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JMS Letters

Dear Sir,

Matrix-assisted Laser Desorption/Ionization with a Tunable Mid-infrared Optical Parametric Oscillator

Matrix-assisted laser desorption ionization (MALDI) is usually performed using an ultraviolet laser, either a nitrogen laser at 337 nm or the third or fourth harmonic of an Nd:YAG laser at 355 or 266 nm, respectively.[1,2] Although not as prevalent, infrared lasers have been used since early in the development of MALDI. The Er:YAG laser with a wavelength of 2.94 μm has been used extensively by Hillenkamp and co-workers.[3–8] A pulsed CO_2 gas laser operating at 10.6 μm has also been used for MALDI.[9] General features of IR MALDI mass spectra are a propensity for multiple charging of the analyte, a greater quantity of sample material removed on each laser shot and an increased propensity to fragmentation with some analytes. The large quantity of material removed with IR lasers can lead to shot to shot instability and difficulties with mass spectra averaged over a large number of laser shots. More recently, tunable infrared sources such as the free-electron laser[10] and a tunable Cr:LiSAF laser pumped optical parametric oscillator[11] (OPO) have been employed. The free electron laser can be tuned from 2.0 to 9.5 μm, but is large and expensive, requiring an MeV electron beam for operation. The Cr:LiSAF pumped OPO is a promising mid-infrared light source, but currently suffers from low pulse energy and poor shot to shot stability.

There are several possible benefits of IR MALDI. First, a wide range of potential matrix materials is made available which utilize strong mid-IR vibrational absorption bands. One promising matrix material is ice, which would appear to be the natural matrix for biological molecules.[8] A second advantage is the increased propensity for fragmentation with some analytes, e.g. oligonucleotides, which can be used to obtain additional structural information.[6,7] Another advantage is that IR MALDI mass spectra can in some cases be obtained with almost complete suppression of low m/z signals due to matrix ions[3,10] which can obscure low-mass analyte ions or lead to detector saturation and loss of sensitivity. Finally, IR MALDI can be used to investigate desorption and ionization mechanisms. Comparison of mass spectra obtained at wavelengths corresponding to different matrix vibrational absorption bands may reveal details of how analyte molecules are desorbed from the surface and ionized.

We have recently employed a commercial tunable OPO system (Mirage 3000B, Continuum, Santa Clara, CA, USA) with a MALDI time-of-flight mass spectrometer. The mass spectrometer is a conventional design[12] with a 1 m flight tube and dual 18 mm microchannel plate detector. The OPO is a two-stage device consisting of a non-resonant oscillator cavity (NRO) and an optical parametric amplifier (OPA). The NRO contains two KTP (potassium titanyl phosphate) crystals in a non-resonant cavity pumped by the 532 nm doubled Nd:YAG output. The 1.45–2.12 μm NRO idler beam is directed into the second-stage single-pass OPA which has two KTP crystals pumped by the 1064 nm Nd:YAG fundamental. The OPA idler output is tunable from 2.12 to 4.0 μm and the signal output is tunable from 1.45 to 2.12 μm. The pulse width is 5 ns and the maximum output energy is 10 mJ at 2 μm and decreases to 0.5 mJ at 4 μm. The IR linewidth is approximately 8 cm^{-1}. IR radiation is directed through a sapphire vacuum window and into the mass spectrometer using gold-coated mirrors. The laser is focused using a CaF_2 lens to a spot size of 100 × 200 μm, which represents the 50% energy contour, assuming a Gaussian beam profile.[13] Attenuation of the Nd:YAG pump beam results in shot to shot instability of the OPO, therefore attenuation is accomplished by inserting several microscope slides into the IR beam path. The threshold laser irradiance is estimated to be 10 MW cm^{-2} with pulse energies measured after attenuation.

Samples were prepared using the matrix compounds succinic acid (butane-1, 4-dioic acid) (Fisher Scientific, Fairlawn, NJ, USA) and caffeic acid (3,4-dihydroxycinnamic acid) (Aldrich Chemical, Milwaukee, WI, USA) and analytes bovine insulin (Sigma, St Louis, MO, USA) and horse heart cytochrome c (Sigma), all of which were used without further purification. Nitrocellulose (Alltech, Deerfield, IL, USA) was dissolved at 5 mg ml^{-1} in 80% (v/v) methanol–acetone. It has been shown in a UV MALDI study that nitrocellulose can improve ion yields in addition to sample reproducibility when co-deposited with matrix and analyte.[14] Samples were prepared by first depositing 10 μl of the nitrocellulose solution on the probe tip and allowing it to dry. Following that, 15 μl of saturated matrix solution and 15 μl of 1–2 mM analyte were deposited on the probe and allowed to dry.

A mass spectrum of horse heart cytochrome c in succinic acid matrix at a wavelength of 3.0 μm is shown in Fig. 1. The mass spectrum is the average of 25 laser shots at a laser irradiance of 12 MW cm^{-2}. We found that when succinic acid was used in conjunction with nitrocellulose, all 25 laser shots could be obtained from the same position on the sample (although it was difficult to obtain more than 25 shots). This result is in contrast with previous reports of rapid signal reduction following even a single laser shot.[3,10,11] It appears that a homogeneous sample surface is particularly important with IR MALDI. This is consistent with the observation of Cramer *et al.*[10] that sample durability was improved when steps were taken to assure sample homogeneity. Many of the features seen in Fig. 1 which are unique to IR MALDI have been reported previously. For example, multiple charging is a common feature, although it is highly dependent on both the matrix and the analyte used.[3,10] Tailing of the analyte peaks to high mass was observed in the FEL study, and is probably due to unresolved adduct peaks.[10] The suppression of low-mass matrix and alkali metal impurity peaks is an advantageous feature of IR MALDI.[3,10] An additional reason for the low alkali metal ion signal is the nitrocellulose co-matrix, which has been shown to suppress the signals from free alkali metal ions and alkali metal ion adducts.[14]

Eight mass spectra of bovine insulin (M_r = 5733.6) with a succinic acid matrix taken at wavelengths between 2.88 and 3.5 μm are shown in Fig. 2. Succinic acid was chosen as the matrix for this study because it gave reproducible mass spectra from sample to sample. In this wavelength region sample absorption is likely owing to the carboxylic acid OH of the matrix and possibly to OH and NH stretching vibrations of the analyte. Each mass spectrum in Fig. 2 is an average of 25 laser shots at an energy within 10% of the threshold at the indicated wavelength. Both the $[M + H]^+$ and $[M + 2H]^{2+}$ peaks are observed, although the relative intensity of the doubly charged ion peak is greater near 3 μm where the peak area is half as large as the singly charged ion

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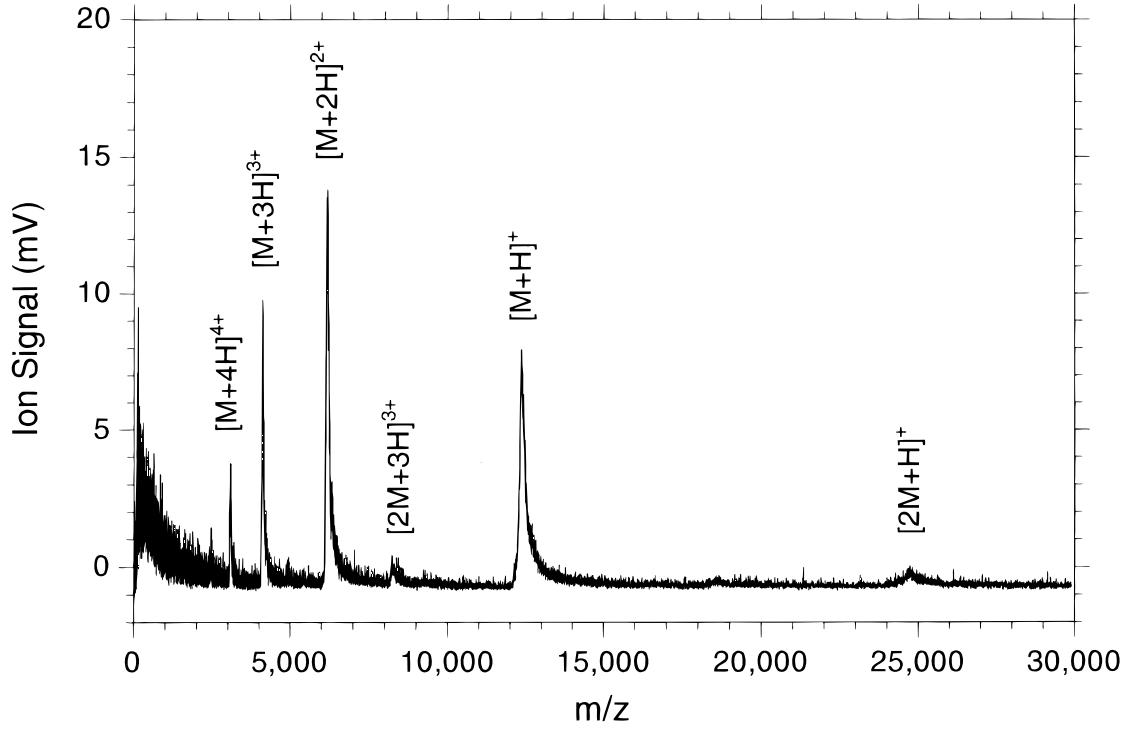

Figure 1. Mass spectrum of cytochrome *c* at 3.0 μm with a succinic acid matrix and nitrocellulose co-matrix (25 shot average).

peak. Below 2.9 and above 3.2 μm, the doubly charged ion peak is less than one fifth as intense as the singly charged ion peak. The mass resolution is the largest at 2.9 μm where it is 200 FWHM, and it decreases to 30 at 3.5 μm. The threshold irradiance for ion production was found to be constant between 2.9 and 3.5 μm at a value of 10 MW cm^{-2}. Below 2.9 μm, the threshold increased by a factor of two and below 2.88 μm no ion signal could be obtained. No analyte signal could be obtained at 2.7, 2.8, 2.85 and 2.875 μm, even when the irradiance was increased to >100 MW cm^{-2}. Note that a matrix signal could be obtained at all wavelengths tested, even when the signal due to analyte ions could not be obtained. The region between 3.6 and 4.0 μm was searched at 100 nm intervals, and the H atom stretch overtone region between 1.45 and 1.7 μm was searched at 50 nm intervals, but also gave no analyte signal.

Preliminary results suggest that the IR MALDI wavelength response depends on the matrix used. A mass spectrum of bovine insulin obtained at 2.73 μm with the caffeic acid matrix is shown in Fig. 3. Caffeic acid has both phenolic and carboxylic acid OH groups, which may extend the matrix absorption further to the blue.[15] Mass spectra obtained with caffeic acid tended to give a greater analyte signal and lower matrix signal than with succinic acid; however, sample to sample consistency was poor by comparison. Insulin samples prepared under otherwise identical conditions with a succinic acid matrix gave no observable analyte signal at 2.73 μm; the shortest wavelength that gave a signal was 2.88 μm (Fig. 2). These results suggest that it may be possible to obtain mid-IR spectra of MALDI signal intensity as a function of wavelength, and that these MALDI response spectra may be unique to a given matrix. At this point, however, it is not possible to exclude other potential effects on wavelength response such as the presence of residual solvent or water, matrix and analyte interactions, or differences in sample crystallization.

In conclusion, we have demonstrated mid-infrared MALDI over a wide range of wavelengths using an OPO which is tunable from 1.4 to 4 μm with more than 100 MW cm^{-2} irradiance. It was found that nitrocellulose added as a co-matrix greatly increased the number of analyte ion-forming laser shots that could be obtained from same sample spot. With a typical sample preparation, at least 25 laser shots could be obtained from a single spot. In a wavelength study using bovine insulin and a succinic acid matrix, it was found that mass resolution declined above 2.9 μm and signal decreased slowly above 3 μm until no analyte signal could be detected above 3.5 μm. The signal decreased more quickly below 2.9 μm, dropping from a signal-to-noise ratio from >10 at 2.88 μm to no detectable signal 5 nm lower at 2.875 μm. Insulin samples prepared with a caffeic acid matrix gave an ion signal down to 2.73 μm. The results from this study and others using tunable infrared sources[10,11] suggest that it may be possible to obtain wavelength spectra of the MALDI response. Such wavelength spectra may provide new insights into the mechanism of infrared desorption and ionization and identify wavelength regions that provide superior analytical performance. Important to such studies will be the ability to obtain reproducible mass spectra over a large number of laser shots. Nitrocellulose has been shown both here and elsewhere to aid in sample durability,[14] and we are investigating both a rotating sample stage and aerosol sample introduction[16,17] as a means to generate reproducible mass spectra over long time periods. Other modifications under way include delayed extraction for improved mass resolution[18] and a cooled sample stage for ice matrix studies.

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Yours,

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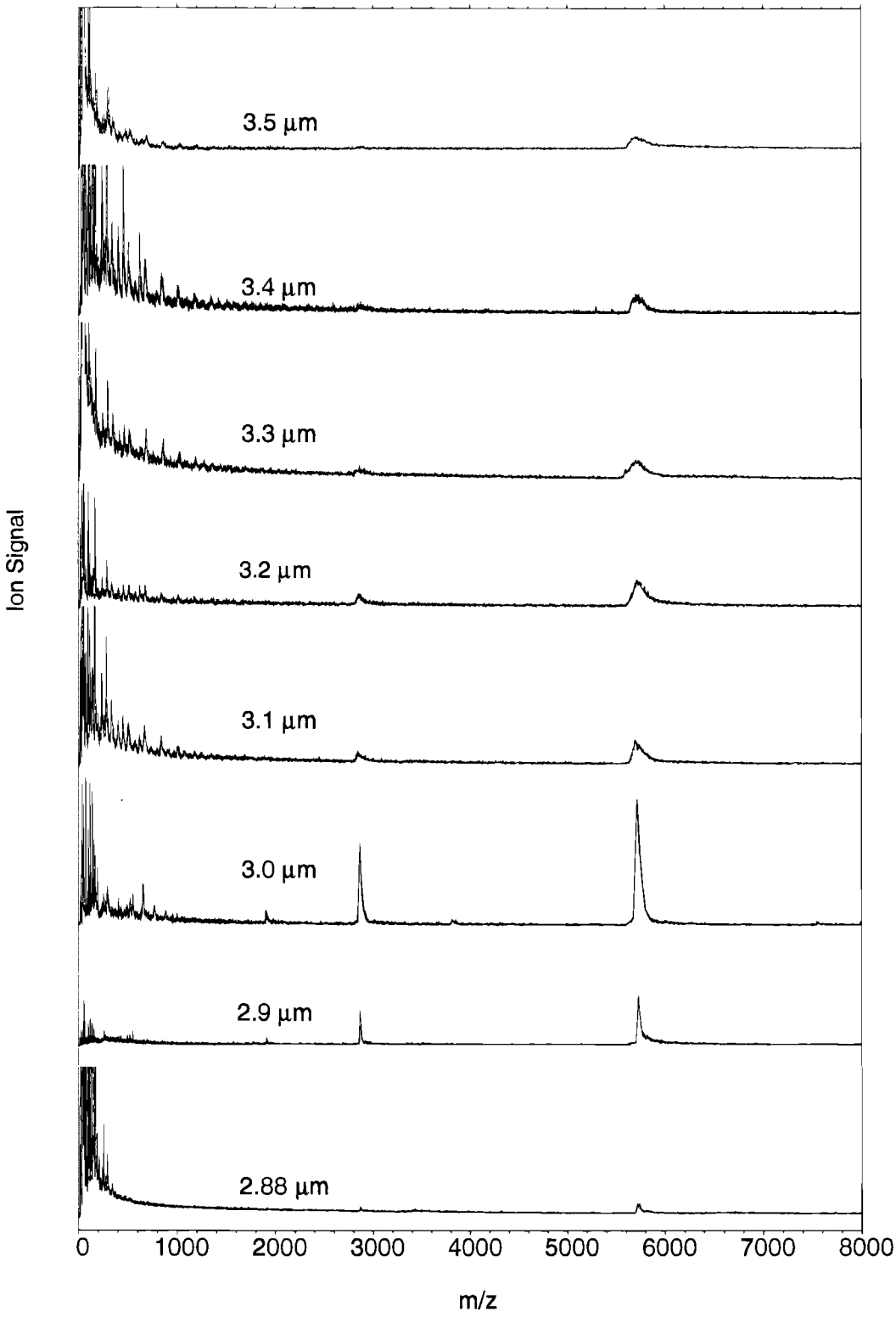

Figure 2. Mass spectra of bovine insulin in a succinic acid matrix at OPO wavelengths between 2.88 and 3.5 mm. Mass spectra are the average of 25 laser shots.

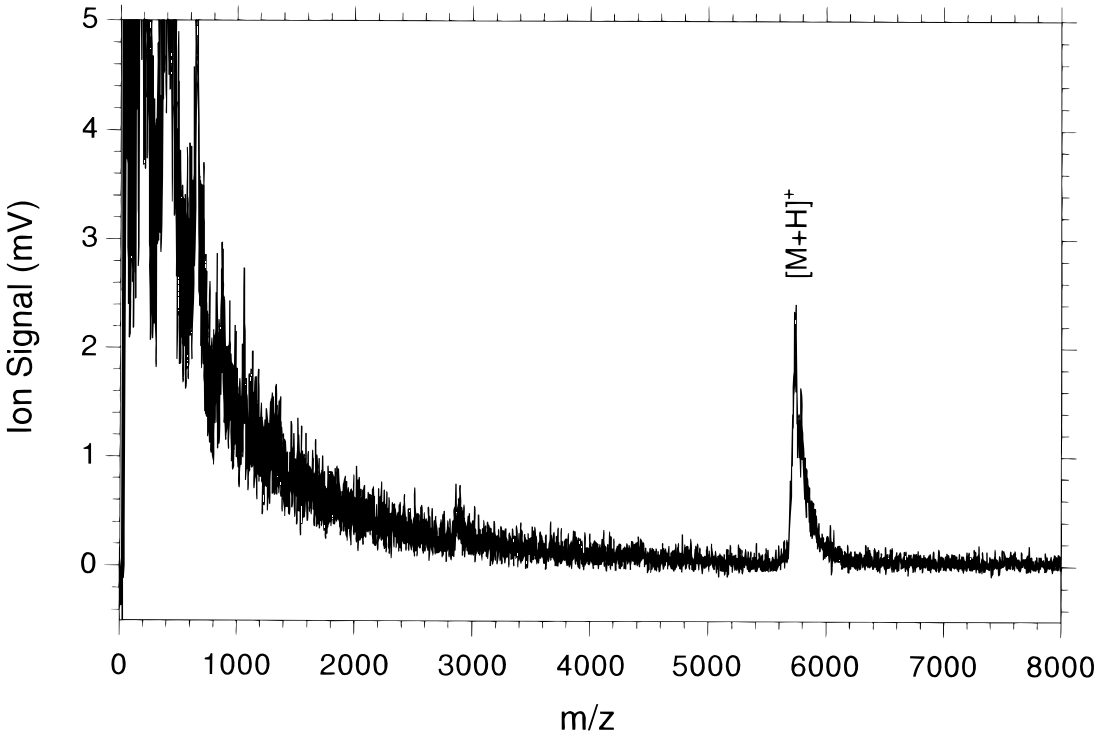

Figure 3. Infrared MALDI mass spectrum of bovine insulin at 2.73 μm with a caffeic acid matrix (25 shot average).

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