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Mass Spectrometry of Low Molecular Mass Solids by Matrix-assisted Laser Desorption/Ionization

Steven C. Goheen, Karen L. Wahl, James A. Campbell* and Wayne P. Hess*
Pacific Northwest National Laboratory, P.O. Box 999, Richland, Washington 99352, USA

Matrix-assisted laser desorption/ionization combined with time-of-flight mass spectrometry (MALDI/TOF-MS) was used for the analysis of low molecular mass compounds. Three classes of molecules were studied: organic acids, salts of oxyanions and amine-based chelating compounds. Mass spectra from samples of citric, propionic, butyric, oxalic and stearic acid; ethylenediaminetetraacetic acid (EDTA), *N*-(2-hydroxyethyl) ethylenediaminetriacetic acid (HEDTA), ethylenediamine *N,N'*-diacetic acid (EDDA) and nitrilotriacetic acid (NTA); and sulfate, nitrate, nitrite and phosphate salts were obtained. These species were analyzed alone and as mixtures in both the positive and negative ion modes. The organic acids and oxyanion salts displayed much stronger signals in the negative ion detection mode whereas chelating compounds, which contain basic amine functional groups, yielded stronger signals in the positive ion mode. This implies that detection sensitivity is often better for a particular ion mode in the analysis of small molecules containing limited classes of functional groups. In all analyses, the presence of high concentrations of sodium was found to quench the MALDI signals. To increase the detection sensitivity, some samples were processed through an ion-exchange column to remove sodium ions. This step was found to enhance the signal by two orders of magnitude over untreated samples.

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INTRODUCTION

The matrix-assisted laser desorption/ionization (MALDI) technique developed by Karas and Hillenkamp[1] and Tanaka *et al.*[2] was designed to overcome the limitations of laser desorption ionization and provide a simple method for introducing high molecular mass (HMM) (>5000 Da) species directly into the gas phase in both neutral and ionic form. For HMM molecules, the associated technique of laser desorption/ionization (LDI) often yields only highly fragmented ion spectra. Indeed, even for many smaller molecules only molecular fragments and elemental constituents are detected. Intact desorption of HMM species by LDI is especially difficult because such species are thermally labile and desorb only at high laser fluences.[3,4] For large molecules, laser ionization competes poorly with fragmentation owing to rapid intermolecular energy transfer. The rapid energy transfer dramatically reduces ionization efficiency[5] and can result in severe fragmentation. MALDI solves the problems associated with intact vaporization and ionization for large molecules and has had much success in analysis of HMM species.[6]

Using MALDI, the mass spectrometry (MS) of very large polymers, biomolecules[7–10] and a variety of thermally labile materials has been accomplished.[11] Many studies have focused on extending the high-mass regime,[1,2,12] with masses over 10^6 Da detected. An experimental disadvantage of targeting low-mass analytes is that the analyte and matrix masses are similar and may interfere. The prospect of such interferences is significant as the optimum matrix to analyte molar ratio is usually high, of the order of 10^3–10^5 to 1.[13,14] Our interest in extending the MALDI technique to the analysis of complex mixtures of low molecular mass (LMM) molecules is derived from the need for speciation of mixed hazardous wastes. Mixed waste is defined as waste containing both hazardous chemicals and radionuclides. A few recent MALDI studies have focused on the medium to low molecular mass nonvolatile compounds,[11,15–18] but we know of no MALDI studies of mixtures of such compounds.

Chelating compounds such as ethylenediaminetetraacetic acid (EDTA) and *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA) are not part of the Environmental Protection Agency's (EPA) list of hazardous chemicals; nevertheless, they are receiving renewed environmental interest.

Large quantities of EDTA and HEDTA (~240 and 1500 tons, respectively) are stored in mixed hazardous waste tanks at the Department of Energy Hanford site.[19] These chelators form water-soluble complexes with most heavy metals, thereby enhancing the migration of heavy metals in soils. For example, studies at

* *Correspondence to*: J. A. Campbell and W. P. Hess, Pacific Northwest National Laboratory, P.O. Box 999, Richland, Washington 99352, USA.

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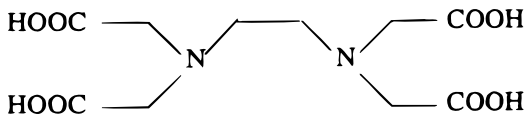
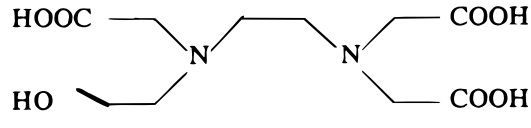


EDTA



HEDTA

Oak Ridge National Laboratory demonstrated that EDTA caused the low-level migration of ^{60}Co from intermediate-level liquid waste disposal pits and trenches.[20] Another study at the Maxey Flats commercial low-level waste disposal site revealed that Pu-EDTA and ^{60}Co-EDTA migrated.[21]

Analytical methods for chelators and organic acids in radioactive wastes generally involve derivatization and gas chromatography,[22,23] which is a time consuming approach. Alternatively, liquid chromatography[24] has been used for organic analysis of radioactive wastes but is hindered by additional hazardous waste generation and co-elution problems in complex mixtures. The MALDI technique should prove valuable in the analysis of LMM waste compounds for several reasons. Owing to the nature of the gentle ionization processes, chelators and other non-volatile species should remain intact during analysis without the need for time-consuming derivatization procedures. When MALDI is combined with time-of-flight (TOF) MS, an entire mass spectrum can be measured in a single analytical step. Since the MALDI process produces both positive and negative molecular ions directly and very little material is required, MALDI is an efficient micro-sampling technique that minimizes radiation exposure in the analysis of highly radioactive tank waste samples. Additional virtues of MALDI-TOF include limited sample preparation and minimal hazardous waste production.

We have used MALDI/TOF to examine anions, organic acids and chelators that are known to be present in storage tanks containing mixed waste.[25] Samples of EDTA, HEDTA, citric acid, nitrilotriacetic acid (NTA) and several of the inorganic anions including sulfate, phosphate, nitrate and nitrite were analyzed using MALDI/TOF in high- and low-salt conditions. Both high-salt and high-pH conditions are features of mixed hazardous tank wastes. Samples containing high salt concentrations can dramatically decrease the MALDI sensitivity, thus severely deteriorating the analytical results.[12] In order to improve the MALDI sensitivity for high salt content samples, we have processed some samples using cation exchange. In addition, we performed MALDI on mixtures of these molecules to reflect the combinations of compounds found in complex mixed wastes. The technical aspects of low- and high-molecular mass MALDI will be considered and the benefits of obtaining both positive and negative ion spectra highlighted.

EXPERIMENTAL

All data were obtained using a commercial linear TOF instrument designed for UV MALDI (Kratos Kompact MALDI II). This instrument has a nominal mass resolution of $m/\Delta m = 600$ (FWHM), and can be operated in the positive or negative ion detection mode. A 337 nm nitrogen laser, of 3 ns pulse duration, is used to induce the desorption/ionization process. The output of the nitrogen laser is focused to a rectangular spot 200 × 100 μm at the sample tray. The laser power was varied using a calibrated attenuator; power was generally set to a range between 40% and 80% above the threshold for ion appearance. This range was found to be optimal for several analytes as higher laser power degraded the signals by reducing the mass resolution and by increasing ion interferences due to fragmentation and matrix ionization. Both positive and negative ions are produced during MALDI and often provide complementary information. Switching between negative and positive ion detection modes is easily accomplished by switching ion optic and detection voltages.

The matrix material 2,5-dihydroxybenzoic acid (DHB) was used in all experiments. In most cases, the DHB matrix was dissolved in water to a concentration of 10 mg ml^{-1} to produce a nearly saturated solution. Analyte molecules were dissolved in water to the same concentration and combined, in various proportions, with the matrix solution. A 1.0 μl volume of sample was applied to each spot on the sample tray. The sample tray consisted of a 8 × 1 cm stainless-steel plate with 20 indentations of 500 μm depth and 2 mm width. The MALDI spectrometer was programmed to scan the focused laser beam along each individual sample spot. In all analyses, the signals from 100 laser pulses were averaged as the laser was scanned. Sample spots were prepared in duplicate, dried in air for 10 min, introduced into the vacuum chamber and immediately analyzed. Samples left in the instrument and analyzed several (>10) h later were found to give less consistent results.

Since we are interested in determining the constituents of complex sample mixtures, we performed MALDI on mixtures of several analytes, including oxalic acid, citric acid, ammonium sulfate and ammonium nitrate. We parameterized the MALDI technique for the analysis of LMM molecules by measuring the effects of varying the matrix to analyte molar ratio, the laser fluence and the sample pH on the analyte base peak ion signal. The sample solution pH was varied for both citric acid and HEDTA analytes before the samples were applied to the sample tray. The pH values were adjusted to ~2, 5 and 10, respectively, using 0.1% trifluoroacetic acid, purified water and 1% ammonia solution. We varied the matrix to analyte molar ratio from 1000:1 to 0.1:1 to determine the optimal concentrations for MALDI of LMM molecules. Our empirical observations indicate that a matrix to analyte molar ratio of 100:1 produced the most consistent ion signals.

Early MALDI studies noted the deleterious effects of small relative concentrations of various salts in the analyte mixture[12] and particular matrix compounds

were marked for their resistance or susceptibility to salt interferences.[13] For our purposes, we wish to analyze samples containing very high sodium salt concentrations. We find that high sodium concentrations reduce the analyte ion signal dramatically in both the positive and negative modes. To enhance the analyte ion signals, we removed the sodium ions by processing some samples using ~2 ml of an ammonium form ion-exchange column generated from the ion-exchange material AG 50W-X8 (20–50 mesh, H-form) and ammonia solution. A 1.0 ml portion of a 100 mg ml^{-1} solution of sodium sulfate was passed through the ion-exchange column, then 9.0 ml of deionized water was used to elute the sulfate through the column. The ion-exchange column was calculated to have more than twice the capacity needed to convert all the sodium sulfate into ammonium sulfate. The eluted material (now at 10 mg ml^{-1}) was mixed with matrix and handled as described previously.

Reagent-grade sodium sulfate was obtained from EM Science (Cherry Hill, NJ, USA) reagent-grade ammonium sulfate from J. T. Baker (Phillipsburg, NJ, USA), ammonium phosphate, ammonium nitrate, high-purity NTA, HEDTA, Na_2EDTA, imminodiacetic acid (IDA), citric acid, and ethylenediamine-*N*,*N*′-diacetic acid (EDDA) from Aldrich Chemical (Milwaukee, WI, USA), oxalic acid from Chem West (Seattle, WA, USA) and fumaric acid, maleic acid and succinic acid from Chem Services (West Chester, PA, USA). The ion-exchange resin AG 50W-X8 (20–50 mesh, H-form) was obtained from Bio-Rad Laboratories (Richmond, CA, USA). The DHB matrix was purchased from Sigma Chemical (St Louis, MO, USA).

RESULTS

We applied MALDI/TOF-MS to the analysis of LMM organic acids, chelators and salts of oxyanions. The positive ion MALDI mass spectra of the chelators typically have strong peaks indicative of the protonated and sodium-adducted intact molecule. Figure 1 shows the positive ion MALDI mass spectra of HEDTA and Na_2EDTA. Each spectrum displays strong protonated $[M + H]^+$ and sodium-adducted $[M + Na]^+$ peaks, where M represents the free acid HEDTA or disodium EDTA. The Na_2EDTA spectrum also displays $[Na_2EDTA - Na + 2H]^+$. At lower *m/z* strong ion signals attributable to the DHB matrix are observed; $[DHB + H - H_2O]^+$ at *m/z* 137, $[DHB + H]^+$ at *m/z* 155 and $[DHB + Na]^+$ at *m/z* 177.

Figure 2 shows the negative ion MALDI mass spectrum of citric acid. The strong negative ion signal is attributed to the deprotonated parent molecule $[M - H]^-$ at *m/z* 191. Figure 2 illustrates how little low-mass matrix interference can be present in the negative ion detection mode as only a single strong matrix peak $[DHB - H]^-$ at *m/z* 153 is observed for *m/z* <300. Ehring *et al.*[26] also noted that negative ion MALDI typically produces such 'clean' mass spectra. The analytes studied and the *m/z* values and assignments of the ions observed in positive and negative ion MALDI/MS are shown in Table 1. Inspection of Table 1 reveals an interesting feature: the amine-based chelating compounds yield strong signals in the positive ion mode whereas the small organic acids and oxyanions yield strong signals in the negative ion mode. We

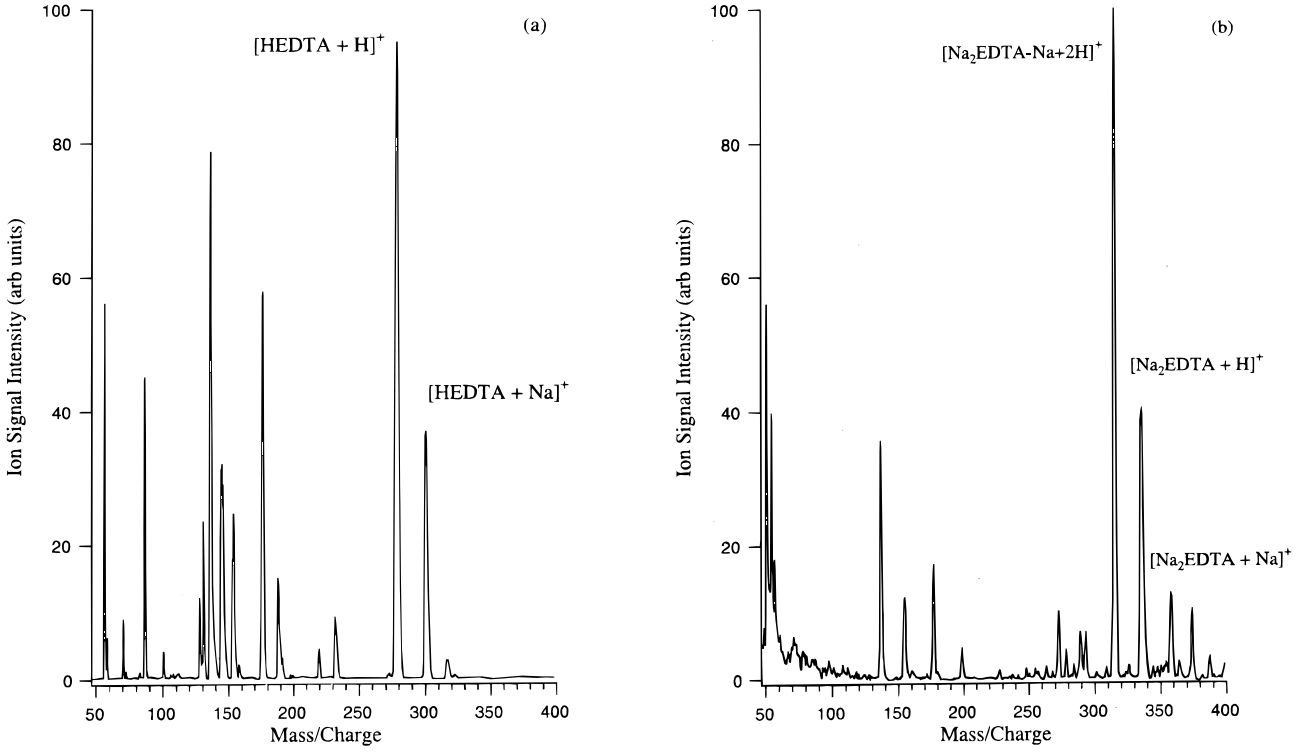


Figure 1. Positive ion MALDI mass spectra for (a) HEDTA and (b) Na_2EDTA in DHB at a matrix:analyte molar ratio of 100:1. The protonated $[M + H]^+$ and sodiated $[M + Na]^+$ peaks are identified. Note the progression of sodiated peaks in (b) at *m/z* 315, 337 and 359. The peaks at *m/z* 137, 155 and 177 are attributed to ionized matrix species as described in the text.

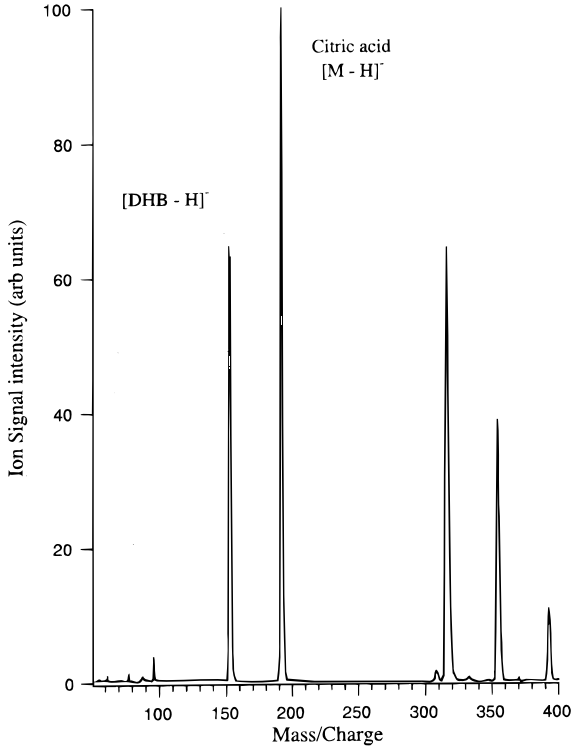


Figure 2. Negative ion MALDI mass spectrum for citric acid in DHB at a matrix:analyte ratio of 100:1. The spectrum displays only two significant peaks for m/z <300: the $[M-H]^-$ base peak at m/z 191 and the single matrix peak $[DHB-H]^-$ at m/z 153. The mass peaks for m/z >300 are associated with the matrix material and are present only in the negative ion mode. The lack of matrix fragmentation is characteristic of the negative ion MALDI of small molecules in this work.

applied both positive and negative ion detection modes to each compound. Whereas only weak mass peaks attributable to the small organic acids and oxyanions could be identified in the positive ion detection mode, intense mass peaks attributable to positive matrix ions were observed. Similarly, only weak mass peaks attributable to the chelating compounds could be identified in the negative ion detection mode, although intense mass peaks attributable to negative matrix ions were also observed. In several cases, unambiguous analyte mass peaks could only be identified in either the negative or positive ion detection mode.

An important experimental consideration in MALDI analysis is the matrix:analyte molar ratio. For smaller molecules, it has been suggested that a ratio of 100:1 to 1000:1 is optimal.[27] This ratio contrasts with optimal ratios of 1000–10 000:1 for HMM species.[28] It is possible that the greater matrix:analyte ratios determined for HMM species are necessary to fully surround the larger molecules. To determine the optimal matrix:analyte ratio of LMM species, we varied the ratio over several orders of magnitude and monitored the effect on the analyte ion signal intensity. Figure 3 shows a graph of the base peak ion signal intensity *vs.* the matrix:analyte molar ratio for the range 1000:1 to 0.1:1. For sulfate ion detection in the negative ion mode, a matrix:analyte molar ratio of 100:1 provides a strong signal, and we find that the best signal-to-noise ratio and most consistent signal response is obtained at this molar ratio. We note, however, that strong but less consistent signals are obtained at the lower matrix to analyte ratios of 10:1 and even at ratios as low as 0.1:1. While such low matrix:analyte ratios are clearly outside the regime normally considered to be MALDI, we must stress that no ions are observed without the minor addition of matrix. The observation of strong MALDI signals from low matrix:analyte samples suggest that current concepts of MALDI vaporization and ionization mechanisms may need to be extended. The possibility exists, however, that the ions observed at very low matrix:analyte ratios are actually derived from local regions containing higher matrix concentrations within the crystallized sample. Further study of the detailed crystallization in such systems is needed to resolve these issues.

For certain environmental analysis applications, such low matrix:analyte ratios may be useful. For example, the analysis of contaminated soil samples may not allow a full dissolution of contaminants adsorbed to soil or rock. In this case, the application of a top layer of matrix material followed by standard MALDI/MS could produce molecular ion signals from the adsorbed contaminant under conditions similar to the lowest

Table 1. Positive and negative ions observed by MALDI/TOF-MS

Analyte	Molecular mass	Ions observed (m/z) (−)	(+)	
Sodium nitrate	85	62 $[NO_3]^-$		
Sodium nitrite	69	46 $[NO_2]^-$		
Oxalic acid	90	89 $[M-H]^-$		
Fumaric acid	116	115 $[M-H]^-$		
Maleic acid	116	115 $[M-H]^-$		
Succinic acid	118	117 $[M-H]^-$		
Ammonium sulfate	132	97 $[HSO_4]^-$		
Citric acid	192	191 $[M-H]^-$		
IDA	134		157 $[M+Na]^+$	173 $[M+K]^+$
HEDTA	278		279 $[M+H]^+$	301 $[M+Na]^+$
EDDA	176		177 $[M+H]^+$	199 $[M+Na]^+$
NTA	191		192 $[M+H]^+$	214 $[M+Na]^+$
Na_2EDTA	336		315 $[Na_2EDTA-Na+2H]^+$	337 $[Na_2EDTA+H]^+$
Na_2EDTA	336		359 $[Na_2EDTA+Na-H]^+$	

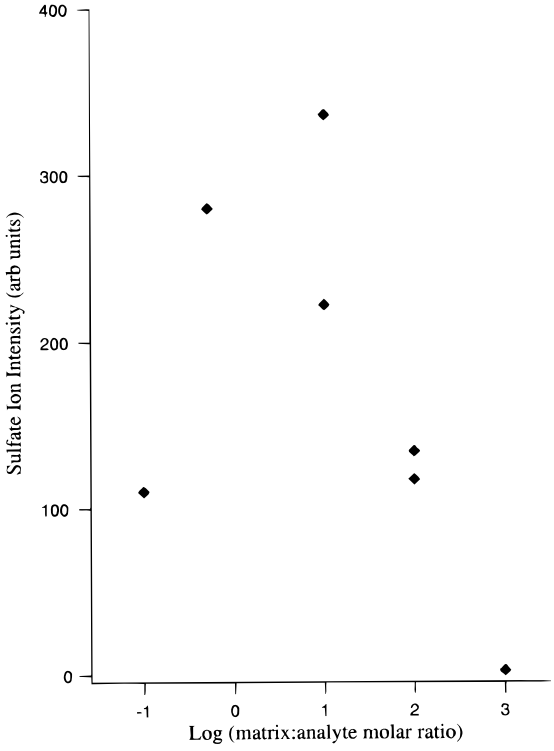


Figure 3. Integrated negative ion signal intensity *vs.* matrix : analyte molar ratio. The ion signal for $[HSO_4]^-$ in DHB is shown over the matrix : analyte molar ratio range of 1000 : 1 to 0.1 : 1. Although strong signals are observed in the range from 10 : 1 to 0.1 : 1, results were more consistent for a molar ratio of 100 : 1.

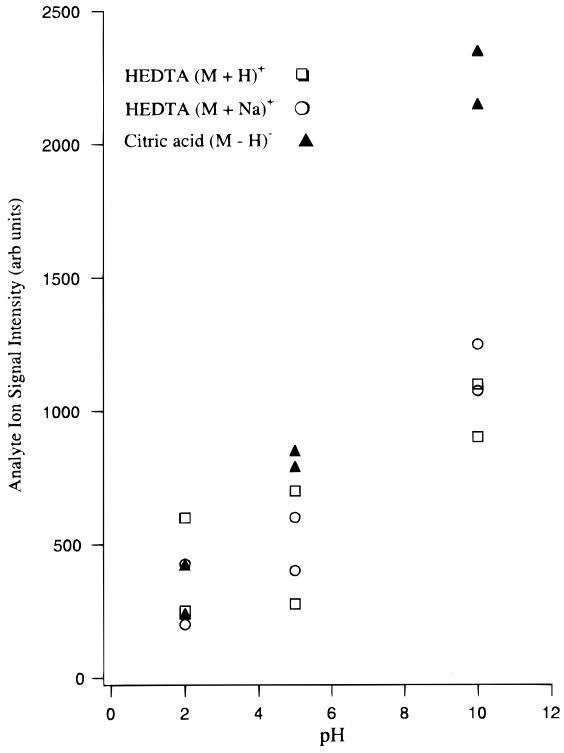


Figure 4. Influence of sample pH on the integrated peak intensity for citric acid and HEDTA analytes. Triangles designate citric acid in the negative ion mode $[M-H]^-$; open squares indicate the protonated HEDTA signal $[M+H]^+$; open circles designate the signal from the HEDTA sodium adduct $[M+Na]^+$. All three peaks increase monotonically with pH over the range 2–10.

matrix : analyte ratio reported here. The optimum matrix : analyte ratio may differ for the various analytes, however, each analyte displayed a correlation similar to that of sulfate, with increasing signal for increasing analyte fraction, over the range 10 000 : 1 to 100 : 1.

In this study, three sample preparation methods were compared. Two layering techniques for sample preparation were examined.[29] The first method involved placing the matrix solution directly on the slide without analyte. The matrix was then air-dried. The sample drop ($\sim$1.0 µl) was applied on top of the matrix and dried. This layering technique was also tried in reverse. That is, analyte was placed on the slide first, then matrix. The third method was the standard preparation using a mixture of analyte and matrix. The highest signal intensity was consistently obtained from the standard mixed analyte and matrix sample preparation method. Although layering methods produced about an order of magnitude less signal than mixing, such techniques may still prove suitable for certain environmental analysis purposes.

We varied the initial sample pH to determine the effect on positive and negative ion modes for HEDTA and citric acid analytes, respectively. A strong base peak was observed for citric acid in the negative ion mode $[M-H]^-$ at *m/z* 191. Ions indicative of HEDTA were observed as $[M+H]^+$ at *m/z* 279 and $[M+Na]^+$ at *m/z* 301. Figure 4 displays the base peak signal strength for samples containing citric acid and HEDTA prepared at pH 2, 5 and 10. All six samples were analyzed using both positive and negative ion detection modes. The strongest signal for all analyte ions was from samples of the highest pH regardless of detection mode polarity. The trend for increasing base peak signal intensity with pH is consistent with results obtained from peptides (*m/z* 800–2000) in the limited pH range from 1.1 to 2.9 using 4-hydroxy-α-cyanocinnamic acid as the matrix.[30]

Hazardous waste is often a very complex mixture containing dozens of compounds. It was anticipated that, at the lowest matrix : analyte molar ratios, multiple analytes might interfere with one another, or that some analytes would preferentially associate with the matrix, resulting in variable sensitivity. Figure 5 displays the negative ion MALDI mass spectrum of a relatively equimolar mixture of citric acid, oxalic acid, ammonium sulfate and sodium nitrate with DHB (matrix : analyte ratio $\approx$1 : 4). All four analytes are readily observable although the nitrate ion signal at *m/z* 62 is considerably weaker than the three other analyte ion signals. Weak nitrate ion intensity is a common feature that has been observed with other similar mixtures. LDI[31] of nitrate compounds is known to fragment the nitrate or nitrite groups, forming NO and O_2 and O atoms,[32,33] although the reasons for low MALDI sensitivity to nitrate are, as yet, unclear.

In an attempt to increase the detection sensitivity in the analysis of samples containing or heavily contaminated with sodium or other alkali metal salts, we used an ion-exchange resin to remove metal cations. Figure 6 displays the MALDI mass spectrum of sodium sulfate (before the ion-exchange process) and ammonium

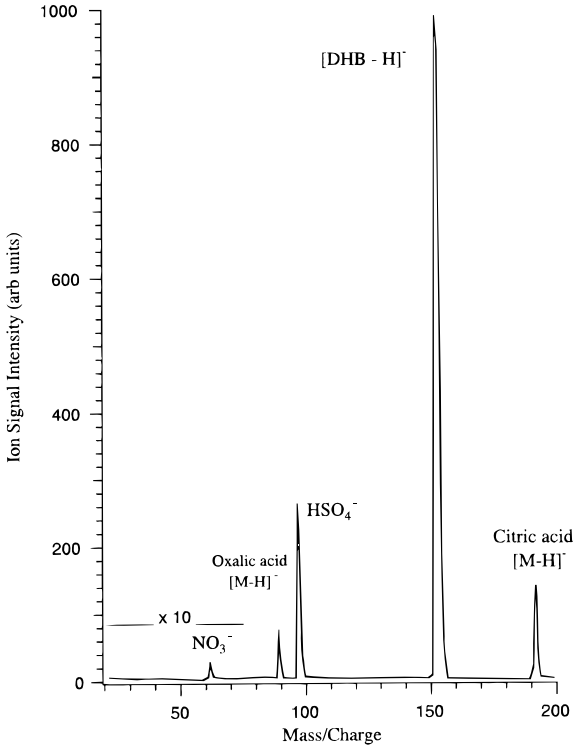


Figure 5. Negative ion MALDI mass spectrum of the analyte mixture consisting of equimolar concentrations of ammonium nitrate, oxalic acid, ammonium sulfate, citric acid and DHB. The matrix : analyte molar ratio is very low at ~1 : 4. It is notable that all four analytes produce detectable signals although the intensity of the NO_3^- peak is significantly weaker than that for the sulfate or organic acid analytes.

sulfate after ion exchange. The absolute ion signal intensity of the HSO_4^- base peak is found to increase by a factor of 140. In addition, the low-mass 'noise' was reduced with respect to the matrix ion signal at m/z 153. The base peak intensity was typically one to two orders of magnitude larger following ion exchange for the same quantity of analyte material.

DISCUSSION

MALDI of LMM compounds

Although many MALDI studies of HMM species have been completed, relatively few studies of small molecules have been reported. Low molecular mass but thermally labile species, while amenable to MALDI analysis, provide unique challenges, and significant technical differences exist between the low- and high-mass regimes. The most significant difference is that the masses of matrix ions, including dimers and fragments, are similar to analyte masses and interferences between matrix and analyte can be significant. Technically, lower extraction and acceleration voltages may be necessary for linear TOF instruments to achieve sufficient mass resolution for LMM compounds, and the high acceleration voltages required for efficient detection of HMM ions are not necessary. If, for these reasons, ions are extracted more slowly, they will experience increased residence time in the desorption plume. The ion chem-

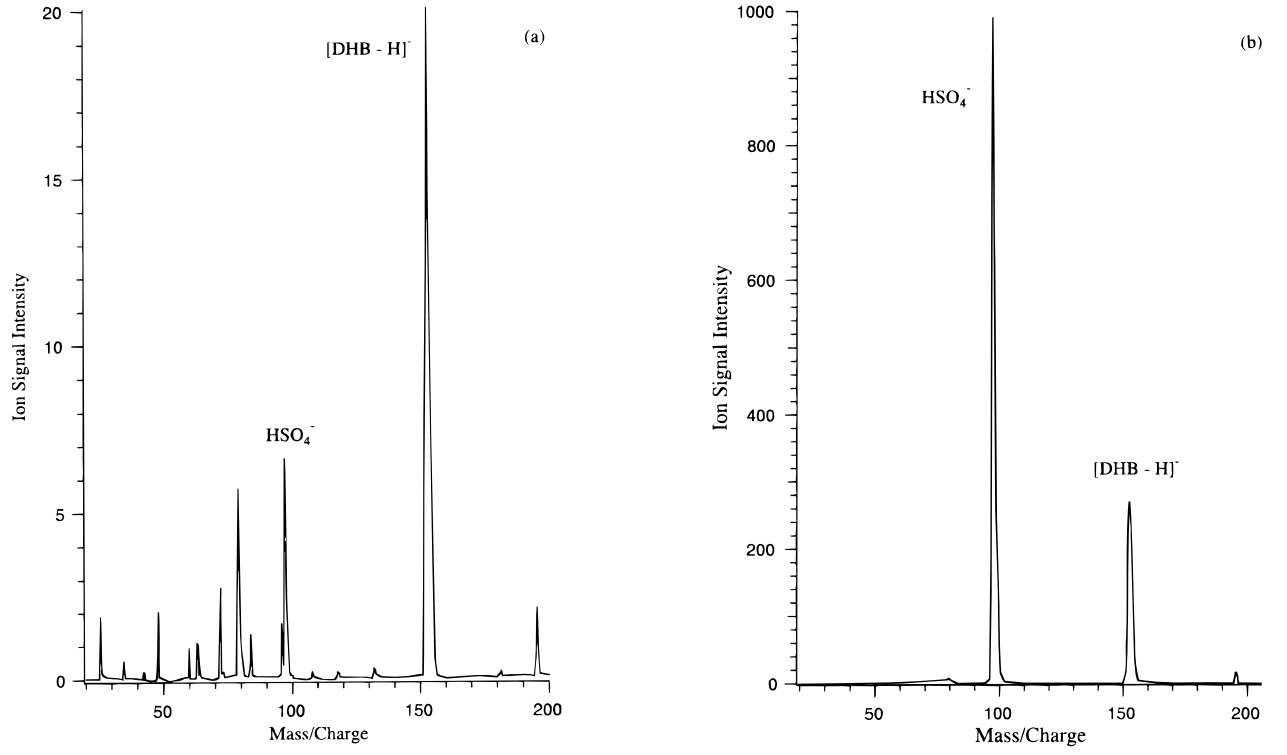


Figure 6. Negative ion MALDI spectrum of sodium sulfate (a) before and (b) after ion exchange. The HSO_4^- base peak increases by a factor of 140 whereas the matrix ion base peak increases by a factor of 15. Note the change in scale between (a) and (b).

istry and yield depends upon desorption plume residence time[34] and hence significant changes in extraction conditions could alter the final ion distribution.

Negative and positive ion modes

The chemistry of large and small molecules provides another significant difference between the two mass regimes. Many HMM biomolecules contain several well separated acidic and basic functional groups. The amphoteric nature of the large molecules probably aids the formation of both negative and positive ions. For smaller molecules either the positive or negative ion mode may be favored. Consider the detection of the organic acids. The acidity of an organic acid is due to the stability of its anion; the more stable the anion, the stronger is the acid. The strong negative ion signals observed for organic acids are a result of the stability of the acid anions. We therefore may reasonably expect strong $[A - H]^-$ base peaks for organic acids. The results in Table 1 confirm this expectation. Similarly, the stable oxyanions nitrate, nitrite and hydrogen sulfate display strong signals in negative ion MALDI.

Since our target chelating compounds EDTA, HEDTA and EDDA are amine derivatives (i.e. strong bases), they form stable positive ions by adduction of a proton (or Na^+) to the basic amine functional groups. Strong parent mass peak signals are observed in the positive ion mode. In studies of small proteins and peptides, Zhu *et al.*[18] found that the positive ion MALDI mode was strongly enhanced by the presence of basic amino acids in the chain. In that work, it was suggested that a proton is transferred in solution from a matrix molecule to a basic amino acid by an acid–base reaction, thus providing a low-energy mechanism for ionization. The concept of pre-formed solid-phase ions leading to gas-phase ion products by direct desorption has been discussed in the fast atom bombardment (FAB) literature. It is thought that an enhanced response is observed because only the physical desorption of the ion need occur, and no ionization step is required.[35] Liao and Allison[35] have recently extended these FAB concepts to MALDI analysis by demonstrating an enhanced response for triphenylphosphium-derivatized peptides in an α-cyano-4-hydroxycinnamic acid matrix.

It is unclear why the negative ion mass peaks indicative of the parent chelating compounds were weak (or undetected). The chelating compounds contain several carboxylic acid functional groups which normally provide suitable sites for the formation of stable negative ions (note the organic acids reported in Table 1). It is possible that the close proximity of the amine groups destabilizes the negative ion sufficiently to prevent its formation. It could also be argued that the formation rate for the positive ion is much more rapid than for the negative ion (for the chelating compounds) and therefore the negative ion yield is exceedingly low. It is unlikely, however, that any such kinetic arguments will prove persuasive because the neutral yield is generally believed to be a factor of 1000 (or more) greater than the ion yield in MALDI.[36,37] Only for a situation where the ion yield surpassed the neutral yield would a kinetic argument appear plausible. The issues surrounding the ion formation step in MALDI are not yet well understood.[36] It is still unclear at what point ionization occurs, whether in the condensed phase,[36] the gas phase[34] or both, and under what particular circumstances.

pH studies

The range of suitable LMM samples was further evaluated by varying the pH of HEDTA and citric acid sample solutions and measuring the positive and negative ion signal intensities, respectively. An increase in base peak signal intensity with pH is evident for both the citric acid $[M - H]^-$ ion at m/z 191 and for the HEDTA ion signals $[M + H]^+$ at m/z 279 and $[M + Na]^+$ at m/z 301. Clearly, sample solution pH had an effect on the ion yield of these two analytes. Ion signals from both positive and negative modes were enhanced at higher pH (although we do not regard the ion signal enhancement for the HEDTA samples as large when compared with signal fluctuations). The pH range covered in this study spans the pK_as of the analytes, and the ion signal increases with pH for both positive and negative ion detection modes. These facts argue against a solution-phase pre-proton transfer as the mechanism responsible for enhanced ionization in these molecules. Our results indicate that the MALDI ion yield is not strongly related to the sample solution oxidation state.

Sodium adduction and interferences

Sodium ion adduction is often observed in MALDI, even for samples that do not contain sodium.[38] In these cases, the sodium impurity is introduced through minor contamination of the matrix material. Nonetheless, sodium adduction competes efficiently with proton adduction at low levels of contamination. For samples containing a significant sodium component, such as Na_2EDTA, we expect and observe a strong $[Na_2EDTA + Na]^+$ peak. We also observe the adduct series $[Na_2EDTA + nNa - (n - 1)H]^+$, $n = -1$ to 1. Although multiple adduction of sodium is rare,[38] such species may reasonably be expected for a tetradentate chelator such as EDTA. The series of peaks at m/z 315, 337 and 359 form a very characteristic pattern which strongly over-determines the data set needed for identification of EDTA in solid samples. This is significant in that prior attempts at detection of chelators have proved difficult as the techniques of LDI and secondary ion mass spectrometry produce severely fragmented mass spectra.[39]

Ion signals obtained from samples heavily contaminated with sodium are very weak compared with the MALDI ion yield obtained after sodium contamination is removed (Fig. 6). A possible mechanism for the reduced analyte ion yield involves the loss of charged species due to interactions with both neutral (Na^0) and ionized (Na^+) sodium atoms. The ionization potential

of isolated sodium neutral is 5.1 eV, significantly lower than that of either the matrix or the analyte. An excess of Na^0 in the desorption matrix or plume could react or exchange charge with positively charged matrix or analyte, thus neutralizing analyte molecules and their ionization source. Since Na^0 requires only $\sim$5 eV to ionize, we may also expect significant Na^+ concentrations in the desorbed plume. Collisions of Na^+ in the matrix plume with negatively charged analyte molecules can rapidly neutralize these species also. Hence high sodium concentrations appear capable of simultaneously degrading both positive and negative ion yields. It is also possible that the high sodium concentration disrupts the matrix itself. Wang *et al.*[34] noted that DHB can separate the analyte from salts by crystallization on the rim of the sample spot. The salt (K^+ and Na^+ ions) congregate in the center of the sample spot leaving the rim crystals as an effective MALDI target.[34] Nonetheless, even a robust matrix such as DHB can tolerate only a limited salt concentration. There are likely other plausible mechanisms for signal degradation by sodium salts.

CONCLUSIONS

We have used MALDI for the analysis of a variety of LMM compounds including organic acids, salts of oxyanions and chelating compounds. The organic acids and oxyanion salts displayed much stronger signals in the negative ion detection mode whereas chelating compounds, which contain basic amine functional groups, yielded stronger signals in the positive ion mode. This implies that MALDI detection sensitivity is often better for a particular ion mode in the analysis of small molecules containing limited classes of functional groups. We varied the matrix:analyte molar ratio and found that parent mass peaks can be detected over a very wide range. This feature of MALDI analysis may prove useful in certain environmental applications. We varied the sample solution pH from 2 to 10 and obtained strong MALDI signals across this range with the most intense signals obtained at the highest pH. In our efforts to apply MALDI to the analysis of high sodium content samples, we removed sodium by cation exchange. The exchange of NH_4^+ for Na^+ yielded a sensitivity increase of a factor of $\sim$100. It is anticipated that by using this ion-exchange process a wide variety of small molecules contained in high salt content mixtures can be easily analyzed by MALDI. Similarly, the simultaneous detection of combinations of analytes is promising for obtaining semiquantitative analyses of more complex mixtures. Future efforts will be directed at advancing quantitative aspects of MALDI for LMM compounds because, at present, ion signals are often erratic. Progress toward quantitative analysis of HMM species has occurred only recently for well characterized samples.[27,40–45] The difficulties encountered in attempts to obtain quantitative yields are due to the many factors which determine MALDI efficiency, such as matrix/analyte effects, laser wavelength and power, morphological effects in sample crystallization[46,47] and the complex nature of the MALDI technique. As yet, most of the operative mechanisms involved in MALDI are not well understood.[6,26,38,48–50] Nonetheless, recent progress using internal standards,[27,31,40,44,45] data analysis techniques[43] and co-matrices[41] is encouraging and we expect that, with more work, MALDI will become a reliable quantitative analysis technique for a wide range of molecular species.

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