Anal. Chem. **2002,** *74,* 4841−4844

Technical Notes

Matrix Addition by Condensation for Matrix-Assisted Laser Desorption/Ionization of Collected Aerosol Particles

Shelley N. Jackson and Kermit K. Murray*

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

Condensation of an ultraviolet absorbing liquid matrix onto aerosol particles was used to enhance the ionization efficiency of large molecules. Laboratory-generated particles were coated with matrix, deposited on a sample target, and analyzed by laser desorption mass spectrometry with no other matrix addition. The aerosol was generated in a Collison nebulizer, and the particles were dried in a diffusion dryer before entering a heated region saturated with the liquid matrix 3-nitrobenzyl alcohol (NBA) and then entering a cooled condensation region. Matrix-coated particles were collected on a sample target and analyzed using a 337-nm laser and a time-of-flight mass spectrometer. Particles containing the peptides gramicidin S and gramicidin D were analyzed both with and without the matrix addition step. Condensation addition of matrix increased the biomolecule ion signal and resulted in mass spectra with less fragmentation and low-mass ion interference.

Bioaerosols are airborne particles that contain biologically active compounds, such as bacteria, viruses, fungal spores, and pollen.[1] These particles are a public health concern, because they can cause infection or an allergic or toxic reaction in people and animals. Furthermore, aerosol particles are used as the dissemination method for many biological warfare agents. Traditional bioaerosol analysis involves collection of particles with later culturing and counting.[2] If the microorganisms in the bioaerosol cannot be cultured, other techniques such as microscopy, endotoxin assay, immunoassays, and gene probes can be used. The main drawback of these methods is the time required for analysis, particularly for those methods in which culturing is necessary. In many cases, multiple tests are needed for positive identification.

Mass spectrometry has shown particular promise for the rapid analysis of biomolecules contained in microorganisms.[3,4] One of the most widely used methods for microorganism identification is pyrolysis mass spectrometry, in which the sample is heated, and the resulting volatile molecules are ionized by electron impact[5] or chemical ionization.[6] Pyrolysis MS has been used to identify mass spectral biomarkers associated with bacteria,[5−7] fungi,[8] and viruses.[9] On-line pyrolysis MS has been used to analyze aerosol particles composed of single bacteria,[10] and a field-portable instrument with in situ thermal hydrolysis and fatty acid methylation has been developed.[11,12] Laser pyrolysis mass spectrometry is a variation of laser desorption/ionization (LDI), in which a pulsed laser is used to rapidly heat the sample and generate ions from a sample deposited on a surface. LDI has the advantages of high spatial resolution, low sample consumption, and a higher mass range when compared with electron or chemical ionization.[13,14] LDI microprobe mass spectrometry has been used to analyze both aerosols [15,16] and microorganisms.[17,18] Despite its advantages, LDI-MS has a mass range limited to at most several thousand Daltons and cannot be used to detect large-molecule constituents of bioaerosols.

Matrix-assisted laser desorption ionization (MALDI) has emerged as the mass spectrometric method of choice for the

(1) Hirst, J. M. In *Bioaerosols Handbook*; Cox, C. S., Wathes, C. M., Eds; CRC Press: Boca Raton, FL, 1995; Chapter 2.
(2) Jensen, P. A.; Schafer, M. P. *Sampling and Characterization of Bioaerosols*, NIOSH Manual of Analytical Methods; National Institute for Occupational Safety and Health: Washington, DC; 1998.
(3) Fenselau, C. In *Mass Spectrometry for the Characterization of Microorganisms*; Fenselau, C., Ed; American Chemical Society: Washington, DC., 1994; Chapter 1.
(4) Spurny, K. R. In *Bioaerosols Handbook*; Cox, C. S., Wathes, C. M., Eds; CRC Press: Boco Raton, FL, 1995; Chapter 12.
(5) Anhalt, J. P.; Fenselau, C. *Anal. Chem.* **1975**, *47*, 219−225.
(6) Barshick, S. A.; Wolf, D. A.; Vass, A. A. *Anal. Chem.* **1999**, *71*, 633−641.
(7) DeLuca, S.; Sarver, E. W.; Harrington, P. B.; Voorhees, K. J. *Anal. Chem.* **1990**, *62*, 1465−1472.
(8) Weijman, A. C. M. *J. Microbiol. Serol.* **1976**, *42*, 315−324.
(9) Madonna, A. J.; Voorhees, K. J.; Hadfield, T. L.; Hilyard, E. J. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 502−511.
(10) Sinha, M. P.; Platz, R. M.; Friedlander, S. K.; Vilker, V. L. *Appl. Environ. Microbiol.* **1985**, *49*, 1366−1373.
(11) Basile, F.; Beverly, M. B.; Voorhees, K. J.; Hadfield, T. L. *Trends Anal. Chem.* **1998**, *17*, 95−109.
(12) Hendricker, A. D.; Abbas-Hawks, C.; Basile, F.; Voorhees, K. J.; Hadfield, T. L. *Int. J. Mass Spectrom.* **1999**, *190*/*191*, 331−342.
(13) Spurny, K. R. In *Analytical Chemistry of Aerosols*; Spurny, K. R.; Ed; CRC Press: Boca Raton, FL, 1999; Chapter 17.
(14) Cotter, R. J. *Anal. Chim. Acta* **1987**, *195*, 45−59.
(15) Kaufmann, R.; Hillenkamp, F.; Wechsung, R.; Heinen, H. J.; Schuermann, M. *Scanning Electron Microsc.* 1979, 279−290.
(16) Wieser, P.; Wurster, R.; Haas, U. *Fresenius' Z. Anal. Chem.* **1981**, *308*, 260−269.
(17) Seydel, U.; Linder, B. *Fresenius' Z. Anal. Chem.* **1981**, *308*, 253−257.
(18) Bohm, R. *Fresenius' Z. Anal. Chem.* **1981**, *308*, 258−259.

10.1021/ac020181i CCC: $22.00
Published on Web 08/21/2002

analysis of large biomolecules in whole cells.[19−22] In MALDI, the sample is mixed with a matrix on the sample target prior to mass analysis. MALDI offers several advantages for the analysis of microorganisms, including a low detection limit, high tolerance for impurities, and a large mass range with little fragmentation. MALDI mass spectra of bacteria contain mass spectral biomarker peaks that are generally species-specific and often strain-specific; however, careful sample preparation is critical to obtaining reproducible results.[22] Methods for bacterial identification using algorithms for mass spectral fingerprint matching[23,24] or on protein database matching[25] have been developed. MALDI-MS analysis of viral proteins[26,27] and of whole fungal cells[28−30] has also been reported.

To perform a MALDI analysis of aerosol particles, the particles must be brought into contact with the matrix prior to laser desorption. There are three ways to add matrix: collected particles (or extracted material) can be mixed with matrix and deposited on the target, particles can be collected on a matrix-coated target, or the matrix can be added to the particles by condensation prior to deposition. Analysis of collected particulates affords the most freedom in sample treatment prior to analysis, but information associated with the individual particles is lost.[31] Deposition on a matrix-coated target is an attractive approach, since individual particles can be analyzed and the process requires no additional steps following deposition. However, initial results suggest that deposition on a thin film of matrix can lead to an increase in background ion signal.[32] Addition of matrix by condensation has been demonstrated previously with on-line particle detection and ionization. In one study, a MALDI matrix was condensed on laboratory-generated particles and aerosolized bacterial spores.[33] Small biomolecules were detected when these particles were irradiated with a 308-nm excimer laser. In another study, the efficiency of 193-nm ionization of ammonium sulfate particles was enhanced when they were coated with a 1-naphthyl acetate "matrix."[34] Both of these studies were performed on-line: particles were ionized without deposition. The possibility also exists for matrix coating and subsequent collection of particles for off-line analysis by MALDI.

In this work, we report a method for the addition of matrix to aerosol particles by condensation prior to collection and analysis of particles using a commercial MALDI mass spectrometer. The liquid matrix 3-nitrobenzyl alcohol (NBA) was added to laboratory-generated particles in a heated saturator and condenser prior to deposition on the MALDI target. The target was inserted into the mass spectrometer without any further treatment, and ions were generated using a 337-nm nitrogen laser. Condensation matrix addition was demonstrated, and mass spectra were obtained for aerosol particles containing small peptides.

EXPERIMENTAL SECTION

The mass spectrometer used in this study was a Voyager DE STR MALDI time-of-flight mass spectrometer (Applied Biosystems, Foster City, CA). Data were obtained in reflectron mode of operation with delayed ion extraction. A laser wavelength of 337 nm at a repetition rate of 20 Hz was used for ionization, and an accelerating voltage of 20 kV was employed. All mass spectra are a sum of 100 laser shots and underwent advanced baseline correction and smoothing using Voyager's "Data Explorer" software. An aerodynamic particle sizer (APS) spectrometer (model 3321, TSI, St. Paul, MN) was employed to measure the size distribution of the generated particles. The APS uses light scattered from two spatially separated laser beams from a 675-nm laser diode to obtain the aerodynamic diameter of particles between 300 nm and 20 μm.

Aerosol particles were generated using nitrogen carrier gas at a flow rate of 5 L/min in a Collison nebulizer (BGI, Waltham, MA). The particles were dried with a silica gel diffusion dryer (TSI). Addition of matrix was accomplished using a heated saturator and condenser similar in design to those employed in condensation nucleus counters.[35] The heated saturator consisted of a 2-cm-i.d. copper tube 20 cm in length. The interior of the tube was lined with felt, which was wetted with the matrix and in contact with a matrix reservoir. Heating tape was wrapped around the exterior of the saturator tube, and a thermocouple and thermostat were used to maintain the temperature at 80 °C. The condenser was a 5-mm-i.d. copper tube 7.5 cm in length, maintained at 15 °C by a recirculating water bath. Upon exiting the condenser, the particles entered a 5-mm-i.d. copper tube 12 cm in length that directed the flow of the particles downward onto the MALDI sample target. Particles were collected for 5 min, and the resulting deposit was white in appearance rather than the yellow color of liquid 3-nitrobenzyl alcohol. The above conditions were also used for the collection of particles without matrix, except for the absence of 3-nitrobenzyl alcohol in the saturator.

Analytes gramicidin S (G-5001, Sigma, St. Louis, MO) and gramicidin D (G-5002, Sigma) and the matrix 3-nitrobenzyl alcohol (Aldrich, Milwaukee, WI) were used without further purification. Sample solutions of gramicidin S and gramicidin D were prepared in methanol at concentrations of 1 mg/mL.

RESULTS AND DISCUSSION

Initial work was conducted to gather particle size and concentration information for generated aerosols. Total particle concentration observed for gramicidin D particles in the size range between 300 nm and 20 μm was ~5000 particles/cm^3. To determine the effectiveness of matrix addition by condensation, particle size measurements were taken with and without the NBA

(19) Fenselau, C.; Demirev, P. A. *Mass Spectrom. Rev.* **2001**, *20*, 157−171.
(20) Lay, J. O., Jr. *Mass Spectrom. Rev.* **2001**, *20*, 172−194.
(21) Lay, J. O., Jr. *Trends Anal. Chem.* **2000**, *19*, 507−516.
(22) Van Baar, B. L. M. *FEMS Microbiol. Rev.* **2000**, *24*, 193-219.
(23) Arnold, R. J.; Reilly, J. P. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 630−636.
(24) Jarman, K. H.; Cebula, S. T.; Saenz, A. J.; Petersen, C. E.; Valentine, N. B.; Kingsley, M. T.; Wahl, K. L. *Anal. Chem.* **2000**, *72*, 1217−1223.
(25) Demirev, P. A.; Lin, J. S.; Pineda, F. J.; Fenselau, C. *Anal. Chem.* **2001**, *73*, 4566−4573.
(26) Siuzdak, G. *J. Mass Spectrom.* **1998**, *33*, 203-211.
(27) Yao, X.; Freas, A.; Ramirez, J.; Demirev, P. A.; Fenselau, C. *Anal. Chem.* **2001**, *73*, 2836−2842.
(28) Welham, K. J.; Domin, M. A.; Johnson, K.; Jones, L.; Ashton, D. S. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 307−310.
(29) Li, T. Y.; Liu, B. H.; Chen, Y. C. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 2393−2400.
(30) Amiri-Eliasi, B.; Fenselau, C. *Anal. Chem.* **2001**, *73*, 5228−5231.
(31) Johnston, M. V.; Wexler, A. S. *Anal. Chem.* **1995**, *67*, 721A−726A.
(32) Bogan, M. J.; Agnes, G. R. *Anal. Chem.* **2002**, *74*, 489−496.
(33) Stowers, M. A.; van Wuijckhuijse, A. L.; Marijnissen, J. C. M.; Scarlett, B.; van Barr, B. L. M.; Kientz, Ch. E. *Rapid Commun. Mass. Spectrom.* **2000**, *14*, 829−833.
(34) Kane, D. B.; Johnston, M. V. *Anal. Chem.* **2001**, *73*, 5365−5369.
(35) Agarwal, J. K.; Sem, G. J. *J. Aerosol Sci.* **1980**, *11*, 343−357.

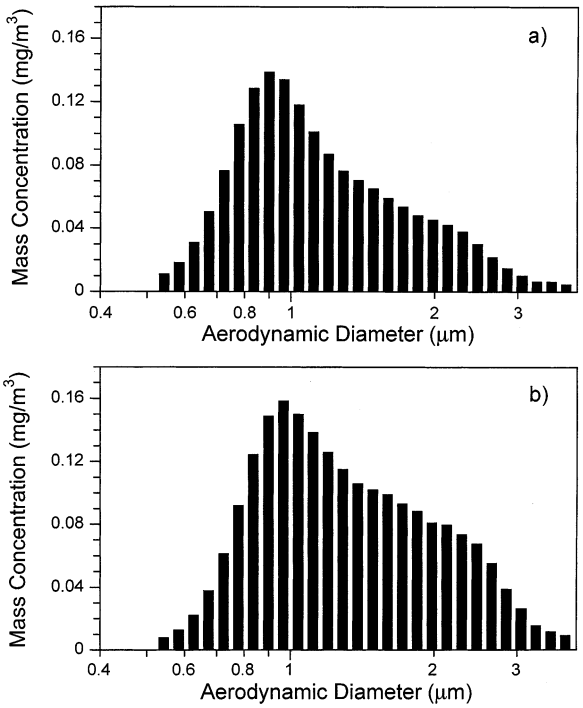

Figure 1. Particle mass concentration distribution of gramicidin D particles (a) before and (b) after matrix addition.

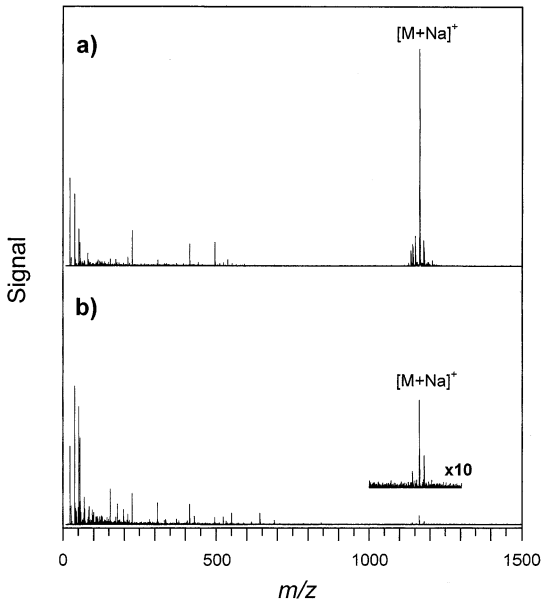

Figure 2. Mass spectra of deposited gramicidin S particles (a) with and (b) without matrix addition by condensation. Data are plotted on the same intensity scale.

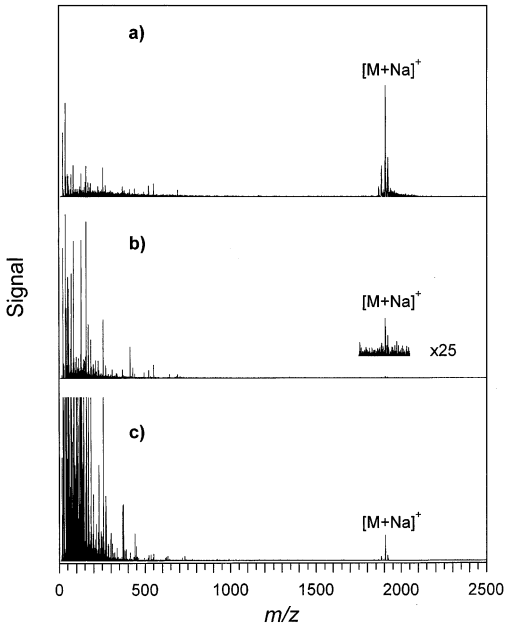

Figure 3. Mass spectra of deposited gramicidin D particles (a) with matrix condensation and (b) without matrix condensation, and (c) without matrix at a higher laser energy. Data are plotted on the same intensity scale.

matrix addition step. Figure 1 illustrates the particle mass concentration distribution for gramicidin D particles before and after the addition of matrix. The mean of the mass weighted particle size distribution increases from 1.34 μm for uncoated particles to 1.54 μm for coated particles. Correspondingly, the mean aerodynamic particle size increases from 0.85 μm for uncoated particles to 0.95 μm for coated particles (data not shown). This size change corresponds to a matrix-to-analyte molar ratio of ~5:1. Previous studies have shown that the optimum matrix to analyte molar ratio for MALDI of aerosol particles is in the range of 10 to 100.[36,37] In a previous aerosol MALDI study, an increase in particle size from 0.8 to 1.25 μm was reported, corresponding to a matrix-to-analyte molar ratio of 20.[33] In the previously reported work, particles were size-selected using a differential mobility analyzer prior to coating.

To test the utility of condensation matrix addition for biomolecule ionization, a solution containing the peptide gramicidin S was used to generate particles. Figure 2a shows a mass spectrum of gramicidin S particles collected with NBA present in the saturator. In this spectrum, the base peak is the peptide sodium adduct $[M + Na]^+$, and there is little interference in the low-mass region from fragment or matrix ions. Peaks associated with protonated gramicidin S and the potassium adduct are also observed. Figure 2b is plotted on the same scale as Figure 2a and shows a mass spectrum obtained from gramicidin S particles collected under the same conditions as for Figure 2a, but without NBA present in the saturator. In this spectrum, the most intense peaks result from alkali metal ions, impurities, and analyte fragment ions, with only a weak gramicidin S sodium adduct peak $[M + Na]^+$ being observed.

Gramicidin D, a mixture of four peptides with molecular weights near 1880 Da, was used for additional tests, because it is larger and somewhat more difficult to ionize than gramicidin S. Mass spectra of coated and uncoated gramicidin D particles are illustrated in Figure 3 (Note that the data are plotted on the same intensity scale). A mass spectrum of gramicidin D particles coated with NBA matrix is shown in Figure 3a. The most intense peak in the mass spectrum is the sodium adduct, $[M + Na]^+$. The protonated molecule, $[M + H]^+$, and the potassium adduct, $[M + K]^+$, are also observed. Figure 3b shows a mass spectrum of uncoated gramicidin D particles, in which the analyte peak has been reduced significantly and the mass spectrum is dominated

(36) Murray, K. K.; Russell, D. H. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 1−9.
(37) Beeson, M. D.; Murray, K. K.; Russell, D. H. *Anal. Chem.* **1995**, *67*, 1981−1986.

by peaks in the low-mass region. The mass spectrum in Figure 3c was obtained under the same conditions as Figure 3b, but with a higher laser energy optimized for the production of analyte signal. The $[M + Na]^+$ peak was somewhat larger, but is still considerably smaller than the low-mass peaks.

CONCLUSIONS

When the liquid matrix nitrobenzyl alcohol was added to aerosol particles by condensation, the ionization efficiency of the deposited particles was significantly enhanced. Particles containing the peptides gramicidin S and gramicidin D produced a strong analyte ion signal when coated with 3-nitrobenzy alcohol matrix. Particles that were not coated with matrix displayed a weak analyte ion signal and were dominated by low-mass ions. Future studies will focus on optimizing the coating procedure and testing solid compounds for matrix addition by condensation. An on-line single particle mass spectrometer that uses condensation matrix addition for large molecule ionization is also under development.

ACKNOWLEDGMENT

This research is supported by the National Institutes of Health under Grant No. R01ES10497. The Voyager DE STR mass spectrometer was funded by the National Institute of Health under Grant No. S10RR14645, K. Murray PI.

Received for review March 21, 2002. Accepted July 12, 2002.

AC020181l