The Development of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry for the Detection of Biological Warfare Agent Aerosols

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The best defensive measures against a biological weapons attack are to determine the agent(s) used and rapidly identify the exposed populations. However, technologies to accomplish these goals are only in their infancy. Through Defense Advanced Research Projects Agency sponsorship, the APL Tiny Time-of-Flight (TOF) Mass Spectrometer Program is attempting to close this window of vulnerability by developing a miniature, field-portable, and automated system for the rapid point detection of biological warfare agent aerosols. This article compares chemical and biological weapons properties to describe why matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) is an attractive tool for a biological weapons detection system. Experimental MALDI-TOF-MS spectra acquired from bacterial spore aerosol particles are presented to illustrate the approaches used in developing this system. (Keywords: Bioaerosols, Biological warfare agent detection, MALDI-TOF-MS.)

INTRODUCTION

Research and development of technologies for detecting weapons of mass destruction have intensified since 1991 when chemical and biological weapons were discovered in Iraq's arsenal. The deaths of 138 people from anthrax in Sverdlovsk, Russia, and 12 from sarin in Tokyo, the intentional *Salmonella* poisoning of 751 people in Oregon, and seizures of anthrax vaccine, bubonic plague cultures, and ricin from militia organizations in the United States suggest that the threat of attack on both military and civilian populations is real.[1–4] However, even the most highly prepared combat force will not be able to detect an attack with biological warfare agents (BWAs) until 25 to 40 min after it has been initiated. Through Defense Advanced Research Projects Agency (DARPA) sponsorship, the APL Tiny Time-of-Flight (TOF) Mass Spectrometer Program is working to close this window of vulnerability by developing a miniature, field-portable, and automated system for the more rapid point-detection of BWA aerosols.[5,6] The goal of this program is to provide

the ability to remotely detect BWAs in less than 10 min so that proactive measures can be taken to avoid exposure, maintain unit effectiveness, and minimize casualties.

As proposed by Bryden et al.,[5,6] the Tiny TOF system is being developed to monitor the chemical composition of airborne particles for the presence of bacteria, viruses, and toxins using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). This multidisciplinary task requires the identification of biomolecules that uniquely identify threat agents and incorporation of their mass spectra into an interpretive software package. The identification of simulant and pathogen biomarkers is a collaborative effort among APL, Dr. Fenselau at the University of Maryland, and Dr. Jackman at the U.S. Army Institute of Infectious Diseases (USAMRIID), Ft. Detrick, Maryland.[7] A MALDI-TOF-MS library containing the spectra of biological warfare simulants, authentic threat agents, interferents, and different environmental backgrounds is being constructed. In a parallel effort, members of the APL Systems Information Science Group are developing signal processing and recognition algorithms for the automated interpretation of these spectra. The successful implementation of these systems requires the development of effective bioaerosol sample collection and preparation methods for MALDI-TOF-MS analysis.

This article provides information about BWAs and bioaerosol sample preparation, and describes why aerosol sampling with MALDI-TOF-MS shows promise for complementing existing BWA detection platforms. Preliminary data generated using BWA simulant aerosols and a commercial MALDI-TOF mass spectrometer are presented, and current research and development areas are discussed.

METHODS TO DETECT CHEMICAL AND BIOLOGICAL WARFARE AGENTS

The physical properties of chemical and biological weapons are very different. Chemical warfare agents, which are dispersed as gases, are generally low in molecular weight (<300 Da), highly reactive, and volatile. Ion mobility chromatography and technology for the rapid gas chromatography, electron impact ionization, and mass spectroscopic detection of these agents are mature.[8–10] Ionization of gaseous samples via collision with 20- to 70-eV electrons can be performed to yield mass spectra that exhibit molecular ions and numerous fragment ions from which the chemical structure of the analytes can be deduced. In contrast, biological weapons can be very complex mixtures of high molecular weight (1- to 200-kDa) components that are chemically similar to those found in bacteria, viruses, and fungi which occur naturally in indoor and outdoor aerosols. BWAs are inherently nonvolatile and require aerosolization for delivery as discrete particles (Table 1). These properties preclude their direct detection by mass spectroscopic methods traditionally used to detect chemical weapons.

Table 1. Chemical and biological warfare agents, potential target analytes, and their properties.

Agent	Examples	Potential analyte classes	Molecular weight range of analytes (Da)
Chemical	Nerve agents GA, GB (sarin), GD, GF, VX	Intact molecules, degradation products, or derivatives	162 to >267
	Vessicants HD, H (sulphur mustards) L (lewisite), CX (phosgene oxime)		114 to >207
	Cyanide AC (hydrocyanic acid) CK (cyanogen chloride)		27 to >61
Biological	Bacteria Anthrax, cholera Plague (pneumonic, bubonic) Tuleremia, Q fever	Unique lipids, oligosaccharides, peptides, or proteins	700 to 145,000
	Viruses Smallpox Venezuelan equine encephalitis Hemorrhagic fevers (e.g., Ebola)	Viral glycolipids, coat-associated proteins	700 to 200,000
	Toxins Botulinum Staphylococcal enterotoxin-B (SEB) Ricin, T-2 mycotoxins, Aflatoxin B_1	Intact toxin molecules or their subunits, proteins, glycoproteins	300 to 150,000

PYROLYSIS AND LASER DESORPTION/IONIZATION MASS SPECTROMETRY

Pyrolysis MS is currently used in the Army's Biological Integrated Detection System to detect chemical and biological aerosols. Aerosol particles are concentrated and pyrolized to yield low molecular weight vapors that are ionized via electron impact.[11] Pyrolysis mass spectra exhibit ions with mass-to-charge ratios (m/z) of less than ≈300 Da. Neural network algorithms are available to interpret these spectra and distinguish among different bacterial strains.[12] However, the more direct detection of a wider range of biological threat agents may be realized when less destructive sample processing and ionization techniques are used. Laser ionization methods make it possible to preserve useful structural information and directly obtain mass spectra from polar, high molecular weight compounds encountered in microbiological studies without resorting to pyrolysis.

Laser desorption (LD) and other "soft" ionization techniques, such as fast atom bombardment and plasma desorption, were developed in the 1970s and 1980s to address the problem of ionizing polar, thermally labile, nonvolatile compounds for mass spectrometric analysis. These properties are typical of bio-organic molecules (proteins, nucleic acids, oligosaccharides) and preclude or interfere with the acquisition of their spectra using a "hard" ionization technique such as electron impact. Laser desorption is considered a soft ionization technique because the resulting spectra are dominated by molecular ions instead of fragment ions.

In 1978, Posthumus et al.[13] showed that oligosaccharide molecular ions (m/z ≈1 kDa) were readily obtained using LD/MS. In studies that followed, other classes of previously inaccessible biomolecules were desorbed and ionized by the application of a brief (pico- to nanosecond) pulse of UV or IR radiation. The mechanisms through which IR- and UV-induced desorption/ionization occur have been studied.[14,15]

Single- and dual-wavelength laser techniques have been used to obtain spectra from aerosol particles introduced directly into TOF mass spectrometers.[16–20] Inorganic species relevant to the study of atmospheric chemistry and organic species such as polycyclic aromatic hydrocarbon ions have been detected in candle, cigarette, and wood smoke; however, these reports have been limited to the detection of species with molecular weights <300 Da. Although this mass range is suitable for the detection of chemical warfare agents, it is not amenable to the development of a miniature detection system. The direct introduction of air into the spectrometer creates the need for large pumps to maintain a vacuum (≈1 mTorr) in the instrument. This inhibits miniaturization efforts by increasing the system's power demands, complexity, and weight.

The main requirement and perhaps the main drawback of LD/MS is that analytes must absorb at the wavelength emitted by the laser. The application of IR-based methods to higher molecular weight compounds is limited by problems with thermal degradation at laser beam densities required to produce sample desorption. These requirements limit the range of compounds studied using LD/MS and motivated research that led to the discovery and exploitation of the MALDI technique by Hillenkamp and Karas.[21–23] Today, MALDI-TOF-MS is routinely used for the analysis of high molecular weight biomolecules (1 to 150 kDa) and synthetic polymers.[24–26]

MALDI-TOF-MS

MALDI-TOF-MS is the method of choice for the rapid structural characterization of high molecular weight compounds.[27] Relative to gas or liquid chromatographic systems employing mass spectrometric detection, sample preparation is minimal because this technique is tolerant of buffers, salts, and many other contaminants. As a result, each analysis requires only a few seconds to perform. Under ideal conditions, spectra can be obtained from femto- and attomole quantities of analyte. In addition to providing molecular weight information, the amino-acid sequence of unpurified peptides of less than ≈3 kDa can be determined using post-source decay and collision-induced dissociation methods.[28–30]

In a typical MALDI experiment, 0.5 μL (2 to 25 pmol) of sample solution is placed on a metal probe, mixed with 0.5 to 2.0 μL of matrix solution (50 mM), and air-dried. Intimate mixing, if not co-crystallization of the matrix and analyte, is critical to obtaining a spectrum.[31–35] Low molecular weight aromatic organic acids such as 2,5-dihydroxybenzoic acid and α-cyano-4-hydroxycinnamic acid are commonly used matrices. Irradiation of the sample with a pulsed UV or IR laser beam in a vacuum chamber produces desorption and ionization of matrix and analyte molecules. The laser ablation track across the surface of a bacterial spore sample is presented in Fig. 1. A complete spectrum can be acquired with one laser shot; however, 10 to 100 spectra are normally averaged. The purpose of the matrix is to nondestructively transfer energy from the laser beam to the sample, thereby producing intact, large molecular ions in the gas phase. The MALDI process is not well understood, and experiments attempting to elucidate the physical and chemical mechanisms through which it works are frequently reported.[22,36,37]

Positively or negatively charged ions produced by irradiation are selected and accelerated to approximately the same kinetic energy for analysis using a TOF mass

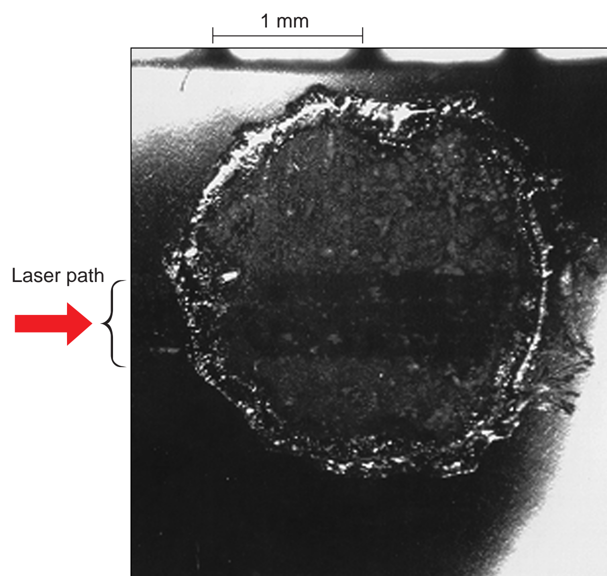

Figure 1. After concentrating aerosol particles (aerodynamic dia. = 0.3 to 10.0 μm) in a circular spot on video recorder tape, a UV-absorbing matrix solution (≈0.3 mL) is applied to the sample for MALDI-MS analysis. Absorption of the incident beam (N_2, 337 nm, 5–10 MW/cm^2) by the matrix and the transfer of this energy to the sample produce ions in the gas phase. This photograph shows the ablation track produced by rastering the laser across a *Bacillus globigii* spore sample during MALDI-TOF-MS analysis.

spectrometer. Measurement of the time of ion flight between the ion source and detector is used to calculate the m/z ratio of each ion. Fundamental MALDI-TOF-MS instrumentation concepts are presented by Cotter,[38] Cornish and Cotter,[39,40] and Cornish and Bryden elsewhere in this issue.

Although oligosaccharides, nucleic acids, and synthetic polymers are analyzed by MALDI-TOF-MS, it has been most intensively applied to the analysis of peptides.[26,41] The success of this technique in the structural characterization of high molecular weight biomolecules has resulted in its ambitious application to the direct analysis of entire organisms such as viruses and bacteria. Typically, a single colony or an aliquot (<1 μL) from a homogeneous culture is directly analyzed after mixing with a MALDI matrix.

Viral proteins have been detected and characterized using MALDI-MS.[7,42] However, the detection of specific proteins in the mass spectra of whole bacteria or extracts has proven more difficult. The mass spectra of bacterial reference standards have been recorded and empirically compared to test samples in only a few published studies.[43–48] Although differences exist in the appearance of ions between 1 and 35 kDa, the chemical identities of ions that distinguish one bacterium from another were not reported in these early studies. Because organisms used as BWAs contain or produce biomolecules responsible for their pathogenic activity, their detection in the mass spectra of reference BWA standards is an important and growing area of research.

BIOAEROSOL PARTICLE SIZE DISTRIBUTIONS AND THE SIOUTAS COLLECTOR

BWAs are most generally effective when deposited in the respiratory tract. For this reason, aerosols are engineered to contain particles with an aerodynamic diameter d_a between 0.3 to 10.0 μm. The aerodynamic diameter is defined as the diameter of a unit-density spherical particle with a gravitational settling velocity equal to that of the particle under study. The physical diameter of a particle is not always equal to its aerodynamic diameter. Particles with $d_a > 15$ μm rapidly settle out of the air column and are effectively filtered by the upper respiratory tract. Particles with $d_a < 10$ μm differentially deposit in the upper respiratory tract, and those with $d_a < 3$ to 5 μm penetrate deep into the lung. Thus, the front end of the Tiny TOF-MS system is designed to deposit aerosol particles with a d_a from 0.5 to 10.0 μm via inertial impaction on video recorder tape for mass spectrometric analysis.

This particle collector design is based on the high-volume inertial impactors developed by Sioutas.[49] Air is drawn through a plenum of 10 acceleration nozzles, each operating at 3 L/min with a total pressure drop of 22 in. of water. Particles are accelerated through a 0.85-mm ID nozzle to 88 m/s and deposited by inertial impaction on video recorder tape located approximately 1 mm away. The 50% cutoff diameter is 0.5 μm, and collection efficiency increases to 90% for particles greater than 0.8 μm. Figure 2 is a scanning electron micrograph of two sample spots deposited by the impactor during a trial conducted at the Army's Dugway Proving Grounds, Utah. After collection, an inkjet printer head is used to apply matrix solution to each sample spot. Following crystallization of the MALDI matrix and sample, the tape is conveyed through a vacuum lock into the Tiny TOF mass spectrometer. The chamber is sufficiently evacuated after 15 s to begin data acquisition. The analytical strategy is to concentrate ambient air particles on a spot and characterize them by MALDI-TOF-MS. More detailed information about the design of the Tiny TOF air collection subsystem is available in a companion article by Anderson and Carlson, this issue.

BIOLOGICAL WARFARE SIMULANTS

BWA simulants that are nonpathogenic to humans are used to test aerosol particle collection devices and detectors at The Johns Hopkins University School of Hygiene and Public Health. The gram-positive endospore-forming bacterium *Bacillus globigii* (variant

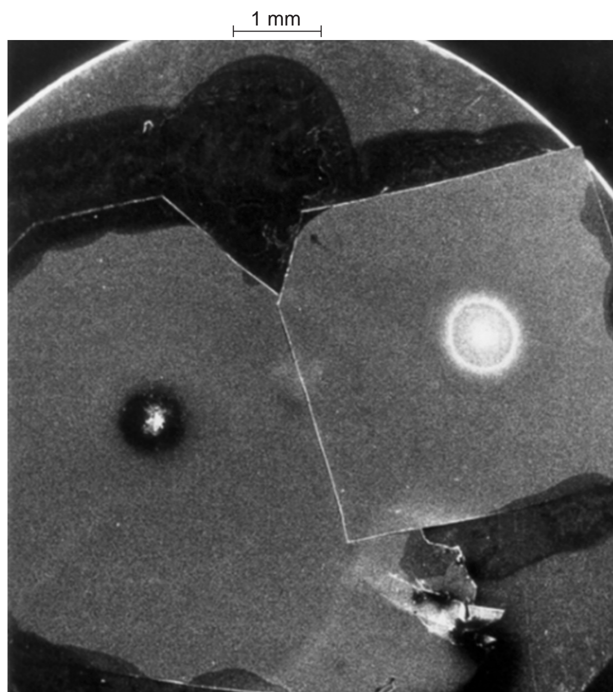

Figure 2. Scanning electron micrograph of diesel smoke (left spot) and smoke grenade (right spot) particles collected on video recorder tape by the Sioutas collector on the outdoor test grid at Dugway Proving Grounds. Separate tape samples were mounted on a sample stub using a conductive adhesive. Inertial impaction of aerosol particles on tape concentrates samples into a circular spot (≈1-mm dia.) for subsequent mass spectroscopic analysis. Matrix was not applied to these samples.

niger) is used to simulate *Bacillus anthracis* spores.[50] *Bacillus globigii* spores exhibit an aerodynamic diameter of ≈0.9 μm and appear as 0.5 × 1.0 μm rods when viewed by scanning electron microscopy.[51] The abundant chicken egg protein ovalbumin (formula weight ≈44.3 kDa) is used to simulate ricin and botulinum toxins. The small RNA virus MS-2 and the gram-negative bacterium *Erwinia herbicola* simulate pathogenic viruses and nonsporulating bacteria, respectively. These simulants are provided by Dugway Proving Grounds. The ultimate sensitivity required to pass Army trials at Dugway is the detection of 20 colony (or plaque) forming units per liter of air and ≈300 picograms of ovalbumin per liter of air, given a 5-min sampling interval.

At the USAMRIID, threat agents (anthrax, plague, Venezuelan equine encephalitis, botulism toxin, and others) are undergoing MALDI analysis in biosafety level 3 and 4 containment facilities. Experiments involving the MADLI-TOF-MS analysis of aerosolized anthrax spores deposited on video recorder tape are currently under way.

The bioaerosol test chamber at the JHU School of Hygiene and Public Health (1.2 m^3) is equipped with a Model 3300 aerosol particle size (APS) spectrometer (TSI, Inc., St. Paul, Minnesota), an AGI-30 reference air sampler (Ace Glass, Vineland, New Jersey), and the Sioutas collector (Fig. 3). A Collison nebulizer (BGI, Inc., Boston, Massachusetts) is used to generate aerosols from simulant solutions. Alternatively, aerosols can be generated from dry *B. globigii* spores and ovalbumin. The concentration of viable and nonviable simulants and the size distribution of these aerosols are measured using different instruments.

The size distribution of aerosol particles between 0.3 and 15.0 μm is determined using the APS spectrometer by measuring the time it takes particles to travel between two beams of light. Typical APS data acquired from *B. globigii* spore and ovalbumin aerosols are presented in Fig. 4. The AGI-30 (all-glass impinger) is a highly characterized reference sampler that traps particles in solution.[52] It is used to measure the concentration of simulant aerosols presented to the Sioutas collector. Bacteria and viruses are quantified by enumerating the number of colonies or plaque-forming units appearing, respectively, following their growth in culture.

Bacterial and viral enumeration enables characterization of aerosols in terms of agent-containing particles per liter of air. The viability of *B. globigii* spores is practically unaffected following capture by the AGI-30 or Sioutas collectors. However, the viability of *E. herbicola* and MS II decreases during collection to the extent that they cannot be quantitatively cultured from

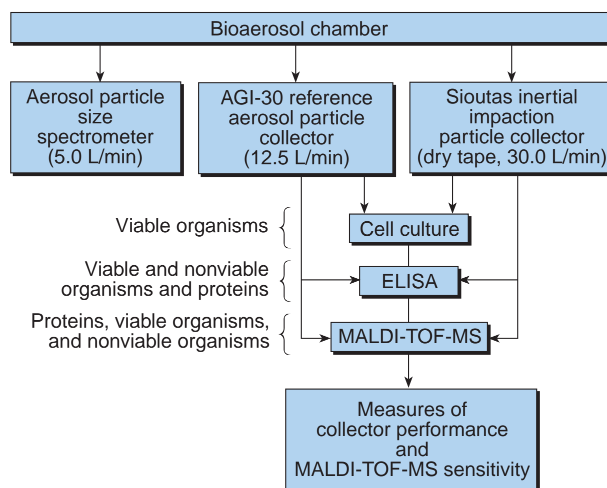

Figure 3. Schematic representation of the bioaerosol test facility at the JHU School of Hygiene and Public Health. Simulant aerosols are generated in a static chamber and simultaneously withdrawn by three different sampling instruments. Particle size distributions are measured using an APS 3300 aerodynamic particle size spectrometer. Simulant aerosol concentrations presented to the Sioutas collector are measured using a reference all-glass liquid impinger (AGI-30). The amount of simulant captured by the AGI-30 and experimental (Sioutas) collectors is measured using enzyme-linked immunosorbent assays, MALDI-TOF-MS, and the enumeration of viable bacteria and viruses.

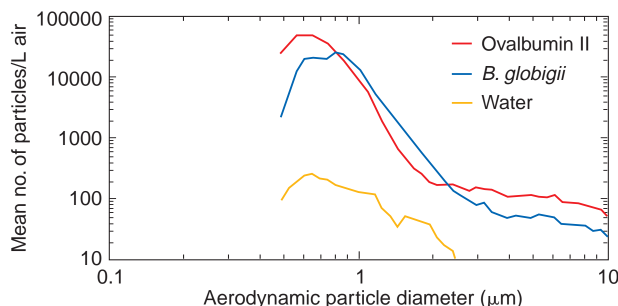

Figure 4. An aerosol particle size spectrometer (APS 3300) is used to measure the aerodynamic particle size distributions of *Bacillus globigii* and ovalbumin II aerosols. *Bacillus globigii* spores exhibit a numerical median diameter of 0.8 to 1.2 μm. Ovalbumin aerosol particle sizes are smaller (≈0.7 μm) and are partially determined by the simulant concentration loaded into the nebulizer. Because the efficiency of aerosol particle collection depends heavily on the aerodynamic diameter of the particles, the particle size distribution is an important parameter in the design and testing of bioaerosol collection devices.

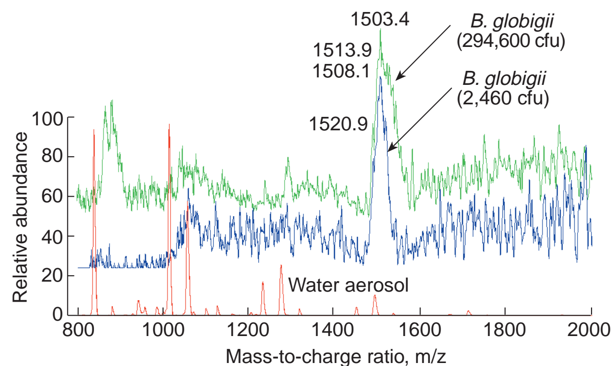

Figure 5. MALDI-TOF mass spectra of *B. globigii* spores collected on video recorder tape using the Sioutas aerosol collector. These spores exhibit an unidentified but characteristic ion cluster at m/z = 1503. As few as 2460 colony forming units (cfu) are sufficient to yield this clearly recognizable *B. globigii* spore spectrum. (Instrument: Kratos MALDI IV, positive ionization, 20-kV extraction, linear TOF-MS modes. Matrix: sinapinic acid and 3-methoxy-cinnamic acid, 50 mM each. Solvent: 70% acetonitrile, 30% water, with 5% trifluoroacetic acid, v/v.)

tape. To preserve the viability of these agents by minimizing shear and drying, an agar slit impaction collector is used to capture *Erwinia* and MS-2 for enumeration. The MALDI-MS spectra of *B. globigii* spores collected on tape by the Sioutas collector are presented in Fig. 5.

Ovalbumin, *B. globigii* spores, *E. herbicola*, and MS-2 can be quantified using enzyme-linked immunosorbent assays (ELISA) developed at Dugway Proving Grounds and the Naval Medical Research Institute (Bethesda, Maryland).[53] Both ELISA and MALDI-TOF-MS have the advantage over culture-based methods of detecting both viable and nonviable organisms. The performance of the Sioutas collector and MALDI-MS detection protocols can be evaluated using these analytical techniques.

Unlike the preparation of liquid samples, the direct analysis of dry bioaerosol particles on tape presents fundamental problems to the acquisition of MALDI spectra. While separate matrix and sample solutions are routinely mixed and spotted on a probe, a matrix solution must be applied to the inertially deposited dry particle spot. This does not allow the same intimate mixing of the matrix and sample to occur as it does when they are mixed as liquids. Sample preparation techniques such as the precoating of tapes with matrix-doped aerogels or adhesives and the co-deposition of MALDI–matrix with aerosol particles are being evaluated to address this problem.

Although MALDI-MS is modestly tolerant of buffer salts, detergents, and other interferents, these adversely affect detection sensitivity. To the extent that more abundant sample components suppress target analyte ionization, sample cleanup procedures must be applied. However, because it is intended for deployment on the battlefield, a design goal of the Tiny TOF system is to minimize the use of fluids. This makes traditional liquid-intensive sample cleanup procedures such as chromatographic separation and solid-phase extraction unacceptable. Fortunately, a variety of micro-volume techniques have evolved for the rapid "on-probe" cleanup of MALDI samples.[54,55] In general, these methods rely on the use of nitrocellulose, polyethylene, polyvinylidene difluoride, ion exchange, or immunoaffinity membranes. Crude MALDI samples deposited on these membranes can be washed with a few microliters of solution to selectively remove interferents from the sample.

CONCLUSIONS

Following an attack with chemical or biological weapons, early recognition of the attack, determination of the agent(s) used, and the rapid identification of exposed populations are the best possible defensive measures. This information can minimize casualties by enabling the prompt delivery of prophylactic and emergency medical care and by limiting additional exposure by focusing decontamination operations. Conceptually, the MALDI-TOF-MS analysis of aerosol particles will enable the rapid, sensitive, and specific detection of chemical and biological warfare agents present in the context of an atmospheric particle background. The practical application of this concept demands the discovery and implementation of new techniques that adapt current aerosol sampling and mass spectrometric sample preparation methods for this nontraditional application. The achievement of this technically challenging goal will provide methods for detecting bioaerosols both on and off the battlefield.

Although the primary focus of the APL/JHU School of Hygiene and Public Health collaboration is defense-related, Tiny TOF-MS technology may also be used to addresses several important public health research needs being pursued by JHU. One such research area stems from uncertainties in the organic composition of ambient airborne particulate matter believed to be responsible for increased morbidity and mortality. The National Research Council has recently identified a research agenda that lists the chemical characterization of airborne particles in 2 of 10 research areas of highest priority.[56] They recommended that Congress make available $40 million (for FY98), and that this level of funding be sustained every year through 2010 to pursue this agenda. A second public health research need concerns children's exposure to pollutants in an inner-city environment. MALDI-TOF-MS holds the potential for detecting and identifying multiple organic constituents including polycyclic aromatic hydrocarbons, pesticides, and higher molecular weight allergens, making it possible to assess aggregate exposures and allowing for the investigation of particle-associated chemical mixtures in disease etiology.

REFERENCES

[1] *JAMA* **278** (6 Aug 1997).

[2] Henderson, D. A., "Bioterrorism as a Public Health Threat," *Emerging Infectious Diseases* **4**, 488–492 (1998).

[3] National Research Council, *Chemical and Biological Terrorism: Research and Development to Improve Civilian Medical Response*, National Academy Press, Washington, DC (1999).

[4] U.S. Congress, Office of Technology Assessment, *Technology Against Terrorism: Structuring Security*, OTA-ISC-511, U.S. Govt. Printing Office, Washington, DC (1992).

[5] Bryden, W. A., Benson, R. C., Ecelberger, S. A., Phillips, T. E., Cotter, R. J., and Fenselau, C., "The Tiny-TOF Mass Spectrometer for Chemical and Biological Sensing," *Johns Hopkins APL Tech. Dig*. **16**, 296–310 (1995).

[6] Bryden, W. B., Benson, R. C., Ko, H. W., and Donlan, M., "Universal Agent Sensor for Counterproliferation Applications," *Johns Hopkins APL Tech. Dig*. **18**, 302–308 (1997).

[7] Thomas, J., Falk, B., Fenselau, C., Jackman, J., and Ezzell, J., "Viral Characterization by Direct Analysis of Capsid Proteins," *Anal. Chem*. **70**, 3863–3867 (1998).

[8] Kientz, C., "Chromatography and Mass Spectrometry of Chemical Warfare Agents, Toxins and Related Compounds: State of the Art and Future Prospects," *J. Chromatogr. A* **814**, 1–23 (1998).

[9] Mulholland, D., "Shrinking Chemical-Weapon Sensors Speed Analysis," *Defense News* (3–9 Aug 1998).

[10] "Technologies," Chap. 2, in *Worldwide Chemical Detection Equipment Handbook*, N. Brletich, M. Waters, G. Bowen, and M. Tracy (eds.), Defense Technical Information Service, Ft. Belvoir, VA, pp. 6–25 (1995).

[11] Beverly, M. B., Basile, F., Voorhees, K. J., and Hadfield, T. L., "A Rapid Approach for the Detection of Dipicolinic Acid in Bacterial Spores Using Pyrolysis/Mass Spectrometry," *Rapid Comm. Mass Spectrom*. **10**, 455–458 (1996).

[12] Timmins, E. M., and Goodacre, R., "Rapid Quantitative Analysis of Binary Mixtures of *E. coli* Strains Using Pyrolysis Mass Spectrometry with Multivariate Calibration and Artificial Neural Networks," *J. Appl. Microbiol*. **83**, 208–218 (1997).

[13] Posthumus, M. A., Kistenmaker, P. G., and Meuzelaar, H. L., "Laser Desorption-Mass Spectrometry of Nonvolatile Bio-Organic Molecules," *Anal. Chem*. **50**, 985–991 (1978).

[14] Van der Peyl, G., Haverkamp, J., and Kistenmaker, P. G., "Thermal Aspects of Laser Desorption Mass Spectrometry," *Int. J. Mass Spectrom. Ion Phys*. **42**, 125–141 (1982).

[15] Van Breeman, R. B., Snow, M., and Cotter, R., "Time-Resolved Laser Desorption Mass Spectrometry: I. Desorption of Preformed Ions," *Int. J. Mass Spectrom. Ion Phys*. **49**, 35–50 (1983).

[16] Morrical, B. D., Fergenson, D. P., and Prather, K. A., "Coupling Two-Step Laser Desorbtion/Ionization with Aerosol Time-of-Flight Mass Spectrometry for the Analysis of Individual Organic Particles," *J. Am. Soc. Mass Spectrom*. **9**, 1068–1073 (1998).

[17] Nordmeyer, T., and Prather, K., "Real-Time Measurement Capabilities Using Aerosol TOF-MS," *Anal. Chem*. **66**, 3540–3542 (1994).

[18] Hinz, H., Kaufmann, R., and Spengler, B., "Simultaneous Detection of Positive and Negative Ions from Single Airborne Particles by Real-Time Laser Mass Spectrometry," *Aerosol Sci. Tech*. **24**, 233–242 (1996).

[19] Murray, K., and Russell, D., "Aerosol MALDI-MS," *J. Am. Soc. Mass Spectrom*. **5**, 1–9 (1994).

[20] Murphy, D. M., Thomas, D. S., and Mahoney, M. J., "*In Situ* Measurements of Organics and Other Elements in Aerosols at 5 to 19 Kilometers," *Science* **282**, 1664–1669 (1998).

[21] Karas, M., Bachmann, D., and Hillenkamp, F., "Influence of the Wavelength in High-Irradiance UV Laser Desorption Mass Spectrometry of Organic Molecules," *Anal. Chem*. **57**, 2935–2939 (1985).

[22] Karas, M., Bachmann, D., Bahr, U., and Hillenkamp, F., "Matrix-Assisted Ultraviolet Laser Desorption of Non-Volatile Compounds," *Int. J. Mass Spectrom. Ion Proc*. **78**, 53–68 (1987).

[23] Karas, M., and Hillencamp F., "Laser Desorption of Proteins with Molecular Masses Exceeding 10 kDa," *Anal. Chem*. **60**, 2299–2301 (1988).

[24] Mechref, Y., and Novotny, M., "Mass Spectrometric Mapping and Sequencing of N-Linked Oligosaccharides Derived from Submicrogram Amounts of Glycoproteins," *Anal. Chem*. **70**, 455–463 (1998).

[25] Hillenkamp, F., and Karas, M., "Mass Spectrometry of Peptides and Proteins by Matrix-Assisted Laser Desorption/Ionization," *Methods Enzymol*. **193**, 280–295 (1990).

[26] Koster, H., Tang, K., Fu, D., Braun, A., van den Boom, D., et al., "A Strategy for Rapid and Efficient DNA Sequencing by Mass Spectrometry," *Nature Biotech*. **14**, 1123–1128 (1996).

[27] Stults, J. T., "Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry," *Curr. Opinion Structural Biol*. **5**, 691–698 (1995).

[28] Russell, D. H., and Edmondson, R. D., "High Resolution Mass Spectrometry and Accurate Mass Measurements with Emphasis on the Characterization of Peptides and Proteins by MALDI-TOF-MS," *J. Mass Spectrom*. **32**, 263–276 (1997).

[29] Cotter, R. J., and Cornish, T. J., "A Tandem TOF Mass Spectrometer," *Anal. Chem*. **65**, 1043–1047 (1993).

[30] Fabris, D., Vestling, M. M., Cordero, M. M., Doroshenko, V. M., Cotter, R. J., and Fenselau, C., "Sequencing Electroblotted Proteins by Tandem Mass Spectrometry," *Rapid Comm. Mass Spectrom*. **9**, 1051–1055 (1995).

[31] Nelson, R. W., McLean, M. A., and Hutchens, T. W., "Quantitative Determination of Proteins by MALDI-TOF-MS," *Anal. Chem*. **66**, 1408–1415 (1994).

[32] Preston, L. M., Murray, K. K., and Russell, D. H., "Reproducibility and Quantitation of MALDI Mass Spectrometry: Effects of Nitrocellulose on Peptide Ion Yields," *Biol. Mass Spectrom*. **22**, 544–550 (1993).

[33] Hensel, R. R., King, R. C., and Owens, K. G., "Electrospray Sample Preparation for Improved Quantitation in MALDI-TOF-MS," *Rapid Comm. Mass Spectrom*. **11**, 1785–1793 (1997).

[34] Nicola, A. J., Gusev, A. I., Proctor, A., Jackson, E. K., and Hercules, D. M., "Application of the Fast-Evaporation Sample Preparation Method for Improving Quantification of Angiotensin II by MALDI," *Rapid Comm. Mass Spectrom*. **9**, 1164–1171 (1995).

[35] Cohen, S. L., and Chait, B. T., "Influence of Matrix Solution Conditions on the MALDI-MS Analysis of Peptides and Proteins," *Anal. Chem*. **68**, 31–37 (1996).

[36] Niu, S., Zhang, W., and Chait, B. T., "Direct Comparison of Infrared and Ultraviolet Wavelength MALDI-MS of Proteins," *J. Am. Soc. Mass Spectrom*. **9**, 1–7 (1997).

[37] Lehmann, E., Knochenmuss, R., and Zenobi, R., "Ionization Mechanisms in MALDI MS Contribution of Preformed Ions," *Rapid Comm. Mass Spectrom*. **11**, 1483–1492 (1997).

[38] Cotter, R. J., *Time of Flight Mass Spectrometry*, American Chemical Society (1997).

[39] Cornish, T. J., and Cotter, R. J., "A Curved Field Reflectron TOF-MS for the Simultaneous Focusing of Metastable Product Ions," *Rapid Comm. Mass Spectrom*. **8**, 781–785 (1994).

[40] Cornish, T. J., and Cotter, R. J., "A Compact TOF-MS for the Structural Analysis of Biological Molecules Using Laser Desorption," *Rapid Comm. Mass Spectrom*. **6**, 242–248 (1992).

[41] Yates, J. R., "Mass Spectrometry and the Age of the Proteome," *J. Mass Spectrom*. **33**, 1–19 (1998).

[42] Siuzdak, G., "Probing Viruses with Mass Spectrometry," *J. Mass Spectrom*. **33**, 203–211 (1998).

[43] Cain, T. C., Lubman, D. M., and Weber, W. J., "Differentiation of Bacteria Using Protein Profiles from MALDI-TOF-MS," *Rapid Comm. Mass Spectrom*. **8**, 1026–1030 (1994).

[44] Krishnamurthy, T., and Ross, P. L., "Rapid Identification of Bacteria by Direct MALDI-MS Analysis of Whole Cells," *Rapid Comm. Mass Spectrom*. **10**, 1992–1996 (1996).

[45]Holland R. D., Wilkes, J. G., Rafii, F., Sutherland, J. B., Persons, C. C., et al., "Rapid Identification of Intact Whole Bacteria Based on Spectral Patterns Using MALDI-TOF-MS," *Rapid Comm. Mass Spectrom.* **10**, 1227–1232 (1996).
[46]Chong, B. E., Wall, D. B., Lubman, D. M., and Flynn, S. J., "Rapid Profiling of *E. coli* Proteins up to 500 kDa from Whole Cell Lysates Using MALDI-TOF-MS," *Rapid Comm. Mass Spectrom.* **11**, 1900–1908 (1997).
[47]Claydon, M. A., Davey, S. N., Edward-Jones, V., and Gordon, D. B., "The Rapid Identification of Intact Microorganisms Using Mass Spectrometry," *Nature Biotech.* **14**, 1584–1586 (1996).
[48]Welham, K. J., Domin, M. A., Scannell, D. E., Cohen, E., and Ashton, D. S., "The Characterization of Micro-organisms by MALDI-TOF-MS," *Rapid Comm. Mass Spectrom.* **12**, 176–180 (1998).
[49]Sioutas, C., Koutrakis, P., Ferguson, S. T., and Burton, R. M., "Developments and Evaluation of an Ambient Particle Concentrator for Inhalation Exposure Studies," *Inhalation Toxicol.* **7**, 633–644 (1995).
[50]Zilinskas, R. A., "Iraq's Biological Weapons: The Past As Future?" *JAMA* **278**, 418–424 (1997).
[51]Bulla, L. A., Julian, G. S., Rhodes, R. A., and Hesseltine, C. W., "Scanning Electron and Phase-Contrast Microscopy of Bacterial Spores," *Appl. Microbiol.* **18**, 490–495 (1969).
[52]Jensen, P. A., Todd, W. F., Davis, G. N., and Scarpino, P. V., "Evaluation of 8 Bioaerosol Samplers Challenged with Aerosols of Free Bacteria," *Am. Ind. Hyg. Assoc. J.* **53**, 660–667 (1992).
[53]Martin, D., Winters, D., Mohr, A., and Harper, B., "Characterization of Aerosolized Ovalbumin," *Aerosol Sci. Technol.* **23**, 534–540 (1995).
[54]Worral, T., Cotter, R., and Woods, A., "Purification of Contaminated Peptides and Proteins on Synthetic Membrane Surfaces for MALDI-MS," *Anal. Chem.* **15**, 750–756 (1998).
[55]Blackledge, J. A., and Alexander, A. J., "Polyethylene Membrane As a Sample Support for Direct MALDI-MS Analysis of High Mass Proteins," *Anal. Chem.* **67**, 843–848 (1995).
[56]National Research Council, *Research Priorities for Airborne Particulate Matter*, National Academy Press, Washington, DC (1998).

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