



Miniature Time-of-Flight Mass Spectrometer for a Field-Portable Biodetection System

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The mass spectrometer is one of the most powerful laboratory tools for analyzing a broad spectrum of chemical and biological materials, but its applicability to field detection problems has been limited given its large size, heavy weight, and prohibitive power requirements. This situation is rapidly changing, however, as the need for field-portable detection systems becomes more critical. This article reports on an effort to bring small time-of-flight (TOF) mass spectrometer technology to bear on the chemical and biological detection problem. We will describe the development of the Pseudo-Tandem TOF Mass Spectrometer for field-portable biodetection. Incorporated into the analyzer's design is an ion reflector that uniquely records ions which are not detectable in conventional TOF instruments. We will also examine the operating principles of the mass analyzer, the miniaturization of the mass analyzer and other system components, and mass spectral biosignatures. (Keywords: Curved field, Field-portable, Mass spectrometer, Pseudo-tandem, Reflectron, TOF.)

INTRODUCTION

Mass spectrometry (MS) is a technique used to determine the masses of molecules and specific fragmentation products formed following vaporization and ionization. Detailed analysis of the mass distribution of the molecule and its fragments leads to molecular identification. The combination of specific molecular identification and extreme sensitivity makes MS one of the most powerful analytical laboratory tools available.

Time-of-flight (TOF) MS is a deceptively simple technique, yet it has the sensitivity and specificity to identify chemical species with concentrations in the attomolar (10^{-18} moles) range.¹ During the past 10

years, this tool has enjoyed a resurgence. By far the most significant reason for its current market presence is the TOF mass analyzer's ability to measure the mass of biomolecular ions by using matrix-assisted laser desorption/ionization (MALDI).² With MALDI, the analyte is mixed with an appropriate organic matrix, inserted into the ionization region, and desorbed from a surface into the TOF drift region. The matrix absorbs the radiative energy and causes a phase transition from solid to gas. In the process, the analyte gains a H^+ ion and can thus be accelerated within an electric field. Although the smaller ions produced can be measured

on a variety of mass spectrometers, a TOF mass spectrometer is particularly qualified for MALDI applications because it has no theoretical upper mass limit.

MALDI is especially well suited to the desorption of the larger macromolecules required for the application of chemotaxonomic methods. Larger mass ions, such as proteins and fragments of DNA strands, simply take more time to reach the detector. Consequently, both the absence of any scanning requirement and an unlimited mass range make TOF-MS the most popular method for biomolecular analysis using MALDI.

The widespread use and rapid refinement of MALDI/TOF analysis have resulted in significant interest in its development for the detection of biological weapons whose mass signatures are often found in the 10- to 100-kDa range. Moreover, the non-scanning TOF analyzer allows for very short data acquisition times, further enhancing its benefit for rapid threat assessment.

A particularly valuable application of MALDI-TOF mass analysis is its ability to identify peptides and proteins with very high specificity and sensitivity. This field of research, in fact, constitutes the greatest driving force for the commercial development of TOF instruments, particularly in the pharmaceutical industry, where they are being used for drug development. Thus, TOF mass analyzers are exceptionally well suited for the biological threat detection of midrange (1,000- to 50,000-Da) toxins in which subfemtomole sensitivity is required. While this capability has existed in the laboratory for the past few years, the development of a small, portable mass spectrometer for potential field detection of chemical and biological substances is a new direction for TOF applications.

OVERVIEW OF LASER DESORPTION TOF-MS

In its most common configuration, TOF-MS employs a pulsed ultraviolet laser to simultaneously desorb and ionize an analyte from a probe surface. The ions formed are accelerated across a short extraction region and allowed to drift down a flight tube, which is usually about 1 m long. As they pass through the drift region, ions disperse in time, with flight times proportional to the square root of their respective masses. An ion detector at the end of the drift region is used to monitor the flight times by recording the signal on a digital oscilloscope. It is in this acquisition mode that the true power of the TOF technique lies since the entire mass spectrum is recorded for every ionization event. Unlike other types of mass spectrometers, a TOF mass spectrometer has no scanning mass analyzer and therefore does not experience loss of signal due to scanning. This multichannel advantage has for years been exploited in TOF analyzers, but other factors limiting the mass

resolution have restricted its use mainly to research laboratories. Recent advances in high-speed electronics, miniaturized vacuum systems, and ultraviolet laser systems have enabled significant size reductions.

Linear TOF Configuration

A linear TOF instrument is one of the simplest chemical analyzers and comprises an ion source, a field-free tube for a drift region, and an ion detector (Fig. 1). Although the production of low-resolution mass spectra is almost trivial, the challenge is to design an instrument with sufficient resolution to uniquely identify the targeted analyte. Mass resolution is expressed in time units as $t/2\Delta t$, where t is the total flight time and Δt is the peak width of each TOF mass peak in the recorded spectrum. Therefore, assuming a constant peak width for each ion packet (constant Δt), a longer total flight time will produce a larger dispersion of ions and hence increased instrument resolving power. This factor has motivated many manufacturers to rely on long drift regions to maximize mass resolution.

Reflector TOF Configuration

The achievable resolution of TOF analyzers was significantly improved when Mamyrin et al.³ proposed that the energy spread (hence time spread) of the ion packets could be corrected for if the ions were allowed to penetrate an electrostatic "mirror" (Fig. 2), which would serve to focus the ions in time at the detector plane. This ion reflectron consists of a series of equally spaced conducting rings that form a retarding/reflecting field in which the ions penetrate, slow down gradually, and reverse direction, thereby reflecting the ions' trajectory back along the incoming path. Ions of a given mass pass into the reflector and are turned around at the same nominal depth within the retarding field.

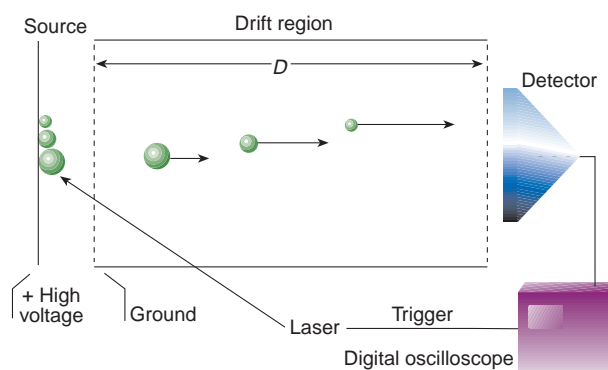


Figure 1. Diagram of a linear time-of-flight (TOF) mass spectrometer showing the principle of time dispersion of ions accelerated into a drift region: $t = \sqrt{m/2eV} (D)$, where t = total flight time, m = mass, D = length of the drift region, and eV = the acceleration energy.

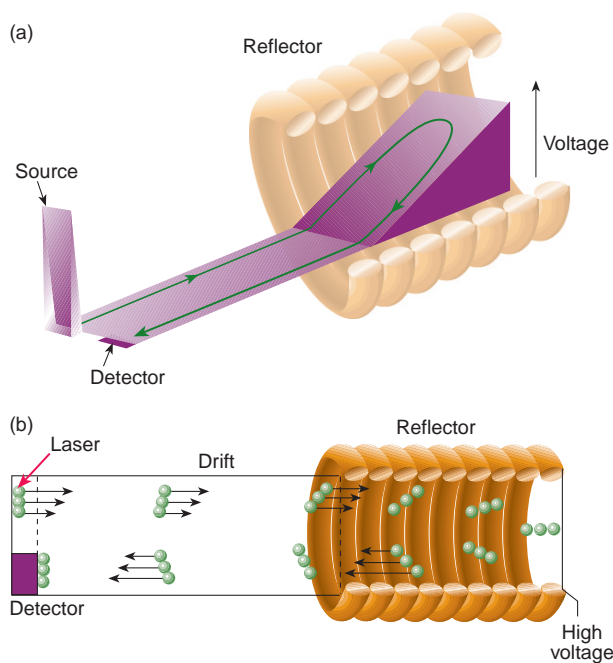


Figure 2. Reflectron TOF-MS. (a) Principles of energy focusing using a linear potential gradient ion reflector. (b) Higher-velocity ions for a given mass follow longer paths through the reflector, lag behind, then catch up with the slower ions at the detector surface.

However, the path traveled is slightly longer for ions with higher kinetic energies E_k .

Because these higher-velocity ions penetrate deeper into the opposing field, they will lag behind the lower-energy ions upon exiting the mirror. The position in the drift region at which the lagging ions catch up with the slower ions corresponds to the focal plane of the reflector. This position is independent of the ion mass and is normally the point chosen to locate the detector. Determining the focal point of this type of reflector (single-stage, constant field) is accomplished by simply satisfying the condition that $L = 4D$, where L is the total TOF drift length, and D is the depth of ion penetration within the reflector.

In addition to its energy focusing properties, the reflector also increases the total drift length by folding the flight path back onto itself. Hence, the total flight time t is increased, while the time spread Δt for ions of a given mass is reduced. Resolving powers on such reflectron TOF mass spectrometers are found to increase manyfold.⁴

Post-Source Decay–Product Ions

Normally, the term “energy correction” applies to the typical circumstance in which all ions are formed *during* the laser pulse (“prompt” fragmentation), not subsequently. Those fragment ions formed *after* the laser pulse are referred to as “metastable” and are the product of either slow unimolecular decay or bimolecular

collisions. If these late-forming fragment ions are created before they exit the extraction region, the resulting TOF mass peaks are asymmetrical in the time domain and exhibit skewed peak shapes. If, on the other hand, the metastable ions are formed during their flight through the drift region (e.g., by collisions with background gas), they are called post-source decay (PSD) ions.⁵

Although PSD peaks are rare in most TOF-MS data, one extremely important class of compounds—peptides (small fragments of proteins)—has been found to produce spectra with many PSD peaks. This propensity of peptides to form fragment ions long after initial acceleration can be explained by a breaking of the peptide linkage along the amino-acid backbone, resulting in a kind of “self-sequencing” decomposition. The PSD product ion peaks are attributable to amino-acid chain fragments of the original peptide precursor.

Detection of PSD ions has been found to be extremely useful in biochemical analysis, mainly for the sequencing information they yield. But detection of PSD ions can be difficult. Relying on the property that all ions acquire the same *energy* within the source, traditional TOF mass spectrometers function by causing dispersion of ion velocities proportional to the ions’ respective masses. However, PSD product ions are formed during the drift period, so their velocities equal that of the precursor. Hence, their energies, rather than their velocities, are dispersed in direct proportion to their masses. Under these circumstances, a linear TOF cannot detect the presence of product ions, since their arrival at the detector occurs simultaneously with that of their parent ions; i.e., no field gradient exists to separate the ions in time.

For example, if unimolecular decomposition splits the molecular ion in half during passage through the drift region, the fragment will retain half the initial E_k of the precursor ion. Hence, the fragment now penetrates only halfway into the reflector. Considering again the focal point relationship ($L = 4D$), D has been reduced by a factor of 2, so L is likewise reduced, and the focal point is shifted closer to the reflector. Each PSD fragment ion is therefore focused to a different point in space (Fig. 3a).

In several commercial TOF instruments, focusing across the entire PSD spectrum is accomplished by stepping the voltage of the reflectron using 10 to 20 segments. Progressively lower mass portions of the PSD spectrum are focused as the reflector voltage is decreased. The entire spectrum is then reconstituted by “stitching” together the individual spectral fragments. This brute-force method of acquiring PSD spectra presents several serious problems. Most importantly, the TOF mass spectrometer is converted into a scanning instrument, thereby defeating the primary strength of TOF mass analyzers, namely, the ability to rapidly acquire a complete mass spectrum without the need for

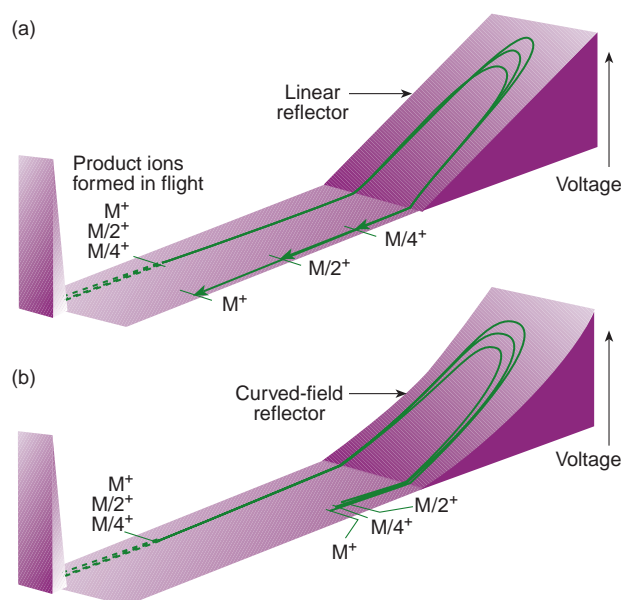


Figure 3. Comparison of focal properties between linear and curved-field reflectrons. (a) Post-source decay (PSD) fragment ions (M^+) focus at different points in space after passage through the reflector. For this special case, the focal plane of the reflector is dependent on mass. (b) Focal points of all PSD fragments are located at the same point in space after exiting the curved-field reflector, thereby eliminating the need to scan the reflector voltage.

any type of scanning procedure. As a result, precious sample may be consumed by the laser desorption process during the time required for the reflector scanning process. Calibration is also difficult since each segment of the PSD spectrum corresponds to a different calibration curve. Although useful in principle, the reflector scanning technique for acquiring PSD data has proven to be difficult and cumbersome in practice.

Curved-Field Reflectron

To circumvent the scanning procedure for PSD data collection, a unique⁶ type of ion reflector was designed⁷ which enables all product ion peaks to be focused simultaneously, thereby recovering the multichannel advantage of the TOF instrument. This is accomplished by shaping the retarding field so that ions experience a progressively steeper potential gradient as they penetrate more deeply into the field of the reflector. Unlike the focusing condition of the linear gradient reflector ($L = 4D$), the curved-field reflector has a single geometric focusing condition. Once the overall reflector depth D and the field free-drift distance L are fixed (in this case, L and D are approximately equal), nearly all of the ions of all energies are focused at the detector plane, no matter how deeply they penetrate the ion reflector (Fig. 3b).⁷ Entire product ion spectra are thus acquired in a few seconds, sample consumption is greatly reduced, and the complete calibration procedure, based on an experimentally determined curve, is instantaneous.⁸

Since precursors and their respective product ions move together at the same velocity through the drift region, this fortuitous characteristic of PSD fragmentation can be exploited for spectral simplification by inserting an appropriate gating mechanism into the ion path. By selecting (gating) a particular precursor ion, and hence ejecting all others, the resulting spectrum consists exclusively of the selected precursor and only those products associated with that selected precursor. Using this technique to isolate the precursor and fragment ions is similar to, if not exactly like, tandem MS.

For a "true" tandem, two separate mass analyzers are linked together in series, separated by a cell in which a mass selected ion is fragmented by collision with a neutral gas. The first analyzer is tuned specifically to transmit only a single mass, and the second is used to record the spectrum of the collision-induced product ions. Similarities between this method and the gated/curved-field combination have led the latter to be referred to as a "pseudo-tandem," because only a single mass analyzer is employed and no gas is added to induce collisions. The distinguishing advantage of the true tandem is the high performance of the first mass analyzer. For a magnetic sector mass analyzer, mass selection capabilities are very narrow ($\pm 0.5\%$ of selected mass), while the gate on the Pseudo-Tandem TOF instrument exhibits far less selectivity ($\pm 4\%$). However, for a rugged, compact, field-portable instrument, the Pseudo-Tandem TOF has an appreciable amount of analytical potential in its own right.

Figure 4 shows the components of the Pseudo-Tandem TOF Mass Spectrometer. Figure 5 is a survey mass spectrum showing a mixture of peptides. Low-mass ions found below 500 Da are attributable to the organic matrix required for the MALDI process, in this case α -cyano-4-hydroxy-cinammic acid. A demonstration of the gating function for the pseudo-tandem mode is shown in the figure. First, the gate is set to isolate the TOF spectrum of angiotensin by adjusting a pulse generator to drop the voltage on the gate from ± 500 V to ground at precisely the time this peptide ion is passing through the gate. The resulting spectrum (angiotensin II, Fig. 5) shows that the matrix ions have been eliminated, as have the other peptides present in the sample. What is found to emerge from the now-reduced background signal, however, is the product ions of angiotensin II, which in this case represent those fragments comprising the sequence ions.

Also shown in Fig. 5 are the other isolated components of the mixture. Of particular note is the PSD spectrum of substance P, which is an excellent test case for the performance of the instrument since its product ion spectrum is normally weak, and the reduced drift length of the mini-TOF makes its detection particularly challenging. The signal quality demonstrated here

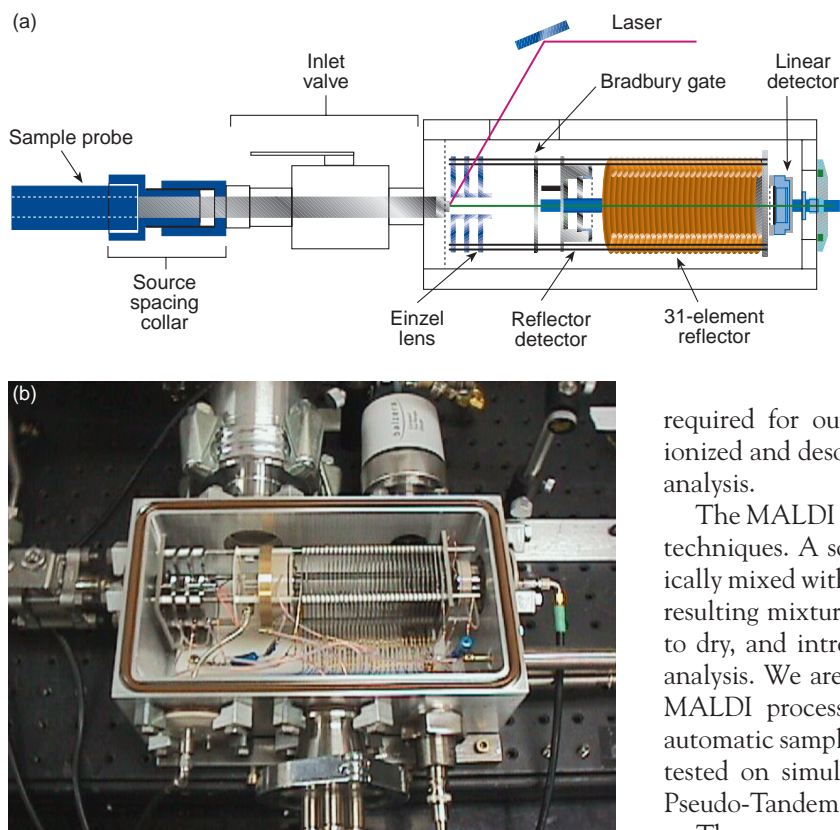


Figure 4. A diagram (a) and photograph (b) of the prototype Pseudo-Tandem TOF Mass Spectrometer.

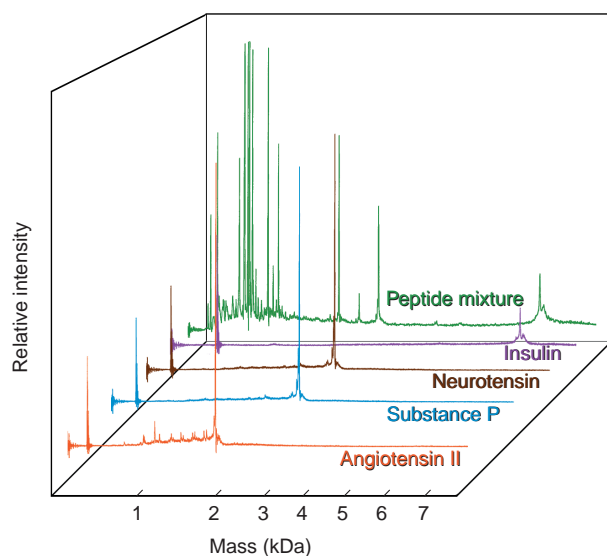


Figure 5. Data showing spectral simplification by using the pulsed ion gate.

compares well with the performance of significantly longer commercial-grade TOF mass spectrometers.

BIODETECTION USING MINIATURE TOF-MS

We are currently developing a miniaturized TOF mass spectrometer system for use in field-portable bio-agent detection systems (Fig. 6). The MALDI methods we apply are a much softer ionization technique, requiring much lower laser irradiances (typically 1 MW/cm^2). With this technique, biomolecules as large as 300,000 Da, which are

required for our chemotaxonomic approach, can be ionized and desorbed intact into the gas phase for mass analysis.

The MALDI process generally involves wet chemical techniques. A solution of the matrix molecule is physically mixed with a solution containing the analyte. The resulting mixture is applied to a sample probe, allowed to dry, and introduced into the mass spectrometer for analysis. We are developing an alternative concept for MALDI processing that is much more amenable to automatic sample preparation. These methods are being tested on simulants using a prototype version of the Pseudo-Tandem TOF Mass Spectrometer.

The spectra of four simulants acquired on the miniature TOF are plotted in Fig. 7. (Field tests for this analysis will be conducted at the Dugway Proving Grounds.) These benchmark tests are used to characterize the mass spectral properties of the simulants using conventional laboratory sample preparation methods and rather high analyte concentrations. Obviously, using MALDI-TOF-MS to distinguish between the bio-agent simulants (ovalbumin, MS-2, BG spores, and *Erwinia herbicola*) is straightforward since the mass

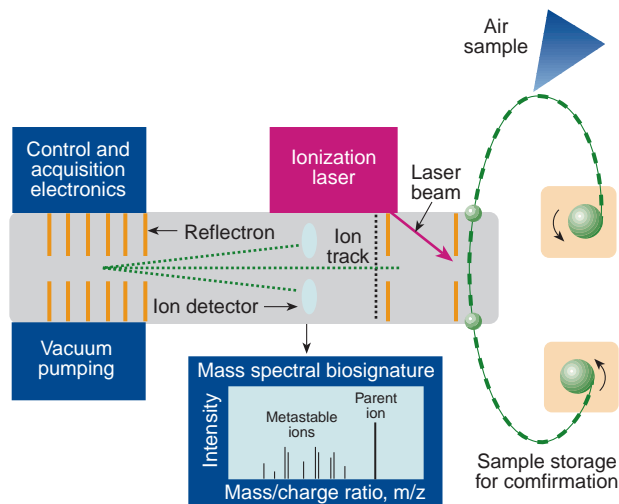


Figure 6. Schematic of a miniature TOF biodetection system.

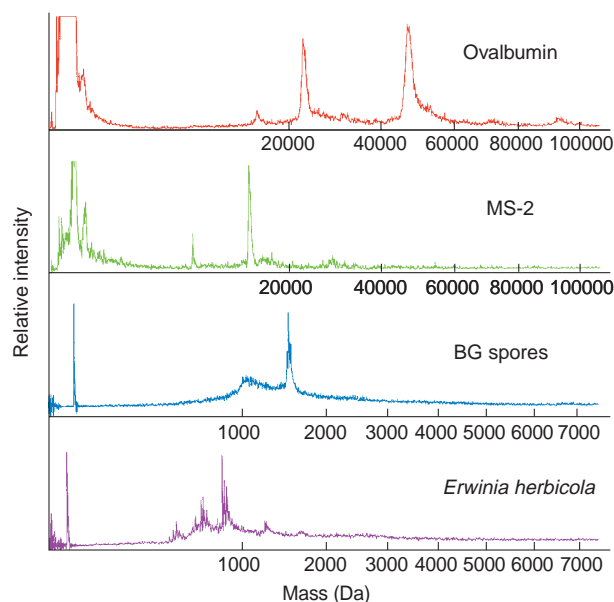


Figure 7. Four spectra of the Dugway simulants. Data were collected from the Pseudo-Tandem TOF Mass Spectrometer.

differences among the agents are substantial. The challenge, however, is to identify the signature mass spectral peaks from trace aerosol concentrations deposited onto a flexible substrate, in this case mylar VCR tape. Substantial efforts are currently under way to achieve this goal.

MASS SPECTRAL BIOMARKER DEVELOPMENT

Although MS is a prime candidate for rapid identification of microorganisms, the instrumentation cannot be employed without the development of mass spectral signatures of the materials of interest. We are using a "chemotaxonomic" approach to this problem, which recognizes that all materials of biological origin are composed of common building blocks, including inorganic chemicals, small organic molecules, carbohydrates, amino acids, and nucleic acids.⁹ (Whereas conventional techniques for microbe identification have relied on morphological and metabolic characteristics, advances in biochemistry, molecular biology, and chemical instrumentation have created new avenues of taxonomy based on the chemical makeup of the cells of the organism. Such an approach is commonly described as chemotaxonomy, which Priest and Austin¹⁰ defined as the study of chemical variation in living organisms, and the use of these chemical characters for classification and identification.) Such building blocks are linked precisely to form all manner of biological materials. These larger macromolecules are the prime signatures that can be used to identify materials of interest.

Chemical markers are being developed that are characteristic of broad classes of microorganisms as well as those that are specific for specific pathogens. These markers can be extremely rapidly quantified using the techniques of modern MS.

The polar lipids present in bacterial cell (and several other) membranes are classically useful chemotaxonomic biomarkers. These biochemicals are also attractive (and useful) as mass spectral biomarkers. A straightforward approach to their mass spectra is to record the molecular ions of the intact phospholipids, including both the polar head groups and the two fatty acid sidechains. Classical treatises¹¹⁻¹³ consider the polar head groups to be more closely under genetic control and the fatty acids to vary quantitatively, reflecting nutritional history and health. The structures and relative amounts of polar head groups and fatty acids in a mixture of phospholipid families may be separately deduced by recording and interpreting phospholipid ion fragmentation. This high-definition step is readily automated. Using fragmentation in this way takes advantage of the high information content of mass spectra and provides information on both the chemotaxonomy and the production of environmental microorganisms. The structures and relative abundances of polar head groups vary among gram-negative and gram-positive bacteria, fungi, and algae, and our earlier studies indicated that semiquantitative considerations based on mass spectra would enable the broad classification of microorganisms.⁹

Phospholipid biomarker libraries include both molecular ion fingerprints and polar head group analyses derived from fragment ions. We are determining the extent to which polar head group ratios can be used to distinguish species and, in particular, target organisms. These quantitative analyses are based on constant neutral loss spectra, which are measured with sophisticated laboratory instruments. These instruments are used to unambiguously determine the molecular identity of the ions and to test the reliability of signal processing algorithms that may be able to extract polar head group information from full-spectrum TOF data.

Although polar lipids are present in bacterial cells and enveloped viruses, they are absent in most viruses and all proteinaceous toxins. A more universal class of biomarker is based on peptides and proteins. The use of this type of biomarker is complicated by the large number (several thousand) of proteins present in a typical cell. However, proteins are being identified that are characteristic of specific bacteria and viruses.

Finally, the genetic pattern (DNA and RNA) of bacteria and viruses is an absolutely characteristic biomarker. Nevertheless, current mass spectrometer technology does not allow the measurement of long-chain DNA and RNA. We are evaluating the chemical and enzymatic production of smaller fragments

(oligonucleotides) for directly fingerprinting viruses. Nucleotide mapping is more difficult for bacteria, where the genetic machinery constitutes a smaller fraction of the cellular mass, and amplification is required.¹⁴ Nucleic analysis using MS is best accomplished by first enzymatically degrading the nucleic acid using probes immobilized on a surface that capture specific sequences and then measuring the mass distribution on the surface. With properly designed probes, efficient generation of genetic information is provided by a mass spectrometer.

CONCLUSIONS

We have discussed the development of a TOF mass spectrometer suitable for integration into a field-portable biodetection system. This instrument includes an advanced reflector design for improved mass resolution and range, and an ion gate for the measurement of PSD ions. We have demonstrated in a laboratory prototype performance rivaling that of commercial systems that are significantly larger than our design. Therefore, the chemotaxonomic methods that are now under development in the laboratory using commercial TOF-MS systems can be readily translatable to the miniaturized system. Using simulants of biological warfare agents, we have demonstrated the utility of this system for analyzing a variety of biological compounds of interest to the biological defense community. When integrated into a biodetection system that will include automated air sampling, miniaturized electronics, as well as an automated

biomarker classification system, the TOF mass spectrometer will represent a significant advancement over the current generation of biodetection systems.

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