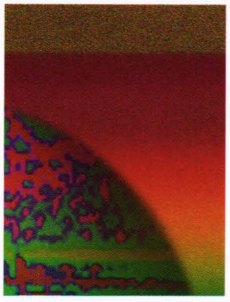

The Tiny-TOF Mass Spectrometer for Chemical and Biological Sensing

Wayne A. Bryden, Richard C. Benson, Scott A. Ecelberger, Terry E. Phillips, Robert J. Cotter, and Catherine Fenselau

Mass spectrometry is the most sensitive and specific detection methodology ever developed for a broad range of chemical substances. It is widely used in environmental, medical, military, and industrial applications, and would see even broader use if the equipment could be made portable enough for field measurements. To date, however, the equipment used for high-performance mass spectrometry has been large, heavy, and power-hungry, precluding its use in remote field measurements. The Applied Physics Laboratory has developed a small, powerful, time-of-flight mass spectrometer in a collaborative program with the Johns Hopkins Medical Institutions and the University of Maryland. Miniaturization of the equipment required the development of new techniques for ion formation in the ion source region and the use of kinetic-energy spread correction schemes based on ion reflectrons. Sampling and ionization schemes for solids, liquids, and vapors have led to promising conceptual designs for microorganism identification, environmental monitoring, and law enforcement analytical tools.

INTRODUCTION

At its most basic level, mass spectrometry is an extremely-high-resolution microbalance that allows determination of the mass of a molecule. From detailed analysis of the masses of the molecule and its fragments, molecular identification is accomplished. These molecular measurements can be carried out at the attomole level of material (10^{-18} mole) using specialized laboratory-based instruments. The combination of specific molecular identification and extremely high sensitivity makes mass spectrometry one of the most powerful analytical laboratory tools ever developed for detection and identification of chemical and biological substances.

Figure 1 shows a simple block diagram of a mass spectrometer system. Mass spectrometry is a vacuum-based technique that first requires the vaporization of the sample into the vacuum chamber. The gas molecules are then ionized using one of several different energetic interactions in the source region. The ions are separated in the mass analyzer according to their mass-to-charge ratio (m/z) and are sequentially

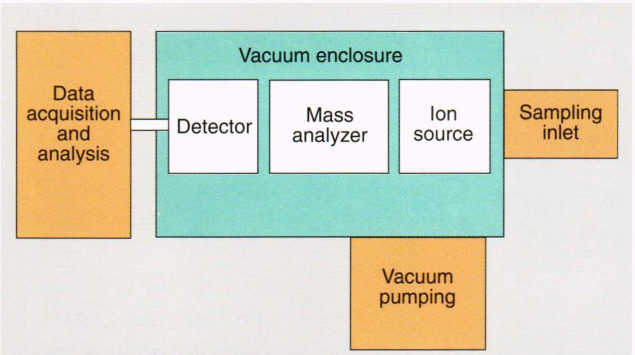

Figure 1. Block diagram of a mass spectrometer system.

Tandem mass spectrometers are undoubtedly the most sensitive, powerful, and flexible analytical tools available for chemical analysis and are crucial to the development and validation of advanced analytical methods.

detected, often with a high-gain electron multiplier. Finally, the data are acquired to form a mass spectrum (a plot of ion intensity versus m/z) and analyzed using computer-aided techniques. Each of the subsystems—vacuum, source, mass analyzer, detector, and data handling—is subject to significant variability based on the intended application.

Figure 2 shows the mass spectrum of a simple organic compound, caffeic acid. The spectrum was acquired using a small mass spectrometer developed at APL and is typical of spectra for pure chemical substances. It is characterized by the molecular peak—a peak corresponding to the molecular weight of the substance—and a series of fragment peaks that are related to the molecular ion by loss of stable molecular fragments. Analysis of such mass spectral patterns generally leads to unambiguous molecular identification for *pure* substances. For impure samples, however, as the ratio of target analyte to environmental impurity is diminished, the mass spectra rapidly become indecipherable. Impure samples, containing many compounds, are usually analyzed using a mass spectrometer coupled with a chromatographic technique that temporally separates the chemical compounds in the sample. These chromatographically pure compounds are then presented sequentially to the inlet of the mass spectrometer for analysis. Alternatively, two or more mass analyzers can be connected in tandem to provide separation capability that is superior in speed and resolution to common chromatographic techniques.

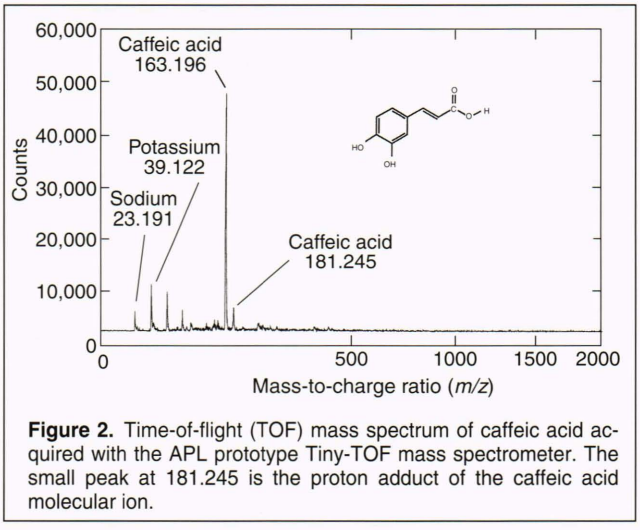

Figure 2. Time-of-flight (TOF) mass spectrum of caffeic acid acquired with the APL prototype Tiny-TOF mass spectrometer. The small peak at 181.245 is the proton adduct of the caffeic acid molecular ion.

Tandem mass spectrometers are undoubtedly the most sensitive, powerful, and flexible analytical tools available for chemical analysis and are crucial to the development and validation of advanced analytical methods. We are fortunate to have access to such an instrument, located at the Structural Biochemistry Center at the University of Maryland, Baltimore County. However, as seen in Fig. 3, tandem mass spectrometers are large, heavy, power-hungry instruments that are inappropriate for portable field applications.

This article describes the development of a small, portable, pseudotandem mass spectrometer for potential field detection of chemical and biological substances. Three principal topics are discussed: development of a miniature mass analyzer based on time-of-flight (TOF) technology; sampling and ionization schemes for liquid, solid, and gaseous substances; and conceptual detection systems for health care, military, environmental, and law enforcement applications.

TOF MASS ANALYZERS

The linear TOF mass spectrometer has a conceptually simple design (Fig. 4). Ions are formed in a short source region of length s, generally defined by a backing plate and an extraction grid. A voltage V placed on the backing plate imposes an electric field E across the source region, where $E = V/s$. The electric field accelerates all of the ions to the same kinetic energy U, which is given by

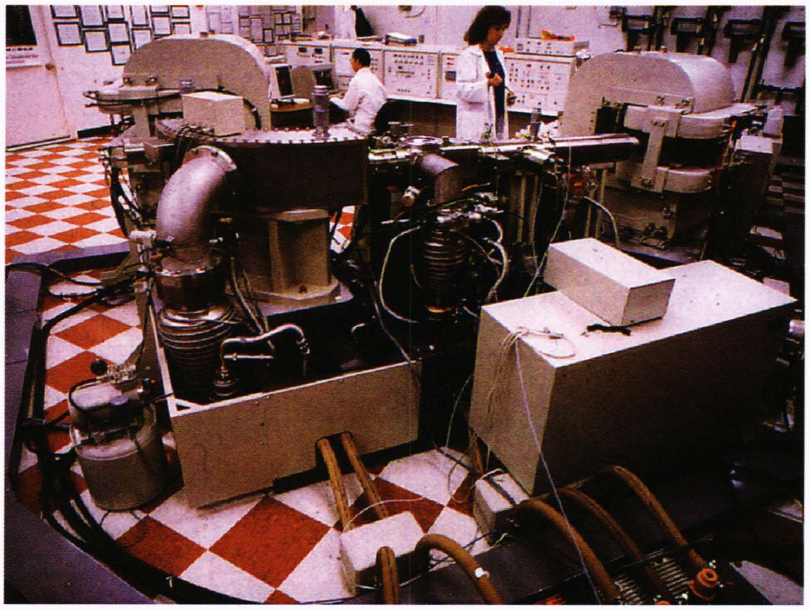

Figure 3. JEOL four-sector mass spectrometer located at the Structural Biochemistry Center at the University of Maryland, Baltimore County.

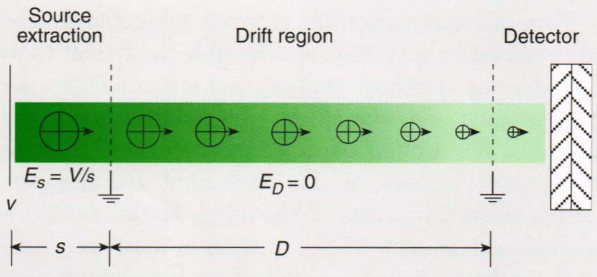

Figure 4. Schematic of a simple linear TOF mass analyzer. (Adapted from Ref. 1.)

$$\frac{mv^2}{2} = zeV = zeEs\,, \tag{1}$$

where m is the mass of the ion, v is the ion velocity, e is the charge on an electron, and z is the number of charges on the ion. The charge, z, is 1 for most ions, though higher values are occasionally seen, especially for larger ions. As the ions pass through the extraction grid, their velocities depend inversely on the square root of their mass-to-charge ratio:

$$v = \left(\frac{2zeEs}{m}\right)^{1/2}. \tag{2}$$

The ions then pass through a much longer drift region of length D. Because they spend most of their time in this region, their TOF, t, measured at the detector, is given approximately by

$$t = \left(\frac{m}{2zeEs}\right)^{1/2} D. \tag{3}$$

The TOF spectrum can be converted directly to a mass spectrum using known values of the accelerating voltage and drift length:

$$\frac{m}{z} = 2eEs\left(\frac{t}{D}\right)^2. \tag{4}$$

Historically, TOF mass spectrometers have employed long flight tubes (up to 8 m) and high accelerating voltages (up to 30 kV), and they have required low background vacuum pressures (10^{-7} to 10^{-8} Torr). Long flight tubes result in longer flight times (that are more easily measured) and, along with high acceleration potentials, they yield increased mass resolution. However, the long tubes, in addition to making the instrument large, require lower pressure levels to minimize gas-phase interactions that can degrade the mass spectrum.

More recently, TOF technology has evolved rapidly along with high-speed electronics, lasers, and vacuum technology. These advances made it clear that we could construct a small, rugged, yet extremely powerful TOF mass spectrometer by carefully tailoring ion creation in the source region, using kinetic energy correction schemes, and employing state-of-the-art analog to digital converters and pulsed lasers. Furthermore, the use of a short flight tube would reduce the stringent vacuum requirements such that modern, low-powered vacuum equipment could be used to achieve the necessary vacuum level of about 10^{-6} Torr. The next section explores the design considerations that led to the successful development of the small TOF mass spectrometer (the Tiny-TOF).

DEVELOPMENT OF THE TINY-TOF

Spatial, Temporal, and Kinetic Energy Effects on Mass Resolution

In simple linear TOF mass spectrometers, mass resolution is generally on the order of 1 in 300 to 400. (Mass resolution is defined here as $m/\Delta m$, where Δm is the minimum difference that can be detected at a

particular mass m). Although such mass resolution is sufficient for some applications, higher resolution is necessary for advanced analytical techniques. Of crucial importance to the performance of TOF analyzers is the capability to create a monoenergetic, tightly spaced ion bunch that is injected into the mass spectrometer at a well defined starting time. Wiley and McLaren,[2] in designing a pulsed-electron impact TOF spectrometer, reported on the deleterious effects of uncertainties in the time of ion formation, location in the extraction field, and initial kinetic energy on mass resolution. These phenomena are illustrated in Fig. 5.

Figure 5a shows how two ions of the same mass that are formed at different times but have the same kinetic energy will traverse the drift region, maintaining a constant difference in time and space. Because mass resolution on the TOF mass spectrometer is given by

$$\frac{m}{\Delta m} = \frac{t}{2\Delta t}, \tag{5}$$

and the time interval Δt is fixed by the ion formation time and measurement electronics, mass resolution can be improved by increasing the flight time t, that is, by using low accelerating voltages, long drift lengths, or both. In the Wiley and McLaren instrument, the electron beam pulses used for ionization were on the order of 0.5 to 5.0 μs (quite long compared with the expected drift time), necessitating the use of pulsed ion extraction to reduce Δt. At the other extreme, laser desorption instruments generally employ lasers with pulse widths from 300 ps to 10 ns. In such cases, the width of Δt may be limited by the detector response or the signal digitizer rather than by uncertainties in the time of ion formation.

When ions of the same mass are formed in different locations in the extraction field (Fig. 5b), the ions formed near the back of the source will be accelerated to a higher kinetic energy than ions formed closer to the extraction grid. The ions formed at the rear of the source enter the drift region later but have higher velocities, so they eventually pass the ions formed closer to the extraction grid and arrive at the detector sooner. It is possible to adjust the extraction field so that ions of any given mass arrive at a space focus plane, located at a distance $2s$ from the source, at the same time. In a properly adjusted instrument, the space focus plane is positioned at the detector. The location of the space focus plane is independent of mass, and ions of different masses arrive at this plane at different times. For desorption techniques, in which ions are formed at a surface rather than in the gas phase, the spatial distribution is greatly minimized.

Ions formed with different initial kinetic energies (Fig. 5c) will have different final velocities after acceleration and will arrive at the detector at different times. This is the most difficult initial condition to correct in a linear instrument because increases in drift length increase peak widths along with the separation of peaks of different mass. Reflectrons and other energy-focusing devices described later in this article are used in the mass analyzer to compensate for differences in kinetic energy in the source.

In summary, using an approach similar to that described by Wiley and McLaren,[2] it is possible to derive an equation for the arrival time of an ion, formed at time t_0, that reflects the initial temporal, spatial, and kinetic energy conditions. That equation is

$$t = t_s + t_D + t_0, \tag{6}$$

where t_s is the time spent in the source and t_D is the time spent in the drift region. The initial conditions reflected in Eq. 6 are given by Cotter:[3,4]

$$t = \frac{(2m)^{1/2}}{eE}\left[\left(U_0 + eEs_0\right)^{1/2} \mp U_0^{1/2}\right] + \frac{(2m)^{1/2} D}{2\left(U_0 + eEs_0\right)^{1/2}} + t_0 . \tag{7}$$

The quantity $U_0 + eEs_0$ is the final kinetic energy of an ion having an initial kinetic energy U_0 and accelerated from an initial position s_0 in the source. In the

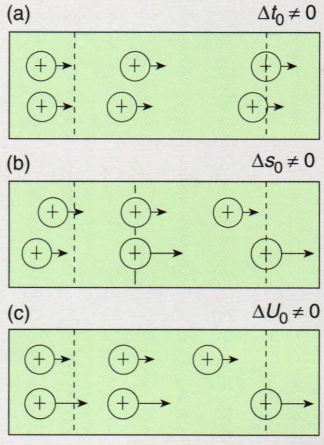

Figure 5. Illustration of sources of error in the TOF mass spectrometer source region. Ions form (a) at slightly differing times, (b) at different locations within the source region, and (c) with different initial kinetic energies. (Adapted from Ref. 1.)

first term in Eq. 7, this same kinetic energy is reached by ions on exiting the source, regardless of the initial direction of ion velocity, whereas the time spent in the source reflects the turnaround time $\mp U_0^{1/2}$. In the second term in Eq. 7, the time spent in the drift region depends again on the initial position s_0 and kinetic energy U_0 in the source, but not on the initial direction of velocity. Thus, longer drift lengths D increase the magnitude of t_D and decrease the effects of turnaround time as well as uncertainties in the time of ion formation t_0. It may also be noted that large extraction voltages ($V = Es$) minimize the effects of U_0.

As noted previously, mass resolution is determined by the peak widths for a given mass, relative to the separation of peaks of different mass. In the TOF mass spectrometer, peak widths Δt are determined by uncertainties in the temporal, spatial, and initial kinetic energy distributions and can be determined by differentiating Eq. 7 with respect to Δt_0, Δs_0, and ΔU_0. In practice, mass resolution is achieved using a combination of three approaches: (1) eliminating or minimizing the initial distribution, (2) correcting the initial distribution during ion extraction from the source, and (3) compensating for the effects of the initial distribution in the mass analyzer.

The Reflectron Energy-Focusing Device

The discussion so far has clarified the possibility of minimizing the temporal and spatial distribution problems using short-pulsed ionization sources, desorption from equipotential surfaces, and fast detectors. However, the initial kinetic energy distribution, which is a property of the ionization technique used, remains the primary initial condition not easily corrected in a simple linear instrument.

We have derived a solution to the initial kinetic energy problem from the work of Mamyrin et al.,[5] who introduced an energy-focusing device known as a reflectron. This device consists of a series of retarding lenses (Fig. 6). The reflectron uses a linear electrical field to turn the ion trajectories around 180°, that is, back along their initial flight axis. In practice, the ions return at a slight angle to permit the detector to be located adjacent to the ion source in an off-axis configuration (Fig. 6a) or in a coaxial configuration (Fig. 6b). With both of these designs, the more energetic ions penetrate the retarding field of the reflectron to a greater depth, so they travel a longer path, arriving at the detector at the same time as the less energetic ions.

Ion Behavior in Reflectron Instruments

Ions spend part of their time in reflectron instruments in two field-free regions having lengths L_1 and L_2, and they spend the rest of the time passing into and out of the reflectron, where they turn around at a penetration depth d. If the combined length L of the drift regions is equal to $L_1 + L_2$, then the time that an ion spends in the field-free regions, t', is given by

$$t' = \left(\frac{m}{2eV}\right)^{1/2} L, \tag{8}$$

which is equivalent to Eq. 3 (assuming that, as in most cases, $z = 1$). When the ions enter a single-stage reflectron, they decelerate in a time given by

$$t'' = \left(\frac{m}{2eV}\right)^{1/2} 2d. \tag{9}$$

After turning around, they are reaccelerated in the same time. Thus the total time to traverse both drift regions and pass through the reflectron distance twice is given by

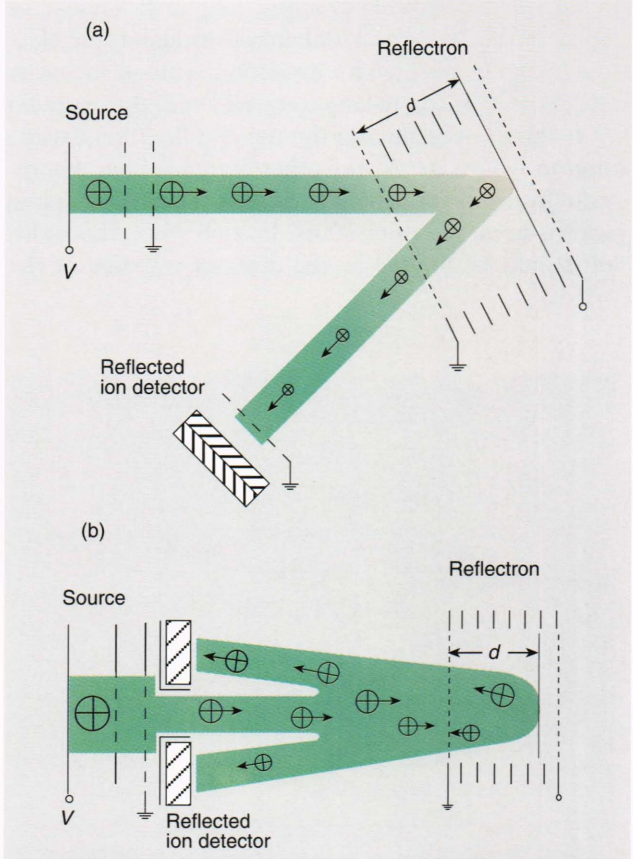

Figure 6. Off-axis (a) and coaxial (b) configuration of a reflectron TOF mass spectrometer. This device corrects the problem of uncertainty in initial kinetic energy distribution. (Adapted from Ref. 1.)

$$t = \left(\frac{m}{2eV}\right)^{1/2} \left(L_1 + L_2 + 4d\right) = km^{1/2}, \qquad (10)$$

where the factor of 4 results from two passes through the reflectron with an average velocity of half of that in the linear regions. Given that Eq. 10 follows a square root law, the same empirical equations for calibrating the mass scale are equally valid for reflectron and linear instruments.

For the single-stage reflectron, Tang et al.[6] noted that optimal focusing is achieved when $L_1 + L_2 = 4d$, which means that ions spend equal amounts of time in the field-free and reflectron regions (i.e., $t' = t''$). Using Eq. 10, it is possible to illustrate in a simple fashion how the reflectron achieves energy focusing. For an ion with an initial kinetic energy U_0 in the forward direction, the total flight time will now be given by

$$t = \left[\frac{m}{2\left(eV + U_0\right)}\right]^{1/2} \left(L_1 + L_2 + 4d\right). \qquad (11)$$

The increase in ion kinetic energy means that the ion will spend less time in the field-free regions (L_1 and L_2), which have fixed distances. However, the penetration depth d will increase, so that the total time in Eq. 11 will be exactly the same as the time in Eq. 10, and the ions will be in focus.

In addition to serving as an energy focusing element, the reflectron has added a powerful new dimension to TOF mass analyzers. Although ions that fragment in the drift region of a linear TOF analyzer produce a signal that is characteristic, in time, of the parent species, such fragmentation has other consequences in the reflectron instrument. For this type of instrument, an ion that fragments in the first field-free region (L_1) produces (1) a neutral product that is not reflected, and which therefore does not contribute to the ion signal as in linear instruments, and (2) a fragment ion whose arrival time at the detector corresponds to neither the precursor nor the product ion mass. The flight times of products from such metastable transitions are predictable, however, and they can be interpreted if one can identify the precursor. If a relationship can be established between the precursor and fragment ion peaks, particularly in a complex spectrum with many components, molecular identification is greatly enhanced. This powerful technique, akin to tandem mass spectrometry, is at the heart of the application of the Tiny-TOF to analysis of samples acquired from the natural environment.

One approach to identifying molecular parent–daughter relationships was introduced by Della-Negra and LeBeyec[7] using a coaxial dual-stage reflectron and developed by Standing et al.[8] using an ion mirror. In this approach, all ions are permitted to enter the reflectron. A detector is also located at the rear of the reflectron and records neutral species resulting from metastable decay in the first field-free drift length. Because these neutral species appear at times corresponding to the mass of the precursor ion, it is possible to register ions in the reflectron detector only when a neutral species corresponding to the precursor mass is received. The resultant spectrum, known as a correlated reflex spectrum,[7] can be obtained using single-ion pulse counting methods.

We are developing another approach that relies on rapid switching of the reflectron from the linear field mode just described to a mode known as a curved field mode. The curved field reflectron was recently invented and patented by Cornish and Cotter[9] at the Johns Hopkins Medical Institutions. It allows simultaneous recording, with high resolution, of all product ions in the mass spectrum. Correlation between linear field spectra and curved field spectra can result in the same sort of pseudotandem mass spectrometry that is displayed by the correlated reflex technique.

The Tiny-TOF

When designing the Tiny-TOF mass spectrometer, we considered all of the TOF enhancement paradigms described earlier and, in addition, the constraint of portable operation. The design of a prototype instrument, now functional at APL, is shown in Fig. 7. A pulsed nitrogen laser is used to desorb ions from a smooth conducting surface parallel to the extraction grid. This arrangement minimizes the uncertainties in the time and location of ion formation. The ions are then accelerated toward the extraction grid and focused by an Einzel lens. The focused ions traverse the first field-free region and enter the reflectron, where they are energy-corrected and turned around. They then traverse the second field-free region and strike the fast multichannel plate detector, producing a mass spectrum.

Figure 8 shows a potential energy diagram of the elements of the analyzer. The electrostatic model was produced using SIMION, a code authored by D. Dahl of the Idaho National Engineering Laboratory. An ion track is superimposed on the potential energy surface to illustrate the effects of the accelerating potential, the Einzel lens, and the reflectron.

Although the mass analyzer itself is small and lightweight (20 cm long, with a 60-cm^2 cross section, and weighing 500 g), it is only part of the overall mass spectrometer system (Fig. 1). The current laboratory

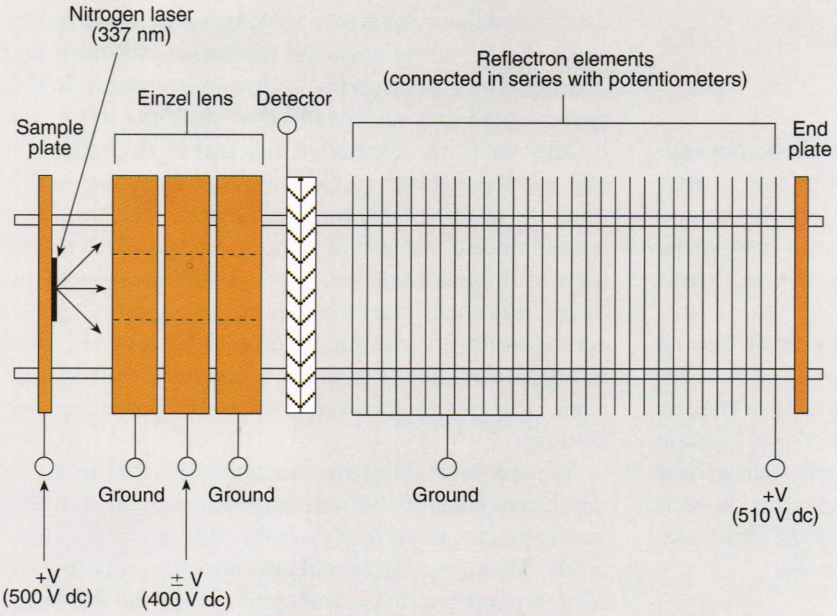

Figure 7. Schematic of the APL prototype Tiny-TOF mass spectrometer. This instrument incorporates recent advances in TOF and vacuum technologies, high-speed electronics, and lasers.

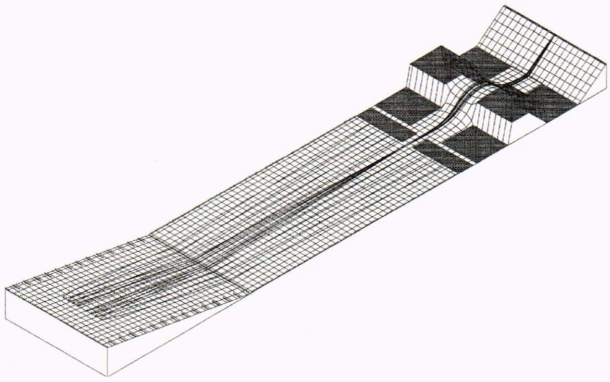

Figure 8. Electrostatic simulation of the electrode assembly of the prototype Tiny-TOF. A representative ion track is superimposed to show ion motion due to the electrostatic fields.

(<1000 V) and detector voltages (<2.5 kV) are generated by small nuclear instrumentation module (NIM) high-voltage power supplies. Ions are generated by interaction of the solid sample with short pulses from an ultraviolet laser (337 nm wavelength, PSI Model PL 2300) with power densities of about 1 MW/cm^2 at the sample. The TOF spectra are acquired using a coaxial microchannel plate detector (Galileo Model LPD-25) coupled to a fast digital oscilloscope (up to 2×10^9 samples/s, LeCroy Model 9354M). The triggering for the data acquisition is acquired using a fast photodiode optical detector (EGG Model FND-100Q) to sense the laser pulse. The TOF spectra are processed into mass spectra and then displayed and stored using TOFWARE (Ilys Software) on a personal computer.

Power requirements for the prototype Tiny-TOF are a modest 120 W, not including the personal computer and digital oscilloscope. The size of the system is currently somewhat large for portable use. However, gains in portability can easily be achieved using an advanced pumping system with a smaller enclosure, miniaturization of electronics (the size and power consumption of the computer, power supplies, and digitizing electronics could readily be reduced employing off-the-shelf hardware), and alternative ionization sources such as small lasers or other directed energy devices. Depending on the application and type of sample, the Tiny-TOF can thus be reduced from the current prototype to a small, field-deployable unit and even, for specialized applications, a battery-powered handheld device.

prototype Tiny-TOF system is shown in Fig. 9. The mass analyzer is inside a custom-designed vacuum enclosure and is pumped using a split-flow turbomolecular-drag pump (Balzers Model TMH-065) and a diaphragm backing pump (Balzers Model MVD12). Vacuum levels of about 10^{-6} Torr are easily obtained. The sample is placed on a metal sample fixture and introduced into the mass spectrometer through a custom-designed vacuum load-lock. Typical acceleration, Einzel lens, reflectron voltages

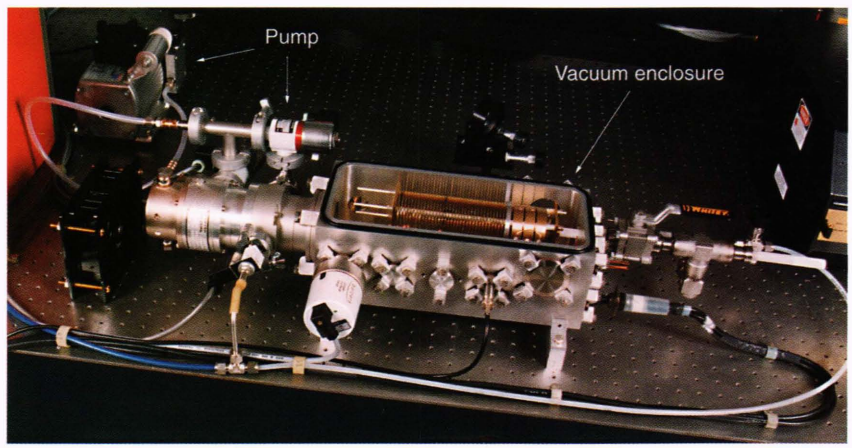

Figure 9. The Tiny-TOF system, showing the vacuum enclosure and pumping arrangement.

SAMPLING AND IONIZATION SCHEMES FOR SOLID, AQUEOUS, AND VAPOR SUBSTANCES

If the Tiny-TOF is to be used for applications in the field, it must possess capabilities for investigating solid, aqueous, and vapor-based samples. This section describes the techniques developed to handle these different inputs.

Analyzing Solid Samples Using Laser Desorption Techniques

When a solid sample is placed on a conducting surface that is in contact with an accelerating potential and is exposed to laser irradiation of sufficient power density, ions are formed and accelerated into a mass spectrometer for analysis. Depending on the peak power density of the laser, different ionization and molecular fragmentation conditions are obtained. For example, Hillenkamp et al.[10] introduced a high-peak-power laser microprobe that permitted elemental analysis of biological samples at high spatial resolution. Using somewhat lower powers, Posthumus, Kistenmaker, and Meuselaar[11] introduced laser desorption techniques whereby nonvolatile and thermally labile substances could be desorbed into the gas phase with moderate fragmentation that allowed the molecular identity to be determined.

Finally, Karas et al.[12] and Tanaka et al.[13] introduced matrix-assisted laser desorption/ionization (MALDI), which was a much softer ionization technique, using lower laser irradiances (typically 1 MW/cm^2). With this technique, biomolecules as large as 300,000 daltons could be ionized and desorbed intact into the gas phase for mass analysis. The basis of MALDI is the interaction of a pulsed laser beam with a laser-absorbing matrix material into which analyte molecules are dispersed (Fig. 10). Pulsed laser energy is absorbed by the matrix and transferred to the analyte, causing it to be ionized and desorbed into the gas phase. In the process, the analyte chemically interacts with fragment ions of the matrix, forming molecular adduct ions.

The MALDI process generally involves wet chemical techniques, whereby 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 in which an ultraviolet-absorbing polymeric tacky substance is used as a combination sampling/sample treatment device. A substrate coated with the tacky substance is placed in contact with the solid surface being tested, causing particulate matter to adhere. The sampling agent provides the necessary adhesion for sampling and also acts as the matrix for the MALDI technique, which is employed for analysis (Fig. 11). An alternative approach is to use a common tacky adhesive (for sampling) in conjunction with a separately applied matrix molecule (for MALDI) to accomplish the same purpose.

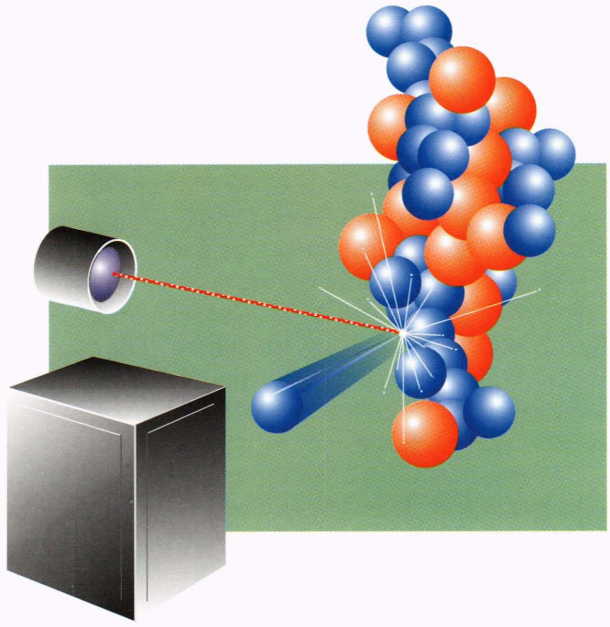

Figure 10. Schematic of the MALDI process. This approach uses a laser-absorbing matrix to ionize and desorb biological molecules into the gas phase for mass analysis.

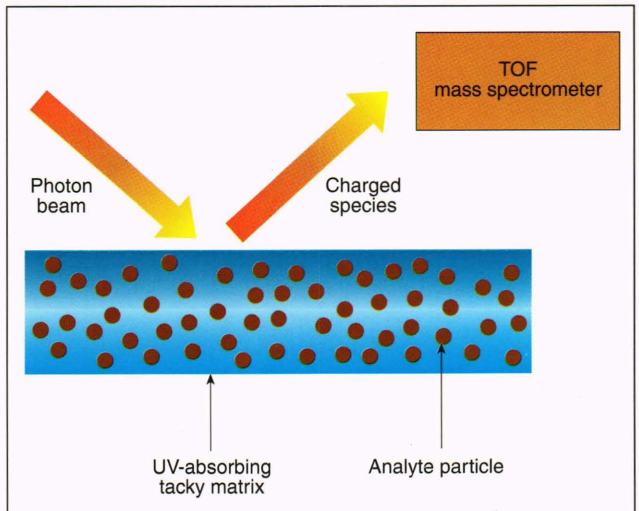

Figure 11. Conceptual scheme of the tacky matrix approach. This concept permits preparation of solid samples for analysis using the MALDI technique.

Analyzing Aqueous Samples Using Electromembrane Techniques

The electromembrane ion source is a newly discovered technique for sample preparation and ionization of species in the aqueous phase.[14] With this technique, a sample is placed on a porous membrane that acts as the interface between the sample in the aqueous phase and the vacuum of the mass spectrometer (Fig. 12). The pores in the membrane are created by chemical etching of ion tracks previously formed in the polymer by a controlled dose of ion irradiation. The pore size is uniformly controlled across the entire membrane with extremely close tolerances, which leads to high selectivity for a given type of chemical.

In operation, a high voltage is imposed across the membrane, and certain types of chemical species (depending on the chemical makeup and pore size of the membrane) cross the barrier, whereupon they enter the mass spectrometer as ions (Fig. 13). Unlike other types of inlet systems, the one used here does not require auxiliary ionization schemes because the ions are created in the transmembrane electric field itself.

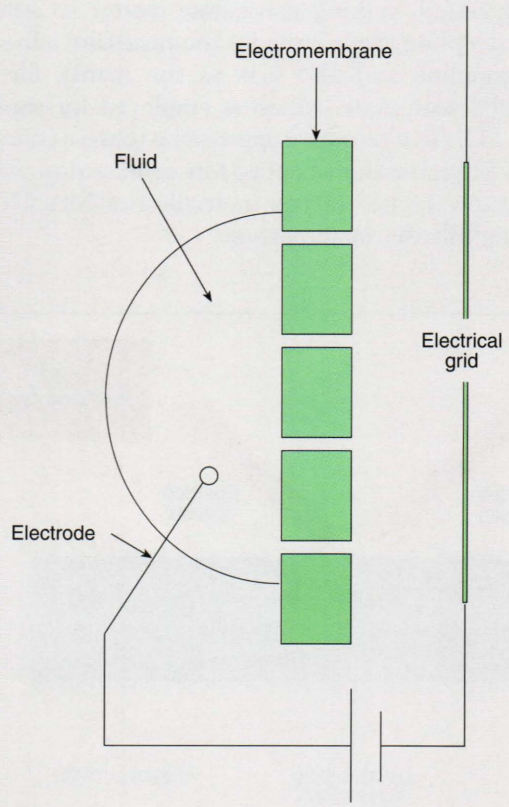

Figure 12. Electric field biasing of the electromembrane in aqueous solution. This recently discovered technique permits selection of different classes of chemicals.

The key potential advantage of this technique is the ability to tailor membrane composition and pore size to select for different classes of chemical compounds. This concept is being further developed in a collaborative effort.

Analyzing Vapor Samples Using Pulsed-Ion Extraction

In their early report, Wiley and McLaren[2] described a method for improving the mass resolution of ions formed in the gas phase by relatively broad pulses (0.5 to 5.0 μs) from an electron beam. Their method, known as time-lag focusing, was intended to compensate for the initial time, space, and energy distributions using delayed, low-voltage, pulsed-ion extraction. The ions were actually formed in a field-free ion source and extracted by a fast drawout pulse (10-ns rise time) to provide time correction. Thus, the TOF was measured as the time after application of the drawout pulse rather than the ionization pulse.

Unfortunately, although time-lag focusing provided correction for the initial kinetic energy spread, the optimal length of the time delay is mass dependent. Furthermore, the method was developed for the boxcar integration method of data acquisition and is not fully compatible with modern time-to-digital or analog-to-digital data acquisition. However, many aspects of the Wiley–McLaren scheme to focus ions formed in the gas phase are now being revisited. At the Johns Hopkins Medical Institutions, time-delayed, pulsed-ion extraction has been used to focus ions formed off-axis by infrared laser desorption,[15] observe metastable fragmentation,[16] and permit the use of broad-pulse primary ion beams with a liquid matrix,[17] approximating the conditions of fast-atom bombardment or dynamic secondary-ion mass spectrometry. The latter can be used with a continuous-flow probe interface connected to a high-performance liquid chromatograph.[18] In this case, the fast extraction pulse provides temporal focusing, whereas the low-voltage, multiple-stage extraction provides space focusing for ions formed from a liquid matrix.

More important, initial formation of the ions in a field-free ion source provides a crude trapping device, since ions of relatively high mass will drift very slowly from the extraction volume. Thus, ionization pulses of 10 μs are practical and, if the cycle time is 10 kHz, they provide a high duty cycle that can be used to increase the sensitivity of the instrument. These techniques, combined with the pseudotandem nature of the Tiny-TOF mass analyzer, can be used to great advantage in the detection of parts per million (or better) concentrations of a volatile chemical in a complicated environmental background.

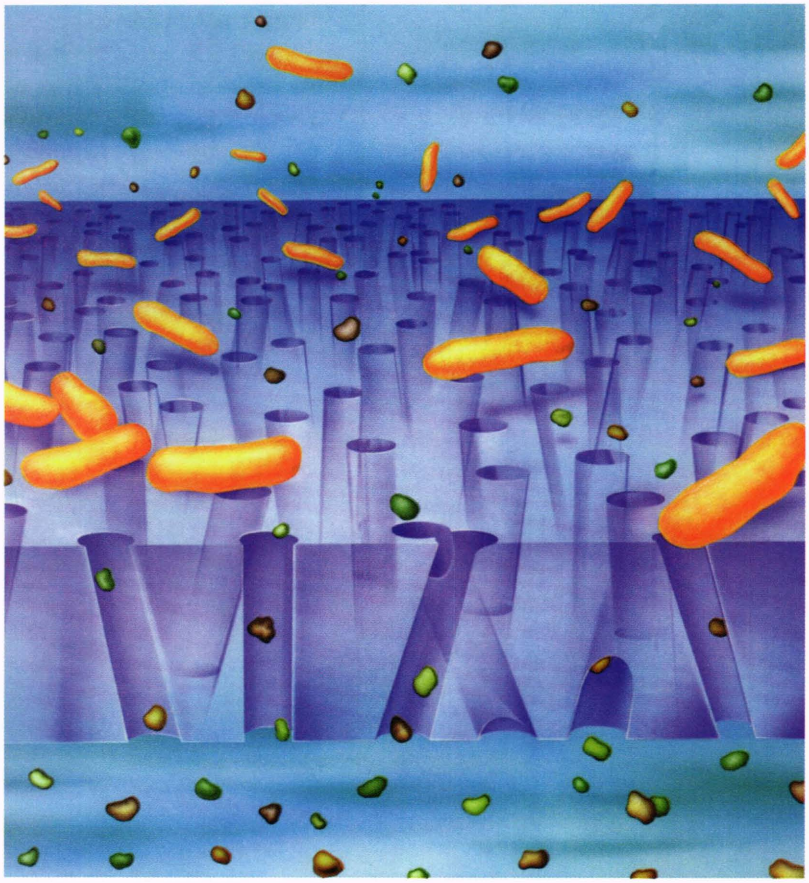

Figure 13. Artist's concept of the Poretics track-etched membrane, which is used in the electromembrane ion source. The membrane's narrow pore-size distribution permits high selectivity of different compounds. (Reproduced with permission of Poretics Corporation.)

APPLICATIONS OF THE TINY-TOF

Mass spectrometry lends itself to many potential applications because of its extreme sensitivity and selectivity. Although much sample analysis can be carried out using laboratory-based mass spectrometers, it is often advantageous to have a portable system for analysis in the field. The Tiny-TOF can be designed for portability and, given the pseudotandem nature of the instrument, it can be used to identify small concentrations of analyte in a large amount of environmental background. This section describes several potential field-installed systems incorporating Tiny-TOF technology.

Microorganism Detection and Identification for Health Care and Military Applications

The identification of microorganisms has been of prime importance to the health care community since the connection between microorganisms and disease was first established. Currently, clinical practice often requires time-consuming analysis of cultured organisms to confirm the disease source. The several-days delay in identification may either cause a delay in treatment or lead to overprescription of antibiotics for treatment of a condition that does not exist in the patient. In the military arena, the detection, identification, and quantification of biological warfare agents for both treaty verification and battlefield scenarios have become increasingly important. A prime candidate technology for microbe identification is advanced biological mass spectrometry using a chemotaxonomic approach.[19]

Conventional techniques for microbe identification have relied on morphological and metabolic characteristics. Advances in biochemistry, molecular biology, and chemical instrumentation, however, have opened new avenues of

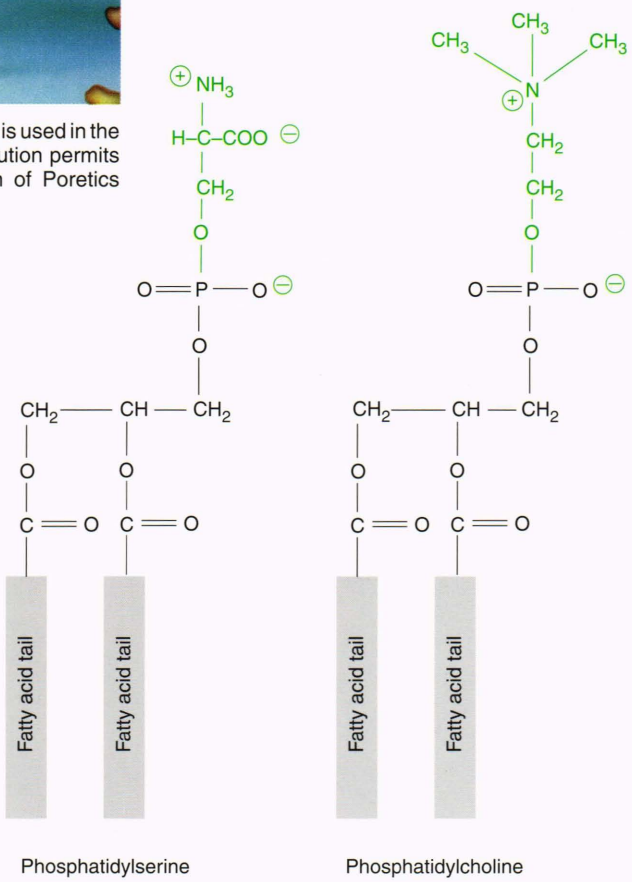

Figure 14. Chemical formulas of common phospholipid compounds. Determining the distribution of phospholipids in cell membranes holds promise as a rapid means of identifying microorganisms. (Redrawn from Ref. 21 with permission.)

taxonomy based on the chemical makeup of cells. Such an approach is commonly described as chemotaxonomy, which Priest and Austin[20] define as the study of chemical variation in living organisms, and the use of chemical characters for classification and identification.

The work described here focuses on a class of endogenous chemical markers called the complex polar lipids, of which the phospholipids are predominant (Fig. 14). These compounds are arranged in a continuous bilayer that provides the basic structure of a cell membrane. Cell membranes contain bound proteins that provide specific receptor sites and enable the transport of specific molecules, necessary for cellular function, across the membrane. To function, a transmembrane protein must be surrounded by specific lipid molecules. Thus, the polar lipid distribution in the cell is responsible for the structure and function of cellular membranes and, hence, the morphology and metabolism of the organism. Although free fatty acids and fatty acid components of complex polar lipids vary with health, life cycle, and nutrition, the distribution of polar head groups of phospholipids is qualitatively and quantitatively stable enough to be considered taxonomically characteristic.[22,23] Furthermore, these polar lipids are abundant, constituting as much as 5% of the dry weight of an organism, and their amphiphilic properties make them easy to recover and analyze by desorption mass spectrometry.[24]

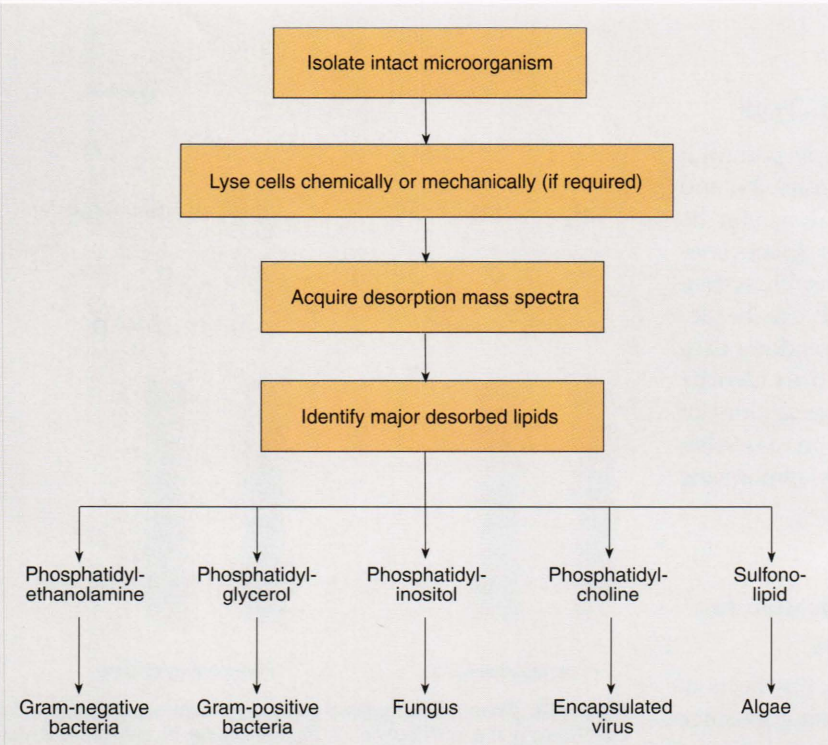

Figure 15. Mass spectrometry–based chemotaxonomic scheme for rapid identification of microorganisms.

Figure 15 shows a chemotaxonomic method utilizing mass spectrometric analysis of polar phospholipid biomarkers. This technique was pioneered at The Johns Hopkins University by Fenselau, Cotter, and others in the late 1980s.[24–26] The method consists of four steps. First, the cells of the intact microorganism are isolated from culture. Then, the cells are chemically lysed (generally by treatment with methanol or a methanol–chloroform mixture) or mechanically lysed to expose the phospholipid molecules. Several different desorption techniques, including fast-atom bombardment, plasma desorption, and laser desorption are then used to preferentially examine the amphiphilic phospholipids. Polar lipids in the cell walls and membranes undergo fragmentation in which the characteristic polar head group is cleaved from the lipid, allowing selective molecular characterization using tandem mass spectrometry. Finally, detailed analysis of the polar head distribution and fatty acid profile provides a taxonomic classification of the organism, and signal processing techniques provide enhanced identification of infectious species in mixed populations. There is also significant potential for the use of protein, peptide, and toxin compounds as a methodology for enhanced identification. Figure 16 shows an implementation of the chemotaxonomic approach for batch sampling.

Environmental Analysis

A small, highly sensitive, specific detector for trace levels of contamination in air and water would have obvious environmental applications. Examples include continuous monitoring of potable water quality at treatment facilities; evaluation of contamination in groundwater at dump sites; monitoring remediation of wastewater prior to discharge; measuring contamination in streams, rivers, and other bodies of water; and determining vapor levels in factories and waste dump settings. Environmental testing systems have been conceived that may take advantage of the aqueous (Fig. 17) and vapor (Fig. 18) sampling methodologies discussed previously.

Law Enforcement Applications

To establish a link between physical evidence gathered in a police investigation and the perpetrators of a crime, the materials and objects composing the evidence must be identified, compared, and linked to a specific individual. Much of this

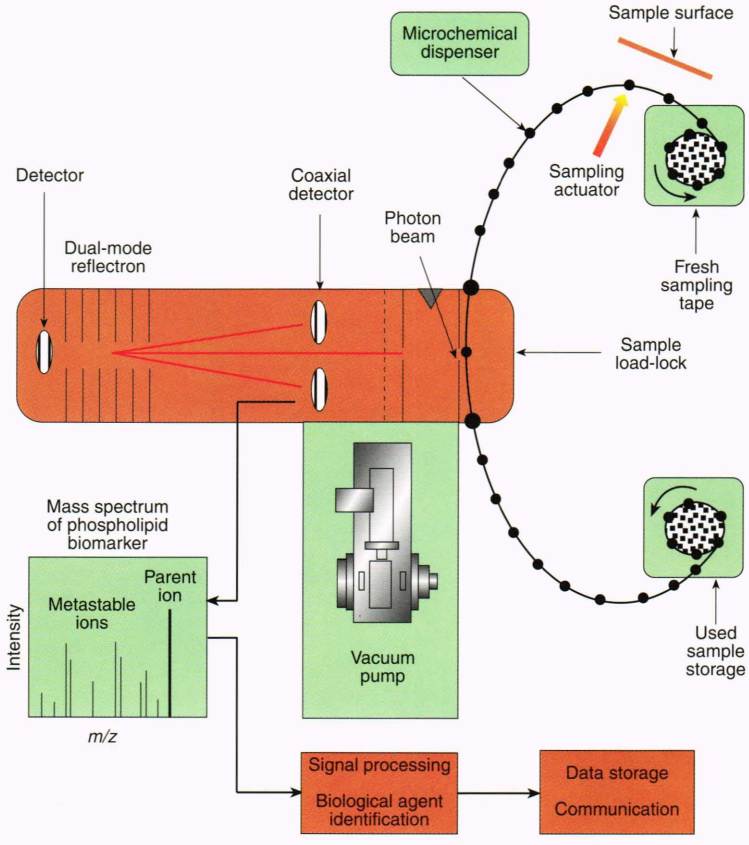

Figure 16. System concept for in-field detection of biological material. Taxonomic classification is provided by phospholipid distribution, and enhanced identification of pathogenic species in mixed populations is achieved by signal processing.

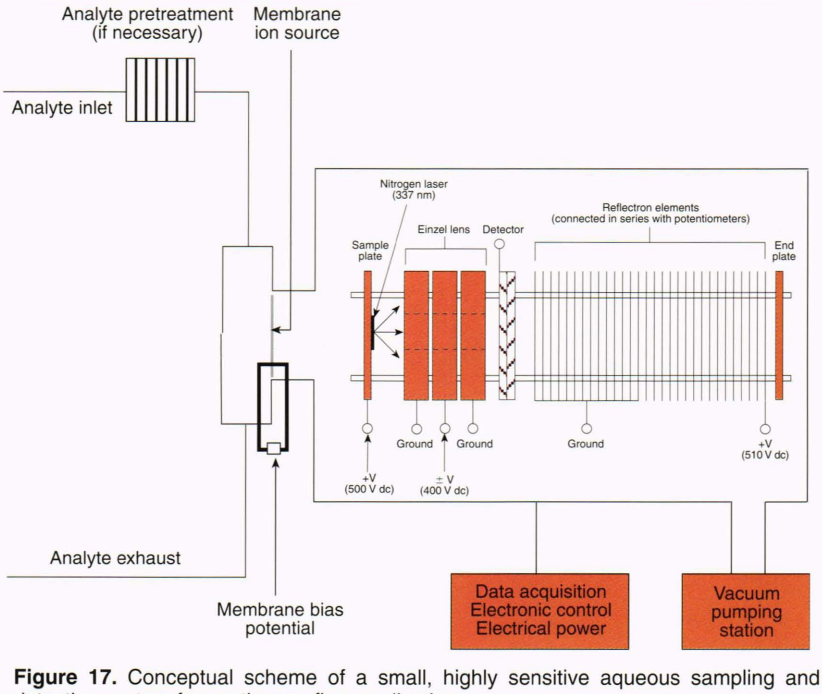

Figure 17. Conceptual scheme of a small, highly sensitive aqueous sampling and detection system for continuous flow applications.

work is carried out by the forensic chemist. One of the primary tools for these investigations is mass spectrometry. Substances of interest to the forensic chemist include low-vapor-pressure solid substances such as contraband (drugs and explosives), bomb blast residue, and materials used in arson. Also of concern are more volatile compounds such as higher-vapor-pressure drugs, solvents used in drug laboratories, arson initiators, and drug breakdown products. Two different systems are of potential interest in law enforcement: a MALDI-based technique for investigating small molecules, and a highly portable vapor sniffer.

While the MALDI technique has been widely employed for biological investigations, potential applications for contraband detection are in their infancy. We are investigating the application of MALDI to small molecules having low vapor pressure, such as drugs and explosives. Using the MALDI technique with solutions, we have measured mass spectral signatures for cocaine (Fig. 19), heroin, and the explosive RDX in the subnanogram range. A final system embodiment of this technique would likely use the MALDI approach with solids.

For volatile compounds, a vapor sniffer technology is appropriate. The conceptual design for a handheld vapor-detection device uses a fast-pulsed valve for vapor-sample acquisition (see Fig. 18). This type of sampling enables passive pumping using sorption or gettering material surrounding the mass analyzer, which greatly reduces the weight and power consumption of the device. These handheld devices could be used until a minimum vacuum level is reached, at which point they would be returned to a base station for battery recharging and vacuum material regeneration.

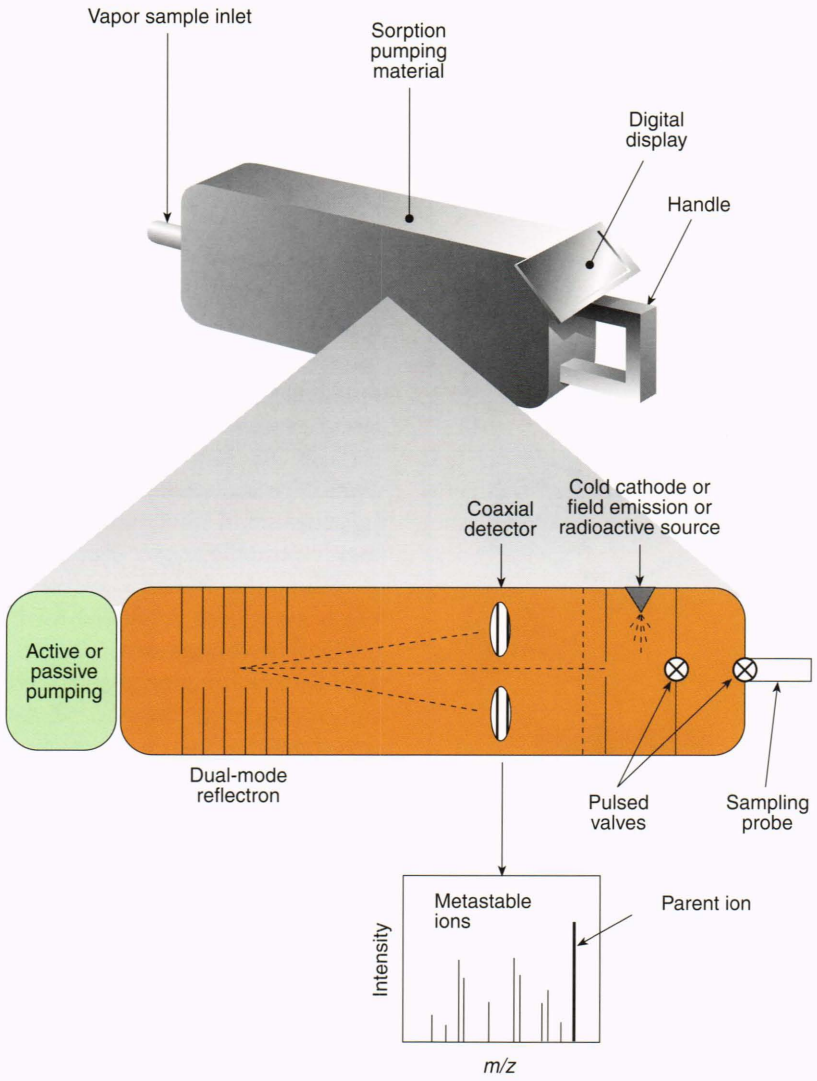

Figure 18. Conceptual scheme of a handheld vapor detector using the Tiny-TOF analyzer. This device could be employed in law enforcement to detect volatile solvents used in drug laboratories, drug breakdown products, and arson initiators. A recharging station is used to recharge the batteries and regenerate sorption material.

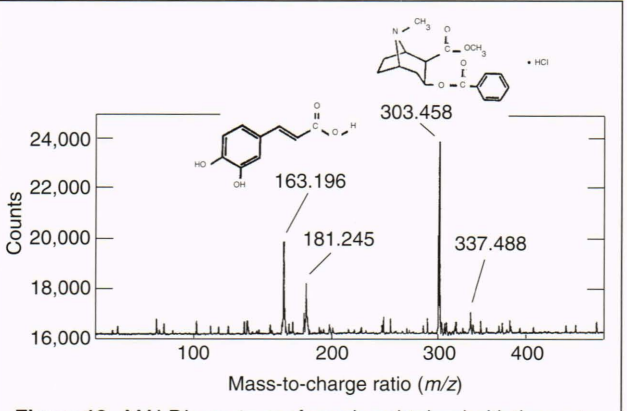

Figure 19. MALDI spectrum of cocaine obtained with the prototype Tiny-TOF mass spectrometer. The MALDI technique has also been used to identify heroin and the explosive RDX in subnanogram amounts. The spectrum shown here was obtained using caffeic acid as the laser-absorbing matrix.

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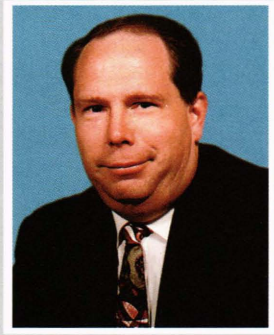

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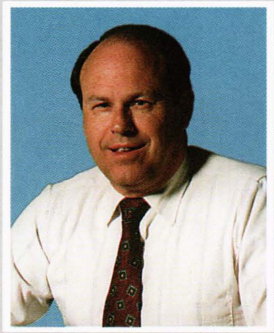

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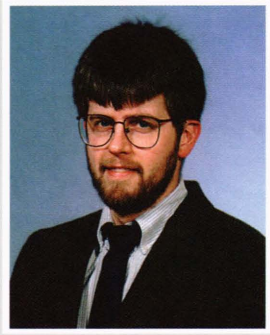

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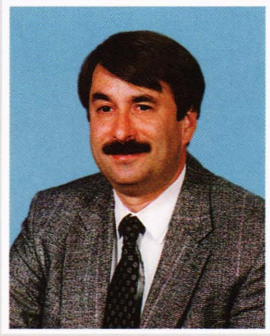

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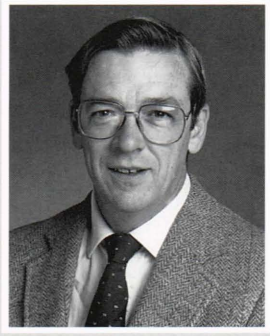

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