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RAPID RESPONSE CHEM/BIO DETECTION SYSTEM BASED ON PHOTOIONIZATION MASS SPECTROMETRY

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ABSTRACT

This work describes a high-speed detection system for identifying chemical and biological weapons. The system is based on new innovations in mass spectrometry (MS) to achieve target molecular identification with minimal false positive responses. The core technologies are: (1) a highly efficient photoionization source and (2) a high performance mass analyzer based on the hybrid combination of quadrupole ion trap and time-of flight technologies (QitTof). The benefits of photoionization for CW detection are near universal detection efficiency, minimal ion fragmentation, and near absence of signal from air constituents and many common matrix background compounds. The detection system being developed is compact, robust and permits automated, unattended operation for real-time early warning systems, point-of-entry screening, and emergency first responder applications.

1. INTRODUCTION

This work describes a promising new chemical and biological (CB) point-detection system based on photoionization mass spectrometry (PI MS). The technology has been extensively tested on chemical agents and is giving initial promising indications for biological agent signatures.

A variety of chemical agent sensors, monitors, and point-detection systems have been developed to meet different requirements.[1,2,3,4,5] Less adequate solutions exist for biological agent detection. The U.S. Army began the Biological Integrated Detection System (BIDS) in 1993. This project employed a battery of instruments, such as a high volume particle sizer, liquid sampler, and flow cytometer in a mobile laboratory mated to a Humvee vehicle. The BIDS vehicle was tested in June 1997 and proved capable of detecting a variety of pathogens in about 5-min, a marked improvement over previous field instruments. Added to this suite of instruments was the Chemical Biological Mass Spectrometer (CBMS). This instrument is an ion trap mass spectrometer (ITMS) capable of biological or chemical detection when connected to an aerosol collector/concentrator or chemical probe, respectively.

The above developments are large mobile laboratories. Efforts to provide man-portable detectors require innovative new technologies. Ion Mobility Spectrometry (IMS), which is used extensively for chemical detection, is being adapted for biological detection by Environmental Technology Group and by Graseby Dynamics. Mass spectrometry offers improved specificity, although developing compact, portable instruments has been a challenge. Accepted methods of biological detection include pyrolysis mass spectrometry; however, improve-

ments are needed for this method to provide high specificity, especially for civilian use. Emerging mass spectrometry technologies for biological detection include matrix assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). MALDI is a widely used technique for large biomolecule identification; however, introducing sample is difficult, normally requiring manual preparation and loading through a vacuum load lock. This makes MALDI challenging for automated field operation. ESI also requires a reliable and automated method of preparing and cleaning biological samples.

2. EXPERIMENTAL

2.a. PI background

Figure 1 illustrates the principle of PI MS. The Syagen PI source imparts an energy that is higher than the ionization potentials (IPs) of typical target molecules, but lower than the IPs of virtually all constituents of air as well as most common solvents. These potential interferents are not ionized, enabling significantly greater dynamic range for detecting and measuring molecules at very low concentrations. Furthermore, because molecules of interest are ionized near their IP thresholds there is minimal fragmentation to clutter a mass spectrum. These performance features provide tremendous benefits for analyzing mixtures and samples in complex matrices. The method of electron ionization (EI), by contrast, imparts energy that greatly exceeds the dissociation thresholds of most molecules. This leads to extensive fragmentation. Figure 2 compares EI MS and PI MS for the nerve agent simulant molecules DIMP and DMMP (diisopropyl methylphosphonate and dimethyl methylphosphonate). EI leads to highly fragmented spectra and weak molecular ion signal. PI, on the other hand, leads to very simple spectra with strong molecular ion signal. The simple mass spectra provided by PI is crucial to analyzing the overlapping spectra of a complex mixture of compounds.

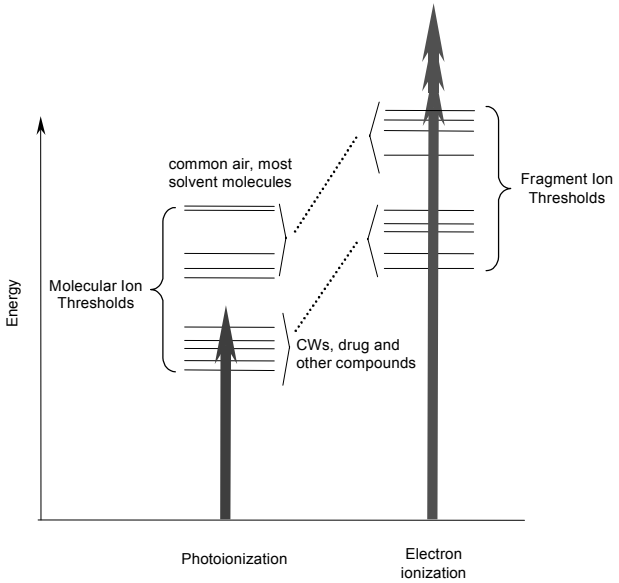


Figure 1.

Diagram showing the principle of photoionization for achieving threshold ionization of molecules. The process of electron ionization is shown for comparison.

The PI source has been described before.[6,7] The PI light source uses a high-output gas discharge tube with a MgF_2 window for transmission of vacuum ultraviolet light into the ionization region. The fill gas is generally a rare gas and the choice of gases depends on the wavelength range of interest. Narrow band energy is available from about 8 to 12 eV, although we usually operate near 10 eV.

The principal mechanism for photoionization of molecule M is photon absorption and electron ejection to form the molecular ion M^+. In the presence of water vapor or protic solvents, the molecular ion can extract H to form MH^+. This tends to occur if M has a high proton affinity. This does not affect quantitation accuracy as the sum of M^+ and MH^+ is constant.

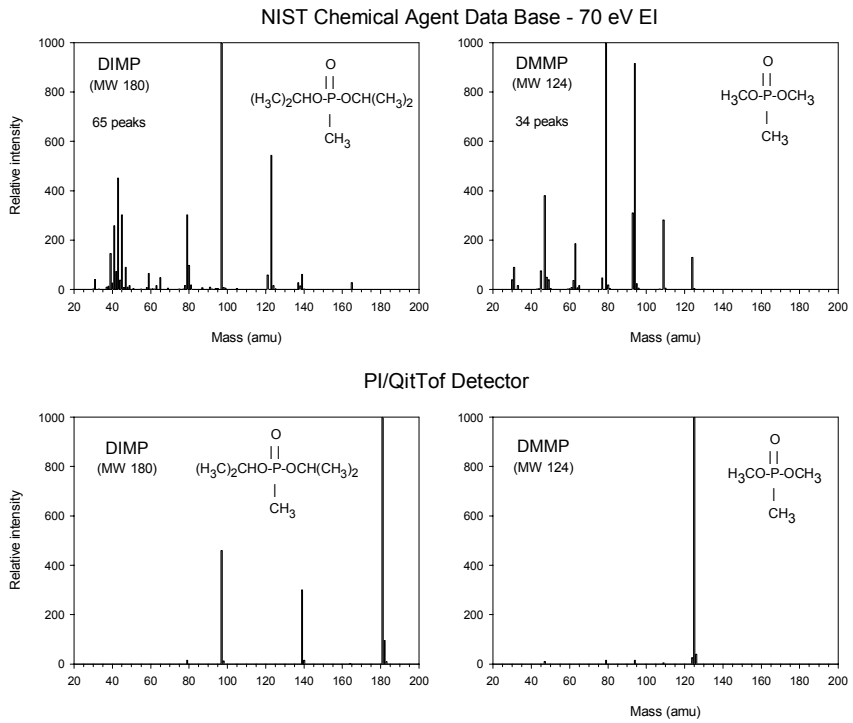


Figure 2.

Comparison of PI vs. EI mass spectra of DMMP and DIMP showing significantly reduced fragmentation and distinct signature peaks for PI mass spectra.

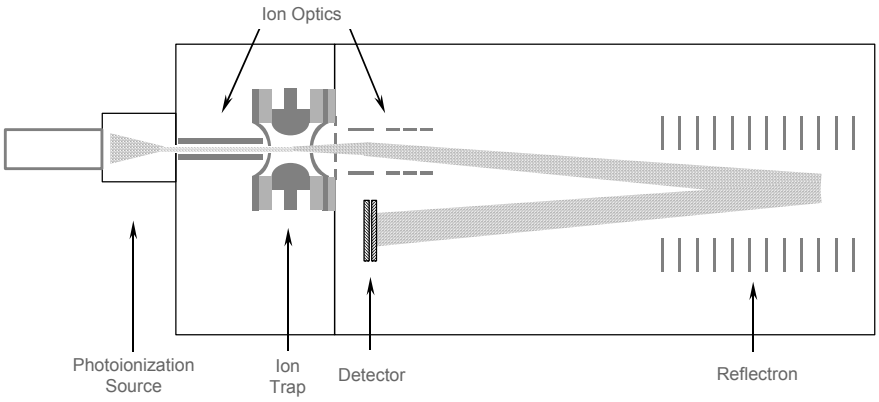


Figure 3.

Schematic diagram of the QitTof mass analyzer shown with a photoionization source.

2.b. QitTof Mass Analyzer

The ChemBio MS system employs a combination of a quadrupole ion trap and a time-of-flight mass analyzer to achieve high-speed, high-performance molecular analysis. This combination is referred to as Qit-Tof and is also well suited for use with the PI source. A schematic showing the basic functions of PI/QitTof system is shown in Figure 3. The major benefits are outlined below:

Quadrupole Ion Trap (QIT): [8,9,10]

- Ability to store ions formed by a continuous ionization source followed by pulsed extraction into the TOFMS
- Option to manipulate ions, such as mass-selective isolation, followed by collisional-induced dissociation in order to fingerprint compounds (e.g., MS/MS)

- Operation at relatively high pressure, thus forming a differential pressure stage between the AS-TPI source and the TOFMS

Time-of-Flight Mass Spectrometry (TOFMS):[11]

- Multichannel mass detection leading to efficient collection of all ions
- Large mass range of detection
- Exceptionally low instrument noise because each mass peak is detected in a narrow time window (about 20 ns)

QIT/TOFMS Combination (QitTof):[12]

- Space-charge repulsion degrades resolution in scanning QITs (ion trap mass spectrometers, ITMS), limiting trap capacity to 300-500 ions per period. Pulsed extraction and TOF analysis remove this restriction enabling >5000 ions per period (with reflectron)
- QitTof can operate at repetition rates >100 Hz vs. 20 Hz max for ITMS enabling increase in dynamic range

Furthermore, in combination with PI, the QIT can achieve significantly improved dynamic range because PI avoids ionization of common atmospheric constituents. As a result, the trap does not fill with atmospheric ions.

2.c. Deployable ChemBio MS System

Syagen has developed a pre-production quality detection system for CW and related compound detection. The system was originally developed for the Defense Threat Reduction Agency (DTRA) for Chemical Weapons Convention (CWC) treaty compliance monitoring. The currently accepted method for validating the presence of alleged CWC scheduled compounds is gas chromatography/mass spectrometry (GC/MS). The slow speed of this method limits the number of samples that can be analyzed in a given inspection time period, potentially compromising the thoroughness of a challenge inspection. The throughput can be greatly increased if a large number of samples could be rapidly screened for the presence of alleged compounds. Only those samples that test positive are then certified by GC/MS.

Figure 4 shows the mobile benchtop PI/QitTof instrument for high-speed screening of liquid samples. The integrated autosampler allows automated and unattended analysis of hundreds to thousands of samples at a time. The current sample cycle time is 45 seconds. The instrument may also be configured for air sampling by dispensing with the autosampler and making a routine change to the sample inlet. Figure 5 shows a configuration that permits rapid deployment to remote sites.

The instrument is capable of MS/MS "on-the-fly" whereby the detection of a targeted compound can trigger an excitation waveform to provide a confirmatory fragment spectrum. The entire instrument is transportable and occupies a 5 sq ft footprint. The specifications of the deployable MS system are summarized in Table I.

Another Syagen system that is currently under development (partially funded by SBCCOM) is a field-portable CB/MS system with a pyrolysis-gas chromatography (Py-GC) interface. This instrument is merging the successful methods of PI MS with the front-end Py-GC used for IMS. The PI MS system weighs between 30-40 lbs depending on configuration. A picture of the first prototype PI/QitTof MS is given in Figure 6. This paper focuses on results obtained on the portable benchtop system in Figure 4; however, the technology can be transitioned to a field-portable size with some modest reduction in specifications (e.g., factor of 3 less sensitivity. The early prototype field-portable system has been described elsewhere.[13,14]

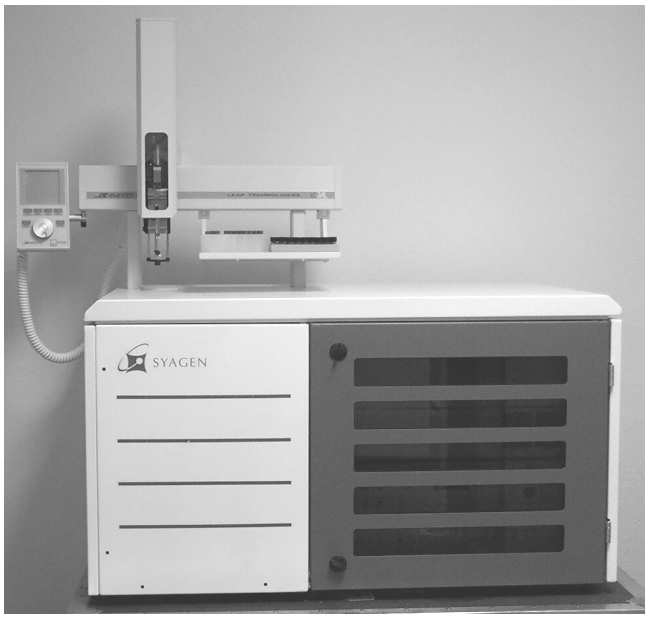


Figure 4

PI/QitTof instrument with autosampler and exterior panels.

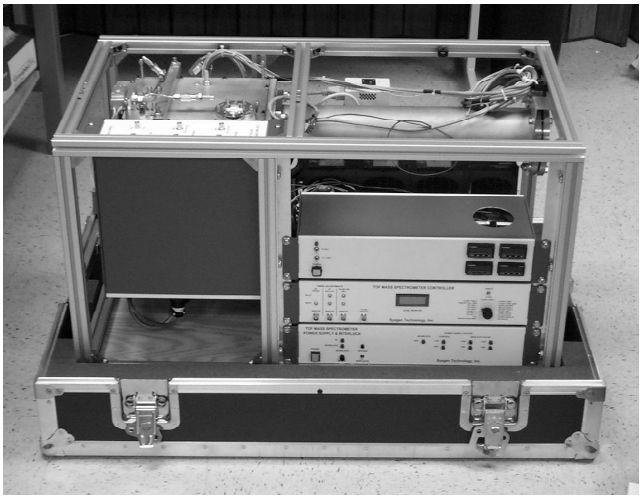


Figure 5

The PI/QitTof without panels or autosampler configured for “fly-away” applications. Computer and roughing pump not shown.

Table I. Specifications of CW analysis system

Property	Specification
Sensitivity 2-5 s sampling time, 3σ LOD	10-100 ppb (air sampling) <1μg/mL, typ. (liquid sampling)
Mass Range	50-800 m/z
Mass Resolution Full width half height	~3000 at 361 m/z
Sample cycle time	45 sec for liquid autosampling 10 s for direct air sampling
Dynamic Range	3-decade linear 4-decade total
Carryover	2 to ~10%
Repeatability	short term RSD of 5% long term (hours) RSD 18-20%

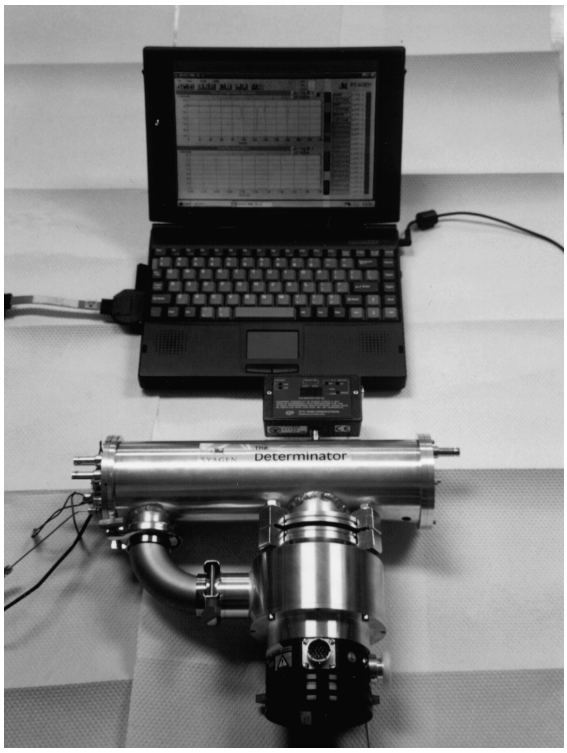


Figure 6

Picture of prototype PI/QitTof mass spectrometer and processor units. Not shown are the backing pump and electronics.

3. RESULTS AND DISCUSSION

3.a. Spectral signatures

PI mass spectra are noted for dominant molecular ion signal and minimal fragmentation. Figures 7-10 show PI mass spectra for representative nerve, blister, riot, and hallucinogenic agents. These compounds were dissolved in solvent and introduced into the MS detector by syringe injection or by direct capillary infusion. In previous experiments with live agents, sample was introduced as vapor using permeation or sorbent tubes in a mixed flow apparatus. Because the liquid samples are vaporized in the ionizer, the spectral characteristcs for liquid and vapor sampling are similar. The primary difference is that protic solvents can serve as a hydrogen source that can react with molecular ion M^+ to form MH^+.

Figure 7 shows spectra of VX recorded in conventional MS mode and using selected ion excitation of the molecular ion MH^+ (269 m/z) to give a MS/MS spectrum yielding the daughter fragment at 128 m/z. The distinct signature provided by the parent/daughter ion signals gives a definitive identification of the molecule VX. Figure 8 shows PI mass spectra of nitrogen and sulfur mustard. Both spectra show strong molecular ion signal with a characteristic 3-peak distribution for a molecule containing two chlorine atoms (^{35}Cl, ^{37}Cl). For sulfur mustard, an additional 3-peak series is observed for MH^+, along with M^+, due to the presence of protic solvent. Figures 9 and 10 show the riot agent CR and the hallucinogen BZ. The predominant signal for CR is the molecular ion while for BZ, the MH^+ ion dominates.

The PI/QitTof was tested for a 48-compound library of CWC treaty-scheduled compounds. 45 molecular ion signals were detected with minimal fragmentation. Table II shows the list of compounds that have been recorded by PI MS.

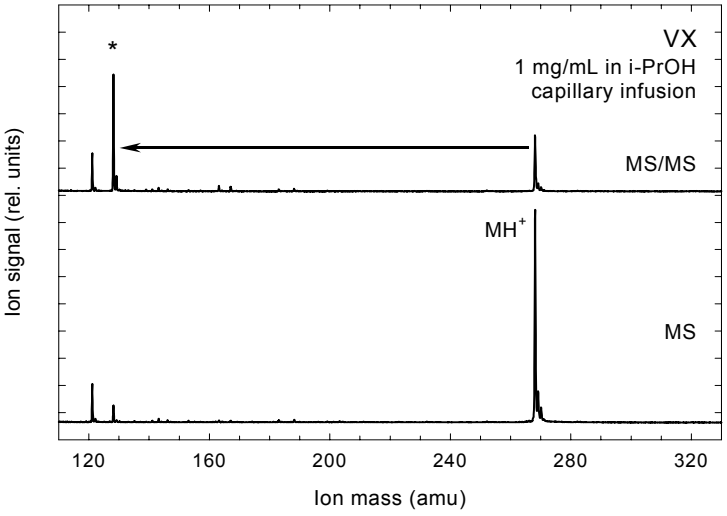
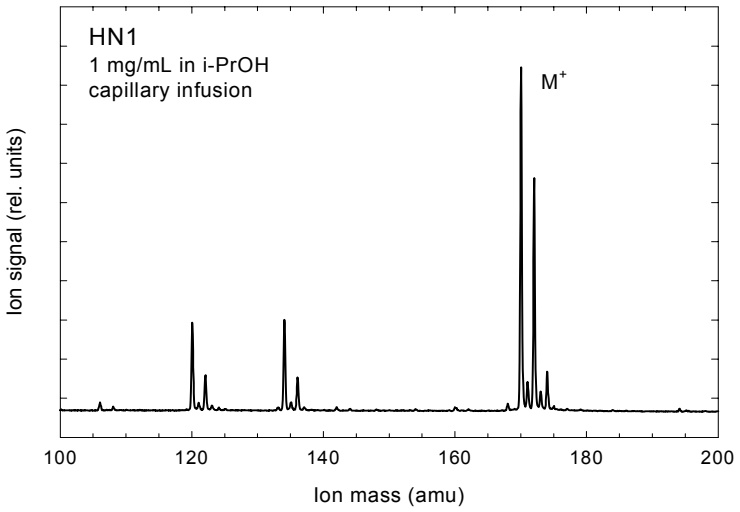


Figure 7

PI mass spectra of VX. (Bottom) MS spectrum shows a predominant molecular ion for VX. (Top) MS/MS spectrum shows mass-selective dissociation to give the confirmatory fragment signal, marked by asterisk.



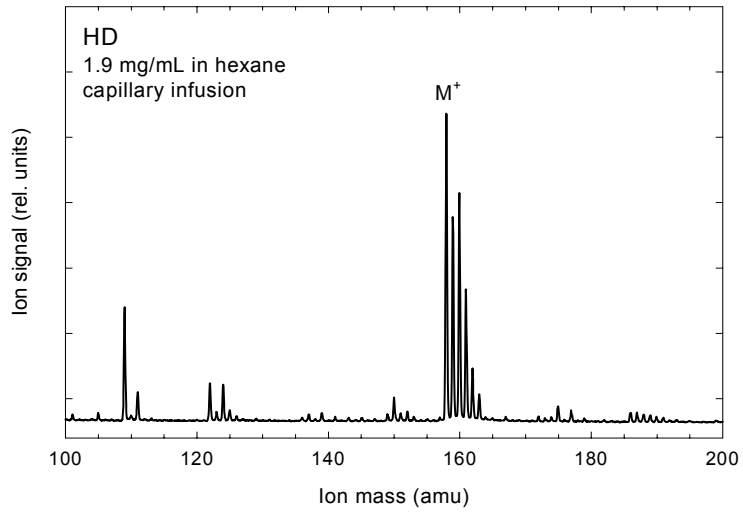


Figure 8

PI MS spectrum of HN1 and HD showing predominant molecular ion signal (isotope distribution for two chlorines) and weak singly chlorinated fragments. Note that HD also forms MH^+.

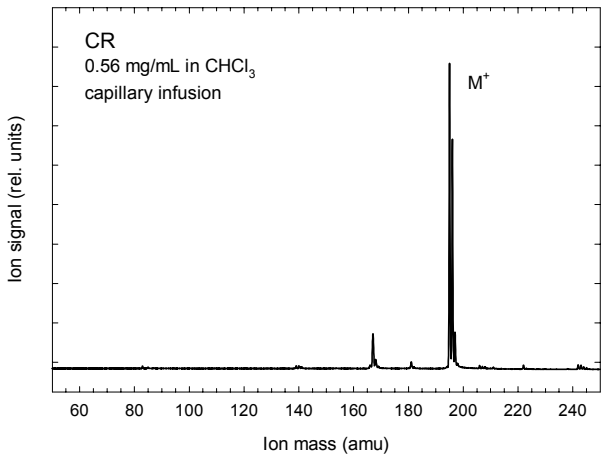


Figure 9

PI MS spectrum of CR, a riot gas. CR does not protonate in $CHCl_3$.

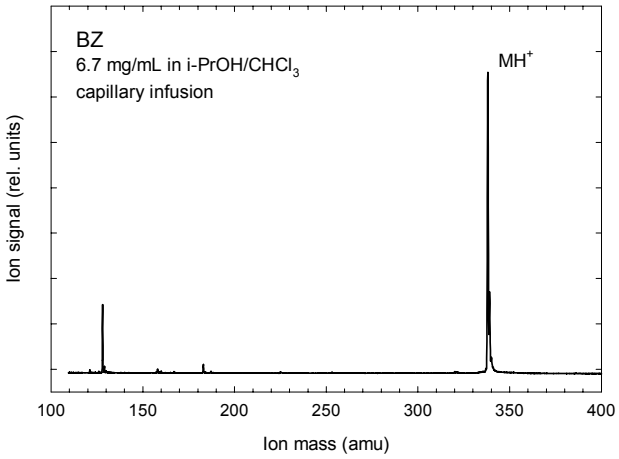


Figure 10

PI MS spectrum of BZ, a central nervous system depressant. Note that BZ ionizes and protonates.

Table II. Library of CWC-relevant compounds tested using Syagen's PI MS detector.

Schedule-1 compounds

Toxic
VX
GA (Tabun)
GB (Sarin)
GD (Soman)
GF

HD (sulfur mustard)
HN-1 (Nitrogen mustard)
HN-3

CR
CS

Precursors (Binary)
QL
DF

Schedule-2 compounds

Toxic
BZ

Precursors
Diisopropyl methylphosphonate
Diethyl methylphosphonate
Dimethyl methylphosphonate
Diethyl ethylphosphonate
Diethyl methylphosphonothioate
Isopropyl methylphosphonic acid
Cyclohexyl methylphosphonic acid
Pinacolyl methylphosphonic acid
Methyl phosphonyl dichloride
3-quinuclidinol
Benzilic acid
Pinacolyl alcohol
Thiodiglycol
N,N-diethylethanolamine
1,4-dithiane
Thiodiglycol sulfoxide
Methylamine
Isopropylamine
Thioxane

Schedule-3 compounds

Precursors
Phosphorus oxychloride
Dimethyl phosphite
Trimethyl phosphite
Diethyl phosphite
Triethyl phosphite
Triethanolamine
N-ethyldiethanolamine
N-methyldiethanolamine

Decomposition Products

Methylphosphonic acid
Ethylmethylphosphonic acid
EMPTA
2-chlorodiethylsulfide

Other relevant compounds

VX disulfide
YL

Not detected: Phosgene, chloropicrin

3.b. Application to biological agents

The key to bacterial differentiation by mass spectrometry is the release and identification of molecular signatures that are specific to different types of bacteria and other pathogens. These include DNA, proteins, lipids, and carbohydrates. A major challenge is breaking down the cell material into detectable molecular constituents. Pyrolysis mass spectrometry (Py-MS) has been demonstrated to be an effective, if not ideal, method for differentiating microorganisms.[15,16] The principal advantages of Py as a sampling method are minimal sample preparation, speed, sensitivity, and low cost. The primary disadvantage of the Py method is that the degradation of bacterial material into a nearly undecipherable collection of molecular fragments. This problem can be compounded by current methods of MS that can further fragment these signatures, thereby destroying much of the uniqueness of the original molecular signatures.

In this work we are implementing two significant improvements to the conventional method of Py-MS. These are:

- Flash pyrolysis developed by A. P. Snyder and coworkers at Edgewood Chemical and Biological Center (ECBC). This method more rapidly heats and desorbs the cell material creating larger molecular fragments with unique signature masses.[17,18]
- Photoionization, which is a more universal ionization source than chemical ionization (CI). PI also gives significantly less fragmentation than electron ionization (EI) for the molecular constituents of bacteria and spore pyrolysis.

The principal pyrolysis products are fatty acids and phospholipids. In addition, BG spores generate picolinic acid. A method has been developed to rapidly methylate the fatty acids to their corresponding fatty acid methyl esters (FAME).[19] This in-situ derivation is important for GC separations of the pyrolysis products, a method that has been successfully demonstrated for ion mobility spectrometry (IMS).[20]

Figure 11 shows examples of FAME compounds recorded using PI MS. These spectra show very clean ion signatures that are dominated by molecular ion signal. These results indicate that an accurate measure of different FAME relative abundances can be made, thereby providing strong signatures for identifying different bacteria.

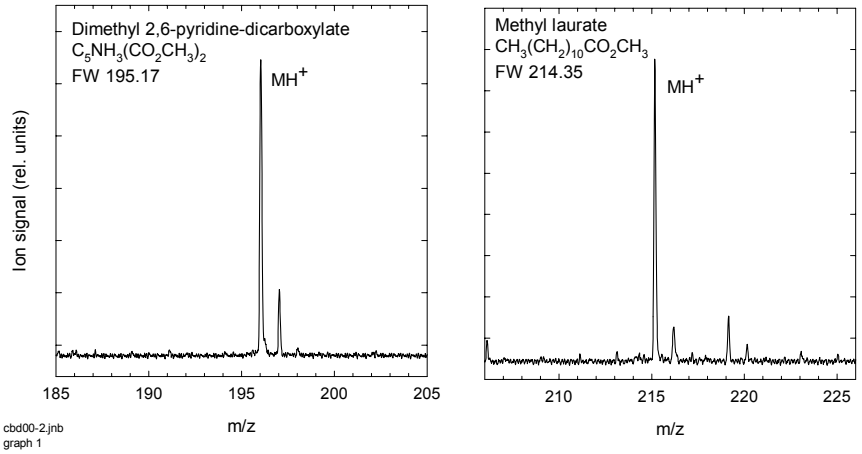


Figure 11.

PI MS of fatty acid methyl esters (FAME). These are signatures for pyrolysis of gram positive spores, such as anthrax.

3.c. Sensitivity

The various embodiments of the PI/QitTof MS instruments tested to date achieve detection limits of about 10-100 ppb with 5 s sampling periods for air sampling. Liquid sampling detection limits were <1 μg/mL in a variety of solvents (e.g., methanol and water) for direct syringe injection with typical volumes of <1 μL. Live agent testing was conducted for nerve agents, mustards and riot gas compounds at the ECBC Surety Lab. Figure 12 shows a plot of sensitivities for a variety of CWC relevant compounds. The bar intensities represent the concentration of compound in air required to give a fixed instrument signal strength (hence larger intensities represent poorer sensitivities). It should be noted that the range of sensitivities spans only about one order of magnitude and the typical variation in sensitivity between two random compounds is about a factor of 2. This is a relatively uniform response compared to other ionization methods such as ESI and APCI.

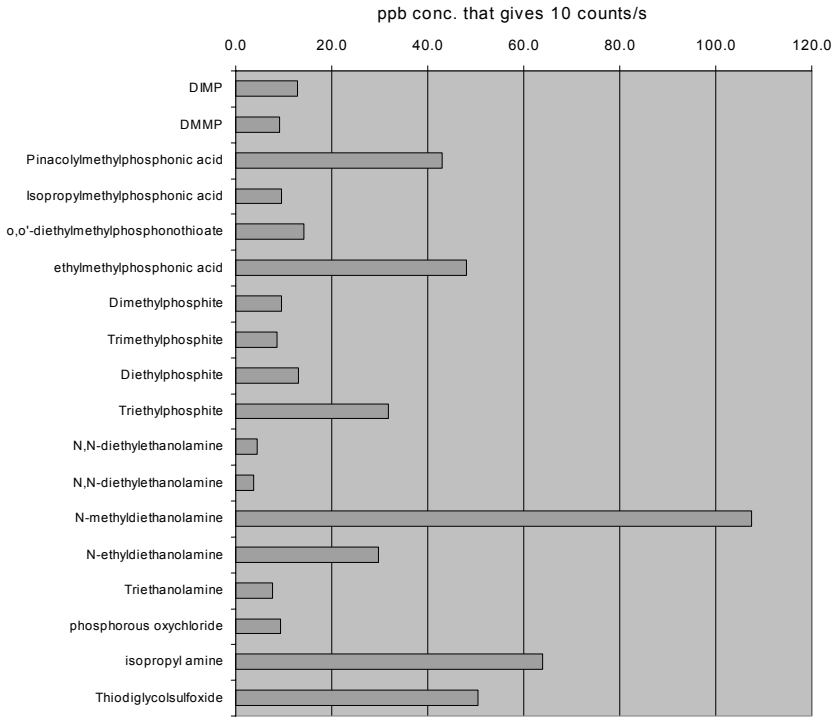


Figure 12

Plot of sensitivities for representative CWC-relevant compounds. Bar intensities represent concentration in air required to give 100 detected ion counts per second.

3.d. Dual Use Benefits

With increasing governmental regulations, environmental concerns, and military and civilian defense needs, the demand for real-time multispecies monitoring systems is growing rapidly. The specifications of the Syagen ChemBio MS instrument meet the requirements for a broad range of military and civilian applications including:

- Military force protection from threat of CB terrorism
- Chemical demilitarization, biological/toxin weapons convention verification and counterproliferation
- Emergency first response for hazardous materials
- Law enforcement for crime scene investigation requires portable detection systems
- Air quality monitoring of large public venues for civilian defense

- Environmental pollution monitoring in municipal water facilities and public waterways
- Medical diagnostics for infectious disease monitoring and for biomolecular analysis

CONCLUSIONS

Chemical and biological agents are weapons of mass destruction that are accessible to terrorists and rogue organizations because of their simplicity and low cost. In order to develop better means to counter these threats, economical, high-resolution, field-portable biological and chemical detection systems are needed. High speed is needed for rapid response in threat situations and for high throughput analysis for treaty verification and nonproliferation monitoring.

In this paper we have focused on Syagen's progress toward fielding a low-cost, high-resolution, field-portable detection systems for rapid and reliable chemical and biological (CB) agent monitoring. Effective sensors must integrate the requirements for efficient recovery of microorganisms, processing of microorganisms for detection, and molecular analysis for accurate pathogen identification. For in-field detection, the sensors must be compact, rugged, reliable, and low maintenance while achieving exceptional levels of sensitivity, specificity, and speed. The instrument criteria that this work addresses are:

- Real-time (1 min), field-portable, molecular detection system for identifying chemicals, pathogens and biological agents
- Dual chemical and biological (CB) air sampling capability with high sensitivity and specificity for low false positive and negative responses
- Automated, rugged, low maintenance operation and low upfront and recurring costs

Syagen has demonstrated the feasibility of a field-portable, ultrasensitive, real-time monitor. The ionizer/mass analyzer technology is readily adapted to front-end sample collectors, concentrators, and/or separators such as aerosol and particle collectors, thermal desorbers, gas chromatographs, etc.

ACKNOWLEDGMENTS

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