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Development of Presumptive and Confirmatory Analytical Methods for the Simultaneous Detection of Multiple Improvised Explosives

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

DEVELOPMENT OF PRESUMPTIVE AND CONFIRMATORY ANALYTICAL
METHODS FOR THE SIMULTANEOUS DETECTION OF MULTIPLE
IMPROVISED EXPLOSIVES

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Kelley L. Peters

2014

To: Interim Dean Michael Heithaus
College of Arts and Sciences

This dissertation, written by Kelley L. Peters, and entitled Development of Presumptive and Confirmatory Analytical Methods for the Simultaneous Detection of Multiple Improvised Explosives, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: November 7, 2014

The dissertation of Kelley L. Peters is approved.

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Florida International University, 2014

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DEDICATION

I dedicate this dissertation to my parents. Without their understanding, unconditional support, guidance, and all those trips to China I never would have been able to complete this process.

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I would like to thank the members of my dissertation committee for their support and assistance. Dr. Bruce McCord has provided me with opportunities to conduct research in the explosive field, providing a great deal of support and assistance along the way. He has also trusted me to deal with instrumental issues and supported me in attending a variety of conferences, both nationally and abroad. Thank you to Dr. John Berry, Dr. Yong Cai, Dr. Chenzhong Li, and Dr. Alexander Mebel for their support and insight throughout this process.

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ABSTRACT OF THE DISSERTATION

DEVELOPMENT OF PRESUMPTIVE AND CONFIRMATORY ANALYTICAL
METHODS FOR THE SIMULTANEOUS DETECTION OF MULTIPLE
IMPROVISED EXPLOSIVES

by

Kelley L. Peters

Florida International University, 2014

Miami, Florida

Professor Bruce McCord, Major Professor

In recent years, there has been a dramatic increase in the use of improvised explosive devices (IEDs) due to ease of synthesis and improved controls placed on commercial/military explosives. Commonly used materials for IED preparations include fertilizers and industrial chemicals containing oxidizers such as ClO_3^- , ClO_4^- , and NO_3^- , as well as other less stable compounds, such as peroxides. Due to these materials having a wide range of volatility, polarity, and composition, detection can be challenging, increasing the amount of time before any analytical information on the identity of the explosive can be determined. Therefore, this research project developed two analytical methods to aid in the rapid detection of multiple explosive compounds.

The use of microfluidic paper-based analytical devices (μPADs) allows for the development of inexpensive paper devices utilizing colorimetric reactions, which can perform five or more simultaneous analyses in approximately five minutes. Two devices were developed: one for the detection of inorganic explosives including ClO_3^- , ClO_4^- , NH_4^+ , NO_3^- , and NO_2^- , and the second device detects high/organic explosives including

RDX, TNT, urea nitrate, and peroxides. Limits of detection ranged from 0.4 μg – 20 μg of explosive residue with an analysis time of less than five minutes.

Development of a confirmatory method utilizing infusion electrochemical detection-electrospray ionization-time-of-flight mass spectrometry (EC-ESI-TOF MS) and 18-crown-6 ethers to produce guest/host complexes with inorganic ions has also been completed. Utilizing this method the inorganic ions present in many IEDs can be successfully detected as ion pairs, while still allowing for the detection of other high explosives¹. Placing an electrochemical detector before the mass spectrometer permits the detection of hydrogen peroxide, an analyte normally difficult to detect through mass spectrometry. Limits of detection ranged from 0.06 ppm - 2 ppm with an analysis time of less than two minutes.

The development of these presumptive and confirmatory analytical methods permits the detection of a wide range of components present in IEDs. These methods decrease the amount of time required to relay information on the type of explosives present by simplifying the analysis process in the field and in a laboratory.

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LIST OF ABBREVIATIONS AND ACRONYMS

18Cr6: 18-Crown-6 Ether

AN: Ammonium Nitrate

ANAL: Ammonium Nitrate and Aluminum

ANFO: Ammonium Nitrate and Fuel Oil

C4: Composition-4

CE: Capillary Electrophoresis

CI: Chemical Ionization

CPE: Carbon Paste Electrode

DC: Direct Current

DESI: Desorption Electrospray Ionization

DMSO: Dimethyl Sulfoxide

DNA: Deoxyribonucleic Acid

EC-ESI-TOF MS: Electrochemical Detection-Electrospray Ionization-Time-of-Flight
Mass Spectrometry

EI: Electron Ionization

ESI: Electrospray Ionization

FAB: Fast Atom Bombardment

FTIR: Fourier Transform Infrared Spectroscopy

GC/ECD/TEA: Gas Chromatography/Electron Capture Detection/Thermal Energy
Analyzer

GC-MS: Gas Chromatography-Mass Spectrometry

HMTD: Hexamethylene Triperoxide Diamine

HMX: Cyclotetramethylene Tetranitramene

HPLC: High Performance Liquid Chromatography

HPLC-EC: High Performance Liquid Chromatography-Electrochemical Detection

HPLC-UV: High Performance Liquid Chromatography-Ultraviolet Detection

IC: Ion Chromatography

IC-CD: Ion Chromatography-Conductivity Detection

IC-IPD: Ion Chromatography-Ion Photometric Detection

ICR: Ion Cyclotron Resonance

IEDs: Improvised Explosive Devices

IMS: Ion Mobility Spectrometry

IR: Infrared Spectroscopy

LC-MS: Liquid Chromatography-Mass Spectrometry

LED: Light Emitting Diode

MALDI: Matrix-Assisted Laser Desorption Ionization

MW: Molecular Weight

MS-MS: Tandem Mass Spectrometry

NG: Nitroglycerin

p-DMAC: *para*-dimethylaminocinnamaldehyde

PDMS: Polydimethylsiloxane

PETN: Pentaerythritol Tetranitrate

PTFE: Polytetrafluoroethylene

PVC: Polyvinyl Chloride

RDX: Cyclotrimethylenetrinitramene

SEM/EDS: Scanning Electron Microscope/Energy Dispersive Spectroscopy

S/N: Signal-to-Noise Ratio

SPME: Solid-Phase Microextraction

TATP: Triacetone Triperoxide

TNB: Trinitrobenzene

TNT: 2,4,6-Trinitrotoluene

TOF: Time-of-Flight

UHPLC-MS: Ultra High Performance Liquid Chromatography-Mass Spectrometry

UN: Urea Nitrate

μPADs: Microfluidic Paper-Based Analytical Devices

US EPA: United States Environmental Protection Agency

UV: Ultraviolet

XRD: X-Ray Diffraction

CHAPTER 1. INTRODUCTION

A. Overview of Explosives

An explosive is a material capable of such rapid decomposition that tremendous energy is released instantaneously generating an explosion by its own energy. It consists of a mixture of a fuel and an oxidizer, strongly electron deficient compounds that provide a source of oxygen to produce rapid combustion-like reactions when fuels are added².

These components can be present as heterogeneous mixtures, such as ammonium nitrate and fuel oil (ANFO), or may exist in the same molecule, such as trinitrotoluene (TNT)³⁻⁵. Explosives commonly require some stimulus (e.g., heat, shock, friction, etc.) to generate the explosion, but the stimulus does not contribute to the energy of the explosion.

In general, explosives can be classified into three groups: low explosives, high explosives, and blasting agents. Low explosives are combustible materials which have a subsonic reaction rate (less than 3000 m/s). Some examples are black powder and smokeless powder. When black powder burns, a chemical reaction takes place generating hot gases and inorganic residues. If this reaction is confined, the pressure rises and heat moves quickly through the powder causing extremely rapid combustion known as deflagration. This pressure builds until it overcomes the containment device, fracturing it at the weakest point, and generating an explosion. Therefore, low explosives are commonly contained in a sealed casing to generate an explosion³.

High explosives react at a rate faster than the speed of sound (greater than 3000 m/s). This process is known as detonation and an explosion is not dependent on confinement. High explosives can be further divided into two classes: primary and secondary explosives. Primary explosives are highly sensitive to friction, shock, and heat, such as

mercury fulminate, lead azide, and triacetone triperoxide (TATP). Secondary explosives require a larger energy input for detonation, typically supplied by a suitable primary explosive, due to their increased stability. Secondary explosive materials do not explode readily by heat or shock, and are generally more powerful than low explosives. Some examples are TNT, nitroglycerin (NG), and cyclotrimethylenetrinitramene (RDX)³.

A third class of explosives is referred to as blasting agents, which are less sensitive fuel and oxidizer mixtures that can be prepared from fertilizers. This type of explosive will fail to detonate when tested with a number 8 detonator (0.4 g pentaerythritol tetranitrate (PETN)). Instead, they require a booster to detonate, where the size of the booster depends on the blasting agents formulation, ranging up to 1 pound or more of high explosives⁶. This class of explosives consists of ammonium nitrate (AN) and urea nitrate (UN).

B. Explosives of Interest

Explosives detection is performed for two general classes of explosives: improvised/homemade explosives and commercial/military explosives. Improvised explosives can be further divided into three categories: low explosives containing inorganic salts, fertilizer based explosives, and peroxides. Low explosives containing inorganic salts consist of formulations including oxidizers such as chlorate, perchlorate, or nitrate mixed with a fuel source (e.g., sugar, sulfur, etc.). Black powder is a common example of a low explosive consisting of potassium nitrate, charcoal, and sulfur. Potassium nitrate and perchlorate are also common in the pyrotechnic industry and when mixed with metal fuels such as aluminum or magnesium are known as flash powders. Potassium chlorate can be mixed with appropriate metal fuels (Ba, Sr, Cu, etc.) and

nitrate salts to create colored flames and fireworks⁷. However, due to potassium chlorate's relatively low melting point and sensitivity, it is being replaced with the more stable compound potassium perchlorate⁵. Potassium chlorate flash powders have been used in a number of terrorist incidents, most notably, a bombing outside of a popular tourist venue in Bali⁸ and at the Boston Marathon⁹.

Fertilizer-based explosives are a second class of improvised explosives consisting of AN and UN. Ammonium nitrate is commonly mixed with a fuel oil, such as diesel or kerosene, to generate a bulk explosive or blasting agent; while urea nitrate can be used in its pure state or mixed with aluminum powder³. Ammonium nitrate and UN are easily obtained from fertilizers, which until recently have not been strictly controlled¹. Agricultural fertilizer containing UN was used in the first World Trade Center attack in 1993 in New York City, NY¹⁰ and AN was used in a truck-bomb explosion outside the Alfred P. Murrah Federal Building in Oklahoma City, OK on April 19, 1995¹¹.

A third class of improvised explosive materials consists of explosives based on organic and inorganic peroxides. These explosives have been encountered more frequently over recent years in IEDs due to the fact that they are easily synthesized from readily obtainable products¹². Highly concentrated hydrogen peroxide (H_2O_2) can be used as an explosive when it is combined with fuels, such as flour or pepper. Peroxides can also be present as functional groups, R-O-O-R, in organic compounds¹³. Organic peroxides are typically found as cyclic compounds such as TATP and hexamethylene triperoxide diamine (HMTD) (Figure 1.1).

Figure 1.1. Structures of two organic peroxide based explosives: (a) triacetone triperoxide (TATP) and (b) hexamethylenetriperoxide diamine (HMTD).

Home-made organic peroxide-based explosives were used in the London bombing on July 7, 2005¹⁴ and have also appeared in the Middle East.

Peroxides are dangerous primary explosives, readily initiated by heat, friction, shock, or impact, making them difficult to manufacture safely as a result of their instability^{13,15-20}. In addition, TATP and other peroxide explosives can be readily synthesized from easily obtained starting materials and can be difficult to analyze. There are a limited number of methods available for detection due to organic peroxide explosive's poor ability to absorb ultraviolet (UV) light, nonexistent fluorescent characteristics, and lack of a nitro group²⁵⁻²⁹.

In recent years, commercial/military explosives, such as Composition 4 (C4, consisting mainly of RDX, stabilizers, and plasticizers) and Semtex (consisting of PETN, RDX, and plasticizers), have become an increasing concern for use in terrorist activities²¹. These explosive materials can be gleaned and altered from munitions, manufactured, or stolen from military facilities. Therefore, an analytical method for the purpose of explosives detection needs to screen for common improvised explosives along with organic high explosives.

C. Explosives Detection

Explosives detection relies on being able to determine the fuel and oxidizer that are present. There are three classes of fuels: (1) hydrocarbons consisting of gas, diesel, charcoal, sugar, etc. (2) energetic hydrocarbons such as nitromethane, nitrocellulose, and nitrobenzene and (3) elemental fuels such as aluminum, magnesium, sulfur, etc.².

Historically, most explosive compounds contain organic nitro (NO_2^-) groups as oxidizers; so many analytical detection methods have focused on being able to detect the presence of nitrates (NO_3^-), a common inorganic components of explosive mixtures, and nitro groups²²⁻²⁷. However, more recently, explosive mixtures have started containing oxidizers such as chlorates (ClO_3^-), perchlorates (ClO_4^-), and peroxides (O_2^-). Less commonly utilized oxidizers include chromates (CrO_4^{2-}), dichromates ($\text{Cr}_2\text{O}_7^{2-}$), iodates (IO_3^-), and permanganates (MnO_4^-)²⁸. With the additional use of these oxidizers, explosives detection has become a more complicated process (Figure 1.2).

Explosive Residue Detection Flow Chart

Figure 1.2. Explosives detection scheme in forensic analysis². Gas chromatography/electron capture detector/ thermal energy analyzer (GC/ECD/TEA), liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), tandem mass spectrometry (MS-MS), Fourier transform infrared spectroscopy (FTIR), scanning electron microscope/energy dispersive spectrometer (SEM/EDS), x-ray diffraction (XRD), ion chromatography-conductivity detection (IC-CD), ion chromatography-indirect photometric detection (IC-IPD).

The complicated detection scheme necessary for comprehensive explosives analysis ultimately increases analysis time and overall cost of analysis as a result of multiple complicated, expensive pieces of equipment being utilized for the determination of the explosive present.

D. Research Goals

To decrease the analysis time and complication of the explosive detection process, two analytical methods, a presumptive and confirmatory method, were developed. The first procedure involved the development of microfluidic paper-based analytical devices

(μ PADs) for the on-site detection of multiple explosive compounds. Paper microfluidics allows for the development of simple devices, which can perform five or more simultaneous analyses while costing only pennies. Wax patterns are printed and melted into chromatography paper generating channels, which allow solvent to be directed to specific areas containing colorimetric reagents²⁹. To cover the widest possible range of explosive materials, two devices were created: one for the detection of inorganic explosive components such as chlorate (ClO_3^-), nitrate (NO_3^-), ammonium (NH_4^+), nitrite (NO_2^-), and perchlorate (ClO_4^-) while the second device detects high/organic explosives such as nitroaromatics (e.g. TNT), nitroamines (e.g. RDX), urea nitrate, and H_2O_2 .

The second procedure involved the development of a single confirmatory detection technique utilizing non-aqueous infusion and electrochemical detection coupled to electrospray ionization time-of-flight mass spectrometry (EC-ESI-TOF MS). Since inorganic explosives are difficult to detect utilizing mass spectrometry, 18-crown-6 ethers were added to form guest/host complexes with the inorganic ions and increase their molecular weight¹. Therefore, this method allows ionic compounds to be detected as ion pairs along with other organic explosives, such as HMTD. One analyte, H_2O_2 , is also low in molecular weight and difficult to detect with a mass spectrometer since it does not complex with 18-crown-6 ether. Therefore, an electrochemical detector is placed prior to the mass spectrometer to aid in the detection of H_2O_2 .

CHAPTER 2. PRESUMPTIVE ON-SITE DETECTION OF EXPLOSIVES

A. Introduction

In recent years there has been a dramatic increase in the use of IEDs due to improved controls placed on commercial and military explosives. Improvised explosive devices were once limited to war zones, but have become an increasing concern for law enforcement officials who may encounter terrorists manufacturing homemade explosives³⁰. In these situations, fast and accurate on-site identification of the explosive material is paramount.

Commonly used materials for improvised explosive preparations include fertilizers and industrial chemicals containing oxidizers such as ClO_3^- , ClO_4^- , and NO_3^- as well as other less stable compounds, such as peroxides². These materials encompass a wide range of properties, such as volatility, polarity, and composition, which require a variety of different analytical techniques to identify the explosive materials. For example, the combination of gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are often used to identify organic compounds, while ion chromatography (IC) and capillary electrophoresis (CE) are used to determine inorganic ions^{31,32,23,33}.

Metals are detected using scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) or X-ray diffraction (XRD)^{34,35}. Each of these techniques requires large, expensive pieces of instrumentation that are not readily portable, so the samples are commonly sent to a laboratory for testing. This cumbersome process increases the amount of time before any analytical information on the identity of the explosive can be provided to on-site personnel. Therefore, a simple, rapid, and

inexpensive analytical method needs to be developed to detect multiple explosive compounds in the field.

B. On-Site Detection Techniques

On-site explosive detection techniques need to be user friendly, fast, reliable, selective, and sensitive. Explosives detection canines are the most common on-site explosive detection technique. These trained dogs do not react to a particular scent, but to a combination of scent compounds that are given off by an explosive or narcotic. The disadvantages of this technique are that the dogs cannot state which explosive they are reacting to, have a high cost of maintaining and training, require a skilled handler, cannot work around the clock, and have behavioral and mood variations³⁶.

On-site detection can be performed using various analytical instrumentation, such as ion mobility spectrometry (IMS)³⁷, Fourier transform infrared spectroscopy, Raman spectroscopy³⁸, and spectrometric assays³⁹. Methods for standoff or remote detection have also been suggested for RDX and PETN through the use of fluorescence detection resulting in limits of detection at the picogram level for both compounds⁴⁰. These analytical detection devices commonly rely on the detection of volatile components and can be costly and bulky, making them unavailable in many situations. Portable instruments also require a power source such as a battery, which can be drained before the field work is completed⁴¹.

Colorimetric and immunoassay based tests have also been developed, but the current procedures are not multiplexed and may require multiple tests and reagents for proper use. This extends the time of analysis and increases the amount of sample that is needed if an unknown explosive is present^{42,43}. Three classes of commercially available

colorimetric test kits are currently available. The first type requires the use of multiple liquid reagents for detection⁴⁴⁻⁴⁶. An example of this is the ETK Five involving the use of glass ampoules in protective plastic tubes, which are broken in order to apply a few drops of reagent to an absorbent paper. Once the ampoules are broken, the colorimetric reagents are only stable for up to two weeks requiring the frequent replacement of these kits⁴⁴. A second type involves the use of aerosol sprays such as EXPRAY, where chemical reagents are sprayed onto absorbent chemical pads used for swipe sampling. This kit only detects polynitroaromatics, nitrate esters, nitramines, and inorganic nitrate compounds⁴⁷ leaving out a large set of improvised explosives, such as peroxides, chlorates, and perchlorates. The last type of on-site detection kit available involves swipe analysis and a portable colorimetric detector. An example of this is the XCAT, which consists of specially formulated optical inks present on detection cards. These cards are swiped on the sample area and inserted into the XCAT, where software is used to identify the explosive present⁴⁸.

These commercially available kits have major drawbacks such as needing to carry liquid reagents for testing, which can be easily spilled or glass ampoules broken before they are able to be used. They also require multiple liquid reagents which only last a short period of time before another kit needs to be obtained for use. If an unknown explosive sample is being analyzed with certain kits, multiple tests may need to be run requiring multiple samples, which may not be available. They are also fairly expensive and can cost anywhere from a few dollars per test strip to hundreds of dollars for test kits. Most test kits also do not supply a test for perchlorate, which has replaced chlorate in pyrotechniques and can be found in many improvised explosive mixtures.

C. Microfluidic Paper-Based Analytical Devices (μ PADs)

Microfluidics involves the application of lithographic and other microfabrication techniques such as embossing to produce devices which can move nanoliter quantities of liquids on small glass or plastic supports. These glass or plastic chips are connected to micro-scale pumps, valves, and electrophoretic power systems to transport the sample to different locations on the device for extraction, separation, and detection. By miniaturizing the system and exploiting the properties of fluids at the micro level, the sample volume is minimized, the reagent volume is reduced, and the overall separation efficiency of the system is increased⁴⁹. Commercial microfluidic systems are beginning to appear for applications in proteomics and deoxyribonucleic (DNA) separations where small sample volumes are necessary, or where multiplexed analyses are needed and laboratory space is at a premium.

Currently, microfluidic systems utilize narrow channels etched or embossed in glass and plastic devices. Typically, these systems are not reused due to their high susceptibility to clogging from dust or precipitates. The initial prototyping of these devices can be very expensive and the complex systems of valves and fluidic channels can be difficult to engineer.

As a result of these issues, paper has become an increasingly attractive substrate for on-site microfluidic testing since it provides many advantages over traditional microfluidic devices including:

- (1) Inexpensive compared to traditional microfluidic devices
- (2) Samples can be easily segregated into different regions
- (3) Multiple assays can be performed simultaneously making it suitable for

biological applications

- (4) Movement of liquid occurs due to capillary action and does not require external pumps or other electrical equipment⁵⁰
- (5) Combustible and biodegradable makeup allowing for easy eliminations of hazardous waste through incineration⁵¹
- (6) Applicable for trace detection, since it is compatible with small volumes of samples
- (7) Circumvents problems associated with bubbles that plague traditional microfluidic systems⁵⁰
- (8) Paper surface can be easily manipulated through printing, coating, and impregnation
- (9) Easy to fabricate in large quantities in a small amount of time
- (10) Can be easily stored and transported
- (11) Paper properties can be changed to suit different applications

Microfluidic paper-based analytical devices have primarily found use as healthcare diagnostic devices in developing regions of the world that do not have the infrastructure to support traditional diagnostic equipment⁵². These devices utilize small amounts of antibodies combined with enzymatic or other colorimetric detection systems to detect the presence of disease states, diabetes, or parasitic infections^{53,54}. The results are photographed and the image can be quickly transmitted to an expert in a hospital or laboratory setting who can evaluate the results and recommend a course of treatment²⁹. Devices have also been created in a similar manner for pharmaceutical testing⁵⁵ and environmental monitoring⁵⁶.

Paper can be made from many different sources such as wood, cotton, jute, flax, hemp, bamboo, ramie, sisal, bagasse, grass, and straw. Wood is used in 90% of the raw material for the paper industry, since processing of non-wood plants is more expensive. The main issue with this type of paper is the high concentration of lignin in the wood, which causes the paper to discolor, affecting the reading of colorimetric tests. Therefore, for paper microfluidic purposes, paper made from 100% cotton is used. Cotton based papers are commonly used for filter paper and chromatography paper, which are manufactured using high-quality cotton linters with a minimum α -cellulose content of 98%⁵¹. There is no single type of paper that possesses the desired characteristics for all paper microfluidics applications, but the most frequently used paper is Whatman no. 1 chromatography paper. This is a result of the paper's smooth surface, low cost, uniformity on both sides, hydrophilic character, medium flow rate, and 0.18 mm thickness, which meets the standard for commercially available printers⁵⁷⁻⁵⁹.

Microfluidic paper-based analytical devices permit the development of inexpensive analytical devices through the fabrication of hydrophobic patterns on chromatography paper²⁹. There are many different ways of fabricating μ PADs such as photolithography, plotting, inkjet etching, plasma treatment, wax printing, ink jet printing, flexography printing, screen printing, cutting, and laser treatment⁶⁰ (Table 2.1).

Table 2.1. Advantages and Disadvantages of Different Fabricating Methods for the Development of μ PADs^{51,57,60}.

Fabrication Techniques	Advantages	Disadvantages
Photolithography	<ul style="list-style-type: none"> • Can be adapted to use low-cost instruments such as a hot plate or sunlight • Can pattern a wide variety of papers • High resolution of microfluidic channels 	<ul style="list-style-type: none"> • Requires multistep process and extensive equipment • Contamination with photoresist • Barriers are susceptible to damage when bent or folded • Can clog paper pores
Plotting	<ul style="list-style-type: none"> • Low cost of polydimethylsiloxane (PDMS) patterning agent • Less demanding viscosity ranges than in printing • Can work with any surface • Flexible devices 	<ul style="list-style-type: none"> • Inconsistent control over hydrophobic barrier formation • Requires customized plotter (hard to obtain) • Deteriorated barrier definition • Cannot be used for high throughput production
Ink Jet Etching	<ul style="list-style-type: none"> • Requires a single printing apparatus to create channels by etching and to print bio/chemical sensing reagents 	<ul style="list-style-type: none"> • Printing apparatus must be customized • Not suitable for mass fabrication
Plasma Treatment ⁶¹	<ul style="list-style-type: none"> • Low cost due to use of cheap patterning agent • Useful for labs with a plasma cleaner wishing to make many replicates of a few simple patterns 	<ul style="list-style-type: none"> • Requires different metal masks for different microfluidic patterns • Cannot produce arrays of free-standing hydrophobic patterns
Wax Printing	<ul style="list-style-type: none"> • Produce massive amount of devices with simple and fast fabrication process • Bio-degradability of barriers • Computer designed patterns 	<ul style="list-style-type: none"> • Expensive wax printers • Requires extra heating step after wax deposition • Designs of patterns must account for spreading of the wax in paper

Ink Jet Printing	<ul style="list-style-type: none"> • Applicable with modified office printers of extremely low cost and high availability • Computer designed patterns • Produce massive amount of devices with simple and fast fabrication process 	<ul style="list-style-type: none"> • Nozzle clogging • Requires extra heating step after deposition • Designs for microfabrication are costly
Flexography Printing ⁶²	<ul style="list-style-type: none"> • Low ink usage • Avoids heat treatment of printed patterns • Compatible with large scale production 	<ul style="list-style-type: none"> • Requires sequentially printing layers of polystyrene solution to create waterproof barriers • Requires different printing plates • Print quality relies on the smoothness of paper surface • Limited compatibility of solvents so requires frequent cleaning to avoid contamination
Screen Printing	<ul style="list-style-type: none"> • Low cost • Deposition of thick layers is possible • No heat treatment 	<ul style="list-style-type: none"> • Inconsistent deposition of ink deposited through the screen results in low resolution • Different printing screens needed for creating different patterns
Cutting ⁵⁴	<ul style="list-style-type: none"> • No contamination from chemicals • Fabrication of 3D structures from paper and tape 	<ul style="list-style-type: none"> • Manual method results in low resolution • Devices must be enclosed in tape • Cannot produce arrays of free-standing hydrophobic patterns
Laser Treatment	<ul style="list-style-type: none"> • High resolution 	<ul style="list-style-type: none"> • Requires specialized laser equipment • Channels do not allow lateral flow of liquids • Requires extra coating for fluid flow

One of the most effective ways to produce μ PADs is wax printing due to the ease of application and minimal instrumentation required (commercially available printer and laminator). This printing method allows for a low-cost production of the devices, as low as \$0.001 or less for devices made from inexpensive papers, approximately \$0.60 for an 8.5 in x 11 in sheet of μ PADs on Whatman no. 1 chromatography paper⁶³. The wax channels are used to compartmentalize chemical reactions and direct liquid samples toward individual sections of paper containing test reagents⁶³. In this method, wax ink is printed onto chromatography paper using a computer design and then the paper is run through a laminator to allow the wax to melt and diffuse into the paper forming hydrophobic barriers. When the wax is melted, it spreads vertically and horizontally into the paper. This vertical spreading creates a hydrophobic barrier across the thickness of the paper, while the lateral spreading decreases the resolution of the printed pattern resulting in patterns that are wider than those originally printed⁶³⁻⁶⁵. The entire printing process is completed within a few minutes and is capable of producing tens to hundreds of copies of the device⁵¹. The reproducibility of this printing technique is controlled by the width of the wax line and the heating temperature, so if these remain the same for each device they can easily be reproducible⁵⁹.

Detection is most commonly performed using colorimetric schemes, however, electrochemical, chemiluminescence, and fluorescence techniques can also be used (Table 2.2).

Table 2.2. Advantages and Disadvantages of Techniques Used for Analyte Detection on μ PADs.

Detection Techniques	Advantages	Disadvantages
Colorimetric Sensing ⁶⁶⁻⁶⁸	<ul style="list-style-type: none"> • Simple • Most adopted 	<ul style="list-style-type: none"> • Judgment of final color can be challenging • Influenced by background noise of paper or sample
Electrochemical Sensing ⁶⁹	<ul style="list-style-type: none"> • Fast sensor responses • Higher sensitivity (nM range) • Independent of ambient light • Less prone to interference from the color/deterioration of the paper 	<ul style="list-style-type: none"> • Requires reading equipment increasing complexity and cost per test
Chemiluminescence ⁷⁰	<ul style="list-style-type: none"> • Based on the emission of light by a chemical reaction • Increased specificity and sensitivity 	<ul style="list-style-type: none"> • Requires darkness or reading equipment making interpretation of results more complicated
Fluorescence ⁷¹	<ul style="list-style-type: none"> • Increased specificity and sensitivity 	<ul style="list-style-type: none"> • Scattering of light on the cellulose fibers • Influence of index refraction between cellulose and air • High cost

Colorimetric detection is a well-established, fundamental technique that has been the most adopted sensing mechanism for paper microfluidics⁵¹. Using physical and chemical properties of the chosen analyte, reagents and analytes interact to produce a visible color change when the analyte of interest is present. Color spot tests may be presumptive or specific, and are used in the detection many different types of analytes.

They respond to intact compounds⁷², ions/functional groups⁷³, metals⁷⁴, or biomarkers⁷⁵ and can be based in classical wet chemistry methods⁷⁶, nanoparticle aggregation⁷⁷, or antibody/aptamer detection^{75,78}. By using multiple indicators for the same compound, the user's ability to discriminate between different shades and intensities of color indicating concentration is increased and data can be accurately interpreted⁶⁴. For ions and small molecules, multiple indicators for the same compound may also help avoid a false positive result, since the interferences for tests using different reagents or mechanisms for the same analyte are unlikely to be the same for both. Changing the chemistry would permit detection of the same compound but differing responses. Therefore as a result of their applicability to paper, versatility, and ease of analysis, colorimetric spot tests will be used for detection on μ PADs.

Colorimetric detection can be performed using visual detection along with scanners, digital cameras, camera phones, and spectrophotometers. Detection using the human eye can be subjective and influenced by lighting and conditions of dry and wet paper, so color charts are commonly included with the devices. When using instrumental techniques, differences in illumination may cause variations in color intensity and hue which can be solved by background correction, subtraction of the background color, or comparison with a calibration curve of standards of known color and intensity. An office scanner can also be utilized to provide high resolution and focus of the digitalized image. These scanners can be portable, easy to use, and are widely used all over the world. Digital cameras with automatic white balance can also be used. After the image is photographed, it is typically analyzed using computer programs such as Adobe Illustrator, Adobe Photoshop, or Image J. Using this software, the total value or

individual channels of the color space can be utilized for quantification purposes. This response is given as the average of the pixel intensity in the detection zone after subtraction from the average intensity for the control (white) region. Software for cell phone cameras is also being developed⁵¹ to allow for quantification in the field. Once captured, images can also be easily sent to a specialist for further analysis using email or text messaging. Lastly, spectrophotometers have been utilized, mostly as a way to optimize the fabrication process in the laboratory due to their large size^{51,57}.

Researchers have been developing new on-site colorimetric tests. Ercag et al. has developed a sensing method for nitroaromatic and nitramine based explosives. Explosives such as TNT, PETN, and RDX, were pre-adsorbed on a poly vinyl chloride surface and sprayed with different reagents for each group of explosives generating different colors⁷⁹. Explosives detection has also been developed utilizing μ PADs. Taudte et al. utilized a μ PAD with circular patterns that required the explosive sample to be placed in the small circular area containing pyrene. Under UV light the μ PAD fluoresced, but when an explosive sample was present the fluorescence was quenched⁷¹. Pesenti et al. published a method to detect three types of trinitroaromatic explosives on μ PADs with 6 mm circular patterns. Potassium hydroxide was used to detect trinitroaromatics through a colorimetric reaction with limits of detection determined to be 7.5 ng for trinitrobenzene (TNB), 12.5 ng for TNT, and 15.0 ng for tetryl⁶⁸. Salles et al. developed a μ PAD for the detection of organic peroxides and nitrobenzenes. The analysis time was 15 minutes with a limit of detection at 0.2 μ g of explosives. This method required the use of a smart phone, custom-made software, and a closed chamber in order to differentiate the color profiles⁸⁰. All of these procedures focused on detecting

a small subset of explosives utilizing spectroscopic detection but failed to present a method for the detection of improvised explosives, particularly those developed from fertilizers and pyrotechnic materials.

CHAPTER 3. CONFIRMATORY DETECTION OF EXPLOSIVES

A. Laboratory Detection Techniques

In previous studies, explosives have been detected via a variety of analytical methods. Bulk detection of explosive compounds is commonly done through Raman and Infrared (IR) spectroscopy.

Trace detection is done through analytical techniques such as GC-MS, LC-MS, IC, and CE. Inorganic compounds, such as AN, are typically detected by IC^{23,24} or CE^{25,26,33,81-84} while organic explosives, such as TNT, are commonly analyzed by GC-MS³¹ or LC-MS^{13,32,85}. However, there are few analytical methods developed which are capable of rapidly detecting inorganic and organic explosives simultaneously, particularly at trace levels. Examples of these selected methods include FTIR, Raman, and specific types of mixed mode ion chromatography.

Inorganic nitrate, chlorate, and perchlorate salts are ionic species; therefore many methods have been developed utilizing ion chromatography with conductivity detection, which measures the electrical conductance between two electrodes and allows for detection of ionic species. Ion chromatography has also been utilized, but perchlorate is strongly retained by anion-exchange stationary-phase materials and requires special eluents for detection. Ion-pairing and ion-exclusion reagents can be used to adjust the retention of late-eluting peaks²⁴; however, the best procedure for inorganic explosives

screening is gradient ion chromatography⁸⁶. Capillary electrophoresis can also be used to separate ionic species, typically using indirect UV detection^{25,84} or contactless conductivity detection. Ion chromatography-mass spectrometry instruments have recently been developed and proven useful in explosive analysis. For example, an anion-exchange HPLC column coupled to a mass spectrometer and negative electrospray ionization has been used to successfully detect chlorate, chlorite, perchlorate, and nitrate ions⁸⁷.

Portable techniques utilizing microfluidic systems have also been developed to quickly detect ions^{25,33,83,88}. These systems use indirect fluorescence detection with laser or light emitting diode (LED) excitation. An approach known as dual opposite injection permits the simultaneous measurements of both cations and anions by CE or microfluidic capillary electrophoresis^{81,83}. Methods have also been developed to detect ions from home-made explosive residue samples, emulsion explosive residues, and firework perchlorate-based explosives^{22-24,89}.

Peroxide-based explosives can be difficult to analyze due to problems with thermal stability and the lack of a UV chromophore⁹⁰. While low injector temperature can be used for analysis of peroxide-based explosives to avoid the problems of decomposition in gas chromatographic injectors, liquid based methods have been developed to detect TATP or HMTD through determination of H₂O₂, a precursor and degradation product. Most of these procedures have utilized either UV irradiation or a short-burst laser treatment to degrade peroxide-based explosives post-column, but prior to being introduced to the detector. Infrared and Raman spectroscopy have been utilized to screen for peroxide based explosives^{85,90-92}. Multiple mass spectrometry methods have also been developed

including headspace GC-MS and solid phase microextraction (SPME) GC-MS⁹³ along with ESI-MS⁹⁴, desorption electrospray ionization (DESI)-MS⁹⁵, and LC-MS^{12,31,87,96,97}. Electrochemical methods have been unable to detect TATP, although they successfully detected its precursor and degradation product H₂O₂^{82,98-101}. There have been no methods reported using a form of electrochemical detection for the direct detection of HMTD and TATP along with H₂O₂. Nanotube array sensors have also been developed to detect organic peroxide explosives¹⁰².

No methods have been reported for a comprehensive analysis of improvised explosives including inorganic salts and peroxide based explosives. While there have been analyses completed utilizing ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) for the detection of high explosives and organic peroxides³², these methods are not capable of detecting inorganic explosives and H₂O₂. The current standard for the analysis of explosive compounds is US EPA method 8330, which utilizes HPLC-UV to detect nitroaromatic and nitroamine compounds,^{32,103,104} but this method does not include a procedure for the detection of peroxide explosives and inorganic oxidizers. Therefore, a method capable of detecting a wide variety of both organic and inorganic materials needs to be developed to rapidly screen unknown debris from a post-blast scene.

B. Infusion Electrochemical Detection Electrospray Ionization Time-of-Flight Mass Spectrometry (EC-ESI-TOF MS)

A method utilizing non-aqueous infusion electrochemical detection electrospray ionization time-of-flight mass spectrometry has the potential to detect multiple explosive compounds using one analytical method. For the detection of H₂O₂, a procedure was

previously established by Tarvin et al. utilizing high performance liquid chromatography with electrochemical detection (HPLC-EC). The optimized mobile phase was determined to be 150 mM aqueous sodium acetate at a pH of 10.5. The electrochemical detector consisted of a 3 mm gold working electrode, a palladium-hydrogen reference electrode, and a palladium auxiliary electrode with an applied potential of +400 mV. The detection limit was determined to be 0.6 μ M for hydrogen peroxide⁹⁹.

To detect inorganic explosive components such as AN and UN, a mass spectrometry method was previously developed. Ammonium and nitrate ions are common endogenous ions in the environment and the detection of these compounds separately does not necessarily mean that an explosive is present. To improve the reliability of the detection, ionic compounds should be detected as ion pairs. This can be accomplished through the use of a non-aqueous mobile phase, ensuring that the ions do not dissociate, and 18-crown-6-ethers to form an ion pair complex capable of being detected using a mass spectrometer¹. These crown ethers do not interact with organic explosives allowing for both inorganic and organic explosives to be detected using one analytical method. de Perre et al. previously developed a procedure utilizing 18-crown-6 ether complexation and mass spectrometry for UN and AN detection. A non-aqueous solvent (methanol) was utilized since the presence of water reduced specificity for the uronium ion by allowing protonation of urea. Detection limits were determined to be 0.16 ppm for AN and 0.25 ppm for UN¹. It was also noted that inorganic ions such as chlorate and perchlorate could be detected as well as organic explosives via direct detection or through the formation of adduct ions.

These two previously developed methods will be combined and the parameters optimized to create one analytical method for the simultaneous identification of improvised explosives. An electrochemical detector will be placed in front of the mass spectrometer to aid in detection of H_2O_2 , while the mass spectrometer will detect inorganic complexation and organic ions.

18-Crown-6 Ethers

Crown ethers are cyclic organic compounds that can contain oxygen, nitrogen, sulfur, or other heteroatoms. The heteroatoms alternate with carbon bridges to form these cyclic compounds, such as 18-crown-6 ether¹⁰⁵ (Figure 3.1).

Figure 3.1. Structure of 18-crown-6 ether

Crown ethers were discovered by Charles Pedersen, who received a Nobel Prize for his work in 1987. They have a macrocycle ring (generally larger than 12 members) where four or more heteroatoms are separated by a carbon-containing unit of two or more atoms. Oxygen is typically the heteroatom that binds alkali metals, while nitrogen is present for the binding of transition metals. The exterior of the macrocycle ends up being hydrophobic, while the interior is hydrophilic and with the appropriately sized cation, an ion complex can be formed. If the macroring hole and the cation diameter are identical, the cation will be embedded in the ring. If the ring is larger than the cation, the ring can

pucker to allow the donor-group-to-ion contacts to be the appropriate size and position. When the cation is larger than the ring, the cation can sit on the ring or be held between two rings. These complexes can also be formed using organic cations, such as ammonium (NH_4^+), through the oxygen atoms in the macroring, which can function as hydrogen bond acceptors. Therefore, 18-crown-6 ethers will form ion complexes with inorganic explosives such as ammonium nitrate and urea nitrate along with ammonium perchlorate and potassium chlorate¹⁰⁵.

Electrochemical Detector

Since hydrogen peroxide has a low molecular weight (MW = 34.01) it is difficult to detect utilizing a mass spectrometer, so an electrochemical detector is used. This type of detection involves the use of three electrodes: a working electrode, reference electrode, and auxiliary electrode. During the oxidation process the electrochemical reaction allows for the release of electrons into the working electrode. This flow of electrons is the current, which is detected by the electrochemical detector. Through the use of an amperometric electrode, the current is proportional to the concentration of the analyte. In this method, the working electrode is a carbon paste electrode (CPE), which is highly sensitive and gives the highest signal to noise (S/N) ratios for all electrodes¹⁰⁶. To construct the CPE, chemically cleaned graphite particles (10-25 μm diameter) are combined with binder and packed tightly into the cavity of a plastic electrode block and the surface is smoothed to a glossy finish¹⁰⁷.

Since it is not possible to determine the absolute potential of an electrode, the electrode potential must always be compared to an arbitrary zero point determined through a reference electrode. The most widely available reference electrode is a

silver/silver chloride electrode. In this electrode the internal electrolyte and external electrolyte are in ionic contact through a small porous ceramic plug sealed into the end of the glass tubing. An auxiliary electrode is also present to specifically collect the current flowing in the circuit and allows the reference electrode to remain at its reference potential¹⁰⁷.

These three electrodes are encased in a thin-layer cell. The liquid enters the detector cell through a micro-port and then flows over a solid working electrode. The flow continues past the reference and auxiliary electrodes. The working electrode is grounded through the detector electronics and the voltage between the auxiliary and reference electrode is controlled to regulate the potential required for the electrochemical reaction. The body of the cell is formed from two solid blocks, one PTFE block containing the working electrode, while the second is made of stainless steel and forms the auxiliary electrode. Connection of these electrodes to the electronics of the system is through gold pins and electrical connectors¹⁰⁷.

Ionization Source

The analyte flow then continues from the electrochemical detector into the ionization source of the mass spectrometer. There are many types of ionization sources for mass spectrometers such as electron ionization, chemical ionization, electrospray ionization, and fast atom bombardment (Table 3.1).

Table 3.1. Comparison of Different Ionization Sources Commonly Used in Mass Spectrometry.

Ionization Method	Typical Analytes	Sample Introduction	Typical Mass Range	Method Highlights
Electron Ionization (EI)	Relatively small and volatile	GC or LC Solid Probe	1-1,000	<ul style="list-style-type: none"> • Hard method • Used for small molecules • Versatile • Provides structural info • Standardized, allowing for libraries
Chemical Ionization (CI)	Relatively small and volatile	GC or LC Solid Probe	60-1,200	<ul style="list-style-type: none"> • Soft method • Used for small molecules • See molecular ion peak
Electrospray Ionization (ESI)	Peptides, proteins, non-volatile	LC or CE	100-50,000	<ul style="list-style-type: none"> • Soft method • Ions can be multiply charged • See molecular ion peak
Fast Atom Bombardment (FAB)	Carbohydrates, organometallics, peptides, non-volatile	Sample mixed in viscous matrix	300-5,000	<ul style="list-style-type: none"> • Soft method but harder than ESI or MALDI
Matrix Assisted Laser Desorption Ionization (MALDI)	Peptides, proteins, nucleotides	Sample mixed in solid matrix	500-100,000	<ul style="list-style-type: none"> • Soft method • Very high mass range • Molecule needs to absorb at the wavelength of the laser to ionize it

Since ion pair complexes are being detected, a soft ionization technique is used to ensure the ion complexes do not break apart¹. Therefore, electrospray ionization is used because it allows for a large mass range and is a soft ionization technique. It also allows for interfacing with an autosampler for a LC, which is required for interfacing with an electrochemical detector.

Electrospray ionization is produced by applying a strong electric field under atmospheric pressure to a liquid passing through a capillary tube. This electric field creates a charge accumulation at the end of the capillary, which will break and form highly charged droplets. These droplets pass through a drying gas in order to desolvate the ions and then are directed into the mass analyzer¹⁰⁸.

Mass Analyzer

The ions expelled from the ionization process are then focused using lenses and accelerated towards a mass analyzer such as a quadrupole, time-of-flight, or orbitrap.

Table 3.2. Advantages and Disadvantages of Mass Analyzers for a Mass Spectrometer¹⁰⁸.

Analyzer	Advantages	Disadvantages
Quadrupole	<ul style="list-style-type: none"> • Unit mass resolution • Fast scan • Low cost 	<ul style="list-style-type: none"> • Lower resolution
Sector (magnetic and/or electrostatic)	<ul style="list-style-type: none"> • High resolution • Exact mass 	<ul style="list-style-type: none"> • Limited resolving power
Time-of-Flight (TOF)	<ul style="list-style-type: none"> • Theoretically no limitation for m/z maximum • High throughput • High resolution 	<ul style="list-style-type: none"> • More expensive • High vacuum
Ion Cyclotron Resonance (ICR)	<ul style="list-style-type: none"> • Very high resolution • Exact mass • Perform ion chemistry 	<ul style="list-style-type: none"> • Very expensive • High vacuum
Orbitrap	<ul style="list-style-type: none"> • High resolution • Exact mass 	<ul style="list-style-type: none"> • Slower analysis than others • Most expensive • High vacuum

A reflectron time-of-flight (TOF) mass analyzer was selected due to its large mass range allowing for detection of ion complexes with crown ether. It also allows for exact mass calculations to determine the ion complexes that are being formed.

In a TOF, ions are accelerated toward a flight tube by a difference of potential applied between an electrode and extraction grid. The ions acquire the same kinetic energy, and when leaving the acceleration region enter a field-free region where they are separated according to their velocities. Smaller ions will move faster while larger ions move slower allowing for separation. The separated ions approach a reflectron, acting like an ion mirror, and are sent back through the flight tube before reaching the detector positioned at the other end of the flight tube. The advantage of utilizing a reflectron is the correction of the kinetic energy dispersion of the ions leaving the source with the same m/z ratio. Ions with a higher kinetic energy will penetrate the reflectron more

deeply than ions with lower kinetic energy. This allows the faster ions to spend more time in the reflectron and reach the detector at the same time as slower ions with the same m/z ¹⁰⁹.

The mass-to-charge ratios are then determined by measuring the time that ions take to move through the field-free region between the source and the detector, a photomultiplier tube. This is then sent to a computer where a mass spectrum is generated¹⁰⁸.

CHAPTER 4. EXPERIMENTAL PROCEDURES

A. Introduction

The goal of this project was to develop a presumptive and confirmatory method for the detection of multiple explosive compounds, focusing on the wide variety of explosive materials utilized in improvised explosive devices. The presumptive method involved the development of microfluidic paper-based analytical devices (μ PAD) for the inexpensive, on-site colorimetric detection of explosive compounds. Two devices were developed: one for the detection of inorganic explosives including ClO_3^- , ClO_4^- , NH_4^+ , NO_3^- , and NO_2^- , and the second device detects high/organic explosives including RDX, TNT, UN, and peroxides.

The confirmatory method involved a procedure that combines amperometric detection for peroxides with electrospray ionization time-of-flight mass spectrometry for inorganic and organic explosives. In the mass spectrometer, inorganic ions were detected using 18-crown-6 ether to capture ions and ion pairs. When switched to negative ion mode, military grade explosives, such as TNT and RDX, can also be detected. These

procedures provide detection of the widest possible variety of explosives, precursors, and reaction products.

B. Development of Microfluidic Paper-Based Analytical Devices (μ PADs) For On-Site Explosive Detection

1. Chemicals

All reagents and chemicals were analytical grade. Explosive standards such as TNT, RDX, and urea nitrate were obtained as dilute solutions in acetonitrile from law enforcement sources. Potassium chlorate, ammonium nitrate, potassium nitrite, and potassium perchlorate were all purchased from Sigma-Aldrich. 30% aqueous hydrogen peroxide solution was purchased from Fisher Scientific.

The handling of explosives can be hazardous and should be performed with appropriate laboratory safeguards. All materials were stored as dilute solutions in sealed plastic vials in an explosion proof freezer. All experiments involving explosives were conducted with appropriate protection including face shield, gloves, and lab coat. Laboratory fume hoods were used when appropriate.

2. μ PAD Fabrication

The μ PADs were designed using Microsoft paint and printed using blue ink on Whatman no. 1 chromatography paper with a wax-based printer (Xerox ColorQube 8750). The paper was then placed into an aluminum foil carrier and run through a laminator at 160°C, speed 1. This melting process was repeated and the μ PADs were cut to appropriate size for use. Two microliters of each colorimetric reagent were spotted onto the μ PADs and allowed to dry for approximately 1 minute.

This process was used for all colorimetric reagents on both the single lane μ PADs and five lane μ PADs (Figure 4.1).

Figure 4.1. Design and placement of colorimetric reagents for the μ PADs. (a) Single lane μ PADs (2.6 cm x 1.7 cm, lane size: 2.3 cm x 0.4 cm, bulb size: 0.6 cm x 0.9 cm) used for optimization of colorimetric tests and (b) five lane μ PADs (4.1 cm x 4.9 cm, lane size: 1.5 cm x 0.3 cm) for multiple explosive analysis. (c) Testing set up for single lane and five lane μ PADs.

After all of the colorimetric reagents were added to their respective lanes on each devices, the μ PADs were laminated using 3 mm lamination sheets at 110°C, speed 4. Certain reagents, notably Nessler's reagent, are heat sensitive and care must be taken to avoid heating the region around this reagent during the lamination step.

Inorganic Explosives μ PAD

The five lane inorganic explosives detection μ PAD included a test for chlorate, nitrate, ammonium, nitrite, and perchlorate in each of their individual lanes. To detect chlorate, an aniline sulfate reagent was spotted at the midpoint of the sample lane and 50% H_2SO_4 was spotted at the top of the sample lane. For the nitrate test, 3 steps were involved: (1) a solid reducing mixture consisting of 0.08 g sulfanilic acid, 1.87 g sodium acetate, and 0.37 g zinc powder was made into a slurry using a saturated trehalose

solution in ethanol and pressed into the bottom of the sample lane using a small metal spatula; (2) 20% H₂SO₄ was spotted midway up the lane; (3) 2.5% 1-naphthol in ethanol was spotted at the top of the sample lane. To detect ammonium, Nessler's reagent was spotted at the top of the sample lane. For the nitrite test, the Griess test was prepared by spotting 0.5% aqueous sulfanilic acid midway up the sample lane and 0.1% aqueous 1-naphthylamine at the top of the sample lane. To detect perchlorate, 0.05% aqueous methylene blue solution was spotted at the top of the sample lane. This μ PAD was run using deionized water as the solvent.

High/Organic Explosives μ PAD

The five lane high/organic explosives μ PAD includes tests for RDX/HMX/PETN, TNT/TNB/Tetryl, urea nitrate, nitrate, and hydrogen peroxide. For the detection of RDX/HMX/PETN, three steps were utilized: (1) A solid reducing mixture of zinc powder was made into a paste using 50% acetic acid and pressed into the bottom of the sample lane using a small metal spatula; (2) midway up the sample lane 0.1% sulfanilic acid in water was spotted; (3) 0.05% 1-naphthylamine in water was spotted at the top of the sample lane. To detect TNT/TNB/Tetryl, 1.5 M potassium hydroxide was spotted at the top of the sample lane. For the urea nitrate test, 0.023 M *para*-dimethylaminocinnamaldehyde in ethanol was spotted at the top of the sample lane. To test for hydrogen peroxide, 1 M aqueous ammonium titanyl oxalate was spotted at the top of the sample lane. This μ PAD utilized 50% acetone/50% water as the solvent.

3. Portable Testing System

A portable testing system was developed for use with the microfluidic devices. The kit included plastic 1 mL vials or reduced volume 250 μ L vials with a slit cut in the lid, allowing the insertion of the μ PAD device into the solvent (Figure 4.1 c). Plastic pipettes and two solvents (water and 50% acetone/50% water) were also included. To perform the analysis, a small amount of powder is placed into the solvent and allowed to dissolve. The μ PAD is then inserted through the slit in the lid and the lid is placed on the vial. The bottom tip of the paper device in the solvent allows capillary action to carry the solvent and the analytes up the μ PAD into the lanes containing the colorimetric test reagents. This entire process, including run time, takes approximately 5 minutes to complete.

C. Validation of μ PADs for Explosive Detection

1. Interference Testing

Gold Bond blue powder, Gold Bond yellow powder, Gold Bond white powder, Publix baking powder, Publix baking soda, salt, iodized salt, Publix powdered whip topping, Publix laundry detergent (powder form), Publix flour, Crystal Light Pink Lemonade mix, and Crystal Light Iced Tea mix were purchased from supermarkets in Miami, FL, United States. Codeine, ephedrine, methamphetamine, and cocaine were obtained in powder form from the International Forensic Research Institute at Florida International University. One thousand ppm samples were prepared for each interferant in the appropriate solvent depending on which μ PAD was being tested to determine if any of these commonly encountered white household powders produced interferences when present.

2. Post-Blast Detection

The Hodgdon Pyrodex and Triple Seven black powder substitutes were both obtained from Hodgdon Powder Company, Inc. GOEX black powder was obtained from GOEX and Red Dot smokeless powder was obtained from Alliant Powder. American Pioneer Powder FFG and Jim Shockey's Gold were obtained from American Pioneer Powder, Inc. The Lemon Drop firework was obtained from a retail store.

To perform post-blast analysis for these samples, a small amount of each powder was placed onto a watch glass and burned with a match. This step was performed in a fume hood and with a face shield to ensure safety. The resulting burned powder was collected using a metal spatula and deposited into a small vial containing the respective solvent.

3. On-Site Post-Blast Detection

With the assistance of the Miami Police Department bomb squad, on-site and post-blast analysis was conducted using the μ PADs. Small polyvinyl chloride (PVC) pipes filled with the respective explosive material and capped with two PVC end caps were used for each shot. These pipe bombs were then placed into a large plastic container for containment and sampling purposes and detonated using a blasting cap. Eight different pipe bombs were constructed and contained different explosive mixtures including ammonium nitrate and aluminum (ANAL), Vaseline and potassium chlorate, triacetone triperoxide, Helix (nitromethane and aluminum), gel dynamite, detasheet, urea nitrate boosted with sheet, and Composition-4.

Figure 4.2. On-site sampling performed (a) pre-blast by adding powder samples to a small amount of solvent and (b) post-blast by swiping the tab of the μ PAD across a piece of large plastic container.

Sampling was performed both pre- and post-blast. Pre-blast testing consisted of collecting a small amount of the explosive material or mixture before detonation (Figure 4.2a). This material was placed into a small plastic vial containing the μ PAD's respective solvent and allowed to develop for approximately five minutes. Results were then photographed and documented. After the blast was completed, a piece of the large plastic container was collected and the bottom tab of each respective μ PAD was swiped over the plastic to collect any post-blast residue present. These tabs were laminated on the back, but not the front allowing for swipe samples to be collected (Figure 4.2b). These μ PADs were then placed in their respective solvents and allowed to develop for approximately five minutes. Results were photographed and documented.

D. Infusion Electrochemical Detection Mass Spectrometry (EC-ESI-TOF MS) for the Detection of Explosives

1. Chemicals

All reagents and chemicals were analytical grade. Explosive samples such as TNT, RDX, and urea nitrate were prepared as dilute solutions in acetonitrile from law enforcement sources. Potassium chlorate, ammonium nitrate, and ammonium perchlorate were all purchased from Sigma-Aldrich. Thirty percent aqueous hydrogen peroxide solution and 18-crown-6 ether was purchased from Fisher Scientific.

The handling of explosives can be hazardous and should be performed with appropriate laboratory safeguards. All materials were stored as dilute solutions in sealed plastic vials in an explosion-proof freezer. All experiments with explosives were conducted with appropriate protection including face shield, gloves, and lab coat. Laboratory fume hoods were used when appropriate.

2. Instrumentation

Figure 4.3. Detector set-up for the Infusion EC-ESI-TOF MS method utilized for analysis.

To detect multiple explosive compounds in one analytical method, a detector scheme was developed consisting of infusion into an electrochemical detector followed by analysis with a mass spectrometer (Figure 4.3). This detection system first starts with an

inlet system such as a direct probe, chromatography, or capillary electrophoresis system. In this case, sample introduction was done using an Agilent 1100 (G1313A) liquid chromatograph with an autosampler (G1312A; Agilent). No HPLC column was used for this infusion based procedure, as the goal was to operate in a non-aqueous environment to maintain the composition of ion pairs. The output tubing from the autosampler was connected to an electrochemical detector (Bioanalytical systems connected to CHI (8107) amperometric detector) consisting of a carbon paste working electrode, silver/silver chloride reference electrode, and a steel auxiliary electrode. A 50 μm spacer was used in the flow cell. Cyclic voltammetry, performed previously, determined the optimal working potential for the detection of hydrogen peroxide to be +400 mV in DC mode⁹⁹.

Following amperometric measurement, the sample is carried into an Agilent G1969A ESI-TOF mass spectrometer (Santa Clara, CA, USA) to perform the analysis of explosives in the presence of complexing agents. Optimization of the fragmentor and skimmer voltages was performed previously¹, where the best conditions were determined to be positive ion mode, capillary voltage 3000 V, fragmentor voltage 125 V, skimmer voltage 40 V, octapole voltages 300 V, gas temperature 150°C, drying gas (nitrogen) flow rate 5 L/min, and nebulizer gas (nitrogen) pressure 10 psig. Two reference compounds [purine (m/z 121.0509) and HP0921 standard (m/z 922.0098)] were added to the sheath solution to improve the mass accuracy during the analysis.

The following complexation ions were used to determine the presence of UN, AN, potassium chlorate (KClO_3), and ammonium perchlorate (NH_4ClO_4): $[\text{18Cr6} + (\text{NH}_2)_2\text{COH}]^+$, $[\text{2(18Cr6)} + (\text{NH}_2)_2\text{COH}]^+$, $[\text{2(18Cr6)} + \text{NH}_4\text{NO}_3 + \text{NH}_4]^+$, $[\text{2(18Cr6)} + \text{KClO}_3 + \text{K}]^+$, and $[\text{2(18Cr6)} + \text{NH}_4\text{ClO}_4 + \text{NH}_4]^+$. HMTD was detected as $[\text{HMTD} + \text{H}]^+$, since it did not complex with the 18-crown-6 ether.

3. Sample Preparation

Samples were prepared by dissolving the respective solid in a 1 mM 18-crown-6 solution in methanol. This stock solution was used for all dilutents and made fresh for optimal results.

E. Validation of Infusion Electrochemical Detection Mass Spectrometry (EC-ESI - TOF MS) for the Detection of Explosives

1. Interference Testing

Corn oil, coffee, Gatorade, Pepsi, Windex, bleach, lubricating oil (WD-40), hand sanitizer, Sweets, diesel, and gasoline, were purchased from supermarkets in Miami, FL, United States. One hundred microliters of the respective interferant were used to spike 395 μL of the respective 100 ppm solution of explosive in methanol. Five microliters of 100 mM 18-crown-6 in methanol was then added to the mixture and the samples were vortexed for 10 seconds to ensure mixing.

2. Collection of Post-Blast Samples

With the assistance of the Miami Police Department bomb squad detail, a pipe bomb study was conducted to collect post-blast samples for analysis utilizing the EC-ESI-TOF MS method. Eight different shots were completed consisting of ammonium nitrate and aluminum (ANAL), Vaseline and potassium chlorate, triacetone triperoxide, Helix

(nitromethane and aluminum), gel dynamite, Detasheet, urea nitrate boosted with Detasheet, and C4.

Figure 4.4. Post-blast sample collection using gauze wetted with 200 μ L of methanol and swabbed over plastic container remnants generated from the blast.

After the blast was completed, a piece of the large plastic container was collected and sampled using a 2x2 inch sterile gauze wetted with 200 μ L methanol. Two different swabs were collected from two different plastic remnants. These swabs were then placed into separate 20 mL amber vials and sealed with a screw top lid and parafilm to prevent contamination. Soil samples were also collected from directly under the plastic container blast area. The sample was placed into a 20 mL amber vial and sealed with a screw top lid and parafilm. Plastic pieces were also collected and sealed with a heat sealer in plastic nylon bags in case additional testing was needed.

3. Extraction/Analysis of Post-Blast Samples

The pieces of gauze used to sample the post blast explosives were extracted by

centrifugation utilizing spin baskets. This involved placing a gauze sample wet with 450 μL methanol (dry swabs 550 μL methanol) in a plastic spin basket that was inserted in a 1.5 mL tube. After centrifugation (Beckman microfuge 12, 3000 g, 5 min), 495 μL of extract was then removed and transferred into 1 mL glass vials along with 5 μL of 1 mM 18-crown-6 ether in methanol. The samples were then injected into the infusion EC-ESI-TOF MS system run in positive and negative ion mode (for high explosives detection) using the optimized conditions described previously.

CHAPTER 5. DEVELOPMENT OF μPADs FOR RAPID, ON-SITE EXPLOSIVES DETECTION

A. Introduction

A presumptive method for the on-site detection of multiple explosive compounds was developed utilizing μPADs . Two different μPADs were created: the first was designed to detect inorganic materials including important oxidizers used in pyrotechnic manufacturing such as nitrates, perchlorates, and chlorates. In addition, this μPAD contained test lanes for ammonium, to detect the common fertilizer based explosive ammonium nitrate, and a lane for nitrite, which is a post blast reaction product that appears following the deflagration of nitrate salts. The second μPAD was designed for the detection of military explosives such as TNT, RDX and PETN. It also contained tests for UN, a common fertilizer based explosive, and peroxide based explosives.

B. Development of μPADs

A tree shaped template consisting of a stem and five branches has been utilized to

allow for a uniform flow along the branches and for multiple tests to be run simultaneously in a minimal amount of time.⁵⁹ The external size of these devices during developmental stages was 45 mm x 38 mm with lane sizes of 13 mm x 4 mm. This size was later reduced to 24 mm x 17 mm with lane sizes of 7 mm x 2 mm to minimize analysis time. Single lane μ PADs were also designed for the development and testing of individual colorimetric tests.

Printing of the μ PADs was completed using a wax based printer on Whatman no. 1 chromatography paper. This type of paper is an ideal substrate for fabricating μ PADs to allow faster transfer of solutions, better analytical performance, and high color intensities for colorimetric tests compared to filter paper and other thicker substrates⁵⁸. To ensure that the wax ink was fully embedded into the paper, the μ PADs were run twice through the laminator set at 160°C, speed 1.

The μ PADs were originally printed using black ink, but significant bleeding of the ink occurred as a result of the organic solvents that were used. Therefore, a comparison of the effects of the wax ink colors and solvent composition was performed to optimize the devices. Solvents chosen were selected based on their ability to maintain the solubility of the explosive compounds being detected. The optimal wax color chosen was bright blue since none of the subsequent colorimetric tests generated this color and it produced minimal problems with solvent induced bleeding. White ink was not an option since it is not readily available and lighter colors (light blue, light pink, lavender, light grey) did not provide a sufficiently solid barrier to solvent flow.

Deionized water was used as the optimal solvent for all experiments using the

inorganic explosives μ PAD, since all of the inorganic explosives are soluble in water. For detection with the high/organic explosives μ PAD, multiple solvents were tested including acetone, acetonitrile, methanol, ethanol, deionized water, 50% dimethyl sulfoxide (DMSO)/50% water, 50% acetone/50% water, 75% acetone/25% water, 50% methanol/50% water, and 75% methanol/25% water. When the percentage of organic solvent was above 50%, a noticeable increase in the bleeding of wax ink occurred due to dissolution of the dye, affecting visualization of color changes. The optimal solvent for this μ PAD was determined to be 50% acetone/50% deionized water to maintain the solubility of all tested compounds and minimize bleeding of the wax ink. The optimized solvent and wax color were used for all further experiments.

The first μ PAD was developed for the detection of inorganic explosives such as pyrotechnic mixtures, black powders, and ammonium nitrate (Figure 5.1).

Figure 5.1. (a) The inorganic explosives μ PAD device prior to analysis. (b) The same μ PAD following analysis of a 1000 ppm mixture of potassium chlorate, ammonium nitrate, potassium nitrite, and potassium perchlorate in deionized water. Color changes started after 5 minutes, with a total run time of approximately 18 minutes.

Table 5.1 lists the relevant tests and reagents for each lane in the inorganic μ PAD.

Table 5.1. Colorimetric tests and reagents used for the detection of inorganic explosive compounds. These μ PADs were prepared using 2 μ L of each reagent spotted onto the μ PAD and allowed to dry (see Experimental Procedures section).

All tests were run using 1000 ppm of the corresponding explosive compound dissolved in deionized water. Deionized water was used as the blank. The reaction time for the single lane μ PADs is about 5 minutes.

Compound Targeted	Reagents	Color Change	Results Blank / Sample
Chlorate ¹¹⁰ (ClO ₃ ⁻)	(1) Aniline sulfate (2) 50% sulfuric acid	Colorless to dark green
Nitrate ^{111,112} (NO ₃ ⁻)	(1) Paste of saturated trehalose solution with solid reducing mixture of sulfanilic acid, sodium acetate, and zinc powder (2) 20% sulfuric acid (3) 1-naphthol	Colorless to orange
Ammonium ¹¹³ (NH ₄ ⁺)	Nessler's Reagent	Pale yellow to brown
Nitrite ¹¹⁰ (NO ₂ ⁻)	Griess test: (1) sulfanilic acid (2) 1-naphthylamine	Colorless to orange/brown
Perchlorate ¹¹³ (ClO ₄ ⁻)	0.05% methylene blue	Blue to Purple

Initially, the tests performed on these μ PADs were chosen based on a literature study of previously developed liquid based colorimetric tests used in qualitative analysis. However, many of these original colorimetric tests required acid concentrations that were high enough to digest paper. Therefore, reagents were modified to permit the development of distinctive color changes without the use of strong acids. For example, the first colorimetric test for chlorate utilized concentrated sulfuric acid and an aniline sulfate solution. To adapt this test for use with a μ PAD, concentration of the concentrated sulfuric acid (H_2SO_4) was varied from 0 to 80% and the colorimetric test was performed in a test tube to determine the minimum acid concentration that could be used, while still being able to detect a color change for chlorate. The optimal level was determined to be approximately 50% H_2SO_4 and then tested on the μ PAD. Fifty percent concentrated H_2SO_4 was spotted onto multiple μ PADs and allowed to sit in the ambient environment for about 1 month. After 1 month, there was no visible degradation of the μ PADs and the resulting colorimetric test successfully detected chlorate.

For the detection of nitrate, which is present in black powder as well as fertilizer based explosives (ammonium, potassium, and urea nitrates), a sequential three step test was chosen based on a modified Griess test^{111,112}. A solid mixture of sulfanilic acid, sodium acetate, and zinc powder was placed at the bottom of the sample lane. A saturated trehalose solution was then used to make a paste with the solid mixture for two reasons: (1) facilitated an easier application to the paper devices and (2) the trehalose slows down migration of the liquid sample on the μ PAD allowing more time for the nitrate to interact with the solid reducing mixture.

The powdered zinc is present to reduce the nitrate (NO_3^-) to nitrite (NO_2^-). The nitrite is converted to nitrous acid using the 20% H_2SO_4 , facilitating the formation of a diazonium salt in the presence of sulfanilic acid.

Figure 5.2. Modified Griess reaction used for the detection of nitrate containing compounds such as ammonium nitrate and black powders. This colorimetric test results in a color change from colorless to orange.

The diazonium salt continues to travel up the sample lane to 1-naphthol spotted at the end of the sample lane (Figure 5.2). The reaction between the diazonium salt and 1-naphthol produces an azo dye, resulting in formation of an orange color¹¹⁴.

The ammonium test involves the use of Nessler's reagent, which consists of potassium iodide and mercuric iodide. Potassium hydroxide (KOH) is present to reduce ammonium (NH_4^+) to ammonia (NH_3). Ammonia then reacts with Nessler's reagent (K_2HgI_4) to produce a brown color complex ($\text{NH}_2\text{Hg}_2\text{I}_3$)^{115,116}.

Figure 5.3. Chemical equation for ammonium test with Nessler's reagent. Ammonia is produced from the reduction of ammonium with potassium hydroxide. Reaction results in a color change from pale yellow to brown.

An additional test lane was developed specifically for nitrite and did not include a reducing agent. This procedure utilized the Griess test where nitrite first reacted with sulfanilic acid to form a diazonium salt followed by visualization with 1-naphthylamine present at the end of the sample lane (Figure 5.4)^{111,117}.

Figure 5.4. Griess test for the detection of nitrite containing compounds. Reaction results in a color change from colorless to orange/brown.

Following the development of these two lanes, a study was performed to determine if nitrate could be differentiated from nitrite utilizing a single μ PAD. As shown in Figure 5.5, when only nitrite was present, the nitrite channel appeared orange/brown and the nitrate channel was purple, presumably due to reduction of nitrite. When only nitrate was present, the orange color appeared only in the nitrate channel while the nitrite channel was blank. When both salts were present, both channels

appeared colored, with an orange/brown color visible in the nitrite lane, and purple and orange colors in the nitrate lane. Therefore, the device successfully differentiated nitrate and nitrite based explosive compositions as well as detected the presence of nitrite in post blast samples resulting from the reduction of nitrate.

Figure 5.5. Nitrite and nitrate tests run with (a) 1000 ppm potassium nitrite in deionized water. The nitrate spot test generates a dark purple color, while the nitrite spot test produces an orange-brown color. (b) 1000 ppm potassium nitrate in deionized water. The nitrate spot test generates an orange color while the nitrite spot test does not show any color change. (c) 1000 ppm mixture of potassium nitrate and potassium nitrite. The nitrite spot test produces an orange-brown color, while the nitrate spot test shows a dark purple and orange color.

The perchlorate test utilizes a common biological reaction with methylene blue (Figure 5.6).

Figure 5.6. Methylene blue compound used for detection of perchlorate. The reaction results in a blue to purple color change.

When perchlorate is present, it binds with the methylene blue complex and causes a weighting effect, precipitating out a purple color complex. Therefore, if perchlorate is present a blue to purple color change will occur^{113,118}.

The second μ PAD was designed for the detection of organic explosives, urea nitrate, and peroxides (Figure 5.7).

Figure 5.7. (a) High/organic explosives μ PAD prior to analysis. (b) The same μ PAD following analysis of a 1000 ppm mixture of RDX, TNT, UN, and H_2O_2 in 50% acetone/50% water. Color changes begin to occur after 5 minutes with a total run time of approximately 18 minutes.

The overall composition of the high/organic explosives μ PAD is shown in Table 5.2 along with the color changes observed for a positive result.

Table 5.2. Colorimetric tests and reagents used for the detection of high/organic explosive compounds. These μ PADs were prepared using 2 μ L of each reagent spotted onto the μ PAD and allowed to dry (see Experimental Procedures section).

All tests were run using 1000 ppm of the corresponding explosive compound dissolved in 50% acetone/50% deionized water and 50% acetone/50% deionized water was used as the blank. The reaction time for single lane μ PADs is about 5 minutes.

Compound Targeted	Reagents	Color Change	Results Blank/Sample
RDX, HMX, PETN ¹¹⁹	(1) Zinc powder in 50% acetic acid (2) Sulfanilic acid (3) 1-naphthylamine	Colorless to Red/Pink
Trinitrotoluene (TNT), Trinitrobenzene (TNB), Tetryl ¹¹⁰	1.5 M Potassium hydroxide	Colorless to Red-orange
Urea Nitrate ⁷³ (UN)	<i>p</i> -DMAC (<i>para</i> -dimethylaminocinnamaldehyde)	Yellow to Red
Nitrate ^{111,112} (NO ₃ ⁻)	(1) Paste of saturated trehalose solution and solid reducing mixture of sulfanilic acid, sodium acetate, and zinc powder (2) 20% sulfuric acid (3) 1-naphthol	Colorless to orange
Hydrogen Peroxide ¹²⁰ (H ₂ O ₂)	Ammonium titanyl oxalate	Colorless to Yellow

The test for military explosives RDX, HMX, and PETN involves a Griess test with sulfanilic acid and 1-naphthylamine. A zinc paste is generated using acetic acid and placed at the bottom of the sample lane. This zinc and acetic acid paste allows for the generation and acidification of NO_2^- to nitrous acid (Figure 5.8).

Figure 5.8. Reduction to nitrous acid and Griess test for colorimetric detection of RDX/HMX/PETN. Reaction results in a color change from colorless to pink/red.

Nitrous acid continues up the lane to interact with the sulfanilic acid. A diazonium salt is generated and continues traveling up the lane to the sample loop where it reacts with 1-naphthylamine. This results in the formation of a water soluble pink/red azo dye^{119,121}.

Since a form of the Griess test is used for the nitrate and nitrite tests, a study was completed to determine if nitrite or nitrate would cause a false positive. It was determined that nitrite and nitrate both produce an orange-brown color change, while RDX, HMX, and PETN will produce a red/pink color change allowing for the compounds to be readily differentiated.

Nitroaromatics, such as TNT, were detected through the use of 1.5 M potassium hydroxide. When polynitroaromatic compounds react with alkaline solutions (such as potassium hydroxide), a Janowsky reaction occurs (Figure 5.9).

Figure 5.9. Janowsky reaction for the colorimetric detection of TNT, TNB, and tetryl. This reaction results in the formation of a red color for trinitroaromatics and a blue/purple color for dinitroaromatics.

This reaction involves the removal of a hydrogen from the acetone solvent that is present. Then, a nucleophilic aromatic addition occurs and generates a red color for trinitroaromatics while a blue to purple color is generated for dinitroaromatics^{110,119,122}.

The detection of urea nitrate involves detection of the uronium ion, which is only present if urea nitrate is present. As the uronium complex travels to the sample loop, it reacts with *para*-dimethylaminocinnamaldehyde (p-DMAC) to produce a color change from yellow to red (Figure 5.10)⁷³. Uronium must be present to supply the hydrogen necessary to protonate the p-DMAC complex, resulting in the formation of water as a leaving group. This allows for a nucleophilic substitution to occur, where uronium is added to p-DMAC with the loss of water.

Figure 5.10. Uronium reaction with *para*-dimethylaminocinnamaldehyde (*p*-DMAC). This reaction results in a color change from yellow to red when uronium is present.

If only urea, a common component in fertilizer, is present in the sample, no color change will be produced, allowing for this test to be specific for the detection of uronium (Figure 5.11).

Figure 5.11. Urea nitrate test using *p*-DMAC. (a) Blank run with 50% acetone/50% water. (b) 1000 ppm urea nitrate in 50% acetone/50% water producing a red color. (c) 1000 ppm urea in 50% acetone/50% deionized water resulting in no color change.

Urea nitrate is strongly acidic (pH 1-2) compared to urea, as a result of the nitric acid moiety in urea nitrate. Therefore, neutral urea will not react with *p*-DMAC while urea nitrate will, by providing the necessary acidity for the colorimetric reaction to occur⁷³.

The addition of the aforementioned nitrate test on the five lane μ PAD permits the user to distinguish between urea and ammonium nitrate. If the nitrate test is positive but the urea nitrate test is not, ammonium nitrate could be present. However, this test is not specific for ammonium nitrate and will show an orange color in the presence of any nitrate salt, while nitrite salts will appear purple. The nitrate test also permits the detection of nitrocellulose and nitroglycerin containing smokeless powders.

Hydrogen peroxide was detected using ammonium titanyl oxalate and the formation of a yellow color (Figure 5.12)¹²⁰. This test will also produce a weak orange color in the presence of triacetone triperoxide (TATP).

Figure 5.12. Test for hydrogen peroxide (H₂O₂) using ammonium titanyl oxalate ((NH₄)₂TiO(C₂O₄)₂). Reaction results in a colorless to yellow color change.

C. Validation of μ PADs

Sensitivity

Limits of detection were determined for these μ PADs as the lowest concentration that a color change could still visibly be detected (Table 5.3). Instrumental limits of detection were determined through the use of a Camag Scanner 3 color densitometer plate reader or through a digital photograph and Image J software. It was then calculated by determining the concentration equal to three times the standard deviation of ten replicates at the lowest visible concentration.

Table 5.3. Limits of detection determined for each individual colorimetric test using single lane μ PADs. Visible limits of detection were determined based on the lowest color change that could be detected on the paper based on the human eye. Instrumental LODs were determined using a color scanner. Those marked with an asterisk (*) were determined using Image J software due to orientation problems with the color scanner. Experiments were run in triplicate with a run time of about 5 minutes for each sample.

Compound being Detected	Visually Minimum Detectable Amount (μg)	Visible LOD (ppm)	Instrumental Minimum Detectable Amount (μg)	Instrumental LOD (ppm)
Chlorate	2.64	100	1.40	53
Nitrite	2.64	100	1.37	52
Ammonium*	7.92	300	7.13	270
Nitrate	21.12	800	19.8	750
Perchlorate*	10.56	400	8.18	310
TNT	1.31	50	1.31	50
Hydrogen Peroxide*	2.62	100	0.39	15
RDX*	7.86	300	7.34	280
Urea Nitrate	10.48	400	9.17	350

Three different procedures were used for the determination of limits of detection for the colorimetric tests. For visual detection, single lane μ PADs were run for each colorimetric test from 50 ppm to 1000 ppm and the lowest visible color change compared to the blank was determined. Two instrumental procedures were also utilized, the first involved a Camag TLC Scanner 3 and the second a digital camera (Canon Rebel EOS T3i, 18-135 mm lens), followed by processing with Image J software.

For calculations using the Camag Scanner 3 color scanner, measurements were determined by absorbance detection. The intensity of the color that develops in the test zone is a function of the concentration of the analyte. Therefore, the more analyte present, the higher the intensity of the color and the higher the absorbance detected. The

wavelength used for analysis was determined by scanning a test zone area at 500 nm wavelength to identify the location of the test zone with highest color intensity. This location was then fixed as wavelengths were scanned from 200 nm to 700 nm. The wavelength generating the highest absorbance at this location was used for all future measurements for that analyte. The absorbance was then measured for each μ PAD and plotted versus concentration.

For calculations using Image J, measurements were based on the number of pixels counted from pictures of the μ PADs using a specific analyte. μ PADs were run from 50 ppm to 1000 ppm and one picture was taken of all μ PADs for each analyte. This picture was then loaded into Image J and a pixel color range was selected for the measurements by determining the range in which the highest concentration generated the most pixels, while the blank generated no pixel count in that specified range. This range was fixed for all measurements for the specified analyte and the pixel count was measured and plotted versus concentration.

Selectivity: Interference Testing

Common white household powders (see Experimental Procedures) were tested as interferences for all colorimetric tests, since they are similar in appearance to many explosive powders. It was determined that no false positives were produced. These powders were also run with individual explosives and explosive mixtures and did not produce false negatives for any of the colorimetric tests.

Controlled substances, such as methamphetamine, cocaine, codeine, and ephedrine were also tested as possible interferences for both μ PADs in the same manner as the household powders. None of these substances produced any false positives or negatives.

Influence of μ PAD Dimensions on Reaction Time

The μ PAD size and set up was also adjusted to facilitate an inexpensive, portable testing system, and to reduce analysis times. The μ PAD size was reduced from 45 mm x 38 mm to 24 mm x 17 mm for future testing analysis. This reduced the time to obtain results from approximately 18 minutes to less than 5 minutes. The colorimetric changes were still clearly visible and the amount of solvent used was significantly reduced because of the shortened time needed to run the μ PAD. Therefore, the decrease of size reduced both analysis time and cost.

Stability Testing

The μ PADs were initially stored in an ambient environment, but this resulted in slight color changes and deterioration of colorimetric reagents within a week. They were then sealed in nylon bags with a heat sealer and placed in small plastic Ziploc bags. These storage techniques both resulted in slight color changes and deterioration of the colorimetric reagents within one month. The powder pastes were also falling off of the μ PADs during the storage time as a result of the drying of the liquids used to create the paste. The next storage technique involved lamination of the μ PADs at 110°C, speed 4 using 3 mm lamination sheets. This allowed for the pastes to be securely attached to the μ PADs and resulted in a storage time of about three months. The addition of the plastic

cover also prevented evaporation of the solvents, protected the surface from contamination, and eliminated dehydration during analysis⁵⁹.

Real Samples

Real samples were tested using fireworks, black powder, black powder substitutes, and smokeless powders. These powders were tested pre-burned and post-burned to determine which compounds were present (Table 5.4) and the results were compared to those previously obtained through a variety of analytical techniques⁸⁶.

Table 5.4. Real sample analysis with the inorganic explosives μ PAD. All tests were performed at 1000 ppm of powder in deionized water.

Powder name	Non-Burned Powder	Burned Powder	Powder content⁸⁶
Hodgdon Pyrodex (The FFFG equivalent)	NO_3^- , ClO_4^-	NO_3^- , NO_2^-	KNO_3 , KClO_4
Alliant Powder Red Dot Smokeless Powder	NO_3^-	NO_2^- , NO_3^-	Nitrocellulose, nitroglycerin
FFFg GOEX Black Rifle Powder	NO_3^-	NO_2^- , NO_3^-	KNO_3
FFG Hodgdon Triple Seven	NO_3^- , ClO_4^-	NO_2^- , NO_3^-	KNO_3 , KClO_4 , 3-nitrobenzoic acid
American Pioneer	NO_3^- , ClO_4^-	NO_2^- , NO_3^-	KNO_3 , KClO_4
Jim Shockey's Gold FFG	NO_3^- , ClO_4^-	NO_2^- , NO_3^-	KNO_3 , KClO_4
Lemon Drop Firework	NO_3^- , ClO_4^-	ClO_4^- , NO_2^- , NO_3^-	KNO_3 , KClO_4

Nitrate was detected in all of the powders, both non-burned and burned. Nitrite was only detected in the burned powders, while perchlorate was detected in the firework powder and all black powder substitutes. The Alliant Powder Red Dot smokeless powder produced a positive result for nitrate, which could be due to nitrocellulose, nitroglycerin, or a combination of the aforementioned two. A pure nitroglycerin or nitrocellulose

sample at suitable concentrations for detection was not able to be obtained to determine which compound is causing the positive result. All of the results obtained for the μ PADs correlated with previous analysis done using varying analytical instrumentation⁸⁶.

Pre- and post-blast detection was also performed on-site with the assistance of the Miami Police Departments bomb squad. The first blast consisted of ammonium nitrate and aluminum powder (ANAL). AN was successfully detected in the NH_4^+ lane and the NO_3^- lanes on the μ PADs in both a pre-blast and post-blast scenario (Figure 5.13).

Figure 5.13. Pre- and post-blast results for μ PADs tested with ANAL. (a) Pre-blast inorganic μ PAD displays an orange color change for the presence of NO_3^- and a brown color change for the presence of NH_4^+ . (b) Pre-blast high/organic μ PAD displaying an orange color change for the presence of NO_3^- . The lane for RDX did produce a dark orange color change showing the presence of NO_3^- , not the presence of RDX/HMX/PETN. (c) Post-blast inorganic μ PAD displaying a brown color change for the present of NH_4^+ . No color change was seen for the presence of NO_3^- or NO_2^- . (d) Post blast high/organic μ PAD displaying no color change for NO_3^- .

Urea nitrate was also successfully detected in both pre-blast and post-blast analyses (Figure 5.14).

Figure 5.14. On-site analysis for urea nitrate. (a) Inorganic μPAD for pre-blast testing displaying the presence of NO_3^- through an orange color change. (b) High/organic μPAD for pre-blast testing showing a positive pink color change for UN. (c) Slight orange color change seen for NO_3^- for the post-blast analysis done with an inorganic μPAD. (d) Pink color change seen for UN on the high/organic μPAD in post-blast analysis.

This was observed through the positive color changes displayed for the nitrate test and the urea nitrate test.

Nitrate (NO_3^-) was also successfully detected in the analysis completed for Helix (a mixture of nitromethane and aluminum), gel dynamite, and Composition-4 in pre-blast situations only. No NO_3^- or NO_2^- responses were seen in a post-blast situation. TATP and ClO_3^- were not able to be detected on site. This could be due to the minute amount of TATP used for pre-blast testing and the sample age. TATP has a high vapor pressure allowing it to readily sublime at room temperature, so the presence of this material in the sample matrix may evaporate over time. Chlorate was not detected due to a solubility issue created by the Vaseline present in the mixture as a fuel source. In addition, no

results were seen for Detasheet due to the insolubility of the rubberized backing. Future work will involve developing methods to deal with insoluble explosives formulations.

D. Conclusions

Two different five lane μ PADs were developed for the analysis of unknown, suspected explosive materials. The first device is able to identify multiple components of inorganic explosives, such as chlorate, perchlorate, nitrate, nitrite, and ammonium, using deionized water as the solvent. The second device is capable of identifying high/organic explosives, such as TNT, RDX, H_2O_2 , and UN using 50% acetone/50% water as the solvent. Limits of detection ranged from 0.4-20 μg of explosive compound, making the devices well suited to characterize unknown powders recovered from improvised explosive manufacturing sites. Through the use of lamination, these devices can be stored for a period of three months before discoloration begins to occur. They are laminated to ensure that the powder pastes remain on the device, limit interferences and dehydration during analysis, and to reduce contact with the colorimetric reagents. The total analysis time including processing and interpretation of results is approximately 5 minutes.

The devices were also tested on-site in a pre- and post-blast scenario with the help of the Miami Police Department bomb squad. In a pre-blast scenario, multiple improvised explosive compounds were successfully detected on-site. These included ammonium nitrate contained in ANAL, nitrate in nitromethane for Helix, gel dynamite, Composition-4, and urea nitrate. These compounds were also successfully detected in a post-blast scenario. Potassium chlorate was not detected pre- or post-blast most likely

due to the interference from Vaseline contained in the improvised explosive mixture. Detasheet also did not produce any pre-blast results due to solubility issues with the respective solvents for testing. Future work will involve developing methods to deal with insoluble explosives formulations.

These newly designed μ PADs are simpler, smaller, less expensive, and easily portable compared to current on-site colorimetric detection techniques for explosives. The devices facilitate the identification of combinations of explosive compounds by permitting simultaneous multiplex testing. Therefore, these μ PADs will provide law enforcement and military personnel with inexpensive and portable chemical tests for rapid, on-site determination of suspected explosive samples.

CHAPTER 6. CONFIRMATORY DETECTION UTILIZING INFUSION EC-ESI-TOF MS

A. Introduction

A wide variety of materials can be utilized in explosive compositions. Military explosives typically consist of organic nitrates, while homemade or improvised explosive preparations may contain fertilizers and industrial chemicals containing oxidizers, such as chlorates, perchlorates, and nitrates, as well as other less stable compounds, such as peroxides. Due to the wide range of volatilities, polarities, and compositions encountered, multiple analyses must be run in a lab to identify the unknown explosive material present. GC-MS and LC-MS¹²³ can be used to identify organic compounds while IC⁹³ and CE²⁶ can be used to identify inorganic ions. These devices are large, expensive pieces of instrumentation, and multiple tests must be run for identification.

This process increases the amount of time before any analytical information on the identity of the explosive can be provided to personnel. An additional complication with the current analytical techniques is that improvised explosives, such as urea nitrate, can be difficult to discriminate from environmental matrices due to a metathetic exchange between ions, as well as decomposition of the uronium ion in water^{124,125}.

The goal of this study was to develop one, single confirmatory method for the analysis of multiple improvised explosive compounds ranging from inorganic ions to organic molecules. This method utilizes infusion electrochemical detection electrospray ionization time-of-flight mass spectrometry (EC-ESI-TOF MS) to detect guest/host complexes generated through complexation of 18-crown-6 ether with inorganic ions (ammonium nitrate, urea nitrate, potassium chlorate, and ammonium perchlorate). These inorganic salts can be successfully detected as ion pairs using electrospray ionization in positive ion mode while still permitting the analysis of other high explosives such as PETN, RDX, HMX, and HMTD¹ using negative and positive electrospray ionization. An electrochemical detector placed before the mass spectrometer permits identification of hydrogen peroxide, an analyte normally difficult to detect through mass spectrometry due to its low mass (MW = 34 g/mol). Overall, this infusion EC-ESI-TOF MS technique allows for the detection of a wide range of components present in IEDs, including fertilizers, inorganic oxidizers, high explosives, and peroxides.

B. Development of Method

Compounds of Interest

Table 6.1 contains a list of compounds being detected and their expected m/z ratio

in the mass spectrometer. H_2O_2 is detected using an electrochemical detector, so it will not have a peak in the mass spectrometer.

Table 6.1. Compounds of interest and their corresponding m/z when complexes are formed. H_2O_2 is difficult to detect in a mass spectrometer due to its low molecular weight and does not complex with 18-crown-6 ether, therefore it is detected with an electrochemical detector. HMTD does not complex with 18-crown-6 ether and is detected as $[\text{HMTD}+\text{H}]^+$. 1:1 corresponds to 1 crown ethers for 1 ion pair while 1:2 corresponds to 2 crown ethers for 1 ion pair.

Chemical Name	Formula	Structure	MW (g/mol)	MS Peaks (m/z)
Hydrogen Peroxide (30% solution)	H_2O_2		34.01	Electrochemical Detector
Hexamethylene Triperoxide Diamine (HMTD)	$\text{C}_6\text{H}_{12}\text{O}_6\text{N}_2$		208.17	+H – 209.0683
Ammonium Nitrate	NH_4NO_3		80.04	1:2 – 626.3661
Urea Nitrate	$((\text{NH}_2)_2\text{C}=\text{OH})^+\text{NO}_3^-$		123.07	1:1 – 325.1946 1:2 – 589.3505
Ammonium Perchlorate	NH_4ClO_4		117.49	1:2 – 689.1918
Potassium Chlorate	KClO_3		122.55	1:2 – 663.3287
18-Crown-6 Ether	$\text{C}_{12}\text{H}_{24}\text{O}_6$		264.32	+H – 265.3278 +NH ₄ – 282.1965 +Na – 287.1454 +K – 303.1195

Theoretical Calculation of Complex Formation

Due to high mass accuracy, the formation of the 18-crown-6 complexes could be determined. To determine the probable mechanism for formation of the complexes computer based calculations were performed using density functional theory with B3LYP and a basis set of 6-31G.

The complex consisting of potassium chlorate with crown ether was determined to be a 1:2 complex, $[2(18\text{Cr}6)+\text{KClO}_3+\text{K}]^+$. This complex is seen as a sandwich complex where the potassium ions are embedded in the macrorings of the 18-crown-6 ethers, while the chlorate ion is sandwiched between them (Figure 6.1).

Figure 6.1. Most stable formation for the potassium chlorate complex with 18-crown-6 ether, $[2(18\text{Cr}6)+\text{KClO}_3+\text{K}]^+$. The grey lines are not bonds; they are ionic interactions occurring between the positively charged potassiums and negatively charged chlorates.

Potassium ions have the best selectivity for the 18-crown-6 ether¹²⁶ since its ion radius of 1.33 Å is the closest to the radius of the 18-crown-6 macroring (1.45 Å)¹²⁷. The anion can also effect the selectivity, where nitrate > thiocynate > perchlorate, iodide > bromide^{128,129}. Therefore, potassium nitrate will complex better than potassium perchlorate.

Ammonium complexes, such as ammonium nitrate ($[2(18\text{Cr}6)+\text{NH}_4\text{NO}_3+\text{NH}_4]^+$) and ammonium perchlorate ($[2(18\text{Cr}6)+\text{NH}_4\text{ClO}_4+\text{NH}_4]^+$), form sandwich complexes through hydrogen bonding. Alternate oxygen atoms in the 18-crown-6 are positioned to form three N – H - - O bonds with the ammonium ion¹⁰⁵.

Figure 6.2. Most stable structure for (a) the ammonium nitrate complex with 18-crown-6 ether ($[2(18\text{Cr}6)+\text{NH}_4\text{NO}_3+\text{NH}_4]^+$) and (b) the ammonium perchlorate complex with 18-crown-6 ether ($[2(18\text{Cr}6)+\text{NH}_4\text{ClO}_4+\text{NH}_4]^+$).

Ammonium can also rotate rapidly in the cavity of the crown ether allowing for the energy to remain low and the complex to remain stable¹³⁰.

For urea nitrate, a complex with the uronium ion is formed, not urea nitrate as an ion pair. The 1:1 complex was determined to be $[18\text{Cr6}+(\text{NH}_2)_2\text{COH}]^+$ and the 1:2 complex was $[2(18\text{Cr6})+(\text{NH}_2)_2\text{COH}]^+$. Formations of the complexes in the most stable form are shown in Figure 6.3.

Figure 6.3. Most stable structure for (a) the 1:1 uronium complex with 18-crown-6 ether ($[18\text{Cr6}+(\text{NH}_2)_2\text{COH}]^+$) and (b) the 1:2 uronium complex with 18-crown-6 ether ($[2(18\text{Cr6})+(\text{NH}_2)_2\text{COH}]^+$).

The hydrogens on the uronium, hydrogen bond with oxygens in the macrocyclic ring contained in 18-crown-6 ether¹³¹, allowing for stable complex formation.

Electrochemical Detection

For the analysis of hydrogen peroxide in an acidic solution, the standard reduction potential for H_2O_2 is $E_0=+1.80\text{V}$, while the standard reduction potential for water is $E_0=-0.83\text{V}$.

When dissolved oxygen in the mobile phase is present at high working potentials, a problem occurs with the detection of hydrogen peroxide. Therefore, detection is better in the oxidative mode.

When an acidic pH is used (pH=0), the oxidation of hydrogen peroxide occurs (3) at $E_0 = -0.66 \text{ V}$. Since H_2O_2 is a weak acid with a pK_a of 11.65, in a strongly basic solution (pH=14) it will form the perhydroxyl ion (HOO^- , 4). In the presence of excess hydroxyl ions (OH^-) an oxidation reaction occurs at $E_0 = +0.08 \text{ V}$ (5). Therefore in the overall reaction (pH > 7), hydrogen peroxide is oxidized to oxygen while the hydroxyl ions are reduced to water (6). This is the oxidizing reaction that occurs at the surface of the working electrode for the detection of hydrogen peroxide⁹⁹.

Solvent Composition

Ammonium acetate was used to buffer the eluent, since it should contain a supporting electrolyte and weakly buffering the eluent will provide the necessary conductivity. Eluents containing salt concentrations of 10 mM are suitable for use with electrochemical detection. The pH was adjusted to 8 with ammonium hydroxide to allow for the detection of hydrogen peroxide¹⁰⁷. When the results with this solvent obtained with the electrochemical detector were compared to results obtained with methanol as the solvent, significant differences were not seen in the intensities (Figure 6.4).

Figure 6.4. Comparison of response generated by the electrochemical detector with varying mobile phases such as methanol (red), 10 mM sodium acetate in water (blue), 10 mM ammonium acetate in water (black), and 10 mM ammonium acetate pH adjusted with ammonium hydroxide (pink).

Therefore, to reduce the amount of extraneous salts present in the mobile phase capable of complexing with the 18-crown-6 ether, methanol was selected as the solvent of choice for analysis.

Infusion EC-ESI-TOF MS

Figure 6.5 presents the results for a 30 ppm sample mixture of hydrogen peroxide, ammonium nitrate, potassium chlorate, ammonium perchlorate, urea nitrate, and HMTD in 1 mM 18-crown-6 ether in methanol.

Figure 6.5. EC-ESI-TOF MS results for a 30 ppm mixture of ammonium nitrate ($[2(18\text{Cr}6)+\text{NH}_4\text{NO}_3+\text{NH}_4]^+$), urea nitrate ($[18\text{Cr}6+(\text{NH}_2)_2\text{COH}]^+$), $[2(18\text{Cr}6)+(\text{NH}_2)_2\text{COH}]^+$, potassium chlorate ($[2(18\text{Cr}6)+\text{KClO}_3+\text{K}]^+$), ammonium perchlorate ($[2(18\text{Cr}6)+\text{NH}_4\text{ClO}_4+\text{NH}_4]^+$), HMTD ($[\text{HMTD}+\text{H}]^+$) and hydrogen peroxide (H_2O_2) in 1 mM 18-crown-6 ether in methanol.

The large excess peaks are generated from the presence of sodium (m/z 287), potassium (m/z 303) and ammonium (m/z 282). The complexation peaks between uronium and 18-crown-6 ether are present as a lower intensity with respect to the other complexation peaks are a results of the selectivity difference of varying cations with 18-crown-6 ether. Eighteen-crown-6 ether is more selective for potassium and ammonium than it is for uronium.

Table 6.2. Ion radii of cations determining selectivity of the 18-crown-6 ether.

Ion	Radius (Å)
Na ⁺	0.95
K ⁺	1.33
NH ₄ ⁺	1.48

The closer the radius of the cation is to the macroring circle of the 18-crown-6 (1.45 Å), the more likely it is to bind. Based on the radius of ions available for complexation, 18-crown-6 will be more selective for potassium than for uronium (Table 6.2).

In explosives detection, urea nitrate will be present in a higher concentration compared to other salts since it is a blasting agent. Therefore, this selectivity difference will not cause an issue. However the presence of additional ions in solution can cause an interference and careful attention should be made to the composition of the background matrix when running this method.

C. Validation of Method

Sensitivity

Calibration curves were generated for each compound across the range of 30 -100 ppm increasing at increments of 10 ppm. The 18-crown-6 ether concentration was held at 1 mM for all samples including HMTD. For concentrations above 500 ppm, the calibration curve levels off due to a limited crown ether concentration. The limit of detection was calculated by determining the inorganic compound concentration equal to

three times the standard deviation of eight replicates of the lowest detectable concentration of the respective compound (Table 6.3).

Table 6.3. Figures of Merit for the explosive compounds detected using EC-ESI-TOF MS. Calibration curves were run for each individual compound from 30-100 ppm, increasing at increments of 10 ppm. LODs were calculated by determine the lowest detectable concentration of the respective compound, performing 8 replicates, and determining the concentration equal to three times the standard deviation.

Compound	R ²	Slope (Intensity/ppm)	LOD (ppm)
Ammonium Nitrate	0.9760	4719	1.1
Ammonium Perchlorate	0.9696	1506.5	0.19
HMTD	0.9877	630.43	2.0
Potassium Chlorate	0.9904	3644.9	0.82
Urea Nitrate (325)	0.8891	14151	0.076
Urea Nitrate (589)	0.8810	9247.9	0.058
Hydrogen Peroxide	0.9942	5.0E-9	0.85

In this method, the selectivity strength for crown ethers is ordered as follows: potassium complexes > ammonium complexes > uronium. Therefore care must be taken when analyzing unknown sample matrices. High concentrations of salts, particularly potassium ions can overwhelm the crown ether complexation. This problem is somewhat minimized through the use of organic solvent extracts, which limit the solubility of potassium salts.

Since a time-of-flight mass analyzer was used, the identity of the compounds could be confirmed through analysis of the Δppm value, which shows the measurement error for the anticipated compound using the following equation:

$$\Delta\text{ppm} = \frac{\text{Calculated } m/z - \text{Experimental } m/z}{\text{Calculated } m/z} * 1,000,000$$

Table 6.4. Mass accuracies determined for the ions formed during analysis with the time-of-flight mass spectrometer.

Compound	Calculated m/z	Experimental m/z	Δm	Δppm
Ammonium Nitrate [2(18Cr6)+NH ₄ NO ₃ +NH ₄] ⁺	626.3711	626.3661	5.0E-3	7.98
Ammonium Perchlorate [2(18Cr6)+NH ₄ ClO ₄ +NH ₄] ⁺	663.3318	663.3287	3.1E-3	4.67
HMTD [HMTD+H] ⁺	209.0774	209.0785	1.1E-3	5.26
Potassium Chlorate [2(18Cr6)+KClO ₃ +K] ⁺	689.1960	689.1918	4.2E-3	6.09
Urea Nitrate – 325 [18Cr6+(NH ₂) ₂ COH] ⁺	325.1975	325.1946	1.1E-3	3.38
Urea Nitrate – 589 [2(18Cr6)+(NH ₂) ₂ COH] ⁺	589.3548	589.3505	4.3E-3	7.30

If this number is low (i.e. below 10) the mass accuracy is acceptable and the structure of the complex can be determined.

Stability

Studies were conducted to determine the repeatability of results over time and how long samples could be stored in 1 mM 18-crown-6 ether in methanol. All injections were performed in triplicate. After approximately five days, the complex samples started to deteriorate and could no longer be detected at concentrations below 100 ppm. Therefore, to ensure trace detection, samples were made fresh daily for the best results.

Selectivity: Interference Testing

Common drinks and products were chosen as interferences based on substances that could be present on people's hands. These ranged from drinks such as Gatorade and coffee to bleach and Windex. Interferences were tested through a spiking process (see experimental procedures), where a small amount of interferent was added directly into the analyte liquid and then analyzed using the EC-ESI-TOF MS procedure described earlier (Figure 6.6).

Figure 6.6. Common interference compounds that could be present on people's hands. The interference compounds were tested by adding 100 μ L of the interferent to 395 μ L of 100 pm of the analyte. Five microliters of 100 mM 18Cr6 in methanol was added for a total of 500 μ L. This sample was then analyzed using the infusion EC-ESI-TOF MS described previously.

Major interferences were seen for bleach, Gatorade, Pepsi, and WD-40 for all analytes being tested. This is due to the high concentration of salts present the interferent samples competing with the explosive analytes of interest for complexation with the 18-crown-6 ethers. Since these salts complexed more readily with the ethers, the explosive analytes of interest were not able to be complexed and therefore were not readily detected. It should be noted that these materials were introduced directly as aqueous solutions, thus a higher level of ions was present in the mixture than would normally be seen with a methanol wetted swab where ion solubility is more limited. Thus, this type of interference testing is not fully representative of how interferences would affect the

explosive analytes of interest. To obtain a more representative view of how this method would work for swabbing, hand swabbing should be completed to test for trace amounts of explosives present on people's hands when these interferences are also present. This will be the subject of future work.

Real Samples

Post-blast samples were collected for multiple shots sampled using sterile gauze wetted with methanol (see experimental section). These samples were analyzed utilizing the previously described EC-ESI-TOF MS method.

In positive ion mode, complexation ions were readily generated and detected for AN in ANAL and KClO_3 in a mixture of Vaseline and KClO_3 (Table 6.5). When switched to negative ion mode, RDX present in C4 was detected along with Sheet. TATP was not able to be determined through this analysis since the peak of interest could not be confirmed. This could be due to the fact that the post-blast residue of TATP does not stay in the environment for long periods of time²⁸. Therefore, by the time the swab was analyzed, the post-blast residue for TATP was no longer present. This issue can be solve by immediately heat sealing remaining pieces in nylon bags and sampling in the lab immediately before analysis.

Table 6.5. Mass error for the results obtained for post-blast analysis utilizing the infusion EC-ESI-TOF MS method.

Compound	Calculated m/z	Experimental m/z	Δm	Δppm
Ammonium Nitrate [2(18Cr6) + NH ₄ NO ₃ + NH ₄] ⁺	626.3711	626.3634	7.7E-3	12.3
Potassium Chlorate [2(18Cr6) + KClO ₃ + K] ⁺	689.1960	689.1956	4E-3	0.58
Urea Nitrate [18Cr6+(NH ₂) ₂ COH] ⁺	325.1975	325.1147	8.3E-2	255
Composition C4 – RDX [M+CH ₂ (OH)CO ₂] ⁻	297.0431	297.0417	1.4E-3	4.71

A peak at m/z of 325.1147 was present during the analysis of the post-blast samples for urea nitrate. The Δppm was calculated to be 255, which is too high of a mass difference to confirm the presence of the uronium ion. Therefore, this analysis did not definitively detect the presence of uronium in a post-blast setting.

D. Conclusions

One, single analytical method utilizing infusion and electrochemical detection coupled with electrospray ionization time-of-flight mass spectrometry has been developed to detect multiple explosives in a run time of approximately two minutes. The electrochemical detector is utilized to detect the presence of H₂O₂, since it is difficult to detect with a mass spectrometer. Eighteen crown six ether is added to allow for complex formation between the 18-crown-6 ether and inorganic explosives such as ammonium nitrate, urea nitrate, potassium chlorate, and ammonium perchlorate. The crown ether does not interact with organic explosives such as HMTD, RDX, and HMTD. If the ionization mode is switched to negative mode, then military grade explosives such as TNT and RDX can also be detected. The development of this method creates one

analytical method that is capable of detecting multiple explosives, such as the high/military grade explosives, along with inorganic explosives such as blasting agents and pyrotechnique mixtures.

This method has also been used for post-blast detection, where swabbing was done with a sterile gauze wetted with methanol and swiped over plastic pieces containing post-blast residue. Soil samples were also collected after each blast for analysis. Some of these samples resulted in successful detection of post-blast explosive residues ranging from improvised explosives, such as ANAL, to military explosives, such as RDX in C4.

Overall, this method has proven useful for the rapid, confirmatory detection of multiple types of explosive compounds with the use of one analytical method utilizing electrochemical detection and time of flight mass spectrometry.

CHAPTER 7. CONCLUSIONS

Two methods have been developed for on-site presumptive and laboratory based confirmatory detection of multiple explosive compounds. The presumptive method utilizes microfluidic paper-based analytical devices (μ PADs) for the rapid, on-site analysis of suspected, unknown explosive materials. The first μ PAD is able to identify multiple components of inorganic explosives, such as chlorate, perchlorate, nitrate, nitrite, and ammonium, using deionized water as the solvent. The second device is capable of identifying high/organic explosives such as TNT, RDX, hydrogen peroxide, and urea nitrate, using 50% acetone/50% water as the solvent. Limits of detection ranged from 0.39-19.8 μ g of explosive compound, making the devices well suited for identification of unknown powders recovered from explosive manufacturing sites. These

devices can be stored for a period of three months before discoloration starts to occur. They are laminated to ensure that the powder pastes remain on the device, to increase storage time, to limit interferences and dehydration during analysis, to reduce the contact time with the colorimetric reagents, and to allow for the use of swabbing techniques for post-blast detection. Total analysis time is 5 minutes with very few steps needed to process the μ PADs.

These newly designed μ PADs are simpler, smaller, and easily portable compared to current on-site colorimetric detection techniques for explosives,. They facilitate the identification of combinations of explosive compounds by permitting simultaneous multiplex testing. Therefore, these μ PADs will provide law enforcement and military personnel with inexpensive and portable chemical tests for rapid, on-site determination of suspected explosive samples.

Future work for these devices will include extensive testing utilizing common improvised explosive mixtures such as ANFO and potassium chlorate mixtures with various fuel sources. This will help determine if these devices are applicable to on-site detection in theater. Testing will also be done to determine the cause of the false positives seen for urea nitrate during on-site and post-blast testing. Utilizing p-DMAC in its powder form as a paste will be explored to eliminate this false positive result.

A confirmatory technique was also developed consisting of one analytical method utilizing infusion and electrochemical detection coupled with electrospray ionization time-of-flight mass spectrometry to detect multiple explosives in a run time of approximately two minutes. The electrochemical detector is utilized to detect the presence of hydrogen peroxide since it is difficult to detect with a mass spectrometer.

Eighteen crown six ether is added to allow for complex formation between the 18-crown-6 ether and inorganic explosives, such as ammonium nitrate, urea nitrate, potassium chlorate, and ammonium perchlorate. These crown ethers do not interact with organic explosives, such as HMTD and TATP. If the ionization mode is switched to negative mode, then military grade explosives, such as TNT and RDX, can also be detected. The development of this method creates one analytical method that is capable of detecting multiple explosives such as the high/military grade explosives along with inorganic explosives such as blasting agents and pyrotechnique mixtures.

On-site pre- and post-blast detection was performed with improvised and military grade explosives with the assistance of the Miami Police Department bomb squad. The μ PADs were successfully able to detect multiple improvised explosive compounds, such as ammonium nitrate contained in ANAL, nitrate in nitromethane for Helix, gel dynamite, and C4, and urea nitrate. These compounds were also successfully detected in a post-blast scenario. Potassium chlorate was not detected due to the interference from Vaseline contained in the improvised explosive mixture. Sheet did not produce any pre-blast results due to solubility issues with the respective solvents for testing.

For the confirmatory method, swabbing was done with a sterile gauze wetted with methanol and swiped over plastic pieces containing post-blast residue. Soil samples were also collected after each blast for analysis. All of these samples resulted in successful detection of post-blast explosive residues ranging from improvised explosives such as ANAL and potassium chlorate and Vaseline. High/military grade explosives were also successfully detected, such as RDX in C4 and gel dynamite along with PETN contained in the detonator used for the blasts and Sheet.

Future work will be done to improve post-blast analysis utilizing a mixture of 50% acetonitrile/50% methanol as the extraction solvent to determine if that will aid in the detection of commercial/military explosives along with the inorganic explosives. Future work will also be done to add a separation method utilizing HPLC or CE, limiting the amount of water utilized in the mobile phase to keep the complexes together.

Overall, both of these methods have proven useful for the rapid detection of multiple explosive compounds. The presumptive method utilizing μ PADs allows for a rapid, inexpensive, and simplistic method for detection of multiple explosive compounds on-site. This will speed up the results obtained on-site and assist bomb squad and military personnel with a simpler detection technique. The confirmatory method utilizing infusion EC-ESI-TOF MS allows for a simple swabbing and extraction technique to be used along with one analytical method for the determination of multiple explosives. This minimizes the time needed to determine what explosive was present on-site in a pre- or post-blast scenario.

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