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DEVELOPMENT OF MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICES FOR THE DETECTION OF LOW EXPLOSIVES

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To: Dean Michael R. Heithaus
College of Arts, Sciences, and Education

This dissertation, written by Kathryn R. Chabaud, and entitled Development of Microfluidic Paper-Based Analytical Devices for the Detection of Low 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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Florida International University, 2019

DEDICATION

This dissertation is dedicated to my mom, my sister, and my wonderful fiancé. Without their love and support, I would not be the scientist I am today. They always pushed me to keep going even in the darkest of times and the most difficult situations. Thank you for everything.

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ABSTRACT OF THE DISSERTATION

DEVELOPMENT OF MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICES FOR THE DETECTION OF LOW EXPLOSIVES

by

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Florida International University, 2019

Miami, Florida

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Incidents of terrorism have been on the rise despite increased government regulation of explosives. These regulations and the internet have made the application of improvised explosive devices (IEDs) such as fireworks and smokeless powders more prevalent. These devices contain low explosives which are easier to acquire than high explosives, which are closely monitored by law enforcement agencies. Pipe bombs typically contain smokeless powders, which are mostly comprised of energetics such as nitroglycerine (NG) and organic additives such as diphenylamine. Another type of easily obtainable material containing low explosives is pyrotechnics. Pyrotechnics are usually made up of a mixture of inorganic chemical oxidizers and carbon, sulfur, or metal fuels that are used to produce different types of sound and lighting effects. Because of the wide range of compounds contained in IEDs, it makes detection difficult and time consuming as many different methods must be used to determine composition. This research project worked to develop a method for the rapid detection of a variety of low explosive components.

Through the use of microfluidic paper-based analytical devices (µPADs), tests can be performed for multiple compounds simultaneously via colorimetric reactions. The first of the two µPADs was developed for the detection of inorganic compounds commonly contained in low explosives, such as pyrotechnics. The second device was developed for the detection of energetics and organic additives contained within smokeless powders. Visual limits of detection ranged from 0.025-0.5 µg of the target compounds with an analysis time of less than 10 minutes for both devices. These methods allow for rapid, on-site detection of a range of different low explosives from pyrotechnics to smokeless powders.

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2,3-DNT	2,3-Dinitrotoluene
2,4’-DNDPA	2,4’-Dinitrodiphenylamine
2,4-DNT	2,4-Dinitrotoluene
2,6-DNT	2,6-Dinitrotoluene
2-NDPA	2-Nitrodiphenylamine
2-NT	2-Nitrotoluene
3,4-DNT	3,4-Dinitrotoluene
3-NT	3-Nitrotoluene
4,4’-DNDPA	4,4’-Dinitrodiphenylamine
4-NDPA	4-Nitrodiphenylamine
4-NsDPA	4-Nitrosodiphenylamine
4-NT	4-Nitrotoluene
% m/m	Mass percent
ATF	Bureau Alcohol, Tobacco, Firearms and Explosives
C18	Octadecylsilane
CE	Capillary Electrophoresis
DBP	Dibutyl Phthalate
DEP	Diethyl Phthalate
DMP	Dimethyl Phthalate
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DNT	Dinitrotoluene

DPA	Diphenylamine
EC	Ethyl Centralite
EDX	Energy Dispersive X-Ray Analyzer
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
GC-TEA	Gas Chromatography-Thermal Energy Analyzer
GSR	Gunshot Residue
IC	Ion Chromatography
IC/MS	Ion Chromatography/Mass Spectrometry
IED	Improvised Explosive Device
IMS	Ion Mobility Spectroscopy
IR	Infrared Spectroscopy
LIGA	Lithographie, Galvanoformung, Abformung
LC	Liquid Chromatography
LC/MS	Liquid Chromatography/Mass Spectrometry
LC-TEA	Liquid Chromatography-Thermal Energy Analyzer
LOC	Lab-On-A-Chip
LOD	Limit of Detection
MC	Methyl Centralite
MeOH	Methanol
µPADs	Microfluidic Paper-Based Analytical Devices
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry

µTAS	Micro Total Analysis System
NC	Nitrocellulose
N-NsDPA	N-Nitrosodiphenylamine
NG	Nitroglycerin
PETN	Pentaerythritol Tetranitrate
PLM	Polarizing Light Microscopy
ppm	parts per million
ppb	parts per billion
RDX	Royal Demolition Explosive
s.d.	Standard Deviation
SERS	Surface-enhanced Raman Spectroscopy
SLM	Stereo Light Microscopy
START	Study of Terrorism and Response to Terrorism
TLC	Thin Layer Chromatography
TNB	Trinitrobenzene
TNT	2,4,6-Trinitrotoluene
TWGFEX	Technical Working Group for Fire and Explosion Analysis
UPLC	Ultra Performance Liquid Chromatography
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence

I. INTRODUCTION

Terror attacks have been on the rise globally over the last few decades. The average number of attacks in the 1980s was approximately 3000 while from 2010-2017, the average number of attacks increased to almost 11,000 per year.[1] With this increase, there has been an effort by governments to regulate dangerous materials, such as explosives. Despite these efforts, access to the internet has made the application of alternative and improvised explosives materials, such as smokeless powders in pipe bombs and fireworks in pressure cookers, prevalent.[2] Current on-site detection methods for explosives range from canine detectors to ion mobility spectrometry (IMS). These methods, among others, have proven to be sensitive, but they are also bulky, expensive, and require an operator with prior knowledge to be utilized. These pitfalls make challenging for field detection applications.[3] Development of rapid, on-site detection methods and lab-based confirmatory methods for these explosives is imperative for forensic investigations.

Microfluidic paper-based analytical devices (μPADs) have been adapted for use in many different fields from medicine to forensics. These devices have the capability of performing laboratory operations in the field, detecting analytes with sensitivities from parts per million (ppm) to parts per billion (ppb) in concentration.[4] Multiple studies have used μPADs coupled with colorimetric testing for forensics purposes such as detection of explosives, drugs, and body fluids.[5–7] These compact, inexpensive devices are ideal for rapid, on-site detection in a forensics environment.

Low explosives utilized in many modern-day terrorist incidents contain a variety of compounds that can be utilized for identification. Inorganic low explosives are primarily comprised of inorganic chemical oxidizers and some type of metallic or organic fuel.[8] On the other hand, organic low explosives, such as smokeless powders, are composed mostly of organic compounds, such as nitrocellulose, additive packages containing other nitro-organics, and stabilizers, such as diphenylamine.[9,10] Colorimetric testing has been employed in the past for the analysis of many of these compounds individually. For instance, the Rhodizonate test for the presence of lead has been in use since the 1930s as a way to estimate the distance from which a firearm was discharged.[11] Compounds originating from organic and inorganic nitrates, have colorimetric tests available such as the Griess reagent.[11] These colorimetric tests can be coupled with paper based devices for rapid isolation and analysis making them a powerful investigative tool.

The goal of the project was to develop microfluidic paper-based analytical devices (µPADs) for the rapid, on-site detection of compounds contained in pyrotechnics and other low explosives devices. Through the combination of these devices with colorimetric tests, these compounds can be presumptively detected in an efficient and cost-effective manner. Testing was conducted on a variety of samples of known composition that were fabricated in the laboratory or samples obtained from law enforcement sources. Composition of unknown smokeless powder samples were verified via the Technical Working Group for Fire and Explosion Analysis (TWGFEX) Smokeless Powders Database. A variety of inorganic low explosives and smokeless powder samples were tested with these devices. To simulate samples that could be collected at a crime scene after an explosion, samples were also tested after being burned inside a fume hood. The result of the current study was two different paper microfluidic devices capable of detection of both inorganic and organic compounds in low explosives.

A. Overview of Explosives

An explosion takes place when a large amount of energy is released instantaneously from a relatively small amount of material.[12,13] For purposes of the project, a chemical explosion is the result of the reaction of an unstable compound or mixture that, once initiated, undergoes a rapid exchange of ions, releases a large amount of energy, and generally also releases a large amount of gas.[12,14] Explosives are primarily composed of a mixture of oxidizers and fuels. These compounds can be combined to generate explosives in one of two ways: through the physical mixing of the necessary components or through a reaction to create a new chemical. An example of a mixture is the stable combination of potassium nitrate, charcoal, and sulfur which form black powder. Trinitrotoluene (TNT) is an unstable molecular explosive where the fuel and oxidizer are contained within the same molecule.[15] Once created, most explosive compounds require an input of energy such as heat or shock in order to generate the decomposition that results in an explosion.[16] Pyrophoric materials are extremely sensitive to heat and often only need to be mixed together to react while the majority of explosives require an initiating heat or shock to begin the chemical reaction.[14]

While some explosives are oxygen dependent or devoid, the majority contain carbon, hydrogen, nitrogen, and oxygen. In the explosion process, there are a few major reactions that take place. For example, carbon can react to form carbon dioxide, hydrogen can react to form steam and/or water, nitrates can produce nitrogen gas, and metals can produce metal oxides. Typically, the unstable union of nitrogen and oxygen breaks down and reforms into more stable compounds like nitrogen gas, carbon dioxide gas, and water. In compounds with low thermodynamic stability, these explosive reactions occur quickly.[13] Some of the most powerful explosives are those that are oxygen rich and can convert all of their atoms into gaseous products. This conversion leads to the rapid and substantial energy release required to create an explosion (Figure 1.1).[17]

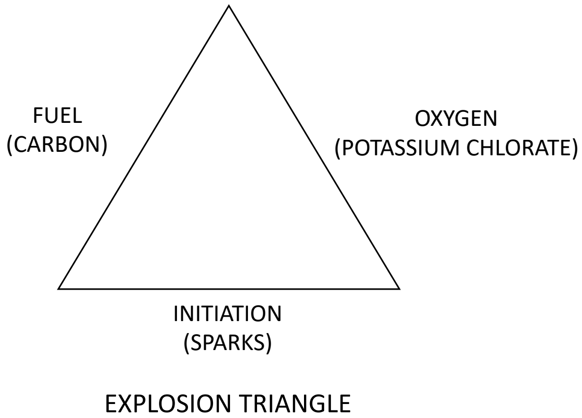


Figure 1.1: Requirements to create an explosion.[14]

Explosives are generally organized into two categories: high and low explosives. The classification is dependent on the means of propagation of the explosion through the surrounding areas. Low explosives are those that burn or deflagrate, a process which takes place at a speed slower than the speed of sound.[13,16,18,19] They are more commonly found as mixtures, and can be dangerous to handle because they are sensitive to friction and sparks. When burned, the subsequent chemical reaction results in the production of large amounts of gases.[16] The burning process is usually quite violent with a characteristic flame and sparks being produced. The gas production is not enough to create an explosion on its own as it is relatively slow compared to other types of explosives. [12] Low explosives can be placed inside of a closed container and the pressure created by the formation of the gas will cause the vessel to fracture at a weak point. The fragmentation of the container creates projectiles which cause the majority of the damage.[16] These fragments can be as small as dust particles or large pieces of the container that may be broken apart, ricochet off of surfaces, or embed themselves into surrounding materials.[14] Common examples of low explosives include black powder, smokeless powder, and pyrotechnics. Their compositions and the residues typically left behind post-blast can be seen in the table below (Table 1.1).[20]

Table 1.1: List of main types of low explosives, their pre-burn composition, and the residues left behind after burning.[20]

Type	**Composition**	**Residues**
Black Powder	KNO_3, Charcoal, Sulfur	K^+, NO_3^-, SO_4^{2-}, HCO_3^-, SCN^-, HS^-, NO_2^-, OCN^-
Smokeless Powder	Nitrocellulose, Nitroglycerine, Nitrotoluenes, Diphenylamines, Centralites	NO_2^-, NO_3^-, nitrated degradation products
Pyrotechnics	Charcoal, Aluminum, Sulfur Magnesium, $KClO_3$, $KClO_4$, NH_4ClO_4, color agents	NO_3^-, SO_4^{2-}, Cl^-, K^+, Na^+, Sr^{2+}, Ba^{2+}, Cu^{2+}, Ca^{2+}, Fe^{2+}, Mg^{2+}, ClO_3^-, C(s), CO_2 (g)

High explosives create shockwaves that travel faster than the speed of sound (3000 m/s) and produce a detonation.[13,19] Unlike low explosives, these types of reactions are not dependent on a container to produce the maximum amount of damage. The way in which the explosive applies its pressure to the surrounding areas is termed brisance (shattering effect). Brisance is caused by the characteristic velocity at which each explosive's shock front propagates. These blast waves cause the bulk of the damage from the detonation of a high explosive.[13] High explosives can be further categorized into primary and secondary explosives. Primary explosives are extremely sensitive to friction, heat, or shock. They are typically used in initiating devices or in low explosive mixtures in order to initiate a second, more stable explosive. Secondary explosives are stable under normal conditions and require activation from a primary explosive in order to propagate a reaction.[12,16] Any combination of the discussed types can be used to create a device.

B. Overview of Smokeless Powders

Smokeless powders are a type of propellant that was introduced between 1870 and 1890 as a replacement for black powder. Black powder is typically a 15:75:10 mixture of charcoal, saltpeter, and sulfur, respectively.[9,16,18] When smoke is not a concern, black powder is considered to be the best compound for efficient fire transmission and for producing a fast, hot flame.[16] Black powder is sensitive to impact making it suitable for controlled blasting.[18] However, black powder suffers from a few downfalls. The production of large amounts of solid residues attracts moisture and can cause rust affecting firearm function. Additionally, smoke generation can obscure a shooter's view or alert those nearby to their presence.[9] These issues led to the widespread use of smokeless powders in small arms and shotgun ammunition.[17] These new propellants had many advantages and provided an improved stability, much more controlled pressure release, decreased smoke and muzzle flash, and less erosion of the barrels of firearms.[21]

Smokeless powders are primarily composed of a nitrocellulose (guncotton) base as the main oxidizer and other organic compounds. These compounds are part of an additive package that can vary among brands (Appendix 1).[11,22] Smokeless powders may be classified on the basis of their energetic content. A smokeless powder that only contains nitrocellulose as the main energetic compound is called a single base powder. Double base powders contain both nitrocellulose and nitroglycerine while triple base powders, which are less common than single and double base, also contain nitroguanidine.[9,11,17] In addition to these energetic compounds used to initiate and sustain the reaction, a number of supplementary chemicals are added to provide different performance enhancing aspects. The majority of these compounds can be classified into one of the following categories: deterrents, flash suppressants, plasticizers, stabilizers, opacifiers, and dyes. Deterrents are used to reduce the initial burning rate, flame temperature, and ignitability while also widening the pressure peak and increasing the efficiency. Some common examples of deterrents include dibutyl phthalate, dinitrotoluene, ethyl centralite, and methyl centralite. Flash suppressants are typically alkali or alkaline earth salts that reduce the free-radical chain reaction that take place in the gases produced as a result of burning. These compounds, utilized to minimize muzzle flash, can include potassium sulfate and barium nitrate, among others. Plasticizers are added during the manufacturing process in order to reduce the amount of volatile solvents needed to colloid the nitrocellulose, to soften the propellant, and to reduce hygroscopicity. Some of these additive compounds can have dual uses. For example, dibutyl phthalate, dinitrotoluene, ethyl centralite, and methyl centralite, mentioned above as deterrents, can also be utilized as plasticizers.[9,10]

One of the most important classes of additives contained in smokeless powders are the stabilizers. Nitrocellulose and nitroglycerine are nitric esters which are manufactured through the nitration of cellulose and glycerine, respectively. Over time, these nitric esters go through a spontaneous thermal decomposition caused by the hydrolysis of the ester functional groups and the production of nitrous and nitric acid.[23] The production of these compounds further catalyze the decomposition process, accelerating the breakdown of the energetics. Stabilizers slow down these reactions by binding the nitric acid produced and preventing it from continuing to catalyze the degradation reactions. Diphenylamine is a stabilizer commonly added to single base powders that produces multiple nitro- and nitroso- products during its decomposition (Figure 1.2).[9,16,20,23] Ethyl centralite (1,3-diethyl-1,3-diphenylurea) is another frequently used stabilizer in double base powders. In many propellant powders, diphenylamine and ethyl centralite are also used in conjunction with methyl centralite.[23]

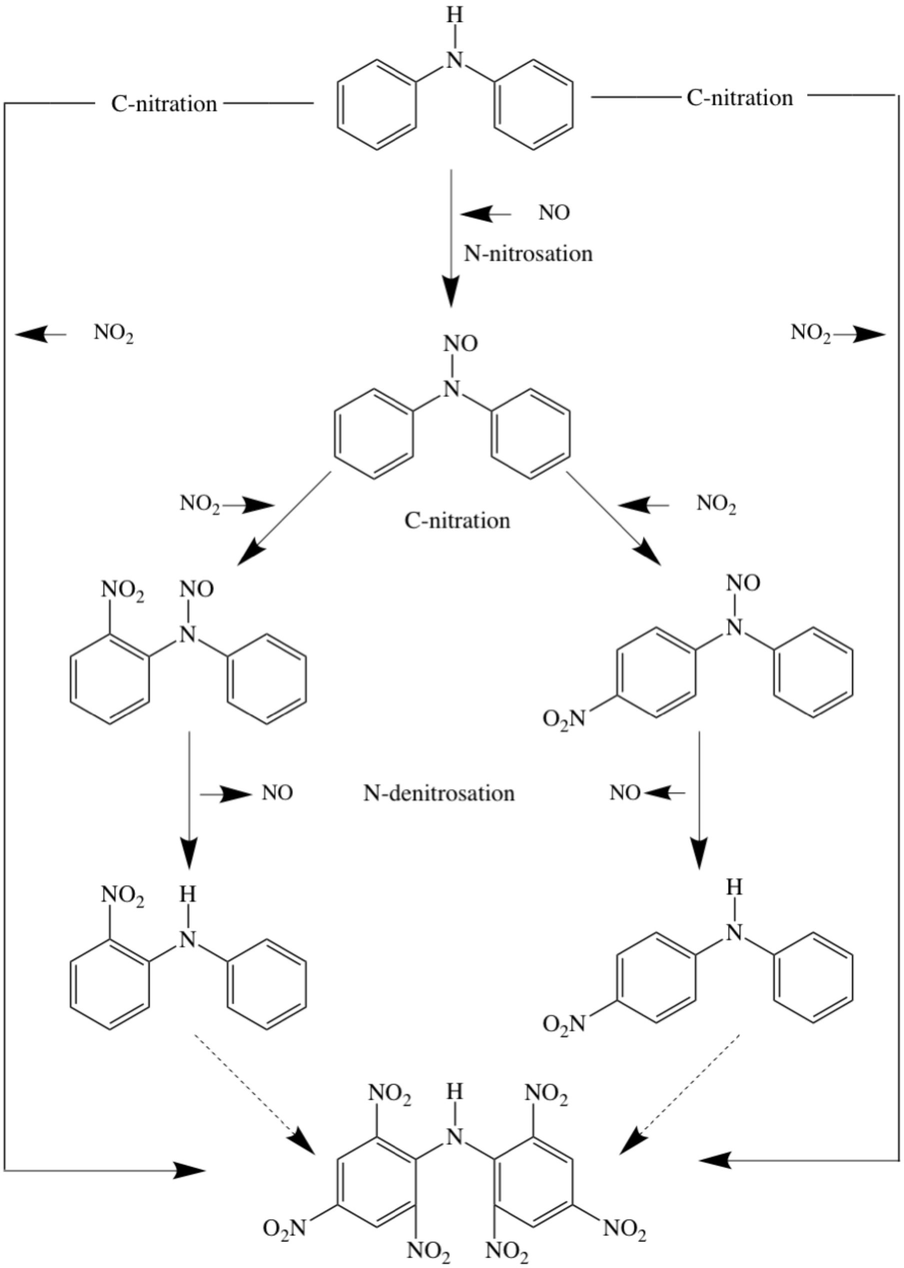


Figure 1.2: Formation of nitro- and nitroso- adducts with diphenylamine.[23]

Table 1.2: Typical smokeless powder additives and decomposition products[20]

Additives	**Decomposition Products**
Diphenylamine (DPA)	2-nitrodiphenylamine (2-NDPA)
Dibutyl phthalate (DBP)	4-Nitrodiphenylamine (4-NDPA)
Dimethyl phthalate (DMP)	N-Nitrosodiphenylamine (N-NsDPA)
Diethyl phthalate (DEP)	2,4'-Dinitrodiphenylamine (2,4'-DNDPA)
2,4-Dinitrotoluene (2,4-DNT)	4,4'-Dinitrodiphenylamine (4,4'-DNDPA)
2,6-Dinitrotoluene (2,6-DNT)	
Trinitrotoluene (TNT)	
Methyl centralite (MC)	
Ethyl centralite (EC)	
Nitroglycerine (NG)	
Potassium sulfate	
Potassium nitrate	
Graphite	

When smokeless powders are utilized as the propellant in a firearm, these organic compounds can be found in the gunshot residue (GSR). Additionally, when the inorganic primer is subjected to percussion from the firing pin of the weapon and ignites the powder, gaseous products of this reaction can coalesce and form inorganic particulates.[9] While the majority of the inorganic residues are produced in this manner, some are also from the bullet and the weapon themselves.[11] Traditional primers contain a mixture of lead styphnate (the initiator), barium nitrate (the oxidizer), and antimony trisulfate (the fuel).[24] In new lead-free primers, these heavy metals are being replaced with metal substitutes such as aluminum and zinc.[11] Other compounds that may be found in the primer cup include sensitizers, frictionators, binders, coloring materials, and certain high explosives (Figure 1.3).[24] All of these compounds come together to produce the organic and inorganic residues that result from the firing of a weapon.

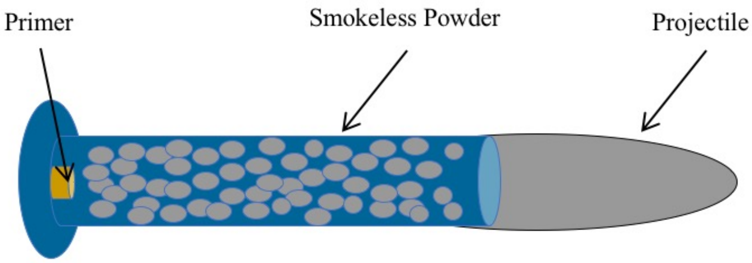


Figure 1.3: Example of ammunition showing where the primer, smokeless powder, and projectile are located within the cartridge

C. Overview of Pyrotechnics

Another commonly used and easily accessible class of low explosive is pyrotechnics, or fireworks. Pyrotechnics came into use around the same time as black powder. These incendiaries containing potassium nitrate, sulfur, and combustible materials were used for both war and amusement. The addition of compounds such as barium nitrate and iron filings added color and sparks to these mixtures when burned.[16] Modern day fireworks are typically composed of a mixture of inorganic chemical oxidizers and carbon, sulfur, or metal fuels. Pyrotechnics reactions are considered to be self-contained, self-sustained, and exothermic. But these properties do not guarantee that the mixture will burn completely once lit. In order for the propagation to continue, the heat transferred to the unburned layer of the mixture must be enough to reach the ignition temperature at which the new layer will begin to burn (Figure 1.4).[8]

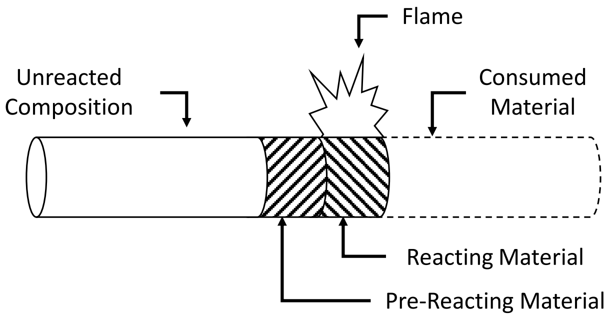


Figure 1.4: Propagation of flame through pyrotechnic material[8]

Chemical mixtures that make up pyrotechnics are used to produce visual, thermal, audible, or mechanical effects when the low explosive is deflagrated via the lighting of a fuse.[8,25] Similar to black powder, these low explosives leave behind up to 60% of their residue as inorganic salts which can aid in their detection. Certain metals are commonly associated with specific colored flames and are used to produce a variety of color or spark effects. With the exception of sodium, the elements themselves are not the species that generate the color. Color is achieved when vaporized metal salts combine with hydroxide or chlorine to produce particular salts. For example, strontium is typically associated with a red flame, which is produced when strontium carbonate ($SrCO_3$) is utilized as a color agent. Initially the $SrCO_3$ is vaporized followed by production of the colored species through reaction with hydrochloric acid (HCl) or water (H_2O) to produce chloride or oxide salts. Subsequently it undergoes excitation, and finally de-excitation which causes the light production. The process can be seen below (Figure 1.5).[8]

Vaporization of color agent: $SrCO_3 + \text{heat} \rightarrow SrO(g) + CO_2$

Production of color species: $SrO(g) + HCl \rightarrow SrCl(g) + OH$

Electron excitation: $SrCl(g) + \text{heat} \rightarrow SrCl(g)^*$

De-excitation, light production: $SrCl(g)^* \rightarrow SrCl(g) + \text{photon}$ (~630nm, red)

Figure 1.5: Chemistry for the production of a red flame adapted from Kosanke[8]

Other common coloring agents include calcium for the production of an orange flame (~600nm), sodium for the production of a yellow flame (~589nm), barium for the production of a green flame (~520nm), copper for the production of a blue flame (~450), and combinations of the above to create more specific colors. Some coloring agents have a dual use as oxidizer, fuel, or color enhancer. In addition to colorants, some metals can be used to produce sparks. Elements that are less electronegative than oxygen combine with it readily to raise the spark's temperature and shift towards bright white sparks. Some examples of spark generators include iron, aluminum, and magnesium.[8] When combined in the correct ratios, these compounds produce the visual effects seen when fireworks are burned.

D. Overview of Improvised Explosive Devices

An improvised explosive device (IED) is defined as a homemade device created by a criminal using destructive, lethal, noxious, pyrotechnic, or incendiary chemicals with the intent of destruction or death.[26] Improvised explosives are a threat in the modern world but many of the common recipes have been around for hundreds of years.[15] Cargo ships carrying explosives can be traced back as far as the 1500s, while homemade bombs and mines were utilized as early as the Civil War.[27] The expansion of the internet in the 1990s brought easy access to texts and knowledge that had previously existed only on the edges of society where the general population had very limited access.[15] As time progressed, the number of crimes involving IEDs increased drastically both in the number and variety of devices.

Improved explosive devices can be classified into one of three categories: blast, fragmentation, and incendiary. Blast IEDs have either a light container or no container at all. Therefore, they produce an explosion but leave little to no shrapnel behind. Fragmentation IEDs, typically involve explosives in metal or glass containers whose purpose is the production of shrapnel to cause additional damage to the surroundings. These devices may also contain items intended to produce projectiles such as nails, nuts, bolts, or ball bearings. Incendiary IEDs are those that combust with the goal of igniting their surroundings. An example of this is the Molotov cocktail, a glass container filled with flammable liquid that is lit and thrown to burn upon impact. In order to function as intended, most IEDs are made of multiple components: an explosive, an energy source used as an initiator, an initiating system to activate the energy source, and sometimes, a container. First, the initiating system is triggered which then activates the energy source followed by the initiation of the explosive charge causing an energy release, shattering the optional container.[26]

In a report released by the Bureau Alcohol, Tobacco, Firearms and Explosives (ATF) in 2016, the majority of main charges found in explosion incidents from 2012-2016 were black powder, black powder substitutes, flash powder/pyrotechnic mixture, pyrotechnics/fireworks, and smokeless powders.[28] These types of explosives are easily accessed and can be made into an IED, like the pipe bomb below (Figure 1.6), with very little prior knowledge or experience required. These facts make IEDs an accessible option for the majority of the population with very minimal controls regulating the sale and purchase of these compounds. Ease of access also illustrates the need for better on-site detection methods.

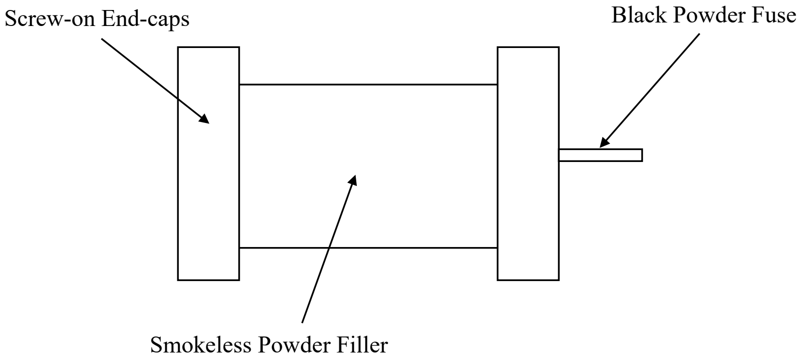


Figure 1.6: Example of a pipe bomb containing smokeless powder (adapted from reference 10)

II. DETECTION OF EXPLOSIVES

A. Introduction

In recent years, access to commercial and military explosives has become controlled by governments around the world. Terrorists have moved away from military grade explosives and have begun using compounds and devices that can be easily manufactured from readily acquirable materials. The presence of alternatives to traditional explosives has resulted in the need for updated detection methods to contend with these improvised devices.[29]

With the majority of terrorist bombings from 2012-2016 involving black powder, black powder substitutes, flash powders, pyrotechnic mixtures, and smokeless powders, detection of low explosives has become increasingly important.[28] If devices are found prior to detonation, these pre-blast compounds can be tested to determine the danger associated with handling of the device by law enforcement personnel.[5] By conducting a detailed analysis of these compounds, it is also possible to trace the explosive back to the manufacturer, and potentially the buyer, by developing a chemical fingerprint of main ingredients and additives.[30] Post-blast analysis of samples taken from the site of a terrorist attack is more complex. Such samples contain both leftover unburned material in addition to burned and degraded particulates. The burned and unburned residue may also be mixed with matrix components from the surrounding environment. In such cases, additional extraction steps must be added to the process to remove potential interferents.[20] It is also important to test samples on-site using portable analytical systems in order to have timely information on potential hazards, sources of materials, and potential leads. While portable detection equipment can provide very specific information, many current analytical techniques such as infrared or ion mobility spectroscopy utilize bulky instrumentation that is expensive and requires extensive knowledge to operate. In addition to laboratory methods that give very detailed information on the samples in question, it is also important to have on-site testing available to provide a rapid and inexpensive analysis that can be easily performed by law enforcement or military personnel.

The Technical Working Group for Fire and Explosion Analysis (TWGFEX) gives recommended guidelines for both pre- and post-blast explosives analysis. Methods for examination of explosive samples are split into four categories: (1) those that provide significant structural and/or elemental information, (2) those that provide limited structural or elemental information, (3) those that provide a high degree of selectivity, and (4) those that are useful but do not fall in either of the other categories. The table below (Table 2.1) lists all of the methods and their classifications for both pre- and post-blast analysis.[31,32] Pre-blast analysis is defined as detection of explosives prior to detonation. It can involve bulk analysis of the charge or trace analysis on a variety of surfaces the charge came into contact with. Post-blast analysis relates to the determination of explosives after detonation and is more often trace analysis of either non-combusted or combusted materials at the scene.[33] The next section will detail the most common laboratory techniques utilized in forensic laboratories in addition to common field techniques.

Table 2.1: Categories of analytical techniques for explosive analysis[31,32]

Categories 1 and 2	Category 3	Category 4
Infrared Spectroscopy (IR)	Gas Chromatography (GC)	Burn Test*
Raman Spectroscopy	Gas Chromatography Thermal Energy Analyzer (GC-TEA)	Flame Test
Gas Chromatography/Mass Spectrometry (GC/MS)	Liquid Chromatography (LC)	Spot Test
Liquid Chromatography/ Mass Spectrometry (LC/MS)	Liquid Chromatography Thermal Energy Analyzer (LC-TEA)	Melting Point
Ion Chromatography / Mass Spectrometry (IC/MS)**	Ion Chromatography (IC)	
Energy Dispersive X-Ray Analyzer (EDX)	Capillary Electrophoresis (CE)	
X-Ray Diffraction (XRD)*	Thin Layer Chromatography (TLC)	
X-Ray Fluorescence (XFR)**	Ion Mobility Spectrometry (IMS)	
	Polarizing Light Microscopy (PLM)	
	Stereo Light Microscopy (SLM)	

* Only present in pre-blast table
** Only present in post-blast table

B. Laboratory Detection Techniques

According to TWGFEX guidelines, some of the most common forensic laboratory techniques include but are not limited to infrared (IR) spectroscopy, Raman spectroscopy, gas chromatography mass spectrometry (GC/MS), liquid chromatography mass spectrometry (LC/MS), ion chromatography mass spectrometry (IC/MS), X-ray analysis, capillary electrophoresis (CE), and ion mobility spectroscopy (IMS).

The most common vibrational spectroscopic techniques utilized in forensics laboratories for pre- and post-blast explosives detection are IR and Raman spectroscopy.[34] The two techniques provide complementary results with differences in spectra due to the fact that IR measures absorbed photons while Raman measures inelasticly scattered photons. Infrared and Raman vibrational bands are characterized by energy, intensity, and band shape. The frequencies of these vibrations are characterized by mass, arrangement, and strength of bonds within the molecules of interest.[34] For IR measurements, Fourier Transform technology allows for the simultaneous measurement of all transmitted wavelengths instead of separation into the discrete wavelengths prior to detection.[35] Both IR and Raman spectra can provide useful analysis of bulk explosives with high specificity because of the ability to detect individual molecular features as well as mixtures.[36] They have been used to analyze a large variety of energetic compounds including both high and low explosives.[37]

Gas chromatography mass spectrometry (GC/MS) is widely used because of its reproducibility, sensitivity, accuracy, and precision.[38,39] Gas chromatography allows for the separation of volatilized samples in a heated column through a partitioning of the sample between a gaseous mobile phase and either a liquid or solid column stationary phase.[40] The combination of GC with mass spectrometry (MS) allows for separation and detection of complex samples.[41] The downfall of GC is the degradation seen in many explosives compounds because of their thermal lability. For this reason, liquid chromatography mass spectrometry is a preferred method of analysis. Liquid chromatography allows for the separation of liquid samples through a partitioning of the sample between a liquid mobile phase and a solid column stationary phase. The LC procedure permits for sample analysis with minimal preparation and cleanup.[42] When paired with mass spectrometry, these chromatography methods become useful confirmatory methods for analysis of trace level explosives.[43] In order to achieve a high selectivity, tandem mass spectrometry (MS/MS) can be used which permits selective detection of targeted compounds or compound groups even in complex matrices.[43,44] Studies have found that despite trace levels in collected samples, the sensitivity and specificity of these instruments still allows for detection of multiple types of explosives both pre- and post-blast.[43,45] Recent work in this laboratory has demonstrated a method using UPLC-MS/MS capable of separating 18 out of 21 compounds in a 100 mg/ml mixture of organic components present in the additive package of pre-blast smokeless powders (Figure 2.1).[30]

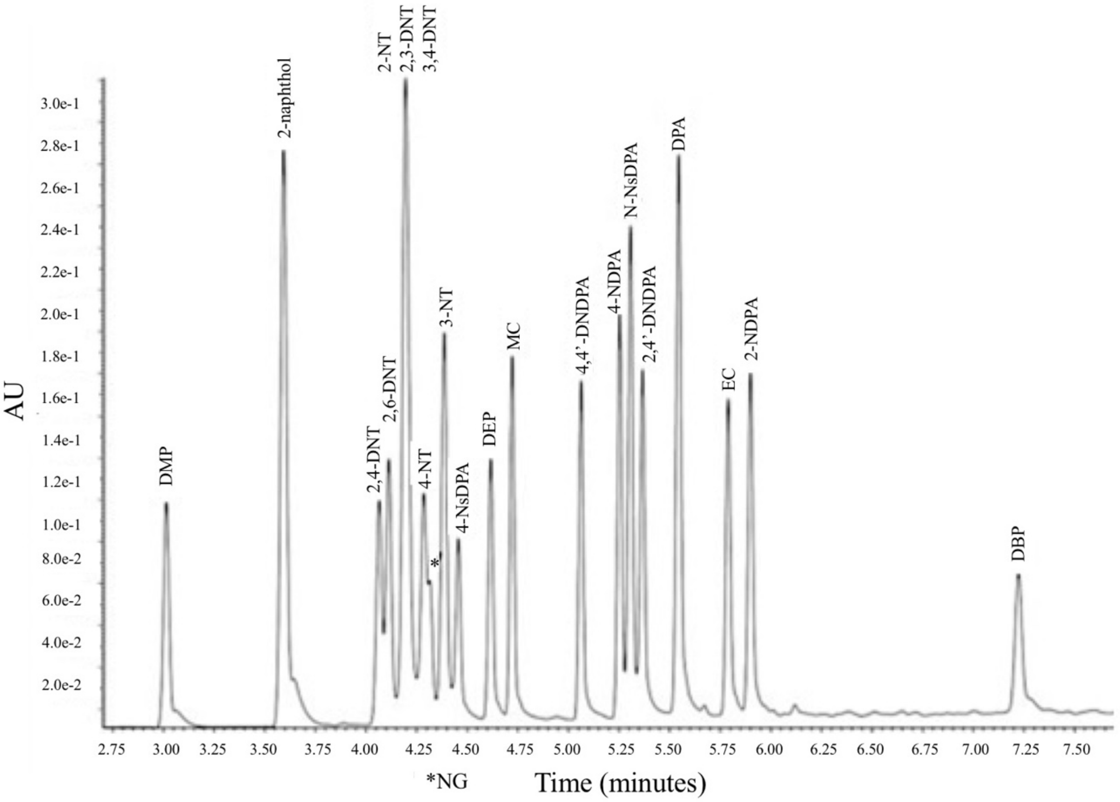


Figure 2.1: C18 Separation of a 100 mg/mL standard mixture[30]

Ion chromatography is another method commonly coupled with mass spectrometry for post-blast detection of explosives. The method separates ions using their interaction with a charged phase.[33] The advantages of IC are its sensitivity and retention of compounds typically lost as a result of evaporation in other methods. This retention can be particularly important for post-blast samples where formation of potassium and sodium salts is seen.[20] It is considered to be one of the most sensitive and specific methods for inorganic anions and organic cations with the ability to detect sub ppm levels of compounds left behind by a blast.[42] When coupled with MS, the method can yield confirmatory quantification of explosives materials found at trace levels post-blast.[33]

Capillary electrophoresis (CE), similar to IC, is an ion separation method that differentiates ions on the basis of their electrophoretic mobility while a voltage is applied. The separation is dependent on the differential mobility of molecules. Mobility is a function of a solvated ion's charge, size and the applied voltage.[40] Capillary electrophoresis has the advantage of having high efficiencies which allows for quick separation of complex ion mixtures and an ability to automate sample pretreatment. When compared to IC, CE has an inferior dynamic range. The inferior dynamic range is caused by its small injection sizes and concentration effects which can produce peak mobility shifts.[42]

X-Ray spectroscopy methods are also commonly utilized for detection of explosives compounds. These methods use the measurement of electromagnetic radiation through its emission, absorption, scattering, fluorescence, or diffraction.[40] Energy dispersive x-ray (EDX) is typically used for elemental analysis through x-ray excitation of a sample.[46] It is an ideal method for initial analysis of intact particles. If sufficient sample is available, x-ray fluorescence (XRF) can also be used to categorize the major elements through emission of their characteristic fluorescence x-rays after absorption of a beam of primary x-rays.[40,47] Depending on the detection system used with these devices, light elements, such as carbon, can potentially go undetected. However, information from elements, such as potassium, chlorine, aluminum, and sulfur, can give a good indication of the presence of pyrotechnics. Crystalline structures can be determined from diffraction data using x-ray powder diffraction (XRD) when coupled with chemical tests for ions, which allows for the non-destructive detection of a variety of inorganic and organic explosives.[47]

Lastly, ion mobility spectroscopy (IMS) is common in explosives analysis because of its high specificity, and low limits of detection.[48] The method was mainly developed for use in the detection of explosives and chemical warfare agents.[49] Ion mobility spectroscopy detectors separate ions on the basis of differential migration down an electrostatic drift tube, therefore, producing low peak resolution. However, because these systems utilize negative ion chemical ionization methods, there are relatively few interferences and the operation is fully automated.[50] Certain types of materials, such as perfumes and organic nitrates, can cause interferences with resulting false positives.[36] Picogram levels of RDX, PETN, TNT, and nitroglycerine have been detected with this instrument at an analysis time of six to eight seconds.[51] While IMS is praised for its sensitivity and ruggedness, the procedure has a limited peak capacity and must be configured to detect positive ions in order to screen for peroxide explosives.[49]

C. On-site Detection Techniques

1. Instrumental

As previously mentioned, IMS is one of the most common methods of explosives detection in security situations.[48] Work has been done to miniaturize these devices for use in the field as a consequence of its popularity. The main concern in miniaturization of these devices is the decrease in sensitivity that comes with the shortening of the drift tube and minimization of the ionization source. However, recent advancements have included the use of an amplifier which has resulted in a good resolution and sensitivity in these smaller devices through an amplification of the signal output.[48] Handheld versions weigh in at only 2.6 kg and have an analysis time of ten to fifteen seconds.[51]

Other methods gaining popularity as a possible on-site detection procedures include infrared and Raman spectroscopy which analyze vibrational transitions after the sample has experienced infrared or laser excitation.[40] Raman has been used to detect DNT at 5 ppb in portable form, making it potentially useful for vapor detection of hidden explosives. More recently, surface-enhanced Raman spectroscopy (SERS) has been used for the enhancement of portable Raman systems and can detect certain nerve agents and explosives at ppb levels making these systems a useful tool for field detection.[52]

Another method that yields low response times and high specificity for on-site detection is mass spectrometry.[53] When utilizing a quadrupole or an ion trap mass spectrometer, devices can be miniaturized into detectors weighing less than 15 kg.[54] An instrument with a triple-quadrupole has been developed for the detection of explosives on airline boarding passes. Detection limits for TNT, RDX, PETN, and NG were on the order of 100 pg with the method. The procedure has the capability of scanning 1000 boarding passes per hour. An MS/MS personnel screening portal has also been developed for explosives detection.[51] As a result of its high selectivity and sensitivity, MS is an ideal candidate for creating on-site detection devices capable of detecting both vapor and trace explosives.[55] It should be noted that many explosives have very low vapor pressures, thus it is important to test wipe samples as well as vapor samples.

2. Canines

Canines have a long history of being used as detectors for a wide variety of substrates ranging from the use of hunting dogs, as long as 12,000 years ago, to detection of explosives during and after World War II. They have been used to detect guns, pipeline leaks, cancerous cells, particular breeds of snakes, drugs, ignitable liquid residues, and explosives, among others.[56] A major advantage of the use of canines is the idea that they are an integrated sampling system that starts with sample collection and ends with alarm notification if a positive result is detected. They can also be trained to more than one target through operant conditioning, which allows detection of a variety of compounds by one animal.[29] The general schema for the detection of an explosive compound is as follows: the odor is taken in via sniffing, the vapor or particulates dissolve into the mucus layers inside the nasal passage where they can contact the specific olfactory receptor, the interaction between the chemical and the receptor produces a messenger cascade via a chemical reaction, and finally a message is sent to the brain that a smell has been detected.[56] Although canines have many practical advantages, such as their sensitivity and ease of use, they have many disadvantages as well. In explosives detection, there are said to be three possible signatures the dog could be alerting to: the explosive itself, a contaminant, or a decomposition product.[57] The mechanism by which the explosive target is being alerted to is still not understood, which increases the difficulty of training and shows that the canines are not capable of confirming a positive result of a specific compound.[48] In addition to these mechanical limitations, they also require extensive training and an experienced handler.[57]

3. Immunoassays

Sensors with the ability to collect and detect explosives in a quick and reliable fashion have been on the rise in recent years. The demand for these devices can be seen across many different industries including transportation, environmental, and national security applications.[58] Immunoassays provide a specific detection technique which take advantage of coupling interactions between a target analyte and an antibody.[51,59] Quantitation is typically accomplished through analyzing changes in fluorescence, radioactivity, or color.[51] A number of different methods have been proposed for the detection of TNT, among others, through fluorescence, chemiluminescence, and electrochemistry.[60–63] One method utilized a fluorescent displacement immunoassay and was able to detect TNT at 0.5 ppb when dissolved in phosphate buffered saline and at 0.05 ppb when dissolved in artificial seawater.[63] Another method utilizes enzyme-based electrochemiluminescence for the detection of TNT and PETN with detection limits of 0.11 ppb and 19.8 ppb respectively.[62]

4. Microfluidics

A field that has seen immense growth over the last few decades is the development of micro total analysis systems (µTAS) or lab-on-a-chip (LOC) technology. The first microfluidic device described in a publication was developed in 1975 as a procedure evolved from thin layer chromatography methods.[4] These devices transport small amounts of liquid samples into chemical reaction and sensing chambers through the use of microscale channels. Microfluidics pose many advantages including simplicity of use, ability to rapidly analyze small amounts of samples, and high portability.[64,65]

Microfluidic devices are miniaturized systems that have the capability of performing lab operations using nanoliter quantities of samples. There are two major classifications of devices seen in literature. The first class, µTAS, consists of microfluidic devices which are attached to outside units, such as sampling devices and detectors. While µTAS units are small, convenient, and inexpensive devices, they can be difficult to produce. These units require outside systems for operation such as an injectors, pumps, high voltage sources, and detector components in order to obtain results.[4] For example, capillary electrophoresis (CE) microdevices have been explored for the quick and effective detection of explosives in the field by military personnel such as TNT and DNTs.[66,67] Table 2.2 demonstrates these and other types of microfluidic devices.

Table 2.2: Basic materials and components for assembling of typical micro total analysis systems (µTAS) working under different separation and detection modes[4]

µTAS Fabrication Method	**Device Body Materials**	**Separation Processes**	**Detection Systems**	**Device Main Components**
Molding	Polymers	Capillary Electrophoresis	Conductometry	Inlets, outlets, connectors, microchannels, microchambers
Micromolding in capillaries	Ceramic	Micellular electrokinetic chromatography	Laser – induced florescence	Valves, pumps
LIGA (Lithographie, Galvanoformung, Abformung)	Glass	Capillary electrochromatography	Electrochemical	Mixers
Etching	Silicon	Gas chromatography	Fluorescence	Electromagnets
Lithography	Quartz	Liquid chromatography	Absorbance	Microheaters
Phase-changing sacrificial layers		Solid–phase extraction	Atomic fluorescence spectrometry	Droplet and bubble generators
Imprinting		Isotachoforesis		
Injection molding			Chemiluminescence	
Conventional machining		Isoelectric focusing	Normal Raman spectroscopy	
Laser ablation			Mass spectrometry	
Hot embossing				

An alternative embodiment of a microfluidic device is the microfluidic paper-based analytical device (µPAD).[4] These devices are completely self-contained and can be used to sample and detect chemical and biological materials. The µPADs were first reported in a publication by the Whitesides Group at Harvard. Over the last couple of decades, these devices have received increased interest because of their many practical advantages. The creation of hydrophobic barriers in the paper permits the development of millimeter-sized, capillary channels that utilize capillary action to propel analyte solutions up a hydrophilic paper channel to a detection zone.[4] These channels can be created through a variety of different methods including photolithography, wax- or inkjet-based printing, knife cutting, and laser treatment.[68] The μPADs are relatively inexpensive, simple to create, portable, self-contained, and able to simultaneously perform multiple analytical tests.[65] These advantages give μPADs an edge over μTAS as a method for on-site detection.

Paper is an optimal substrate for microfluidic devices because: 1. Production is inexpensive, 2. Paper's ability to wick aqueous solutions creates a passive movement of these liquids from beginning to end without need for outside instrumentation, 3. Paper has been extensively utilized in analytical chemistry techniques, therefore, previous methods can be incorporated to develop new applications, 4. Paper is thin and can be found in a variety of thicknesses allowing it to be easy to stack, store, and transport, 5. Paper is usually made of cellulose or cellulose blends so it can be used for biological sample testing, 6. Paper is white, which allows for a good contrast when utilized in colorimetric testing, 7. Paper has a variety of forms and compositions giving it an assortment of usable properties.[69]

An important component of μPAD manufacturing is the method by which the hydrophobic barriers are created. There are a variety of options including photolithography, inkjet etching, plotting, cutting, and wax printing.[69] Each fabrication method has its advantages and can affect the desired outcome of the devices (Table 2.3).

For manufacturing devices in bulk, wax printing has many benefits. Wax printing permits rapid computer design and printing of a large number of devices in the fewest number of steps compared to other methods.[70] Once printed, the paper is passed through a laminator which melts the wax into the paper, both vertically and horizontally, forming a hydrophobic barrier. The horizontal spreading does decrease the resolution of the design during laminator heating.[71,72] While printed devices are not as highly resolved as those created through photolithography, the spread of the wax can be managed during the design stages.[70]

Detection methods for µPADs include colorimetric, electrochemical, chemiluminescence, and fluorescence techniques.[68] While most of these techniques require outside equipment, colorimetric assays can be utilized independently. The procedure is also well-established, and therefore, frequently adopted for use with µPADs.[68] In colorimetric detection, the analyte of interest must bind to or react with the indicator to produce a color change that can be visualized. Colorimetric sensing relies heavily on the ability of the compound, or class of compounds in question, to react selectively with the color indicator.[36] Colorimetric reagents can react with an entire compound, particular functional groups, or individual elements.[5,36,73] When properly developed, the intensity of the color in the test zone is proportional to the concentration of the analyte on the paper.[69] In addition to visual detection, other devices, such as scanners, cameras, and spectrophotometers, can aid in detection. For example, a photograph of the final color produced by the reagent can be analyzed by a color imaging program, such as ImageJ (ImageJ bundled with Java 1.8.0_112), by looking at the pixel intensity and developing a calibration curve as it relates to color saturation.[73]

Table 2.3: The advantages and disadvantages of some µPAD fabrication methods.[74,75]

Fabrication Methods	Advantages	Disadvantages
Wax Printing	• Fast fabrication and ability to produce large number of devices • Bio-degradable • Simple, computer-designed patterns	• Expensive and low popularity printers • Requires secondary heating step after wax deposition • Patterns must account for wax spread during heating
Photolithography	• High resolution with small microfluidic channels • Can utilize low-cost options for implementation (hot plate, sunlight, etc.)	• Extensive/expensive equipment • Requires extra washing step (a multi-step process) • Susceptible to contamination • Easily bent/damaged
Plotting	• Polydimethylsiloxane is cheap as patterning agent • Has less demanding viscosity ranges than printing • Devices have flexibility • Works on any surface	• Difficult to control liquid dispensed • Lack of barrier definition • No ability for high output production processes
Ink Jet Etching	• Can use single printing apparatus both to create channels by etching and to apply/print bio/chemical sensing reagents	• Time consuming to create channels • Requires custom printing apparatus • Not suitable for high output production processes
Ink Jet Printing	• Can use several print heads simultaneously • Can utilize widely available and inexpensive office printers • Simple, computer-designed patterns • High output due to speed and ease of process	• Nozzle easily clogs • Thermal printers can affect ink • Requires secondary heating step after ink deposition • Designs for microfabrication are costly
Cutting	• No contamination from chemicals • Allows for fabrication of 3D structures from paper and tape • Applicable in areas with limited resources	• Low resolution • Resolution and cost vary • Some methods not suitable for high output production processes

Recently, colorimetric testing has gained popularity for the detection of explosives, whereas, gunshot residue-based tests have been utilized for distance estimation since the 1930s.[11,76,77] Colorimetric methods used for the detection of gunshot residue present a potential sensing method that can be applied to low explosives including smokeless powders and pyrotechnic mixtures. For example, the sodium rhodizonate test for the detection of lead and the Griess reagent for the detection of nitrites and nitrates could potentially be adapted as sensors for the detection of organic and inorganic low explosives residues.[11]

The µPADs were originally developed as alternative devices for point-of-care testing in developing countries.[69] Today, their use has expanded to multiple fields including forensic science. In forensics, they can be utilized for diagnostic work in the detection of drug residue, compounds in biofluids, DNA, and explosive residue.[4] One method encompassed the detection of three trinitroaromatic explosives on µPADs utilizing 6 mm circular patterns. These trinitroaromatics were detected through the use of potassium hydroxide to produce a colorimetric reaction with limits of detection ranging from 7.5 ng to 15 ng for trinitrobenzene (TNB), TNT, and tetryl.[78] Another µPAD was developed for the detection of organic peroxides and nitrobenzenes with an analysis time of 15 minutes and a limit of detection of 0.2 µg.[79] Prior work in the McCord laboratory demonstrated the applicability of these devices for the determination of inorganic oxidizers used in pyrotechnics and organic explosives with analysis time of under five minutes and limits of detection from 0.39–19.8 µg.[5]

D. Forensic Relevance of Low Explosives Analysis

Bombings and other terrorist attacks require quick identification of the explosive materials to aid in processing evidence. Thus, on-site detection methods can provide critical data to investigators in the field, assisting in identifying suspects and determining the safety of a crime scene. It is relatively easy for individuals to obtain items that are typically used in IEDs, such as bullets, smokeless powders, and fireworks. Rapid determination of the presence of these items is best done in the field. Improvised explosives contain a wide variety of different compounds. These may include inorganic chemical oxidizers and metal fuels found in pyrotechnics or an assortment of organic additives present in smokeless powders.

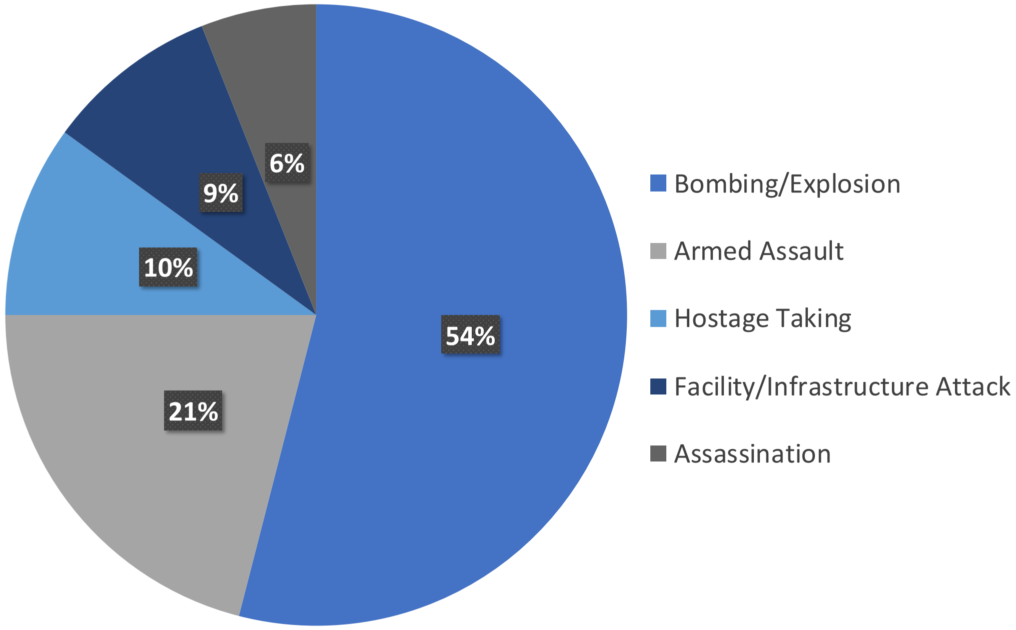


Figure 2.2: Graph showing the percentage of tactics used in worldwide terrorist attacks in 2016.[80]

A recent terrorist attack that utilized pyrotechnics/fireworks as its main source of explosives is the Boston Marathon Bombing.[81] As previously stated, fireworks are usually comprised of a mixture of chemical oxidizers with carbon, sulfur, or metal fuels.

These materials can provide distinguishable residues that can be used for detection and differentiation of the original explosive.[8,25] Other terrorist incidents, such as the attempted attack at Port Authority in New York, involved the use of smokeless powders.[82] Commercial brands of smokeless powders can vary widely in both the composition and quantity of these compounds. They can be used to create a distinct chemical profile for each powder.[83]

The ability to detect and differentiate low explosives compounds is increasingly important in today's world where terror attacks can happen with little warning. A highly portable detection method that provides minimal interference with a first responder's daily duties is needed now more than ever.

E. Research Goals

The overall purpose of this research project was to develop a presumptive, on-site method for the detection of low explosives and their residues. In order to achieve this goal, the project was separated into two sections:

1. Develop a µPAD for the detection of inorganic low explosives
2. Develop a µPAD for the detection of energetics and organics in smokeless powders

The µPADs are simple, yet versatile, devices that can perform multiple analyses at once while still remaining inexpensive. They use wax printing to create hydrophobic barriers that guide analyte solutions up a paper channel through capillary action.[4] Two devices were created: one for the detection of metallic fuels, such as lead, barium, antimony, aluminum, zinc, magnesium, and iron while the second detects energetics and organic additives in smokeless powders, such as nitroglycerine (NG), diphenylamines (DPAs), dinitrotoluenes (DNTs), and nitrates.

When compared to previous methods, these devices have the ability to be multiplexed in order to test for multiple analytes simultaneously. They are also extremely compact, easy to use, do not entail much sample preparation, and require minimal reagents and solvents to run samples. Separation of the reagents into two devices allows for detection of many different analytes under different conditions. The use of multiple devices creates a comprehensive mode of detection for a wide variety of compounds contained in explosives that can be paired with other similar devices. The devices allow for quick processing of on-site samples to give investigators an idea of what is present at a scene. Having the ability to categorize explosives on-site gives investigators knowledge that can be used to protect themselves when handling dangerous compounds and also allows laboratories to have a preliminary idea of what they can expect in samples they receive from a crime scene.

III. EXPERIMENTAL PROCEDURES

Some parts published in: “Chabaud, K. R.; Thomas, J. L.; Torres, M. N.; Oliveira, S.; McCord. B. R.; Simultaneous colorimetric detection of metallic salts contained in low explosives residue using a microfluidic paper-based analytical device (µPAD); *Journal of Forensic Chemistry*, 9, 35-41. doi: 10.1016/j.forc.2018.03.008”. Supported in part by NIJ2012-DN-BX-K048 awarded by the National Institute of Justice, Office of Justice Programs, and U.S. Department of Justice.

A. Introduction

The project investigated the detection of a variety of metallic fuels and organic smokeless powder compounds via colorimetric testing. The metallic fuels included lead, barium, antimony, zinc, iron, and aluminum. The organic compounds included diphenylamine (DPA), N-nitrosodiphenylamine (N-NsDPA), 2-nitrodiphenylamine (2-NDPA), 4-nitrodiphenylamine (4-NDPA), 2,4’-dinitrodiphenylamine (2,4’-DNDPA), 4,4’-dinitrodiphenylamine (4,4’-DNDPA), 4-nitrosodiphenylamine (4-NsDPA), methyl centralite (MC), ethyl centralite (EC), dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), nitroglycerin (NG), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-nitrotoluene (4-NT), 2,3-nitrotoluene (2,3-DNT), 2,4-dinitrotoluene (2,4-NT), 2,6-dinitrotoluene (2,6-NT), and 3,4-nitrotoluene (3,4-DNT). Each standard was dissolved in solvent and then diluted to the specified concentration.

The project had two major goals. The first was the development of a paper-based analytical device (µPAD) for the detection of metallic fuels. These metals were chosen on the basis of their consistent appearance in low explosives materials. Once tests were chosen for each metal, different parameters were investigated for use on paper including concentration, location of the reagent on the device, use of pH modifiers for certain reactions, storage methods, limits of detection, and possible interferences. The second goal was the development of a paper-based analytical device (µPAD) for the detection of organic compounds typically found in smokeless powders. Three reagents were investigated, and the same parameters as above were manipulated. The µPADs themselves were also examined to determine optimal shape, lane length and shape, and the effect of the chemicals used in testing on the ink used to create each device.

B. Development of Microfluidic Paper-Based Analytical Devices (µPADs) For On-Site Low Explosives Detection

1. Chemicals

All reagents and chemicals used in these experiments were analytical grade. All chemicals used were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA), MCB Reagents (Cincinnati, OH, USA), Spectrum Chemical (New Brunswick, NJ, USA), Tokyo Chemical Industry Co. (Portland, OR, USA), ACE Fingerprint Equipment Laboratories (Wake Forest, NC, USA), Cerilliant Corporation (Round Rock, TX), Fluka Chemika (Buchs, Switzerland), Restek Corporation (Bellefonte, PA), or Sigma-Aldrich (St. Louis, MO, USA). Swabbed bullet casings, fireworks, smokeless powders, and low explosive powders were acquired from law enforcement sources or purchased locally.

Pyrotechnic µPAD

The chemicals used to create the reagents for the pyrotechnic µPAD were sodium rhodizonate, sodium bitartrate, sodium sulfide, *p*-aminophenol, ammonium acetate, aluminon, xylidyl blue, disodium phosphate, and sodium hydroxide, which were all purchased from Thermo Fisher Scientific; Fair Lawn, NJ, USA, and tartaric acid and dithizone, which were purchased from Sigma-Aldrich; St. Louis, MO, USA.

The deflagration of pyrotechnic mixtures can be hazardous if not performed in a safe and properly equipped laboratory setting. All low explosives compositions were stored in solution for safety. Experiments were performed using open burning on ceramic substrates with minimal quantities of materials. In addition, face shields, goggles, gloves, and lab coats were utilized when appropriate.

Smokeless Powder µPAD

The chemicals used to create the reagents for the energetic and organic additive (smokeless powder) µPADs were potassium hydroxide, which was purchased from Spectrum Chemical (New Brunswick, NJ, USA), sodium hydroxide, sulfuric acid, and methanol which were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA), 1-napthylamine which was purchased from Tokyo Chemical Industry Co. (Portland, OR, USA), and sulfanilic acid which was purchased from Sigma-Aldrich (St. Louis, MO, USA). The running solvent used for the µPAD was dimethylsulfoxide from Thermo Fisher Scientific (Fair Lawn, NJ, USA).

The diphenylamine (DPA), N-nitrosodiphenylamine (N-NsDPA), 2-nitrodiphenylamine (2- NDPA), 4-nitrodiphenylamine (4-NDPA), 2,4'-dinitrodiphenyl-amine (2,4'-DNDPA), 4,4'-dinitrodiphenylamine (4,4'-DNDPA), 4-nitrosodiphenylamine (4-NsDPA), methyl centralite (MC), ethyl centralite (EC), dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), nitroglycerine (NG), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-nitrotoluene (4-NT), 2,3-dinitrotoluene (2,3-DNT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), and 3,4-dinitrotoluene (3,4-DNT) 1000 µg/mL stock solutions were initially prepared in DMSO and refrigerated. These organic standards were obtained from Acros Organics (Fair Lawn, NJ), Cerilliant Corporation (Round Rock, TX), Fluka Chemika (Buchs, Switzerland), Restek Corporation (Bellefonte, PA), and Sigma-Aldrich (St. Louis, MO, USA).

The deflagration of smokeless powder mixtures can be hazardous if not performed in a controlled, properly equipped laboratory setting. Experiments were completed utilizing open burning on ceramic substrates with small quantities of smokeless powders. In addition, face shields, goggles, gloves, and lab coats were utilized when appropriate.

2. µPAD Fabrication

The paper-based microfluidic devices were designed using Microsoft Paint (Microsoft; Redmond, WA, USA) prior to printing. The designs were printed on Whatman no. 1 chromatography paper (GE Healthcare, UK) using a Xerox wax-based printer (Xerox ColorQube 8580; Xerox, USA). The particular paper was superior to thicker substrates because of its lower resistance to flow, higher color intensity, and better overall analytical performance.[5,84] The paper was placed into a protective aluminum foil casing before being passed through a laminator (Tah Hsin Industrial Corp, TCC-600) three times at speed 1 and a temperature of 160°C. The heating process formed the hydrophobic barriers by ensuring that the wax ink had set into the chromatography paper and created a proper barrier for liquid to flow. The µPADs were cut to size prior to use.

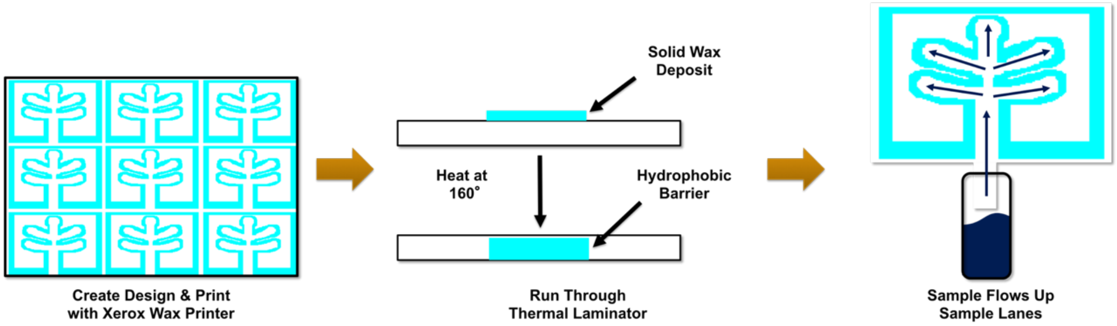


Figure 3.1: Schematic of design, printing, laminating, and use of µPADs

Each multiplexed µPAD was made up of multiple lanes, which contained individual color tests. Other devices were designed with a single lane for use as a quick method for analyzing individual tests. One microliter of the liquid colorimetric reagents were spotted in their respective channels. A slurry reagent was deposited by smearing it onto its respective channel. All reagents were allowed to dry for at least one minute prior to use and analytes were tested for consistency and shelf life for up to 1 month. The preparation process was the same for both one lane and multi-lane microfluidic devices. Each of the three devices used can be seen below.

Figure 3.2: Single Lane µPAD Designs

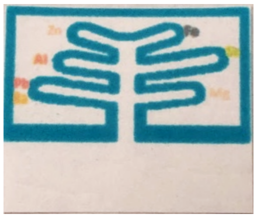


Figure 3.3: Multiplexed Metallic Fuel µPAD Design

Figure 3.4: Multiplexed Organic Compound µPAD Design

3. µPAD Preparation

Pyrotechnic µPAD

A six-lane µPAD was designed to provide tests for lead, barium, antimony, iron, aluminum, zinc, and magnesium. Chip designs were developed in the form of a tree trunk with branches (Figure 3.5). The design permits sample to flow into multiple channels with each reagent was isolated in a separate channel. Lead and barium were both detected using a spot test prepared by depositing a mixture of 0.06 g of sodium rhodizonate, 0.6 g of sodium bitartrate, and 0.5 g of tartaric acid in water. The mixture of these powders created a buffered slurry. Three mg of that mixture was smeared onto µPAD Lane 1 with a small metal spatula. For the aluminum test, 1 µL of a 50:50 solution of 0.5% ammonium acetate and 5% aluminon in water was spotted at the top of Lane 2. Zinc was detected by placing 1 µL of a saturated dithizone solution in methanol at the top of sample Lane 3. Iron was detected using 1 μL of a 0.2 M solution of *p*-aminophenol in water spotted at the top of Lane 4. To detect antimony, 1 μL of a 5 M sodium sulfide solution in water was spotted at the top of Lane 5. Magnesium was detected using a 50:50 mixture of 1 mM xylidyl blue and 0.3 M Na_2HPO_4/NaOH buffer with a pH of 12. For this test, three 1 μL aliquots of xylidyl blue were spotted consecutively after allowing each spot to dry followed by three consecutive 1 μL aliquots of the buffer all at the top of Lane 6. Standards used for testing of this μPAD were all prepared in water.

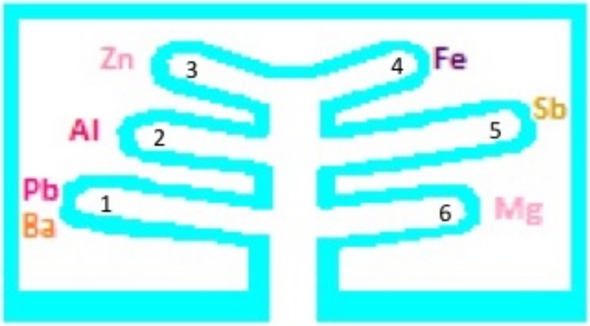


Figure 3.5: Diagram of pyrotechnic μPAD showing lane placement of reagents: 1. Sodium rhodizonate, sodium bitartrate, and tartaric acid paste in water 2. 50:50 solution of 0.5% ammonium acetate and 5% aluminon, 3. saturated dithizone solution, 4. 0.2 M solution of p-aminophenol in water, 5. 5 M sodium sulfide solution in water, 6. 50:50 mixture of 1 mM xylidyl blue and 0.3 M Na2HPO4/NaOH buffer

Smokeless Powder μPAD

A three-lane μPAD was designed to contain tests for diphenylamines, nitrated diphenyalmines, centralites, dinitrotoluenes, nitrates, and nitroglycerine (Figure 3.6). To determine 4-nitrodiphenylamine, 2,4-dinitrodiphenylamine, 4,4’-dinitrodiphenylamine, 4-nitrosodiphenylamine, 2,4-dinitrotoluene, 2,6-dinitrotoluene, and 3,4-dinitrotoluene, a 1 μL spot of 5 M KOH was placed at the top of the Sample Lane 1. A modified Griess reagent was used to detect nitrate ions and nitroglycerine.[5] A slurry was created with zinc metal by mixing it with a 50:50 glycerin:water solution. The material was smeared in sample lane 2c. Next, two 1 µL spots of 0.1% sulfanilic acid in a 70:30 water:acetic acid solution were placed midway up the lane at 2b. Two 1 µL spots of 0.5% 1-naphthylamine in methanol was placed at the top of the sample lane at 2a. Diphenylamine, N-nitrosodiphenylamine, 2-nitrodiphenylamine, 4-nitrodiphenylamine, 4-nitrosodiphenylamine, methyl centralite, and ethyl centralite were detected by placing a 1 µL spot of 0.1 M cerium (IV) sulfate in a 1 M sulfuric acid solution in Lane 3. Standards used for testing of this µPAD were prepared in dimethylsulfoxide (DMSO) excluding nitroglycerine as the prepared standard was already diluted to 1000 µg/mL in methanol.

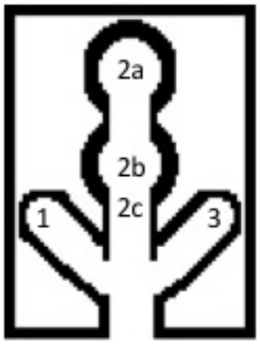


Figure 3.6: Diagram of smokeless powder µPAD showing lane placement of reagents: 1. 5 M KOH in water, 2a. 0.5% 1-naphthylamine in methanol, 2b. 0.1% sulfanilic acid in a 70:30 water:acetic acid, 2c. Zinc slurry in 50:50 glycerin:water, 3. 0.1 M cerium (IV) sulfate in a 1 M sulfuric acid

4. Portable Testing System

Pyrotechnic µPAD

The testing system involved the use of a glass autosampler vial (Thermo Fisher Scientific; Fair Lawn, NJ, USA) with a 500 µL glass vial insert (Thermo Fisher Scientific; Fair Lawn, NJ, USA) that allowed for the insertion of the paper portion (tab) of the µPAD into the solvent (Figure 3.7). To utilize the device, a small amount of sample was placed into the vial insert and allowed to dissolve. The end of the µPAD was then placed into the vial and the solution moved up from the insert via capillary action.

The solvent carried the sample up the sample lanes to each reagent test area where the sample interacted with each test. The process of running the µPAD took less than ten minutes in total.

Figure 3.7: Vial insert and vials utilized for portable testing system

Smokeless Powder µPAD

The testing system involved the use of a glass autosampler vial (Thermo Fisher Scientific; Fair Lawn, NJ, USA) with a 500 µL glass vial insert (Thermo Fisher Scientific; Fair Lawn, NJ, USA) that allowed for the insertion of the paper portion (tab) of the µPAD into the solvent. Smokeless powder samples were placed into methanol and allowed to extract. To utilize the testing system, 2 µL of extracted sample was spotted onto the µPAD. The paper portion (tab) of the µPAD was then placed into the solvent where the solution moved up from the insert via capillary action. The solvent carried the sample up the sample lanes to each reagent test area where the sample interacted with each test. The process of running the µPAD took less than ten minutes.

C. Validation of µPADs for Low Explosives Detection

1. Limits of Detection

Pyrotechnic µPAD

These tests were performed on single lane µPADs by placing the reagent at the top of the sample lane. A 1000 µg/mL standard of the compound of interest was created and 1 µL of this solution was placed in the sample lane directly below the reagent. The µPAD was then placed into a running solution that carried the standard solution up the remaining amount of the sample lane until the entire sample reacted with the reagent. Dilutions of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, and 10 µg/mL were initially created. If the reagent was determined to have an LOD less than 500 µg/mL, but greater than 250 µg/mL, additional standards at 400 µg/mL and 300 µg/mL were prepared and analyzed. A group of a dozen people was polled to determine the minimum amount of each reagent which produced a color change. On the basis of these results, the limit of detection was determined to be the lowest concentration at which 90% of the people detected that a color change took place.[85] Those polled were not alerted to the concentration of each test. Tests were run in triplicate at each concentration.

Smokeless Powder µPAD

These tests were completed on single lane µPADs by placing the reagents in the respective sample lanes. A 1000 µg/mL standard of the compound of interest was created and 1 µL of this solution was place in the sample lane directly below the lowest point the reagents occupied. The µPAD was then placed into a running solution that carried the standard solution up the remaining amount of the sample lane until the entire sample reacted with the reagent. Dilutions of 1000 µg/mL, 750 µg/mL, 500 µg/mL, 250 µg/mL, and 100 μg/mL were initially created in either DMSO for the organic additives or water for the energetics. Photos were taken with an iPhone 8 camera after running each μPAD. In order to maintain a consistent lighting environment, the results were photographed with a white background in an indoor environment where the lighting was controlled. The phone was placed at a distance of 10 cm from the device while capturing the photo. The magnification was kept consistent throughout. A group of a dozen people was polled for each reagent, and on the basis of these results, the limit of detection was determined to be the lowest amount at which 90% indicated a color change took place.[85] Those polled were not alerted to the concentration of each test. Tests were run in triplicate at each amount.

Photos were analyzed via the densiometry feature on ImageJ Software (ImageJ bundled with Java 1.8.0_112). The images were imported into the program where an area was selected that encompassed the most saturated color and the "Measure" feature was used and the "mean" value was recorded. The analysis was repeated for each standard dilution with the same area that was selected for the first image. The "mean" values for each dilution were averaged and graphed using Excel.

To calculate the instrumental limit of detection, 10 image replicates of the visual limit of detection were taken. These images were used to collect two densiometry measurements: the most saturated color area and a blank area of the same size. The blank was subtracted from its respective color area and each of these values were averaged in order to determine the noise. Finally, the standard deviation of the noise is taken and the formula below (Figure 3.8) is utilized to calculate the limit of detection.

$$Limit\ of\ Detection = \frac{3s.d._{Noise}}{Slope}$$

Figure 3.8: Formula for the calculation for instrumental limit of detection

2. Interference Testing

Pyrotechnic µPAD

A variety of metallic compounds were run with each reagent to determine cross reactivity of each test. Metallic salts selected for the current study were sodium chloride, copper chloride, and calcium chloride from Thermo Fisher Scientific; strontium chloride from MCB Reagents; and potassium chloride from Spectrum Chemical. The metallic salt solutions were prepared at a concentration of 1000 µg/mL and tested with all reagents to determine cross reactivity of the metals and possible interferents that could produce false positives during testing.

Smokeless Powder µPAD

A variety of organic compounds were run with each reagent to determine cross reactivity of each test. The organic compounds selected for this study were dimethyl phthalate, diethyl phthalate, dibutyl phthalate, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, ethyl centralite, methyl centralite, and 2,6-dinitrotoluene. These organic standards were obtained from Acros Organics (Fair Lawn, NJ), Cerilliant Corporation (Round Rock, TX), Fluka Chemika (Buchs, Switzerland), Restek Corporation (Bellefonte, PA), and Sigma-Aldrich (St. Louis, MO, USA). These solutions were prepared at a concentration of 1000 µg/mL and all reagents were tested to determine if any compound cross reacted which would indicate possible interferents that could produce false positives during real sample testing.

3. Stability Study

Pyrotechnic μPAD

A stability study was performed to determine the shelf life of the reagents in the present study. Single lane μPADs were prepared with each reagent as described above before being heat sealed inside of nylon bags and placed in a location with no light for storage. The reagents were observed and tested with the respective 1000 μg/mL standard after storage for one week, two weeks, and four weeks. For each reagent, a blank was run in addition to three replicates of the 1000 μg/mL standard for the analyte of interest.

Smokeless Powder μPAD

A stability study was performed to determine the shelf life of the reagents used in the current study. Single lane μPADs were prepared with each reagent as described above before being heat sealed inside of nylon bags. These bags were then stored in three separate ways: in a dark location, with Drierite Desiccant® in the nylon bag in a dark location, and in a freezer. The reagents were observed and tested with the respective 1000 μg/mL standard after storage at one day, three days, one week, two weeks, and four weeks. The standards were chosen on basis of the contrast of the resultant color to the μPAD design and the color of the paper. 4,4'-nitrodiphenylamine was used as the standard for the potassium hydroxide reagent as it yields a distinguishable blue color change. Diphenylamine was used at the standard for the ceric sulfate reagents as it yields a distinguishable black-gray color change. Nitrate was used at the standard for the Griess test as it produces a red color change as compared to the light pink produced by nitroglycerine. For each reagent, a blank was run in addition to three replicates of the 1000 μg/mL standard for the analyte of interest.

4. Real Samples

Pyrotechnic μPAD

For testing of primer residues, cartridges were tested by placing 40 μL of a 50:50 acetone:water solution into each cartridge with a pipette and then placing this solution directly onto the bottom of the μPAD. Pyrotechnic mixtures were made and burned in small amounts in a fume hood. Amounts of these compounds ranging from 50 to 150 mg were mixed together in particular ratios following recommendations in a lab manual produced by Washington College: "The Chemistry of Pyrotechnics & Explosives: Laboratory Notebook."[86] The chemicals used for the present study include: magnesium ribbon, barium sulfate, barium nitrate, potassium perchlorate, zinc powder, potassium nitrate, activated charcoal, sulfur, and iron filings from Thermo Fisher Scientific; and aluminum powder from ACE Fingerprint Equipment Laboratories.

The pyrotechnic mixtures were placed in ceramic containers and burned with a small torch prior to being transferred into testing containers. Post-burn samples were tested in both water and 0.7 M hydrochloric acid to account for the fact that, depending on the compounds present, some mixtures would produce insoluble metal-oxide complexes that could then produce a false negative. A few milligrams of the burned samples were placed into 500 μL of both water and 0.7 M hydrochloric acid before placing the end of the μPAD lane into the solution for testing.

Smokeless Powder μPAD

For smokeless powder sample testing, smokeless powder samples obtained from law enforcement sources or through local purchases were used. Unburned samples were prepared by creating a 10 mg/mL of the sample in methanol for extraction. Burned samples were prepared in amounts of 1 g and burned in the fume hood before being placed into methanol for extraction. The burned samples were weighed and diluted in methanol at a concentration of 50 mg/mL. Samples were allowed to extract for 5 minutes, 10 minutes, 30 minutes, and 60 minutes to determine shortest extraction time necessary to produce results. Once extraction was complete, 2 μL of the solution was placed on the bottom of the sample lane to be run with the selected running solvent, DMSO. Composition of major components of smokeless powder samples were verified via the Technical Working Group for Fire and Explosion Analysis (TWGFEX) Smokeless Powders Database.[87]

IV. μPAD FOR INORGANIC LOW EXPLOSIVES DETECTION

Some parts published in: Chabaud, K. R.; Thomas, J. L.; Torres, M. N.; Oliveira, S.; McCord. B. R.; Simultaneous colorimetric detection of metallic salts contained in low explosives residue using a microfluidic paper-based analytical device (μPAD); *Forensic Chem*. Supported in part by NIJ2012-DN-BX-K048 awarded by the National Institute of Justice, Office of Justice Programs, and U.S. Department of Justice.

A. Introduction

In a study done by Study of Terrorism and Response to Terrorism (START), it was determined that approximately 90% of terrorist attacks globally from 1970 to 2014 utilized either firearms or a variety of explosives.[88] A major concern for law enforcement are improvised explosive devices (IEDs). As military explosives become more controlled, criminals have turned to alternate methods for manufacturing IEDs that typically involve the use of explosive fillers such as black powder, smokeless powder, and fireworks.[2]

Development of rapid, on-site detection methods for these low explosives is imperative. An important class of low explosives is the pyrotechnics. Pyrotechnics can contain inorganic chemical oxidizers and fuels that are used to produce visual, thermal, audible, or mechanical effects when the low explosive is deflagrated. Determination of the oxidizer used in these compositions can be important in the determination of the explosive used.[5] There are also a variety of metallic components used in pyrotechnics including metallic powders for producing flash and sparks, and metallic salts for color production.[25,89] In situations when pyrotechnics are involved, it is also important to determine the source of the metallic fuel. Metallic salts are also present in explosive primers where components such as lead, barium and antimony may be present in cartridges and detonators.[90] Trace levels of these components can be found post-blast as gunshot and primer residues.

For forensics purposes, there is a need for quick and efficient detection of low explosives in the field. On-site instrumental techniques can be bulky and difficult for military or law enforcement personnel to operate. As a result, there is an emphasis on the development of presumptive tests that are small, portable, and easily performed in the field. Such tests include colorimetric spot tests and lateral flow immunoassays.[6] Examples of colorimetric reagents used in explosive residue analysis include the sodium rhodizonate test for the detection of lead, and the Greiss test for nitrate.[11] Unfortunately, most tests that are currently in use are only capable of testing for a single component at a time, limiting the flexibility of the approach.[6] This can be detrimental in situations where only little sample is present, and the composition of the explosive device is unknown.

A recent alternative for presumptive testing involves the use of paper-based microfluidic devices (µPADs) for the detection of both high and low explosives in the field. In previous work performed by the McCord laboratory, paper microfluidic devices were proposed as a quick and useful method for the determination of oxidizers utilized in low explosives and pyrotechnics. These devices can be multiplexed and allow for the detection of five or more target components simultaneously.[5] Paper microfluidic devices can also provide a useful platform for the future development of colorimetric testing in the analysis of additional components in inorganic low explosives residues.[11]

In the current project, a µPAD was developed for the on-site detection of metallic fuels in pyrotechnics and primer residues. When combined with previously developed methods for the determination of inorganic oxidizers, the test permits a comprehensive method for the analysis of components in improvised explosive devices.

B. Results and Discussion

The goal of the present project was to develop a µPAD for the detection of metallic components in low explosives residues, mainly those used in IEDs and pyrotechnic devices. The µPAD was developed for the detection of lead, barium, antimony, aluminum, iron, zinc, and magnesium. Lead, barium, and antimony are the three main components in heavy metal primer residues while aluminum and iron may also appear in primer residues.[11] Metals such as aluminum, barium, iron, and magnesium are also commonly used as metallic fuels or to influence the brightness and color of a variety of fireworks.[25]

The µPADs were designed for multiplexed testing and for running individual tests. The blue color of the wax ink channels for the multiplexed design was chosen because of results from a previous study that analyzed the effect of organic solvents on particular ink colors.[5] Blue ink produced the least amount of bleeding with applicable solvents. The blue is ideal because none of the colorimetric tests produced blue upon reaction with their respective analytes. Black channels were used during initial development and testing with single lane devices as the color was easy to distinguish from any possible color changes that could be observed and it photographed well for documentation purposes.[6] Both µPAD designs can be seen in Figures 4.1 and 4.2 with scales. Deionized water was used as the solvent for all experiments. The µPAD was developed for the detection of inorganic components of low explosives residues, such as in fireworks and primer residues. Table 4.1 shows the results of each reagent tested with a respective 1000 µg/mL metallic salt solution.

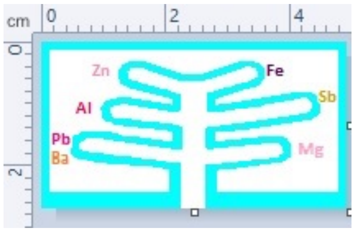


Figure 4.1: Multiplexed pyrotechnic µPAD design with scale

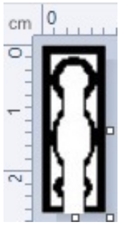


Figure 4.2: Single lane µPAD design[6] with scale

Table 4.1: Colorimetric color test results for the detection of inorganic primer residue components and inorganic low explosives components. Each test was performed by placing 1 µL of test solution or a small amount of paste at the top of each channel and then allowed to dry. Tests pictured below were run using a 1000 µg/mL standard solution of the corresponding metallic salt compound dissolved in a dilute hydrochloric acid solution. This solution was also used for the blank. Reaction time for these single lane µPADs was less than five minutes.

Target	**Lead**	**Barium**	**Antimony**	**Zinc**	**Aluminum**	**Iron**	**Magnesium**
Color Change	Tan to Pink/ Purple	Tan to Orange/ Red	Colorless to Brown	Pale Yellow to Pink	Light Pink to Red	Colorless to Purple	Blue to Pink
Blank/ Sample							

The sodium rhodizonate reagent is commonly used in a firing distance test to determine how far the fired weapon was from its target.[11] Sodium rhodizonate is very unstable in liquid form and was prepared fresh for every use. The problem was solved by using this reagent in paste form so that it was only slightly dampened prior to deposition and drying on the μPAD. The sodium rhodizonate was also mixed with sodium bitartrate and tartaric acid to remove the background color caused by the sodium rhodizonate, leading to an acidic buffer that caused a pink color formation in the presence of lead and an orange color in the presence of barium.[91] When both lead and barium were present in the sample, these two colors were seen separately in the channel as pictured below in Figure 4.11.

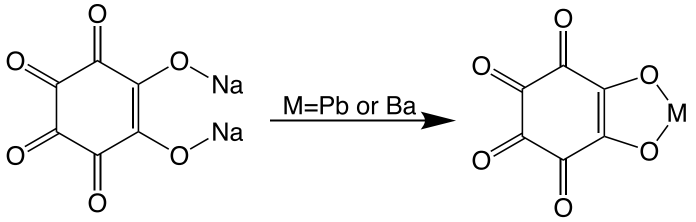


Figure 4.3: Reaction of sodium rhodizonate with lead and barium to form metal chelation complex

Sodium sulfide is known to react with antimony to produce a brown/orange colored compound. Initially, a 1 μL aliquot of this reagent was spotted at the top of the sample lane at a concentration of 1 M and analyzed with a 1000 μg/mL antimony standard. Little to no color change was observed on the paper. The preparation was then modified to utilize a 1 μL spot of a 5 M solution producing a brown/gold color that was clearly visible. It was determined that the test also produced a gray/black color change in the presence of lead, which was remedied by moving the antimony detection channel to the top lane of the multiplexed μPAD. No interference then occurred presumably because of excessive retention of lead ions on the paper device, and their inability to move a large distance on paper.

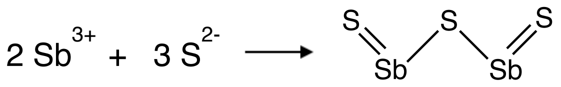


Figure 4.4: Reaction of sodium sulfide with antimony

Dithizone yields a light pink color change in the presence of zinc ions in solution. When originally tested on paper, 1 µL of a 1 M reagent was spotted at the top of the sample lane and run with a 1000 µg/mL zinc standard. No color change was observed. The concentration was increased until it was determined that a saturated solution was needed to see a color change. The reagent was prepared in methanol as dithizone was not soluble in water.

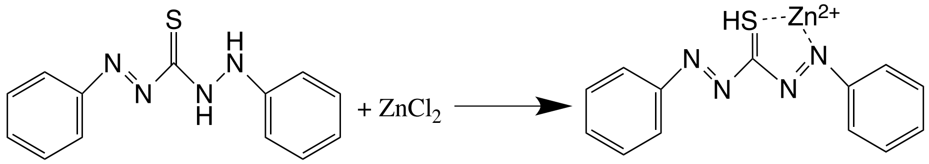


Figure 4.5: Reaction of dithizone with zinc to form metal chelation complex

The combination of ammonium acetate and aluminon is known to yield a red color in the presence of aluminum. A 50:50 mixture of these two compounds yielded an acidic environment that permitted the detection of the presence of aluminum ions in a sample. Aluminon was prepared as a 0.5% water solution and ammonium acetate as a 5% water solution prior to mixing in a 50:50 ratio.

$$Al^{3+}(aq) + 3NH_3(g) + 3H_2O(l) + \text{aluminon} \longrightarrow [Al(OH)_3\text{•aluminon}](s) + 3NH_4\text{+}(aq)]$$

Figure 4.6: Reaction of aluminon with aluminum to form metal chelation complex

The test for iron uses *p*-aminophenol, which yields a purple color on paper when iron is present. A 1 µL aliquot at a concentration of 0.2 M yielded a bright color change when run with a 1000 µg/mL iron solution in water.

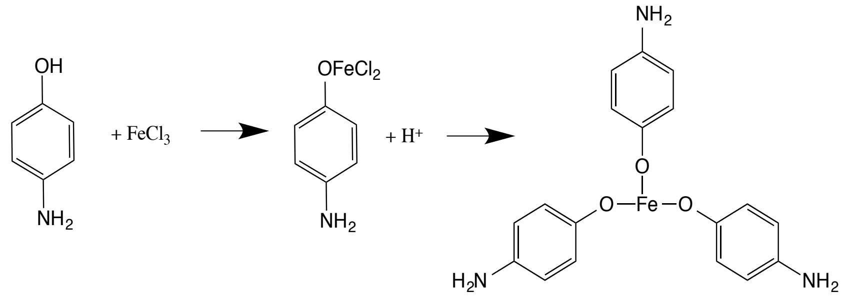


Figure 4.7: Reaction of *p*-aminophenol with iron to form metal chelation complex

Xylidyl blue is a known metal chelating agent that can be modified to be more specific with the adjustment of pH levels. Initially, a xylidyl blue concentration of 0.1 mM was used for the magnesium spot test. A 50:50 mixture of xylidyl blue and a pH 12 buffer of Na_2HPO_4/NaOH was also prepared as it only complexes in basic solution. The test was initially blue/purple and turned pink in the presence of magnesium. The concentration was determined to be too low when placed on paper, so it was increased to 1 mM, which yielded a bright color change.

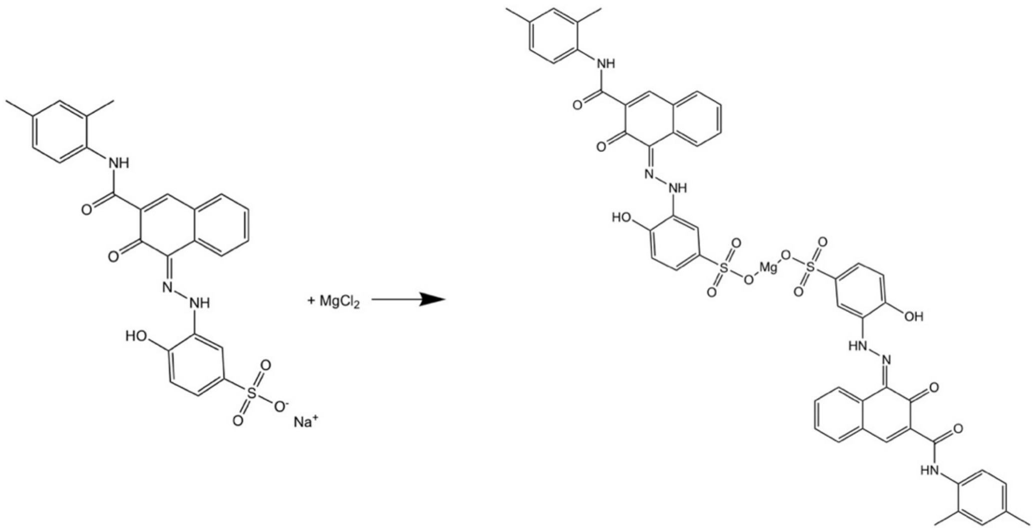
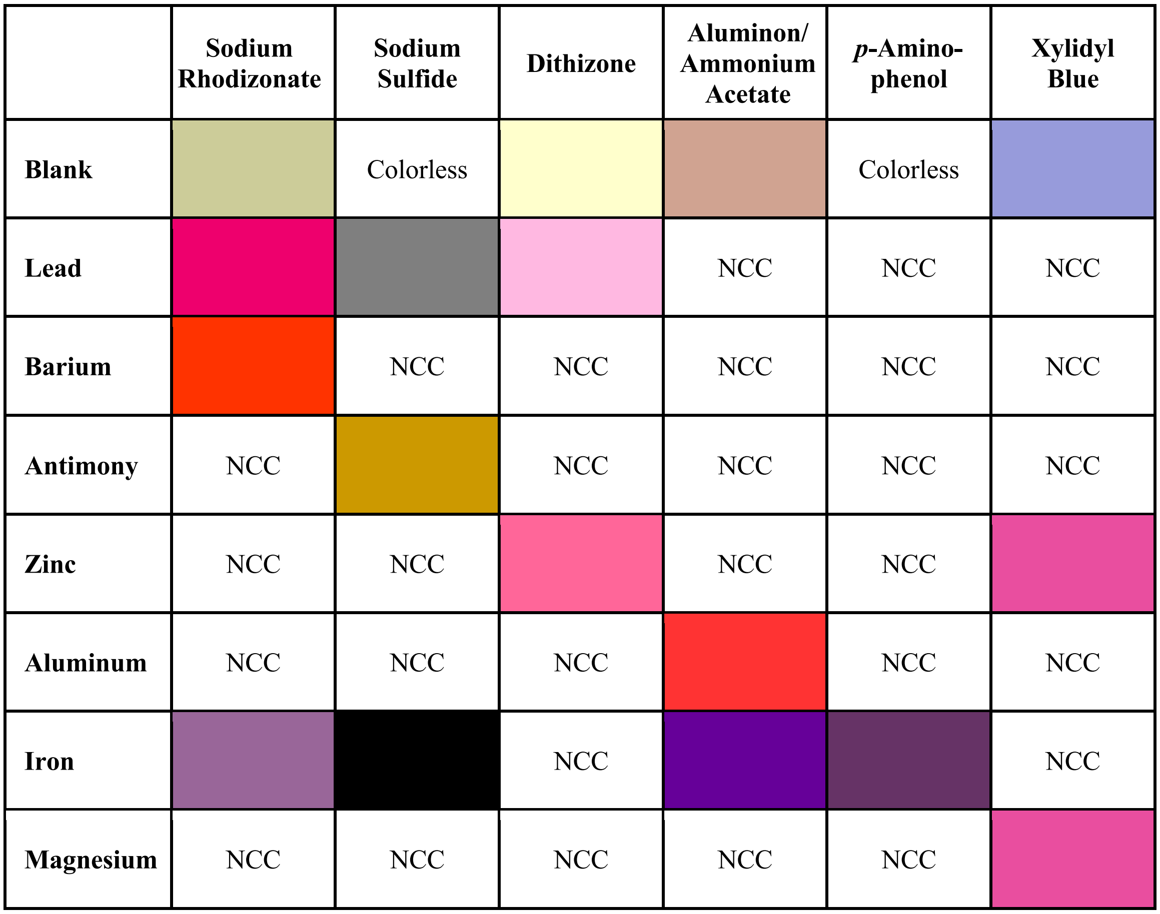


Figure 4.8: Reaction of xylidyl blue with magnesium to form metal chelation complex

1. Cross Reactivity

Cross reactivity was determined by testing each analyte of interest with each reagent present on the µPAD. Iron reacted with all tests except dithizone and xylidyl blue. All color changes seen can be easily distinguished from the analyte of interest for each reagent. The xylidyl blue test for magnesium also yielded multiple pink color changes. Aluminum and zinc both caused a color change, therefore, magnesium cannot be confirmed unless the tests for aluminum and zinc yield negative results.

Table 4.2: Test results for the cross reactivity of metals. Each test was performed by placing 1 µL of test solution or a small amount of paste as described at the top of each channel and then allowed to dry. Tests were run using a 1000 µg/mL standard solution of the corresponding metallic salt compound dissolved in deionized water. This solution was also used for the blank. NCC indicates no color change occurred.



	Sodium Rhodizonate	**Sodium Sulfide**	**Dithizone**	**Aluminon/ Ammonium Acetate**	***p*-Amino-phenol**	**Xylidyl Blue**
Blank	🟨	Colorless	🟨	🟫	Colorless	🟦
Lead	🟪	◼	🟪	NCC	NCC	NCC
Barium	🟧	NCC	NCC	NCC	NCC	NCC
Antimony	NCC	🟨	NCC	NCC	NCC	NCC
Zinc	NCC	NCC	🟪	NCC	NCC	🟪
Aluminum	NCC	NCC	NCC	🟥	NCC	NCC
Iron	🟪	⬛	NCC	🟪	🟪	NCC
Magnesium	NCC	NCC	NCC	NCC	NCC	🟪

2. Limits of Detection

Limits of detection were determined visually by analyzing specific concentrations of each compound of interest. A visual minimum detectable amount was determined using a 1 µL aliquot of each standard spotted in the sample lane. The 1 µL value can be used along with the respective concentration to calculate the amount of the metal present in the measured 1 µL of the standard. This is the visual minimum detectable amount and ranges from 0.025 µg to 0.4 µg (Table 4.3). Typically, these metal salts and powders make up at least 5 mass percent (% m/m) of a pyrotechnic sample, and thus they should be easily detected in powder samples and most residues.[25] To verify this issue, pyrotechnic mixtures were prepared and tested.

Table 4.3: Test results for the limit of detection of each metal with its respective colorimetric test. Each test was performed by placing 1 µL or a small amount of paste of each respect reagent or at the top of each channel and then allowed to dry. Tests were performed by spotting 1 µL of the 1000 µg/mL corresponding metallic salt standard solution dissolved in deionized water directly below the reagent. These test solutions were diluted and spotted until a color change could no longer be confirmed after running the µPAD.

Compound of Interest	Visual LOD (µg/mL)	Visual Minimum Detectable Amount (µg)
Lead	250	0.25
Barium	250	0.25
Antimony	250	0.25
Zinc	25	0.025
Aluminum	250	0.25
Iron	400	0.4
Magnesium	250	0.25

3. Interference Testing

A group of common metallic components found in low explosives residues were tested using these devices. These compounds included copper, strontium, calcium, potassium, and sodium. All interferent solutions were prepared as 1000 µg/mL standards and analyzed. With the exception of dithizone, which produced a pale pink color change with sodium and calcium, none of the tested reagents produced positive results on the µPADs.

Table 4.4: Common metallic components contained in low explosives were tested as 1000 µg/mL solutions to determine possible interferences.

	Sodium Rhodizonate	**Sodium Sulfide**	**Dithizone**	**Aluminon/ Ammonium Acetate**	***p*- Amino- phenol**	**Xylidyl Blue**
Blank	🟫	Colorless	🟨	🟫	Colorless	🟦
Strontium	NCC	NCC	NCC	NCC	NCC	NCC
Sodium	NCC	NCC	🟪	NCC	NCC	NCC
Copper	NCC	NCC	NCC	NCC	NCC	NCC
Potassium	NCC	NCC	NCC	NCC	NCC	NCC
Calcium	NCC	NCC	🟪	NCC	NCC	NCC

4. Stability Study

Testing of stored µPADs at one and two weeks revealed no noticeable discoloration of any reagents and all tests produced the proper color change when reacted with the corresponding analyte of interest. However, at four weeks, discoloration could be seen for the rhodizonate mixture, dithizone, and *p*-aminophenol. The rhodizonate mixture was a dark brown color and distinction between a blank test and a µPAD run with a 1000 μg/mL standard of both barium and lead did not yield a distinguishable positive result. Dithizone was golden brown as opposed to the tan previously seen in the blank. When run with a zinc standard, a distinguishable pink color change was still visible. A *p*-aminophenol blank when viewed at four weeks was brown in color. A color change that produced dark purple ring around the brown spot was observed when run with iron. Sodium sulfide only produced a color change through the second week testing period.

5. Real Samples

Three different brands of ammunition cartridges were tested by adding 40 μL of a 50:50 acetone:water solution into each cartridge with a pipette and then placing this solution directly onto the bottom of the μPAD. A pink/purple color change was seen for the sodium rhodizonate mixture for both 39 mm cartridges indicating a positive result for lead, and a slight pink color change was observed for the dithizone solution for all three cartridges that indicated a positive result for zinc.

The μPAD was also used to test burned pyrotechnic samples of different compositions. Upon testing of real samples in water, it was determined that many of these compounds can be present as metal powders in the samples and are not soluble in water alone. Upon burning, some metals form insoluble metal oxide compounds.[20,92] However, acidification of samples greatly improves solubility, which led to the decision to use dilute hydrochloric acid as an additional solvent. Care was taken to dilute samples sufficiently to prevent degradation of the chromatography paper, which lead to using a concentration of approximately 0.7 M HCl. Blanks of the μPAD were run in both water and the acidic solution to avoid possible false positive identification resulting from changes caused by the difference in pH. The water and hydrochloric acid blanks can be seen in Figures 4.9 and 4.10 respectively. It can be seen that the acidic HCl solution caused the xylidyl blue reagent to yield a result that looks similar to a positive result; therefore, the test must be discarded when run in an acidic medium. The aluminon reagent also changes yielding an orange color, but this is different from the positive pink seen when aluminum is present.

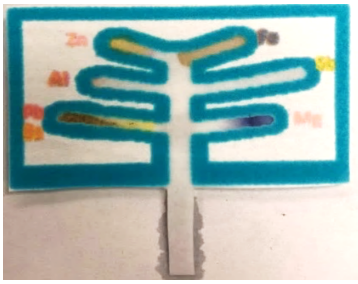


Figure 4.9: Blank run of metallic fuel µPAD in deionized water

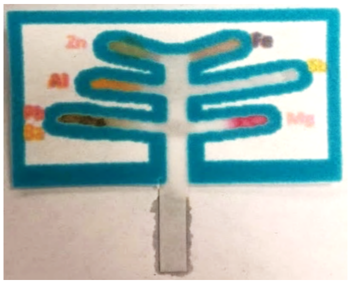


Figure 4.10: Blank run of metallic fuel µPAD in 0.7M hydrochloric acid

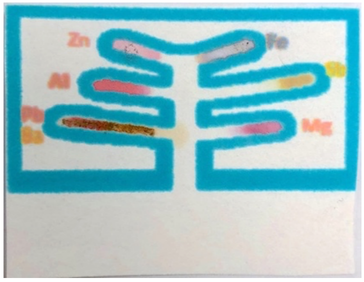


Figure 4.11: Metallic fuel µPAD positive results

Five different pyrotechnic compositions were tested in triplicate: underwater flare mix 1, photoflash mix 1, spreader star mix 1, mild flash 1, and volcano mix 1.[86] These compositions were chosen so different mixtures of the inorganic compounds in question could be tested. See Table 4.5 for mixture components.

Table 4.5: Sample compositions for each of the five tested pyrotechnic mixtures. Each mixture was made on the order of milligrams in order to obtain enough sample for testing. Results can also be seen for the µPADs that were run in water and 0.7M HCl.

Name	**Composition**[86]	**Quantity (mg)**[86]	**Results**
Underwater Flare Mix 1	Magnesium Aluminum Barium Sulfate Barium Nitrate	16 12 40 32	• Positive for magnesium in water • Positive for aluminum in HCl • Positive for barium in both solvents
Photoflash Mix 1	Aluminum Barium Nitrate Potassium Perchlorate	40 30 30	• Positive for aluminum in both solvents • Positive for barium in both solvents
Spreader Star Mix 1	Zinc Potassium Nitrate Charcoal Sulfur	80 28 14 5	• Positive for zinc in HCl
Mild Flash Mix 1	Aluminum Barium Nitrate Sulfur	21 75 4	• Positive for aluminum in HCl • Positive for barium in both solvents
Volcano Mix 1	Charcoal Sulfur Potassium Nitrate Iron Filings	4 4 24 10	• Positive for iron in both solvents

In all sample sets containing barium that were run in acidic solvent, a pink color was also seen when observing the sodium rhodizonate reagent zone. This is as a result of the sensitivity of this reagent to pH changes, which cause different colors to be observed. When the pH is acidic, lead yields a purple color change when present instead of the pink observed in a neutral solution. A positive for barium in a neutral or basic solution yields an orange color change while the pink change observed during real sample testing can be attributed to the acidity of the running solution.

For all tests run in the 0.7 M HCl, the xylidyl blue reagent produced a pink color change, but this was not indicative of a positive result for magnesium. Xylidyl blue was also sensitive to changes in pH and the acidic solution overwhelmed the included phosphate buffer. Thus, the test is not operable and should not be applied when an acidic wash solution is used.

On basis of the results, it would be pertinent for all samples to be run in both water and hydrochloric acid. When burned, some of these metals form metal oxides that are insoluble in water and therefore do not produce a color change with their corresponding test reagent. This would prevent potential false negatives.

C. Concluding Remarks

A six-lane multiplexed µPAD was developed for the detection of particular low explosives metallic components. The µPAD was able to identify components present in pyrotechnic devices, including barium, zinc, aluminum, iron, and magnesium, while also identifying components present in varying compounds contained in primer residue including lead, barium, antimony, zinc, and aluminum. The inorganic compounds were detectable both as standards and in real samples. The device was tested using both water and dilute hydrochloric acid as the solvent. The use of HCl does affect the color changes seen in both the sodium rhodizonate test and the xylidyl blue test. During testing, a pink color can be seen for barium in addition to the expected orange. The xylidyl blue test is overwhelmed by the acidity of the HCl solution and cannot be used in these conditions. Limits of detection of the metallic components ranged from 0.025 to 0.4 µg showing the capability of these devices for detecting low explosive residue components with analysis times under ten minutes. These devices are cost effective and can easily be operated by military or law enforcement personnel for a rapid determination of possible explosive compounds.

V. µPAD FOR SMOKELESS POWDER ENERGETICS AND ORGANIC ADDITIVES DETECTION

A. Introduction

Terrorist attacks are a common occurrence in present times. A recent report by the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) reported that 439 bombings occurred in the United States in 2016. Of these 439 cases, the second most common main charges, behind pyrotechnics and pyrotechnic mixtures, were smokeless powders and black powder substitutes.[28] The following year, there was an attempted attack on New York Port Authority with a pipe bomb containing smokeless powder that could have been deadly.[82] These improvised explosive devices (IEDs) are a continuing concern for law enforcement around the world as they can be easily constructed from items available to the general public. In the late 1800s, smokeless powders were introduced as black powder replacements that burned cleaner and produced less smoke than their black powder counterparts.[9] Since then, they have been widely utilized in different type of ammunition, pipe bombs, and other IEDs.[17] Smokeless powders are chiefly composed of energetic compounds that are utilized to initiate a reaction, such as nitrocellulose and nitroglycerine. Additionally, they contain additive packages of organic compounds, which perform specific functions, such as stabilizers, plasticizers, flash suppressants, etc.[9,10] These compositions are indicative of smokeless powders and can also be utilized to differentiate between brands and lots of these powders.[30]

While it has been shown that these compounds can be differentiated in a laboratory setting, methods for on-site detection of smokeless powders can prove useful in the field of forensics. Miniaturized, on-site instruments are still being developed, but these devices can be difficult to use for non-scientific personnel. Therefore, a need has developed for a compact, simple, and efficient test that can be utilized by law enforcement and crime scene investigators. Current methods include colorimetric tests and lateral flow immunoassays. An ongoing issue is that these methods typically only test for one compound at a time severely limiting their testing capabilities.[7] The need to prepare multiple samples for different testing methods can be problematic when only small amounts of sample are available to begin with.

Recently, a viable alternative to these types of tests has been on the rise. A group of devices called microfluidic paper-based analytical devices (μPADs) have been developed for various forensic purposes. Previous research done in the McCord laboratory has led to the development of μPADs for the detection of illicit drugs, bodily fluids, and multiple different types of explosives.[5–7,93] The types of explosives that can be detected range from military grade high explosives to metallic fuels contained in low explosive devices. These devices are multiplexed and allow for the detection of up to six compounds simultaneously.[5,93] μPADs have a variety of current and future applications in the field of forensics.

In the current project, a μPAD was developed for the on-site detection of energetics and organic compounds contained in smokeless powders and other low explosives. The device could be used in conjunction with those previously mentioned to create an all-inclusive testing method for a variety of explosive compounds that can be utilized in IEDs.

B. Results and Discussion

The goal of the project was to develop a μPAD for the detection of energetics and organic compounds commonly found in smokeless powder and other low explosives. The μPAD was developed for the detection of diphenylamines, dinitrotoluenes, nitroglycerine, and nitrates/nitrites. All of these compounds are commonly found in smokeless powders or their degradation products.[11,30] Nitroglycerine, nitrates, and nitrites in particular can be found in many low explosives. Nitroglycerine is a common energetic or plasticizer while many compounds contained in both smokeless powders and other types of low explosives contain nitrated compounds.[10]

Multiple μPADs were designed for multiplexed testing and for running individual tests. Black wax channels were used both for the multiplexed design and during initial development and testing with single lane devices. The color of the wax design was easy to distinguish from any possible color changes that could be observed and it photographed well for documentation purposes.[6] The wax design also showed no bleed coinciding with the use of organic solvents. The μPAD designs can be seen in Figures 5.1 and 5.2 with scales. DMSO was used as the solvent for most experiments. The nitroglycerine standard utilized came pre-mixed in methanol, but all subsequent tests were run in DMSO. Other organic solvents were tested such at methanol, acetone, and acetonitrile. In pure form, these solvents evaporated off of the paper surface before they could travel to the end of the lanes. When mixed with water, the compounds of interest will not dissolve as they are not soluble in water. Thus, DMSO was chosen as the solvent for the bulk of analysis. The μPAD was developed for the detection of energetics and organic components of low explosives residues, such as those in smokeless powders.

Tables 5.1, 5.2, and 5.3 show the results of each reagent tested with 1000 µg/mL solutions of the organic compounds examined that produced a color change.

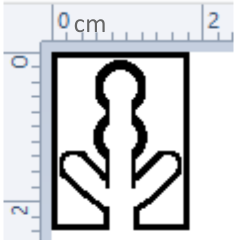


Figure 5.1: Multiplexed smokeless powder µPAD design with scale

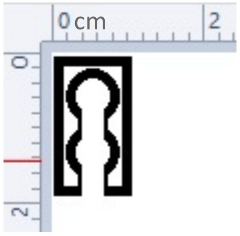
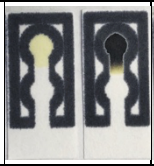
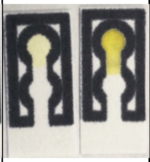
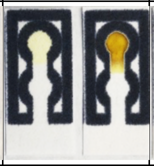
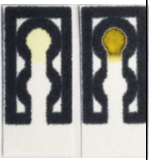
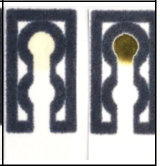


Figure 5.2: Single lane µPAD design with scale

Cerium (IV) sulfate is known to chelate with compounds containing multiple phenyl rings to produce different colored compounds depending on the ring configuration and substituents.[94] A 1 µL aliquot of the reagent was spotted at the top of the sample lane at 0.1% in 1 M sulfuric acid and analyzed with a 1000 µg/mL diphenylamine standard. A dark gray color change was noted. Higher concentrations of the reagent were prepared but the cerium (IV) sulfate did not readily dissolve at 1M sulfuric acid concentration. The acid concentration could not be raised further as it was detrimental to both the paper and the wax channels. The 0.1% mixture in a 1 M sulfuric acid was utilized for future experiments. It was determined that the cerium (IV) sulfate test also produced color changes for N-nitrosodiphenylamine, 2- nitrodiphenylamine, 4- nitrodiphenylamine, and 4-nitrosodiphenylamine, as shown in Table 5.1. These compounds produced a less saturated gray color change than DPA. 2-NDPA produced a tan color change. The difference in the gray color saturation could be a result of some molecules being more sterically hindered than others. DPA has no substituents whereas all the other compounds tested contain a substituent. These color changes could all be differentiated from the yellow color that N-NsDPA, 2-NDPA, 4-NPDA, and 4-NsDPA produce when dissolved in solution. DMSO was utilized as a blank and to create all standard solutions tested.

Table 5.1: Colorimetric test results for the detection of organic low explosives residue components with cerium (IV) sulfate. Each test was performed by placing 1 µL of test solution at the top of each channel and then allowed to dry. Tests pictured below were run using a 1000 µg/mL standard solution of the corresponding compound. Reaction time for these single lane µPADs was less than five minutes.

Target	**DPA**	**N-NsDPA**	**2-NDPA**	**4-NDPA**	**4-NsDPA**
Color Change	Light yellow to dark gray	Light yellow to light gray	Light yellow to tan	Light yellow to light gray	Light yellow to gray
Blank/ Sample					

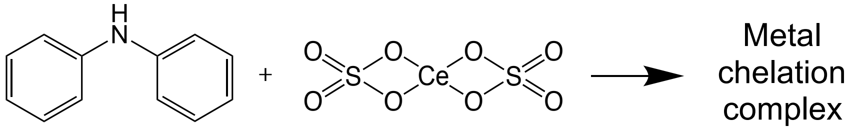
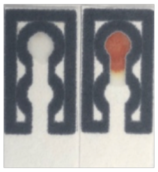
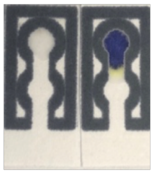
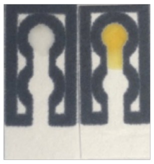
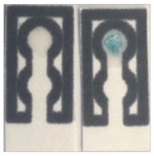


Figure 5.3: Reaction of diphenylamine with cerium (IV) sulfate in dilute sulfuric acid

Potassium hydroxide is known to react with nitroaromatics to produce colored compounds. In a situation when polynitroaromatic compounds are placed in alkaline solutions, a Janowsky reaction occurs to produce a colored complex. An example can be seen below in Figure 5.4 where the polynitroaromtic compound is dinitrotoluene. Initially, a 1 µL aliquot of the reagent was spotted at the top of the sample lane at a concentration of 1.5 M and analyzed with a 1000 µg/mL 2,4-dinitrotoluene standard.[5] A green color change was observed but rapidly disappeared preventing documentation. The KOH concentration was increased to 5 M and two 1 µL spots of the solution were added to the sample lane. A green color change was produced that was clearly visible and stayed on the paper for a longer period of time. It was determined that the potassium hydroxide test also produced a color change when contacted with 4-nitrodiphenylamine, 2,4-dinitrodiphenylamine, 4,4’-dinitrodiphenylamine, and 4-nitrosodiphenylamine. Different colors were produced depending on the placement of the substituents on the rings. 4-NDPA and 2,4-DNDPA both produce a red color change. 4,4’-DNDPA produces a blue color change. 4-NsDPA produces an orange color change. These color changes could all be differentiated from the yellow color that N-NsDPA, 2-NDPA, 4-NPDA, and 4-NsDPA produce when dissolved in solution. These colors can be observed in Table 5.2. DMSO was utilized as a blank and to create all standard solutions tested.

Table 5.2: Colorimetric test results for the detection of organic low explosives residue components with potassium hydroxide. Each test was performed by placing 1 µL of test solution at the top of each channel and then allowed to dry. Tests pictured below were run using a 1000 µg/mL standard solution of the corresponding compound. Reaction time for these single lane µPADs was less than five minutes.

Target	**4-NDPA**	**2,4-DNDPA**	**4,4’-DNDPA**	**4-NsDPA**	**2,4-DNT**
Color Change	Colorless to red	Colorless to red	Colorless to blue	Colorless to orange	Colorless to green
Blank/ Sample					

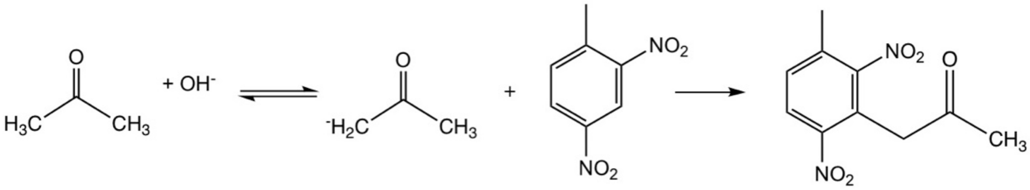
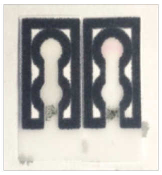
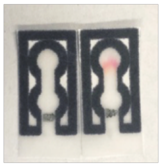
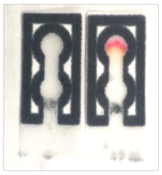


Figure 5.4: Janowsky reaction of dinitrotoluene

The Griess reagent is commonly used for the detection of nitrites and nitrates as they relate to explosive compounds.[11] An example of the reaction can be seen in Figure 5.5 with the Griess reagent being used to detect nitrite. A 1 µL aliquot of each reagent was spotted in its respective place on the sample lane. First, a 0.5% 1-naphthylamine solution was placed at the top of the sample lane. Next, a solution of 0.1% sulfanilic acid was placed midway up the channel. Last, a zinc paste made with sulfanilic acid, sodium acetate, and a saturated trehalose solution was smeared at the bottom of sample lane.[5] Upon drying, the paste easily fell off the device preventing proper detection. The test was modified later as a zinc paste made with a 50:50 glycerine:water mixture, which was smeared at the bottom of the sample lane. A 1000 µg/mL standard of nitroglycerine was used to test the Greiss reagent. A very light pink color change formed at the top of the sample lane after running the device. The solutions were increased from a single 1 µL spot each to two 1 µL spots each which produced a more saturated pink color change. It was determined that the test also produced a red color change when reacted with nitrite and a pink color change when reacted with nitrate. These color-producing reactions can be seen below in Table 5.3. The 1000 µg/mL standard of nitroglycerine was bought already diluted in methanol. As such, it was prepared this way throughout. The reagent was tested with both methanol and DMSO throughout to rule out possible interferences. The nitrate and nitrite solutions were prepared in water as they are not readily soluble in DMSO. The devices themselves were analyzed with a running solution of DMSO which is miscible with both water and methanol. DMSO was utilized as a blank due to its use as the running solvent for the device and it produced no noticeable interferences.

Table 5.3: Colorimetric test results for the detection of low explosives residue components with a modified Griess reagent. Each test was performed by placing 1 µL of the test solutions in their respective places in the channel, smearing the paste at the bottom of the channel, and then allowing them to dry. Tests pictured below were run using a 1000 µg/mL standard solution of nitroglycerine and nitrate/nitrite. Reaction time for these single lane µPADs was less than five minutes.

Target	**NG**	**Nitrate**	**Nitrite**
Color Change	Colorless to pale pink	Colorless to pink	Colorless to red
Blank/ Sample			

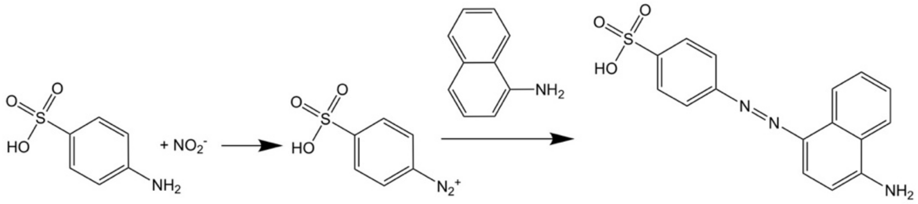


Figure 5.5: Reaction of nitrite with sulfanilic acid followed by 1-naphthylamine

1. Cross Reactivity Testing

Cross reactivity for the reagents was determined by testing all analytes of interest with each reagent present on the multiplexed μPAD. Classes of compounds reacted similarly to the same tests. All diphenylamines with a single nitro or nitroso group reacted with cerium (IV) sulfate while those with two nitrate groups did not. Each produced some variation of a gray or tan color change allowing the class of compounds to be distinguished from others. The cerium (IV) sulfate can therefore detect diphenylamines.

When reacted with potassium hydroxide, dinitrotoluenes with a nitro group at the fourth carbon both produced a green color change though only 2,4-dinitrotoluene produced a color change on paper. The diphenylamines with nitro or nitroso groups produced different colors depending on their arrangement. 4-NDPA and 2,4-DNDPA, which both contain a nitro or nitroso constituent at the fourth carbon, produced a red color change. 4,4’-DNDPA, which has a nitro group at both the fourth carbon and the four-prime carbon, produced a blue color change. 4-NsDPA, which has a nitroso group at the fourth carbon, produced an orange color change. These four different colors allow for distinction of these different compounds.

When utilizing the modified Griess reagent, the only analytes that produced a color change were nitroglycerine and the salt solutions of nitrate and nitrite.

Nitroglycerine produces a light pink color change while the pure nitrate and nitrite ion solutions produced a deep red/pink color change.

Table 5.4: Test results for the cross reactivity of organic smokeless powder additives. Each test was performed by placing test solution in its respective channel and then allowed to dry. Tests were run using a 1000 µg/mL standard solution of the corresponding additive dissolved in DMSO, methanol, or water. This solution was also used for the blank. NCC indicates no color change.

	Cerium (IV) Sulfate	Potassium Hydroxide	Modified Griess Reagent
Blank	🟨		
Diphenylamine	⬛	NCC	NCC
N-nitrosodiphenylamine	▨	NCC	NCC
2-nitrodiphenylamine	🟫	NCC	NCC
4-nitrodiphenylamine	▨	🟥	NCC
2,4’-dinitrodiphenylamine	NCC	🟥	NCC
4,4’-dinitrodiphenylamine	NCC	🟦	NCC
4-nitrosodiphenylamine	⬛	🟧	NCC
Nitroglycerine	NCC	NCC	🟪
Nitrate	NCC	NCC	🟪
Nitrite	NCC	NCC	🟥
2,4-dinitrotoluene	NCC	🟩	NCC

While these tests produced definitive color changes for different compounds or classes of compounds, they were also tested to determine if mixtures of these analytes allowed for distinction between compounds or classes (Table 5.5). When viewing color changes produced by the cerium (IV) sulfate reagent, 2-NDPA’s tan color change could not be distinguished when in solution with any of those compounds that produced a gray color change. Mixtures of DPA, N-NsDPA, 4-NDPA, and 4-NsDPA all produced similar gray color changes and would therefore not be able to be separately identified in a mixture.

When mixtures of analytes were tested that produced color changes with KOH, the orange color formed by 4-NsDPA was always masked by any other compound present. A green color change (2,4-DNT) was only favorably visible when mixed with 4-

NsDPA. The red and blue color changes produced by 4-NDPA, 2,4-NDPA, and 4,4-NDPA always presided over any other possible color changes. When a blue color producer and a red color producer were mixed together, a purple color change occurred allowing the assumption that 4,4-NDPA is present along with 4-NDPA and/or 2,4-NDPA.

Mixtures of solutions tested with the modified Griess reagent revealed that any blend of nitroglycerine, nitrate, and nitrite produced a dark pink/red color. Nitroglycerine can only be confirmed if a light pink color resides on the sample area. Otherwise, a deeper red/pink indicated some combination of nitrate, nitrite, and nitroglycerine in the analyzed sample.

Table 5.5: Test results of mixtures of organic smokeless powder additives. Each test was performed by placing 1 µL of test solution at the top of each channel and then allowed to dry. Tests were run using a 1000 µg/mL standard solution of the corresponding compound dissolved in DMSO, methanol, or water (as previously described).

Mixture	Reagent Tested	Color Observed
DPA & N-NsDPA	Cerium (IV) Sulfate	⬛
DPA & 2-NDPA		⬛
N-NsDPA & 2-NDPA		⬜
DPA, N-NsDPA, & 2-NDPA,		⬛
2,4-DNDPA & 4,4’-DNDPA	KOH	🟪
2,4-DNDPA & 4-NsDPA		🟥
2,4-DNDPA & 2,4-DNT		🟥
4,4’-DNDPA & 4-NsDPA		🟦
4,4’-DNDPA & 2,4-DNT		🟦
4-NsDPA & 2,4-DNT		🟩
2,4-DNDPA, 4,4’-DNDPA, 4-NsDPA, & 2,4-DNT		🟪
NO_3^- & NO_2^-	Modified Griess Reagent	🟥
NO_3^-, NO_2^-, & NG		🟥

2. Limits of Detection

Limits of detection were determined visually by analyzing specific concentrations of each compound of interest. A visual minimum detectable amount was determined based on the use of a 1 µL aliquot of each standard spotted in the sample lane. The 1 µL value was used along with the respective concentration to calculate the amount of the compound present in the measured 1 µL of the standard. The visual minimum detectable amount ranged from 0.025 to 0.5 µg (Tables 5.6, 5.7, and 5.8).

The instrumental limit of detection was determined utilizing images taken of the same devices used to determine the visual limit of detection. These photographs were analyzed for each compound in ImageJ Software via the densiometry function. Once plotted in excel, it was determined that the best fit for the results was linear. Examples of the plotted graphs can be seen below (Figures 5.6, 5.7, and 5.8) for each compound of interest. Instrumental limits of detection were determined through a measure of the noise between the lowest visual detection limit and the blank (Tables 5.6, 5.7, and 5.8). The instrumental limits of detection ranged from 0.011 to 0.48 µg.

Table 5.6: Test results for the limit of detection of each compound with cerium (IV) sulfate. Each test was performed by placing 1 µL of cerium (IV) sulfate at the top of the channel and then it was allowed to dry. Tests were performed by spotting 1 µL of the 1000 µg/mL corresponding standard solution directly below the reagent. These test solutions were diluted and spotted until a color change could no longer be confirmed after running the µPAD.

Compound of Interest	**Visual LOD (µg/mL)**	**Visual Minimum Detectable Amount (µg)**	**Instrumental LOD (µg)**
Diphenylamine	250	0.25	0.17
N-nitrosodiphenylamine	250	0.25	0.22
2-nitrodiphenylamine	500	0.50	0.48
4-nitrodiphenylamine	500	0.50	0.45
4-nitrosodiphenylamine	100	0.10	0.11

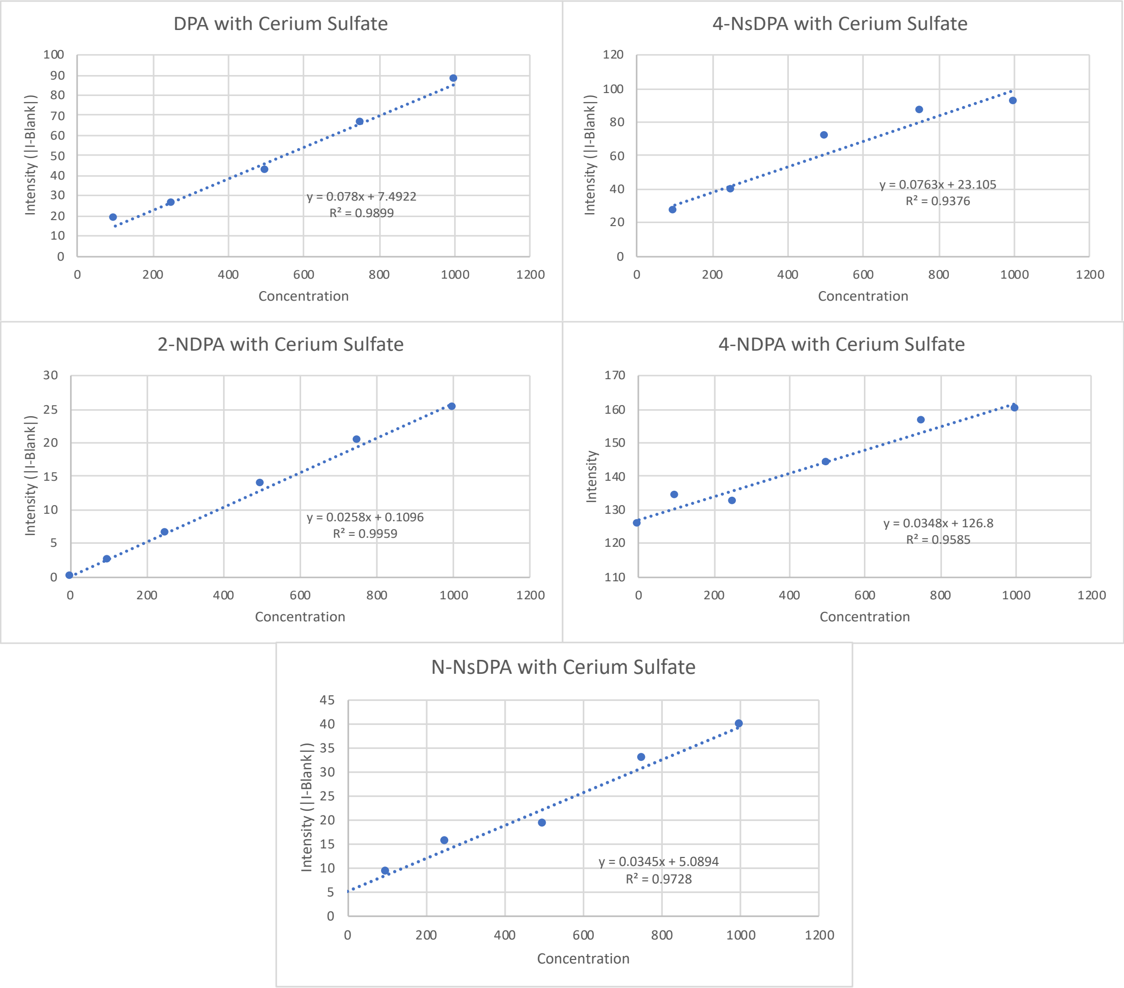


Figure 5.6: ImageJ linear fit plots of concentration (µg/mL) vs. color intensity for cerium sulfate

Table 5.7: Test results for the limit of detection of each compound with potassium hydroxide. Each test was performed by placing 2 µL of potassium hydroxide at the top of each channel and then it was allowed to dry. Tests were performed by spotting 1 µL of the 1000 µg/mL corresponding standard solution dissolved in DMSO directly below the reagent. These test solutions were diluted and spotted until a color change could no longer be confirmed after running the µPAD.

Compound of Interest	**Visual LOD (µg/mL)**	**Visual Minimum Detectable Amount (µg)**	**Instrumental LOD (µg)**
4-nitrodiphenylamine	250	0.25	0.13
2,4’-dinitrodiphenylamine	25	0.025	0.018
4,4’-dinitrodiphenylamine	25	0.025	0.011
4-nitrosodiphenylamine	500	0.50	0.28
2,4-dinitrotoluene	500	0.50	0.25

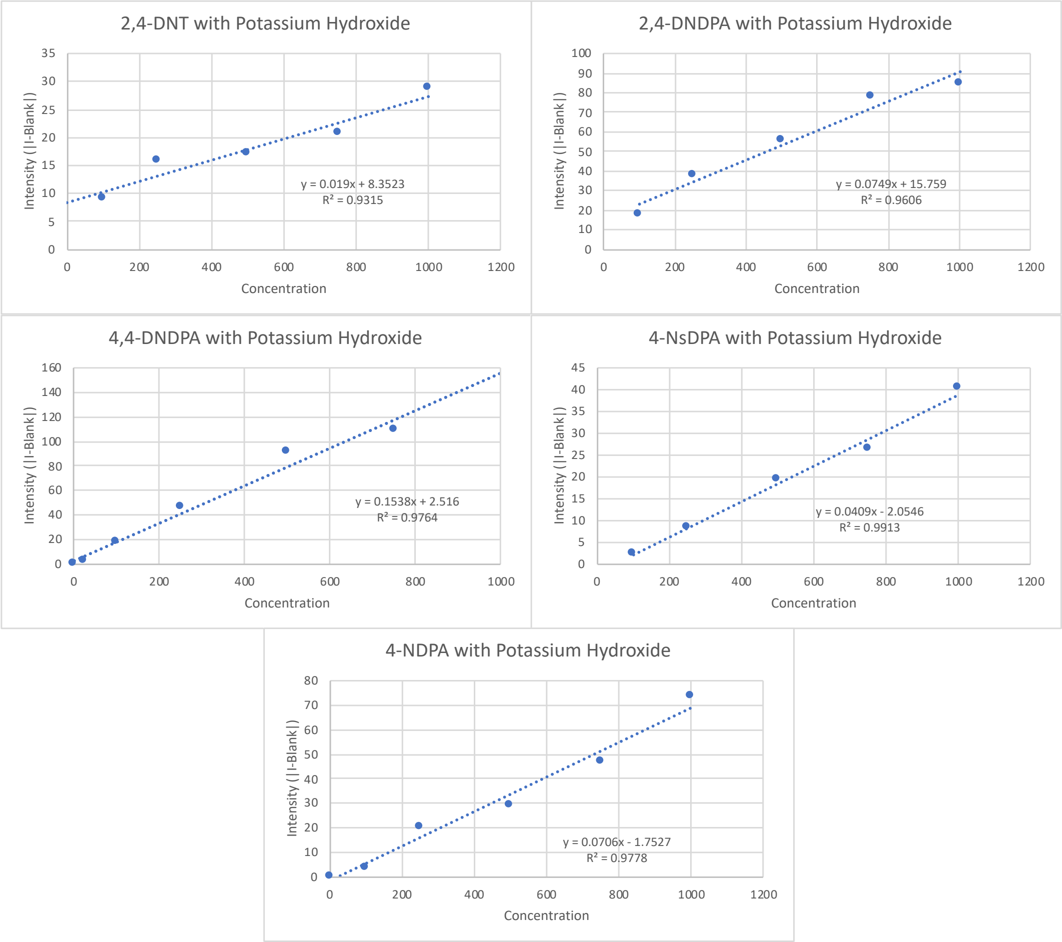


Figure 5.7: ImageJ linear fit plots of concentration (µg/mL) vs. color intensity for potassium hydroxide

Table 5.8: Test results for the limit of detection of each compound with modified Griess reagent. Each test was performed by placing 2 µL of 1-naphthylamine at the top of the channel, followed by 2 µL of sulfanilic acid in the middle of the channel, and finally a zinc metal slurry was smeared at the bottom of the lane before it was allowed to dry. Tests were performed by spotting 1 µL of the 1000 µg/mL corresponding standard solution dissolved in water or methanol directly below the zinc paste. These test solutions were diluted and spotted until a color change could no longer be confirmed after running the µPAD.

Compound of Interest	Visual LOD (µg/mL)	Visual Minimum Detectable Amount (µg)	Instrumental LOD (µg)
Nitroglycerine	500	0.50	0.42
Nitrate	500	0.50	0.26
Nitrite	100	0.10	0.079

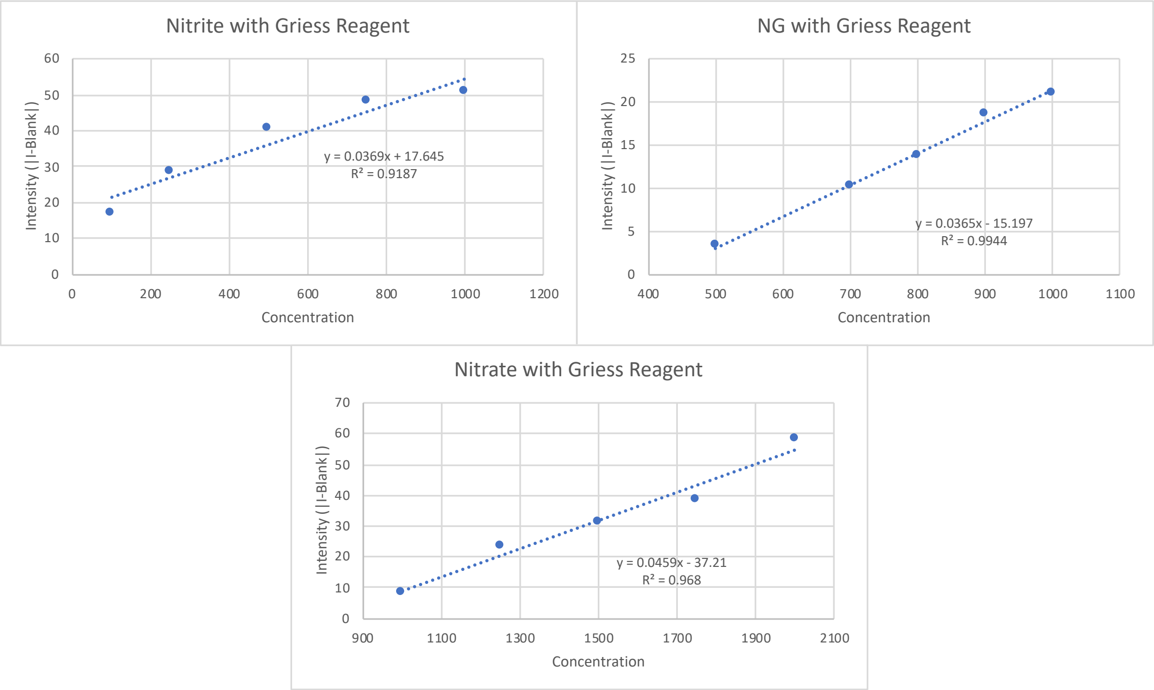


Figure 5.8: ImageJ linear fit plots of concentration (µg/mL) vs. color intensity for Griess Reagent

3. Interferences

A group of common organic compounds found in smokeless powders were tested using the device to determine if they would produce a color change. These compounds included dimethyl phthalate, diethyl phthalate, dibutyl phthalate, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, methyl centralite, ethyl centralite, 2,3-dinitrotoluene, 2,6-dinitrotoluene, and 3,4-dinitrotoluene. All interferent solutions were prepared as 1000 µg/mL standards and run utilizing DMSO as the solvent. None of the tested reagents produced positive results on the µPAD and therefore, no interferents were determined based on the specific analytes tested.

Table 5.9: Common organic compounds contained in smokeless powders and other low explosives were tested as 1000 µg/mL solutions to determine interferences. NCC indicates no color change.

	Cerium Sulfate	**Potassium Hydroxide**	**Modified Griess Reagent**
Blank	🟨		
Dimethyl phthalate	NCC	NCC	NCC
Diethyl phthalate	NCC	NCC	NCC
Dibutyl phthalate	NCC	NCC	NCC
2-nitrotoluene	NCC	NCC	NCC
3-nitrotoluene	NCC	NCC	NCC
4-nitrotoluene	NCC	NCC	NCC
Methyl Centralite	NCC	NCC	NCC
Ethyl Centralite	NCC	NCC	NCC
2,3-dinitrotoluene	NCC	NCC	NCC
2,6-dinitrotoluene	NCC	NCC	NCC
3,4-dinitrotoluene	NCC	NCC	NCC

4. Stability Study

The initial method of storing the µPADs consisted of sealing them inside of a nylon bag and placing the bags in a dark location as previously described. After being stored for one week in these conditions, KOH and cerium (IV) sulfate showed no discoloration indicating degradation but did not produce a color change. The modified Griess reagent showed discoloration at both reagent spots. The 1-naphthylamine spot showed an orange color while the sulfanilic acid spot presented a pink color. Results of testing these devices yielded no distinguishable color change and the blank was not able to be differentiated from the positive test. In order to create a stable environment, further testing was needed which consisted of two storage methods: addition of Drierite Desiccant ® to the nylon bag before storage in the dark (Figure 5.9) and storage of sealed nylon bags in a freezer. Both methods provided more stability than the original technique.

Figure 5.9: Single lane µPADs stored in sealed nylon bag with Drierite ®

When stored with desiccant, KOH produced the expected color change through the two-week testing period, but the color produced at weeks three and four was minimal. The cerium (IV) sulfate reagent, which has a light-yellow coloration after spotting, was not showing its light-yellow color after seven days of storage with desiccant. Additionally, it did not produce any color change when tested with 1000 µg/mL standards. The same trend continued through the other testing time periods. The modified Griess reagent showed much less discoloration at one week than during storage without desiccant present. When initially tested, no color change occurred. The zinc paste was removed from the µPADs before storing and retesting the devices. At one week, an orange color change was seen but was not distinguishable from the blank (Figure 5.11 and 5.13). The trend also continued through the other testing periods.

Figure 5.10: Griess reagent stored in nylon bag for 1 week before run

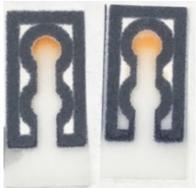


Figure 5.11: Blank and tested Griess reagent stored in nylon bag for 1 week

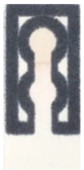


Figure 5.12: Griess reagent stored in nylon bag with Drierite for 1 week before run

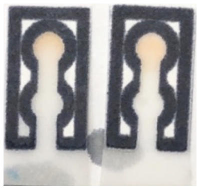


Figure 5.13: Blank and tested Griess reagent stored in nylon bag with Drierite for 1 week

When stored in the freezer, both the cerium (IV) sulfate and KOH reagents showed no discoloration from one day up to four weeks. As was previously noted, the modified Griess reagent did not produce a color change when the zinc paste was frozen. When only the liquid reagents were frozen, the reagent showed stability over the four-week period. The liquid reagents for the Griess test only showed minimal discoloration that began at week 3 and was visually distinguishable from a positive result. The cerium (IV) sulfate produced a comparable color change to a freshly tested device through the week 3 testing phase. At week four, the color was less saturated than the previous week. KOH and the Griess reagent maintained the same color saturation for the duration of the four-week test period. These results indicate that storage in the freezer will maintain the performance of these µPADs for the longest period of time of the methods tested.

5. Real Samples

Three different brands of smokeless powders were tested pre-burn and post burn. Pre-burn samples were prepared by placing 20 µg of each powder into 2 mL of methanol to create a 10 µg/mL solution. Four solutions were prepared for each powder and allowed to extract for 5 minutes, 10 minutes, 30 minutes, and 60 minutes prior to testing. The solution was then placed directly onto the bottom of the µPAD at the end of the extraction period. For all 3 powders, the color changes were visible after 10 minutes of extraction and longer. The samples tested at 5 minutes produced little to no color change. As a result, all subsequent experiments were performed with a 10-minute extraction time. A pink color change was seen for the Griess Reagent for both Alliant Red Dot and Hercules Bullseye powders indicating a positive result for nitroglycerine/nitrate/nitrite. Dupont IMR-4895 did not yield a positive result for the Griess reagent, which was consistent with the fact that the powder is single-based and does not contain nitroglycerine, only nitrocellulose. All three powders produced a light gray color with the cerium (IV) sulfate. Of the three, the Bullseye revealed the least saturation at the cerium (IV) sulfate testing zone which is consistent with the information obtained from the TWGFEX Smokeless Powder Database. Red Dot and IMR contain high amounts of diphenylamine whereas Bullseye contains small amounts of diphenylamine and 2-nitrodiphenylamine.

The µPAD was also used to test burned smokeless powder samples of the same brands listed above. These samples were prepared by burning 1 g of each powder in a fume hood. For Red Dot, the average recovery was 1.5%. For Bullseye, the average recovery was 2.5%. For IMR, the average recovery was 7.5%. Each sample was diluted to 50 mg/mL in methanol and allowed to extract for 10 minutes. Red Dot produced a pink color change for the Griess Reagent. Bullseye produced a light orange color change for the Griess Reagent. The color change indicates the presence of nitroglycerine/nitrate/nitrite in both samples. None of the samples produced a color change for the cerium sulfate reagent. All of the samples produced a brown color change in potassium hydroxide that is different from any color changes seen through the duration of the testing. The blank run and a run containing positive results for diphenylamine at potassium hydroxide, nitrite at the Griess Reagent, and 4,4-dinitrodiphenyaline (Figures 5.14 and 5.15) can be seen below.

Figure 5.14: Blank run of Smokeless Powder µPAD in DMSO

Figure 5.15: Smokeless Powder µPAD positive results

C. Concluding Remarks

A three-lane multiplexed µPAD was developed for the detection of energetics and organic compounds found in smokeless powders. The µPAD was capable of identifying a common energetic, nitroglycerine. It can also detect organic compounds typically utilized in smokeless powder additive packages including diphenylamine, N-nitrosodiphenylamine, 2-nitrodiphenylamine, 4-nitrodiphenylamine, 4-nitrosodiphenylamine, 2,4-dinitrodiphenylamine, 4,4'-dinitrodiphenylamine, and 2,4-dinitrotoluene. Due to the low concentrations of some of these compounds in smokeless powders, only some were visualized in real samples, but all compounds were detectable as standard solutions. The device utilized DMSO as the standard solvent and running solution as these compounds are not soluble in water (with the exception of nitroglycerine as it was pre-diluted in methanol). The visual minimum detectable amount of nitroglycerine was 0.5 µg while the organic compounds ranged from 0.025 to 0.5 µg. Instrumental limits of detection for all compounds ranged from 0.011 to 0.48 µg which showed the ability of these devices to detect small amounts of the aforementioned compounds. The extraction and run time for the device was under 20 minutes. The µPAD is cost efficient, easy to utilize, and can be employed in the field for quick detection of compounds contained in smokeless powders, which are commonly utilized in IEDs.

VI. CONCLUSIONS

In recent years, terrorist attacks have become a part of everyday life with the death toll due to these attacks approaching 20,000 victims in 2017.[95] As has been seen in previous studies, data shows that the large majority of terrorist attacks worldwide continue to involve the use of explosives.[88] Additionally, the majority of devices utilized in these attacks contain black powder, black powder substitutes, flash powder/pyrotechnic mixtures, pyrotechnics/fireworks, and smokeless powders. This indicates a need for improved detection of low explosives and low explosive-containing devices.[28] In order to meet the need, the goal of the present project was to create easily accessible, on-site detection methods for a variety of low explosives. The ability to do rapid on-site testing of potential explosive compounds can give vital information that will protect law enforcement personnel at a pre- or post-blast explosive scene.

In the current project, two devices were developed for the detection of low explosives and related compounds. The method utilized microfluidic paper-based analytical devices (µPADs) as a means of on-site detection for a variety of compounds. The first µPAD is capable of identifying metals contained in different low explosives as the metallic fuel or as a color producing agent in pyrotechnic devices. The metals identified with the device include lead, barium, antimony, zinc, aluminum, iron, and magnesium utilizing either water or 0.7 M hydrochloric acid as the solvent. The second µPAD is capable of identifying energetics and organic additives typically found in smokeless powders and other low explosive materials. The compounds identified with the device include nitroglycerine, nitrate, nitrite, diphenylamine, N-nitrosodiphenylamine, 2-nitrodiphenylamine, 4-nitrodiphenylamine, 4-nitrosodiphenylamine, 2,4-dinitrodiphenylamine, 4,4’-dinitrodiphenylamine, and 2,4-dinitrotoluene utilizing DMSO as the running solvent. Visual minimum detectable amounts range from 0.025 to 0.5 µg allowing for these devices to detect these low explosives components in samples obtained from or at explosion sites. Minimal interferents were determined with the variety of compounds tested. These devices can be stored in a freezer for at least a month with little to no deterioration of the tests with a possibility of longer storage times. Both µPADs can be run in under 10 minutes with no extraction time needed for the metallic device and extraction of smokeless powders being possible in 10 minutes. Pre- and post-blast detection of an assortment of samples was performed with these devices. The metallic µPAD was successfully able to detect metals in spent bullet casings in addition to a variety of different pyrotechnic compositions including Underwater Flare Mix, Photoflash Mix, Spreader Star Mix, Mild Flash Mix, and Volcano Mix. The smokeless powder µPAD was able to detect nitroglycerine or nitrate/nitrite ions, and diphenylamine and its nitrated derivatives in multiple brands of smokeless powders. It was also able to differentiate a single base powder from a double base powder by indicating the presence of nitroglycerine.

These µPADs have many advantages for use in forensics, especially explosives detection. As compared to other on-site methods, they do not require any external equipment to run which cuts down on cost and no specialized knowledge is needed to operate the equipment. µPADs utilize paper as the main substrate allowing for cheap mass-production of these devices that only require a wax printer and heat laminator to be manufactured. They are also able to be multiplexed so that multiple target analytes can be detected simultaneously as opposed to test tube colorimetric analysis that can only detect one compound at a time. These multiplexed µPADs are small and can easily be carried in a crime scene investigator kit or even in a wallet. They provide rapid, simple, inexpensive, portable testing to law enforcement and military personnel at crime scenes and terrorist attack sites.

There are some limitations to the study and others involving the presumptive detection of compounds contained in low explosives. Certain compounds may be present in very low concentrations and extraction may be required increasing the amount of time needed for testing. These compounds may be present in even smaller amounts post-blast or post-burn further inhibiting on-site presumptive testing. It also must be kept in mind that these devices are presumptive and should therefore be coupled with confirmatory detection techniques.

Future work might involve improving the long-term stability of these μPADs through lamination to provide a better method to maintain the integrity of the paste reagents. Lamination would also allow for the utilization of other zinc pastes for the smokeless powder detection device. Additionally, a kit could be produced that utilizes previous μPADs for high explosives detection in conjunction with those produced in the study. In order to produce commercially available kits, a more extensive interference study would be necessary. It should encompass everyday materials that could be mistaken for these types of explosives. A blind study to determine reproducibility of the devices could also be performed. Lastly, combining these devices with instrumental methods for confirmation of results would result in a comprehensive testing method for a variety of explosives.

Overall, these devices could prove useful for the detection of compounds contained in low explosives in the field. These two μPADs allow personnel to perform quick explosives testing on-site with little to no training. They are cost effective and, when coupled with previous devices created in the McCord group, could be an all-inclusive presumptive testing method for a variety of explosive compounds. They minimize time and effort needed by law enforcement and military personnel must be in touch with potentially dangerous creating a safer environment in tumultuous times.

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APPENDICES

Appendix 1:

List of characteristic organic smokeless powder constituents and their usage in smokeless powders.[10,22]

Compound	Abbreviation	Usage
Diphenylamine	DPA	Stabilizer
N-Nitrosodiphenylamine	N-NsDPA	Stabilizer reaction product
4-Nitrosodiphenylamine	4-NsDPA	Stabilizer reaction product
2-Nitrodiphenylamine	2-NDPA	Stabilizer reaction product
4-Nitrodiphenylamine	4-NDPA	Stabilizer reaction product
2,4'-Dinitrodiphenylamine	2,4'-DNDPA	Stabilizer reaction product
4,4'-Dinitrodiphenylamine	4,4'-DNDPA	Stabilizer reaction product
Dibutyl phthalate	DBP	Plasticizer, Deterrent
Diethyl phthalate	DEP	Plasticizer
Dimethyl phthalate	DMP	Plasticizer
Ethyl centralite	EC	Stabilizer, Deterrent, Plasticizer
Methyl centralite	MC	Stabilizer, Deterrent
Nitroglycerin	NG	Energetic, Plasticizer
2-Nitrotoluene	2-NT	Product
3-Nitrotoluene	3-NT	Product
4-Nitrotoluene	4-NT	Product
2,3-Dinitrotoluene	2,3-DNT	Flash inhibitor
2,4-Dinitrotoluene	2,4-DNT	Flash inhibitor
2,6-Dinitrotoluene	2,6-DNT	Flash inhibitor
3,4-Dinitrotoluene	3,4-DNT	Flash inhibitor

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