AMBIENT IONIZATION MASS SPECTROMETRY: ADVANCES IN MONITORING CLANDESTINE ACTIVITIES, SUPPORTING THE WARFIGHTER, AND CHEMICAL LABORATORY EDUCATION REDEVELOPMENT

by

Patrick Walter Fedick

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Chemistry

West Lafayette, Indiana

December 2018

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. R. Graham Cooks, Chair
Department of Chemistry
Dr. Hilkka I. Kenttämaa
Department of Chemistry
Dr. Marcy H. Towns
Department of Chemistry
Dr. Mary J. Wirth
Department of Chemistry

Approved by:
Dr. Christine A. Hrycyna
Head of the Graduate Program

To Anne "Dee Dee" Rufino

ACKNOWLEDGMENTS

First and foremost, I would like to thank my family for their unwavering support of my academic endeavors and for always encouraging and reassuring me through this expedition. From a young age my parents, Walter and Nancy, instilled the importance of education, hard work, perseverance, and challenged me to constantly expand my horizons. Through their inspiration and motivation, I have learned to embrace chemistry and the scientific method. My siblings, Anastasia and Andrew, gave me the determination to complete this degree by always providing me with a healthy dose of competition, all while cheering me on and lifting me up. Grandma Fedick and Dee Dee, even when I was a troublemaker as a child, never let me, or anyone else, forget my potential, all while providing me the luxury to explore things in my own and many times backwards way. To my two wonderful sibling-in-laws, Brandon and Deanna, thank you for all the support over this process. Finally, to my new godson, Carson James, thank you for giving me the welcomed distractions from writing this document the last few months.

My wife, Alissa, thank you for being my pillar of strength on the bad days, your words of encouragement provided me the strength to get through these times. Additionally, for every proofed essay, manuscript, poster and oral presentation, if it was not for you, I would not have succeeded at half of them. Most importantly thank you for your constant love and for showing me there is a reason to get out of the lab. To Alissa's family, who I now have the pleasure of calling my own, thank you Michael, Twila, Jordan, David, and Dana for all the support and care from the start of my graduate career. Thank you for always being understanding of my crazy schedule and allowing me to break from the activity at hand to write a paper at your kitchen table or on the couch while everyone else was doing something more enjoyable.

Before I move onto my Purdue family, I first must thank the wonderful professors at Monmouth University who without them, I would have never arrived at Purdue. Professors Tsanangurayi Tongesayi, Greg Moehring, Danuta Szwajkajzer, Catherine Duckett, Robin Kucharczyk, Jamie Kretch, Joe Tang, Dmytro Kosenkov, Azzam Elayan, Kayla Lewis, and Jonathan Ouellet, thank you for providing the foundation to build upon. Monmouth University will forever be a second home to me. Additionally, I must thank Drs. Richard Vachet, Vincent Rotello, Bo Yan, and all of members of the Vachet and Rotello groups for the introduction to mass

spectrometry during my research experience for undergraduates (REU) at the University of Massachusetts Amherst.

The largest thank you to my research advisor, Professor R. Graham Cooks, for providing me with the opportunity to work in Aston labs. Dr. Cooks not only taught me the world of mass spectrometry, gave a healthy skepticism of chromatography and the unnecessary overuse of high-resolution mass spectrometry, but most importantly demonstrated how a truly great scientist should think and conduct themselves. Not only have you been an excellent academic advisor, but also a great mentor outside of the laboratory and a gracious host over the many social functions over the last four years. I am grateful for my time in Aston Labs and look forward to coming back and working with the group in the future through collaborations. I am grateful for the help of Ms. Brandy McMasters who has made life in Aston Labs possible, through helping with orders, fixing my travel, and helping me plot when the best time to speak with Dr. Cooks was if I was trying to get away with something he may say no to. Beyond the professional, I truly enjoyed our constant chats in your office.

I am grateful to the former lab members who gave me my initial trainings in the laboratory, Drs. Ryan Bain, Christopher Pulliam, Stephen Aytron, Adam Hollerbach, Valentina Pirro, Alan Jarmusch, Zane Baird, Michael Wleklinski, Caitlian Falcone, and Karen Yannel. The guidance and influence of Drs. Ryan Bain, Kinsey Bain, Stephen Ayrton, Christopher Pulliam, and Adam Hollerbach can be seen throughout this dissertation and they vastly contributed to my personal development both as a scientist and an individual. Working with the rest of Aston labs has provided me the opportunity to learn from: Dr. Christina Ferreira, Dr. Pu Wei, Dr. Xin Yan, Dr. Zhenwei Wei, Dr. Brett Marsh, Clint Alfaro, Dalton Snyder, Fabien Thery, Kiran Iyer, Fan Pu, David Logsdon, Kiran Iyer, Zhuoer Xie, Xingshou Chen, Tsdale Mehari, Yangjie Li, Robert Schrader, Sangeeta Pandey, Lucas Szalwinski, Rong Chen, Hannah Brown, and Nicolás Gutiérrez. Finally, a major thanks to Stephen Ayrton and Robert Schrader for the many figures and graphical abstracts they have created for me over this process, many which will appear in this document.

I would also like to thank my committee members, Dr. Hilkka I. Kenttämaa, Dr. Marcy H. Towns, and Dr. Mary J. Wirth for all the help and advice they provided me throughout this process. Most notably Professor Towns for her intellectual discussions of chemical education and the navigation of the institutional review board (IRB). I must also thank Mr. Paul Bower, Mrs. Jeanne

Meyer, Mrs. Heidi Bagnall, Dr. Jiangou Mei, and Dr. David Thompson for their assistance in the chemical education projects described in Chapter 8.

I would also like to thank the many internship opportunities that I was fortunate enough to participate in during my graduate career. These helped shape my career goals, and the scope of research that I went on to perform during graduate school detailed in the following chapters. First working for the Federal Bureau of Investigation through the Honors Internship Program (HIP) during my first year of graduate school. Secondly, working for the Department of Homeland Security, Customs Border Protection Agency, through the Homeland Security Science, Technology, Engineering, and Mathematics (HS-STEM) program, especially, Dr. Renee Stevens, Dr. Eugene Bondoc, Dr. Jun-Ling You and Dr. Jusheng Qi. Thirdly, the Naval Surface Warfare Center, Crane through the Naval Research Enterprise Internship Program (NREIP), especially, Dr. Jonathan Dilger, Dr. Jack Caldwell, Dr. Kelly Thoreson, Dr. Douglas Papenmeier, Mr. Robert "Bud" Hoerter, Mr. Brian R. Sabo, and Mr. David Acton. Two microgrants from the Naval Surface Warfare Center, Crane provided funding for parts of three of the chapters in this dissertation. Finally, the Naval Air Warfare Center, China Lake through the Science Mathematics and Research for Transformation (SMART) program, especially, Mrs. Roxanne Quintana, Dr. Stephen Fallis, Dr. Lee Cambrea, Ms. Alicia Hughes, Mrs. Michelle Wade, and Mrs. Catherine Kusznir. The Department of Defense SMART program is also thanked for my funding the last year and a half of my PhD.

This dissertation also must recognize Dr. Christopher Mulligan and Dr. Nicholas Manicke for allowing me to collaborate with them and work in their laboratories on a variety of projects described below. I have also had the extreme pleasure of working on several experiments described in the subsequent chapters with the support of Dr. Wie Yu from Diagnostic anSERS and Metrohm, Mr. Brandon Milan from Neoteryx, and Dr. Brian Laughlin and Dr. Justin Wiseman from Prosolia. Finally, Applied Ballistics who permitted Ryan Bain and I to conduct our study on organic gunshot residues at their location.

To all my co-authors, without your collaboration I would not be able to write this document. In alphabetical order by last name I want to thank: Stephen Ayrton, Kinsey Bain, Ryan Bain, Bjorn Berendsen, Brandon Bills, Brian Bohrer, Jessica Coleman, Jonathan Dilger, Marcos Eberlin, William Fatigante, Tanya Flick, Seth Hall, Adam Hollerbach, Kiran Iyer, Zachary Lawton, Joseph Ludwig, Nicolas Manicke, Tsdale Mehari, Shunshun Miao, Eric Miklaszewski, Christopher

Mulligan, Michel Nielen, Adam O'Leary, Heather Osswald, Douglas Papenmeier, Valentia Pirro, Christopher Pulliam, Robert Schrader, Dalton Snyder, Sebastiaan Teunissen, Kelly Thoreson, and Arian van Asten.

Finally, a heartfelt thank you to my Purdue friends most notably: Hilary Brown, Heather Siebert, Omkaran "Omi" Menon, Janny Dinh, Samantha Ebner, and Gary Prato. These individua ls not only proofed papers, listened to presentations and discussed research ideas, but also kept me sane through the process. The amount of times we spent together studying for cumes, preparing for the original research proposal, or practicing presentations could only be matched by nights out and following morning brunches. When I made the trip from New Jersey to Indiana I was not sure what to expect or if I would enjoy the change in scenery and culture, but you all made it feel like home and for this I will be forever grateful.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF REACTION SCHEMES

ABSTRACT

Author: Fedick, Patrick, W. PhD
Institution: Purdue University
Degree Received: December 2018
Title: Ambient Ionization Mass Spectrometry: Advances in Monitoring Clandestine Activities, Supporting the Warfighter, and Chemical Laboratory Education Redevelopment.
Committee Chair: R. Graham Cooks

Ambient ionization mass spectrometry enables rapid *in-situ* analysis of a plethora of analytes that are relevant to the forensic and defense communities. As the arsenal of ambient ionization techniques, aimed at solving specific targeted problems, continues to expand, the adoption of these techniques into non-academic settings has been relatively slow. At times, although the technique can provide answers in a more rapid and cheaper manner, the technique does not pass all of the required legal rules for a particular analysis when dealing with forensic evidence. This can be demonstrated with the rapid detection of drugs by paper spray ionization mass spectrometry. Paper spray ionization mass spectrometry can have drugs deposited onto the paper substrate, the paper can wipe a surface for trace analytes, and there are commercial and automated ionization sources for this process. While analysis by paper spray is rapid, the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) states that a minimum of two instrumental techniques need to be utilized. Utilizing paper substrates that have nanoparticles embedded for surface enhanced Raman spectroscopy, that can also be utilized for paper spray ionization mass spectrometry, makes ambient ionization more appealing as it completes that first legal requirement.

Other times, the slow adoption of these new ambient ionization techniques is due to specific communities not being aware of ambient ionization, and specific applications have not yet been demonstrated. Swab touch spray ionization mass spectrometry follows similar processes as paper spray ionization, as the swab acts both as the sampling substrate and the ionization source and can swab for analytes in a manner where the paper substrate may be damaged and unable to perform the ionization for analysis. This can be seen for the swabbing of organic gunshot residues and explosives, both of which current methods already use a swab for sampling but then need lengthy extraction techniques. The applicability of paper spray ionization and swab touch spray ionization

for these forensic and defense analyses is only furthered by the fact that they both couple extremely well with portable mass spectrometers for analysis in the field.

There are also many fields that ambient ionization is just starting to take its place in the analytical toolbox. Two such defense fields that are just beginning to expand into ambient ionization are the analysis of pyrotechnics and microelectronics. Pyrolysis gas-chromatography mass spectrometry methods have been developed and utilized for environmental tests for pyrotechnic formulation, but they are slow and there is an abundance of cleaning steps between analyses to prevent carry over and contamination. Using paper and swabs as the collection device and ionization source for environmental analysis of these pyrotechnics allow for them to be functioned at ambient conditions at the scale at which will be utilized in the field by the Warfighter. Similarly, authenticating microelectronics by desorption electrospray ionization mass spectrometry removes the subjectivity of the current methods, while rendering the integrated circuit intact enabling future use if deemed as a genuine part. By taking slower or more subjective tests, in a field that has not utilized ambient ionization heavily in the past and adding these new capabilities to their tool chest expands the acceptance and future applications of the technique.

As acceptance and utilization of ambient ionization grows, the next generation of scientists need to have hands on training in these techniques. Through the development of new teaching laboratories that couple both the fundamentals of the technique at hand, while also examining an interesting application to better engage the students, a number of laboratory exercises have been developed. The creation of new laboratory exercise utilizing the next generation of instrumentation and analytical techniques is vital for the future and rapid application of these techniques. The work discussed herein chronicles the utilization and demonstration of ambient ionization mass spectrometry in monitoring clandestine activities, supporting the Warfighter, and redeveloping chemical laboratory education.

CHAPTER 1. INTRODUCTION.

1.1 Overview

This dissertation is a culmination of efforts utilizing ambient ionization mass spectrometry for three major topic areas: monitoring clandestine activities (Chapters 2-3), supporting the Warfighter (Chapters 4-5), and redeveloping chemical laboratory education (Chapter 6). Chapter 7 deals with reaction acceleration of a prominent pharmaceutical reaction and has applications to all three topic areas, hence it was also included as a separate chapter. All of these chapters are tied together by the broad range of scientific challenges that ambient ionization mass spectrometry techniques aim to solve.

Mass spectrometry (MS) is an analytical technique that focuses on the analysis of ionized chemical species.[1-2] A mass spectrometer is constructed of three parts: the ion source, the mass analyzer and the detector.[3] The ion source generates the analyte ions (positively or negatively charged) that are separated by the mass analyzer based on their mass-to-charge-ratio. Where there are many types of mass analyzers, this work has primarily been performed using ion traps. However, there will be mentions of triple quadrupole mass spectrometers, orbitrap mass spectrometers, and time of flight mass spectrometers throughout the dissertation, as well as, portable mass spectrometers which will be introduced in Section 1.3. Once the ions have been separated by the mass analyzer, the ions interact with the detector and a mass spectrum is generated. The mass spectrum is a plot of the ion signal or intensity versus the mass-to-charge ratio. Much of what ties this dissertation together is not the analyzer or detector, but instead are how the ions are generated prior to entering the instrument. Every project discussed utilizes ambient ionization techniques and innovative ways of applying these techniques to tackle new problems.[4]

1.2 Ambient Ionization

Ambient ionization was disclosed with the first publications of desorption electrospray ionization (DESI)[5] in 2004 and of direct analysis in real time (DART) in 2005,[6] which permitted mass spectral analysis of analytes in the open environment in a rapid manner, with little to no sample preparation.[7-8] Since the disclosure of these first two ambient ionization techniques, a plethora of variants and new techniques have been developed.[8] Depending on the problem at hand,

a specific ion source could readily be employed or if no source existed, individuals can be inspired to develop their own. With rapid analysis times and simple techniques, ambient ionization has had a large impact on the forensic community.[9] Additionally, these ambient ionization techniques are customizable to the targeted problem, couple easily with portable mass spectrometers, enabling efficient *in-situ* analyses, and are ideal for defense and homeland security applications. Much of this dissertation will focus on three ambient ionization techniques: DESI,[5] paper spray ionization,[10] and swab touch spray ionization,[11] all of which will be described in the following subsections.

1.2.1 Desorption Electrospray Ionization

Desorption electrospray ionization (DESI), the first ambient ionization mass spectrometry technique, was developed in the Cooks group in 2004.[5] During DESI, a stream of charged microdroplets are directed to a surface where droplets impact and sputter off smaller charged "secondary" droplets which contain species desorbed from the surface. In the secondary droplets these species desorbed from the surface are ionized and delivered to the mass spectrometer. As the charged secondary droplets enter the mass spectrometer they are transformed from sub-micron sized droplets to fully desolvated ions. Figure 1.1 shows a pictorial representation of the DESI process. This process can be thought of as being similar to SIMS, specifically variants or SIMS that use large water clusters of Bucky balls as the impacting particle.[12-13] DESI has been demonstrated in a variety of forensic applications[14-15] such as detecting trace drugs from latent finger prints.[16] The forensic capabilities of DESI in regard to differentiating counterfeit and genuine microelectronics will be explored in Chapter 5.

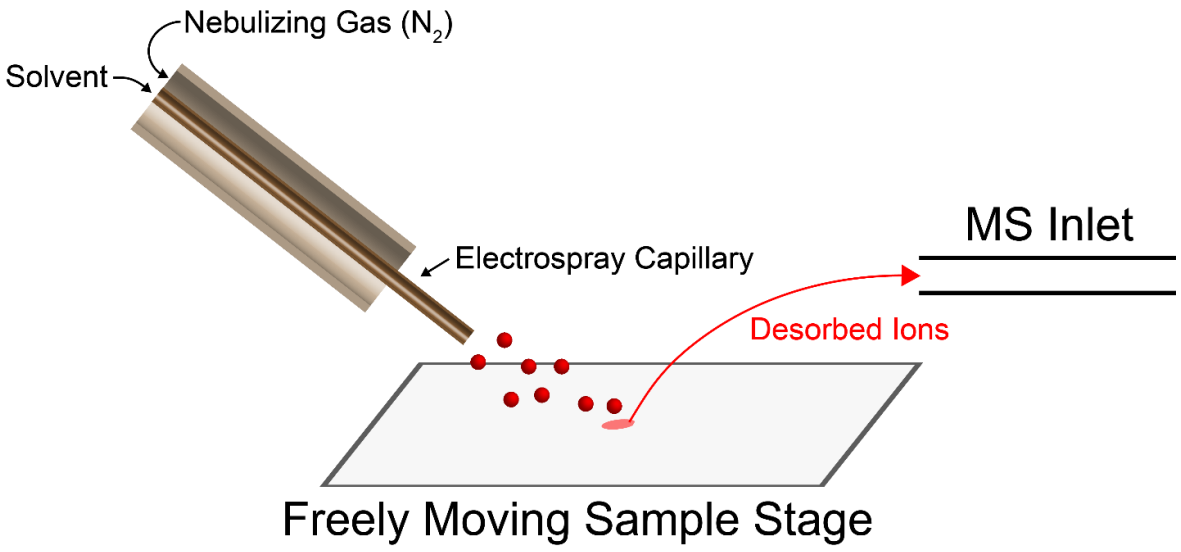


Figure 1.1 Desorption electrospray ionization (DESI) mass spectrometry schematic where the primary droplets are directed towards a surface and the analyte containing secondary droplets enter the mass spectrometer for mass analysis.

Reactive DESI, a variant also used in Chapter 5, incorporates derivatization reactions to improve the analysis of hard to ionize or easily suppressed species. In reactive DESI, a derivatizing reagent is doped into the primary solvent spray and the reaction between the doped in reagent and the material desorbed from the surface can be performed during the time it takes the secondary charged droplet to reach the mass spectrometer. Derivatization is a common method for selectively increasing the ionization efficiency of particular classes of analyte in complex mixtures.[17]

1.2.2 Paper Spray Ionization

Paper spray (PS) ionization utilizes a paper or porous substrate cut to a sharp point. The analytes are deposited via pipetting, swabbing or dipping, and are wicked through the substrate by a solvent that is applied to the back of it. When high voltage is applied, there is an enhanced electric field at the tip of the paper enabling the ionization of the analyte molecules.[18] Since there is no need for pneumatics or sample preparation, it is one of the simplest techniques to couple to a portable mass spectrometer. PS has been applied greatly in the forensic community, from quantitative determination of drugs in whole blood,[19] detection of explosives,[20] and detection of chemical warfare simulants.[21] Some of the most studied work with PS coupled to a portable mass spectrometer is detection of drugs of abuse.[22-25] Figure 1.2 shows a pictorial representation of paper

spray ionization with vendor and part numbers when coupled to a FLIR AI-MS 1.2 portable mass spectrometer, as well as a photograph of the same setup.[26]

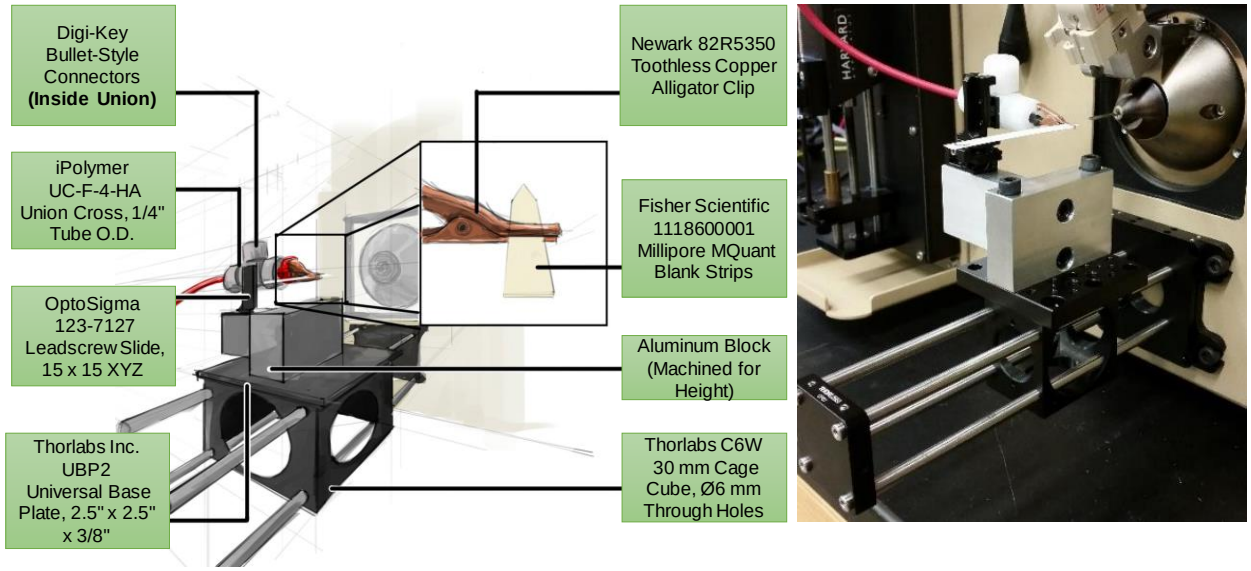


Figure 1.2 Paper spray ion source setup used on a FLIR AI-MS 1.2 portable mass spectrometer. Vendor and parts are labeled. The most notable aspect is the sharp tip of the paper and the copper clip to apply the voltage.

While most of the manuscripts involving PS use home-built sources (as is the case for most of Chapter 2), there is a commercially available PS ion source sold by Prosolia (Indianapolis, IN) named the Velox 360. The Velox 360 PS source attaches directly onto the mass spectrometer and utilizes cartridges with precut paper. The ion source will apply the spray solvent and high voltage in an automated fashion. Figure 1.3 shows a picture of the Velox 360 source and the paper cartridges. This a variant of the paper cartridges used by the Velox 360 was also utilized in Chapter 2.

Figure 1.3 Commercial paper spray ion source sold by Prosolia. The cartridges stack in the center tower and are analyzed one at a time. The solvent and the potential are applied through the software and after the method is developed it is fully automated.

1.2.3 Swab Touch Spray Ionization

A relatively new ambient ionization method developed by the Cooks group is swab touch spray ionization.[27] Swab touch spray ionization utilizes a rayon-tipped swab, or alternative swabbing substrates, to collect the analytes of interest by swabbing the dry or wetted swab over the area of interest. The surface analytes are adsorbed onto the swab surface after moving the swab over the surface of interest. The swab is constructed with an aluminum handle which allows a high voltage lead to be connected directly to the swab to promote ionization when solvent is applied.[28] Other handles like wood or plastic are nonconductive and require additional steps for ionization.[11] The solvent systems for swab touch spray ionization are robust and can be modified with derivatizing agents if the reactive form of this ionization system is required. Figure 1.4 shows a pictorial representation of swab touch spray ionization.

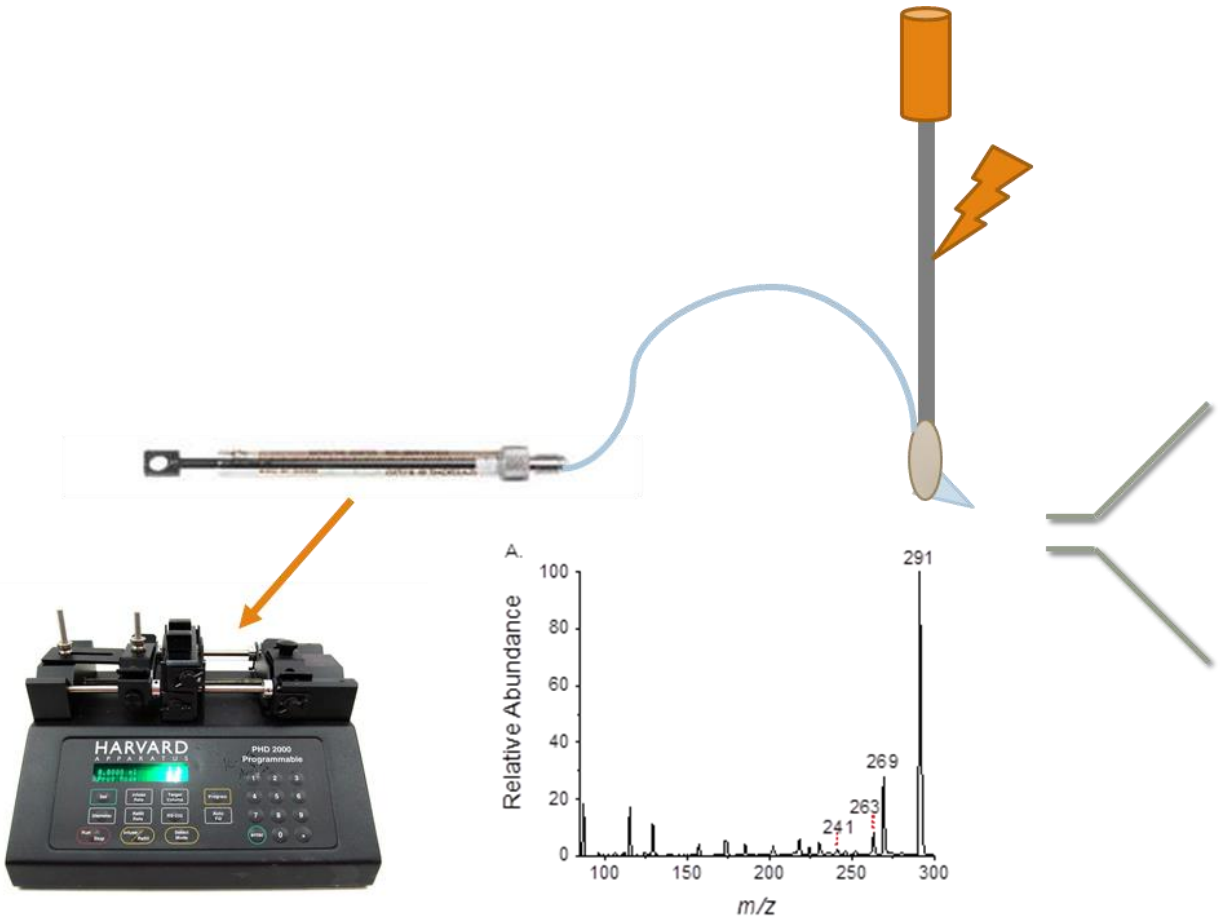


Figure 1.4 Overall setup of swab touch spray. A swab with a metallic handle has solvent applied to it via a syringe and syringe pump. A high voltage is applied to the handle of the swab and a Taylor cone is produced ionizing analyte molecules into the inlet of the mass spectrometer resulting in a mass spectrum.

Like PS, no pneumatics or sample preparation is required. The benefits of swab touch spray ionization are its ability to collect samples in areas in which PS can have damage to the sampling surface, swabs are already used by the forensic community so there is less of a conceptual barrier, and its ability to have a very stable spray for prolonged periods of time. Additionally, the swabs are wrapped in a tamperproof packaging that is ideal for forensic, defense, and homeland security applications, where chain of custody issues may arise. Swab touch spray ionization mass spectrometry has been applied to bacterial analysis from the back of human throats,[29] drugs of abuse[27] detection in oral fluids, analysis of human gliomas,[30] as well as, the detection of organic

gunshot residues[31] and explosives[32] from a variety of surfaces including hands which will be discussed in Chapter 3.

1.3 Portable Mass Spectrometers

Portable mass spectrometers are becoming more prevalent due to improved instrumentation, commercialization, and the robustness of new ionization methodologies. Ambient ionization methodologies have evolved to target specific real-world problems, fulfill requirements of the analysis at hand and couple readily to portable MS systems. Portable instrumentation, such as MS, has been of increased interest to the broader scientific community due to the inherent capability of performing *in-situ* analyses.[33] These MS have smaller footprints compared laboratory-based "benchtop" counterparts allowing for the transportation to locales of interest.[34]

A review of current literature shows particularly high activity with regards to portable MS instrumentation and application development amongst the environmental, forensic, and defense communities.[22, 35-36] Portable MS systems are of particular interest due to the sensitivity, specificity, and rich chemical information provided by the technique, particularly tandem MS analysis.[37-38] A recent review of miniaturized MS systems outlined and compared the major research directions and entities offering commercial platforms.[39] Interestingly, a major uptick in applicability of portable MS systems has been recently seen, not from major instrumental modification (e.g. vacuum system, mass analyzer), but from advances in ionization source technology, particularly ambient ionization.[40] Ambient ionization allows sample examination in its native state with little to no sample preparation, such as explosive residues on luggage[41] or fingerprints[42] or drug residues from clandestine drug lab apparati,[23] the forensic community continues to pursue adoption and expansion of these techniques towards portable devices.[43-44]

Since the first report of ambient ionization on a portable MS system, researchers have sought to provide the fieldability, ruggedness, and usability that is required for practical *in-situ* analysis by non-technical operations (e.g. forensic practitioners, law enforcement officers). Subsequently, validation efforts have sought to assess competency towards adoption in the forensic sciences,[24] for which there is an inherent need for court admissibility and method standardization. The rigor and variable nature of field-borne sample processing can be burdensome, but ambient

MS techniques continue to advance towards higher performance, with specific sources showing chemical and/or matrix-specific proficiency depending on the application area.

1.3.1 Purdue Homebuilt Mini 12 Mass Spectrometer

One of the portable MS that has been utilized in Chapter 3 is the homebuilt Mini 12 mass spectrometer.[45] The Mini 12 which weighs approximately 25 kg with its outer case and its paper spray cartiage holder attached, both which have been removed for the analyses performed in the later chapter making it slightly lighter (Figure 1.5). With the outer case attached the Mini 12 has the outer dimensions of 19.6 inches in length, 12.1 inches in width and 16.5 inches in height. The Mini 12's mass analyzer is a rectilinear ion trap that operates in both positive and negative ion mode and can perform multiple stages of mass analysis. Its power consumption is much less than that of a commercial benchtop instrument, consuming less than 100 W of power. It has the capability of being operated either from a 110 V AC outlet or from a battery attachement.

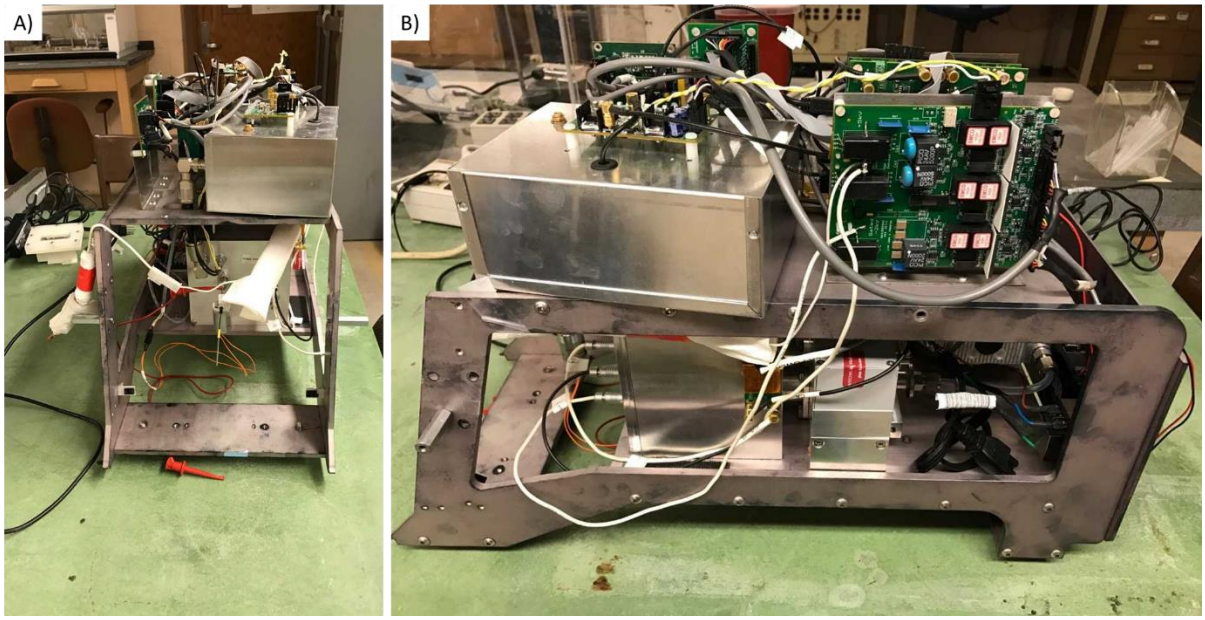


Figure 1.5 The portable Mini 12 mass spectrometer with its protective cover removed. The Mini 12 weighs 25kg and can perform both positive and negative ionization, as well as, multiple stages of mass analysis. A) The front view and inlet of the Mini 12. B) The side view of the Mini 12.

The low power consumption is is achieved by using significantly smaller pumps on the Mini 12 when compared to benchtop models. One HiPace 10 (Pfeiffer Vacuum, Nashua, NH) and one two-stage diapgragm rough pump (KNF Beuberger, Trenton, NJ) keep the operational pressure of the mass specrtrometer as low as 1×10^{-5} Torr. To overcome the difference in pressure of the ambient environment and the pressure within the ion trap, a discontinous atmoshperic pressure interfeace (DAPI) is utilized to compensate for the reduced pumping speed.[46] The DAPI is a pinch valve which is primarily closed and opens for ~10 ms to allow ions to enter the trap. After the ions enter, the pinch valve is again closed to allow the pressure to fall to an appropriate level for mass analysis.

1.3.2 PURSPEC Technologies Mini β Mass Spectrometer

The next generation of the Mini 12 MS has been commercialized by PURSPEC Technologies (Beijing, China) with the release of the Mini β which was utilized in Chapter 2. This commercialized portable MS weighs 22 kg, 3 kg lighter than its predecessor. The dimensions of the Mini β are slightly smaller than the Mini 12 with an outer dimension of 18.5 inches in length, 9.4 inches in width and 11.8 inches in height. Figure 1.6 shows the Mini β with the outer cover removed. Similar to the Mini 12 the DAPI is utilized to maintain the pressure below 1×10^{-5} Torr. One HiPace 30 turbomolecular pump (Pfeiffer Vacuum, Nashua, NH) and one two-stage diaphragm roughing pump (KNF Neuberger, Trenton, NJ) were used to achieve these pressures. The Mini β operates in both negative and positive ion mode and is capable of performing multiple stages of mass analysis.

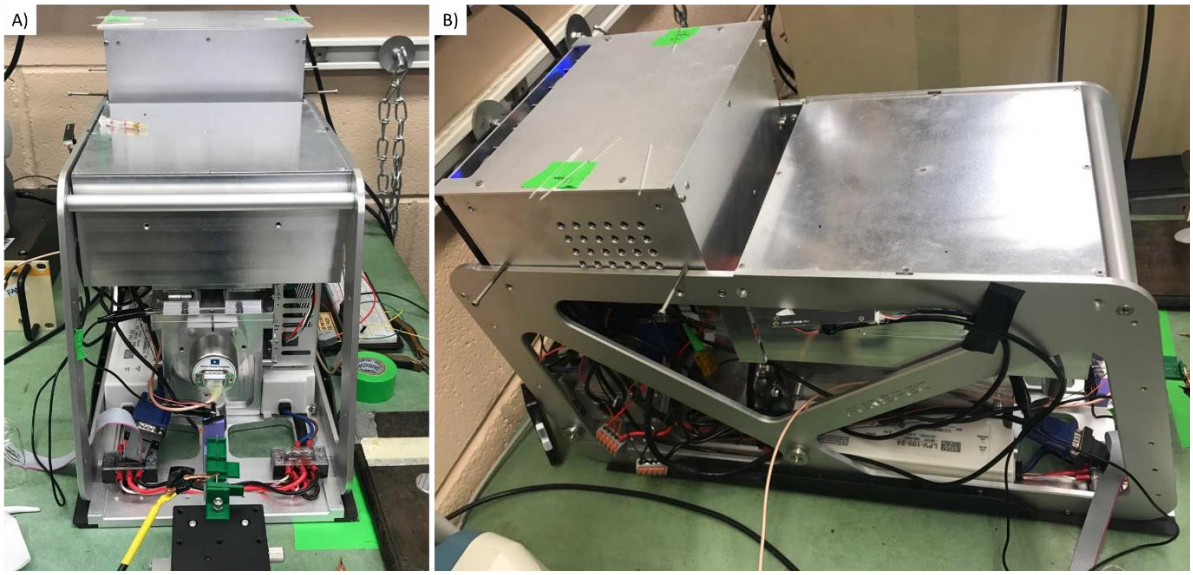


Figure 1.6 The portable Mini β mass spectrometer with its protective cover removed. The Mini β weighs 22kg and can perform both positive and negative ionization, as well as, multiple stages of mass analysis. A) The front view and inlet of the Mini β B) The side view of the Mini β.

1.3.3 FLIR Systems AI-MS 1.2 Mass Spectrometer

Another commercialized portable MS system that was utilized in Chapter 3 is the FLIR Systems AI-MS 1.2 (West Lafayette, IN) cylindrical ion trap mass spectrometer.[25] While heavier and larger than the previous two described portable mass spectrometers, this commercial system has been ruggedized for *in-situ* analysis in harsh environments (Figure 1.7).[23] The AI-MS 1.2 weighs ~ 45kg and has an outer dimension of 23.6 inches in length, 19.6 inches in width and 15.7 inches in height. The AI-MS 1.2 can also be operated in both positive and negative ion mode and can perform multiple stages of mass analysis. Unlike the Mini 12 and β, the AI-MS 1.2 does not utilize a DAPI and maintains a constant atmospheric pressure inlet due to the larger pumps that maintain the pressure of the trap. It still has relatively low power requirements and is also capable of being run with a battery supply.

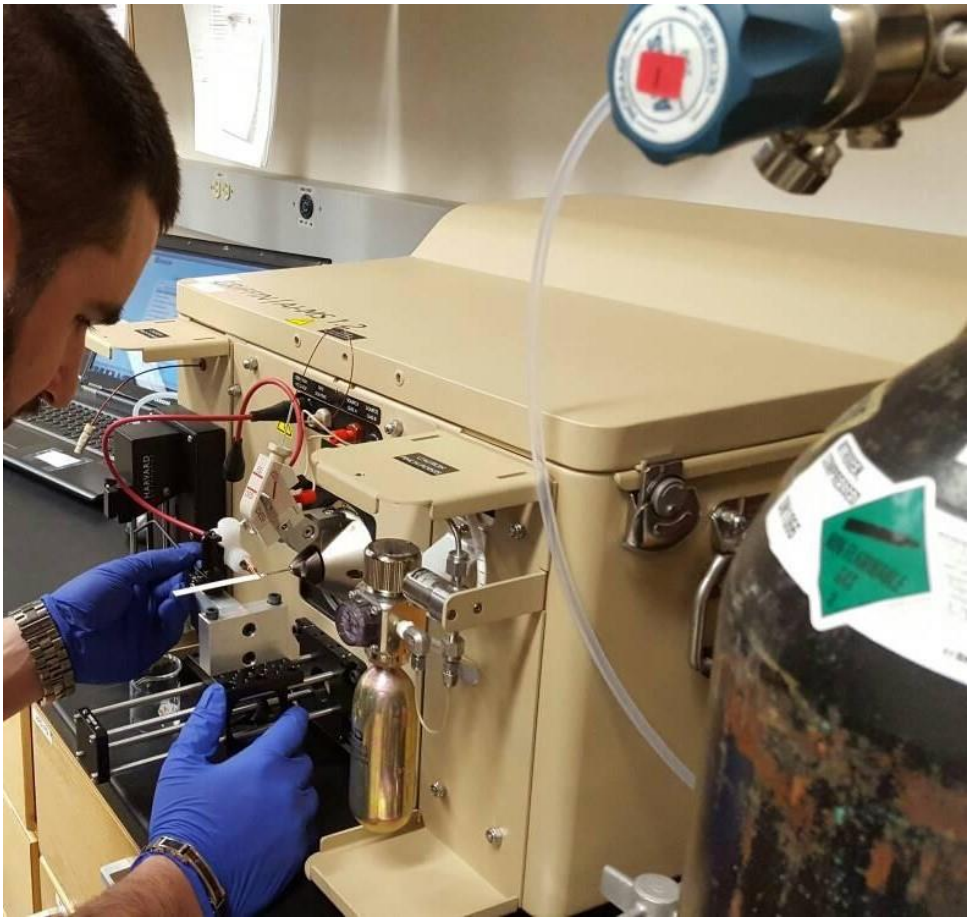


Figure 1.7 The FLIR System AI-MS 1.2 portable mass spectrometer. The AI-MS 1.2 weighs 45 kg and can perform both positive and negative ionization, as well as, multiple stages of mass analysis. Paper spray ionization is being performed in the photograph.

The AI-MS 1.2 has a centralized mounting system that was developed to incorporate modular, rapidly interchangeable ionization sources that were comprised of low-cost, commercially-available parts (Figure 1.8).[24] No extraneous power supplies or pressurized gases are needed, enhancing overall portability for the system. To date, the Mulligan group has developed paper spray ionization, paper cone spray ionization, swab touch spray ionization, and atmospheric pressure chemical ionization (APCI) sources to interface with the AI-MS 1.2 platform.[26] Chapter 3 will describe work with the swab touch spray ionization source done in collaboration with the Mulligan group.

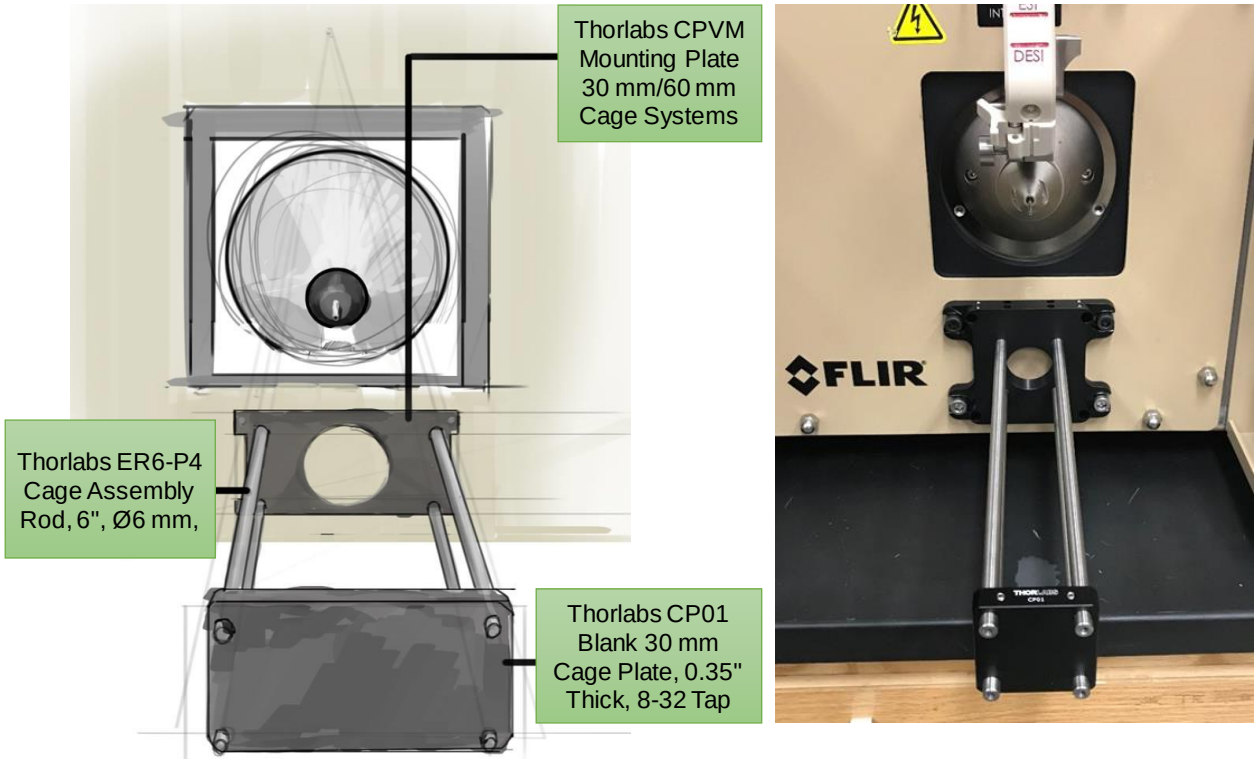


Figure 1.8 The FLIR System AI-MS 1.2 portable mass spectrometer's centralized mounting system that ambient ionization sources were built upon.

1.4 Raman Spectroscopy

While this dissertation primarily focuses on applications and utilizations of mass spectrometry, Chapter 2 incorporates the pairing of two instrumental techniques, mass spectrometry and Raman spectroscopy. The non-destructive Raman spectroscopy was performed first and was followed by the destructive mass spectrometry analysis. Raman spectroscopy is a light scattering technique in which a laser light source irradiates a sample and the scattered light that is shifted in energy from the laser frequency is recorded.[3] This process is extremely weak compared to the Rayleigh scattering in which the measured light has the same frequency of the laser light.[47] This Raman shift is the basis for the vibrational spectroscopic technique and provides information on molecular vibrations, which is the basis of for the sample identification.[47] Plotting the intensity of Raman shifted light versus frequency results in a Raman spectrum of the sample. On this scale, the band positions will lie at frequencies that correspond to the energy levels of different functional group vibrations.[48]

1.4.1 Surfaced Enhanced Raman Spectroscopy

Surface-enhanced Raman spectroscopy (SERS) is an extension of Raman spectroscopy, where metallic nanostructures are used to enhance the intensity of the otherwise weak Raman scattering.[49] In practice, this means that the sample is placed on the nanostructured metal surface and then it is illuminated with a laser, just like in the case of normal Raman spectroscopy. However, when the metallic structure interacts with the light, surface plasmons are excited, which consequently increases the measurable Raman signal.[50] The increase in Raman signal has made SERS an appealing instrumental method for forensic analyses.[51-53]

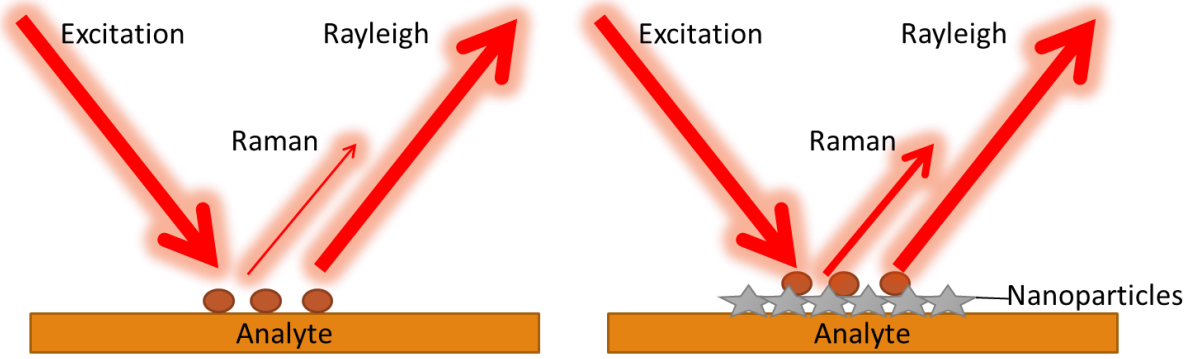


Figure 1.9 Visual depiction of the enhancement of the Raman scattering by the deposited nanoparticles on the surface enhanced Raman spectroscopy substrate.

While nanoparticles can be deposited on a number of substrates, one SERS substrate of particular interest is paper.[54] A particular advantage of paper SERS (pSERS)substrates is the ease with which they can be created using inkjet printers.[55] Fabrication of paper SERS substrates is straight-forward compared to the typical micro-fabrication of SERS substrates lowering the entrance barrier for utilization and it minimizes the cost of fabrication.[56] These pSERS substrates have been used to detect drugs[57], fungicides[58], pesticides[57] and polymerase chain reaction (PCR) products.[59] An additional major benefit for pSERS substrates are the ability to after taking a Raman spectrum, is it can be utilized for PS mass spectrometry. Finally, these substrates are commercially available from Metrohm (Riverview, FL). Figure 1.10 shows both the gold and silver versions of the two commercial systems which have been utilized in Chapter 2.

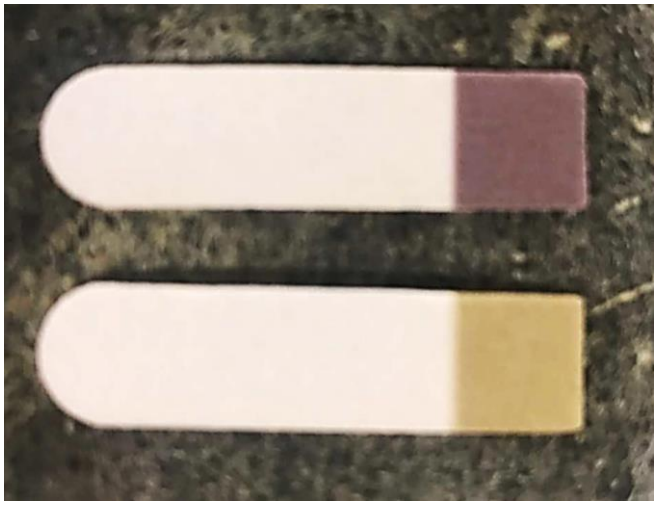


Figure 1.10 Commercial pSERS substrate. The top pSERS substrate has gold nanoparticles inkjet printed onto it and the bottom has silver nanoparticles inkjet printed onto it.

1.4.2 Portable Metrohm Mira Raman Spectrometer

Much like the benefits seen in utilizing a portable mass spectrometer for forensic, defense and homeland security applications, the same is applied to performing Raman spectroscopy in the field. The Metrohm Instant Raman Analyzer (Mira) (Riverview, FL), a handheld Raman spectrometer, has been developed for *in-situ* analyses (Figure 1.11). This commercialized portable Raman spectrometer weighs 750 g and has an outer dimension of 3 inches in length, 1.5 inches in width and 5 inches in height. It has a 3.7-inch touch screen display that is compatible with wearing gloves. The Mira Raman operates with a laser that has a wavelength of 785 nm with the output power of less than or equal to 100 mW, all while only being powered by two AA batteries. This handheld Raman has a wavenumber range from 400 to 2,300 cm^{-1}. There are a number of attachments that the Mira Raman can utilize including the calibration standard and the SERS attachment that couples with the paper SERS substrates that Metrohm also sells, both of which will be utilized in Chapter 2.

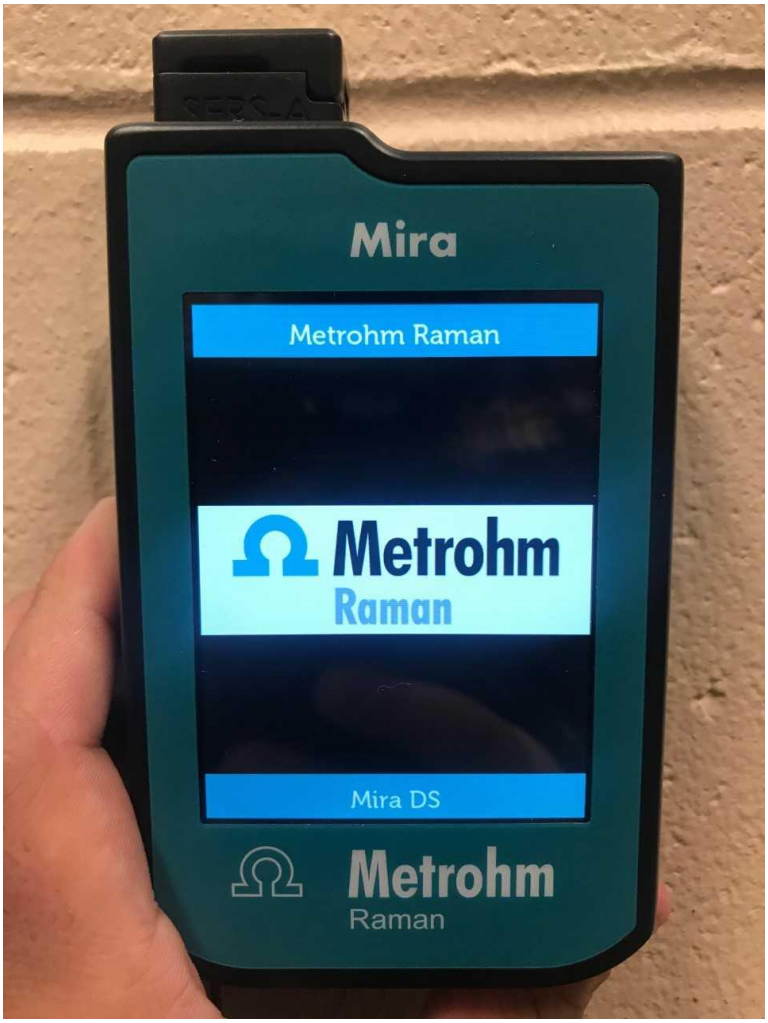


Figure 1.11 Portable and handheld Metrohm Mira Raman spectrometer with the pSERS attachment.

CHAPTER 2. FORENSIC SAMPLING BY A SINGLE PAPER SUBSTRATE FOLLOWED BY DUAL ANALYSIS BY SURFACE ENHANCED RAMAN SPECTROSCOPY AND MASS SPECTROMETRY FOR IDENTIFICATION AND CONFIRMATION.

Portions of this chapter have been published in Analytical Chemistry.

1. Fedick, P.W.*, Bills, B.J., Manicke, N.E., Cooks, R.G. "Forensic Sampling and Analysis from a Single Substrate: Surface-Enhanced Raman Spectroscopy Followed by Paper Spray Mass Spectrometry" *Analytical Chemistry*, (2017), **89**, 10973-10979. DOI: 10.1021/acs.analchem.7b02798.

2.1 Abstract

Sample preparation is the most common bottle-neck in the analysis and processing of forensic evidence. Time-consuming steps in many forensic tests involve complex separations such as liquid and gas chromatography or various types of extraction techniques, typically coupled with mass spectrometry (e.g. LC-MS). Ambient ionization ameliorates these slow steps by reducing or even eliminating sample preparation. While some ambient ionization techniques have been adopted by the forensic community, there is significant resistance to discarding chromatography as most forensic analyses require both an identification and a confirmation technique. Here we describe the use of a paper substrate, the surface of which has been inkjet printed with silver nanoparticles, for surface enhanced Raman spectroscopy (SERS). The same substrate can also act as the paper substrate for paper spray mass spectrometry. The coupling of SERS and paper spray ionization creates a quick, forensically feasible combination. Finally, the paper SERS substrates can be analyzed easily by portable Raman spectrometers and portable mass spectrometers making *in-situ* analysis for forensically relevant systems, that pass forensic requirements, a possibility.

2.2 Introduction

Forensic science relies heavily on so-called "hyphenated techniques" such a gas chromatography mass spectrometry (GC-MS) or liquid chromatography mass spectrometry (LC-MS) due to their long history of providing reliable, reproducible and validated information.[60-61] Whilst reliable, these hyphenated analytical techniques suffer from relatively long analysis times and they are typically not amenable to *in-situ* analysis. The standards set by ASTM International[62]

and Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations[63] follow these practices, which makes non-chromatographic approaches a challenge unless a more attractive capability is newly available. These standards state that mass spectrometry is a validated "Category A" technique, the highest category based on discriminating power, but that a secondary technique must also be utilized, for example, nuclear magnetic resonance (NMR) spectroscopy, Raman spectroscopy, any of a number of separation techniques, or even colorimetric tests.[62]

Desorption electrospray ionization (DESI)[5], along with direct analysis in real-time (DART)[6], the first ambient ionization techniques, gave the mass spectrometry community the ability to sample analytes in the open atmosphere transforming how sampling is performed. Two major benefits of ambient ionization are its definitive features: first, formation of ions in the ambient environment and second, limited (if any) sample preparation. Ambient ionization mass spectrometry applications in forensic science are outlined in two recent reviews.[9, 64] Paper spray ionization is an ambient ionization method which makes use of a paper substrate cut to a sharp tip.[65] Ions are generated with the application of a high voltage and solvent, and this simple technique can be used for direct sampling of complex mixtures.[18] Paper spray ionization has proven useful in the analysis of a wide variety of samples including dried blood spots[66], drugs of abuse[67], chemical warfare agents[21] and bacteria[68]. Recent advances in paper spray ionization are ongoing, for example surface modifications for improved ionization or reactive applications[69-73]. Although paper spray excels as a rapid, cost-effective and easy-to-use method, forensic applications require a secondary technique for analyte confirmation.

Another paper based method that has been developed, not for mass spectrometry but rather for Raman spectroscopy, involves the use of paper surface Raman substrates[55]. Raman spectroscopy has gained popularity in forensics due to the increased sensitivity achieved in Surface Enhanced Raman Spectroscopy (SERS).[51] A particular advantage of paper SERS substrates is the ease with which they can be created using inkjet printers.[55] Fabrication of paper SERS substrates is straight-forward compared to the typical micro-fabrication of SERS substrates and it minimizes the cost of fabrication.[56] While pSERS substrates are commercially available and attractive for forensics, this method too requires a second instrumental technique for confirmation.[62-63]

Recognizing the complementary nature of these two methods, we demonstrate here the utilization of a commercial pSERS substrate for Raman spectroscopy analysis followed by mass spectrometry. The amalgamation of the two techniques provides a simple and fast forensic

methodology requiring minimal sample preparation (Figure 2.1). The first proof of principal demonstrations were performed on benchtop instruments, however, the ability to perform *in-situ* on a portable mass spectrometer[44] and portable Raman spectrometer is ideal for forensic analyses. The ability to perform this technique *in-situ* enables these forensic analyses to be performed in the field and mitigate the need for samples to be transported back to the forensic laboratory.

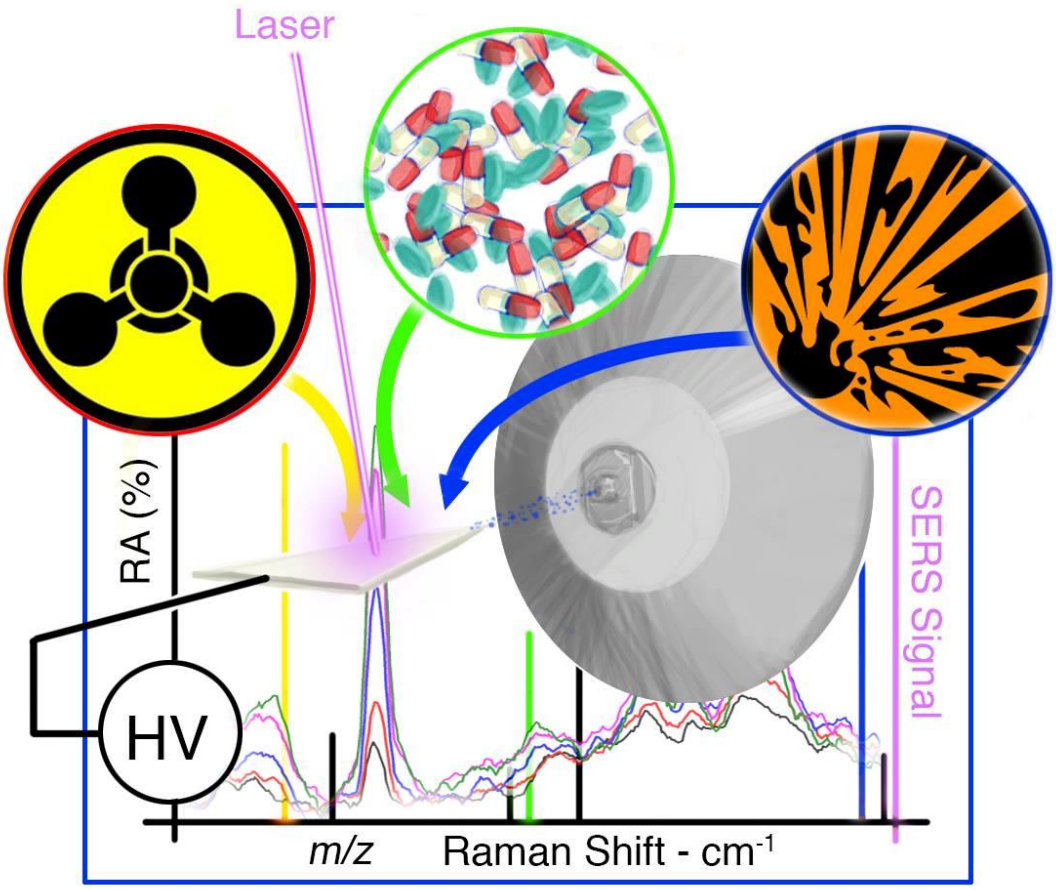


Figure 2.1 A pictorial representation of the paper surfaced enhanced Raman spectroscopy substrate being analyzed by Raman spectroscopy followed by paper spray ionization mass spectrometry for the analysis of chemical warfare agent simulants, drugs, and explosives.

2.3 Experimental

2.3.1 Reagents and Workflow

HPLC-grade methanol, acetonitrile, and the chemical warfare agent simulants (DMMP, DIMP, dichlorvos) were purchased from Sigma-Aldrich (St. Louis, MO). The drugs and explosives were purchased from Cerilliant (Round Rock, Texas). All samples on the benchtop

systems were analyzed after placing 3µg, a forensically relevant mass, of material on the pSERS substrate. The portable systems placed only 1µg onto the surface of the substrate. Unmounted Gold 2.0 and silver pSERS substrates were purchased from Diagnostic anSERS (College Park, MD), which was later acquired by Metrohm (Riverview, FL), and future studies purchased the substrates from Metrohm. For the benchtop system the paper spray analysis was conducted using a paper spray cartridge. A Biopsy punch with plunger, 3mm, was purchased from Integra Miltex (York, PA) to enable coupling the pSERS substrate with the spray cartridge. Paper spray cartridges were machined from Delrin plastic from McMaster-Carr, (Elmhurst, IL) on a bench-top mini milling machine by Sherline (Vista, CA). Whatman grade 31 ET chromatography paper was used for the spray substrate and was purchased from GE Healthcare Life Sciences (Pittsburgh, PA).

The analysis workflow for the benchtop systems is described in Figure 2.2. Standard solutions were pipetted onto the pSERS substrate, and a Raman spectrum was recorded after drying. The pSERS substrate was then placed in the paper spray cartridge, and a paper spray mass spectrum was recorded for the same sample. The entire analysis time was a few minutes. The pSERS substrates were biopsy punched because it allowed five samples to be analyzed from one commercial substrate, which is a cost saving step. The biopsy punch is not a requirement, and to show that the commercial pSERS substrate could be used without that step, the drugs hydromorphone and morphine were analyzed on the full pSERS strip.

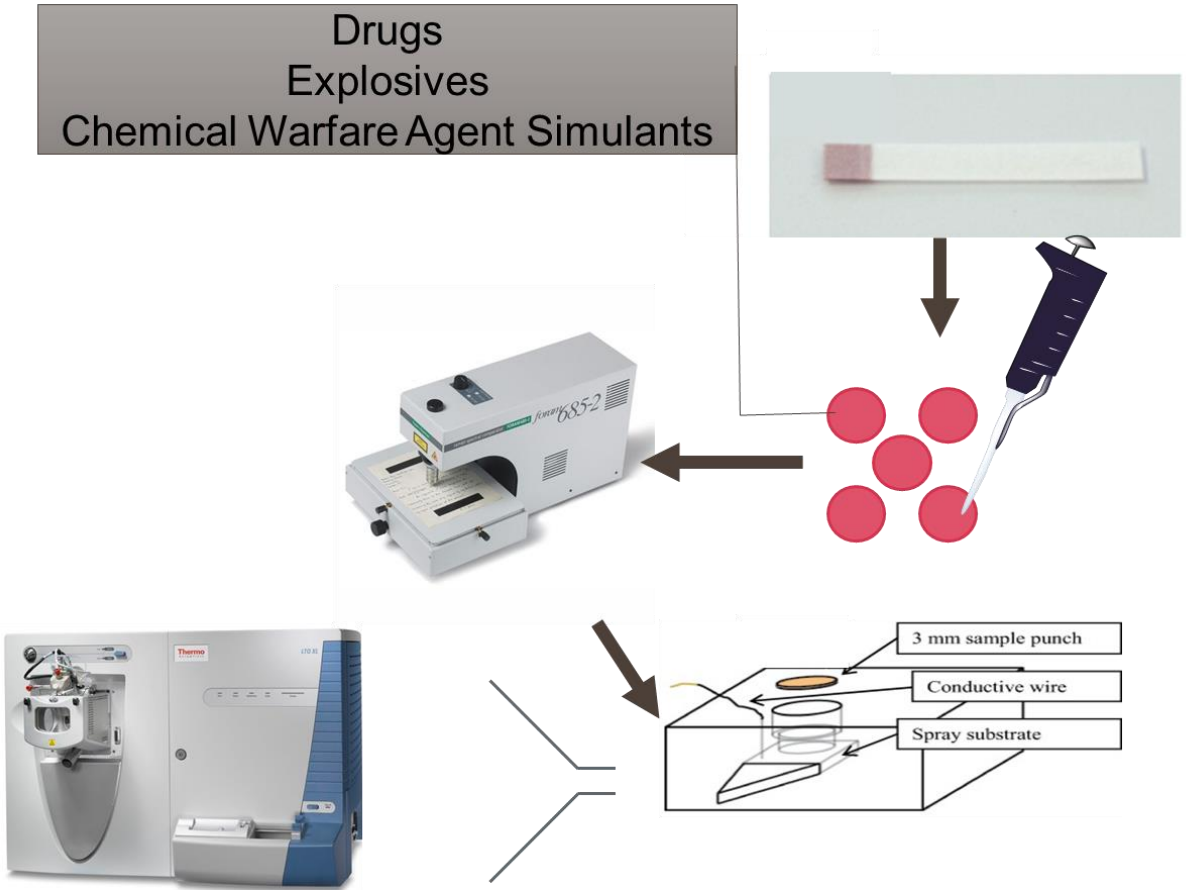


Figure 2.2 Experimental workflow for this dual instrumental analysis system on benchtop systems, starting from the biopsy punch and sample deposition, then to Raman spectroscopy and finally to paper spray ionization mass spectrometry.

The analysis workflow for the portable systems is described in Figure 2.3. Standard solutions were pipetted onto the pSERS substrate, and a Raman spectrum was recorded after drying. The pSERS substrate was then cut to a sharp tip and a paper spray mass spectrum was recorded for the same sample. The entire analysis time was a few minutes.

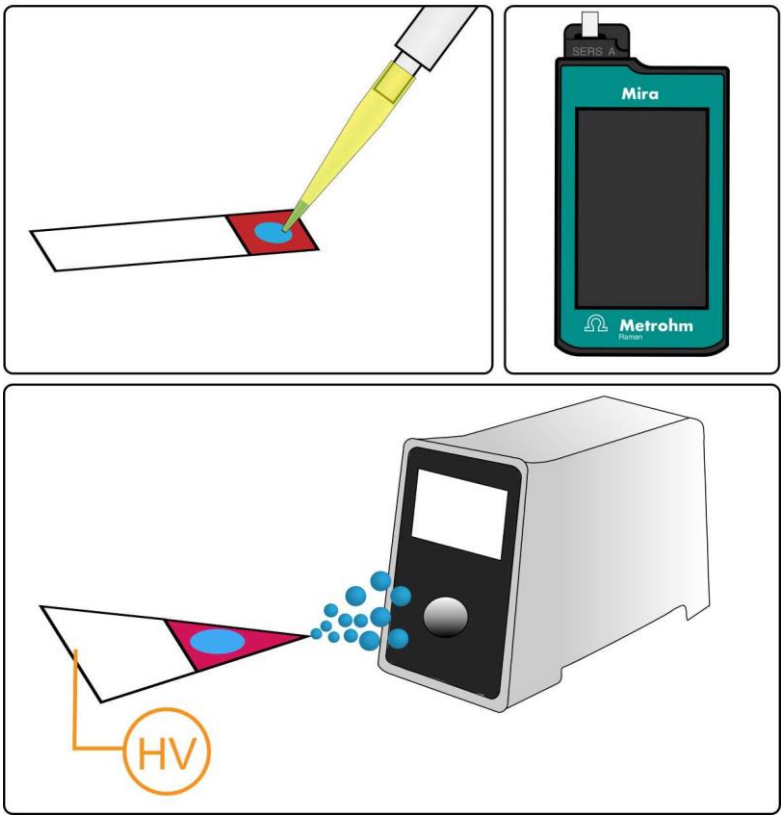


Figure 2.3 Experimental workflow for this dual instrumental analysis system on portable systems, starting from the sample deposition, then to Raman spectroscopy and finally to paper spray ionization mass spectrometry.

2.3.2 Raman Spectroscopy

2.3.2.1 Benchtop Raman

Raman spectra were collected on a Foster + Freeman FORAM 785 HP spectrometer (Evesham, Worcestershire, UK). The excitation source used a 785nm 30mW laser. Each spectrum was collected over 5 scans at 2 seconds a scan. Raman shifts were determined using polystyrene beads as a calibration standard. The compounds at 3µg were not detectable by Raman spectroscopy without the pSERS substrate. To test this, the nonSERS coated portion of the paper substrate was analyzed with the 3µg added which resulted in no interpretable signal.

2.3.2.2 Portable Raman

Raman spectra were collected on a Metrohm Instant Raman Analyzers (Mira) (Riverview, FL), a handheld Raman spectrometer. The excitation source used a 785nm 100mW laser with the

laser power set to 5mW. Each spectrum was averaged over 5 scans at a 1 second integration time. Raman shifts were determined using the commercial calibration standard tool that is provided with the Mira. The compounds at 1µg were not detectable by Raman spectroscopy without the pSERS substrate. To test this, the nonSERS coated portion of the paper substrate was analyzed with the 3µg added which resulted in no interpretable signal.

2.3.3 Mass Spectrometry

2.3.3.1 Benchtop Mass Spectrometry

All spectra were recorded using a Thermo LTQ XL mass spectrometer (San Jose, CA). The drugs and chemical warfare agent simulants were analyzed in positive ion mode, whereas the explosives were analyzed in negative ion mode. All MS/MS product ion scans were generated through collision-induced dissociation (CID). Normalized collision energies in the range of 10-35 (arbitrary manufacturer's unit on the LTQ XL) were applied to achieve sufficient fragmentation. The spray cartridge consisted of a block of Delrin plastic with three milled holes, as described in a previous manuscript.[74] A 0.5x5 mm slot on the front of the cartridge contained the paper spray substrate, while a 3 mm diameter hole in the top of the cartridge contained the sample on a three mm punch of SERS paper in contact with the spray substrate. The third 0.5 mm diameter hole in the top of the cartridge contained a wire in contact with the spray substrate to allow a voltage to be applied. HPLC-grade methanol or acetonitrile was applied to the pSERS substrate via pipette to ensure that the paper was completely wetted. A voltage of +4.5kV or -3.0kV was applied to the wire contact.

2.3.3.2 Portable Mass Spectrometry

All spectra were recorded using a PURSPEC Technologies Mini β mass spectrometer (Beijing, China). The drugs were analyzed in positive ion mode. All MS/MS product ion scans were generated through collision-induced dissociation (CID). Once the paper substrate was cut to a sharp point and positioned in front of the inlet of the mass spectrometer, HPLC-grade methanol was applied to the pSERS substrate via pipette to ensure that the paper was completely wetted. A voltage of +4.5kV was applied to a copper clip that was attached to the back of the paper substrate.

2.4 Results and Discussion

2.4.1 Benchtop Results

2.4.1.1 Drugs of Abuse

With the SWGDRUG guidelines[63] being explicit on the requirement of two different methods, four drugs of abuse were tested by the combined pSERS Raman/PS-MS method. The selection of drugs encompassed relevant samples.[75] The increased use of synthetic designer drugs worldwide was the reason why 4-methylethcathinone was selected.[76] As seen, in Figure 2.4a, the Raman shifts at 1012.5 cm^{-1}, 1125 cm^{-1}, 1390 cm^{-1} and 1455 cm^{-1} have been assigned to the in-phase 2,4,6 radial carbon stretch, C-N-C out of phase stretch, CH rock in the O=C-H, and the aromatic semicircle stretch, respectively. The product ion scan of protonated 4-methylethcathinone (Figure 2.4b) (*m/z* 192) shows two product ions at *m/z* 174 and *m/z* 147 corresponding to the neutral loss of water and the loss of C_2H_7N respectively.

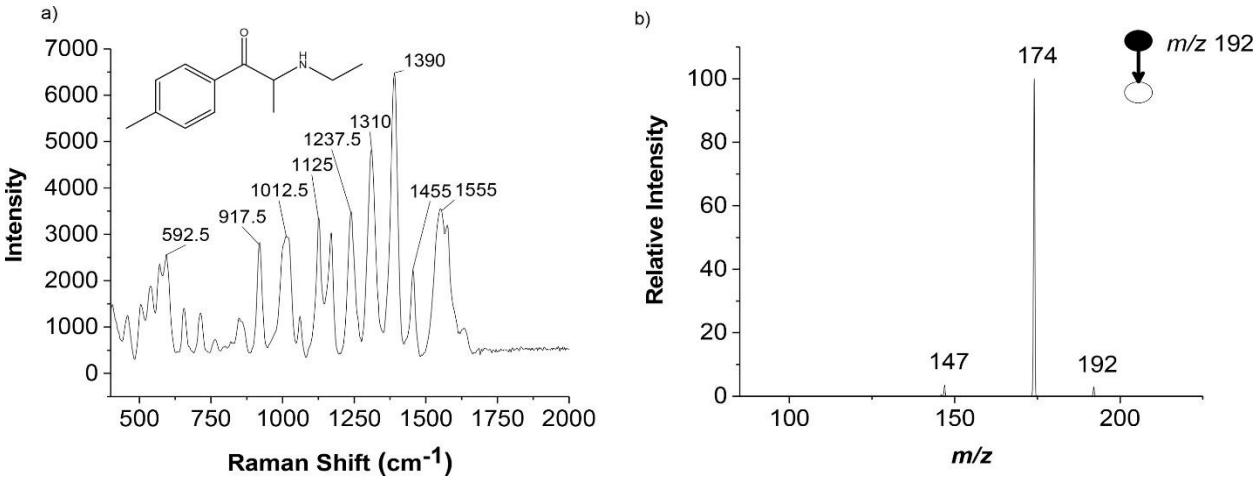


Figure 2.4 Raman (a) and subsequent positive polarity paper spray MS/MS (b) spectra for 4-methylethcatinone.

Hydromorphone and morphine were selected because they are isobars and the Raman spectra aid in distinguishing them. These two drugs were not sampled via the 3 mm punched pSERS substrate but rather using the standard commercial rectangular design. When it was time to perform the mass spectrometric analysis, the paper was cut to a sharp triangular tip. As seen, in Figure 2.5a, the Raman shifts differ for the two isobaric compounds. The red and blue traces

indicate the subtle differences between these two structurally similar compounds. The MS/MS scans also show differences, that of morphine having a product ion at *m/z* 201, which corresponds to the loss of $C_5H_{11}N$, that does not appear in hydromorphone (Figure 2.5b). Similarly, the product ion scan of hydromorphone includes ions at *m/z* 243, *m/z* 227 and *m/z* 185, which do not appear in morphine. While both techniques can reliably differentiate the two compounds, the complementary nature of the two gives increased confidence of the analysis.

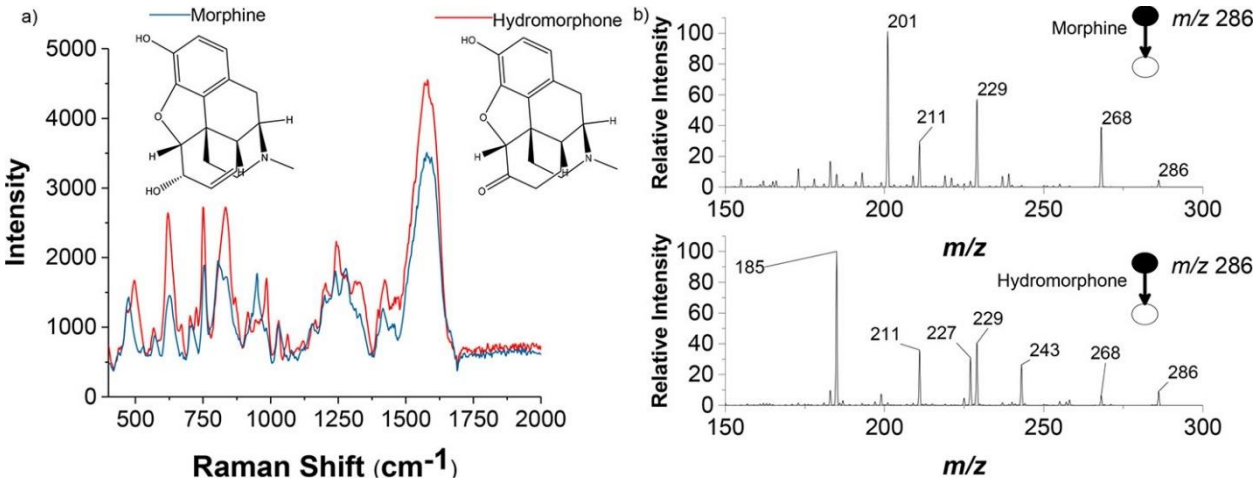


Figure 2.5 a) Raman spectra for morphine and hydromorphone where there are differences in Raman shifts due to the structural differences. b) Product ion MS/MS scans for morphine and hydromorphone.

Two drugs that have caused an increase in substance abuse and overdoses, especially in young adults, are heroin and fentanyl.[77-78] Production of fentanyl is a low cost operation and it is typically used to cut heroin; this has caused numerous overdoses.[79] Figure 2.6a shows the Raman spectra for heroin and fentanyl in their pure form as well as a simulated street sample where the heroin is cut with fentanyl in a 10 to 1 ratio. The characteristic Raman shifts for the two compounds can be seen in the street sample. The product ion scans of the street sample also can be used to identify and readily distinguish the presence of both compounds (Figure 2.6b). The product ion scan of protonated heroin (*m/z* 370) shows fragments at *m/z* 328, *m/z* 310, *m/z* 268, *m/z* 211, *m/z* 193 while that of protonated fentanyl (*m/z* 337) shows product ions at *m/z* 281 and *m/z*188.

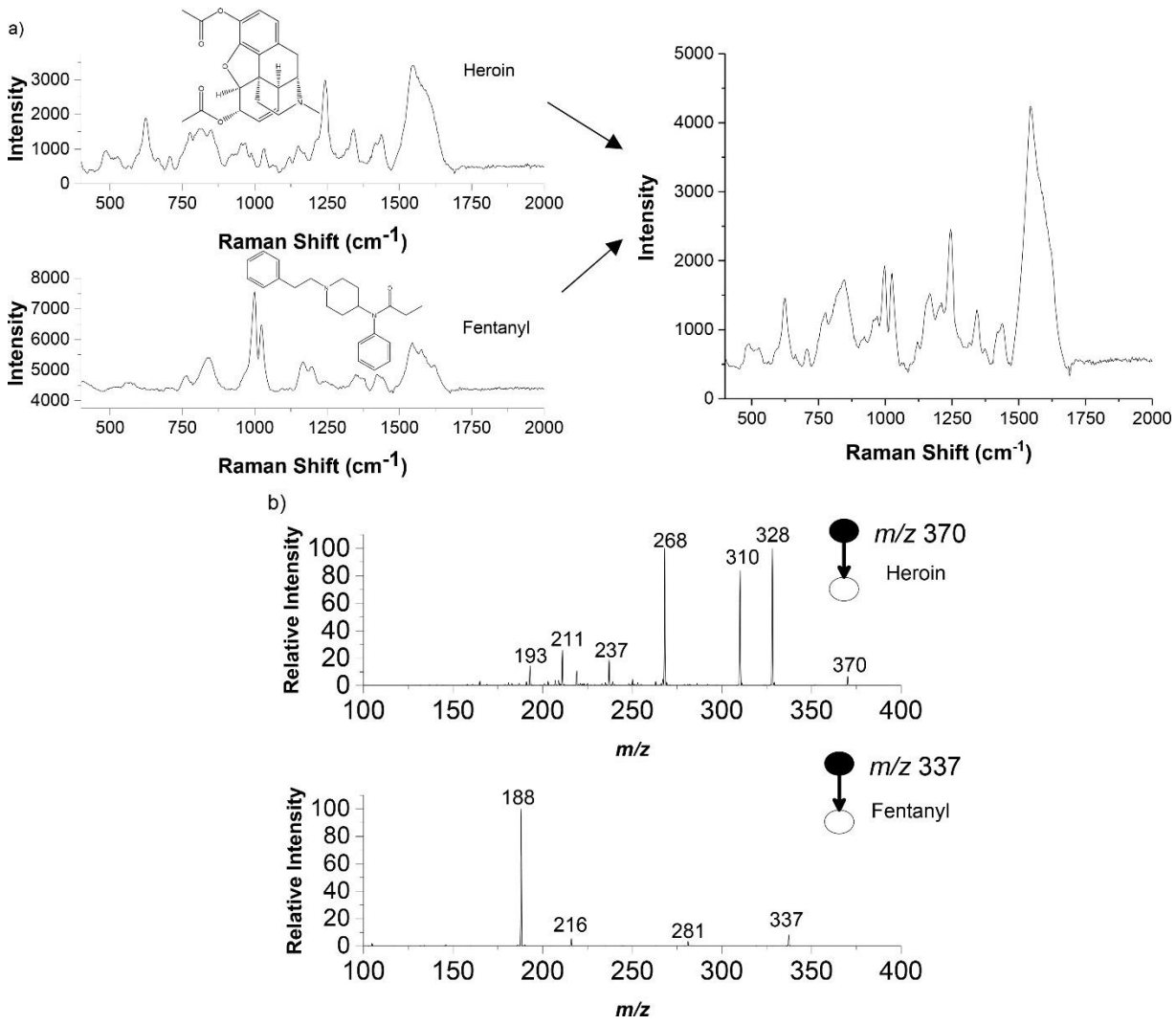


Figure 2.6 a) Raman spectra for heroin and fentanyl as well as the street sample of heroin that was cut with fentanyl. b) The product ion spectra for heroin and fentanyl from the street sample of heroin that was cut with fentanyl.

2.4.1.2 Explosives

Certain nitro-explosives are difficult to identify due to their uninformative MS/MS spectra.[80] As a result, ambient MS methods for compounds like cyclotrimethylenetrinitramine (RDX) and cyclotetramethylenetetranitramine (HMX) are limited to performing identification from the full scan MS or from the MS/MS spectra of the dimers, which only fragment back to the monomers.[81-82] In our approach, identification of these explosives by full scan MS without MS/MS confirmation is mitigated by complementary detection using Raman spectroscopy. As seen, in Figure 2.7a and 2.7c each Raman spectrum has the standard Raman shifts for a nitro-explosive at

~840 cm^{-1} and ~1350 cm^{-1}, which corresponds to a NO stretch and a NO_2 stretch. The full scan mass spectrum of RDX matches the literature,[81-83] showing peaks at *m/z* 257, *m/z* 259, and *m/z* 284 which correspond to RDX adducts with anions ^{35}Cl, ^{37}Cl and NO_3 respectively (Figure 2.7b). The full scan mass spectrum of HMX likewise agrees with the literature,[80-82, 84] showing peaks at *m/z* 331, *m/z* 333, and *m/z* 358 which correspond to HMX adducts with the anions ^{35}Cl, ^{37}Cl and NO_3 respectively (Figure 2.7d). Trinitrotoluene (TNT) however, does fragment well and can be identified by MS alone, but the complementary nature of Raman is used as a confirmatory test. As seen, in Figure 2.7e, the Raman shifts at 850 cm^{-1} and 1342.5 cm^{-1} correspond to the NO and NO_2 stretches, respectively. The product ion scan of radical anion TNT (*m/z* 227) matches literature data[41, 85-86] (Figure 2.7f) showing fragment peaks at *m/z* 209 and *m/z* 196, which correspond to the loss of water and HNO. To test the viability of the pSERS substrates to swab a surface, 10µg of TNT was dried on a glass slide. The pSERS substrate was wetted with acetonitrile and the surface was swabbed. The spectra matched that of the pipetted TNT onto the pSERS substrate, showing that the dual instrumental analysis could also be applied in swabbing collection scenarios.

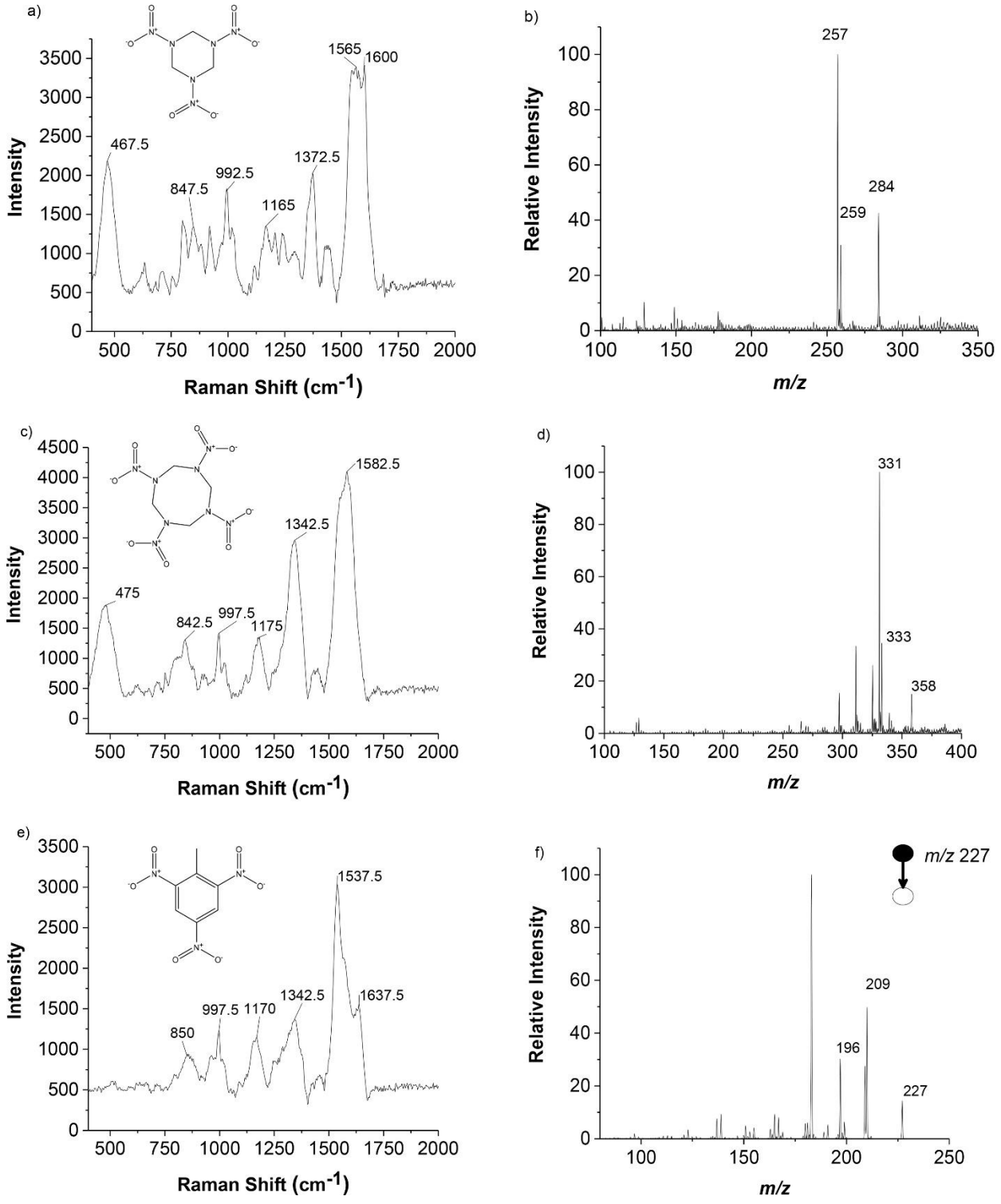


Figure 2.7 Raman and subsequent negative polarity paper spray full scan spectra for a-b) RDX c-d) HMX, and the Raman and subsequent negative polarity MS/MS paper spray spectra for e-f) TNT.

2.4.1.3 Chemical Warfare Simulants

Chemical warfare agents have had significant news coverage in recent years.[87] The uses of chemical warfare agents, while infrequent, are highly monitored and publicized events.[88-89] Three chemical warfare agent simulants were analyzed by the pSERS Raman PS-MS system. They were the nerve agent simulants diisopropyl methylphosphonate (DIMP), dimethyl methylphosphonate (DMMP), and dichlorvos. DIMP showed Raman shifts (Figure 2.8a) at 842.5 cm^{-1}, 997 cm^{-1}, 1120 cm^{-1}, 1377 cm^{-1}, and 1460 cm^{-1}which correspond to in phase P-O-C stretch, out of phase P-O-C stretch, P=O Stretch, P-CH_3 stretch, and a C-CH_3 stretch, respectively. The product ion scan of protonated DIMP (Figure 2.8b) (*m/z* 181) showed product ions at *m/z* 139 and *m/z* 97 which correspond to losses of C_3H_6 and C_6H_{12} respectively. The ion at *m/z* 160 is believed to be background interference. DMMP showed Raman shifts (Figure 2.8c) at 842.5 cm^{-1}, 992.5 cm^{-1}, 1177.5 cm^{-1}, and 1377.5 cm^{-1} which correspond to in phase P-O-C, out of phase P-O-C, P=O, and P-CH_3 stretches, respectively. Protonated DMMP (Figure 2.8d) (*m/z* 125) as well as sodiated DMMP (not shown) could be isolated in the ion trap. However, no product ions were observed even at maximum CID energy, although the precursor ion was highly abundant. The combination of Raman and mass spectrometry did confirm the presence of DMMP, however. As a final example, dichlorvos showed Raman shifts (Figure 2.8e) at 852.5 cm^{-1}, 995 cm^{-1}, 1165 cm^{-1} which correspond to in phase P-O-C stretch, out of phase P-O-C stretch, and P=O stretch. The product ion scan of protonated dichlorvos (Figure 2.8f) (*m/z* 222) showed product ions at *m/z* 204, *m/z* 176, *m/z* 145, *m/z* 112 and *m/z* 109 which corresponded to loss of water, CH_2O_2, $C_2H_5O_3$, $C_2H_6O_3P$, and C_2HCl_2O, respectively.

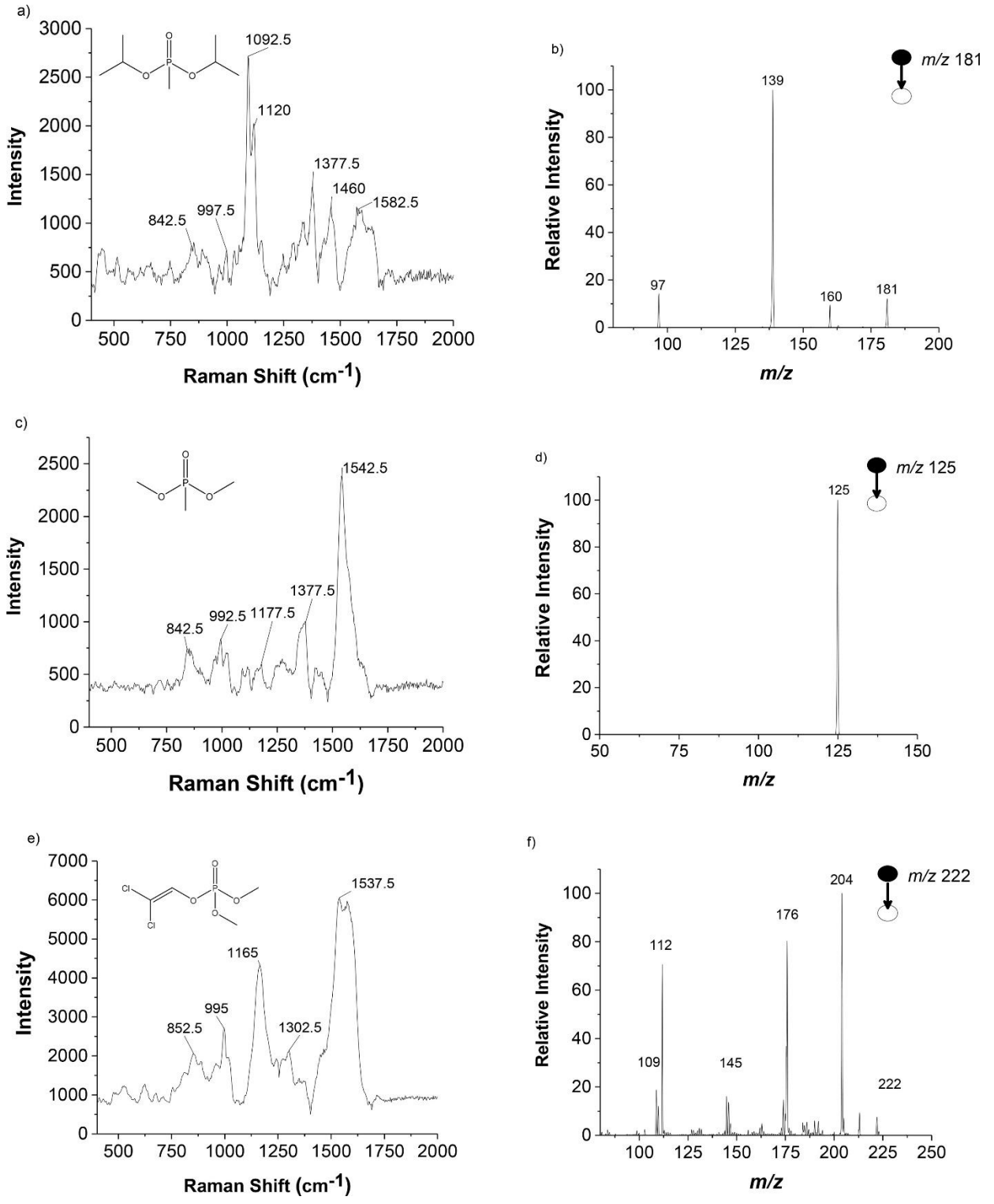


Figure 2.8 The Raman and subsequent positive polarity paper spray MS/MS spectra for a-b) DIMP c-d) DMMP and e-f) dichlorvos.

2.4.1.4 Portable Results
2.4.1.5 Drugs of Abuse

As roadside testing for seized drugs continues to be of importance to the forensic community, and the recent insurgence of fentanyl and fentanyl derivatives in the United States,[90] coupling this identification and confirmation technique on two portable systems removes the barrier of transporting samples back to the laboratory for analysis. Fentanyl has been causing overdoses and killing people in unprecedented numbers in recent years.[91] Figure 2.9 shows the spectra taken on the Mira with the pSERS attachment and Mini β for trace fentanyl residues, both which showed identical spectra as seen on the benchtop system above.

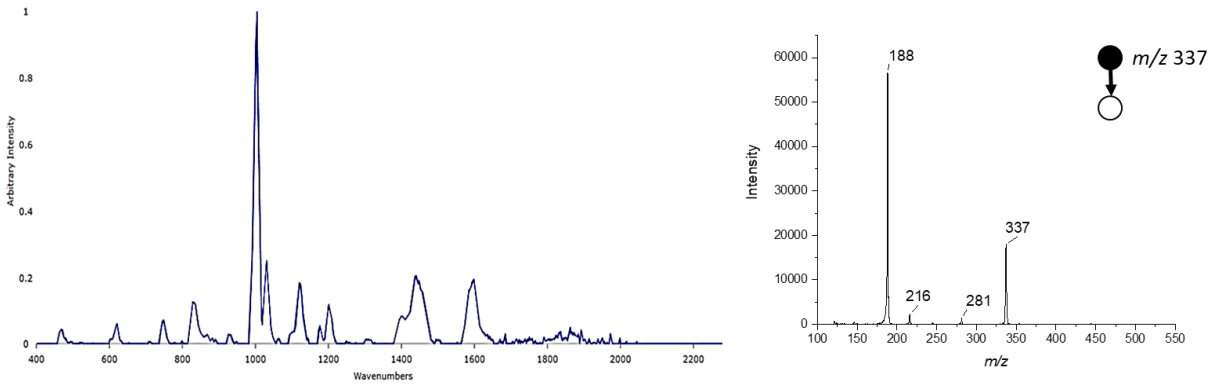


Figure 2.9 The Raman spectrum and subsequent positive polarity paper spray MS/MS scan spectrum for trace fentanyl.

When mixed with heroin in a 1:1 mixture, the Raman spectrum again could identify both drugs based on their characteristic shifts. The full scan mass spectrum showed both protonated precursor ions for both fentanyl (*m/z* 337) and heroin (*m/z* 370) very prominently (Figure 2.10) and subsequent fragmentation confirmed their identities.

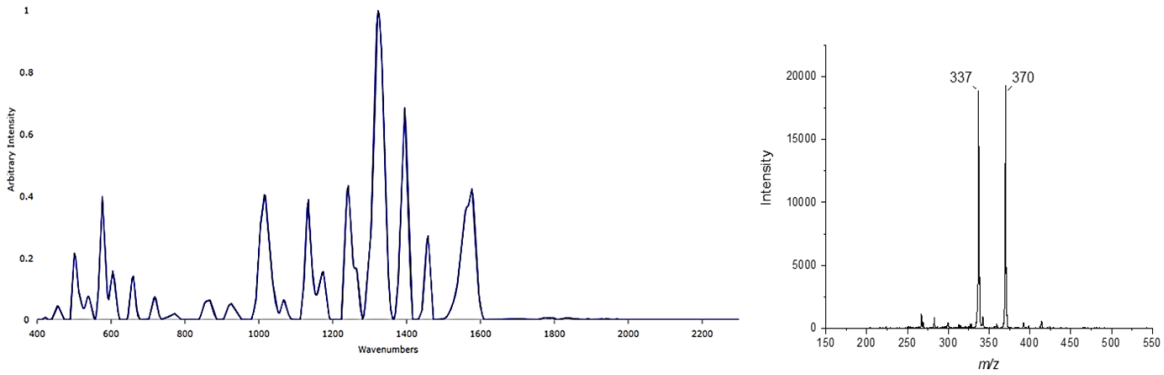


Figure 2.10 The Raman spectrum and subsequent positive polarity paper spray full scan spectrum for a 1:1 mixture of trace fentanyl and heroin.

Interestingly, since heroin is a substance in the factory supplied database of controlled substances in the Mira, an alert was shown on the screen of the Mira, as seen in Figure 2.11, warning the first responder that an opioid was present. These databases can be expanded to include new synthetic drugs as they come out on the market.

Figure 2.11 Database hit on Mira portable Raman spectrometer indicating that the presence of an opioid was detected.

As clandestine chemists know that much of analytical detection methods rely on databases for rapid analysis, the changing of functional groups to evade detection is seen in an increasing prevalence. Figure 2.12 shows two Raman and MS/MS spectra taken on the portable systems for two different derivatives of fentanyl, remifentanil and sufentanil.

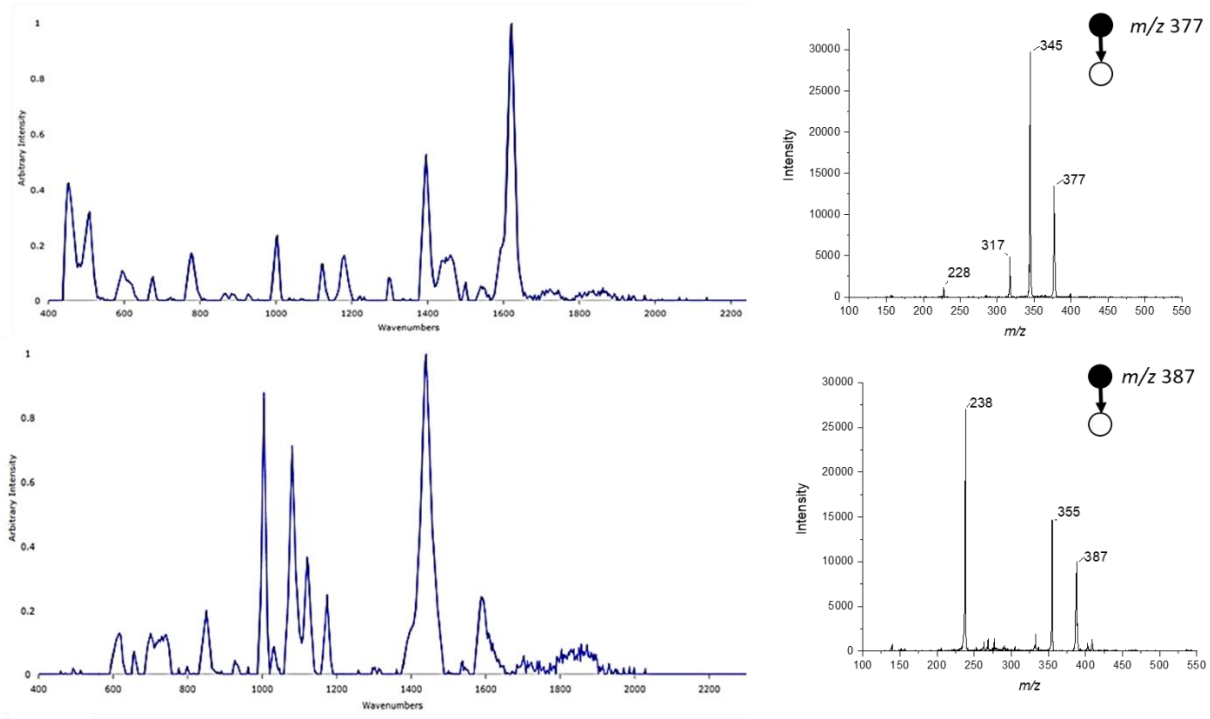


Figure 2.12 The Raman and subsequent positive polarity paper spray MS/MS spectra for both remifentanil and sufentanil.

2.5 Conclusions

The use of a pSERS substrate for both Raman spectroscopy and PS-MS allows rapid analyte identification and confirmation without sample preparation steps and with the use of a single substrate for complementary spectroscopic measurements. Both Raman spectroscopy and PS-MS can be performed in the ambient environment which makes the coupling of the two instrumental techniques so appealing. The decrease in analysis time as compared to the hyphenated chromatography techniques could help to decrease forensic sample backlogs. The substrates are low cost and readily integrated into a forensic laboratory workflow. These substrates work for both SERS and PS-MS and as a cost saving method, a biopsy punch can be employed to create five pSERS substrates from one test strip.

Additionally, because the substrates are inkjet printed, highly customizable patterning could be employed to fit the needs of the study. This study has shown the range of compound types to which this dual instrumental method is applicable. The ability to help distinguish isobaric compounds, confirm compounds that do not readily provide informative tandem mass spectra, and finally the ability to swab a surface and analyze the compounds all add to the strength of this technique. Finally, by using both a portable Raman spectrometer and a portable mass spectrometer, the need to even bring samples back to the laboratory is beginning to be mitigated.

CHAPTER 3. SWAB TOUCH SPRAY IONIZATION MASS SPECTROMETRY FOR THE DETECTION AND IDENTIFICATION OF EXPLOSIVE AND ORGANIC GUNSHOT TRACE RESIDUES.

Portions of this chapter have been published in the following three journals: Forensic Chemistry, Instruments, and Propellants, Explosives, Pyrotechnics.

1. Fedick, P.W.*, Bain, R.M.* "Swab Touch Spray Mass Spectrometry for Rapid Analysis of Organic Gunshot Residue from Human Hand and Various Surfaces Using Commercial and Fieldable Mass Spectrometry Systems" *Forensic Chemistry*, (2017), **5**, 53-57. DOI: 10.1016/j.forc.2017.06.005.

2. Fedick, P.W., Fatigante, W.L., Lawton, Z.E., O'Leary, A.E., Hall, S.E., Bain, R.M., Ayrton, S.T., Ludwig, J.A., Mulligan, C.C. "A Low-Cost, Simplified Platform of Interchangeable, Ambient Ionization Sources for Rapid, Forensic Evidence Screening on Portable Mass Spectrometric Instrumentation" *Instruments*, (2018), **2**, 1-15. DOI: 10.3390/instruments2020005.

3. Bain, R.M.*, Fedick, P.W.*, Dilger, J.M., Cooks, R.G. "Analysis of Residual Explosives by Swab Touch Spray Ionization Mass Spectrometry" *Propellants, Explosives, Pyrotechnics*, (2018), **43**, Just Accepted. DOI: 10.1016/j.forc.2017.06.005.

3.1 Abstract

Swab touch spray ionization mass spectrometry, an ambient ionization technique, has been applied to the analysis of six explosives from various surfaces including glass, metal, Teflon, plastic, human hands, and three types of gloves (nitrile, vinyl and latex). Additionally, organic gunshot residues, specifically methyl centralite (1,3-dimethyl-1,3-diphenylurea) and ethyl centralite (1,3-diethyl-1,3-diphenylurea) were also analyzed. These are characteristic compounds forensic analysts test for when determining if an individual has discharged a firearm. These compounds were identified by swab touch spray ionization mass spectrometry. All of these compounds have long been analyzed by several instrumental techniques, many of which involve extensive sample preparation or have lengthy analysis times. This chapter will demonstrate an ambient ionization method that requires no sample preparation, offers real-time analysis, and can be paired with a portable ion trap mass spectrometer for *in-situ* analysis. Swab touch spray ionization utilizes a rayon-tipped swab that has an aluminum wire handle which can simply be swabbed over the area of interest. After contacting a surface, solvent is applied to the rayon tip of

the swab and a high voltage applied to the aluminum handle to generate ions for analysis. Additional benefits of swab touch spray ionization are that the swabs are commercially available, sterile, and they are individually packaged to prevent contamination. The swabs are also forensically feasible because they are sealed prior to delivery by the vendor with a tamper-proof label. Here we have shown swab touch spray ionization for the direct swabbing of surfaces for trace residues and the confirmation of their presence to aid in investigative procedures.

3.2 Introduction

Many forensic, defense, and homeland security investigations involve trace residues. Trace residues such as explosives and organic gunshot residues are typically analyzed by mass spectrometry through sampling with a swab, stub, or wipe followed by a lengthier sample preparation procedure.[92-96] Liquid chromatography or gas chromatography coupled to mass spectrometry [97-99] and ion mobility [100-101] represent the current state of the art methods for these residue detections.[102] Forensic staples like gas chromatography[103] and liquid chromatography[104] are reliable analytical techniques, but they suffer from lengthy analysis times and are not amenable to *in-situ* analysis. In order for these chromatographic methods to be utilized, an extraction technique, such as a solid phase microextracton,[105] has to be performed which adds to the analysis time. Speed of analysis is important and alternative methods have been explored.

To improve on *in-situ* analyses, colorimetric detection methods have been developed but typically suffer from low specificity.[106] Additionally, in regards to gunshot residue analysis, many of the colorimetric tests only detect inorganic residues. Forensic investigations into the possibility of a suspect discharging a firearm in recent years has expanded from elemental inorganic gunshot residue analyses to molecular organic gunshot residue analyses (OGSR).[107] Protocols have been developed for the collection and analysis of OGSR[107] which focus heavily on the stabilizers present in many ammunition types such as methyl centralite (MC) (1,3-dimethyl-1,3-diphenylurea) and ethyl centralite (EC) (1,3-diethyl-1,3-diphenylurea). These two compounds are commonly selected from the expansive list of OGSRs because they are not commonly used in any other application and would therefore be among the most discriminatory compounds for determining if a person has potentially discharged a firearm.[108] However a recent comprehensive review identified over 136

compounds to be associated with OGSR, many of which current colorimetric tests are not testing for.[102]

Ambient ionization mass spectrometry,[109] starting with desorption electrospray ionization (DESI)[5] and direct analysis in real-time (DART)[6], changed how mass spectrometry sampling can be performed. Since the onset of ambient ionization, there have been ample forensic advances because of these techniques, as outlined in two recent reviews. [9, 64] Two major benefits of ambient ionization as it relates to forensic science are the lack of sample preparation and the ability to form ions in the open environment. As rapid analysis of explosive residues is desirable, both DESI [41-42, 81, 110] and DART [111-113] have been employed for both of these applications. [114] These methods have proven especially useful in the analysis on skin, clothing, and other surfaces; however, both methodologies require specialized equipment. A recent review has detailed the current advances of ambient ionization to the trace detection of explosive residues. [115] Similarly, DESI mass spectrometry has been shown to be capable of identifying and distinguishing OGSR in a simple and noninvasive method.[14-15] OGSR has been shown to be detectable on skin for hours after discharging a firearm;[116] however, there is degradation over time and improved *in-situ* analysis would greatly benefit the forensic community.[117] Although providing rapid detection times, the issues that these two ion sources, in regards to analysis, include the DART's plasma generator and the DESI's spray setup , of which neither is easily coupled to a portable instrument which would be desirable for *in-situ* analysis.[86]

Low temperature plasma (LTP) ionization, another ambient ionization, has been applied extensively to explosive residues. [80, 83-84] The low temperature characteristic of the plasma, as well as being safe to the touch, enables residues to be sampled from a variety of surfaces, including human skin without physical damage or sample degradation. [85] This technique has also been used with portable mass spectrometers, where a backpack MS showed promise for *in-situ* measurements.[118-119] While LTP is promising, it does require that a specialized ionization source be used and it utilizes high AC potentials which have an extra level of safety concern. [85] By contrast, one of the simplest ambient ionization techniques, PS, uses only a low current DC potential to ionize analyte from a sharply pointed piece of paper wetted with solvent. [120] As shown previously, this allows detection of 2-methyl-1,3,5-trinitrobenzene (TNT), 2,2-bis[(nitrooxy)methyl]propane-1,3-diyl dinitrate (PETN), 1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX) and 1,3,5-trinitro-1,3,5-triazinane (RDX). [121] PS has a low barrier of entrance into a forensic setting as the paper acts as

both as the sampling device and the ionization source. While PS shows great promise, the stability of the tip is essential for successful ionization, and this can make swabbing with the paper substrate difficult. [122] If the tip of the paper substrate is damaged during swabbing, subsequent analysis will be adversely affected, although proper training can alleviate this problem. [24]

As swabs are currently the convenient methodology utilized for sampling by forensic analysts, there have been attempts to directly analyze the swab without the extraction techniques which amplifies analysis time. This extends the room for operator error and sources of contamination as noted earlier. There have been reports of swabs that have removed the sample preparation steps through the use of TOF-SIMS directly on the swab,[123] but the ability of *in-situ* analysis is nonexistent due to the complexity of the instrumental setup. Similar to paper spray ionization, the swab acts both as the sampling device and the ionization source. Swab touch spray ionization has the added advantage over PS of being able to perform analysis from an individually-packed, tamperproof swab, rather than a fragile hand-cut paper triangle. [124] The surface of interest is touched with either the dry or wetted swab and moved in a circular motion atop the surface. The analyte is adsorbed onto the surface of the swab, which can then be positioned in front of the mass spectrometer inlet. A steady flow of solvent is applied to the swabbing material which will act as the extraction and spray solvent. Once the swab is wetted a high voltage is applied to the metallic handle of the swab. The metallic handle allows for the voltage to permeate throughout the swab material and form a Taylor cone upon the tip of the swab. Analyte molecules are directly ionized and detected by the mass spectrometer (Figure 3.1). The ease of swab touch spray ionization, its forensic feasibility for explosives and organic gunshot residues, and its ability to be coupled to a portable mass spectrometer for *in-situ* analysis has been realized.

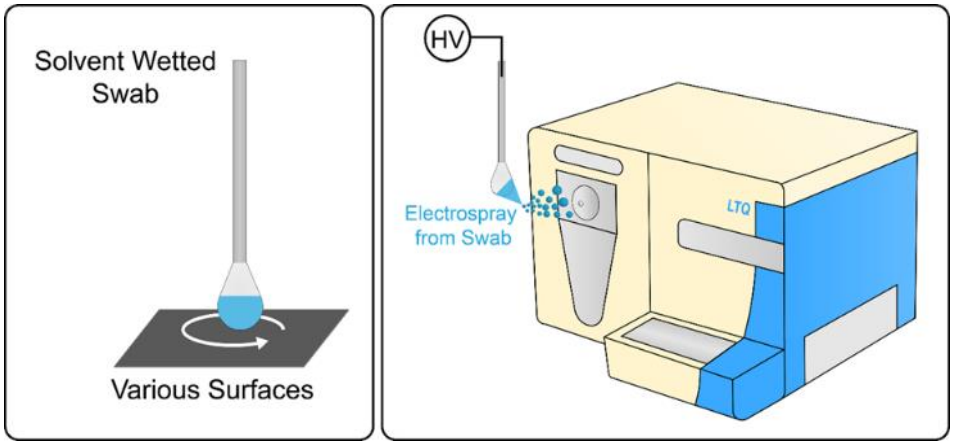


Figure 3.1 Pictorial representation of swab touch spray ionization mass spectrometry. The first panel shows the swab being utilized as a sampling device and the second panel shows the swab being utilized as the ionization source.

3.3 Experimental

3.3.1 Swab Touch Spray Ionization

All spectra were recorded in positive ion mode using a Thermo LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap mass spectrometer (San Jose, CA), a home-built Mini 12 rectilinear ion trap (Purdue University, West Lafayette, IN),[125] or a FLIR Systems AI-MS 1.2.[26] All MS/MS product ion scan mass spectra were generated through collision-induced dissociation (CID). Normalized collision energies from 8-15 units on the ion trap were used. Explosive residue spectra were collected in negative ion mode, except when analyzing tetrabutylammonium perchlorate, which was collected in both positive and negative ion modes. Spectra for OGSR were collected in positive ion mode.

Medical grade sterile swabs (Copan Diagnostics, Murritea, CA) constructed with aluminum handles and rayon swabs were utilized for all experiments. Each swab was individually packaged with a tamper-proof label and removed from packing only to swab and was then returned to the casing. Each surface (bare hands, gloved surfaces, clothing and spent casings) was swabbed in a circular motion. Prior to swabbing, the swabs were pre-wetted with 20 µL of acetonitrile for explosive analysis. This volume was selected as the swabs remained saturated with solvent during swabbing. The OGSR analyses were performed with a dry swab. Approximately 20 circular motion passes were performed over the area of interest, for example the top side of the right hand between the thumb and the pointer finger.

The swabs were positioned vertically (approximately 8 mm) above the inlet of the mass spectrometer. The position is an important variable. If the swab was too close to the inlet, discharge occurs. Conversely, if the swab was too far away, a stable cone would not be produced resulting in an unstable signal or no signal at all. HPLC-grade methanol was applied to the swab via a pipette to ensure that the swab was completely wetted. Then a continual flow of a solvent was delivered to the swab by a Harvard Apparatus standard infusion only PHD 22/2000 syringe pump (Holliston, MA) using a Hamilton syringe (Reno, NV) at a varied flow rate (10-30 µl/min) to maintain a steady spray. A high voltage of 5.5kV, supplied by the instrument, was applied to the aluminum handle and the generation of a spray could be visually observed. The entire time from swabbing through the collection of the spectra is ca 2 minutes. Efforts have been made previously to achieve robust signal with 3D printed parts and a camera to aid in alignment. [124] Similarly, a rail system has been used to aid in alignment for non-expert users and in-field applications with the FLIR Systems AI-MS 1.2 mass spectrometer (Figure 3.2).[32]

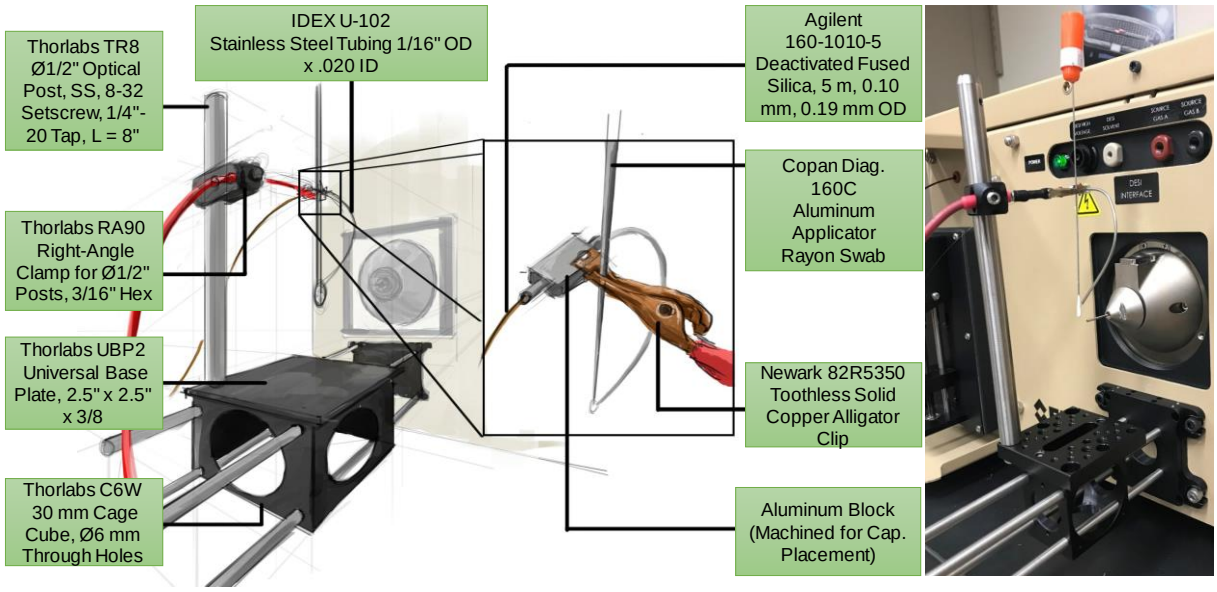


Figure 3.2 Touch swab spray ionization mass spectrometry on a portable FLIR Systems AI-MS 1.2 mass spectrometer. Company and part numbers are listed for reproducibility and ease of replication.

3.3.2 Explosive Analysis

The explosives TNT, PETN, HMX and RDX were purchased from Cerilliant (Round Rock, Texas), while sodium perchlorate and tetrabutylammonium perchlorate were purchased from

Sigma-Aldrich (St. Louis, MO). HPLC grade acetonitrile was purchased from Fisher Scientific (Hampton, NH). Samples were prepared from 1.0 mg/mL stock solutions in acetonitrile. Direct analysis of explosives was performed by depositing 5.0 μL of solution on the swab using a pipette. Lower concentrations for limit of detection studies were prepared by serial dilutions (orders of magnitude) from the stock solutions to provide conservative limits of detection from the surface. This method created a conservative approximate limit of detection that can be expected in the field and is not determined by extrapolation.

Surfaces bearing trace explosives were prepared by spotting 1.0 μL of reagent onto each surface. Surfaces included black nitrile exam gloves (Ammex, Seattle, WA), diamond grip latex gloves (Microflex, Reno, NV), anti-static vinyl gloves (OAK Technical, Matteson, IL), glass microscope slides (Gold Seal, Portsmouth, NH), polytetrafluoroethylene (PTFE) plugs (Swagelok, Indianapolis, IN), stainless-steel plugs (Swagelok, Indianapolis, IN), blue polyethylene flat-top screw cap (Fisher Scientific, Hampton, NH) and human hands (those of the authors). Once the sample had been spotted on the surface, it was allowed to dry prior to swabbing with a swab pre-wetted with 20 μL of acetonitrile.

3.3.3 Organic Gunshot Residue Analysis

HPLC-grade methanol, methyl centralite (1,3-dimethyl-1,3-diphenyl-urea) and ethyl centralite (1,3-diethyl-1,3-diphenyl-urea) were purchased from Sigma-Aldrich (St. Louis, MO). Anti-static vinyl gloves (OAK Technical, Matteson, IL), Diamond Grip Latex gloves (Microflex, Reno, NV) and Black Nitrile Exam Gloves (Ammex, Seattle, WA) were used in these experiments. Initial tests swabbed the standards from the reagent bottles of the OGSRs and were analyzed by swab touch spray ionization mass spectrometry. After the validity of performing OGSR analysis was determined, swabbing was performed after discharging real ammunition by a firearm.

Four different 9mm handguns, with four different ammunitions were used in this study. Each of the ammunitions were discharged by only one of the four different handguns. Federal Ammunition 9mm Luger 115 Grain Full Metal Jacket rounds (Anoka, MN) were discharged by a Heckler & Koch VP9 (Newington, NH). Independence 9mm Luger 124 Grain Full Metal Jacket rounds (Lewiston, ID) were discharged by a Beretta M9 (Accokeek, MD). Winchester 9mm Luger 147 Grain Full Metal Jacket rounds (East Alton, IL) were discharged by a Glock 17 (Smyrna, GA). Federal Ammunition American Eagle 147 Grain Full Metal Jacket Flat Point rounds (Anoka, MN)

were discharged by a SIG Sauer P320 (Newington, NH). Different firearms and ammunitions were selected to determine if this could be a universal method.

To minimize the confounded variables of shooters, ammunition and firearm the casing of each expended ammunition was swabbed and analyzed for the presence of MC and EC. Each firearm was discharged by one individual and each individual only fired one ammunition and firearm per session at the range. For each experiment, unless otherwise stated, 10 rounds were fired from the handgun and then the shooters hands were immediately swabbed once the weapon was cleared. For all ammunitions where EC and MC could be detected by swab touch spray mass spectrometry after 10 rounds were discharged could also be detected after a single discharging of the firearm on both mass spectrometers. Figure 3.3 is a pictorial representation of the workflow of the experiment.

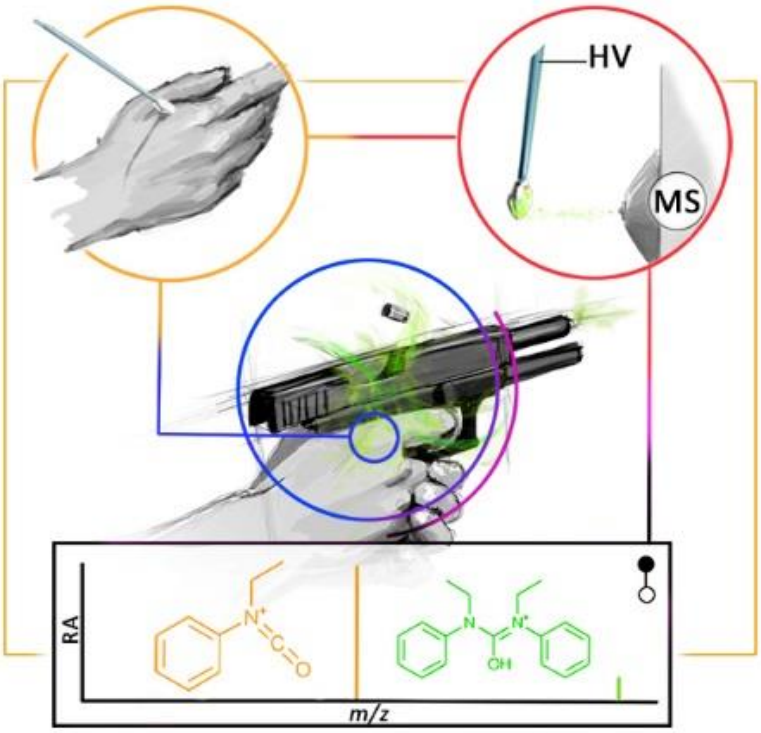


Figure 3.3 Experimental workflow for the analysis of organic gunshot residue. First, a firearm was discharged. Second, the hand of the individual who discharged the firearm was swabbed. Third, the swab was positioned in front of the mass spectrometer inlet for swab touch spray ionization.

3.4 Results and Discussion

3.4.1 Explosive Residues

Direct analysis of 5 μg of TNT using the product ion scan (Figure 3.4) gave a spectrum rich in fragment ions. The fragmentation of TNT has been previously studied by DESI [41] and LTP [86] and both the radical anion (*m/z* 227) and deprotonated (*m/z* 226) TNT were observed. Using an isolation width of 3 Th on the LTQ and a peak centered at *m/z* 226.5, both the radical anion and deprotonated TNT were isolated and fragmented. Product ions at *m/z* 210, *m/z* 197, and *m/z* 196 correspond to the loss of OH, the loss of NO, and the loss of HNO, respectively.

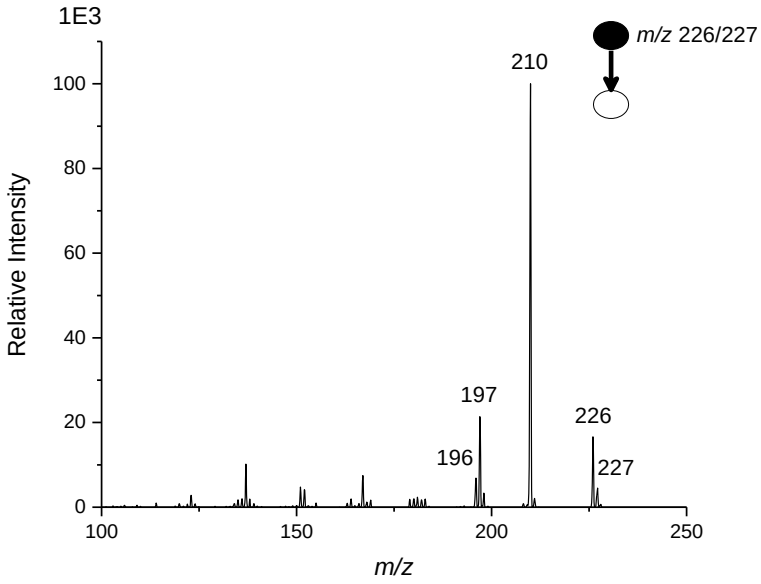


Figure 3.4 MS/MS Spectrum of TNT with an isolation width of 3 Th centered at m/z 226.5. Product ions at m/z 210, m/z 197, and m/z 196 correspond to the loss of OH, the loss of NO, and the loss of HNO, respectively.

Full scan mass spectra recorded for the direct analysis of 5.0 μg of RDX, HMX, and PETN from a swab are shown in Figure 3.5. In agreement with the literature, [82, 84] RDX was identified by four ions that form adducts with RDX; $[RDX+Cl_{35/37}]^-$ at *m/z* 257 and *m/z* 259, $[RDX+NO_2\text{-}H]^-$ at *m/z* 267 and $[RDX+NO_3]^-$ at *m/z* 284. Similarly, HMX and PETN were identified by the corresponding four adducts. HMX was identified by $[HMX+Cl_{35/37}]^-$ at *m/z* 331 and *m/z* 333, $[HMX+NO_2\text{-}H]^-$ at *m/z* 341 and $[HMX+NO_3]^-$ at *m/z* 358. PETN was identified by $[PETN+Cl_{35/37}]^-$ at *m/z* 351 and *m/z* 353, $[PETN+NO_2\text{-}H]^-$ at *m/z* 361 and $[PETN+NO_3]^-$ at *m/z* 378. These three

explosives were identified by their adducts rather than their fragmentation profiles as no measurable fragments are detected on the ion trap mass spectrometer, as is consistent with previous results. [81]

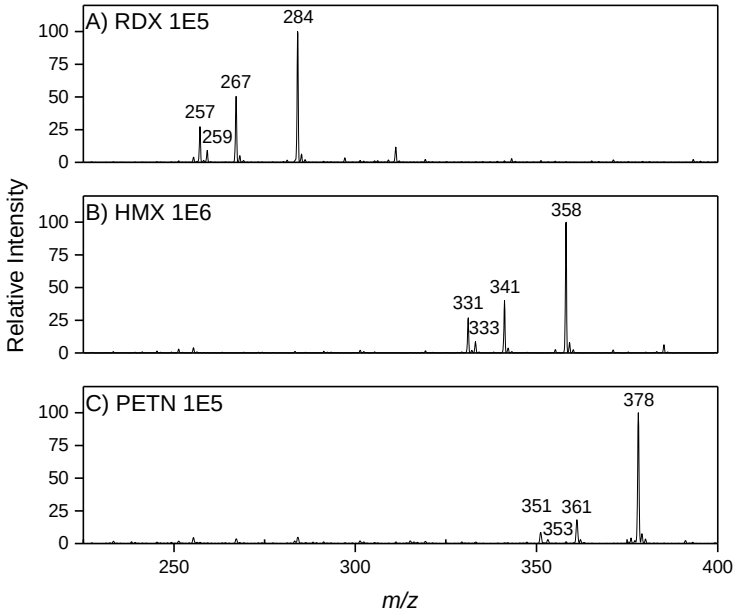


Figure 3.5 Full scan mass spectra recorded for RDX, HMX, and PETN. All three explosives were identified by four ions that form adduct with the explosives. The ions from left to right correspond to [explosive+Cl35/37]-, [explosive+NO2-H]- and [explosive+NO3]- for all three explosives.

Explosive perchlorate compounds were also of interest in this study because of their widespread use.[126] The direct analysis of 5.0 µg of tetrabutylammonium perchlorate was analyzed in full scan negative ion mode to look for perchlorate ions, as well as in the positive ion mode to measure tetrabutylammonium (Figure 3.6). Tetrabutylammonium at *m/z* 242 produced fragments at *m/z* 186, *m/z* 184, *m/z* 142, *m/z* 130 and *m/z* 100 corresponding to tributylammonium, dehydrogenated tributyl ammonium, dehydrogenated methyl dibutylammonium, dibutylammonium and loss of ethane from dehydrated dibutyl ammonium cation.

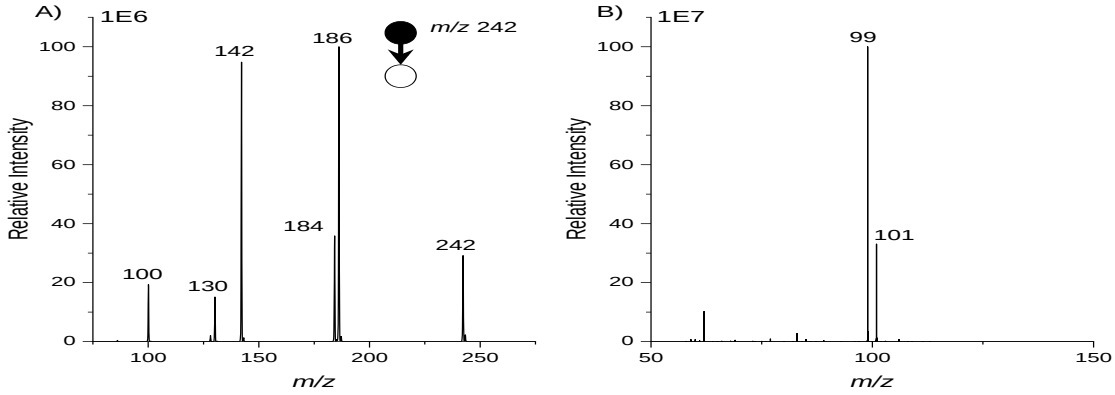


Figure 3.6 A) Swab touch spray ionization mass spectrometry product ion scan mass spectrum of tetrabutylammonium perchlorate in positive ion mode shows tetrabutylammonium at m/z 242 and characteristic fragment ions including those of m/z 186, 184, 142, 130, and 100. B) Swab touch spray ionization mass spectrometry full scan mass spectrum in negative ion mode of tetrabutylammonium perchlorate shows the perchlorate ions at m/z 99 and m/z 101.

In addition to characterizing explosives applied directly to the swab, a variety of forensically applicable surfaces were also screened for explosive residues. A known amount was spotted onto the substrate and the sample was allowed to dry. The swab, pre-wetted with 20 µL of acetonitrile, was rastered over the surface in a circular motion to collect the explosives residue. Surfaces and their corresponding limits of detection can be found in Table 3.1 for each solid surface. Additionally, for screening and forensic purposes, gloves and human hands were swabbed for all explosives and limits of detection for each explosive can be found in Table 3.2.

Table 3.1 Approximate limits of detection for explosives from various surfaces by swab touch spray ionization mass spectrometry.

Explosive	Glass	PTFE	Stainless Steel	Polyethylene
TNT	10 pg	10 pg	10 pg	10 pg
RDX	10 ng	10 ng	10 ng	10 ng
HMX	10 ng	10 ng	10 ng	10 ng
PETN	10 ng	10 ng	10 ng	10 ng
Sodium perchlorate	100 pg	100 pg	100 pg	100 pg
Tetrabutylammonium perchlorate	1 pg	1 pg	1 pg	1 pg

Table 3.2 Approximate limits of detection for explosives from gloves and human hands by swab touch spray ionization mass spectrometry.

Explosive	Vinyl Glove	Nitrile Glove	Latex Glove	Exposed Hand
TNT	10 pg	10 pg	10 pg	10 pg
RDX	10 ng	10 ng	10 ng	10 ng
HMX	10 ng	10 ng	10 ng	10 ng
PETN	10 ng	100 ng	10 ng	10 ng
Sodium perchlorate	100 pg	100 pg	100 pg	100 pg
Tetrabutylammonium perchlorate	1 pg	1 pg	1 pg	1 pg

Analysis from common surfaces for residual amounts of explosives is applicable to forensic and security analyses. [81, 118] The ability to screen people by analyzing human hands is also of great interest in situations such as airports screenings. Similarly, finding gloves near a crime scene that have residual explosives can be traced back to a perpetrator using fingerprints [16] inside the glove or using cameras between the crime scene and the location where the gloves were discovered. Additionally, the successful swabbing of a variety of surfaces shows the robustness of this technique and suggests feasibility across a variety of other surfaces as well. The surfaces studied all gave similar results except that the limit of detection for PETN was an order of magnitude higher for nitrile gloves than other surfaces or other explosives. This is likely a result of chemical selectivity specifically related to PETN and nitrile.

3.4.2 Organic Gunshot Residue

To test the viability of swab touch spray for OGSR analysis the inside of the bottles of MC and EC standards were swabbed and analyzed by the LTQ ion trap (Figure 3.7). The MS/MS spectra of both ions correlating to $[MC+H]^+$ (*m/z* 241) and $[EC+H]^+$ (*m/z* 269) matched the literature [14-15] and had reproducible and stable spectra with distinctive fragment peaks that were observable through the subsequent experiments.

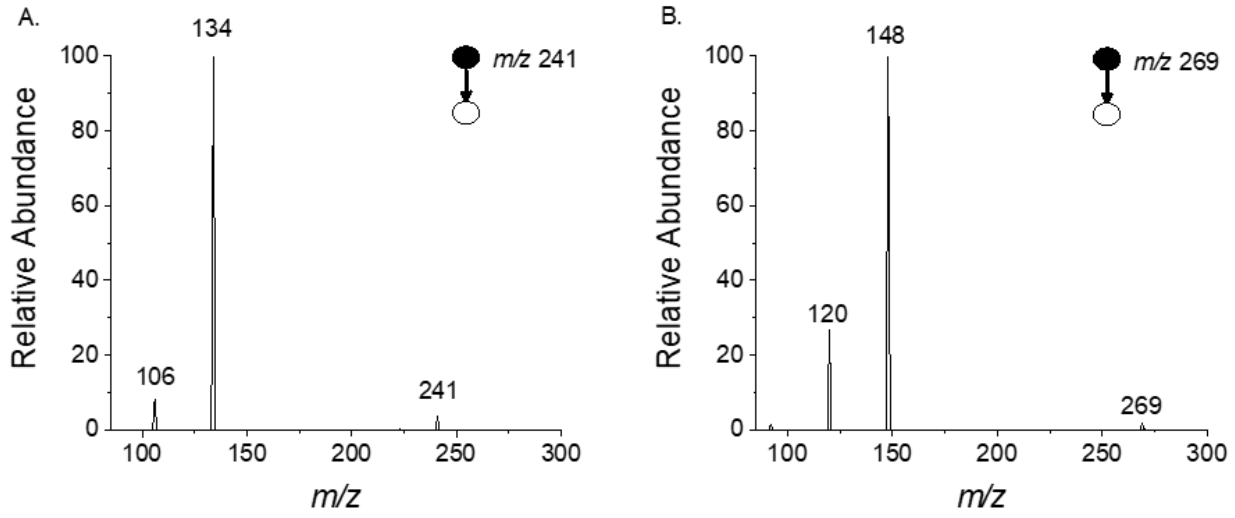


Figure 3.7 . Swab touch spray product ion spectra of standards. A) protonated MC is seen at m/z 241, with the loss of neutral methylaniline to form the fragment at m/z 134, and a subsequent neutral loss of CO to form the fragment at m/z 106 and B) protonated EC at m/z 269, with the loss of neutral ethylaniline to form the fragment at m/z 148 and a subsequent loss of CO to form the fragment at m/z 120. Both spectra were obtained on a Thermo LTQ XL Ion Trap.

After these experiments had demonstrated that swab touch spray is capable of ionizing MC and EC standards, swab touch spray was utilized to analyze OGSR produced from discharging a firearm. The researchers shot four different 9mm full metal jacket ammunitions, with varying grains, and tested the ability of the dry swab to extract the OGSR off the right hand of the shooter when swabbed. Four surfaces were tested, an exposed bare hand, a hand covered in a vinyl glove, a nitrile glove, and a latex glove. The variety of gloved surfaces were selected based on the general availability of these to the public who may try and cover up a crime they committed. This also shows the robust nature of the swab at extracting the OGSR from a variety of surfaces. Table 3.3 describes the ability of the swab to extract the OGSR off the surface.

Table 3.3 Ability to detect MC and EC from bare hands, vinyl, nitrile, and latex gloves by swab touch spray. The top three ammunitions were able to be detected on all surfaces, whereas there was no detectable MC or EC from the fourth ammunition.

Ammunition	Compound	Exposed Hand	Vinyl Glove	Nitrile Glove	Latex Glove
Federal Ammunition 9mm Luger 115 Grain Full Metal Jacket	Methyl Centralite	✓	✓	✓	✓
	Ethyl Centralite	✓	✓	✓	✓
Independence 9mm Luger 124 Grain Full Metal Jacket	**Methyl Centralite**	✓	✓	✓	✓
	Ethyl Centralite	✓	✓	✓	✓
Winchester 9mm Luger 147 Grain Full Metal Jacket	Methyl Centralite	✓	✓	✓	✓
	Ethyl Centralite	✓	✓	✓	✓
Federal Ammunition American Eagle 147 Grain Full Metal Jacket Flat Point	Methyl Centralite	Not Detected	Not Detected	Not Detected	Not Detected
	Ethyl Centralite	Not Detected	Not Detected	Not Detected	Not Detected

As seen in Table 3.3, MC and EC was detected in all four surfaces for the first three ammunitions; however, MC and EC were not detected in the American Eagle 147 Grain full metal jacket flat point ammunition discharged from a SIG Sauer P320. As the composition of the bullets are not public knowledge, the researchers also swabbed the inside of the spent casing to determine if the lack of detection of MC and EC was a result of the swab or the lack of the two compounds found in the ammunitions. There was no signal of MC or EC for the American Eagle casing either. We propose that there may not be any MC or EC in this ammunition, or with the increased grain potentially the MC and EC is in a more limited quantity and the quantity transferred to the surface is below the limit of detection of the technique. For each of the three ammunitions that were found to contain MC and EC, a single discharge of the firearm provided enough of both compounds to be detected by swab touch spray mass spectrometry. The lower limit of detection for both MC and EC were lower than 50ng on the LTQ XL.

The researchers checked the inside of the other ammunitions' spent rounds and were able to detect both MC and EC in all three ammunitions (Figure 3.8). The EC spectrum looks identical to the standards, however in the MC spectrum a peak at *m/z* 181 and at *m/z* 223 are also present in the Winchester 9mm Luger ammunition, both in the swabs of the gloves, bare hands and the casing. The authors propose that these peaks in the MC spectrum at *m/z* 181 and *m/z* 223 arises from an isobaric compound at *m/z* 241 also present in the ammunition. The proposed explanation is the peak at *m/z* 223 is created by the loss of water while the peak at *m/z* 181 is created by the neutral loss of acetate potentially to form the ion at *m/z* with the molecular formula $C_{12}H_7NO^-$ which would have arisen from the parent mass of $C_{14}H_{10}NO_3^-$. The chemical formulae were confirmed by exact mass using the Thermo LTQ-orbitrap mass spectrometer (San Jose, CA) which provided high resolution data with a mass error of less than 5 ppm. The identification of MC however is still performed by the prominent fragment peaks at *m/z* 134 and *m/z* 106.

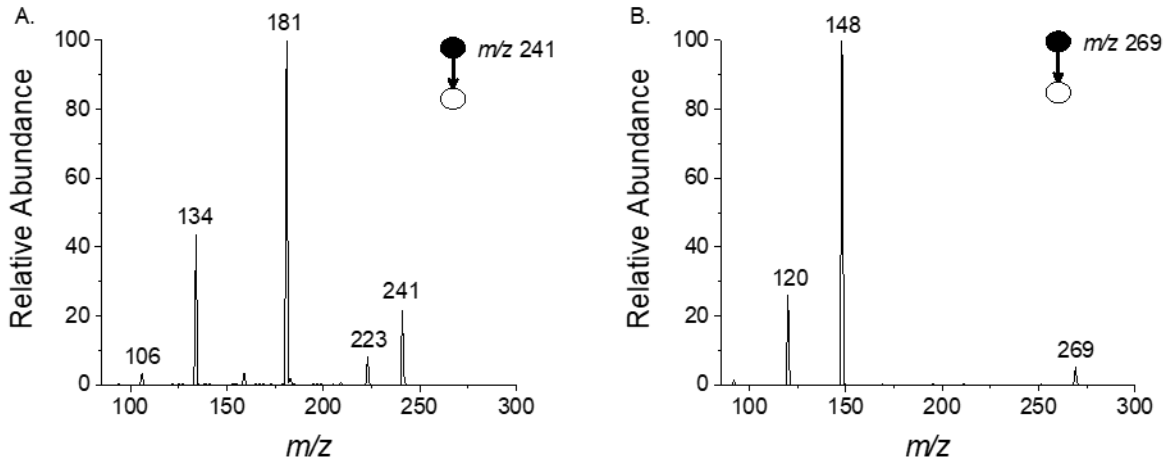


Figure 3.8 Swab touch spray product ion mass spectra of a Winchester 9mm Luger 147 Grain Full Metal Jacket spent casing for A) protonated MC at m/z 241, which shows the loss of neutral methylaniline to form the fragment at *m/z* 134, and a subsequent neutral loss of CO to form the fragment at *m/z* 106. The fragments at *m/z* 181 and *m/z* 223 arises from an isobaric compound at *m/z* 241. B) protonated EC at *m/z* 269, with the loss of neutral ethylaniline to form the fragment at *m/z* 148 and a subsequent loss of CO to form the fragment at *m/z* 120. Both spectra were obtained on a Thermo LTQ XL Ion Trap.

Another surface on which swab touch spray was utilized was the clothing of the shooter (Figure 3.9). Both MC and EC are present in the full scan, with the more prominent species being sodiated, however the sodiated species does not fragment as efficiently. Again, the protonated MC and EC were easily isolated and fragmented, and identified that the wearer of the clothing had

discharged a firearm. A hybrid Thermo LTQ-Orbitrap mass spectrometer (San Jose, CA) was used for analysis of Winchester 9mm Luger 147 Grain Full Metal Jacket samples. The Orbitrap results confirm the chemical formula of $C_{17}H_{21}N_2O$ and $C_{15}H_{17}N_2O$ with errors of 6.8 and 2.0 ppm, respectively. The MS/MS product ion scan also confirmed the chemical formulae of the product ion with errors under 5.0 ppm for all product ions.

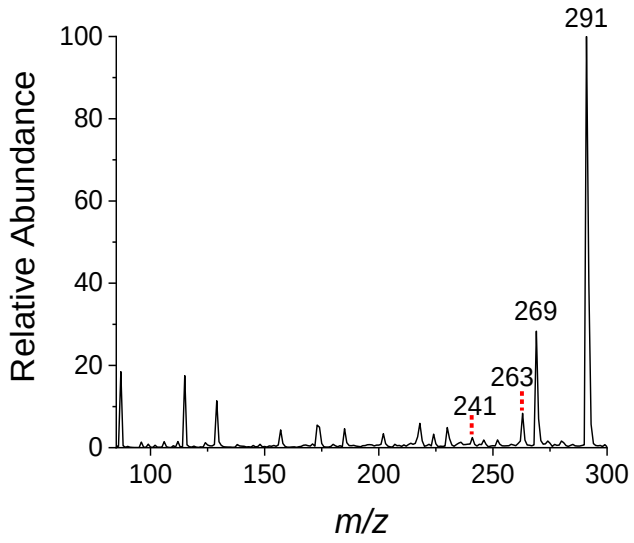


Figure 3.9 Full scan swab touch spray spectrum off the clothing of a shooter who fired Winchester 9mm Luger 147 Grain Full Metal Jacket ammunition showing both protonated and sodiated MC and EC.

3.4.3 Portable Mass Spectrometry

Detection of MC and EC by swab touch spray on a homebuilt Mini 12 was also successful. As the Mini 12 does not consistently have pneumatic assistance due to the discontinuous atmospheric pressure inlet (DAPI),[125] the experiment required the flow rate on the syringe pump to be altered. This ensured that a buildup of solvent did not occur on the capillary. Regardless of the challenge of altering the flow rate over time to ensure a stable spray, the Mini 12 was also capable of detecting MC and EC by swab touch spray from hands of the shooter (Figure 3.10). The Mini 12 was also able to detect both MC and EC after the discharge of a single round of ammunition.

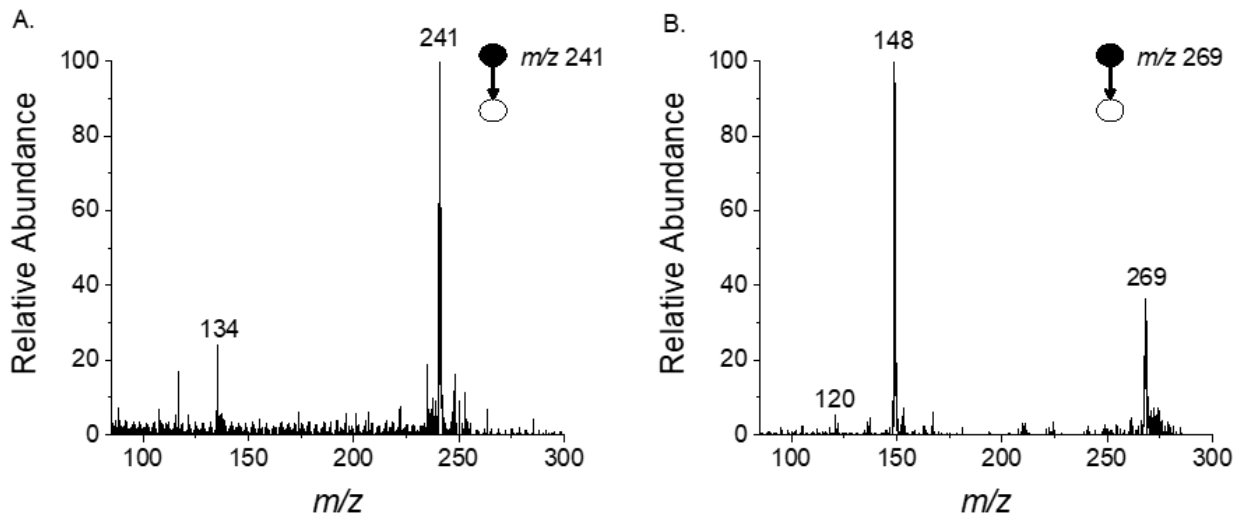


Figure 3.10 Swab touch spray product ion scan mass spectra off the hands of a shooter who fired Independence 9mm Luger 124 Grain Full Metal Jacket ammunition A) MS/MS of MC and B) MS/MS of EC, analyzed by a field portable Mini 12 mass spectrometer.

In addition to the utilization of the Mini 12, the detection of MC and EC was performed on the FLIR AI-MS 1.2 portable MS. As seen in Figure 3.2, the AI-MS 1.2 has a homebuilt mounting rack for reproducible and efficient analysis. Figure 3.11 shows the full scan spectrum of EC, which shows both the protonated and sodiated ions. The insert shows the MS/MS spectrum was also collected to ensure the identification of the OGSR. As a commercial portable this demonstration on an additional MS showed the broad applicability to forensic applications.

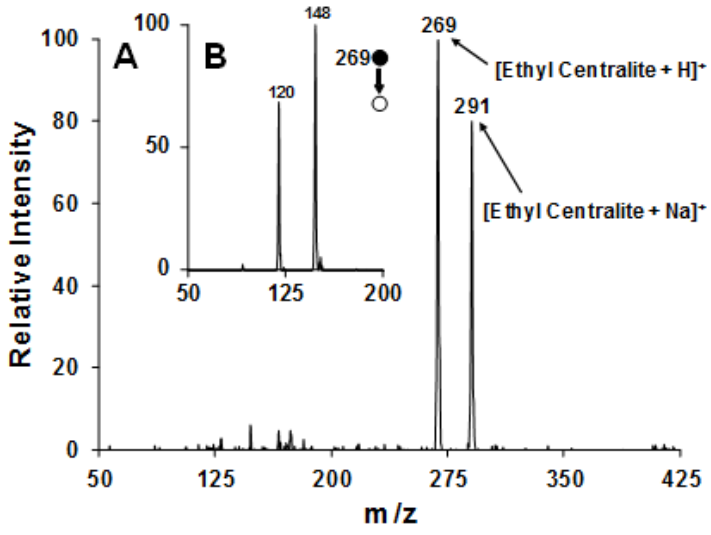


Figure 3.11 Swab touch spray ionization spectra from a FLIR AI-MS 1.2 portable MS collected after swabbing a trace, (A) surface-bound residue of EC. Both protonated (m/z 269) and sodiated (m/z 291) EC is observed. (B) MS/MS spectrum of protonated EC (m/z 269).

3.5 Conclusions

Swab touch spray has been shown to be an effective method for identifying OGSR from a variety of surfaces: hands, gloves, clothing, and spent shell casings. Determining if a potential suspect had discharged a firearm is a time sensitive matter.[116] Similarly, real time detection of explosive residues can aid in forensic, defense, and homeland security applications. The analysis of six different explosive residues from a variety of surfaces: glass, stainless steel, polyethylene, PTFE, human hands, nitrile gloves, vinyl gloves, and latex gloves furthered the scope of this ionization technique. The ability to detect these compounds can have direct applications on forensic investigations with regard to OGSR. Additionally, airport security could employ this technology in conjunction with the current ion mobility systems for explosive residues. The Mini 12 and the AI-MS 1.2, which has both been shown to be capable of *in-situ* analysis[127], has been demonstrated to be able to provide law enforcement with an answer in a more rapid and simple sampling method.

The advantages of swab touch spray are the ease of use, contained commercial packaging, low entrance barrier, and the fact that your sampling device is also your ionization source. Swab touch spray ionization requires no sample preparation, no lengthy analysis times, and is capable of in-field analysis. The swabs being tamper-proof sealed, individually wrapped, and requiring no preparatory steps, which can be a point at which contamination can be introduced, is a potentially ideal forensic sampling device. The low detection limits for explosives, and the ability to detect OGSR after a single discharge of a firearm are just two demonstrations of how swab touch spray ionization can improve forensic analyses.

CHAPTER 4. ENVIRONMENTAL MONITIORING OF PYROTECHNICS FOR POTENTIAL TOXIC BY-PRODUCTS.

4.1 Abstract

Smoke dyes are complex molecular systems often made of highly-conjugated aromatic hydrocarbon rings that have the potential to form many molecular derivatives and fragments when deployed. The chemical analysis of smoke samples is challenging due to the adiabatic temperature of the pyrotechnic combustion and the molecular complexity of the physically dispersed reaction products. Presented here is the characterization of the reaction byproducts of Mk124 smoke signals, which contain the red dye Disperse Red 9 (1-(methylamino)anthraquinone), using ambient ionization mass spectrometry. To achieve this, Mk124 smokes were functioned in the presence of sampling swabs that collected byproduct residues from the smoke plume in the ambient environment. These swabs were then analyzed using mass spectrometry to identify the expended pyrotechnic residues, with particular interest in halogenated species. Alongside the investigation of the currently fielded US Navy Mk124 smoke signal, results from the thermal decomposition of a simplified smoke system consisting of Disperse Red 9 (dye), potassium chlorate (oxidizer), and sucrose (fuel) are also presented. By understanding the chemical composition of smokes and their reaction products, potential toxicity effects can be easily assessed, which can lead to safer formulations with improved performance. Additionally, identifying pollutants can help guarantee compliance with stringent environmental regulations. These results can help the US Navy assess how smoke byproducts may impact Warfighter performance, personnel health, and the environment.

4.2 Introduction

Colored smokes are used extensively by the military for both signaling and obscurant applications.[128] These obscurants are generated by suspending particles in the atmosphere that obstruct parts of the electromagnetic spectrum in a mechanism similar to fog or mist.[129] The desired color and the pyrotechnic application (i.e., concealing things from view or signaling allies) is determined by the chemical composition of the pyrotechnic dye that is sublimed.[130] While signaling smokes are critical to military applications, there are health concerns for the Warfighter

as well as long-term environmental concerns.[131-133] Recently, heavy metals and perchlorate compounds are being phased out of pyrotechnic formulations in favor of "green" versions due to health concerns.[134] In the process of removing less environmentally friendly reagents such as perchlorate oxidizers, the compositions were reformulated to meet environmental and health criteria while maintaining performance.[135-136] These new "green" replacements meet the criteria for production reagents, but analysis is still necessary to ensure that no additional harmful byproducts are created when the items are functioned.

Pyrolysis gas chromatography mass spectrometry (GC/MS) is a current method utilized to characterize the side products and chemical reactions occurring when functioning a pyrotechnic.[137-138] This method easily identifies the chemical species in the pyrotechnic composition with any side products that are generated from deploying the devices.[139] As with any methodology, there are trade-offs when employing pyrolysis GC/MS. First, the overall run time of the pyrolysis GC/MS is relatively long (~45 minutes per sample). Second, the line from the pyrolysis chamber to the GC/MS requires cleaning between each run due to carry-over contamination concerns. Third, the pyrolysis chamber operates under an inert helium atmosphere, whereas the pyrotechnic is designed to be functioned in an ambient environment. Ambient ionization mass spectrometry is used as it improves both the problems of analysis time and sampling conditions.[4, 8, 140]

With the advent of desorption electrospray ionization (DESI)[5] in 2004 and direct analysis in real-time (DART)[6] in 2005, sampling and ionization of analytes were possible in ways never achieved before. Ionization sources classified as ambient typically follow three criteria: the sample is ionized in the open ambient environment, there is minimal or no sample preparation, and the analysis is rapid, usually due to the lack of chromatographic separations prior to analysis.[4] The work presented here demonstrates the adoption of ambient ionization techniques to the analysis of pyrotechnic compounds, specifically the currently fielded US Navy Mk124 smoke signal. Three ionization techniques are utilized: nanoelectrospray ionization,[141-142] paper spray ionization,[10, 18] and swab touch spray ionization.[27, 29] All three are spray-based ionization techniques with the latter two also being ambient ionization techniques.

Nanoelectrospray ionization is a rapid analysis technique that does not require chromatography and features ionization in the open environment; however, it also requires sample preparation through the extraction of the compounds from the collection device.[141] A variant of

electrospray, nanoelectrospray produces ions at the tip of a glass capillary drawn to an outer diameter of a few micrometers. There is no sheath gas and the flow rate is much lower than in electrospray, and additionally each glass capillary is individually used and then disposed so there is no cleaning of sample lines.[142] Paper spray ionization utilizes a piece of filter paper cut into a triangle with a sharp tip.[10] When a spray solvent and a high voltage is applied to the paper, a Taylor cone is produced at the tip of the paper and analytes that were deposited onto the paper are ionized.[122] Swab touch spray ionization utilizes a rayon-tipped collection swab that has an aluminum wire handle where high voltage can be applied to generate ions.[31] Similar to paper spray ionization, a spray solvent is applied to the tip of the swab. With both paper spray ionization and swab touch spray ionization techniques, the substrate acts as both the sampling device and the ionization source, undergoing an electrospray-like ionization through the production of a Taylor cone from the tip of the paper or swab.[11, 122]

In this study, the three collection and ionization methodologies were assessed to determine their practicality for screening the chemical composition and reaction products of pyrotechnic formulations in the environment that they would be functioned in. Figure 4.1 shows the Mk124 smoke being functioned in a burn cage to collect the smoke products. The results are useful for identifying smoke byproducts that may impact Warfighter performance, personnel health, and the environment.

Figure 4.1 Mk124 smoke being functioned in a burn cage. Filter paper, swabs, and glass wool were positioned around the smoke to collect samples.

4.3 Experimental

4.3.1 GCMS

Pyrolysis GC/MS was performed for comparison to the ambient ionization methods. Following the procedure by Dilger et. al.,[138] GC/MS measurements of pyrolyzed samples were performed via a Pyroprobe 2000 (CDS Analytical, Inc., Oxford, PA) connected in-line to a Finnigan PolarisQ GC/MS^n ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Mock smoke compositions of Disperse Red 9 and potassium chlorate were placed within a quartz fire tube plugged at both ends with quartz wool (4 μm, Quartz Scientific, Inc., Fairport Harbor, OH). The pyrolysis probe was inserted into a chamber filled with helium carrier gas and underwent pyrolysis for 30 seconds. The gaseous analytes were transferred from the chamber to the GC column by helium carrier gas. Eluted compounds were directly injected into the source region of the mass spectrometer and subjected to electron impact ionization prior to mass analysis.

4.3.2 Ambient Ionization

A Mk124 smoke signal was positioned on a table within an outdoor chain-link fence enclosure. Above the pyrotechnic device, three pieces of glass wool were arranged such that they would be within the plume of the smoke about 70 cm from the source. These pieces of glass wool were held in place by two ring stands using one clamp per piece. Above the glass wool, two pieces of paper filter paper were positioned, each held in place by a clamp. Suspended from the ceiling of the chain-link fence enclosure, three wire racks were hung in the path of the smoke at distances of approximately 85 cm, 160 cm, and 250 cm from the source. Attached to each of these three wire racks were four swabs and three filter papers. After setting up the collection apparatus, the Mk124 was deployed and the smoke composition was deposited onto the surface of the three types of sampling devices (Figure 4.2). Each sampling device was then individually sealed and transported to the laboratory to perform mass spectrometry analysis using the three ionization methodologies noted above.

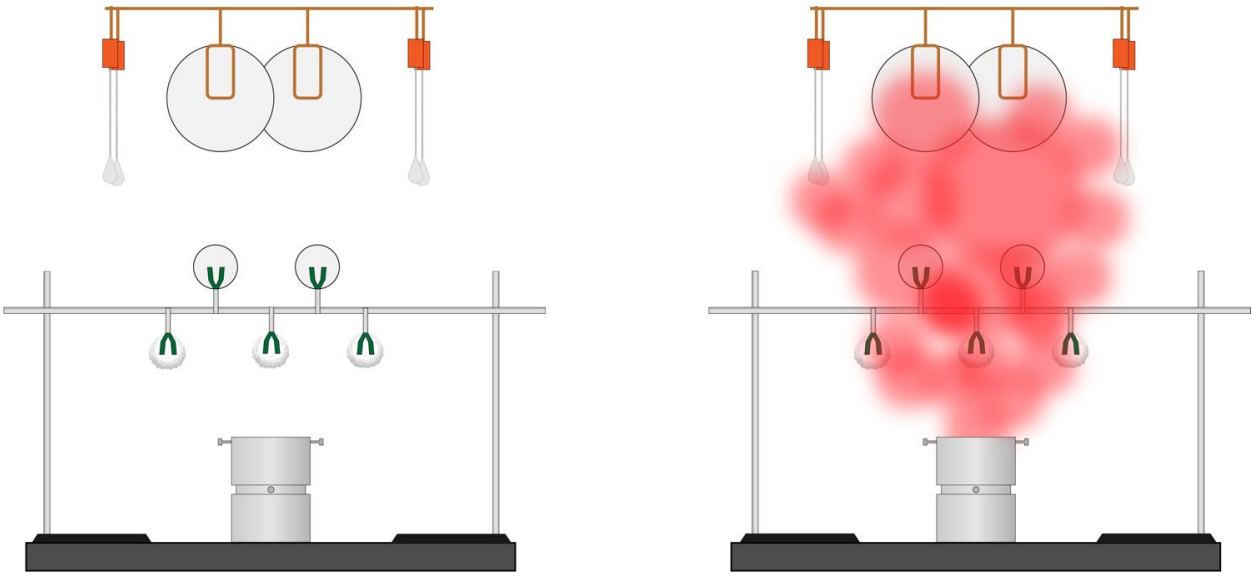


Figure 4.2 Experimental setup. The pyrotechnic was set on a table with three pieces of glass wool positioned above it. On the same rack two paper substrates were positioned above the glass wool. Suspended in the path of the red smoke plume were three sets of wire hangers each holding four swabs and three paper substrates. Both the paper and the swabs acted as the collection devices as well as the subsequent ionization sources.

All spectra were recorded in positive and negative ion modes using a Thermo LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap mass spectrometer (San Jose, CA). All tandem mass spectrometry (MS/MS) product ion scan mass spectra were generated through collision-induced dissociation (CID). Normalized collision energies from 5 to 30 (arbitrary units) on the ion trap were used. High resolution spectra were collected as well through the Orbitrap.

Glass wool was placed in a solution of HPLC-grade methanol with 1.0% modifier (to promote ionization) and sonicated. This sample solution was loaded into a nanoelectrospray emitter via pipette. The nanoelectrospray emitters were formed from borosilicate glass capillaries (1.5 mm o.d., 0.86 mm i.d., Sutter Instrument Co., Novato, CA) pulled to a 5 μm tip using a Flaming/Brown micropipette puller (Sutter Instrument Co. model P-97, Novato, CA). An electrode was placed into the back of the pulled glass capillary, and the ion source was positioned in front of the mass spectrometer inlet. Ions in nanoelectrospray were generated by applying ~2 kV to the electrode for both positive and negative ion modes (Figure 4.3A).

Whatman 1 chromatography paper was cut into a triangular shape approximately 10 mm long and 5 mm wide at the base. The paper triangle was held in place by a copper clip attached to

the instrument high voltage lead. The paper was wetted via pipette with a solution of HPLC-grade methanol with 1.0% modifier to promote ionization. Approximately 10 µL of spray solvent was added to the paper, and then a high voltage (4-5 kV) was applied to the paper through the copper clip. Solvent was continually added via pipette to maintain a stable spray (Figure 4.3B).

Medical grade sterile swabs (Copan Diagnostics, Murritea, CA) constructed with aluminum handles and rayon swabs were utilized for all experiments. Each swab was individually packaged with a tamper-proof label and removed from the packaging only to sample and then was returned to the casing. The swabs were positioned vertically, approximately 8 mm above the inlet of the mass spectrometer. HPLC-grade methanol with 1.0% modifier (to promote ionization) was applied to the swab via pipette to ensure that the swab was completely wetted. Then a continual flow of the methanol solvent was sprayed onto the swab by a Harvard Apparatus standard infusion only PHD 22/2000 syringe pump (Holliston, MA) using a Hamilton syringe (Reno, NV) at a varied flow rate (10–30 µl/min) to maintain a steady spray. A high voltage of 5.5 kV was applied to the aluminum handle, and the generation of a spray could be visually observed (Figure 4.3C).

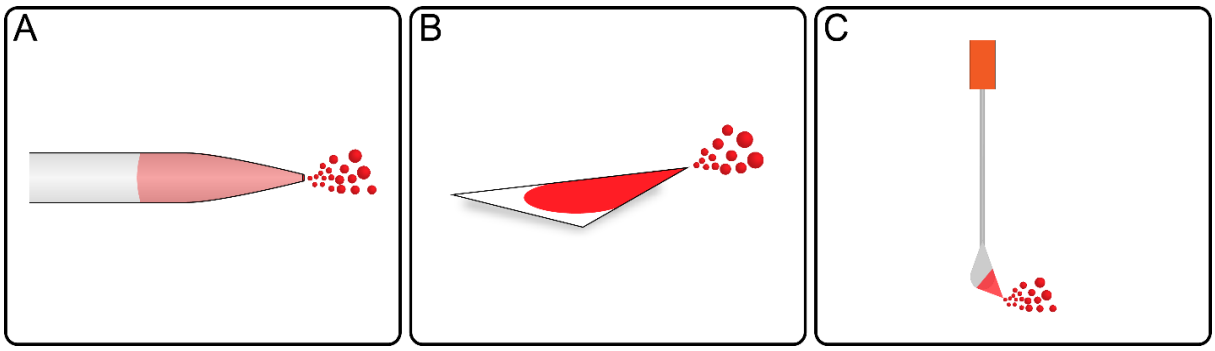


Figure 4.3 Three ion sources that operate under the ambient environment. A) nanoelectrospray ionization, B) paper spray ionization, and C) swab touch spray ionization.

4.4 Results and Discussion

4.4.1 Fielded Mk124 Pyrotechnic Smoke

To aid in the identification of potential byproducts of functioning the Mk124 smoke signal, two dyes in the composition, Disperse Red 9 and Sudan II (1-(2,4-xylylazo)-2-naphthol), were analyzed by nanoelectrospray ionization mass spectrometry. Figure 4.4 shows the MS/MS spectra of Disperse Red 9 in positive ion mode and Sudan II in both positive and negative ion modes. The

fragments that occur from CID during mass analysis are suspected to be side products that can occur when the Mk124 is functioned in the ambient environment.

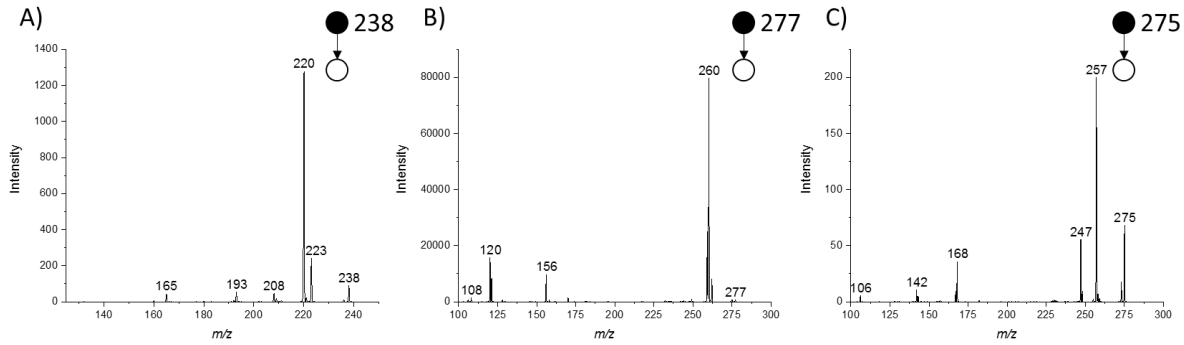


Figure 4.4 MS/MS spectra of A) Disperse Red 9 in positive ion mode, B) Sudan II in positive ion mode, C) Sudan II in negative ion mode. All three spectra were collected by nanoelectrospray ionization.

The fragmentation pattern of the Disperse Red 9 dye was similar to the fragmentation pattern observed by the pyrolysis GC/MS under pyrolytic conditions. As the two instruments operate under very different conditions (ionization energy, gas, and chromatographic influences), differences in the relative intensity of product ions are to be expected as well as alternative fragmentation pathways (Figure 4.5).

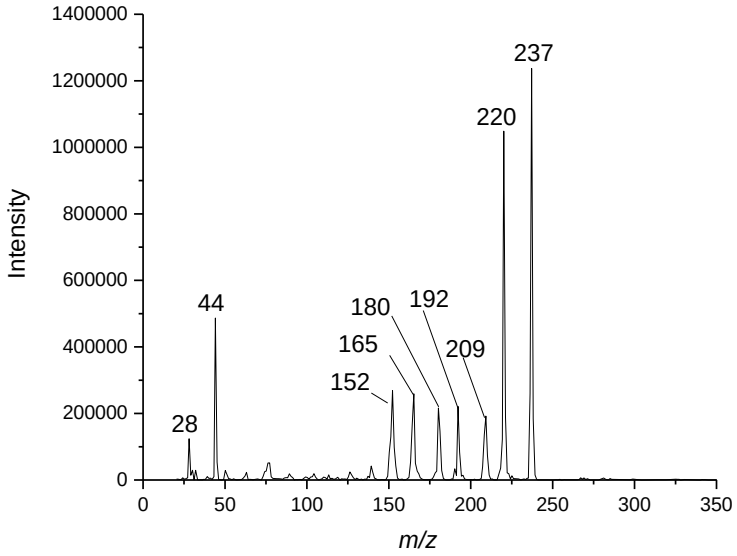


Figure 4.5 Pyrolysis GC/MS spectrum of Disperse Red 9. The fragments generated by the electron impact ionization correlate to the ambient ionization fragmentation data.

As the dyes typically used in smokes are cyclic, and the fragments produced during CID or through the electron impact ionization show cyclic compounds, the potential for these compounds to become chlorinated in the presence of the potassium chlorate is of concern. The MS/MS spectrum of chlorinated Disperse Red 9 $[M-H+Cl]^-$ by paper spray ionization is shown in Figure 4.6. To fragment both isotopes of the chlorinated Disperse Red 9, an isolation width of 4 was utilized with a CID energy of 7 (arbitrary units)

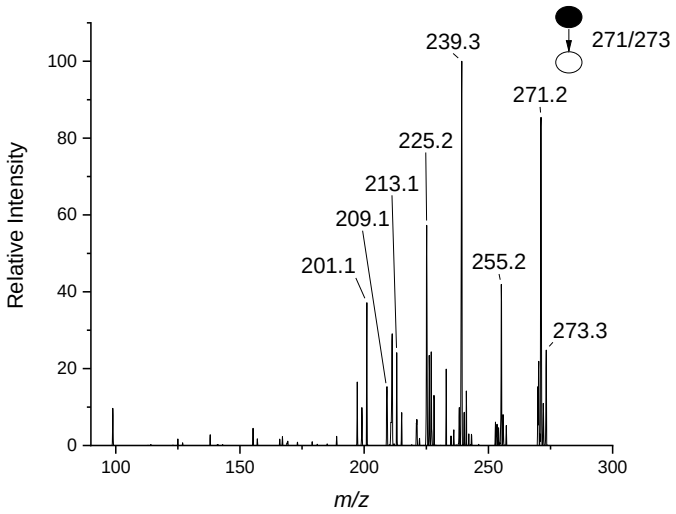


Figure 4.6 MS/MS spectrum of the chlorinated Disperse Red 9 [M-H+Cl]- by paper spray ionization.

While ambient ionization utilizes both targeted isolation of suspect chlorinated species found in the full scan data, the pyrolysis GC/MS method relies on single scans related to retention time. Figure 4.7 shows the mass spectrum corresponding to chlorinated Disperse Red 9 as it is eluted from the GC.

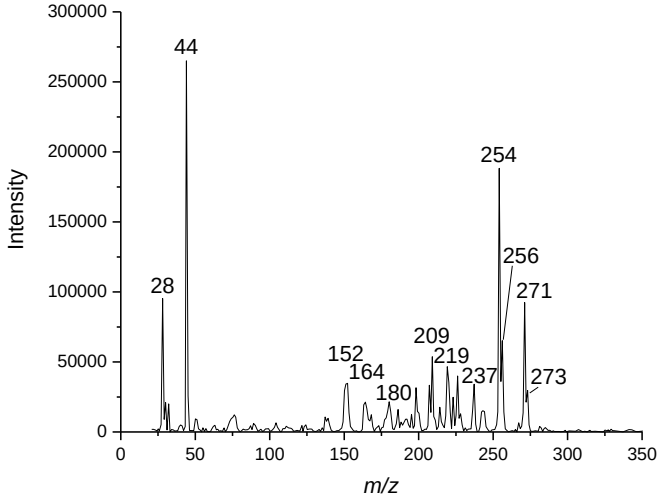


Figure 4.7 Pyrolysis GC/MS spectrum of chlorinated Disperse Red 9.

4.5 Conclusions

Ambient ionization mass spectrometry is a promising technique for the analysis of pyrotechnic smokes and obscurants. Functioning of the Mk124 smokes in the open environment, which is also the way they are utilized in the field, allows for the analysis to be conducted under relevant conditions. As reaction pathways can differ in aerobic and anaerobic environments, these ambient ionization techniques can provide a multigram analysis for full systems that complement the milligram pyrolysis GC/MS methodology for formulation development. Through the use of an ion trap, full scan spectra chlorinated species can be identified, while MS/MS spectra can be used for structural confirmation of species. The Orbitrap can produce high-resolution spectra to help confirm the identity of any byproducts seen in the spectra. The rapid analysis times of the three ionization techniques allow for many samples to be quickly analyzed, which can aid in the testing and development of novel pyrotechnic compositions. The analysis presented herein demonstrates that ambient ionization mass spectrometry can be used to monitor the chemical species generated by a functioned colored smoke pyrotechnic device.

CHAPTER 5. AUTHENTICATION OF MICROELECTRONICS BY MASS SPECTROMETERIC SURFACE TECHNIQUES.

5.1 Abstract

A major challenge in the electronics industry is counterfeit electronic components as they impact quality and security. These parts can be introduced into the supply chain in a multitude of ways varying from surplus parts that have been modified, or through the use of salvaged scrap parts that are refurbished. The susceptibility to counterfeiting poses a threat to Department of Defense systems. If malicious or subpar microelectronics are used in critical devices that support the Warfighter, malfunctions can occur. Currently, to combat counterfeits, electronics components are examined using solvent wipes and microscopy, which are time consuming, require an expert user, and are not based on molecular signatures. Mass spectrometry can greatly improve the forensic analyses of these microelectronics. The Naval Surface Warfare Center, Crane provided 29 plastic encapsulated integrated circuits suspected as being counterfeit in addition to certified genuine parts. Two ionization methods were explored: desorption electrospray ionization (DESI) and secondary ion mass spectrometry (SIMS). Reactive DESI, a variant of traditional DESI where there are derivatization reagents in the spray solvent, provided discrimination between the two sample classes, through the aid of principal component analysis (PCA). Molecular signatures provided by the DESI analysis were not subjective like other microscopy methodologies, but gave distinctive spectra which allowed the separation. SIMS images were collected of the integrated circuits after performing focused ion beam sputtering (FIB). The FIB, utilizing a 45-degree angled cut, provided the underlying surface for SIMS analysis, while not damaging the integrity of the integrated circuit. SIMS, utilizing a Bi^+ ion source, provided imaging data of the morphology of the underlaying particles. The differences in the underlying morphologies of the encapsulated particles allow discrimination, while not disrupting the performance of the IC.

5.2 Introduction

Counterfeit electronic components have become a major challenge for the entire electronics industry as it impacts quality and, more importantly, security.[143] Counterfeits can be produced in a variety of different ways including new or surplus parts that have been modified in some manner.

Furthermore, they can be salvaged scrap parts that are refurbished to look as though they are new.[144] Usually, the packaging of these parts is altered to modify their identity or disguise the effects of salvaging. The modification is typically the simple removal of markings and addition of new markings. Additionally, the alterations can be more complicated such as the recovery of a dye and repackaging.[145] The main point of susceptibility to counterfeiting in the electronics industry lies in the supply chain. Management of this weakness tends to focus on visible disruptions of the supply chain instead of the covert entrance of counterfeits.[146] Currently counterfeit electronics are identified using microscopy which is time consuming, requires an expert user, and is not based on molecular signatures.[145] Mass spectrometry, with its excellent sensitivity and ability to be automated, is an excellent choice for developing techniques for detecting the differences between counterfeit and authentic integrated circuits. Hieftje and co-workers developed a flowing atmospheric pressure afterglow (FAPA) ionization source, a plasma based ambient ionization source, which was able to discriminate between classes of counterfeit and genuine plastic encapsulated integrated circuits. [147-148] While discrimination of the plastic encapsulated integrated circuits (IC) was possible, one issue however with this method was if the ion source was positioned on top of the IC for extended periods of time, the highly energetic plasma would destroy the top of the IC potentially damaging its functioning.[149]

Desorption electrospray ionization,[5] like FAPA has the advantage of working with native samples, in the ordinary environment; which increases speed and simplifies the process of chemical analysis. As previously described in the DESI process, a stream of solvent microdroplets is directed to and impacts upon the analyte surface from which micron-sized secondary droplets, which contain molecules removed from the surface, are splashed.[5] These droplets are delivered to the mass spectrometer where they are transformed into fully desolvated ions. This can be used to qualitatively explore the distribution of analytes in the sample as they generally relate to ion abundances at a given point. This technique has been used for a variety of applications. Most notably this technique has been applied for the imaging of compounds in biological tissue by several research group.[150-152] These images (with training sets) have been used to discriminate between different classes of samples (e.g. cancerous and non-cancerous tissue) an application which is in principle analogous to that planned here.[153-154]

A major advantage DESI is there reactive forms, which uses a derivatization reaction in the plasma or spray. These reactive forms can be implemented in conjunction with ionization so

that hard-to-ionize compounds can be selectively observed.[155] In reactive DESI, a reagent ion is doped into the primary spray and the reaction between the doped in reagent and the material desorbed from the surface can be performed during the time it takes the secondary droplet to reach the mass spectrometer. Derivatization is a common practice for selectively increasing the ionization efficiency of particular classes of analyte in complex mixtures.[17] Specifically, with DESI, it has been shown that reactions can be performed at faster rates than their corresponding bulk-phase reaction by Girod et al.[156] Often, these rate accelerations can be dramatic (ca 1000) compared to the bulk-phase reaction.[157] Reactive DESI has been demonstrated for the selective detection of hydrolysis products by our group.[158] By combining ion/molecule chemistry with mass spectrometry the ability to find discriminating features on a surface is potentially enhanced.

Finally, another very powerful mass spectrometric tool, which was long used for elemental analysis before a molecular version was developed, is secondary ion mass spectrometry.[159] The key to obtaining molecular information from SIMS is to use low primary ion fluxes, a method known as static SIMS. Hence, even though energy deposition at the point of impact may be large, and molecularly destructive, a much larger area is gently activated, and ionized molecules are desorbed. These ions provide molecular information (molecular mass and some features of structure). Molecular SIMS is not as gentle ('soft') a method as DESI, but it still gives useful information. It also provides high quality 2D images. The availability of elemental and complementary molecular information, including distributions across surface by imaging, is a major advantage of SIMS. Note the special applicability of SIMS to polymers, which is a topic studied in detail by numerous authors including Benninghoven, Hercules and Cotter.[160-162] The understanding of molecular SIMS, including the ionization processes leading to emission of precharged ions, cation attached molecules and radical cations, and the role of matrix in these processes owes much to early studies at Purdue.[163-167]

Reported in this chapter are two mass spectrometric ionization methods that have been explored to aid in distinguishing between authentic and counterfeit microelectronics. SIMS can provide both molecular and elemental information to generate images of both authentic and counterfeit plastic encapsulated integrated circuits. While the SIMS process is lengthy for analysis, the clear differences in the morphology of the underlying layers of the ICs remove some of the subjectivity of the solvent tests. The second ionization source is an ambient technique that provide rapid molecular information in the open atmosphere. The untreated surface of the ICs can be

analyzed by DESI and through multivariate statistical analyses DESI can separate authentic and counterfeit ICs. Both of these techniques will add to the forensic analyst's toolbox in preventing counterfeit microelectronics from entering our mission critical systems at the Department of Defense.

5.3 Experimental

5.3.1 Integrated Circuits

Authentic Integrated Circuits were purchased from SMT Corporation (Sandy Hook, CT). Counterfeit ICs were donated by SMT Corporation for testing and from seized materials by the Department of Homeland Security. A total of 29 unique part types and one certified genuine part were examined. To determine their classification, the gold standard solvent tests were performed on each of the seized samples.

5.3.2 Solvent Tests

The authenticity of the ICs were determined by two solvent tests, an acetone and Dynasolve 750 screening. The acetone solvent test had a cotton swab with wooden handle wetted with acetone and then vigorously rastered over the top of the IC. The cotton swab would remove the top layer of poorly made counterfeits, usually ICs that were blacktopped with a paint substrate. The cotton swab and the top of the IC was then observed under an optical microscope to observe the discoloration and any potential underlaying markers. Authentic or more sophisticated counterfeits would not discolor the swab or have any layers removed. Unfortunately, as the ICs could be dirty and slight discoloration becomes a judgement call based on the operator's experience leading to a third category of "maybe".

The Dynasolve 750 test submerged half of the IC in a beaker of the D750 and heated for ~30 minutes. Dynasolve 750, is a commercial system for plastic de-encapsulation. After it was swabbed with a new cotton swab, the IC was placed in a water bath and sonicated. The sonication aids in bringing out any underlying defects in the materials, such as the original markings on a counterfeit. The IC and the swab were analyzed under the microscope. This test is destructive for the IC so after the analysis the IC will not work.

5.3.3 Desorption Electrospray Ionization

Reactive DESI was utilized by adding KOH to a MeOH:Water spray solvent. The addition of KOH allowed the spray solvent to mimic Dynasolve 750 as KOH is a major component of it (Figure 5.1). The DESI ion source was coupled to a Thermo LTQ ion trap mass spectrometer (San Jose, CA). Spectra were recorded in both positive and negative ion mode. For consistency, each IC had 30 seconds of positive ion spectra and 30 seconds of negative ion spectra recorded for the PCA analysis.

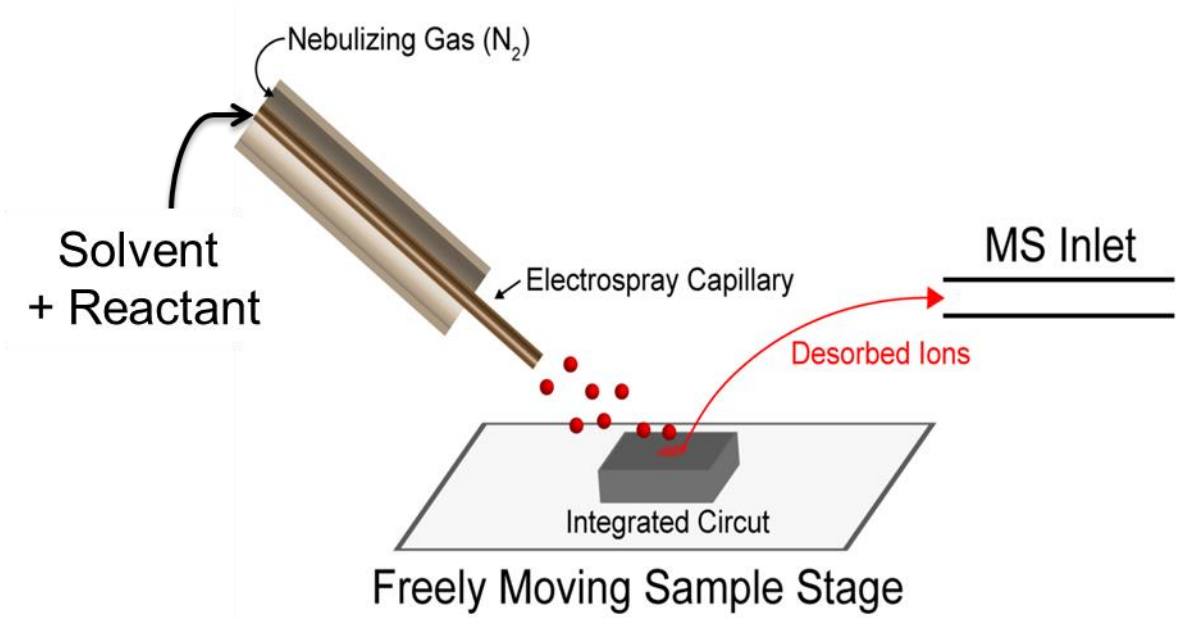


Figure 5.1 Pictorial representation of desorption electrospray ionization mass spectrometry (DESI-MS) schematic, where primary droplets are directed to a surface and the analyte contained in desorbed secondary droplets enter the mass spectrometer for mass analysis. The reactant and spray solvent consisted of 50:50 MeOH:Water with 0.1% KOH.

5.3.4 TOF-SIMS

An IONTOF-SIMS 5 mass spectrometer (location) was utilized to collect all spectra in both positive and negative ion mode (Figure 5.2). A liquid metal ion gun Bi_3^+ operating at 30kV in spectrometry mode was used to collect all ion images. A sawtooth rastering mode was used in a 128 x 128 pixel at 5 shots per pixel. The mass range of 0 to 450 amu was collected. Samples were introduced into the load lock chamber and pumped down to 10^{-7} torr and then introduced inot the main chamber which was held at 10^{-9} torr.

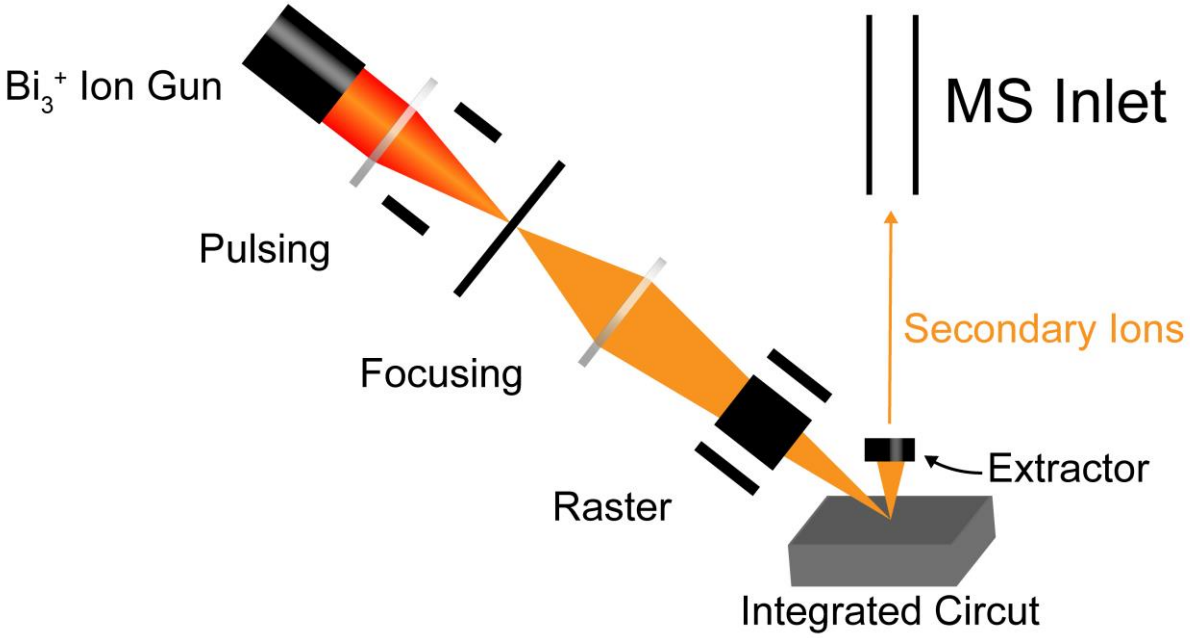


Figure 5.2 Pictorial representation of the time of flight secondary ion mass spectrometry (TOF-SIMS) schematic, where high energy Bi3+ primary ions bombard the surface of an integrated circuit to sputter secondary ions (elemental and molecular) to be analyzed by a time of flight mass spectrometer.

To generate the surface for the TOF-SIMS analysis, a gallium ion beam used to sputter material away was utilized (Figure 5.3). A ~ 700nm aperture was used for the initial bulk sputtering and then the IC surface was polished with a 300nm and a 100n, aperture. The dwell time was 50 µs with a crater size of ~ 200 nm x 200 nm square. This rastering was performed over a 100 µm surface and each focused ion beam (FIB) cutting took approximately 3 hours per IC. The resulting image after the FIB cut of the IC surface can be seen in Figure 5.4.

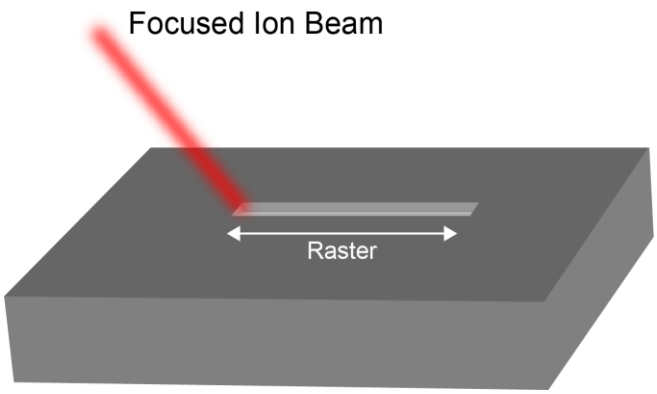


Figure 5.3 Focused ion beam (FIB) sputtering utilized to access the underlying layers of the polymer plastic encapsulate. This creates a face that can be analyzed and imaged.

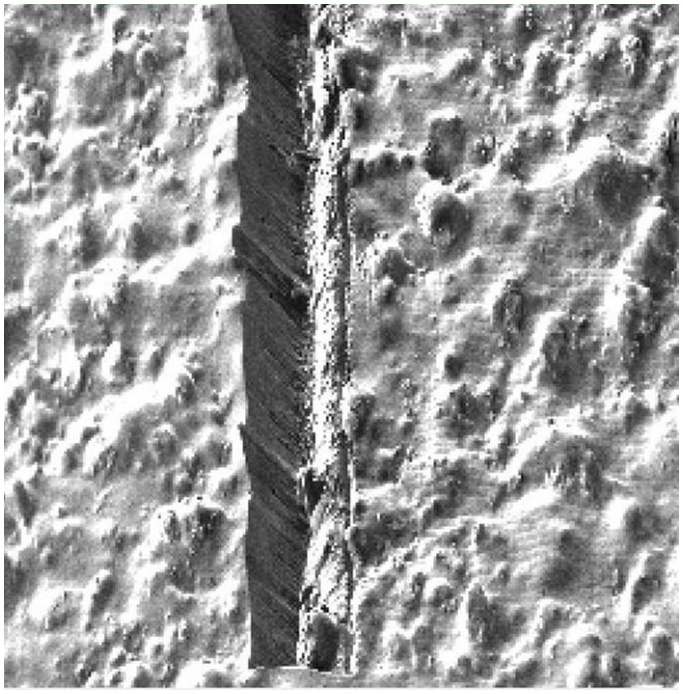


Figure 5.4 A image of the top of the IC after FIB sputtering. The wall that is created from the FIB cut will be imaged by the TOF-SIMS.

5.3.5 PCA

Many techniques are available for pattern recognition analysis. They are divided between unsupervised and supervised methods.[168-169] Principal component analysis, (PCA) provides information about the presence of relationships among samples and/or variables studies. By means

of PCA, the data information content is reorganized and compacted into few principal components (PCs), which are orthogonal and describe independent contributions to part of the variance in the data. The projections of the data objects (i.e. the samples) onto the PCs are called scores, while the importance of each original spectral variable (*m/z* peak) in defining a certain PC is given by the loading coefficient. Both score and loading values can be represented in two-dimensional scatter plots. Simultaneous examination of the plots reveal information enclosed in the datasets useful for characterizing the samples. In more detail, in the score plot, it is possible to visualize groupings that indicate similarities among samples on the basis of the information derived from the mass spectra, and these can be associated with particular characteristics of the samples analyzed. Subsequently, an examination of both the loading plot and the score plot allows chemical characterization of the samples to be achieved, revealing which *m/z* peaks are the most important in defining each sub-set of samples under consideration. The relationship between the score and loading plots is evident from the co-directionality of objects and variables in related score and loading plots. A Matlab script was written in our laboratory to take the full scan mass spectra and analyze them by PCA for visual interpretation.

5.4 Results and Discussion

5.4.1 Solvent Tests

After performing the acetone solvent test, 12 of the ICs were deemed as counterfeits due to the extreme discoloring of the swab and the IC surface (Figure 5.5). There were 9 ICs that did not have any discoloration on either the swab or the IC surface allowing them to pass the initial acetone test. Although the ICs passed the acetone test, this did not guarantee that the ICs were genuine. Of the remaining ICs, 8 had slight discoloration of the swab but no discoloration of the IC surface. This third category was created as there was no clear distinction of whether the IC passed or failed the acetone test. The discoloration of the swab could simply be due to the surface of the ICs being dirty from the manufacturing or transportation process.

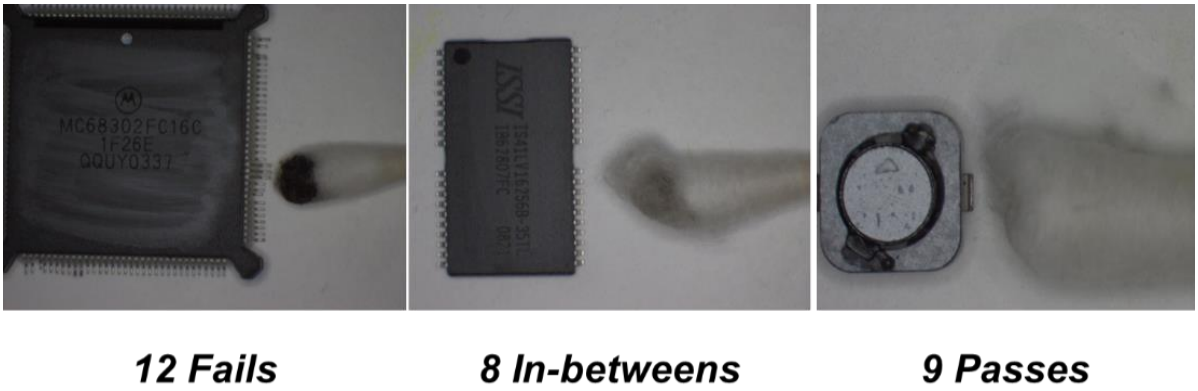


Figure 5.5 Integrated circuits (IC) after conducting the acetone solvent test. ICs fell into one of three categories: fails due to extreme discoloration of both the swab and the IC surface, in-betweens which had minor discoloration of the swab but no discoloration of the IC surface, and passes which neither the swab or the IC surface was discolored.

Following the acetone solvent test, the Dynasolve 750 solvent test was performed. There were 23 ICs that were deemed failures. Figure 5.6 shows that the underlying "original" markings for the counterfeit IC has been revealed and the determination that this IC was unquestionably resurfaced was made. The remain 6 ICs passed the more rigorous Dynasolve 750 solvent test. These final categories that the ICs were placed into based on the two solvent tests can be seen in Table 5.1.

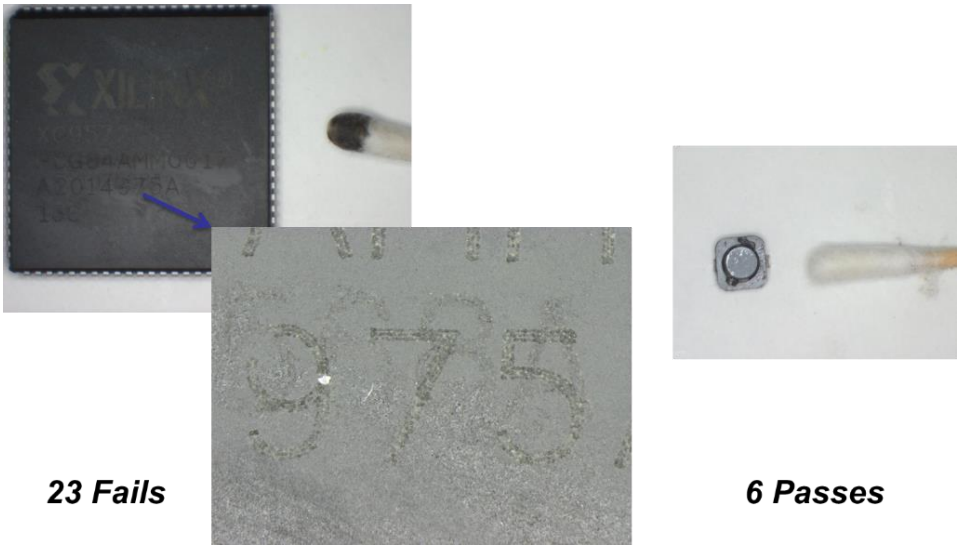


Figure 5.6 Integrated circuits (IC) after conducting the Dynasolve 750 solvent test. ICs fell into two categories: fails due to extreme discoloration of both the swab and the IC surface and passes which neither the swab or the IC surface was discolored. For ICs that failed, some ICs revealed their original markings after the Dynasolve 750 solvent test.

Table 5.1 Categories of the integrated circuits (IC) after solvent tests were performed. These categories were later utilized in the statistical methods for discrimination.

Category	Number of Parts
Failed Both Solvent Tests	12
Maybe Acetone, Fail D750	7
Pass Acetone, Fail D750	5
Maybe Acetone, Pass D750	1
Passed Both Solvent Tests	4
Genuine Part	1

5.4.2 Reactive Desorption Electrospray Ionization

Spectra were recorded for both genuine and counterfeit ICs through reactive DESI MS utilizing KOH in the MeOH:Water spray solvent to mimic Dynasolve 750. Figure 5.7 shows the overlain spectra of a known counterfeit and a known genuine IC. The differences in the spectra can be interpreted with a Matlab script that was written in our laboratory and a score plot was generated to visually observe the degree of dissimilarity between the two mass spectra. The visual score plots of the datasets of ICs can be seen in Figures 5.8 and 5.9. The six colored categories represent: authentic, integrated circuits that passed both the acetone and Dynasolve tests, integrated circuits that were labeled “dirty” in the acetone test but passed the more rigorous Dynasolve test, integrated circuits that passed the acetone test but failed the Dynasolve test, integrated circuits that were labeled “dirty” for the acetone test but failed the Dynasolve test, and integrated circuits that failed both the acetone and Dynasolve tests.

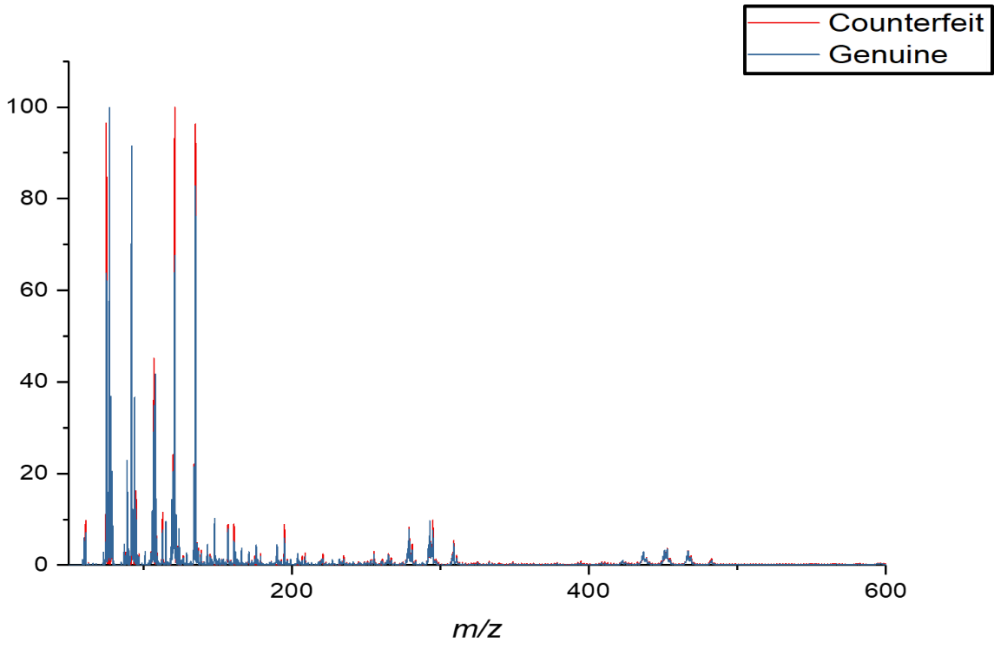


Figure 5.7 Overlain full scan mass spectra of a known counterfeit and a known genuine IC acquired in negative ion mode through reactive DESI MS.

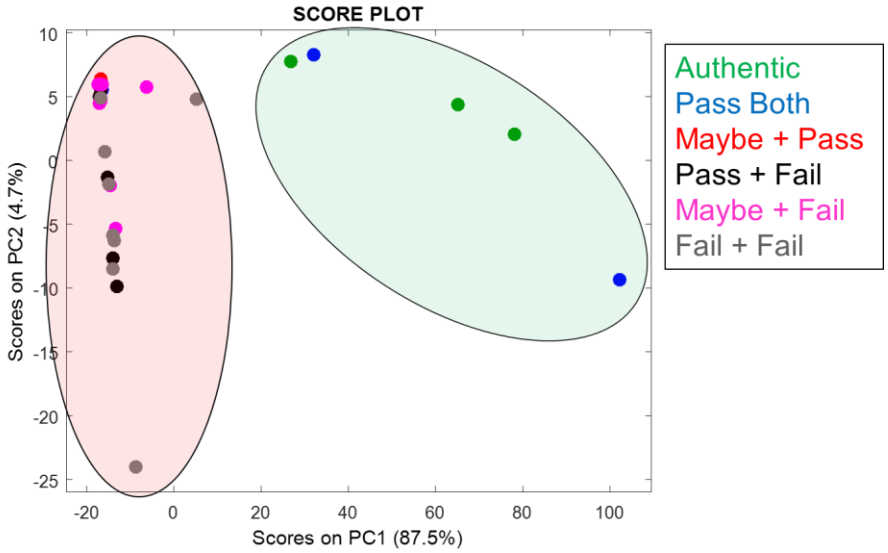


Figure 5.8 The visual score plots of the datasets of plastic encapsulated integrated circuits using principal component 1 and principal component 2. The six colored categories represent: authentic, integrated circuits that passed both the acetone and Dynasolve tests, integrated circuits that were labeled "dirty" in the acetone test but passed the more rigorous Dynasolve test, integrated circuits that passed the acetone test but failed the Dynasolve test, integrated circuits that were labeled "dirty" for the acetone test but failed.

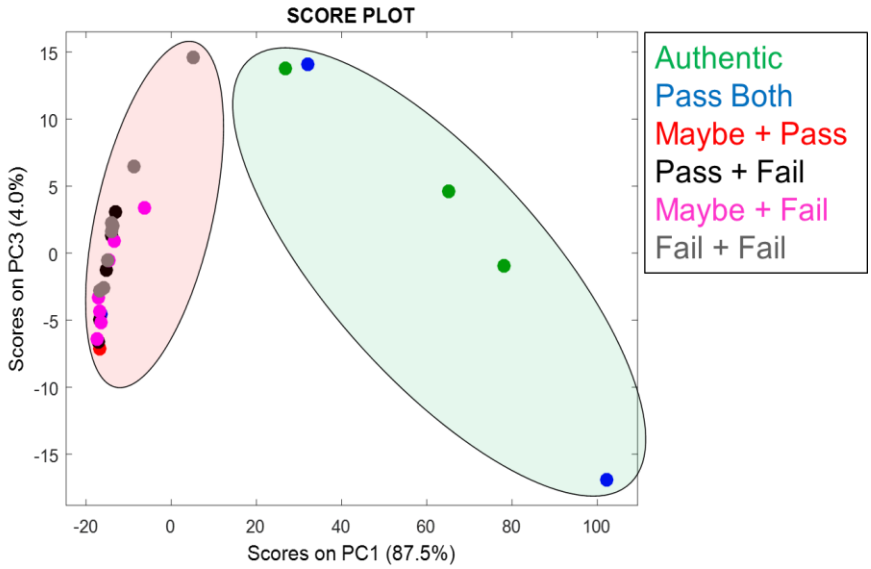


Figure 5.9 The visual score plots of the datasets of plastic encapsulated integrated circuits using principal component 1 and principal component 3. The six colored categories represent: authentic, integrated circuits that passed both the acetone and Dynasolve tests, integrated circuits that were labeled "dirty" in the acetone test but passed the more rigorous Dynasolve test, integrated circuits that passed the acetone test but failed the Dynasolve test, integrated circuits that were labeled "dirty" for the acetone test but failed.

5.4.3 TOF-SIMS Images

Surface analysis by TOF-SIMS alone did not provide separation. However, the images that were created after performing focused ion beam sputtering (FIB) provided potentially discriminating features. The FIB provided a new surface by utilizing a 45-degree angled cut that provided a new surface for SIMS analysis, while not damaging the integrity of the IC. The high quality 2D images (Figure 5.10) show both elemental and molecular information, including distributions across the surface by imaging. The images in Figure 5.9 show both positive and negative ion modes of both genuine and counterfeit plastic encapsulated integrated circuits. The differences in the underlying morphologies of the encapsulated particles seem to be a distinguishing differentiation between the two classes of ICs. The characterization of these underlying morphologies is still unknown and need to be explored further, but the hypothesis is that when resurfacing the counterfeit ICs, the underlying particles are packed tighter and lose their spherical shape. Where this technique is also subjective like the solvent tests, a major benefit is the non-destructive nature of this test.

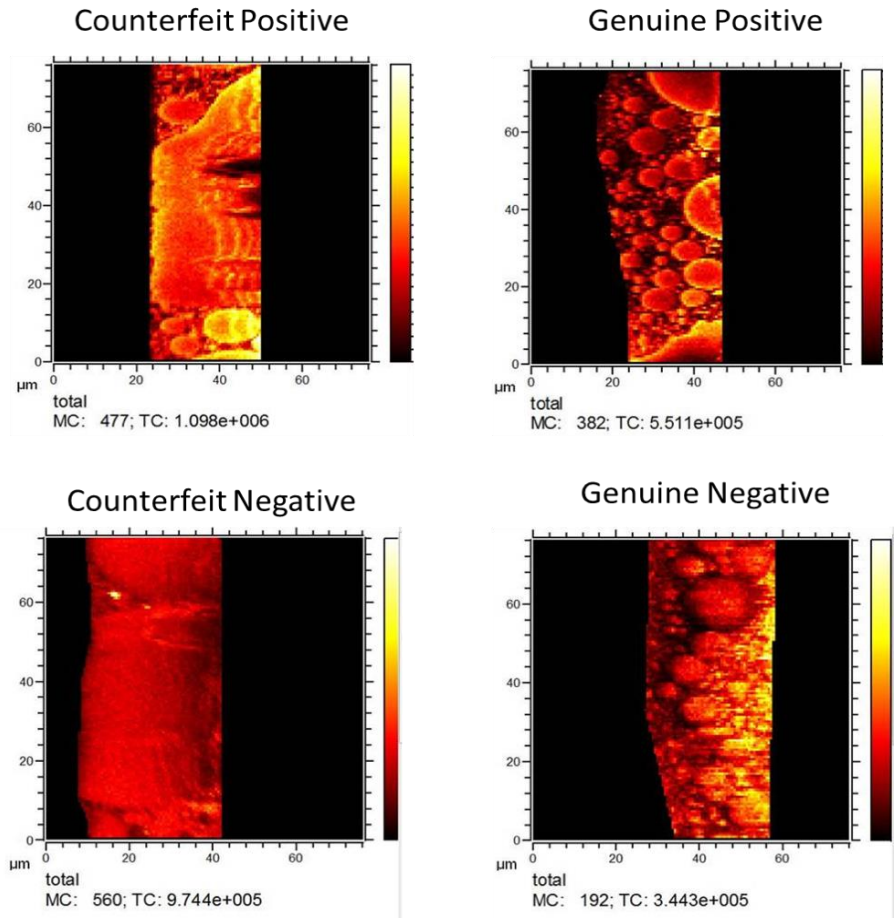


Figure 5.10 TOF-SIMS images of the face of an integrated circuit that was exposed by FIB sputtering. The morphology of the underlying particles differs between the genuine and counterfeit plastic encapsulates. This may be a method for distinguishing between the two classes.

5.5 Conclusion

A methodology based on mass spectrometric (MS) analyses to distinguish counterfeit electronics components from those of certified manufacturers has been developed. Two different MS ionization methods, secondary ion mass spectrometry (SIMS) and desorption electrospray ionization (DESI) were utilized to distinguish authentic and counterfeit integrated circuits. Morphological differences were observed by SIMS-MS imaging after a focused ion beam oblated the top layers of the surface of the integrated circuit. The cuts made by the focused ion beam did not effect the function of the integrated circuit, enabling the use if deemed an authentic part. The morphological differences showed that the counterfeit resurfaced integrated circuits did not have

cylindrical underlayers like in the authentic parts, but instead flattened and depressed images. While the SIMS-MS methodology was a strong technique for discrimination, rapid screening was performed by reactive DESI-MS. Reactive DESI-MS enabled the distinction of authentic and counterfeit integrated circuits by using multivariate discrimination methods, specifically principal component analysis (PCA). Reactive DESI-MS permitted discrimination based on spectra as compared to the traditional visually determined methods.

CHAPTER 6. REDEVELOPING CHEMISTRY LABORATORY EDUCATION THROUGH THE INCORPORTAION OF INNOVATIVE EXPERIMENTATION

Portions of this chapter have been published in the following two journals: the Journal of Chemical Education and the International Journal of Mass Spectrometry.

1. Fedick, P.W., Bain, R.M., Miao, S., Pirro, V., Cooks, R.G. "State-of-the-art mass spectrometry for point-of-care and other applications: A hands-on intensive short course for undergraduate students" *International Journal of Mass Spectrometry*, (2017), **417**, 22-28. DOI: 10.1016/j.ijms.2017.04.008.

2. Fedick, P.W., Bain, R.M., Bain, K., Tsdale, M.F., Cooks, R.G. "Accelerated *tert*-butyloxycarbonyl deprotection of amines in microdroplets produced by a pneumatic spray" *International Journal of Mass Spectrometry*, (2018), **430**, 98-103. DOI: 10.1016/j.ijms.2018.05.009.

3. Fedick, P.W., Bain, R.M., Bain, K., Cooks, R.G. "Chiral Analysis by Tandem Mass Spectrometry Using the Kinetic Method, by Polarimetry, and by ^{1}H NMR Spectroscopy" *Journal of Chemical Education*, (2017), **94**, 1329-1333. DOI: 10.1021/acs.jchemed.7b00090.

6.1 Abstract

Laboratory exercises often teach the fundamentals that students should know about the current subject area. These laboratory exercises are usually reused for years, at times making the pre-laboratory, post-laboratory and data analysis obsolete as the answers are readily available from previous students or on the internet from various student help websites. Developing novel laboratory exercises to still demonstrate the fundamentals of a subject and utilizes new advances in the literature helps maintain students' interest and rigor. Through these laboratory exercise redevelopments, students learn cutting edge science while making them delve into the recent literature. These new laboratory exercises still test students on the fundamental knowledge and laboratory goals, while introducing students to the real-world applications. The new laboratory exercises described herein draw from recent publications from the Cooks group and have real-world applications to prepare students for their careers after their undergraduate education. These laboratory exercises demonstrate new emerging technologies and techniques that were developed for organic chemistry I and II and analytical chemistry II.

6.2 Introduction

Outdated laboratory exercises, while often demonstrating the fundamentals of a subject, typically do not maintain a substantial level of interest to the students.[170] The Cooks group, for the last five years, has taken new, basic, and applied research that our group has recently published and redeveloped the methodologies to fit within a teaching laboratory exercise for a class period.[171-176] Doing this allows us to both provide the fundamental knowledge and demonstrate interesting applications that maintain student interest. As these laboratory exercises are completely novel, the students are challenged in their pre- and post-laboratory exercises to explore the current literature and form their own ideas. By doing so, students are challenged to learn more as the pre- and post-laboratory questions are not easily searchable online - unlike the laboratory exercises that have been implemented for 20+ years.[177] As the Cooks group is mass spectrometry focused, our laboratory exercises utilize mass spectrometry in the teaching laboratory, as the detection method, to measure chemical properties or as a synthetic tool.

For each redeveloped laboratory exercise described herein, a set of pre-laboratory readings are provided that include the most recent literature, so students are required to delve into new research. The pre-laboratory questions assess a combination of fundamental knowledge, laboratory goals, and relevance to the real world. Post-laboratory exercises required data manipulation and interpretation, demonstration of fundamental concepts, and out-of-the box thinking, such as listing possible limitations of this technique or improvements to make this technique more efficient. A week after the laboratory exercise student were verbally interviewed to see what knowledge they retained and how this affected their overall understanding of the topic.

The inspiration for the development of these new laboratory exercises were drawn from the hands-on workshop organized every year by the Cooks group. This workshop demonstrates new emerging technologies and techniques.[175] There is attendance from Purdue undergraduate and graduate students, from multiple departments, as well as faculty members from other institutions. It is open to anyone who has an interest, especially those from nearby universities, such as Indiana University, Indiana University Purdue University Indianapolis, Notre Dame, Ohio State, and University of Evansville, to name a few. Our research collaborators and industrial partners frequently attend this meeting as well. The course is free to all and usually spans a two to three-day period. There are typically six hands-on demonstrations where participants actively engage in experimentation, being led by the graduate students and post-docs who developed these methods.

The hands-on workshop and the redevelopment of Purdue's laboratory curricula attempt to highlight recent achievements of cutting-edge science and provide students a window into state-of-the-art instrumentation and ambient ionization mass spectrometry prior to them joining the workforce as discussed in Section 6.3.

These new laboratories exercises presented herein explored accelerating reactions through alternative synthetic methods utilizing mass spectrometry ionization sources as generators of confined volumes.[157] The Cooks group discovered that reactions in confined volume systems can undergo accelerated product formation as compared to their traditional counterparts, such as a reaction under reflux in a round bottom flask. A few laboratory exercises have been performed on these topics in the past at Purdue.[172-173, 178] One of the most recent developed for organic chemistry II was a reaction in these accelerated systems that had previously never been published.[176] As seen in verbal interviews, the students quite enjoyed being on the cutting edge of unpublished science described in Section 6.4. The next laboratory redevelopment that will be discussed was developed for organic chemistry I and brought analytical figures of merits into focus while discussing chiral analysis for a synthesis that had been performed in a prior laboratory exercise.[174] This laboratory exercise introduced students to the kinetic method for chiral analysis by mass spectrometry and allowed students to explore the advantages and disadvantages of each instrumental technique it was compared to as discussed in Section 6.5. Finally, a laboratory redevelopment was created for analytical chemistry II where process analytical technologies were explored, enabling two instruments to be explored within on laboratory exercise, while providing a real-world application for the students as seen in Section 6.6.

6.3 State-of-the-Art Mass Spectrometry for Point-of-Care and other Applications: A Hands-on Intensive Short Course for Undergraduate Students

Mass spectrometry is a core component of many chemistry curricula, with in-depth coverage often occurring in analytical chemistry courses.[179] Mass spectrometry, however, is often introduced in an undergraduate student's first year, as seen in the American Chemical Society's Anchoring Concept Content Map of General Chemistry[180] and Organic Chemistry[181] and a number of laboratory exercises targeted at these students.[173, 178, 182] While there is no formal Anchoring Concept Content Map for Analytical Chemistry, one would imagine that mass spectrometry would

be a 'level one' anchoring concept and that ionization sources would be considered an important component.

Ambient ionization was first reported in 2004 with the introduction of desorption electrospray ionization (DESI).[5] DESI-MS requires solvent to be sprayed, using an electrospray, at a surface on which analyte is present. The impact of charged solvent droplets on the surface generates secondary droplets which carry the analyte ions from the surface into the mass spectrometer.[5] In the 13 years since the first publication on DESI, ambient ionization has expanded rapidly and is the topic of a number of reviews and books.[7, 40, 109, 183-186] Traditionally, students are taught the fundamentals of mass spectrometry in undergraduate courses, however, emerging techniques are typically omitted.[19] With course and laboratory time being limited, especially in a course such as Instrumental Analysis where a plethora of instrumental techniques are often covered, topics such as ambient ionization may not get sufficient coverage, if they receive any.[179]

With mass spectrometry now so crucial to analytical chemistry courses, as well as being an important tool for most chemists and biologists, Purdue University's Center of Analytical Instrumentation Development (CAID) has been holding two to three-day graduate student organized workshops open to internal and external students since the summer of 2008. The logo for the CAID hands-on workshop can be seen in Figure 6.1. A hallmark of these workshops is the emphasis on methodology, fundamental theory, and current applications. This workshop has become a set piece in Purdue's undergraduate Instrumental Analysis course, where students get firsthand experience with recent mass spectrometry developments while reinforcing the fundamentals learned in the classroom. The assessment exercise developed as part of this short course, focused on students registered for Instrumental Analysis at Purdue University, but students from other courses, other universities, and industrial scientists also attend the CAID workshop. Similar to chemistry summer camps[187-188] and short courses[189] which blend hands-on research, lectures, and discussions of chemistry over meals, the annual CAID meetings attempt to showcase novel research while covering the fundamentals. Students were guided through CAID by graduate students, who are currently performing research on these topics, through a peer led style that contrasts with traditional course structures.[190] This allowed the students to become comfortable with the advanced materials and facilitated questions about the material.

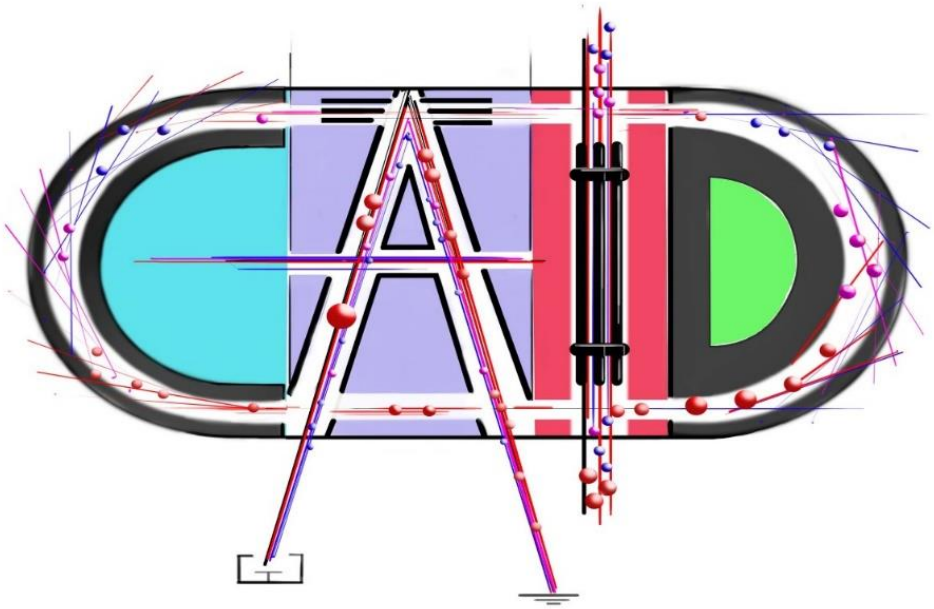


Figure 6.1 Workshop logo consisting of mass analyzers and ions spelling out CAID which stands for the Center of Analytical Instrumentation Development.

CAID provides an out of the classroom, hands-on period for the students to experience mass spectrometry and learn about recent advances in the field. CAID takes the fundamentals learned by the students in the classroom and provides a "crash course" on relevant applications, information on how to operate the instrumentation, and a firsthand look into new areas of research in mass spectrometry. The effectiveness of the CAID short course to deeply immerse, motivate, and educate students was confirmed through formal student assessment and feedback. The recruitment flyer for the CAID workshop can be seen in Figure 6.2.

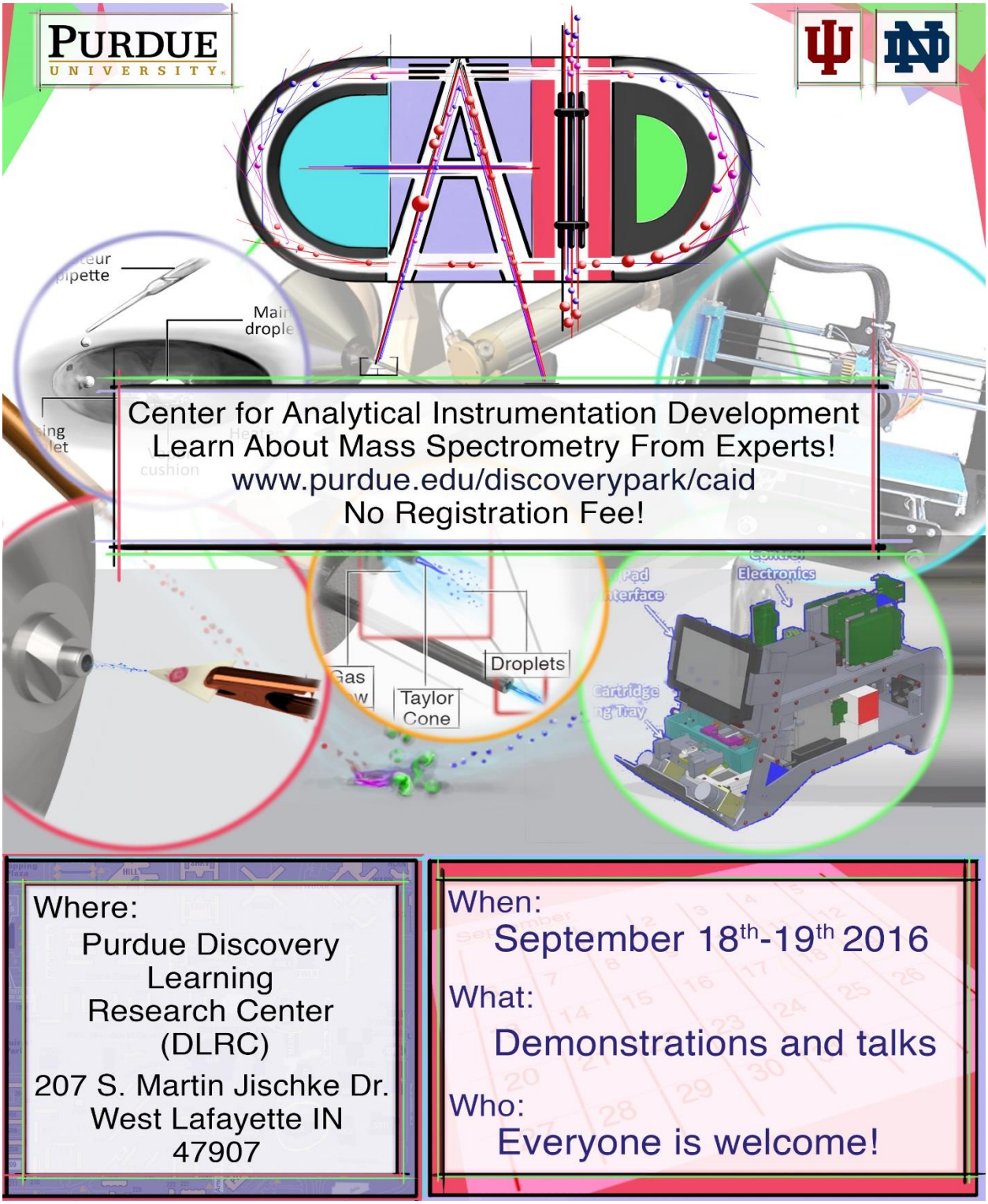


Figure 6.2 CAID hands-on workshop recruitment flyer for the 2016 workshop.

6.3.1 Experimental

Upper division undergraduate students at Purdue University enrolled in CHM 42400 Instrumental Analysis, participated in the 2016 Center for Analytical Instrumentation Developments Annual Workshop "State-of-the-Art Mass Spectrometry for Point-of-Care and other Applications" as a course requirement. The students focused on mass spectrometry in the early weeks of the course, covering fundamentals of the topic, the plethora of mass analyzers, and how ions are created, manipulated, and detected. The CAID workshop consisted of six experiments focused on different areas of mass spectrometry, which are highlighted below. The twenty-three undergraduate students from the analytical chemistry II course, split into six groups, which also included additional undergraduates, graduates, and professionals from Purdue, other universities and a variety of companies, rotated through each experiment.

Current mass spectrometry techniques and applications, especially those which achieve high speed by using ambient ionization and/or miniature instruments are presented with emphasis on the nature of the instrumentation and methodology. Multiple ionization sources, types of mass spectrometers, and ion manipulation techniques are covered. The students rotated through the six experiments in a random order according to their assigned groups. Each experiment lasted forty-five minutes, with three experiments taking place before lunch and three after.

In no particular order, the first experiment was titled "Analysis of Human Brain Cancer Using Tissue Smears by Desorption Electrospray Ionization – Mass Spectrometry". During this laboratory exercise the students were introduced to DESI and how it is being applied to cancer diagnostics. The second experiment was titled "Biofluid Analysis by Paper Spray Mass Spectrometry". During this laboratory exercise the students were introduced to PS ionization and how it can aid in rapid clinical tests. The third experiment was titled "Touch Spray Mass Spectrometry Using Medical Swabs for the Detection of Strep Throat Causing Bacterium and Illicit Drugs in Oral Fluid". During this laboratory exercise students were introduced to swab touch spray ionization and how it is an optimal sampling and ionization technique, especially in areas where PS may not be applicable due to the environment in which the sample needs to be collected. The fourth experiment was titled "Reaction Acceleration by the Leidenfrost Method and Reaction Monitoring Using a Miniature Mass Spectrometer". This laboratory exercise introduced students to three major topics, reaction acceleration, reaction monitoring and portable mass spectrometers. The fifth experiment was titled "Fundamental Exploration of Electrospray Ionization Methods".

This laboratory exercise allowed students to visually observe spray plumes from a variety of ionization sources through the aid of a laser and camera setup. The last experiment was titled "Ambient Pressure Ion Mobility Spectrometry Using a 3D-Printed Ion Mobility Spectrometer". In this laboratory exercise students were introduced to 3D printing and how it aids in rapid development of novel parts. They then learned about ion mobility and witnessed an entirely 3D-printed ion mobility spectrometer separating analytes.

The first day began at 8:30am for check in and breakfast, then an hour-long tutorial by Dalton Snyder (Purdue University) on "Overview of characteristics of different mass analyzers", followed by three lab rotations, and hour and a half lunch and finally three more lab rotations. The second day began with check in and breakfast, followed by three forty-five minute research talks and ended with a lunch.

The first research talk by Arash Zarrine-Afsar (University of Toronto) was titled "Optimization of Mass Spectrometry Imaging Workflow: How Multimodality Imaging Can Help."[191] Students learned how polarimetry can help guide imaging mass spectrometry by swiftly indicating relevant sections of tissue to scan and thus decrease the overall time of the imaging experiment. The second research talk by Abraham Badu-Tawiah (The Ohio State University) was titled "Disease Management through a Mass Spectrometry-based On-Demand Diagnostic Approach."[192] This talk focused on low cost paper devices that are currently being developed to detect diseases in areas of the world that are resource deficient. The third talk by Amar Basu (Wayne State University) was titled "Microfluidics: Fundamentals and Applications in Mass Spectrometry."[193] Students were given an overview of microfluidic devices and how they are typically interfaced with mass spectrometers. These three talks highlighted current research and helped build upon the students' knowledge of recent advances in mass spectrometry.

Many of the CAID experiments cross-referenced one another, one example of this was three experiments that used 3D printed parts and one experiment that highlighted the applicability of 3D printing for rapid prototyping. In addition to overlapping methods, all of the write-ups for the experiments and recordings of the talks were placed online for the students. The constant blending of similar methods, all approached from different experimental viewpoints, and the different methods of conveying the concepts, helped students understand the material better.[194-195]

6.3.2 Results and Discussion

The students enrolled in Purdue course CHM 42400, were required to answer Pre-Workshop questions that were generated to inspire students to perform the required reading of journal articles associated with the CAID experiments. Upon completion of the workshop the students were given a week to complete post laboratory questions to assess their retention of information. Finally, the students were verbally interviewed following the laboratory period to assess their understanding of mass spectrometry, ambient ionization, and the experiments in which they participated. All questions are available in the Appendix A.

The following laboratory period, approximately a week after CAID, the students were interviewed orally to assess what they had retained from the workshop. Students were interviewed in groups of 3 (and in one group of 2) to maximize participation.[196] Table 6.1 summarizes the responses to the eight questions that the students were asked to answer. Students all agreed that CAID was a helpful and beneficial educational tool. Only minimal changes were suggested to the CAID format which included changing the start time and altering the starting date. One group wanted additional educational techniques beyond mass spectrometry. Overall, the majority of the groups provided detailed information on the fundamentals of the experiments in which they participated. These interviews of the students confirmed that CAID is beneficial as a short course to reinforce the fundamentals that the students learned in the classroom and to demonstrate novel techniques that are current in the research community.

Table 6.1 Comparison of Student Post-Laboratory Verbal Interview Questions and Responses for the Hands-on Workshop.

Questions/Statements for Response[a]		Groups, *N*[b]	Response Characterization — Students in the Group:
1	Was the CAID short course a worthwhile learning experience for the class in your opinion? Do you feel like it enhanced your knowledge on mass spectrometry and its applications?	8	Viewed CAID as a worthwhile learning experience.
		8	Felt that it enhanced their knowledge on mass spectrometry and its applications.
		1	Was quoted, "Concepts that are hard to understand in class can easily be explained by seeing the instruments. Topics are much more interesting after getting physical implementation of the concepts."
2	Can you describe briefly the advantages of using DESI-MS for brain cancer analysis over traditional Histopathological exams? How are the DESI-MS images created? What markers are analyzed to differentiate healthy and non-healthy tissues?	4	Mentioned that DESI-MS is faster than traditional histopathological exams.
		4	identified that DESI-MS is a more quantitative method.
		5	Five groups correctly described the process of performing DESI-MS.
		5	Five groups brought attention to the process of rastering that occurs in acquiring a DESI-MS image.
		3	Three groups were able to identify the lipid profiles that are used to differentiate healthy and non-healthy tissue.
3	Can you briefly describe Touch Spray and Swab Touch Spray? What are the advantages and disadvantages for specific applications of this ionization technique over other ionization techniques that you have learned about during CAID?	4	Four groups were able to correctly define both Touch Spray and Swab Touch Spray.
		4	Four groups were able to correctly define only Swab Touch Spray.
		5	Five groups stated that an advantage was that it was a cheaper and faster analysis.
		3	Three cited no transfer step and less sample preparation as an advantage
		2	Two groups state a disadvantage was the signal was dependent on the shape of the probe.
4	Can you describe briefly how an electrospray plume is created? Describe ESI in general terms. What are paper and relay spray ionization? Why was the camera and laser used to observe these phenomenon?	3	All eight groups were able to describe some of the fundamentals of electrospray ionization.
		4	Four groups used that term "Taylor Cone" in their description.
		2	Two groups described the inert gas to help direct the ions.
		7	Seven groups described the fundamental components of paper spray.
		3	Three groups mentioned the use of a static gun for the fundamentals of relay spray.
		8	All eight groups understood that the camera and laser were used to visualize the droplets.

Table 6.2 continued

5	Can you briefly describe the advantages of using a 3D-printer for analytical instrumentation prototyping? What is ion mobility spectrometry?	3	All eight groups commented on the flexibility and customizability of 3D printing.
		5	Five groups mentioned that it is cheaper to prototype and it is produced in the laboratory.
		8	All eight groups described the movement and separation of ions in space based on size and charge.
		4	Four groups described the necessity of the electric field for this separation.
6	Can you briefly describe the Leidenfrost effect? What are advantages and disadvantages of using a miniature mass spectrometer over a conventional benchtop instrument?	8	All eight groups were able to correctly describe the Leidenfrost effect.
		1	One group "Say if you have water, and if you have hot plate, you drop water on it, before the droplet reaches the hot plate, it already starts to form vapor, like a cushion, and the droplet does not make physical contact of the surface, the droplet slowly evaporates itself without boiling away."
		8	All eight groups determined that the mini has the advantage of portability and *in-situ* analysis.
		6	Six groups mentioned the trade off in performance compared to a benchtop mass spectrometer.
7	For paper spray ionization does the paper have to be in any specific shape? Why is high voltage applied to the paper?	7	Seven groups stated that the paper must be in a triangle shape.
		4	Four groups elaborated that it relied on a sharp point.
		1	One group was quoted "…at the tip the electric field would seem to be really high…"
		8	All eight groups understood that high voltage was required to induce the spray.
8	Are there any changes that you would make to CAID in future years (please suggest feasible ideas)?	3	Three groups suggested moving the start day from Sunday.
		2	Two groups suggested a later start time.
		2	Two groups said they enjoyed the schedule and would not change anything.
		1	One group suggested a shorter day.
		1	One group preferred more broad analytical techniques in addition to mass spectrometry.
[a] Graduate TAs verbally interviewed students. [b] The 23 total students were assigned to 7 groups of 3 and 1 group of 2.			

The annual CAID workshop has been shown to be a useful educational tool to deeply immerse undergraduate students in mass spectrometry. These intensive workshops for undergraduate students on specialized topics, such as novel ambient ionization techniques, are not only useful, but are enjoyed by the students as seen in the exit interviews. The CAID workshop, which is approaching its tenth anniversary, has been reviewed yearly to see how it can be improved. After reviewing the program, the workshop responses, the format of tutorials, relevant research presentations, and hands-on demos lead by graduate students who are emerging experts in their

field, it was judged to be a worthwhile program that might usefully be adopted by other institutions. The perceived success of the CAID workshop can be seen in the students' abilities to provide the appropriate answers to the interview questions that both covered fundamentals and applications. It will continue to be a staple of the CHM 42400 course at Purdue University. The experiments presented in future CAID workshops will continue to evolve to focus on current and applicable mass spectrometry research. The authors of this paper hope that other universities will take the model of the CAID workshop and adopt it to their specialties, whether they be ambient ionization techniques, instrumental advances, proteomics, or any other fields in which the facilitators are expert.

6.4 Accelerated *tert*-Butyloxycarbonyl Deprotection of Amines in Microdroplets Produced by a Pneumatic Spray

Protection and deprotection of organic compounds in multistep reactions using functional groups such as *tert*-butyloxycarbonyl (Boc), is widely performed in synthetic organic chemistry. Reaction rate acceleration studies in spray-based ionization methods (electrosonic spray, paper spray, nanospray) have become increasingly common. Here, we demonstrate reaction rate acceleration of Boc deprotection using easy ambient sonic-spray ionization (EASI), a pneumatic technique which does not involve an applied voltage, in a teaching laboratory setting (Figure 6.3).

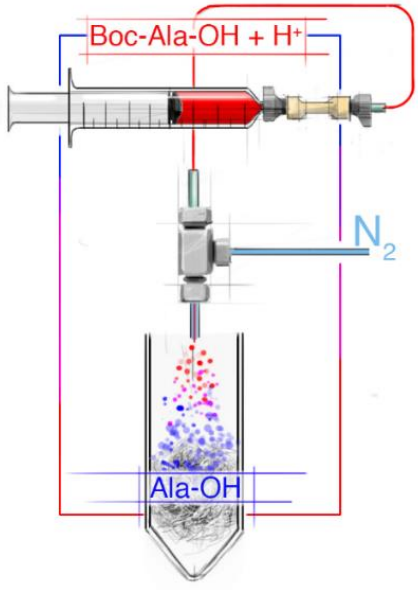


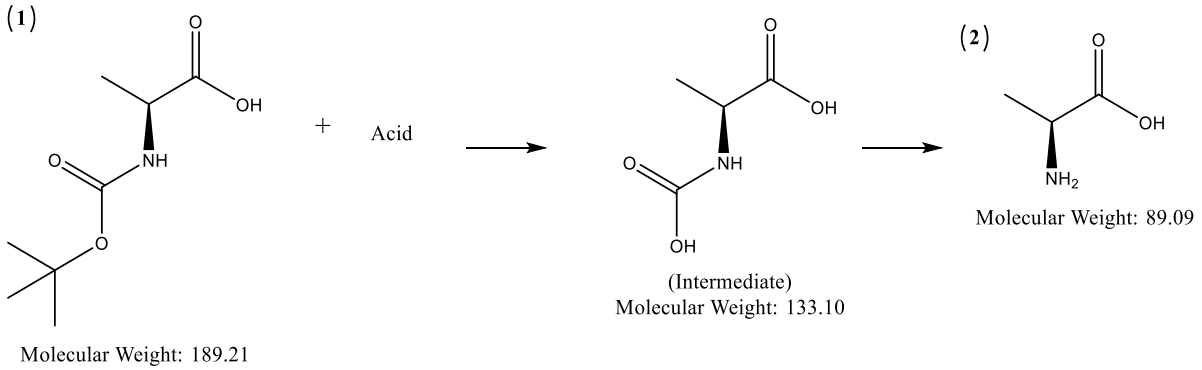
Figure 6.3 Pictorial representation of the easy ambient sonic-spray ionization (EASI) used as an accelerated reaction technique.

The goal of this laboratory exercise was to explore acceleration in a previously unexplored spray-based reaction, while emphasizing the importance of protecting groups for multistep synthesis in a pedagogic setting. Rate acceleration factors of more than an order of magnitude were observed in the uncharged micron-sized droplets generated by EASI. The effect of reaction conditions on reaction acceleration was examined including changes in the type of acid, reagent concentration ratios and syringe pump flow rates. Student knowledge was assessed by pre-laboratory assignments, post-laboratory reports, and oral interviews. Multistep organic synthesis involves a sequence of reactions from starting materials to product, frequently with use of column chromatography or other means to purify intermediates.[197] There are many possible reaction routes to any one desired product or intermediate. Cost, time, and yield are criteria in choosing the best synthetic route.[198] Throughout a multistep synthesis, functional groups may need to be conserved. A protecting group is often introduced prior to a particular reaction step and later removed, in order to preserve a specific group that otherwise would not survive.[199] To undergraduate chemistry students, knowledge of the use of protection and deprotection reactions is pivotal to the understanding of multistep synthesis.[200-202]

Protecting and deprotecting a functional group adds two additional steps to a multistep synthetic scheme. There are a plethora of protecting groups, each with requirements that need to be met for their removal once the chemical entity has progressed to a stage in the reaction scheme where the functional group can be safely maintained through the subsequent steps to the final product.[203] The protecting group used in this experiment is the *tert*-butyloxycarbonyl functional group, commonly referred to as "Boc."[204] It is primarily utilized to protect amines in multistep syntheses and is extensively used in peptide synthesis and medicinal chemistry.[205] The Boc group can be removed by relatively strong acids, such as hydrochloric acid or trifluoroacetic acid, or by the combination of heat and milder acids.[204]

Time is a major consideration in multistep reactions, so chemists typically increase the rate of reaction by using elevated temperatures. Refluxing is usually used to achieve this increase in rate without losing sample or solvent.[206] While thermal acceleration is widely used, another common method of acceleration is through catalysis.[207] Catalysts accelerate reactions by providing an alternative mechanistic pathway with a lower activation energy. More recently, there has been a growing recognition that reactions can be accelerated by other means, specifically by performing reactions at interfaces.[208-210] Modest acceleration has been reported for some reaction mixtures in

microfluidics,[211-212] while electrospray ionization,[213-216] and other spray methods produce droplets which sometimes yield very large acceleration factors. In cases of reactions in small confined volumes (i.e. thin films or microdroplets) it is thought that partial solvation of the reagents at the air-solvent interface is the cause of reaction rate acceleration.[157] We explore the use of a spray-based method to accelerate the deprotection of an amine in this laboratory exercise, specifically, the deprotection of Boc-Ala-OH (**1**) by acid to produce free Ala-OH (**2**) (Scheme 6.1).



Scheme 6.1 Deprotection of Boc-Ala-OH (1) using strong acid to form the amino acid (2) via the intermediate carbamic acid.

The deprotection occurs by initial protonation of the tert-butyl carbamate and subsequent loss of the cationic butyl group as the carboxylic acid with production of the free amine. Reaction rate acceleration has been demonstrated using on-line mass spectrometric analysis of reaction mixtures ionized by electrospray ionization and other spray-based ionization methods.[198, 214-220] This phenomenon has been highlighted in recent reviews.[157, 221-222] Experiments can be performed using electrospray ionization to spray and collect appreciable amounts of material in minutes.[213] Using a continuous thin film variant of droplet chemistry, Wei et al. collected nearly 100 mg/hr of reaction product with a steady state rate acceleration factor of 100.[223] We use a variety of electrospray and reaction conditions to explore both the kinetics of the reaction and the processes of electrospray reaction rate acceleration. Various factors influence reaction rate acceleration in electrospray including: solution flow rate, gas flow rate, collection surface and reagent concentration.[157] Factors such as solvent evaporation will increase reaction rates but may not change the rate constant. On the other hand, increasing the surface/volume ratio may increase rate constants if surface reactivity differs from bulk reactivity. Reaction rate acceleration can be calculated by comparing the rate for the bulk material to that recorded using the accelerated method.

This is approximated by simply taking the ratio of product to starting material ratio for the sprayed material divided by the ratio for the bulk after the same reaction time. This calculated rate acceleration factor is only an approximate as it assumes equal ionization efficiencies for the reagent and product as well as assuming the same form of reaction kinetics (Equation 6.1).[223-224]

Equation 6.1 Reaction acceleration is determined by the ratio of ratios of product to reactant of spray and bulk.

$$\textbf{Reaction Rate Acceleration} = \frac{\left(\frac{\textbf{Intensity of Product}}{\textbf{Intensity of Reactant}}\right)_{\textbf{Accelerated System}}}{\left(\frac{\textbf{Intensity of Product}}{\textbf{Intensity of Reactant}}\right)_{\textbf{Bulk}}}$$

The first learning objective of this laboratory exercise was for students to gain a better understanding of how spray-based reactions can be accelerated when compared to their solution-phase counterparts. Unlike previously spray-based accelerated reaction exercises performed, developed and implemented by our research group,[157, 172-173] this chemical system uses no voltage during the acceleration, a modification that is more amenable to a teaching laboratory environment, while maintaining the mission of bringing cutting-edge research to the teaching laboratory.[175] This "no-voltage" spray-based method, also known as easy ambient sonic-spray ionization (EASI), has been the topic of a recent review.[19] The chemical system of *tert*-butyloxycarbonyl (Boc) deprotection has been selected for its common and important use in medicinal chemistry[205, 225] and for the relatively low reagent cost. One learning objective centered on students considering how experimental parameters influence the acceleration of the formation of the deprotected product. Some of these experimental parameters - variation in the flow rate, concentration of the Boc-protected compound relative to the reactant acid, and the choice of acid itself - changed the measured rate acceleration factor. Note that the exercise does not measure intrinsic rate constants. The second learning objective was for students to understand the purpose and importance of protecting groups in multistep syntheses. By conducting part of a multistep synthesis in an accelerated fashion, students learned how and why protecting groups have such an important role in organic synthesis and how time-saving steps could benefit synthesis.

6.4.1 Experimental

All chemicals (Boc-Ala-OH, hydrochloric acid (HCl), and trifluoracetic acid (TFA), and methanol) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol (MeOH) was purchased from Fisher Scientific (Pittsburgh, PA). EASI spray emitters were constructed with fused silica lines with 100-micron I.D. and 360-micron O.D. (PolyMicro, Phoenix, AZ), one tee assembly, one union assembly, two NanoTight sleeves, and a stainless-steel capillary (IDEX Health and Science, Oak Harbor, WA). To control the flow of reagent solution, infuse syringe pumps (Standard Infusion PHD 22/2000, Harvard Apparatus, Holliston, MA) were utilized with gastight chemseal syringes (Hamilton Robotics, Reno, NV). Nitrogen (Indiana Oxygen, Lafayette, IN) was used as the nebulizing gas. The construction and part numbers can be found in Figure 6.4. A photograph of the reaction acceleration setup can be found in Figure 6.5.The reaction mixtures were sprayed into 15mL Falcon conical centrifuge tubes (Fisher Scientific, Pittsburgh, PA) from which the bottom had been removed, so as to avoid pressure build-up, with glass wool in its place to collect the product.

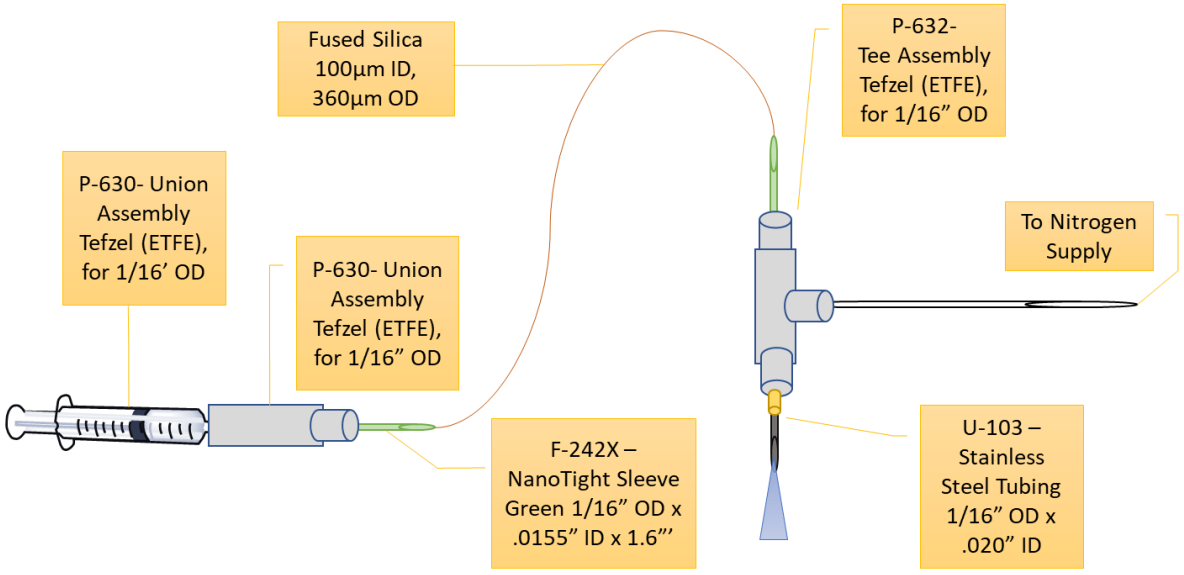


Figure 6.4 Easy ambient sonic-spray ionization (EASI) droplet generation system consisting of a gastight syringe, fused silica lines, Teflon unions, nanotight sleeves and a stainless-steel capillary. Part numbers have been supplied where applicable.

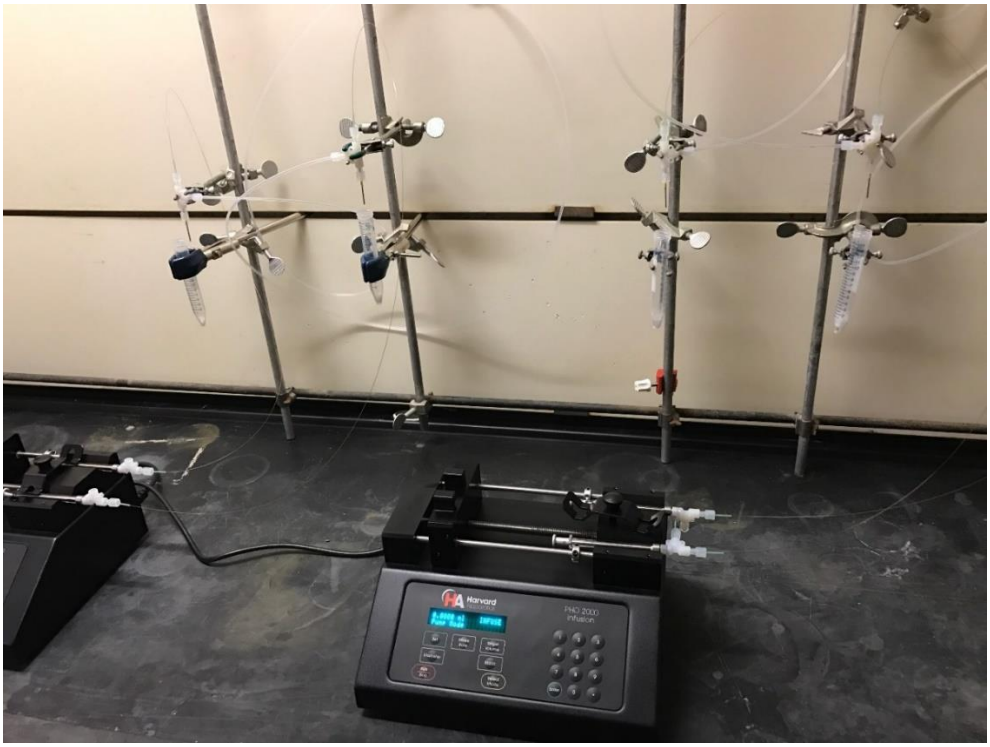


Figure 6.5 Photograph of the EASI reaction acceleration set up for the deprotection reaction.

This three-hour laboratory exercise was performed by 15 undergraduate students enrolled in an organic chemistry II honors section. The students were divided into three groups, and each group performed the same set of six reactions using a range of conditions, with varying flow rates, acids and ratios of acid to Boc-Ala-OH (Table 6.2). The laboratory handout is available in Appendix B. No voltage was applied to the EASI spray emitter. All spectra were recorded in positive ion mode using a LTQ XL ion trap mass spectrometer (Thermo Instruments, San Jose, CA). All mass spectra recorded for product analysis utilized nanospray ionization as the ionization source at 2.0 kV and set 3 mm from the inlet to the vacuum system. The nanospray emitters were constructed from borosilicate glass capillaries (1.5 mm o.d., 0.86 mm i.d., Sutter Instrument Co.) that were pulled to a tip using a Flaming/Brown micropipette puller (Sutter Instrument Co. model P-97, Novato, CA, U.S.A.).

Table 6.3 Student's Reaction Conditions for Reaction Acceleration of the Deprotection of Boc-Ala-OH. Six sets of conditions, altering flow rate, acid type, and acid to Boc-Ala-OH ratio were explored.

Flow Rate	HCl	TFA
5µl/min	10:1 HCl to Boc-Ala-OH	10:1 TFA to Boc-Ala-OH
5µl/min	1:1 HCl to Boc-Ala-OH	1:1 TFA to Boc-Ala-OH
20µl/min	10:1 HCl to Boc-Ala-OH	10:1 TFA to Boc-Ala-OH

6.4.2 Results and Discussion

6.4.2.1 Reaction Acceleration of the Deprotection of Boc-Ala-OH

To determine the acceleration factor for spray conditions relative to bulk conditions, students performed reactions under each of the six conditions listed in Table 6.2 in both bulk and spray. Upon completion of the spray reactions, the product was extracted from the glass wool by running 2mL of MeOH through the tube. Reactions that were conducted under bulk conditions were all allowed to react for a time equal to the entire spray time and sample workup time. Both the spray based and bulk reactions were analyzed by nanospray ionization mass spectrometry. Figure 6.6 shows student-collected spectra of both the spray and the bulk conditions using HCl in a 10:1 ratio acid to Boc-Ala-OH, with a flow rate of 5µl/min condition. The reactant was observed at *m/z* 190, corresponding to protonated Boc-Ala-OH, and the product was observed at *m/z* 90, corresponding to protonated Ala-OH with removal of the Boc protecting group. The carbamic acid intermediate in the Boc deprotection process was also observed at *m/z* 134, which corresponds to the loss of the tert-butyl group and formation of protonated carbamic acid.

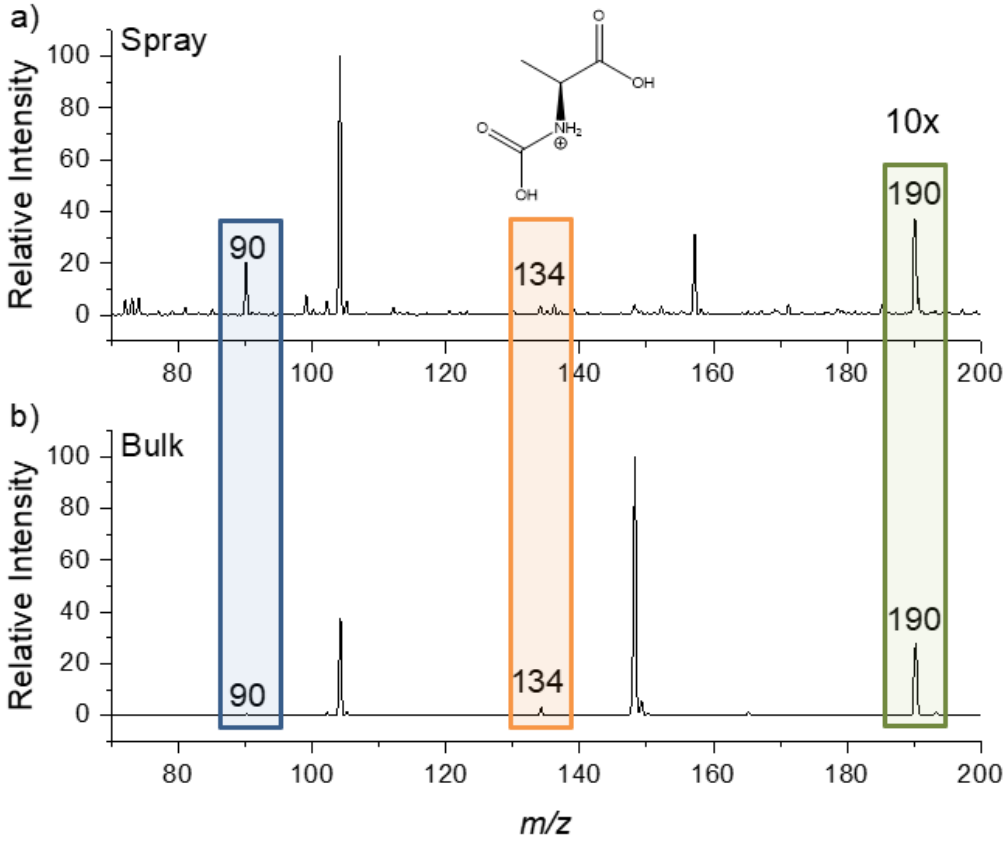


Figure 6.6 Full scan positive ion mode mass spectra. The blue box indicates the Ala-OH which has had the BOC group removed, whereas the green indicates the reactant BOC-Ala-OH. The reactant peak has been scaled by a factor of ten to aid in visualization. The orange box indicates the intermediate, which the students did not use in their calculations. (a) Spray reaction utilizing HCl in a 10:1 ratio of acid to Boc-Ala-OH at a flow rate of 5 µl/minute. (b) Corresponding bulk reaction mixture. Ion signals at m/z 104 and 148 are the product and intermediate of a side reaction (esterification of the carboxylic acid of the BOC-Ala-OH) and for the purpose of the teaching laboratory exercise were not brought to the attention of the student.

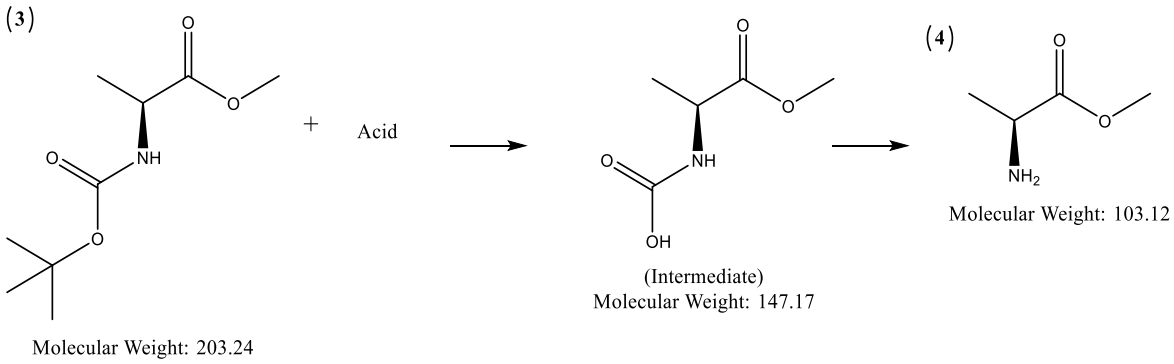
Students collected spectra for each condition and calculated the acceleration factors (uncorrected for ionization efficiencies) of each condition (Table 6.3). They then compared the acceleration factors for each condition to determine which provided the largest acceleration factor. Every condition that the students utilized demonstrated spray-based acceleration. The most significant acceleration was observed from the TFA acid condition in a 10:1 acid to Boc-Ala-OH ratio, with a flow rate of 5 µL/min during spray reaction.

Table 6.4 Student reaction acceleration factors for Boc-Ala-OH for each spray condition. Increase in ratio of the acid to the Boc-Ala-OH causes the product to form at an accelerated rate. Similarly, both acids had the highest acceleration rate with an EASI flow rate of 5 µL/min. TFA in a 10:1 ratio to Boc-Ala-OH at a flow rate of 5 µL/min had the largest acceleration factor overall.

Acid	Mole Ratio of Acid to Boc-Ala-OH	Flow Rate	Acceleration Factor
HCl	1:1	5 µL/min	1.2
	10:1		18
		20 µL/min	12
TFA	1:1	5 µL/min	2.0
	10:1		35
		20 µL/min	4.2

6.4.2.2 Reaction Acceleration of the Deprotection of Boc-Ala-OMe

While not explored by the students in this laboratory exercise, the deprotection of Boc-Ala-OH led to a side reaction where the carboxylic acid on the alanine was converted into the methyl ester (**3**). This reaction was accelerated to form the deprotected Ala-OMe (**4**) as seen in Scheme 6.2. To determine the acceleration of deprotection of the Boc-Ala-OMe without the convoluting effect of the methylation of the carboxylic acid in-situ, the same experiment was performed using Boc-Ala-OMe as the starting material. Figure 6.7 shows the spray and bulk spectra for bulk conditions for the HCl acid condition in a 10:1 ratio acid to Boc-Ala-OMe, with a flow rate of 5 µl/min condition. There is a larger acceleration factor as seen in Table 6.4 likely due to the removal of the intermediate step of the methyl-ester formation. The trend in acceleration factor for Boc-Ala-OMe is the same as Boc-Ala-OH. Boc-Ala-OMe was not used initially in the teaching laboratory exercise because it costs three times as much as Boc-Ala-OH and the fundamentals of spray-based reaction acceleration were still demonstrated. However, if funding allows, the authors suggest utilizing the Boc-Ala-OMe as the reactant as it yields a larger acceleration rate for the students to observe and may increase their understanding of the concept. Similarly, depending on the time allotted for the laboratory exercise comparing both the Boc-Ala-OH and the Boc-Ala-OMe could be a worthwhile learning exercise to discuss side reactions and by-products.



Scheme 6.2 Deprotection of Boc-Ala-OMe (3) using strong acid to form the amino acid ester (4) via the intermediate carbamic ester.

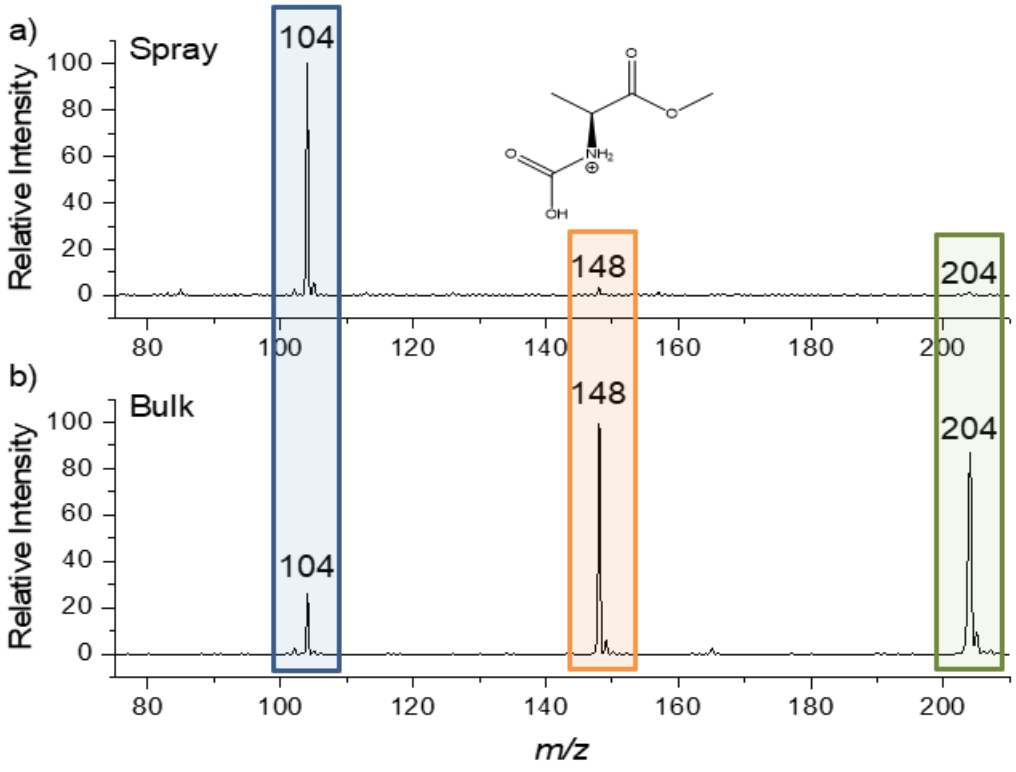


Figure 6.7 Full scan positive mode spectra. The blue box indicates the Ala-OMe from which the BOC group is removed, the green indicates the reactant BOC-Ala-OMe. The orange box indicates the intermediate. (a) The spray reaction utilizing HCl in a 10:1 ratio of acid to Boc-Ala-OMe at a flow rate of 5 µl/minute. (b) Mass spectrum for corresponding bulk reaction mixture.

Table 6.5 Reaction Acceleration Factors for Boc-Ala-OMe for Each Spray Condition. Increase in ratio of the acid to the Boc-Ala-OMe causes the product to form at an accelerated rate. Similarly, the highest acceleration rate with an EASI flow rate of 5 µL/min. The larger acceleration factors may provide the justification of spending more on the reagents to more clearly demonstrate the fundamentals of reaction acceleration to students.

Acid	Mole Ratio of Acid to Boc-Ala-OMe	Flow Rate	Acceleration Factor
HCl	1:1	5 µL/min	59
	10:1		216
		20 µL/min	183

6.4.2.3 Student Interviews

During an ungraded exercise a week after the completion of the laboratory exercise, the students were interviewed orally to assess their understanding of mass spectrometry, reaction rate acceleration, and the experiment in which they participated. Students were interviewed in three groups of 5 to maximize participation and promote discussion that may not have been elicited through individual written or verbal prompting.[196] Table 6.5 summarizes the responses to the five questions that the students were asked to answer.

Table 6.6 Comparison of Student Post-Laboratory Verbal Interview Questions and Responses for Boc Ala Lab.

Questions/Statements for Response[a]		Groups, *N*[b]	Response Characterization — Students in the Group:
1	This is probably the first time that EASI has been used to spray and collect accelerated reaction products in an undergraduate teaching laboratory. Could you please explain why you believe this was or was not a valuable laboratory experiment? How could this experiment be improved?	3	Thought the experiment was a valuable laboratory exercise
		3	Stated that data processing was difficult. (Although stated to be difficult all the students correctly determined their acceleration factors.)
		1	Suggested that only one variable should be assigned to a group and then the data pooled.
2	Please describe the collection set-up, EASI parameters, as well as syringe pump parameters.	3	Described the overall experimental setup
		3	Explained why the bottom of the collection tube was removed to allow gas flow and the use of glass wool as a collection mechanism.
		3	Described the effect of flow rate on the syringe pump had on the rate of acceleration and droplet size.
3	Why are reactions able to be accelerated by spray-based methods? What factors contribute to this?	3	Described surface effects of the partially desolvated droplets
		2	Described the change in activation energy in partially desolvated droplets.
4	Please explain how you calculate acceleration factors?	3	Stated that it was a ratio of the bulk and spray based methods' ratio of intensities of the product and reactants
5	Why does the nanospray analysis not effect the acceleration?	3	Described how both the bulk and sprayed products are analyzed using nanospray so the effect is negated.

[a] Graduate TAs verbally interviewed students. [b] The 15 total students were assigned to 3 groups of 5 each.

As seen in Table 6.5, all the students thought this was a valuable experiment, sharing excitement about the novelty of this experiment since this reaction had not previously been reported in spray. For example, one student stated that this was an "…alternative method that may be more commonly used in the future… We are ahead of the game." In their post-laboratory reports, the students most commonly suggested multiplexing the spray emitters to increase product

formation. The students showed a fundamental understanding on how reactions are accelerated by spray-based methods and how to calculate acceleration factors based on their pre-laboratory, post-laboratory, and verbal interviews.

This laboratory exercise provided organic II students with the opportunity to learn about the importance of protecting groups and multistep synthesis within the framework of spray-based accelerated reactions. As seen in the laboratory reports and the verbal interviews, students developed a grasp of the fundamental theories explaining why reactions accelerate in spray-based methods, their importance to time and ease of synthesis, all while exploring new chemistry. The benefit of no voltage being applied during the spray process, as well as the low cost of the reagents, make this laboratory exercise appealing for instructional settings.

The students were also able to probe the new reaction acceleration that has not been previously reported in the literature. The results acquired by students were not pre-tested and provided a true experiment, rather than a "cookbook" laboratory, and in turn they demonstrated the first case of acceleration of a deprotection reaction and the first demonstrated case of reaction acceleration using EASI. The additional study on neutral spray droplets for reaction acceleration adds to the growing literature on accelerated methods such as Leidenfrost droplets,[226] thin films,[223] desorption electrospray ionization (DESI)[156] and other charged spray droplet systems.[216] Post-laboratory interviews showed that the students found this laboratory exercise both valuable and a worthwhile experiment.

6.5 Chiral Analysis by Tandem Mass Spectrometry Using the Kinetic Method, by Polarimetry, and by ^{1}H NMR Spectroscopy

The goal of this laboratory exercise was for students to understand the concept of chirality and how enantiomeric excess (*ee*) is experimentally determined using the analysis of ibuprofen as an example. Students determined the enantiomeric excess of the analyte by three different instrumental methods: mass spectrometry, nuclear magnetic resonance and optical polarimetry. This laboratory exercise introduced mass spectrometry into the first-semester organic chemistry curriculum. Further, it allowed the students to compare and contrast the analytical figures of merit for each technique, a topic usually discussed later in chemistry courses. The *ee* resolution, sensitivity, limits of detection, and required sample preparation were considered. Furthermore, this laboratory experiment taught students how to use the kinetic method for chiral analysis by mass

spectrometry, a new technique in a student laboratory exercise. Students' knowledge of how to select the appropriate technique for enantiomeric excess determination was broadened, as was their knowledge of the general concept of chirality, as seen in their laboratory reports and exit interviews.

Chirality and the underlying subtopic of enantiomeric excess (*ee*) are major topics taught in organic chemistry.[227] The ACS Exams Institute's Anchoring Concept Content Map II: Organic Chemistry (ACCM II) lists chirality as a major topic within the anchoring concept of "Structure and Function".[181] These concepts are often encountered in experimental laboratory activities in which students determine the enantiomeric purity of a compound and calculate the enantiomeric excess.[228] These chirality measurements are often performed in teaching laboratories using polarimetry[229] or nuclear magnetic resonance (NMR) spectroscopy.[230] While these two instrumental techniques diversify a student's knowledge base, a third instrumental technique, mass spectrometry, is also suitable for enantiomeric excess determination.[231-232]

Mass spectrometry (MS) chiral determinations utilize the relative kinetics of dissociation of cluster ions that include the analyte and a chiral reference, a procedure known as the kinetic method (Figure 6.8).[233] Typically, trimeric cluster ions are used to determine the percent enantiomeric excess.[234-235] The trimeric cluster ion is formed by binding a central metal ion (M, in the 2^+ oxidation state), two optically pure reference ligands (ref), and the analyte molecule (A) to form the $[M(ref)_2(A)\text{-}H]^+$ complex.[236] This complex has two diastereomeric forms which include both (*R*) and (*S*) versions of the analyte. It is isolated by mass and fragmented by collision-induced dissociation (CID). The dissociation kinetics of the competitive loss of the analyte vs. reference is measured as the ratio of product ion abundances.

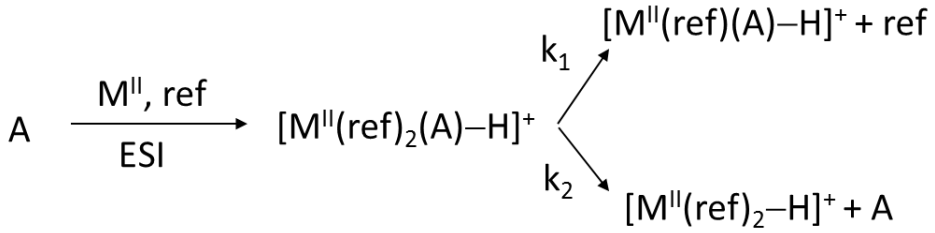


Figure 6.8 The branching ratio, the ratio of the two product ions, is dependent on the enantiomer of the analyte, the reference and the metal ions interactions.

These ion abundances will differ as a function of analyte *ee* and the ratio of abundances can therefore be calibrated in a plot of analyte *ee* vs. the natural logarithm of the ratio of these two product ion intensities.[237] The natural logarithm is used to generate a linear relationship. Variations

in this product ion abundance ratio arise from the different proportions of the two enantiomers, (*R*) or (*S*), in the cluster ion. Fundamentally, the relative rates of ligand loss (either ibuprofen or ref) during CID from the main cluster ion arise from differences in ligand-ligand interactions (Figure 6.9). The kinetic method has been the topic of a number of reviews,[238-240] any or all of which could be supplemental reading for students prior to a pre-laboratory discussion.

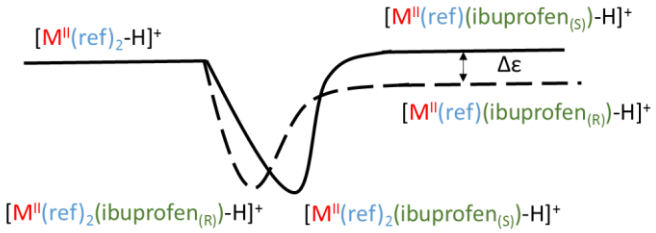


Figure 6.9 Potential energy surface for the fragmentation process of both the (R) and (S) forms of ibuprofen in the [MII(ref)2(ibuprofen(R/S))-H]+ cluster precursor ion. The (R/S) ratio is categorized by the competitive losses of (ref) and (ibuprofen(R/S)) where the energy difference Δε is dictated by sterics and competitive kinetics.

The inclusion of MS as a method for enantiomeric excess determination incorporates another sublevel of the ACCM II[181] into the laboratory exercise. This addition allows students to develop connections between chirality and kinetics, a connection that is not well studied in the chemistry education research literature.[241] This laboratory exercise also introduces students to the ideas of limits of detection and how a sample matrix can affect measurements in analytical chemistry.[242] Reinforcing core organic chemistry concepts while developing students' skills to consider the conditions based on the figures of merit and sample requirements of each technique facilitates the expansion of students' abilities to construct sound arguments when selecting the appropriate instrumental method. This objective was inspired by studies on undergraduate research experiences where students engage in real scientific practice, such as developing the skills to ask questions and select the appropriate methods for investigation.[243]

6.5.1 Experimental

An organic chemistry I honors section from Purdue University consisting of 14 students participated in this three-hour laboratory experiment. Students were placed into three groups and cycled through the three instrumental stations, following a pre-laboratory lecture. The laboratory

handout is available in the Appendix C. The three instrumental stations were MS, NMR spectroscopy, and polarimetry. These three techniques were selected based on the resources available at Purdue University, however, other chiral analysis tools could also be used, such as chiral HPLC. While this laboratory exercise was performed at the introductory organic level, it would also be suitable for second semester or advanced organic courses, as well as, courses in analytical chemistry. The learning objectives of this laboratory exercise were for students to develop a deeper understanding of chirality, enantiomeric excess determination, understand the advantages and disadvantages of each instrumental technique, and achieve the ability to determine how circumstances call for the selection of a particular instrumental technique.

In order to determine the enantiomeric excess of a compound by MS, a trimeric cluster needs to be prepared prior to analysis. Students analyzed three standard solutions which generated the trimeric cluster of Cu^{II}, two L-Tryptophan and one ibuprofen at 0, 50, and 99 % *ee* (*S*) using a Thermo Fisher LTQ mass spectrometer (San Jose, CA, USA). The trimeric cluster, $[Cu(L\text{-}Trp)_2ibuprofen–H]^+$ *m/z* 676, was fragmented and the ratio of two distinct fragment peaks at *m/z* 470 and 472, which correspond to the loss of ibuprofen or tryptophan, respectively, was calculated (Equation 6.2). A calibration curve was generated by plotting the natural logarithm of the ratio of those two peak intensities against the known % *ee*. Students analyzed an unknown % *ee*, and an unknown % *ee* in a sample that had Pepto-Bismol® chewable tablets and Tylenol Extra Strength® added to the unknown. The scan parameters of the LTQ were as follows: isolation width (m/z) 10, normalized collision energy 8, activation qz 0.250, and activation time (ms) 30.

Equation 6.2 Calculation of the intensity of the signal due to loss of analyte divided by that for competitive loss of the reference.

$$\ln(R) = \ln\left(\frac{k_2}{k_1}\right) = \ln(\frac{[M^{II}(ref)_2 - H]^+}{[M^{II}(ref)(A) - H]^+}$$

In order to determine the enantiomeric excess of a compound by NMR spectroscopy, diastereomers need to be prepared prior to analysis[230]. Students analyzed three standard solutions of the diastereomeric ibuprofen derivative, formed by the reaction of ibuprofen with (1*S*, 2*S*)-(-)-1,2-diphenylethylenediamine, on a Varian 300 MHz NMR spectrometer (Palo Alto, California, USA) with ibuprofen at 0, 50, and 99 % *ee* (*S*).

In a previous laboratory period, students had analyzed ibuprofen by polarimetry. To build student awareness on choice of technique depending on particular circumstances, two samples of ibuprofen were analyzed by the polarimeter in this exercise. The two Ibuprofen samples had concentrations matching the MS and NMR spectroscopy experiments, both which are too low for the polarimeter to detect. Since students had already analyzed higher concentration ibuprofen earlier in the semester, they were better able to recognize how concentration can influence the selection of analytical methodology.

6.5.2 Results and Discussion

6.5.2.1 Polarimetry

Students compared their previously analyzed higher concentration 99 % *ee* (*S*) ibuprofen from a laboratory exercise earlier in the semester with the results that they obtained from utilizing the same enantiomerically pure sample but at mass spectrometry and NMR concentrations. Figure 6.10 demonstrated the reproducibility and the accuracy of polarimetry when working at relatively high concentrations and how inaccurate and irreproducible it was when working at a concentration suitable for NMR and mass spectrometry. This allowed students to delve into how selecting an analytical methodology can, at times, depend on the concentration of the sample. Similarly, a discussion of how the sample needed to be extremely pure for polarimetry ensued between the students, making polarimetry challenging if the analyte is trapped within a dirty matrix.

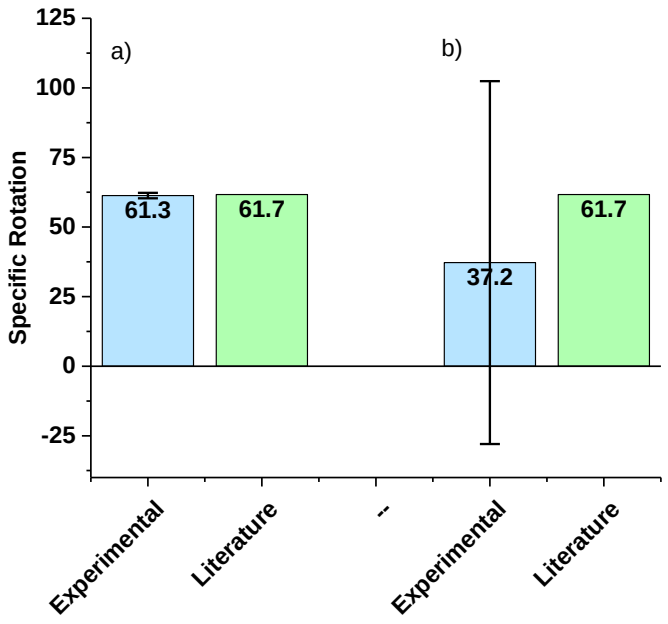


Figure 6.10 Polarimetry of 99 % ee (S) ibuprofen performed in triplicate a) at high concentration (0.2 M) b) at the concentration used for NMR spectroscopy (0.05 M).

6.5.2.2 NMR Spectroscopy

Students observed the differences in peak splitting in the region of 1.3-1.5 ppm (Figure 6.11). The spectrum of the (*S*,*S*) ibuprofen derivative consisted of a doublet, whereas the (*R*,*S*) ibuprofen derivative consisted of a quartet. The integration of these peaks allowed students to determine the % *ee* of the samples. Students also analyzed a sample at 25% *ee* (*S*) in a sample that had Pepto-Bismol® chewable tablets and Tylenol Extra Strength® added to the unknown. The student's observed no change to the spectrum in the 1.3-1.5 ppm range, however, they did observe a change in the overall spectrum, and they recognized how impurities could potentially inhibit % *ee* determination by NMR spectroscopy.

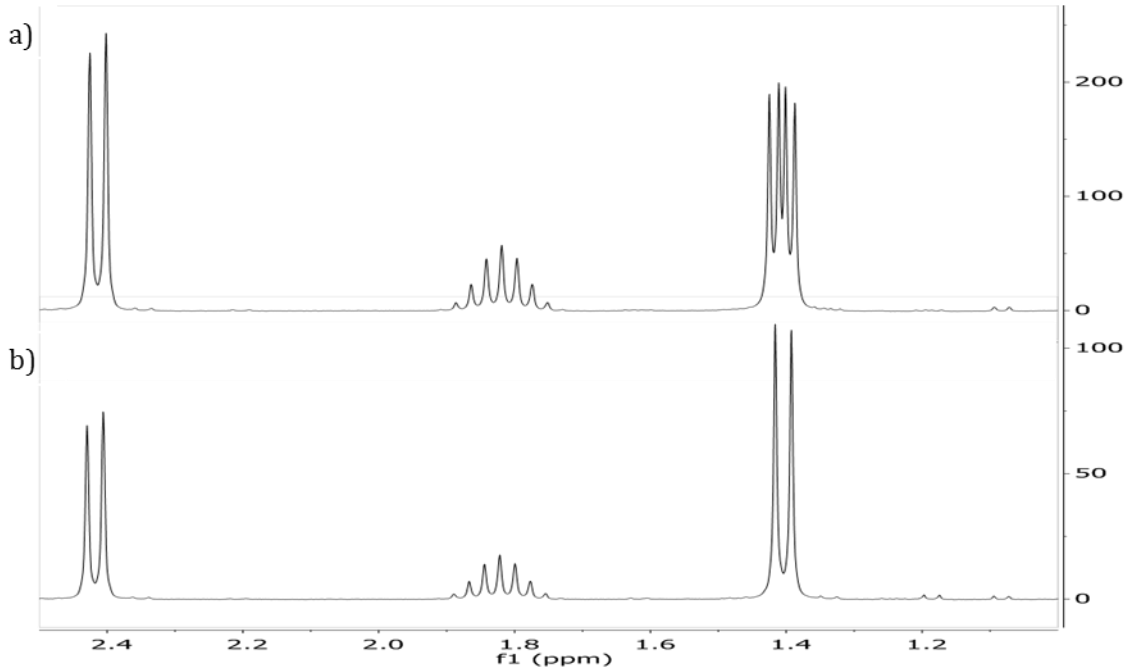


Figure 6.11 NMR spectra of diastereomeric ibuprofen derivative formed by the reaction of ibuprofen with (1S, 2S)-(-)-1,2-diphenylethylenediamine a) NMR spectrum of (R,S) ibuprofen derivative b) NMR spectrum of (S,S) ibuprofen derivative.

6.5.2.3 Mass Spectrometry

Students recorded mass spectra of the trimeric cluster, $[Cu(L\text{-}Trp)_2ibuprofen–H]^+$ at *m/z* 676. This ion was isolated and fragmented and the ratio of two distinct fragment peaks at *m/z* 470 and *m/z* 472, which correspond to the loss of ibuprofen or tryptophan, respectively, was calculated (Figure 6.12). A calibration curve was generated by plotting the natural logarithm of the ratio of those two peak intensities against the known % *ee* for ibuprofen at 0, 50, and 99 % *ee* (*S*). Students analyzed an unknown % *ee*, and an unknown % *ee* in a sample that had Pepto-Bismol® chewable tablets and Tylenol Extra Strength® added to the unknown. Both unknowns were able to be determined by mass spectrometry. For the unknown of 75% *ee* (*S*), students measured the intensity at *m/z* 470 as 4245 and divided it by the intensity at *m/z* 472, which was 957 (Figure 6.12 A). The natural log of this R value was calculated and the students utilized their calibration curve to determine the % *ee* (*S*) (Figure 6.12 B).

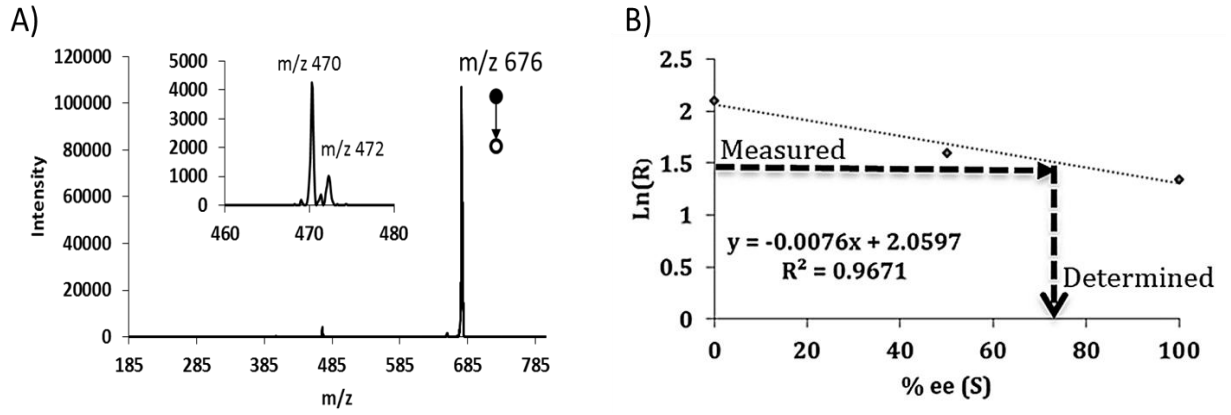


Figure 6.12 a) Student generated MS/MS spectrum of m/z 676, the trimeric cluster [MII(A)(ref)2–H]+ at 75% ee (S). The inlaid spectrum is a zoom in of the fragment peaks at m/z 470 and 472, which corresponds to ([MII(ref)2–H]+) and ([MII(A)(ref)– H]+) respectively. b) Calibration curve of the ratio R against % ee. Students measured their calculated ln(R) and then determined their % ee (S). The error on the calibration curve is ~ 3-4% ee.

6.5.2.4 Student Interviews

Upon the completion of the laboratory session, students were verbally interviewed in their groups by the graduate teaching assistants to assess what they had retained from the laboratory period. Table 6.6 summarizes the student responses to the five interview questions. Each group rated this experiment as a worthwhile exercise, while demonstrating a strong grasp of the advantages and disadvantages of each instrumental technique. In a recent study, DeKorver and Towns discuss the importance of targeting the cognitive, affective, and psychomotor learning domains in order to achieve meaningful learning.[244] Taking all the student data into consideration, the students' demonstrated excitement about and satisfaction with the experiment, while also achieving the learning objectives. This reveals that this laboratory exercise successfully targeted the three learning domains, affording the students the opportunity to achieve meaningful learning.

Table 6.7 Comparison of Student Post-Laboratory Verbal Interview Questions and Responses for Chirality Lab.

Questions/Statements for Response[a]		Groups, *N*[b]	Response Characterization —Students in the Group:
1	This is probably the first time that mass spectrometry and the kinetic method has been explored in an undergraduate teaching laboratory, as well as comparing its analytical figures of merit to NMR spectroscopy and polarimetry. Could you please explain why you believe this was or was not a valuable laboratory experiment? How could this experiment be improved?	3	Thought the experiment was a worthwhile exercise
		3	Appreciated exposure to mass spectrometry
		3	Appreciated the exposure to learning about the pros and cons of choosing an instrumental technique for analysis
		2	Would have liked more background on mass spectrometry prior to the experiment, since this was the first time they worked with a mass spectrometer
2	Please explain chirality, enantiomer, diastereomers, and enantiomeric excess and why these are important in relation to pharmaceuticals.	3	Could correctly describe chirality, enantiomers, diastereomers, and enantiomeric excess
		3	Explained the difference that enantiomers can have on biological activity of drugs
		3	Described the potential harmful side effects of giving a patient the wrong enantiomer
3	Please explain the kinetic method as it applies to *ee* determination by mass spectrometry and the advantages and disadvantages of this method.	3	Described the derivatization of the ibuprofen to form the trimeric cluster for mass spectrometry
		3	Described how the difference in enantiomers causes different fragmentation patterns
		3	Described the small sample volume and low concentration, as well as the ability to analyze impure samples as advantages
		2	Mentioned that the mass spectrometer was not as precise as NMR spectroscopy
		1	Said that the kinetic method measurement by mass spectrometry had more room for error
4	Please explain the how NMR spectroscopy data can be used to determine % *ee* and the advantages and disadvantages of this method.	3	Explained the need for diastereomers for NMR spectroscopy analysis
		3	Stated that impurities could cause problems in NMR spectroscopy
		2	Mentioned that it requires less sample than polarimetry
		1	Stated that NMR spectroscopy is more sensitive to changes in *ee* than Mass Spectrometry
5	Please explain how polarized light interacts with ibuprofen and how the measurement is made.	3	Described how polarized light interacts with a chiral [stereogenic] center
		3	Explained how the specific enantiomer will change the rotation of the light and thus determine the enantiomeric excess

[a] Graduate TAs verbally interviewed students. [b] The 14 total students were assigned to 3 groups of 4 or 5 each.

This experiment exposed organic chemistry I students to MS, the kinetic method, and how MS can be useful in enantiomeric excess determinations. It provided the opportunity to consider the various preparation and derivatization steps involved in analyses, as well as what benefit each technique affords. Most importantly, this laboratory exercise gave students an opportunity to develop the ability to critically consider various analytical methods and determine which would be optimal depending on their sample conditions. Student data show that not only did students enjoy this experiment, but that they also were largely able to achieve the above stated learning objectives.

6.6 Process Analytical Technology for On-Line Monitoring of Organic Reactions by Mass Spectrometry and UV-Vis Spectroscopy

Process analytical technology (PAT) monitors chemical processes in real-time using analytical instrumentation.[245] *In-situ* monitoring of manufacturing processes are relied upon heavily by the pharmaceutical industry.[246-249] PAT has seen increased importance after the US Food and Drug Administration (FDA) provided its "Guidance for Industry PAT" report which outlined the regulatory framework of PAT to help assure quality by improved process design and monitoring.[183] While pharmaceutical companies are heavily invested in these applications, there are also prominent PAT applications in the soil management,[250] food quality assurance,[251] animal cell culture[252] and in the chemical industry. Qualities required of a PAT tool are the capability to track the process in real time, preferably on-line with minimal human interaction, and to be tailored to the specific manufacturing process.[245] Multiplexing PAT analyzers can be advantageous as this can provides confirmatory analysis,[253] as well as increasing confidence in these measurements to serve as in-process control measures.

Depending on the specific chemical process, different analytical technologies may be selected.[245] Examples of analytical methods utilized in PAT include Raman Spectroscopy,[254] Infrared Spectroscopy,[255] and particle size analysis,[256] each with their own analytical advantages and disadvantages. UV-Vis Spectroscopy has been reported for on-line flow chemistry for both transition metal oxide catalyzed reactions[257] and for the determination of chemical reaction kinetics.[258] Mass spectrometry (MS) has recently become important as a PAT tool to monitor batch quality on-line,[259] to elucidate reaction mechanisms[260] and synthetic pathways[261] and can be easily multiplexed to allow monitoring of multiple reaction vessels at the same time.[262] On-line

chemical derivatization to facilitate the determination of enantiomeric excess,[231] as well as, more common derivatization experiments to promote ionization efficiency have been reported.[262] Catalytic reactions[263-264] and air and water sensitive reactions[265] have been successfully monitored by mass spectrometry demonstrating the breadth of reactions this PAT tool can be utilized for. In the context of a laboratory exercise, on-line reaction monitoring enables the instructor to delve into topics such as MS ionization efficiency while presenting the students with a detailed understanding of the fundamentals of MS operations in addition to coupling the lesson with a real world application and state-of-the-art technology.[266-267] Similarly, many chemistry students seek a career in industry,[267-268] giving practical value to an introduction to the fundamentals of PAT.

The analytical instrumental techniques used in PAT are often covered in the analytical teaching laboratory curriculum,[269] and are typically introduced earlier in organic teaching laboratories.[173-174, 178] To the author's knowledge this is the first reported multiplexed PAT on-line reaction teaching monitoring laboratory exercise. The coupling of these two specific instruments allowed students to utilize two instruments within one laboratory exercise, explore the fundamentals behind each and their requirements for analysis, and contemplate the benefits and disadvantages of having these two instruments in tandem (Figure 6.13).[270-271]

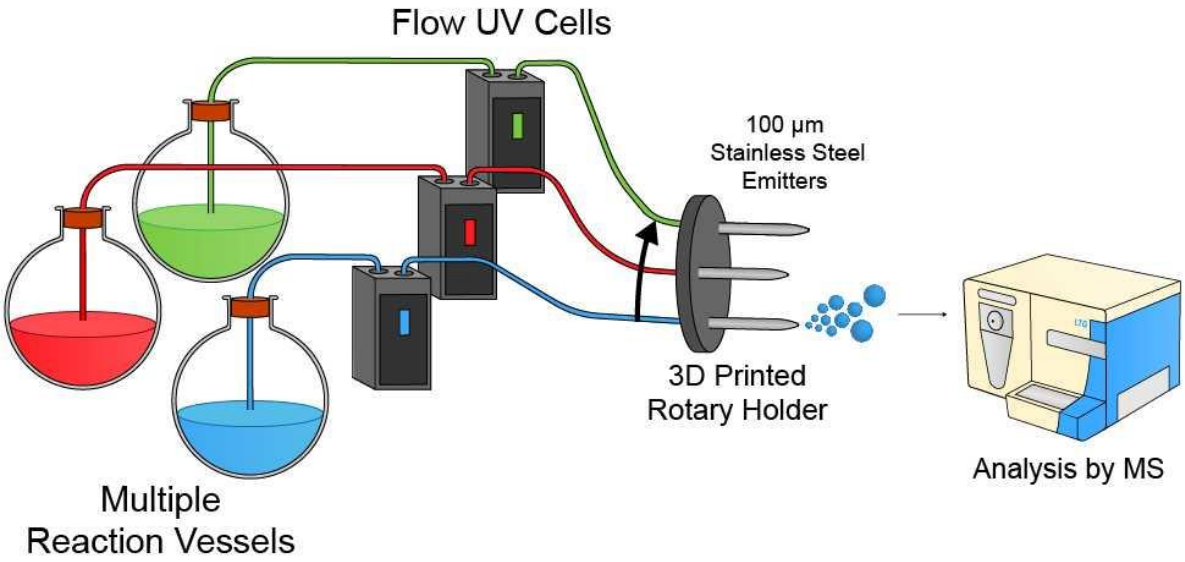


Figure 6.13 Pictorial representation of the coupling of flow UV-Vis spectroscopy and mass spectrometry for on-line reaction monitoring of chemical reactions.

Coupling the two instruments presented challenges in the instrumental set up which required the use of 3D printing to construct three pieces for this laboratory: the UV-Vis cover to allow the flow cuvette to remain in darkness, a holder for the unions of the flow lines, and the rotating coupler to sample the spray in turn from each of the several reaction vessels.[262] The utilization of 3D printing in chemistry laboratory exercises has increased[272-275] as it allows the inexpensive creation of highly customizable parts by rapid prototyping. Additionally, pedagogical value comes from the fact that a microcontroller was utilized to move the 3D printed rotating coupler[262] when monitoring multiple reactions in rapid sequence.

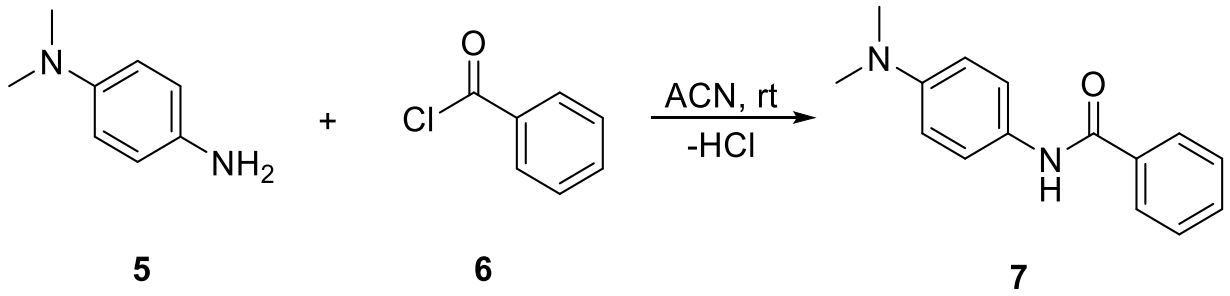
The first learning objective of this laboratory exercise was for students to gain a better understanding of how PAT can provide real-time information on the course of a chemical process. An associated objective was to show how the specific measurements are made and what instrumental and molecular properties determines their success as judged by dynamic range and duty cycle. The second major learning objective was that students recognize how rapid prototyping can enable the development of new scientific procedures and innovation as seen in addressing the rate- and quality-limiting process steps through customization of analytical instrumentation. Both pedagogical targets were combined with the mission of bringing cutting-edge research to the teaching laboratory so that students are better prepared for future developments in chemical analysis.

6.6.1 Experimental

A total of 29 undergraduate students enrolled in analytical chemistry II laboratory, were split into three laboratory sections. Each section performed this laboratory exercise in the three-hour allotted laboratory period. The students in each section were split into two groups resulting in five groups of five and one group of four between all sections. The two groups in each section rotated between the PAT experiment and the 3D printing demonstration. Prior to the start of the laboratory exercise students turned in their pre-laboratory reports. Pre-laboratory questions can be found in Appendix D and were provided a pre-laboratory lecture and safety demonstration.

The students in this laboratory exercise monitored the amide bond formation (Scheme 6.3) by flow UV-Vis spectroscopy followed by down-stream mass spectrometric analysis. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade acetonitrile (ACN) was purchased from Fisher Scientific (Hampton, NH). All UV-Vis spectra were recorded in the

Scanning Kinetics Mode with a wavelength range from 600 to 200nm with a cycle of one spectrum per minute using a Cary 50 UV-Vis spectrometer (Agilent Technologies, Santa Clara, CA) and all mass spectra were recorded in the positive ion mode using a LTQ XL Ion Trap mass spectrometer (Thermo-Fisher Scientific, San Jose, CA).



5 **6** **7**

Scheme 6.3 Amide bond formation between para-substituted aniline p-(N,N-dimethylamino)aniline (5) with benzoyl chloride (6) to form the amide (7).

On-line reaction monitoring was performed utilizing fused silica lines, unions assemblies, tee assemblies, and tubing sleeves, which are outlined in Figure 6.14. In addition to all of the parts shown in Figure 6.14, a nitrogen tank (Indiana Oxygen, Lafayette, IN), a flow regulator (Brooks Instrument, Hatfield, PA) which was set to 1 mL/min, a 12 x 4.5 mm stir bar (Fisher Scientific, Hampton, NH) in the reaction vessel, a magnetic stir plate (Fisher Scientific), a round bottom flask for waste collection (Corning Life Sciences, Tewksbury MA), a Arduino Uno IDE microcontroller (Adafruit, New York City, NY) and a EasyDriver Stepper Motor Driver (Sparkfun, Niwot, CO) were also utilized. The specifications for each of the parts were utilized to control the flow rate, alternative diameters and volumes can be used but this may effect the overall flow and performance. The specifications for the UV-Vis cuvette were chosen to match the specifications of the Cary 50 UV-Vis spectrometer and alternative systems may need alternative specifications.

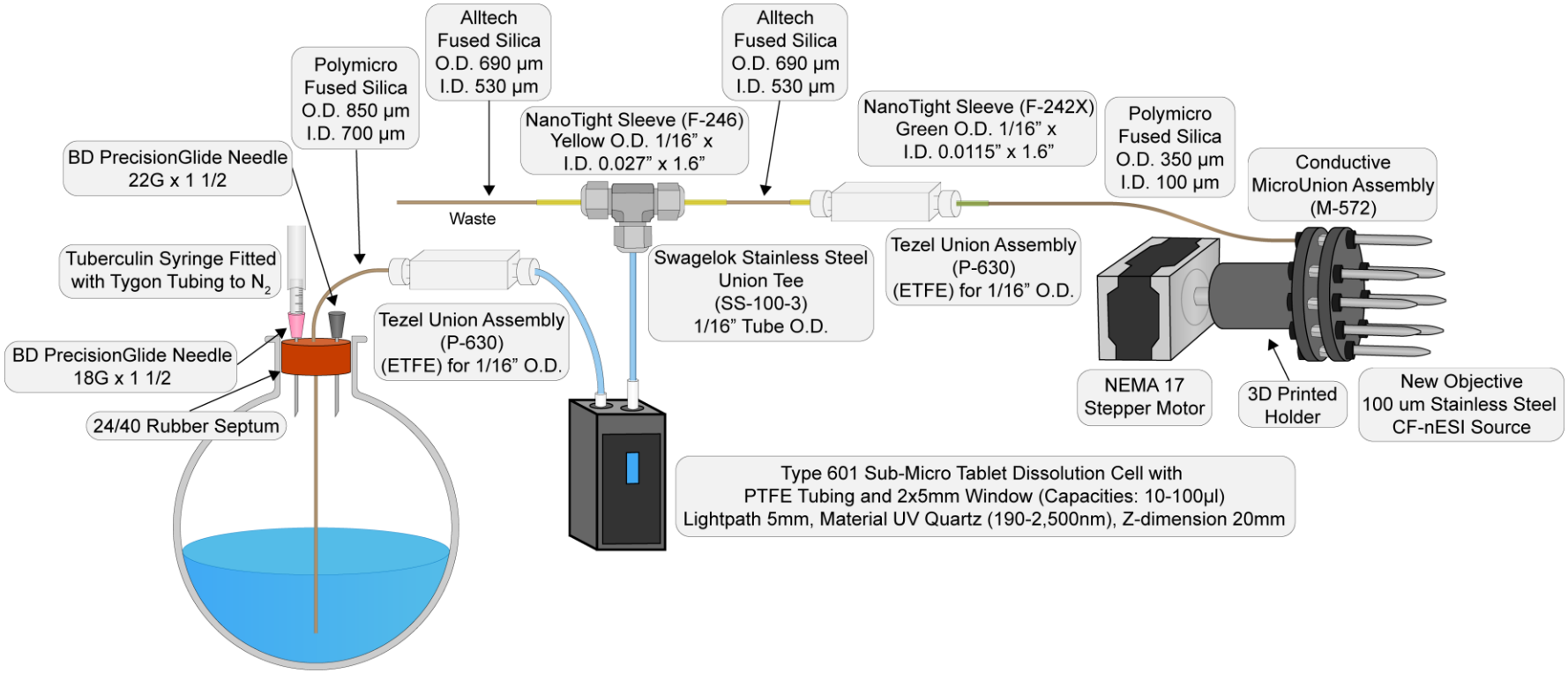


Figure 6.14 . Instrumental setup for the on-line reaction monitoring of the amide bond formation by flow UV-Vis spectroscopy followed by mass spectrometry. Where applicable, each part is labeled with the company, item name, item specifications, and product number for future reproducibility and open access.

As described previously, [262] a custom 3D printed holder was designed using Autodesk Inventor® and converted to an STL file and sliced using Simplify3D . Parts were 3D printed with polylactic acid (PLA)/polyhydroxyalkanoate (PHA) filament (ColorFabb, Belfeld, Netherlands) by a Mendelmax 3 (Makers Tool Works, Oklahoma City, OK). Additionally, a UV-Vis cover for the flow cuvette, and a holder for the unions of the flow lines were also 3D printed. Figure 6.15 shows the rendering of the 3D printed parts from the STL files.

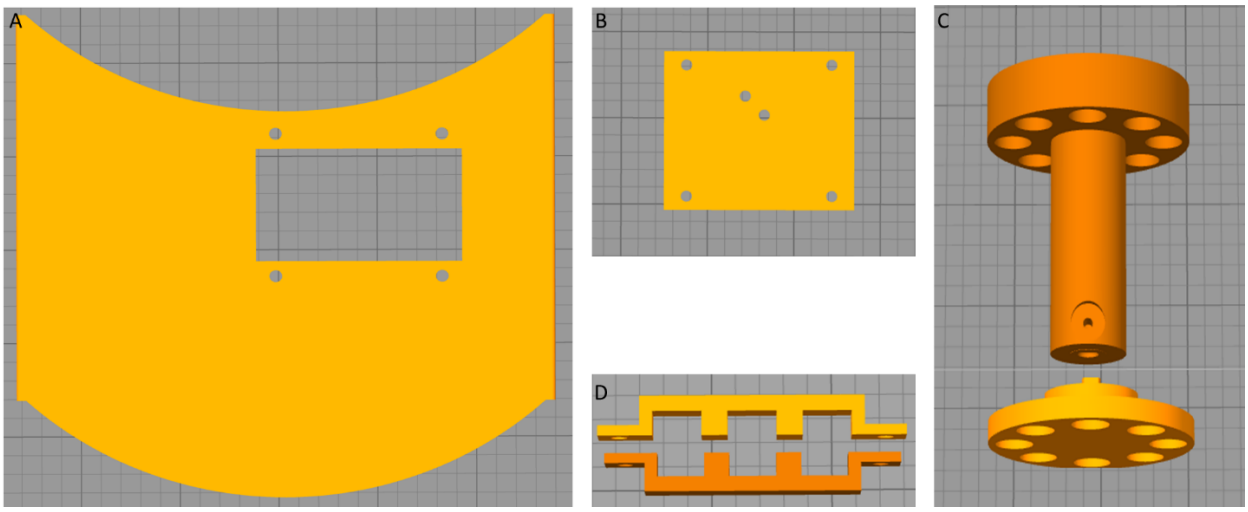


Figure 6.15 Files for 3D printing parts utilized in the laboratory exercise. A) The UV-Vis cover, B) the secondary cover for the flow lines to go through, enabling fast removal, C) two piece rotating continuous flow nanoelectrospray holder, and D) the flow line holders.

6.6.2 Results and Discussion

6.6.2.1 Online Reaction Monitoring by Flow UV-Vis Spectroscopy

Students monitored the formation of **7** through flow UV-Vis (Figure 6.16). The UV-Vis spectra of the two starting materials **5** and **6** were collected, and they showed distinctive peaks at 215 nm to 260 nm and 225 nm to 295 nm, respectively. Upon the injection of **5** and **6** into the round bottom flask, the two peaks related to **5** and **6** coalesced into a peak in the range from 215 nm to 265 nm and a second much broader peak at longer wavelength. Over the course of the reaction, the intensities in the 215 nm to 230 nm and 260 nm to 320 nm regions began to rise to form a single broad peak. To explore the fundamentals of UV-Vis, the students were provided the thought exercise of contemplating what they would change in the instrumental setup if the concentrations of the reactants were altered or if the reactants or product did not have conjugated pi-bonds. The students reached conclusions on the issues related to dynamic range and reagent selection. The

resolution of the UV-Vis spectra was compared to that of mass spectrometry by the students and they deduced that although UV-Vis spectrometers are much cheaper than mass spectrometers, the loss in resolution is a significant disadvantage.

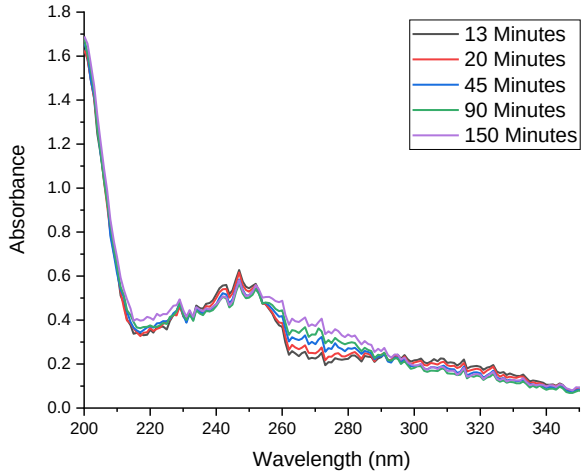


Figure 6.16 UV-Vis spectra for reaction: 5 + 6 → 7. Five colored spectra measured at time points corresponding to 13, 20, 45, 90, and 150 minutes. The signal in the region of 215 nm to 230 nm and 260 nm to 300 nm increases over time.

6.6.2.2 Online Reaction Monitoring by Mass Spectrometry

Students monitored the formation of the amide **7** through continuous flow-nanoelectrospray ionization (CF-nESI) mass spectrometry (Figure 6.17). Upon the injection of **5** into the round bottom flask, the intensity of the ion at *m/z* 137, corresponding to $[M+H]^+$, the protonated form of **5**, began to rise. After the injection of **6** no additional signals where observed by CF-nESI mass spectrometry due to its poor ionization efficiency. As time goes on, the ion at *m/z* 241, corresponding to $[M+H]^+$ of product **7**, is observed in increasing abundances. After ~45 minutes the intensity of both *m/z* 137 and *m/z* 241 are equal. At longer time the *m/z* 137 decreases in relative intensity as more product is being formed. To explore the fundamentals of mass spectrometry the students were asked to predict what they would observe if 4-dimethylaminoaniline were replaced with 4-fluoroaniline. The issue of ionization efficiencies was discussed, and they deduced that only the 4-fluoroaniline starting material would be able to be observed by CF-nESI mass spectrometry. No product would be observed due to the low ionization efficiency of the amide moiety rendering the technique impractical. However, students inferred the high resolution and the characterization

of molecules were both major advantages for mass spectrometry. This thought exercise could be adapted for further laboratory exercises to delve into substituent groups and their effect on reaction rates. Finally, students were shown how they could monitor multiple reactions simultaneously through the aid of the microcontroller and the 3D printed rotating source holder, which was a major advantage for mass spectrometry.

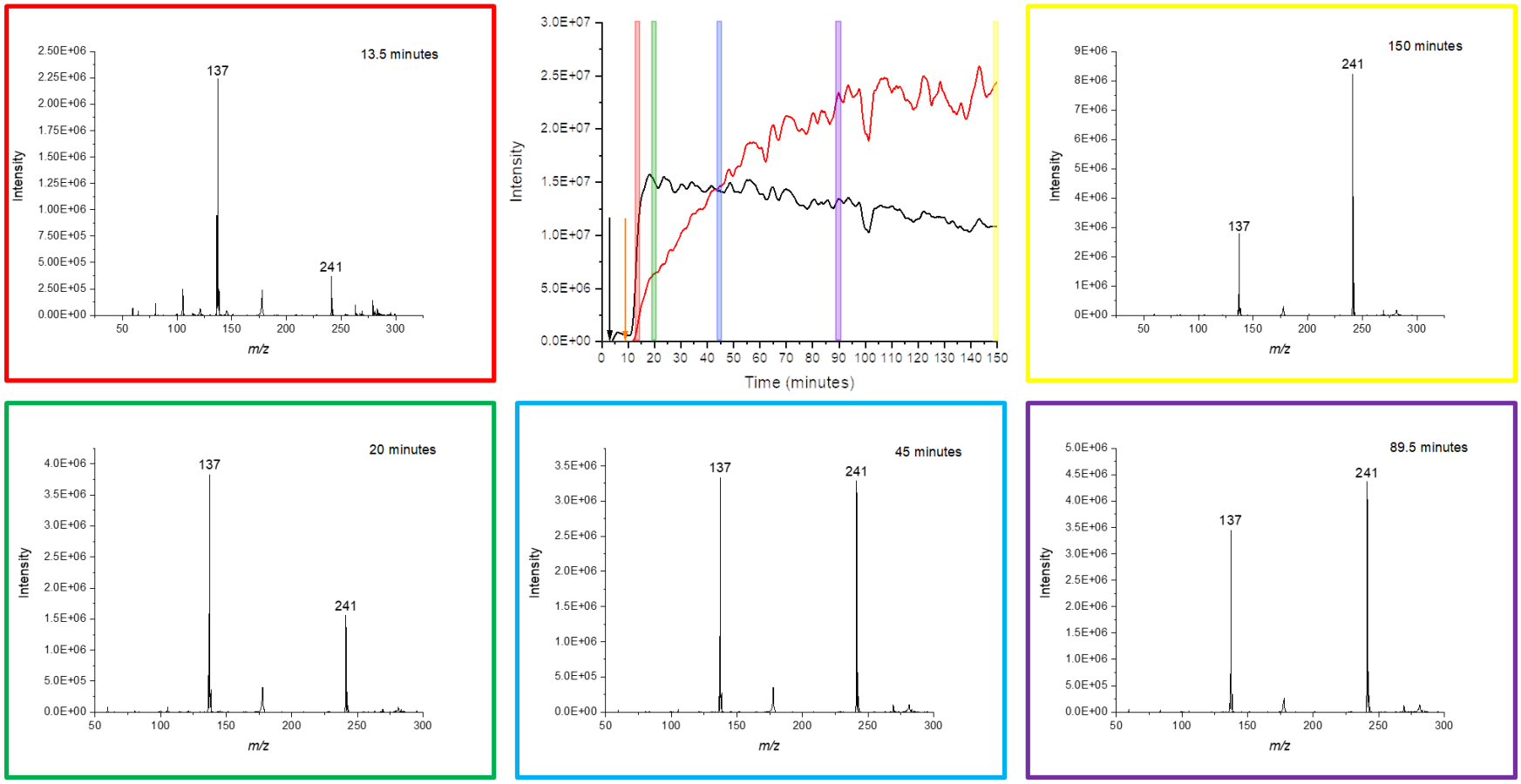


Figure 6.17 Selected ion chronograms for reaction: 5 + 6 → 7 with a lowess filter applied at a 0.02 span to smooth data for easier visual interpretation. The black trace is the ion chronogram of 5 and the red trace is the ion chronogram of 7. Notice that reactant 6 is not tracked by mass spectrometry due to its poor ionization efficiency in positive ion mode CF-nESI MS. The black arrow corresponds to 5 being injected into the round bottom flask, while the red arrow corresponds to the injection of 6. The five colored boxes have the spectra that correspond to the markers of the same color on the ion chronograms.

6.6.2.3 Student Interviews

Upon the completion of the laboratory session, students were verbally interviewed in their groups by the graduate teaching assistants to assess what they had retained from the laboratory period. Table 6.7 summarizes the student responses to the seven interview questions. As seen in Table 6.7, the students thought this was a valuable laboratory exercise due to the practicality and applicability to industry, while learning about figures of merits for the two instrumental procedures. The students made several excellent suggestions to modify this laboratory; if other instructors replicate this study they may want to consider implementing these suggestions. The most noteworthy was to provide students with data from a previous run that shows the reaction going to completion, as this reaction had not gone to completion within the laboratory exercise. Additionally, the request for a pre-laboratory question that gave input into the experimental design, such as "Design and draw your own 3D Printed CF-nESI holder that could move to accommodate multiple reaction monitoring. Be cognizant of the experimental design and the fused silica lines that will that you will have to protect." could be a noteworthy addition. Finally, the suggestion of a more hands-on portion where they build their own setup either in a previous laboratory exercise, or while the current set up is running was also a valuable suggestion and potential improvement.

Table 6.8 Comparison of Student Post-Laboratory Verbal Interview Questions and Responses for PAT Lab.

Questions/Statements for Response[a]		Groups, N^b	Response Characterization — Students in the Group:
1	This is probably the first time that PAT has been explored in a undergraduate teaching laboratory, as well as the first time a Flow UV-Vis has been coupled to a Mass spectrometer for on-line reaction monitoring. Could you please explain why you believe this was or was not a valuable laboratory experiment? How could this experiment be improved?	6	All agreed that this was valuable teaching experience with the emphasis of the practical real world pharmaceutical applications described in the laboratory exercise.
		4	Suggested having an additional station with the experimental components on hand to let the students physically build the flow setup and have a more hands-on experience.
		3	Suggested adding additional PAT instruments into the workflow.
		2	Suggested giving students reaction data from a longer period, i.e. just past reaction completion.
		2	Suggested adding a prelab question to which the student had to design a part to 3D print to aid in the experimental design.
		1	Suggested adding a prelab question requiring them to look up the exact masses and UV-Vis absorption peaks for the reaction.
		1	Suggested moving this to the organic chemistry laboratory so students can early on learn about PAT and how it differs from how organic laboratory exercises typically only look at the starting materials and final product.
2	Please describe the experimental setup.	6	Explained the overall experimental setup from the round bottom flask, the nitrogen line that pressurized the flask, the fused silica to flow the UV-Vis and then the Mass Spectrometer.

Table 6.7 Continued.

3	What are the strengths for UV-Vis as a PAT. What are its weaknesses?	5	Stated that a strength of UV-Vis is that it is relatively cheap instrumentation that can sample relatively quickly.
		3	Stated that a strength of UV-Vis is that it is a non-destructive instrumental method.
		3	Stated that a strength of UV-Vis is that is relatively simple to operate.
		2	Stated that a strength of UV-Vis is that it does not take a lot of sample preparation.
		2	Stated that a strength of UV-Vis is that it can perform quantitative analysis.
		1	Stated that a strength of UV-Vis is that it can analyze compounds that do not ionize and may be "mass spectrometry blind".
		6	Stated that a weakness of UV-Vis is its concentration restrictions and limited linear dynamic range.
		5	State that a weakness of UV-Vis is its resolution.
		4	Stated that a weakness of UV-Vis is its reliance on conjugation of molecules.
		1	Stated that a weakness of UV-Vis is certain analytes may be photosensitive and will photodegrade.
		1	Stated that a weakness of UV-Vis is that the current setup only one reaction can be monitored at a time.
4	What are the strengths for Mass Spectrometry as a PAT. What are its weaknesses?	5	Stated that a strength of Mass Spectrometry is it high resolution compared to UV-Vis.
		4	Stated that a strength of Mass Spectrometry is its ability to characterize molecules
		2	Stated that a strength of Mass Spectrometry is its ability to monitor intermediates, trace impurities, and side products.
		2	Stated that a strength of Mass Spectrometry is its ability to utilize isotopic ratios during analysis.
		1	Stated that a strength of Mass Spectrometry is its ability to analyze compounds in complex mixtures.
		1	Stated that a strength of Mass Spectrometry is it is extremely sensitive.
		1	Stated that a strength of Mass Spectrometry is that it does not rely on any change in conjugation to differentiate the reaction.
		3	Stated that a weakness of Mass Spectrometry is its inability to distinguish between isomers in full scan mode.
		2	Stated that a weakness of Mass Spectrometry is that it is relatively expensive.
		2	Stated that a weakness of Mass Spectrometry is that it is a destructive technique.
		2	Stated that a weakness of Mass Spectrometry is the effect of ionization efficiencies.
		1	Stated that a weakness of Mass Spectrometry is the instrumental maintenance and difficulty of data interpretation.
5	If you were going to improve this setup what would you change?	4	Suggested running separate fused silica lines to the UV-Vis and Mass Spectrometer, with a in line dilution for UV-Vis to run higher concentrations.
		1	Suggested to use stronger tubing than the fused silica.
		1	Suggested changing the setup from solution to gas phase reaction monitoring.
		1	Suggested using a flow cell for the reaction as compared to the round bottom flask.

Table 6.7 Continued.

6	How does 3D printing aid in experimental designs?	6	Spoke about the capability for rapid and cheap prototyping.
		6	Spoke about the customizability and easy modification of parts, some that have never existed before.
		5	Spoke about the difference in design time and wait time compared to custom manufacturing from a commercial source.
7	Please explain the issues when deciding how to orient an object on the print bed. What other major difficulties are there involving 3D printing? How can you mitigate these problems?	6	Discussed the importance of having a high surface area on the printbed and the minimalization of scaffolding material.
		5	Discussed the importance of selecting the proper plastic depending on your application. Further they discussed the how the extent of the plastic expanding upon cooling can be a major problem.
		4	Discussed the importance of having the printer bed level prior to printing.
		3	Discussed the importance of having adhesion on the printer bed surface prior to printing and an optimal extruder temperature for the specific plastic being utilized.
		2	Discussed the difficulties when choosing a fill time, as well as, the damaging effects of warping and stringing.
		2	Discussed the trial and error learning process typically involved in 3D printing.
		2	Discussed the high degree of technical knowledge required to be able to design and successfully print an item.

[a] Graduate TAs verbally interviewed students. [b] The 23 total students were assigned to 7 groups of 3 and 1 group of 2.

This laboratory exercise demonstrated the importance of PAT, the considerations of selecting an instrument for the task at hand, and how rapid prototyping allows new analytical processes to be explored. Students had a strong understanding of PAT, the strengths and weaknesses of the two PAT techniques that they explored, and a broader sense of how 3D printing can aid in experimental design. As seen in the suggested improvements to the laboratory setup, the students gave significant thought to how they may do things differently, as seen in their responses to the verbal interviews.[269] This laboratory exercise is appealing because it has a low entrance barrier as many institutions have UV-Vis spectrometers and mass spectrometers. Additionally, the open source format for the CAD files for 3D-printing and the code for the Arduino aids instructors in implementing this laboratory exercise. Overall, the students showed an understanding of PAT, analytical figures of merit for the different PAT methods and the benefits and weaknesses of 3D printing in aiding experimental designs as demonstrated in their pre-laboratory, post-laboratory, and verbal interviews.

6.7 Conclusions

Teaching laboratory exercise redevelopment projects for both organic and analytical chemistry courses taught cutting edge science to the undergraduate students, while developing an understanding of the fundamentals that underlie these new methodologies. As mass spectrometry becomes more dominant in all fields of science, the incorporation of the analytical technique into the teaching laboratory exercises is crucial for students for a well-rounded education, preparing them for a career after graduation. The student's pre- and post-laboratory reports showed the students' increase knowledge of the laboratory learning objectives at hand, as well as, added to their overall understanding of mass spectrometry. All experiments were performed on a relatively low-cost Thermo LTQ ion trap mass spectrometer, which lowers the barrier of acceptance and inclusion into the syllabi in other universities. Across each experiment the student groups rated the experiments as worthwhile exercises, due to the overall setup and the novel nature of the experiments. Additionally, the verbal interviews showed that the students demonstrated a strong grasp of the of the goals of experiment at hand. Taking all the student data into consideration, the students demonstrated excitement about and satisfaction with the experiments, while also achieving the learning objectives.

CHAPTER 7. REACTION ACCELERATION OF PALLADIUM CATALYZED SUZUKI CROSS COUPLING REACTIONS BY THE LEIDENFROST EFFECT.

7.1 Abstract

The Suzuki cross coupling reaction is one of the most widely performed reactions in synthetic organic chemistry. While metal catalyzed reactions and acceleration due to heat have been well studied, confined volume reaction acceleration has been studied to a much lesser extent. Demonstrated here, is the utilization of Leidenfrost droplets for the acceleration of the Suzuki cross coupling reaction. Substituent effects for the Suzuki cross coupling were also explored. The most prominent reaction acceleration observed was when 3-bromopyridine was used as the starting material. The largest acceleration factor, averaged over three trials, was reported as 73.83 ± 2.03 when compared to the bulk. This is the first time that metal catalyzed reactions were probed by Leidenfrost droplets. Reactions were performed in triplicate and the relatively small deviations between runs shows the potential of the Leidenfrost droplet acceleration in reaction screening and trend predictivity using different substrates.

7.2 Introduction

The Nobel Prize winning reaction – the Suzuki cross coupling reaction,[276] named after its inventor Akira Suzuki, is one of the most widely used reactions in the pharmaceutical industry today.[225] The formation of a carbon-carbon bond by coupling partners, boronic acid, and an organohalide was first reported in 1979.[277-278] The carbon-carbon bond is formed through the aid of a metal catalyst, most commonly by a palladium complex; however, other metals have been utilized as well.[279-280] Similar to other metal-catalyzed reactions, like the two other cross coupling named reactions that Suzuki shared the Nobel Prize with, the Negishi reaction,[281] and the Heck reaction,[282] these metal catalyzed reactions have been well studied.

The field of speeding up chemical reactions through a catalyst, as well as, through heat, has a long-standing and well-studied history.[283] A relatively new form of accelerating reactions comes through the mechanism of thin films and other forms of reagent confinement.[17, 218, 284] Chemical synthesis and reaction acceleration through the use of mass spectrometry ionization

sources was discovered by the Cooks group in 2006.[285] Since then, reaction acceleration has been probed using a number of reactions such as Claisen-Schmidt condensation,[173] Katritsky transamination,[286] Hydrazone formation,[287] and Boc-deprotection,[176] to name a few. A number of different ionization sources can be used to generate droplets that may vary in size for reaction acceleration.[288] Some of these include electrospray,[289] nano-electrospray,[219, 287] paper spray,[178, 286] easy ambient sonic-spray,[176] desorption electrospray,[156] and theta tips[290] to name a few.

Although the mechanism of reaction acceleration is not completely known, there are a number of competing theories for the mechanisms for acceleration. First, is the degree of desolvation of the droplet, which was explored during the Hantzch synthesis. As the spray source was moved further away from the mass spectrometer inlet, increasing the product formation was found to be increased, perhaps due to increased desolvation of the droplets.[219] Confined volume droplets and surface effects have also been studied with base-catalyzed condensation between an indanone and an aromatic aldehyde, which showed that there is a direct significant surface effect on reaction acceleration when comparing different substituents on the aromatic aldehyde via a Hammett plot.[291] Another droplet study, performed by the spray-based ambient ionization source known as easy ambient sonic-spray ionization (EASI), which produces uncharged droplets, generated the free amine product of a "Boc" deprotection reaction at an accelerated rate of up to 200 when compared to the bulk reaction.[176] The generation of thin-films on a substrate has also been studied and is thought to be the combination of the above mentioned factors.[223]

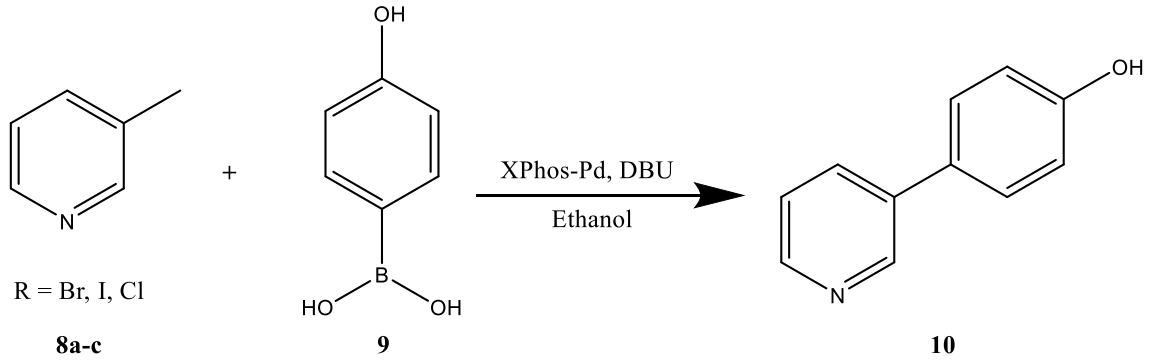
These alternative synthetic techniques have generated milligram amounts of product in several minutes and can be useful in determining new reaction pathways and synthetic schemes.[292] To calculate the reaction rate acceleration, the ratio of the intensity of the product ion to the intensity of the reactant ions for the accelerated system is divided by the same ratio for the bulk system (Equation 6.1).

It is important to note that this is a mass spectrometric ratio and is not the same as the rate constants for the reaction. The reaction rate acceleration is not identical but is certainly related to the rate constants of the two systems. As seen, many of these non-traditional accelerated synthesis methods are performed through the aid of these ambient ionization sources. Metal-catalyzed systems have also been studied in these accelerated systems, such as the copper-catalyzed C-O and C-N coupling reactions that were performed by both electrospray and paper spray.[293] Microdroplet

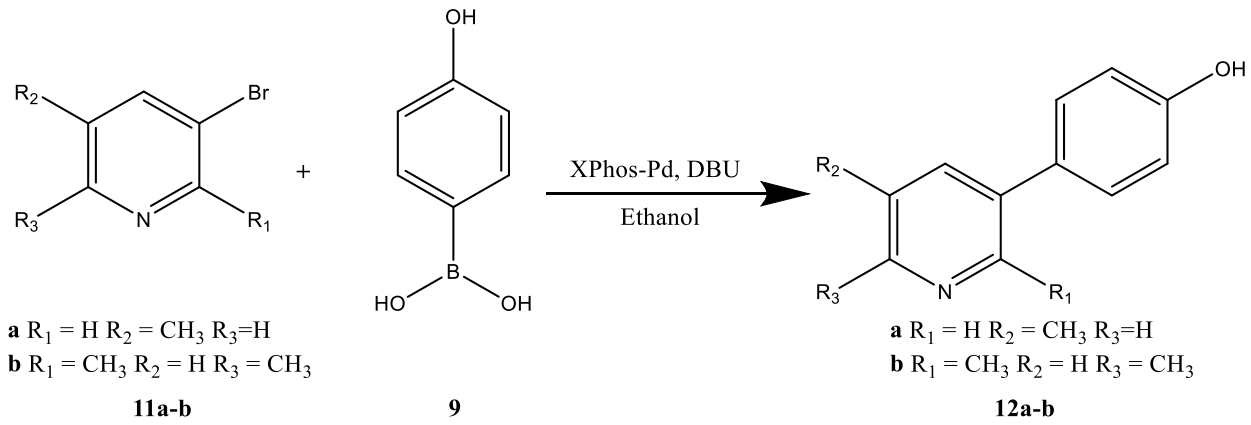
reactions of the Suzuki cross coupling reaction have been screened with DESI[294] and the ability to scale up reactions after these initial accelerated screenings have been reported.[295]

Another way of accelerating reactions, which has not been studied as extensively as acceleration using ambient ionization sources, is through the use of the Leidenfrost effect.[296] The Leidenfrost effect occurs when a liquid droplet is levitated by its own vapor cushion when dropped on a surface that is at a significantly higher temperature than the liquid's boiling point.[297] The vapor cushion creates an insulating layer which prevents the liquid from boiling rapidly. The droplet size can therefore be maintained through the continuous addition of solvent. While the phenomenon was first reported in the 18th century, this system was first realized for its accelerated synthetic potential in the Bain et. al. manuscript where they looked at a number of reaction schemes.[296] Katritzky transamination, Hydrazone formation, Claisen-Schmidt formation, and the synthesis of diazepam have all been explored.[266, 296, 298] More recently, Li et. al utilized the Leidenfrost effect to accelerate pharmaceutical degradation with reaction acceleration factors of up to 188.[299] As Leidenfrost has shown promise in accelerating reactions it was selected to screen the ability to accelerate metal-catalyzed reactions.

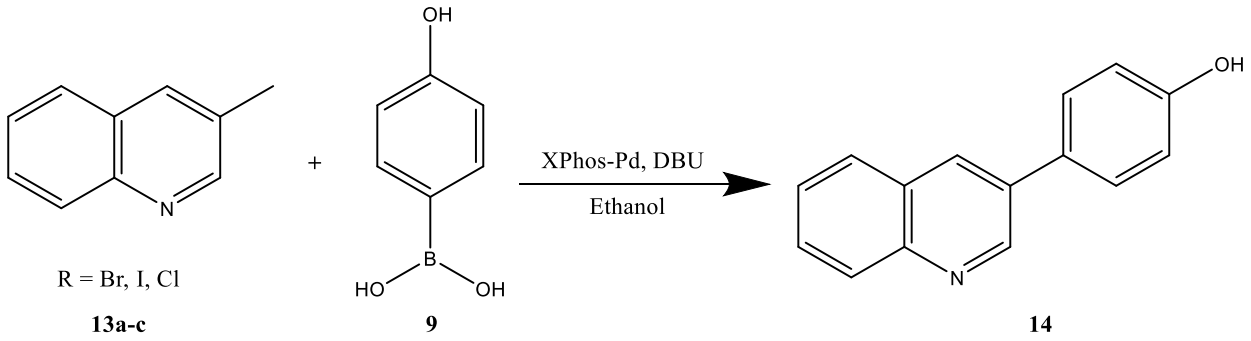
To explore metal catalyzed reactions within a Leidenfrost system, the Suzuki cross coupling reaction was performed. A total of 8 reactions were screened, using different substituents on the halopyridine and haloquinoline (Scheme 7.1 – 7.3).



Scheme 7.1 Suzuki cross-coupling between meta-substituted pyridine: 3-bromopyridine (8a) 3-iodopyridine (8b) and 3-chloropyridine (8c) with 4-hydroxyphenylboronic acid (9) to form 4-(pyridin-3-yl)phenol (10).



Scheme 7.2 Suzuki cross-coupling between substituted meta-substituted bromo pyridines: 3-bromo-5-methylpyridine (11a) and 3-bromo-2,6-dimethylpyridine (11b) with 4-hydroxyphenylboronic acid (9) to form 4-(5-methylpyridin-3-yl)phenol (12a) and 4-(2,6-dimethylpyridin-3-yl)phenol (12b).



Scheme 7.3 Suzuki cross-coupling between meta-substituted quinolines: 3-bromoquinoline (13a) 3-iodooquinoline (13b) and 3-chloroquinoline (13c) with 4-hydroxyphenylboronic acid (9) to form 4-(quinolin-3-yl)phenol (14).

Reaction mixtures were dropped onto a hot plate using a Pasteur pipette. The droplet size was maintained at constant volume of 50 µl for 10 minutes using a syringe pump that dispensed solvent at a constant flow rate (Figure 7.1). Accelerated product formation was observed in all cases, except for the two compounds that had chlorine substituents. No product formation was observed for both the pyridine and the quinoline systems in the Leidenfrost droplet or their respective bulk systems. In order to calculate acceleration factors, product formation in the Leidenfrost droplet was compared to a bulk system that was heated to reflux and maintained at reflux for the same 10-minute period.

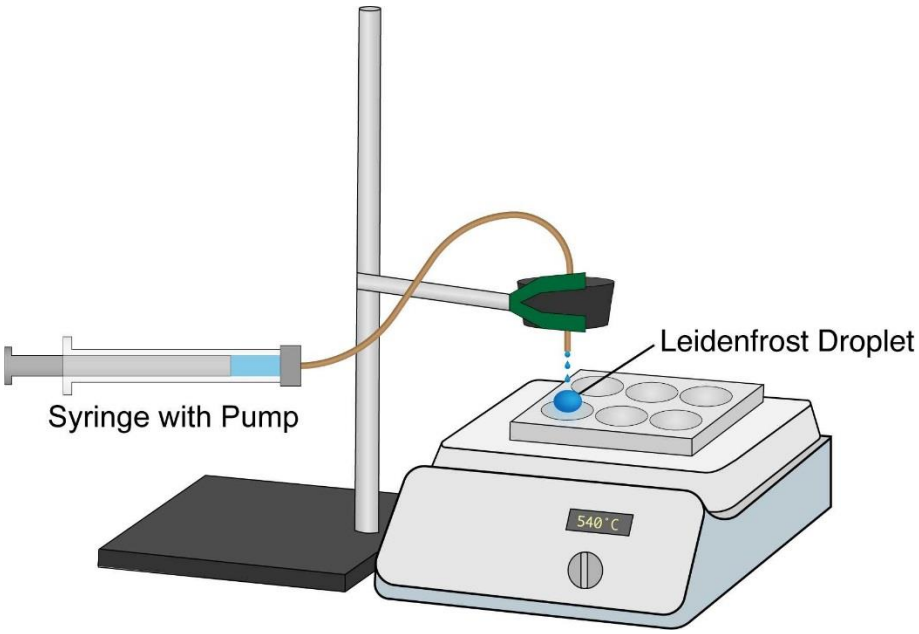


Figure 7.1 Setup of the Leidenfrost system. The ceramic well plate was placed on top of the hot plate set to 540°C. The initial reaction mixture was added with a glass transfer pipette and then the syringe pump applied solvent at a rate of 275 µl/min to maintain the size of a 50 µl droplet. PEEK tubing delivered the solvent and was held in place by a rubber stopper that had a hole drilled in the center of it.

7.3 Experimental

7.3.1 Leidenfrost Conditions

The Leidenfrost droplets were maintained at a constant volume of 50 µL by continuously adding solvent via a Harvard Apparatus PHD 22/2000 syringe pump (Holliston, MA) at 275 µl/min. (Figure 1) The solvent was delivered by a Popper and Sons Perfektum Micro-Mate Interchangeable 20cc syringe (New Hyde Park, NY) outfitted with PEEK tubing 1/16" OD x .030" ID (IDEX Health and Science, Oak Harbor, WA). The PEEK tubing was held in place by a rubber stopper with a hole punched in the center. The PEEK tubing was attached to the syringe using a female luer adapter attached to a 10-32 male luer adapter, a one-piece fingertight 10-32 coned, for 1/16' OD, and a stainless-steel union body true ZDV .062 thru hole (IDEX Health and Science, Oak Harbor, WA). The reaction mixture was maintained over a 10-minute period through the addition of pure ethanol in a porcelain spotting / color plate (Thomas Scientific, Swedesboro, NJ) atop a Fisher Scientific hotplate with a surface temperature 540 °C. The volume was kept constant by monitoring the diameter of the droplet over the time course of the experiment.

Figure 7.2 Photograph of the Leidenfrost setup for a Suzuki cross-coupling reaction.

The internal temperature of the Leidenfrost droplet was measured by a Thermoworks 1/8" Dia. High Temp Handheld Probe, 5" long (K-212) that was connected to a Themoworks Therma K Professional Thermocouple (American Fork, UT) which recorded the temperature at ~77°C slightly below ethanol's boiling point of 78.37°C. The reaction mixture starts at a volume of 1 ml and decreases to 50 µl which is the volume at which the droplet is held constant. This corresponds to a concentration factor of ~20. Hence the bulk-phase reactions were performed at a twentyfold higher concentration to correct for the concentration effect in the Leidenfrost droplets. Comparisons to bulk reactions were performed with the bulk reactions set to reflux for 10 minutes and then sampled.

7.3.2 Substituents

XPhos Pd G3, 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), 4-hydroxyphenylboronic acid, 3-bromopyridine, 3-iodopyridine, 3-chloropyridine, 3-bromoquinoline and anhydrous pure 200 proof ethyl alcohol was purchased from Sigma Aldrich (St Louis, MO). The ethyl alcohol was used as the solvent system for all experiments. 3-iodoquinoline was purchased from EnamineStore

(Monmouth Junction, NJ), 3-chloroquinoline was purchased from Accela (San Diego, CA), 3-bromo-2,6-dimethylpyridine was purchased from Matrix Scientific (Columbia, SC) and 3-bromo-5-methylpyridine was purchased from Asymchem (Morrisville, NC). Leidenfrost reactions were conducted with **8a-c**, **11a-b**, and **13a-c** at a concentration of 0.1mM, **9** at 0.1mM, XPhos-Pd G3 at 0.01mM and DBU at 0.2mM. Bulk experiments were conducted with all reactants at 20 times the concentrations of the Leidenfrost experiments.

7.3.3 Mass Spectrometric Analysis

Mass spectrometric analyses were performed in negative ionization mode on an LTQ ion trap (Thermo Fisher Scientific, San Jose, CA). The LTQ had the following parameters: capillary voltage of -15V, capillary temperature of 150°C, and a tube lens voltage of -65V. Nanoelectrospray ionization (nESI) was utilized at 2.0kV. To construct the nESI tips, borosilicate glass capillaries (1.5 mm O.D., 0.86 mm I.D., Sutter Instrument Co.), were pulled to a tip using a Flaming/Brown micropipette puller (Sutter Instrument Co. model P-97, Novato, CA, USA) with an outer diameter of 2 μm.

7.4 Results and Discussion

The Suzuki cross-coupling utilizing the brominated compounds provided the largest acceleration factors of the substituents studied. Figure 7.3 shows the spectra of 3-bromopyridine (**8a**) and 3-bromoquinoline (**13a**) when reacted with 4-hydroxyphenylboronic acid (**9**) to form 4-(pyridin-3-yl)phenol (**10**) and 4-(quinolin-3-yl)phenol (**14**) respectively. The bulk reaction when performed at room temperature and under reflux produced negligible amounts of product. The calculated average acceleration factors of when utilizing the two bromo substituents (**8a**) and (**13a**) were 73.83 ± 2.03 and 68.09 ± 1.34 respectively (Table 7.1).

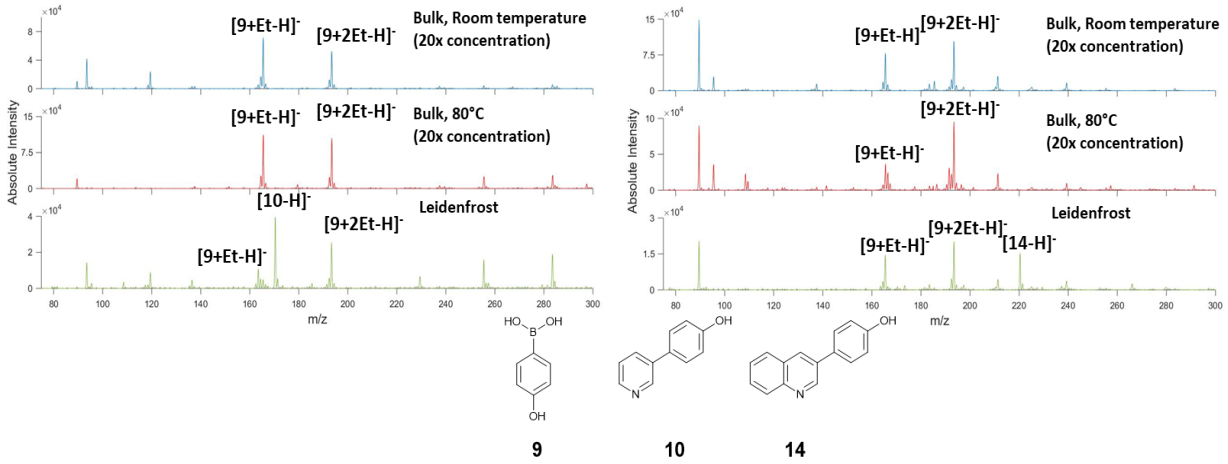


Figure 7.3 Suzuki cross-coupling between - 3-bromopyridine (8a) (left) and 3-bromoquinoline (13a) (right) with 4-hydroxyphenylboronic acid (9) to form 4-(pyridin-3-yl)phenol (10) and 4-(quinolin-3-yl)phenol (14). Top Blue: Spectrum of the bulk reaction at room temperature at a concentration 20x higher than the subsequent Leidenfrost experiments after ten minutes. Middle Red: Spectrum of the bulk reaction refluxed for ten minutes at a concentration 20x higher than the subsequent Leidenfrost experiments. Bottom Green: Spectrum of the accelerated reaction by Leidenfrost droplets after ten minutes of continual solvent addition.

The Suzuki cross-coupling utilizing the iodo species provided modest acceleration factors. Figure 7.4 shows the spectra of 3-iodopyridine (**8b**) and 3-iodoquinoline (**13b**) when reacted with 4-hydroxyphenylboronic acid (**9**) to form 4-(pyridin-3-yl)phenol (**10**) and 4-(quinolin-3-yl)phenol (**14**) respectively. The bulk reaction when performed at room temperature and under reflux produced negligible amounts of product. The calculated average acceleration factors when utilizing the two iodo substituents (**8b** and **13b**) were 12.70 ± 0.19 and 13.31 ± 0.30 minutes respectively (Table 7.1).

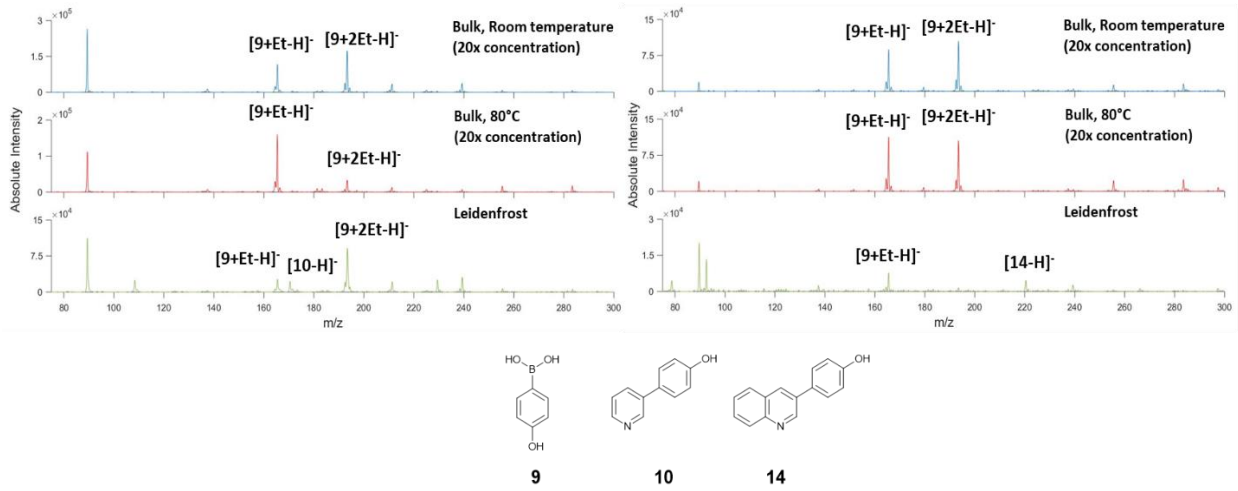


Figure 7.4 Suzuki cross-coupling between - 3-iodopyridine (8b) (left) and 3-iodoquinoline (13b) (right) with 4-hydroxyphenylboronic acid (9) to form 4-(pyridin-3-yl)phenol (10) and 4-(quinolin-3-yl)phenol (14). Top Blue: Spectrum of the bulk reaction at room temperature at a concentration 20x higher than the subsequent Leidenfrost experiments after ten minutes. Middle Red: Spectrum of the bulk reaction refluxed for ten minutes at a concentration 20x higher than the subsequent Leidenfrost experiments. Bottom Green: Spectrum of the accelerated reaction by Leidenfrost droplets after ten minutes of continual solvent addition.

When switching the substituents to the chloro species (**8c** and **13c**) no product was formed in any of the three conditions: room temperature bulk, refluxed bulk, or in the Leidenfrost droplets (Figure 7.5). This trend in substituents is consistent with what is mimicked in published bulk reactions.[300]

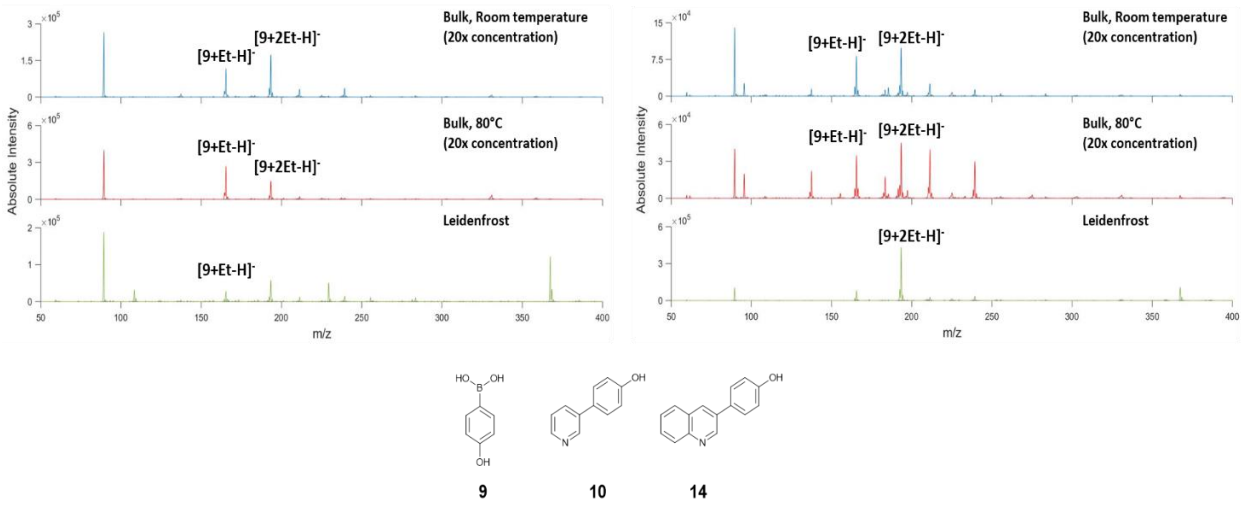


Figure 7.5 Suzuki cross-coupling between - 3-chloropyridine (8c) (left) and 3-chloroquinoline (13c) (right) with 4-hydroxyphenylboronic acid (9) Top Blue: Spectrum of the bulk reaction at room temperature at a concentration 20x higher than the subsequent Leidenfrost experiments after ten minutes. Middle Red: Spectrum of the bulk reaction refluxed for ten minutes at a concentration 20x higher than the subsequent Leidenfrost experiments. Bottom Green: Spectrum of the accelerated reaction by Leidenfrost droplets after ten minutes of continual solvent addition. No product was formed under any condition. Additionally, no dimers or trimers were seen in the higher mass range.

To further explore substituent effects, two 3-bromopyridine compounds with additional methyl substituents on the ring were analyzed. Figure 7.6 shows Suzuki cross coupling product when 3-bromo-5-methylpyridine (**11a**) reacts with 4-hydroxyphenylboronic acid (**9**) to form 4-(5-methylpyridin-3-yl)phenol (**12a**) and when 2,6-dimethyl-3-bromopyridine (**11b**) reacts with 4-hydroxyphenylboronic acid (**9**) to form 4-(2,6-dimethylpyridin-3-yl)phenol (**12b**). With the addition of the methyl groups to the ring, the reaction acceleration is decreased over an order of magnitude with the average calculated factor for 4a being 2.02 ± 0.10 and 4b producing negligible product (Table 7.1).

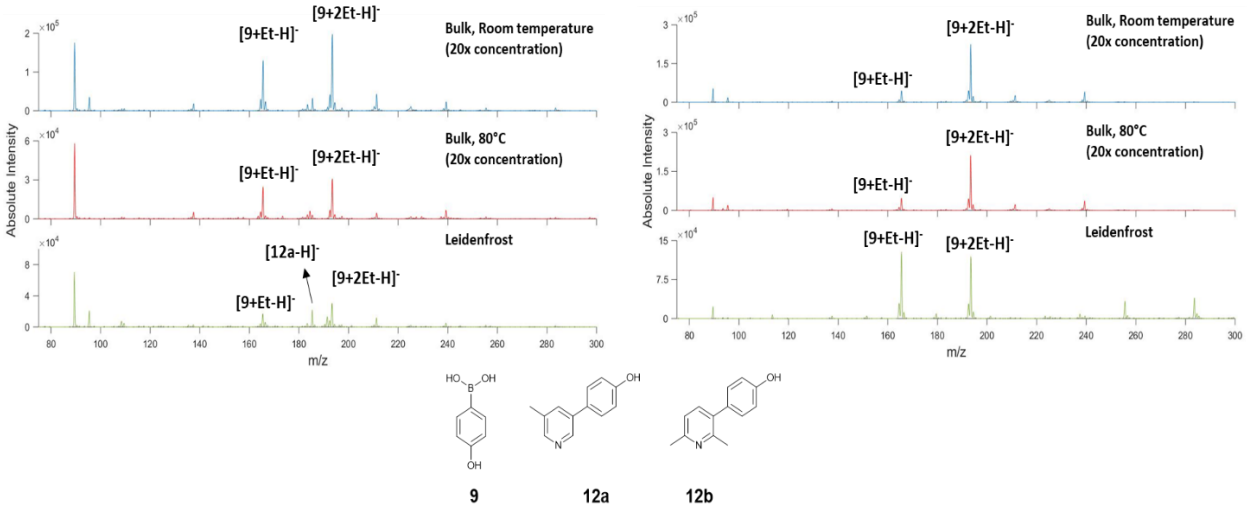


Figure 7.6 Left: Suzuki cross-coupling between 3-bromo-5-methylpyridine (11a) with 4-hydroxyphenylboronic acid (9) to form 4-(5-methylpyridin-3-yl)phenol (12a), Right: Suzuki cross-coupling between 2,6-dimethyl-3-bromopyridine (11b) with 4-hydroxyphenylboronic acid (9) (No reaction observed). Top Blue: Spectrum of the bulk reaction at room temperature at a concentration 20x higher than the subsequent Leidenfrost experiments after ten minutes. Middle Red: Spectrum of the bulk reaction refluxed for ten minutes at a concentration 20x higher than the subsequent Leidenfrost experiments. Bottom Green: Spectrum of the accelerated reaction by Leidenfrost droplets after ten minutes of continual solvent addition.

To explore the reproducibility of these acceleration factors, every reaction was performed in triplicate. Table 7.1 shows each substituent's acceleration factor for the three runs. The relatively small standard deviation between the three runs for all substrates, demonstrates how these Leidenfrost droplets could be utilized for rapid screening of potential substrates for reaction completion because of their reproducibility.

Table 7.1 Acceleration factors for each substituent. Each experiment was performed in triplicate.

Substituent	Acceleration factors*			
	Run 1	Run 2	Run 3	Average ± Stdev
3-bromopyridine	71.59	74.37	75.54	73.83 ± 2.03
3-iodopyridine	12.85	12.49	12.76	12.70 ± 0.19
3-chloropyridine	N/A#			
3-bromoquinoline	68.95	66.54	68.77	68.09 ± 1.34
3-iodoquinoline	13.65	13.19	13.08	13.31 ± 0.30
3-chloroquinoline	N/A#			
3-bromo-5-methylpyridine	2.06	2.09	1.91	2.02 ± 0.10
3-bromo-2,6-dimethylpyridine	N/A#			

*Acceleration factors reported in Table 7.1 are calculated using the ratios of the mass spectrometry signals of the products to those of the starting materials in the Leidenfrost droplet relative to the bulk. This is not identical to the ratio of the rate constants of the 2 systems but may be related #No product formation

Additionally, Figure 7.7 shows the formation of product with 3-bromopyridine (**8a**) over 10 minutes under Leidenfrost conditions even when no base is added. However, no product is observed in the bulk conditions without the base.

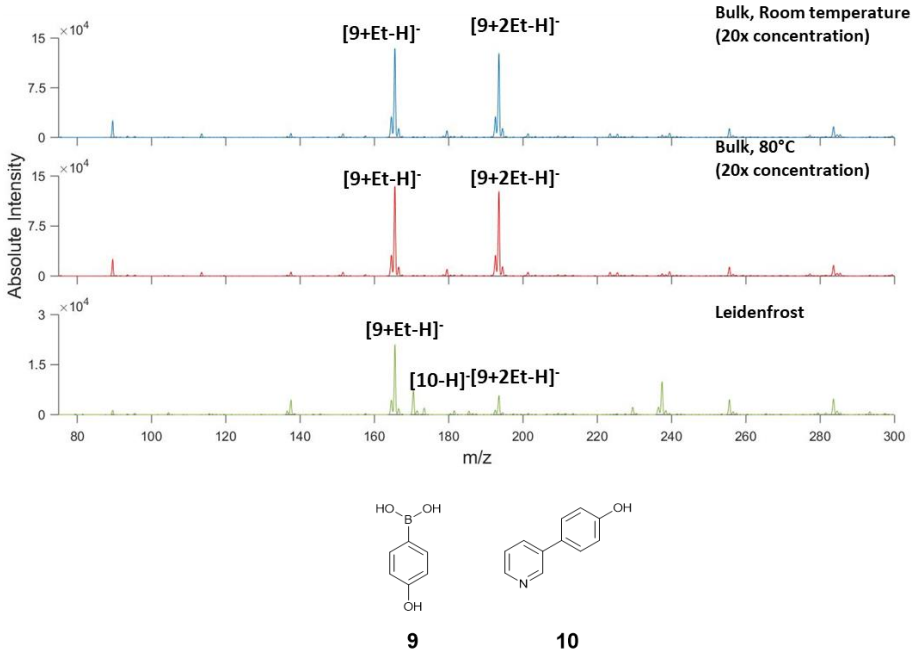


Figure 7.7 Suzuki cross-coupling between 3-bromopyridine (8a) with 4-hydroxyphenylboronic acid (9) to form 4-(pyridin-3-yl)phenol (10) without the addition of a base. No product is formed in the bulk conditions. However, there is product formation in the Leidenfrost droplets. Top Blue: Spectrum of the bulk reaction at room temperature (10 min) at a concentration 20x higher than the Leidenfrost experiments. Middle Red: Spectrum of the bulk reaction refluxed (10 min) at a concentration 20x higher than the Leidenfrost experiments. Bottom Green: Spectrum of the accelerated reaction by Leidenfrost droplets after ten minutes of continual solvent addition.

7.5 Conclusions

Droplets generated using the Leidenfrost technique was shown to be suitable for accelerating metal-catalyzed cross coupling reactions. The largest measured reaction acceleration for the Suzuki cross coupling was shown with the 3-bromo substituents. Additional substituents on the ring severely hindered reaction acceleration by Leidenfrost perhaps due to steric effects. While not all reactions were accelerated, the trend in reactivity in the Leidenfrost droplets remained consistent with the trend observed in the bulk solutions. In addition, the reproducibility of the Leidenfrost droplet acceleration using the continuous solvent addition as a means of maintaining droplet size, demonstrates that Leidenfrost droplets could be a potential screening tool for new reactions. Complete conversion to product in the Leidenfrost droplet can be achieved by increasing the solvent addition time. This is particularly useful when dealing with potentially less favorable reactions with slower reaction kinetics.

APPENDIX A. STATE-OF-THE-ART MASS SPECTROMETRY FOR POINT-OF-CARE AND OTHER APPLICATIONS: A HANDS-ON INTENSIVE SHORT COURSE FOR UNDERGRADUATE STUDENTS LABORATORY INFORMATION

Tutorial Title:

1. Overview of Characteristics of Different Mass Analyzers.

Guest Speaker Talks:

1. Optimization of Mass Spectrometry Imaging Workflow: How Multimodality Imaging Can Help.
2. Disease Management through a Mass Spectrometry-based On-Demand Diagnostic Approach.
3. Microfluidics: Fundamentals and Applications in Mass Spectrometry.

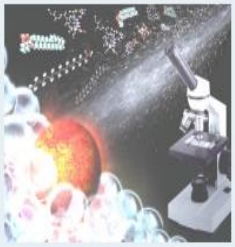
Hands-on Experiment List:

1. Analysis of Human Brain Cancer Using Tissue Smears by Desorption Electrospray Ionization – Mass Spectrometry.
2. Biofluid Analysis by Paper Spray Mass Spectrometry.
3. Touch Spray Mass Spectrometry Using Medical Swabs for the Detection of Strep Throat Causing Bacterium and Illicit Drugs in Oral Fluid.
4. Reaction Acceleration by the Leidenfrost Method and Reaction Monitoring Using a Miniature Mass Spectrometer.
5. Fundamental Exploration of Electrospray Ionization Methods.
6. Ambient Pressure Ion Mobility Spectrometry Using a 3D-Printed Ion Mobility Spectrometer.

Hands-on Trifold Welcome Brochure and Schedule:

"Mass spectrometry (MS) is arguably the preeminent tool for life science research and is accelerating in its role for patient care. MS has a rich diversity across the parameters of specificity, lower limits of quantitation, speed to result, physical size, cost and expertise required. No other approach covers the range from anesthetic gases, to lipids, to drugs, to peptides and proteins, to the metabolome and more. A number of new ideas have evolved from the basic science over the last decade. Several have become inventions and are evolving to innovations that matter for patients. There is plenty of room for applying more imagination to instrument configurations as well as applications. This experience will help YOU contribute to the revolution"

Peter T. Kissinger



Analytical Instrumentation

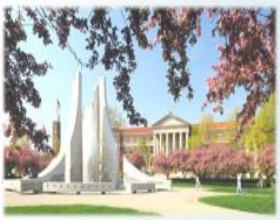
Regional Synergy

Purdue University
Indiana University
University of Illinois
University of Notre Dame

Center for Analytical Instrument Development

Co-directors:
Prof. R. Graham Cooks
Email: cooks@purdue.edu
Phone: 765-494- 5263

Prof. Peter T. Kissinger
Email: kissingp@purdue.edu
Phone:765-494-0717



CAID 2016

Discovery Learning Research Center

Purdue University
West Lafayette, IN 47907

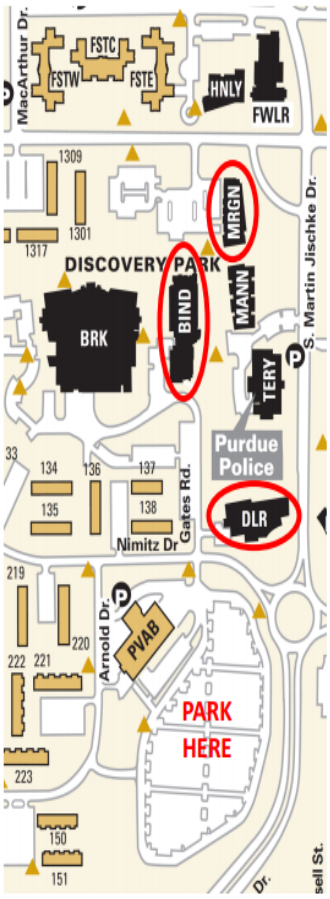
Center for Analytical Instrumentation Development
207 South Martin Jischke Drive

Bindley Bioscience Center
1203 W. State Street

Purdue University Center for Cancer Research
201 S. University Street

PURDUE UNIVERSITY
Discovery Park

MAP



App: Purdue Maps. Look for buildings DLR, MRGN, and WTHR

Schedule

Sunday, September 18th

8:30 AM. Breakfast, Introduction and Registration (MRGN hall)

9:30 AM. Tutorial. Dalton Snyder (Purdue) "*Overview of the characteristics of different mass analyzers*" (MRGN 121)

10:30 AM. Lab 1 (DLR 415)

11:15 AM. Lab 2 (DLR 415)

12:00 PM. Lab 3 (DLR 403)

1:00 PM Lunch break (MRGN hall)

2:15 PM. Lab 4 (WTHR 63)

3:00 PM. Lab 5 (BRWN B102)

3:45 PM. Lab 6 (BRWN B102)

Monday, September 19th

8:30 AM. Breakfast & coffee (MRGN hall)

9:30 AM. Talk 1 — Prof. Arash Zarrine-Afsar, University of Toronto (MRGN 121)

10:15 AM. Talk 2 — Prof. Abraham Badu-Tawiah, The Ohio State University (MRGN 121)

11:00 AM. Talk 3 — Prof. Amar Basu, Wayne State University (MRGN 121)

11—1 PM. Open doors in Lab (DLR & WTHR).

If you did not have a chance to see the demos on Sunday, stop by the lab and talk to the exhibitors!

1 PM. **Lunch and closing event (MRGN hall)**

Program # 1

Lab 1 (DLR 415)

Analysis of Human Brain Cancer Using Tissue Smears by Desorption Electrospray Ionization Mass Spectrometry

Lab 2 (DLR 415)

Biofluid Analysis by Paper Spray Mass Spectrometry

Lab 3 (DLR 403)

Touch Spray Mass Spectrometry Using Medical Swabs for the Detection of Strep Throat Causing Bacterium and Illicit Drugs in Oral Fluid

Lab 4 (WTHR 63)

Reaction Screening and Monitoring on a Portable Mass Spectrometer

Lab 5 (BRWN B102)

Fundamental Exploration of Electrospray Ionization Methods

Lab 6 (BRWN B102)

Ambient Pressure Ion Mobility using a 3D-printed Ion Mobility Spectrometer

Pre-Lab Questions:

1. Many of the ionization sources that you will see at CAID fall under the category of "ambient ionization", please explain what is meant by this term and list 2 examples of this category of ionization and briefly describe the techniques that you have selected.
2. Desorption Electrospray Ionization (DESI), first published in Science in 2004, began the ambient ionization explosion that is seen today. Please draw a labeled cartoon of the DESI setup and describe the DESI process.
3. Touch spray (TS) ionization has been developed as a complimentary test for strep throat detection to the current standards: rapid antigen detection test (RADT) and throat cultures. What advantages and disadvantages does TS have compared to RADT? And compared to throat cultures?
4. For the invention of Electrospray Ionization, John Fenn was awarded the 2002 Nobel Prize in Chemistry. Please draw out the ionization mechanism and explain the process of ion creation and the transformation from solution-phase to gas-phase.
5. The lab material for "Ambient Pressure Ion Mobility Spectrometry Using a 3D-Printed Ion Mobility Spectrometer" talks about fused deposition modeling (FDM) 3D-Printing and CAD models. Please describe what both of these are.
6. Please explain what ion mobility is and draw a labeled diagram of how an ion mobility spectrometer operates from 1st principles.
7. Please describe the Leidenfrost effect. What is reaction acceleration occurring in Leidenfrost droplets? Why is it important? How could this technique be used in commercial and industrial settings?
8. In order for the Mini 12 to interface with atmospheric pressure ionization sources it utilizes a DAPI. What does DAPI stand for and what does it control?
9. During CAID you will see a homebuilt paper spray set up, however Prosolia Inc., Indianapolis makes a commercial paper spray source. Watch the video found at http://www.prosolia.com/resources/videos/velox-360. What is the name of the commercial source?

Post-Lab Questions:

1. During CAID, DESI-MS imaging was performed on brain samples. Report two other organs that DESI-MS has analyzed and provide citations.
2. Two demos of TS and Swab TS for the identification of strep throat and detection of illicit substances were performed during CAID. Think of another analyte and system for which TS or Swab TS could be useful.
3. The "Fundamental Exploration of Electrospray Ionization Methods" lab uses a piezoelectric gun to produce ions through relay electrospray ionization. Please explain the process of ionization and why the piezoelectric gun is used.
4. Three different experiments during CAID utilized parts that were 3D printed. What parts where they?
5. During CAID you saw reaction acceleration due to the Leidenfrost Effect. Describe one other instrumental setup that have been used to accelerate reactions? Explain the setup and provide a reference.
6. The Mini 12 that was used in CAID is a home-built instrument from Purdue University. However, there are commercial portable mass spectrometers, one company was even started by a Purdue faculty member in Biomedical Engineering and Chemistry. List a commercial portable mass spectrometer, company name and provide the link to their website.
7. During CAID you operated a Triple Quadrupole Mass Spectrometer. Please explain what each quadrupole is used for.

Exit Interview Questions:

1. Was the CAID short course a worthwhile learning experience for the class in your opinion? Do you feel like it enhanced your knowledge on mass spectrometry and its applications?
2. Can you describe briefly the advantages of using DESI-MS for brain cancer analysis over traditional Histopathological exams? How are the DESI-MS images created? What markers are analyzed to differentiate healthy and non-healthy tissues?
3. Can you briefly describe Touch Spray and Swab Touch Spray? What are the advantages and disadvantages for specific applications of this ionization technique over other ionization techniques that you have learned about during CAID?

4. Can you describe briefly how an electrospray plume is created? Describe ESI in general terms. What are paper and relay spray ionization? Why was the camera and laser used to observe these phenomena?
5. Can you briefly describe the advantages of using a 3D-printer for analytical instrumentation prototyping? What is ion mobility spectrometry?
6. Can you briefly describe the Leidenfrost effect? What are advantages and disadvantages of using a miniature mass spectrometer over a conventional benchtop instrument?
7. For paper spray ionization does the paper have to be in any specific shape? Why is high voltage applied to the paper?
8. Are there any changes that you would make to CAID in future years (please suggest feasible ideas)?

Tips for Organizers and Instructors:

1. Assign Groups – Place students into their respective "Track" by color-coding their welcome packets and folders. By distinguishing their "Track", it enables a smooth start to the short course.
2. Printed Material - Printing the laboratory procedures and giving the students the material (in their color-coded folder) helps make CAID run smoother, rather than relying on the students to remember to bring the materials themselves. Prior to the start of CAID, all of the material was posted on the CAID website, however, the students were instructed that paper copies would be provided at the meeting.
3. Limit Number of Demos – This year, CAID was structured with three demos in the morning, lunch, and then three demos after. Limiting the number of back to back demos helps retain the student's attention and motivation.
4. Practice Tailored Demos – Prior to the start of CAID, the presenters practiced the set ups, made sure that all of the reagents were in good working order, and the instruments were functioning properly. Further, the experiments that the presenters are teaching are experiments that they have a copious amount of practice from their own research. Make sure to pick experiments that the presenters are comfortable with and experiments that if something malfunctions, the presenters have enough experience to work through the demo. Practice also helps make sure the presenters realize the time constraints of their ~45 minute time slot for introduction, experiment and questions.

5. Start Planning Early – CAID planning meetings usually start around May for our September meeting. Website design, reservations, experiments and their introduction and procedural notes, etc. take longer than one would think to plan.

APPENDIX B. ACCELERATED DEPROTECTION OF TERT-BUTOXYCARBONYL (BOC) GROUP BY SPRAY-BASED METHODS LABORATORY HANDOUT

Required Readings:

1) Müller, T., Badu-Tawiah, A. and Cooks, R. G. (2012), Accelerated Carbon-Carbon Bond-Forming Reactions in Preparative Electrospray. Angew. Chem. Int. Ed., 51: 11832–11835. DOI:10.1002/anie.201206632

2) Yan, X., Bain, R. M. and Cooks, R. G. (2016), Organic Reactions in Microdroplets: Reaction Acceleration Revealed by Mass Spectrometry. Angew. Chem. Int. Ed., 55, 12960-12972. DOI:10.1002/anie.201602270

3) Coffey, D. S., Hawk, M. K. N., Pedersen, S. W., Ghera, S. J., Marler, P. G., Dodson, P. N. and Lytle, M. L. (2004), Large Scale Deprotection of a tert-Butoxycarbonyl (Boc) Group Using Aqueous HCl and Acetone. Org. Proc. Res. Dev., 8, 945–947. DOI: 10.1021/op049842z

4) Ashworth, I. W., Cox, B. G. and Meyrick, B. (2010), Kinetics and Mechanism of N-Boc Cleavage: Evidence of a Second-Order Dependence upon Acid Concentration. J. Org. Chem., 75, 8117-8125. DOI: 10.1021/jo101767h

Purpose:

The focus of this laboratory exercise is to gain a better understanding of how spray-based reactions can be accelerated compared to their solution phase counterparts. The chemical system of tert-Butoxycarbonyl (Boc) deprotection has been selected due its common and important use in medicinal chemistry. Students will focus on how experimental parameters influence the acceleration of the formation of the deprotected product. Some of these experimental parameters will be the flow rate, concentration of the Boc protected compound relative to the acid it reacts with, and the how varying the acid itself changes the acceleration.

Learning Objectives:

1. Gain a deeper understanding of how spray-based methods can accelerate reactions.
2. Understand the parameters that need to be considered for optimizing reaction acceleration.
3. Comprehended the potential advantages of accelerating reactions.

Introduction:

Multistep organic synthesis utilizes starting materials and stepwise reacting them until the desired product is synthesized. There are many reaction routes for one desired product, and typically cost, time, and yield are factors in choosing the synthetic route. In the middle of a multistep route from reactants to product there is the possibility for a number of functional groups that may need to be conserved throughout the process. A protecting group can be introduced into a reaction step that will later have to be removed, in order to preserve a specific part of the molecule that otherwise may not be capable of surviving some of the other reagents or chemical steps. Protecting and deprotecting molecules add two additional steps in multistep reactions. There are lists of the vast number of protecting groups and the requirements that are needed to remove the protecting group once the molecule has progressed to a place where the functional group will be safe and can continue onto the final product. The protecting group that this experiment will focus on is the tert-butyloxycarbonyl commonly referred to as BOC group. BOC protects amines in multistep syntheses and has an extensive role in peptide synthesis and medicinal chemistry. The BOC group can be removed by concentrated strong acids, such as hydrochloric acid or trifluoroacetic acid, or the addition of heat to the reaction.

With time a concern with multistep reactions, typically the drive towards products is helped by the addition of heat or a catalyst. Organic reactions typically accelerate at higher temperatures and are usually set to reflux to achieve this increase in rate while not losing sample or solvent. While thermal acceleration is a large part of organic chemistry, a second large acceleration area is the use of a catalysis. The catalysis will accelerate the reaction due to decrease in the required activation energy. There are other ways of accelerating reactions in solution, but one way that has been shown is spray based method.

Reaction rate acceleration has been demonstrated in electrospray ionization and other spray-based ionization events when performed at particularly high concentrations for mass spectrometry. With this fact, it is apparent that simple dilution would alleviate this acceleration if reaction monitoring would be desired rather than acceleration. Experiments can be performed using electrospray ionization to spray and collect appreciable amounts of material in minutes. This will be performed in this laboratory with a variety of electrospray and reaction conditions to both explore the kinetics of the reaction and the processes of electrospray reaction rate acceleration. These rates can be compared to bulk simply by taking the ratio of product to starting material ratio

for the sprayed material divided by the ratio for the bulk. This calculated factor depends on the ability to ionize both the starting material and the product and is typically called an "acceleration factor."

A variety of factors influence reaction rate acceleration in electrospray including: solution flow rate, gas flow rate, collection surface and concentration. Most of these factors can be related to the possible causes of reaction acceleration. These causes include: desolvation, surface reactivity and compartmentalization within the droplet. Specifically, with the pH dependency of this reaction, the pH at the surface is likely lower than the bulk droplet as the charged species (hydronium ions) are thought to be at the surface.

Pre-Laboratory Questions:

1. What is the Boc group usually used to protect? Why is successful protection and deprotection of compounds important in organic synthesis?
2. Explain three mechanisms of reaction acceleration in droplets.
3. Explain the mechanism of easy ambient sonic spray ionization (EASI). What is the role of the nebulizing gas?
4. Draw out the schematic of an easy ambient sonic spray ionization (EASI) source.
5. Why is glass wool at the bottom of the tubes that the reaction mixture is sprayed down? What is its function?
6. What are some experimental variables that control whether a reaction is accelerated?

Hazards:

The SDS for all of the chemicals should be read by the students prior to their arrival for the laboratory. Nanospray ionization uses pulled capillaries that are extremely sharp and should be handled carefully when loading the sample. The electrode at the back of the nanospray capillary is supplied with high voltage (low current) and the group should be cognizant of the scan indicator light on the mass spectrometer identifying when the potential is being applied. While the instrument is scanning students should refrain from touching the electrode. The operator of the LTQ should warn the rest of the group when the mass spectrometer is switched from standby to scan. The mass spectrometer should not be operated without a TA present.

Instrumentation:

All mass spectrometry experiments will be performed on a linear ion trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA). EASI Spray emitters were constructed with fused silica lines with 100 I.D. and 360 O.D. (PolyMicro, Phoenix, AZ), a Tee assembly, a union assembly, two nanotight sleeves, and a stainless steel capillary (IDEX Health and Science, Oak Harbor, WA). To control the flow of reaction solution infuse syringe pumps (Standard Infusion PHD 22/2000, Harvard Apparatus, Holliston, MA) were utilized with gastight chemseal syringes (Hamilton Robotics, Reno, NV).

Procedure:

This laboratory will be conducted in three separate parts: the acceleration of the Boc-deprotection reaction by EASI, mass spectrometric analysis of the product of the accelerated spray based system and the bulk solution, and analysis of accelerated reaction mixture by refluxing. Students will work in groups of 4 and will complete the three sections in one laboratory period.

Reaction Acceleration by EASI:

1. Prepare a 1:10 molar ratio of Boc-Ala-OH to HCl by weighing out 0.095 grams of Boc-Ala-OH and dissolve it with 5 ml of 1M HCl (previously diluted with methanol from concentrated HCL) in a 20 ml scintillation vial.
2. Take a syringe and withdraw 150ul of the reaction mixture.
3. Place a stir bar into the scintillation vial and place it on the magnetic stir plate. Stir on low.
4. Take your syringe and place it into the EASI set-up.
5. Set the flow-rate of the Hamilton Syringe Pump to 5ul/minute with a total volume of 100ul.
6. Obtain a 15ml Falcon tube with the bottom cutoff so gas can escape, and place a small amount of glass wool in the bottom of the tube to collect your product.
7. Place the falcon tube (with the help of a ring stand) under your EASI source emitter to properly collect your reaction mixture.
8. When everyone is ready sequentially turn on the nitrogen gas, and then the syringe pump.
9. When the 20 minutes has passed, turn off the syringe pump and the nitrogen gas.
10. Remove the falcon tube and take a pipette with 2ml of methanol and wash the glass wool into a new scintillation vial.

11. Take your bulk product scintillation vial and your accelerated product scintillation vial to the mass spectrometer and perform those steps sequentially.
12. Repeat for TFA, a flow rate of 20ul/minute for both acids, and for a 1:1 molar ratio of Boc-Ala-OH to acid.

Reaction Acceleration by Refluxing:

1. Obtain a round bottom flask and weigh out 0.095 grams of Boc-Ala-OH.
2. Add 5 ml of 1M HCl (previously diluted with methanol) to the flask.
3. Reflux the reaction mixture.
4. One group will sample after 30mins, one after 60mins, and one after 90mins.
5. Take the refluxed products to the mass spectrometer and perform those steps sequentially.

Mass Spectrometry:

1. Pipette 10 µl of solution from the scintillation vial into the open end of the nanospray capillary.
2. Place the electrode into the open end of the nanospray capillary and then place the electrode and nanospray emitter into the 3D printed holder on the 3D stage.
3. The distance of the tip of the nanospray emitter should be approximately 100 mm (or less) from the inlet of the mass spectrometer. This can be controlled by turning the z-dimension stage dial. If the nanospray emitter is too far away from the inlet, there will be a reduction in signal, whereas if the emitter is too close, a discharge may occur to the mass spectrometer, which could damage the instrument. Warning: The inlet to the mass spectrometer is kept at a constant 200□C.
4. Check that the tube lens voltage is set to 65V and the capillary voltage is set to 15V.
5. Turn on the voltage by switching the instrument from standby to scan by clicking the yellow pause box in the top left corner of the screen, this will turn to a green arrow. Please warn your lab members when applying high voltage. (The voltage in this experiment is applied at +1.5 kV)
6. Click on the Acquire Data button (looks like a camera) to save data. Click on Folder, and select the appropriate folder to save your data on the desktop. Label file name with the date, your name, and sample information. Press start to begin recording data.
7. After you have acquired the data, turn off the voltage and stop scanning and turn the instrument onto standby by clicking the green arrow in the top left corner of the screen, which will into yellow pause sign.

8. Click on the acquire data again and click on view. The new program that open has two graphs, the top is the total ion current, and the lower is the mass spectrum.
9. Select the pushpin in the top right of the corner of the bottom graph. Then left click and drag across the top spectrum.
10. Export the data as a .csv file with the help from the TA.
11. Repeat steps 2-12 for your various conditions.
12. Calculate the acceleration factor for each condition.

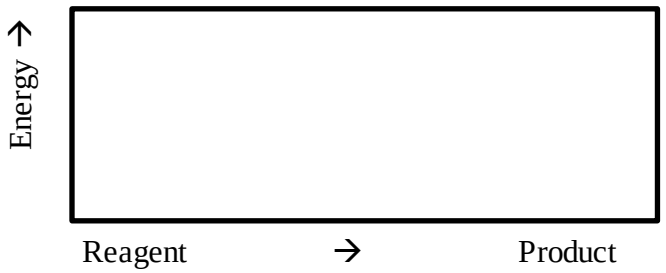
Laboratory Report Requirements:

Please turn in two copies of your laboratory report. The first, your typical report including laboratory notebook pages. The second, the same report without your name on it or laboratory notebook pages. Both reports should include your pre and post experimental questions as well as:

• An introduction explaining the objectives of the experiment.
• Full interpretation of all spectra and data collected.
• Calculated acceleration factors for each condition.

Post-Laboratory Questions:

1. Calculate the relative abundance of your product compared to your reactant for each spray condition. Calculate the acceleration factor for each method compared to the bulk. What trends did you see in regards to concentration of the acid? What trends did you see with changing of the acid?
2. Please complete the energetics of the droplets compared to the bulk solution phase.



3. You successfully prepared small quantities of product. How would you suggest scaling this method up to rapidly produce more product.
4. Describe how the results of refluxing the reaction differed from the spray based method.

Exit Interview Questions:

1. This is probably the first time that EASI has been used to spray and collect accelerated reaction products in an undergraduate teaching laboratory. Could you please explain why you believe this was or was not a valuable laboratory experiment? How could this experiment be improved?
2. Please describe the collection set-up, EASI parameters, as well as syringe pump parameters.
3. Why are reactions able to be accelerated by spray-based methods? What factors contribute to this?
4. Please explain how you calculate acceleration factors?
5. Why does the nanospray analysis not effect the acceleration?

APPENDIX C. CHIRAL ANALYSIS BY TANDEM MASS SPECTROMETRY USING THE KINETIC METHOD, BY POLARIMETRY, AND BY ^{1}H NMR SPECTROSCOPY LABORATORY HANDOUT

Required Readings:

1) Bain, R. M.; Yan, X.; Raab, S. A.; Ayrton, S. T.; Flick, T. G.; Cooks, R. G. On-line chiral analysis using the kinetic method. Analyst 2016, 141, 2441-2446.

2) Ranc, V.; Havlíček, V.; Bednar, P.; Lemr, K. Nano-desorption electrospray and kinetic method in chiral analysis of drugs in whole human blood samples. Eur. J. Mass Spectrom. 2008, 14, 411–417.

3) Sen, S. E. and Anliker, K. S. 1H NMR Analysis of R/S ibuprofen by the formation of diastereomeric pairs: microscale stereochemistry experiment for the undergraduate organic laboratory. J. Chem. Educ. 1996, 73 (6), 569-572

4) Purdue University Department of Chemistry CHM25501 Fall 2016 Laboratory Manual – Experiment Resolution of the Enantiomers of Ibuprofen

Purpose:

The focus on this lab is to gain a better understanding of chirality and enantiomeric excess (*ee*) and how they are experimentally determined. Ibuprofen in different enantiomeric excess percentages will be analyzed by optical polarimetry, NMR spectroscopy and mass spectrometry. Students will focus on analytical figures of merit and how they compare across the methods, including *ee* resolution/sensitivity, limits of detection, and time required to perform the analysis, so that students can understand how to pick the proper technique for chiral analysis in the future.

Experimental Techniques:

Mass Spectrometry, Nuclear Magnetic Resonance Spectrometry, Polarimetry

Learning Objectives:

1. Gain a deeper understanding of chirality and enantiomeric excess.
2. Learn how to perform chiral analysis on a number of instrumental setups.
3. Develop selection criteria for instrumental techniques based on the experimental challenge.
4. Determine the pros and cons of each instrumental setup based on analytical figures of merit.

Introduction:

Enantiomeric purity is critical for producing safe pharmaceuticals. Many drugs only have one enantiomer that is therapeutic, whereas, the other enantiomer is not and may even be harmful to the health of the patient. Given the risks involved with patient exposure to inactive enantiomers, commercial drugs are typically only approved for sale if the enantiomeric purity is known and can be analyzed to meet a target purity that was specified at the time of FDA approval. One of a handful of exceptions to this trend is ibuprofen, which is sold as a racemate, but undergoes chiral inversion due to enzymatic activity on the inactive form. The body processes the inactive (*R*)-(-)-enantiomer and creates the active (*S*)-(+)-enantiomer in vivo. Since this chiral inversion occurs naturally within the body, it does not matter whether the tablet taken by the patient is the pharmacologically active (*S*)-(+)-enantiomer, the inactive (*R*)-(-)-enantiomer, or the racemic mixture. To probe the enantiomeric purity of solutions containing differing (*S*)-(+)-ibuprofen content, a set of samples containing 50%, 75%, and 100% (*S*)-(+)-enantiomer will be prepared and analyzed by various instrumental techniques. Each of these techniques will vary in terms of limits of detection, ability to analyze mixtures, and sample preparation steps.

The first technique that will be studied is optical polarimetry. Optical polarimetry utilizes polarized light to interact with the chiral center of a molecule. As the light interacts with the chiral center, the plane of polarization rotates and the degree of the rotation corresponds to the enantiomeric enrichment of the species and the specific rotation of the compound. Each optically active substance has its own specific rotation as defined in Biot's Law:

$$Specific\ Rotation = [\alpha]_{your\ sample} = [\alpha]_{\lambda}^{T} = \frac{\alpha_{obs}}{C * l}$$

[α] = specific rotation, T = temperature, λ = wavelength,

c = concentration in g/mL, l = optical path length in dm.

To determine the optical rotation of a sample, subtract the angle of maximum illumination of the blank from the angle of maximum illumination of the sample.

$$[\alpha]_{sample} = [\alpha]_{measured} - [\alpha]_{blank}$$

The enantiomeric purity can be calculated by determining the percent optical purity. Optical purity is calculated by dividing the measured specific rotation of your sample by the literature value of the specific rotation of a sample containing only one enantiomer at the same concentration.

$$\%\ optical\ purtiy\ = \frac{[\alpha]_{sample}}{[\alpha]_{literature\ value}} * 100$$

Ideally, each stereoisomer contributes to the total optical rotation, such that the amount they contribute is proportional to the amount present. For instance, if a solution contains only one enantiomer, the maximum rotation is observed and the optical purity is 100%. The percent optical purity for the R enantiomer for example, can also be calculated by taking the difference in the percentages of each enantiomer.

$$\%\ optical\ purtiy\ (R) = \%R - \%S = \frac{mol\ R - mol\ S}{mol\ R + mol\ S}$$

The percent of one stereoisomer in a sample can be calculated from the percent optical purity using the following equation:

$$\%R = 50 + \frac{\%\ optical\ purity}{2}$$

The second technique that will be used to probe chirality is Nuclear Magnetic Resonance Spectroscopy (NMR). Enantiomers have identical physical properties in an achiral solvent such as $CDCl_3$, thus ^1H NMR fails to show differences in the spectra due to the presence of chirality. However, ^1H NMR is able to determine the enantiomeric excess of chiral compounds by derivatizing the enantiomers to form diastereomers. In this lab, ibuprofen is converted into a mixture of diastereomeric amides by acid activation with 1,1'-carbonyldiimidazole followed by reaction with (*S*)-(-)-α-methylbenzylamine or by the formation of diastereomeric salts by reaction with (1*S*,2*S*)-(-)-1,2-diphenylethylenediamine. Due to the presence of two chiral centers the modified structures, the set of products expected are the (*R*,*R*), (*S*,*S*), (*R*,*S*), and (*S*,*S*) isomers. The (*R*,*R*) and (*S*,*S*)-diastereomers are mirror images, and thus enantiomers, as are the (*R*,*S*) and (*S*,*R*) pairs. However, the (*R*,*R*)/(*S*,*S*) pair and the (*R*,*S*)/(*S*,*R*) pair have different ^1H NMR spectra; the spectra may be similar in terms of chemical shift range and approximate coupling constants, but overall the spectra of the diastereomeric pairs will be different and the compounds will be distinguishable.

The third and final analytical technique that will be used to probe chirality is mass spectrometry. The mass spectrometry technique used here is similar to the NMR spectroscopy approach in that it requires sample modification in order for the enantiomers to have different spectra. Mass spectrometry utilizes the kinetic method to convert experimental data on dissociation of the ion signals for these trimeric diastereomeric clusters to determine the percent of

enantiomeric excess. The trimeric diastereomeric clusters are formed by binding a central metal ion, two optically pure reference ligands, and the analyte of interest together to form the $[M(ref)_2(A)\text{-}H]^+$ complex in the gas phase. In this experiment, the central metal ion will be Cu(II) and the reference ligands will be L-Trp. This trimeric diastereomeric cluster will fragment to two distinct product ions $[M(ref)_2\text{-}H]^+$ and $[M(ref)A\text{-}H]^+$. The ratio of the ion signals for these two competitive processes is related to the ratio of enantiomers in the given sample. This ratio will then be compared to a calibration curve of ratios where the %*ee* of the analyte of interest was varied. For the kinetic method to be chirally selective the reference ligand and the metal choice are critical. The competitive fragmentations of the cluster must involve a difference in energetics induced by the change in chirality, otherwise the population of species after fragmentation will not be a function of the chirality and a calibration curve will not be log-linear. When comparing these three techniques, it is important to keep in mind the amount of material needed for each experiment, the sample preparation required and the analytical figures of merit.

Pre-Laboratory Questions:

1. Define and explain the following terms: chirality, enantiomers, enantiomeric excess, and diastereomers.
2. Name a drug that is sold as only one enantiomer, draw out both enantiomers and circle the pharmaceutically active enantiomer.
3. In a previous laboratory experiment you synthesized ibuprofen. Draw the (R) and (S) enantiomers of this compound.
4. Why is ibuprofen able to be sold as a racemic mixture if only one of the enantiomers is pharmaceutically active?
5. Explain how the kinetic method is applied to enantiomeric excess determination.
6. Why did ibuprofen have to be derivatized to its diastereomeric analog for 1H NMR analysis? Does this relate to the MS technique for mass spectrometry or polarimetry?
7. This will be the second time you have performed an experiment with polarized light, please explain and illustrate in detail how this process of using polarized light determines the enantiomeric excess.

Hazards:

The SDS should be read by students for all of the chemicals used in the laboratory before arriving to the laboratory. Nano-spray ionization uses pulled capillaries that are extremely sharp and should be handled carefully when loading the sample. The electrode at the back of the Nano-spray capillary is supplied with high voltage (low current) and the group should be cognizant of the scan indicator light on the mass spectrometer identifying when the potential is being applied. While the instrument is scanning students should refrain from touching the electrode. The operator of the LTQ should warn the rest of the group when the mass spectrometer is switched from standby to scan. The mass spectrometer should not be operated without a TA present. The NMR spectrometer should also not be operated with the TA present.

Instrumentation:

All mass spectrometry experiments will be performed on a linear ion trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA). All NMR spectroscopy experiments will be performed on a 300 MHz spectrometer (Varian 300 MHz, Palo Alto, CA). All polarimetry experiments will be performed on Vernier LabQuest 2.

Procedure:

This laboratory will be conducted in three separate parts: polarimetry of ibuprofen, NMR spectroscopy of ibuprofen derivatives, and mass spectrometry of ibuprofen complexes. Students will work in groups of 4 and will cycle through each of the three experiments in one laboratory period.

Polarimetry:

1. Obtain the 2 standards of 100% (*S*)-ibuprofen (one at the MS concentration 10^{-3}M and one at the NMR spectroscopy concentration 10^{-2}M), and 1 sample of 100% D-glucose (2g/20ml) found in the labeled scintillation vials in the hood.
2. Remove the polarimeter cell and fill it with 20ml of methanol as a blank.
3. Place the cell back into the polarimeter.
4. Open LabQuest and tap the **graph icon** in the upper right corner to make the graph visible.
5. To collect data, click the green arrow icon in the lower left corner.

6. Rotate the analyzer clockwise in a slow fluid motion. Continue rotating the analyzer until the curve on the screen has been completed.
7. To analyze the data, drag the stylus over the first peak to select the data.
8. Click on **Analyze** and then **illumination** which will open a new screen.
9. Select **curve fit**, and then **Gaussian**. This will calculate the B coefficient, which is the angle at maximum illumination. Record this value.
10. Clean the cell will methanol.
11. When ready to run the next sample click **Ok**. This will bring you back to the graph screen. Tap the **Folder Cabinet** icon on the left slide. A new graph labeled "Run 2" will appear.
12. Repeat steps 2-10 for your both Ibuprofen samples.
13. Using the procedure above switch methanol for water and repeat your blank.
14. Now place D-Glucose into the cell and take a time point every 5 minutes. Record and observe how the specific rotation changes over time.

NMR Spectroscopy:

1. Obtain the NMR tubes containing 3 standards (50%, 75% and 100% (*S*)-ibuprofen), 1 impure mixture and 1 unknown % *ee* sample from your TA. Record the letter of your unknown sample. Each sample the derivatized ibuprofen is at a concentration of 0.05 M.
2. The derivatized ibuprofen was prepared prior to lab by combining 0.5 mL ibuprofen solution (20 mg/mL) with 0.5 mL of (1*S*, 2*S*)-(-)-1,2-diphenylethylenediamine solution (10 mg/ml) which were placed in a clean NMR tube.

Mass Spectrometry:

1. Obtain the 3 standards (50%, 75%, and 100% (*S*)-ibuprofen), 1 impure mixture and 1 unknown % *ee* sample found in the labeled scintillation vials in the hood. Record the letter of your unknown sample. Each sample the ibuprofen is at a concentration of 1.0×10^{-3} M, a metal complexing agent $CuCl_2$ at 5.0×10^{-3} M, and reference ligand L-Trp at 5.0×10^{-3} M.
2. Pipette 10 µl of solution from the scintillation vial into the open end of the nanospray capillary.
3. Place the electrode into the open end of the nanospray capillary and then place the electrode and nanospray emitter into the 3D printed holder on the 3D stage.

4. The distance of the tip of the nanospray emitter should be approximately 100 mm (or less) from the inlet of the mass spectrometer. This can be controlled by turning the z-dimension stage dial. If the nanospray emitter is too far away from the inlet, there will be a reduction in signal, whereas if the emitter is too close, a discharge may occur to the mass spectrometer, which could damage the instrument. Warning: The inlet to the mass spectrometer is kept at a constant 200°C.
5. Turn on the voltage by switching the instrument from standby to scan by clicking the yellow pause box in the top left corner of the screen, this will turn to a green arrow. Please warn your lab members when applying high voltage. (The voltage in this experiment is applied at +1.5 kV)
6. Click on the **Acquire Data** button (looks like a camera) to save data. Click on Folder and select the appropriate folder to save your data on the desktop. Label file name with the date, your name, and sample information. Press start to begin recording data.
7. Click on the **Define Scan** button (looks like four circles) to isolate and fragment your clusters. In the **Parent Mass (*m/z*)** column insert the mass of your ibuprofen complex, change your **Isolation Width (*m/z*)** to 10, and your **Normalized Collision Energy** to 8. Press **apply** and then **okay**.
8. After you have acquired the data, turn off the voltage and stop scanning and turn the instrument onto standby by clicking the green arrow in the top left corner of the screen, which will into yellow pause sign.
9. Click on the **acquire data** again and click on **view**. The new program that open has two graphs, the top is the total ion current, and the lower is the mass spectrum.
10. Select the pushpin in the top right of the corner of the top graph. The right click on the top spectrum and select **Autofilter**.
11. Select the pushpin in the top right of the corner of the lower graph. Now you can select the MS/MS data by selecting the mass spectrum on the top plot that correlates to when the isolation and fragmentation occurred.
12. Click in the bottom graph and copy and paste your spectrum from the software into a new Microsoft Word document. Then export the data as a .csv file with the help from the TA.
13. Repeat steps 2-12 for your standards, unknown, and mixture.
14. Create a plot of % *ee* versus the Ln(R) of your standards. Determine the % *ee* of your unknown.

Laboratory Report Requirements:

Please turn in two copies of your laboratory report. The first, your typical report containing including laboratory notebook pages. The second, the same report without your name on or laboratory notebook pages. Both reports should include your pre and post experimental questions as well as:

- An introduction explaining the objectives of the experiment.
- Full interpretation of all spectra and data (NMR, MS, Polarimetry) collected.
- Plots of all results and identification of unknowns (label your unknown).

Post-Laboratory Questions:

1. Construct a table for the three methods (Mass spectrometry, NMR spectroscopy, and Optical Polarimetry) with the benefits and limitations of using the specific technique to determine the chirality of molecules.
2. Why did the mass spectrometry system need the metal-ligand system to determine the chirality of molecules? How does this relate to the NMR spectroscopy method for chiral analysis?
3. Why is polarimetry able to separate pure enantiomers without any derivatization?
4. If your chiral compound was in a complex mixture, which of the three techniques would you be most likely to use for determination of enantiomeric excess? Why?
5. If your chiral compound was very dilute, which of the three techniques would you be most likely to use for determination of enantiomeric excess? Why?

Exit Interview Questions:

1. This is probably the first time that mass spectrometry and the kinetic method has been explored in an undergraduate teaching laboratory, as well as comparing its analytical figures of merit to NMR spectroscopy and polarimetry. Could you please explain why you believe this was or was not a valuable laboratory experiment? How could this experiment be improved?
2. Please explain chirality, enantiomer, diastereomers, and enantiomeric excess and why these are important in relation to pharmaceuticals?
3. Please explain the kinetic method as it applies to *ee* determination by mass spectrometry and the advantages and disadvantages of this method.

4. Please explain the how NMR spectroscopy data can be used to determine % *ee* and the advantages and disadvantages of this method.
5. Please explain how polarized light interacts with ibuprofen and how the measurement is made.

APPENDIX D. PROCESS ANALYTICAL TECHNOLOGY FOR ON-LINE MONITORING OF ORGANIC REACTIONS BY MASS SPECTROMETRY AND UV-VIS SPECTROSCOPY LABORATORY HANDOUT

Required Readings:

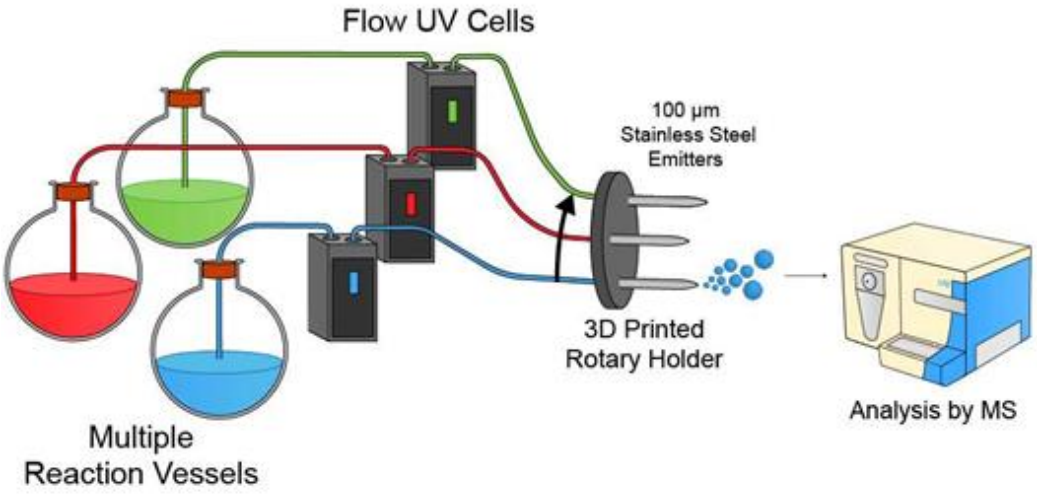
1) Simon, L. L.; Pataki, H.; Marosi, G.; Meemken, F.; Hungerbühler, K.; Baiker, A.; Tummala, S.; Glennon, B.; Kuentz, M.; Steele, G.; Kramer, H. J. M.; Rydzak, J. W.; Chen, Z.; Morris, J.; Kjell, F.; Singh, R.; Gani, R.; Gernaey, K. V.; Louhi-Kultanen, M.; O'Reilly, J.; Sandler, N.; Antikainen, O.; Yliruusi, J.; Frohberg, P.; Ulrich, J.; Braatz, R. D.; Leyssens, T.; von Stosch, M.; Oliveira, R.; Tan, R. B. H.; Wu, H.; Khan, M.; O'Grady, D.; Pandey, A.; Westra, R.; Delle-Case, E.; Pape, D.; Angelosante, D.; Maret, Y.; Steiger, O.; Lenner, M.; Abbou-Oucherif, K.; Nagy, Z. K.; Litster, J. D.; Kamaraju, V. K.; Chiu, M.-S., Assessment of Recent Process Analytical Technology (PAT) Trends: A Multiauthor Review. Organic Process Research & Development 2015, 19 (1), 3-62.

2) Workman, J.; Lavine, B.; Chrisman, R.; Koch, M., Process Analytical Chemistry. Analytical Chemistry 2011, 83 (12), 4557-4578.

3) Pulliam, C. J.; Bain, R. M.; Osswald, H. L.; Snyder, D. T.; Fedick, P. W.; Ayrton, S. T.; Flick, T. G.; Cooks, R. G., Simultaneous Online Monitoring of Multiple Reactions Using a Miniature Mass Spectrometer. Analytical Chemistry 2017, 89 (13), 6969-6975.

Purpose:

This laboratory will introduce students to process analytical technologies (PAT), how they can be applied to on-line reaction monitoring, and how 3D-printing can enable new technologies to be more rapidly employed. The two PAT instruments that this laboratory will focus on are mass spectrometry and UV-Vis spectroscopy, both which are capable of monitoring chemical synthesis on-line and in real time. Students will focus on the instrumentation behind PAT tools, the challenges that are associated with on-line monitoring and the benefits that can accompany successful PAT tools. Finally, as some of the critical pieces of this experiment were designed in-house and through the aid of 3D printers, students will be exposed to how to set up design files and explore how 3D printing can aid in experimental designs, specifically through the lens of PAT tools.



Learning Objectives:

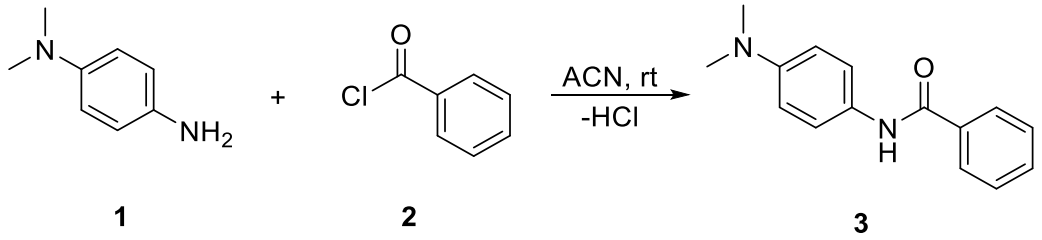
1. Gain an introduction to process analytical technology.
2. Gather an understanding of how on-line monitoring is employed by mass spectrometry and UV-Vis spectroscopy.
3. Get an understanding of how 3-D Printing can help with rapid development and PAT.
4. Apply the techniques from Analytical I and II to a real-world problem.

Introduction:

Process analytical technology (PAT) monitors chemical processes in real-time using analytical instrumentation. In-situ monitoring of manufacturing processes are relied upon heavily by the pharmaceutical industry. PAT has seen increased importance after the US Food and Drug Administration (FDA) provided its "Guidance for Industry PAT" report which outlined the regulatory framework of PAT to help assure quality by improved process design and monitoring. While pharmaceutical companies are heavily invested in these applications, there are also prominent PAT applications in the soil management, food quality assurance, animal cell culture and in the chemical industry. Qualities required of a PAT tool are the capability to track the process in real time, preferably on-line with minimal human interaction, and to be tailored to the specific manufacturing process. Multiplexing PAT analyzers can be advantageous as this can provides confirmatory analysis, as well as increasing confidence in these measurements to serve as in-process control measures.

Depending on the specific chemical process, different analytical technologies may be selected. Examples of analytical methods utilized in PAT include Raman Spectroscopy, Infrared Spectroscopy, and particle size analysis, each with their own analytical advantages and disadvantages. UV-Vis Spectroscopy has been reported for on-line flow chemistry for both transition metal oxide catalyzed reactions and for the determination of chemical reaction kinetics. Mass spectrometry (MS) has recently become important as a PAT instrument to monitor batch quality on-line, to elucidate reaction mechanisms and synthetic pathways and is easily multiplexed to allow monitoring of multiple reaction vessels at the same time. On-line chemical derivatization to facilitate the determination of enantiomeric excess as well as more common derivatization experiments to promote ionization efficiency have been reported.

In this laboratory exercise, you will monitor an amide bond formation (Scheme 1) by flow UV-Vis spectroscopy followed by down-stream mass spectrometric analysis. The coupling of these two specific instruments allowed you to utilize two instruments within one laboratory exercise, explore the fundamentals behind each and their requirements for analysis, and contemplate the benefits and disadvantages of having these two instruments in tandem.



Scheme 1. Amide bond formation between para-substituted aniline p-(N,N-dimethylamino)aniline (1) with benzoyl chloride (2) to form the amide 3.

Coupling the two instruments can present challenges in the instrumental set up. To overcome these challenges the use of 3D printing was utilized to construct three pieces for this laboratory exercise: the UV-Vis cover to allow the flow cuvette to remain in darkness, a holder for the unions of the flow lines, and the rotating coupler to sample the spray in turn from each of the several reaction vessels. The utilization of 3D printing in chemistry laboratory exercises has increased as it allows the inexpensive creation of highly customizable parts by rapid prototyping. In this laboratory exercise you will explore the advantages and disadvantages of 3D printing, the

challenges that accompany 3D printing, the software to construct these parts and finally you will print a part that is utilized in this laboratory exercise.

Pre-Laboratory Questions:

1. What is process analytical technology (PAT)? Name four technologies that are commonly used in PAT and what information will these techniques provide?
2. Name two advantages and two disadvantages of using UV-Vis spectroscopy for process analytical technology.
3. Name two advantages and two disadvantages of using mass spectrometry for process analytical technology.
4. Look at the diagram below. Why do the diameters of the silica capillaries change? What are some engineering challenges when constructing an on-line reaction monitoring setup?
5. What benefits does 3D Printing add to a laboratory setup? Are there any disadvantages?

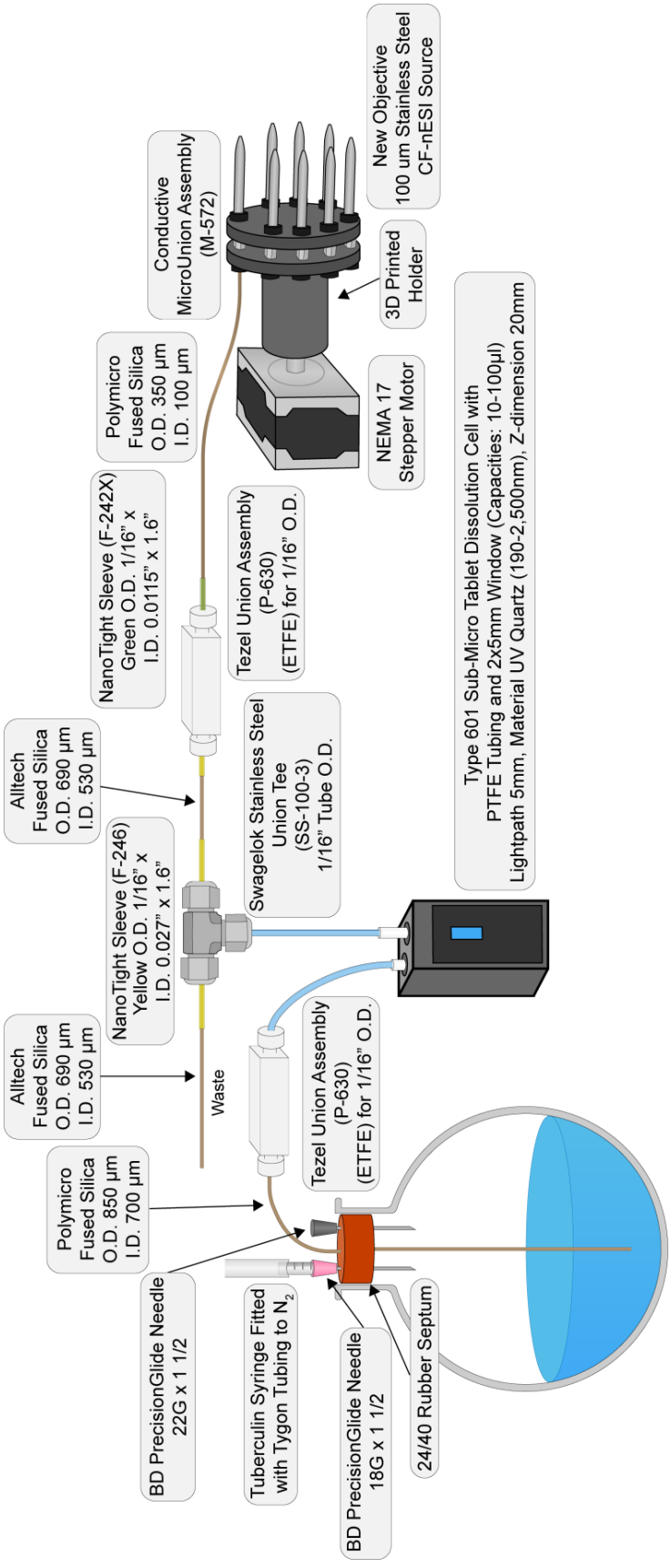
Hazards:

All safety data sheets (SDS) for the chemicals utilized in this laboratory exercise should be read by the student prior to the commencement of the laboratory period. The mass spectrometer utilizes a high voltage (low current) lead to create measurable ions. The high voltage lead is located above the rotating actuator and is applied to the emitter at the 12 o'clock position. Once the reaction has begun the voltage will be applied for the duration of the laboratory exercise and students should be cognizant that the high voltage is on. The mass spectrometer should not be left unattended by TA. The reaction vessels are pressurized by nitrogen gas to induce flow. When the flow of the reaction is initiated, the sash on the hood should be closed as a precaution. The syringes that are used to inject the reaction are sharp and should be handled with care. After injection place the syringe needle into the sharps container. The 3D printer has a heated extruder and bed that could burn students if handled improperly. Caution should be used around the printer.

Instrumentation:

A Cary 50 UV-Vis spectrometer will be utilized to collect all UV-Vis spectra. The instrument will be operated in Scanning Kinetics Mode. A Flow UV-Vis cell will be plumbed directly from the pressurized reaction vessel. To help with the access to the flow UV-Vis cell while

in the UV-Vis spectrometer, a cover with a removable window was 3-D printed. All mass spectrometry measurements will be performed on a Thermo-Fisher Scientific LTQ XL Ion Trap mass spectrometer. Ions will be formed through a positive potential applied to the continuous flow nanospray emitters. To hold the lines to the nanospray emitters in place, a holder for the unions was 3D printed. To monitor multiple reactions on one mass spectrometer simultaneously, a 3D printed rotating holder was developed, and powered by a stepper motor, as described in Pulliam et. al. (above). A Mendlemax 3D printer will be utilized in the demonstration of how 3D printing is performed.


New Objective
100 um Stainless Steel
CF-nESI Source
3D Printed
Holder
NEMA 17
Stepper Motor
Conductive
MicroUnion Assembly
(M-572)
Polymicro
Fused Silica
O.D. 350 µm
I.D. 100 µm
NanoTight Sleeve (F-242X)
Green O.D. 1/16" x
I.D. 0.0115" x 1.6"
Tezel Union Assembly
(P-630)
(ETFE) for 1/16" O.D.
Alltech
Fused Silica
O.D. 690 µm
I.D. 530 µm
Swagelok Stainless Steel
Union Tee
(SS-100-3)
1/16" Tube O.D.
Type 601 Sub-Micro Tablet Dissolution Cell with
PTFE Tubing and 2x5mm Window (Capacities: 10-100µl)
Lightpath 5mm, Material UV Quartz (190-2,500nm), Z-dimension 20mm
NanoTight Sleeve (F-246)
Yellow O.D. 1/16" x
I.D. 0.027" x 1.6"
Alltech
Fused Silica
O.D. 690 µm
I.D. 530 µm
Waste
Tezel Union Assembly
(P-630)
(ETFE) for 1/16" O.D.
Polymicro
Fused Silica
O.D. 850 µm
I.D. 700 µm
BD PrecisionGlide Needle
22G x 1 1/2
Tuberculin Syringe Fitted
with Tygon Tubing to N_2
BD PrecisionGlide Needle
18G x 1 1/2
24/40 Rubber Septum

Procedure:

This laboratory exercise will be broken into two sections after the injection of the reaction material and the flow of reaction mixture has begun. The two sections: first monitoring by UV-Vis spectroscopy and mass spectrometry and second the basics of 3D-printing for rapid prototyping and incorporation in experimental designs. The two sections will be completed in one laboratory period.

Reaction Preparation:

Prior to arrival in the laboratory, the TAs prepared 2000 mL of acetonitrile solvent in the round bottom reaction vessels, as well as, has the tubing and fused silica setup. After the pre-laboratory lesson, the groups will proceed with the amide bond formation reaction (Scheme 1) and inject with a syringe the first reactant (p-(N,N-dimethylamino)aniline) into the round bottomed reaction vessel. After three, stable, reproducible spectra are recorded by the UV-Vis spectrometer and the mass spectrometer, the second reactant (benzyl chloride) will be added. At this point the groups will separate into the three laboratory groups.

Reaction Monitoring by UV-Vis:

1. Locate the UV-Vis spectrum of the reactions being performed in your class session on the desktop of the computer. (Paper copies will also be handed out to you by your TA).
2. Open the UV-Vis spectroscopy software by clicking the Cary scanning kinetics program located on the Desktop.
3. Zero the UV-Vis spectrometer.
4. Click on the “Setup” tab.
5. Change your Start (nm) to 600 and your Stop (nm) to 200.
6. Change your Cycle (min) to 1.0 and your Stop (min) to 240.0.
7. Click OK.
8. Click Start.
9. Save the file name with your lab group and reaction.
10. Enter you sample name and click OK.

Reaction Monitoring by Mass Spectrometry:

1. Open the mass spectrometer software by clicking on the Tune program on the desktop.
2. Load the scan method. (Your TA will show you which is the current method).
3. Click on the Define Scan button (looks like four circles) to set the mass range from *m/z* 50 to *m/z* 300. Press apply and then okay.
4. When everyone is ready begin the run and make sure you communicate with the entire lab that you are turning on the high voltage. To turn on the voltage click the yellow pause box in the top left corner of the screen, this will turn to a green arrow.
5. Record the data by clicking on the Acquire Data button (looks like a camera) to save data with your lab group and reaction. Press start to begin recording data.

3D-Printing to Rapid Prototyping and Reaction Monitoring:

This exercise will be led by a teaching assistant. The following topics will be investigated.

1. Explore the program Inventor. Look over the following features:
    a. 2D Sketch.
    b. Extrude function for making 3D images.
    c. Mirror function for symmetrical operations.
    d. The functions in the ribbon such as Fillet and Chamfer.
2. View the 3D printed continuous nanospray holder file, the union holder file, and the UV-Vis cover file.
3. Explore Simplify 3D. Figure out what is a slicer.
    a. Learn about G-Code.
4. Explore the 3D Printer. Think of the following:
    a. Bed leveling.
    b. Bed adhesion.
    c. What are common 3D Printing issues
        i. Extrusion multiplier
        ii. Stringing
        iii. Warping
5. Print a new union holder.

Laboratory Report Requirements:

Please turn in two copies of your laboratory report. The first, your typical report and the second, the same report without your name on it. Both reports should include your processed data and your post experimental questions as well as.

Post-Laboratory Questions:

1. Calculate and plot the extracted ion chronogram of a reaction of each reaction from the mass spectrometry dataset. What trend does your reactant and product follow? What does this extracted ion chromatogram tell you? How long until the reactant and product ions were at equal intensity? Label at which time points you added your first and second reactant.
2. Plot a both an overlay and waterfall graph of the UV-Vis spectra. Label the spectra at which you added the first and second reactants. What trend did you observe?
3. Make a table of advantages and disadvantages for each method. In what cases would mass spectrometry be a more beneficial technic than UV-Vis spectroscopy, and in what cases would UV-Vis spectroscopy be more beneficial than mass spectrometry?
4. After exploring 3D printing, what advantages does this technique add to the development of new technologies? What are its limitations?

Exit Interview Questions:

1. This is probably the first time that PAT has been explored in a undergraduate teaching laboratory, as well as the first time a Flow UV-Vis has been coupled to a Mass spectrometer for on-line reaction monitoring. Could you please explain why you believe this was or was not a valuable laboratory experiment? How could this experiment be improved?
2. Please describe the experimental setup.
3. What are the strengths for UV-Vis as a PAT. What are its weaknesses?
4. What are the strengths for Mass Spectrometry as a PAT. What are its weaknesses?
5. If you were going to improve this setup what would you change?
6. How does 3D printing aid in experimental designs?
7. Please explain the issues when deciding how to orient an object on the print bed. What other major difficulties are there involving 3D printing? How can you mitigate these problems?

Arduino Code for Optional Multiple Reaction Monitoring:

```
// LTQ Reaction Monitoring
// Last Modified 3/12/18 RLS

//Edit these parameters for different number of emitters and the distance moved between each turn
int distance = 230;
int emitters = 6;
int delaytime = 2000; //Sampling time in microseconds

int scan = 1;
int steps = 0;

void setup() {
  pinMode(8, OUTPUT); // Stepper Motor Direction Pin
  pinMode(9, OUTPUT); // Stepper Motor Step Pin
  digitalWrite(8, HIGH);
  digitalWrite(9, LOW);
}

void loop() {
if (scan < emitters) {
  delay(delaytime);
  digitalWrite(8, HIGH);
  while (steps < distance) {
  digitalWrite(9, HIGH);
  delayMicroseconds(700);
  digitalWrite(9, LOW);
  delayMicroseconds(700);
  steps = steps + 1;
  }
  steps = 0;
```

```
  scan = scan + 1;
  }

else {
  delay(delaytime);
  steps = distance * (emitters - 1);
  while (steps > 0) {
  digitalWrite(8, LOW);
  digitalWrite(9, HIGH);
  delayMicroseconds(700);
  digitalWrite(9, LOW);
  delayMicroseconds(700);
  steps = steps - 1;
  }
  scan = 1;
  }
}
```

REFERENCES

1. Watson, J. T.; Sparkman, O. D., *Introduction to Mass Spectrometry - Instrumentation, Applications, and Strategies for Data Interpretation*. 4 ed.; Wiley, John & Sons, Incorporated: 2007.

2. de Hoffmann, E.; Stroobant, V., *Mass Spectrometry: Principles and Applications*. 3 ed.; Wiley: 2007.

3. Harris, D. C., *Quantitative Chemical Analysis*. W. H. Freeman and Co.: 2015.

4. *Ambient Ionization Mass Spectrometry*. Royal Society of Chemistry: 2014.

5. Takáts, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G., Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization. *Science* **2004,** *306* (5695), 471.

6. Cody, R. B.; Laramée, J. A.; Durst, H. D., Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions. *Analytical Chemistry* **2005,** *77* (8), 2297-2302.

7. Huang, M.-Z.; Yuan, C.-H.; Cheng, S.-C.; Cho, Y.-T.; Shiea, J., Ambient Ionization Mass Spectrometry. *Annual Review of Analytical Chemistry* **2010,** *3* (1), 43-65.

8. Weston, D. J., Ambient ionization mass spectrometry: current understanding of mechanistic theory; analytical performance and application areas. *Analyst* **2010,** *135* (4), 661-668.

9. Correa, D. N.; Santos, J. M.; Eberlin, L. S.; Eberlin, M. N.; Teunissen, S. F., Forensic chemistry and ambient mass spectrometry: A perfect couple destined for a happy marriage? *Analytical Chemistry* **2016,** *88* (5), 2515-2526.

10. Liu, J.; Wang, H.; Manicke, N. E.; Lin, J. M.; Cooks, R. G.; Ouyang, Z., Development, characterization, and application of paper spray ionization. *Analytical Chemistry* **2010,** *82* (6), 2463-2471.

11. Jarmusch, A. K.; Pirro, V.; Logsdon, D. L.; Cooks, R. G., Direct ion generation from swabs. *Talanta* **2018,** *184*, 356-363.

12. Pacholski, M. L.; Winograd, N., Imaging with Mass Spectrometry. *Chemical Reviews* **1999,** *99* (10), 2977-3006.

13. Mahoney, J. F.; Perel, J.; Ruatta, S. A.; Martino, P. A.; Husain, S.; Cook, K.; Lee, T. D., Massive cluster impact mass spectrometry: A new desorption method for the analysis of large biomolecules. *Rapid Communications in Mass Spectrometry* **1991,** *5* (10), 441-445.

14. Morelato, M.; Beavis, A.; Ogle, A.; Doble, P.; Kirkbride, P.; Roux, C., Screening of gunshot residues using desorption electrospray ionisation–mass spectrometry (DESI–MS). *Forensic Science International* **2012,** *217* (1–3), 101-106.

15. Zhao, M.; Zhang, S.; Yang, C.; Xu, Y.; Wen, Y.; Sun, L.; Zhang, X., Desorption Electrospray Tandem MS (DESI-MSMS) Analysis of Methyl Centralite and Ethyl Centralite as Gunshot Residues on Skin and Other Surfaces. *Journal of Forensic Sciences* **2008,** *53* (4), 807-811.

16. Ifa, D. R.; Manicke, N. E.; Dill, A. L.; Cooks, R. G., Latent Fingerprint Chemical Imaging by Mass Spectrometry. *Science* **2008,** *321* (5890), 805.

17. Espy, R. D.; Wleklinski, M.; Yan, X.; Cooks, R. G., Beyond the flask: Reactions on the fly in ambient mass spectrometry. *TrAC Trends in Analytical Chemistry* **2014,** *57*, 135-146.

18. Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z., Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry. *Angewandte Chemie International Edition* **2010,** *49* (5), 877-880.

19. Teunissen, S. F.; Fernandes, A. M. A. P.; Eberlin, M. N.; Alberici, R. M., Celebrating 10 years of easy ambient sonic-spray ionization. *TrAC Trends in Analytical Chemistry* **2017,** *90* (Supplement C), 135-141.

20. Tsai, C.-W.; Tipple, C. A.; Yost, R. A., Application of paper spray ionization for explosives analysis. *Rapid Communications in Mass Spectrometry* **2017,** *31* (19), 1565-1572.

21. McKenna, J.; Dhummakupt, E. S.; Connell, T.; Demond, P. S.; Miller, D. B.; Michael Nilles, J.; Manicke, N. E.; Glaros, T., Detection of chemical warfare agent simulants and hydrolysis products in biological samples by paper spray mass spectrometry. *Analyst* **2017,** *142* (9), 1442-1451.

22. Hall, S. E.; #x; Leary, A. E.; Lawton, Z. E.; Bruno, A. M.; Mulligan, C. C., Trace-Level Screening of Chemicals Related to Clandestine Desomorphine Production with Ambient Sampling, Portable Mass Spectrometry. *Journal of Chemistry* **2017,** *2017*, 7.

23. O'Leary, A. E.; Hall, S. E.; Vircks, K. E.; Mulligan, C. C., Monitoring the clandestine synthesis of methamphetamine in real-time with ambient sampling, portable mass spectrometry. *Analytical Methods* **2015,** *7* (17), 7156-7163.

24. Lawton, Z. E.; Traub, A.; Fatigante, W. L.; Mancias, J.; O'Leary, A. E.; Hall, S. E.; Wieland, J. R.; Oberacher, H.; Gizzi, M. C.; Mulligan, C. C., Analytical Validation of a Portable Mass Spectrometer Featuring Interchangeable, Ambient Ionization Sources for High Throughput Forensic Evidence Screening. *Journal of The American Society for Mass Spectrometry* **2017,** *28* (6), 1048-1059.

25. O'Leary, A. E.; Oberacher, H.; Hall, S. E.; Mulligan, C. C., Combining a portable, tandem mass spectrometer with automated library searching - an important step towards streamlined, on-site identification of forensic evidence. *Analytical Methods* **2015,** *7* (8), 3331-3339.

26. Fedick, W. P.; Fatigante, L. W.; Lawton, E. Z.; O'Leary, E. A.; Hall, E. S.; Bain, M. R.; Ayrton, T. S.; Ludwig, A. J.; Mulligan, C. C., A Low-Cost, Simplified Platform of Interchangeable, Ambient Ionization Sources for Rapid, Forensic Evidence Screening on Portable Mass Spectrometric Instrumentation. *Instruments* **2018,** *2* (2).

27. Pirro, V.; Jarmusch, A. K.; Vincenti, M.; Cooks, R. G., Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry. *Analytica Chimica Acta* **2015,** *861*, 47-54.

28. Fedick, P. W.; Bain, R. M., Swab touch spray mass spectrometry for rapid analysis of organic gunshot residue from human hand and various surfaces using commercial and fieldable mass spectrometry systems. *Forensic Chemistry* **2017,** *5* (Supplement C), 53-57.

29. Jarmusch, A. K.; Pirro, V.; Kerian, K. S.; Cooks, R. G., Detection of strep throat causing bacterium directly from medical swabs by touch spray-mass spectrometry. *Analyst* **2014,** *139* (19), 4785-4789.

30. Pirro, V.; Llor, R. S.; Jarmusch, A. K.; Alfaro, C. M.; Cohen-Gadol, A. A.; Hattab, E. M.; Cooks, R. G., Analysis of human gliomas by swab touch spray-mass spectrometry: applications to intraoperative assessment of surgical margins and presence of oncometabolites. *Analyst* **2017,** *142* (21), 4058-4066.

31. Fedick, P. W.; Bain, R. M., Swab touch spray mass spectrometry for rapid analysis of organic gunshot residue from human hand and various surfaces using commercial and fieldable mass spectrometry systems. *Forensic Chemistry* **2017,** *5*, 53-57.

32. Bain, R. M.; Fedick, P. W.; Dilger, J. M.; Cooks, R. G., Analysis of Residual Explosives by Swab Touch Spray Ionization Mass Spectrometry. *Propellants, Explosives, Pyrotechnics* **2018,** *0* (0).

33. Guo, Q.; Gao, L.; Zhai, Y.; Xu, W., Recent developments of miniature ion trap mass spectrometers. *Chinese Chemical Letters* **2017**.

34. de Araujo, W. R.; Cardoso, T. M. G.; da Rocha, R. G.; Santana, M. H. P.; Muñoz, R. A. A.; Richter, E. M.; Paixão, T. R. L. C.; Coltro, W. K. T., Portable analytical platforms for forensic chemistry: A review. *Analytica Chimica Acta* **2018,** *1034*, 1-21.

35. Wells, J. M.; Roth, M. J.; Keil, A. D.; Grossenbacher, J. W.; Justes, D. R.; Patterson, G. E.; Barket, D. J., Implementation of DART and DESI Ionization on a Fieldable Mass Spectrometer. *Journal of the American Society for Mass Spectrometry* **2008,** *19* (10), 1419-1424.

36. Plocoste, T.; Jacoby-Koaly, S.; Petit, R.-H.; Molinié, J.; Roussas, A., In situ quantification and tracking of volatile organic compounds with a portable mass spectrometer in tropical waste and urban sites. *Environmental Technology* **2017,** *38* (18), 2280-2294.

37. Gao, L.; Song, Q.; Patterson, G. E.; Cooks, R. G.; Ouyang, Z., Handheld Rectilinear Ion Trap Mass Spectrometer. *Analytical Chemistry* **2006,** *78* (17), 5994-6002.

38. Badman, E. R.; Graham Cooks, R., Miniature mass analyzers. *Journal of Mass Spectrometry* **2000,** *35* (6), 659-671.

39. Snyder, D. T.; Pulliam, C. J.; Ouyang, Z.; Cooks, R. G., Miniature and Fieldable Mass Spectrometers: Recent Advances. *Analytical Chemistry* **2016,** *88* (1), 2-29.

40. Cooks, R. G.; Ouyang, Z.; Takats, Z.; Wiseman, J. M., Ambient Mass Spectrometry. *Science* **2006,** *311* (5767), 1566.

41. Takats, Z.; Cotte-Rodriguez, I.; Talaty, N.; Chen, H.; Cooks, R. G., Direct, trace level detection of explosives on ambient surfaces by desorption electrospray ionization mass spectrometry. *Chemical Communications* **2005,** (15), 1950-1952.

42. Justes, D. R.; Talaty, N.; Cotte-Rodriguez, I.; Cooks, R. G., Detection of explosives on skin using ambient ionization mass spectrometry. *Chemical Communications* **2007,** (21), 2142-2144.

43. Brown, H.; Oktem, B.; Windom, A.; Doroshenko, V.; Evans-Nguyen, K., Direct Analysis in Real Time (DART) and a portable mass spectrometer for rapid identification of common and designer drugs on-site. *Forensic Chemistry* **2016,** *1*, 66-73.

44. Keil, A.; Talaty, N.; Janfelt, C.; Noll, R. J.; Gao, L.; Ouyang, Z.; Cooks, R. G., Ambient Mass Spectrometry with a Handheld Mass Spectrometer at High Pressure. *Analytical Chemistry* **2007,** *79* (20), 7734-7739.

45. Li, L.; Chen, T. C.; Ren, Y.; Hendricks, P. I.; Cooks, R. G.; Ouyang, Z., Mini 12, miniature mass spectrometer for clinical and other applications--introduction and characterization. *Anal Chem* **2014,** *86* (6), 2909-16.

46. Chen, C.-H.; Lin, Z.; Tian, R.; Shi, R.; Cooks, R. G.; Ouyang, Z., Real-Time Sample Analysis Using a Sampling Probe and Miniature Mass Spectrometer. *Analytical Chemistry* **2015,** *87* (17), 8867-8873.

47. Rostron, P.; Gaber, S.; Gaber, D., Raman Spectroscopy, Review *International Journal of Engineering and Technical Research* **2016,** *6*, 50-64.

48. Socrates, G., *Infrared and Raman Characteristic Group Frequencies*. Third ed.; Wiley: 2001.

49. Haynes, C. L.; McFarland, A. D.; Van Duyne, R. P., Surface-Enhanced Raman Spectroscopy. *Analytical Chemistry* **2005,** *77* (17), 338 A-346 A.

50. Le Ru, E. C.; Blackie, E.; Meyer, M.; Etchegoin, P. G., Surface Enhanced Raman Scattering Enhancement Factors: A Comprehensive Study. *The Journal of Physical Chemistry C* **2007,** *111* (37), 13794-13803.

51. Muehlethaler, C.; Leona, M.; Lombardi, J. R., Review of Surface Enhanced Raman Scattering Applications in Forensic Science. *Analytical Chemistry* **2016,** *88* (1), 152-169.

52. Doty, K. C.; Lednev, I. K., Raman spectroscopy for forensic purposes: Recent applications for serology and gunshot residue analysis. *TrAC Trends in Analytical Chemistry* **2018,** *103*, 215-222.

53. Fikiet, M. A.; Khandasammy, S. R.; Mistek, E.; Ahmed, Y.; Halámková, L.; Bueno, J.; Lednev, I. K., Surface enhanced Raman spectroscopy: A review of recent applications in forensic science. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2018,** *197*, 255-260.

54. Pilot, R.; Signorini, R.; Fabris, L., Surface-Enhanced Raman Spectroscopy: Principles, Substrates, and Applications. In *Metal Nanoparticles and Clusters: Advances in Synthesis, Properties and Applications*, Deepak, F. L., Ed. Springer International Publishing: Cham, 2018; pp 89-164.

55. Yu, W. W.; White, I. M., Inkjet Printed Surface Enhanced Raman Spectroscopy Array on Cellulose Paper. *Analytical Chemistry* **2010,** *82* (23), 9626-9630.

56. Betz, J. F.; Yu, W. W.; Cheng, Y.; White, I. M.; Rubloff, G. W., Simple SERS substrates: powerful, portable, and full of potential. *Physical Chemistry Chemical Physics* **2014,** *16* (6), 2224-2239.

57. Yu, W. W.; White, I. M., Inkjet-printed paper-based SERS dipsticks and swabs for trace chemical detection. *Analyst* **2013,** *138* (4), 1020-1025.

58. Hoppmann, E. P.; Yu, W. W.; White, I. M., Highly sensitive and flexible inkjet printed SERS sensors on paper. *Methods* **2013,** *63* (3), 219-224.

59. Hoppmann, E. P.; Yu, W. W.; White, I. M., Inkjet-Printed Fluidic Paper Devices for Chemical and Biological Analytics Using Surface Enhanced Raman spectroscopy. *IEEE Journal of Selected Topics in Quantum Electronics* **2014,** *20* (3), 195-204.

60. Peters, F. T., Recent advances of liquid chromatography–(tandem) mass spectrometry in clinical and forensic toxicology. *Clinical Biochemistry* **2011,** *44* (1), 54-65.

61. Brettell, T. A., Forensic Science Applications of Gas Chromatography. In *Modern Practice of Gas Chromatography*, John Wiley & Sons, Inc.: 2004; pp 883-967.

62. International, A., Standard Practice for Identification of Seized Drugs. ASTM International: 2014.

63. SWGDRUG, Recommendations Edition 7.1 (2016-June-09). SWGDRUG: 2016.

64. Hoffmann, W. D.; Jackson, G. P., Forensic Mass Spectrometry. *Annual Review of Analytical Chemistry* **2015,** *8* (1), 419-440.

65. Liu, J.; Wang, H.; Manicke, N. E.; Lin, J.-M.; Cooks, R. G.; Ouyang, Z., Development, Characterization, and Application of Paper Spray Ionization. *Analytical Chemistry* **2010,** *82* (6), 2463-2471.

66. Teunissen, S. F.; Fedick, P. W.; Berendsen, B. J.; Nielen, M. W.; Eberlin, M. N.; Cooks, R. G.; van Asten, A., Novel selectivity-based forensic toxicological validation of a paper spray mass spectrometry method for the quantitative determination of eight amphetamines in whole blood. *Submitted to JASMS* **2017**.

67. Espy, R. D.; Teunissen, S. F.; Manicke, N. E.; Ren, Y.; Ouyang, Z.; van Asten, A.; Cooks, R. G., Paper Spray and Extraction Spray Mass Spectrometry for the Direct and Simultaneous Quantification of Eight Drugs of Abuse in Whole Blood. *Analytical Chemistry* **2014,** *86* (15), 7712-7718.

68. Pulliam, C. J.; Wei, P.; Snyder, D. T.; Wang, X.; Ouyang, Z.; Pielak, R. M.; Graham Cooks, R., Rapid discrimination of bacteria using a miniature mass spectrometer. *Analyst* **2016,** *141* (5), 1633-1636.

69. Narayanan, R.; Sarkar, D.; Cooks, R. G.; Pradeep, T., Molecular Ionization from Carbon Nanotube Paper. *Angewandte Chemie International Edition* **2014,** *53* (23), 5936-5940.

70. Damon, D. E.; Maher, Y. S.; Yin, M.; Jjunju, F. P. M.; Young, I. S.; Taylor, S.; Maher, S.; Badu-Tawiah, A. K., 2D wax-printed paper substrates with extended solvent supply capabilities allow enhanced ion signal in paper spray ionization. *Analyst* **2016,** *141* (12), 3866-3873.

71. Banerjee, S.; Basheer, C.; Zare, R. N., A Study of Heterogeneous Catalysis by Nanoparticle - Embedded Paper-Spray Ionization Mass Spectrometry. *Angewandte Chemie International Edition* **2016,** *55* (41), 12807-12811.

72. Resende, S. F.; Teodoro, J. A. R.; Binatti, I.; Gouveia, R. L.; Oliveira, B. S.; Augusti, R., On-surface photocatalytic degradation of methylene blue: In situ monitoring by paper spray ionization mass spectrometry. *International Journal of Mass Spectrometry* **2017,** *418*, 107-111.

73. Ji, J.; Nie, L.; Liao, L.; Du, R.; Liu, B.; Yang, P., Ambient ionization based on mesoporous graphene coated paper for therapeutic drug monitoring. *Journal of Chromatography B* **2016,** *1015*, 142-149.

74. Bills, B. J.; Manicke, N. E., Development of a prototype blood fractionation cartridge for plasma analysis by paper spray mass spectrometry. *Clinical Mass Spectrometry* **2016,** *2*, 18-24.

75. Crime, U. N. O. o. D. a. *World Drug Report 2015*; 2015.

76. Addiction, E. M. C. f. D. a. D. *Perspectives on Drugs: Injection of synthetic cathinones*; 2015.

77. Cedarbaum, E. R.; Banta-Green, C. J., Health behaviors of young adult heroin injectors in the Seattle area. *Drug and Alcohol Dependence* **2016,** *158*, 102-109.

78. Hedegaard, H.; Warner, M.; Minino, A., Drug Overdose Deaths in the United States, 1999–2015. *NCHS Data Brief* **2017,** *273*.

79. Frank, R. G.; Pollack, H. A., Addressing the Fentanyl Threat to Public Health. *New England Journal of Medicine* **2017,** *376* (7), 605-607.

80. Garcia-Reyes, J. F.; Harper, J. D.; Salazar, G. A.; Charipar, N. A.; Ouyang, Z.; Cooks, R. G., Detection of Explosives and Related Compounds by Low-Temperature Plasma Ambient Ionization Mass Spectrometry. *Analytical Chemistry* **2011,** *83* (3), 1084-1092.

81. Cotte-Rodríguez, I.; Takáts, Z.; Talaty, N.; Chen, H.; Cooks, R. G., Desorption Electrospray Ionization of Explosives on Surfaces: Sensitivity and Selectivity Enhancement by Reactive Desorption Electrospray Ionization. *Analytical Chemistry* **2005,** *77* (21), 6755-6764.

82. Cotte-Rodriguez, I.; Cooks, R. G., Non-proximate detection of explosives and chemical warfare agent simulants by desorption electrospray ionization mass spectrometry. *Chemical Communications* **2006,** (28), 2968-2970.

83. Zhang, Y.; Ma, X.; Zhang, S.; Yang, C.; Ouyang, Z.; Zhang, X., Direct detection of explosives on solid surfaces by low temperature plasma desorption mass spectrometry. *Analyst* **2009,** *134* (1), 176-181.

84. Chen, W.; Hou, K.; Xiong, X.; Jiang, Y.; Zhao, W.; Hua, L.; Chen, P.; Xie, Y.; Wang, Z.; Li, H., Non-contact halogen lamp heating assisted LTP ionization miniature rectilinear ion trap: a platform for rapid, on-site explosives analysis. *Analyst* **2013,** *138* (17), 5068-5073.

85. Harper, J. D.; Charipar, N. A.; Mulligan, C. C.; Zhang, X.; Cooks, R. G.; Ouyang, Z., Low-Temperature Plasma Probe for Ambient Desorption Ionization. *Analytical Chemistry* **2008,** *80* (23), 9097-9104.

86. Sanders, N. L.; Kothari, S.; Huang, G.; Salazar, G.; Cooks, R. G., Detection of Explosives as Negative Ions Directly from Surfaces Using a Miniature Mass Spectrometer. *Analytical Chemistry* **2010,** *82* (12), 5313-5316.

87. Droste, D. J.; Shelley, M. L.; Gearhart, J. M.; Kempisty, D. M., A systems dynamics approach to the efficacy of oxime therapy for mild exposure to sarin gas. *Am J Disaster Med* **2016,** *11* (2), 89-118.

88. Haines, D. D.; Fox, S. C., Acute and Long-Term Impact of Chemical Weapons: Lessons from the Iran-Iraq War. *Forensic Sci Rev* **2014,** *26* (2), 97-114.

89. Tokuda, Y.; Kikuchi, M.; Takahashi, O.; Stein, G. H., Prehospital management of sarin nerve gas terrorism in urban settings: 10 years of progress after the Tokyo subway sarin attack. *Resuscitation* **2006,** *68* (2), 193-202.

90. Rudd, R. A.; Aleshire, N.; Zibbell, J. E.; Matthew Gladden, R., Increases in Drug and Opioid Overdose Deaths—United States, 2000–2014. *American Journal of Transplantation* **2016,** *16* (4), 1323-1327.

91. Ciccarone, D., Fentanyl in the US heroin supply: A rapidly changing risk environment. *International Journal of Drug Policy* **2017,** *46*, 107-111.

92. Dalby, O.; Butler, D.; Birkett Jason, W., Analysis of Gunshot Residue and Associated Materials—A Review. *Journal of Forensic Sciences* **2010,** *55* (4), 924-943.

93. Moore, D. S., Instrumentation for trace detection of high explosives. *Review of Scientific Instruments* **2004,** *75* (8), 2499-2512.

94. Barron, L.; Gilchrist, E., Ion chromatography-mass spectrometry: A review of recent technologies and applications in forensic and environmental explosives analysis. *Analytica Chimica Acta* **2014,** *806*, 27-54.

95. Gassner, A.-L.; Ribeiro, C.; Kobylinska, J.; Zeichner, A.; Weyermann, C., Organic gunshot residues: Observations about sampling and transfer mechanisms. *Forensic Science International* **2016,** *266*, 369-378.

96. Gassner, A.-L.; Weyermann, C., LC–MS method development and comparison of sampling materials for the analysis of organic gunshot residues. *Forensic Science International* **2016,** *264*, 47-55.

97. Perr, J. M.; Furton, K. G.; Almirall, J. R., Gas chromatography positive chemical ionization and tandem mass spectrometry for the analysis of organic high explosives. *Talanta* **2005,** *67* (2), 430-436.

98. Palit, M.; Pardasani, D.; Gupta, A. K.; Dubey, D. K., Application of Single Drop Microextraction for Analysis of Chemical Warfare Agents and Related Compounds in Water by Gas Chromatography/Mass Spectrometry. *Analytical Chemistry* **2005,** *77* (2), 711-717.

99. Xu, X.; Koeberg, M.; Kuijpers, C.-J.; Kok, E., Development and validation of highly selective screening and confirmatory methods for the qualitative forensic analysis of organic explosive compounds with high performance liquid chromatography coupled with (photodiode array and) LTQ ion trap/Orbitrap mass spectrometric detections (HPLC-(PDA)-LTQOrbitrap). *Science and Justice 54* (1), 3-21.

100. Lee, J.; Park, S.; Cho, S. G.; Goh, E. M.; Lee, S.; Koh, S.-S.; Kim, J., Analysis of explosives using corona discharge ionization combined with ion mobility spectrometry–mass spectrometry. *Talanta* **2014,** *120*, 64-70.

101. Du, Z.; Sun, T.; Zhao, J.; Wang, D.; Zhang, Z.; Yu, W., Development of a plug-type IMS-MS instrument and its applications in resolving problems existing in in-situ detection of illicit drugs and explosives by IMS. *Talanta* **2018,** *184*, 65-72.

102. Goudsmits, E.; Sharples, G. P.; Birkett, J. W., Recent trends in organic gunshot residue analysis. *TrAC Trends in Analytical Chemistry* **2015,** *74*, 46-57.

103. Kabir, A.; Furton, K. G., Chapter 25 - Applications of Gas Chromatography in Forensic Science A2 - Poole, Colin F. In *Gas Chromatography*, Elsevier: Amsterdam, 2012; pp 563-604.

104. Taudte, R. V.; Roux, C.; Bishop, D.; Blanes, L.; Doble, P.; Beavis, A., Development of a UHPLC method for the detection of organic gunshot residues using artificial neural networks. *Analytical Methods* **2015,** *7* (18), 7447-7454.

105. Dalby, O.; Birkett, J. W., The evaluation of solid phase micro-extraction fibre types for the analysis of organic components in unburned propellant powders. *Journal of Chromatography A* **2010,** *1217* (46), 7183-7188.

106. Almog, J.; Zitrin, S., Chapter 4 - Colorimetric Detection of Explosives. In *Aspects of Explosives Detection*, Marshall, M.; Oxley, J. C., Eds. Elsevier: Amsterdam, 2009; pp 41-58.

107. Taudte, R. V.; Roux, C.; Blanes, L.; Horder, M.; Kirkbride, K. P.; Beavis, A., The development and comparison of collection techniques for inorganic and organic gunshot residues. *Analytical and Bioanalytical Chemistry* **2016,** *408* (10), 2567-2576.

108. Goudsmits, E.; Sharples, G. P.; Birkett, J. W., Preliminary classification of characteristic organic gunshot residue compounds. *Science & Justice* **2016,** *56* (6), 421-425.

109. Monge, M. E.; Harris, G. A.; Dwivedi, P.; Fernández, F. M., Mass Spectrometry: Recent Advances in Direct Open Air Surface Sampling/Ionization. *Chemical Reviews* **2013,** *113* (4), 2269-2308.

110. Talaty, N.; Mulligan, C. C.; Justes, D. R.; Jackson, A. U.; Noll, R. J.; Cooks, R. G., Fabric analysis by ambient mass spectrometry for explosives and drugs. *Analyst* **2008,** *133* (11), 1532-1540.

111. Rowell, F.; Seviour, J.; Lim, A. Y.; Elumbaring-Salazar, C. G.; Loke, J.; Ma, J., Detection of nitro-organic and peroxide explosives in latent fingermarks by DART- and SALDI-TOF-mass spectrometry. *Forensic Science International* **2012,** *221* (1), 84-91.

112. Sisco, E.; Dake, J.; Bridge, C., Screening for trace explosives by AccuTOF™-DART®: An in-depth validation study. *Forensic Science International* **2013,** *232* (1), 160-168.

113. Nilles, J. M.; Connell Theresa, R.; Stokes Sarah, T.; Dupont Durst, H., Explosives Detection Using Direct Analysis in Real Time (DART) Mass Spectrometry. *Propellants, Explosives, Pyrotechnics* **2010,** *35* (5), 446-451.

114. Forbes, T. P.; Sisco, E., Recent Advances in Ambient Mass Spectrometry of Trace Explosives. *Analyst* **2018**.

115. Forbes, T. P.; Sisco, E., Recent advances in ambient mass spectrometry of trace explosives. *Analyst* **2018,** *143* (9), 1948-1969.

116. Moran, J. W.; Bell, S., Skin Permeation of Organic Gunshot Residue: Implications for Sampling and Analysis. *Analytical Chemistry* **2014,** *86* (12), 6071-6079.

117. Taudte, R. V.; Roux, C.; Beavis, A., Stability of smokeless powder compounds on collection devices. *Forensic Science International* **2017,** *270*, 55-60.

118. Hendricks, P. I.; Dalgleish, J. K.; Shelley, J. T.; Kirleis, M. A.; McNicholas, M. T.; Li, L.; Chen, T.-C.; Chen, C.-H.; Duncan, J. S.; Boudreau, F.; Noll, R. J.; Denton, J. P.; Roach, T. A.; Ouyang, Z.; Cooks, R. G., Autonomous in Situ Analysis and Real-Time Chemical Detection Using a Backpack Miniature Mass Spectrometer: Concept, Instrumentation Development, and Performance. *Analytical Chemistry* **2014,** *86* (6), 2900-2908.

119. Dalgleish, J. K.; Hou, K.; Ouyang, Z.; Cooks, R. G., In Situ Explosive Detection Using a Miniature Plasma Ion Source and a Portable Mass Spectrometer. *Analytical Letters* **2012,** *45* (11), 1440-1446.

120. Teunissen, S. F.; Fedick, P. W.; Berendsen, B. J. A.; Nielen, M. W. F.; Eberlin, M. N.; Graham Cooks, R.; van Asten, A. C., Novel selectivity-based forensic toxicological validation of a paper spray mass spectrometry method for the quantitative determination of eight amphetamines in whole blood. *Journal of The American Society for Mass Spectrometry* **2017,** *28* (12), 2665-2676.

121. Tsai, C. W.; Tipple Christopher, A.; Yost Richard, A., Application of paper spray ionization for explosives analysis. *Rapid Communications in Mass Spectrometry* **2017,** *31* (19), 1565-1572.

122. Espy, R. D.; Muliadi, A. R.; Ouyang, Z.; Cooks, R. G., Spray mechanism in paper spray ionization. *International Journal of Mass Spectrometry* **2012,** *325-327*, 167-171.

123. Castellanos, A.; Bell, S.; Fernandez-Lima, F., Characterization of firearm discharge residues recovered from skin swabs using sub-micrometric mass spectrometry imaging. *Analytical Methods* **2016,** *8* (21), 4300-4305.

124. Jarmusch, A. K.; Pirro, V.; Logsdon, D. L.; Cooks, R. G., Direct Ion Generation from Swabs. *Talanta* **2018**.

125. Li, L.; Chen, T.-C.; Ren, Y.; Hendricks, P. I.; Cooks, R. G.; Ouyang, Z., Mini 12, Miniature Mass Spectrometer for Clinical and Other Applications—Introduction and Characterization. *Analytical Chemistry* **2014,** *86* (6), 2909-2916.

126. Sokol, E.; Jackson, A. U.; Cooks, R. G., Trace detection of inorganic oxidants using desorption electrospray ionization (DESI) mass spectrometry. *Central European Journal of Chemistry* **2011,** *9* (5), 790-797.

127. Pulliam, C. J.; Bain, R. M.; Wiley, J. S.; Ouyang, Z.; Cooks, R. G., Mass Spectrometry in the Home and Garden. *Journal of The American Society for Mass Spectrometry* **2015,** *26* (2), 224-230.

128. National Research, C., *Toxicity of Military Smokes and Obscurants: Volume 1*. The National Academies Press: Washington, DC, 1997.

129. National Research, C., *Toxicity of Military Smokes and Obscurants: Volume 2*. The National Academies Press: Washington, DC, 1999.

130. Conkling, J. A.; Mocella, C., *Chemistry of Pyrotechnics: Basic Principles and Theory, Second Edition*. Second Edition ed.; 2010.

131. Medinsky, M. A.; Cheng, Y. S.; Kampcik, S. J.; Henderson, R. F.; Dutcher, J. S., Disposition and metabolism of 14C-solvent yellow and solvent green aerosols after inhalation. *Fundamental and Applied Toxicology* **1986,** *7* (1), 170-178.

132. Marrs, T. C.; Colgrave, H. F.; Edginton, J. A. G.; Cross, N. L., Repeated dose inhalation toxicity of cinnamic acid smoke. *Journal of Hazardous Materials* **1989,** *21* (1), 1-13.

133. Marrs, T. C.; Colgrave, H. F.; Rice, P.; Edginton, J. A. G.; Morris, B., The repeated dose toxicity of a smoke containing Disperse Blue 180, an anthraquinone dye mixture. *Journal of Hazardous Materials* **1989,** *21* (1), 73-88.

134. Steinhauser, G.; Klapötke Thomas , M., "Green" Pyrotechnics: A Chemists' Challenge. *Angewandte Chemie International Edition* **2008,** *47* (18), 3330-3347.

135. Glück, J.; Klapötke, T. M.; Küblböck, T., Development of a Sustainable Perchlorate-Free Yellow Pyrotechnical Strobe Formulation. *ACS Sustainable Chemistry and Engineering* **2018,** *6* (3), 4400-4404.

136. Klapötke Thomas, M.; Rusan, M.; Sabatini Jesse, J., Chlorine-Free Pyrotechnics: Copper(I) Iodide as a "Green" Blue-Light Emitter. *Angewandte Chemie International Edition* **2014,** *53* (36), 9665-9668.

137. Cropek, D. M.; Kemme, P. A.; Day, J. M.; Cochran, J., Use of pyrolysis GC/MS for predicting emission byproducts from the incineration of double-base propellant. *Environmental Science and Technology* **2002,** *36* (20), 4346-4351.

138. Dilger, J. M.; Miklaszeski, E. J.; Papenmeier, D. M.; Wilharm, C. K.; Neiswinger, M. A., Pyrolysis / Gas Chromatography / Mass Spectrometry: A New Tool to Detect Toxic Byproducts of Pyrotechnic Reactions. In *42nd IPS Seminar*, 2016.

139. Ralph, J.; Hatfield, R. D., Pyrolysis-Gc-Ms Characterization of Forage Materials. *Journal of Agricultural and Food Chemistry* **1991,** *39* (8), 1426-1437.

140. Chen, H.; Gamez, G.; Zenobi, R., What Can We Learn from Ambient Ionization Techniques? *Journal of the American Society for Mass Spectrometry* **2009,** *20* (11), 1947-1963.

141. Wilm, M.; Mann, M., Analytical properties of the nanoelectrospray ion source. *Analytical Chemistry* **1996,** *68* (1), 1-8.

142. Juraschek, R.; Dülcks, T.; Karas, M., Nanoelectrospray - More than just a minimized-flow electrospray ionization source. *Journal of the American Society for Mass Spectrometry* **1999,** *10* (4), 300-308.

143. Huang, H.; Boyer, A.; Ben Dhia, S., Electronic counterfeit detection based on the measurement of electromagnetic fingerprint. *Microelectronics Reliability* **2015,** *55* (9–10), 2050-2054.

144. Shrivastava, A.; Pecht, M., Counterfeit capacitors in the supply chain. *Journal of Materials Science: Materials in Electronics* **2014,** *25* (2), 645-652.

145. Sood, B.; Das, D.; Pecht, M., Screening for counterfeit electronic parts. *Journal of Materials Science: Materials in Electronics* **2011,** *22* (10), 1511.

146. DiMase, D.; Collier, Z. A.; Carlson, J.; Gray, R. B.; Linkov, I., Traceability and Risk Analysis Strategies for Addressing Counterfeit Electronics in Supply Chains for Complex Systems. *Risk Analysis* **2016,** *36* (10), 1834-1843.

147. Andrade, F. J.; Shelley, J. T.; Wetzel, W. C.; Webb, M. R.; Gamez, G.; Ray, S. J.; Hieftje, G. M., Atmospheric Pressure Chemical Ionization Source. 2. Desorption−Ionization for the Direct Analysis of Solid Compounds. *Analytical Chemistry* **2008,** *80* (8), 2654-2663.

148. Andrade, F. J.; Shelley, J. T.; Wetzel, W. C.; Webb, M. R.; Gamez, G.; Ray, S. J.; Hieftje, G. M., Atmospheric Pressure Chemical Ionization Source. 1. Ionization of Compounds in the Gas Phase. *Analytical Chemistry* **2008,** *80* (8), 2646-2653.

149. Pfeuffer, K. P.; Caldwell, J.; Shelley, J. T.; Ray, S. J.; Hieftje, G. M., Detection of counterfeit electronic components through ambient mass spectrometry and chemometrics. *Analyst* **2014,** *139* (18), 4505-4511.

150. Jarmusch, A. K.; Pirro, V.; Baird, Z.; Hattab, E. M.; Cohen-Gadol, A. A.; Cooks, R. G., Lipid and metabolite profiles of human brain tumors by desorption electrospray ionization-MS. *Proceedings of the National Academy of Sciences* **2016,** *113* (6), 1486.

151. Eberlin, L. S.; Tibshirani, R. J.; Zhang, J.; Longacre, T. A.; Berry, G. J.; Bingham, D. B.; Norton, J. A.; Zare, R. N.; Poultsides, G. A., Molecular assessment of surgical-resection margins of gastric cancer by mass-spectrometric imaging. *Proceedings of the National Academy of Sciences* **2014,** *111* (7), 2436.

152. Garza, K. Y.; Feider, C. L.; Klein, D. R.; Rosenberg, J. A.; Brodbelt, J. S.; Eberlin, L. S., Desorption Electrospray Ionization Mass Spectrometry Imaging of Proteins Directly from Biological Tissue Sections. *Analytical Chemistry* **2018,** *90* (13), 7785-7789.

153. Alfaro, C. M.; Jarmusch, A. K.; Pirro, V.; Kerian, K. S.; Masterson, T. A.; Cheng, L.; Cooks, R. G., Ambient ionization mass spectrometric analysis of human surgical specimens to distinguish renal cell carcinoma from healthy renal tissue. *Analytical and Bioanalytical Chemistry* **2016,** *408* (20), 5407-5414.

154. Jarmusch, A. K.; Alfaro, C. M.; Pirro, V.; Hattab, E. M.; Cohen-Gadol, A. A.; Cooks, R. G., Differential Lipid Profiles of Normal Human Brain Matter and Gliomas by Positive and Negative Mode Desorption Electrospray Ionization – Mass Spectrometry Imaging. *PLOS ONE* **2016,** *11* (9), e0163180.

155. Nyadong, L.; Hohenstein, E. G.; Galhena, A.; Lane, A. L.; Kubanek, J.; Sherrill, C. D.; Fernández, F. M., Reactive desorption electrospray ionization mass spectrometry (DESI-MS) of natural products of a marine alga. *Analytical and Bioanalytical Chemistry* **2009,** *394* (1), 245-254.

156. Girod, M.; Moyano, E.; Campbell, D. I.; Cooks, R. G., Accelerated bimolecular reactions in microdroplets studied by desorption electrospray ionization mass spectrometry. *Chemical Science* **2011,** *2* (3), 501-510.

157. Yan, X.; Bain, R. M.; Cooks, R. G., Organic Reactions in Microdroplets: Reaction Acceleration Revealed by Mass Spectrometry. *Angewandte Chemie International Edition* **2016,** *55* (42), 12960-12972.

158. Song, Y.; Cooks, R. G., Reactive desorption electrospray ionization for selective detection of the hydrolysis products of phosphonate esters. *Journal of Mass Spectrometry* **2007,** *42* (8), 1086-1092.

159. Honig, R. E., Sputtering of Surfaces by Positive Ion Beams of Low Energy. *Journal of Applied Physics* **1958,** *29* (3), 549-555.

160. Benninghoven, A., Analysis of Submonolayers on Silver by Negative Secondary Ion Emission. *physica status solidi (b)* **1969,** *34* (2), K169-K171.

161. Xu, K.; Proctor, A.; Hercules, D. M., Time-of-flight secondary ion mass spectrometry (TOF-SIMS) of polyisoprenes. *Microchimica Acta* **1996,** *122* (1), 1-15.

162. Demirev, P.; Olthoff, J. K.; Fenselau, C.; Cotter, R. J., High-mass ion fragmentation as a function of time and mass. *Analytical Chemistry* **1987,** *59* (15), 1951-1954.

163. Pachuta, S. J.; Cooks, R. G., Mechanisms in molecular SIMS. *Chemical Reviews* **1987,** *87* (3), 647-669.

164. Unger, S. E.; Day, R. J.; Cooks, R. G., Possitive and negative secondary ion mass spectra and mass-analyzed ion kinetic energy spectra of some amides, amines and related compounds: Mechanisms in molecular sims. *International Journal of Mass Spectrometry and Ion Physics* **1981,** *39* (2), 231-255.

165. Detter, L. D.; Pachuta, S. J.; Cooks, R. G.; Walton, R. A., Effects of vacuum on tetrakis-isocyanide complexes of silver(I) and copper(I): Implications for sims analyses. *Talanta* **1986,** *33* (11), 917-918.

166. Cooks, R. G.; Busch, K. L., Matrix effects, internal energies and MS/MS spectra of molecular ions sputtered from surfaces. *International Journal of Mass Spectrometry and Ion Physics* **1983,** *53*, 111-124.

167. Unger, S. E.; Cooks, R. G.; Steinmetz, B. J.; Delgass, W. N., Molecular SIMS at surfaces: Thiophene on silver. *Surface Science* **1982,** *116* (2), L211-L217.

168. Oliveri, P.; Chiara Casolino, M.; Forina, M., Chapter 2 - Chemometric Brains for Artificial Tongues. In *Advances in Food and Nutrition Research*, Steve, L. T., Ed. Academic Press: 2010; Vol. Volume 61, pp 57-117.

169. Al-Saffar, Y.; Stephanson, N. N.; Beck, O., Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-experience from the Swedish population. *J Chromatogr B Analyt Technol Biomed Life Sci* **2013,** *930*, 112-20.

170. Gibbins, L.; Perkin, G., *Laboratories for the 21st century in STEM higher education: a compendium of current UK practice and an insight into future directions for laboratory-based teaching and learning*. Centre for Engineering and Design Education © Loughborough University: 2013.

171. Bain, R.; Pulliam, C.; Raab, S.; Cooks, G., Chemical Synthesis Accelerated by Paper Spray: The Haloform Reaction. *Journal of Chemical Education* **2016,** *93* (2), 340-344.

172. Bain, R. M.; Pulliam, C. J.; Raab, S. A.; Cooks, R. G., On-Line Synthesis and Analysis by Mass Spectrometry. *Journal of Chemical Education* **2015,** *92* (12), 2146-2151.

173. Bain, R. M.; Pulliam, C. J.; Yan, X.; Moore, K. F.; Müller, T.; Cooks, R. G., Mass Spectrometry in Organic Synthesis: Claisen–Schmidt Base-Catalyzed Condensation and Hammett Correlation of Substituent Effects. *Journal of Chemical Education* **2014,** *91* (11), 1985-1989.

174. Fedick, P. W.; Bain, R. M.; Bain, K.; Cooks, R. G., Chiral Analysis by Tandem Mass Spectrometry Using the Kinetic Method, by Polarimetry, and by 1H NMR Spectroscopy. *Journal of Chemical Education* **2017,** *94* (9), 1329-1333.

175. Fedick, P. W.; Bain, R. M.; Miao, S.; Pirro, V.; Cooks, R. G., State-of-the-art mass spectrometry for point-of-care and other applications: A hands-on intensive short course for undergraduate students. *International Journal of Mass Spectrometry* **2017,** *417* (Supplement C), 22-28.

176. Fedick, P. W.; Bain, R. M.; Bain, K.; Mehari, T. F.; Cooks, R. G., Accelerated tert-butyloxycarbonyl deprotection of amines in microdroplets produced by a pneumatic spray. *International Journal of Mass Spectrometry* **2018,** *430*, 98-103.

177. Sevian, H.; Fulmer, G. W., Student Outcomes from Innovations in Undergraduate Chemistry Laboratory Learning. *Educación Química* **2012,** *23*, 149-161.

178. Bain, R. M.; Pulliam, C. J.; Raab, S. A.; Cooks, R. G., Chemical Synthesis Accelerated by Paper Spray: The Haloform Reaction. *Journal of Chemical Education* **2016,** *93* (2), 340-344.

179. Salzer, R., Eurocurriculum II for Analytical Chemistry approved by the Division of Analytical Chemistry of FECS. *Analytical and Bioanalytical Chemistry* **2004,** *378* (1), 28-32.

180. Holme, T.; Luxford, C.; Murphy, K., Updating the General Chemistry Anchoring Concepts Content Map. *Journal of Chemical Education* **2015,** *92* (6), 1115-1116.

181. Raker, J.; Holme, T.; Murphy, K., The ACS Exams Institute Undergraduate Chemistry Anchoring Concepts Content Map II: Organic Chemistry. *Journal of Chemical Education* **2013,** *90* (11), 1443-1445.

182. Sneha, M.; Dulay, M. T.; Zare, R. N., Introducing mass spectrometry to first-year undergraduates: Analysis of caffeine and other components in energy drinks using paper-spray mass spectrometry. *International Journal of Mass Spectrometry*.

183. Administration, F. a. D., Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance. Information, D. o. D., Ed. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070305.pdf, 2004.

184. Badu-Tawiah, A. K.; Eberlin, L. S.; Ouyang, Z.; Cooks, R. G., Chemical Aspects of the Extractive Methods of Ambient Ionization Mass Spectrometry. *Annual Review of Physical Chemistry* **2013,** *64* (1), 481-505.

185. Ifa, D. R.; Wu, C.; Ouyang, Z.; Cooks, R. G., Desorption electrospray ionization and other ambient ionization methods: current progress and preview. *Analyst* **2010,** *135* (4), 669-681.

186. Hollerbach, A.; Ayrton, S.; Jarmusch, A.; Graham Cooks, R., Desorption Electrospray Ionization: Methodology and Applications A2 - Lindon, John C. In *Encyclopedia of Spectroscopy and Spectrometry (Third Edition)*, Tranter, G. E.; Koppenaal, D. W., Eds. Academic Press: Oxford, 2017; pp 401-408.

187. Schwarz, G.; Frenzel, W.; Richter, W. M.; Täuscher, L.; Kubsch, G., A Multidisciplinary Science Summer Camp for Students with Emphasis on Environmental and Analytical Chemistry. *Journal of Chemical Education* **2016,** *93* (4), 626-632.

188. Sheridan, P. M.; Szczepankiewicz, S. H.; Mekelburg, C. R.; Schwabel, K. M., Canisius College Summer Science Camp: Combining Science and Education Experts To Increase Middle School Students' Interest in Science. *Journal of Chemical Education* **2011,** *88* (7), 876-880.

189. Rosado, D. A.; Masterson, T. S.; Masterson, D. S., Using the Mini-Session Course Format To Train Students in the Practical Aspects of Modern Mass Spectrometry. *Journal of Chemical Education* **2011,** *88* (2), 178-183.

190. Gosser, D. K.; Roth, V., The Workshop Chemistry Project: Peer-Led Team-Learning. *Journal of Chemical Education* **1998,** *75* (2), 185.

191. Tata, A.; Gribble, A.; Ventura, M.; Ganguly, M.; Bluemke, E.; Ginsberg, H. J.; Jaffray, D. A.; Ifa, D. R.; Vitkin, A.; Zarrine-Afsar, A., Wide-field tissue polarimetry allows efficient localized mass spectrometry imaging of biological tissues. *Chemical Science* **2016,** *7* (3), 2162-2169.

192. Chen, S.; Wan, Q.; Badu-Tawiah, A. K., Mass Spectrometry for Paper-Based Immunoassays: Toward On-Demand Diagnosis. *Journal of the American Chemical Society* **2016,** *138* (20), 6356-6359.

193. Trivedi, V.; Doshi, A.; Kurup, G. K.; Ereifej, E.; Vandevord, P. J.; Basu, A. S., A modular approach for the generation, storage, mixing, and detection of droplet libraries for high throughput screening. *Lab on a Chip* **2010,** *10* (18), 2433-2442.

194. Boyle, T.; Bradley, C.; Chalk, P.; Jones, R.; Pickard, P., Using Blended Learning to Improve Student Success Rates in Learning to Program. *Journal of Educational Media* **2003,** *28* (2-3), 165-178.

195. Sharma, P., Blended learning. *ELT Journal* **2010,** *64* (4), 456-458.

196. Kitzinger, J., Qualitative research. Introducing focus groups. *BMJ : British Medical Journal* **1995,** *311* (7000), 299-302.

197. Peters, D. G.; Ji, C., A Multistep Synthesis for an Advanced Undergraduate Organic Chemistry Laboratory. *Journal of Chemical Education* **2006,** *83* (2), 290.

198. Wleklinski, M.; Falcone, C. E.; Loren, B. P.; Jaman, Z.; Iyer, K.; Ewan, H. S.; Hyun, S.-H.; Thompson, D. H.; Cooks, R. G., Can Accelerated Reactions in Droplets Guide Chemistry at Scale? *European Journal of Organic Chemistry* **2016,** *2016* (33), 5480-5484.

199. Young, P. E.; Campbell, A., The synthesis of a dipeptide from its component amino acids: Protecting groups in the elementary organic laboratory. *Journal of Chemical Education* **1982,** *59* (8), 701.

200. Demchenko, A. V.; Pornsuriyasak, P.; De Meo, C., Acetal Protecting Groups in the Organic Laboratory: Synthesis of Methyl 4,6-O-Benzylidene-α-D-Glucopyranoside. *Journal of Chemical Education* **2006,** *83* (5), 782.

201. Baar, M. R.; Russell, C. E.; Wustholz, K. L., The Ethylene Ketal Protecting Group Revisited: The Synthesis of 4-Hydroxy-4,4-diphenyl-2-butanone. *Journal of Chemical Education* **2005,** *82* (7), 1057.

202. Alber, J. P.; DeGrand, M. J.; Cermak, D. M., Synthesis of 5,5-Diphenyl-4-penten-2-one: A Variation on a Classic Organic Synthesis Laboratory. *Journal of Chemical Education* **2011,** *88* (1), 82-85.

203. Kocienski, P. J., *Protecting Groups*. Thieme: 2005.

204. Ashworth, I. W.; Cox, B. G.; Meyrick, B., Kinetics and Mechanism of N-Boc Cleavage: Evidence of a Second-Order Dependence upon Acid Concentration. *The Journal of Organic Chemistry* **2010,** *75* (23), 8117-8125.

205. Roughley, S. D.; Jordan, A. M., The Medicinal Chemist's Toolbox: An Analysis of Reactions Used in the Pursuit of Drug Candidates. *Journal of Medicinal Chemistry* **2011,** *54* (10), 3451-3479.

206. Lu, X.; Pellechia, P. J.; Flora, J. R. V.; Berge, N. D., Influence of reaction time and temperature on product formation and characteristics associated with the hydrothermal carbonization of cellulose. *Bioresource Technology* **2013,** *138* (Supplement C), 180-190.

207. Pindur, U.; Lutz, G.; Otto, C., Acceleration and selectivity enhancement of Diels-Alder reactions by special and catalytic methods. *Chemical Reviews* **1993,** *93* (2), 741-761.

208. Rideout, D. C.; Breslow, R., Hydrophobic acceleration of Diels-Alder reactions. *Journal of the American Chemical Society* **1980,** *102* (26), 7816-7817.

209. Narayan, S.; Muldoon, J.; Finn, M. G.; Fokin, V. V.; Kolb, H. C.; Sharpless, K. B., "On Water": Unique Reactivity of Organic Compounds in Aqueous Suspension. *Angewandte Chemie International Edition* **2005,** *44* (21), 3275-3279.

210. Breslow, R., Hydrophobic effects on simple organic reactions in water. *Accounts of Chemical Research* **1991,** *24* (6), 159-164.

211. Mellouli, S.; Bousekkine, L.; Theberge, A. B.; Huck, W. T. S., Investigation of "On Water" Conditions Using a Biphasic Fluidic Platform. *Angewandte Chemie International Edition* **2012,** *51* (32), 7981-7984.

212. Fallah-Araghi, A.; Meguellati, K.; Baret, J.-C.; Harrak, A. E.; Mangeat, T.; Karplus, M.; Ladame, S.; Marques, C. M.; Griffiths, A. D., Enhanced Chemical Synthesis at Soft Interfaces: A Universal Reaction-Adsorption Mechanism in Microcompartments. *Physical Review Letters* **2014,** *112* (2), 028301.

213. Müller, T.; Badu-Tawiah, A.; Cooks, R. G., Accelerated Carbon□Carbon Bond-Forming Reactions in Preparative Electrospray. *Angewandte Chemie International Edition* **2012,** *51* (47), 11832-11835.

214. Lee, J. K.; Kim, S.; Nam, H. G.; Zare, R. N., Microdroplet fusion mass spectrometry for fast reaction kinetics. *Proceedings of the National Academy of Sciences* **2015,** *112* (13), 3898-3903.

215. Bain, R. M.; Pulliam, C. J.; Ayrton, S. T.; Bain, K.; Cooks, R. G., Accelerated hydrazone formation in charged microdroplets. *Rapid Communications in Mass Spectrometry* **2016,** *30* (16), 1875-1878.

216. Bain, R. M.; Sathyamoorthi, S.; Zare, R. N., "On-Droplet" Chemistry: The Cycloaddition of Diethyl Azodicarboxylate and Quadricyclane. *Angewandte Chemie International Edition* **2017,** *56* (47), 15083-15087.

217. Banerjee, S.; Zare, R. N., Syntheses of Isoquinoline and Substituted Quinolines in Charged Microdroplets. *Angewandte Chemie International Edition* **2015,** *54* (49), 14795-14799.

218. Banerjee, S.; Gnanamani, E.; Yan, X.; Zare, R. N., Can all bulk-phase reactions be accelerated in microdroplets? *Analyst* **2017,** *142* (9), 1399-1402.

219. Bain, R. M.; Pulliam, C. J.; Cooks, R. G., Accelerated Hantzsch electrospray synthesis with temporal control of reaction intermediates. *Chemical Science* **2015,** *6* (1), 397-401.

220. Ewan, H. S.; Iyer, K.; Hyun, S.-H.; Wleklinski, M.; Cooks, R. G.; Thompson, D. H., Multistep Flow Synthesis of Diazepam Guided by Droplet-Accelerated Reaction Screening with Mechanistic Insights from Rapid Mass Spectrometry Analysis. *Organic Process Research & Development* **2017**.

221. Espy, R. D.; Wleklinski, M.; Yan, X.; Cooks, R. G., Beyond the flask: Reactions on the fly in ambient mass spectrometry. *TrAC Trends in Analytical Chemistry* **2014,** *57* (Supplement C), 135-146.

222. Lee, J. K.; Banerjee, S.; Nam, H. G.; Zare, R. N., Acceleration of reaction in charged microdroplets. *Quarterly Reviews of Biophysics* **2015,** *48* (4), 437-444.

223. Wei, Z.; Wleklinski, M.; Ferreira, C.; Cooks, R. G., Reaction Acceleration in Thin Films with Continuous Product Deposition for Organic Synthesis. *Angewandte Chemie International Edition* **2017,** *56* (32), 9386-9390.

224. Bain, R. M.; Pulliam, C. J.; Thery, F.; Cooks, R. G., Accelerated Chemical Reactions and Organic Synthesis in Leidenfrost Droplets. *Angewandte Chemie International Edition* **2016,** *55* (35), 10478-10482.

225. Brown, D. G.; Boström, J., Analysis of Past and Present Synthetic Methodologies on Medicinal Chemistry: Where Have All the New Reactions Gone? *Journal of Medicinal Chemistry* **2016,** *59* (10), 4443-4458.

226. Li, Y.; Liu, Y.; Gao, H.; Helmy, R.; Wuelfing, W. P.; Welch, C.; Cooks, R. G., Accelerated Forced Degradation of Pharmaceuticals in Levitated Microdroplet Reactors. *Chem. Eur. J.* **2018**.

227. Luján-Upton, H., Introducing Stereochemistry to Non-science Majors. *Journal of Chemical Education* **2001,** *78* (4), 475.

228. Fossey, J. S.; Anslyn, E. V.; Brittain, W. D. G.; Bull, S. D.; Chapin, B. M.; Le Duff, C. S.; James, T. D.; Lees, G.; Lim, S.; Lloyd, J. A. C.; Manville, C. V.; Payne, D. T.; Roper, K. A., Rapid Determination of Enantiomeric Excess via NMR Spectroscopy: A Research-Informed Experiment. *Journal of Chemical Education* **2017,** *94* (1), 79-84.

229. McCullagh, J. V., The Resolution of Ibuprofen, 2-(4'-Isobutylphenyl)propionic Acid. *Journal of Chemical Education* **2008,** *85* (7), 941.

230. Sen, S. E.; Anliker, K. S., 1H NMR Analysis of R/S Ibuprofen by the Formation of Diasteriomeric Pairs: Microscale Stereochemistry Experiment for the Undergraduate Organic Laboratory. *Journal of Chemical Education* **1996,** *73* (6), 569.

231. Bain, R. M.; Yan, X.; Raab, S. A.; Ayrton, S. T.; Flick, T. G.; Cooks, R. G., On-line chiral analysis using the kinetic method. *Analyst* **2016,** *141* (8), 2441-2446.

232. Ranc, V.; Havlíček, V.; Bednar, P.; Lemr, K., Nano-desorption electrospray and kinetic method in chiral analysis of drugs in whole human blood samples. *European Journal of Mass Spectrometry* **2008,** *14* (6), 411-417.

233. Wu, L.; Andy Tao, W.; Cooks, R. G., Kinetic method for the simultaneous chiral analysis of different amino acids in mixtures. *Journal of Mass Spectrometry* **2003,** *38* (4), 386-393.

234. Cooks, R. G.; Kruger, T. L., Intrinsic basicity determination using metastable ions. *Journal of the American Chemical Society* **1977,** *99* (4), 1279-1281.

235. McLuckey, S. A.; Cameron, D.; Cooks, R. G., Proton affinities from dissociations of proton-bound dimers. *Journal of the American Chemical Society* **1981,** *103* (6), 1313-1317.

236. Tao, W. A.; Gozzo, F. C.; Cooks, R. G., Mass Spectrometric Quantitation of Chiral Drugs by the Kinetic Method. *Analytical Chemistry* **2001,** *73* (8), 1692-1698.

237. Tao, W. A.; Zhang, D.; Wang, F.; Thomas, P. D.; Cooks, R. G., Kinetic Resolution of d,l-Amino Acids Based on Gas-Phase Dissociation of Copper(II) Complexes. *Analytical Chemistry* **1999,** *71* (19), 4427-4429.

238. Cooks, R. G.; Wong, P. S. H., Kinetic Method of Making Thermochemical Determinations: Advances and Applications. *Accounts of Chemical Research* **1998,** *31* (7), 379-386.

239. Yu, X.; Yao, Z.-P., Chiral recognition and determination of enantiomeric excess by mass spectrometry: A review. *Analytica Chimica Acta* **2017,** *968*, 1-20.

240. Graham Cooks, R.; Patrick, J. S.; Kotiaho, T.; McLuckey, S. A., Thermochemical determinations by the kinetic method. *Mass Spectrometry Reviews* **1994,** *13* (4), 287-339.

241. Bain, K.; Towns, M. H., A review of research on the teaching and learning of chemical kinetics. *Chemistry Education Research and Practice* **2016,** *17* (2), 246-262.

242. Parsons, M. L., The definition of detection limits. *Journal of Chemical Education* **1969,** *46* (5), 290.

243. Laursen, S.; Hunter, A.-B.; Seymour, E.; Thiry, H.; Melton, G., *Undergraduate Research in the Sciences: Engaging Students in Real Science*. John Wiley & Sons: San Francisco, USA, 2010.

244. DeKorver, B. K.; Towns, M. H., General Chemistry Students' Goals for Chemistry Laboratory Coursework. *Journal of Chemical Education* **2015,** *92* (12), 2031-2037.

245. Simon, L. L.; Pataki, H.; Marosi, G.; Meemken, F.; Hungerbühler, K.; Baiker, A.; Tummala, S.; Glennon, B.; Kuentz, M.; Steele, G.; Kramer, H. J. M.; Rydzak, J. W.; Chen, Z.; Morris, J.; Kjell, F.; Singh, R.; Gani, R.; Gernaey, K. V.; Louhi-Kultanen, M.; O'Reilly, J.; Sandler, N.; Antikainen, O.; Yliruusi, J.; Frohberg, P.; Ulrich, J.; Braatz, R. D.; Leyssens, T.; von Stosch, M.; Oliveira, R.; Tan, R. B. H.; Wu, H.; Khan, M.; O'Grady, D.; Pandey, A.; Westra, R.; Delle-Case, E.; Pape, D.; Angelosante, D.; Maret, Y.; Steiger, O.; Lenner, M.; Abbou-Oucherif, K.; Nagy, Z. K.; Litster, J. D.; Kamaraju, V. K.; Chiu, M.-S., Assessment of Recent Process Analytical Technology (PAT) Trends: A Multiauthor Review. *Organic Process Research & Development* **2015,** *19* (1), 3-62.

246. Wu, H.; Dong, Z.; Li, H.; Khan, M., An Integrated Process Analytical Technology (PAT) Approach for Pharmaceutical Crystallization Process Understanding to Ensure Product Quality and Safety: FDA Scientist's Perspective. *Organic Process Research & Development* **2015,** *19* (1), 89-101.

247. Chanda, A.; Daly, A. M.; Foley, D. A.; LaPack, M. A.; Mukherjee, S.; Orr, J. D.; Reid, G. L.; Thompson, D. R.; Ward, H. W., Industry Perspectives on Process Analytical Technology: Tools and Applications in API Development. *Organic Process Research & Development* **2015,** *19* (1), 63-83.

248. Bordawekar, S.; Chanda, A.; Daly, A. M.; Garrett, A. W.; Higgins, J. P.; LaPack, M. A.; Maloney, T. D.; Morgado, J.; Mukherjee, S.; Orr, J. D.; Reid, G. L.; Yang, B.-S.; Ward, H. W., Industry Perspectives on Process Analytical Technology: Tools and Applications in API Manufacturing. *Organic Process Research & Development* **2015,** *19* (9), 1174-1185.

249. Streefland, M.; Martens Dirk, E.; Beuvery, E. C.; Wijffels René, H., Process analytical technology (PAT) tools for the cultivation step in biopharmaceutical production. *Engineering in Life Sciences* **2013,** *13* (3), 212-223.

250. Sever, N. E.; Warman, M.; Mackey, S.; Dziki, W.; Jiang, M., Chapter 35 - Process Analytical Technology in Solid Dosage Development and Manufacturing A2 - Qiu, Yihong. In *Developing Solid Oral Dosage Forms*, Chen, Y.; Zhang, G. G. Z.; Liu, L.; Porter, W. R., Eds. Academic Press: San Diego, 2009; pp 827-841.

251. van den Berg, F.; Lyndgaard, C. B.; Sørensen, K. M.; Engelsen, S. B., Process Analytical Technology in the food industry. *Trends in Food Science & Technology* **2013,** *31* (1), 27-35.

252. Craven, S.; Whelan, J., Process Analytical Technology and Quality-by-Design for Animal Cell Culture. In *Animal Cell Culture*, Al-Rubeai, M., Ed. Springer International Publishing: Cham, 2015; pp 647-688.

253. Fedick, P. W.; Bills, B. J.; Manicke, N. E.; Cooks, R. G., Forensic Sampling and Analysis from a Single Substrate: Surface-Enhanced Raman Spectroscopy Followed by Paper Spray Mass Spectrometry. *Analytical Chemistry* **2017,** *89* (20), 10973-10979.

254. Esmonde-White, K. A.; Cuellar, M.; Uerpmann, C.; Lenain, B.; Lewis, I. R., Raman spectroscopy as a process analytical technology for pharmaceutical manufacturing and bioprocessing. *Analytical and Bioanalytical Chemistry* **2017,** *409* (3), 637-649.

255. Simpson Michael, B., Near-Infrared Spectroscopy for Process Analytical Technology: Theory, Technology and Implementation. *Process Analytical Technology* **2010**.

256. Shekunov, B. Y.; Chattopadhyay, P.; Tong, H. H. Y.; Chow, A. H. L., Particle Size Analysis in Pharmaceutics: Principles, Methods and Applications. *Pharmaceutical Research* **2007,** *24* (2), 203-227.

257. Brückner, A.; Kondratenko, E., Simultaneous operando EPR/UV–vis/laser–Raman spectroscopy — A powerful tool for monitoring transition metal oxide catalysts during reaction. *Catalysis Today* **2006,** *113* (1), 16-24.

258. Benito-Lopez, F.; Verboom, W.; Kakuta, M.; Gardeniers, J. G. E.; Egberink, R. J. M.; Oosterbroek, E. R.; van den Berg, A.; Reinhoudt, D. N., Optical fiber-based on-line UV/Vis spectroscopic monitoring of chemical reaction kinetics under high pressure in a capillary microreactor. *Chemical Communications* **2005,** (22), 2857-2859.

259. Wang, L.; Zeng, S.; Chen, T.; Qu, H., Direct analysis in real time mass spectrometry, a process analytical technology tool for real-time process monitoring in botanical drug manufacturing. *Journal of Pharmaceutical and Biomedical Analysis* **2014,** *91*, 202-209.

260. Yan, X.; Sokol, E.; Li, X.; Li, G.; Xu, S.; Cooks, R. G., On-Line Reaction Monitoring and Mechanistic Studies by Mass Spectrometry: Negishi Cross-Coupling, Hydrogenolysis, and Reductive Amination. *Angewandte Chemie International Edition* **2014,** *53* (23), 5931-5935.

261. Yan, X.; Bain, R. M.; Li, Y.; Qiu, R.; Flick, T. G.; Cooks, R. G., Online Inductive Electrospray Ionization Mass Spectrometry as a Process Analytical Technology Tool To Monitor the Synthetic Route to Anagliptin. *Organic Process Research & Development* **2016,** *20* (5), 940-947.

262. Pulliam, C. J.; Bain, R. M.; Osswald, H. L.; Snyder, D. T.; Fedick, P. W.; Ayrton, S. T.; Flick, T. G.; Cooks, R. G., Simultaneous Online Monitoring of Multiple Reactions Using a Miniature Mass Spectrometer. *Analytical Chemistry* **2017,** *89* (13), 6969-6975.

263. Vikse, K. L.; Ahmadi, Z.; Manning, C. C.; Harrington, D. A.; McIndoe, J. S., Powerful Insight into Catalytic Mechanisms through Simultaneous Monitoring of Reactants, Products, and Intermediates. *Angewandte Chemie International Edition* **2011,** *50* (36), 8304-8306.

264. Theron, R.; Wu, Y.; Yunker, L. P. E.; Hesketh, A. V.; Pernik, I.; Weller, A. S.; McIndoe, J. S., Simultaneous Orthogonal Methods for the Real-Time Analysis of Catalytic Reactions. *ACS Catalysis* **2016,** *6* (10), 6911-6917.

265. Vikse, K. L.; Woods, M. P.; McIndoe, J. S., Pressurized Sample Infusion for the Continuous Analysis of Air- And Moisture-Sensitive Reactions Using Electrospray Ionization Mass Spectrometry. *Organometallics* **2010,** *29* (23), 6615-6618.

266. Fedick, P. W.; Bain, R. M.; Miao, S.; Pirro, V.; Cooks, R. G., State-of-the-art mass spectrometry for point-of-care and other applications: A hands-on intensive short course for undergraduate students. *International Journal of Mass Spectrometry* **2017,** *417*, 22-28.

267. Brown, M. E.; Cosser, R. C.; Davies-Coleman, M. T.; Kaye, P. T.; Klein, R.; Lamprecht, E.; Lobb, K.; Nyokong, T.; Sewry, J. D.; Tshentu, Z. R.; van der Zeyde, T.; Watkins, G. M., Introducing Chemistry Students to the "Real World" of Chemistry. *Journal of Chemical Education* **2010,** *87* (5), 500-503.

268. Dunn, J. G.; Kagi, R. I.; Phillips, D. N., Developing Professional Skills in a Third-Year Undergraduate Chemistry Course Offered in Western Australia. *Journal of Chemical Education* **1998,** *75* (10), 1313.

269. Fahey, A.; Tyson, J., Instrumental Analysis in the Undergraduate Curriculum. *Analytical Chemistry* **2006,** *78* (13), 4249-4254.

270. Hao, H.; Barrett, M.; Hu, Y.; Su, W.; Ferguson, S.; Wood, B.; Glennon, B., The Use of in Situ Tools To Monitor the Enantiotropic Transformation of p-Aminobenzoic Acid Polymorphs. *Organic Process Research & Development* **2012,** *16* (1), 35-41.

271. Proctor, L. D.; Warr, A. J., Development of a Continuous Process for the Industrial Generation of Diazomethane1. *Organic Process Research & Development* **2002,** *6* (6), 884-892.

272. Kosenkov, D.; Shaw, J.; Zuczek, J.; Kholod, Y., Transient-Absorption Spectroscopy of Cis−Trans Isomerization of N,N-Dimethyl-4,4′-azodianiline with 3D-Printed Temperature-Controlled Sample Holder. *Journal of Chemical Education* **2016,** *93* (7), 1299-1304.

273. Porter, L. A.; Washer, B. M.; Hakim, M. H.; Dallinger, R. F., User-Friendly 3D Printed Colorimeter Models for Student Exploration of Instrument Design and Performance. *Journal of Chemical Education* **2016,** *93* (7), 1305-1309.

274. Porter, L. A.; Chapman, C. A.; Alaniz, J. A., Simple and Inexpensive 3D Printed Filter Fluorometer Designs: User-Friendly Instrument Models for Laboratory Learning and Outreach Activities. *Journal of Chemical Education* **2017,** *94* (1), 105-111.

275. Davis, E. J.; Jones, M.; Thiel, D. A.; Pauls, S., Using Open-Source, 3D Printable Optical Hardware To Enhance Student Learning in the Instrumental Analysis Laboratory. *Journal of Chemical Education* **2018**.

276. Suzuki, A., Cross-Coupling Reactions Of Organoboranes: An Easy Way To Construct C□C Bonds (Nobel Lecture). *Angewandte Chemie International Edition* **2011,** *50* (30), 6722-6737.

277. Miyaura, N.; Yamada, K.; Suzuki, A., A new stereospecific cross-coupling by the palladium-catalyzed reaction of 1-alkenylboranes with 1-alkenyl or 1-alkynyl halides. *Tetrahedron Letters* **1979,** *20* (36), 3437-3440.

278. Miyaura, N.; Suzuki, A., Stereoselective synthesis of arylated (E)-alkenes by the reaction of alk-1-enylboranes with aryl halides in the presence of palladium catalyst. *Journal of the Chemical Society, Chemical Communications* **1979,** (19), 866-867.

279. Han, F.-S., Transition-metal-catalyzed Suzuki-Miyaura cross-coupling reactions: a remarkable advance from palladium to nickel catalysts. *Chemical Society Reviews* **2013,** *42* (12), 5270-5298.

280. Yang, C. T.; Zhang, Z. Q.; Liu, Y. C.; Liu, L., Copper-Catalyzed Cross-Coupling Reaction of Organoboron Compounds with Primary Alkyl Halides and Pseudohalides. *Angewandte Chemie International Edition* **2011,** *50* (17), 3904-3907.

281. Baba, S.; Negishi, E., A novel stereospecific alkenyl-alkenyl cross-coupling by a palladium- or nickel-catalyzed reaction of alkenylalanes with alkenyl halides. *Journal of the American Chemical Society* **1976,** *98* (21), 6729-6731.

282. Heck, R. F., Acylation, methylation, and carboxyalkylation of olefins by Group VIII metal derivatives. *Journal of the American Chemical Society* **1968,** *90* (20), 5518-5526.

283. Leadbeater, N. E.; Marco, M., Ligand-Free Palladium Catalysis of the Suzuki Reaction in Water Using Microwave Heating. *Organic Letters* **2002,** *4* (17), 2973-2976.

284. Yan, X.; Bain Ryan, M.; Cooks, R. G., Organic Reactions in Microdroplets: Reaction Acceleration Revealed by Mass Spectrometry. *Angewandte Chemie International Edition* **2016,** *55* (42), 12960-12972.

285. Augusti, R.; Chen, H.; Eberlin, L. S.; Nefliu, M.; Cooks, R. G., Atmospheric pressure Eberlin transacetalization reactions in the heterogeneous liquid/gas phase. *International Journal of Mass Spectrometry* **2006,** *253* (3), 281-287.

286. Yan, X.; Augusti, R.; Li, X.; Cooks, R. G., Chemical Reactivity Assessment Using Reactive Paper Spray Ionization Mass Spectrometry: The Katritzky Reaction. *ChemPlusChem* **2013,** *78* (9), 1142-1148.

287. Bain Ryan, M.; Pulliam Christopher, J.; Ayrton Stephen, T.; Bain, K.; Cooks, R. G., Accelerated hydrazone formation in charged microdroplets. *Rapid Communications in Mass Spectrometry* **2016,** *30* (16), 1875-1878.

288. Hollerbach, A.; Logsdon, D.; Iyer, K.; Li, A.; Schaber, J. A.; Graham Cooks, R., Sizing sub-diffraction limit electrosprayed droplets by structured illumination microscopy. *Analyst* **2018,** *143* (1), 232-240.

289. Bain, R. M.; Ayrton, S. T.; Cooks, R. G., Fischer Indole Synthesis in the Gas Phase, the Solution Phase, and at the Electrospray Droplet Interface. *Journal of The American Society for Mass Spectrometry* **2017,** *28* (7), 1359-1364.

290. Bain Ryan, M.; Sathyamoorthi, S.; Zare Richard, N., "On-Droplet" Chemistry: The Cycloaddition of Diethyl Azodicarboxylate and Quadricyclane. *Angewandte Chemie International Edition* **2017,** *56* (47), 15083-15087.

291. Li, Y.; Yan, X.; Cooks, R. G., The Role of the Interface in Thin Film and Droplet Accelerated Reactions Studied by Competitive Substituent Effects. *Angewandte Chemie International Edition* **2016,** *55* (10), 3433-3437.

292. Falcone, C. E.; Jaman, Z.; Wleklinski, M.; Koswara, A.; Thompson, D. H.; Cooks, R. G., Reaction screening and optimization of continuous-flow atropine synthesis by preparative electrospray mass spectrometry. *Analyst* **2017,** *142* (15), 2836-2845.

293. Iyer, K.; Yi, J.; Bogdan, A.; Talaty, N.; Djuric, S. W.; Cooks, R. G., Accelerated multi-reagent copper catalysed coupling reactions in micro droplets and thin films. *Reaction Chemistry & Engineering* **2018,** *3* (2), 206-209.

294. Wleklinski, M.; Loren, B. P.; Ferreira, C. R.; Jaman, Z.; Avramova, L.; Sobreira, T. J. P.; Thompson, D. H.; Cooks, R. G., High throughput reaction screening using desorption electrospray ionization mass spectrometry. *Chemical Science* **2018,** *9* (6), 1647-1653.

295. Wleklinski, M.; Falcone Caitlin, E.; Loren Bradley, P.; Jaman, Z.; Iyer, K.; Ewan, H. S.; Hyun, S. H.; Thompson David, H.; Cooks, R. G., Can Accelerated Reactions in Droplets Guide Chemistry at Scale? *European Journal of Organic Chemistry* **2016,** *2016* (33), 5480-5484.

296. Bain Ryan, M.; Pulliam Christopher, J.; Thery, F.; Cooks, R. G., Accelerated Chemical Reactions and Organic Synthesis in Leidenfrost Droplets. *Angewandte Chemie International Edition* **2016,** *55* (35), 10478-10482.

297. Quéré, D., Leidenfrost Dynamics. *Annual Review of Fluid Mechanics* **2013,** *45* (1), 197-215.

298. Ewan, H. S.; Iyer, K.; Hyun, S.-H.; Wleklinski, M.; Cooks, R. G.; Thompson, D. H., Multistep Flow Synthesis of Diazepam Guided by Droplet-Accelerated Reaction Screening with Mechanistic Insights from Rapid Mass Spectrometry Analysis. *Organic Process Research & Development* **2017,** *21* (10), 1566-1570.

299. Li, Y.; Liu, Y.; Gao, H.; Helmy, R.; Wuelfing, W. P.; Welch Christopher, J.; Cooks, R. G., Accelerated Forced Degradation of Pharmaceuticals in Levitated Microdroplet Reactors. *Chemistry – A European Journal* **2018,** *0* (0).

300. Kotha, S.; Lahiri, K.; Kashinath, D., Recent Applications of the Suzuki—Miyaura Cross-Coupling Reaction in Organic Synthesis. *ChemInform* **2003,** *34* (9).

VITA

Patrick Walter Fedick
www.linkedin.com/in/pfedick

Education

Purdue University, West Lafayette, IN (2018)
PhD in Analytical Chemistry,
Department of Defense SMART Scholar

Monmouth University, West Long Branch, NJ (2014) ***Summa Cum Laude***
B.S in Chemistry (ACS Certified), Minor: Physics
B.A. in Psychology, Second Minor: Information Technology
Certification – Information Technology

Scientific Research Experience

Purdue University, West Lafayette, IN
Graduate Research Assistant for Dr. R. Graham Cooks *Fall 2014 – Fall 2018*

- PhD committee members: Professors R. Graham Cooks (chair), Hilkka I. Kenttämaa, Marcy H. Towns and Mary A. Wirth
- Thesis title: "Ambient Ionization Mass Spectrometry: Advances in Monitoring Clandestine Activities, Supporting the Warfighter, and Chemical Laboratory Education Redevelopment."
- Research primarily in areas of defense and forensic-based analytical mass spectrometry, reactive mass spectrometry, portable mass spectrometry, and chemical education mass spectrometry projects:
  - Reactive paper spray ionization mass spectrometry for the improved ionization efficiency and fragmentation of illicit substances (GHB and THC), as well as explosives (TNT, RDX, HMX and PETN).
  - Multi-vessel on-line reaction monitoring and advances to process analytical technology through miniature mass spectrometry.
  - Forensic applications of multigenerational CID using a miniature mass spectrometer.
  - Screening panel for amphetamines in whole blood using a miniature mass spectrometer.
  - Identification of organic gunshot and explosive residues through touch swab spray ionization using both benchtop and miniature mass spectrometers.
  - Authentication of integrated circuits through ambient ionization using both benchtop and miniature mass spectrometers.
  - Dual-Analyzer interrogation of drugs of abuse, explosives and chemical warfare agent simulants through paper surfaced enhanced Raman spectroscopy followed by paper spray ionization mass spectrometry.
  - Assessment of harmful byproducts (chloride type agents) that can be formed during combustion in pyrotechnic military smokes through ambient ionization mass spectrometry.
  - Reaction acceleration through spray, thin film and Leidenfrost droplets
  - Chemical education, novel ways of integrating mass spectrometry into the undergraduate teaching laboratory.
- Engaged in both government funded (National Science Foundation, Department of Energy, Naval Surface Warfare Center Crane) and privately funded (ConocoPhillips and Amgen) projects gaining experience with a variety of diverse types of scientific research and goals.
- Graduate mentor for undergraduate student Saerom Kim, working on reactive paper spray projects.

Department of Navy, China Lake, CA *Summer 2018*

SMART (Science Mathematics and Research for Transformation) Scholar

- Twelve-Week Summer Graduate Research Appointment with the Naval Air Warfare Center China Lake, Weapons Division, Analytical Chemistry Branch.
  - Battlefield forensics for the trace detection of explosive compounds by paper surfaced enhanced Raman followed by paper spray mass spectrometry on portable systems.

Department of Navy, Crane, IN *Summer 2017*

Naval Research Enterprise Internship Program (NREIP) Intern for Dr. Jack Caldwell and Dr. Jonathan Dilger

- Ten-Week Summer Graduate Internship with the Naval Surface Warfare Center Crane, Materials Division.
  - Forensic analysis of integrated circuits by TOF-SIMS, DESI, and LTP.
    - Rapid screening for counterfeit and authentic integrated circuits
    - Origin of foundry diagnostics.
  - Environmental impact of pyrotechnic "smokes"
    - Looked at by harmful byproducts (chloride type agents) that can be formed during combustion through pyrolysis GC-MS.
    - Utilize swab touch spray for a rapid ambient monitoring of these harmful byproducts.

Department of Homeland Security, Springfield, VA *Summer 2015*

Homeland Security Science, Technology, Engineering, and Mathematics (HS-STEM) Intern for Dr. Jun-Ling You and Dr. Jusheng Qi

- Ten-Week Summer Graduate Internship with the U.S. Customs and Border Protection, Laboratories and Scientific Services Directorate, Office of Information and Technology (OIT).
  - Rapid screening of synthetic cannabinoids and designer drugs by paper spray mass spectrometry.
  - Employed a Prosolia Velox 360 Paper Spray ion source incorporated with an AB Sciex 4500 Qtrap mass spectrometer.

Monmouth University, West Long Branch, NJ

Chemistry Researcher for Dr. Tsanangurayi Tongesayi, *Fall 2011 – Spring 2014*

- Team leader for multiple research projects:
  - Investigation of alternative reagents to perform Fenton's reaction through photochemical reactions monitored by UV-Vis.
  - Heavy metal analysis in rice monitored by XRF spectroscopy and flame atomic absorption.
  - Arsenic remediation in natural water monitored by graphite furnace and cyclic voltammetry.
  - Chromium speciation monitoring in natural water by UV-Vis.
  - Analysis of chlorine in water through UV-Vis.
  - Iron oxide nanoparticle synthesis and characterization for environmental applications.

University of Massachusetts Amherst, Amherst, MA *Summer 2013*

Chemistry Researcher for Dr. Richard Vachet and Dr. Vincent Rotello, Graduate Mentor – Bo Yan,

- Ten-Week Summer Undergraduate Research Experience (SURE) – funded by the National Science Foundation
  - Detection method development of gold nanoparticle ligand replacement by glutathione using matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF).
  - Monitoring host-guest chemistry, specifically Cucurbit[7]uril gold nanoparticle complexes, in cells using MALDI-TOF.

Seton Hall University, South Orange, NJ *Spring 2011*

Biology Researcher for Dr. Heping Zhou,

- Preparation, optimization, and analysis of microarray slides and standards to examine gene expression.

Other Science Experience

Purdue University, West Lafayette, IN
Chemistry Graduate Teaching Assistant *Fall 2014 – Fall 2016*

- Attended lectures by the instructor whom I assisted, held office hours, led recitation sections and laboratory sections, demonstrated laboratory equipment, and graded students' laboratory reports.
  - Fall 2016 – Three sections of Advanced Analytical Chemistry (analytical chemistry II)
  - Fall 2015 – Two sections of Advanced Analytical Chemistry (analytical chemistry II)
  - Spring 2015 – Two sections of General Chemistry 115 (engineering majors)
  - Fall 2014 – Two sections of General Chemistry 115 (engineering majors)

Private Chemistry Tutor *Fall 2015 – Fall 2018*

- Held private individual and group tutoring sessions for students in General Chemistry 115.

Monmouth University, West Long Branch, NJ
Chemistry Lab Assistant *Spring 2012 – Spring 2014*

- Facilitated laboratory set up, prepared reagents, assisted professor, and answered student's questions.
  - Spring 2014 – Two sections of Analytical Chemistry I and two sections of Analytical Chemistry II
  - Fall 2013 – General Chemistry I Honors
  - Spring 2013 –General Chemistry I and Analytical Chemistry I
  - Fall 2012 – General Chemistry I and Environmental Chemistry
  - Spring 2012 – General Chemistry II

Supplemental Instruction Leader *Fall 2013 – Spring 2014*

- Spring 2014 – Analytical Chemistry II (Instrumental Analysis)
- Fall 2013 – Environmental Chemistry

Peer Tutor *Fall 2012 – Spring 2014*

- Physics I and II, Environmental Chemistry, Quantitative Analysis, Instrumental Analysis, Information Technology, Windows Applications: Program Design and Implementation, Information Systems Project Management, and Internet and Network Technology.

Peer Learning Assistant *Fall 2012 and Fall 2013*

- First Year Seminar Course "Dinosaurs and DNA: Biology in the Movies"

Peer Mentor Coordinator for the School of Science *Spring 2013 – Spring 2014*
Peer Mentor for the School of Science *Summer 2012 – Spring 2013*
Information Technology Lab Assistant *Spring 2013 – Spring 2014*

- Spring 2014 – Windows Applications: Program Design and Implementation
- Fall 2013 – Two sections of Information Technology
- Summer 2013 – Information Systems Project Management
- Spring 2013 – Information Technology and two sections of Information Systems Project Management

Other Work Experiences

FBI Honors Internship Program (HIP) Intern *Summer 2014 – Spring 2015*
Newark Division, Garret Mountain Residence Agency, Woodland Park, NJ *Summer 2014*

- Worked on Squad 1 of the Garret Mountain Residence Agency dealing primarily with cases related to crimes against children, human trafficking, and prostitution.

Indianapolis Division, Field Office, Indianapolis, Indiana *Fall 2014 – Spring 2015*

- Worked on Squad C7 of the Indianapolis Field Office dealing primarily with cases related to public corruption, human trafficking, color of law abuse, and prostitution.

Presentations (as Presenting Author)

1. **Fedick, Patrick**; Schrader, Robert; Hoerter, Robert; Pirro, Valentina; Dilger, Jonathan; Caldwell, Jack; Cooks, R. Graham. "Determination of Authenticity of Plastic Encapsulated Integrated Circuits in the

Supply Chain." Dawn or Doom Conference, West Lafayette, IN, United States, November 2018. (Poster)

2. **Fedick, Patrick**; Thoreson, Kelly; Papenmeier, Douglas; Dilger, Jonathan; Cooks, R. Graham. "Chemical Analysis of Red Smokes By-products Using Ambient Ionization Mass Spectrometry" Turkey Run Conference on Analytical Chemistry, Marshall, IN, United States, November 2018. (Poster)
3. **Fedick, Patrick**; Bain, Ryan; Fatigante, William; Mulligan, Christopher; Cooks, R. Graham. "Swab Touch Spray Ionization Mass Spectrometry for Rapid Analysis of Trace Residues of Forensic Relevance." 256th ACS National Meeting and Exposition, Boston, MA, United States, August 2018, ANYL – 557. (Oral)
4. **Fedick, Patrick**; Bain, Ryan; Bain, Kinsey; Schrader, Robert; Mehari, Tsdale; Pulliam, Christopher; Ayrton, Stephen; Cooks, R. Graham. "Redeveloping Chemistry Laboratory Exercises to Bring State-of-the-Art Novel Chemistry and Mass Spectrometry into the Teaching Laboratory." 256th ACS National Meeting and Exposition, Boston, MA, United States, August 2018, CHED – 439. (Oral)
5. **Fedick, Patrick**; Bain, Ryan; Bain, Kinsey; Schrader, Robert; Mehari, Tsdale; Pulliam, Christopher; Ayrton, Stephen; Cooks, R. Graham. "Redeveloping Chemistry Laboratory Exercises to Bring State-of-the-Art Novel Chemistry and Mass Spectrometry into the Teaching Laboratory." 256th ACS National Meeting and Exposition, Boston, MA, United States, August 2018, CHED - 439. Sci-Mix. (Poster)
6. **Fedick, Patrick**; Schrader, Robert; Hoerter, Robert; Pirro, Valentina; Dilger, Jonathan; Caldwell, Jack; Cooks, R. Graham. "Determination of Authenticity of Plastic Encapsulated Integrated Circuits in the Supply Chain." ASMS Conference & Exposition, San Diego, CA, United States, June 2018. (Poster)
7. **Fedick, Patrick**; Bain, Ryan. "Swab touch spray mass spectrometry for rapid analysis of organic gunshot residue from human hand and various surfaces using commercial and fieldable mass spectrometry systems." Impression, Pattern and Trace Evidence Symposium (IPTES), Arlington, VA, United States, January 2018 (Oral)
8. **Fedick, Patrick**; Bills, Brandon; Manicke, Nicholas; Cooks, R. Graham. "Forensic Sampling and Analysis from a Single Substrate: Surface Enhanced Raman Spectroscopy Followed by Paper Spray Mass Spectrometry" Impression, Pattern and Trace Evidence Symposium (IPTES), Arlington, VA, United States, January 2018 (Oral)
9. **Fedick, Patrick**; Bain, Ryan. "Swab Spray Mass Spectrometry for Rapid Analysis of Organic Gunshot Residue from Human Hand and Various Surfaces Using Commercial and Fieldable Mass Spectrometry Systems." Dawn or Doom Conference, West Lafayette, IN, United States, September 2017. (Poster)
10. **Fedick, Patrick**; Pulliam, Christopher; Snyder, Dalton; Bain, Ryan; Cooks, R. Graham. "Monitoring Clandestine Activities by the Mini 12" The 11th Harsh-Environment Mass Spectrometry Workshop, Oxnard, CA, September 2017. (Oral)
11. **Fedick, Patrick**; Bain, Ryan. "Swab Spray Mass Spectrometry for Rapid Analysis of Organic Gunshot Residue from Human Hand and Various Surfaces Using Commercial and Fieldable Mass Spectrometry Systems." 254th ACS National Meeting and Exposition, Washington, DC, United States, August 2017, Sci-Mix. (Poster)
12. **Fedick, Patrick**; Bain, Ryan. "Swab Spray Mass Spectrometry for Rapid Analysis of Organic Gunshot Residue from Human Hand and Various Surfaces Using Commercial and Fieldable Mass Spectrometry Systems." 254th ACS National Meeting and Exposition, Washington, DC, United States, August 2017, ANYL - 76. (Poster)
13. **Fedick, Patrick**; Bain, Ryan; Miao, Shunshun; Pirro, Valentina; Cooks, R. Graham. "Hands-on Intensive Short Course for Undergraduate Students: State-of-the-Art Mass Spectrometry for Point-of-Care and Other Applications." 254th ACS National Meeting and Exposition, Washington, DC, United States, August 2017, CHED - **77**. (Poster)

14. **Fedick, Patrick**. "DESI, LTP and TOF-SIMS of Integrated Circuits for Determination of Authenticity." Lunch and Learn Seminar, Naval Surface Warfare Center Crane, IN, United States, August 2017. (Oral)
15. **Fedick, Patrick**; Bain, Ryan; Bain, Kinsey; Cooks, R. Graham. "Chiral Analysis by Tandem Mass Spectrometry using the Kinetic Method, Polarimetry, and 1H NMR: An Organic I Laboratory." ASMS Conference & Exposition, Indianapolis, IN, United States, June 2017. (Poster)
16. **Fedick, Patrick**; You, Jun-Ling. "Rapid Screening of Designer Drugs by Paper Spray Mass Spectrometry" Presentation to the Department of Homeland Security, Customs Boarder Protection, August 2015. (Oral)
17. Bellivue, Kimberly; Caputo, Emily; Chace, Peter; **Fedick, Patrick**; Tongesayi, Tsanangurayi. "Magnetic Iron Oxide Nanoparticles: Environmental Impacts and Potential Applications" Monmouth's Thirteenth Annual School of Science Student Research Conference, Monmouth University, April 2014. (Poster)
18. **Fedick, Patrick**; Lechner, Lauren; Patel, Adit; Wioland, Kevin; Szwajkajzer, Danuta; Moehring, Greg. "Successful Chapter Activities of the Monmouth University Chemistry Club" 247th ACS National Meeting and Exposition, Dallas, TX, United States, April 2014, CHED - **1347**. (Poster)
19. **Fedick, Patrick**; Yan, Bo; Vachet, Richard. "Using Mass Spectrometry to Monitor Nanoparticles in Complex Biological Systems" Summer Undergraduate Research Experience (SURE) MassNanoTech, University of Massachusetts Amherst, August 2013. (Oral)
20. Tongesayi, Tsanangurayi; Bray, Chelsea; Brock, Christina; **Fedick, Patrick**; Lechner, Lauren. "Levels of lead in rice food products imported into the United States of America." 245th ACS National Meeting & Exposition, New Orleans, LA, United States, April 2013, Sci-Mix. (Poster)
21. Tongesayi, Tsanangurayi; Bray, Chelsea; Brock, Christina; **Fedick, Patrick**; Lechner, Lauren. "Levels of lead in rice food products imported into the United States of America." 245th ACS National Meeting & Exposition, New Orleans, LA, United States, April (2013), ENVR- **369**. (Poster)
22. Tongesayi, Tsanangurayi; Bray, Chelsea; **Fedick, Patrick**; Le Beau, Arielle; Brock, Christina; Lechner, Lauren. "Levels of arsenic and metals under the Restriction of Hazardous Substances (RoHS) (cadmium, chromium, and lead) in rice food products by country of origin." 244th ACS National Meeting & Exposition, Philadelphia, PA, United States, August 2012, Sci-Mix. (Poster)
23. Tongesayi, Tsanangurayi; Bray, Chelsea; **Fedick, Patrick**; Le Beau, Arielle; Brock, Christina; Lechner Lauren. "Levels of arsenic and metals under the Restriction of Hazardous Substances (RoHS) (cadmium, chromium, and lead) in rice food products by country of origin." 244th ACS National Meeting & Exposition, Philadelphia, PA, United States, August 2012, ENVR- **280.** (Poster)
24. Brock, Christina; **Fedick, Patrick**; Le Beau, Arielle; Lechner, Lauren; Tongesayi Tsanangurayi. "Lead in the Food Chain" Monmouth University's 3rd Annual Summer Research Symposium, August 2012. (Poster)
25. Elashal, Hader; **Fedick, Patrick;** Zhou Heping. "Use of Microarray Technology to Monitor the Expression of Inflammatory Mediators" Department of Chemistry and Biochemistry 15th Annual Departmental Symposium and Poster Session in conjunction with the Petersheim Academic Exposition, Seton Hall University, April 2011. (Poster)

Computer Fluency Skills

Microsoft: Excel, Word, PowerPoint and Access; Scifinder; Adobe Photoshop and Dreamweaver; Visual Basic; ChemDraw; Origin; Excalibur; Statistical Package for the Social Sciences; Autodesk Inventor.

Laboratory Instrumentation Skills

- Variety of Mass Analyzers – Linear Ion Traps, Triple Quadrupoles, Orbitraps, "Miniature" Ion Traps, Time of Flights – Mass Spectrometers.
- Variety of Ionization Techniques – Electrospray, Electrosonic Spray, Nano Spray, Paper Spray (also commercial Prosolia Velox 360 Paper Spray Source), Paper Cone Spray, Desorption Electrospray, Swab Touch Spray, Easy Ambient Sonic-Spray, Matrix Assisted Laser Desorption, Secondary Ion Mass Spectrometry, Atmospheric Pressure Chemical, Electron Impact, Chemical – Ionizations.

- Variety of Spectroscopic Techniques - UV-Vis, Atomic Absorption, X-Ray Fluorescence, Fluorescence, Infrared, Raman – Spectroscopies.

Awarded Honors

Department of Defense SMART Scholar *Fall 2017-Fall 2018*
Harsh Environment Mass Spectrometry Workshop Student Travel Award *Fall 2017*
New Jersey Association of Forensic Scientists Scholarship *Spring 2017*
Novartis Science Scholarship *Fall 2013 – Spring 2014*
Monmouth University Academic Excellence Scholarship *Fall 2011 – Spring 2014*
Seton Hall University Scholarship *Fall 2010 – Spring 2011*
Dean's List *Spring 2011 – Spring 2014*
American Chemical Society National Meeting Travel Grant ($300) Received *Spring 2014*
Monmouth University Department of Chemistry 2014 Chemistry Service Award *Spring 2014*
Monmouth University Department of Psychology 2014 Griffin Award *Spring 2014*
American Chemical Society 2013 Undergraduate Award in Analytical Chemistry *Spring 2013*
Omicron Delta Kappa Honor Society Inducted *Spring 2014*
Phi Lambda Upsilon Honor Society Inducted *Spring 2013*
Psi Chi Honor Society Inducted *Fall 2012*

Professional Affiliations

Member of the International Pyrotechnics Society *Summer 2017 – Present*
Member of the New Jersey Association of Forensic Scientists *Spring 2017 – Present*
Member of the American Academy of Forensic Sciences *Spring 2016 – Present*
- Member of the Criminalistics Subdivision

Member of the American Association for the Advancement of Science *Fall 2014 – Present*
Member of the American Society for Mass Spectrometry *Fall 2014 – Present*
Member of the American Chemical Society *Summer 2012 – Present*
- Member of the Analytical Subdivision

Community Affiliations

Member of the Graduate Student Health Insurance Selection Committee *Spring 2018*
Member of the Outstanding Graduate Faculty Mentor Award Selection Committee *Spring 2018*
Committee Member of the Purdue Votes Coalition *Fall 2017 – Summer 2018*
Committee Member of the Purdue Campus Community Bar Retail Coalition *Fall 2017 – Spring 2018*
Graduate Bill of Rights Committee Member *Summer 2017 – Spring 2018*
Executive Board Member on the Purdue Graduate Student Government *Summer 2017 – Spring 2018*
Chairman of Student Affairs and Community Outreach Subcommittees *Summer 2017 – Spring 2018*
Senate Representative of the Purdue Graduate Student Government *Summer 2017 – Spring 2018*
Elected Member of the Purdue Chemistry Graduate Student Advisory Board *Summer 2017 – Spring 2018*
Member Chemistry Graduate Student Advisory Board Charter Committee *Spring 2017 – Spring 2018*
Member of the Graduate Student Senate Subcommittee on Student Affairs *Fall 2016 – Spring 2018*
President of the Monmouth University ACS Student Chapter *Summer 2013 – Spring 2014*
Treasurer of the Monmouth University Community Service Club *Fall 2012 – Spring 2014*
Member of the Dean's Advisory Council *Fall 2012 – Spring 2013*

Professional Community Service

Peer Reviewer – Journal of the American Society for Mass Spectrometry *Summer 2018 - Present*
Session Presider – 256th ACS National Meeting ANYL: Advances in Mass Spectrometry *Spring 2018*
National Institute of Justice Grant Reviewer - Research and Evaluation on Drugs and Crime *Spring 2018*
Purdue Undergraduate Research Conference Judge *Spring 2018*
Organizer of the Big Grad Event at Purdue University *Spring 2018*
Organizer of the Next Generation Scholars Event at Purdue University *Fall 2017*
Career Panelist / Speaker for Purdue University Neurological Society *Fall 2016*
Organizer for Center for Analytical Instrumentation Development Workshop *Fall 2015 – Fall 2018*

- Fall 2018 – "Mass Spectrometry Recent Advances"
- Spring 2017 – "Celebration of Mass Spectrometry at Purdue University"
- Fall 2016 – "State-of-the-Art Mass Spectrometry for Point-of-Care and Other Applications"
- Spring 2016 – "Biotechnology Innovation and Regulatory Science (BIRS) CAID: Mass Spectrometry in the Clinic and Surgery"
- Fall 2015 – "Mass Spectrometry in the Clinic and Surgery"

Chemistry Experiment Demonstrator *Spring 2013 – Fall 2018*

- Fall 2018 – National Chemistry Week Demonstrations at Earhart, Burnett Creek, Wea Ridge, and St. Lawrence Grammar Schools x6
- Spring 2018 – Iota Sigma Pi Girl Scout Day
- Fall 2017 – National Chemistry Week Demonstrations at Wea Ridge Grammar School x3
- Fall 2016 – National Chemistry Week Demonstration at St. Boniface Grammar School
- Fall 2015 – National Chemistry Week Demonstration at St. Boniface Grammar School
- Fall 2014 – National Chemistry Week Demonstration at St. Boniface Grammar School
- Spring 2013 – Science Day at Dutch Neck Elementary

References Available Upon Request

PUBLICATIONS

1. Dilger, Jonathan M.; Miklaszewski, Eric J.; Papenmeier, Douglas M.; Thoreson, Kelly M.; **Fedick, Patrick W.**; Coleman, Jessica E.; Bohrer, Brian C. "Pyrolysis/GC/MS as a Method to Rapidly Profile Pyrotechnic Formulations for Objectionable Products of Emission" *ACS Sustainable Chem. and Eng*. 2018, Just Accepted. **DOI**: 10.1021/acssuschemeng.8b04342

2. Hollerbach, Adam; **Fedick, Patrick W.**; Cooks, R. Graham. "Ion Mobility-Mass Spectrometry using a Dual-Gated 3D printed Ion Mobility Spectrometer" *Anal. Chem.* 2018, Just Accepted. **DOI**: 10.1021/acs.analchem.8b02209

3. Bain, Ryan M.*; **Fedick, Patrick W.***; Dilger, Jonathan M.; Cooks, R. Graham. "Analysis of Residual Explosives by Swab Touch Spray Ionization Mass Spectrometry" *Propellants, Explosives, Pyrotechnics*. 2018, *43*, Just Accepted. **DOI**: 10.1002/prep.201800122.

4. **Fedick, Patrick W.**; Bain, Ryan M.; Bain, Kinsey; Mehari, Tsdale F.; Cooks, R. Graham. "Accelerated *tert*-Butyloxycarbonyl Deprotection of Amines in Microdroplets Produced by a Pneumatic Spray" *IJMS*. 2018, *430*, 98-103. **DOI:** 10.1016/j.ijms.2018.05.009

5. **Fedick, Patrick W.;** Fatigante, William L.; Lawton, Zachary E.; O'Leary, Adam E.; Hall, Seth E.; Bain, Ryan M.; Ayrton, Stephen T.; Ludwig, Joseph A.; Mulligan, Christopher C. "A Low-Cost, Simplified Platform of Interchangeable, Ambient Ionization Sources for Rapid, Forensic Evidence Screening on Portable Mass Spectrometric Instrumentation" *Instruments*. 2018, *2*, 5. **DOI:** 10.3390/instruments2020005

6. **Fedick, Patrick W.**; Bills, Brandon J.; Manicke, Nicholas E.; Cooks, R. Graham. "Forensic Sampling from a Single Substrate for Surface-Enhanced Raman Spectroscopy followed by Paper Spray ionization Mass Spectrometry" *Anal. Chem.* 2017, *89*, 10973-10979. **DOI**: 10.1021/acs.analchem.7b02798.

7. Teunissen, Sebastiaan F.; **Fedick, Patrick W.**; Berendsen, Bjorn J.A.; Nielen, Michel W.F.; Eberlin, Marcos N.; Cooks, R. Graham; van Asten, Arian C. "Novel Selectivity-Based Forensic Toxicological Validation of a Paper Spray Mass Spectrometry Method for the Quantitative Determination of Eight Amphetamines in Whole Blood" *J. Am. Soc. Mass Spectrom.* 2017, *28*, 2665-2676. **DOI:** 10.1007/s13361-017-1790-0.

8. **Fedick, Patrick W.***; Bain, Ryan M.*. "Swab Touch Spray Mass Spectrometry for Rapid Analysis of Organic Gunshot Residue from Human Hand and Various Surfaces Using Commercial and Fieldable Mass Spectrometry Systems" *Forensic Chemistry*. 2017, *5*, 53-57. **DOI:** 10.1016/j.forc.2017.06.005.

9. **Fedick, Patrick W.**; Bain, Ryan M.; Bain, Kinsey; Cooks, R. Graham. "Chiral Analysis by Tandem Mass Spectrometry Using the Kinetic Method, Polarimetry, and ^{1}H NMR" *J. Chem. Ed*. 2017, *94*, 1329-1333. **DOI:** 10.1021/acs.jchemed.7b00090.

10. Pulliam, Christopher J.; Bain, Ryan M.; Osswald, Heather L.; Snyder, Dalton T.; **Fedick, Patrick W.**; Ayrton, Stephen T.; Flick, Tawnya G.; Cooks, R. Graham. "Simultaneous On-line Monitoring of Multiple Reactions using a Miniature Mass Spectrometer" *Anal. Chem.* 2017, *89*, 6969–6975. **DOI**: 10.1021/acs.analchem.7b00119.

11. **Fedick, Patrick W.**; Bain, Ryan M.; Miao, Shunshun; Pirro, Valentina; Cooks, R. Graham. "State-of-the-Art Mass Spectrometry for Point-of-Care and other Applications: Development of a Two Day Hands-on Intensive Short Course for Upper Division Undergraduate Analytical Students" *IJMS*. 2017, *417*, 22-28. **DOI:** 10.1016/j.ijms.2017.04.008.

12. Snyder, Dalton T.; **Fedick, Patrick W.**; Cooks, R. Graham. "Multigenerational Collision-Induced Dissociation for Characterization of Organic Compounds" *Anal. Chem.* 2016, *88*, 9572−9581. **DOI:** 10.1021/acs.analchem.6b02209.

13. Yan, Bo; Yesilbag Tonga, Gulen; Hou, Singyuk; **Fedick, Patrick W.**; Yeh, Yi-Cheun; Alfonso, Felix S.; Mizuhara, Tsukasa; Vachet, Richard W.; Rotello, Vincent M. "Mass Spectrometric Detection of Nanoparticle Host-Guest Interactions in Cells" *Anal. Chem.* 2014, *86*, 6710–6714. **DOI:** 10.1021/ac501682y.

14. Tongeasyi, Tsanagurayi; **Fedick, Patrick**; Lechner, Lauren; Brock, Christiana; Le Beau, Arial; Bray Chelsea. "Daily Bioaccessible Levels of Selected Essential but Toxic Heavy Metals from the Consumption of Non-Dietary Food Sources" *Food and Chemical Toxicology* 2013, *62*, 142-147. **DOI:** 10.1016/j.fct.2013.08.052.

analytical chemistry

Article

Cite This: Anal. Chem. 2017, 89, 10973-10979

pubs.acs.org/ac

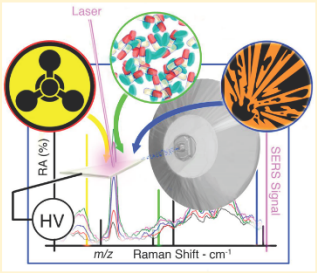
Forensic Sampling and Analysis from a Single Substrate: Surface-Enhanced Raman Spectroscopy Followed by Paper Spray Mass Spectrometry

Patrick W. Fedick,[†,§] Brandon J. Bills,[‡,§] Nicholas E. Manicke,*[,‡] and R. Graham Cooks*[,†]

[†]Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States

[‡]Department of Chemistry and Chemical Biology, Indiana University—Purdue University Indianapolis, Indianapolis, Indiana 46202, United States

ABSTRACT: Sample preparation is the most common bottleneck in the analysis and processing of forensic evidence. Time-consuming steps in many forensic tests involve complex separations, such as liquid and gas chromatography or various types of extraction techniques, typically coupled with mass spectrometry (e.g., LC-MS). Ambient ionization ameliorates these slow steps by reducing or even eliminating sample preparation. While some ambient ionization techniques have been adopted by the forensic community, there is significant resistance to discarding chromatography as most forensic analyses require both an identification and a confirmation technique. Here, we describe the use of a paper substrate, the surface of which has been inkjet printed with silver nanoparticles, for surface enhanced Raman spectroscopy (SERS). The same substrate can also act as the paper substrate for paper spray mass spectrometry. The coupling of SERS and paper spray ionization creates a quick, forensically feasible combination.



Forensic science relies heavily on so-called "hyphenated techniques", such a gas chromatography mass spectrometry (GC-MS) or liquid chromatography mass spectrometry (LC-MS), because of their long history of providing reliable, reproducible, and validated information.[1,2] While reliable, these hyphenated analytical techniques suffer from relatively long analysis times, and they are typically not amenable to in situ analysis. The standards set by ASTM International[3] and Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations[4] follow these practices, which makes nonchromatographic approaches a challenge unless a more attractive capability is newly available. These standards state that mass spectrometry is a validated "Category A" technique, the highest category based on discriminating power but that a secondary technique must be utilized, for example nuclear magnetic resonance (NMR) spectroscopy, Raman spectroscopy, any of a number of separation techniques, or even colorimetric tests.[3]

Desorption electrospray ionization (DESI),[5] along with direct analysis in real-time (DART),[6] the first ambient ionization techniques, gave the mass spectrometry community the ability to sample analytes in the open atmosphere transforming how sampling is performed. Two major benefits of ambient ionization are its definitive features: first, formation of ions in the ambient environment and, second, limited (if any) sample preparation. Ambient ionization mass spectrometry applications in forensic science are outlined in two recent reviews.[7,8] Paper spray ionization is an ambient ionization method which makes use of a paper substrate cut to a sharp tip.[9] Ions are generated with the application of a high voltage and solvent, and this simple technique can be used for direct sampling of complex mixtures.[10] Paper spray ionization has proven useful in the analysis of a wide variety of samples including dried blood spots,[11] drugs of abuse,[12] chemical warfare agents,[13] and bacteria.[14] Recent advances in paper spray ionization are ongoing, for example surface modifications for improved ionization or reactive applications.[15−19] Although paper spray excels as a rapid, cost-effective, and easy-to-use method, forensic applications require a secondary technique for analyte confirmation.

Another paper-based method that has been developed, not for mass spectrometry but rather for Raman spectroscopy, involves the use of paper surface Raman substrates.[20] Raman spectroscopy has gained popularity in forensics because of the increased sensitivity achieved in Surface Enhanced Raman Spectroscopy (SERS).[21] A particular advantage of paper SERS substrates is the ease with which they can be created using inkjet printers.[20] Fabrication of paper SERS substrates is straightforward compared to the typical microfabrication of SERS substrates and it minimizes the cost of fabrication.[22] Paper SERS (pSERS) substrates have been used to detect drugs,[23] fungicide,[24] pesticides,[23] and polymerase chain reaction (PCR) products.[25] While pSERS substrates are commercially available and attractive for forensics, this method too requires a second instrumental technique for confirmation.[3,4]

Received: July 18, 2017
Accepted: September 19, 2017
Published: September 19, 2017

DOI: 10.1021/acs.analchem.7b02798
Anal. Chem. 2017, 89, 10973−10979

Recognizing the complementary nature of these two methods, we demonstrate here the utilization of a commercial pSERS substrate for Raman spectroscopy analysis followed by mass spectrometry. The amalgamation of the two techniques provides a simple and fast forensic methodology requiring minimal sample preparation.

MATERIALS AND METHODS

Chemicals and Supplies. HPLC-grade methanol, acetonitrile, and the chemical warfare agent simulants (DMMP, DIMP, dichlorvos) were purchased from Sigma-Aldrich (St. Louis, MO). The drugs (4-methylethcathinone, morphine, heroin, fentanyl, and hydromorphone) and explosives (RDX, HMX, and TNT) were purchased from Cerilliant (Round Rock, Texas). All samples were analyzed after placing 3 μg, a forensically relevant mass, of material on the pSERS substrate. Unmounted Gold 2.0 pSERS substrates were purchased from Diagnostic anSERS (College Park, MD). A Biopsy punch with plunger, 3 mm, was purchased from Integra Miltex (York, PA). Paper spray cartridges were machined from Delrin plastic from McMaster-Carr, (Elmhurst, IL) on a benchtop mini milling machine by Sherline (Vista, CA). Whatman grade 31 ET chromatography paper was used for the spray substrate and was purchased from GE Healthcare Life Sciences (Pittsburgh, PA).

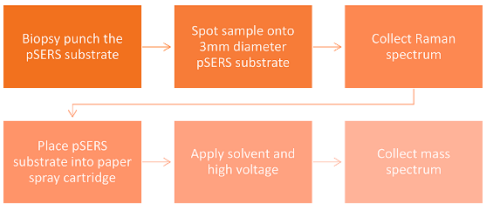


Figure 1. Experimental workflow for this dual instrumental analysis system, starting from the biopsy punch and sample deposition, then to Raman spectroscopy and finally to paper spray mass spectrometry.

Paper Spray Ionization Mass Spectrometry. All spectra were recorded using a Thermo LTQ XL mass spectrometer (San Jose, CA). The drugs and chemical warfare agent simulants were analyzed in positive ion mode, whereas the explosives were analyzed in negative ion mode. All MS/MS product ion scans were generated through collision-induced dissociation (CID). Normalized collision energies in the range of 10−35 (arbitrary manufacturer's unit on the LTQ XL) were applied to achieve sufficient fragmentation. The spray cartridge consisted of a block of Delrin plastic with three milled holes, as described in a previous manuscript.[26] A 0.5 × 5 mm slot on the front of the cartridge contained the paper spray substrate, while a 3 mm diameter hole in the top of the cartridge contained the sample on a three mm punch of SERS paper in contact with the spray substrate. The third 0.5 mm diameter hole in the top of the cartridge contained a wire in contact with the spray substrate to allow a voltage to be applied. HPLC-grade methanol or acetonitrile was applied to the pSERS substrate via pipet to ensure that the paper was completely wetted. A voltage of +4.5 or −3.0 kV was applied to the wire contact.

Raman. Raman spectra were collected on a Foster + Freeman FORAM 785 HP spectrometer (Evesham, Worcestershire, UK). The excitation source used a 785 nm 30 mW laser. Each spectrum was collected over 5 scans at 2 s a scan. Raman shifts were determined using polystyrene beads as a calibration standard. The compounds at 3 μg were not detectable by Raman spectroscopy without the pSERS substrate. To test this, the nonSERS coated portion of the paper substrate was analyzed with the 3 μg added which resulted in no interpretable signal.

Workflow. The analysis workflow is described in Figure 1. Standard solutions were pipetted onto the pSERS substrate, and a Raman spectrum was recorded after drying. The pSERS substrate was then placed in the paper spray cartridge, and a paper spray mass spectrum was recorded for the same sample. The entire analysis time was a few minutes. The pSERS substrates were biopsy punched because it allowed five samples to be analyzed from one commercial substrate, a cost saving step. The biopsy punch is not a requirement, and to show that the commercial pSERS substrate could be used without that step, the drugs hydromorphone and morphine were analyzed on the full pSERS strip.

RESULTS AND DISCUSSION

Drugs. With the SWGDRUG guidelines[4] being explicit on the requirement of two different methods, four drugs of abuse were tested by the combined pSERS Raman/PS-MS method. The selection of drugs encompassed relevant samples.[27] The increased use of synthetic designer drugs worldwide was the reason why 4-methylethcathinone was selected.[28] As seen in Figure 2a, the Raman shifts at 1012.5, 1125, 1390, and 1455 cm^{-1} have been assigned to the in-phase 2,4,6 radial carbon stretch, C−N−C out of phase stretch, CH rock in the O=C−H, and the aromatic semicircle stretch, respectively. The product ion scan of protonated 4-methylethcathinone (Figure

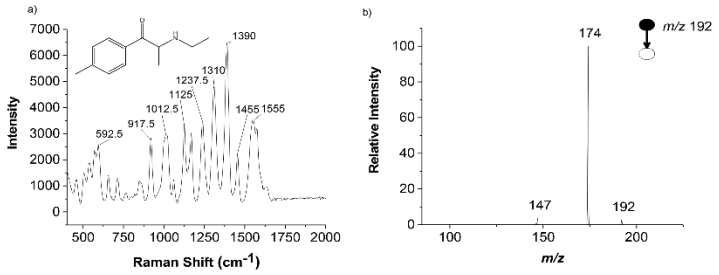


Figure 2. (a) Raman and (b) subsequent positive polarity paper spray MS/MS spectra for 4-methylethcatinone.

DOI: 10.1021/acs.analchem.7b02798
Anal. Chem. 2017, 89, 10973−10979

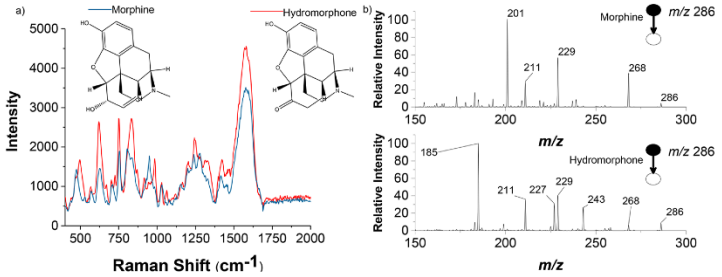


Figure 3. (a) Raman spectra for morphine and hydromorphone, where there are differences in Raman shifts because of the structural differences. (b) Product ion MS/MS scans for morphine and hydromorphone.

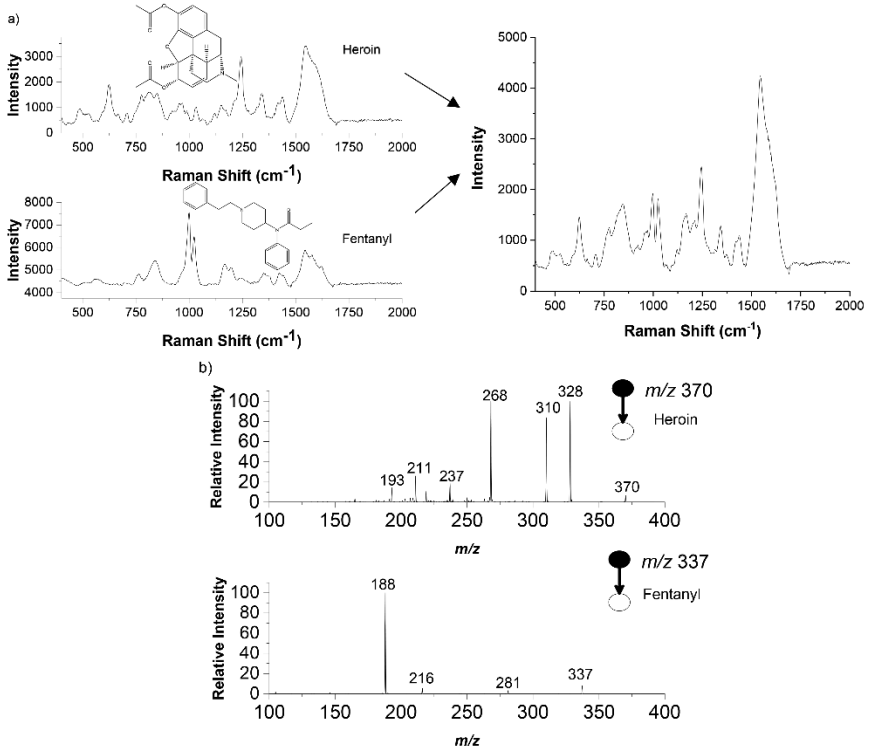


Figure 4. (a) Raman spectra for heroin and fentanyl, as well as the street sample of heroin that was cut with fentanyl. (b) Product ion spectra for heroin and fentanyl from the street sample of heroin that was cut with fentanyl.

2b) (m/z 192) shows two product ions at m/z 174 and m/z 147 corresponding to the neutral loss of water and the loss of C_2H_7N respectively.

Hydromorphone and morphine were selected because they are isobars and the Raman spectra aid in distinguishing them. These two drugs were not sampled via the 3 mm punched pSERS substrate but rather using the standard commercial rectangular design. When it was time to perform the mass spectrometric analysis, the paper was cut to a sharp triangular tip. As seen, in Figure 3a, the Raman shifts differ for the two isobaric compounds. The red and blue traces indicate the subtle differences between these two structurally similar compounds. These subtle and reproducible differences allow differentiation. The MS/MS scans also show differences, that of morphine having a product ion at m/z 201, which corresponds to the loss of $C_5H_{11}N$, that does not appear in hydromorphone (Figure 3b). Similarly, the product ion scan of hydromorphone includes ions at m/z 243, m/z 227, and m/z 185, which do not appear in

DOI: 10.1021/acs.analchem.7b02798
Anal. Chem. 2017, 89, 10973−10979

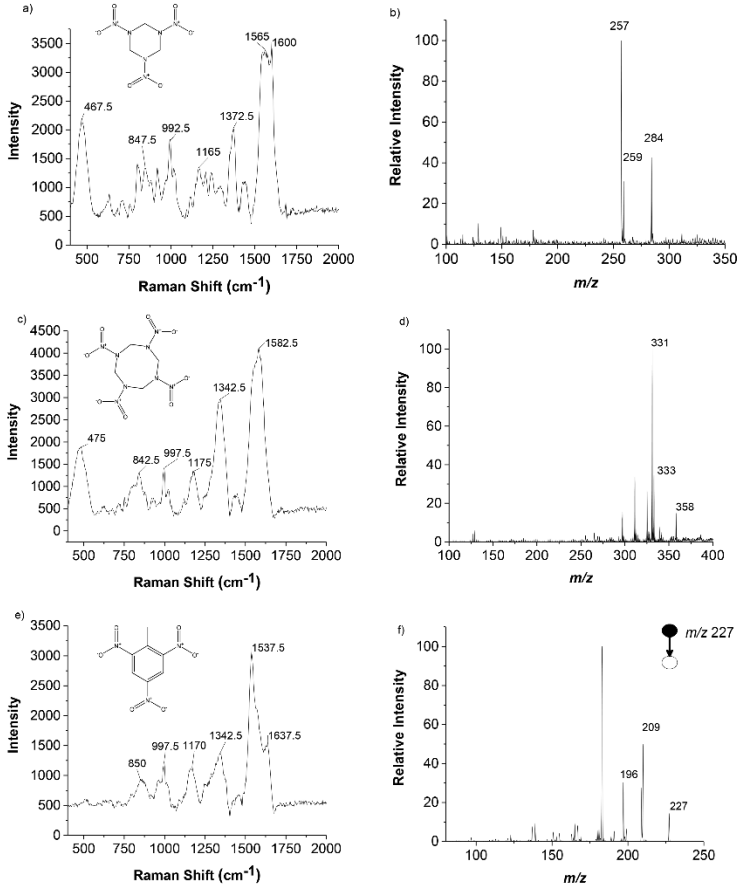


Figure 5. Raman and subsequent negative polarity paper spray full scan spectra for (a, b) RDX (c, d) HMX, and the Raman and subsequent negative polarity MS/MS paper spray spectra for (e, f) TNT.

morphine. While both techniques can reliably differentiate the two compounds, the complementary nature of the two gives increased confidence of the analysis.

Two drugs that have caused an increase in substance abuse and overdoses, especially in young adults, are heroin and fentanyl.[29,30] Production of fentanyl is a low cost operation, and it is typically used to cut heroin; this has caused numerous overdoses.[31] Figure 4a shows the Raman spectra for heroin and fentanyl in their pure form, as well as a simulated street sample, where the heroin is cut with fentanyl in a 10 to 1 ratio. The characteristic Raman shifts for the two compounds can be seen in the street sample. The product ion scans of the street sample also can be used to identify and readily distinguish the presence of both compounds (Figure 4b). The product ion scan of protonated heroin (m/z 370) shows fragments at m/z 328, 310, 268, 211, and 193, while that of protonated fentanyl (m/z 337) shows product ions at m/z 281 and 188.

Explosives. Certain nitro-explosives are difficult to identify because of their uninformative MS/MS spectra.[32] As a result, ambient MS methods for compounds like cyclotrimethylenetrinitramine (RDX) and cyclotetramethylenetetranitramine (HMX) are limited to performing identification from the full scan MS or from the MS/MS spectra of the dimers, which only fragment back to the monomers.[33,34] In our approach, identification of these explosives by full scan MS without MS/MS confirmation is mitigated by complementary detection using Raman spectroscopy. As seen, in Figure 5a and 5c each Raman spectrum has the standard Raman shifts for a nitro-explosive at ~840 and ~1350 cm^{-1}, which corresponds to a NO stretch and a NO_2 stretch. The full scan mass spectrum of RDX matches the literature,[33−35] showing peaks at m/z 257, 259, and 284, which correspond to RDX adducts with anions ^{35}Cl, ^{37}Cl, and $C_3H_7O_2$, respectively (Figure 5b). The full scan mass spectrum of HMX likewise agrees with the literature,[32−34,36] showing peaks at m/z 331, 333, and 358, which correspond to HMX adducts with the anions ^{35}Cl, ^{37}Cl, and NO_3, respectively (Figure 5d). Trinitrotoluene (TNT), however, does fragment well and can be identified by MS/

DOI: 10.1021/acs.analchem.7b02798
Anal. Chem. 2017, 89, 10973−10979

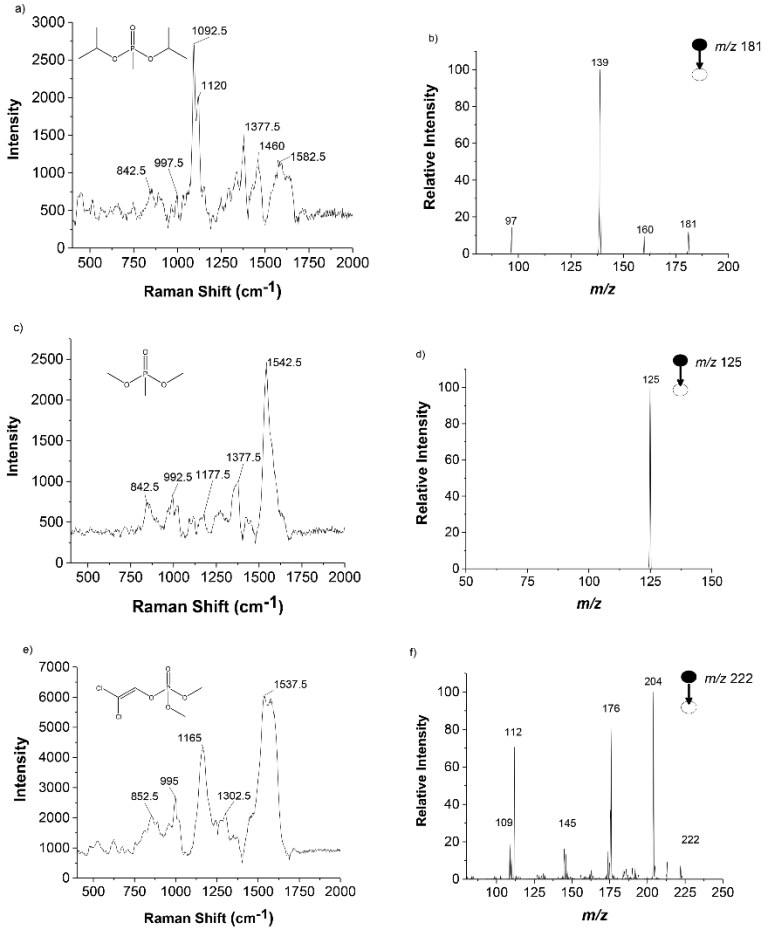


Figure 6. Raman and subsequent positive polarity paper spray MS/MS spectra for (a, b) DIMP, (c, d) DMMP, and (e, f) dichlorvos.

MS alone, but the complementary nature of Raman is used as a confirmatory test. As seen, in Figure 5e, the Raman shifts at 850 and 1342.5 cm^{-1} correspond to the NO and NO_2 stretches, respectively. The product ion scan of radical anion TNT (m/z 227) matches literature data[37−39] (Figure 5f) showing fragment peaks at m/z 209 and m/z 196, which correspond to the loss of water and HNO. To test the viability of the pSERS substrates to swab a surface, 10 μg of TNT was dried on a glass slide. The pSERS substrate was wetted with acetonitrile and the surface was swabbed. The spectra matched that of the pipetted TNT onto the pSERS substrate, showing that the dual instrumental analysis could also be applied in swabbing collection scenarios.

Chemical Warfare Agent Simulants. Chemical warfare agents have had significant news coverage in recent years.[40] The uses of chemical warfare agents, while infrequent, are highly monitored and publicized events.[41,42] Three chemical warfare agent simulants were analyzed by the pSERS Raman PS-MS system. They were the nerve agent simulants diisopropyl methylphosphonate (DIMP), dimethyl methylphosphonate (DMMP), and dichlorvos. DIMP showed Raman shifts (Figure 6a) at 842.5, 997, 1120, 1377, and 1460 cm^{-1}, which correspond to in phase P−O−C stretch, out of phase P−O−C stretch, P═O stretch, P−CH_3 stretch, and a C−CH_3 stretch, respectively. The product ion scan of protonated DIMP (Figure 6b) (m/z 181) showed product ions at m/z 139 and 97, which correspond to losses of C_3H_6 and C_6H_{12}, respectively. The ion at m/z 160 is believed to be background interference. DMMP showed Raman shifts (Figure 6c) at 842.5, 992.5, 1177.5, and 1377.5 cm^{-1}, which correspond to in phase P−O−C, out of phase P−O−C, P═O, and P−CH_3 stretches, respectively. Protonated DMMP (Figure 6d) (m/z 125), as well as sodiated DMMP (not shown) could be isolated in the ion trap. However, no product ions were observed even at maximum CID energy, although the precursor ion was highly abundant. The combination of Raman and mass spectrometry did confirm the presence of DMMP, however. As a final example,

DOI: 10.1021/acs.analchem.7b02798
Anal. Chem. 2017, 89, 10973−10979

dichlorvos showed Raman shifts (Figure 6e) at 852.5, 995, 1165 cm^{-1}, which correspond to in phase P−O−C stretch, out of phase P−O−C stretch, and P═O stretch. The product ion scan of protonated dichlorvos (Figure 6f) (m/z 222) showed product ions at m/z 204, 176, 145, 112, and 109, which corresponded to loss of water, CH_2O_2, $C_2H_5O_3$, $C_2H_6O_3P$, and C_2HCl_2O, respectively.

CONCLUSIONS

The use of a pSERS substrate for both Raman and PS-MS allows rapid analyte identification and confirmation without sample preparation steps and with the use of a single substrate for complementary spectroscopic measurements. Both Raman and PS-MS can be performed in the ambient environment which makes the coupling of the two instrumental techniques so appealing. The decrease in analysis time as compared to the hyphenated chromatography techniques could help to decrease forensic sample backlogs. The substrates are low cost and readily integrated into a forensic laboratory workflow. These substrates work for both SERS and PS-MS and as a cost saving method, a biopsy punch can be employed to create five pSERS substrates from one test strip. Additionally, because the substrates are inkjet printed, highly customizable patterning could be employed to fit the needs of the study. This study has shown the range of compound types to which this dual instrumental method is applicable. The ability to help distinguish isobaric compounds, confirm compounds that do not readily provide informative tandem mass spectra, and finally the ability to swab a surface and analyze the compounds all add to the strength of this technique. The study has not been extended to quantitation but quantitation using added internal standards in PS-MS is detailed in the review cited[11] while modest quantitative performance in SERS without standards is reported.[43] While not shown in this manuscript, paper spray has been performed in situ on a portable mass spectrometer[44] and commercial portable Raman spectrometers are available. The ability to potentially perform this technique in situ will add value to the combined methodology.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: nmanicke@iupui.edu.

*E-mail: cooks@purdue.edu.

ORCID

Nicholas E. Manicke: 0000-0002-2296-0497

R. Graham Cooks: 0000-0002-9581-9603

Author Contributions

§P.W.F. and B.J.B. contributed equally.

Notes

The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This material is based in part upon work supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Separations and Analysis Program, under Award Number DE-FG02-06ER15807 and by Award 2016-DN-BX-0007 from the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. Patrick Fedick would like to acknowledge support from the Department of Defense SMART scholarship. The authors thank Steve Ayrton for artwork for the TOC and Lee Cambrea for her discussions of Raman spectroscopy.

REFERENCES

(1) Peters, F. T. *Clin. Biochem.* **2011**, *44* (1), 54−65.

(2) Brettell, T. A. Forensic Science Applications of Gas Chromatography. In *Modern Practice of Gas Chromatography*; John Wiley & Sons, Inc., 2004; pp 883−967.

(3) ASTM International *Standard Practice for Identification of Seized Drugs*; ASTM International, 2014.

(4) SWGDRUG *Recommendations ed. 7.1 (2016-June-09)*; SWGDRUG, 2016.

(5) Takáts, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science* **2004**, *306* (5695), 471.

(6) Cody, R. B.; Laramée, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77* (8), 2297−2302.

(7) Hoffmann, W. D.; Jackson, G. P. *Annu. Rev. Anal. Chem.* **2015**, *8* (1), 419−440.

(8) Correa, D. N.; Santos, J. M.; Eberlin, L. S.; Eberlin, M. N.; Teunissen, S. F. *Anal. Chem.* **2016**, *88* (5), 2515−2526.

(9) Liu, J.; Wang, H.; Manicke, N. E.; Lin, J.-M.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2010**, *82* (6), 2463−2471.

(10) Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z. *Angew. Chem., Int. Ed.* **2010**, *49* (5), 877−880.

(11) Teunissen, S. F.; Fedick, P. W.; Berendsen, B. J.; Nielen, M. W.; Eberlin, M. N.; Graham Cooks, R. G.; van Asten, A. *J. Am. Soc. Mass Spectrom.* **2017**, DOI: 10.1007/s13361-017-1790-0.

(12) Espy, R. D.; Teunissen, S. F.; Manicke, N. E.; Ren, Y.; Ouyang, Z.; van Asten, A.; Cooks, R. G. *Anal. Chem.* **2014**, *86* (15), 7712−7718.

(13) McKenna, J.; Dhummakupt, E. S.; Connell, T.; Demond, P. S.; Miller, D. B.; Michael Nilles, J.; Manicke, N. E.; Glaros, T. *Analyst* **2017**, *142* (9), 1442−1451.

(14) Pulliam, C. J.; Wei, P.; Snyder, D. T.; Wang, X.; Ouyang, Z.; Pielak, R. M.; Graham Cooks, R. *Analyst* **2016**, *141* (5), 1633−1636.

(15) Narayanan, R.; Sarkar, D.; Cooks, R. G.; Pradeep, T. *Angew. Chem., Int. Ed.* **2014**, *53* (23), 5936−5940.

(16) Damon, D. E.; Maher, Y. S.; Yin, M.; Jjunju, F. P. M.; Young, I. S.; Taylor, S.; Maher, S.; Badu-Tawiah, A. K. *Analyst* **2016**, *141* (12), 3866−3873.

(17) Banerjee, S.; Basheer, C.; Zare, R. N. *Angew. Chem., Int. Ed.* **2016**, *55* (41), 12807−12811.

(18) Resende, S. F.; Teodoro, J. A. R.; Binatti, I.; Gouveia, R. L.; Oliveira, B. S.; Augusti, R. *Int. J. Mass Spectrom.* **2017**, *418*, 107−111.

(19) Ji, J.; Nie, L.; Liao, L.; Du, R.; Liu, B.; Yang, P. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2016**, *1015-1016*, 142−149.

(20) Yu, W. W.; White, I. M. *Anal. Chem.* **2010**, *82* (23), 9626−9630.

(21) Muehlethaler, C.; Leona, M.; Lombardi, J. R. *Anal. Chem.* **2016**, *88* (1), 152−169.

(22) Betz, J. F.; Yu, W. W.; Cheng, Y.; White, I. M.; Rubloff, G. W. *Phys. Chem. Chem. Phys.* **2014**, *16* (6), 2224−2239.

(23) Yu, W. W.; White, I. M. *Analyst* **2013**, *138* (4), 1020−1025.

(24) Hoppmann, E. P.; Yu, W. W.; White, I. M. *Methods* **2013**, *63* (3), 219−224.

(25) Hoppmann, E. P.; Yu, W. W.; White, I. M. *IEEE J. Sel. Top. Quantum Electron.* **2014**, *20* (3), 195−204.

(26) Bills, B. J.; Manicke, N. E. *Clinical Mass Spectrometry* **2016**, *2*, 18−24.

(27) United Nations Office on Drugs and Crime. *World Drug Report 2015*; UNODC Research: Vienna, 2015.

(28) European Monitoring Centre for Drugs and Drug Addiction. *Perspectives on Drugs: Injection of Synthetic Cathinones*; EMCDDA: Lisbon, Portugal, 2015.

(29) Cedarbaum, E. R.; Banta-Green, C. J. *Drug Alcohol Depend.* **2016**, *158*, 102−109.

DOI: 10.1021/acs.analchem.7b02798
Anal. Chem. 2017, 89, 10973−10979

(30) Hedegaard, H.; Warner, M.; Minino, A. *Drug Overdose Deaths in the United States, 1999–2015*, NCHS Data Brief 273; National Center for Health Statistics: Hyattsville, MD, 2017.
(31) Frank, R. G.; Pollack, H. A. *N. Engl. J. Med.* **2017**, *376* (7), 605–607.
(32) Garcia-Reyes, J. F.; Harper, J. D.; Salazar, G. A.; Charipar, N. A.; Ouyang, Z.; Cooks, R. G. *Anal. Chem.* **2011**, *83* (3), 1084–1092.
(33) Cotte-Rodríguez, I.; Takáts, Z.; Talaty, N.; Chen, H.; Cooks, R. G. *Anal. Chem.* **2005**, *77* (21), 6755–6764.
(34) Cotte-Rodriguez, I.; Cooks, R. G. *Chem. Commun.* **2006**, *28*, 2968–2970.
(35) Zhang, Y.; Ma, X.; Zhang, S.; Yang, C.; Ouyang, Z.; Zhang, X. *Analyst* **2009**, *134* (1), 176–181.
(36) Chen, W.; Hou, K.; Xiong, X.; Jiang, Y.; Zhao, W.; Hua, L.; Chen, P.; Xie, Y.; Wang, Z.; Li, H. *Analyst* **2013**, *138* (17), 5068–5073.
(37) Takats, Z.; Cotte-Rodriguez, I.; Talaty, N.; Chen, H.; Cooks, R. G. *Chem. Commun.* **2005**, *15*, 1950–1952.
(38) Harper, J. D.; Charipar, N. A.; Mulligan, C. C.; Zhang, X.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2008**, *80* (23), 9097–9104.
(39) Sanders, N. L.; Kothari, S.; Huang, G.; Salazar, G.; Cooks, R. G. *Anal. Chem.* **2010**, *82* (12), 5313–5316.
(40) Droste, D. J.; Shelley, M. L.; Gearhart, J. M.; Kempisty, D. M. *Am. J. Disaster Med.* **2016**, *11* (2), 89–118.
(41) Haines, D. D.; Fox, S. C. *Forensic Sci. Rev.* **2014**, *26* (2), 97–114.
(42) Tokuda, Y.; Kikuchi, M.; Takahashi, O.; Stein, G. H. *Resuscitation* **2006**, *68* (2), 193–202.
(43) Peksa, V.; Jahn, M.; Štolcová, L.; Schulz, V.; Proška, J.; Procházka, M.; Weber, K.; Cialla-May, D.; Popp, J. *Anal. Chem.* **2015**, *87* (5), 2840–2844.
(44) Pulliam, C. J.; Bain, R. M.; Wiley, J. S.; Ouyang, Z.; Cooks, R. G. *J. Am. Soc. Mass Spectrom.* **2015**, *26* (2), 224–230.

DOI: 10.1021/acs.analchem.7b02798
Anal. Chem. 2017, 89, 10973–10979

Forensic Chemistry 5 (2017) 53–57

Contents lists available at ScienceDirect

Forensic Chemistry

journal homepage: www.elsevier.com/locate/forc

Short Communications

Swab touch spray mass spectrometry for rapid analysis of organic gunshot residue from human hand and various surfaces using commercial and fieldable mass spectrometry systems

Patrick W. Fedick [a,1,*], Ryan M. Bain [b,1,*]

[a] Department of Chemistry, Purdue University, West Lafayette, IN 47907, United States
[b] Department of Chemistry, Stanford University, Stanford, CA 94305, United States

ARTICLE INFO

Article history:
Received 5 May 2017
Received in revised form 21 June 2017
Accepted 26 June 2017
Available online 27 June 2017

Keywords:
Swab touch spray ionization
Mass spectrometry
Organic gunshot residue
In-situ analysis
Portable instrumentation

ABSTRACT

Organic gunshot residues, specifically methyl centralite (1,3-dimethyl-1,3-diphenylurea) and ethyl centralite (1,3-diethyl-1,3-diphenylurea), are characteristic compounds for which forensic analysts test when determining if an individual has discharged a firearm. These distinctive compounds have long been analyzed by several instrumental techniques, many of which involve extensive sample preparation or have lengthy analysis times. Presented here is an ambient ionization method that requires no sample preparation, offers real-time analysis, and can be paired with a portable ion trap mass spectrometer for *in-situ* analysis. Swab touch spray ionization utilizes a rayon-tipped swab that has an aluminum wire handle which can simply be swabbed over the area of interest. After contacting the hands of the shooter, or other surfaces, the swab simply has solvent applied to the rayon tip and a high voltage applied to the aluminum handle. This process generates ions that the mass spectrometer will use to determine if the organic gunshot residues are present. Mass spectrometry allows for the direct confirmation of organic gunshot residue on suspects hands, confirmed through tandem mass spectrometry. Additional benefits of swab touch spray ionization are that the swabs are commercially available, they are sterile, and they are individually packaged to prevent contamination. The swabs are also forensically feasible because they are sealed prior to delivery by the vendor with a tamper-proof label. Here we have shown that this ambient ionization method allows for the direct swabbing of suspected shooters hands for trace residues and confirm the presence of the two organic gunshot residues investigated.

1. Introduction

Forensic investigation into the possibility of a suspect discharging a firearm in recent years has expanded from elemental inorganic gunshot residue analyses to molecular organic gunshot residue analyses (OGSR) [1]. Protocols have been developed for the collection and analysis of OGSR [1] which focus heavily on the stabilizers present in many ammunition types such as methyl centralite (MC) (1,3-dimethyl-1,3-diphenylurea) and ethyl centralite (EC) (1,3-diethyl-1,3-diphenylurea). These two compounds were selected from the expansive list of OGSRs because they are not commonly used in any other application and would therefore be among the most discriminatory compounds for determining if a person has potentially discharged a firearm [2]. The complementary nature of OGSR analysis adds a level of confirmation to the typical inorganic analysis that is traditionally performed. OGSR has been shown to be detectable on skin hours after discharging a firearm [3]; however, there is degradation over time and improved *in-situ* analysis would greatly benefit the forensic community [4]. A recent comprehensive review identified over 136 compounds to be associated with OGSR [5]; however, this current study will only focus on the detection of MC and EC as a proof of principle.

Currently OGRS analysis has been accomplished by a number of analytical techniques, many of which require lengthy chromatography or extraction techniques [5]. Forensic staples like gas chromatography [6] and ultra-high performance liquid chromatography [7] have demonstrated their capabilities of identifying and determining the presence of OGRS. While these methods are reliable analytical techniques, they suffer from lengthy analysis times and are not amenable to *in-situ* analysis. Similarly, the standard sampling methods for OGSR are various forms of swabs and

* Corresponding authors.
E-mail addresses: pfedick@purdue.edu (P.W. Fedick), ryanbain@stanford.edu (R.M. Bain).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.forc.2017.06.005
2468-1709/

stubs [8,9]. In order for these chromatographic methods to be utilized, an extraction technique, such as a solid phase microextraction [10], has to be performed. Where swabs and stubs are convenient methodologies for sampling, when combined with extraction techniques the number of experimental and sample preparation steps increases, amplifying analysis time and extending the room for operator error and sources of contamination. There have been reports of swabs that have removed the sample preparation steps through the use of TOF-SIMS directly on the swab [11], but the ability of *in-situ* analysis is nonexistent due to the complexity of the instrumental setup.

Ambient ionization mass spectrometry [12], starting with desorption electrospray ionization (DESI) [13] and direct analysis in real-time (DART) [14], changed how mass spectrometry sampling can be performed. Since the onset of ambient ionization, there have been ample forensic advances because of these techniques, as outlined in two recent reviews [15,16]. Two major benefits of ambient ionization as it relates to forensic science are the lack of sample preparation and the ability to form ions in the open environment. DESI mass spectrometry has been shown to be capable of identifying and distinguishing OGSR in a simple and noninvasive method [17,18]. While DESI has advantages over the extraction based techniques spoken above, swab touch spray ionization may be better suited for OGSR analysis [19–21]. Swab touch spray utilizes a rayon-tipped swab to collect the analytes of interest by swabbing the dry swab over the area of interest (i.e. the hands of a suspected shooter, or an article of clothing of the suspected shooter). The swab is constructed with an aluminum handle which allows a high voltage lead to be connected directly to the swab to promote ionization when solvent is applied [20]. The aluminum handle is pertinent to swab touch spray because this is how the high voltage is applied; other handles like wood or plastic are nonconductive. Some of the methods above also utilized swabs, but they either required an extraction method or a more complex method of ionization [5]. These extraction methods or more complex methods of ionization diminish the ability for fast turnaround times of the forensic analysis and further limit the technique's ability to be coupled with a portable instrument. This paper will demonstrate the ease of swab touch spray ionization, its forensic feasibility for organic gunshot residues, and its ability to be coupled to a portable mass spectrometer for *in-situ* analysis.

2. Materials and methods

2.1. Chemicals and supplies

HPLC-grade methanol, methyl centralite (1,3-dimethyl-1,3-diphenyl-urea) and ethyl centralite (1,3-diethyl-1,3-diphenyl-urea) were purchased from Sigma-Aldrich (St. Louis, MO). Anti-static vinyl gloves (OAK Technical, Matteson, IL), Diamond Grip Latex gloves (Microflex, Reno, NV) and Black Nitrile Exam Gloves (Ammex, Seattle, WA) were used in these experiments.

2.2. Swab touch spray source and mass spectrometer

All spectra were recorded in positive ion mode using a Thermo LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap mass spectrometer (San Jose, CA) or a home-built Mini 12 rectilinear ion trap (Purdue University, West Lafayette, IN) [22]. All MS/MS product ion scan mass spectra were generated through collision-induced dissociation (CID). Normalized collision energies from 8 to 15 on the ion trap were used. Medical grade sterile swabs (Copan Diagnostics, Murritea, CA) constructed with aluminum handles and rayon swabs were utilized for all experiments. Each swab was individually packaged with a tamper-proof label and removed from packing only to swab and then was returned to the casing. Each surface (bare hands, gloved surfaces, clothing and spent casings) were swabbed in a circular motion after discharging the firearm. Approximately 20 circular motion passes were performed over the area of interest, for example the top side of the right hand between the thumb and the pointer finger. The swabs were positioned vertically (approximately 8 mm) above the inlet of the mass spectrometer. HPLC-grade methanol was applied to the swab via pipette to ensure that the swab was completely wetted. Then a continual flow of methanol spraying solvent was delivered to the swab by a Harvard Apparatus standard infusion only PHD 22/2000 syringe pump (Holliston, MA) using a Hamilton syringe (Reno, NV) at a varied flow rate (10–30 μl/min) to maintain a steady spray. A high voltage of 5.5 kV was applied to the aluminum handle and the generation of a spray could be visually observed [20].

2.3. Ammunition and firearm

Four different 9 mm handguns, with four different ammunitions were used in this study. Each of the ammunitions were discharged by only one of the four different handguns. Federal Ammunition 9 mm Luger 115 Grain Full Metal Jacket rounds (Anoka, MN) were discharged by a Heckler & Koch VP9 (Newington, NH). Independence 9 mm Luger 124 Grain Full Metal Jacket rounds (Lewiston, ID) were discharged by a Beretta M9 (Accokeek, MD). Winchester 9mm Luger 147 Grain Full Metal Jacket rounds (East Alton, IL) were discharged by a Glock 17 (Smyrna, GA). Federal Ammunition American Eagle 147 Grain Full Metal Jacket Flat Point rounds (Anoka, MN) were discharged by a SIG Sauer P320 (Newington, NH). Different firearms and ammunitions were selected to determine if this could be a universal method. To minimize the confounded variables of shooters, ammunition and firearm the casing of each expended ammunition was swabbed and analyzed for the presence of MC and EC. Each firearm was discharged by one individual and each individual only fired one ammunition and firearm per session at the range. For each experiment, unless otherwise stated, 10 rounds were fired from the handgun and then the shooters hands were immediately swabbed once the weapon was cleared. For all ammunitions where EC and MC could be detected by swab touch spray mass spectrometry after 10 rounds were discharged could also be detected after a discharging of the firearm on both mass spectrometers.

3. Results and discussion

3.1. Swab touch spray of various surfaces

To test the viability of swab touch spray for OGSR analysis the inside of the bottles of MC and EC standards were swabbed and analyzed by the LTQ ion trap (Fig. 1). The MS/MS spectra of both ions correlating to $[MC+H]^+$ (m/z 241) and $[EC+H]^+$ (m/z 269) matched the literature [17,18] and had reproducible and stable spectra with distinctive fragment peaks that were observable through the subsequent experiments.

After these results had demonstrated that swab touch spray is capable of ionizing MC and EC standards, swab touch spray was utilized to analyze OGSR produced from the discharging of a firearm. The researchers shot four different 9mm full metal jacket ammunitions, with varying grains, and tested the ability of the dry swab to extract the OGSR off the right hand of the shooter when swabbed. Four surfaces were tested, an exposed bare hand, a hand covered in a vinyl glove, a nitrile glove, and a latex glove. The variety of gloved surfaces were selected based on the general availability of these to the general public who may try and cover up a crime they committed, as well as to show the robust nature of the swab at extracting the OGSR off of a variety of surfaces. Table 1 describes the ability of the swab to extract the OGSR off the surface.

As seen in Table 1, MC and EC was detected in all four surfaces for the first three ammunitions; however, MC and EC were not detected in the American Eagle 147 Grain full metal jacket flat point ammunition discharged from a SIG Sauer P320. As the composition of the bullets are not public knowledge, the researchers also swabbed the inside of the spent casing to determine if the lack of detection of MC and EC was a result of the swab or the lack of the two compounds found in the ammunitions. There was no signal of MC or EC for the American Eagle casing either, to which the researchers propose that there may not be any MC or EC in this ammunition, or with the increased grain potentially the MC and EC is in a more limited quantity and the quantity transferred to the surface is below the limit of detection of the technique. Each of the three ammunitions that were found to contain MC and EC, a single discharge of the firearm provided enough of both compounds to be detected by swab touch spray mass spectrometry. The lower limit of detection for both MC and EC were lower than 50 ng on the LTQ XL. The researchers checked the inside of the

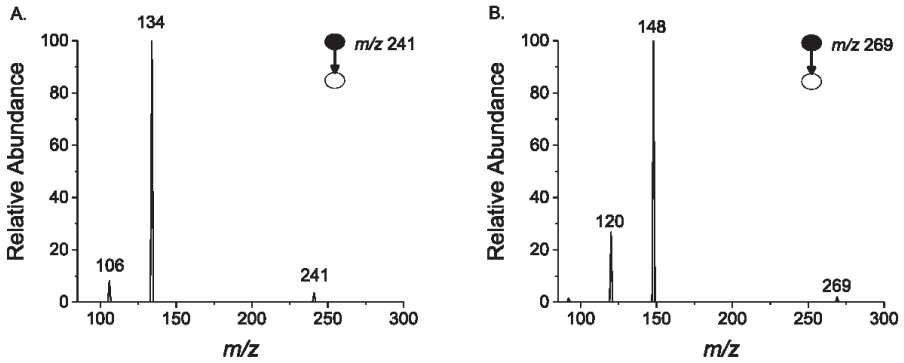


Fig. 1. Swab touch spray product ion spectra of standards. (A) Protonated MC is seen at *m/z* 241, with the loss of neutral methylaniline to form the fragment at *m/z* 134, and a subsequent neutral loss of CO to form the fragment at *m/z* 106 and (B) protonated EC at *m/z* 269, with the loss of neutral ethylaniline to form the fragment at *m/z* 148 and a subsequent loss of CO to form the fragment at *m/z* 120. Both spectra were obtained on a Thermo LTQ XL Ion Trap. Subsequent losses were confirmed by MS^3 scans (data not shown).

Table 1
Ability to detect MC and EC from bare hands, vinyl, nitrile, and latex gloves by swab touch spray. The top three ammunitions were able to be detected on all surfaces, whereas there was no detectable MC or EC from the fourth ammunition.

Ammunition	Compound	Exposed hand	Vinyl glove	Nitrile glove	Latex glove
Federal Ammunition 9 mm Luger 115 Grain Full Metal Jacket	Methyl Centralite	✓	✓	✓	✓
	Ethyl Centralite	✓	✓	✓	✓
Independence 9 mm Luger 124 Grain Full Metal Jacket	Methyl Centralite	✓	✓	✓	✓
	Ethyl Centralite	✓	✓	✓	✓
Winchester 9 mm Luger 147 Grain Full Metal Jacket	Methyl Centralite	✓	✓	✓	✓
	Ethyl Centralite	✓	✓	✓	✓
Federal Ammunition American Eagle 147 Grain Full Metal Jacket Flat Point	Methyl Centralite	Not Detected	Not Detected	Not Detected	Not Detected
	Ethyl Centralite	Not Detected	Not Detected	Not Detected	Not Detected

other ammunitions' spent rounds and were able to detect both MC and EC in all three (Fig. 2). The EC spectrum looks identical to the standards, however in the MC spectrum a peak at *m/z* 181 and at *m/z* 223 are also present in the Winchester 9 mm Luger ammunition, both in the swabs of the gloves, bare hands and the casing. The authors propose that these peaks in the MC spectrum at *m/z* 181 and *m/z* 223 arises from an isobaric compound at *m/z* 241 also present in the ammunition. The proposed explanation, is the peak at *m/z* 223 is created by the loss of water while the peak at *m/z* 181 is created by the neutral loss of acetate potentially to form the ion at *m/z* with the molecular formula $C_{12}H_7NO^-$ which would have arisen from the parent mass of $C_{14}H_{10}NO_2^-$. The chemical formulae were confirmed by exact mass using the LTQ-orbitrap mass spectrometer with a mass error of less than 5 ppm. The identification of MC is still readily performed by the prominent fragment peaks at *m/z* 134 and *m/z* 106.

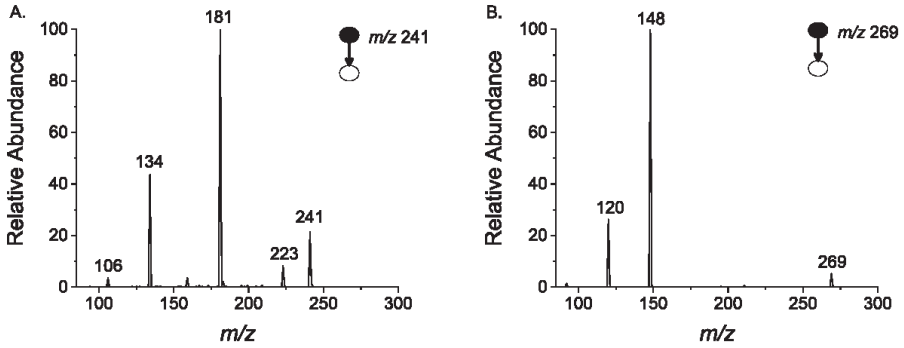


Fig. 2. Swab touch spray product ion mass spectra of a Winchester 9 mm Luger 147 Grain Full Metal Jacket spent casing for (A) protonated MC at *m/z* 241, which shows the loss of neutral methylaniline to form the fragment at *m/z* 134, and a subsequent neutral loss of CO to form the fragment at *m/z* 106. The fragments at *m/z* 181 and *m/z* 223 arises from an isobaric compound at *m/z* 241. (B) protonated EC at *m/z* 269, with the loss of neutral ethylaniline to form the fragment at *m/z* 148 and a subsequent loss of CO to form the fragment at *m/z* 120. Both spectra were obtained on a Thermo LTQ XL Ion Trap.

Another surface on which swab touch spray was utilized was the clothing of the shooter (Fig. 3). Both MC and EC are present in the full scan, with the more prominent species being sodiated, however the sodiated species does not fragment as efficiently. Again, the protonated MC and EC were easily isolated and fragmented, and identified that the wearer of the clothing had discharged a firearm.

3.2. High-resolution MS

A hybrid LTQ-Orbitrap mass spectrometer was used for analysis of Winchester 9mm Luger 147 Grain Full Metal Jacket samples. The Orbitrap results confirm the chemical formula of $C_{17}H_{21}N_2O$ and $C_{15}H_{17}N_2O$ with errors of 6.8 and 2.0 ppm, respectively. The MS/MS product ion scan also confirmed the

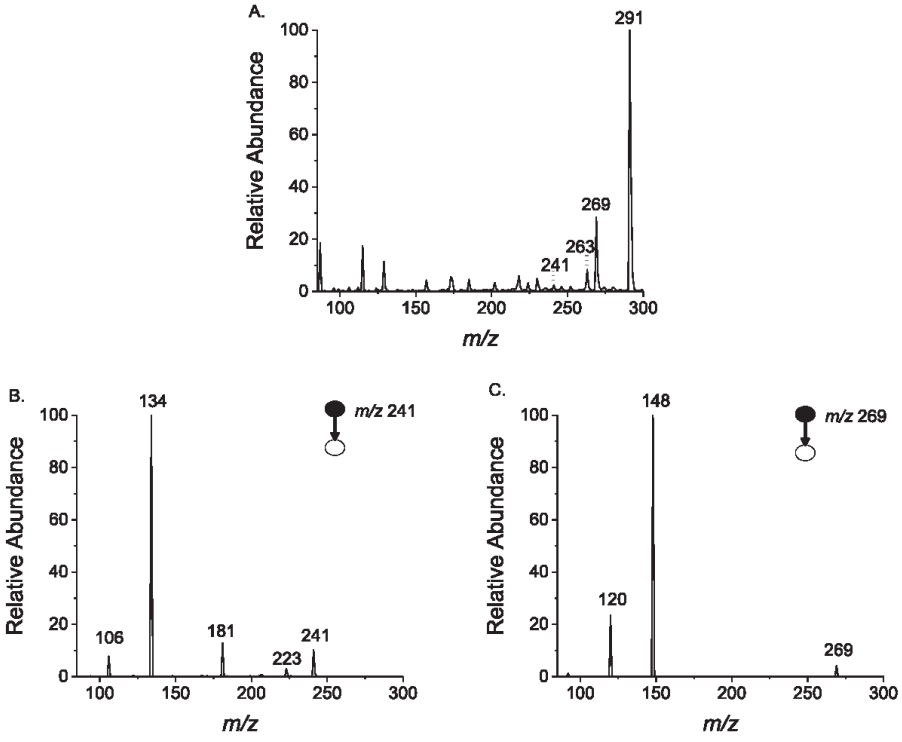


Fig. 3. Swab touch spray spectra off the clothing of a shooter who fired Winchester 9 mm Luger 147 Grain Full Metal Jacket ammunition (A) full scan (B) MS/MS of MC and (C) MS/MS of EC, all analyzed on a Thermo LTQ XI Ion Trap.

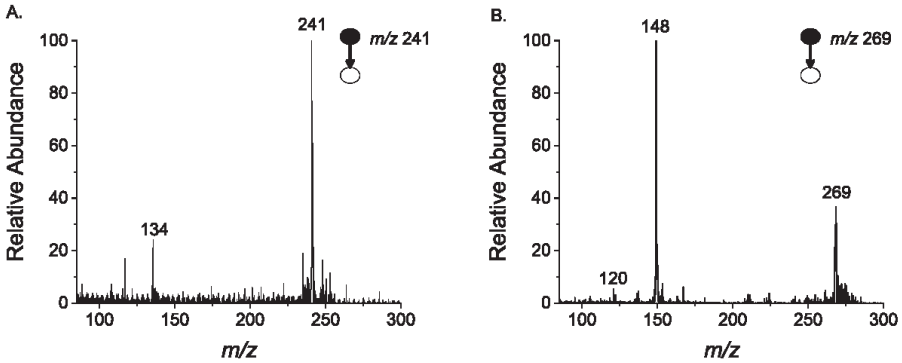


Fig. 4. Swab touch spray product ion scan mass spectra off the hands of a shooter who fired Independence 9 mm Luger 124 Grain Full Metal Jacket ammunition (A) MS/MS of MC and (B) MS/MS of EC, analyzed by a field portable Mini 12 mass spectrometer.

chemical formulae of the product ion with errors under 5.0 ppm for all product ions.

3.3. Mini 12 rectilinear ion Trap

Detection of MC and EC by swab touch spray on a homebuilt Mini 12 was also successful. As the Mini 12 does not consistently have pneumatic assistance due to the discontinuous atmospheric pressure inlet (DAPI) [22] the experiment required the flow rate on the syringe pump to be altered because the experiment was performed to ensure that a buildup of solvent did not occur on the capillary. Regardless of the challenge of altering the flow rate over time to ensure a stable spray, the Mini 12 was also capable of detecting MC and EC by swab touch spray from hands of the shooter (Fig. 4). The Mini 12 was also able to detect both MC and EC after the discharge of a single round of ammunition. Determining if a potential suspect had potentially discharged a firearm or not is a time sensitive matter [3]. The Mini 12, which has been shown to be capable of *in-situ* analysis [23], has been demonstrated to be able to provide law enforcement with an answer in a more rapid, simple sampling method.

4. Conclusions

Swab touch spray has been shown to be an effective method for identifying organic gunshot residue from a variety of surfaces: hands, gloves, clothing, and spent shell casings. This ambient technique requires no sample preparation, no lengthy analysis times, and is capable of in-field analysis. While only two organic gunshot residues were targeted in this proof-of-principle manuscript, this technique can be expanded to encompass more OGSRs and improve the confidence of the forensic analyst that a suspect had or had not discharged a firearm. Both MC and EC were detected after a single discharge of a firearm on both the portable Mini 12 and the benchtop LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap mass spectrometers. The Mini 12 analysis was performed in a laboratory setting; however, future testing could be performed to identify the capability of these swabs *in-situ*. Similarly, since there is a plethora of ammunition manufacturers, it is important that a database of the compounds detectable for each ammunition is an important future research direction. The OGSR provides complementary and confirmatory information to that of elemental inorganic gunshot residue and the method describe in this work can provide that information from a forensically viable swab substrate. The swabs being tamper-proof sealed, individually wrapped, and requiring no preparatory steps, which can be a point at which contamination can be introduced, is a potentially ideal forensic sampling device.

Acknowledgements

The authors would like to thank Applied Ballistics, West Lafayette, Indiana, for allowing the authors to perform experiments at their location. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Authors would also like to thank Christopher J. Pulliam and Valentina Pirro for their intellectual discussions regarding swab touch spray ionization. Authors would also like to acknowledge Steve Ayrton for his creation of the graphical abstract.

References

[1] R.V. Taudte, C. Roux, L. Blanes, M. Horder, K.P. Kirkbride, A. Beavis, The development and comparison of collection techniques for inorganic and organic gunshot residues, Anal. Bioanal. Chem. 408 (10) (2016) 2567–2576.
[2] E. Goudsmits, G.P. Sharples, J.W. Birkett, Preliminary classification of characteristic organic gunshot residue compounds, Sci. Justice 56 (6) (2016) 421–425.
[3] J.W. Moran, S. Bell, Skin permeation of organic gunshot residue: implications for sampling and analysis, Anal. Chem. 86 (12) (2014) 6071–6079.
[4] R.V. Taudte, C. Roux, A. Beavis, Stability of smokeless powder compounds on collection devices, Forensic Sci. Int. 270 (2017) 55–60.
[5] E. Goudsmits, G.P. Sharples, J.W. Birkett, Recent trends in organic gunshot residue analysis, TrAC Trends Anal. Chem. 74 (2015) 46–57.
[6] A. Kabir, K.G. Furton, Chapter 25 – applications of gas chromatography in forensic science A2 – poole, in: F. Colin (Ed.), Gas Chromatography, Elsevier, Amsterdam, 2012, pp. 563–604.
[7] R.V. Taudte, C. Roux, D. Bishop, L. Blanes, P. Doble, A. Beavis, Development of a UHPLC method for the detection of organic gunshot residues using artificial neural networks, Anal. Methods 7 (18) (2015) 7447–7454.
[8] A.-L. Gassner, C. Ribeiro, J. Kobylinska, A. Zeichner, C. Weyermann, Organic gunshot residues: observations about sampling and transfer mechanisms, Forensic Sci. Int. 266 (2016) 369–378.
[9] A.-L. Gassner, C. Weyermann, LC–MS method development and comparison of sampling materials for the analysis of organic gunshot residues, Forensic Sci. Int. 264 (2016) 47–55.
[10] O. Dalby, J.W. Birkett, The evaluation of solid phase micro-extraction fibre types for the analysis of organic components in unburned propellant powders, J. Chromatogr. A 1217 (46) (2010) 7183–7188.
[11] A. Castellanos, S. Bell, F. Fernandez-Lima, Characterization of firearm discharge residues recovered from skin swabs using sub-micrometric mass spectrometry imaging, Anal. Methods 8 (21) (2016) 4300–4305.
[12] M.E. Monge, G.A. Harris, P. Dwivedi, F.M. Fernández, Mass spectrometry: recent advances in direct open air surface sampling/ionization, Chem. Rev. 113 (4) (2013) 2269–2308.
[13] Z. Takáts, J.M. Wiseman, B. Gologan, R.G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, Science 306 (5695) (2004) 471.
[14] R.B. Cody, J.A. Laramée, H.D. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions, Anal. Chem. 77 (8) (2005) 2297–2302.
[15] D.N. Correa, J.M. Santos, L.S. Eberlin, M.N. Eberlin, S.F. Teunissen, Forensic chemistry and ambient mass spectrometry: a perfect couple destined for a happy marriage?, Anal Chem. 88 (5) (2016) 2515–2526.
[16] W.D. Hoffmann, G.P. Jackson, Forensic mass spectrometry, Annu. Rev. Anal. Chem. 8 (1) (2015) 419–440.
[17] M. Zhao, S. Zhang, C. Yang, Y. Xu, Y. Wen, L. Sun, X. Zhang, Desorption electrospray tandem MS (DESI-MSMS) analysis of methyl centralite and ethyl centralite as gunshot residues on skin and other surfaces, J. Forensic Sci. 53 (4) (2008) 807–811.
[18] M. Morelato, A. Beavis, A. Ogle, P. Doble, P. Kirkbride, C. Roux, Screening of gunshot residues using desorption electrospray ionisation–mass spectrometry (DESI–MS), Forensic Sci. Int. 217 (1–3) (2012) 101–106.
[19] A.K. Jarmusch, V. Pirro, K.S. Kerian, R.G. Cooks, Detection of strep throat causing bacterium directly from medical swabs by touch spray-mass spectrometry, Analyst 139 (19) (2014) 4785–4789.
[20] V. Pirro, A.K. Jarmusch, M. Vincenti, R.G. Cooks, Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry, Anal. Chim. Acta 861 (2015) 47–54.
[21] B.-C. Yang, F. Wang, X. Yang, W. Zou, J.-C. Wang, Y. Zou, F.-Y. Liu, H. Liu, O.-P. Huang, Medical swab touch spray-mass spectrometry for newborn screening of nicotine and cotinine in meconium, J. Mass Spectrom. 51 (12) (2016) 1237–1242.
[22] L. Li, T.-C. Chen, Y. Ren, P.I. Hendricks, R.G. Cooks, Z. Ouyang, Mini 12, miniature mass spectrometer for clinical and other applications—introduction and characterization, Anal. Chem. 86 (6) (2014) 2909–2916.
[23] C.J. Pulliam, R.M. Bain, J.S. Wiley, Z. Ouyang, R.G. Cooks, Mass spectrometry in the home and garden, J. Am. Soc. Mass Spectrom. 26 (2) (2015) 224–230.

Full Paper

Propellants, Explosives, Pyrotechnics

DOI: 10.1002/prep.201800122

Analysis of Residual Explosives by Swab Touch Spray Ionization Mass Spectrometry

Ryan M. Bain[+],[a] Patrick W. Fedick[+],[a] Jonathan M. Dilger,[b] and R. Graham Cooks*[a]

Abstract: Swab touch spray ionization mass spectrometry, an ambient ionization technique, has been applied to the analysis of six explosives from various surfaces including glass, metal, Teflon, plastic, human hands and three types of gloves (nitrile, vinyl and latex). A swab, attached to a metallic handle, was used to sample explosive residues and acted as the ion source. The explosives, 1,3,5-trinitro-1,3,5-triazinane (RDX), 1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX), and 2,2-bis[(nitrooxy)methyl]propane-1,3-diyl dinitrate (PETN) had an absolute limit of detection of 10 ng from all the surfaces except for PETN from the nitrile gloves (limit of detection 100 ng). Sodium perchlorate, 2-methyl-1,3,5-trinitrobenzene (TNT) and tetra-butylammonium perchlorate had limits of detection of 100 pg, 10 pg, and 1 pg, respectively from all surfaces. This study demonstrates the feasibility of swab touch spray ionization mass spectrometry for detection of a wide array of explosives from a variety of forensically applicable surfaces with disposable, commercial, tamperproof and individually-wrapped conductive swabs without complicated/lengthy sample preparations or extractions.

Keywords: Mass Spectrometry · Ambient Ionization · Explosives

1 Introduction

Residual and trace explosives analysis by mass spectrometry typically involves the use of a swab or wipe followed by a lengthier sample preparation procedure [1]. Liquid chromatography or gas chromatography coupled to mass spectrometry [2] and ion mobility [3] represent the current state of the art methods in explosives detection. Colorimetric detection methods have been developed but typically suffer from low specificity [4]. Raman spectroscopy methodologies look promising as a good alternative; however, there can be issues with fluorescence and background mitigation [5]. Bulk detection of explosives is commonly performed by x-ray diffraction as highlighted in a recent review [6]. Radioactive sources (typically ^{63}Ni), are also commonly used, but they represent a major cost burden due to additional administrative oversight to track the sources and routine testing for radiation leakages [7]. A more rapid and simpler test for explosives would be useful in a variety of applications, including airport security and forensic crime scenes. The relatively new field of ambient ionization mass spectrometry pertains to ionization under atmospheric pressure with the stipulation of little to no sample preparation (unlike most electrospray ionization or atmospheric pressure chemical ionization experiments) [8]. Desorption electrospray ionization [9] (DESI) and direct analysis in real-time [10] (DART) were the first ambient ionization sources to be reported and many other have followed including low temperature plasma ionization [11] (LTP), paper spray ionization [12] (PS) and swab touch spray ionization [13]. As rapid analysis of explosive residues is desirable, both DESI [14] and DART [15] have been employed for this application [16]. These methods have proven especially useful in the analysis on skin, clothing, and other surfaces; however, both methodologies require specialized equipment. Examples include the DART plasma generator and the DESI spray setup, of which neither is readily coupled to a portable instrument which would be desirable for *in-situ* analysis [17]. A recent review has detailed the current advances of ambient ionization to the trace detection of explosive residues [18].

Low temperature plasma ionization has been applied extensively to explosive residues [19]. The low temperature characteristic of the plasma, as well as being safe to the touch, enables residues to be sampled from a variety of surfaces, including human skin without physical damage or sample degradation [11]. This technique has also been used with portable mass spectrometers, where a backpack MS showed promise for *in-situ* measurements for homeland security and defense applications [20]. While LTP is promising, it does require that a specialized ionization source be used and it utilizes high AC potentials which have an extra level

[a] *R. M. Bain,[−] P. W. Fedick,[+] R. G. Cooks*
Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States
**e-mail: cooks@purdue.edu*
[b] *J. M. Dilger*
Naval Surface Warfare Center Crane Division, Crane, Indiana 47522, United States
[+] *These authors contributed equally*

Supporting information for this article is available on the WWW under https://doi.org/10.1002/prep.201800122

of safety concern [11]. By contrast, one of the simplest ambient ionization techniques, PS, uses only a low current DC potential to ionize analytes from a sharply pointed piece of paper wetted with solvent [21]. As shown previously, this allows detection of 2-methyl-1,3,5-trinitrobenzene (TNT), 2,2-bis[(nitrooxy)methyl]propane-1,3-diyl dinitrate (PETN), 1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX) and 1,3,5-trinitro-1,3,5-triazinane (RDX) [22]. PS has a low barrier of entrance into a forensic setting as the paper acts as both as the sampling device and the ionization source. While PS shows great promise, the stability of the tip is essential for successful ionization, and this can make swabbing with the paper substrate difficult [23]. If the tip of the paper substrate is damaged during swabbing, subsequent analysis will be adversely affected, although proper training can alleviate this problem [24].

Swab touch spray ionization, as performed on this experiment, utilizes a rayon swab or an alternative swabbing substrate attached to a conductive handle [25]. Surface analytes are attached to the swab surface by moving the swab over the surface of interest. Solvent is continually applied to the swab's tip after sampling the surface, and a high voltage is applied to the metallic handle to generate a Taylor cone which then leads to ionization of the sample [25]. Similar to paper spray ionization, the swab in swab touch spray ionization acts both as the sampling device and the ionization source. Swab touch spray ionization has the added advantage over PS of being able to perform analysis from an individually-packed, tamperproof swab, rather than a fragile hand-cut paper triangle [26]. Previously we have demonstrated the field-ability of swab touch spray ionization with portable instrumentation for the analysis of organic gunshot residues 1,3-diethyl-1,3-diphenylurea (ethyl centralite) and 1,3-dimethyl-1,3-diphenylurea (methyl centralite) [27]. Chemical warfare agent simulants have also been analyzed by this methodology [28]. Herein, we demonstrate the feasibility of swab touch spray ionization mass spectrometry for the analysis of six residual explosives from various forensically applicable surfaces.

2 Experimental

2.1 Chemicals and Surfaces Preparation

The explosives TNT, PETN, HMX and RDX were purchased from Cerilliant (Round Rock, Texas), while sodium perchlorate and tetrabutylammonium perchlorate were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade acetonitrile was purchased from Fisher Scientific (Hampton, NH). Samples were prepared from 1.0 mg/mL stock solutions in acetonitrile. Direct analysis of explosives was performed by depositing 5.0 μL of solution on the swab using a pipette. Lower concentrations for limit of detection studies were prepared by serial dilutions (orders of magnitude) from the stock solutions to provide conservative limits of detection from the surface. This method created a conservative approximate limit of detection that can be expected in the field and is not determined by extrapolation. Surfaces bearing trace explosives were prepared by spotting 1.0 μL of reagent onto each surface. Surfaces included black nitrile exam gloves (Ammex, Seattle, WA), diamond grip latex gloves (Microflex, Reno, NV), anti-static vinyl gloves (OAK Technical, Matteson, IL), glass microscope slides (Gold Seal, Portsmouth, NH), polytetrafluoroethylene (PTFE) plugs (Swagelok, Indianapolis, IN), stainless-steel plugs (Swagelok, Indianapolis, IN), blue polyethylene flat-top screw cap (Fisher Scientific, Hampton, NH) and human hands (those of the authors). Once the sample had been spotted on the surface, it was allowed to dry prior to swabbing with a swab prewetted with 20 μL of acetonitrile.

2.2 Swab Touch Spray Ionization

Ionization was performed from rayon medical grade sterile swabs (Copan Diagnostics, Murritea, CA). Each swab was opened by breaking the tamper-proof seal and used only once. Prior to swabbing, the swabs were pre-wetted with 20 μL of acetonitrile. This volume was selected as the swabs remained saturated with solvent during swabbing. To collect samples, the surface was sampled by swabbing using a circular motion (Figure 1). Then swabs were positioned 5–10 mm above the ion transfer capillary of the mass spectrometer. The position was an important variable. If the swab was too close to the inlet, discharge would occur. Conversely, if the swab was too far away, a stable cone would not be produced resulting in an unstable signal or no signal at all. Acetonitrile was added to the positioned swab using a syringe pump (Harvard Apparatus standard infusion only PHD 22/2000 syringe pump, Holliston, MA) connected to a 0.5 mL gastight syringe (Hamilton, Reno, NV) at a flow rate 10–30 mL/min. This flow was varied to maintain a steady spray without over wetting and unnecessarily diluting the sample. A high voltage of +/−5.5 kV was applied to the aluminum handle, and the generation of a spray could be observed by eye. The flow rate as well as the posi-

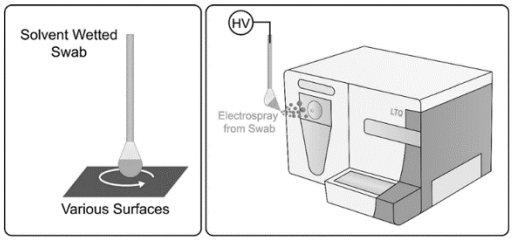


Figure 1. Pictorial representation of swab touch spray ionization source demonstrating sampling (left) and ionization using a commercial ion trap mass spectrometer (right).

tion were both optimized in order to achieve a steady signal. Efforts have been made previously to achieve robust signal with 3D printed parts and a camera to aid in alignment [26]. Similarly, a rail system has been used to aid in alignment for non-expert users and in-field applications with miniature instrumentation [29].

2.3 Mass Spectrometry

All mass spectral analyses were performed using a Thermo LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap mass spectrometer (San Jose, CA). Spectra were collected in negative ion mode, except when analyzing tetrabutylammonium perchlorate, which was collected in both positive and negative ion modes. The instrument also served as the supply of the high voltage applied to the metallic shaft of the swab. The entire time from swabbing through the collection of the spectra is ca 2 minutes.

3 Results and Discussion

Direct analysis of 5 μg of TNT using the product ion scan (Figure 2) gave a spectrum rich in fragment ions. The fragmentation of TNT has been previously studied by DESI [14a] and LTP [17] and both the radical anion (*m/z* 227) and deprotonated (*m/z* 226) TNT were observed. Using an isolation width of 3 Th on the LTQ and a peak centered at *m/z* 226.5, both the radical anion and deprotonated TNT were isolated and fragmented. Product ions at *m/z* 210, *m/z* 197, and *m/z* 196 correspond to the loss of OH, the loss of NO, and the loss of HNO, respectively. The product ion mass spectrum recorded for 10 pg of TNT swabbed from a glass surface (Figure S1) gave this as the limit of detection.

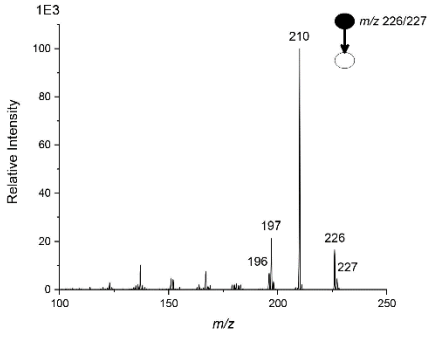


Figure 2. MS/MS product ion scan of TNT using swab touch spray ionization. The ion at *m/z* 226 corresponds to the $[M-H]^-$ species and the ion at *m/z* 227 corresponds to the $[M]^{\cdot}$ as expected. Product ions at *m/z* 210, *m/z* 197, and *m/z* 196 correspond to the loss of OH, the loss of NO, and the loss of HNO, respectively.

Full scan mass spectra recorded for the direct analysis of 5.0 μg of RDX, HMX, and PETN from a swab are shown in Figure 3. The full scan mass spectra at the limit of detection of 10 ng of RDX, HMX, and PETN swabbed off a glass slide are recorded Figure S2. In agreement with the literature [19b, 30], RDX was identified by four ions that form adducts with RDX; $[RDX+Cl_{35/37}]^-$ at *m/z* 257 and *m/z* 259, $[RDX+NO_2-H]^-$ at *m/z* 267 and $[RDX+NO_3]^-$ at *m/z* 284. Similarly, HMX and PETN were identified by the corresponding four adducts. HMX was identified by $[HMX+Cl_{35/37}]^-$ at *m/z* 331 and *m/z* 333, $[HMX+NO_2-H]^-$ at *m/z* 341 and $[HMX+NO_3]^-$ at *m/z* 358. PETN was identified by $[PETN+Cl_{35/37}]^-$ at *m/z* 351 and *m/z* 353, $[PETN+NO_2-H]^-$ at *m/z* 361 and $[PETN+NO_3]^-$ at *m/z* 378. PETN had a limit of detection of 10 ng on all surfaces except for the nitrile glove surface, which had a measured limit of 100 ng. These three explosives were identified by their adducts rather than their fragmentation profiles as no measurable fragments are detected on the ion trap mass spectrometer, as is consistent with previous results [14d].

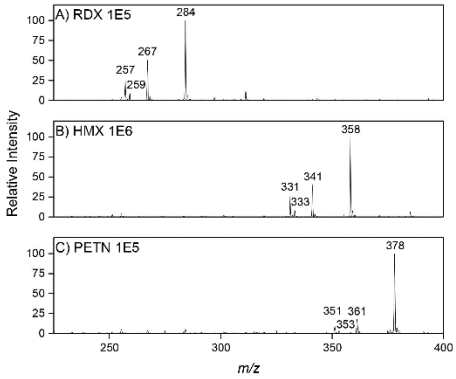


Figure 3. A) Swab touch spray ionization mass spectrum of 5 μg of RDX in negative ion mode. The ions at *m/z* 257 and *m/z* 259 correspond to $[RDX+Cl_{35/37}]^-$ at *m/z* 267 to $[RDX+NO_2-H]^-$ and at *m/z* 284 to $[RDX+NO_3]^-$. B) Mass spectrum of 5 μg of HMX in negative ion mode. The ions at *m/z* 331 and *m/z* 333 correspond to $[HMX+Cl_{35/37}]^-$ at *m/z* 341 to $[HMX+NO_2-H]^-$ and at *m/z* 358 to $[HMX+NO_3]^-$. C): Mass spectrum of 5 μg of PETN in negative ion mode. The ions at *m/z* 351 and *m/z* 353 correspond to $[PETN+Cl_{35/37}]^-$ at *m/z* 361 to $[PETN+NO_2-H]^-$ and at *m/z* 378 to $[PETN+NO_3]^-$.

Explosive perchlorate compounds were also of interest in this study because of their widespread use [31]. The direct analysis of 5.0 μg of tetrabutylammonium perchlorate was analyzed in full scan negative ion mode to look for perchlorate ions, as well as in the positive ion mode to measure tetrabutylammonium (Figure 4). Tetrabutylammonium at *m/z* 242 produced fragments at *m/z* 186, *m/z* 184, *m/z* 142, *m/z* 130 and *m/z* 100 corresponding to tribu-

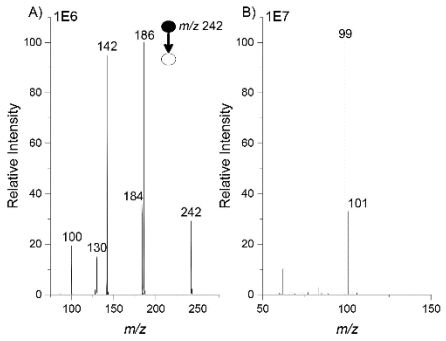


Figure 4. A) Swab touch spray ionization mass spectrometry product ion scan mass spectrum of tetrabutylammonium perchlorate in positive ion mode shows tetrabutylammonium at *m/z* 242 and characteristic fragment ions including those of *m/z* 186, 184, 142, 130, and 100. B) Swab touch spray ionization mass spectrometry full scan mass spectrum in negative ion mode of tetrabutylammonium perchlorate shows the perchlorate ions at *m/z* 99 and *m/z* 101.

tylammonium, dehydrogenated tributyl ammonium, dehydrogenated methyl dibutylammonium, dibutylammonium and loss of ethane from dehydrated dibutyl ammonium cation. The product ion scan mass spectrum establishing the limit of detection as 1 pg of tetrabutylammonium perchlorate swabbed from a glass surface can be found in Figure S3. Sodium perchlorate was also analyzed (Figure S4) and the characteristic perchlorate peaks were used for identification.

In addition to characterizing explosives applied directly to the swab (Figure 2–4) and trace residues on glass (S1–S4), a variety of forensically applicable surfaces were also screened for explosive residues. Identical to the experiments using glass, a known amount was spotted onto the substrate and the sample was allowed to dry. The swab, pre-wetted with 20 µL of acetonitrile, was rastered over the surface in a circular motion to collect the explosive residue. Surfaces and their corresponding limits of detection can be found in Table 1 for each solid surface. Additionally, for screening and forensic purposes, gloves and human hands were swabbed for all explosives and limits of detection for each explosive can be found in Table 2.

Table 1. Approximate limits of detection for explosives from various surfaces by swab touch spray ionization mass spectrometry.

Explosive	Glass	PTFE	Stainless steel	Polyethylene
TNT	10 pg	10 pg	10 pg	10 pg
RDX	10 ng	10 ng	10 ng	10 ng
HMX	10 ng	10 ng	10 ng	10 ng
PETN	10 ng	10 ng	10 ng	10 ng
Sodium perchlorate	100 pg	100 pg	100 pg	100 pg
Tetrabutylammonium perchlorate	1 pg	1 pg	1 pg	1 pg

Table 2. Approximate limits of detection for explosives from gloves and human hands by swab touch spray ionization mass spectrometry.

Explosive	Vinyl glove	Nitrile glove	Latex glove	Exposed hand
TNT	10 pg	10 pg	10 pg	10 pg
RDX	10 ng	10 ng	10 ng	10 ng
HMX	10 ng	10 ng	10 ng	10 ng
PETN	10 ng	100 ng	10 ng	10 ng
Sodium perchlorate	100 pg	100 pg	100 pg	100 pg
Tetrabutylammonium perchlorate	1 pg	1 pg	1 pg	1 pg

Analysis from common surfaces for residual amounts of explosives is applicable to forensic and security analyses, from airports to crime scenes [14d, 20a]. The ability to screen people by analyzing human hands is also of great interest in situations such as airport screenings. Similarly, finding gloves near a crime scene that have residual explosives can be traced back to a perpetrator using fingerprints [32] inside the glove or using cameras between the crime scene and the location where the gloves were discovered. Additionally, the successful swabbing of a variety of surfaces shows the robustness of this technique and suggests feasibility across a variety of other surfaces as well. The surfaces studied all gave similar results except that the limit of detection for PETN was an order of magnitude higher for nitrile gloves than other surfaces or other explosives. This is likely a result of chemical selectivity specifically related to PETN and nitrile.

4 Conclusion

To recapitulate, the analysis of six different explosive residues from a variety of surfaces (glass, stainless steel, polyethylene, PTFE, human hands, nitrile gloves, vinyl gloves and latex gloves) was performed with low absolute limits of detection without the need for complex and time-consuming extraction or sample preparation steps. Limits of detection for RDX, HMX, and PETN were 10 ng from all surfaces, with the exception of PETN on the nitrile gloves. Sodium perchlorate, TNT and tetra-butyl ammonium perchlorate had limits of detection of 100 pg, 10 pg, and 1 pg, respectively from all surfaces examined. The wide breadth of surfaces demonstrates the applicability of swab touch spray ionization for forensic, military and homeland security applications. In addition, as the explosives were detectable from human skin, airport security could employ this technology in conjunction with the current ion mobility systems. With swab touch spray ionization previously being demonstrated using portable mass spectrometers [27, 29],

this technique can be useful for *in-situ* analysis for real time detection of explosive residues.

The advantages of swab touch spray ionization are the ease of use, contained commercial packaging, low entrance barrier, and the fact that your sampling device is also your ionization source. When utilizing PS the paper triangle is the sampling and ionization source; however, swab touch spray does not require the analyst to carefully handle the device to preserve the tip as is needed in PS. Swab touch spray has detection limits comparable to PS [22] while providing a forensically feasible platform. While the detection limits have been shown to be lower for LTP, swab touch spray does not require additional parts such as the plasma generation and AC power supply [19a].

Acknowledgements

We acknowledge support from NSWC-CRANE CRANBAA18-002, the artistic contributions of Robert L. Schrader and discussions with Kiran Iyer. This material is also based on work supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Separations and Analysis Program (DE-FG02-06ER15807). Patrick Fedick acknowledges support from the Department of Defense SMART scholarship.

References

[1] a) O. Dalby, D. Butler, W. Birkett Jason, Analysis of gunshot residue and associated materials-A Review *J. Forensic Sci.* **2010**, *55*, 924–943; b) D. S. Moore, Instrumentation for trace detection of high explosives *Rev. Sci. Instrum.* **2004**, *75*, 2499–2512; c) L. Barron, E. Gilchrist, Ion chromatography-mass spectrometry: A review of recent technologies and applications in forensic and environmental explosives analysis *Anal. Chim. Acta* **2014**, *806*, 27–54.

[2] a) J. M. Perr, K. G. Furton, J. R. Almirall, Gas chromatography positive chemical ionization and tandem mass spectrometry for the analysis of organic high explosives *Talanta* **2005**, *67*, 430–436; b) M. Palit, D. Pardasani, A. K. Gupta, D. K. Dubey, Application of single drop microextraction for analysis of chemical warfare agents and related compounds in water by gas chromatography/mass spectrometry *Anal. Chem.* **2005**, *77*, 711–717; c) X. Xu, M. Koeberg, C.-J. Kuijpers, E. Kok, Development and validation of highly selective screening and confirmatory methods for the qualitative forensic analysis of organic explosive compounds with high performance liquid chromatography coupled with (photodiode array and) LTQ ion trap/Orbitrap mass spectrometric detections (HPLC-(PDA)-LTQOrbitrap) *Sci. Justice* **2014**, *54*, 3–21.

[3] a) J. Lee, S. Park, S. G. Cho, E. M. Goh, S. Lee, S.-S. Koh, J. Kim, Analysis of explosives using corona discharge ionization combined with ion mobility spectrometry-mass spectrometry *Talanta* **2014**, *120*, 64–70; b) Z. Du, T. Sun, J. Zhao, D. Wang, Z. Zhang, W. Yu, Development of a plug-type IMS-MS instrument and its applications in resolving problems existing in in-situ detection of illicit drugs and explosives by IMS *Talanta* **2018**, *184*, 65–72.

[4] J. Almog, S. Zitrin, in *Aspects of Explosives Detection* (Eds.: M. Marshall, J. C. Oxley), Elsevier, Amsterdam, **2009**, pp. 41–58.

[5] D. S. Moore, R. J. Scharff, Portable Raman explosives detection *Anal. Bioanal. Chem.* **2009**, *393*, 1571–1578.

[6] K. Wells, D. A. Bradley, A review of X-ray explosives detection techniques for checked baggage *Appl. Radiat. Isot.* **2012**, *70*, 1729–1746.

[7] R. G. Ewing, D. A. Atkinson, G. A. Eiceman, G. J. Ewing, A critical review of ion mobility spectrometry for the detection of explosives and explosive related compounds *Talanta* **2001**, *54*, 515–529.

[8] D. N. Correa, J. M. Santos, L. S. Eberlin, M. N. Eberlin, S. F. Teunissen, Forensic chemistry and ambient mass spectrometry: A perfect couple destined for a happy marriage? *Anal. Chem.* **2016**, *88*, 2515–2526.

[9] Z. Takáts, J. M. Wiseman, B. Gologan, R. G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization *Science* **2004**, *306*, 471–473.

[10] R. B. Cody, J. A. Laramée, H. D. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions *Anal. Chem.* **2005**, *77*, 2297–2302.

[11] J. D. Harper, N. A. Charipar, C. C. Mulligan, X. Zhang, R. G. Cooks, Z. Ouyang, Low-temperature plasma probe for ambient desorption ionization *Anal. Chem.* **2008**, *80*, 9097–9104.

[12] H. Wang, J. Liu, R. G. Cooks, Z. Ouyang, Paper spray for direct analysis of complex mixtures using mass spectrometry *Angew. Chem. Int. Ed.* **2010**, *49*, 877–880.

[13] A. K. Jarmusch, V. Pirro, K. S. Kerian, R. G. Cooks, Detection of strep throat causing bacterium directly from medical swabs by touch spray-mass spectrometry *Analyst* **2014**, *139*, 4785–4789.

[14] a) Z. Takats, I. Cotte-Rodriguez, N. Talaty, H. Chen, R. G. Cooks, Direct, trace level detection of explosives on ambient surfaces by desorption electrospray ionization mass spectrometry *Chem. Commun.* **2005**, 1950–1952; b) N. Talaty, C. C. Mulligan, D. R. Justes, A. U. Jackson, R. J. Noll, R. G. Cooks, Fabric analysis by ambient mass spectrometry for explosives and drugs *Analyst* **2008**, *133*, 1532–1540; c) D. R. Justes, N. Talaty, I. Cotte-Rodriguez, R. G. Cooks, Detection of explosives on skin using ambient ionization mass spectrometry *Chem. Commun.* **2007**, 2142–2144; d) I. Cotte-Rodríguez, Z. Takáts, N. Talaty, H. Chen, R. G. Cooks, Desorption electrospray ionization of explosives on surfaces: Sensitivity and selectivity enhancement by reactive desorption electrospray ionization *Anal. Chem.* **2005**, *77*, 6755–6764.

[15] a) F. Rowell, J. Seviour, A. Y. Lim, C. G. Elumbaring-Salazar, J. Loke, J. Ma, Detection of nitro-organic and peroxide explosives in latent fingermarks by DART- and SALDI-TOF-mass spectrometry *Forensic Sci. Int.* **2012**, *221*, 84–91; b) E. Sisco, J. Dake, C. Bridge, Screening for trace explosives by AccuTOF™-DART®: An in-depth validation study *Forensic Sci. Int.* **2013**, *232*, 160–168; c) J. M. Nilles, T. R. Connell, S. T. Stokes, D. H. Dupont, Explosives detection using direct analysis in real time (DART) mass spectrometry *Propellants Explos. Pyrotech.* **2010**, *35*, 446–451.

[16] T. P. Forbes, E. Sisco, Recent advances in ambient mass spectrometry of trace explosives *Analyst* **2018**, *143*, 1948–1969.

[17] N. L. Sanders, S. Kothari, G. Huang, G. Salazar, R. G. Cooks, Detection of explosives as negative ions directly from surfaces using a miniature mass spectrometer *Anal. Chem.* **2010**, *82*, 5313–5316.

[18] T. P. Forbes, E. Sisco, Recent advances in ambient mass spectrometry of trace explosives *Analyst* **2018**, *143*, 1948–1969.

[19] a) Y. Zhang, X. Ma, S. Zhang, C. Yang, Z. Ouyang, X. Zhang, Direct detection of explosives on solid surfaces by low temperature plasma desorption mass spectrometry *Analyst* **2009**, *134*, 176–181; b) W. Chen, K. Hou, X. Xiong, Y. Jiang, W. Zhao, L. Hua,

P. Chen, Y. Xie, Z. Wang, H. Li, Non-contact halogen lamp heating assisted LTP ionization miniature rectilinear ion trap: a platform for rapid, on-site explosives analysis *Analyst* **2013**, *138*, 5068–5073; c) J. F. Garcia-Reyes, J. D. Harper, G. A. Salazar, N. A. Charipar, Z. Ouyang, R. G. Cooks, Detection of explosives and related compounds by low-temperature plasma ambient ionization mass spectrometry *Anal. Chem.* **2011**, *83*, 1084–1092.

[20] a) P. I. Hendricks, J. K. Dalgleish, J. T. Shelley, M. A. Kirleis, M. T. McNicholas, L. Li, T.-C. Chen, C.-H. Chen, J. S. Duncan, F. Boudreau, R. J. Noll, J. P. Denton, T. A. Roach, Z. Ouyang, R. G. Cooks, Autonomous in situ analysis and real-time chemical detection using a backpack miniature mass spectrometer: Concept, instrumentation development, and performance *Anal. Chem.* **2014**, *86*, 2900–2908; b) J. K. Dalgleish, K. Hou, Z. Ouyang, R. G. Cooks, In situ explosive detection using a miniature plasma ion source and a portable mass spectrometer *Anal. Lett.* **2012**, *45*, 1440–1446.

[21] S. F. Teunissen, P. W. Fedick, B. J. A. Berendsen, M. W. F. Nielen, M. N. Eberlin, R. Graham Cooks, A. C. van Asten, Novel selectivity-based forensic toxicological validation of a paper spray mass spectrometry method for the quantitative determination of eight amphetamines in whole blood *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 2665–2676.

[22] C. W. Tsai, C. A. Tipple, R. A. Yost, Application of paper spray ionization for explosives analysis *Rapid Commun. Mass Spectrom.* **2017**, *31*, 1565–1572.

[23] R. D. Espy, A. R. Muliadi, Z. Ouyang, R. G. Cooks, Spray mechanism in paper spray ionization *Int. J. Mass Spectrom.* **2012**, *325–327*, 167–171.

[24] Z. E. Lawton, A. Traub, W. L. Fatigante, J. Mancias, A. E. O'Leary, S. E. Hall, J. R. Wieland, H. Oberacher, M. C. Gizzi, C. C. Mulligan, Analytical validation of a portable mass spectrometer featuring interchangeable, ambient ionization sources for high throughput forensic evidence screening *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 1048–1059.

[25] V. Pirro, A. K. Jarmusch, M. Vincenti, R. G. Cooks, Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry *Anal. Chim. Acta* **2015**, *861*, 47–54.

[26] A. K. Jarmusch, V. Pirro, D. L. Logsdon, R. G. Cooks, Direct ion generation from swabs *Talanta* **2018**, *184*, 356–363.

[27] P. W. Fedick, R. M. Bain, Swab touch spray mass spectrometry for rapid analysis of organic gunshot residue from human hand and various surfaces using commercial and fieldable mass spectrometry systems *Forensic Chemistry* **2017**, *5*, 53–57.

[28] D. T. Snyder, L. J. Szalwinski, R. L. Schrader, V. Pirro, R. Hilger, R. G. Cooks, Precursor and neutral loss scans in an RF scanning linear quadrupole ion trap *J. Am. Soc. Mass Spectrom.* **2018**, *29*, 1345–1354.

[29] P. W. Fedick, W. L. Fatigante, Z. E. Lawton, A. E. O'Leary, S. E. Hall, R. M. Bain, S. T. Ayrton, J. A. Ludwig, C. C. Mulligan, A low-cost, simplified platform of interchangeable, ambient ionization sources for rapid, forensic evidence screening on portable mass spectrometric instrumentation *Instruments* **2018**, *2*(2), 5.

[30] I. Cotte-Rodriguez, R. G. Cooks, Non-proximate detection of explosives and chemical warfare agent simulants by desorption electrospray ionization mass spectrometry *Chem. Commun.* **2006**, 2968–2970.

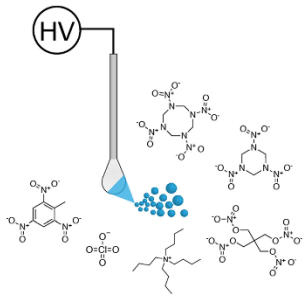
[31] E. Sokol, A. U. Jackson, R. G. Cooks, Trace detection of inorganic oxidants using desorption electrospray ionization (DESI) mass spectrometry *Cent. Eur. J. Chem.* **2011**, *9*, 790–797.

[32] D. R. Ifa, N. E. Manicke, A. L. Dill, R. G. Cooks, Latent fingerprint chemical imaging by mass spectrometry" *Science* **2008**, *321*, 805.

Received: April 19, 2018
Revised: August 13, 2018
Published online: ■■, ■■■

FULL PAPER

Analysis of Explosive Residues by Swab Touch Spray Ionization Mass Spectrometry.



*R. M. Bain, P. W. Fedick, J. M. Dilger, R. G. Cooks**

instruments

MDPI

Article

A Low-Cost, Simplified Platform of Interchangeable, Ambient Ionization Sources for Rapid, Forensic Evidence Screening on Portable Mass Spectrometric Instrumentation

Patrick W. Fedick [1], William L. Fatigante [2], Zachary E. Lawton [2], Adam E. O'Leary [2], Seth. E. Hall [2], Ryan M. Bain [1], Stephen T. Ayrton [1], Joseph A. Ludwig [2] and Christopher C. Mulligan [2,*]

[1] Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA; pfedick@purdue.edu (P.W.F.); rmbain@dow.com (R.M.B.); s.t.ayrton@icloud.com (S.T.A.)
[2] Department of Chemistry, Illinois State University, Normal, IL 61790, USA; wlfatig@ilstu.edu (W.L.F.); zelawton@gmail.com (Z.E.L.); aoleary@fona.com (A.E.O.); seth.edward.hall@gmail.com (S.E.H.); jaludw1@ilstu.edu (J.A.L.)
* Correspondence: mulligan@ilstu.edu; Tel.: +1-309-438-2464

Received: 14 February 2018; Accepted: 19 March 2018; Published: 25 March 2018

Abstract: Portable mass spectrometers (MS) are becoming more prevalent due to improved instrumentation, commercialization, and the robustness of new ionization methodologies. To increase utility towards diverse field-based applications, there is an inherent need for rugged ionization source platforms that are simple, yet robust towards analytical scenarios that may arise. Ambient ionization methodologies have evolved to target specific real-world problems and fulfill requirements of the analysis at hand. Ambient ionization techniques continue to advance towards higher performance, with specific sources showing variable proficiency depending on application area. To realize the full potential and applicability of ambient ionization methods, a selection of sources may be more prudent, showing a need for a low-cost, flexible ionization source platform. This manuscript describes a centralized system that was developed for portable MS systems that incorporates modular, rapidly-interchangeable ionization sources comprised of low-cost, commercially-available parts. Herein, design considerations are reported for a suite of ambient ionization sources that can be crafted with minimal machining or customization. Representative spectral data is included to demonstrate applicability towards field processing of forensic evidence. While this platform is demonstrated on portable instrumentation, retrofitting to lab-scale MS systems is anticipated.

Keywords: ambient ionization; portable mass spectrometry; portable instrumentation; desorption electrospray ionization (DESI); paper spray ionization (PSI); paper cone spray ionization (PCSI); atmospheric pressure chemical ionization (APCI); swab touch spray ionization (STSI); modular ion sources; forensics; drug evidence; arson

1. Introduction

Portable instrumentation, such as mass spectrometry (MS), has been of increased interest to the broader scientific community due to the inherent capability of performing in-situ analyses [1,2]. Additionally, the smaller footprint of portable instrumentation compared to their laboratory-based, "benchtop" counterparts allows for transportation to locales of interest. A review of current literature shows particularly high activity in regards to portable MS instrumentation and application development amongst the environmental [3,4], forensic [5–9] and defense [10,11] communities.

Portable MS systems are of particular interest across fieldable instrumental platforms due to the sensitivity, specificity, and rich chemical information provided by the technique, particularly tandem MS analysis [12,13]. A recent review of miniaturized MS systems outlined and compared the major research directions and entities offering commercial platforms [2].

Interestingly, a major uptick in applicability of portable MS systems has been recently seen, not from major instrumental modification (e.g., vacuum system, mass analyzer), but from advances in ionization source technology, particularly ambient ionization [14]. Ambient ionization mass spectrometry, or "ambient MS," in which analyte ions are created in the ambient environment from unprepared samples, arose with the development of desorption electrospray ionization (DESI) [15] and direct analysis in real time (DART) [16]. As ambient MS allows sample examination in its native state with little to no sample preparation, such as explosive residues on luggage [17] or fingerprints [18] or drug residues from clandestine drug lab apparatus [19], the forensic community continues to pursue adoption [20] and expansion of these techniques towards portable devices [7,21–23]. A number of recent reviews outlining forensic applications of ambient MS have been published [23–27]. Since the advent of DESI and DART, the last decade has seen an influx of new ambient MS methodologies, such as desorption atmospheric pressure chemical ionization (DAPCI) [28,29], paper spray ionization (PSI) [30], paper cone spray ionization (PCSI) [31], swab touch spray ionization (STSI) [32], low temperature plasma (LTP) probe [33], and flowing atmospheric pressure afterglow (FAPA) [34], to identify a few. Recent reviews have chronicled the development of these new ionization methods, with the number of demonstrated sources increasing every year [31,35–38].

Since the first report of ambient ionization on a portable MS system [39], researchers have sought to provide the reliability, ruggedness and usability that is required for practical in-situ analysis by non-technical operations (e.g., forensic practitioners, law enforcement officers). Subsequently, validation efforts in this realm have sought to assess competency towards adoption in the forensic sciences [20,22,40–42], for which there is an inherent need for court admissibility and method standardization. Steering committees such as the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) [43] provide recommendations for method standardization and general classification; note that mass spectrometry (in the form of gas chromatography/mass spectrometry, or GC/MS) is considered a "Category A" technique, which is the highest ranking. Recent efforts to meet the selectivity requirement for Category A classification via ambient MS are of note, such as the coupling of PSI-MS with surface-enhanced Raman spectroscopy [44]. Forward-thinking examinations into the legality of using portable, ambient MS systems in routine law enforcement activities have also been reported [6], as well as the utility afforded by employing interchangeable, ambient ionizations sources on portable MS systems for broad chemical evidence processing [22].

The rigor and variable nature of field-borne sample processing can be burdensome, but ambient MS techniques continue to advance towards higher performance, with specific sources showing chemical and/or matrix-specific proficiency depending on the application area. To realize the full potential and applicability of ambient MS, a selection of sources may be more prudent, showing a need for a low cost, flexible ionization source platform. To this end, a centralized mounting system was developed for portable MS instrumentation that incorporates modular, rapidly interchangeable ionization sources comprised of low-cost, commercially-available parts. Herein, design considerations are reported for a suite of ambient ionization sources that can be crafted with minimal machining or customization. Representative spectral data is included to demonstrate applicability towards field processing of forensic evidence. While this platform is demonstrated on portable instrumentation, retrofitting to lab-scale MS systems is anticipated.

2. Materials and Methods

2.1. Portable MS System and Ambient Ionization Sources

All ionization source development and applications were performed upon the FLIR Systems AI-MS 1.2 cylindrical ion trap (CIT) mass spectrometer (FLIR Mass Spectrometry, West Lafayette, IN USA), a fieldable, commercially-available system that has been ruggedized for in-situ analysis in harsh environments [11]. Figure S1 provides photos for scale, as well as extraneous, instrument specific information. System dimensions (60 × 50 × 40 cm, L × W × H) and weight (~45 kg), including all required peripherals for ambient ionization sources, is in line with fieldability requirements [2,45]. The AI-MS 1.2 can be operated in both positive and negative ion mode, utilizing MS/MS spectra for accurate chemical identification, which is afforded by the CIT mass analyzer. The miniaturized vacuum system of this instrument has relatively low power requirements, yet still maintains a capillary-based, atmospheric pressure inlet, which allows coupling to traditional spray-based ionization methods (e.g., electrospray ionization (ESI)) and newer ambient MS techniques. The AI-MS 1.2 platform comes with a factory-stock ESI/DESI combination ion source [46], which is detailed further in the Supplementary Materials, as it is not the focus of this work. Figure S2–S5 provide additional information, as well as sampling considerations of DESI-MS.

Development of ambient ionization sources focused on techniques that have shown high proficiency in the fields of forensic analysis, but more importantly, are noted by their simplicity of use and minimal consumable requirements. Specifically, PSI, PCSI, STSI, and atmospheric pressure chemical ionization (APCI) sources were developed, as all necessary voltages and syringe pumping requirements (for STSI) are inherent to the AI-MS 1.2 platform. No extraneous power supplies or pressurized gases are needed, enhancing overall portability. Note that the selection of ion sources developed herein, while diverse, do not represent an exhaustive list of methodologies that are or could be made inherently portable. Each constructed ionization source is described in detail below, providing design considerations, part descriptions, and representative data from samples of forensic interest and authentic evidence types. Furthermore, vendor/part information and overall cost breakdowns are delineated in Table S1 for quick reference. Consumable cost per sample (for swabs and cones) can be seen in Table S2.

For all ionization sources that employ spray solvent [ESI, DESI, PSI, PCSI, and STSI], a composition of 1:1 MeOH:H_2O with 0.1% formic acid was used; note that many solvent systems that are employed in LC-MS are typically compatible with ESI, DESI, PSI and PCSI. Spray or discharge voltage (for APCI) was set at 4 kV for all sources. All data reported herein was collected in positive ion mode.

2.2. Centralized Mounting System for Interchangeable Ion Source Modules

To allow quick interchangeability of ionization sources, akin to "plug and play" operation, a centralized, rail-based mounting/positioning system was implemented. Using stock optics parts from Thorlabs Inc. (Newton, NJ, USA), a universal base plate (CPVM) was adhered to the front fascia of the AI-MS 1.2, as depicted in Figure 1. This base plate uses a 4-rod cage assembly (ER6-P4), upon which a modular, 30 mm cage cube (C6W) can quickly slide on and off. This cage cube serves as the base for each ionization source developed, creating independent ionization "modules". The blank cage plate (CP01) seen in Figure 1 is optional, but does serve to add structural support and vibration resistance to the overall cage assembly. This mounting system, while specifically developed for the AI-MS 1.2, could also be implemented on several other commercial (e.g., Thermo (Waltham, MA, USA), Sciex (Washington, D.C, USA), etc.) and portable (e.g., MT Explorer 50 (Masstech, Columbia, MD, USA), Acquity QDa (Waters, Milford, MA, USA), etc.) MS systems. As the overall weight of this mounting system is low (<1 lb), high performance epoxy could potentially be used in lieu of screw mounting. Note that care should be taken so any remaining warranty is not voided or internal components/electronics are damaged. Further, as with all home-built ionization source development, safety precautions could be of interest to users to avoid shock and discharge to MS instrumentation.

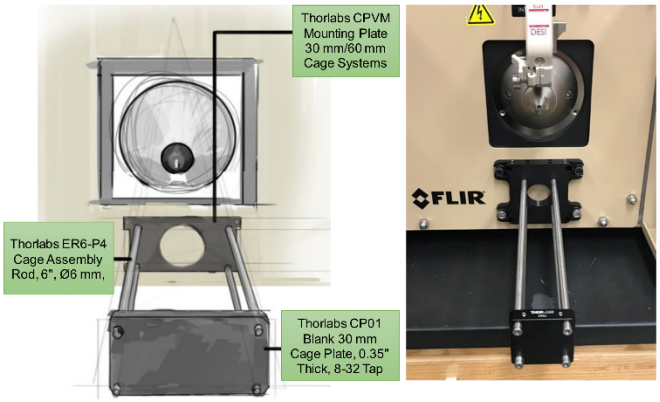


Figure 1. Centralized, rail-based mounting system to allow "plug-and-play" style operation of modular paper spray ionization (PSI), paper cone spray ionization (PCSI), atmospheric pressure chemical ionization (APCI), and swab touch spray ionization (STSI) sources, based upon stock optical consumables from Thorlabs Inc. (Newton, NJ, USA). The vertical mounting plate is attached directly with minor modification to front plate of FLIR AI-MS. The cage assembly rods are attached to the mounting plate and secured by the blank cage plate.

3. Results and Discussion

3.1. Paper Spray Ionization (PSI)

Paper spray ionization (PSI) [47], which employs mechanistic aspects of both paper chromatography and electrospray ionization, utilizes a triangularly-cut paper or porous substrate as a disposable ionization source. Analytes of interest can be investigated through a myriad of sampling strategies, such as deposition via pipetting, swabbing or dipping, and the swab itself can be used as a surface-transfer medium to investigate bound, trace residues [6,19]. Addition of a small aliquot (1–2 μL) of solution (e.g., 1:1 MeOH:H_2O with 0.1% formic acid via pipette) and high voltage allows analyte dissolvation and wicking/elution to the triangular egress of the paper, from which an ESI-like spray is generated [48]. As there is no need for pneumatics or extensive sample preparation, it is one of the simplest techniques for coupling to portable MS systems. PSI has been applied broadly in the forensic community, such as quantitative determination of abused drugs in whole blood [40] and rapid detection of explosives [49] and chemical warfare agent simulants [50]. It has been shown highly proficient towards drug evidence screening on portable MS systems, as well [9,19,22,51]. Current efforts towards substrate modification are also of interest [52,53], as they offer the capability for advanced sampling strategies that should prove interesting to the forensic community.

The constructed PSI-MS module (seen in Figure 2) is built upon a ThorLabs universal base plate (UBP2) fastened to a cage cube (C6W) using four stainless steel (SS) 4-40 thread screws. High voltage application to the employed paper substrate is accomplished with a simple "alligator-style" clamping electrode that is attached to a x-y-z translational positioning system (OptoSigma, Santa Ana, CA, USA); note that there are many suppliers of similar translational stages. This allows positioning of the PSI paper substrate (Millipore MQuant blank testing strips, Fisher Scientific, Waltham, MA, USA) relative to the MS capillary. Bulk 6066 aluminum block was machined to provide adequate height of the positioning system from the universal base plate; this height will be specific to the MS system employed. For additional utility, a polytetrafluoroethylene (PTFE) tube union cross fitting (UC-F-4, iPolymer, Inc., Irvine, CA, USA) was fastened to the vertical (z) positioner of the stage. Inside the union, the HV cable terminates in a bullet-style, quick-snap connector. Soldering of the corresponding

female connector to alligator clamping electrode then allows the user to quickly switch out this part for hygiene/carryover remediation; note that this modification is not required for performing PSI-MS analysis. A side profile view of the source module can be seen in Figure S9.

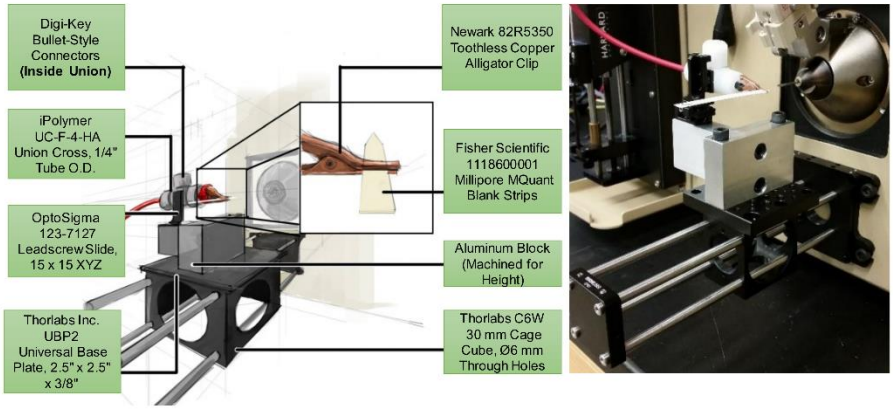


Figure 2. Simplified paper spray ionization (PSI) source module built upon the ThorLabs cage cube for the centralized mounting system. High voltage is supplied via alligator clip, which is held in place and positioned via a stock x-y-z positioner (OptoSigma). A simple Teflon union (iPolymer) is used to insulate the high voltage cable of the MS system, which terminates in a bullet-style connector that allows quick-swapping of alligator clips. Application of solvent by the user via pipette and high voltage to the paper substrate utilized produces analyte ions via electrospray-like processes. Said paper substrate can be used for dipping, spotting or physically transferring via surface swabbing chemicals of interest.

Figure 3 shows representative data collected with modular PSI source. Figure 3 depicts the resultant spectra obtained after using a paper substrate to gently swab the interior of a storage bag containing bulk heroin evidence. PSI-MS spectra are marked by its simplicity, here yielding the protonated molecule, $[M + H]^+$, for heroin at m/z 370. Corresponding PSI-MS/MS spectra (Figure 3B) of the protonated precursor ion produces characteristic fragmentation that can used for accurate chemical identification. Figure 3C depicts the analysis of injectable drug evidence containing morphine sulfate via PSI-MS; here, the liquid phase drug was spotted directly on the paper substrate via the syringe paraphernalia it resided in. PSI-MS/MS collected for both heroin and morphine were highly similar to that reported in literature [54]. Photos of the authentic evidence analyzed via PSI-MS can be seen in Figures S6 and S7 for rock heroin and injectable morphine sulfate, respectively.

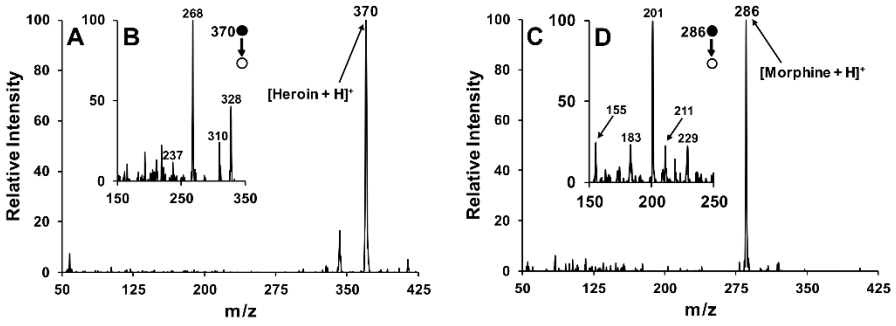


Figure 3. (**A**) PSI-MS spectrum from a swab used to probe heroin evidence, displaying the protonated molecule, $[M + H]^+$, at m/z 370. (**B**) Corresponding PSI-MS/MS spectrum of the protonated precursor ion, yielding characteristic fragmentation used for chemical identification. (**C**) PSI-MS spectrum collected from an injectable solution of morphine sulfate, displaying the protonated molecule at m/z 286. (**D**) Corresponding PSI-MS/MS spectrum of protonated morphine, yielding characteristic fragmentation.

3.2. Paper Cone Spray Ionization (PCSI)

Paper cone spray ionization (PCSI), a three-dimensional variant of PSI, employs a hydrophobic wax paper cone as both the sample holder, extraction chamber, and disposable ionization source [31]. Solid analytes (e.g., bulk powders, psychotropic plants, etc.) can be placed into the triangular pyramidal structure, in which an in-situ solid-liquid extraction occurs upon the addition of solvent (e.g., ~100 µL of 1:1 MeOH:H_2O with 0.1% formic acid); methodologies for hand-folding these cones have been reported by Kim and Cha [31]. Similar to PSI, a high voltage is then applied to perform spray-like ionization of dissolved analytes from the pyramidal egress, with no need for pneumatic assistance. PCSI is a relatively new technique, but it has already been applied to food products such as ground beef and tea leaves [31], abused pharmaceutical tablets [22], and medicinal herbs [55]. As a majority of forensic evidence encountered in the field would be in bulk phase (e.g., powders, crystals), PSCI is a promising in-situ analysis for seized drugs for its simplicity and minimalistic preparation requirements.

The constructed PCSI-MS module (seen in Figure 4) is built upon a ThorLabs universal base plate (UBP2) fastened to a cage cube (C6W) using four SS 4-40 thread screws. To provide the proper height and angle in relation to the inlet capillary of the MS system employed, two optical construction posts (ThorLabs, TR3T) were fastened to the base plate; angular positioning of the PCSI source in relation to the MS inlet was shown to be fairly robust, with 20–60° producing relatively similar ion signal. For routing and securing the HV cable, a ThorLabs right angle clamp (RA90) and threaded adaptor (AI25E8E) was used. Using an alligator clamping electrode fashioned to the HV cable allows one to secure the conical ionization of PCSI, while also delivering the required high voltage.

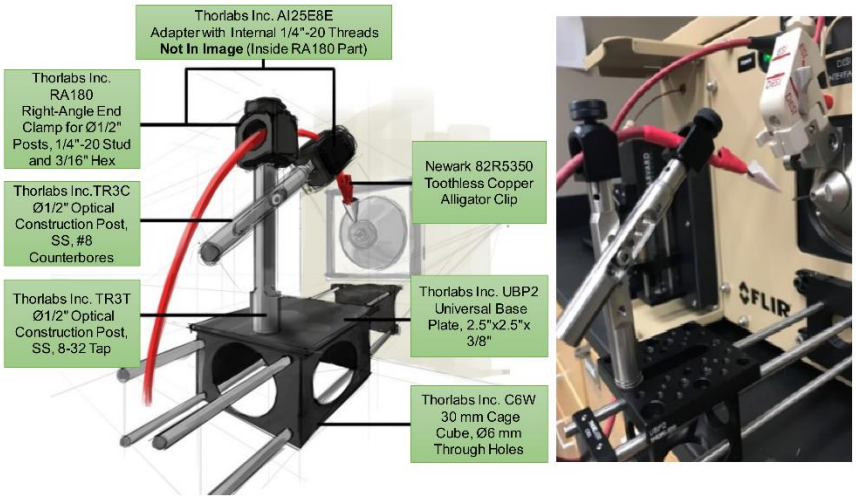


Figure 4. Simplified paper cone spray ionization (PCSI) source module built upon the ThorLabs cage cube for the centralized mounting system. Optical construction posts and adapters allow positioning of the alligator clip that serves to hold the conical ion source volume and supply high voltage. Application of spray solvent to the conical volume allows dissolvation of the bulk sample placed within, followed by spray ionization at the pyramidal egress of the source.

Figure 5 shows PCSI-MS and MS/MS spectra collected directly from Suboxone® sublingual film, a pharmaceutical-grade opioid deterrent. By simply folding the film and placing it into the pyramidal structure, addition of spray solvent generates highly intense ion signatures for the active ingredients naloxone (m/z 328) and buprenorphine (m/z 468). A photo of this evidence can be seen in Figure S8.

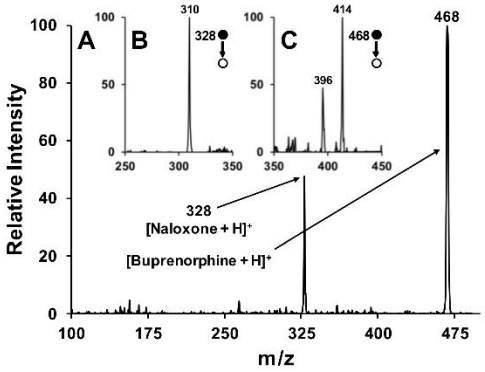


Figure 5. (**A**) PCSI-MS spectrum collected directly from Suboxone® sublingual film, a prescribed treatment for opioid addiction. Addition of the film to the conical volume of the source and spray solvent produces intense ion signatures for protonated naloxone (m/z 328) and protonated buprenorphine (m/z 468). Corresponding PCSI-MS/MS spectra from (**B**) naloxone and (**C**) buprenorphine yield fragmentation patterns similar to that reported in literature [56,57].

3.3. *Swab Touch Spray Ionization (STSI)*

Swab Touch Spray Ionization (STSI) utilizes a rayon swab or alternative swabbing substrate that specifically has a metallic handle. The surface-bound analytes are adsorbed via physical transfer onto said substrate after moving the swab over the surface of interest. A continual flow of solvent is applied to the swab tip along with high voltage through the metallic handle to initiate ionization [32]. Like both PSI and PCSI, there are no pneumatics or sample preparation required, barring the physical transfer event. STSI has been applied to bacterial [58] and drugs of abuse [59] detection in oral fluids, as well as the detection of organic gunshot residues from a variety of surfaces, including the hands of individuals after firearm discharge [32]. Specific benefits of STSI include the ability to collect samples from rough surfaces that can damage PSI paper substrates and the propensity for very stable spray ionization from the swab itself (e.g., extended spray durations). Of note, swab-based sampling is already used extensively by the forensic community, so there is less of a conceptual barrier [32].

The constructed STSI-MS module (seen in Figure 6) is built upon a ThorLabs universal base plate (UBP2) fastened to a cage cube (C6W) using four SS 4-40 thread screws. To provide the required height in relation to the MS inlet capillary, an optical post (ThorLabs, TR8) was used in a similar fashion as the PCSI module. Onto the optical post, a right-angle clamp (RA90) was attached to secure the HV cable, terminating with an alligator clamping electrode, in place. This electrode clamps directly to the swab handle itself, and height is adjusted so that the swab head is ~1 cm above the inlet. Solvent is flowed to the swab through fused silica capillary, which is connected to the onboard syringe pump of the AI-MS 1.2. As seen in the depiction, a slight customization was employed to protect the delicate silica capillary and secure contact to the swab head; note that this feature is not required to perform STSI-MS. A small piece of 6066 grade aluminum block was bored to the outer diameter of 1/16" SS tubing (IDEX, U-102), which is held in place by a set screw. This SS capillary sheath was bent to the appropriate angle to route the silica capillary (which is passed through the inner diameter of the capillary) to the swab head. The aluminum block was then spot-welded to the alligator clamping electrode. As all parts are conductive, HV still routes to the swab head to initiate spray ionization directly from the swab medium.

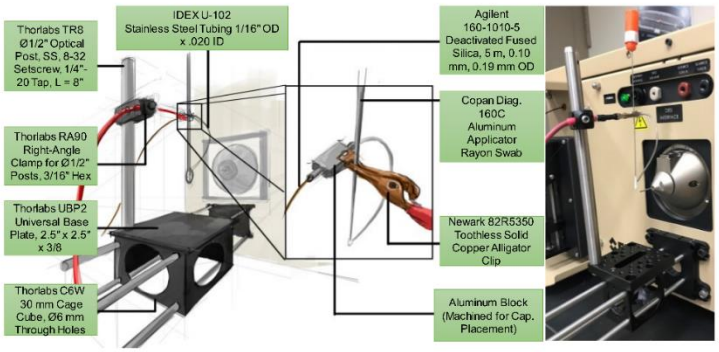


Figure 6. Simplified swab touch spray ionization (STSI) source module built upon the ThorLabs cage cube for the centralized mounting system. Similar to the PCSI source, an optical post is used to adjust positioning height of the alligator clip electrode. Depicted here is a machined clip modification, which is optional, that serves to route the fused silica, solvent delivery line running from the on-board syringe pump. A small section of stainless steel tubing, bent to an appropriate angle to maintain the position of the silica capillary in proximity to the sampling swab, is used to protect the glass-based delivery line. The high voltage applied via the alligator clip transfers directly to the wetted swab via its metallic shaft. Once adequate solvent saturates the swab, electrospray-like mechanisms produce ions from chemicals transferred to said swab during a sampling event.

Figure 7 shows representative STSI-MS spectra obtained from ethyl centralite, an organic gunshot residue commonly found on the hands and clothing of individuals who have discharged a firearm. Here, the STSI swab was used to probe a 400 ng ethyl centralite residue deposited onto the finger pad of an individual. Observed ions include the expected protonated form of ethyl centralite (m/z 269), but also a sodiated adduct at m/z 291. The propensity for alkali earth metal adducts in spray-based, ambient MS sources is commonly seen from real matrices, particularly in fingerprint oils.

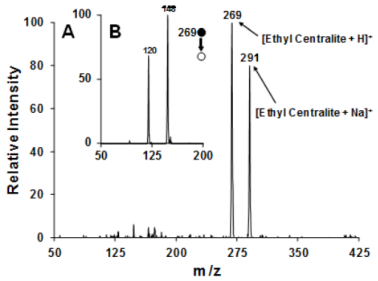


Figure 7. (**A**) STSI-MS spectrum collected after swabbing a trace, surface-bound residue of ethyl centralite, an organic component of gunshot residue. Both protonated (m/z 269) and sodiated (m/z 291) ethyl centralite is observed, as alkali earth metals can be abundant from authentic sample matrices and surfaces. (**B**) STSI-MS/MS of protonated ethyl centralite (m/z 269), yielding a simple, yet highly specific fragmentation pattern.

3.4. Atmospheric Pressure Chemical Ionization (APCI)

Direct air monitoring and headspace analysis has been demonstrated on portable MS systems by utilizing simplified atmospheric pressure chemical ionization (APCI) sources [60,61], including trace detection of toxic industrial compounds from room air [62,63] and headspace vapors of common solvents utilized in clandestine drug manufacture [19,22,64]. APCI, first reported by Horning et al., commonly employs a corona discharge to create primary reagent ions (typically a series of protonated water clusters, depending on relative humidity) via ion/molecule reactions starting with $N_2^{+\cdot}$ and $O_2^{+\cdot}$ [65]. Said protonated water clusters then go on to protonate (in positive ion mode) gas-phase analytes present in the corona region via charge exchange mechanisms, when energetically favorable [66]. Corona discharge can be produced in a minimalistic fashion, as only a discharge needle (e.g., tungsten wire) and DC HV under resistance is needed.

The constructed APCI-MS module (seen in Figure 8; photos can be found in Figures S10–S12) is built upon a ThorLabs universal base plate (UBP2) fastened to a cage cube (C6W) using four SS 4-40 thread screws. The corona discharge capillary (created from a 1 cm section of Hamilton syringe cleaning wire soldered into a 1/16" SS capillary) is housed within a PTFE body (iPolymer FRT-F-42-HA [female] and MRT-F-42-HA [male] run tees) for safety; internal views of the discharge needle and ionization volume can be seen in Figures S13 and S14. The corona discharge needle is held in close proximity (~1 cm) to the MS inlet, which serves as the counter electrode after application of 4 kV DC. Note that caution must be taken in order to prevent direct arcing to the inlet capillary, which could prove detrimental to the MS system. The exposed section of SS tubing (which extends out of the PTFE body) is where the HV cable is attached. This can be done in a variety of ways, including an alligator clip. Note that a more effective, current-controlled corona discharge is generated when resistance is applied in series to the discharge needle (100 MΩ resistor, 1.5 W, 66k6838, Newark, Inc., Chicago, IL, USA); this also offers some protection from unwanted arcing to the MS inlet. To provide the height relative to the MS inlet and rigidity of the ionization chamber, a ThorLabs optical post (TR1) was used to mount a machined aluminum plate to the universal base plate. This aluminum plate can be used to secure the ionization volume via screw clamps (CL3/M, Thorlabs) and inhibit misalignment of the

discharge needed relative to the MS inlet. The exact footprint and thickness of the aluminum plate can be matched to the instrument in question.

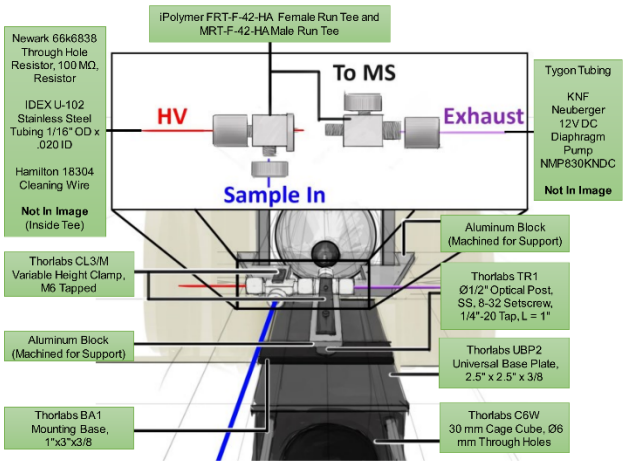


Figure 8. Simplified atmospheric pressure chemical ionization (APCI) source module built upon the ThorLabs cage cube for the centralized mounting system. A PTFE chamber is used to house a corona discharge needle and position it in proximity to the MS inlet capillary. The discharge needle is soldered to a stainless steel capillary for rigidity. High voltage is applied to the exposed portion of the discharge needle through an alligator clip electrode; a 100 MΩ resistor is soldered in series with the high voltage cable to produce a more effective corona discharge between the discharge needle and inlet capillary. The PTFE chamber is produced from tee unions, allowing an inlet for gas samples to be pulled through the source volume and exhausted via a small diaphragm pump and Tygon tubing. The PTFE chamber rests on an aluminum base that was machined to match the form factor on inlet system of the AI-MS 1.2. Overall height is controlled so that the discharge needle and inlet capillary align by an optical post.

To allow for the grab sampling of headspace vapors or emanating gases, a crude gas sampling system can be simply made from tygon or PTFE tubing through the available ports of the ionization chamber employed. Gaseous samples can be pulled through the discharge region for analysis (and subsequently exhausted) via a small external pump. For this source, a miniature, 12 V DC diaphragm pump (NMP830KNDC, KNF Neuberger, Trenton, NJ, USA) capable of ~3 L/min flows was employed for this purpose.

Figure 9 shows representative APCI-MS spectra obtained via grab sampling with the modular source. For Figure 9A, mock arson evidence was generated by burning pine wood in the presence of acetone as an accelerant. The charred wood sample was placed in an enclosed vessel so that headspace vapors could be formed, which were then subsequently sampled via external pumping. Headspace analysis produces rich spectra, including protonated acetone in the monomeric (m/z 59), hydrated (m/z 77), and dimeric (m/z 117) forms. The natural product pinene from the wood materials can be seen as both protonated (m/z 137) and hydrated (m/z 166) ions. Application to carpet-based arson evidence was also examined. Figure 9B shows nylon-based carpeting that was burned in the presence of ethanol as an accelerant. Headspace analysis via APCI-MS yielded the protonated ethanol dimer (m/z 93), but also interesting signatures from the carpet matrix. Spectral peaks at m/z 114 were tentatively identified as protonated caprolactam, which is a known degradation product from the nylon polymer used in construction of the carpet [67].

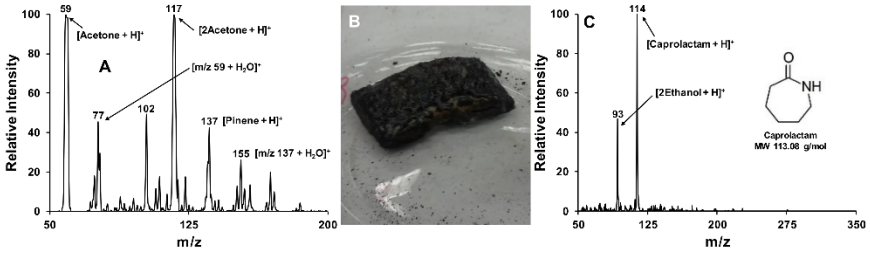


Figure 9. (**A**) APCI-MS spectrum collected from the gaseous headspace emanating from charred pine wood that was burned using acetone as an accelerant. Headspace analysis produces rich spectra, including the protonated acetone in the monomeric (*m*/*z* 59), hydrated (*m*/*z* 77), and dimeric (*m*/*z* 117) forms. The natural product pinene from the wood materials can be seen as both protonated (*m*/*z* 137) and hydrated (*m*/*z* 166) ions. (**B**) Photo showing a charred, nylon carpet sample burned in the presence of ethanol as an accelerant. (**C**) APCI-MS spectrum collected from the gaseous headspace emanating from the charred carpet sample, displaying the protonated ethanol dimer (*m*/*z* 93). Also seen is protonated caprolactam (*m*/*z* 114), which is a degradation product from the nylon polymer used in construction of the carpet.

4. Conclusions

In this work, design considerations are reported for a suite of ambient ionization sources that can be crafted with minimal machining or customization. The novelty of the modular, interchangeable design implemented could prove highly useful when coupled with portable MS instrumentation, particularly in application areas like forensic investigation where samples can be highly variable in nature.

As seen in Table S1, the centralized mounting system and PSI, PCSI, STSI, and APCI modules can be crafted for less than $2000 (per current pricing) and with minimal to no machining required; minor machining of custom parts, as shown herein, can enhance the overall usability of the sources, but is not required. A majority of peripherals used are common scientific parts, and even if the specific part/items reported here become obsolete or unavailable from the specified manufacturer, an analogous, commercial solution should be easily identified. Furthermore, while this platform is demonstrated on portable instrumentation, retrofitting to lab-scale MS systems is anticipated.

As the breadth of known ambient MS techniques increases, many should prove adaptable to this modular mounting system. For instance, techniques like desorption atmospheric pressure chemical ionization (DAPCI) [68], LTP [69], atmospheric pressure photoionization (APPI) [70], and easy ambient sonic spray ionization (EASI) [71] could prove adaptable, but may necessitate external compressed gas and power requirements. Simple platforms that are both frugal and expandable, such as that reported herein, could prove to be a catalyst towards full adoption by field practitioner communities.

Supplementary Materials: The following are available online at http://www.mdpi.com/2410-390X/2/2/5/s1, Table S1: Part List and Estimated Cost for Modular Ionization Sources, Table S2: Estimated Cost (per Sample) for Swab and Cone Consumables, photos of forensic evidence analyzed (Figures S6–S8), and alternate views of the modular ionization sources to guide construction (Figures S9–S14).

Acknowledgments: This project was supported by Award Nos. 2011-DN-BX-K552 and 2015-IJ-CX-K011, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice. Patrick Fedick would like to acknowledge support from the Department of Defense SMART scholarship.

Author Contributions: Patrick W. Fedick led manuscript creation, as well as the development of the touch spray ionization along with Ryan M. Bain, Stephen T. Ayrton, and Joseph A. Ludwig. William L. Fatigante led all evidentiary investigations on the FLIR Systems AI-MS 1.2 and the corresponding data analysis. Zachary E. Lawton, Adam E. O'Leary and Seth E. Hall each individually led the development of the paper cone spray ionization, paper

spray ionization, and atmospheric pressure chemical ionization sources, respectively. Christopher C. Mulligan was primary investigator for this work, and all instrumentation development and application was conducted in his laboratory.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Guo, Q.; Gao, L.; Zhai, Y.; Xu, W. Recent developments of miniature ion trap mass spectrometers. *Chin. Chem. Lett.* **2017**. [CrossRef]
2. Snyder, D.T.; Pulliam, C.J.; Ouyang, Z.; Cooks, R.G. Miniature and fieldable mass spectrometers: Recent advances. *Anal. Chem.* **2016**, *88*, 2–29. [CrossRef] [PubMed]
3. Meng, X.; Zhang, X.; Zhai, Y.; Xu, W. Mini 2000: A robust miniature mass spectrometer with continuous atmospheric pressure interface. *Instruments* **2018**, *2*. [CrossRef]
4. Plocoste, T.; Jacoby-Koaly, S.; Petit, R.-H.; Molinié, J.; Roussas, A. In situ quantification and tracking of volatile organic compounds with a portable mass spectrometer in tropical waste and urban sites. *Environ. Technol.* **2017**, *38*, 2280–2294. [CrossRef] [PubMed]
5. Mach, P.M.; McBride, E.M.; Sasiene, Z.J.; Brigance, K.R.; Kennard, S.K.; Wright, K.C.; Verbeck, G.F. Vehicle-mounted portable mass spectrometry system for the covert detection via spatial analysis of clandestine methamphetamine laboratories. *Anal. Chem.* **2015**, *87*, 11501–11508. [CrossRef] [PubMed]
6. Bruno, A.M.; Cleary, S.R.; O'Leary, A.E.; Gizzi, M.C.; Mulligan, C.C. Balancing the utility and legality of implementing portable mass spectrometers coupled with ambient ionization in routine law enforcement activities. *Anal. Methods* **2017**, *9*, 5015–5022. [CrossRef]
7. Brown, H.; Oktem, B.; Windom, A.; Doroshenko, V.; Evans-Nguyen, K. Direct Analysis in Real Time (DART) and a portable mass spectrometer for rapid identification of common and designer drugs on-site. *Forensic Chem.* **2016**, *1*, 66–73. [CrossRef]
8. Lam, R.; Lennard, C.; Kingsland, G.; Johnstone, P.; Symons, A.; Wythes, L.; Fewtrell, J.; O'Brien, D.; Spikmans, V. Person-portable equipment in environmental forensic investigations: Application to fire scenes. *Aust. J. Forensic Sci.* **2018**, 1–10. [CrossRef]
9. Hall, S.E.; O'Leary, A.E.; Lawton, Z.E.; Bruno, A.M.; Mulligan, C.C. Trace-Level Screening of Chemicals Related to Clandestine Desomorphine Production with Ambient Sampling, Portable Mass Spectrometry. *J. Chem.* **2017**. [CrossRef]
10. Evans-Nguyen, K.M.; Quinto, A.; Hargraves, T.; Brown, H.; Speer, J.; Glatter, D. Transmission mode desorption electrospray ionization (TM-DESI) for simultaneous analysis of potential inorganic and organic components of radiological dispersion devices (RDDs). *Anal. Chem.* **2013**, *85*, 11826–11834. [CrossRef] [PubMed]
11. Wells, J.M.; Roth, M.J.; Keil, A.D.; Grossenbacher, J.W.; Justes, D.R.; Patterson, G.E.; Barket, D.J. Implementation of DART and DESI ionization on a fieldable mass spectrometer. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1419–1424. [CrossRef] [PubMed]
12. Gao, L.; Song, Q.; Patterson, G.E.; Cooks, R.G.; Ouyang, Z. Handheld rectilinear ion trap mass spectrometer. *Anal. Chem.* **2006**, *78*, 5994–6002. [CrossRef] [PubMed]
13. Badman, E.R.; Graham Cooks, R. Miniature mass analyzers. *J. Mass Spectrom.* **2000**, *35*, 659–671. [CrossRef]
14. Cooks, R.G.; Ouyang, Z.; Takats, Z.; Wiseman, J.M. Ambient mass spectrometry. *Science* **2006**, *311*, 1566–1570. [CrossRef] [PubMed]
15. Takáts, Z.; Wiseman, J.M.; Gologan, B.; Cooks, R.G. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **2004**, *306*, 471–473. [CrossRef] [PubMed]
16. Cody, R.B.; Laramée, J.A.; Durst, H.D. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal. Chem.* **2005**, *77*, 2297–2302. [CrossRef] [PubMed]
17. Takats, Z.; Cotte-Rodriguez, I.; Talaty, N.; Chen, H.; Cooks, R.G. Direct, trace level detection of explosives on ambient surfaces by desorption electrospray ionization mass spectrometry. *Chem. Commun.* **2005**, 1950–1952. [CrossRef] [PubMed]
18. Justes, D.R.; Talaty, N.; Cotte-Rodriguez, I.; Cooks, R.G. Detection of explosives on skin using ambient ionization mass spectrometry. *Chem. Commun.* **2007**, 2142–2144. [CrossRef] [PubMed]

19. O'Leary, A.E.; Hall, S.E.; Vircks, K.E.; Mulligan, C.C. Monitoring the clandestine synthesis of methamphetamine in real-time with ambient sampling, portable mass spectrometry. *Anal. Methods* **2015**, *7*, 7156–7163. [CrossRef]
20. Steiner, R.R.; Larson, R.L. Validation of the direct analysis in real time source for use in forensic drug screening. *J. Forensic Sci.* **2009**, *54*, 617–622. [CrossRef] [PubMed]
21. Keil, A.; Talaty, N.; Janfelt, C.; Noll, R.J.; Gao, L.; Ouyang, Z.; Cooks, R.G. Ambient mass spectrometry with a handheld mass spectrometer at high pressure. *Anal. Chem.* **2007**, *79*, 7734–7739. [CrossRef] [PubMed]
22. Lawton, Z.E.; Traub, A.; Fatigante, W.L.; Mancias, J.; O'Leary, A.E.; Hall, S.E.; Wieland, J.R.; Oberacher, H.; Gizzi, M.C.; Mulligan, C.C. Analytical validation of a portable mass spectrometer featuring interchangeable, ambient ionization sources for high throughput forensic evidence screening. *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 1048–1059. [CrossRef] [PubMed]
23. Pavlovich, M.J.; Musselman, B.; Hall, A.B. Direct analysis in real time—Mass spectrometry (DART-MS) in forensic and security applications. *Mass Spectrom. Rev.* **2018**, *37*, 171–181. [CrossRef] [PubMed]
24. Correa, D.N.; Santos, J.M.; Eberlin, L.S.; Eberlin, M.N.; Teunissen, S.F. Forensic chemistry and ambient mass spectrometry: A perfect couple destined for a happy marriage? *Anal. Chem.* **2016**, *88*, 2515–2526. [CrossRef] [PubMed]
25. Ifa, D.R.; Jackson, A.U.; Paglia, G.; Cooks, R.G. Forensic applications of ambient ionization mass spectrometry. *Anal. Bioanal. Chem.* **2009**, *394*, 1995–2008. [CrossRef] [PubMed]
26. Morelato, M.; Beavis, A.; Kirkbride, P.; Roux, C. Forensic applications of desorption electrospray ionisation mass spectrometry (DESI-MS). *Forensic Sci. Int.* **2013**, *226*, 10–21. [CrossRef] [PubMed]
27. Green, F.M.; Salter, T.L.; Stokes, P.; Gilmore, I.S.; O'Connor, G. Ambient mass spectrometry: Advances and applications in forensics. *Surf. Interface Anal.* **2010**, *42*, 347–357. [CrossRef]
28. Jjunju, F.P.M.; Maher, S.; Li, A.; Badu-Tawiah, A.K.; Taylor, S.; Graham Cooks, R. Analysis of Polycyclic Aromatic Hydrocarbons Using Desorption Atmospheric Pressure Chemical Ionization Coupled to a Portable Mass Spectrometer. *J. Am. Soc. Mass Spectrom.* **2015**, *26*, 271–280. [CrossRef] [PubMed]
29. Chen, H.; Zheng, J.; Zhang, X.; Luo, M.; Wang, Z.; Qiao, X. Surface desorption atmospheric pressure chemical ionization mass spectrometry for direct ambient sample analysis without toxic chemical contamination. *J. Mass Spectrom.* **2007**, *42*, 1045–1056. [CrossRef] [PubMed]
30. Liu, J.; Wang, H.; Manicke, N.E.; Lin, J.-M.; Cooks, R.G.; Ouyang, Z. Development, characterization, and application of paper spray ionization. *Anal. Chem.* **2010**, *82*, 2463–2471. [CrossRef] [PubMed]
31. Kim, P.; Cha, S. Paper cone spray ionization mass spectrometry (PCSI MS) for simple and rapid analysis of raw solid samples. *Analyst* **2015**, *140*, 5868–5872. [CrossRef] [PubMed]
32. Fedick, P.W.; Bain, R.M. Swab touch spray mass spectrometry for rapid analysis of organic gunshot residue from human hand and various surfaces using commercial and fieldable mass spectrometry systems. *Forensic Chem.* **2017**, *5*, 53–57. [CrossRef]
33. Harper, J.D.; Charipar, N.A.; Mulligan, C.C.; Zhang, X.; Cooks, R.G.; Ouyang, Z. Low-temperature plasma probe for ambient desorption ionization. *Anal. Chem.* **2008**, *80*, 9097–9104. [CrossRef] [PubMed]
34. Shelley, J.T.; Wiley, J.S.; Hieftje, G.M. Ultrasensitive ambient mass spectrometric analysis with a pin-to-capillary flowing atmospheric-pressure afterglow source. *Anal. Chem.* **2011**, *83*, 5741–5748. [CrossRef] [PubMed]
35. Javanshad, R.; Venter, A.R. Ambient ionization mass spectrometry: Real-time, proximal sample processing and ionization. *Anal. Methods* **2017**, *9*, 4896–4907. [CrossRef]
36. Weston, D.J. Ambient ionization mass spectrometry: Current understanding of mechanistic theory; analytical performance and application areas. *Analyst* **2010**, *135*, 661–668. [CrossRef] [PubMed]
37. Monge, M.E.; Harris, G.A.; Dwivedi, P.; Fernández, F.M. Mass spectrometry: Recent advances in direct open air surface sampling/ionization. *Chem. Rev.* **2013**, *113*, 2269–2308. [CrossRef] [PubMed]
38. Maher, S.; Jjunju, F.P.M.; Taylor, S. Colloquium: 100 years of mass spectrometry: Perspectives and future trends. *Rev. Mod. Phys.* **2015**, *87*, 113–135. [CrossRef]
39. Mulligan, C.C.; Talaty, N.; Cooks, R.G. Desorption electrospray ionization with a portable mass spectrometer: In situ analysis of ambient surfaces. *Chem. Commun.* **2006**, 1709–1711. [CrossRef] [PubMed]

40. Teunissen, S.F.; Fedick, P.W.; Berendsen, B.J.A.; Nielen, M.W.F.; Eberlin, M.N.; Graham Cooks, R.; van Asten, A.C. Novel selectivity-based forensic toxicological validation of a paper spray mass spectrometry method for the quantitative determination of eight amphetamines in whole blood. *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 2665–2676. [CrossRef] [PubMed]
41. McKenna, J.; Jett, R.; Shanks, K.; Manicke, N.E. Toxicological drug screening using paper spray high-resolution tandem mass spectrometry (HR-MS/MS). *J. Anal. Toxicol.* **2018**. [CrossRef] [PubMed]
42. Gurdak, E.; Green, F.M.; Rakowska, P.D.; Seah, M.P.; Salter, T.L.; Gilmore, I.S. VAMAS interlaboratory study for desorption electrospray ionization mass spectrometry (DESI MS) intensity repeatability and constancy. *Anal. Chem.* **2014**, *86*, 9603–9611. [CrossRef] [PubMed]
43. SWGDRUG. SWGDRUG Recommendations Edition 7.1. Available online: http://www.swgdrug.org/Documents/SWGDRUG%20Recommendations%20Version%207-1.pdf (accessed on 20 March 2018).
44. Fedick, P.W.; Bills, B.J.; Manicke, N.E.; Cooks, R.G. Forensic sampling and analysis from a single substrate: Surface-enhanced Raman spectroscopy followed by paper spray mass spectrometry. *Anal. Chem.* **2017**, *89*, 10973–10979. [CrossRef] [PubMed]
45. Giannoukos, S.; Brkić, B.; Taylor, S.; Marshall, A.; Verbeck, G.F. Chemical Sniffing Instrumentation for Security Applications. *Chem. Rev.* **2016**, *116*, 8146–8172. [CrossRef] [PubMed]
46. Vircks, K.E.; Mulligan, C.C. Rapid screening of synthetic cathinones as trace residues and in authentic seizures using a portable mass spectrometer equipped with desorption electrospray ionization. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 2665–2672. [CrossRef] [PubMed]
47. Damon, D.E.; Davis, K.M.; Moreira, C.R.; Capone, P.; Cruttenden, R.; Badu-Tawiah, A.K. Direct biofluid analysis using hydrophobic paper spray mass spectrometry. *Anal. Chem.* **2016**, *88*, 1878–1884. [CrossRef] [PubMed]
48. Wang, H.; Liu, J.; Cooks, R.G.; Ouyang, Z. Paper spray for direct analysis of complex mixtures using mass spectrometry. *Angew. Chem. Int. Ed.* **2010**, *49*, 877–880. [CrossRef] [PubMed]
49. Tsai, C.-W.; Tipple, C.A.; Yost, R.A. Application of paper spray ionization for explosives analysis. *Rapid Commun. Mass Spectrom.* **2017**, *31*, 1565–1572. [CrossRef] [PubMed]
50. McKenna, J.; Dhummakupt, E.S.; Connell, T.; Demond, P.S.; Miller, D.B.; Michael Nilles, J.; Manicke, N.E.; Glaros, T. Detection of chemical warfare agent simulants and hydrolysis products in biological samples by paper spray mass spectrometry. *Analyst* **2017**, *142*, 1442–1451. [CrossRef] [PubMed]
51. O'Leary, A.E.; Oberacher, H.; Hall, S.E.; Mulligan, C.C. Combining a portable, tandem mass spectrometer with automated library searching—An important step towards streamlined, on-site identification of forensic evidence. *Anal. Methods* **2015**, *7*, 3331–3339. [CrossRef]
52. Damon, D.E.; Maher, Y.S.; Yin, M.; Jjunju, F.P.M.; Young, I.S.; Taylor, S.; Maher, S.; Badu-Tawiah, A.K. 2D wax-printed paper substrates with extended solvent supply capabilities allow enhanced ion signal in paper spray ionization. *Analyst* **2016**, *141*, 3866–3873. [CrossRef] [PubMed]
53. Maher, S.; Jjunju, F.P.M.; Damon, D.E.; Gorton, H.; Maher, Y.S.; Syed, S.U.; Heeren, R.M.A.; Young, I.S.; Taylor, S.; Badu-Tawiah, A.K. Direct Analysis and Quantification of Metaldehyde in Water Using Reactive Paper Spray Mass Spectrometry. *Sci. Rep.* **2016**, *6*, 35643. [CrossRef] [PubMed]
54. Pavlic, M.; Libiseller, K.; Oberacher, H. Combined use of ESI–QqTOF-MS and ESI–QqTOF-MS/MS with mass-spectral library search for qualitative analysis of drugs. *Anal. Bioanal. Chem.* **2006**, *386*, 69–82. [CrossRef] [PubMed]
55. Jun, G.; Park, T.-M.; Cha, S. Fast and simple chemical fingerprinting analysis of medicinal herbs by paper cone spray ionization mass spectrometry (PCSI MS). *Bull. Korean Chem. Soc.* **2016**, *37*, 1337–1343. [CrossRef]
56. Fang, W.B.; Chang, Y.; McCance-Katz, E.F.; Moody, D.E. Determination of Naloxone and Nornaloxone (Noroxymorphone) by High-Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry. *J. Anal. Toxicol.* **2009**, *33*, 409–417. [CrossRef] [PubMed]
57. Moody, D.E.; Slawson, M.H.; Strain, E.C.; Laycock, J.D.; Spanbauer, A.C.; Foltz, R.L. A Liquid Chromatographic-Electrospray Ionization-Tandem Mass Spectrometric Method for Determination of Buprenorphine, Its Metabolite, norBuprenorphine, and a Coformulant, Naloxone, That Is Suitable for in Vivo and in Vitro Metabolism Studies. *Anal. Biochem.* **2002**, *306*, 31–39. [CrossRef] [PubMed]
58. Jarmusch, A.K.; Pirro, V.; Kerian, K.S.; Cooks, R.G. Detection of strep throat causing bacterium directly from medical swabs by touch spray-mass spectrometry. *Analyst* **2014**, *139*, 4785–4789. [CrossRef] [PubMed]

59. Pirro, V.; Jarmusch, A.K.; Vincenti, M.; Cooks, R.G. Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry. *Anal. Chim. Acta* **2015**, *861*, 47–54. [CrossRef] [PubMed]
60. Zhai, Y.; Feng, Y.; Wei, Y.; Wang, Y.; Xu, W. Development of a miniature mass spectrometer with continuous atmospheric pressure interface. *Analyst* **2015**, *140*, 3406–3414. [CrossRef] [PubMed]
61. Laughlin, B.C.; Mulligan, C.C.; Cooks, R.G. Atmospheric pressure ionization in a miniature mass spectrometer. *Anal. Chem.* **2005**, *77*, 2928–2939. [CrossRef] [PubMed]
62. Mulligan, C.C.; Justes, D.R.; Noll, R.J.; Sanders, N.L.; Laughlin, B.C.; Cooks, R.G. Direct monitoring of toxic compounds in air using a portable mass spectrometer. *Analyst* **2006**, *131*, 556–567. [CrossRef] [PubMed]
63. Huang, G.; Gao, L.; Duncan, J.; Harper, J.D.; Sanders, N.L.; Ouyang, Z.; Cooks, R.G. Direct detection of benzene, toluene, and ethylbenzene at trace levels in ambient air by atmospheric pressure chemical ionization using a handheld mass spectrometer. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 132–135. [CrossRef] [PubMed]
64. Hall, S.E.; Mulligan, C.C. Application of ambient sampling portable mass spectrometry toward on-site screening of clandestine drug operations. *LCGC* **2014**, *12*, 8–13.
65. Horning, E.C.; Horning, M.G.; Carroll, D.I.; Dzidic, I.; Stillwell, R.N. New picogram detection system based on a mass spectrometer with an external ionization source at atmospheric pressure. *Anal. Chem.* **1973**, *45*, 936–943. [CrossRef]
66. Proctor, C.J.; Todd, J.F.J. Atmospheric pressure ionization mass spectrometry. *Org. Mass Spectrom.* **1983**, *18*, 509–516. [CrossRef]
67. Lehrle, R.S.; Parsons, I.W.; Rollinson, M. Thermal degradation mechanisms of nylon 6 deduced from kinetic studies by pyrolysis-g.c. *Polym. Degrad. Stab.* **2000**, *67*, 21–33. [CrossRef]
68. Jjunju, F.P.M.; Maher, S.; Li, A.; Syed, S.U.; Smith, B.; Heeren, R.M.A.; Taylor, S.; Cooks, R.G. Hand-held portable desorption atmospheric pressure chemical ionization ion source for in situ analysis of nitroaromatic explosives. *Anal. Chem.* **2015**, *87*, 10047–10055. [CrossRef] [PubMed]
69. Dalgleish, J.K.; Wleklinski, M.; Shelley, J.T.; Mulligan, C.C.; Ouyang, Z.; Cooks, R.G. Arrays of low-temperature plasma probes for ambient ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2013**, *27*, 135–142. [CrossRef] [PubMed]
70. Kauppila, T.J.; Syage, J.A.; Benter, T. Recent developments in atmospheric pressure photoionization-mass spectrometry. *Mass Spectrom. Rev.* **2017**, *36*, 423–449. [CrossRef] [PubMed]
71. Lalli, P.M.; Sanvido, G.B.; Garcia, J.S.; Haddad, R.; Cosso, R.G.; Maia, D.R.J.; Zacca, J.J.; Maldaner, A.O.; Eberlin, M.N. Fingerprinting and aging of ink by easy ambient sonic-spray ionization mass spectrometry. *Analyst* **2010**, *135*, 745–750. [CrossRef] [PubMed]