Paper Spray Ionization: Applications and Perspectives

Ethan M McBride*[1], Phillip M. Mach[1], Elizabeth S. Dhummakupt[1], Sarah Dowling [3], Daniel O. Carmany[2], Paul S. Demond[2], Gabrielle Rizzo[2], Nicholas E. Manicke[3], and Trevor Glaros*[1]

[1]U.S. Army Combat Capabilities Development Command Chemical Biological Center, 8198 Blackhawk Rd. (8938), Building E5183, Aberdeen Proving Ground, MD 21010, USA

[2]Excet, Inc. 6225 Brandon Ave, Suite 360, Springfield, VA 22150, USA

[3]Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, 402 N. Blackford St., Indianapolis, IN 46202, USA

*Corresponding Authors

trevor.g.glaros.civ@mail.mil (TG)

ethan.m.mcbride.ctr@mail.mil (EMM)

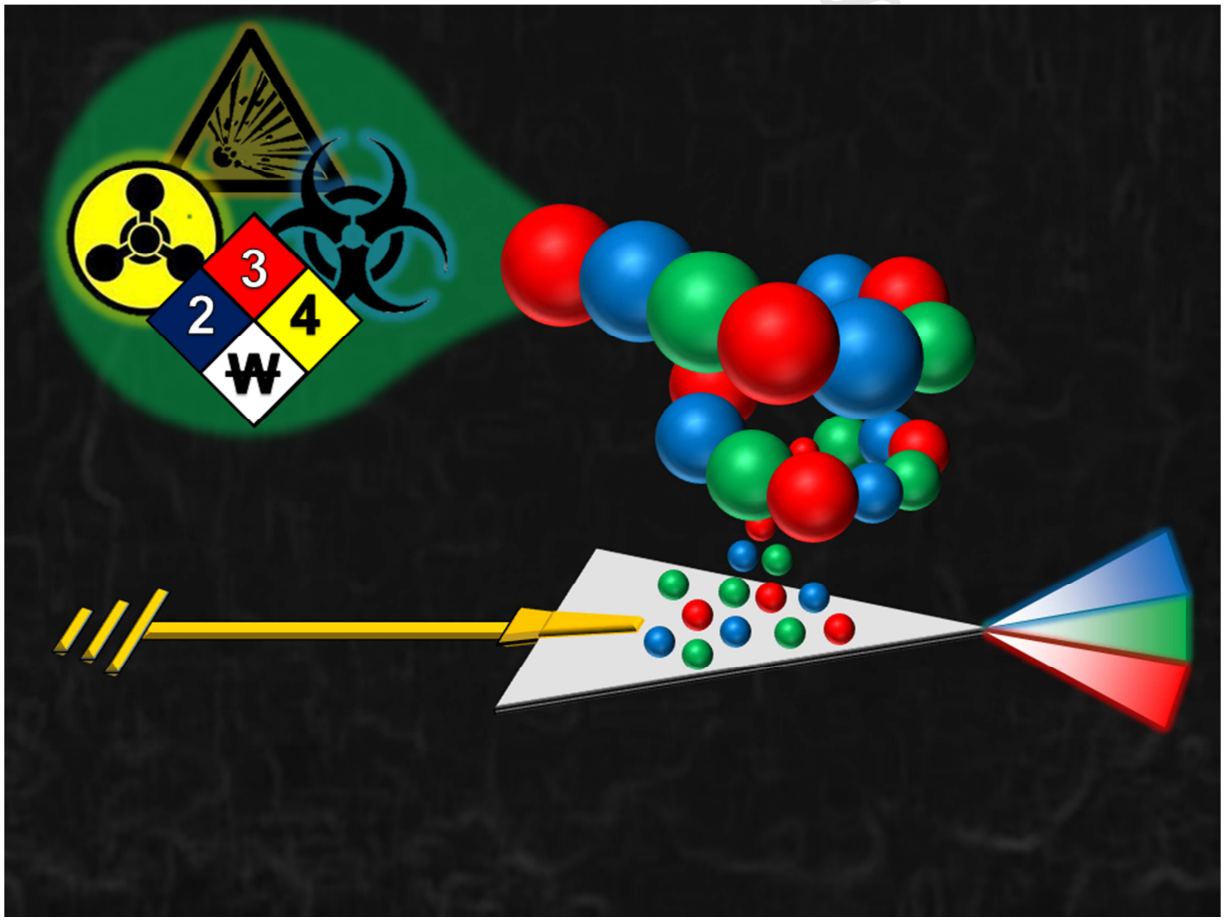
This is the author's manuscript of the article published in final edited form as:

McBride, E. M., Mach, P. M., Dhummakupt, E. S., Dowling, S., Carmany, D. O., Demond, P. S., … Glaros, T. (2019). Paper Spray Ionization: Applications and Perspectives. TrAC Trends in Analytical Chemistry. https://doi.org/10.1016/j.trac.2019.06.028

Abstract:

Paper spray ionization has grown to become one of the most successful ambient ionizations methods within the past decade. Requiring little to no sample preparation and being remarkably simple to construct, this technique has seen application in a wide number of fields. This review approaches the mechanism of how paper spray works, and seeks to better classify what it is and is not in a rapidly expanding field of ambient techniques. Additionally, many applications of the technique in clinical, forensic, environmental, and reaction monitoring regimes are explored. Finally, perspectives towards the future of how paper spray could be utilized will be expanded upon, including unexplored substrates and possibilities for the 'omics space.

Graphical Abstract:



1. Introduction

Sample introduction techniques have undergone numerous transitions since the early days of mass spectrometry. Initially, the only samples that could be analyzed were short-chain, volatile liquid hydrocarbons that could be easily desorbed under vacuum. Over the better part of the last century, these techniques have slowly evolved to improve virtually every aspect of mass spectrometric analysis and unlock new and exciting avenues of research. One of the latest paradigm-shifting innovations in this field, ambient ionization, has opened a number of unique applications. Ambient ionization, defined as the "…formation of ions outside the mass spectrometer directly from samples in their native environment with no or minimal sample preparation,[1]" has found applications in clinical settings, forensics, natural product research, food quality, environmental monitoring, biological analysis, and multiomics. Although electrospray and nanoelectrospray ionization result in the formation of ions at ambient pressures, they often require extensive sample preparation and therefore are not commonly mentioned with near prepless 'ambient ionization' techniques. By eliminating time-consuming sample preparation steps and greatly reducing solvent, vacuum, and power requirements, samples can be ionized on drastically shorter time scales with remarkably simple hardware compared to more traditional ionization methods, like electrospray and electron ionization. Additionally, because of their inherent simplicity, these techniques naturally lend themselves to use by non-scientists such as law enforcement, first responders and medical personnel.

After the emergence in 2004-2005 of ambient ionization with the unveiling of Desorption Electrospray Ionization (DESI) by R.G. Cooks et al [2]. and Direct Analysis in Real Time (DART) by R.B. Cody et al. [3], there was a flurry of new ambient techniques. Indeed, within a few years it became evident that there was perhaps too much "biodiversity in the acronym zoo"[4] and newer techniques should be thoroughly explored beyond proof-of-concept before being named as a "novel" technique. As these techniques began to be evaluated for potential applications, one of the most popular to emerge was paper spray (PS) ionization. PS utilizes a paper substrate cut to a sharp point for which a sample of interest can be applied. Next a spray solvent is applied to the paper substrate that both promotes analyte extraction and ionization. Finally, a high voltage is applied to the paper resulting in an electrospray-like ionization event at the paper tip. Since being introduced in 2010[5], paper spray has been developed into one of the most popular ambient ionization platforms and offers numerous advantages over other ambient techniques. Although paper spray is relatively new in terms of ambient techniques, it has already been well-established and developed into commercial products offered by Prosolia, Inc. and Thermo Scientific.

This review seeks to precisely designate what constitutes paper spray ionization within the broad, overlapping realm of ambient ionization techniques, and to explore the many different areas of research to which it has been applied; including clinical, forensic, mechanistic, and environmental. Notably, to better distinguish and explore paper spray as a singular technique and to avoid detraction, both leaf spray and coated blade spray which are related mechanistically but do not constitute "paper spray" are excluded from this review. Although leaf spray has been used in a number of applications for detecting exogenous and endogenous chemistries on or within plant materials including roots, stems, and leaves, it is inherently limited in its applications and therefore has more niche, limited uses. Coated blade spray (CBS)

also appears similar to paper spray, but more closely resembles solid-phase microextraction (SPME) in terms of analyte enrichment and does not possess any type of porous substrate for ionization. Wooden-tip electrospray ionization, occasionally referred to more generally as "solid-substrate electrospray," is a technique first proposed in 2011[6] that does fit into the category of "porous solid spray substrate" and therefore will be included herein. Thread spray mass spectrometry, a relatively new development[7] in ambient ionization, can consist of cellulosic threads (e.g. cotton thread) inserted through a glass capillary, but also does not possess the characteristic porosity of paper spray mass spectrometry and has also been omitted here. Finally, a perspective on the future applications of the paper spray technique will be put forth in an attempt to explicate the burgeoning relationship between paper spray ionization and biological detection, miniaturized mass spectrometers, and nontraditional spray substrates.

2. Paper Spray Theory and Mechanism

Mechanistically, PS ionization works by applying a sample, typically in solution, to the front of a triangular piece of paper (Figure 1). Prior to mass analysis, a spray solvent is used to wet the paper from the rear and extract the analyte/s of interest. Analysis is initiated by applying a high voltage directly to the paper resulting in an electrospray-like ionization event. The applied high voltage may be either positive or negative that usually ranges from 2.0-5.5 kV. The spray mechanism of PS ionization was originally not well understood, but it was known that factors such as the angle of the paper spray tip, distance of the tip from the mass spectrometer inlet, and solvent composition could drastically affect the stability of ion formation and signal stability. Espy et al.[8] found that the cellulosic fibers of the paper could produce multiple spray jets from the surface and edges of the paper, each contributing to the production of a distribution of solvent droplet sizes with diameters of a few microns or less. Due to this smaller droplet size and the microliter volumes of spray solvent used, PS ionization is better categorized as an "electrospray-like" event most akin to nanoelectrospray (nESI). This relationship was initially discovered when the ions produced from PS resembled those produced from nESI, suggesting that the droplets formed from both techniques has similar internal energies. This is further corroborated by the similar size droplets produced from both techniques,[8] as opposed to those from electrospray ionization which are roughly an order of magnitude larger. Flow rate is dictated by the transport of solvent through the porous cellulosic channels within the paper, and varies with pore size[9], solvent properties[10], and the applied voltage.

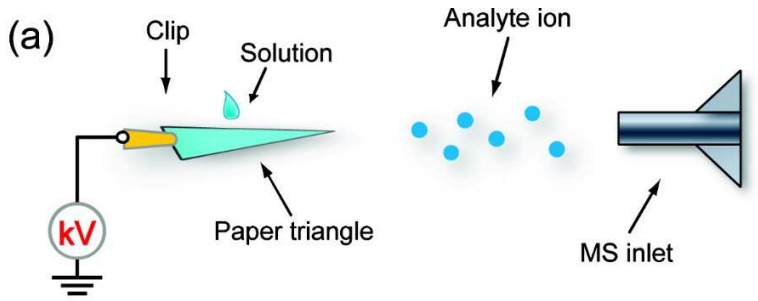


Figure 1 – Schematic representation for paper spray analysis. Applied voltage is supplied through the clip to the paper substrate where the ionization process occurs under ambient conditions. Reprinted (adapted) with permissions from (Liu, J.; Wang, H.; Manicke, N. E.; Lin, J.-M.; Cooks, R. G.; Ouyang, Z., Development, Characterization, and Application of Paper Spray Ionization. Analytical Chemistry 2010, 82 (6), 2463-2471). Copyright (2019) American Chemical Society.

Paper is primarily composed of compressed cellulosic fibers that, when formed into sheets, produces an amorphous series of porous channels within the overall structure. Because of these channels, paper is able to sufficiently absorb and transport solvent via capillarity, hence its traditional use in chromatographic separations. This same transport also allows for solvent to resolubilize analyte that is contained within the paper, either in dried or liquid form. This amorphous channel structure is a vital component of the PS mechanism, and helps distinguish PS from other forms of ambient ionization. The cellulose structure is also capable of being augmented through physical or chemical means, such as chemical derivatization with silanizing agents to alter the normally hydrophilic properties of the paper. However, the paper substrate itself also has other unique properties that allow it to be used in a number of different scenarios. For instance, the natural ability of the paper to absorb liquid makes the paper spray substrate itself an ideal sampling mechanism, whereby liquids can be absorbed and solids can be adsorbed onto the paper.

2.1. Development and History

The development of paper spray and ambient ionization, in general, can trace its roots back to the need for introducing liquid samples into the vacuum of the mass spectrometer most effectively. This problem was addressed in a number of ways over the years, and these developments have been reviewed previously.[11] However, the development of paper spray as a technique came initially from its ability to detect analytes from dried blood spots (DBS) on paper. The ability to analyze complex mixtures in the presence of matrix without the need for sample preparation or cleanup as is typically required for chromatography was the main driving force behind this and the invention of other ambient techniques. The advantage of using PS for DBS analysis over other techniques is attributed to its ability to retain the interfering sample matrix within its porous structure, and the fact that DBS can be applied directly to the paper to be analyzed without the need for a lengthy extraction process from punches of DBS on paper. In addition to DBS, whole blood analysis was also investigated in the presence of coagulants, which allowed for larger sample volumes (≥10 µL) of blood to be analyzed without the addition of any interferents from the coagulant.[12]

2.2. Factors Impacting Ionization

PS ionization, as previously discussed, is most similar to nanoelectrospray ionization in terms of solvent volumes utilized and the size of droplets within the spray plume; however, there are a few distinct advantages over nanoelectrospray. Because the spray solvent added to the paper substrate acts both to extract and solubilize the analyte of interest, there is almost no sample preparation required prior to analysis. This is especially important for biological samples, where matrix can easily confound nanoelectrospray regimes without proper cleanup. The absence of this problem in PS can be partly attributed to the porous nature of the cellulosic paper structure, which can trap some of this matrix but does not lead to diffusion of the analyte throughout the paper. The process of "wicking" by which the solvent system moves through the paper also precludes the need for a pump to introduce sample into the mass spectrometer; a voltage must only be applied to the paper. Similarly, since each PS substrate is discarded after use, there is no possibility of carryover from one sample to the next from the substrate.

There are numerous considerations to make when doing a paper spray analysis. The most common substrates for analysis continue to be chromatography or filter paper. These pure cellulosic substrates comes in many thicknesses and porosities, which can contribute greatly to both ionization efficiency and flow rate. Thinner papers and smaller internal pore diameters tend to decrease flow through the paper, while simultaneously increasing ionization efficiency and decreasing recovery.[9] Additionally, Bills et al. determined that the solvent system used had a greater impact upon limit of detection compared to paper substrate. This tradeoff between ionization efficiency and recovery should generally discourage the use of "universal" substrates, as detection limits can vary drastically.

Initial forays into PS were made with simple methanol or methanol:water solvent systems. As is the case in electrospray, it was found that the addition of some aqueous solvent improves surface tension and spray stability. However, in general, PS ionization has higher onset voltages in positive ionization mode compared to nanoelectrospray. Negative ion mode suffers from sensitivity issues and Taylor cone instability from corona discharge.[8] This can be especially troublesome for analytes that are typically observed in the negative mode, such as explosives. Changing to a more hydrophobic substrate, such as polyethylene, can improve signal in these cases,[13] as well as choosing spray solvent systems including chlorinated solvents, as these have been shown to increase the voltage potential for corona discharge (Figure 2).[14]

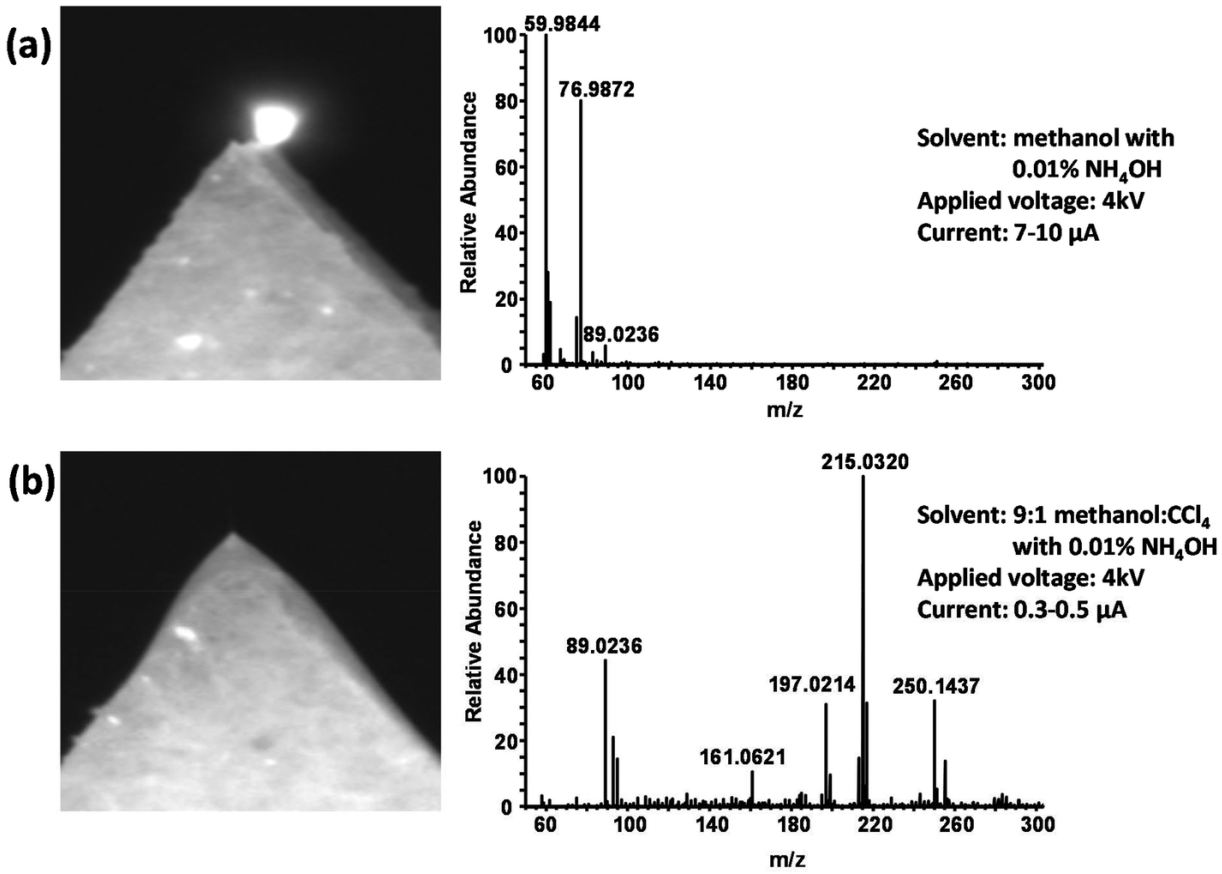


Figure 2 - Corona discharge can be seen during analysis (a) utilizing primarily methanol as the spray solvent, while improvents can be seen when switching to (b) a mixed methanol:CCl_4 system. Note the higher spray current associated with the corona discharge and the visible Taylor cone and lower current for the chlorinated solvent system. [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.] – Published by The Royal Society of Chemistry.

Tip angle and construction also plays a critical role in spray stability. Investigations into the

optimum tip angle have shown that signal intensity, spray current, and electric field at the tip are all dependent on the angle of the paper, with 30° being the most commonly-encountered angle based upon its higher electric field at the tip. PS substrates can also be laser-cut to ensure a reproducible tip angle and construction. Beyond the shape of the portion of the paper that produces the PS event, there is a large degree of flexibility in terms of PS device design. Initial designs consisted of a simple triangle cut from paper with a voltage applied *via* an alligator clamp.

3. Paper Spray Applications

3.1. Clinical

Paper spray has been utilized in diverse clinical applications since its inception, which a large associated body of literature can corroborate. The majority of these applications have revolved around the analysis of biological matrices such as blood[15], plasma[16], and urine[17], but have also been extended to other complex material such as tissue biopsies, cell culture medium, tissue homogenate, and bacterial colonies. PS techniques offer several advantages unique to clinical applications. The ability to use the collection medium (DBS paper) as the ionization medium greatly simplifies transport and storage of samples. Considering the single-use, disposable nature of these PS substrates, they also represent a low-cost consumable for clinicians. Throughput can also be improved with a prep-less ionization technique like PS; liquid chromatography-mass spectrometry (LC-MS) has long been considered the gold standard in this area[18], but can require significant sample preparation and long analysis times characteristic of LC-MS analysis.

Analytes in the clinical setting can be subdivided into endogenous and xenobiotic compounds. Endogenous compounds can be monitored to determine certain disease states.[19] The lack of derivatization steps and incorporation of DBS paper for both sampling and ionization is uniquely advantageous when sampling is performed with infants, such as for neonatal screening of metabolic disorders.[20] Alternatively, xenobiotics such as pharmaceuticals[21] and drugs of abuse[15] can often be quantitated to ng/mL levels directly from these biofluids.

The application of PS in a clinical setting also naturally lends itself to the idea of combining this technology with miniaturized mass spectrometers. Many point-of-care (POC) applications have been considered for this and other ambient ionization techniques[22], which would ideally be able to provide easily interpreted, quantitative results from biological matrices and be operable by non-experts with little training. However, there are significant hurdles still to be addressed for POC analysis. Many compounds lend themselves to analysis via PS by virtue of being relatively hydrophobic and ionizing well; for those that are not, however, there can be difficulties to overcome in terms of ion suppression and matrix effects. The authors would recommend two superb and specialized review articles by Manicke et al., and Zhang et al., that engage xenobiotics[23] and therapeutic applications[24], respectively, in a more detailed manner.

Perhaps one of the best examples of breakthroughs in the clinical space have come about in the past few years with the advent of combining immunoassays with PS. Chen et al.[25] developed an immunoassay technique for malarial proteins utilizing bioconjugation of an ionic probe with an antibody. This setup, designated “touch paper spray ionization,” avoided enzymatic reactions and therefore was more stable and offered quantitative results once the reaction strip containing the assay was combined with the paper spray strip (Figure 3). In another instance, an

enzymatic reaction was performed directly on the paper spray substrate itself. Carmany et al.

performed this enzymatic reaction[26] with acetylcholinesterase (AChE), along with a non-native

cleavable substrate specific to its activity known as MATP+, for the detection not only of AChE

activity, but also the class of inhibiting chemical warfare agent present in diluted whole human

blood. This represented the first instance of detecting both the reduced enzymatic activity of

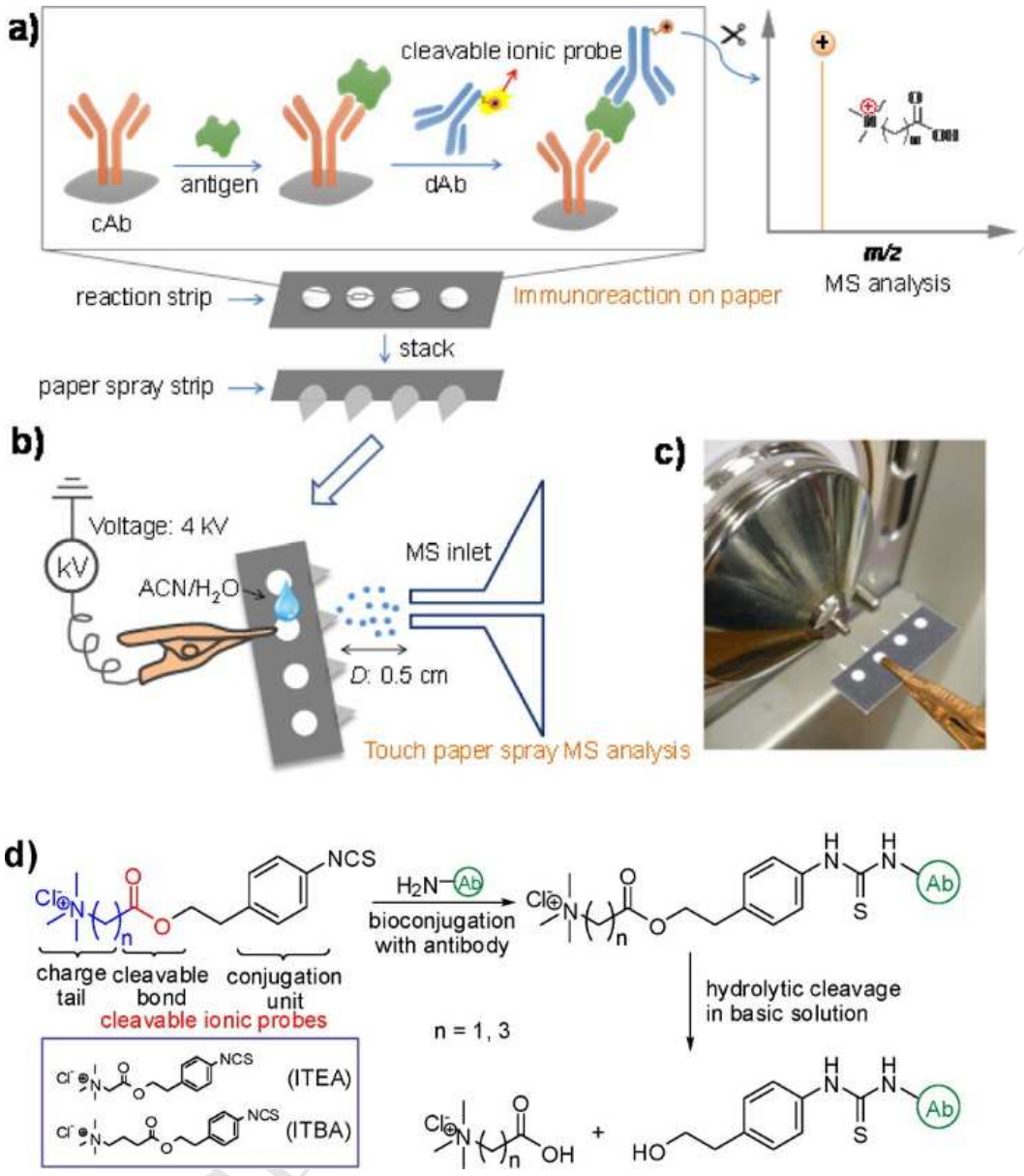


Figure 3 - Overview of touch paper spray mass spectrometry (TPS-MS) system. The immunoassay shown in a) consists of immobilized capture antibody (cAb) for *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) malarial or cancer antigens which are reacted with a cleavable ionic probe and sandwiched onto the paper substrate for analysis. The ionic probes are cleaved using a basic solution and release low molecular weight (MW < 200) hydrolysis products detected by MS. In addition, the TPS-MS substrate was robustly designed for field use. Reprinted (adapted) with permission from (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.). Copyright (2019) American Chemical Society.

AChE as well as the toxicant responsible which is an improvement upon the earlier pH- and

colorimetric-based assays. By utilizing the substrate in this way, complementary information can be acquired without sacrificing the advantages PS can offer. In another instance, immunoaffinity capture was built into the PS device. Combined with a spray substrate that showed improved ionization of proteins, several important clinical protein targets were detected directly from plasma without pretreatment[27].

3.2. Forensic

Beginning with the seminal paper on the PS technique in 2010, there was an obvious desire to not only utilize this technique for clinical diagnostics, but also for forensic sampling. Certainly, the overlap between these areas has become apparent, as the ability to detect traces of drugs of abuse or therapeutics from DBS could be used both in POC diagnostics as well as for forensic toxicology. Perhaps the first truly forensic use of PS was shown in 2012[28], although the term "forensic" does not appear even once in the article. This technique was used to detect contaminants, such as melamine, plasticizers, and food dyes, from within common food stuffs. The PS technique showed good linearity and detection limits in the low ng/g range for several β-agonists. From this initial article, the use of PS has expanded into the detection of many compounds of forensic interest, including explosives[29], drugs of abuse[30], agrochemicals[31], food and drink adulteration[32], ink and questioned documents[33], and chemical warfare agents.[34]

The methods for utilizing the PS substrate itself in a forensic setting do vary from just a means of ambient ionization and sample introduction. The porous nature of the substrate naturally lends itself as a tool for absorbing or adsorbing chemistry from surfaces or bulk samples. Dhummakupt et al. have shown both through incorporation of metal-organic frameworks (MOF)[35] that the highly volatile G-series of chemical warfare agents can be effectively analyzed with PS. The wipe technique originally shown in 2010[5] was found to be highly effective for heroin and thiabendazole from both nonporous (desktop) and porous (fruit) substrates, respectively. This ability to sample from bulk evidence can also be used in situations such as clandestine laboratory investigation. O'Leary et al.[30] showed the use of PS substrate to directly analyze not only bulk powder, but also reaction vessels containing precursors and intermediates for the synthesis of the controlled substance methamphetamine. This technique was combined with other ambient ionization techniques to demonstrate the ability for real-time analysis of an illicit synthesis.

Modifications of the paper substrate can also be used to further improve the utility of the paper. Forensic analysis typically involves a presumptive test taken in the field, followed by a second, more rigorous analysis of the sample back in the laboratory. The initial test can help confirm or deny the presence of an analyte, while the second analysis confirms this and helps quantitate the amount of substance(s) present. One method currently in use in the field as a presumptive test is surface-enhanced Raman spectroscopy (SERS). Fedick et al.[29] combined this portable technology with PS by incorporating silver nanoparticles used in SERS into the paper via inkjet printing, and were able to analyze drugs, explosives, and CWA simulants by SERS and PS-MS from the same paper substrate (Figure 4).

3.3. Catalysis and Reaction Monitoring

The roots of reactive paper spray ionization and synthetic monitoring can be traced back to very similar experiments performed with reactive desorption electrospray ionization (reactive DESI). It was found that reaction rates could be drastically improved with the use of this

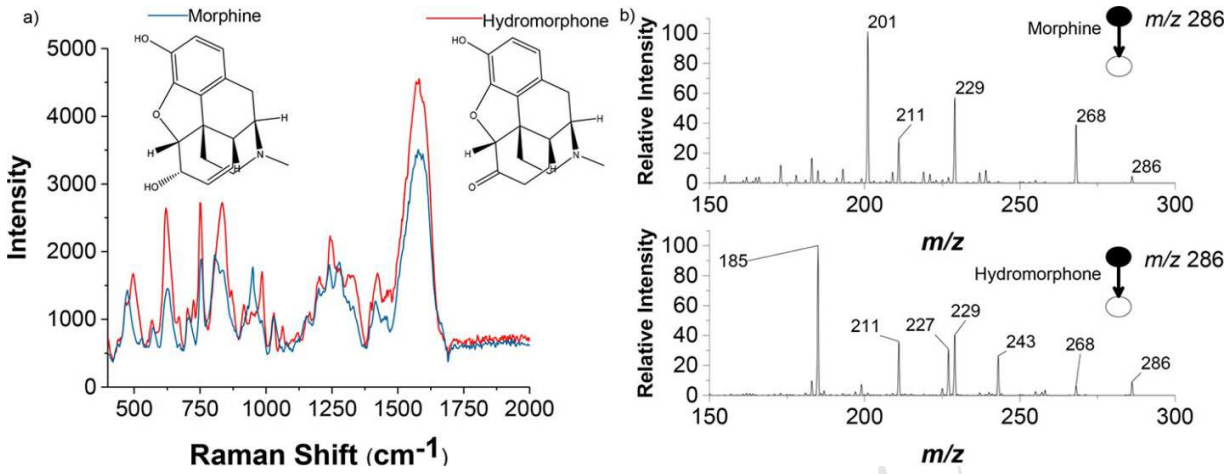


Figure 4 - By combining both Raman and MS capability on a single PS substrate, complementary information is obtained on two isobaric compounds, morphine and hydromorphone. These differences can be detected in both the a) Raman and b) MS/MS spectra shown here. Reprinted (adapted) with permission from (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.). Copyright (2019) American Chemical Society.

reactive DESI source, although the exact mechanism was not fully understood at the time. The formation of micro droplets was found to be critical in this rate increase, and investigations into accelerated reactions with paper spray have yielded similar results[36]. Reactive paper spray involves incorporating a reagent either into the spray solvent or substrate that can react with the target analyte. In addition, the paper substrate can also be chemically modified to provide some enhanced capture or derivatization capability. Banerjee et al.[37] developed nanoparticle-embedded paper for investigating heterogeneous catalysis of palladium, silver, and gold nanoparticle-catalyzed Suzuki cross-coupling reactions, achieving reactions that occurred on the time scale of the analysis (~30 seconds). Sarkar et al. showed[38] that 2,4,6-trinitrotoluene, or TNT, could be readily converted to 2,4,6-triaminotoluene with the addition of sodium borohydride to the spray solvent and the use of platinum NP-coated nanotubes embedded within the paper (Figure 5). This amino derivative was far more easily detected in the positive mode using PS, and represents an instance where both spray solvent and substrate can be modified to enhance a particular reaction.

Most of the advances in exploring the mechanisms of reaction enhancement via PS were directly influenced from studies done with reactive DESI. Because of this, there have been significant improvements in our understanding of the processes involved in droplet formation that contribute to these drastic increases in reaction rate. The reader is encouraged to explore some of the reviews[39] of these investigations to more fully understand experimental design and progress towards on-line reaction monitoring and improved ionization efficiencies for difficult analytes.

3.4. Environmental

Environmental analysis, not unlike forensic chemistry, has long been dominated by chromatography coupled to mass spectrometers, such as GC-MS and LC-MS for “gold standard”

analysis. Volatile organic compounds (VOC), industrial chemicals, and other relatively volatile

compounds are easily separated and analyzed by GC-MS, with this technique dating back in

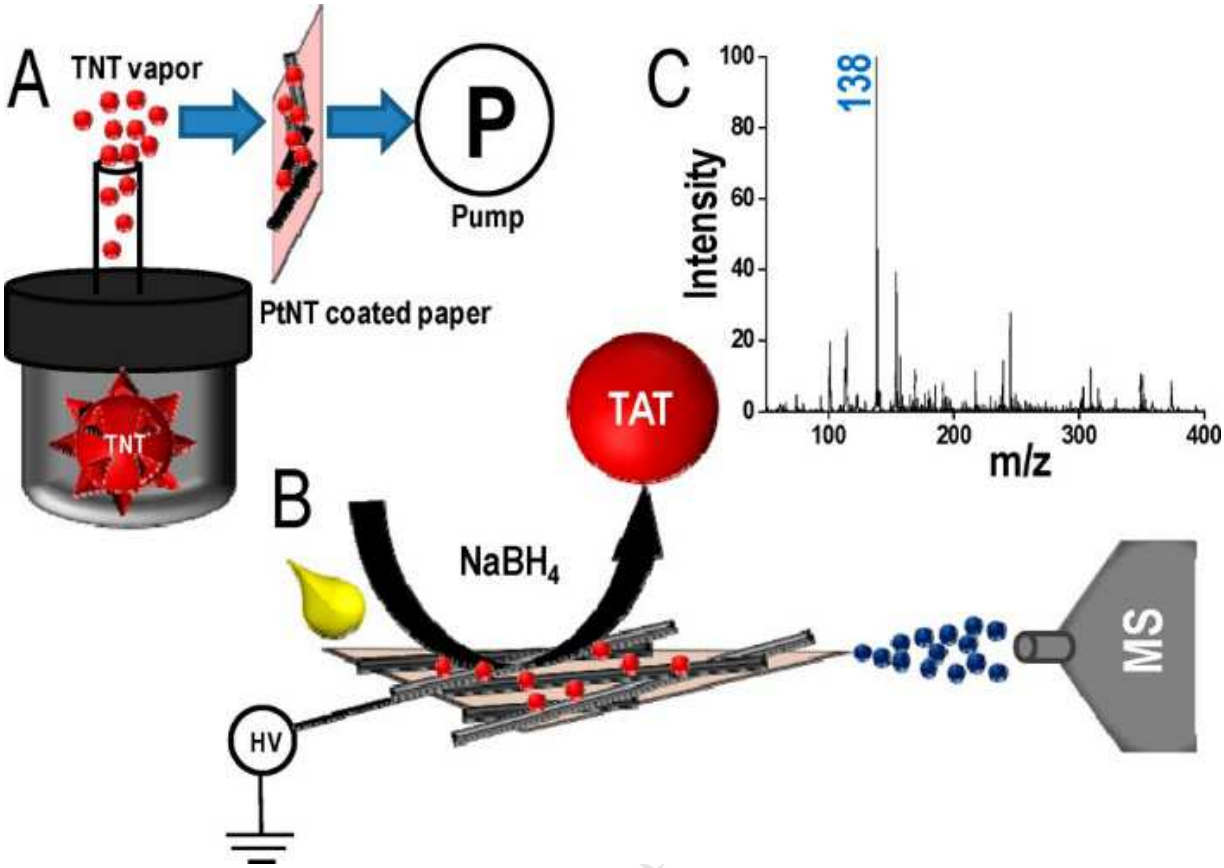


Figure 5 – Trinitrotoluene (TNT) sample is heated gently and subjected to vacuum to introduce TNT vapors onto the platinum nanoparticle-nanotube (Pt NP-NT)-coated paper substrate. When exposed to sodium borohydride solution, this was then reduced to triaminotoluene (TAT) for detection in positive-mode MS as protonated *m/z* 138. This reactive-PS application allows for explosives detection in the positive mode. Reprinted (adapted) with permission from (Sarkar, D.; Som, A.; Pradeep, T., Catalytic Paper Spray Ionization Mass Spectrometry with Metal Nanotubes and the Detection of 2,4,6-Trinitrotoluene. Analytical Chemistry 2017, 89 (21), 11378-11382.). Copyright (2019) American Chemical Society

usage for the better part of a century. Correspondingly, LC-MS analysis has been used

extensively for water- and soil-based chemistries, such as pharmaceuticals and agrochemicals.

However, as has been shown previously, these compounds can also be easily detected (and

often quantified) by ambient ionization methods. Although the breadth of literature pertaining

to the PS technique in environmental chemistry lags behind other areas, it has still been shown

to be a simple, powerful technique in this realm. Jjunju et al. showed that corrosion inhibitors

commonly used in oil refineries and wastewater treatment plants, whether quaternary

ammonium salts[40] or long chain aliphatic diamines (Figure 6)[41], can be detected *in situ* at

relatively low (ppb) concentrations utilizing a Mini-12 portable rectilinear ion trap or benchtop

linear ion trap with a PS interface, respectively. The Mini-12 portable system in particular would

allow for monitoring of these chemicals directly in the field, without the need for lengthy

workup and laboratory analysis. In another instance, reactive PS was used to improve the

detection of metaldehyde[42], a common molluscicide and possible pollutant, with LOD below 0.1

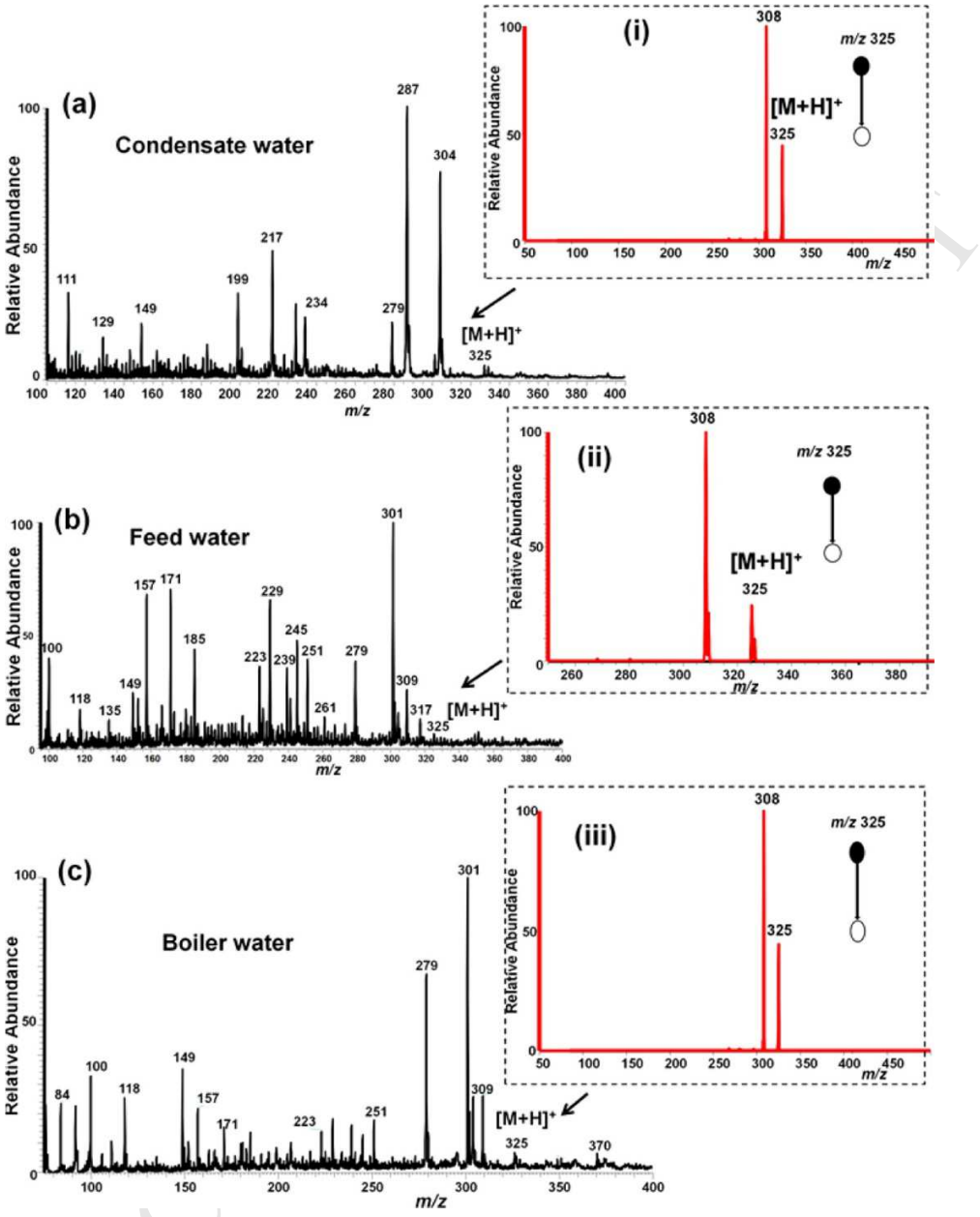


Figure 6 - Water samples taken from three different parts of water boiler plants, all indicating the presence of the long chain aliphatic primary diamine Duomeen O (n-oleyl-1,3-diaminopropane). Limits of detection for these raw water samples were below 0.1 pg. Reprinted (adapted) with permission from (Jjunju, F. P. M.; Maher, S.; Damon, D. E.; Barrett, R. M.; Syed, S. U.; Heeren, R. M. A.; Taylor, S.; Badu-Tawiah, A. K., Screening and Quantification of Aliphatic Primary Alkyl Corrosion Inhibitor Amines in Water Samples by Paper Spray Mass Spectrometry. Analytical Chemistry 2016, 88 (2), 1391-1400.). Copyright (2019) American Chemical Society.

ng/mL in raw water samples; this also represents the threshold that water companies in the

European Union are required to maintain for pesticides in water (<0.1 μg/L). Reeber et al. showed[31] that triazine herbicides could also be detected at ppb concentrations, representative of current regulatory standards in the United States, from water and crop extracts in combination with PS. There is also evidence that oxidative byproducts from degradation under environmental conditions can be "fished" from solution by the electrospray process.

Perhaps one of the more unique applications of PS to *in situ* environmental detection of commonly-encountered chemicals comes from the Cooks group[43]. Utilizing PS, as well as leaf spray and low temperature plasma (LTP) as the ionization source coupled to their portable Mini system, they showed detection of a range of chemical classes including vanillin from aerosol air freshener, benzalkonium chloride bactericide from surface wipes, and hydrocortisone from topical ointment applied to skin followed by surface wipes. Utilizing MS/MS capabilities, these compounds showed good selectivity amongst different environmental matrices. Alternatively, our group has pioneered the development of an aerosol capture device that was utilized to improve detection limits for CWA simulants to approach those stated for current worker population limits.[34] The ability to improve detection down to these levels on very short (2 minute) time scales could represent a future capability for maintaining safe working conditions in industries with known volatilized contaminants such as oil and gas refining or manufacturing.

4. Future Applications

Despite the many areas of research that have been improved upon with the use of PS, there are still significant gaps in both the instrumentation development as well as how the substrate itself is utilized. As of the writing of this manuscript, there is only one commercially-available paper spray ionization device which can only be coupled to Thermo Fisher Scientific's benchtop mass spectrometers, and only one commercially-available (in some regions) portable MS that has a dedicated attachment for PS. Virtually all of the innovations associated with PS have been performed on self-constructed apparatuses; this allows for much creativity in terms of solid substrate and experimental conditions, but also introduces problems with reproducibility. Given the economic investment required for a commercialized front-end PS system, 3D printing offers a possible solution to this problem. There have already been numerous innovations in this area, including 3D-printed cartridges integrated with ion optics[44] and continuous solvent application (Figure 7) for extended spray times[45]. 3D-printed microfluidic devices integrated within a PS ionization device[46] are also possible, and several microfluidics capabilities have been previously reviewed[47], as well as various printing techniques for the microfluidics. Murray et al. utilized a unique printing design to concentrate the flow of analyte to the MS inlet and showed a marked increase in analysis time through the use of wax-printed barriers.[48]Specialized or ruggedized 3D-printed front-end systems could be easily manufactured from the same schematic files by multiple laboratories to improve reproducibility or introduce new capabilities that can be combined with PS ionization.

Despite the advantage that PS offers for little to no need for sample preparation or separation, PS has also been coupled with other techniques, such as ion mobility spectrometry (IMS)[49], solid-phase microextraction (SPME)[16], liquid-phase microextraction (LPME)[50], and slug-flow microextraction[51]. These techniques can be applied when the matrix is complex and rapid

extractions or separations can be performed to improve analysis without sacrificing the speed

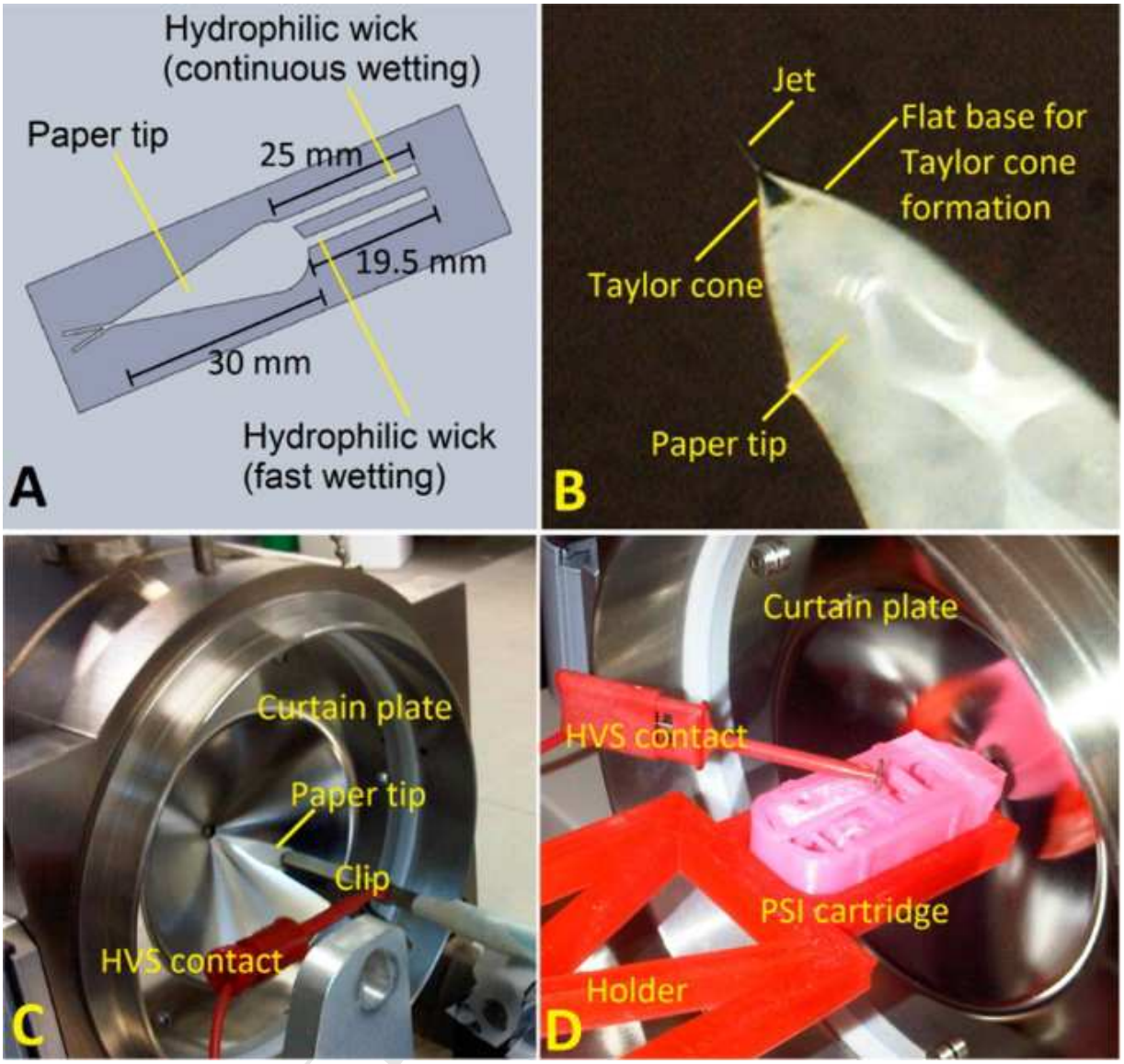


Figure 7 - Design and implementation of designs for PS devices that can be continually wetted. These designs allow for effective ionization an order of magnitude longer than conventional PS analysis. These devices were custom-designed and fabricated. Reprinted (adapted) with permission from (Salentijn, G. I. J.; Permentier, H. P.; Verpoorte, E., 3D-Printed Paper Spray Ionization Cartridge with Fast Wetting and Continuous Solvent Supply Features. Analytical Chemistry 2014, 86 (23), 11657-11665.). Copyright (2019) American Chemical Society.

and simplicity of the PS technique. Understanding how impactful the conjugation of a

tangential technique can be with a PS substrate has already resulted in great progress, but is only now in its infancy. Another area that has not been fully explored is the use of alternative porous substrates. Applications such as leaf spray has certainly been derived from the PS technique, but there are many other porous substrates that could boast significant advantages over cellulosic paper material. Metal foam, commonly used in the heat exchanger and filter industries, possesses the right porosity and conductivity for PS, with the added benefit of being far more rugged than paper. Similarly, extruded silica-based substrates such as Teslin are manufactured similarly to paper but possess other interesting properties such as excellent chemical resistance and longevity.

One of the most unexplored but perhaps most exciting fields which paper spray has only just begun to enter would undoubtedly be the 'omics space. Although the seminal research article on paper spray showed that a protein could be analyzed by the technique, it was not until Zhang et al.[52] combined PS with an IMS-MS system that is was shown that PS can ionize proteins even in their native state. By incorporating a relatively hydrophobic material such as polystyrene[53], complex matrix such as raw blood samples can be analyzed and various proteins and peptides can be detected. This technique has even been extended to extracting in-gel proteins subjected to electrophoresis directly on a paper substrate coated with carbon nanotubes (CNT) with limits of detection of as little as 10 ng for a mixture of proteins[54]. The ability to directly analyze this intact gel without pre-treatment could offer a facile alternative to matrix-assisted laser desorption ionization (MALDI) and perfectly complements the top-down proteomics workflow. A further innovation of this CNT-coating was developed to allow for selective enrichment of plasma proteins with a 3D-printed cartridge integrated with CNT-coated polyethylene and an antibody enrichment column[27]. The ability to investigate post-translational modifications (PTM), such as the cysteine, glutathione, and sulfonate PTMs shown, are crucial to top-down proteomics and represent a great leap forward in the PS technique. An example combining many of the advances made in PS is exemplified in a recent paper[55] from Wichert et al. Utilizing a porous nylon membrane for wiping and CNT sputter coated porous polyethylene PS substrate for analysis, they were able to collect and detect a proteinaceous toxin surrogate from a laboratory benchtop. In addition, a prototype "pen" is shown for the potential to further improve the ability to wipe from a surface with increased reproducibility. This represents not only a move forward in environmental biological detection, but showcases how versatile PS can be when applied in multiple ways.

5. Conclusions

Despite the many advances made utilizing paper spray ionization in the past decade, there has not been a comprehensive review to properly delineate what truly defines "paper spray" as well as explore all of the fields in which it has (and could) be utilized. Herein we have explored how paper spray has been used in the past and what the path forward will likely entail. Being so popular without the support of commercial off-the-shelf devices has forced the paper spray community to innovate and design in ways not often seen with ionization sources, and continues to provide novel avenues to solving problems in complex matrices and environments. Further improvements in instrumentation, portable or otherwise, as well as the burgeoning field of biodetection offer new and exciting possibilities for this technique.

1. Murray, K. K.; Boyd, R. K.; Eberlin, M. N.; Langley, G. J.; Li, L.; Naito, Y., Definitions of terms relating to mass spectrometry (IUPAC Recommendations 2013). *Pure and Applied Chemistry* **2013,** *85* (7), 1515-1609.

2. Takats, Z.; Wiseman, J. M.; Cooks, R. G., Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms and applications in forensics, chemistry, and biology. *J Mass Spectrom* **2005,** *40* (10), 1261-75.

3. 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.

4. 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.

5. 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.

6. Hu, B.; So, P.-K.; Chen, H.; Yao, Z.-P., Electrospray Ionization Using Wooden Tips. *Analytical Chemistry* **2011,** *83* (21), 8201-8207.

7. Jackson, S.; Swiner, D.; C. Capone, P.; Badu-Twaiah, A., *Thread Spray Mass Spectrometry for Direct Analysis of Capsaicinoids in Pepper Products*. 2018; Vol. 1023.

8. 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.

9. Bills, B. J.; Kinkade, J.; Ren, G.; Manicke, N. E., The impacts of paper properties on matrix effects during paper spray mass spectrometry analysis of prescription drugs, fentanyl and synthetic cannabinoids. *Forensic Chemistry* **2018,** *11*, 15-22.

10. Washburn, E. W., The Dynamics of Capillary Flow. *Physical Review* **1921,** *17* (3), 273-283.

11. McBride, E. M.; Verbeck, G. F., A Mass Spectrometer in Every Fume Hood. *J Am Soc Mass Spectrom* **2018,** *29* (8), 1555-1566.

12. Espy, R. D.; Manicke, N. E.; Ouyang, Z.; Cooks, R. G., Rapid analysis of whole blood by paper spray mass spectrometry for point-of-care therapeutic drug monitoring. *Analyst* **2012,** *137* (10), 2344-2349.

13. Wong, M. Y.-M.; Man, S.-H.; Che, C.-M.; Lau, K.-C.; Ng, K.-M., Negative electrospray ionization on porous supporting tips for mass spectrometric analysis: electrostatic charging effect on detection sensitivity and its application to explosive detection. *Analyst* **2014,** *139* (6), 1482-1491.

14. 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.

15. Jett, R.; Skaggs, C.; Manicke, N. E., Drug screening method development for paper spray coupled to a triple quadrupole mass spectrometer. *Analytical Methods* **2017,** *9* (34), 5037-5043.

16. Zhang, C.; Manicke, N. E., Development of a Paper Spray Mass Spectrometry Cartridge with Integrated Solid Phase Extraction for Bioanalysis. *Analytical Chemistry* **2015,** *87* (12), 6212-6219.

17. Michely, J. A.; Meyer, M. R.; Maurer, H. H., Paper Spray Ionization Coupled to High Resolution Tandem Mass Spectrometry for Comprehensive Urine Drug Testing in Comparison to Liquid Chromatography-Coupled Techniques after Urine Precipitation or Dried Urine Spot Workup. *Analytical Chemistry* **2017,** *89* (21), 11779-11786.

18. Adaway, J. E.; Keevil, B. G., Therapeutic drug monitoring and LC–MS/MS. *Journal of Chromatography B* **2012,** *883-884*, 33-49.

19. Bag, S.; Hendricks, P. I.; Reynolds, J. C.; Cooks, R. G., Biogenic aldehyde determination by reactive paper spray ionization mass spectrometry. *Analytica Chimica Acta* **2015,** *860*, 37-42.

20. Yang, Q.; Manicke, N. E.; Wang, H.; Petucci, C.; Cooks, R. G.; Ouyang, Z., Direct and quantitative analysis of underivatized acylcarnitines in serum and whole blood using paper spray mass spectrometry. *Analytical and Bioanalytical Chemistry* **2012,** *404* (5), 1389-1397.

21. Manicke, N. E.; Yang, Q.; Wang, H.; Oradu, S.; Ouyang, Z.; Cooks, R. G., Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots. *International Journal of Mass Spectrometry* **2011,** *300* (2), 123-129.

22. 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.

23. Manicke, N. E.; Bills, B. J.; Zhang, C., Analysis of biofluids by paper spray MS: advances and challenges. *Bioanalysis* **2016,** *8* (6), 589-606.

24. Zhang, W.; Wang, X.; Xia, Y.; Ouyang, Z., Ambient Ionization and Miniature Mass Spectrometry Systems for Disease Diagnosis and Therapeutic Monitoring. *Theranostics* **2017,** *7* (12), 2968-2981.

25. 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.

26. Carmany, D. O.; Mach, P. M.; Rizzo, G. M.; Dhummakupt, E. S.; McBride, E. M.; Sekowski, J. W.; Benton, B.; Demond, P. S.; Busch, M. W.; Glaros, T., On-substrate Enzymatic Reaction to Determine Acetylcholinesterase Activity in Whole Blood by Paper Spray Mass Spectrometry. *Journal of The American Society for Mass Spectrometry* **2018**.

27. Zhang, C.; Glaros, T.; Manicke, N. E., Targeted Protein Detection Using an All-in-One Mass Spectrometry Cartridge. *Journal of the American Chemical Society* **2017,** *139* (32), 10996-10999.

28. Zhang, Z.; Cooks, R. G.; Ouyang, Z., Paper spray: a simple and efficient means of analysis of different contaminants in foodstuffs. *Analyst* **2012,** *137* (11), 2556-2558.

29. 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.

30. 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.

31. Reeber, S. L.; Gadi, S.; Huang, S.-B.; Glish, G. L., Direct analysis of herbicides by paper spray ionization mass spectrometry. *Analytical Methods* **2015,** *7* (23), 9808-9816.

32. Garrett, R.; Rezende, C. M.; Ifa, D. R., Coffee origin discrimination by paper spray mass spectrometry and direct coffee spray analysis. *Analytical Methods* **2013,** *5* (21), 5944-5948.

33. Jurisch, M.; Augusti, R., Detection of signature forgery with erasable pens using paper spray mass spectrometry (PS-MS). *Analytical Methods* **2016,** *8* (23), 4543-4546.

34. Dhummakupt, E. S.; Mach, P. M.; Carmany, D.; Demond, P. S.; Moran, T. S.; Connell, T.; Wylie, H. S.; Manicke, N. E.; Nilles, J. M.; Glaros, T., Direct Analysis of Aerosolized Chemical Warfare Simulants Captured on a Modified Glass-Based Substrate by "Paper-Spray" Ionization. *Analytical Chemistry* **2017,** *89* (20), 10866-10872.

35. Dhummakupt, E. S.; Carmany, D. O.; Mach, P. M.; Tovar, T. M.; Ploskonka, A. M.; Demond, P. S.; DeCoste, J. B.; Glaros, T., Metal–Organic Framework Modified Glass Substrate for Analysis of Highly Volatile Chemical Warfare Agents by Paper Spray Mass Spectrometry. *ACS Applied Materials & Interfaces* **2018,** *10* (9), 8359-8365.

36. 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.

37. 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.

38. Sarkar, D.; Som, A.; Pradeep, T., Catalytic Paper Spray Ionization Mass Spectrometry with Metal Nanotubes and the Detection of 2,4,6-Trinitrotoluene. *Analytical Chemistry* **2017,** *89* (21), 11378-11382.
39. Cooks, R. G.; Mueller, T., Through a glass darkly: glimpses into the future of mass spectrometry. *Mass spectrometry (Tokyo, Japan)* **2013,** *2* (Spec Iss), S0001.
40. Jjunju, F. P. M.; Li, A.; Badu-Tawiah, A.; Wei, P.; Li, L.; Ouyang, Z.; Roqan, I. S.; Cooks, R. G., In situ analysis of corrosion inhibitors using a portable mass spectrometer with paper spray ionization. *Analyst* **2013,** *138* (13), 3740-3748.
41. Jjunju, F. P. M.; Maher, S.; Damon, D. E.; Barrett, R. M.; Syed, S. U.; Heeren, R. M. A.; Taylor, S.; Badu-Tawiah, A. K., Screening and Quantification of Aliphatic Primary Alkyl Corrosion Inhibitor Amines in Water Samples by Paper Spray Mass Spectrometry. *Analytical Chemistry* **2016,** *88* (2), 1391-1400.
42. 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. *Scientific Reports* **2016,** *6*, 35643.
43. 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.
44. Salentijn, G. I.; Oleschuk, R. D.; Verpoorte, E., 3D-Printed Paper Spray Ionization Cartridge with Integrated Desolvation Feature and Ion Optics. *Analytical Chemistry* **2017,** *89* (21), 11419-11426.
45. Salentijn, G. I. J.; Permentier, H. P.; Verpoorte, E., 3D-Printed Paper Spray Ionization Cartridge with Fast Wetting and Continuous Solvent Supply Features. *Analytical Chemistry* **2014,** *86* (23), 11657-11665.
46. Zhang, Y.; Li, H.; Ma, Y.; Lin, J.-M., Paper spray mass spectrometry-based method for analysis of droplets in a gravity-driven microfluidic chip. *Analyst* **2014,** *139* (5), 1023-1029.
47. Lim, W. Y.; Goh, B. T.; Khor, S. M., Microfluidic paper-based analytical devices for potential use in quantitative and direct detection of disease biomarkers in clinical analysis. *Journal of Chromatography B* **2017,** *1060*, 424-442.
48. Murray, I.; Walker, G.; Bereman, M. S., Improving the analytical performance and versatility of paper spray mass spectrometry via paper microfluidics. *Analyst* **2016,** *141* (13), 4065-4073.
49. Jingjing, Z.; Jie, J.; Xiaolin, Q., Development of an ion mobility spectrometer with a paper spray ionization source. *Review of Scientific Instruments* **2013,** *84* (6), 065103.
50. Fang, L.; Deng, J.; Yu, Y.; Yang, Y.; Wang, X.; Liu, H.; Luan, T., Coupling liquid-phase microextraction with paper spray for rapid analysis of malachite green, crystal violet and their metabolites in complex samples using mass spectrometry. *Analytical Methods* **2016,** *8* (36), 6651-6656.
51. Deng, J.; Wang, W.; Yang, Y.; Wang, X.; Chen, B.; Yao, Z.-P.; Luan, T., Slug-flow microextraction coupled with paper spray mass spectrometry for rapid analysis of complex samples. *Analytica Chimica Acta* **2016,** *940*, 143-149.
52. Zhang, Y.; Ju, Y.; Huang, C.; Wysocki, V. H., Paper Spray Ionization of Noncovalent Protein Complexes. *Analytical Chemistry* **2014,** *86* (3), 1342-1346.
53. Li, J.; Zheng, Y.; Mi, W.; Muyizere, T.; Zhang, Z., Polystyrene-impregnated paper substrates for direct mass spectrometric analysis of proteins and peptides in complex matrices. *Analytical Methods* **2018,** *10* (24), 2803-2811.
54. Han, F.; Yang, Y.; Ouyang, J.; Na, N., Direct analysis of in-gel proteins by carbon nanotubes-modified paper spray ambient mass spectrometry. *Analyst* **2015,** *140* (3), 710-715.
55. Wichert, W. R. A.; Dhummakupt, E. S.; Zhang, C.; Mach, P. M.; Bernhards, R. C.; Glaros, T.; Manicke, N. E., Detection of Protein Toxin Simulants from Contaminated Surfaces by Paper Spray Mass Spectrometry

Journal of the American Society for Mass Spectrometry **2019**.

- Paper spray has become one of the most popular ambient ionization techniques.
- Significant progress has been made recently in developing methods and applications.
- Herein these methods and applications are explored in a variety of research spaces.
- Future efforts will include ‘omics applications and further design developments.