STRUCTURED CONDUCTIVE PROBES FOR MASS SPECTROMETRY

STRUCTURED CONDUCTIVE PROBES FOR MASS SPECTROMETRY.

By PETR NALIVAIKA, B. Sc.

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment
of the Requirements for the Degree Master of Science

McMaster University MASTER OF SCIENCE (2019) Hamilton, Ontario

(Chemistry)

TITLE: Structured conductive probes for mass spectrometry

AUTHOR: Petr Nalivaika, B. Sc. (McMaster University)

SUPERVISOR: Dr. Jose Moran-Mirabal

NUMBER OF PAGES: 93

ABSTRACT

The introduction of ionization under ambient conditions has greatly simplified mass spectrometric analysis. Over past decade, ambient ionization mass spectrometry (MS) methods have revolutionized the way complex samples are analyzed under environmental conditions without requiring, in most cases, any sample pretreatment. Ambient ionization MS gained popularity among other analytical techniques due to its simplicity and its suitability for analysis of small and large molecules. However, ambient ionization methods can suffer from low accuracy and sensitivity due to matrix effects and interferences within complex samples, as well as from poor ionization efficiency. Matrix effects in ambient ionization are usually caused by ion suppression and may depend on different factors, e.g. matrix-to-analyte concentration ratios, proton affinities of analyte and matrix species.

To overcome these challenges, in this thesis we present a new approach where a probe is used both as a direct sampling device and as an efficient ambient ionization source. This approach leverages high surface area gold electrodes, fabricated through low-cost bench-top fabrication methods and functionalized using self-assembled alkyl thiol monolayers, as functional conductive sampling probes (FCSPs) for the extraction and concentration of analytes from a sample solution. FCSPs loaded with the targeted analytes were then used to demonstrate a new and highly efficient ionization approach, called Primary Ion Mass Spectrometry Source (PIMSS). In this approach, following capture, the bound analytes are directly desorbed into the mass spectrometer, where ionization is achieved solely through the extraction voltage applied to the probe. 3D-printing was used to design an interface to couple FCSPs to the mass spectrometer. In this work, we discuss a detailed method development and optimization stage and present capabilities of the proposed assay.

ACKNOWLEDGEMENTS

First of all, I would like to thank my research supervisor, Dr. Jose Moran-Mirabal, for his support throughout my time at McMaster University. This project would not have been possible without his inspiration and encouragement. I am very grateful to him for the opportunity to become a part of the great research team and meet all the wonderful people.

I would like to express my deepest gratitude to all the current and former group members of Dr. Moran-Mirabal group for always being supportive and ready to help. I am very grateful to Ayodele Fatona, Yujie Zhu, Kevin Saem and Baweleta Isho for their contributions to this work and provided training, as well as to other graduate students, Urooj Gill, Saeid Rahimi-Razin, Mouhanad Babi, Sara Makaremi, Markus Rose, Helen Luu and Xiuping Ding, for their friendship and amazing work environment they created.

Many thanks to my committee members, Dr. Dragan Vuckovic, Dr. Philip Britz-McKibbin, Dr. Peter Kruse and Dr. Kirk Green, for always being helpful and professional. I would like to acknowledge everyone at the department of Chemistry and Chemical Biology for their assistance, McMaster University and VBM Science for funding the research project.

Finally, I wish to thank my friends and family for their support during these years. And a special thanks to my wife, Iryna Alshakova, for always being caring and supportive.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABMC	Acetyl-β-methylcholine chloride
APCI	Atmospheric pressure chemical ionization
ASAP	Atmospheric pressure solids analysis probe
APGD	Atmospheric pressure glow discharge ionization
APPeI	Atmospheric pressure Penning ionization
BT	Benzenethiol
CBS	Coated blade spray
CNT	Carbon nanotube
CV	Cyclic voltammetry
DAPCI	Desorption atmospheric pressure chemical ionization
DAPPI	Desorption atmospheric pressure photoionization
DART	Direct analysis in real time
DBDI	Dielectric barrier discharge ionization
DEP	Direct electrospray probe
DESI	Desorption electrospray ionization
DIS	Daughter ion spectrum
DoE	Design of experiment
DT	Decanethiol
ELDI	Electrospray laser desorption ionization
ESI	Electrospray ionization
FD-ESI	Fused-droplet electrospray ionization
FCSP	Functional conductive sampling probe
GC	Gas chromatography
HV	High voltage
LA-ESI	Laser ablation electrospray ionization
LA-FAPA	Laser ablation/flowing atmospheric pressure afterglow
LC	Liquid chromatography
LPI	Liquid-surface Penning ionization
LTPI	Low-temperature plasma ionization
MALDI-MS	Matrix-assisted laser desorption/ionization mass spectrometry
MALDESI	Matrix-assisted laser desorption electrospray ionization
MRM	Multiple reaction monitoring
MS	Mass spectrometry
OT	1-Octadecanethiol
PADI	Plasma-assisted desorption/ionization
PESI	Probe electrospray ionization

PFOS	Heptadecafluorooctanesulfonic acid potassium salt
PIMSS	Primary ion mass spectrometry source
PMMA	Poly(methyl methacrylate)
PS	Polystyrene
PT	Propanethiol
PTFE	Polytetrafluoroethylene
REDOX	Oxidization and reduction
SAM	Self-assembled monolayer
SCP	Structured conductive probe
SEM	Scanning electron microscopy
SPME	Solid-phase microextraction
TIC	Total ion count
V_C	Capillary voltage
V_E	Extraction voltage
WCA	Water contact angle

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. INTRODUCTION

Over past decade, ambient ionization mass spectrometry (MS) methods[1] have revolutionized the way complex samples are analyzed under environmental conditions without requiring, in most cases, any sample pretreatment.[2] Ambient ionization MS has gained popularity among other analytical techniques due to its simplicity and high sensitivity, and for being suitable for analysis of both small and large molecules. MS can be performed using a wide range of ambient ion sources and ionization substrates that will be discussed in detail below. Such versatility has made ambient ionization a rapidly growing field with major environmental, forensic, pharmaceutical and imaging applications.[3] Due to the above mentioned advantages, ambient ionization presents an ideal platform for high-throughput analysis.[4-5] Application of such ambient ionization technique as desorption electrospray ionization (DESI) demonstrated high sample rates (2-3 samples per second), high matrix tolerance and low cross-contamination between samples.[4] Acceptable sample rates can be achieved with an application of sample plates that can carry multiple analytes, similar to sample plates used in matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS).[6]

Ambient ionization sources can be combined with miniaturized MS instrumentation to allow field use. For example, ambient ionization sources with direct sampling are used for miniaturization of existing MS equipment by replacing sample preparation part of the instrument (gas and liquid chromatography).[7] Ambient ionization methods combined with miniaturized MS equipment have been recently applied for the analysis of cosmetics,

foodstuff, and illegal substances.[8] Nowadays, ambient ionization methods are represented by over a hundred techniques based on different desorption/ionization principles,[9] according to which they can be classified. A classification by ionization methods utilized in ambient ionization sources[10] will be discussed below.

1.1 Ambient ionization methods based on electrospray ionization (ESI)

1.1.1 Methods based on the direct introduction of the analyte into an ESI plume

Electrospray ionization (ESI)[11] is the most widely utilized technique in ambient ionization. ESI MS is a "soft" ionization technique with no or little fragments formed as a result of the ionization process. The sample is introduced in the form of a solution into the ionization source through a capillary and is constantly sprayed. Application of high electric potential to the capillary (3-5 kV) and a nebulizing gas results in formation of highly charged droplets. High voltage application leads to the formation of Taylor Cone which ejects liquid in the form of spray (plume). The droplets are transferred to the mass spectrometer with the assistance of electric field gradient and a hot stream of inert gas. During transfer process, droplets break down and ion desolvation occurs. Due to the droplet shrinking, coulombic forces become very high and cause ions to desorb into the gas phase. ESI became popular due to its great transfer and ionization efficiency, ability to analyze large biomolecules (such as proteins) and compatibility with chromatographic separation techniques.[12]

Electrospray is widely applied in ambient ionization MS. In ambient ionization, ions are formed outside the mass spectrometer and, unlike in ESI-MS, the sample is not

introduced via the electrospray capillary. In ESI-assisted ambient ionization, methods like thermal evaporation, pyrolysis, pneumatic ultrasonic nebulization, laser desorption are used to introduce or desorb the sample into the ESI plume, where the ionization occurs. The ionization process, when the sample introduction or desorption and following ionization are two different processes, is also referred as post-ionization.

ESI was first used as a post-ionization source in 1998 when it was combined with chromatography to ionize analyte molecules exiting GC and LC columns under ambient conditions (GC-ESI and secondary ESI).[13-14] Compared to conventional GC-MS with electron ionization that has a high fragmentation rate, GC-ESI provided a way to "softly" ionize analyte molecules with acceptable efficiency.[15] However, because of the nature of GC, GC-ESI is limited only to analysis of volatile components. Another method, secondary ESI, is based on the ability of an electrospray plume to produce ions from ambient vapours that can be present at very small levels.[14] This ability makes secondary ESI a suitable tool for analysis of volatile species such as explosives. One of the reported limitations for this method was a high chemical background noise and long baseline recovery to zero after analyte injection.[16] The ionization efficiency for these methods was further enhanced by increasing the number of electrosprayers (multiple-channel ESI), which can be useful when dealing with high LC flow rates.[17]

Other ESI-based ambient methods that were successfully developed for the analysis of large molecules, such as proteins and peptides, include fused-droplet electrospray ionization (FD-ESI)[18], extractive ESI,[19] and neutral desorption extractive ESI.[20] In FD-ESI,

3

an analyte is detected via a two-step process that involves introduction of the fine mist solution of analyte into the ESI plume, followed by protein ion formation as a result of fusion with the solvent droplets in the plume. FD-ESI can be characterized as an efficient method for detection of peptides and proteins with high salt tolerance compared to conventional ESI-MS. Extractive and neutral desorption extractive ESI are variations of FD-ESI suitable for analysis of volatile[21] and non-volatile compounds[22], that can be applied for remote analysis since analyte molecules can be transported over 1 m.[23] One of the main advantages of post-ionization in the ESI plume is high ionization efficiency. This is due to the large amount of generated charged particles available for interaction with the analyte. Overall, this group of methods presents an attractive way of ionization from liquid and gaseous phases. However, ionization from a solid sample is limited.

1.1.2 Laser irradiation- and IR-based ESI

Another category of ESI-based ambient ionization techniques that addresses ionization of solid samples, involves laser irradiation of a surface with a pulsed nitrogen laser and post-ionization in the ESI plume (ELDI).[24] Ionization in this group of methods occurs through the interaction of sample molecules with ESI-generated charged particles. Laser irradiation in that particular case is used for desorption of molecules from the solid surface (Figure 1.1). Currently there are more than a dozen laser-based ionization methods. Some of the most popular techniques are electrospray laser desorption ionization (ELDI), matrix-assisted laser desorption electrospray ionization (MALDESI) and laser ablation ESI (LA-ESI). In ELDI sample molecules are desorbed from the solid surface via laser

irradiation and gaseous particles are generated. Ions are generated from the desorbed species directly in the ESI plume. Unlike matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), ELDI does not use organic matrix for ionization. The role of the organic matrix in MALDI is to absorb laser energy, required for desorption, and transfer it to the analyte; it prevents the analyte from fragmentation and required for its ionization (e.g. via proton transfer mechanism).[25] Thus, without presence of organic matrix ELDI generates multiply charged molecules of a studied analyte. Similar to MALDI, analytes of interest are large biomolecules such as proteins and peptides. ELDI-MS is a powerful tool for spatial mapping and has been applied to molecular imaging of plant and fungus slices and animal tissues. One of the main ELDI drawbacks is that in many cases direct laser irradiation might cause fragmentation of some analytes, making the analysis very complex.[26] MALDESI uses conventional organic MALDI desorption matrices, mixed with analyte (at a 1000:1 ratio or lower), instead of direct laser irradiation of the sample, and it has similar ionization and desorption principles with ELDI.[27-29] The method suffers from drawbacks related with the organic matrix use: detector saturation in the low MW range (<500 Da), introduction of background ions into the mass spectrometer, high matrix ion interference, co-desorption and formation of cluster ions.[30-31] In LA-ESI, similarly to ELDI, desorption and ionization are two discrete steps, where an IR laser is used to desorb analyte molecules from a liquid or a solid sample, and ionization occurs via ESI-like mechanism in the plume. LA-ESI has been applied for identification of proteins, lipids, and metabolites. It was reported that LA-ESI is capable of metabolite detection from untreated whole blood samples, while DESI and ELDI require moderate blood treatment.[32-33] LA-ESI tissue

imaging can suffer from sample handling issues such as moisture condensation and constant temperature control over a long period of time.[34] Despite of all the positive features, laser-based ambient ionization techniques have certain limitations. Sample size is limited because of physical desorption. Furthermore, laser irradiation can generate neutral particles of solid samples that results in undesirable background for the MS analyzer.[35] However, the main drawback is a destructive nature of laser-based desorption. Fragmentation is undesirable if the goal is to identify a mass of a biomolecule and preference is given to "soft" ionization techniques.
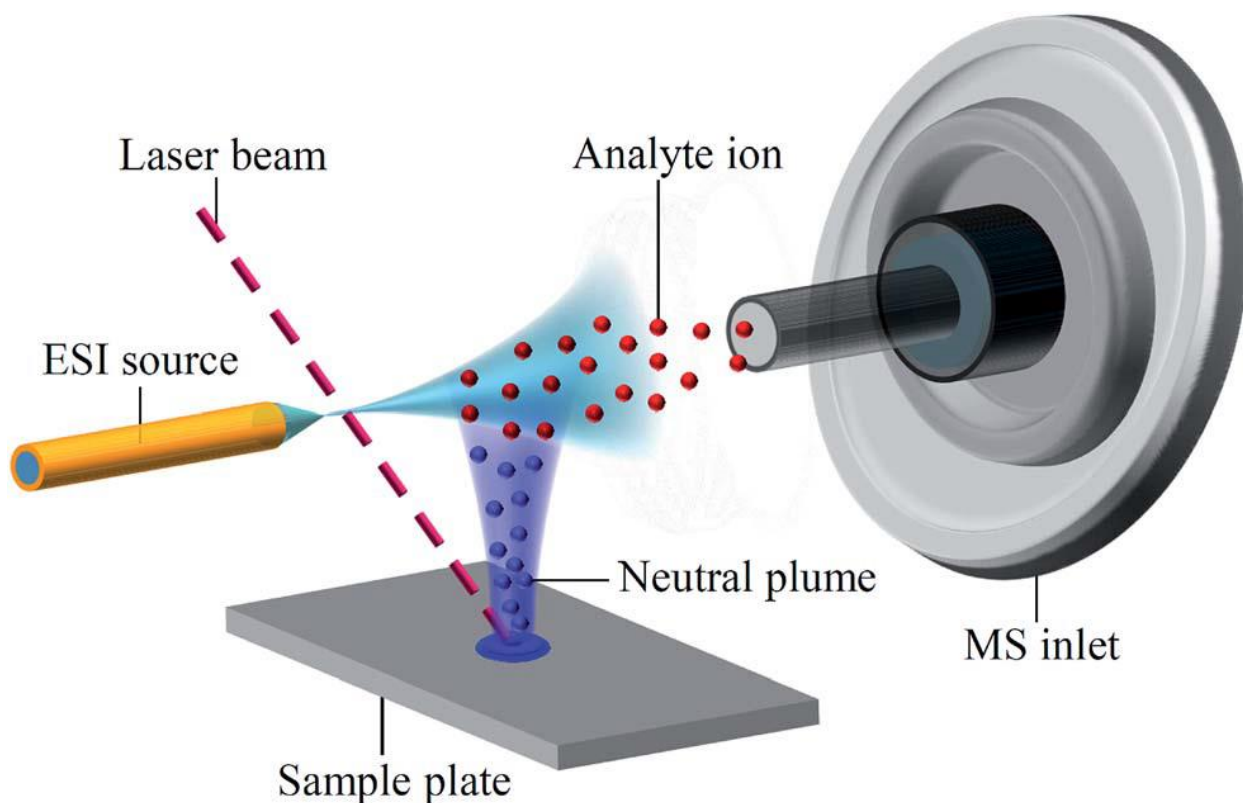
Figure 1.1. Schematic of a two-step ionization process in laser ionization-based ESI. Analyte molecules are desorbed from a sample surface via laser irradiation. Next, desorbed species are ionized in ESI plume (adapted from Ref. **35**).

1.2 Ambient ionization methods with electrospray desorption
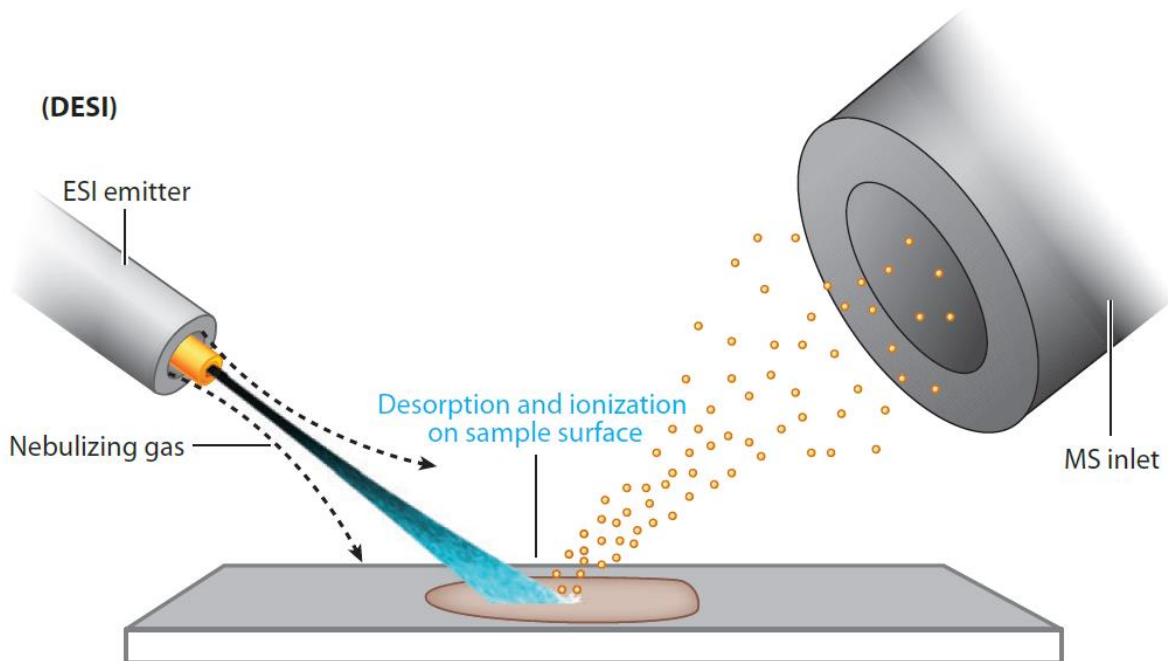
Figure 1.2. Schematic of the ionization process in desorption electrospray ionization MS (DESI) (adapted from Ref. **10**).

All the techniques mentioned above involve introduction of the sample molecules into the ESI plume and subsequent post-ionization in it. A second major category of techniques uses electrospray for the simultaneous desorption and ionization from a surface of interest. Desorption electrospray ionization (DESI) has been thoroughly studied over the past 15 years. In DESI-MS, charged solvent droplets generated by the emitter source collide with the sample surface with the assistance of a nebulizing gas (Figure 1.2).[10] As a result of collisions, new micro droplets are generated and directed into the spectrometer inlet. Microdroplets can be expelled at different angles, velocities and sizes, and it has been

suggested that a two-step droplet-pickup mechanism is involved. According to this mechanism, a solvent film that contains analyte molecules is initially formed. Next, incident droplet collisions desorb the surface liquid and carry the sample molecules into the instrument.[36-39]

DESI and DESI-like methods are unique because they do not require sample pretreatment, one of the main reasons why ambient mass spectrometry has become so popular. DESI-MS has been applied in forensics, including analysis of illicit drugs[40], explosives[41], chemical warfare agents[42] and other substances, as well as studies of pharmaceutical drugs[4], polymers[43] and imaging of biological tissues,[44-45] showcasing its simplicity and wide applicability. However, according to many published articles, DESI has lower sensitivity than common analytical techniques and, because of low reproducibility, quantification can be a challenge.[46]

1.3 Ambient ionization methods with electrospray generation at the probe

More recently developed methods include techniques where electrospray is generated directly from the probe. Electrospray is typically generated from a liquid sample or solution droplet which is deposited onto a probe connected to a high voltage (HV) source. In the direct electrospray probe (DEP) method,[48] a sample droplet is deposited on a metal ring connected to the HV source, while in the paper spray (PS) ionization method, a sample droplet and wetting solution are deposited onto a triangular piece of paper (Figure 1.3). Voltage is applied (typically 3-5 kV) to the paper wetted with a small amount of methanol/water (1:1 v/v) solution. The HV creates a high electric potential between the

substrate and MS inlet which is necessary to create an electric field that induces a charge at the tip of the probe. For such techniques no nebulizing gas is required, since Coulombic forces break the liquid to form charged droplets, causing the subsequent desolvation processes to generate dry ions.[47, 49] The ionization mechanism for this group of methods is still largely unknown, but it has been proposed that ions are formed in a similar fashion as in the plume of ESI-MS.[50-51] This group of methods will be discussed in detail in the Direct Spray Ionization Methods section below.
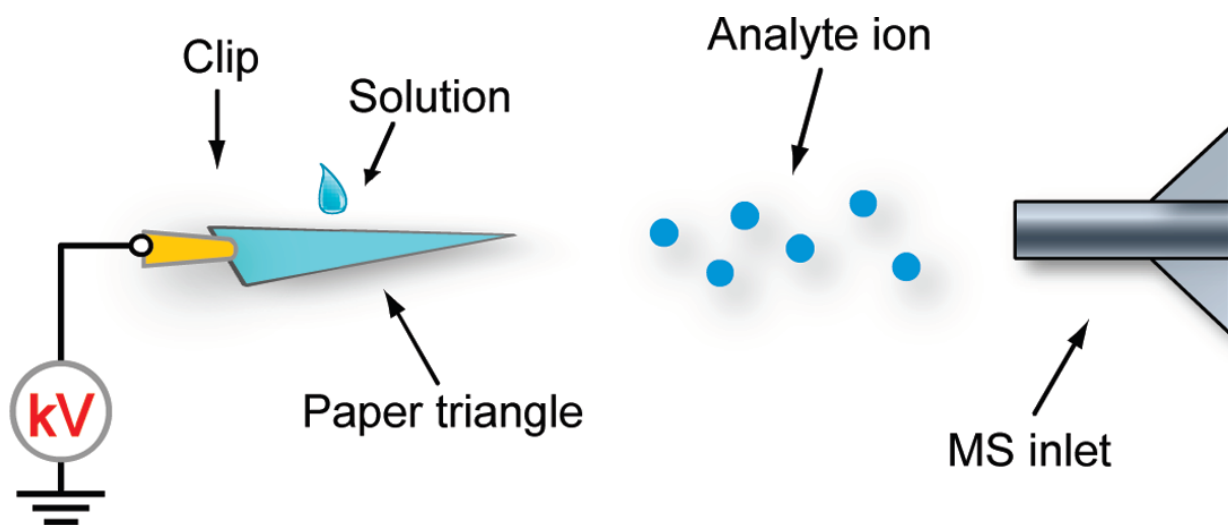
Figure 1.3. Schematic of paper spray ionization mass spectrometry (adapted from Ref. **47**).

1.4 Atmospheric pressure chemical ionization-based techniques

1.4.1 Corona discharge-based methods

Finally, there is a category of atmospheric pressure chemical ionization-based techniques (APCI). APCI methods can be divided into two subcategories: corona

discharge-based methods and plasma-based methods. In corona discharge-based APCI, ion formation occurs through a series of ion-molecule interactions between analyte molecules and reagent species. Corona discharge-based APCI includes such methods as atmospheric pressure solids analysis probe (ASAP), desorption atmospheric pressure chemical ionization (DAPCI) and desorption atmospheric pressure photoionization (DAPPI). Compared to ESI-based methods that were mentioned above, APCI-based ionization techniques are more suitable for analysis of less polar and more volatile compounds. In corona discharge APCI, analyte ions are formed as a result of ion-molecule interactions between the analyte and an activated reagent species. In ASAP, a hot nitrogen gas stream is used to bring molecules from the surface of a solid sample to the APCI needle to be ionized under corona discharge APCI conditions (Figure 1.4).[52] ASAP has been applied for detection of lipids, capsaicins, and carotenoids in biological tissues.[53] In DAPCI (Figure 1.5), the sample surface is hit with charged species generated by APCI source (e.g. electrons, protons, metastable atoms, solvent ions) to desorb and also ionize analyte molecules.[54] While ASAP and DAPCI provide low ionization efficiency for molecules with low polarity, DAPPI is capable of efficient ionization of such molecules.[55] APCI-based techniques have limited mass range (<2000 Da) and very sensitive to contaminants. Some of the more recent methods are mainly plasma-based ambient ionization techniques, *e.g.* dielectric barrier discharge ionization (DBDI),[56] low-temperature plasma (LTP)[57] and plasma-assisted desorption/ionization (PADI), and will be discussed in the next section.[58]
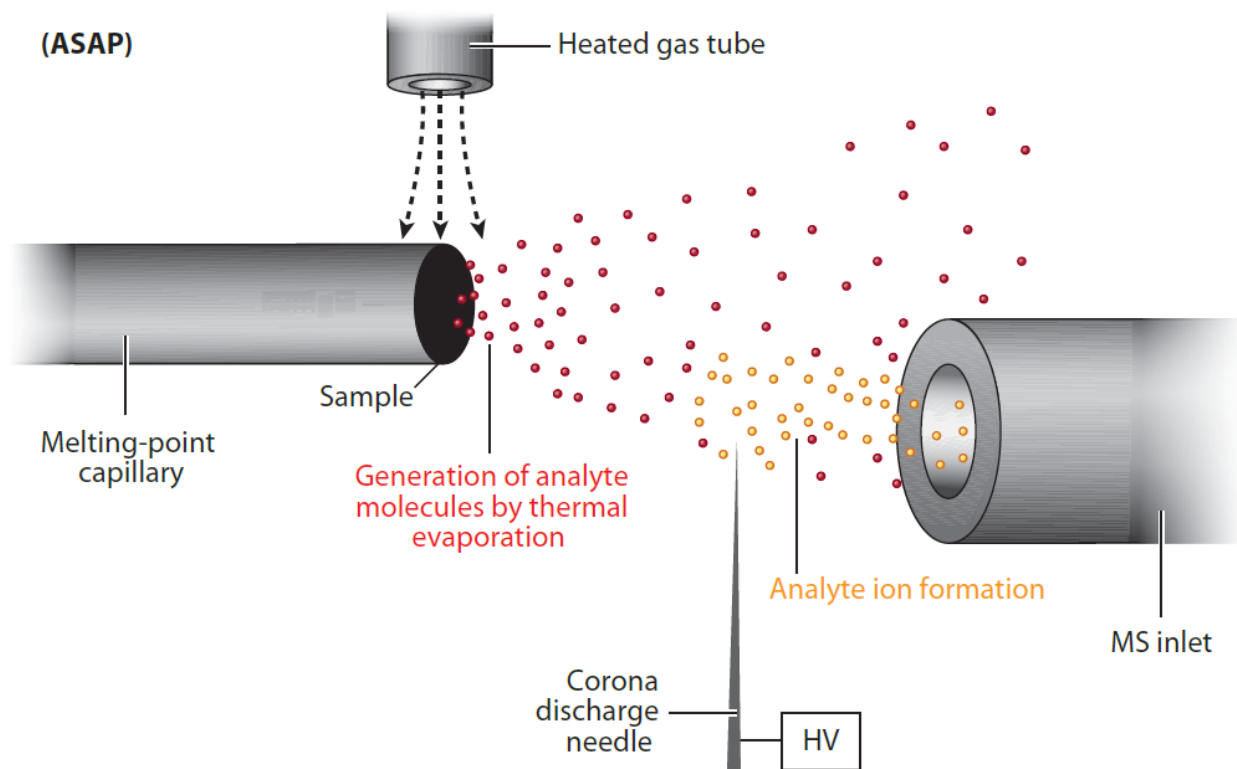
Figure 1.4. Schematic illustration of atmospheric pressure solids analysis probe (ASAP) (adapted from Ref. **52**).
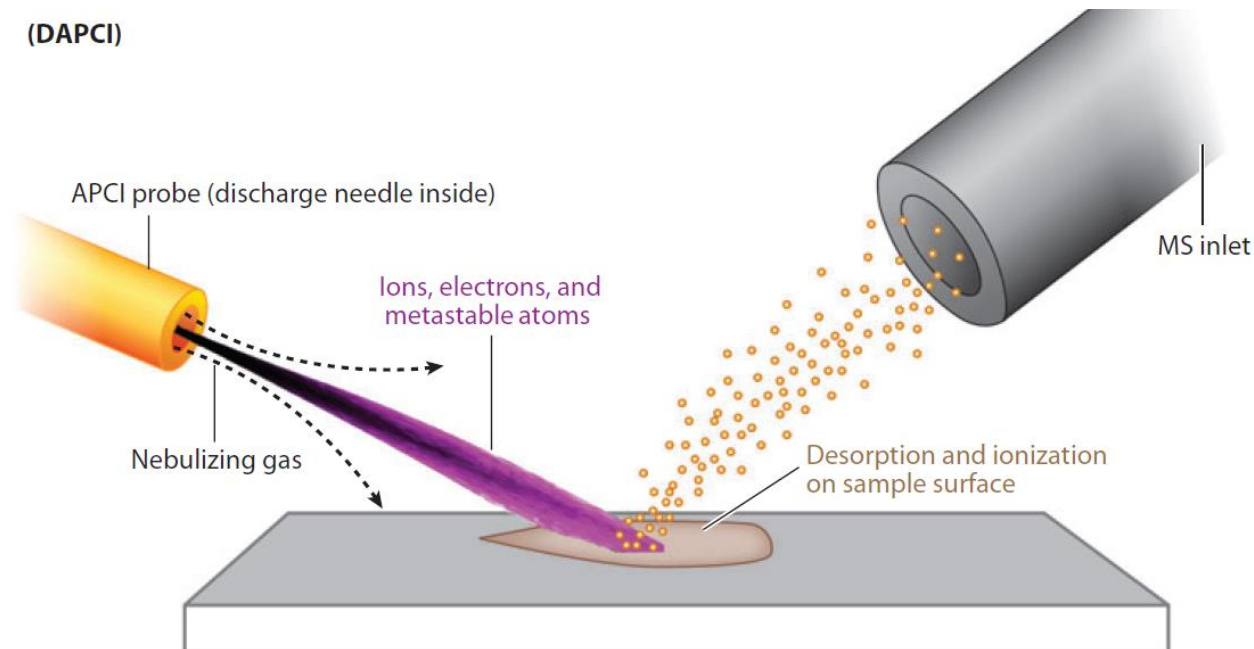
Figure 1.5. Schematic illustration of desorption atmospheric pressure chemical ionization (DAPCI) (**54**).

1.4.2 Plasma-based methods

Plasma based techniques gained a lot of popularity for being able to analyze a wide range of analytes in a rapid fashion. This group of methods is based on the Penning ionization mechanism, where analyte ions are formed through energy transfer from an excited species with higher ionization energy.[59] Direct analysis in real time (DART)[60] (Figure 1.6) was introduced in 2005 and has become a well-established since then. DART is a versatile tool, it allows rapid analysis of a large number of analytes, including high-throughput screening in quality control. In DART, the analyte is ionized when exposed to a stream of excited He species. As a result of interaction, ions, electrons and metastable particles can be produced. Several ionization mechanisms have been suggested for DART, [37, 61] of which the most common is the Penning ionization[59] or energy transfer (from excited

species to sample molecules) mechanism. DART has been applied to analysis of volatile organic compounds,[62-63] quantification of drugs in biological matrixes,[64] chemical warfare agents[65] and illicit drugs.[66] Other techniques that have similar ionization principle are liquid-surface Penning ionization (LPI),[67] atmospheric pressure Penning ionization (APPeI),[68] atmospheric pressure glow discharge ionization (APGD),[69] and laser ablation/flowing atmospheric pressure afterglow (LA-FAPA).[70] Overall, APCI-based techniques present an attractive way of detection of non-polar and volatile compounds. It should be mentioned that APCI-based techniques are very sensitive to contaminants and have limited mass range (<2000 Da). Thus, these methods are not applicable to analysis of large biomolecules. APCI-based techniques require complex instrumentation and high-level technical expertise.
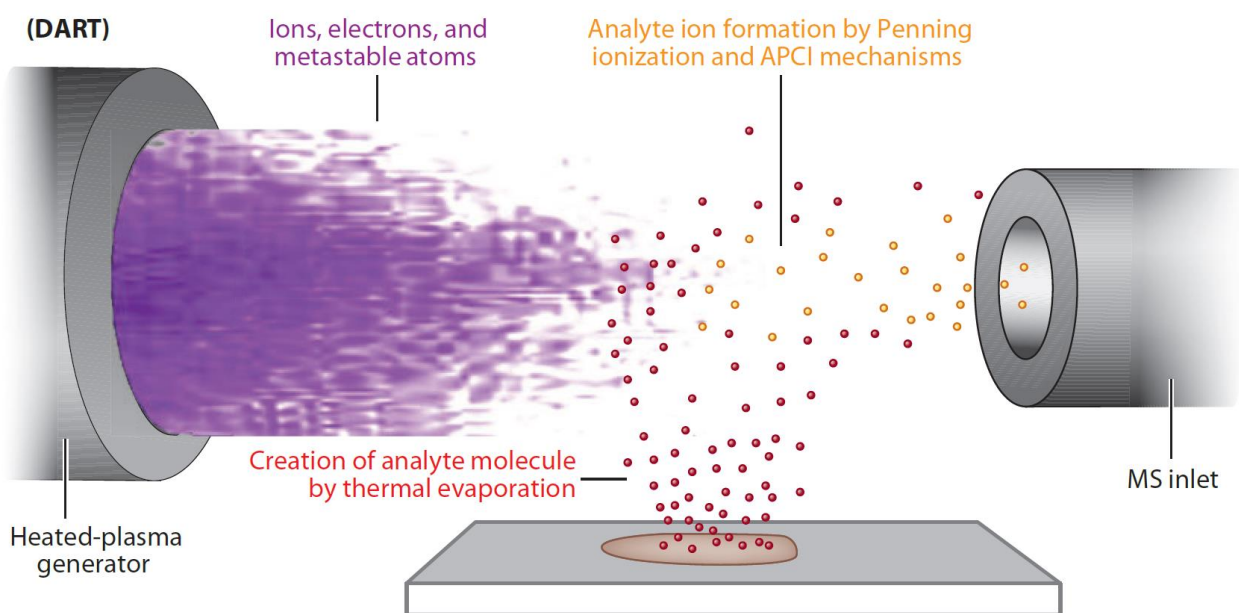
Figure 1.6. Schematic of direct analysis in real time method (DART) (adapted from Ref. **60**).

1.5 Direct spray mass spectrometry

1.5.1 Paper-spray ionization

Direct spray mass spectrometry methods present special interest due to the ability to generate electrospray directly from a solid substrate or a probe. The method's efficiency depends highly on the characteristics of the substrate. The selectivity and sensitivity of the technique can be fine-tuned by manipulating the selected substrate (e.g. type of material, surface coating, shape, voltage application). Some of the methods that are part of this group have been already discussed here (DEP and PS-MS). Direct ionization techniques can be performed on different metal and polymer substrates, commercial or modified paper, plant leaves, animal tissues, and even living organisms. All of these techniques are multistep processes. Steps can involve analyte deposition, desolvation, extraction, transport, ionization or electrospray formation. Paper-spray ionization, for example, is a three-step process that includes extraction of the analyte on the paper by the solvent, transport to the tip of the substrate and generation of charged droplets that leads to ionization and delivery of the analyte ions to the MS. Several different types of paper-based substrates have been applied, including unmodified chromatographic paper,[71] silica-coated paper,[72] silanized paper,[73] print paper[74] and carbon nanotube (CNT) paper.[75] The chemical and physical properties of substrates can be significantly changed when functionalized. Examples of substrate properties that can be changed are conductivity, separation ability, hydrophobicity/hydrophilicity. Surface modification can significantly enhance the properties of the substrate. For example, it has been demonstrated that a porous filter paper

substrate, uniformly dispersed with the CNTs, has larger surface area and enhanced conductivity compared to the unmodified paper substrate.[76] Spraying from CNT-paper can be achieved at a low voltage of 3 V, while chromatography paper requires up to 5 kV for stable spraying. In another study, chromatographic paper was coated with silica to improve the efficiency of recovery of the analytes.[72] Paper spray ionization has been widely applied in the fields of forensics, drug and foodstuff analysis. One of the first applications of PS-MS was determination of therapeutic and illicit drugs in fresh and dried blood spots (Figure 1.7).[77-79] More applications of PS were found for identification of food adulteration: *in situ* analysis of residual agrochemicals on fruits, analysis of spices,[80-81] caramel samples,[82] tea[83] and coffee.[84] There are many more applications to which PS can be applied.[85-88] Wood, as another cellulose-based substrate, was also used for direct spray ionization.[89] Commercially available wooden sharpened toothpicks were applied for the detection of amino acids, lipids, and proteins from different biological samples.[90-92] To summarize, paper- and cellulose-based substrates present a low-cost and simple, yet efficient, platform for rapid analysis of various compounds. However, there several drawbacks in regard to PS-MS. Sensitivity can be an issue in some cases, e.g. limits of detection for some drugs are not within the therapeutic range and it is challenging to perform semi-quantitative and quantitative analysis of drugs in complex biological matrices such as blood and urine using PS-MS. Additionally, cellulose-based substrates contain hydroxyl groups and this may hinder the elution of polar components due to strong hydrogen bonding and Wan der Waals forces between polar sample molecules and the paper substrate.[93]
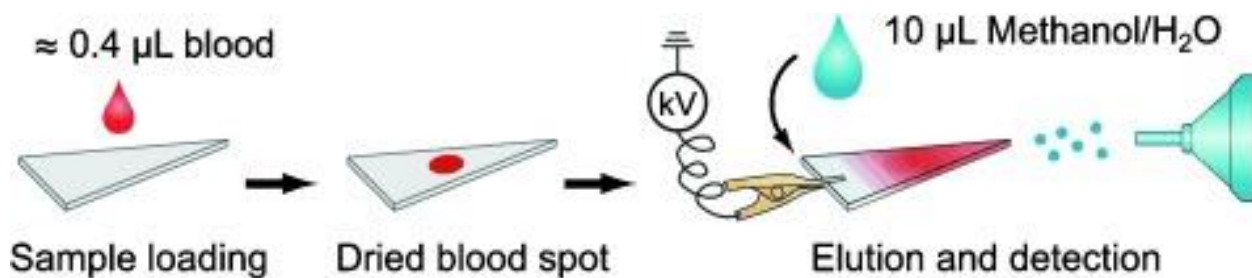
Figure 1.7. Schematic of dried blood spot using paper spray ionization mass spectrometry (taken from Ref. **71**).

1.5.2 Tissue-spray ionization

Direct spray ionization can be performed directly from the tissues, without sample pretreatment and without an extraction step. Spray can be generated from leaves cut in the shape of a triangle under conditions similar to PS-MS. Leaf-spray provides information on compounds contained in the plants[94-95] and agrochemicals.[96] Tissues of living organisms can be also analyzed via direct ionization MS.[97-99] While PS-MS is precise enough for generation of quantitative data, tissue-spray methods can only provide semi-quantitative information. but are promising tools for rapid screening of sugars, amino acids, organic acids, pesticides and biomolecules.

Tissue-spray ionization also presents a very attractive platform for medical applications, e.g. in situ diagnosis with minimal sample pretreatment. The method was successfully applied for differentiation between healthy and cancer tissues from kidneys from deceased patients.[100] Tissue-spray was also recently applied to analysis of human lung squamous cell carcinoma tissue and adjacent normal lung tissue. It was reported that method had better sensitivity and number of detected analytes was higher compared to

16

DESI, wooden tip spray and paper spray.[101] The technique may become a rapid medical identification tool for pathologies in human tissues.

1.5.3 Direct electrospray probe and probe electrospray ionization

Finally, direct ionization can be performed on conductive substrates via high voltage application (direct ionization probe and probe electrospray ionization). Substrates used for these methods allow greater tunability due to the availability of a wide range of materials, coatings and compatible solvents. Copper[102] and platinum[103] wires were the first substrates used for direct electrospray probe (DEP). Later, a solid-phase microextraction (SPME) graphite fiber was used as a substrate and a concentration probe.[104] The combination of SPME and direct ionization led to the successful detection of surfactants at a ppb level. Gold coated optical fibers and tungsten oxide nanowires have also been applied to the investigation of protein solutions.[105-106] The DEP principle of operation was mentioned earlier in this chapter. In probe electrospray ionization (PESI), electrospray is generated by application of HV to a stainless steel needle which contains a small amount of the liquid sample on the tip.[48] The PESI approach provides high tolerance to buffers and contaminated samples and is suitable for the analysis of amino acids, proteins and peptides in biological samples.[107] They provide almost unlimited potential for further development due to the great amount of substrates and modifications available. Probe-based methods do not require a dedicated ionization source, which is an advantage and disadvantage at the same time. Applicability of such methods in pharmaceutical and clinical areas is very limited because of instrumental simplicity. Probes and substrates in one ionization method,

produced by different laboratories or companies may vary one from another and are not regulated by any standards.

1.6 Thesis overview

This thesis presents a novel ambient ionization technique called Primary Ion Mass Spectrometry Source (PIMSS), that incorporates use of high surface area gold electrodes as functional structured conductive probes for mass spectrometry. The concept proposed in this work is also a probe-based ambient ionization method. Although, it utilizes a completely different ionization mechanism without formation of electrospray from the probe. Chapter 2 describes the method development stage of the project and highlights the most important parameters of the ambient ionization technique as well as challenges that might be encountered during this step. The chapter shows a progression from an early stage method with low efficiency to the highly reproducible and sensitive technique with advanced design. Chapter 3 demonstrates a potential and applications of the developed method.

A simple 3D-printing based probe-to-MS coupling design was demonstrated. Optimal mass spectrometry parameters were verified and applied. The developed method was used as an extraction probe and as an ion source for mass spectrometry. Advantages, limitations, and future work and applications are discussed in Chapter 4.

1.7 References

1.   Van Berkel, G. J.; Pasilis, S. P.; Ovchinnikova, O., Established and emerging atmospheric pressure surface sampling/ionization techniques for mass spectrometry. *Journal of mass spectrometry : JMS* **2008,** *43* (9), 1161-80.

2.   Chen, H.; Ouyang, Z.; Cooks, R. G., Thermal production and reactions of organic ions at atmospheric pressure. *Angew Chem Int Ed Engl* **2006,** *45* (22), 3656-60.

3.   Harris, G. A.; Galhena, A. S.; Fernandez, F. M., Ambient sampling/ionization mass spectrometry: applications and current trends. *Analytical chemistry* **2011,** *83* (12), 4508-38.

4.   Chen, H.; Talaty, N. N.; Takats, Z.; Cooks, R. G., Desorption electrospray ionization mass spectrometry for high-throughput analysis of pharmaceutical samples in the ambient environment. *Analytical chemistry* **2005,** *77* (21), 6915-27.

5.   Li, L.-P.; Feng, B.-S.; Yang, J.-W.; Chang, C.-L.; Bai, Y.; Liu, H.-W., Applications of ambient mass spectrometry in high-throughput screening. *Analyst* **2013,** *138* (11), 3097-3103.

6.   Manicke, N. E.; Kistler, T.; Ifa, D. R.; Cooks, R. G.; Ouyang, Z., High-throughput quantitative analysis by desorption electrospray ionization mass spectrometry. *J Am Soc Mass Spectrom* **2009,** *20* (2), 321-5.

7.   Xu, W.; Manicke, N. E.; Cooks, G. R.; Ouyang, Z., Miniaturization of Mass Spectrometry Analysis Systems. *JALA Charlottesv Va* **2010,** *15* (6), 433-439.

8.   Ma, Q.; Bai, H.; Li, W.; Wang, C.; Li, X.; Cooks, R. G.; Ouyang, Z., Direct identification of prohibited substances in cosmetics and foodstuffs using ambient ionization on a miniature mass spectrometry system. *Anal Chim Acta* **2016,** *912*, 65-73.

9.   Harris, G. A.; Nyadong, L.; Fernandez, F. M., Recent developments in ambient ionization techniques for analytical mass spectrometry. *Analyst* **2008,** *133* (10), 1297-301.

10.   Huang, M. Z.; Yuan, C. H.; Cheng, S. C.; Cho, Y. T.; Shiea, J., Ambient ionization mass spectrometry. *Annu Rev Anal Chem (Palo Alto Calif)* **2010,** *3*, 43-65.

11.   Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M., Electrospray ionization for mass spectrometry of large biomolecules. *Science* **1989,** *246* (4926), 64-71.

12.   Wilm, M., Principles of electrospray ionization. *Mol Cell Proteomics* **2011,** *10* (7), M111 009407.

13.   Lee, C. Y.; Shiea, J., Gas chromatography connected to multiple channel electrospray ionization mass spectrometry for the detection of volatile organic compounds. *Analytical chemistry* **1998,** *70* (13), 2757-61.

14.   Wu, C.; Siems, W. F.; Hill, H. H., Jr., Secondary electrospray ionization ion mobility spectrometry/mass spectrometry of illicit drugs. *Analytical chemistry* **2000,** *72* (2), 396-403.

15.   Li, D. X.; Gan, L.; Bronja, A.; Schmitz, O. J., Gas chromatography coupled to atmospheric pressure ionization mass spectrometry (GC-API-MS): review. *Anal Chim Acta* **2015,** *891*, 43-61.

16.   Martinez-Lozano, P.; Rus, J.; Fernandez de la Mora, G.; Hernandez, M.; Fernandez de la Mora, J., Secondary electrospray ionization (SESI) of ambient vapors for explosive

detection at concentrations below parts per trillion. *J Am Soc Mass Spectrom* **2009,** *20* (2), 287-94.

17.   Schneider, B. B.; Douglas, D. J.; Chen, D. D., Multiple sprayer system for high-throughput electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom* **2002,** *16* (20), 1982-90.

18.   Lee, C. C.; Chang, D. Y.; Jeng, J.; Shiea, J., Generating multiply charged protein ions via two-step electrospray ionization mass spectrometry. *Journal of mass spectrometry : JMS* **2002,** *37* (1), 115-7.

19.   Chen, H.; Venter, A.; Cooks, R. G., Extractive electrospray ionization for direct analysis of undiluted urine, milk and other complex mixtures without sample preparation. *Chem Commun (Camb)* **2006,** (19), 2042-4.

20.   Chen, H.; Wortmann, A.; Zenobi, R., Neutral desorption sampling coupled to extractive electrospray ionization mass spectrometry for rapid differentiation of biosamples by metabolomic fingerprinting. *Journal of mass spectrometry : JMS* **2007,** *42* (9), 1123-35.

21.   Chingin, K.; Gamez, G.; Chen, H.; Zhu, L.; Zenobi, R., Rapid classification of perfumes by extractive electrospray ionization mass spectrometry (EESI-MS). *Rapid Commun Mass Spectrom* **2008,** *22* (13), 2009-14.

22.   Marquez, C. A.; Wang, H.; Fabbretti, F.; Metzger, J. O., Electron-transfer-catalyzed dimerization of trans-anethole: detection of the distonic tetramethylene radical cation intermediate by extractive electrospray ionization mass spectrometry. *J Am Chem Soc* **2008,** *130* (51), 17208-9.

23.   Chen, H.; Hu, B.; Hu, Y.; Huan, Y.; Zhou, Z.; Qiao, X., Neutral desorption using a sealed enclosure to sample explosives on human skin for rapid detection by EESI-MS. *J Am Soc Mass Spectrom* **2009,** *20* (4), 719-22.

24.   Shiea, J.; Huang, M. Z.; Hsu, H. J.; Lee, C. Y.; Yuan, C. H.; Beech, I.; Sunner, J., Electrospray-assisted laser desorption/ionization mass spectrometry for direct ambient analysis of solids. *Rapid Commun Mass Spectrom* **2005,** *19* (24), 3701-4.

25.   Knochenmuss, R., Ion formation mechanisms in UV-MALDI. *Analyst* **2006,** *131* (9), 966-86.

26.   Huang, M. Z.; Hsu, H. J.; Lee, J. Y.; Jeng, J.; Shiea, J., Direct protein detection from biological media through electrospray-assisted laser desorption ionization/mass spectrometry. *J Proteome Res* **2006,** *5* (5), 1107-16.

27.   Sampson, J. S.; Hawkridge, A. M.; Muddiman, D. C., Generation and detection of multiply-charged peptides and proteins by matrix-assisted laser desorption electrospray ionization (MALDESI) Fourier transform ion cyclotron resonance mass spectrometry. *J Am Soc Mass Spectrom* **2006,** *17* (12), 1712-6.

28.   Sampson, J. S.; Hawkridge, A. M.; Muddiman, D. C., Direct characterization of intact polypeptides by matrix-assisted laser desorption electrospray ionization quadrupole Fourier transform ion cyclotron resonance mass spectrometry. *Rapid Commun Mass Spectrom* **2007,** *21* (7), 1150-4.

29.   Sampson, J. S.; Hawkridge, A. M.; Muddiman, D. C., Development and characterization of an ionization technique for analysis of biological macromolecules:

liquid matrix-assisted laser desorption electrospray ionization. *Analytical chemistry* **2008,** *80* (17), 6773-8.

30.   Karas, M.; Kruger, R., Ion formation in MALDI: the cluster ionization mechanism. *Chem Rev* **2003,** *103* (2), 427-40.

31.   Cohen, L. H.; Gusev, A. I., Small molecule analysis by MALDI mass spectrometry. *Analytical and bioanalytical chemistry* **2002,** *373* (7), 571-86.

32.   Nemes, P.; Barton, A. A.; Li, Y.; Vertes, A., Ambient molecular imaging and depth profiling of live tissue by infrared laser ablation electrospray ionization mass spectrometry. *Analytical chemistry* **2008,** *80* (12), 4575-82.

33.   Nemes, P.; Vertes, A., Laser ablation electrospray ionization for atmospheric pressure, in vivo, and imaging mass spectrometry. *Analytical chemistry* **2007,** *79* (21), 8098-106.

34.   Nemes, P.; Woods, A. S.; Vertes, A., Simultaneous imaging of small metabolites and lipids in rat brain tissues at atmospheric pressure by laser ablation electrospray ionization mass spectrometry. *Analytical chemistry* **2010,** *82* (3), 982-8.

35.   Cheng, S.-C.; Shiea, C.; Huang, Y.-L.; Wang, C.-H.; Cho, Y.-T.; Shiea, J., Laser-based ambient mass spectrometry. *Anal Methods-Uk* **2017,** *9* (34), 4924-4935.

36.   Venter, A.; Sojka, P. E.; Cooks, R. G., Droplet dynamics and ionization mechanisms in desorption electrospray ionization mass spectrometry. *Analytical chemistry* **2006,** *78* (24), 8549-55.

37.   Harris, G. A.; Fernandez, F. M., Simulations and experimental investigation of atmospheric transport in an ambient metastable-induced chemical ionization source. *Analytical chemistry* **2009,** *81* (1), 322-9.

38.   Costa, A. B.; Cooks, R. G., Simulation of atmospheric transport and droplet-thin film collisions in desorption electrospray ionization. *Chem Commun (Camb)* **2007,** (38), 3915-7.

39.   Douglass, K. A.; Jain, S.; Brandt, W. R.; Venter, A. R., Deconstructing desorption electrospray ionization: independent optimization of desorption and ionization by spray desorption collection. *J Am Soc Mass Spectrom* **2012,** *23* (11), 1896-902.

40.   Leuthold, L. A.; Mandscheff, J. F.; Fathi, M.; Giroud, C.; Augsburger, M.; Varesio, E.; Hopfgartner, G., Desorption electrospray ionization mass spectrometry: direct toxicological screening and analysis of illicit Ecstasy tablets. *Rapid Commun Mass Spectrom* **2006,** *20* (2), 103-10.

41.   Cotte-Rodriguez, I.; Takats, 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), 6755-64.

42.   Cotte-Rodriguez, I.; Cooks, R. G., Non-proximate detection of explosives and chemical warfare agent simulants by desorption electrospray ionization mass spectrometry. *Chem Commun (Camb)* **2006,** (28), 2968-70.

43.   Nefliu, M.; Venter, A.; Cooks, R. G., Desorption electrospray ionization and electrosonic spray ionization for solid- and solution-phase analysis of industrial polymers. *Chem Commun (Camb)* **2006,** (8), 888-90.

44.   Wiseman, J. M.; Ifa, D. R.; Song, Q.; Cooks, G. R., Tissue imaging at atmospheric pressure using desorption electrospray ionization (DESI) mass spectrometry. *Angew Chem Int Ed Engl* **2006,** *45*, 7188–7192.

45.   Wiseman, J. M.; Ifa, D. R.; Zhu, Y.; Kissinger, C. B.; Manicke, N. E.; Kissinger, P. T.; Cooks, R. G., Desorption electrospray ionization mass spectrometry: Imaging drugs and metabolites in tissues. *Proc Natl Acad Sci U S A* **2008,** *105* (47), 18120-5.

46.   Morelato, M.; Beavis, A.; Kirkbride, P.; Roux, C., Forensic applications of desorption electrospray ionisation mass spectrometry (DESI-MS). *Forensic Sci Int* **2013,** *226* (1-3), 10-21.

47.   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), 2463-71.

48.   Hiraoka, K.; Nishidate, K.; Mori, K.; Asakawa, D.; Suzuki, S., Development of probe electrospray using a solid needle. *Rapid Commun Mass Spectrom* **2007,** *21* (18), 3139-44.

49.   Chen, L. C.; Nishidate, K.; Saito, Y.; Mori, K.; Asakawa, D.; Takeda, S.; Kubota, T.; Hori, H.; Hiraoka, K., Characteristics of probe electrospray generated from a solid needle. *J Phys Chem B* **2008,** *112* (35), 11164-70.

50.   Huang, M. Z.; Cheng, S. C.; Cho, Y. T.; Shiea, J., Ambient ionization mass spectrometry: a tutorial. *Anal Chim Acta* **2011,** *702* (1), 1-15.

51.   Li, A.; Wang, H.; Ouyang, Z.; Cooks, R. G., Paper spray ionization of polar analytes using non-polar solvents. *Chem Commun (Camb)* **2011,** *47* (10), 2811-3.

52.   McEwen, C. N.; McKay, R. G.; Larsen, B. S., Analysis of solids, liquids, and biological tissues using solids probe introduction at atmospheric pressure on commercial LC/MS instruments. *Analytical chemistry* **2005,** *77* (23), 7826-31.

53.   McEwen, C.; Gutteridge, S., Analysis of the inhibition of the ergosterol pathway in fungi using the atmospheric solids analysis probe (ASAP) method. *J Am Soc Mass Spectrom* **2007,** *18* (7), 1274-8.

54.   Song, Y.; Cooks, R. G., Atmospheric pressure ion/molecule reactions for the selective detection of nitroaromatic explosives using acetonitrile and air as reagents. *Rapid Commun Mass Spectrom* **2006,** *20* (20), 3130-8.

55.   Haapala, M.; Pol, J.; Saarela, V.; Arvola, V.; Kotiaho, T.; Ketola, R. A.; Franssila, S.; Kauppila, T. J.; Kostiainen, R., Desorption atmospheric pressure photoionization. *Analytical chemistry* **2007,** *79* (20), 7867-72.

56.   Na, N.; Zhao, M.; Zhang, S.; Yang, C.; Zhang, X., Development of a dielectric barrier discharge ion source for ambient mass spectrometry. *J Am Soc Mass Spectrom* **2007,** *18* (10), 1859-62.

57.   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), 9097-104.

58.   Ratcliffe, L. V.; Rutten, F. J.; Barrett, D. A.; Whitmore, T.; Seymour, D.; Greenwood, C.; Aranda-Gonzalvo, Y.; Robinson, S.; McCoustra, M., Surface analysis under ambient conditions using plasma-assisted desorption/ionization mass spectrometry. *Analytical chemistry* **2007,** *79* (16), 6094-101.

59.   Bell, K. L.; Dalgarno, A.; Kingston, A. E., Penning ionization by metastable helium atoms. *J. Phys. B* **1968,** *1*, 18-22.

60.   Cody, R. B.; Laramee, 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), 2297-302.

61.   Song, L.; Dykstra, A. B.; Yao, H.; Bartmess, J. E., Ionization mechanism of negative ion-direct analysis in real time: a comparative study with negative ion-atmospheric pressure photoionization. *J Am Soc Mass Spectrom* **2009,** *20* (1), 42-50.

62.   Maleknia, S. D.; Bell, T. L.; Adams, M. A., Eucalypt smoke and wildfires: Temperature dependent emissions of biogenic volatile organic compounds. *Int. J. Mass Spectrom.* **2009,** *279*, 126-33.

63.   Haefliger, O. P.; Jeckelmann, N., Direct mass spectrometric analysis of flavors and fragrances in real applications using DART. *Rapid Commun Mass Spectrom* **2007,** *21* (8), 1361-6.

64.   Yu, S.; Crawford, E.; Tice, J.; Musselman, B.; Wu, J. T., Bioanalysis without sample cleanup or chromatography: the evaluation and initial implementation of direct analysis in real time ionization mass spectrometry for the quantification of drugs in biological matrixes. *Analytical chemistry* **2009,** *81* (1), 193-202.

65.   Nilles, J. M.; Connell, T. R.; Durst, H. D., Quantitation of chemical warfare agents using the direct analysis in real time (DART) technique. *Analytical chemistry* **2009,** *81* (16), 6744-9.

66.   Jagerdeo, E.; Abdel-Rehim, M., Screening of cocaine and its metabolites in human urine samples by direct analysis in real-time source coupled to time-of-flight mass spectrometry after online preconcentration utilizing microextraction by packed sorbent. *J Am Soc Mass Spectrom* **2009,** *20* (5), 891-9.

67.   Yuan, M.; Kaneko, T.; Yokoyama, Y.; Tsuchiya, M., Liquid ionization mass spectrometry of some triorganotin carboxylates. *Anal. Sci.* **2001,** *17*, 1405–11.

68.   Hiraoka, K.; Fujimaki, S.; Kambara, S.; Furuya, H.; Okazaki, S., Atmospheric-pressure Penning ionization mass spectrometry. *Rapid Commun Mass Spectrom* **2004,** *18* (19), 2323-30.

69.   Zhao, J.; Zhu, J.; Lubman, D. M., Liquid sample injection using an atmospheric pressure direct current glow discharge ionization source. *Anal. Chem.* **1992,** *64*, 1426–33.

70.   Shelley, J. T.; Ray, S. J.; Hieftje, G. M., Laser ablation coupled to a flowing atmospheric pressure afterglow for ambient mass spectral imaging. *Analytical chemistry* **2008,** *80* (21), 8308-13.

71.   Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z., Paper spray for direct analysis of complex mixtures using mass spectrometry. *Angew Chem Int Ed Engl* **2010,** *49* (5), 877-80.

72.   Zhang, Z.; Xu, W.; Manicke, N. E.; Cooks, R. G.; Ouyang, Z., Silica coated paper substrate for paper-spray analysis of therapeutic drugs in dried blood spots. *Analytical chemistry* **2012,** *84* (2), 931-8.

73.   Ren, Y.; Wang, H.; Liu, J.; Zhang, Z.; McLuckey, M. N.; Ouyang, Z., Analysis of Biological Samples Using Paper Spray Mass Spectrometry: An Investigation of Impacts by the Substrates, Solvents and Elution Methods. *Chromatographia* **2013,** *76* (19-20), 1339-1346.

74.   Wang, H.; Ren, Y.; McLuckey, M. N.; Manicke, N. E.; Park, J.; Zheng, L.; Shi, R.; Cooks, R. G.; Ouyang, Z., Direct quantitative analysis of nicotine alkaloids from biofluid samples using paper spray mass spectrometry. *Analytical chemistry* **2013,** *85* (23), 11540-4.

75.   Narayanan, R.; Sarkar, D.; Cooks, R. G.; Pradeep, T., Molecular ionization from carbon nanotube paper. *Angew Chem Int Ed Engl* **2014,** *53* (23), 5936-40.

76.   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.

77.   Manicke, N. E.; Abu-Rabie, P.; Spooner, N.; Ouyang, Z.; Cooks, R. G., Quantitative analysis of therapeutic drugs in dried blood spot samples by paper spray mass spectrometry: an avenue to therapeutic drug monitoring. *J Am Soc Mass Spectrom* **2011,** *22* (9), 1501-7.

78.   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), 2344-9.

79.   Su, Y.; Wang, H.; Liu, J.; Wei, P.; Cooks, R. G.; Ouyang, Z., Quantitative paper spray mass spectrometry analysis of drugs of abuse. *Analyst* **2013,** *138* (16), 4443-7.

80.   Taverna, D.; Di Donna, L.; Mazzotti, F.; Policicchio, B.; Sindona, G., High-throughput determination of Sudan Azo-dyes within powdered chili pepper by paper spray mass spectrometry. *Journal of mass spectrometry : JMS* **2013,** *48* (5), 544-7.

81.   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), 2556-8.

82.   Li, A.; Wei, P.; Hsu, H. C.; Cooks, R. G., Direct analysis of 4-methylimidazole in foods using paper spray mass spectrometry. *Analyst* **2013,** *138* (16), 4624-30.

83.   Deng, J.; Yang, Y., Chemical fingerprint analysis for quality assessment and control of Bansha herbal tea using paper spray mass spectrometry. *Anal Chim Acta* **2013,** *785*, 82-90.

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

85.   Hamid, A. M.; Jarmusch, A. K.; Pirro, V.; Pincus, D. H.; Clay, B. G.; Gervasi, G.; Cooks, R. G., Rapid discrimination of bacteria by paper spray mass spectrometry. *Analytical chemistry* **2014,** *86* (15), 7500-7.

86.   Oradu, S. A.; Cooks, R. G., Multistep mass spectrometry methodology for direct characterization of polar lipids in green microalgae using paper spray ionization. *Analytical chemistry* **2012,** *84* (24), 10576-85.

87.   Liu, W.; Wang, N.; Lin, X.; Ma, Y.; Lin, J. M., Interfacing microsampling droplets and mass spectrometry by paper spray ionization for online chemical monitoring of cell culture. *Analytical chemistry* **2014,** *86* (14), 7128-34.

88.   Cody, R. B.; Dane, A. J., Paper spray ionization for ambient inorganic analysis. *Rapid Commun Mass Spectrom* **2014,** *28* (8), 893-8.

89.   Hu, B.; So, P. K.; Chen, H.; Yao, Z. P., Electrospray ionization using wooden tips. *Analytical chemistry* **2011,** *83* (21), 8201-7.

90.   Yang, Y.; Deng, J.; Yao, Z. P., Pharmaceutical analysis by solid-substrate electrospray ionization mass spectrometry with wooden tips. *J Am Soc Mass Spectrom* **2014,** *25* (1), 37-47.

91.   So, P.-K.; Ng, T.-T.; Wang, H.; Hu, B.; Yao, Z.-P., Rapid detection and quantitation of ketamine and norketamine in urine and oral fluid by wooden-tip electrospray ionization mass spectrometry. *Analyst* **2013,** *138* (8), 2239-2243.

92.   Hu, B.; So, P. K.; Yao, Z. P., Analytical properties of solid-substrate electrospray ionization mass spectrometry. *J Am Soc Mass Spectrom* **2013,** *24* (1), 57-65.

93.   Zhang, Z.-P.; Liu, X.-N.; Zheng, Y.-J., Ambient Ionization-Paper Spray Ionization and Its Application. *Chinese Journal of Analytical Chemistry* **2014,** *42* (1), 145-152.

94.   Chan, S. L.; Wong, M. Y.; Tang, H. W.; Che, C. M.; Ng, K. M., Tissue-spray ionization mass spectrometry for raw herb analysis. *Rapid Commun Mass Spectrom* **2011,** *25* (19), 2837-43.

95.   Liu, J.; Wang, H.; Cooks, R. G.; Ouyang, Z., Leaf spray: direct chemical analysis of plant material and living plants by mass spectrometry. *Analytical chemistry* **2011,** *83* (20), 7608-13.

96.   Malaj, N.; Ouyang, Z.; Sindona, G.; Cooks, R. G., Analysis of pesticide residues by leaf spray mass spectrometry. *Anal Methods-Uk* **2012,** *4* (7), 1913-1919.

97.   Hu, B.; Lai, Y. H.; So, P. K.; Chen, H.; Yao, Z. P., Direct ionization of biological tissue for mass spectrometric analysis. *Analyst* **2012,** *137* (16), 3613-9.

98.   Liu, J.; Cooks, R. G.; Ouyang, Z., Biological tissue diagnostics using needle biopsy and spray ionization mass spectrometry. *Analytical chemistry* **2011,** *83* (24), 9221-5.

99.   Hu, B.; Wang, L.; Ye, W.-C.; Yao, Z.-P., In Vivo and Real-time Monitoring of Secondary Metabolites of Living Organisms by Mass Spectrometry. *Scientific Reports* **2013,** *3*, 2104.

100.   Hu, B.; Wang, L.; Ye, W. C.; Yao, Z. P., In vivo and real-time monitoring of secondary metabolites of living organisms by mass spectrometry. *Sci Rep* **2013,** *3*, 2104.

101.   Wei, Y.; Chen, L.; Zhou, W.; Chingin, K.; Ouyang, Y.; Zhu, T.; Wen, H.; Ding, J.; Xu, J.; Chen, H., Tissue spray ionization mass spectrometry for rapid recognition of human lung squamous cell carcinoma. *Sci Rep* **2015,** *5*, 10077.

102.   Hong, C.-M.; Lee, C.-T.; Lee, Y.-M.; Kuo, C.-P.; Yuan, C.-H.; Shiea, J., Generating electrospray from solutions predeposited on a copper wire. *Rapid Communications in Mass Spectrometry* **1999,** *13* (1), 21-25.

103.   Kuo, C.-P.; Yuan, C.-H.; Shiea, J., Generation of electrospray from a solution predeposited on optical fibers coiled with a platinum wire. *Journal of the American Society for Mass Spectrometry* **2000,** *11* (5), 464-467.

104.   Kuo, C. P.; Shiea, J., Application of direct electrospray probe to analyze biological compounds and to couple to solid-phase microextraction to detect trace surfactants in aqueous solution. *Analytical chemistry* **1999,** *71* (19), 4413-7.

105.   Jeng, J.; Shiea, J., Electrospray ionization from a droplet deposited on a surface-modified glass rod. *Rapid Commun Mass Spectrom* **2003,** *17* (15), 1709-13.

106.   Jeng, J.; Lin, C. H.; Shiea, J., Electrospray from nanostructured tungsten oxide surfaces with ultralow sample volume. *Analytical chemistry* **2005,** *77* (24), 8170-3.

107.   Mandal, M. K.; Chen, L. C.; Hashimoto, Y.; Yu, Z.; Hiraoka, K., Detection of biomolecules from solutions with high concentration of salts using probe electrospray and nano-electrospray ionization mass spectrometry. *Anal Methods-Uk* **2010,** *2* (12), 1905-1912.

CHAPTER 2. STRUCTURED CONDUCTIVE PROBES FOR AMBIENT IONIZATION IN MASS SPECTROMETRY

2.1 Abstract

Ambient ionization mass spectrometry (MS) techniques are attractive due to their simplicity and ability to rapidly analyze samples with minimal or no sample preparation. However, ambient ionization methods can suffer from low accuracy and sensitivity due to matrix effects and interferences within complex samples, as well as from poor ionization efficiency. One way to overcome these challenges is to implement a solution for enhanced method sensitivity and introduce an extraction step for analysis of complex samples. In this work we present a new approach where a probe can be used both as a direct sampling device and as an efficient ambient ionization source. This approach leverages high surface area gold electrodes, fabricated through low-cost bench-top fabrication methods and functionalized using self-assembled alkyl thiol monolayers, as functional structured conductive probes (FCSPs) for the extraction and concentration of analytes from a sample solution. FCSP loaded with the targeted analytes were then used to demonstrate a new and highly efficient ionization approach, called Primary Ion Mass Spectrometry Source (PIMSS). Following capture on the probe, the bound analytes were directly desorbed into the mass spectrometer, where ionization was achieved solely through the extraction voltage applied to the probe. 3D-printing was used to design an interface to couple FCSPs to the mass spectrometer. Experiments with three model compounds demonstrate that the assay can be used for the analysis of cationic, anionic and uncharged molecules. Our results showed that this technique has high reproducibility and low detection limits (pg levels). We anticipate that this sensitive and cost-efficient approach could find wide application in bioanalytical, environmental, forensic and other biomedical analyses.

2.2 Introduction

Mass spectrometry is a powerful and sensitive technique applied for identification and quantification of a wide range of analytes, but it requires extensive technical knowledge and is often associated with time consuming preparative steps. Introduction of ionization under ambient conditions has greatly simplified mass spectrometric analysis. Over past decade, ambient ionization mass spectrometry (MS) methods[1] have revolutionized the way complex samples are analyzed under environmental conditions without requiring, in most cases, any sample pretreatment.[2] Ambient ionization MS gained popularity among other analytical techniques due to its simplicity and its suitability for analysis of small and large molecules. An additional advantage is that ambient ionization sources can be combined with miniaturized MS instrumentation to develop portable systems that are appropriate for field use.[3] The introduction of desorption electrospray ionization (DESI)[4] and direct analysis in real time (DART)[5] in the mid-2000s spurred the development of other new ambient ionization methods. Nowadays ambient ionization methods are represented by a set of varied techniques based on different desorption and ionization mechanisms.[6-10] These methods are now widely used in environmental, forensic, biomedical, clinical and other applications.[10-11] Of particular interest in these areas are methods where the targeted analytes can be desorbed and ionized from a variety of substrates (*e.g.,* glass, PMMA, PTFE, stainless steel, gold, paper)[12-14] by the application of solvent and high voltages (HV) because they facilitate the implementation of simple field-deployable methods.

The probes used to introduce samples into the MS are key components of ambient ionization methods because they influence the capture, ionization, and coupling efficiency, which ultimately determine the sensitivity of these MS methods. In the most common approach, Solid Phase Micro-Extraction (SPME) probes,[15] structured materials with micron to nanometer size

pores that can be used to extract organic analytes from solution, are used as thermal desorption sources for MS. Some studies have used SPME fibers as desorption substrates for MS with an independent ionization source, e.g. low-temperature plasma ionization (LTPI)[16] or dielectric barrier discharge ionization (DBDI),[17] which increases the ion yield, but makes the process more complicated. Recently, the coated blade spray (CBS) method, proposed by Pawliszyn in 2014, incorporated the use of a stainless-steel substrate covered with a thin SPME coating to serve as both a desorption and ionization source.[18] While the porous material used in SPME presents a large surface that the analyte can be adsorbed to, it also presents a serious disadvantage, since the penetration of the analytes into the porous structure leads to a reduction of the material that can be desorbed from the probe or results in long analysis times. Because of these limitations, new methods and materials are needed to increase the surface area for material adsorption while retaining an efficient and timely recovery during the desorption step.

This work presents the Functional Conductive Sampling Probe – Primary Ion Mass Spectrometry Source (FCSP-PIMSS) as a new concept for ambient ionization mass spectrometry. In this approach, a high surface area structured electrode, fabricated through bench top methods on a polystyrene (PS) substrate,[19] is functionalized with an alkyl thiol self-assembled monolayer (SAM),[20] resulting in a functional conductive sampling probe or FCSP. The nature of the SAM was chosen to enable the partitioning and concentration of targeted molecules (e.g., a 1-octadecanethiol or C_{18} SAM enables the recovery of non-polar molecules) onto the dipstick FCSP from a sample solution, such as water, urine or blood. Following capture, the bound analytes can be desorbed, ionized and coupled into the mass spectrometer through the application of an extraction voltage to the probe, turning the FCSP into a primary ion source. The FCSP-PIMSS concept was demonstrated in this work through the desorption of model analytes, analysis of

daughter ions in Multiple-Reaction-Monitoring mode, and measurement of the total ion intensity recorded by the MS detector. The experimental conditions of probe positioning with respect to the MS inlet and electrospray capillary, and capillary and probe voltages were optimized to yield the highest signal from the desorbed analytes. Desorption experiments showed limits of detection at the picogram level for model analytes methacholine chloride, caffeine and perfluorooctanesulfonic acid (PFOS) absorbed onto the probe. Due to the unique characteristics of the approach, the FCSP-PIMSS concept presents several advantages over traditional ambient ionization methods, such as low cost and tunable functionalization of the sampling probe, more efficient extraction of adsorbed analytes from the probe, and efficient ionization and ion transfer into the MS through the direct application of a desorption/ionization voltage on the probe. We anticipate that this new approach could significantly reduce the complexity of MS use by replacing the sample pretreatment and separation steps by a rapid solid phase extraction onto the sampling probe, and turning the sampling probe into the ionization source. This in turn could improve the sample-to-diagnosis turnaround time by enabling the direct analysis of samples applied onto the sampling probe.

2.3 Experimental section

Reagents. 1-octadecanethiol (OT, 98%), acetyl-β-methylcholine chloride (ABMC, ≥98%), perfluorooctanesulfonic acid potassium salt (PFOS, ≥98%), and caffeine (99%), standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfuric acid (H_2SO_4, 98%) was obtained from Caledon (Georgetown, ON, Canada), Ethanol (95% and 100%) from Comalc (Brampton, ON, Canada). All reagents were of analytical grade and were used without further purification. Ultra-pure water (18.2 MΩ·cm, TOC < 10 ppm, from a Reference A+ MilliQ water purification

system, Millipore, Etobicoke, ON, Canada) was used to prepare ABMC, PFOS and caffeine solutions.

Probe Fabrication: All devices were fabricated on pre-stressed polystyrene (PS) shrink films (Graphix Shrink Film, Graphix, Maple Heights, OH, USA). Shrink film sheets were cleaned in isopropanol, ethanol, and ultra-pure water under orbital agitation (60 rpm) for 5 min, and were dried using a dry nitrogen stream. Self-adhesive vinyl films (FDC-4300, FDC graphic films, South Bend, IN, USA) and PS sheets were cut out using a Robo Pro CE5000-40-CRP blade cutter (Graphtec America Inc., Irvine, CA, USA). All designs were prepared using CAD software. Self-adhesive vinyl masks were transferred to PS cut-outs using transfer tape. Gold (99.999% purity, LTS Chemical Inc., Chestnut Ridge, NY, USA) films with 50 nm thickness were deposited using a manual planar magnetron sputtering system (Torr Compact Research Coater CRC-600, New Windsor, NY, USA) at deposition rate of 1.2 Å/s. After sputtering the vinyl masks were removed and shrinking was performed in an Isotemp vacuum oven (Fisher Scientific, Ottawa, ON, Canada) at 160°C for 5 minutes.

Scanning Electron Microscopy (SEM) Characterization. SEM images of the gold films before and after shrinking were obtained using a JSM-7000S scanning electron microscope (JEOL USA, Peabody, MA, USA) with an accelerating voltage of 3 kV, working distance of 6 mm, and 3 mA probe current.

General Procedure for Probe Functionalization. Probes were cleaned in Novascan Ultraviolet PSD-UV Ozone Cleaner for 15 min and rinsed with a small amount of 95% ethanol after and dried using a nitrogen stream. Clean probes were masked with self-adhesive vinyl films, exposing only the sample pad region, and were incubated with 1 mM OT solution (40 mL) in anhydrous ethanol in polystyrene dishes (100×15 mm); a maximum of 5 probes were prepared per

dish. The dishes were kept in a glove bag purged with nitrogen for 2 hours, after which the devices were copiously rinsed with anhydrous ethanol. The vinyl mask was then removed and the probes were stored in anhydrous ethanol until further used.

Electrochemical Characterization. Cyclic voltammetry (CV) was performed using a CHI 660D electrochemical workstation (CH Instrument, Austin, TX, USA) and a standard three-electrode set-up consisting of an Ag/AgCl reference electrode, a platinum wire counter electrode, and the gold probe on polystyrene as the working electrode. For assessing the electroactive surface area, CV was performed in a 50 mM H_2SO_4 solution. The potential was swept from 0 to 1.5 V at a scan rate of 0.1 V s^{-1} for 10 CV scans.

Contact Angle Characterization. The wettability of the functionalized gold surfaces was assessed through static contact angle measured by the sessile drop method (OCA 20 Future Digital Scientific system, Garden City, NY, USA). Briefly, 1 µl of ultra-pure water was dropped onto the thiol functionalized gold surface and digital images were acquired.

3D-printing. A fused-deposition modelling 3D printer was used to prototype an interface (referred to as sample holder) for coupling the fabricated probes to a commercially available mass spectrometer (Waters Micromass Quattro Ultima, Mississauga, ON, Canada). The holder design was created using Fusion 360 software (Autodesk, San Rafael, CA, USA). The sample holders were printed on a MakerBot Replicator 2X 3D Printer (Brooklyn, NY, USA), using the supplied ABS filament as the printing material. 3D-printed sample holders were modified with a metallic clip so the probes could be connected to an external 20 kV high voltage power supply (Spellman, Valhalla, NY, USA).

Mass Spectrometry (MS). Samples were analyzed using **a triple quadrupole** Waters Micromass Quattro Ultima instrument equipped with an electrospray ionization (ESI) source.

50:50 water methanol solution was used as a solvent at a 30 µL/min flow rate and the cone voltage was set to 50 V. Waters MassLynx software was used for data processing. Other MS parameters used in data acquisition are listed in Table S1. The sample holder angle, capillary and extraction voltages were identified using a set of optimization experiments. Optimal MS parameters and calibration curves of ABMC, caffeine and PFOS were obtained in multiple reaction monitoring (MRM) mode. Major MRM transitions are shown in Table 2.1. OT-functionalized probes were placed on a sample holder coupled to the MS instrument and covered by a glass lid. A circular opening was cut out the lid in order to perform the probe switching. Prior to the analysis, the opening was sealed with Parafilm M film (Sigma-Aldrich) and the atmosphere within the glass lid was purged with nitrogen for two minutes. This procedure was repeated every time a new probe was installed in order to eliminate possible ambient contaminants and interferences. After data acquisition had been started, the solvent flow and voltage applied to the probe were turned on simultaneously. The voltage applied to the ESI source capillary was turned on as soon as the data acquisition started (programmed by the software used). The acquired signal intensities were averaged for the first 15 seconds of desorption, and the signal was corrected by background subtraction.

Table 2.1. Mass spectrometer MRM parameters.

Compound	ESI mode	MRM transition	Cone voltage (V)	Collision energy (eV)
ABMC	+	$160 \rightarrow 101$	50	15
Caffeine	+	$195 \rightarrow 138$	50	20
PFOS	-	$499 \rightarrow 99$	50	30

2.4 Results and discussions

2.4.1 SCP Fabrication and characterization

The structured gold films used as the basis for the development of the functional conductive sampling probes (FCSPs) were fabricated using a simple and low-cost method previously developed in our lab.[19] Briefly, self-adhesive vinyl film stencils were fabricated using a blade cutter, adhered onto clean pre-stressed PS sheets, and a 50-nm gold coating was sputtered onto them. Lift-off of the adhesive stencil revealed patterned gold electrodes that contained a contact area and a circular sample pad, connected by a thin narrow connecting region (Figure 2.1A). The gold film thickness was selected to ensure that the resulting electrodes were continuous and highly conductive, while minimizing the fabrication costs (~$0.20/probe based on academic cost of materials and fabrication). The PS substrates containing the patterned electrodes were then heated at 160°C, causing the PS substrates to biaxially shrink to 16% of their original size (Figure 2.1B, Video S1). The compressive stress introduced by the shrinking substrate resulted in wrinkled gold films (Figure 2.1C), which have been shown to exhibit ~6-fold larger electroactive areas than flat electrodes with a comparable footprint.[21] The large surface area presented by the structured electrodes is attractive because it can enhance the amount of analyte that can be adsorbed/desorbed from the probes when used as sampling devices.
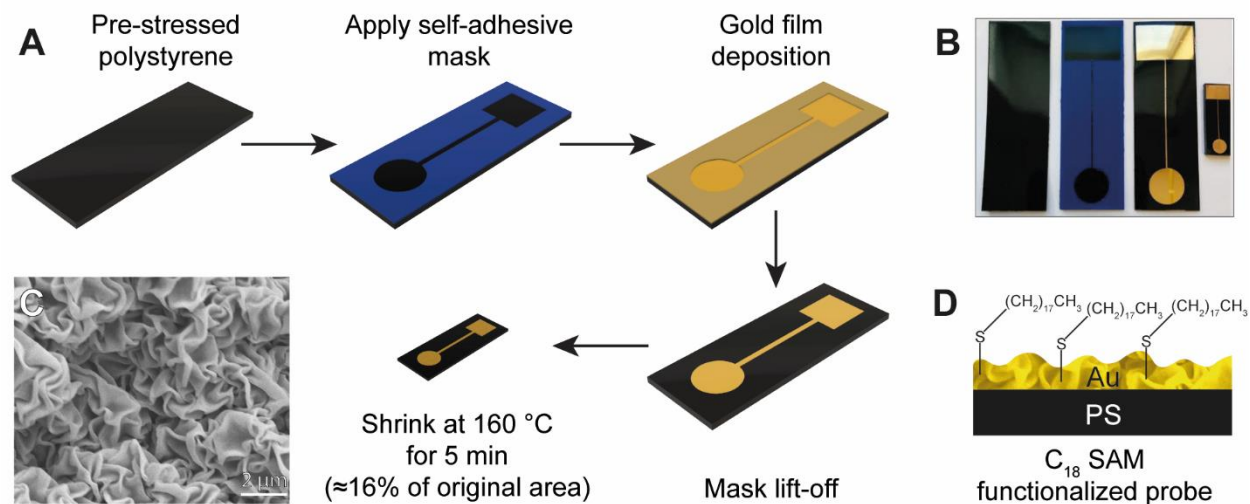
Figure 2.1. Functional Conductive Sampling Probe fabrication. A) Patterning using craft cutter, gold deposition and thermal shrinking of pre-stressed PS. B) SEM image of 50 nm shrunken Au film. C) Results of the individual steps in the fabrication of the structured conductive probes. Dimensions of pre-stressed PS are 60×20 mm, shrunken probe – 24×8 mm. D) Schematic of the structured conductive probe functionalized with 1-octadecanethiol (OT) SAM.

The interfacial properties of the structured probes were tuned by modification through thiol self-assembled monolayers (SAMs). Thiol chemistry is attractive because it has been widely used in the development of microanalytical devices and because a wide range of compounds with different chemical functionalities that can be covalently linked to gold surfaces are commercially available. Structured and flat probes were functionalized by applying an adhesive mask that protected the connecting section and exposed only the sample pad to the thiol solution (Figure S1). The functionalization was first tested through the formation of SAMs from propanethiol (PT), benzenethiol (BT), or decanethiol (DT) solutions, as described in the Supplemental Information. The properties of the modified surfaces were then evaluated through water contact angle (WCA) measurements, to determine surface wettability. Flat electrodes functionalized with PT, BT and DT exhibited maximum WCA values of 88°, 101°, and 104°, respectively, at 24 hours of incubation

in the thiol solution (Figure S4). On the other hand, structured electrodes functionalized with PT, BT and DT exhibited maximum WCA values of 134º, 133º, and 134º, respectively, after 24 hours of incubation in the thiol solution (Figure S5). The stark difference in contact angles can be attributed to the wrinkles on the gold surface, which make the surface more hydrophobic, as previously reported,[19] regardless nature or the surface coverage of the SAM.

To directly determine the gold surface area that could undergo oxidation/reduction cycles and thus calculate the surface coverage of the SAMs, CV was performed in a dilute acid solution using the functionalized probes. Structured electrodes functionalized with PT, BT, and DT exhibited electroactive gold surface areas of 98%, 60%, and 1%, respectively, at 24 hours of incubation in the thiol solution (Figure S2). This suggested that the PT, BT and DT SAMs covered approximately 2%, 40%, and 99% of the electrode surfaces, respectively. The electro active surface area of PT and BT could be significantly reduced to 44% and 1%, respectively, by incubating the functionalized electrodes with a 1-octadecanethiol (OT) solution to backfill the available gold surface with OT molecules (Figure S3). These results indicate that the SAMs formed by PT and BT are not tightly packed and therefore allow the oxidation of the gold surface. Conversely, DT and OT SAMs pack densely and cover the electrode surface uniformly. In the subsequent experiments performed to demonstrate the FCSP-PIMSS concept, the fabricated probes were modified with OT SAMs (Figure 1D), to form a uniform non-polar surface that could mimic the C_{18} stationary phases routinely used in solid phase extractions and reversed phase liquid chromatography.

## 2.4.2 Mass spectrometry interface design

The interface, or probe holder, for the ambient ionization MS implementation of the PIMSS concept was developed through computer aided design and implemented using fused deposition

modeling 3D printing. The guiding principles for the design were that the probe holder should allow: i) the adjustment of the distance between the probe, the electrospray capillary and the MS inlet, ii) control over the angle at which the sample pad on the probe lies with respect to the MS components, iii) electrical connection of the FCSPs to a high voltage supply, iv) rapid switching between probes, and v) reproducible positioning of the probe in the MS. The use of 3D printing allowed us to rapidly prototype through multiple iterations (Figure 3.6) of the interface to couple the SCPs to a commercially available Waters Micromass Quattro Ultima Mass Spectrometer (Figure 2B-C).

The evolution of the probe holders responded to the need to accurately position the probes in the MS inlet space with minimal effort. Thus, the initial holders (Figure 3.6-i) were mounted on translation stages that allowed the movement of the probe to a determined position; however, they proved to be too cumbersome and difficult to rapidly implement on a shared MS instrument. This led to a series of static probe holders (Figure 3.6-ii-iv) that positioned the probe accurately and were referenced internally to the MS instrument, but fixed the relative location of the probe to the ESI capillary and MS inlet. These holders were designed with probe angles of 0-90º, as defined by running the probe in either the *Surface Reflection* or *Surface Skimming* desorption modes detailed below. As detailed below, the position of the probe with respect to the spectrometer elements was critical to ensure that solvent assisted with the desorption (Figure 2D), and to maximize the capture of the ionized species into the MS inlet cone. The final holder design also incorporated a clip-on feature that allowed the rapid exchange of probes, connecting the probe to an external HV source that provided the extraction voltage, and a guiding slot to ensure the reproducible positioning of the probe. For the Quattro Ultima MS instrument used in this study, the static probe holders were affixed to the site normally occupied by the ESI shield. While the holders presented in this study

44

were designed for a Waters MS instrument, the interface could be customized to suit instruments from different vendors. The designed probe holders present "plug-and-play" interfaces with a slot for FCSPs that are easily accessible and allow user to switch between probes within several seconds as it is demonstrated in Video S2. Having FCSPs "plugged-into" the interface, it only takes the press of a button to turn on the HV and solvent to perform the analysis.
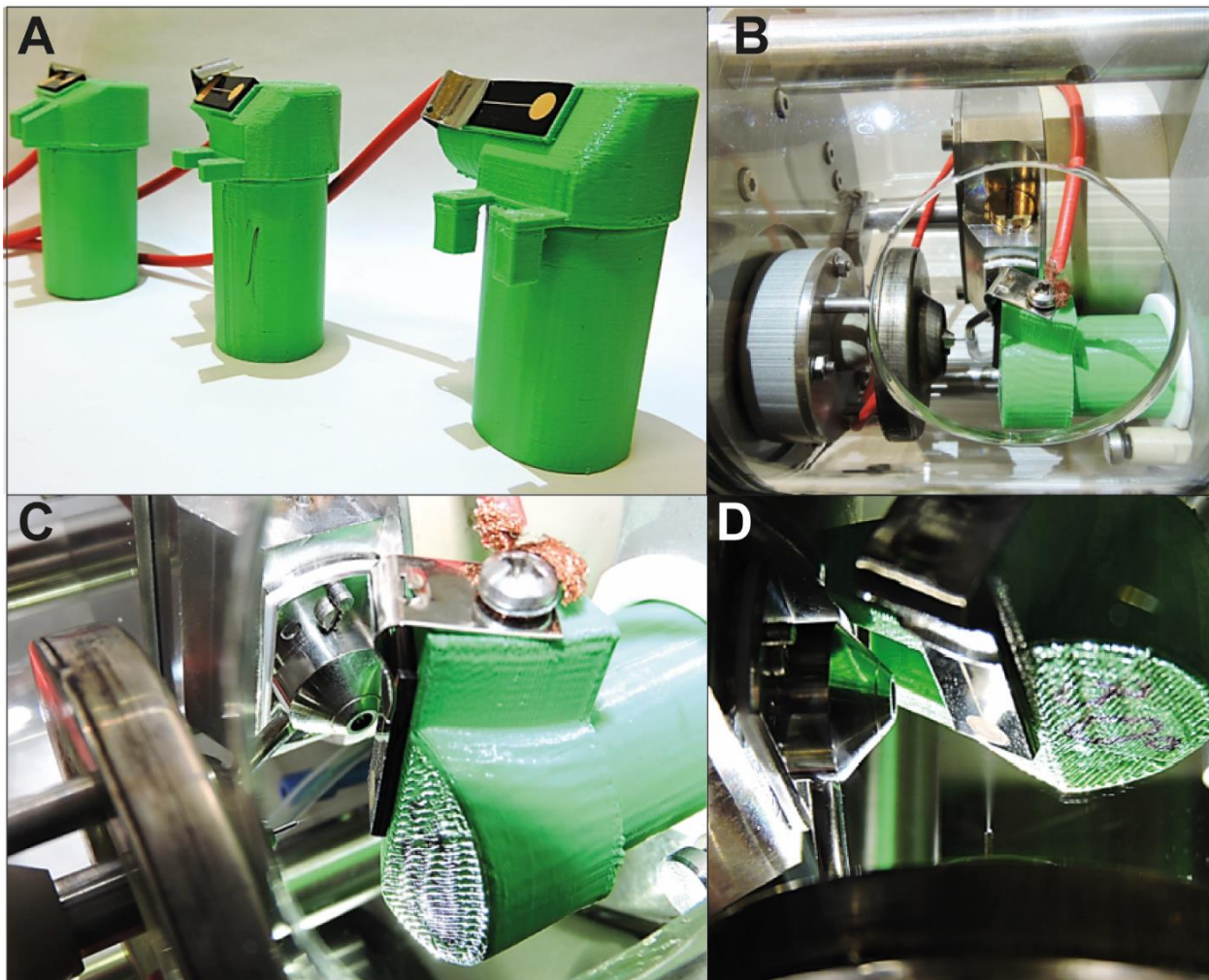

Figure 2.2. The probe was coupled to the MS instrument using 3D-printed holders. (A) The holders were printed with three different probe angles (30°, 45°,60°) and connected to an HV source. (B) Probe holder with a mounted probe coupled to a Waters Micromass Quattro Ultima MS. (C) A close-up image of the probe coupling in *Surface Skimming* (*S*) position. (D) Running the sample with solvent applied.

2.4.3 Method optimization

Ambient ionization methods that combine analyte desorption and ionization (*e.g.*, DESI) require much optimization to ensure the acquisition of the maximum signal possible with the minimum amount of sample. There is a wide range of parameters that can have a large impact on the desorption and ionization efficiency, as well as the final signal intensity. To optimize the operation of the probes in the PIMSS concept, three main parameters were studied: i) the position and angle of the probe with respect to the ESI capillary and the analyzer inlet cone, ii) the voltage applied to the ESI capillary and the extraction voltage applied to the probe, and iii) the use of solvent for assisted desorption. These parameters were used in the desorption of ABMC from OT-functionalized probes, and the optimization was aimed at obtaining the largest total ion signal possible over a predefined time window of 15 s.

At first, the effect of probe positioning was investigated. For this study, solvent spray was used to assist in desorption with no voltage applied to the capillary, and the voltage applied to the probe was set constant at 4.5 kV (referred to as extraction voltage). Two different positions of the probe relative to the MS inlet were studied: a) *Surface Reflection* (*R*, Figure 3A), where the probe sensing pad is positioned directly in front of the inlet, and the solvent stream (incident at an angle α from the probe normal) is assumed to desorb the analyte in a direction corresponding to a reflection of the stream, and b) *Surface Skimming* (*S*, Figure 3B), where the solvent (incident at angle α) skims the surface of the sensing pad, which is positioned in line with the inlet cone and subtends an angle $\beta = 90 - \alpha$ with its normal. Initially, sample holders for four different angles ($\alpha = 0, 30, 45, 60°$ angles) were 3D-printed and tested in *R* configuration for the desorption of ABMC deposited onto the probes. The probe sample pads were functionalized with OT-SAMs and 0.2 or 0.02 µg of ABMC were loaded onto the probe from stock solutions. The evaporation of the solvent

on these electrodes was carried out at room temperature for 10 minutes prior to desorption in *R* mode under the parameters specified in the experimental section. The results obtained (Figure S5) showed that adequate desorption resulted for sample holders with $\alpha = 30°$, $45°$, and $60°$, while little to no signal was obtained for $0°$ in *R* configuration. Therefore, angles $30°$, $45°$, and $60°$ were used for further optimization experiments on probes with similar surface functionalization and sample deposition protocol. It must be noted that the construction of the Waters ESI source did not allow application of angles higher than $\alpha = 60°$ in *R* configuration and lower that $\alpha = 30°$ in *S* configuration due to the limited distance between MS inlet and ESI capillary.
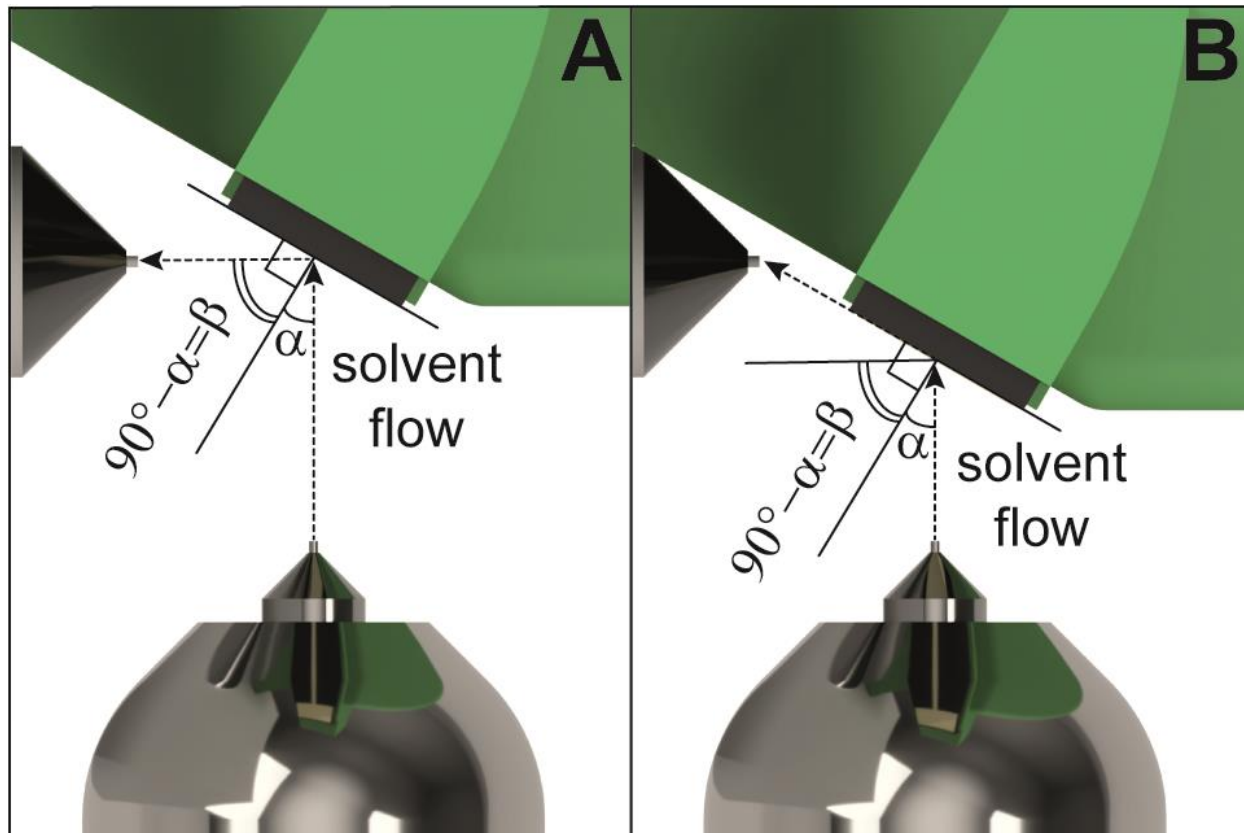
Figure 2.3. Schematic of probe positioning and angle optimization. (A) *Surface Reflection* (*R*) position, (B) *Surface Skimming* (*S*) position. Sample holders with $\alpha = 30°$ for both configurations were chosen to demonstrate the probe positioning. Direction of a solvent flow and proposed direction of the desorbed analyte are illustrated using dashed-line arrows.

Once a range of suitable probe angles with respect to the solvent stream from the ESI capillary had been defined, the placement with respect to the MS inlet was assessed. These desorption experiments were run at a constant extraction voltage of 4.5 kV, using OT-functionalized probes loaded with 2 µg of ABMC, and at solvent stream incident angles of α = 30°, 45°, and 60°. In the *Surface Reflection* desorption mode (*R,* Figure 2.3A), the initial hypothesis was that the solvent stream would help eject ionized analytes from the probe surface and that these would be efficiently captured by the MS inlet being placed at a reflection angle in front of the probe. From the desorption experiments, it was observed that the total ion count (TIC) signal in the *R* configuration was maximal (~1×10^3 count level) at angles of α = 45 or 60° (Figure 2.4). However, the observed signals were low, which suggested that the molecules desorbed from the probe surface were not being efficiently routed to and captured by the MS inlet. This led us to propose the *Surface Skimming* desorption mode (*S*, Figure 2.3B), where it was anticipated that the hydrodynamic solvent flow would help guide the molecules desorbed form the probe surface into the MS inlet. In the ABMC desorption experiments, the TIC signals generated in the *S* configuration were 2-3 orders of magnitude larger than those for the *R* configuration, indicating that this placement of the probe led to the optimal capture of desorbed ions. The large difference in signal observed between the two configurations can be explained by the hydrodynamic flow of ions induced by the solvent stream from the ESI capillary in the *Surface Skimming* configuration, which significantly enhances the desorption and transfer from the probe surface into the MS inlet. In contrast, the incident spray droplet collisions on the probe surface that happen in the *Surface Reflection* configuration (akin to the droplet-pickup mechanism[22] that takes place in DESI), was not very efficient in desorbing the molecules and transferring them to the MS inlet. Furthermore, it was observed that the TIC signal in the *S* configuration was maximal (~1×10^6 count level) at an

angle of $\alpha = 30°$ (Figure 2.4). Thus, the *S* desorption configuration with a probe angle of $\alpha = 30°$ was thus used in all subsequent experiments.
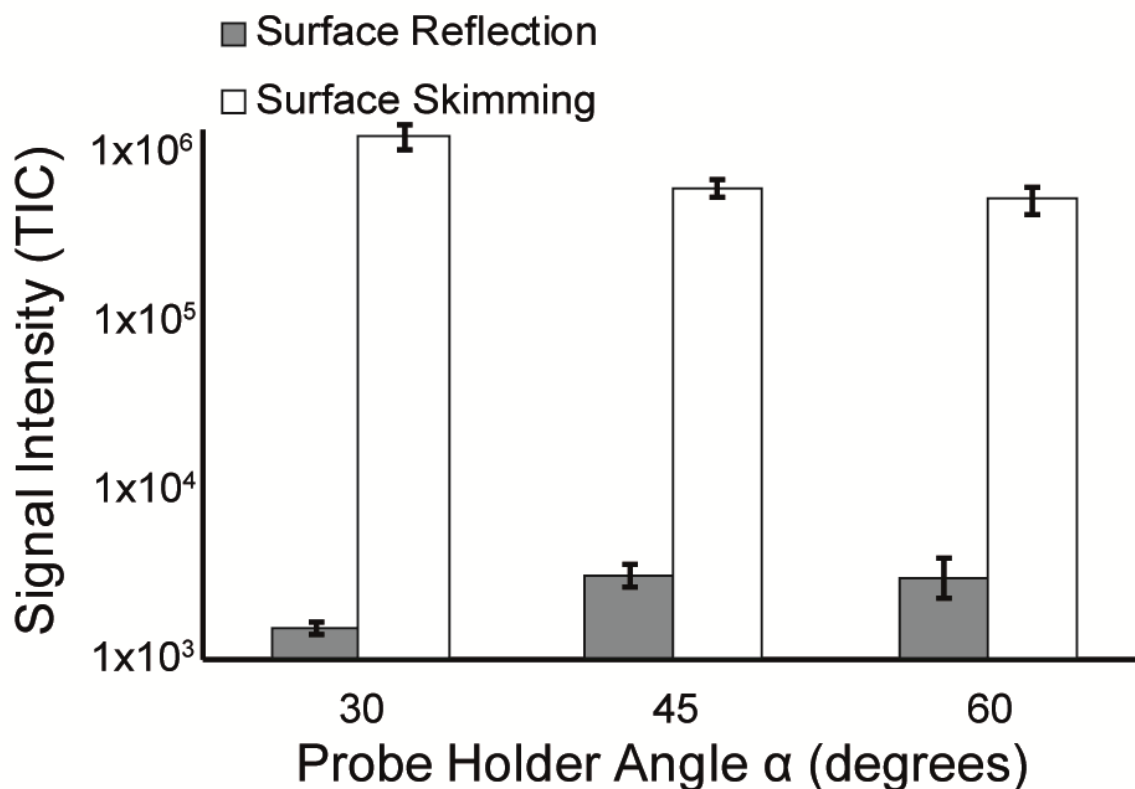

Figure 2.4. Comparison of probe positioning configurations at a constant extraction voltage of 4.5 kV. Desorption from FCSPs loaded with 2 μg of ABMC and mounted onto probe holders in *Surface Reflection* (*R*) and *Surface Skimming* (*S*) configurations with angles $\alpha = 30°, 45°, 60°$. Error bars in the graph represent the standard error of the mean, with n = 5 probe replicates.

Next, the effect of the voltages applied to the ESI capillary and the probe on the efficiency of desorption, ionization and collection of analytes was studied. Experiments were performed under 3 different conditions: i) voltage applied to the ESI source capillary only (capillary voltage, V_C); ii) voltage applied to the FCSP only (extraction voltage, V_E); iii) capillary and extraction voltages applied simultaneously with identical strengths. Note that the baseline experiment with

no voltages applied is the same for all three conditions, and resulted in negligible signal (Figure 2.5), indicating that in the absence of voltage, a minimal number of ions are produced and detected when there is solvent sprayed onto the probe. Additionally, to demonstrate the ability to run the analysis using the PIMSS concept in both positive or negative ion detection mode, the experiments were run for three different analytes that are in cationic (ABMC), anionic (PFOS), or uncharged (caffeine) form while in aqueous solution. All FCSPs used in the experiments were functionalized with OT SAMs and loaded with 2 μg of the analyte of interest. It is also worth noting that the experiments where only V_C is applied are similar to the conventional mode of operation of DESI-MS, where the solvent is ionized by the application of a voltage to the ESI capillary and the solvent impinging upon the probe holder is then responsible for desorbing and ionizing the analyte prior to its introduction to the MS analyzer. On the other hand, the case where only V_E is applied corresponds to the novel FCSP-PIMSS concept proposed here, where the functionalized structured conductive probe acts simultaneously as the sample collecting device and ionization source. Finally, the simultaneous application of V_C and V_E corresponds to a hybrid mode, where ionization can occur at the solvent source and at the probe holder.

These experiments showed that the desorption, ionization and collection efficiency resulted in higher TIC signals for experiments run under the FCSP-PIMSS conditions, regardless of the charge state of the analyte. Figure 5 shows that for all analytes, the signal collected was lowest in the DESI-like mode, where only the capillary voltage was applied. Remarkably, the application of the extraction voltage alone (FCSP-PIMSS mode), resulted in the highest signals for all conditions tested, indicating a significant improvement over the DESI-like mode when both experiments were run in the same geometrical configuration. On the other hand, the simultaneous application of capillary and extraction voltages resulted in signals that were larger than the DESI-like mode, but

lower than or equal to those obtained in FCSP-PIMSS mode. This suggests that the inclusion of a capillary voltage of equal magnitude to the extraction voltage is counterproductive in this configuration. Furthermore, it was observed that in the FCSP-PIMSS desorption mode, the optimal extraction voltages for ABMC, caffeine and PFOS were 4.5, 4.5, and -3.5 kV, respectively, and resulted in TIC signals in the 10^5-10^6 range for the three analytes. It should be noted that while higher voltages could potentially lead to higher signals, the experimental setup limits the highest voltage possible due to arcing between the ESI capillary and the FCSP. Overall, these experiments showcase the ability of the FCSP-PIMSS mode to analyze anionic, cationic and neutral analytes adsorbed onto the probes with excellent sensitivity and under ambient ionization conditions.
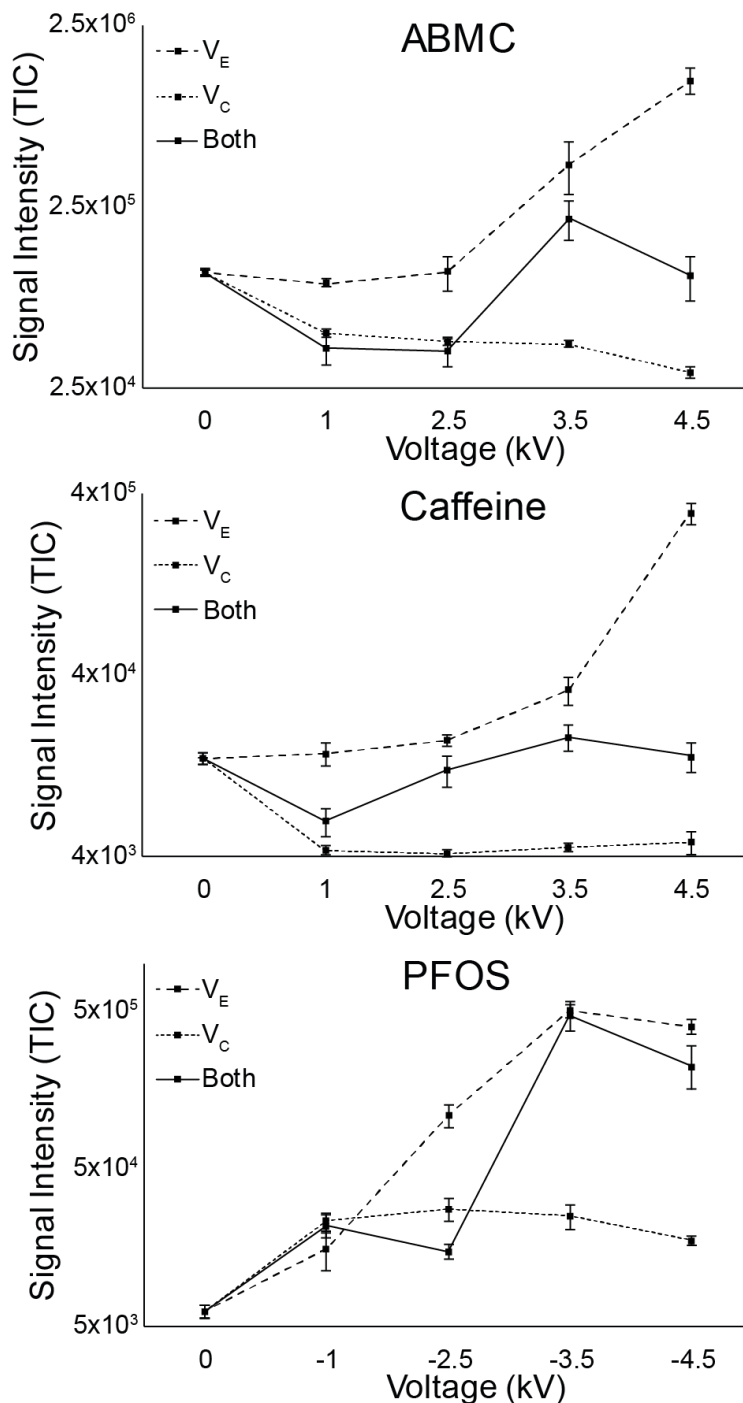
Figure 2.5. Effect of the voltages applied on the desorption, ionization, and collection efficiency from FCSPs. The voltage study was performed using probes positioned in the *S* configuration with $\alpha = 30°$ and loaded with 2 μg of the analyte of interest (ABMC, caffeine, or PFOS). V_C corresponds to the capillary voltage (applied to the ESI source) and V_E corresponds to extraction voltage (applied to the probe), while both corresponds to the application of the same potential for V_C and V_E. Error bars represent the standard error of the mean, with n = 5.

2.4.4 FCSP-PIMSS of Cationic, Neutral and Anionic Analytes

After identifying the optimal experimental parameters to generate the maximum intensity from the probes using the PIMSS concept, calibration curves were generated for the three targeted analytes. The probes were functionalized with OT SAMs and loaded with 200, 20, 2, 0.2, 0.02 and 0.002 ng of each of the analytes as previously described. The analytes were then desorbed from the sample pads using the *S* configuration and applying the optimal V_E for each analyte (4.5 kV for ABMC and caffeine, and -3.5 kV for PFOS). The calibration curves (Figure 2.6) show high reproducibility for analyte desorption at each of the tested concentrations (n = 5 probes for each concentration), as shown by the relatively small error associated with the measurements. These measurements also display high linearity over several orders of magnitude of analyte concentration, demonstrating a wide linear dynamic range of over 4 orders of magnitude. From these experiments, the lower limits of detection calculated for the analytes using desorption and ionization with the PIMSS concept were in the picogram range, with LODs of 20 pg for ABMC and 200 pg for caffeine and PFOS. Reviews on the analysis of low MW substances by DESI[23] and DART[24] techniques report linear ranges comparable to the values demonstrated here and LODs ranging from microgram to picogram levels. Signal intensity acquired for desorption of 2 μg of caffeine ~10-fold lower than signal intensity for the same amount of methacholine chloride. This was attributed to methacholine chloride being a cationic analyte that does not require to be ionized, while caffeine is a neutral molecule that has to be ionized prior to the detection, with the lower signals implying that only some portion of the caffeine is ionized.
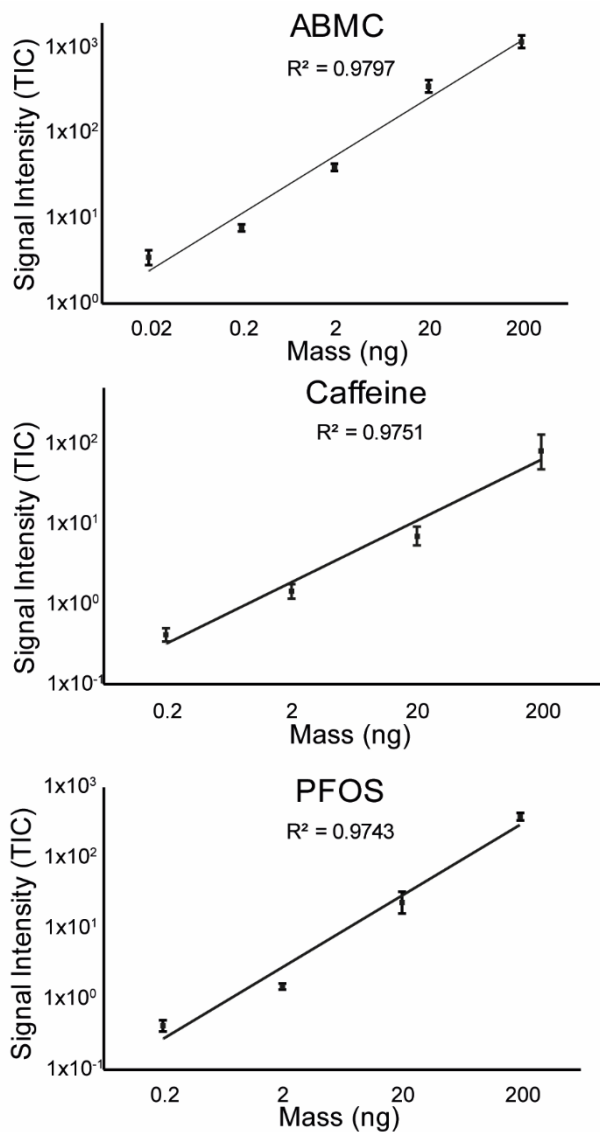
Figure 2.6. Calibration curves for OT-functionalized probes loaded with different amounts of ABMC, caffeine or PFOS. The desorption experiments were carried out in the S configuration $\alpha = 30°$, and $V_E = 4.5$ kV for ABMC and caffeine, and -3.5 kV for PFOS. Error bars represent the standard error of the mean, with n=5.

2.4.5 Application of FCSPs to Extraction of Analytes

Finally, the ability to use the FCSPs as microextraction devices that could them be coupled to the MS through the PIMSS concept was tested with caffeine as the target analyte. FCSPs containing OT-modified sampling pads were submerged in 0.5 mL of aqueous solutions containing caffeine at concentrations of 10, 1 and 0.1 µg/mL, and incubated under constant agitation for periods of up to 12 hours. Bare gold structured probes (no functionalization) and bare PS substrates were used as controls for the non-specific adsorption of the analyte to the different components of the probe. After extraction, the FCSPs were dried under ambient conditions and the analyte was desorbed using the optimized PIMSS conditions (S configuration, $\alpha = 30°$, $V_E = 4.5$ kV), and TIC signals recorded for 15 s. The total mass extracted by the probe was calculated using the calibration curve in Figure 2.6, and corresponded to 2.1 ± 0.2, 2.2 ± 0.2, and 1.1 ± 0.2 ng for the 10, 1 and 0.1 µg/mL solutions respectively. This shows that the maximum load for the functionalized probes (with the surface area characteristic for our design) is on the order of 2 ng, and that they become quickly saturated in solutions with concentrations > 100 ng/mL, but can effectively extract and concentrate materials form more dilute solutions. While further optimization is required, these preliminary results open the possibility of using the functionalized probes as microextraction and concentration devices that can then be directly coupled to the MS through the PIMSS concept.

2.5 Conclusion

A new all-in-one approach, FCSP-PIMSS, which can be used for sample extraction and as ion source for MS was developed and optimized. The approach presented leverages structured electrodes, functionalized with an alkyl thiol SAM as probes for the deposition or capture of an analyte that can then be desorbed and coupled into the MS inlet using an extraction voltage applied

directly to the probe. A new interface for coupling versatile SCP to the MS instrument was designed and 3D printed to allow us to reproducibly position the probe with respect to the solvent stream and the MS inlet, and maximize the total ion count signal. The extraction voltage applied to the probe was then optimized for cationic, anionic and neutral analytes. The FCSP-PÏMSS method has a wide linear dynamic range and allows the detection of analytes down to the picogram levels. The method due to the simplicity of the fabrication is low cost (~\$0.20/probe) and versatile. Future steps in the development of this technique include tuning the surface chemistry of the probe to increase the efficiency of extraction and gain selectivity towards targeted analytes. We anticipate that this method could find use in point-of-need sampling in environmental or biomedical applications, where the FCSPs can be used as dip-stick probes to gather and concentrate targeted species that can then be directly desorbed and analyzed through MS.

2.6 Supporting Information

**2.6.1. Probe functionalization**
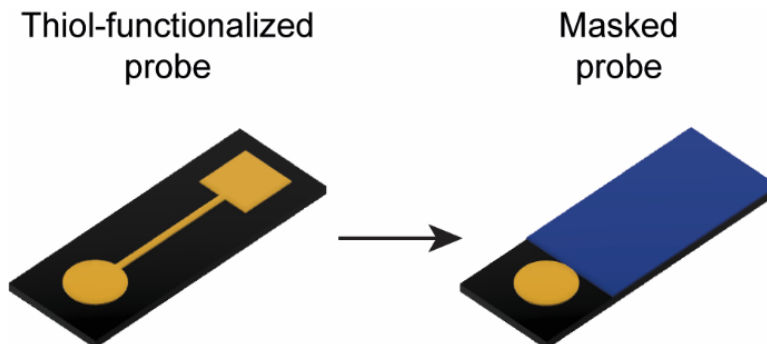
Figure S1. Functionalization process of structured and flat probes using an adhesive mask that protected the connecting section and exposed only the sample pad to the thiol solution.

2.6.2. Electrochemical characterization of flat and shrunken PS probes modified with thiol SAMs

Flat electrodes fabricated on pre-stressed PS substrates were incubated in solutions of PT, DT, or BT for intervals of time between 0 and 24 hours in an inert nitrogen environment. Following incubation, the surface coverage of the monolayers was evaluated using CV (Figure S2a). As devices are made with gold films, any available gold surface will undergo oxidization and reduction (REDOX). To determine the amount of gold undergoing REDOX, the reduction peaks in the cyclic voltammograms were integrated using the CHI600E Electrochemical Analyzer and the electrochemically active surface area of the modified devices was calculated. These electroactive surface area measurements were normalized to the electroactive surface area of unmodified devices. In order to ensure that the detected surface was uncovered and available to other SAMs, devices were incubated in one of the aforementioned thiols for a given length of time, and were then incubated in a solution of OT for 2 hours. This process is termed backfilling and

consists of initial incubation in one thiol, followed by a secondary incubation in another thiol of interest. Surface coverage of these devices was again analyzed using CV (Figure S2b).
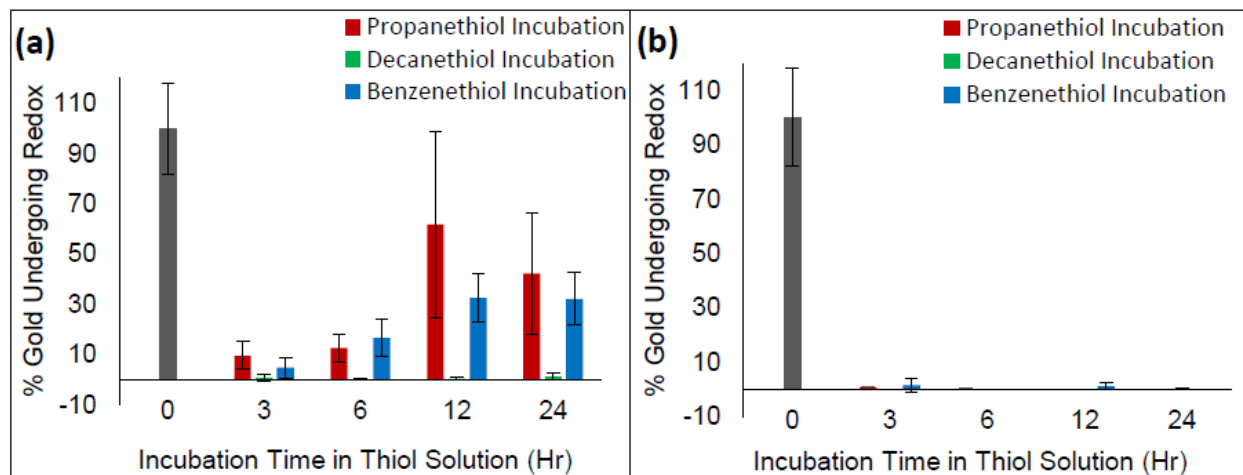
Figure S2. Percent gold undergoing REDOX on modified unshrunken devices. A) Devices incubated in 1 mM solutions of PT, DT, or BT prior to CV. B) Devices incubated in 1 mM solutions of PT, DT, or BT followed by a 2-hour backfilling with OT. All CV scans were performed in a 50 mM sulfuric acid solution. The potential was swept positively from 0 to 1.5 V at a scan rate of 0.1 Vs-1 for 5 CV scans. The grey bar represents the negative control (bare gold, no exposure to thiol).
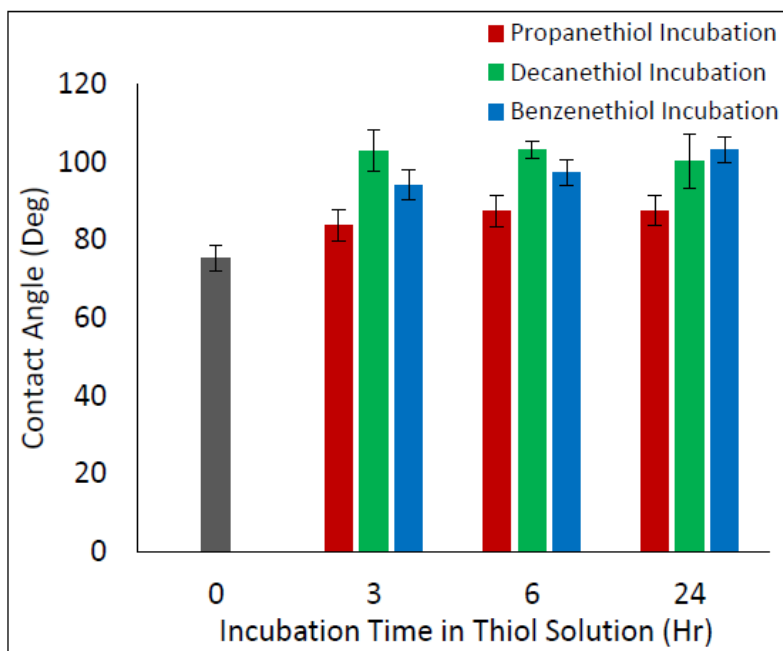
Shrunken devices were incubated in 1 mM solutions of PT, DT, or BT for time intervals between 0 and 24 hours, with CV performed following the incubations (Figure S3a). To adjust the surface coverage and alter the polarity of the monolayers, devices were backfilled with a 2-hour incubation in the hydrophobic OT (Figure S3b) prior to electrochemical analysis.
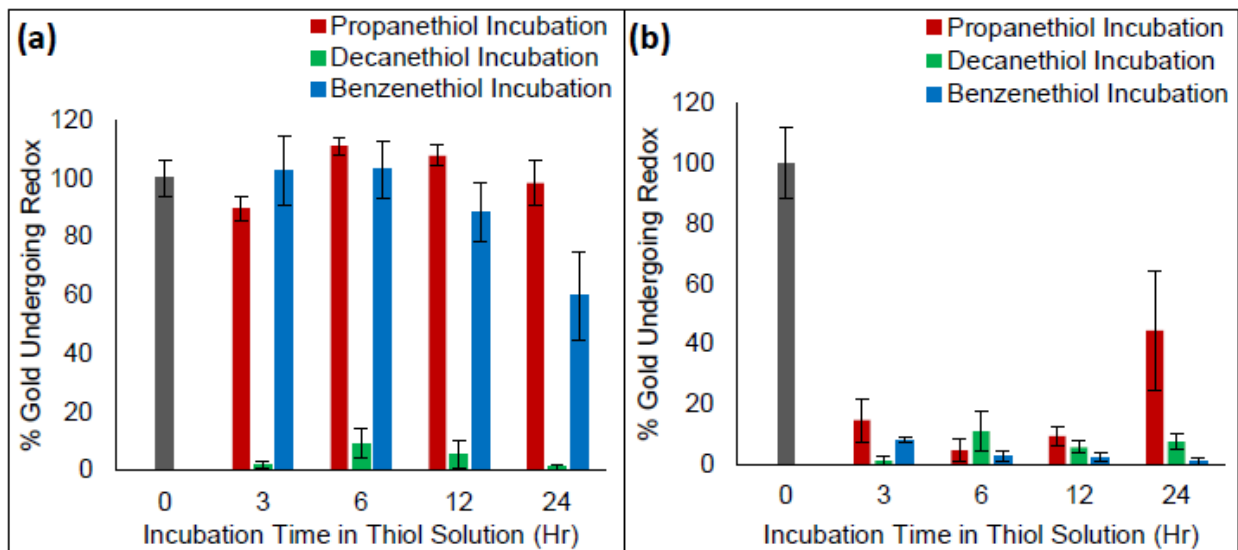
Figure S3. Percent gold undergoing REDOX on modified shrunken devices. A) Devices incubated in 1 mM solutions of PT, DT, or BT prior to CV. B) Devices incubated in 1 mM solutions of PT, DT, or BT followed by a 2 hour backfilling with OT. CV was performed in a 0.05 M sulfuric acid solution. The potential was swept positively from 0 to 1.5 V at a scan rate of 0.1 Vs-1 for 5 CV scans. The grey bar represents the negative control (bare gold, no exposure to thiol).

2.6.3. Wettability characterization of flat and shrunken PS probes modified with thiol SAMs.

Further characterization consisted of determining the wettability of flat gold electrodes incubated in 1 mM solutions of PT, DT, or BT for a varying length of time. This method involves determining the equilibrium contact angle of the interaction between a water droplet and the solid surface it is applied onto. Contact angle measurements were made with a high-speed contact angle measurer. Wettability measurements were used to confirm the quality of monolayer formation by characterizing the surface polarity of devices incubated in the thiol solutions (Figure S4). Successful surface modification of flat electrodes allowed us to begin the modification of shrunken pre-stressed PS devices using a similar modification protocol.
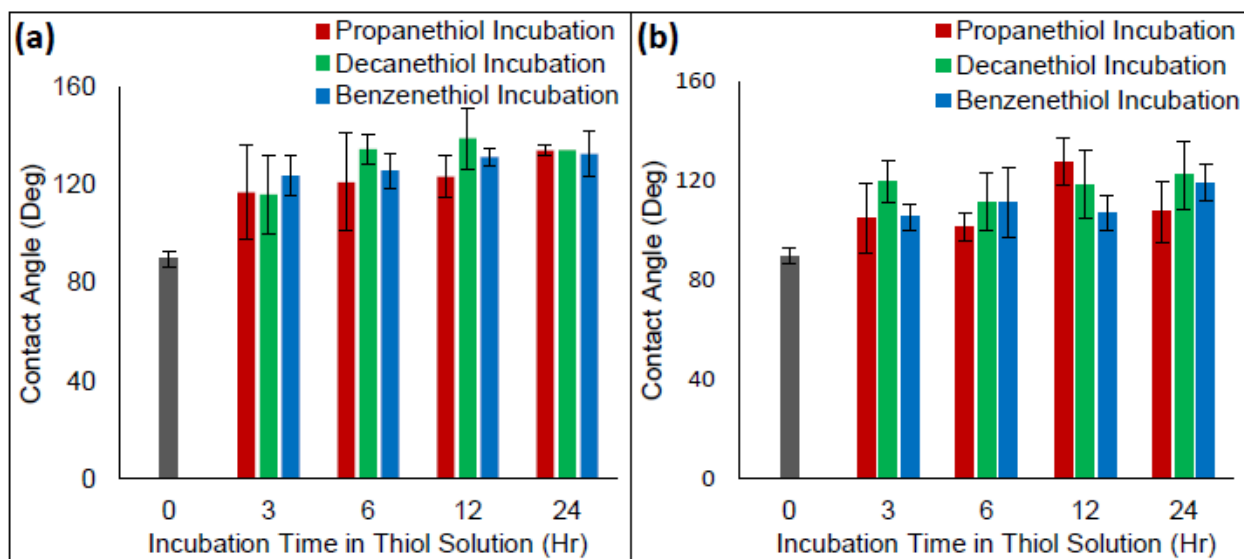
Figure S4. Contact angle measurements of modified flat devices. Flat gold films were incubated in 1 mM solutions of PT, DT, or BT for 0-24 hours prior to analysis of surface polarity. Contact angle measurements were made in triplicate; 1 μl droplets of MilliQ grade water onto the sample pad region of the fabricated probes. The grey bar represents the negative control (bare gold, no exposure to thiol).

2.6.4. Characterization of shrunken PS devices modified with thiol SAMs.

Additional surface characterization included measuring the wettability of the SAMs on the shrunken PS devices. Following initial functionalization in 1 mM solutions of PT, DT, or BT, contact angle measurements were taken and compared to negative controls; shrunken devices with bare gold films (Figure S5a). To determine the effect of mixed monolayers on surface wettability, contact angle measurements were taken for devices backfilled with OT (Figure S5b). The efficiency of the shrunken devices as sampling probes for MS was tested following the characterization of the various thiol SAM formation.

Figure S5. Contact angle measurements of modified shrunken devices. A) Devices incubated in 1 mM solutions of PT, DT, or BT. B) Devices incubated in 1 mM solutions of PT, DT, or BT followed by a 2 hour backfilling with OT. Contact angle measurements were made in duplicate; 1 µl droplets of MilliQ grade water onto the sample region of the fabricated electrodes.

2.6.5. Mass spectrometry parameters

Capillary voltage, extraction voltage and probe holder angle were tested during the optimization experiments. Other parameters were constant throughout the optimization experiments.

Table S1. MS parameters used for desorption experiments

MS Parameter	Value
Capillary Voltage (kV)	0; 1.0; 2; 3.5, 4.5
Extraction Voltage (kV)	0; 1.0; 2; 3.5, 4.5
Probe holder angle (º)	30, 45, 60
Solvent (water/methanol ,v/v)	50/50
Solvent Flow Rate (μL/min)	30
Capillary (kV)	0.00
Cone (V)	50
Source Temperature (˚C)	80
Desolvation Temperature (˚C)	200
Cone Gas Flow (L/Hr)	OFF
Desolvation Gas Flow (L/Hr)	OFF
Nebuliser Gas Flow	ON

2.6.6. Preliminary probe angle selection study
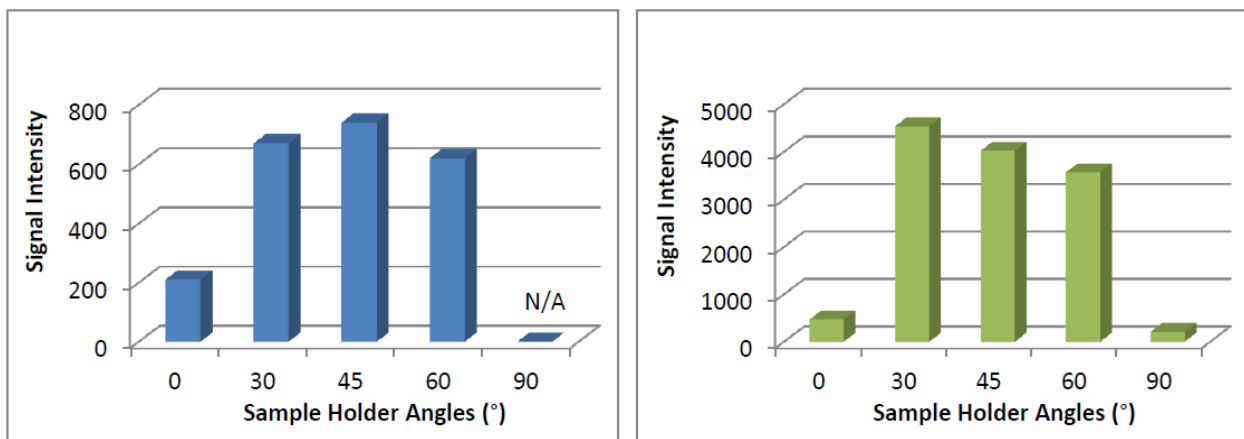
Figure S6. Desorption of 10 ng and 100 ng of ABMC in *Surface Reflection* configuration.

The optimal MS parameters were found in Daughter Ion Spectrum (DIS) mode. Calibration curves were obtained in mode Multiple Reaction Monitoring (MRM) mode. In comparison to the DIS mode, MRM mode is much more sensitive. As both MS detectors are static, this allows greater "dwell time" on the ions of interest and therefore better sensitivity ($\sim100\times$) compared to MS/MS scanning.

2.7. References

1.   Van Berkel, G. J.; Pasilis, S. P.; Ovchinnikova, O., Established and emerging atmospheric pressure surface sampling/ionization techniques for mass spectrometry. *Journal of mass spectrometry : JMS* **2008,** *43* (9), 1161-80.

2.   Chen, H.; Ouyang, Z.; Cooks, R. G., Thermal production and reactions of organic ions at atmospheric pressure. *Angew Chem Int Ed Engl* **2006,** *45* (22), 3656-60.

3.   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), 2909-16.

4.   Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G., Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **2004,** *306* (5695), 471-3.

5.   Cody, R. B.; Laramee, 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), 2297-302.

6.   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), 9097-104.

7.   Nemes, P.; Vertes, A., Laser ablation electrospray ionization for atmospheric pressure, in vivo, and imaging mass spectrometry. *Analytical chemistry* **2007,** *79* (21), 8098-106.

8.   Ratcliffe, L. V.; Rutten, F. J.; Barrett, D. A.; Whitmore, T.; Seymour, D.; Greenwood, C.; Aranda-Gonzalvo, Y.; Robinson, S.; McCoustra, M., Surface analysis under ambient conditions using plasma-assisted desorption/ionization mass spectrometry. *Analytical chemistry* **2007,** *79* (16), 6094-101.

9.   Shiea, J.; Huang, M. Z.; Hsu, H. J.; Lee, C. Y.; Yuan, C. H.; Beech, I.; Sunner, J., Electrospray-assisted laser desorption/ionization mass spectrometry for direct ambient analysis of solids. *Rapid Commun Mass Spectrom* **2005,** *19* (24), 3701-4.

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

11.   Harris, G. A.; Galhena, A. S.; Fernandez, F. M., Ambient sampling/ionization mass spectrometry: applications and current trends. *Analytical chemistry* **2011,** *83* (12), 4508-38.

12.   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. *Journal of mass spectrometry : JMS* **2005,** *40* (10), 1261-75.

13.   Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z., Paper spray for direct analysis of complex mixtures using mass spectrometry. *Angew Chem Int Ed Engl* **2010,** *49* (5), 877-80.

14.   Manicke, N. E.; Wiseman, J. M.; Ifa, D. R.; Cooks, R. G., Desorption electrospray ionization (DESI) mass spectrometry and tandem mass spectrometry (MS/MS) of phospholipids and sphingolipids: ionization, adduct formation, and fragmentation. *J Am Soc Mass Spectrom* **2008,** *19* (4), 531-43.

15.   Arthur, C. L.; Pawliszyn, J., Solid phase microextraction with thermal desorption using fused silica optical fibers. *Analytical chemistry* **1990,** *62* (19), 2145–2148.

16.   Dumlao, M. C.; Jeffress, L. E.; Gooding, J. J.; Donald, W. A., Solid-phase microextraction low temperature plasma mass spectrometry for the direct and rapid analysis of chemical warfare simulants in complex mixtures. *Analyst* **2016,** *141* (12), 3714-21.

17.   Mirabelli, M. F.; Wolf, J. C.; Zenobi, R., Direct Coupling of Solid-Phase Microextraction with Mass Spectrometry: Sub-pg/g Sensitivity Achieved Using a Dielectric Barrier Discharge Ionization Source. *Analytical chemistry* **2016,** *88* (14), 7252-8.

18.   Gomez-Rios, G. A.; Pawliszyn, J., Development of coated blade spray ionization mass spectrometry for the quantitation of target analytes present in complex matrices. *Angew Chem Int Ed Engl* **2014,** *53* (52), 14503-7.

19.   Gabardo, C. M.; Zhu, Y.; Soleymani, L.; Moran-Mirabal, J. M., Bench-top fabrication of hierarchically structured high-surface-area electrodes. *Adv. Fund. Mater.* **2013,** *23*, 3030-3039.

20.   Ulman, A., Formation and Structure of Self-Assembled Monolayers. *Chem Rev* **1996,** *96* (4), 1533-1554.

21.   Saem, S.; Zhu, Y.; Luu, H.; Moran-Mirabal, J., Bench-Top Fabrication of an All-PDMS Microfluidic Electrochemical Cell Sensor Integrating Micro/Nanostructured Electrodes. *Sensors (Basel)* **2017,** *17* (4).

22.   Venter, A.; Sojka, P. E.; Cooks, R. G., Droplet Dynamics and Ionization Mechanisms in Desorption Electrospray Ionization Mass Spectrometry. *Analytical chemistry* **2006,** *78* (24), 8549-8555.

23.   Morelato, M.; Beavis, A.; Kirkbride, P.; Roux, C., Forensic applications of desorption electrospray ionisation mass spectrometry (DESI-MS). *Forensic Science International* **2013,** *226* (1), 10-21.

24.   Chernetsova, E. S.; Morlock, G. E.; Revelsky, I. A., DART mass spectrometry and its applications in chemical analysis. *Russian Chemical Reviews* **2011,** *80* (3), 235-255.

CHAPTER 3. METHODOLOGY

Since ambient ionization mass spectrometry heavily relies on the conditions of the experiment, each parameter of the experiment should be thoroughly tested and optimized in order to obtain adequate data. Typical optimization for ambient ionization methods like DESI MS include electrospray and geometrical parameters. Mass spectrometry parameters have to optimized accordingly to the analyte's nature (expected m/z value, MS detection mode, optimal collision voltage for tandem MS detection). Electrospray parameters are capillary and cone voltages, source and desolvation temperatures, cone gas, desolvation gas, and nebulizer gas flows. Geometrical parameters include incidence angle, distances and angles between sprayer tip and probe surface, and between MS inlet and probe surface and other related measurements (including probe positioning and probe dimensions). Incidence angle (angle between the solvent source and the sample or solvent delivery angle) has a special place among other parameters since the correct selection of this angle is crucial for efficient ionization and ion delivery to the mass spectrometer. All these parameters define whether analyte will be successfully desorbed from the surface, picked up by the solvent, transferred into the mass spectrometer and detected. In order to find optimal parameters for the proposed SCSP-PIMSS (Structured Conductive Sampling Probe - Primary Ion Mass Spectrometry Source) ionization concept, each parameter has been verified and tested by performing multiple preliminary experiments and by more complex statistical methods often applied in chemometrics, *e.g.,* design of experiment. Principles of the concept were described in Chapter 2. Optimization was performed in a systematic manner starting with a proof-of-concept study, followed by the verification of the spray and geometrical parameters.

3.1 Proof of concept study

In the beginning of the study it was necessary to test whether it is possible to perform desorption from the proposed structured conductive probes or not. Probes, fabricated and functionalized as described in chapter 2, were coupled to the mass spectrometer by a clamp holder and an alligator clip connected to the connection pad. Methacholine (acetyl-β-methylcholine) chloride was chosen as an analyte for initial studies because it is a positively charged analyte. It allowed to estimate maximum desorption and ion transfer efficiency from the surface of the sample pad to the MS inlet because the compound did not have to be ionized. Methacholine chloride is also a metabolically stable synthetic analog of acetylcholine that is used to assess bronchial reactivity in the laboratory[1] and clinically.[2]
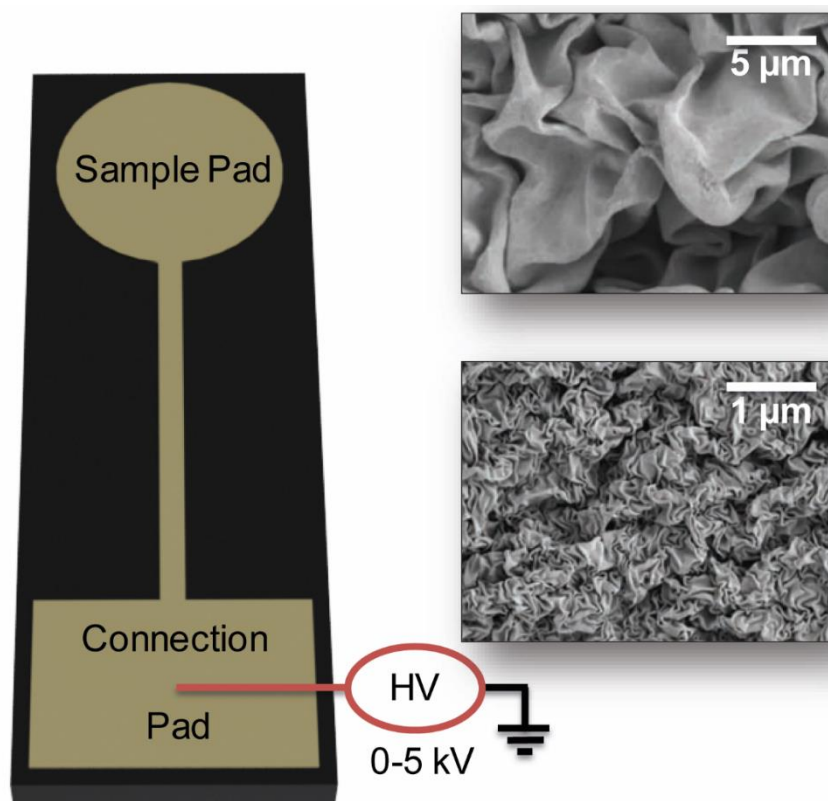

Figure 3.1. Structured Conductive Sampling Probe design. The connection pad is used for HV application; sample pad region – for analyte deposition. SEM micrographs of 50 nm structured thin gold film demonstrate wrinkled features.

In the first set of experiments the goal was to find optimal conditions for desorption experiments. First, we performed optimization of the MS parameters. ESI-MS method with general settings for Micromass Quattro Ultima Mass Spectrometer was provided by the lab manager. The parameters included capillary voltage, source temperature, desolvation temperature and solvent flow rate. Cone gas and desolvation gas flows were switched off because it was found that the probe surface was drying too fast and it did not provide enough time for the analyte to dissolve. Nebuliser gas flow was left on for constant spraying. According to the droplet-pickup mechanism that takes place in DESI (discussed in Chapter 1), a solvent film that contains analyte molecules is initially formed. After, incident droplet collisions desorb the surface liquid and carry the sample molecules into the instrument.[3-6] It was proposed that the FCSP-PIMSS method might benefit from similar phenomena. The solvent flow rate was adjusted accordingly in order to evenly cover the sample pad surface with a thin liquid film throughout the measurement and was set to 30 µL/min. A methanol/water (1:1, v/v) was selected as a desorption solvent as it is the most common composition used for conventional ESI-MS. Desolvation and source temperature were set to the values normally used for ESI-MS measurements for this particular instrument. Change of these temperatures did not show any significant difference during trial measurements. Cone voltage was also not changed from the original ESI-MS method. Collision voltage for MS/MS (Multiple Reaction Monitoring mode) was found for each analyte using direct injections of the analyte of interest. Measurements were done in Daughter Ion Spectrum mode. At the optimal collision voltage ratio of a parent ion and major daughter ion should be 1:1. Finally, the voltage applied to the probe or extraction voltage was set to a constant value of 4.5 kV for probe positioning studies.

The samples were analyzed using a Waters Quattro Ultima ESI-MS instrument (Figure 3.2). All experiments were performed in MS/MS mode. Ion mode was set to ES+ (positively

charged electrospray mode). The optimal collision voltage was found in Daughter Ion Spectrum mode, with the parent m/z of 160 and daughter m/z of 101. Calibration curves were obtained in MRM (Multiple Reaction Monitoring) mode. In comparison to the Daughter Ion Spectrum mode MRM mode is much more sensitive. This mode is equal to MS/MS mode of SIR (Selected Ion Recording). As both MS detectors are static, this allows greater "dwell time" on the ions of interest and therefore better sensitivity ($\sim$100$\times$) compared to MS/MS scanning. MRM mode is a helpful tool for analysis of "dirty" samples, including environmental samples, drugs, forensic and toxicology samples. Mass spectrometry parameters used in the optimization experiments can be found in Table 3.1.
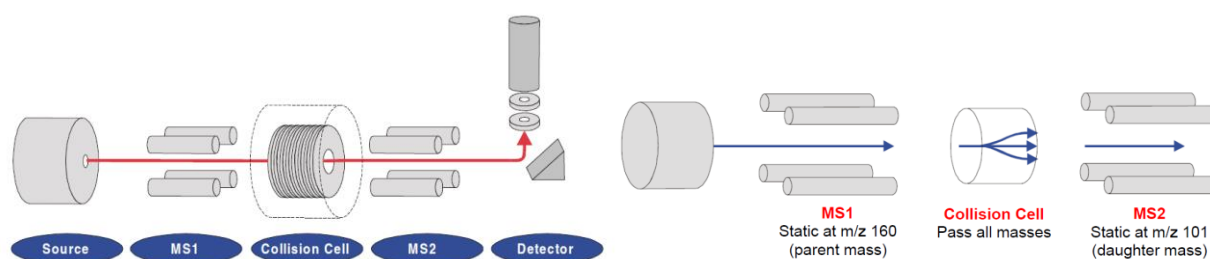
Figure 3.2. MS/MS setup (Adapted from "Operator's guide, Micromass Quattro Ultima Pt Mass Spectrometer." Waters Corporation, 2003).

Two microliters of the sample solution in MilliQ water were deposited onto the sample pad regions of functionalized electrodes (probe dimensions specified in the Figure S3.1) that had been pre-wetted with 0.5 µL of ethanol. This was needed in order to distribute the sample solution evenly on the sample pad surface, which due to the structuring was hydrophobic (water contact angle of $\sim$110º). Solutions of 4 different concentrations were tested (1000, 100, 10 and 1 µg/mL). Probes with the deposited analyte were dried at room temperature for 30 minutes. The probe was positioned in *Surface Reflection* position (*R*) as described in Chapter 2 (Figure 2.3A). The center

of the sample pad was placed directly opposite of the mass spectrometer inlet. This positioning

was used in optimization studies for other experiments as well.

Table 3.1. MS parameters used for initial optimization experiments with solvent assistance.

MS Parameter	Settings
Capillary (kV)	0.00
Cone (V)	50
Source Temperature (˚C)	80
Desolvation Temperature	200
Cone Gas Flow (L/Hr)	OFF
Desolvation Gas Flow (L/Hr)	OFF
Nebuliser Gas Flow	ON
Collision Voltage (V)	15
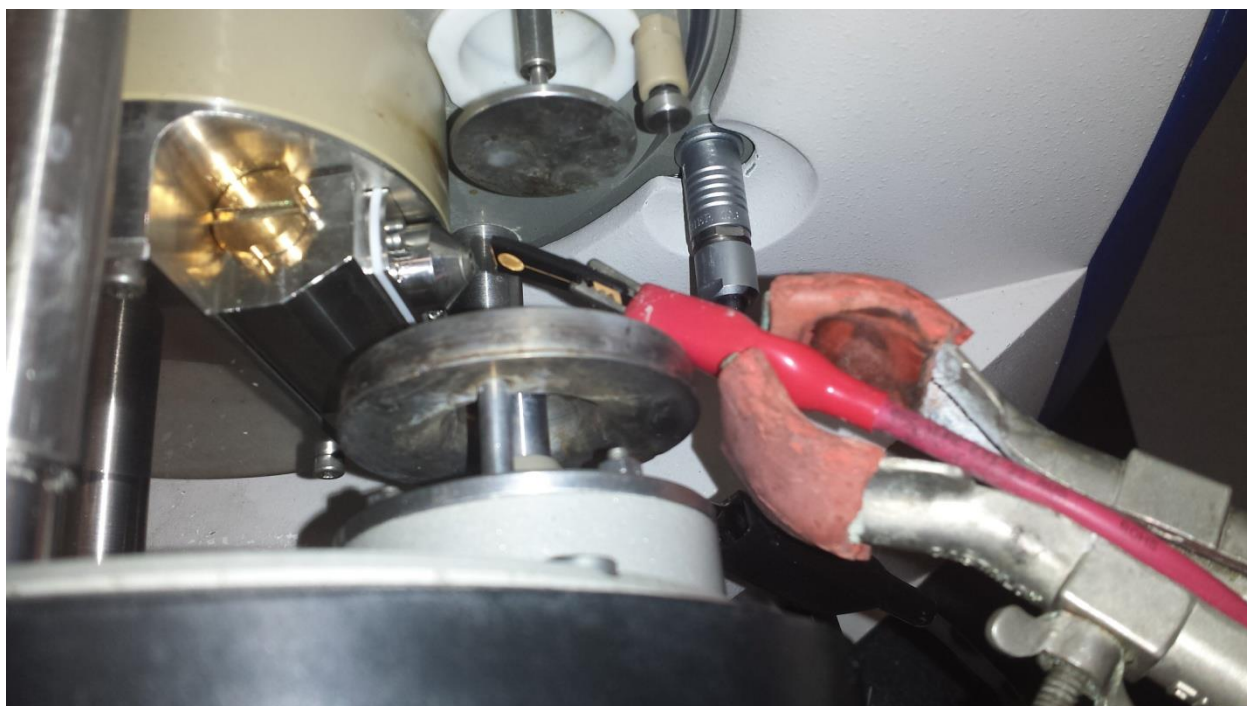
Figure 3.3. Image of the initial testing arrangement to hold a structured Conductive Sampling Probe and couple the desorbed material into to the mass spectrometer inlet.

The initial probe holder and MS coupling setup is shown in Figure 3.3. The probes were connected to an external HV source. The setup shown allowed the rapid switching between probes, but it did not allow us to position the probe reproducibly with respect to the ESI capillary and the MS inlet. As a result, signal intensities significantly varied from probe to probe. In addition, it was found that a new way to connect to the probe was needed, since the alligator clip would occasionally damage the probe contacting pad. This decreased the probe reusability, which was a very important consideration at the beginning of the method development stage. Another consequence of the irreproducible probe placement was that arcing between the probe and spray capillary was frequently seen due to the HV applied and the small distance between the metallic surfaces. These initial experiments indicated that desorption from the probes was possible, but the observation of high variability indicated the need for a way to ensure the reproducible placement of the probe and motivated the improvement steps detailed below.

To achieve higher reproducibility in probe placement and easier connection to the external voltage source, a new sample holder was designed. In this holder, the concept was to have a long positionable arm that could bring the sample to a precise location referenced to an external mount. The holder was designed to have a size-matched slot where the probe could be inserted reproducibly. Given the need to optimize the probe holder, it was decided that as a first iteration the holder could be 3D printed. This also conveyed the advantage of having an insulating material that would minimize arcing. The probe holder was first designed using Autodesk Fusion 360 software and fabricated using a MakerBot Replicator 2X 3D Printer, loaded with ABS (acrylonitrile butadiene styrene) filament. Next, the probe holder was attached to an XYZ-axis stage from Thorlabs (Figure 3.4) via a recessed hole that accommodated a screw. The probes were connected to the external voltage source with alligator clips as described earlier. The new holder

design allowed generation of more reproducible data individual probes, such as that shown in Figure 3.5. Limits of detection (LoD) for methacholine chloride that were obtained with the second version of the holder were at nanogram levels. In DESI-MS, the reported LoD values for low MW compounds are similar or higher,[7] which indicated that there was promise for the PIMSS approach implemented using the conductive structured probes. The goal of the proof of concept study was to show the ability to transfer analyte from the probe into the spectrometer. This was successfully demonstrated for methacholine chloride desorption using two sample holder designs. In order to enable real applications of the PIMSS concept, many parameters still had to be studied and verified. Overall, the sensitivity for methacholine was satisfactory, but the detected signal was not always reproducible, and some probe failures were observed (signals much lower than expected or no signal at all). Furthermore, the entire setup was large and inconvenient, particularly for a shared the MS instrument, because setting up the equipment was time consuming and not always accurate. Thus, a simpler method to position the probe reliably and quickly with minimal equipment modification was sought, and new probe holders were designed

Figure 3.4. Image of a second version of the probe holder. A 3D-printed probe holder was coupled to a commercial ThorLabs platform for better probe positioning and more reproducible desorption and coupling to the MS inlet.
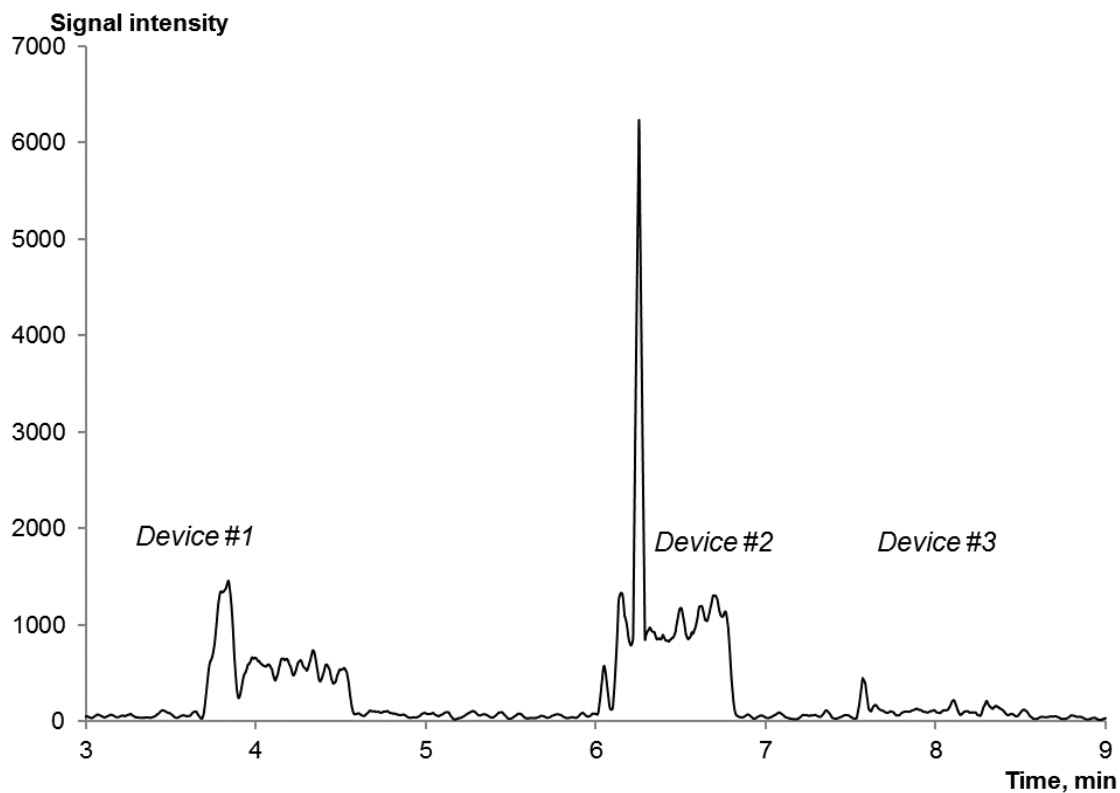

Figure 3.5. Desorption of 100 pg of acetyl-β-methylcholine from three separate devices using the second version of the sample holder.

3.2 Stationary sample holder design and optimization of MS parameters

3.2.1 Stationary sample holder development

In order to minimize the variability for the probe positioning, a stationary sample holder design was adopted where probes could effortlessly be swapped in or out to generate replicate data. During the sample holder development stage, more than a dozen of different designs were created and tested (Figure 3.6, **1-5**). The goal here was to miniaturize the sample holder setup, get rid of the bulky ThorLabs platform and alligator clips that occasionally damaged the probes, and simplify as much as possible the probe switching process. The very first design of a 3D-printed holder (**1**) had a slot that matched the dimensions of the probes, which was extremely helpful in ensuring reproducible positioning during switching between electrodes. Thus, this feature was incorporated in the final design. We also identified an opening for a metallic electrospray shield (seen as a metallic disk behind the probes in Figure 3.3) in the Waters MS instrument that was used for the experiments, which could be used as an ideal point of attachment for the probe holder. Thus, the shield was removed, and the opening was used to insert a newly designed sample holder. In this way, designs **3-5** eliminated the need to use the adjustable and bulky ThorLabs platform. The final probe holder design (**5**) contains an electrode slot and a conductive metallic clip to connect to the HV source, which would not damage the probes during the switching process. Once the reproducibility in probe positioning was resolved, the other consideration that needed to be addressed was the positioning of the probe with respect to the MS inlet and ESI capillary. This is an important property of the probe holder design because optimal positioning would result in the maximum coupling efficiency and thus higher MS signal from the desorbed sample.
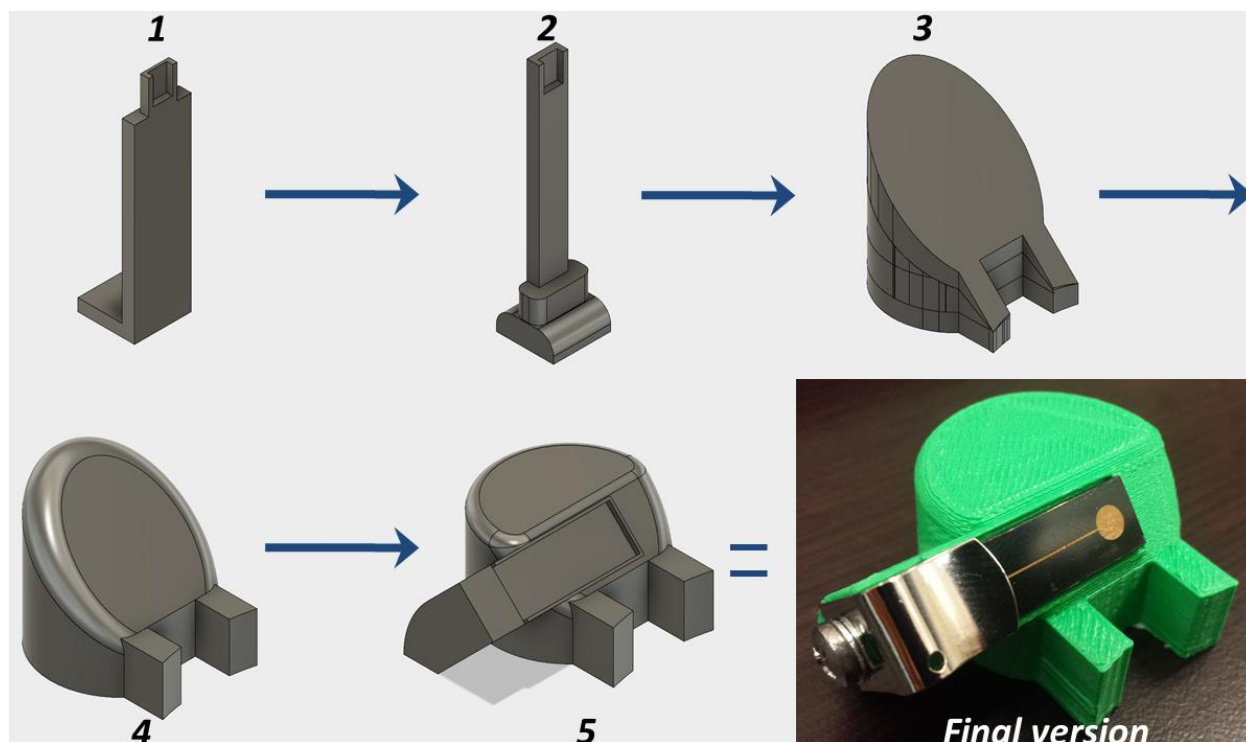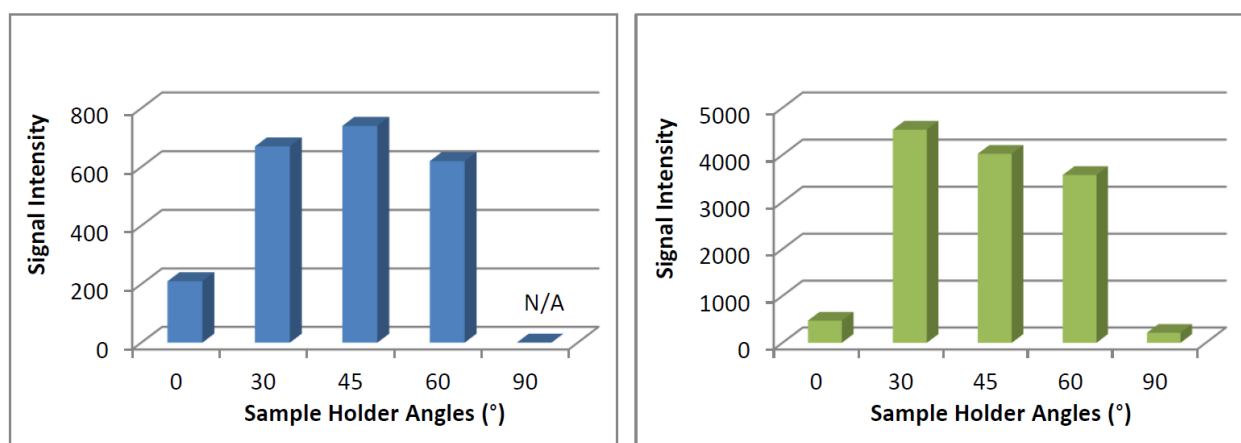
Figure 3.6. Evolution of 3D-printed sample holder designs including sample holder #1 that was used in the proof of concept study and a final miniaturized version.

3.2.2 Optimization of probe positioning and MS parameters.

Two parameters were optimized for the probe positioning with the fixed sample holder design: probe angle with respect to the electrode normal and electrode position with respect to the MS inlet. For the angle optimization study, five different sample holders with 0°, 30°, 45°, 60° and 90° angles were 3D-printed and tested in *Surface Reflection* mode (*cf.* Figure 2.4), where the electrode was placed directly in front of the MS inlet. The holders with angles of 0° and 90° were eliminated right from the beginning. The first one significantly hindered the access of the solvent spray to the probe, while the second produced little to no signal (Figure 3.7). The remaining three angles were tested with probes containing acetyl-β-methylcholine drop cast from 0.1 and 0.01 μg/L solutions in MilliQ water. Briefly, 2 μl of this solution was pipetted onto the sample pad regions

experiments (in our case the total number of experiments used to map the parameter space was reduced from 27 to 9). The DoE approach helps to find correlation between the parameters of an experiment and the measured response (in our case, daughter ion signal intensity). Our experiments showed that probe positioning, solvent composition, capillary and electrode voltages had a significant impact on the MS signal generated from molecules desorbed form the electrode surfaces.

Table 3.2. Specification of Factors and Experimental Domain.

Factors	-1-Level	0-Level	1-Level
x1: Sample Holder Angle (deg)	30	45	60
x2: Capillary and Electrode Voltages (kV)	1	2.25	3.5
x3: Methanol conc. (%)	25	50	75

To explore the parameter space, a two-level, full factorial design (23) was applied. Two factor levels were set as 'high' and 'low' in accordance to preliminary experimental results and instrumentation limitations (Table 3.2). These factors defined the experimental space explored (Figure S3.2). For the experimental design study, a 10 ng/L solution of acetyl-β-methylcholine chloride in MilliQ water was used. The sample deposition procedure was the same as indicated above and the experiments were performed in the *Surface Reflection* position. DoE data processing is explained in detail in Supporting Information section. As a result of DoE data processing, correlations between parameters of the experiment and measured response were found (Figure S3.3). The results of 9 trials (8 "corners" within the experimental space, which represent all possible combinations of "high" and "low" factor levels and 1 central point) acquired are presented in Figure 3.8. From these experiments, the highest signal intensity was obtained at 1 kV of capillary

and electrode voltages, 75% MeOH/ 25% H_2O solvent composition and 60° angle sample holder. This data was confirmed by DoE data processing (Figure S3.3).
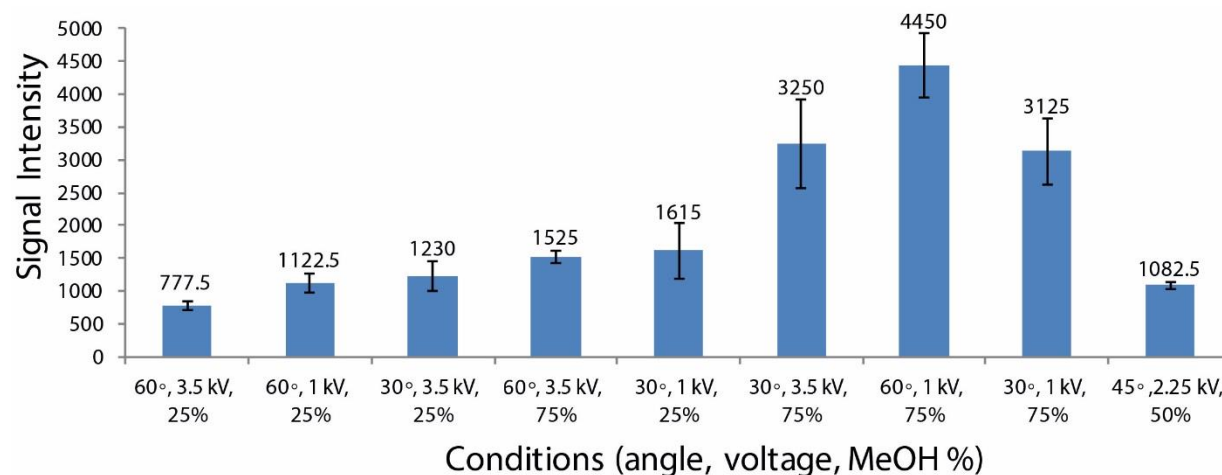
Figure 3.8. DoE results obtained by placing the probe in the *Surface Reflection* position. Bars represent means and errors represent standard error of the mean; n=5.

Finally, a series of trials was performed for the *Surface Skimming* position, using the parameters that were found to yield the four highest for the *Surface Reflection* position. A comparison of signal intensities for both positions is presented in Figure 3.8. For the *Surface Skimming* position the signal intensities were significantly higher than those for the *Surface Reflection* position. The highest signal intensity obtained was at 1 kV, 75% MeOH and 60° sample holder angle, which is ~30 times higher than the signal obtained using the same parameters for the *Surface Reflection* position. Overall, the acquired data demonstrates that 1 kV, 75% MeOH and 60° angle provide the best signal intensity for the desorption of cations adsorbed by drop casting on the structured conducting sample probes.
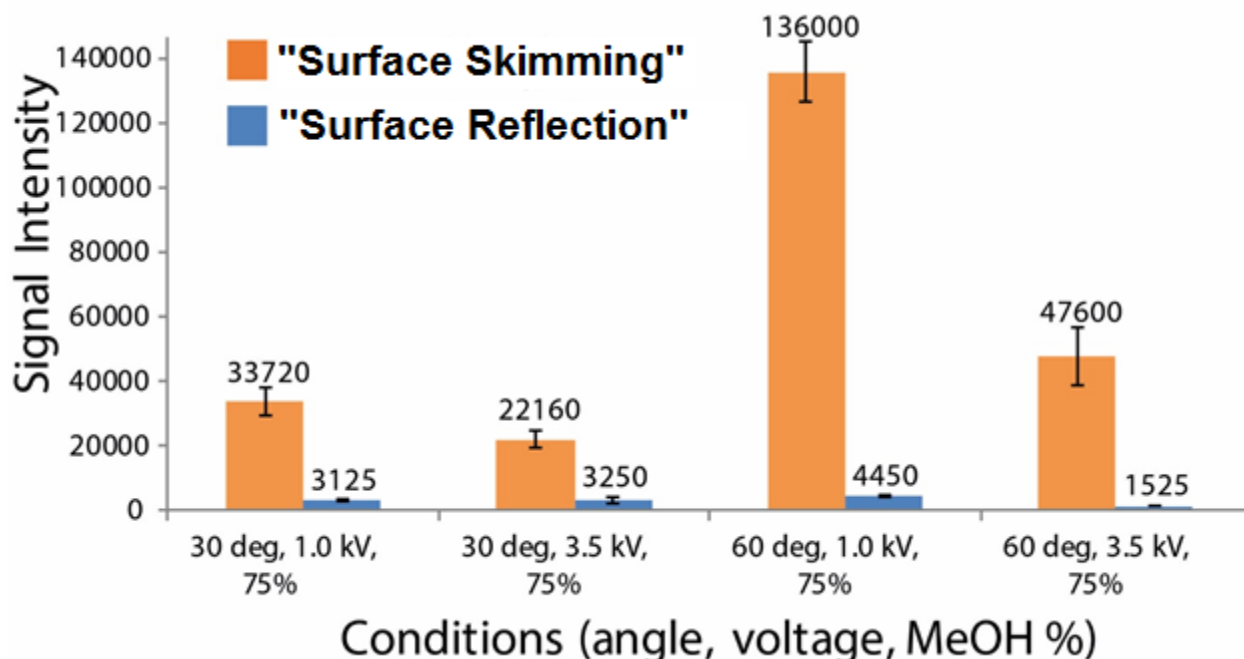
Figure 3.9. Comparison of the efficiency of desorption and coupling to the MS between the *Surface Skimming* and *Surface Reflection* positions (bars represent means and errors represent standard error of the mean; n=5).

After the impact of the key experimental parameters on the signal intensity was evaluated, the method was used to detect different species at a range of concentrations. Calibration curves were generated for three analytes with different molecular charge: positively charged acetyl-β-methylcholine chloride, neutral caffeine, and negatively charged heptadecafluorooctanesulfonic acid potassium salt (PFOS). Solutions were prepared in MilliQ water at different concentrations of the analyte (1 g/L – 1 μg/L), and total masses of 2 μg, 200 ng, 20 ng, 2 ng, 200 pg, 20 pg and 2 pg were deposited onto the electrodes. 2 pg amounts of caffeine and PFOS were not detected, while 2 μg amounts provided a signal overload and were not included in the calibration. As observed from the calibration curves presented in Figure 2.7, the method demonstrated a broad linear range and lower limits of detection at picogram levels. This set of data provided an idea on the ionization efficiency of the neutral analytes on the example of caffeine. Signal intensity for

desorption of 200 ng of caffeine was ~10-fold lower than ABMC signal. From assumption that all ABMC delivered into the MS was detected because it is a cationic molecule,.one can conclude that one small part of caffeine was ionized. It is important to note that the DoE studies were performed in electrospray modality (capillary voltage on). Later it was confirmed that the electrospray voltage has a negative effect on the signal intensity and method sensitivity. The final optimization steps were already discussed in detail in Chapter 2 in a peer reviewed manuscript format.

Overall, the studies performed in Chapter 3 demonstrated the importance of thorough and systematic optimization of the experimental parameters and how drastically the sensitivity can vary when only one parameter or positioning is changed. The DoE study proved to be a useful optimization tool that required a minimal number of experiments, and it helped to figure out the effect of each parameter on the final signal intensity. However, the optimal parameters obtained for one analyte are not guaranteed to be suitable for other molecules. As further studies showed, such parameters as electrode voltage and collision voltage have to be individually verified for each analyte.

3.4 Supplementary information.
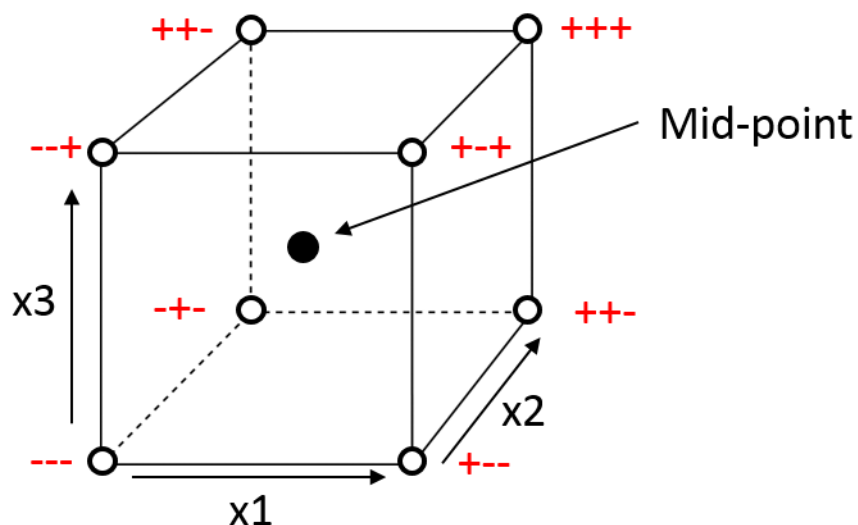
3.4.1 Probe dimensions.
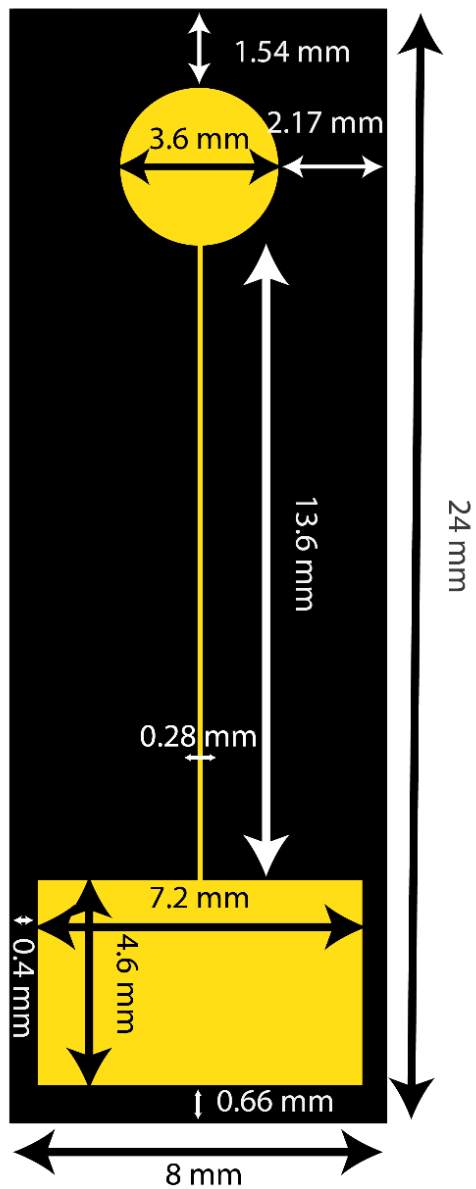
Thickness = 1.4 mm
Connecting region length = 13.6 mm
Connecting region width = 0.28 mm

Figure S3.1. Dimensions of the fabricated probe. Sample pad diameter is adjusted to the area covered by the solvent spray.

3.4.2. Design of experiments

Figure S3.2. Experimental space of a two level, full-factorial design.

For our purposes, a series of experiments were set up so that every possible combination of factor levels was run (Figure S3.3). The resulting data was processed using the built-in Microsoft Excel multiple linear regression function.

Figure S3.3. DoE data processing.

The correlation between the experimental parameters and the measured response is described by the coefficients obtained from the multiple linear regression analysis. For instance, the data tells us that the increase of MeOH concentration in the solvent by one level (from 50% to 75%) will lead to overall increase in signal intensity by 957.55. Conversely, the signal intensity would drop by 434.25 if the voltage was increased from 2.25 to 3.5 kV. It can be observed that correlation between parameters is also important and should be taken into consideration. When we change both parameters (x1x3), despite the positive correlation and high individual effect on final signal intensity of solvent composition, the effect of the sample holder angle prevails over it. Thus, sample holder angle should be considered as the most significant parameter in the experiments. However, soon after DoE tests, the mass spectrometer was not functional for several months and after it was repaired, optimization experiments described in Chapter 2 were performed. Acquired data did not quite match the information that was provided by DoE. It was found that optimal desorption angle was 30º, while DoE showed ~4-fold lower intensity for this angle compared to the best result (60º).

3.5 References

1.   Martin, J. G.; Opazo-Saez, A.; Du, T.; Tepper, R.; Eidelman, D. H., In vivo airway reactivity: predictive value of morphological estimates of airway smooth muscle. *Can J Physiol Pharmacol* **1992,** *70* (4), 597-601.

2.   JAMES, A.; RYAN, G., Testing airway responsiveness using inhaled methacholine or histamine. *Respirology* **1997,** *2* (2), 97-105.

3.   Venter, A.; Sojka, P. E.; Cooks, R. G., Droplet dynamics and ionization mechanisms in desorption electrospray ionization mass spectrometry. *Analytical chemistry* **2006,** *78* (24), 8549-55.

4.   Harris, G. A.; Fernandez, F. M., Simulations and experimental investigation of atmospheric transport in an ambient metastable-induced chemical ionization source. *Analytical chemistry* **2009,** *81* (1), 322-9.

5.   Costa, A. B.; Cooks, R. G., Simulation of atmospheric transport and droplet-thin film collisions in desorption electrospray ionization. *Chem Commun (Camb)* **2007,** (38), 3915-7.

6.   Douglass, K. A.; Jain, S.; Brandt, W. R.; Venter, A. R., Deconstructing desorption electrospray ionization: independent optimization of desorption and ionization by spray desorption collection. *J Am Soc Mass Spectrom* **2012,** *23* (11), 1896-902.

7.   Morelato, M.; Beavis, A.; Kirkbride, P.; Roux, C., Forensic applications of desorption electrospray ionisation mass spectrometry (DESI-MS). *Forensic Sci Int* **2013,** *226* (1-3), 10-21.

8.   Analytical Methods Committee, A. N., Experimental design and optimisation (4): Plackett–Burman designs. *Anal Methods-Uk* **2013,** *5* (8), 1901-1903.

9.   Gong, W. J.; Zhang, Y. P.; Choi, S. H.; Zhang, Y. J.; Lee, K.-P., Application of response surface methodologies in capillary electrophoresis. *Microchimica Acta* **2006,** *156* (3), 327-335.

10.   Kaiser, C.; Segui-Lines, G.; D'Amaral, J. C.; Ptolemy, A. S.; Britz-McKibbin, P., Electrokinetic probes for single-step screening of polyol stereoisomers: the virtues of ternary boronate ester complex formation. *Chemical Communications* **2008,** (3), 338-340.

CHAPTER 4: CONCLUSION AND FUTURE DIRECTIONS

4.1 Conclusions.

A new highly efficient Primary Ion Mass Spectrometry Source (PIMSS) concept was introduced. The concept is based on the application of high surface area gold electrodes as Functionalized Conductive Sampling Probes (FCSPs). The PIMSS concept combines use of FCSPs as an extraction technique and the ion source (Figure 4.1). In comparison to other DESI-like (electrospray applied to the sample surface) and probe-based methods (electrospray formed from the probe), the PIMSS concept does not utilize electrospray in any way. Instead, ions are generated directly from the surface of the probe through an application of high voltage and solvent. Voltage study performed in Chapter 2, where method is operated in DESI-like conditions (voltage applied only to the spray capillary) and PIMSS conditions (voltage applied only to the probe) clearly demonstrates better sensitivity of the proposed approach. Limits of detection acquired for the studied analytes were at the same level or higher compared to DESI-MS. Developed method has a wide linear dynamic range and allows quantitation of remarkably small amounts of analyte (picogram levels). Important method development steps as well as major challenges and limitations were described in detail in Chapter 3.
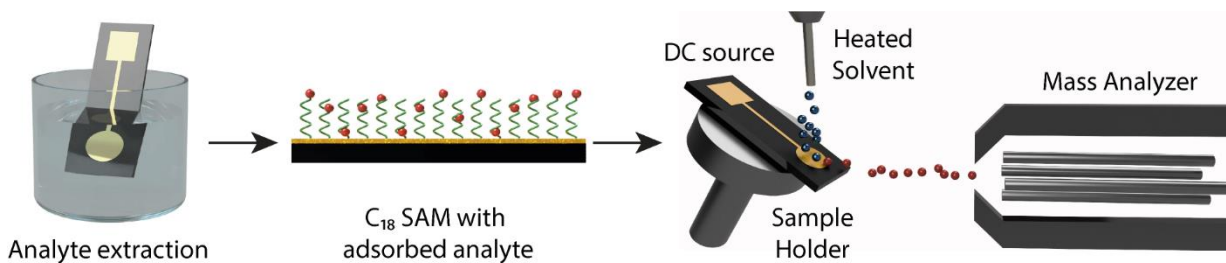
Figure 4.1. A schematic representation of Structured Conductive Sampling Probe - Primary Ion Mass Spectrometry Source (SCSP-PIMSS) approach.

Fused deposition modeling 3D printing has shown to be an efficient tool for simple and rapid prototyping of a MS coupling device. The proposed probe holder design allowed multiple important features: i) the adjustment of the distance between the probe, the electrospray capillary and the MS inlet, ii) control over the angle at which the sample pad on the probe lies with respect to the MS components, iii) electrical connection of the FCSPs to a high voltage supply, iv) rapid switching between probes, and v) reproducible positioning of the probe in the MS. During the probe holder development, several holder iterations were introduced and each new design contained new improvements and demonstrated better results. The final version of the probe holder allowed to desorb analytes of interest with the highest efficiency.

The PIMSS concept is a very simple and cost-efficient approach. Implementation of concept in the lab does not require a purchase of an expensive external ion source in comparison to DESI or DART. It would only take an available mass spectrometer with an ESI source and a probe holder designed specifically for that instrument. Many mass spectrometers allow enough space in the ESI chamber to implement an external probe holder. Fused deposition modeling 3D-printing can be used to combine the PIMSS concept

with other commercially available mass spectrometers. Thus, this factor can positively affect method applicability. Overall, the PIMSS concept can take its place among other popular probe-based desorption methods due to the versatility and simplicity.

4.2 Future Directions

Future development steps of the PIMSS concept may include investigation of alternative probe materials to improve overall method performance and increase extraction capacity of the analyte. First problem that has to be addressed, is ionization efficiency of the method. As the calibration curves demonstrate in Chapter 2 (Figure 2.6), signal intensity acquired for desorption of 2 μg of caffeine at optimized conditions is ~10-fold lower than signal intensity for the same amount of methacholine chloride. Methacholine chloride is a cationic analyte and does not require to be ionized, while caffeine is a neutral molecule and has to be ionized prior to the detection. It means that only small part of caffeine is ionized and analyzed. The answer to this problem might also be an application of an alternative desorption substrate. Various materials have been applied as desorption substrates in probe electrospray ionization (PESI)[1] and direct electrospray probe (DEP)[2] methods until now. In this group of methods electrospray is generated upon an application of voltage directly from the probe. The materials used for PESI and DEP include copper[2] and platinum[3] (analysis of protein solutions), stainless steel in a form of acupuncture needles [4-6] (analysis of protein solutions, lipids, peptides and soft drinks), tungsten[7] and tungsten oxide[8] (analysis of illicit drugs, protein solutions and metabolites in live cells). Second group of materials presents modified substrates. For example, surface modified acupuncture needle as a DEP substrate

was reported.[9] The surface area of the probe and surface hydrophilicity was enhanced through an oxidation treatment. Another example of surface modified probes describes a solid-phase microextraction probe modified with nanosized TiO_2 used for extraction enhancement of phosphopeptides from complex samples.[10] This is only a small part of probe-based desorption methods that exist nowadays. It demonstrated that probes can be fabricated using all kinds of conductive material with a combination of surface modifications. The PIMSS concept is not limited to the analysis of low molecular weight analytes. Based on the example of abovementioned methods, probe-based methods can be applied to the analysis of small analytes as well as large biomolecules. Investigation of bioanalytical, environmental, forensic applications presents interest in future.

[11] At the moment, the probes applied in PIMSS concept are capable of extracting very small amount of the analyte from the solution of interest. Application on porous substrates presents interest due to their large surface area. Due to the increased total surface area of porous substrates, extraction capacity of the probes can be significantly improved. Inorganic porous materials (silicon, gold, alumina) have been previously applied in surface-enhanced desorption MS techniques.[11]

Alternative desorption solutions have never been really applied for the PIMSS concept. A methanol/water mixture, a typical ESI solution, has been used as the desorption solvent. Methods in the previous section use different spray solvents depending on the investigated analyte. Solvent compositions typically include common ESI and LC solvents: methanol, ethanol, water, chloroform, acetonitrile. Some water-based mixtures also contain acetic acid or ammonia in small amounts (1-2%). It prompts an idea to investigate more

compositions. Although, materials used in the probe fabrication can be a major limitation here since polystyrene is dissolvable in some organic solvents.

The PIMSS extraction feature presented in Chapter 2 still requires more thorough investigation. There is no clear understanding of kinetics in the solid-liquid extraction process and what parameters may provide optimal extraction conditions. It has been demonstrated that probes can extract only 2 ng of analyte because of a small amount of the extracting material. Empirically, it was determined that the probes become saturated in water solution with concentration of caffeine higher than 100 ng/mL, but it still can effectively extract materials from more dilute solutions. In order to fully understand the process, a series of systematic experiments is required. The study may as well include mathematical modelling that was previously applied to kinetics study of solid-phase extraction and solid-phase microextraction.[12] Optimal extraction time, temperature and solution volume is also a subject for further clarification.

4.3 References

1.   Yoshimura, K.; Chen, L. C.; Asakawa, D.; Hiraoka, K.; Takeda, S., Physical properties of the probe electrospray ionization (PESI) needle applied to the biological samples. *Journal of mass spectrometry : JMS* **2009,** *44* (6), 978-85.

2.   Kuo, C. P.; Shiea, J., Application of direct electrospray probe to analyze biological compounds and to couple to solid-phase microextraction to detect trace surfactants in aqueous solution. *Analytical chemistry* **1999,** *71* (19), 4413-7.

3.   Kuo, C.-P.; Yuan, C.-H.; Shiea, J., Generation of electrospray from a solution predeposited on optical fibers coiled with a platinum wire. *Journal of the American Society for Mass Spectrometry* **2000,** *11* (5), 464-467.

4.   Hiraoka, K.; Nishidate, K.; Mori, K.; Asakawa, D.; Suzuki, S., Development of probe electrospray using a solid needle. *Rapid Communications in Mass Spectrometry* **2007,** *21* (18), 3139-3144.

5.   Chen, L. C.; Nishidate, K.; Saito, Y.; Mori, K.; Asakawa, D.; Takeda, S.; Kubota, T.; Terada, N.; Hashimoto, Y.; Hori, H.; Hiraoka, K., Application of probe electrospray to direct ambient analysis of biological samples. *Rapid Communications in Mass Spectrometry* **2008,** *22* (15), 2366-2374.

6.   Otsuka, Y.; Naito, J.; Satoh, S.; Kyogaku, M.; Hashimoto, H.; Arakawa, R., Imaging mass spectrometry of a mouse brain by tapping-mode scanning probe electrospray ionization. *Analyst* **2014,** *139* (10), 2336-2341.

7.   Gong, X.; Zhao, Y.; Cai, S.; Fu, S.; Yang, C.; Zhang, S.; Zhang, X., Single Cell Analysis with Probe ESI-Mass Spectrometry: Detection of Metabolites at Cellular and Subcellular Levels. *Analytical chemistry* **2014,** *86* (8), 3809-3816.

8.   Jeng, J.; Lin, C.-H.; Shiea, J., Electrospray from Nanostructured Tungsten Oxide Surfaces with Ultralow Sample Volume. *Analytical chemistry* **2005,** *77* (24), 8170-8173.

9.   Yu, Z.; Chen, L. C.; Mandal, M. K.; Yoshimura, K.; Takeda, S.; Hiraoka, K., Direct Electrospray Ionization Mass Spectrometric Profiling of Real-World Samples via a Solid Sampling Probe. *Journal of The American Society for Mass Spectrometry* **2013,** *24* (10), 1612-1615.

10.   Zhao, Y.; Gong, X.; Si, X.; Wei, Z.; Yang, C.; Zhang, S.; Zhang, X., Coupling a solid phase microextraction (SPME) probe with ambient MS for rapid enrichment and detection of phosphopeptides in biological samples. *Analyst* **2015,** *140* (8), 2599-2602.

11.   Law, K. P.; Larkin, J. R., Recent advances in SALDI-MS techniques and their chemical and bioanalytical applications. *Analytical and bioanalytical chemistry* **2011,** *399* (8), 2597-622.

12.   Semenov, S. N.; Koziel, J. A.; Pawliszyn, J., Kinetics of solid-phase extraction and solid-phase microextraction in thin adsorbent layer with saturation sorption isotherm. *Journal of Chromatography A* **2000,** *873* (1), 39-51.