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MINIATURE MASS SPECTROMETER SYSTEM WITH A SAMPLING PROBE

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MINIATURE MASS SPECTROMETER SYSTEM WITH A SAMPLING PROBE

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To my dear wife Chen-I, and my daughters Chi-Anne, and Vivian

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ABSTRACT

Chen, Chien-Hsun. Ph.D., Purdue University, August 2015. Study of Miniature Mass Spectrometer System with a Sampling Probe. Major Professor: Zheng Ouyang.

This thesis focuses on the development of miniature mass spectrometry systems and the sampling probes. Mass spectrometers are usually installed and used in the analytical laboratory. It requires complex sample preparation before the analysis. Use of miniature mass spectrometer enables the chemical analysis outside the lab and a sampling probe provides convenience for real-time analysis. Two miniature mass spectrometer systems have been developed in this work.

A backpack mass spectrometer with a handheld probe was designed for on-site forensic analysis. The handheld probe consists of a mass analyzer, high vacuum pump, and in-line low temperature plasma (LTP) source. The wearable backpack unit contained other components can be easily carried by the user. A miniature pumping system with a 130g drag pump and a 350g scroll pump was used in this system. Direct surface analysis of illicit drug and explosives in tens of nanogram was demonstrated.

Another portable mass spectrometer with a remote sampling probe was designed for *in vivo* tissue analysis in surgical and endoscopic procedures. The remote sampling probe has a dual-channel configuration, with one channel (0.5mmID) for providing the

desorption spray, and one channel (1.5m-long, 1.6mmID) for guiding the charged species back to the mass spectrometer. No high voltage was applied to generate the spray and pure water was used as the solvent for compatibility with medical operation. The sensitivity of the whole system was improved by efficient desolvasion using a heated capillary and ion accumulation with multiple ion introductions. Lipid profiles of high signal to noise ratio have been obtained from direct analysis of rat brain, liver, lung, and intestine tissue sections on this platform. The compatibility of this system would allow it be used in the operating surgical room to provide molecular information that potentially could guide the surgeons to make a prompt decision during the surgery. Other applications such as a quick screening of chemicals on a Pelican case and aging study of pen ink were also demonstrated.

CHAPTER 1. INTRODUCTION

1.1 Mass Spectrometry

The origin of mass spectrometry can be traced back to the first observation of positively charged anode rays in 1886 by a physicist, E. Goldstein.[1] W. Wien later found that these rays can be deflected by a strong magnetic or electric field and separated them according to the mass to charge (m/z) ratio. The mass spectrograph was obtained by J. J. Thomson, after pressure in the chamber was much reduced. F.W. Aston[2-3] followed Thomson's work on discovery of isotopes and developed modern techniques for mass spectrometry. Different ionization sources and mass analyzers were introduced, and one of the significant developments was the ion trap technique developed by H. Dehmelt and W. Paul[4] in the 1950s and 1960s. While early developments were mainly achieved by physicists, mass spectrometry was generally used by chemists due to its superior analytical values. The following important developments were achieved by J. B. Fenn and K. Tanaka for their discovery of electrospray ionization (ESI)[5] in 1989 and matrix-assisted laser desorption/ionization (MALDI)[6] in 2005, respectively. These two ionization methods opened the gates for analysis of biomolecules due to the production of intact biomolecular ion. It made the analysis of complex biological samples possible, and provided biologists an unique tool for the systematic study of the biological systems.[7]

The main components in a mass spectrometer include the ionization source, analyzer, and ion detector. The ionization source is responsible for changing molecules from the neutral state to the ionic state, and the ions can then be analyzed. The choice of the ionization source depends on the property of the sample and the information to be obtained from the sample. Generally, small or volatile molecules are usually ionized through electron ionization (EI)[8] or plasma/discharge-based ionization methods.[9] They can be directly ionized in the gas phase. Large or non-volatile molecules require MALDI-based or ESI-based ionization methods. They can be ionized in the solid or liquid phase with the assistant of matrix or solvents most of the time. Different types of mass analyzers have been developed for the analysis of mass-to-charge ratios (m/z) of ions that are produced in the ionization sources. Linear Time-of-Flight,[10] Quadrupole Ion Trap,[11] and Triple Quadrupole[12] are basic mass analyzers. Orbitrap,[13] Fourier Transform Ion Cyclotron Resonance (FTICR),[14] Magnetic Sector,[15] and Time-of-Flight with Reflectron[16] come with higher mass resolution. High resolution mass spectrometers[17] provide the advantage of direct information of molecular formula via accurate mass. Ion detectors can be divided into image current detector and particle multiplier. Image current detectors[18-19] measure the induced current. This type of detectors can be operated in the low vacuum environment and the detection efficiency doesn't change with the mass of the ions. Electron multipliers, such as the channeltron, and microchannel plate (MCP), provide amplification of about six orders of the signal via electron multiplications.[20] This type of detectors is more sensitive but has to work in the high vacuum environment.

1.1.1 Ambient mass spectrometry

Mass spectrometry (MS) has become the gold standard for chemical analysis. On-site, real-time mass spectrometry analysis is still limited since the regular procedures of MS analysis usually require complex sample collection preparation, and separation.[21] Ambient mass spectrometry was introduced in 2004 for real-time chemical analysis of samples in their native states in the ambient environment.[22] It allows direct ionization of untreated samples for mass analysis.[23] The methods provide several benefits, high throughput[24], simple procedures[25], and native analysis[26].

In the last decade, many different ambient ionization methods have been developed to target different analytical problems.[23,27-30] In this study, they are divided into three categories, plasma-based, spray-based and laser-based[31-32] methods. Since the laser was not used in the thesis work, the introduction of ambient ionization methods focuses on the plasma-based and spray-based methods.

1.1.1.2 Plasma-based Ambient Ionization Methods

Several plasma-based ambient ionization methods have been developed and they are different in production of plasma, geometry, and power.[9,33-35] Its application has been demonstrated for a broad range of applications, including the analysis of explosives,[36] illicit drugs,[37] agrochemicals,[38] writing ink,[39] bacterial cells,[40] and blood or serum samples[41] from different surfaces.

One of the most commonly used methods is Direct Analysis in Real Time (DART) which was introduced in 2005 and has been commercialized.[33] The ionization region is separated from the discharge region, and heated helium gas is used as discharge gas.[42]

The ionic plasma species produced from discharge is filtered and the metastable species (He*) interact with the analytes (M) leading to penning ionization (schematic 1.1).[43]

$$He^* + M \rightarrow He + M^{+*} + e^- \qquad (1.1)$$

People also found that reactant ions (N_2^+, H_2O^+, $(H_2O)_n^+$) could be produced via reaction of He* with nitrogen or water vapor in the air. Analytes in the gas phase with higher proton affinity could be ionized through proton transfer from the reactant ions (schematic 1.2).

$$(H_2O)_nH^+ + M \rightarrow (H_2O)_n + MH^+ \qquad (1.2)$$

Another plasma-based ambient ionization method, low temperature plasma (LTP), was introduced in 2008.[35] The characteristic of low temperature makes it a better fit for direct analysis of a delicate surface of an object. One of the examples is the direct analysis of trace amount of chemicals on human finger (Figure 1.1 b),[35] and the other example is imaging of inkpads of seal on works of art.[44] The other advantages of LTP include the easy construction and low power consumption that are of high potential for on-site analysis coupling with miniature mass spectrometer.[45-47]

The configuration of LTP probe is shown in Figure 1.1. The plasma was produced with a dielectric barrier discharge using an ac electric field across the wall of a glass tube. Helium, argon, nitrogen, and ambient air could be used as the discharge gas. The sample was directly desorbed and ionized from the surface, and ions were taken into the inlet of the mass spectrometer for the subsequent MS analysis. The ionization mechanism of LTP is related to schematic 1.1 and 1.2, and the plasma can directly react with the sample.[48] It has been demonstrated the wide range of applications including analysis of explosives,[36,49] agrochemicals,[38] drugs,[35] food,[46] crude oil[50], olive oil,[51] and bacteria.[52]

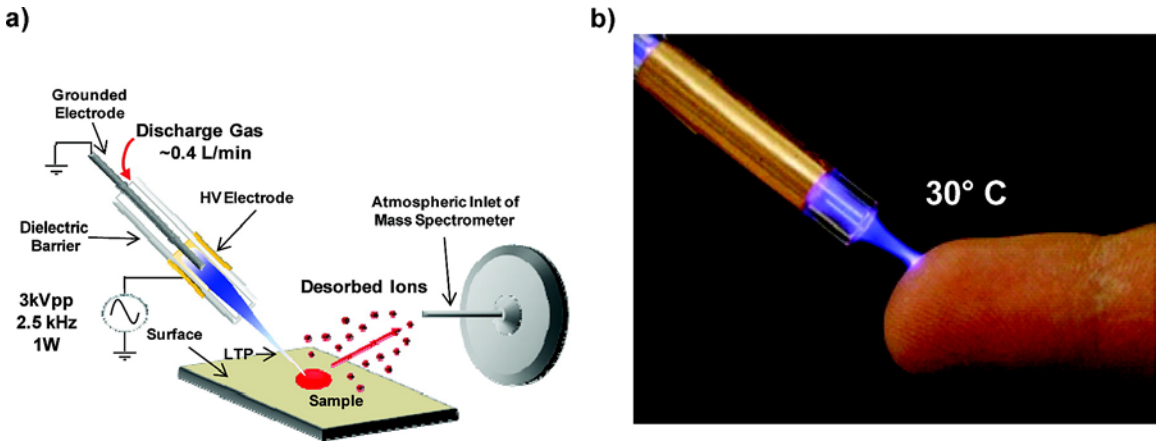

Figure 1.1 LTP probe for ambient mass spectrometry (a) Schematic and (b) photo of the LTP probe. Trace amount of the sample was directly analyzed on a human finger. Figures are from [35].

1.1.1.3 Spray-based Ambient Ionization Methods

Several spray-based ambient ionization methods have been developed. Compared to plasma-based methods, spray-based methods extend the applicable range of chemical compounds to low volatility and high molecular weights.[53-54]

The first spray-based ambient ionization method, DESI, was introduced in 2005.[22] The analytes was picked up by the charged droplets which were produced by an electrosonic spray ionization (ESSI) source[55] using nitrogen curtain gas. Initial droplets produced a thin liquid film on the sample surface, and subsequent droplets produced the emission of the smaller droplets carrying the analytes.[56] There are two ionization mechanisms for DESI. For small molecules, ionization comes with charge transfer; for large molecules, ionization is similar to ESI.[57] The set-up of DESI is shown in Figure 1.2.

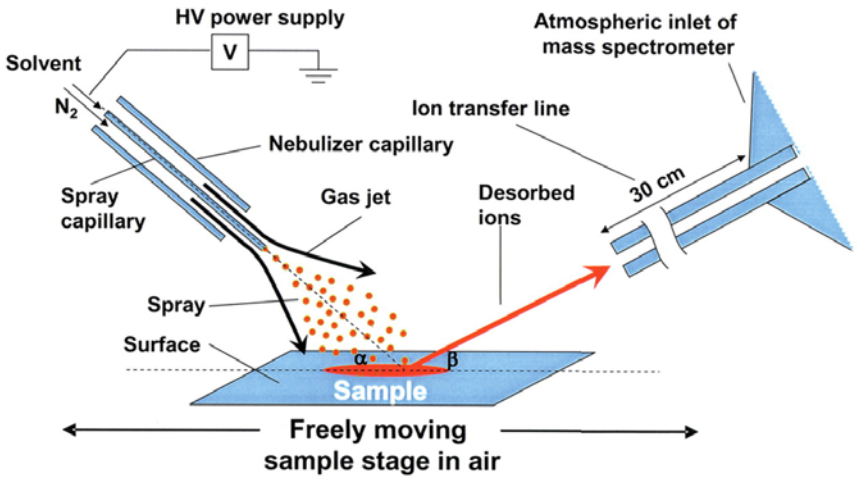

Figure 1.2 Schematic of Desorption Electrospray Ionization (DESI). The figure is from [22].

DESI is a soft desorption ionization method producing low internal energy of ions with 1.7~1.9 eV.[58] Little or no fragmentation of analyte ions occur, which is important for direct analysis of complex mixtures. It covers a broad range of molecules in terms of molecular weight, volatility, and polarity.[57] When used for analysis of urine sample, it has better tolerance to high concentration of salt as compared to ESI, and the cleaning process for the sample can be eliminated.[59] Good quantification could be obtained with the internal standard; semi-quantitative analysis can be achieved without the internal standard.[60-61] It is a sensitive ambient ionization method and the absolute detection limits of pure compounds are usually in the range of 1 to 1,000 pg.[57] It's also a high throughput approach with total analysis time less than five seconds.[24,61]

The solvent choice of DESI plays an important role in ionization.[62] Methonal/water (1:1) is a standard solvent for polar molecules, while different solvents are chosen for different species of analytes.[63] Non-aqueous solvents, such as CH_3Cl_3/THF (1:1) and

CH_3Cl_3/CH_3CN (1:1), were used for the analysis of hydrophobic compounds.[64] The addition of surfactants to the spray solvent is a strategy to increase the sensitivity due to a reduction in surface tension.[65] Additions of small amounts of acid to the solvent can help the formation of positive ions.[57] Addition of a reactant to the solvent can modify the analyte and increase its ionization efficiency and this approach is called reactive DESI.[66-67]

DESI was used in different fields such as environment,[68] food,[69] forensic,[70] homeland security,[71] pharmaceutical,[24] and imaging.[72] The biological tissue samples have been directly analyzed using DESI[73] and the lipid profiles were obtained in both positive and negative ion modes.[74] Difference in lipid profiles between normal and cancer tissue was observed. Human-liver adenocarcinoma tissue and human-liver normal tissue could be discriminated by DESI analysis.[75] The cancerous tissue was shown to have a higher level of sphingomyelin, which is related to the dysfunctional ceramide-mediated apoptosis pathway. More studies have shown that tissue analysis with DESI MS could be used for cancer diagnosis.[76-78]

A typical negative-ion mass spectrum of tissue section is shown in Figure 1.3 for the white matter of the rat brain tissue section. In the low mass range (m/z 200~400), the main components are fatty acids such as palmitic acid, oleic acid, stearic acid, arachidonic acid, and docosohexaenoic acid. In the high mass range (m/z 700~1000), the main components are identified as phospholipids, such as sulfatides (ST), phosphatidylserines (PS), phosphatidylinositols (PI), and plasmenyl glycerophospho ethanolamines (plasm-PE), etc.

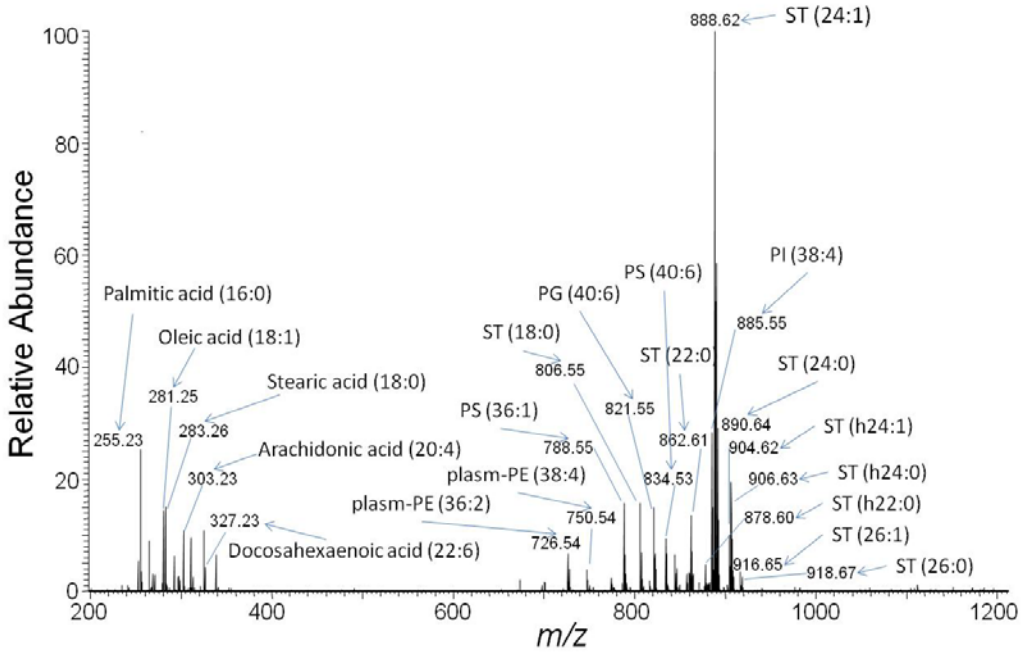

Figure 1.3 DESI Mass spectrum of white matter of rat brain tissue section in the negative ion mode.

Many efforts have been put on cancer diagnosis using DESI MS. The studied samples include the human brain tumors,[76] human breast cancer,[79-81] human prostate cancer,[82] human papillary renal cell carcinoma,[78] human bladder carcinomas,[77,83] and canine urinary bladder cancer.[84] For the human brain tumors, differentiations was achieved in diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (grade IV). It was found that a smaller quantity of sulfatides and galactoceramides were detected in the negative and positive ion modes, respectively, as the stage of cancer becomes higher. This observation was consistent with a previous report using regular lipids extraction method.[76] For the canine urinary bladder cancer, profiles of glycerophospholipids, sphingolipids, and fatty acids could be used to separate the cancerous region and non-cancerous region, which have been confirmed with the

H&E-staining method.[84] For the human prostate cancer, a cholesterol sulfate was clearly observed in cancerous tissues, but not in normal tissue.[82]

Other spray-based ambient ionization methods include neutral desorption extractive electrospray ionization (ND-EESI)[85], nano-DESI[86], liquid micro-conjunction surface sampling probe (LMJ-SSP)[87], paper spray[25], and easy ambient sonic-spray ionization (EASI)[88], etc.

1.1.2 Long-distance Ion Transfer

Ambient ionization serves as a solution for real-time analysis of objects in their native states.[22] The samples of analysis typically need to be very close to the inlet of mass spectrometer, so the signal produced can be taken into the mass spectrometer. Otherwise the signal loss might be significant. Once the object of analysis is big, or unmovable or not easily accessible, it can be challenging for application of the ambient ionization methods.

Efficient transfer of ions from a non-proximate point with a sampling probe back to mass spectrometer might be a solution to the problem. The first demonstration of this concept was the non-proximate DESI.[89] Ions produced by DESI from a sample surface were transferred via a stainless steel capillary (1.8mmID, 1-3m long), facilitated by the gas flow driven by the vacuum of the mass spectrometer (Figure 1.4). A flexible tube made with conductive silicone was also tested and similar transfer efficiency was obtained. Cocaine of 1 ng on a human finger was directly detected using LTQ mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA) with a 1m-long tube.

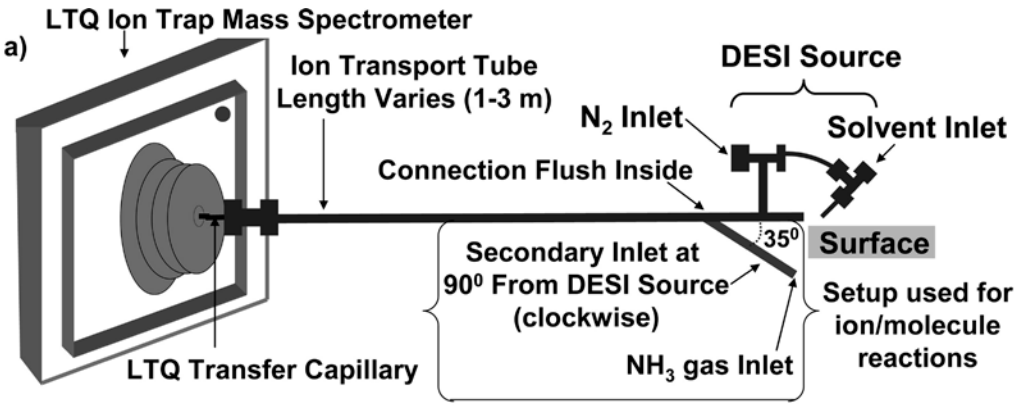

Figure 1.4 Schematic of non-proximate DESI. The figure is from [89].

A previous study on the ion transfer over a long-distance showed that a laminar flow was formed at the 5 cm length of the transfer tube (Figure 1.5c).[90] The difference in ion signal loss is only 16% when comparing the length of transfer over 10cm and 60cm (Figure 1.5e). The diffusion along the radial axis could be the main reason for ion loss during the transfer. Higher gas flow velocity and larger internal diameter of the tube could provide higher transfer efficiency (Figure 1.5d).

If the gas flow from a sampling device has been provided by the ambient ionization source such as remote DESI, simple configuration could be used (Figure 1.6a). If gas was not used in the ionization source, an additional pump might be used to overcome the mismatch between the desirable gas flow for ion transfer and intake gas (Figure 1.6b).

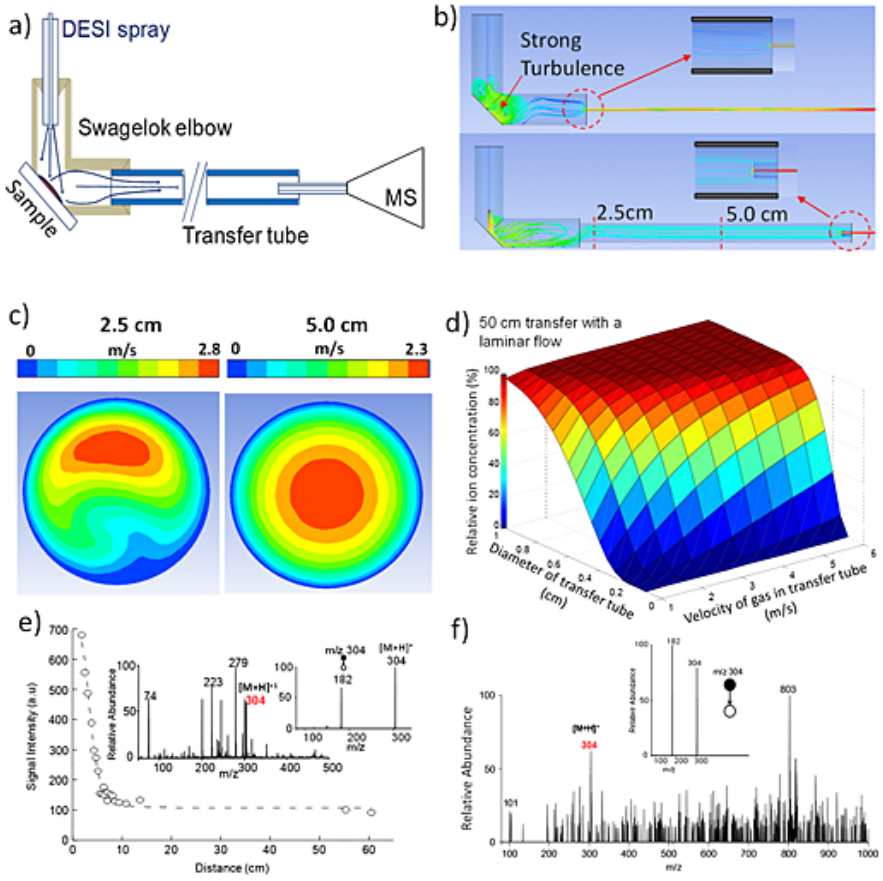

Figure 1.5 Model for the long-distance ion transmission via laminar gas flow (a) Schematic of remote DESI setup (b) Simulation of fluid dynamics of long-distance ion transmission through a 0.55mmID tube and a 4.3mmID tube (c) Map of flow velocity at the cross section at the length of 2.5 and 5 cm (d) Relative ion concentration at the transfer distance of 50cm as the function of tube diameter and gas velocity. (e) Signal intensity of cocaine ion along different transfer distance (f) Mass spectrum from the analysis of 5µg cocaine using a 2m-long 4.3mmID Tygon tubing. These figures are from [90].

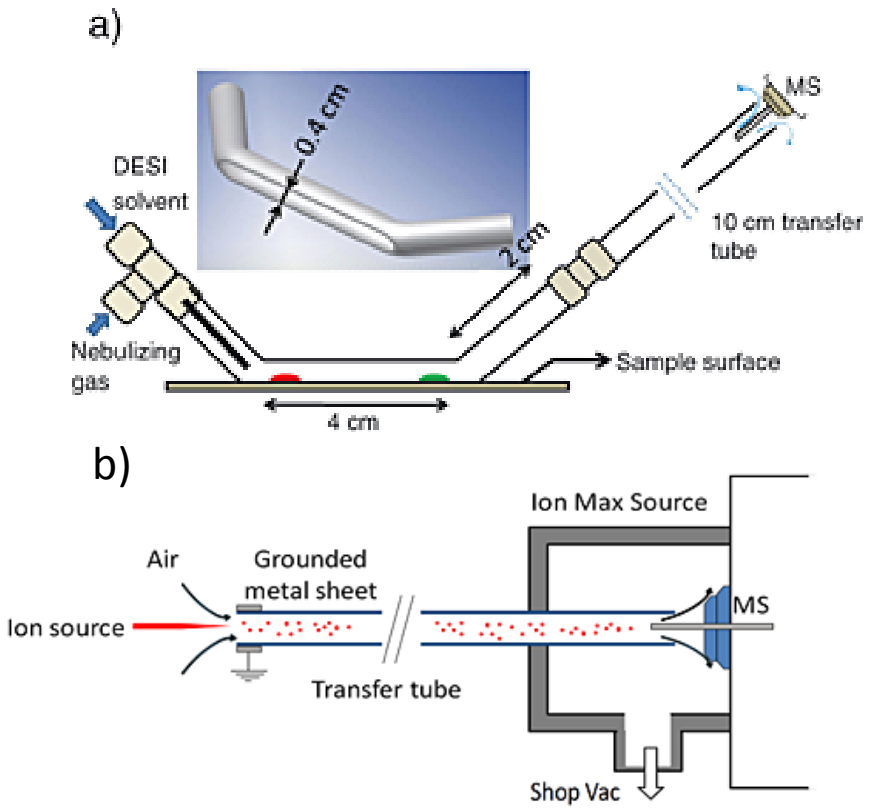

Figure 1.6 (a) Configuration of remote DESI. The front end of the transfer tube is close. (b) Configuration of the ion source without the use of gas. The front end of the transfer tube is open. These figures are from [90].

Competitive study has been performed for transferring ion produced by plasma-based and spray-based ambient ionization methods. Cocaine of 1.7μg[90] and RDX of 10μg[47] have been detected by plasma-based method, LTP, via ion transfer using a Tygon tube. Better sensitivity has been obtained for the spray-based ambient ionization methods, such as DESI[89], compared to plasma-based ambient ionization methods such as LTP. It was proposed that charged droplets might help to provide better transmission efficiency compared to dry ions in the atmospheric pressure. The solvated ions might survive better than the dry ions over a long-distance transfer. Based on this, long-distance ion transfer

could be maintained in the state of charged droplets and rapid release of the ions through heated capillary or gas when they are close to the vacuum of mass spectrometer.[91]

1.2 Miniature Mass Spectrometer

Mass spectrometer has played an important role among modern analytical instruments based on its high sensitivity and specificity. Due to its bulky size and complicated function, it is usually set up in the analytical lab. This limits its broad applications for on-site analysis.[92] In some situations, quick results from the on-site analysis are necessary, such as for screening of explosives at the checkpoints and disease diagnosis in clinics; in many situations, quick analysis could save time and cost for sample collection and storage, such as for agrochemical analysis in the garden[93] and surface analysis in the MARS[94]. On-site mass spectrometry analysis relies on the availability of portable mass spectrometers and this drives the development of miniature mass spectrometer.[95-96]

1.2.1 Mass Analyzer

Different types of mass analyzers have been used in the mass spectrometers, and most of them have been miniaturized. Ion trap,[97-98] quadrupole,[99] and time-of-flight[100] have been used to build the integrated systems.

1.2.1.1 Ion Trap

The quadrupole ion trap (Paul trap) was developed in 1953.[101] It is consisted of two hyperbolic endcap electrodes and a hyperbolic ring electrode. Linear ion trap mass

analyzer including four hyperbolic quadrupole rods and two end electrodes was also developed, with ion storage capacity improved.[102-103] The good examples of simplification/miniaturization of ion trap mass analyzers include the development of rectilinear ion traps,[97,104] cylindrical ion traps,[98,105] and micro-fabricated quadrupole ion traps.[99] The simplification of the hyperbolic surface of the quadrupole rods to a planar surface was used in the examples of rectilinear ion trap. A good mass resolution still can be obtained.

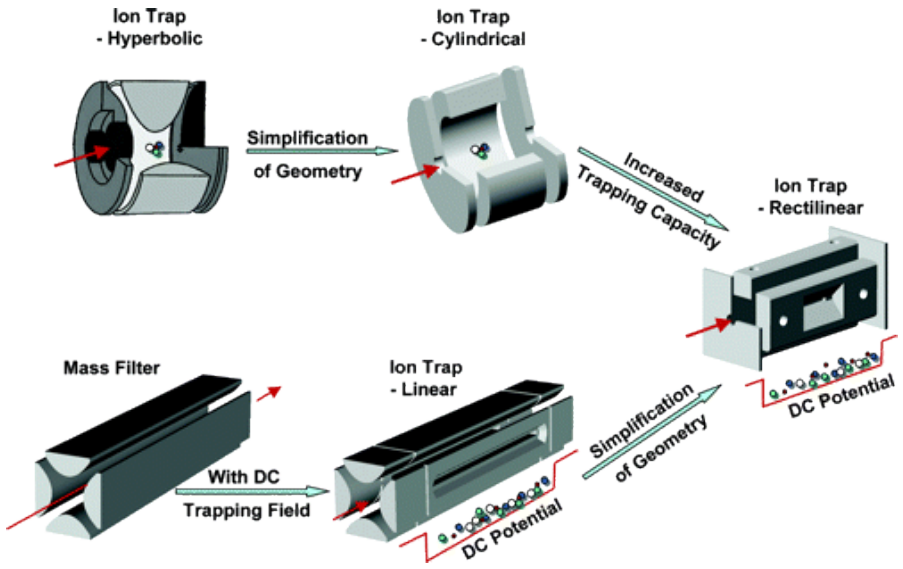

Figure 1.7 Different types of ion trap mass analyzers and the conceptual evolution of rectilinear ion trap mass analyzer. This figure is from [97].

The quadrupole ion trap has been miniaturized to the size of 20μm with the technology of micro electro mechanical system (MEMS). An array of ion traps has been made on a chip (Figure 1.8). The driving rf voltage was lowered down to 45V, and rf frequency was raised to 100MHz.[106]

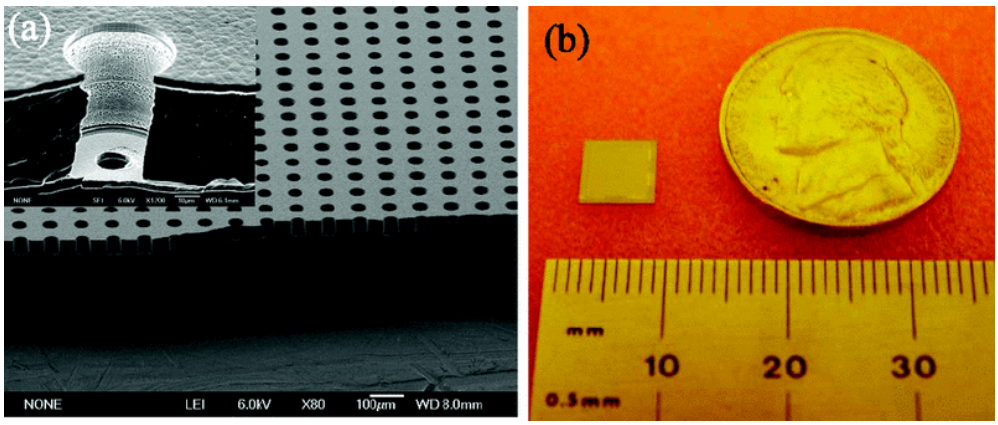

Figure 1.8 Micro-fabricated quadrupole ion trap (a) Imaging of scanning microscope and (b) picture of the quadrupole ion trap chip. This figure is from [106].

1.2.2 Sample Introduction and Atmospheric Pressure Interface

Atmospheric pressure interface (API) is an important component for miniature mass spectrometer with ambient ionization capability. Ions are expected to be sent to the vacuum from the atmospheric pressure environment. The API interfaces can be divided into two main categories, continuous atmospheric pressure interface (CAPI) and discontinuous atmospheric pressure interface (DAPI)[107]. The size of the miniature mass spectrometers and the design of the pumping systems can be determined by the API interfaces.

1.2.2.1 Continuous Atmospheric Pressure Interface

Continuous atmospheric pressure interface (CAPI) was tested in miniature mass spectrometer. The pumping requirement of miniature mass spectrometer is much higher for using the CAPI since the air is continuously brought into the mass spectrometer. One

of the solutions for reduction of the pumping requirement is the minimization of internal diameter of the sampling capillary. A narrow stainless steel capillary with internal diameter of 127μm by direct connection between atmospheric environment and vacuum with continuous sampling was tested (Figure 1.9).[108] A small pumping system including a miniature turbo pump (Pfeiffer TPD 011, 10L/s) and a small diaphragm pump (KNF Neuberger model 1091-N84.0-8.99, 5L/min) was used and 15mtor could be achieved with this configuration. A mass resolution (fwhm) is 3 under this working pressure, and the loss of mass solution is expected to be compensated by tandem mass spectrometry.

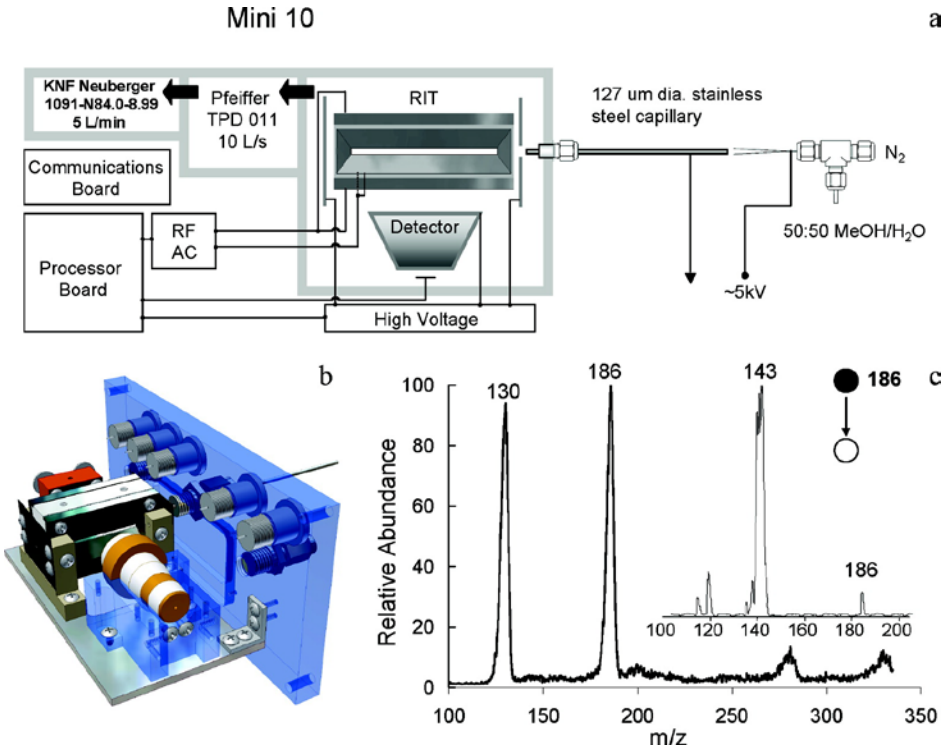

Figure 1.9 Mini 10 mass spectrometer coupled with a 127 μm stainless steel capillary for continuous sampling (a) Schematic of the whole device (b) Three-dimensional diagram of the whole device (c) mass spectrum of mixture of dibutylamine (m/z 130) and tributylamine (m/z 186) using electrospray ionization. The figures are from [108].

1.2.2.2 Discontinuous Atmospheric Pressure Interface

Discontinuous atmospheric pressure interface (DAPI) was developed for miniature mass spectrometer, that allows to couple to a miniature pumping system.[107,109] The sequential control of open and close time of DAPI via a pinch valve[109] or pulsed pinhole[110] could much reduce the need of pumping requirement. The Mini 10 handheld rectilinear ion trap mass spectrometer was developed with a DAPI interface, and the total weight of mass spectrometer is about 10kg by using the miniature pumping system with rough and turbo pumps with 5L/min and 11L/s, respectively (Figure 11.1a).[108] The open time of DAPI depends on the length and internal diameter of the stainless steel capillary and the volume of the manifold, and the pump down time is determined by the volume of the manifold the pumping speed of the turbo pump. Typically, a 13ms open time and 600ms close time are used with a 500μm ID capillary to achieve 1 mtorr for MS analysis in the Mini-10 rectilinear ion trap mass spectrometer (Figure 1.11b).

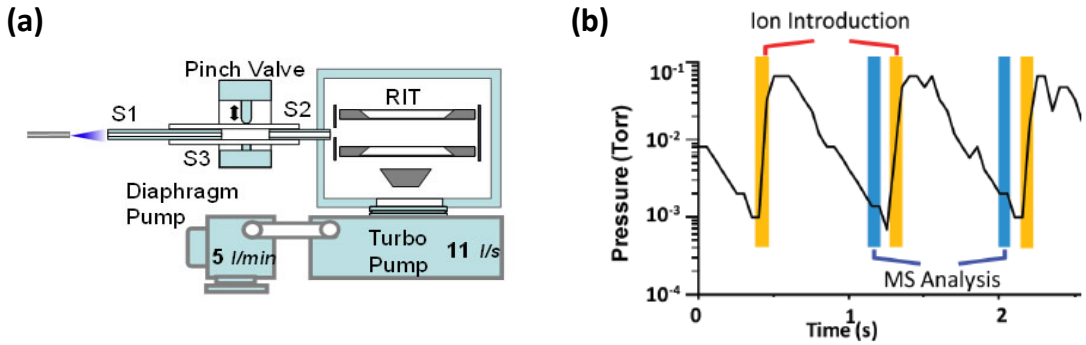

Figure 1.10 Discontinuous atmospheric pressure interface (DAPI) (a) Schematic of a Mini-10 rectangular ion trap mass spectrometer with DAPI and (b) Pressure change was monitored along with the sequential control of open and close of DAPI. The figures are from [107].

1.3 Conclusion

Mass spectrometry has become an important analytical tool for both scientific research and regular chemical analysis. Ambient mass spectrometry provides a way to analyze a sample in its native state with a fast, real-time approach. Different kinds of ambient ionization methods, including the plasma-based, spray-based, and laser-based methods, have been developed in recent years. In the thesis work, one of the plasma-based methods, LTP, was used to analyze semi-volatile compounds, including the agrochemicals, explosives, illicit drugs for the forensics. An in-line LTP was also developed for a handheld probe for easy analysis of the target. One of the spray-based methods, DESI, was used to analyze non-volatile compounds such as lipids for clinical diagnostics. A remote sampling probe for surgical and endoscopic procedures was also developed.

Mass spectrometer is usually used in the analytical laboratories. Miniature mass spectrometer could be easily used in any indoor or outdoor place, where there is a need for continuous monitoring or quick chemical analysis. Different designs of miniature mass spectrometers have been built in the last decade. A strong intent of design is to make it smaller and smaller due to the need of light, handheld analytical instruments. A handheld mass spectrometer provides easy access to the target of analysis, such as direct analysis of fruit on the tree, and quick screening of passenger's luggage at the checkpoint. Another direction in design is to obtain mandatory performance while keep in a compact size at the same time. A mass spectrometer of a compact size but adequate performance

could fit the need of on-site and indoor analysis, such as intra-surgical tissue analysis and monitoring of manufacturing process in the factory.

In order to provide the feasibility of easy operation of this system, the idea of miniature mass spectrometer systems with the sampling probes was proposed. The sampling probe provides easy access to the point of interest and could be easily handled by the operator. In this thesis work, a backpack mass spectrometer system with a handheld LTP has been designed for on-site forensic analysis[111]. An intra-surgical mass spectrometer system with a remote sampling probe[91] has been designed for real-time tissue analysis. More miniature mass spectrometer systems are expected to be designed for more applications in the future.

1.4 References

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CHAPTER 2. DESIGN OF PORTABLE MASS SPECTROMETERS WITH HANDHELD PROBES: ASPECTS OF THE SAMPLING AND MINIATURE PUMPING SYSTEMS

2.1 Introduction

Mass spectrometry (MS) is widely used in chemical and biological analysis. The analysis procedure typically requires samples to be collected from the field and analyzed using mass spectrometers in the lab. In-field, real-time analysis outside the analytical laboratory is of significant interest for environmental monitoring,[1] forensic analysis[2] at the crime scene, detection of explosives[3] and chemical weapons[4] at military and civilian check points, for control of pesticide residues for food safety[5], and for intra-surgical chemical analysis[6] for disease diagnosis. These types of applications can benefit from the miniaturization of the MS analytical systems, a topic which has been actively pursued using the combination of miniature mass spectrometers and ambient ionization sources.[7] Different types of mass spectrometers have been miniaturized, including time of flight,[8] quadrupole,[9] and ion trap systems.[10] The advantages of ion trap analyzers include their small size, relatively high operating pressure and the MS/MS capability. Currently, several portable ion trap miniature MS instruments have been developed using ion trap mass analyzers, such as Mini10,[11] Mini 11,[12] and Mini12[13] from Purdue University, Tridion-9 GC-MS[14] from Torion Inc. (American Fork, UT), GC/QIT[15] from Jet Propulsion Laboratory, Chemsense 600[16] from Griffin Analytical Technology LLC.

(West Lafayette, IN), and MMS-1000 from 1st Detect Inc. (Austin, TX). To use mass spectrometers outside the analytical laboratory, it is also important to have simple analytical procedures that enable complete analysis starting with the samples in their native states. Ambient ionization methods have been developed for this purpose.[17] Currently, several ambient ionization methods, including the low-temperature plasma (LTP),[7b, 18] paper spray,[13] and extraction spray,[13] have been demonstrated with good analytical performance with miniature ion trap mass spectrometers.

The concept of sampling probes for miniature MS systems has also been explored for ease of operation during real-time analysis in the field. Different configurations have been investigated for designing a sampling probe with an ambient ionization source (Figure 2.1). The first approach is to use a long tube (Connection I in Figure 2.1a) to remotely transfer the desorbed ions from the ambient ionization source to the vacuum manifold containing the mass analyzer. This concept was initially demonstrated with desorption electrospray ionization (DESI)[19] using a long stainless steel tubing and later with tubing of larger diameter for flow-assisted ion transfer.[20] The flow dynamics in the ion transfer process was also studied.[21] For clinical applications, a rapid evaporative ionization mass spectrometry system (REIMS) system was developed in which tissue was ionized by a surgical knife and the ions subsequently transferred to a mass spectrometer for analysis.[6a] A sampling probe using DESI and a narrow (1.6 mm i.d.), flexible plastic tubing as long as 4 m was also designed for endoscopic sampling purposes. MS analysis of the remotely transferred ions produced lipid profiles of good quality for tissue sections and intact organs.[22] Transfer of dry ions generated by atmospheric pressure chemical

ionization or LTP over long distances has been shown to have relatively lower efficiency in comparison with sprayed ions. [21, 23]

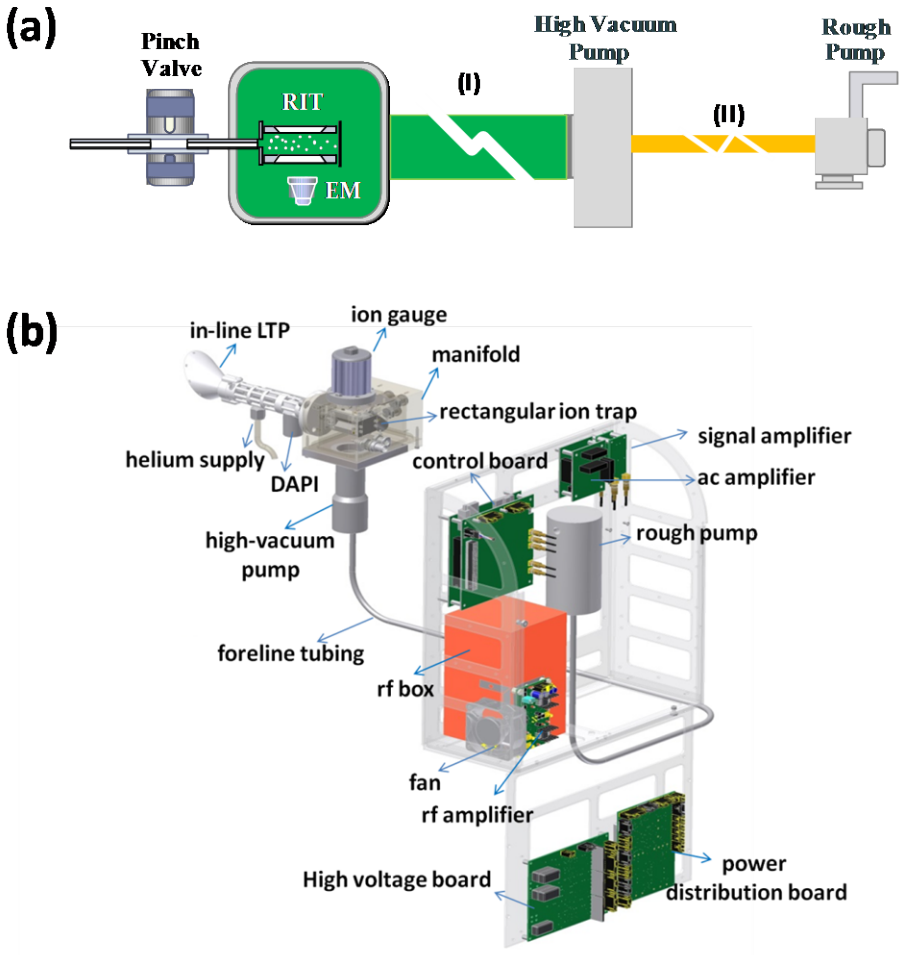

Figure 2.1 Schematic configurations showing (a) general case for pumping system and a miniature ion trap instrument with a DAPI (pinch valve) and (b) specific case of a backpack MS system with the high-vacuum pump integrated into the handheld sampling unit so that connections I and II can be minimized in length while connection III can be made long

The second approach is to keep the sampling/ionization source close to the vacuum manifold containing the mass analyzer, while extending the connection (II in Figure 2.1a) between the manifold and the high vacuum pump, which is packaged together with the control electronics. This concept was first demonstrated using a 1.2 m long bellow (25.4

mm i.d.) with a modified Mini 10 instrument[24] which practically resulted in a much enlarged manifold volume. Using the discontinuous atmospheric pressure interface (DAPI)[25] for ion introduction, a longer time was required for each scan cycle but improved sensitivity was obtained. A backpack MS system has recently been developed using this configuration with a coaxial LTP.[7d]

The third possible configuration is to integrate the high vacuum pump to the handheld sampling unit while using a long tube (Connection III in Figure 2.1a) for connection to the rough pump/control electronics package. The advantage of this configuration is that a much narrower tube (Figure 2.1b) can be used for this foreline connection, since the pressure inside this tube is about 5 torr and molecular flow is by the rough pump.[24] In comparison with the second configuration described above, faster scan speeds can be obtained with DAPI since no additional volume is added on the high vacuum side. This configuration, however, would not be practically applicable unless the high vacuum pump is small enough for integration into the handheld unit, while still providing sufficient pumping. In this study, we use several new prototype miniature pumps developed by Creare Inc. (Hanover, NH) to test this third configuration. The performance of several systems with different combinations of high vacuum and rough pumps was characterized.

2.2 Experimental section

The handheld unit used has a manifold (71x74x108mm) containing a rectilinear ion trap, a channel electron multiplier (DeTech2300, Detector Technology Inc., Palmer, MA) with a conversion dynode, and a DAPI interface. The control electronics of a Mini 11[12]

was used to test MS performance. The flow-constraining stainless steel capillary of the DAPI was 5cm long and of 1.5mm o.d. and 0.5mm i.d. To operate the coaxial LTP probe,[7d] an ac voltage of 1,000V at 30kHz was used to induce a helium discharge (flow rate 0.2L/min). 2,4-Dinitrophenol (DNP) dissolved in pure methanol solution was used with nanoESI for these test analyses. Methanol solutions of an agrochemical (diphenylamine), an illicit drug (cocaine), and explosives (DNP, 2,4,6-trinitrotoluene) were prepared and 1μL of each was deposited on a glass slide to form a dried sample spot for LTP analysis. A Pirani gauge (series 925C, MKS Instruments Inc., Andover, MA) was directly mounted onto the vacuum manifold to record pressure. Fans were used to air-cool the prototype pumps during operation.

A flat and polishing surface around a hole with 30mm diameter was made on the manifold for testing these three high vacuum pumps. Pfeiffer Hipace 10 turbo pump (Pfeiffer Vacuum Inc., Nashua, NH) was connected to the manifold with a connection adapter (DN 25 ISO-KF) and controlled by its integrated electronics with 24V DC power supply. Creare 550g turbo pump was directly connected to the manifold and supply DC voltage was increased slowly after its desired rotation speed 100k rpm was achieved. Creare 130g drag pump was directly connected to the manifold and was driven by a microprocessor-controlled drive and control unit (DCU) to achieve 200k rpm rotation speed. Two rough pumps, 2-stage diaphragm pump KnF N84.3 (KNF Neuberger Inc., Trenton, NJ) and Creare 130g scroll pump were tested. The diaphragm pump was driven by a 24 V DC power and scroll pump was controlled by DCU to achieve 3k rpm rotation speed.

2.3 Results and discussion

The pumps used in this study are shown in Figure 2.2 and their specifications are listed in Table 2.1. Note that the actual pumping speed of a pump changes when the inlet and foreline pressures vary from the specified values. The combination of a HiPace 10 turbo pump and a KnF N84.3 diaphragm pump used for developing the Mini 10[26] and has been a popular selection for commercial handheld instruments. The Creare 550g turbo pump was used for construction of the 4 kg Mini 11 system.[12] Both the HiPace 10 turbo pump and the Creare 550g turbo pump can provide an ultimate vacuum much below 1 mtorr. However, for miniature ion trap mass spectrometers with the DAPI interfaces, the working pressure of the manifold is 1 mtorr with increases up to 1 torr during gas pulse introduction.[25] Thus, a medium to high vacuum pump with lower compression ratio but with a high pumping speed achieved using only molecular drag stages might be more suitable. To explore this, a Creare 130g drag pump with only drag stages was developed. Without the turbo stages, the miniature high vacuum pump could be fabricated at lower cost and potentially can be more robust for operation during movement of the instrument. Both the Creare 550g turbo and 130g drag pumps have better form factors than does the HiPace 10 turbo pump for integration to a handheld unit. A newly developed Creare 350g scroll pump was also tested as an alternative rough pump. In comparison with the diaphragm pump, the scroll pump runs extremely quietly, does not vibrate or require maintenance such as replacement of diaphragms, which makes it very attractive for the future development of miniature MS systems.

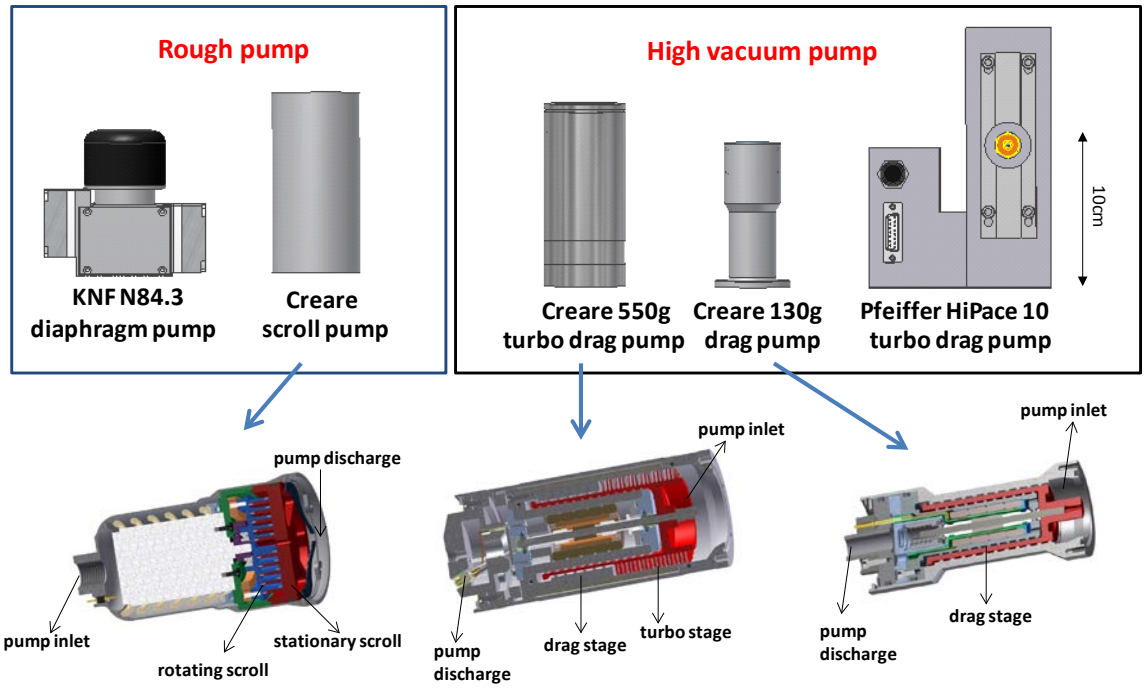

Figure 2.2 Backing (rough) and high-vacuum pumps used in this study

Table 2.1 Specifications of rough and high vacuum pumps used in this study

Pump	Weight (g)	Pumping Speed	Compression Ratio	Max. Foreline Pressure	Max. Power (W)	Rotor Speed (rpm)	Inlet ID (mm)
KnF N84.3 Diaphragm Pump	900	5 L/min (at 1atm)		760 torr	18		
Creare130g Scroll Pump	350	1 L/min (at 1torr)		760 torr	6		
HiPace 10 Turbo Pump	1,800	10L/s (at 0.1mtorr)	$3x10^6$	18 torr	28	90k	24
Creare 550g Turbo Pump	550	>4 L/s (at 0.1mtorr)	$1x10^9$	10 torr	12	100k	53
Creare 130g Drag Pump	130	>4 L/s (at 0.1mtorr)	$1x10^5$	10 torr	12	200k	25

The feasibility of implementing a long, narrow connection between the high vacuum and rough pumps for miniature instrument was first tested with a Mini 10 fitted with the HiPace 10 turbo pump and KnF N84.3 diaphragm pump. Comparison was made between the original configuration using a 10 cm tube of 4.8 mm i.d. (Figure 2.3a) and the new configuration with a 6 m long tube of the same i.d. (Figure 2.3b). By using a DAPI opening time of 13 ms to analyze the ions generated by nanoESI of 5 ppb DNP in methanol, the manifold pressure was raised to 300 mtorr and then pumped down during the closed cycle. The total time of each scan cycle was set as 600ms. For the original configuration with the 10 cm tube, this was sufficiently long to allow the manifold pressure to return to 1 mTorr after the first scan cycle. However, the base pressure increased as the scan cycle was repeated and the signal intensity for the protonated DNP decreased significantly (Figure 2.3e), which is likely due to the loss of ions during trapping and less efficient ion ejection during the RF scan at the elevated pressures.[27] This indicates that the delay time between each two adjacent opening events of the DAPI was not long enough to allow the gas introduced to be fully pumped out. With the same scan time, the base pressure of the manifold was maintained significantly better for the configuration with a 6 m connection between the high vacuum and rough pumps (Figure 2.3d, f) and signal intensity was maintained during the continuous cycles.

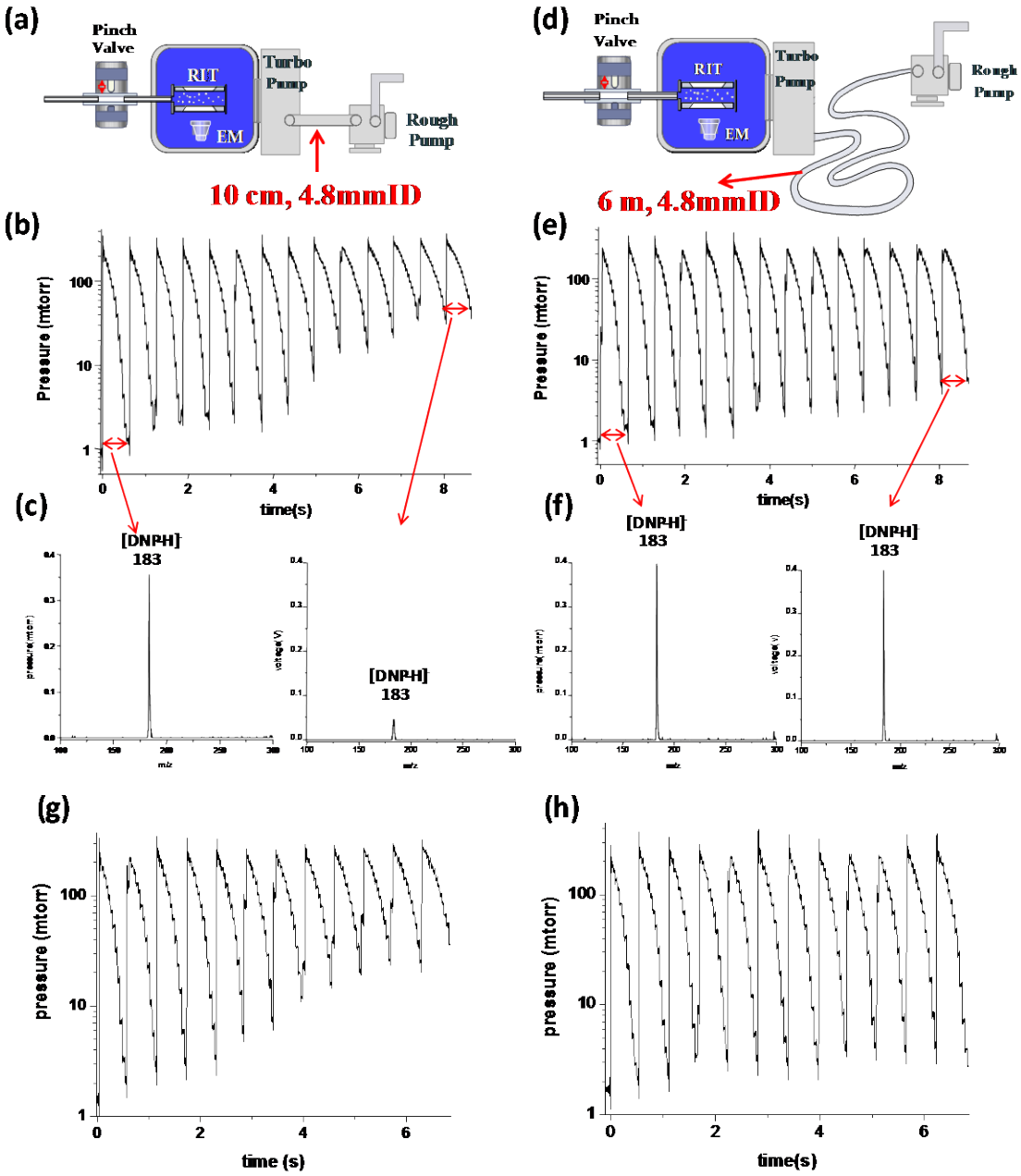

Figure 2.3 Configurations of miniature MS with (a) 10 cm long tube and (b) 6 m long tube as foreline connection between the high vacuum HiPace 10 pump and the diaphragm pump, tube i.d. 4.8 mm. Pressure variations recorded over a number of continuous scans using (c) configuration a and (d) configuration b with 13 ms DAPI opening, 600 ms total time for each scan cycle. Spectra recorded with the 1st and 14th scan for (e) configuration a and (f) configuration b, nanoESI of 5 ppb DNP in methanol

The observed differences are most likely related to the variation of the foreline pressure in the connection tube, which has an impact on the pumping speed of the turbo as well as the rough pump. With a significantly longer turbo-to-rough connecting tube, the foreline volume was much enlarged and the increase of the foreline pressure due to the introduction of the same amount of gas during a DAPI opening event was thereby also much decreased. For a validation of this hypothesis, the same test was done with another configuration, in which a 58 cm long bellow of a larger i.d. of 38 mm was added in series with the original 10 cm long, 4.8mm i.d. tubing. With this additional volume of 635cm^3 added into the foreline connection, the base pressure of manifold could be maintained at 1 mtorr with continuous operation and with each scan cycle 600 ms.

The three high vacuum pumps, HiPace10 turbo pump, Creare 550g turbo pump and Creare 130g drag pump, were then tested with the Creare 350g scroll pump using a 1.3 m long tube of 3.2mm i.d. for the foreline connection. A DAPI opening time of 13 ms was used and the delay time after the DAPI closing was adjusted to find out the shortest scan cycle time that allowed a stable base manifold pressure to be maintained for a large number of continuous scans. The results of this characterization are shown in Figure 2.4. The pressure went up to about 200 mtorr for both the HiPace 10 tubo pump and Creare 550g turbo pump (Figure 2.4a, b). However, the manifold pressure was pumped down to 1 mtorr faster with the Creare 550g turbo pump and a scan time of 300 mscould be used instead of 550 ms for the HiPace 10 turbo pump. Although HiPace 10 turbo pump has a better specified pumping speed for molecular flow at the ultimate pressure, Creare 550 turbo pump is a more efficient pump in the higher pressure range above 1 mtorr.

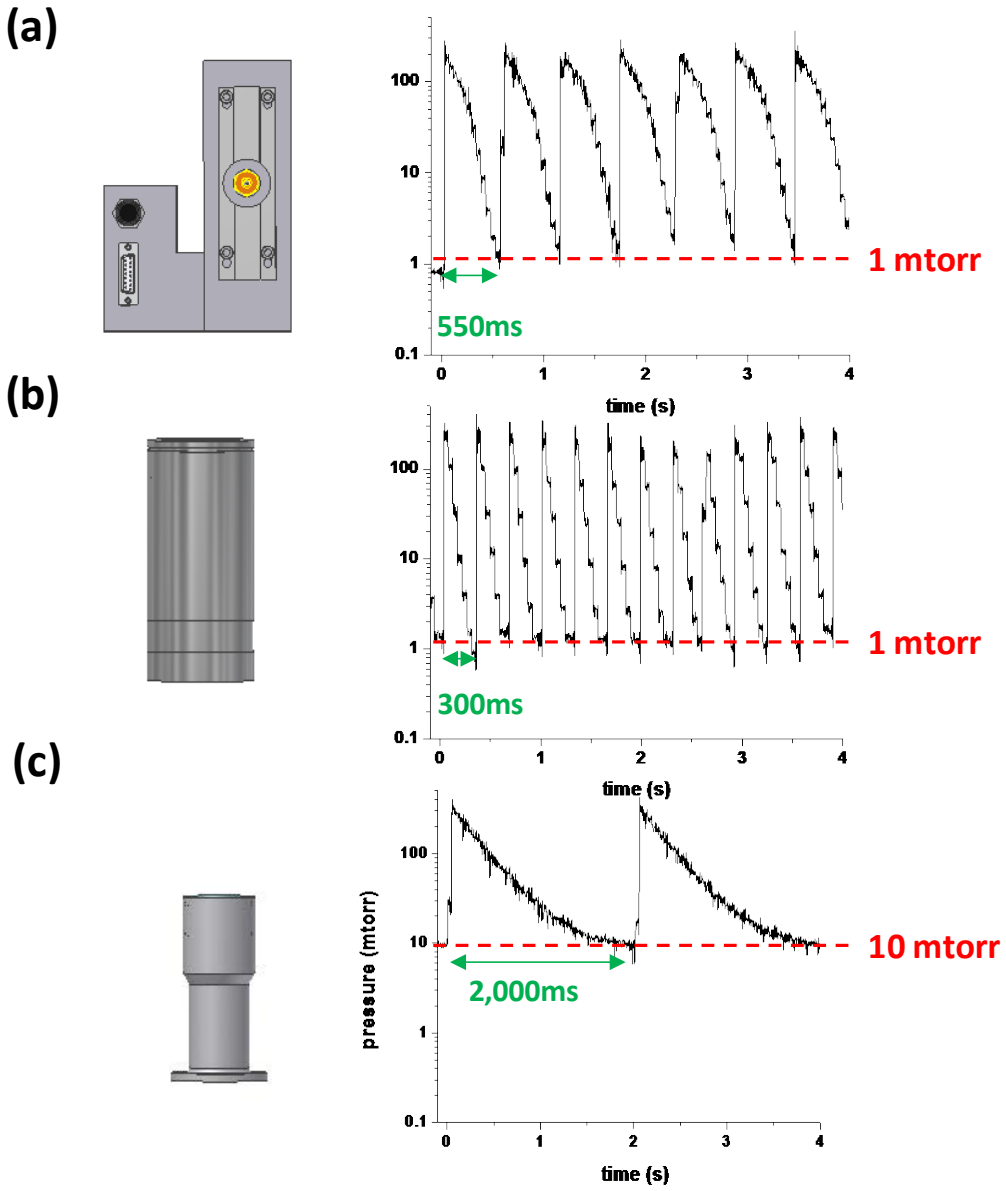

Figure 2.4 Identification of shortest scan cycle time with sustainable base pressure of manifold during continuous scanning for (a) HiPace 10, (b) Creare 550 and (c) Creare 130, each connected via a 1.3 m long tube of 3.2 mm i.d. with the Creare scroll pump

For the smaller Creare 130 drag pump with only pure drag stages, the manifold pressure went up to about 300 mtorr during the DAPI opening. This could be due to a

smaller total volume in high vacuum, which affects the pressure buffer as previously discussed.[24] Note that both the manifold size and the pump size contribute to the volume of the high vacuum region. It also took a longer time (2 s) for the pressure to decrease to 10 mtorr. The Creare 130g drag pump can maintain a vacuum at 10^{-4} torr when its inlet was sealed directly without any leak. When working with the manifold and DAPI of the handheld sampling unit, 10 mtorr was the lowest base pressure it could continuously sustain.

The new backpack MS configuration with high vacuum pump on the sampling probe connected through a long and narrow tube to the rough pump in the backpack was then tested with each of the two Creare high vacuum pumps by connecting with the Creare 350g scroll pump for the direct analysis of low volatility chemicals from the glass slides. The coaxial LTP previously developed with the backpack MS was used as the desorption ionization source. Figure 2.5 shows a collection of mass spectra recorded for 1 ng DPA in positive mode and 10 ng TNT in negative mode using Creare 550g turbo pump (Figure 2.5a, b), 10 ng cocaine in positive mode and 20 ng DNP in negative mode using Creare 130g drag pump (Figure 2.5c, d). Although the MS analysis using the ion trap was performed at 10 mtorr with Creare 130g drag pump, good sensitivity and resolution was still obtained. For the case of DNP, 2ng detection limits could be obtained. The resolution (full width at half maximum) was 0.7 when MS analysis was operated at 1mtorr with Hipace 10 turbo pump or Creare 550g turbo pump. The resolution dropped to 1.8 when MS analysis was operated at 10 mtorr with Creare 130g drag pump. With MS/MS performed for structural confirmation (insets in Figure 2.5c, d), the slight loss in resolution due to the MS analysis at higher pressure might not be a significant concern.

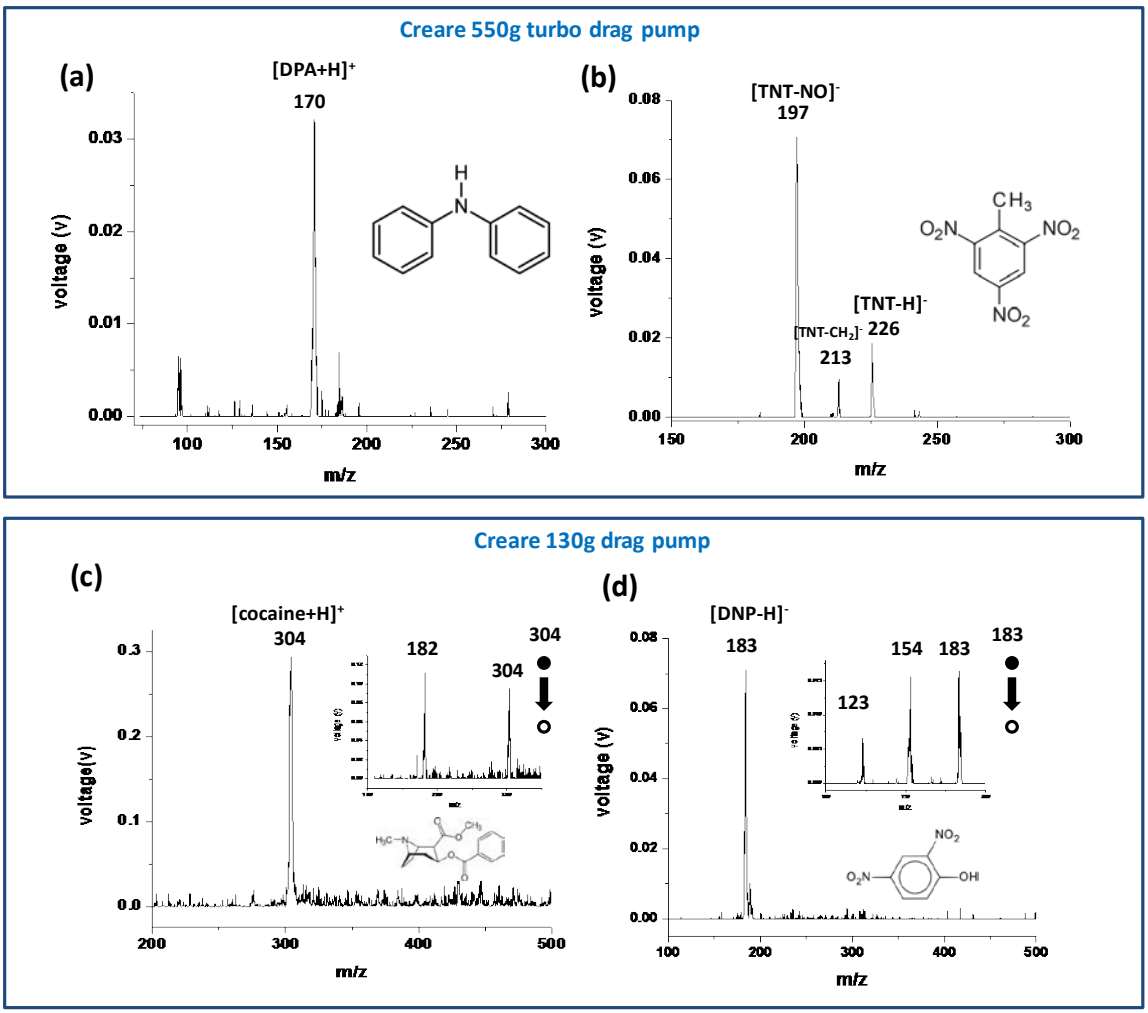

Figure 2.5 Mass spectra recorded using a handheld sampling unit with a coaxial LTP ion source for direct analysis of chemicals from glass slides, (a) 1 ng DNP and (b) 10 ng TNT (negative) with Creare 550 turbo pump; (c) 10 ng cocaine (positive) and (d) 20 ng DNP (negative) with Creare 130 drag pump. Both high vacuum pumps were connected via a1.3mlong tube of 3.2mmi.d. with the Creare scroll pump.

2.4 Conclusions

In this study, we explored the concept of using small prototype pumps for designing miniature MS systems fitted with sampling probes and discontinuous interfaces to ambient ionization sources. The use of a long, narrow tube to extend the connection between the high-vacuum and rough pump was demonstrated to be a feasible solution for

designing a sampling probe using DAPI for ion introduction. Pump configurations using a quiet scroll pump in combination of high vacuum pumps as light as 130g were demonstrated to be promising.

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CHAPTER 3. DEVELOPMENT OF A MASS SPECTROMETRY SAMPLING PROBE FOR CHEMICAL ANALYSIS IN SURGICAL AND ENDOSCOPIC PROCEDURES

3.1 Introduction

Mass spectrometry (MS) is a powerful tool for general purpose analysis of complex mixtures at high sensitivity and selectivity. Routine analytical procedures using mass spectrometry require sample preparation and chromatographic separation[1, 2] although direct analysis of complex mixtures is possible using tandem mass spectrometry provided that the ionization process is soft.[3-5] The recently developed ambient ionization methods allow direct ionization of complex chemical and biological samples in their native state.[6-14] Ambient ionization MS has been shown to provide adequate sensitivity and to be compatible with MS/MS for identification of mixture components.[7, 15-20] High precision in quantitation has been shown recently using the ambient ionization method of paper spray.[18, 21]

For MS imaging of tissue samples, very limited sample preparation is possible both from a perspective of access to the tissue and also so as to preserve the original distribution of the analytes in the sample.[22-25] In future planned applications to diagnostics during surgery, only a limited amount of time is available for sample manipulation[22-25] These considerations and the desire to obtain chemical information at localized positions on the surface of an organ, mean that ambient ionization has particular

advantages for imaging tissue, at atmospheric pressure without pre-treatment and on a dimensional scale compatible with surgery.

Though MS imaging is being developed as an important tool for drug discovery,[26, 27] biomarker discovery,[28] and disease diagnosis,[29-31] quick tissue analysis,[32-35] or profiling[36, 37] with ambient ionization method can also play an important role in biomedicine to provide highly specific chemical information of the sample in a timely fashion.[38] As demonstrated by prior experiments using desorption electrospray ionization on excised tissue sections[39] and by results from the rapid evaporative ionization mass spectrometry (REI-MS) method,[40, 41] ambient ionization can be effectively used for real-time chemical analysis during surgery. In this study, we explored the development of a sampling/ionization probe using a modified form of desorption electrospray ionization (DESI) for surgical and endoscopic procedures. Previous studies on small sample sets of human liver [36], bladder [42], kidney [43], prostate [28], testicular [44] and brain [29, 45] cancers performed using DESI-MS imaging, show correlations of lipid distributions with pathology. A mass spectrometry sampling probe could enable an *in-vivo* tissue characterization to facilitate the diagnosis as well as the decision making during an open or laparoscopic surgery and the endoscopic procedures. Colon rectal (CR) cancer is the 4th leading cause of cancer morbidity in the US and early diagnosis is essential for successful treatment. *In-vivo* chemical analysis could provide important information on the cause of the colon inflammation[46, 47] and subsequent colon cancer. [48-51] To use such as sampling probe, the analytes on tissue surfaces need to be ionized and transferred over several meters in tubes thin enough to insert through probes for laparoscopic or endoscopic procedures. The main concerns relating to design of an ambient ionization

endoscopic probe are the sensitivity for the analysis and the safety of the operation. In the REIMS method, the analytes are evaporated by the surgical tools, transferred to the vicinity of the MS inlet using a gas flow and ionized in the course of analysis. In our design, the desorption ionization event occurs at the sample surface. It has been shown previously that the species ionized by DESI can survive a long distance transfer with the gas flow from a DESI source.[52, 53]DESI has been shown to be effective in desorption ionization of nonvolatile organic compounds and biomolecules directly from tissue samples.[36] High voltage, high velocity gas flow, and organic solvents have been used to facilitate the desorption ionization at high efficiency;[16] however, these conditions are not compatible with the safety requirement for clinical in-vivo endoscopic operations. Recently, *n,n*-dimethylformamide (DMF) / ethanol solvent system has been used for DESI to minimize the damage of the tissue,[54] which allows the same tissue sample to be used for other imaging procedures. Although these and other morphologically friendly solvents are still not biocompatible with *in-vivo* applications,[55, 56] this approach is insightful to show that DESI conditions could be varied while preserving the ionization efficiency. In the course of this work, a probe with an inside diameter as small as 1/16 inch (outside diameter 1/8 inch) were developed in conjunction with a desorption ionization source. This probe easily fits into an endoscopic tube. The conditions for performing DESI were varied systematically and optimized to improve safety during in-vivo operation while retaining the necessary sensitivity for chemical analysis.

3.2 Experimental section

Rat brain tissue sections (thickness=10μm) were sliced by a microtome inside a freezer and put on glass slides. E. coli polar lipid extract was purchased from Avanti polar lipids, Inc. (Alabaster, Al). Tygon tubings (R-3603) were purchased from the VWR Scientific (Sanfrancisco, CA). Intact rat kidneys and intestine were taken after the sacrifice of rat. The intestine was cut open so the mucosal surface could be analyzed. Solvents used in the experiments include pure water (D.I. water from Milli-pore Milli Q system) and methanol/water (1:1), and pure methanol (Mallinckrodt Baker, Inc., Phillipsburg, NJ).

Exactive Orbitrap and LTQ linear ion trap mass spectrometers (Thermo Scientific Inc., San Jose, CA) were used for mass analysis. The original heated capillary was replaced by an extended capillary. A diaphragm pump (four-stage diaphragm pump N813.4 from KNF Inc., free flow rate=13L/min) was connected to the back end of the Tygon tubing when operating intact organ analysis. A vacuum gauge (series 925C micropirani transducer) which offers a measurement range from 10^{-5} torr to atmosphere was used here to measure the pressure at the end of the tubing.

3.3 Results and discussion

The design of the endoscopic probe is shown in Figure 3.1a. A Tygon tubing of 1/8" (3.17 mm) o.d., 1/16" (1.59 mm) i.d. and with a length of up to 4.0 m was used to transfer the ions from the sample to the mass spectrometer. The internal diameter of the working channel can be as large as 5 mm[57] for a laparoscope and 3.7 mm for a colonoscope.[58] For real clinical applications, a custom-designed tubing with proper outside diameter needs to

be made for specific applications. Tygon tubings used in this study for proof-of-concept demonstration were made from the nonconductive material Tygon-3603,[53] which were chosen for the design, due to the good flexibility, softness, and chemical resistance of the material. A coaxial fused silica capillary sprayer was made by inserting a capillary of 50µm i.d. and 150µm o.d. into a capillary of 530µm i.d. and 700µm o.d. The inner capillary was used to deliver the solvent while the outer capillary was used for the auxiliary nitrogen gas flow. The front end of the capillary sprayer was inserted through the wall of the Tygon tubing as shown in the inset of Figure 3.1a. During the sampling for analysis, the end of the Tygon tubing was pushed against the sample surface, with ~3mm between the sprayer and the surface of the sample. When pushed against the sample surface, the soft edge of the Tygon tubing sealed the surface. The spray solvent and the auxiliary gas delivered at 4-8 µL/min and 1.5-5.2 L/min, respectively, were contained inside the tubing without leaking. This is important for a real operation with an endoscopic probe.

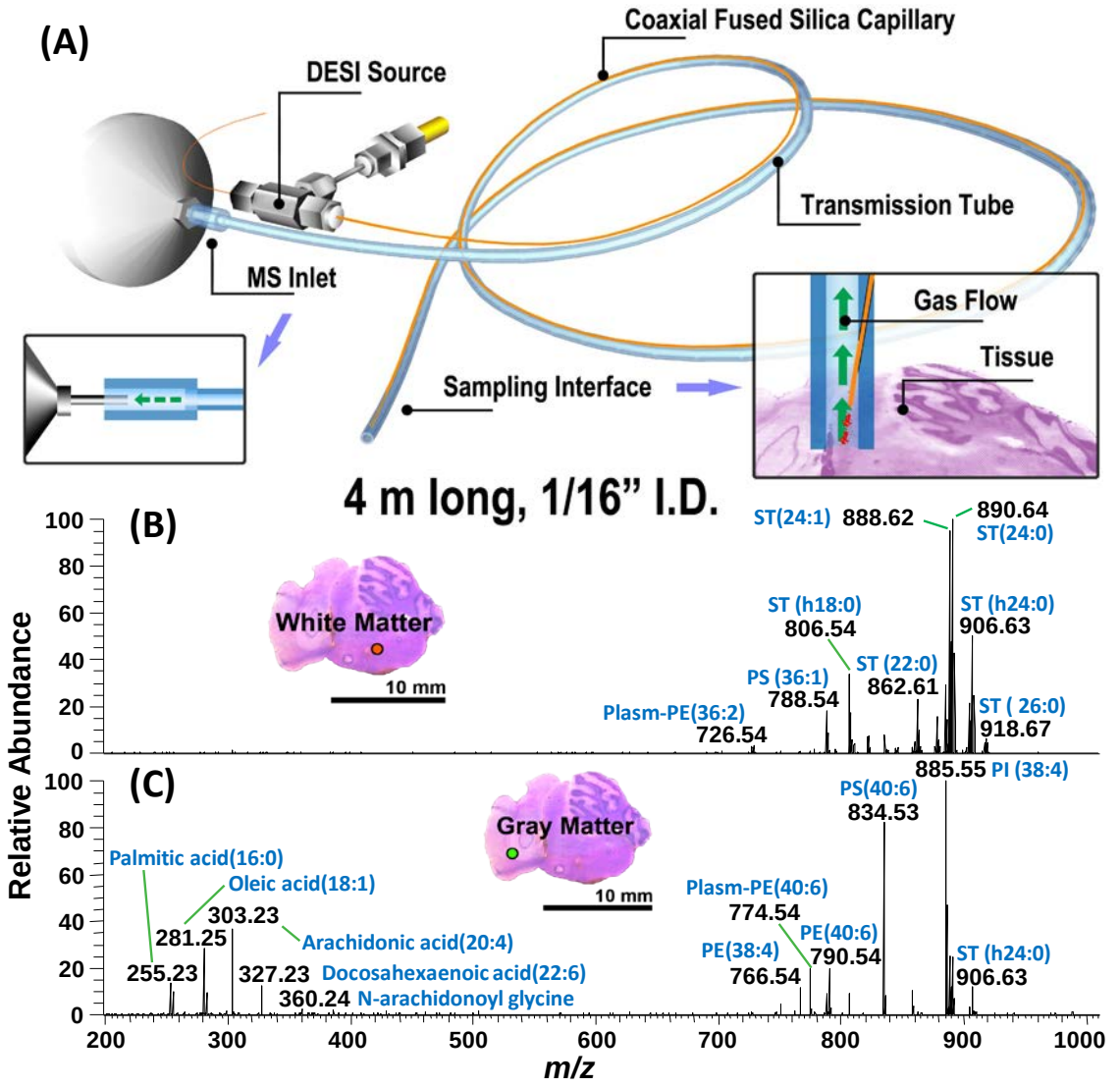

Figure 3.1 (a) Schematics of an endoscopic sampling ionization probe which composed of a coaxial capillary sprayer and a transfer tube. A probe with a 4mlong, 1/16" i.d. tubing was used for the analysis of the rat brain tissue section, with the spectra recorded for (b) the white matter and (c) the grey matter. Gas flow rate of 4.3 L/min, high voltage at -4.5kV, methanol/water 1:1 as spray solvent.

It has been previously demonstrated that the analytes on surface can be sampled and ionized by DESI and efficiently transferred by the gas flow from the DESI source through a flexible bent tubing.[52,53] The transfer efficiency of the ionic species is dependent on the speeds of transfer toward the MS inlet and the radio diffusion towards the inside wall of the tube.[53] With a much smaller 1/16" i.d. of the tubing used for the design of the endoscopic sampling probe, a low transfer efficiency potentially due to the diffusion of the ions to the tube wall was a significant concern. In an initial test, the typical conditions for DESI, viz. high gas flow rate at 4.3 L/min, methanol/water (1:1) solvent as spray solution, and high voltage of -4.5kV, were applied to test the efficiency of the desorption and the transfer over long distance using the Tygon tubing. The sampling probe was coupled with the MS inlet simply using a short Tygon tubing of 1/8"i.d. and ~2cm length (Figure 1a). The MS capillary inlet was located at the center of the opening of the coupling Tygon tubing. The gas flow from the sprayer was also used for ion transfer and allowed to exhaust at the end of the coupling Tygon tubing.

Probes of different lengths were made and tested for analyzing rat brain tissue sections. Surprisingly, excellent signals were obtained from lipids and fatty acids with a probe length of 4 m, which is sufficiently long for an endoscopic probe. The time delay between pushing the sampling probe against the tissue section surface and obtaining the signals was about 0.5 s. The mass spectra of white and gray matter are shown in Figure 1b and c. The profiles of lipids and fatty acids observed are pretty similar to those previously reported for DESI analysis.[22] The fact that the ions survive long distance transfer through a 1/16" i.d, tubing supports the hypothesis previously revealed that the ions might be continuously generated from the charged droplets during the transfer.[53]

This also indicates that ambient methods based on droplet extraction might be more suitable for the design of endoscopic probes for MS analysis. Although adequate transfer efficiency was obtained for the analyte ions from the sample, the conditions for desorption ionization must be altered to become compatible with the safety requirement for the endoscopic operation. A series studies were done to characterize the roles of the organic solvent, high electric voltage, and the gas flow rate, based on which the alternative conditions were suggested and tried experimentally. Ideally, the methanol/water solvent should be replaced by pure water as the spray solvent. It is known from the studies of the spray-based ionization methods, that addition of methanol in the spray solvents helps the formation of smaller droplets during the spray and the subsequent desolvation of the analyte ions.[59] In previously studies, it has been shown that DESI analysis with methanol/water provides significantly higher analyte signals than with pure water.[59] The results of a comparison study using analysis of 0.5μg lipid extract sample on a Teflon slide are shown in Figure 3.2. A high voltage of -4.5kV was used and the rates for the solvent and gas flow were optimized to get the maximum signals in each case. The signals of plasma-PE(38:6) (m/z=747.52) were three times higher with pure methanol than with pure water, when the DESI was performed at the MS inlet (Figure 3.2a); however, the relative intensities were reversed in the case with a 4 m long probe (Figure 3.2b). The signals observed with pure water were three times higher than those with pure methanol.

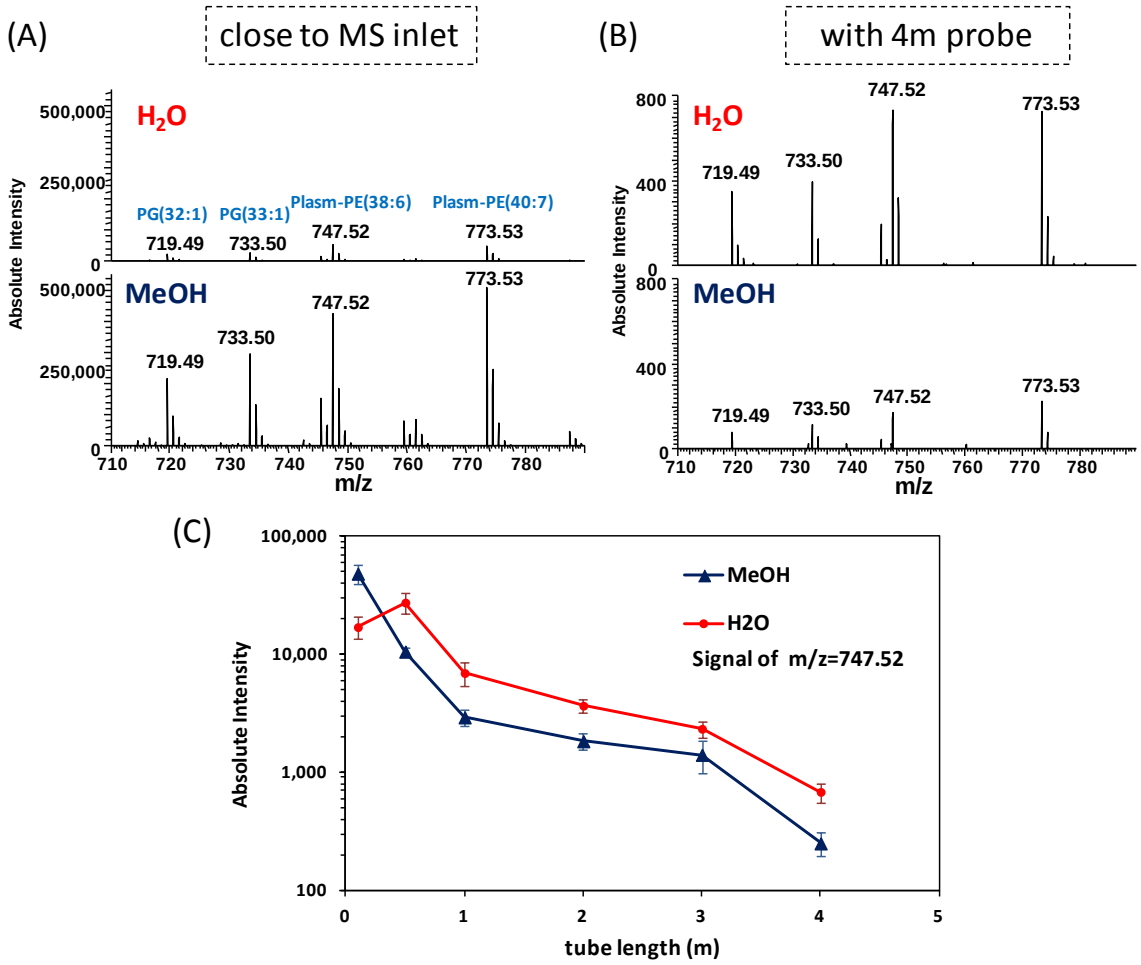

Figure 3.2 DESI analysis of 0.5 µg polar lipid extract deposited on the Teflon slides using pure water or methanol as the spray solvent. (a) Analysis with DESI performed close to the MS inlet, gas flow rate of 1.3L/min, solvent flow rate of 3µL/min. (b) Analysis with 4m probe, gas flow rate at 4.3L/min, solvent flow rate at 8µL/min. (c) Signal intensity of plasma-PE (38:6) (m/z=747.52) recorded with probes of different tube lengths from 0.1 to 4.0m.

To better understand this phenomenon, probes of different lengths were used for this comparison study. As shown in Figure 3.2c, the signal of plasma-PE(38:6) with pure methanol as spray solvent has a monotonic decreasing trend as a function of the probe length. For pure water as the spray solvent, the signal increased when the desorption ionization occurred 50 cm away from the MS inlet. Although the signal intensity also decreased with longer probes, overall the DESI with pure water had a better performance with probes of long lengths. This observation could be explained with the desolvation of the spray droplets and formation of the analyte ions with DESI. Relatively larger primary droplets with water as DESI spray solvent might cause an inefficient desolvation in formation of secondary dry ions for MS analysis, when DESI is performed close to the MS inlet. However, when the droplets containing the analyte ions are transferred over long distance, better desolvation could be achieved through collisions with gas molecules during the transfer and the gradual desolvation might also help to protect the ions from losing charges through reactions. This leads to an overall advantage of using water instead of methanol as spray solution for DESI in the design of an endoscopic probe.

The effectiveness of the high voltage for the spray in the DESI MS analysis has been characterized previously,[16] while desorption ionization also using spray but without applying high voltage, such as easy ambient sonic-spray ionization (EASI), has also been shown to have high efficiency.[60, 61]The efficiency in generation of the secondary dry ions after desorption is dependent on the size and the charge density of the primary droplets, which are subjected to the spray conditions including the voltage and the gas flow speed. A comparison study was first done with the desorption spray source close to the MS inlet without transferring with Tygon tubing, where 0 V or -4.5 kV was used for spray while

the gas flow rate was varied up to 1.75 L/min. The sample of 0.5μg lipid extract on a Teflon slide was used for the analysis. Methanol/water (1:1) was used as the spray solvent. The intensity of plasma-PE(38:6) (m/z=747.52) was monitored as a function of the gas flow rate. As shown in Figure 3.3a and b, at low gas flow rate, the desorption spray ionization benefits significantly (thirty times higher) from the application of a high voltage. However, the impact by the gas flow is much more significant when there is no voltage applied for the spray. At a flow rate of 1.5 L/min or higher, there is no significant difference in desorption ionization efficiency between applying a high voltage for spray or not. This is consistent with the findings in previous studies involving sonic gas flows [62] where high velocity gas flows were found to be helpful to improve the ionization efficiency.

Ideally, use of higher gas flow rates would lead further increase the desorption ionization efficiency; however, practically this was difficult to achieve for desorption ionization performed close to the MS inlet, since the dispersion of secondary ionic species from the sample surface becomes severe that results in a poorer sampling by the MS inlet. Using a sample probe with its end sealed with the sample surface, this is not a concern since the gas containing the ions is forced toward the MS inlet. As shown in Figure 3c, the signal for the analysis with a 1 m probe could be further improved by one order of magnitude when the gas flow increased from 1.5 to 5.5 L/min. There is also no difference for applying a high voltage or not for the spray (Figure 3.3d).

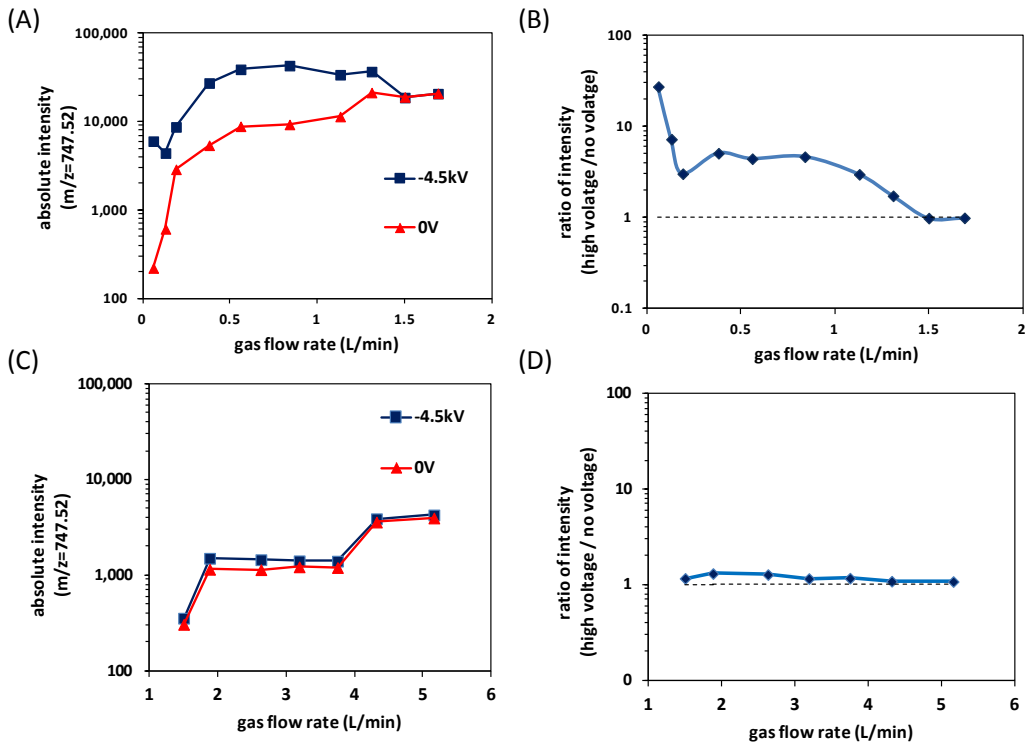

Figure 3.3 (a) Intensities and (b) ratio of the intensities of plasma-PE (38:6) (m/z=747.52) recorded as a function of gas flow rate with or without high voltage, desorption ionization performed close to the MS inlet. (c) Intensities and (d) ratio of the intensities of plasma-PE (38:6) (m/z=747.52) recorded as a function of gas flow rate with or without high voltage, a sampling probe of 1 m used for the analysis.

With the understanding of each role played by the spray solvent, spray voltage and the gas flow in the desorption spray ionization, a 4 m sampling probe operated with pure water, no spray voltage and at a gas flow rate of 5.2 L/min was used in a performance comparison with DESI close to the MS inlet at a typical optimized condition. (Figure 3.4a and b). They were both tested for analysis of rat brain tissue sections. The quality of the spectrum compares well for these two methods in terms of the signal intensity and the species identifiable with the spectrum. As a test for potential applications with endoscopic diagnosis, analysis of the mucosal surface inside the fresh rat intestine was

performed using a 1 m long probe using the biocompatible conditions for desorption ionization. Besides the fatty acids and the lipids, peaks at strong intensities were observed for dimmers of the fatty acids (Figure 3.4c). The potential carryover between analyses was also characterized by moving the sampling probe between the surfaces of the intestine mucosa and a latex glove. It was found that the signals due to the chemicals on the previous sample disappeared completely 4.5 s after the probe was moved to a new surface.

Though efficient analysis using the sampling probe could now be performed without organic solvents or high voltage, which is harmful for in-vivo endoscopic analysis, the damage to the tissues by the gas flow still needs to be addressed. The previously described method of using special organic solvents for DESI to minimize the tissue damage is not a solution for the design of this endoscopic sampling probe. Organic solvents generally are not biologically friendly and the high gas flow is recognized to be important for eliminating the high spray voltage. In addition to its role in the droplet generation and ion desolvation, the gas flow at a higher rate also helps to improve the efficiency in transfer of the ions, either as dry ions, partially solvated or contained in the charged droplets. The gas flow is aiming at the sample surface and pushed back toward the MS inlet, which inevitably results in a worse impact by the gas molecules and the droplets to the tissue at a higher gas flow rate. To make the in-vivo analysis minimally invasive with the sample probe, this issue has to be addressed while retaining good sensitivity for the analysis.

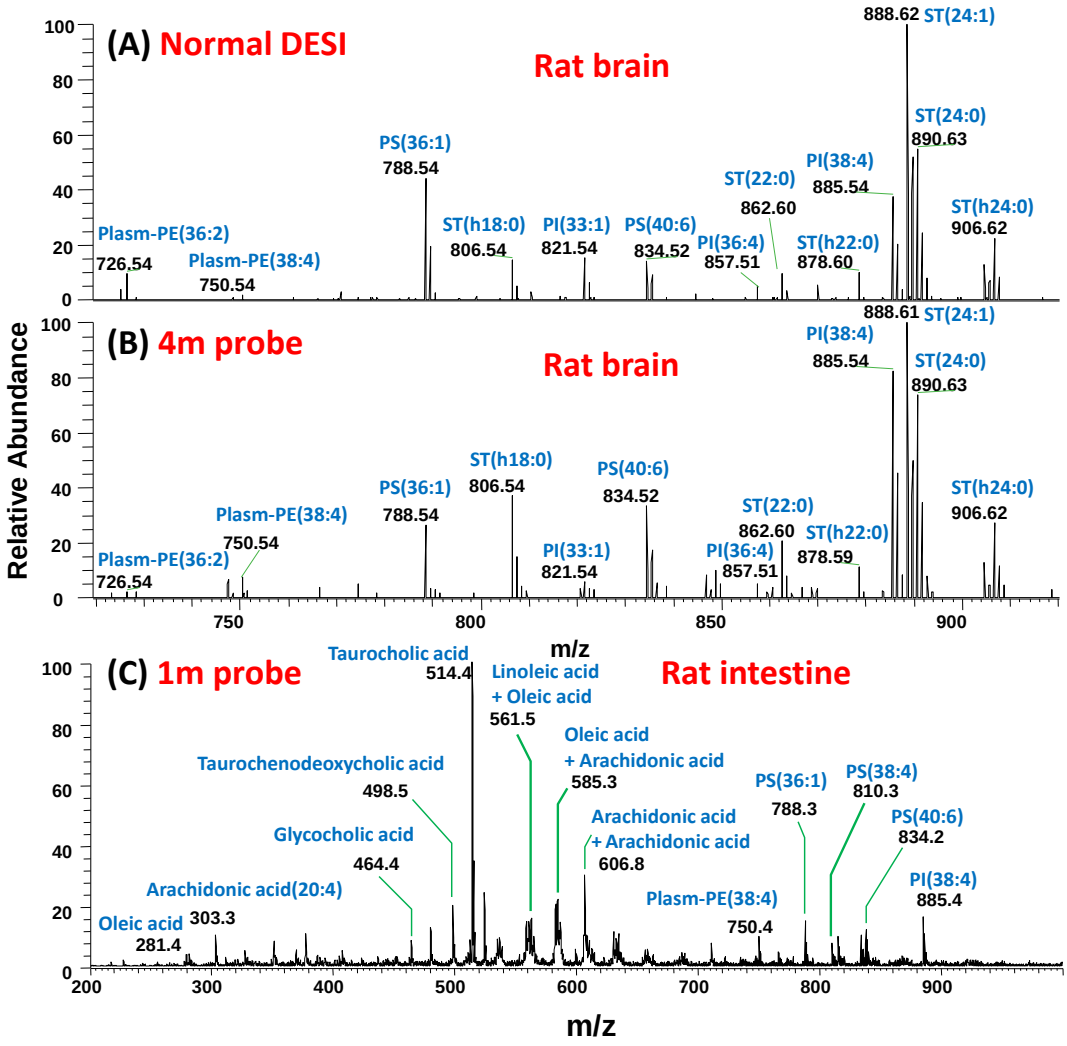

Figure 3.4 Analysis of rat brain tissue sections using (a) a DESI close to the MS inlet with MeOH/H_2O (1:1) as spray solvent and a high voltage of -4.5kV and (b) a 4m probe with water as spray solvent and no high voltage. (c) Analysis of rat intestine using a 1m probe with water as spray solvent and no high voltage. Gas flow rate, 5.2 L/min for 4 m and 1m probes and 1.5 L/min for DESI.

A modification to the coupling of the sampling probe to the MS inlet was developed as shown in Figure 3.5a. A diaphragm pump was used to add a pulling force to drag the gas toward the MS inlet. This revision in the coupling has been found to be significant in terms of preventing the damages to the sample surface. In a test with analysis of fresh rat kidneys, no visible damages were observed using the probe with the pump (Figure 3.5b), while spectra with good signals of the analytes were recorded with water and no high voltage applied for spray (Figure 3.5c). In comparison, marks were made on the kidney surface due to the damages during the sampling ionization when the probe was used without the pump (Figure 3.5b). The pressure inside the probe was found to be significantly changed with the pump. A vacuum gauge connected to the adapter between the end of the tubing and the MS inlet and the local pressure was measure to be 315 torr with the diaphragm pump on. Although 3.8 L/min gas flow rate was used, the pressure inside the probe was lower than the atmospheric pressure. This resulted in a gentle suction helped to form a sealing of the probe end to the sample surface. Although the Tygon material is not as soft as others such as silicone, the kidney tissue is soft and a good sealing was easily achieved without carefully positioning the probe end. Simulations of the front end of the probe were done by ANSYS software as shown in Figure 3.5d to help better understand the experimental observations. The local gas dynamics in the sampling region is changed by the pulling force added with the diaphragm pump. As shown in Figure 3.5e, the average pressure at the sample surface is about 1000 Torr without the pump but is reduced to about 600 Torr with the pump, which

results in a significantly reduced impact by the gas molecules and the droplets onto the sample surface.

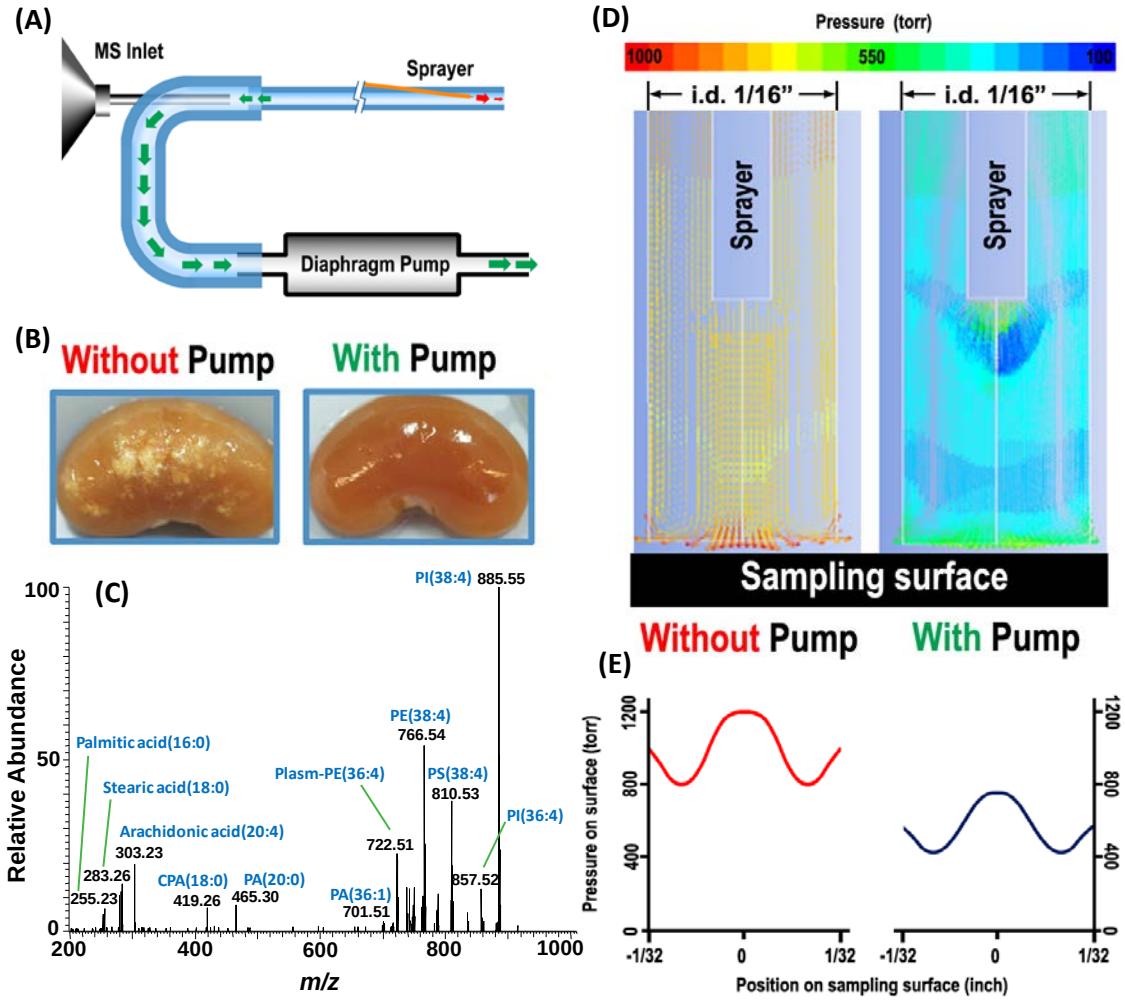

Figure 3.5 (a)Noninvasive sampling probe with the gas flow pulled by a diaphragm pump, 1 m long 1/16" i.d. Tygon tubing, pure water as spray solvent, spray voltage of 0V, gas flow rate from sprayer at 3.8 L/min.(b) Comparison of the surfaces of rat kidneys after sampling without (left) and with (right) the diaphragm pump. (d) Contour maps with streamlines simulated for sampling without (left) and with (right) the diaphragm pump. (e) Pressure distribution along the radius on the sampled surface for sampling without (left) and with (right) the diaphragm pump.

3.4 Conclusions

An attempt has been made to design a sampling probe based on ambient ionization that potentially can be used for endoscopic analysis. Derived from the DESI and the long distance ion transfer method previously studied, the individual roles and overall impacts by applying the electric voltage, spray solvent and gas flow were investigated, which led to the development of performing the sampling analysis at a biocompatible fashion. This work along with other effort in this field shows the potential in the combination of the direct sampling analysis using ambient ionization and the gas flow assisted ion transfer. To be used in clinical diagnosis, much further development is needed to convert this probe to actual devices and operation procedures including cleaning the sample surfaces need to be developed.

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CHAPTER 4. REAL-TIME SAMPLE ANALYSIS USING REMOTE SAMPLING PROBE AND MINIATURE MASS SPECTROMETER

4.1 Introduction

On-site chemical analysis provides information promptly for decision making[1] and can support a wide range of applications, such as the screening of agrochemicals in foodstuffs,[2,3] monitoring of pollution in the environment, and intra-surgical analysis supporting the surgeons' decisions during an operation.[4,5] The implementation of real-time chemical analysis, however, requires that complete analysis procedures be performed at the site of sample collection in a fast and simple fashion. Miniature devices have been developed for different analytical technologies, such as gas chromatography (GC),[6] ion mobility spectrometry (IMS),[7] and mass spectrometry (MS),[8] to enable real-time analysis in the field. MS provides highly specific molecular information for a broad range of compounds; its miniaturization, however, can be challenging due to the vacuum required for mass analysis. Miniaturization of the pumping system has been one of the most critical steps for developing small MS instruments, especially for analysis of non-volatile compounds. Discontinuous atmospheric pressure interface (DAPI) was designed to sample ions from the atmospheric pressure environment with small pumping systems.[9] Ions are introduced with a short opening time (about 13ms) through the DAPI, then trapped in an ion trap over a delay time of several hundred milliseconds, which is required to allow the elevated pressure to decrease back to millitorr level for mass

analysis. A scan cycle as short as 300ms was achieved with a 550g turbo and 350g scroll pump.[10] Various version of integrated ion trap mass spectrometers have been developed with the DAPI interface and small pumping systems,[11-13] with the smallest one weighing only 4 kg.[12]

The significance of having an atmospheric pressure interface for miniature MS instruments lies in its enabling the coupling of ambient ionization methods[14-17] for direct analysis of nonvolatile compounds in complex samples. Ambient ionization allows direct analysis of samples in their native states.[18,19] Ion transfer over a long distance has also been combined with ambient ionization to develop sampling ionization probes that give easy access to the sample.[20-22] Nonvolatile analytes were ionized from the surfaces of the objects of interest and the charged species sent back to a mass spectrometer for MS analysis. It was found that the gas flow[23,24] could facilitate the efficient transfer of the ions over a long distance through a thin tube,[25] which can possibly be inserted in an endoscope for in-vivo analysis during laparoscopic or endoscopic procedures. Real-time analysis of tissue samples was achieved by simply pushing a sealed sampling tip against the tissue and lipid profiles were obtained with the desorbed, charged species transferred over 4m with a 1.6mm i.d. flexible tube. With proper flow of gas applied, no high voltage or organic solvent was required for the desorption ionization, which makes the method compatible for in-vivo analysis. Auxiliary pumping was used to pull the gas for ion transfer, which altered the gas dynamics of the sampling area and minimized the destruction to the tissue surface. [25]

Portable systems with sampling probes have been explored for real-time, in-field chemical analysis. This concept was first demonstrated with backpack mass

spectrometers. A low temperature plasma ionization source and a vacuum manifold containing the mass analyzer were separated from the main body of the instrument but connected via a tube.[26] This configuration was designed based on the idea of bringing the mass analyzer closer to the sample. In a later study,[10] ultra-small turbo pumps were used and incorporated into the handheld sampling unit. In the current study, we attempt to couple a flexible thin-tube sampling probe with a handheld mass spectrometer. The potential advantages of this configuration are the ease of operation with the light-weight and flexible probe and its compatibility with devices like medical endoscopes. The disadvantage, however, mainly is associated with the long distance transfer of the charged species and the potential significant loss in sensitivity for the analysis. An exploratory study was carried out to demonstrate this concept, with an integrated system built and tested for the analysis of chemicals from different sample surfaces.

4.2 Instrumentation

A homebuilt miniature ion trap mass spectrometer, Mini 10,[9,11] was modified for this study. It consisted of a DAPI interface, a rectilinear ion trap (RIT), a channel electron multiplier (DeTech 2300, Detector Technology Inc., Palmer, MA) with a conversion dynode, a Pirani gauge (series 925C, MKS Instrument Inc., Andover, MA), a Pfeiffer Hipace 10 turbo pump (10L/s, Pfeiffer Vacuum Inc., Nashua, NH) and a KnF N84.3 diaphragm pump (5 L/min, KNF Neuberger Inc., Trenton, NJ). The flow constraining capillary for DAPI was 10cm long and of 1.6mm o.d. and 0.5mm i.d. Three rf frequencies (1238kHz, 1038kHz, 760 kHz) were used for trapping and MS analysis of ions in three different mass ranges (m/z 55-460, m/z 78-660, m/z 160-1300, respectively).

The conversion dynode was operated at -3850 V for positive ion detection and +3850 V for negative ion detection.

The sampling probe of 1.5 m length was coupled to the modified Mini 10 as shown in Figure 4.1a. It had a dual-channel configuration, one channel for delivering the nebulizing gas and solvent for spray desorption and the other one for transferring the ions back to the mass spectrometer. The spray tube had a coaxial configuration with an outer tube (510 μm i.d., 1520 μm o.d.) for delivery of nitrogen gas and an inner fused silica capillary (50 μm i.d., 150 μm o.d.) for delivery of solvent. The ion transfer channel was 1.5 m long, 1.6 mm i.d. and 3.2 mm o.d. The sampling head has an opening of 3.2 mm. It was made of a soft material, silicone, so as to provide a seal on the surface. Once the seal is provided, the charged species desorbed from the sample surface by the charged droplets from the sprayer were guided back by the gas flow into the ion transfer channel (Figure 4.1b). An adapter was designed to provide connection between the probe and the Mini 10. An additional diaphragm pump (KnF N84.3, KNF Neuberger Inc., Trenton, NJ) was connected to the adapter to pull the gas inside the ion transfer channel toward the MS. In a previous study,[25] it was shown that a vacuum seal could be produced at the sampling head with the additional pumping and the destruction to the soft samples, such as tissues, was minimized. A flow control meter was used to adjust the pumping flow rate. The nitrogen gas pressure used for desorption ionization was 230 psi, corresponding to a gas flow rate of 0.7 L/min. An additional pump (rough pump 2 in Figure 4.1a) (KnF N84.3, KNF Neuberger Inc., Trenton, NJ) was used to pull the gas inside the tube, which helped to minimize the destruction to soft samples.

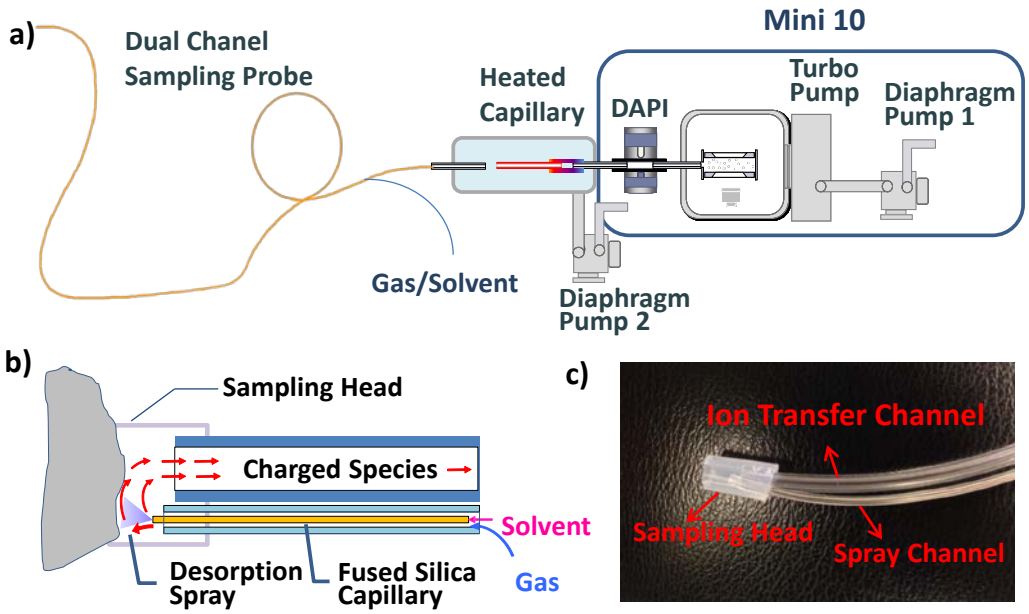

Figure 4.1 a) Schematic of the miniature MS system with integrated sampling probe. b) Configuration of the sampling end of the probe. c) Photo of the sampling end of the probe.

4.3 Experimental

E. Coli polar lipid extracts were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). A sonic spray source[27] was built for ionizing the polar lipid extracts in experiments for optimizing the conditions of the interface between the sampling probe and DAPI. Methanol solutions of agrochemical PCP (2,3,4,5,6-pentachlorophenol) and DNP (2,4-dinitrophenol) were both prepared with an analyte concentration of 100ppm. A blue ballpoint pen (Pilot BPS-GP, Pilot Corp., Jacksonville, FL) was used to write on the print paper (Business 4200, Xerox Corp., Norwalk, CT) to create the ink samples. For the study of the aging of the ink samples, another blue ballpoint pen (S.K.B. SB-1000 0.5 mm, Kaohsiung, Taiwan) was used to write on the print papers to prepare the ink sample, some of which were then exposed to a 100 W incandescent lamp for different lengths of time periods to simulate the aging of ink samples under lights. Pure methanol

(Mallinckrodt Backer Inc., Phillipsburg, NJ) was used as the spray solvent for the analysis of PCP, DNP, and ink. Tissue sections of rat brain, liver, lung, and intestine were prepared at thickness between 10 to 30 μm using a cryostat microtome and mounted on the glass slides. Pure water (D.I. water from Milli-pore Milli Q system) was used as the spray solvent for tissue analysis, and solvent flow rate of 8μL/min was controlled using a syringe pump. No high voltage was used for the desorption spray in any analysis.

4.4 Results and Discussion

In a previous study, it was found that the survival of the ions or the charged species during a transfer in gas flow was dependent on their reactivity.[20,23,28] In this current study, we observed that the types of the materials used for the ion transfer tube had a significant impact on the ion transfer. For an investigation for selection of the transfer tube, six common types of plastic tubes (all 1 m long, 1.6 mm i.d.) were used to construct the sampling probes of 1m. Each of them was coupled to a LTQ (Thermo Scientific Inc., San Jose, CA) and tested for analysis of a rat brain tissue section. Negative ion mode was set for MS analysis. As shown in Figure 4.2, strong signals were obtained for fatty acids and lipids with transfer tubes of conductive silicone, Tygon R-3603, and Tygon ND 100-80, but not for polytetrafluoroehtylene (PTFE), perfluoroalkoxy alkane (PFA) or vinyl chloride (Vinyl). In the previous study[23] using tubes of a larger internal diameter, 4.3 mm, ion transfer was achieved using PTFE, although not as well as Tygon. When thinner tubes were used in these studies, the material effect was much more significant and not even chemical noise was observed with PTFE, PFA or Vinyl tubes. These three materials have a common characteristic: they are known to be easily negatively charged on their

surfaces.[29] In a separate test involving transfer of positive ions using PTFE, the signal was also found to be low. In contrast, conductive silicone is capable of draining the charge. Tygon tubes were designed to have high resistance to the accumulation of chemical residues, which presumably helps to minimize the charging on their surfaces. Among the tested materials, Tygon ND 100-80 is certified for medical applications. We used it for the rest of the experiments reported in this manuscript.

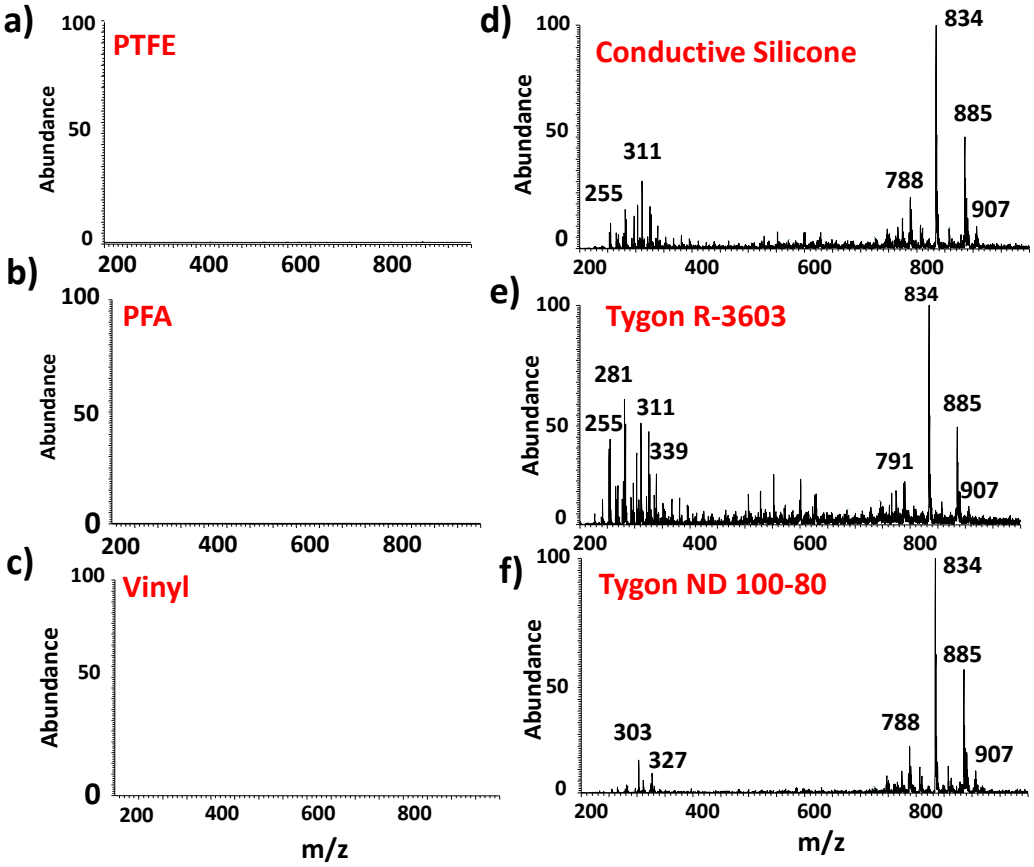

Figure 4.2 Spectra recorded for analysis of rat brain tissue sections using ion transfer tubes made of a) PTFE, b) PFA, c) Vinyl, d) conductive silicone, e) Tygon R-3603 and f) Tygon ND 100-80. Tube lengths of 1 m, 1.6 mm i.d., LTQ in negative ion mode.

The experiments testing the tube material were done in parallel with the optimization of other features of the integrated system to improve the sensitivity for direct sampling chemical analysis. Loss of ions was inevitable over a long-distance transfer. Accumulation of ions in the ion trap analyzer could be one of the solutions. For each ion introduction event, the DAPI open time was limited, since the manifold pressure would otherwise be raised too high and ions could not be effectively trapped. Therefore, multiple ion introductions[30] were used for each MS analysis cycle, as shown in Figure 3a, b. The variation of manifold pressure for a scan cycle with ten ion introduction events is shown in Figure 4.3a. For each ion introduction, 13 ms DAPI open time was used and the manifold pressure rose up to about 100 mtorr. A delay time of 500 ms followed each ion introduction, during which the pressure dropped below 10 mtorr. To test this analysis procedure, a lipid extract sample (100 μg/ml in 90/10 water/methanol) was ionized using a sonic spray source and a 30 cm long tube of 1.6 mm i.d. was used to transfer the ions to the DAPI of the Mini 10. Spectra were recorded for direct analysis of lipid extracts, with the number of ion introduction events was varied. The signal-to-noise ratios (S/N) of plasma-PE (38:6) at m/z 748 were calculated for each spectrum and are plotted as a function of number of ion introduction events in Figure 3b.

Another parameter that was optimized using the same setup for improving the sensitivity was the temperature of the heated capillary. The sampling probe was originally coupled to the DAPI without a heated capillary and it was found the signal intensity was extremely low. In previous studies, it was observed that ions from spray sources could survive much better than low temperature probe or atmospheric pressure chemical ionization sources.[23,28,31] It was proposed that the ions surviving the transfer

process might have been in droplets or as solvent clusters during the transfer and that they became fully desolvated at the inlet of the mass spectrometer. The solvent molecules surrounding the ions could actually protect the ions from loss through reactions. Addition of a heated capillary to the DAPI was expected to improve desolvation significantly. A stainless steel capillary of 10 cm length, 0.5 mm i.d and 1.6 mm o.d. was connected to the front capillary of the DAPI through a ceramic tube (1.6 mm i.d. and 3 mm o.d.) as a heat insulator. The capillary was wrapped with braided fiberglass sleeve for electrical insulation, then coiled with a resistive heating wire (6.75 Ω/ft, part number 30BNC, Consolidated Electronic Wire & Cable, Franklin Park, IL), and coated with alumina adhesive (930HP, Cotronics Corp., Brooklyn, NY) for fixing the wire and heat insulation. Spectra were recorded, with 10 ion introduction events for each scan, for the lipid extracts at different temperature of the heated capillary and the S/N of plasma-PE (38:6) at m/z 748 was calculated and plotted as a function of the temperature as shown in Figure 3d. It was observed that the S/N started to increase significantly once the capillary temperature was raised above 80 °C and continued to increase linearly with the temperature of the heated capillary. The 1.5 m long sample probe was then coupled with the Mini 10 through this heated capillary and tested by analysis of ink on print paper made using the a blue ballpoint pen (Pilot BPS-GP). The signal of crystal violet (m/z 372) appeared when the capillary was heated to 140°C. The S/N was improved by a factor of 2 when the temperature increased to 270°C.

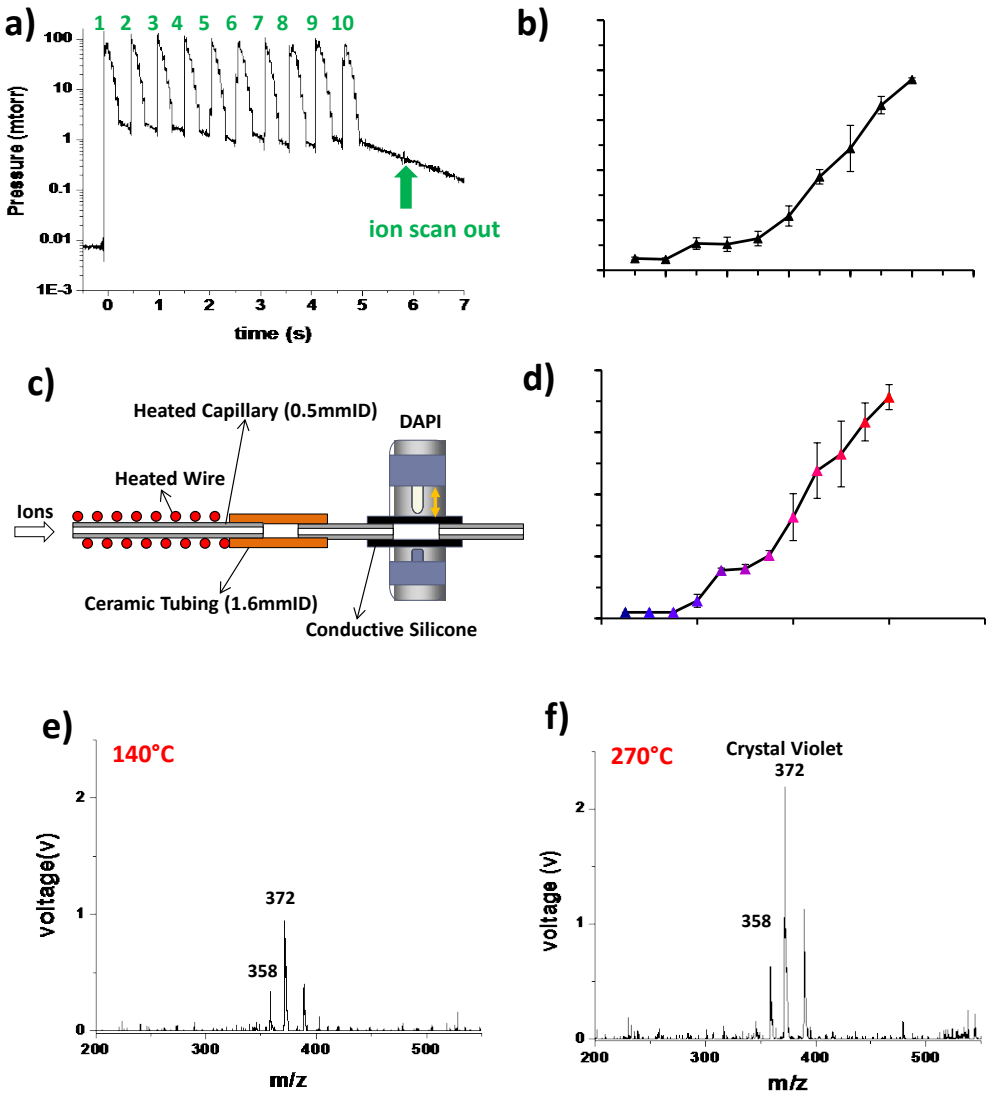

Figure 4.3 a) Variation of the manifold pressure during a scan with ion introduction, DAPI open time 13 ms. b) S/N of peak m/z 748 for plasma-PE (38:6) as a function of the number of ion introduction events for each scan, spectra recorded using a sonic spray ionization source for analysis of lipid extracts, ion transfer tube of 30 cm length and 1.6 mm i.d., heated capillary temperature 270°C. (c) Configuration for coupling of the heated capillary with the DAPI. (d) S/N of peak m/z 748 for plasma-PE(38:6) as a function of the temperature of the heated capillary. (e) Direct analysis of ink on paper using the 1.5m-long dual-channel sampling probe with desorption ionization function, 10 ion introduction events per scan.

The miniature MS system with the 1.5 m-long sampling probe was then tested in a series of applications, such as analysis of agrochemicals, detection of explosives and

signature authentication based on the aging of the ink. Ten ion introductions prior to each mass scan and heating of the capillary to 270°C were implemented. Pentachlorophenol (PCP) is an agrochemical that has been used as fungicide, insecticide, herbicide, and algaecide. Nowadays, it has been banned in ten countries due to its slow biodegradation rate and high toxicity.[32] To demonstrate the idea of quick, on-site analysis, 300 ng of PCP was deposited on a glass slide, and the mass spectrum was obtained immediately after the sampling tip of the probe reached to the point of deposit (Figure 4.4a). The spectrum recorded clearly showed the isotope distribution of $[M-H]^-$ with m/z 263, 265, 267, and 269.

As a demonstration of detection of hazardous substances, DNP, a agrochemical compound that can also be used for making explosives, was detected from a Pelican case (Pelican Storm Case iM2620, 54 x 41 x 27 cm, Pelican Products Inc., South Deerfield, MA) (Figure 4.4b). DNP of 300 ng in 3 µL methanol was deposited in an area of 9 mm^2 on a Pelican case and let dry to form a dried spot. For detection, the Mini 10 was set for MS/MS with $[DNP-H]^-$ m/z 183 as the precursor ion. Ten ion introduction events were used for each mass scan. The sampling head of the probe was moving across the surface of the Pelican case. When it covered the area of the DNP dried spot, the spectrum shown in Figure 4b was obtained, with characteristic peaks of DNP fragment ions at m/z 123 and 125. The use of a flexible probe certainly makes it convenient for checking different surfaces on a large object.

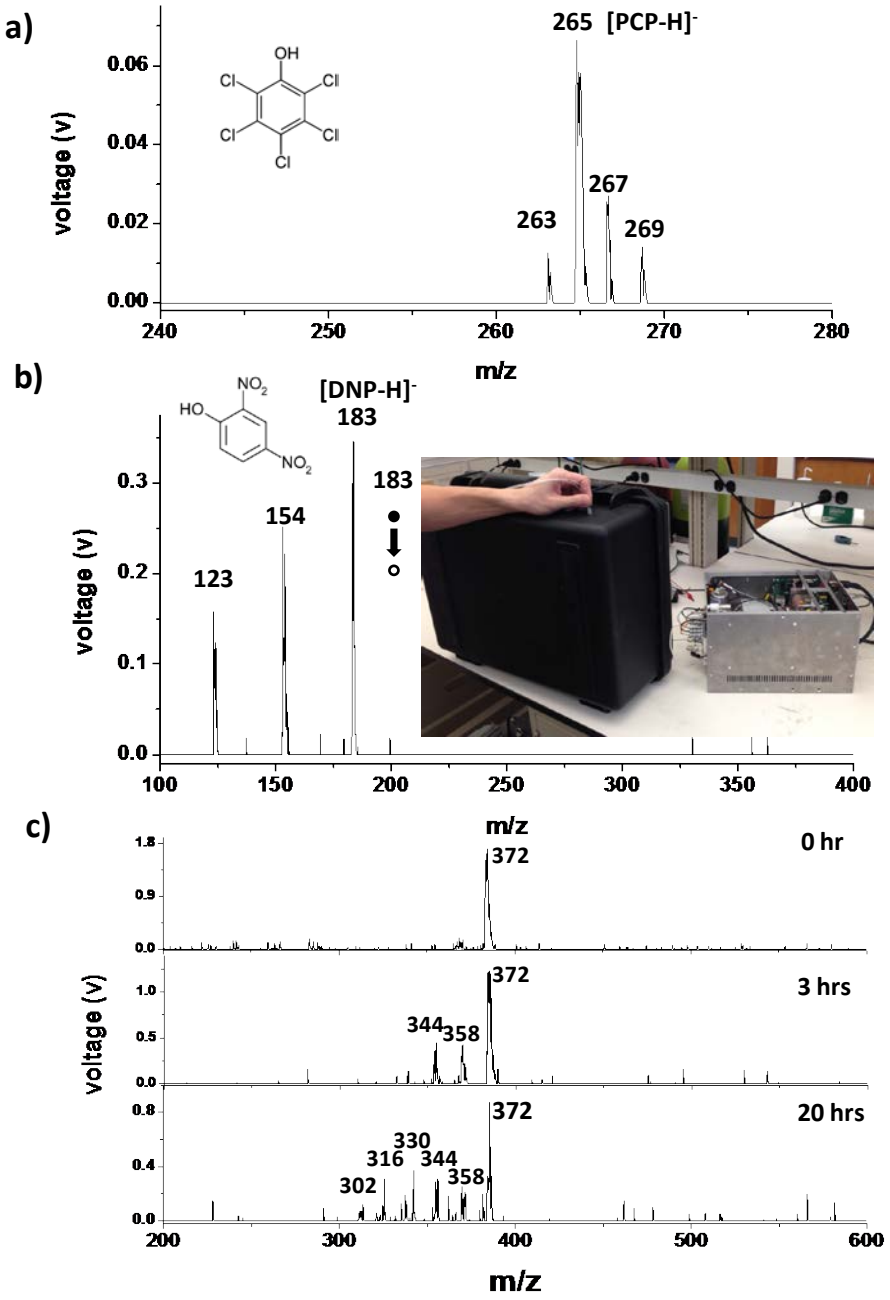

Figure 4.4 Real-time analysis of chemical compounds from the surface using the integrated miniature MS system with a 1.5 m long sampling probe, a) MS spectrum for detection of 300ng PCP on a glass slide, b) MS/MS spectrum for 300ng DNP from the outer surface of Pelican case, c) MS spectra of the blue ballpoint pen ink on print paper after exposure to the light from a 100W incandescent lamp for 0, 3, and 20hrs.

Direct analysis of the document ink, as a means for authentication, has recently been performed using ambient ionization mass spectrometry.[33,34] The photodegradation products of the ink can be observed and used to assess the age of the ink. In this study, ink samples on print papers, prepared using a SKB blue ballpoint pen, were exposed to the light from a 100 W incandescent lamp for 0, 3, and 20 hrs before they were examined using the miniature MS system with the sampling probe. The mass spectra recorded are shown in Figure 4.4c. The major component in the ink, crystal violet (m/z 372) was observed before the photodegradation and two degradation products at m/z 358 and 344 observed after 3 hr exposure to the light, while three additional products m/z 330, 316 and 302 were observed after 20 hr exposure. The mass differences, each of 14 Da, were due to a series of demethylations. The use of a sampling probe for this type of analysis does not damage a document while specific chemical information becomes available for authentication or forensic purposes.

One of the goals for developing a thin and flexible sampling probe for MS analysis was intra-surgical MS specifically in-vivo tissue analysis for use during surgerical or endoscopic procedure.[25] Some recent studies have shown the potential of using real-time MS analysis to provide rich molecular information on tissue to assist in surgical decision making.[5,35,36] The use of a miniature MS system, instead of a lab-scale mass spectrometer, would certainly make the implementation of real-time MS analysis much more convenient in surgical room. We tested the integrated miniature MS system with a 1.5m-long sampling probe using tissue samples from rat brain, liver, lung and intestine (Figure 4.5).

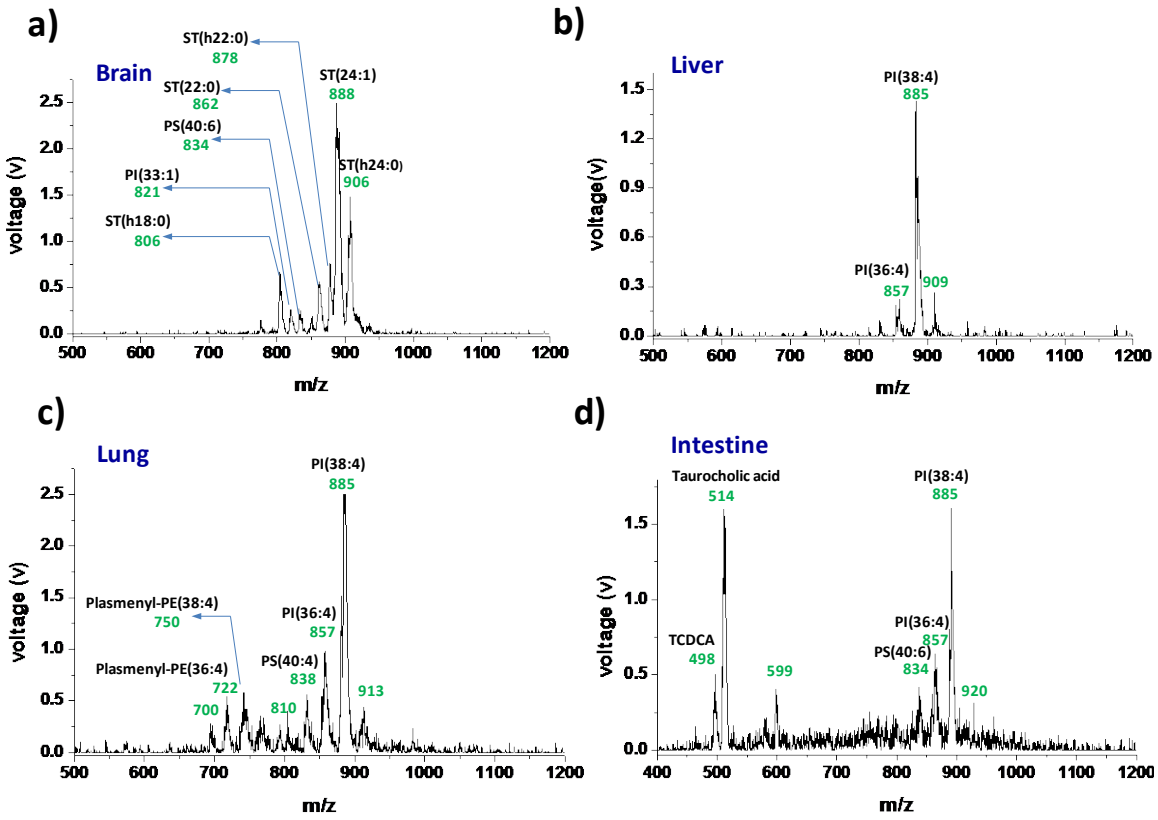

Figure 4.5 MS spectra of tissue sections of rat a) brain, b) liver, c) lung and d) intestine, direct analysis using the miniature MS system with a 1.5 m sampling probe, 10 ion introduction events per scan, 270°C heated capillary, no high voltage or organic solvent applied for the desorption spray, and pure water was used as the spray solvent.

The Mini 10 was operated in negative ion mode with ten ion introductions for each scan, at 270°C heated capillary temperature and recording an extended mass range of m/z 160 to 1300 for analysis of lipids. In order to make sampling compatible with the medical procedure, no high voltage was applied to the spray and pure water was used as the spray solvent for the desorption ionization. The sampling head was pushed against each of the tissue sections on glass slides and the spectra recorded are shown in Figure 4.5a-d. Different profiles of phospholipids were obtained for the rat organs and bile acids were also observed from the intestine. The total time taken to record each mass spectrum was

about 6 seconds. In this study, two separate tubes of 1.5 mm o.d. and 3.2 mm o.d. were used for the proof-of-concept experiment. For future implementation in endoscopic procedures, a dual-channel (1.6 mm i.d. and 0.5 mm i.d.) single tube fitted into the operating channel of an endoscope (<3.7 mm i.d.) or a laparoscope (< 5 mm i.d.) can be easily manufactured by an extrusion process.

4.5 Conclusions

In previous work done by multiple research groups, sampling probes and miniature MS have been proposed and demonstrated independently. The combination of them is of a great interest for future development of integrated MS systems for in-field, real time chemical and biological analysis. In this proof-of-concept study, we investigated the feasibility of combining a thin sampling probe with a miniature ion trap instrument for direct analysis of semi-volatile (e.g. crystal violet, PCP, DNP) as well as non-volatile (e.g. lipids) compounds. The selection of the materials for the sampling probe will be dependent on the efficiency of ion transfer as well as the compatibility to other requirements in the application. Spray-based ambient ionization have been consistently shown to be effective in desorption ionization and protection of ions during the transfer; however, interface to MS with an efficient desolvation would also be critical for the analysis.

4.6 References

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VITA

VITA

Chien-Hsun Chen was born in 1981 in Maioli, Taiwan. He was admitted to National Taiwan University in 1999, majored in Mechanical Engineering. During the third year of the study, he joined Prof. Long-Sun Huang's group and did research in the field of Bio Micro Electro Mechanical Systems (Bio-MEMS). He found interest about using his mechanical engineering background for developing the bio-sensing device, and decided to continue this research as a master's student at Institute of Applied Mechanics, National Taiwan University. The Bio-MEMS sensor developed in this study is capable of detecting trace amount of chemical and biological sample via nanomechanics, and potentially could be implanted into the human body for long-term, real-time monitoring of human health. After getting the master's degree, he went to work at Genomics Research Center, Academia Sinica. His work focused on the development of novel mass spectrometer to solve current challenging analytical problems. One of the successful projects, large bio-molecule mass spectrometer, extends one order of current mass range via charge detection, and provides single large biomolecular ion detection via secondary ion ejection methods. After four years work experience, he came to Purdue University to begin his Ph.D. study and supervised by Prof. Zheng Ouyang on the development of miniature mass spectrometer (mini-MS). He spent two years on the development of mass

spectrometry sampling probe for the chemical analysis in surgical and endoscopic procedures, one year on the development of backpack mass spectrometer, and two years on the development of intra-surgical mass spectrometer system with a remote sampling probe for real-time tissue analysis.

PUBLICATIONS

PUBLICATIONS

Journals

1. **Chien-Hsun Chen**, Z. Lin, R. Graham Cooks, and Zheng Ouyang, "Real-time sample analysis using remote sampling probe and miniature mass spectrometer" (submitted to *Analytical Chemistry*)
2. **Chien-Hsun Chen**, T.-C. Chen, X. Zhou, R. Kline-Schoder, P. Sorensen, R. Graham Cooks, and Zheng Ouyang, "Design of portable mass spectrometers with handheld probes: an aspect of ion introduction and pumping system", *Journal of American Society for Mass Spectrometry* 26, 240 (2015)
3. Paul. I. Hendricks, Jon K. Dalgleish, Jacob T. Shelley, Matthew A. Kirleis, Matthew T. McNicholas, Linfan Li, Tsung-Chi Chen, **Chien-Hsun Chen**, Jason S. Duncan, Frank Boudreau, Robert J. Noll, John P. Denton, Timothy A. Roach, Z. Ouyang and R. Graham Cooks; "Autonomous in-situ analysis and real-time chemical detection using a backpack miniature mass spectrometer: concept, instrumentation development, and performance", *Analytical Chemistry*. 86, 2900 (2014)
4. **Chien-Hsun Chen**, Z. Lin, S. Garimella, L. Zheng, R. Shi, R. Graham Cooks, and Zheng Ouyang, "Development of a mass spectrometric sampling probe for chemical analysis in surgical and endoscopic procedures", *Analytical Chemistry* 85, 11843 (2013)
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Presentations

1. **Chien-Hsun Chen**, Z. Lin, R. Shi, R. Graham Cooks, and Zheng Ouyang, "Development of endoscopic DESI probe and miniature mass spectrometer for real-time tissue analysis" Turkey Run 2014 Analytical Conference (oral presentation)
2. **Chien-Hsun Chen**, Ziqing Lin, Lingxing Zheng, Riyi Shi, R. Graham Cooks, and Zheng Ouyang; "Development of a mass spectrometry sampling probe for chemical analysis in surgical and endoscopic procedures" *Gastroenterology Symposium*, Biomedical Engineering, Purdue University, 2013.
3. Tsung-Chi Chen, **Chien-Hsun Chen**, Xiaoyu Zhou, Robert Kline-Schoder, Paul Sorensen, R. Graham Cooks, and Zheng Ouyang, "Design of portable mass spectrometers with handheld probes: an aspect of ion introduction and pumping system" *61th American Society for Mass Spectrometry Conference*, Minneapolis, 2013.
4. **Chien-Hsun Chen**, Ziqing Lin, Sandilya Garimella, R. Graham Cooks, and Zheng Ouyang, "Development of an endoscopic DESI sampling probe" *59th American Society for Mass Spectrometry Conference*, Denver, 2011.
5. **Chien-Hsun Chen**, J.-L. Lin, M.-L. Chu, Y.-S. Wang, and Chung-Hsuan Chen "Single large biomolecular ion detection" *57th American Society for Mass Spectrometry Conference*, Philadelphia, 2009.
6. **Chien-Hsun Chen**, J.-L Lin; M.-L. Chu, and Chung-Hsuan Chen "Novel MALDI ion trap mass spectrometer for large biomolecule detection" *56th American Society for Mass Spectrometry Conference*, Denver, 2008. (oral presentation)

Patents

1. R. Graham Cooks, Zheng Ouyang, **Chien-Hsun Chen**, L.S. Eberlin, and Z. Lin "Enclosed Desorption Electrospray Ionization Probes and Method of Use Thereof" Application No. US20120312979 A1 (Jun, 2012)
2. Chung-Hsuan Chen, **Chien-Hsun Chen**, Jung-Lee Lin and Ming-Lee Chu; "Mass Spectrometer and Methods for Detecting Large Biomolecules" Application No. US20110284733 A1 and US8258464 B2 (May, 2010)