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On-line laser desorption/ionization mass spectrometry

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ON-LINE LASER DESORPTION/IONIZATION MASS SPECTROMETRY

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DEDICATION

To my family and friends

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The chapters of this dissertation represent different periods in my development as a student and a scholar. Be that as it may, the inclusion and support of my family, friends, teachers and advisors made it possible for me to complete this chapter of my life.

My mother and father, Linda and Johnny Narcisse have gone above and beyond as parents with constant instruction, encouragement and endorsement throughout my educational and personal endeavors. They are living reminders of the type of person I strive to become; I owe them a lifetime of radiating the characteristics they instilled in me. The friendships of my older and younger brothers, Stephen and Bradley, has been a consistent positive influence of my being and has pushed me to maintain confidence while keeping me humble. My daughter, Summer, is the newest inspiration in my life, she will constantly challenge me to get smarter everyday.

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ABSTRACT

Interfaces for on-line laser desorption/ionization mass spectrometry are presented in this dissertation. An on-line laser desorption interface allows samples to be introduced directly into the mass spectrometer for high-throughput applications. For this research, a linear time-of-flight mass spectrometer was constructed with an ionization source designed to accept various laser desorption interfaces. A ball inlet interface was used for continuous analyte deposition at atmospheric pressure and vacuum ionization. A solvent-based cleaning system and a separate capillary for MALDI matrix delivery was utilized for continuous on-line sampling with the ball inlet interface. Microfluidic devices were brought into contact with the ball inlet and eluant was electrokinetically driven and deposited onto the ball inlet sampling surface. Polymer-based microfluidic chips were engineered with open-ended channels for on-line coupling, and peptide mixtures were separated on-chip and mass detected on-line. A non-contact deposition method was studied using the ball inlet interface and a single droplet generator. Single droplets with 100 picoliter volume were ejected by a piezoelectric-actuated droplet generator and deposited onto the ball inlet. Analyte droplets were placed onto a pre-deposited matrix layer during on-line analysis, and protonated molecule signal was obtained from as little as 10 fmol analyte. Two contact methods for fast sampling were studied with the ball inlet interface and a direct desorption/ionization interface. Direct contact with the ball inlet was achieved with an indented ball surface for solid, non-crystalline, samples. Analytes were brought into direct contact with the ball inlet allowing deposits to settle into micromachined wells on the ball surface. The wells prevented scraping-off of the analyte by the vacuum gasket due to mechanical forces. The analysis of plant tissue, which is not suited for ball inlet sampling, was performed with the direct desorption/ionization interface.

CHAPTER 1. INTRODUCTION

1.1. Mass Spectrometry Basic Principles

Mass spectrometry (MS) is one of the most powerful techniques in chemical analysis with applications in a vast array of scientific disciplines. Characteristics of mass spectrometric analysis are high detection sensitivity, accurate mass measurement and molecular specificity. Mass spectrometry has been incorporated into almost every realm of science since the early 1900s with commercialization of these instruments beginning in the 1950s.[1] Today, it is uncommon for an analytical laboratory to operate without a mass spectrometer. Over the years, mass spectrometry has become a common tool in areas such as chemistry, biochemistry, physics, environmental science, forensic science, pharmaceutical, petroleum and military defense industries, encompassing a broad scope of target analytes. The versatility and extensive applicability of mass spectrometry make it a valuable tool for obtaining qualitative or quantitative chemical information.

Mass spectrometers are instruments that measure the mass-to-charge ratio of gas-phase ions.[2] Mass-to-charge ratio, or *m/z*, is obtained by dividing the mass number (*m*) of an ion by its charge number (*z*). A mass spectrometer consists of three fundamental parts: the ion source, the mass analyzer, and the detector. Mass spectrometry analysis begins with ionization of neutral atoms or molecules to form gas phase ions. These ions are separated in space or time according to their *m/z* and the resulting signal, as a function of *m/z*, is the mass spectrum. Other important aspects of mass spectrometers include the sampling mechanism, vacuum system, and data processing. Sample inlets regulate how a sample is introduced to the mass spectrometer's ion source region.[3] Sampling and ionization can be achieved under vacuum or at atmospheric pressure, whereas mass analysis and ion detection is conducted under high vacuum, to limit

collisions with air molecules, and for high voltage operation. Proper integration of all the mass spectrometer components is necessary for overall instrument performance.

1.2. Early Ionization Methods

The method of ionization is determined by sample type and the information desired from the mass spectrum. There are many types of ion sources that use different ionization mechanisms. Ionization for mass spectrometry, like any analytical tool, has evolved and many changes have occurred in recent years. Early ionization techniques, such as electron ionization and chemical ionization, were limited to the analysis of volatile compounds with small molecular weights of a few hundred Daltons (Da).[1, 2] Later ionization methods, such as fast atom bombardment,[4] pushed the upper limits of mass spectrometry to a few thousand Daltons. Not until the emergence of "soft" ionization techniques, matrix-assisted laser desorption ionization (MALDI)[5, 6] and electrospray ionization (ESI)[7], were mass ranges in excess of a few thousand Da achievable. These contrasting, yet complementary, techniques expanded the applicability of mass spectrometry into scientific fields where higher mass limits are practical.

1.2.1. Electron and Chemical Ionization

Electron ionization (EI) has, historically, been the most popular and widely used ionization technique.[3, 8] In EI a gas, solid, or liquid sample vaporized by heating produces gas molecules that are bombarded with an energetic electron beam to generate ions. The ions have high internal energy that causes fragmentation; however, the fragmentation patterns can be interpreted with database searching.[9, 10] On the other hand, the fragmentation can also prevent the detection of some molecules, limiting the usefulness of this technique to volatile molecules with molecular weights below 400 Da.

Chemical ionization (CI) was developed due to the need for softer ionization. This method produces ions with less energy resulting in less fragmentation compared to EI.[2, 11] The CI

ion source is similar to the EI ion source, but the ionization process is different. In chemical ionization, a reagent gas, usually methane, isobutane or ammonia, is bombarded with an electron beam producing reagent ions. The reagent ions ionize the sample molecules through ion-molecule reactions to produce sample ions. The use of different reagent gases can produce different fragmentation patterns. Chemical ionization allows molecules with molecular masses up to 1000 Da to be ionized, but like EI, is limited to volatile compounds. However, the gas phase sampling of EI and CI make these ionization techniques easily adaptable to gas chromatography/mass spectrometry (GC-MS) instrumentation.

1.3. Spray Ionization Methods

Spray methods are used to describe ionization techniques in which the sample is sprayed from solution to form ions.[2, 11] Nonvolatile samples can be ionized without first being converted to the gaseous state. Liquid solutions can be sprayed directly into the ion source at atmospheric pressure. These ion sources allow for the direct handling of analytes from liquid chromatography (LC) or capillary electrophoresis (CE) eluent or biological fluid analysis.

1.3.1. Atmospheric Pressure Chemical Ionization

Atmospheric pressure chemical ionization (APCI) is similar to chemical ionization in that ion-molecule interactions promote ionization, but APCI samples are introduced in solution form.[2, 11] APCI sample solution flows through a capillary and pneumatically nebulized with a nitrogen gas stream. The nitrogen gas carries the sample droplet spray into a heated tube where solvent evaporation and analyte desolvation into the carrier gas occurs. The heated gas mixture is carried through a corona discharge in a reduced pressure region where ionization occurs. The resulting ions pass into successive reduced pressure regions and into the vacuum region of the mass analyzer. APCI can be applied to polar and ionic compounds with molecular weights up to 1500 Da.

1.3.2. Electrospray Ionization

Electrospray ionization was reported by Fenn and co-workers in the late 1980s as an ionization technique for samples in the liquid phase.[7] Prior to Fenn, Dole and co-workers described an electrospray of dilute polymer solutions into an evaporation chamber to produce macroions.[12, 13] ESI produces charged droplets from an analyte solution at the end of a capillary at high voltage. Solvent evaporation causes droplets to shrink to a point where the repelling coulombic forces are large enough to induce explosions of the droplets.[14] Alternatively, the droplets undergo a cascade of ruptures, yielding smaller and smaller droplets until the electric field on their surface becomes large enough to produce the desorption of the analyte molecules.[14] If large molecules with many ionizable sites are present, the resulting ions carry a great number of charges. Because ESI uses liquid sample introduction and ionization is at atmospheric pressure, it is well suited and routinely used for on-line MS detection of liquid separations such as liquid chromatography[15] and capillary electrophoresis.[16]

1.4. Desorption Ionization Methods

Desorption ionization methods are a range of techniques that can volatize and ionize condensed phase samples.[11] These ionization mechanisms require the sample to be mixed with a suitable matrix and bombarded with a high-energy beam. A desorption matrix is a separate compound that absorbs energy and helps ionize the dissolved or incorporated analyte. Samples are introduced to the ion source using an insertion probe, or target, from which the sample is desorbed and ionized.

1.4.1. Fast-Atom Bombardment

In fast-atom bombardment (FAB) ionization,[4] the sample is dissolved in a nonvolatile liquid matrix and is bombarded with a kV energy beam of Xe atoms, Cs^+ ions, or massive glycerol-NH_4^+clusters. The liquid matrix and sample are sputtered from the probe surface as

neutral molecules and ions.[2] The FAB matrix is a nonvolatile solvent and is usually *m*-nitrobenzyl alcohol (NBA) or glycerol.[3] Although masses as high as 30 kDa have been observed, above 4000 Da the signal intensity becomes weak; this is considered the upper mass limit of FAB.[11]

1.4.2. Laser Desorption Ionization and MALDI

Laser desorption/ionization (LDI) is used to describe ionization with a pulsed laser. Proper sample preparation and laser parameters must be chosen to prevent the production of ion fragments rather than intact species. Early LDI experiments were carried out with pulsed infrared (IR) lasers with ultraviolet (UV) lasers introduced shortly thereafter.[17]

The matrix-assisted laser desorption/ionization (MALDI) process was simultaneously developed by Tanaka[5] and Hillenkamp[6] in the mid 1980s as a modification of laser desorption ionization.[3] The MALDI process begins when an analyte is embedded in an excess of a matrix compound. The matrix is responsible for absorbing energy from the laser radiation, isolating analyte molecules, and providing protons for analyte ionization. Sample preparation is very important, and is the first step in a MALDI experiment.[18] Matrix and analyte solutions are deposited on a probe at a matrix to analyte molar ratio between 100:1 and 50,000:1 to reduce analyte molecule association. Upon solvent evaporation, the matrix forms crystals incorporating the analyte. Once the probe is inserted into the ion source of a mass spectrometer, the crystals on the probe are irradiated with a pulsed laser. This process generally produces singly protonated ions, yielding easily interpretable mass spectra.

Use of ESI and MALDI for biological mass spectrometry is widespread and the biological applications are continually growing.[11] The two techniques are complementary, but MALDI has some advantages over ESI in a bioanalytical setting. MALDI has a higher tolerance for impurities that often result when dealing with liquid separations. The singly charged ions

produced in the MALDI process give less complex mass spectra. The multiple charging that occurs in the ESI process can make it difficult to perform crude mixture analysis. MALDI is generally used for off-line detection of liquid separations, and on-line methods are difficult due to the requirement for solid samples.[19]

1.4.3. Atmospheric Pressure MALDI

The combination of MALDI with atmospheric pressure ionization (AP-MALDI) was introduced by Laiko *et. al.*[20] Like ESI, the ionization process in AP-MALDI is carried out at atmospheric pressure and the resulting ions are transported into the mass analyzer. Ion transfer is assisted by an inert carrier gas, usually nitrogen. Ion formation is similar to traditional MALDI but AP-MALDI may allow further specific atmospheric pressure processes, such as ion-molecule reactions, to occur. Since its induction, AP-MALDI has led to the expansion of mass analyzer options for MALDI-MS[20, 21] and brought about advances in molecular imaging with mass spectrometry.[22] However, sensitivity and mass limit suffers due to the transfer of ions from atmospheric pressure to the vacuum region of the mass spectrometer.

1.5. Ambient Ionization

Ambient ionization for mass spectrometry is the acquisition of a mass spectrum from samples in their native environment, without any sample pretreatment, by creating ions outside of the instrument.[23] AP-MALDI is one example and other atmospheric pressure ionization techniques have been recently demonstrated that allow ions to be created under ambient conditions.[23] Desorption electrospray ionization (DESI)[24] and direct analysis in real-time (DART)[25] can both be used to obtain mass spectra from ordinary samples in their native environment. DESI utilizes a fine spray of charged droplets direct toward an analyte surface, the droplets pick up analytes, such as small organics or large biomolecules, the droplets are lifted-off in the gas flow to form ions in an electrospray-like process. Other methods that are similar to

DESI, but use a pulsed laser, have recently been described employ the same general principles: for example, electrospray-assisted laser desorption ionization (ELDI)[26] and matrix-assisted laser desorption electrospray ionization (MALDESI).[27, 28] The DART method uses a plasma of excited-state He atoms and ions directed at an analyte surface; this plasma desorbs and ionizes low-molecular weight molecules directly from the surface.[25] Both DESI and DART eliminate the need for sample preparation while retaining the speed, specificity and sensitivity of MS and have applications in areas such as explosives detection, drug metabolites in biological fluids, intact tissue analysis and active ingredients in pharmaceutical tablets.

1.6. Coupling Liquid Separations to Mass Spectrometry

The ability of ESI and MALDI to analyze large biomolecules and transfer them to the gas phase has made these techniques highly significant in protein and peptide analysis and the study of drugs and their metabolites.[29] Liquid separation techniques combined with MS detection provide a powerful and widely used analytical tool in proteomics and metabolomics. When coupling to liquid separations, ESI has the natural advantage over MALDI because it is a direct liquid introduction technique. Electrospray samples are in liquid solution, making liquid separation coupling to ESI straightforward and yielding powerful two-dimensional analysis methods. There are challenges when coupling MALDI to liquid separations because MALDI samples are typically dried on a surface before MS analysis. However, the advantages of MALDI, including the robustness of laser ionization and easily interpretable mass spectra, make it a desirable approach over ESI. Coupling MALDI-MS to liquid separations is traditionally performed off-line but the high-throughput capabilities of on-line analysis are more suitable for the demands of bioanalytical studies.

Recently, microscale total analysis systems, or “Lab-on-a-Chip” technology, has gained interest in bioanalytical analysis due to rapid analysis times, small size, low sample consumption,

low cost and detection methods that are highly sensitive.[30, 31] Also know as microfluidic chips, their fast development in recent years has led to many advances in chemical and biochemical analysis. Microfluidic chips are used to transport and manipulate nanoliter or picoliter volumes of fluids through micro-channels in the fabricated glass or plastic chips.[32] Functions such as mixing, filtering, polymerase chain reaction (PCR), and electrophoresis and liquid chromatography separations have been developed and applied within microfluidic chips.[33-38] Sample transport through the chip can be achieved using hydrodynamic or electroosmotic flow. Chip microfabrication is inexpensive, and techniques such as photolithography can be used to form channels, reservoirs, and other microscale features, and along with rapid analysis and low sample consumption, small chip size aids in portability.

Detection for microfluidic chips is generally limited to methods that are rapid, sensitive, and are able to be performed on-chip. On-chip detection eliminates the design complexity that arises when interfacing to the outside world. The fluorescence method of detection is popular because of its ability to be performed directly on the chip. A continuous laser, focused on the chip, can either be used with a photomultiplier (PMT) and point detected or with a charge-coupled device (CCD) for area detection.[39] Fluorescence requires sample derivatization or reliance on analyte native fluorescence.[15]

MALDI-MS detection for microfluidic chips is usually performed off-line using various techniques.[40] Off-line coupling of liquid separations to MALDI-MS is a two-step process requiring collection of sample for later MALDI analysis.[19] Liquid chromatography (LC) fractions can be collected in individual vials or the separation effluent directly deposited onto a MALDI target. This two-step process can suffer from instrumental issues concerning separation efficiency and excessive dilution of the analyte when matrix is added. When coupling MALDI to small-scale separations such as capillary electrophoresis (CE) or microfluidic chips, the small

sample volumes and the requirement for a high voltage connection can pose additional challenges. More technologically advanced off-line MALDI detection for microfluidic chips include microfabricated aliquot collection devices such as nano-vials and on-chip spray nozzles and robotics for automated MS analysis.[34, 35, 41] Current MALDI ion sources are designed for manual loading of crystalline samples making off-line approaches more commonplace.

1.7. On-line MALDI

On-line MALDI interfaces are of varied designs.[40, 42, 43] These interfaces can be grouped into three categories: aerosol,[44, 45] capillary,[46, 47] or mechanical.[48-50] In aerosol MALDI, a premixed analyte and matrix mixture is sprayed directly into the mass spectrometer.[51] Particles are passed through a heated tube to accelerate solvent evaporation then irradiated with a pulsed UV laser. Although it is compatible with solid matrices and many experiments can be run without cleaning, aerosol MALDI suffers from excessively high sample consumption. The capillary based continuous flow (CF) MALDI interfaces uses a narrow bore capillary to deliver analyte in a liquid matrix to the mass spectrometer.[47, 52, 53] The mixture of analyte and matrix is desorbed directly from the CF probe. CF-MALDI is limited due to the lack of suitable liquid matrix materials for use with desorption lasers, and the capillary connection can result in excessive suction at the liquid separation interface.

Mechanical interfaces for on-line MALDI are compatible with more commonly used solid matrices. Vacuum deposition, uses a capillary to transport an analyte and matrix solution at a flow rate of hundreds of nL/min.[48] The solution is deposited onto a rotating quartz wheel within the vacuum of the ion source where the solvent evaporates and the resulting thin sample trace is rotated into position for laser desorption. The most significant drawback to this method is the requirement for manual cleaning of the wheel; if cleaning is not efficient a memory effect results. Modifications have been made to the vacuum deposition method in which a rotating

Mylar tape replaces the quartz wheel.[54, 55] This modification addresses the cleaning issue, but for on-line microscale separations, capillary dead volume limits the systems utility.

A second mechanical approach is based on a ball inlet interface.[49, 56] Matrix and analyte solution are delivered to a gasket pressed tightly against the surface of a ball that rotates several times each minute. As the ball rotates, the solution is exposed to vacuum where the volatile solvent evaporates, leaving the matrix and analyte. When the ball has rotated one half turn, it is in position for laser desorption. The ball inlet does not exhibit a memory effect, probably due to the removal of the ball coating by the laser or as the surface scrapes against the cup that holds the ball in place. This interface does not suffer from the drawbacks of the aerosol and CF interfaces, but as with the vacuum deposition interface, capillary dead volume and suction by source vacuum can be problematic.

1.8. Modified Rotating Ball Inlet

A modified rotating ball inlet has been constructed with an open configuration, placing the ball at the atmospheric pressure/vacuum barrier of the ion source.[50, 57, 58] This novel design allows for samples to be deposited onto the ball at atmospheric pressure, outside of the vacuum region of the ion source. The sample is then rotated past a polymer vacuum gasket and into the vacuum region of the ion source for laser desorption. The ball inlet interface is described in Chapter 2. Liquid separation techniques are directly coupled to this interface without suffering the effects of suction from the vacuum of the ion source. Microscale separation devices, such as microfluidic chips and capillaries, can be brought into direct contact with the ball during rotation. Chip or capillary effluent is allowed to flow directly from the separation channel and onto the surface of a stainless steel ball with no intermediate steps. Once deposited, matrix addition occurs with a separate capillary and the analyte/matrix mixture is allowed to air dry before rotation past the polymer vacuum gasket and into the ionization source. A constant rotation

speed of approximately 0.3 rpm is maintained during analysis. After laser desorption the remaining material exits the vacuum region and is cleaned with a solvent saturated felt pad leaving a clean surface for new analyte deposition.

The modified ball inlet system has the advantages of off-line analysis by allowing any separation device to perform atmospheric pressure analysis and deposition while allowing the ionization process to occur under vacuum. This functionality eliminates the drawbacks of ambient ionization methods such as atmospheric pressure (AP) MALDI because there is no need to transfer ions from an atmospheric pressure region to a vacuum region.[20]

1.9. Research Objectives

The objectives of this research were 1) to develop a new direct analysis detector for microfluidic chips and analytes in non-traditional sample forms using MALDI-MS and 2) to explore applications using the new analytical techniques. The modified ball inlet, previously discussed, was designed and the experiments discussed in Chapters 3 through 5 were performed using this interface. In Chapter 6 a novel interface for direct laser desorption and ionization is described.

CHAPTER 2. EXPERIMENTAL

2.1. Overview

This chapter contains a discussion of experimental instrumentation as it relates to this research. A brief overview of time-of-flight mass analysis and a detailed explanation of the "home-built" linear TOF mass spectrometer, constructed specifically for these studies, is presented. Design and concept of novel laser desorption interfacing will be described. System parameters during typical mass analysis will be provided for each project with more detailed materials and methods discussed in the following chapters. All experiments were performed on the home-built linear TOF instrument unless otherwise noted.

2.2. Time-of-flight Mass Spectrometry

Time-of-flight mass spectrometry is the study of separating gas phase ions according to mass-to-charge in a field free region after acceleration to a constant energy. A time-of-flight (TOF) mass analyzer separates ions based on their mass-to-charge ratio (*m/z*).[8] TOF MS is typified by a theoretically unlimited mass range and high sensitivity. Unlike scanning mass analyzers, such as sectors and ion traps, an entire mass spectrum can be obtained simultaneously with a duty cycle limited only by the time it takes for the ions to traverse the flight tube, typically within hundreds of microseconds. A TOF tube consists of a chamber evacuated to a pressure of 10^{-6} torr or lower to prevent collisions with background gas molecules. The length of the TOF tube is based on the desired ion separation resolution over a given mass range. Ions are accelerated through the ion source region with the same kinetic energy therefore the flight times of ions entering the TOF tube are dependant on their mass. This concept is defined by the equation

$$KE = \frac{1}{2}mv^2 \qquad (1)$$

where KE is the kinetic energy, m is ion mass, and v is the velocity.[8] Considering the equation:

$$KE = zeV \tag{2}$$

where z is the charge number of the ion, e is the charge of an electron and V is the acceleration voltage, equation (1) can be solved for velocities in the field free region of the flight tube

$$v = \left[\frac{2eV}{m}\right]^{\frac{1}{2}} \tag{3}$$

assuming a singly charged ion ($z = 1$) and neglecting the time spent in ion source. Subsequently, flight time (t) can be solved using the equation,

$$t = \left[\frac{m}{2eV}\right]^{\frac{1}{2}} D \tag{4}$$

where D is length of the flight tube.

Mass calibration can be obtained from flight times using the flight times of two known masses (constants a and b) using equation:

$$t = a \cdot m^{1/2} + b \tag{5}$$

where a and b are found from fitting the *m/z* values of two known ion peaks with b also considering time offsets due to possible systematic error. Mass resolution (R) in time-of-flight mass spectrometry can be defined as

$$R = \frac{m}{\Delta m} = \frac{t}{2\Delta t} \tag{6}$$

usually reported using the full-width at half-maximum (fwhm) and can be calculated from either flight times or masses.

2.3. Linear Time-of-flight Mass Spectrometer

The MALDI mass spectrometer designed for this research is a laboratory built linear time-of-flight mass spectrometer with static ion extraction. The unique aspect of this instrument

is a specially designed ion source for on-line interfacing. Figure 2-1 is a schematic diagram of the linear time-of-flight mass spectrometer.

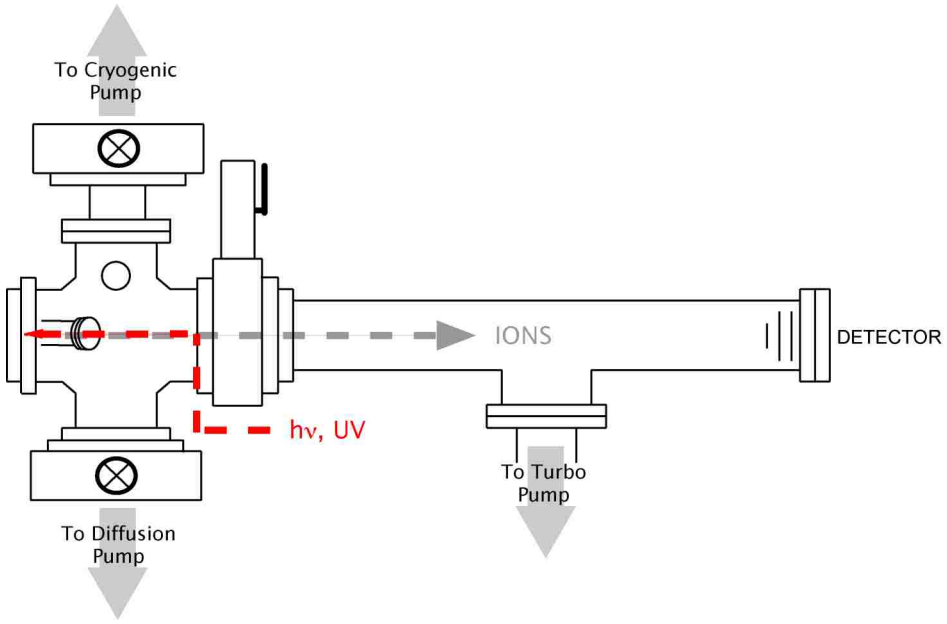

Figure 2-1. Schematic layout of the linear time-of-flight mass spectrometer (TOF-MS). The ion source is adaptable with an ISO 100 flange at the interface. A frequency tripled Nd:YAG laser (355 nm) or a nitrogen laser (337 nm) provides ultra violet radiation. The micro-channel plate detector is connected to a digital oscilloscope, and subsequently, a computer for analysis (not shown).

The ion source consists of a stainless steel flange, ISO NW100 (MDC, Hayward, CA), mounted onto a 8-inch conflat four way stainless steel cross in a manner such that the laser can be focused onto a target at the atmospheric pressure and vacuum interface. The ISO 100 interface flange is electrically isolated using a 1 in. thick Delrin (acetyl resin) insulator flange and held at high voltage during operation. Figure 2-2 is a photograph of the ion source chamber. The flange can be custom machined to interface many different sample probe types that allow for samples to be ionized directly from the vacuum side of the flange. This versatility allows for interfacing specific to each project. The cross is equipped with a quartz viewport for laser introduction, which is at a 45° angle with respect to the interface flange, and various feedthroughs for

electrical connections. The source is evacuated with a 1500 L/s 6-in. water-cooled diffusion pump (M-6, Varian, Lexington, MA). The ion source pressure is maintained at high vacuum. Enhanced pumping for more volatile solvents is achieved with a liquid nitrogen cooled cryogenic pump.

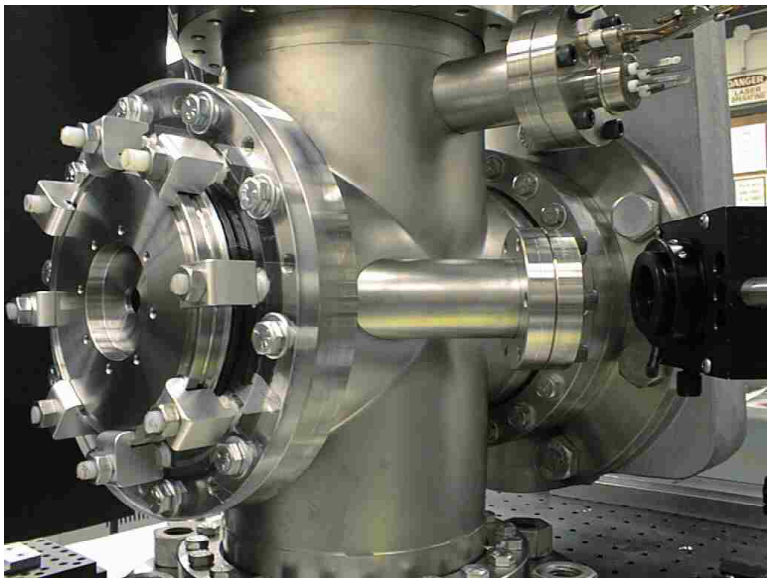

Figure 2-2. Photograph of the linear MALDI ion source chamber. The laser beam path travels through the focusing lens (pictured on the right) to the vacuum side of the interface (far left of image). Here, the insulating flange is Plexiglas.

Positive ions formed by laser irradiation are extracted by a continuous positive voltage of 10 to 20 kV DC. This voltage is supplied with a 30 kV high voltage power supply (Gamma High Voltage, Ormond Beach, FL). For continuous ion extraction (CIE), the acceleration voltage is applied directly to the interface flange. Ions are accelerated from the interface flange and pass a grounded lens which begins the field free region of the time-of-flight tube. The grounded lens is a stainless steel disc with machined orifices for ion transmission. The orifice is covered by 90 % transmission grid, (70 lpi, Buckbee Mears, Cortland, NY) to maintain a uniform, electrically grounded field, with a section removed to allow the laser beam to pass through unimpeded. Ions

are then transmitted into the 1 m linear TOF analyzer for mass analysis. The flight tube operated at high vacuum and evacuated using a 345 L/s turbomolecular pump (Turbovac 360, Leybold, Export, PA).

UV-MALDI ions are generated using a frequency tripled Minilite10 Nd:YAG pulsed laser (Continuum, Santa Clara, CA). A wavelength of 355-nm is used at a repetition rate of 2 to 10 Hz. A pulsed N_2 laser operating at 337-nm at a repetition rate of 10 Hz was used for some experiments (VSL-337ND-S, LSI, Franklin, MA). The UV beam is steered with 1 inch high-reflection UV grade mirrors (CVI, Albuquerque, NM). A Glan-Thompson polarizer mounted on a rotating stage is used for laser attenuation. The laser beam is focused onto the target using a plano-convex CaF_2 lens (Janos, Keene, NH) through a UV grade sapphire window (MDC, Hayward, CA). The laser beam is focused to a spot size of roughly 300 by 200 μm, measured using laser burn paper and an image magnifier.

Positive ion detection was achieved with a bipolar time-of-flight detector (Burle Electro-Optics, Lancaster, PA) or a dual microchannel plate (MCP) detector (Galileo, Sturbridge, MA). The bipolar detector is a hybrid device that contains a MCP and a photomultiplier tube (PMT). This configuration allows ions to be post-accelerated with up to 10 kV of kinetic energy while the overall detector outputs remains fixed at ground potential.

The detector signal was sent directly to the 50 Ω input of a 500 MHz digital oscilloscope (LT372, LeCroy, Chestnut Ridge, NY). The scope is triggered by the laser Q-switch (Nd:YAG) or sync output (N_2). Mass spectra resulted from an average of up to 20 laser shots, and were plotted as a function of peak intensity (volts) versus flight time (seconds). Signal-to-noise ratio can be improved by summing spectra from many individual laser shots taken from the same sample spot. Once a spectrum is averaged, it is transferred via GPIB cable connection to a Power Macintosh G4 computer (Apple Computer, Cupertino, CA) for further analysis. For separation

experiments performed with the ball inlet interface, three-dimensional plots (elution time vs. flight time) result from many sets of ten shot averaged mass spectra acquired throughout the experiment. Mass spectra were analyzed using LabView computer software (National Instruments, Austin, TX). This program enables functions such as mass calibration, mass resolution calculation, and file format conversion. The mass calibration function converts a time spectrum into a mass spectrum. Spectra were calibrated by first determining the flight times of two or more peaks with known mass-to-charge (*m/z*). A linear fit was performed on the flight times and *m/z* values of the peaks, then the time scale of the spectrum was converted to mass to charge ratio.

2.4. Ball Inlet Interface

In this section, a modification of the original ball inlet interface described by Orsnes *et al*.[49] is presented. The design and specifications for the modified ball inlet[50, 57, 58] are presented first followed by experimental parameters and setup for each ball inlet experiment. More specific procedures and reagents for each experiment are presented in the following chapters.

2.4.1. Ball Inlet Design

The ball inlet system is a laser desorption mass spectrometry interface that allows for continuous on-line sampling of solid and liquid phase analytes. This interface was used for multiple experiments that are described in this section. The ball inlet design consists of a 0.75-in. stainless steel ball bearing, which provides the means of continuous introduction and ionization of MALDI and LDI samples. Figure 2-3 illustrates the ball inlet interface. The ball, mounted on a 0.25-in. diameter (6-in. length) stainless steel drive shaft, mechanically rotates a deposited matrix and/or analyte at atmospheric pressure into the vacuum of the mass spectrometer. The shaft rotates on sintered bronze bearings. Analyte can be in solution or solid form. The ball

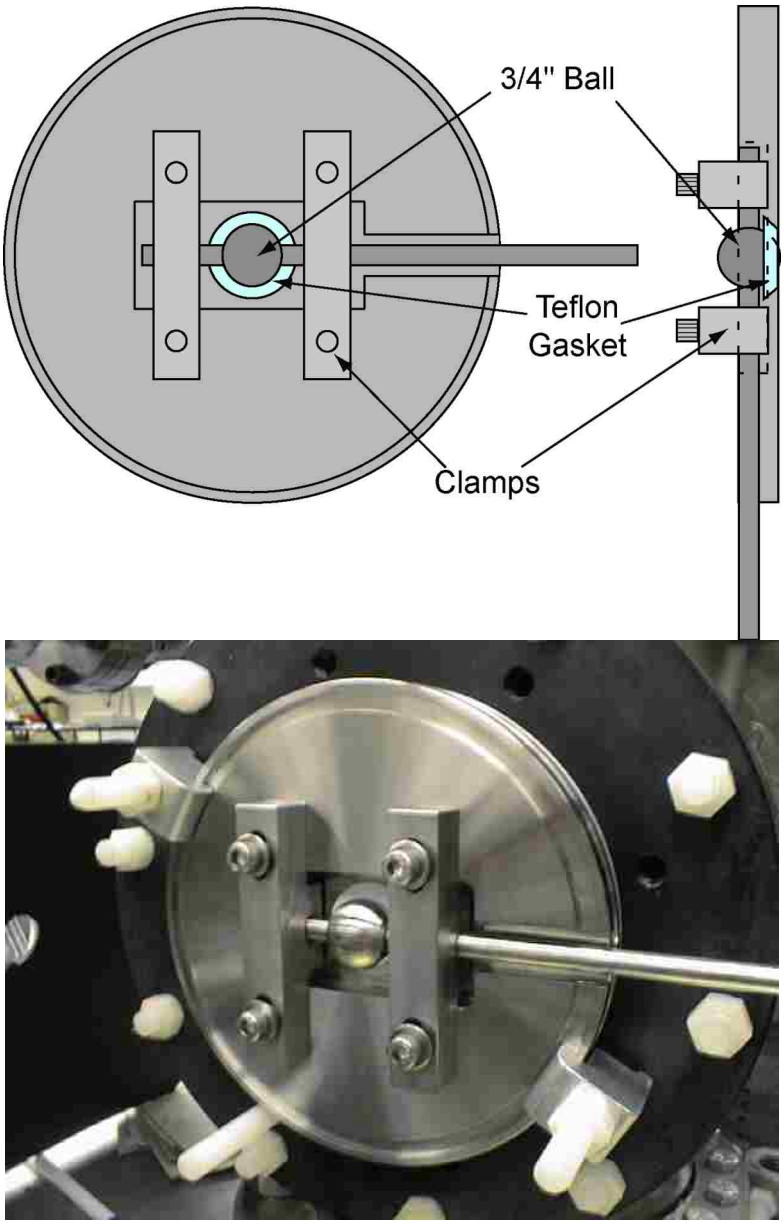

Figure 2-3. Front and top view illustration and digital photograph of the ball inlet interface. The ball and shaft were clamped to the atmospheric pressure side of the stainless steel flange. Ionization occurs on the opposite, vacuum pressure, side of the ball upon rotation.

and shaft are clamped onto the interface flange held at the acceleration potential. When clamped, the ball rests tightly in a specially designed polymer gasket creating a vacuum seal. The gasket allows a sample to be rotated into the source without being scraped off. The ball and shaft are rotated with a motor and gearbox retrieved from a syringe pump (Model 901, Harvard

Apparatus, Holliston, MA). The rotation rate of the multispeed transmission motor was held constant and operated at a speed of 0.1 to 0.3 rpm. Once in the ion source, the sample is ionized and accelerated directly from the surface of the ball.

The interface flange, previously described, was machined to allow the vacuum side of the ball inlet to sit flush when clamped. The electrically conductive properties of all the interface components permit high voltage for ion extraction to be connected the ball inlet interface. The drive shaft was electrically isolated from the rotation motor with a 0.25 in. Delrin joint (Part No. UJD-4/4, Small Parts, Miami Lakes, FL). During ball inlet experiments, extraction voltage potentials were held between 10 and 15 kV.

2.4.2. On-line Electrophoresis MALDI

On-line capillary electrophoresis mass spectrometry (CE-MS) experiments were carried out with the ball inlet interface as previously described.[50] Eluant from micro capillary electrophoretic separations was kinetically driven and deposited directly onto the atmospheric region of the ball inlet. Matrix solution and separation buffer were pressure driven and deposited onto the ball interface surface with the aid of a syringe pump. Once deposited, crystallized analyte and matrix rotated into the vacuum region of the mass spectrometer for laser ablation and ionization. Sample remaining after ablation was rotated out of the vacuum region of the ion source to the atmospheric pressure region. Figure 2-4 is a close-up illustration of the ball inlet during on-line CE-MS experiments. Here, continuous on-line cleaning was achieved with a solvent saturated felt pad in contact with the rotating ball. Constant cleaning allows for on-line electrophoresis separation eluant to be deposited onto a clean ball surface at all times without interruption.

Fused-silica capillaries, 20 cm length and 50 μm i.d. (Polymicron, Phoenix, AZ), were used for on-line CE-MS experiments. The home-built CE system was designed for manual

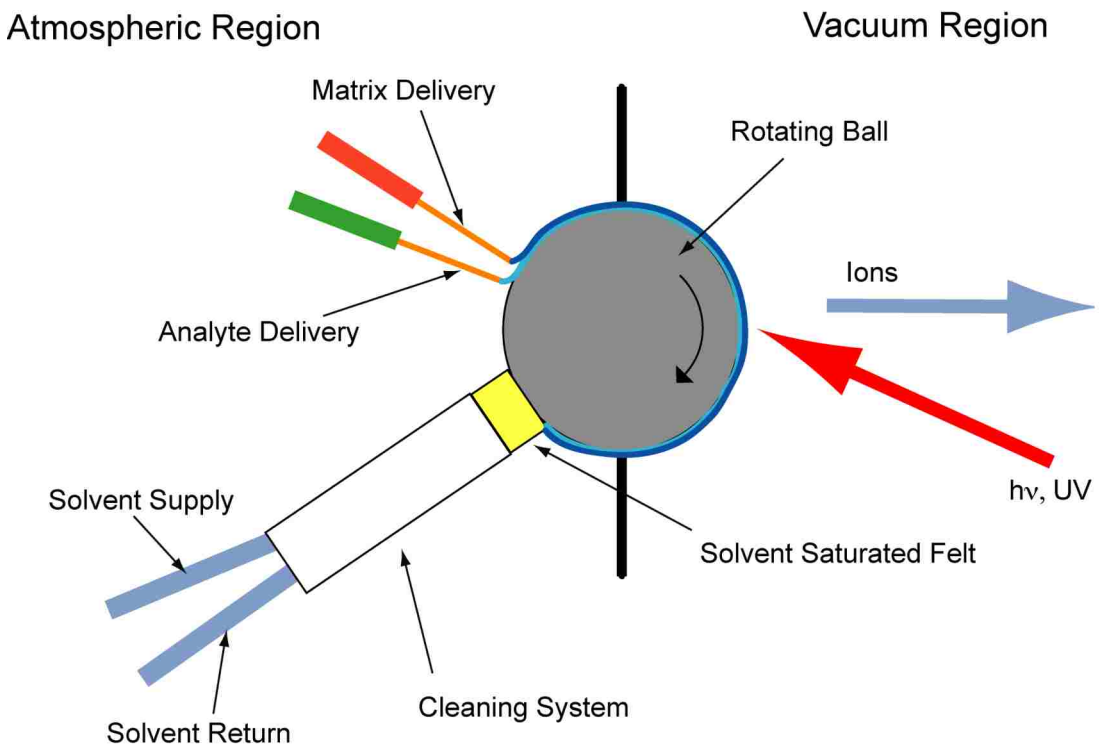

Figure 2-4. Illustration of the ball inlet for on-line capillary delivery. Matrix and analyte were delivered through capillaries onto the rotating ball. Upon drying and crystallization, matrix and analyte were rotated past a Teflon gasket (not shown) and into the vacuum region of the ionization source and exposed to laser irradiation. Remaining sample was cleaned from the ball inlet surface with a solvent saturated felt pad (methanol or ethanol) after exiting the ion source.

injection of biomolecule mixtures. A schematic diagram of the on-line CE-MS experiment is illustrated in Figure 2-5. The 10 kV positive potential applied to the ball inlet for ion acceleration was subsequently used as the cathodic potential for CE. A separate power supply (CZE 1000R, Spellman, Hauppauge, NY) provided an anodic potential of 18-22 kV to a platinum electrode for electrokinetic separations. Plug injections were performed manually by switching the separation capillary from buffer vial to sample vial for 10 s.

Microfluidic chip separations were also performed and analyzed using the ball inlet interface. On-line microfluidic chip detection was performed with the same principles as CE-MS experiments and have been previously described.[57] Figure 2-6 illustrates the method of

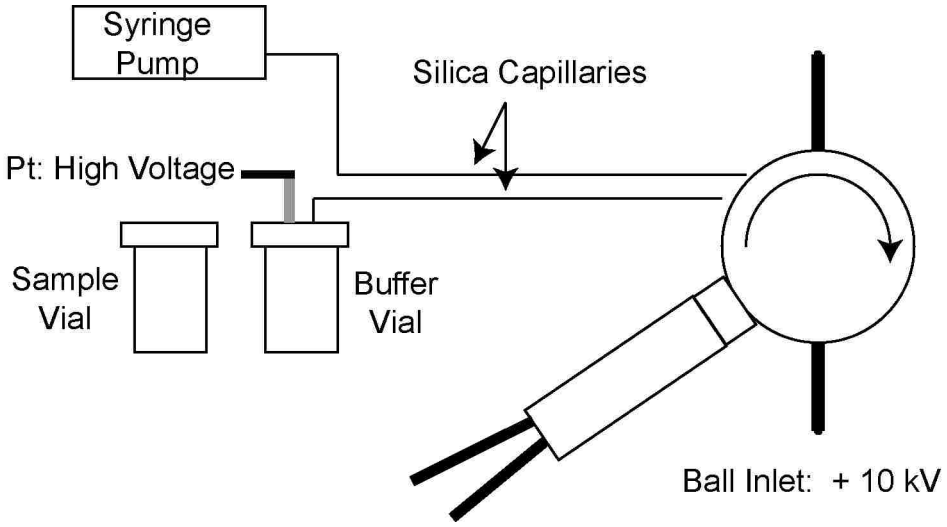

Figure 2-5. Diagram of the on-line capillary electrophoresis setup. A platinum electrode provided anodic potential while the ball inlet provided cathodic potential. A fused silica capillary was manually switched, with electrode, from buffer vial to sample vial for plug injections. A separate capillary was used to deposit MALDI matrix and a third capillary delivered additional buffer when needed (not shown).

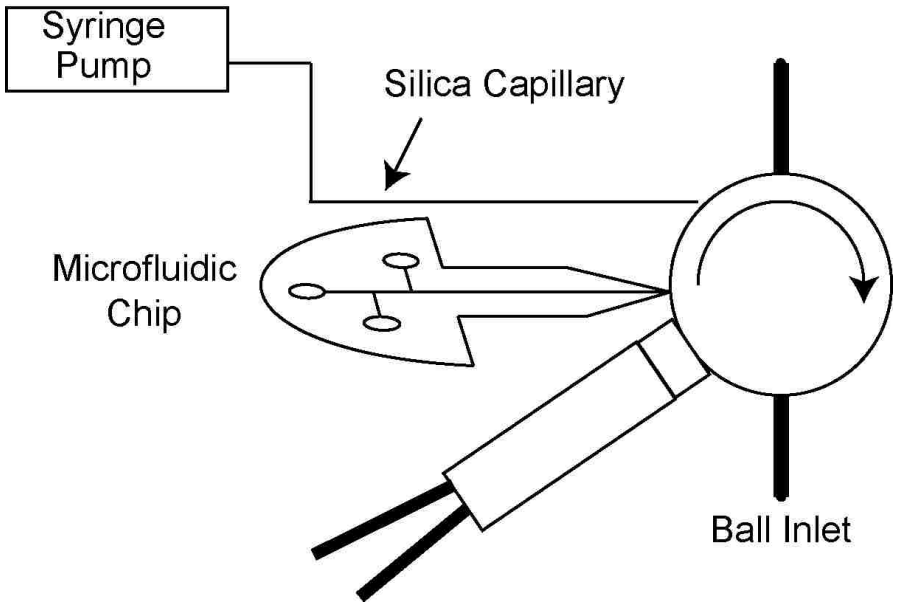

Figure 2-6. Diagram of the on-line microfluidic chip MALDI detection setup. The microfluidic chip was directly butted against the ball inlet.

interfacing microfluidic chips to the ball inlet interface. Microfabricated poly(methylmethacrylate) (PMMA) chips were specially designed so that the fluidic separation channel exit could be directly butted against the ball inlet. Similar to the on-line CE-MS approach, the V-shaped channel exit was aligned, with an XYZ translational stage, so that the chip tip made contact with the ball inlet. The alignment was important for a stable electrical connection between the ball and chip, and to achieve a constant sample deposit (trace) onto the ball surface.

Real-time CE-MS and chip-MS analysis were plotted using a two-dimensional NIH Image software (Image/J, National Institutes of Health, Bethesda, MD). Averaged (10 shots) mass spectra were stored and plotted on a 2-D graph using elution time versus mass-to-charge (*m/z*) ratio of the analytes present. All peptide and protein standards, MALDI matrices, separation buffer and digestion enzyme were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification.

2.4.3. Single Droplet Deposition

On-line droplet deposition studies were carried out with a piezoelectric actuated droplet generator (Model 201, Uni-photon Systems, Brooklyn, NY) and the ball inlet interface as shown in Figure 2-7.[58] Single droplets were deposited onto the ball at atmospheric pressure and rotated into the vacuum region for ionization. The droplet generator tip and matrix capillary were aligned at the center of the ball using an XYZ translation stage (Model LP-1, Newport, Irvine, CA). The alignment was necessary to prevent changing the laser alignment during analysis. Bringing the capillary into contact with the ball intermittently made matrix spots prior to analyte droplet deposition.

Droplets of analyte solution were delivered with a 50-μm i.d. fused silica capillary and syringe pump and deposited onto the MALDI matrix spots. The analyte was delivered into the

droplet generator from a 100 μM solution at a flow rate of 1.5 μL/min using a syringe pump (Model 55-2222; Harvard Apparatus). The droplet generator was pointed downward toward the

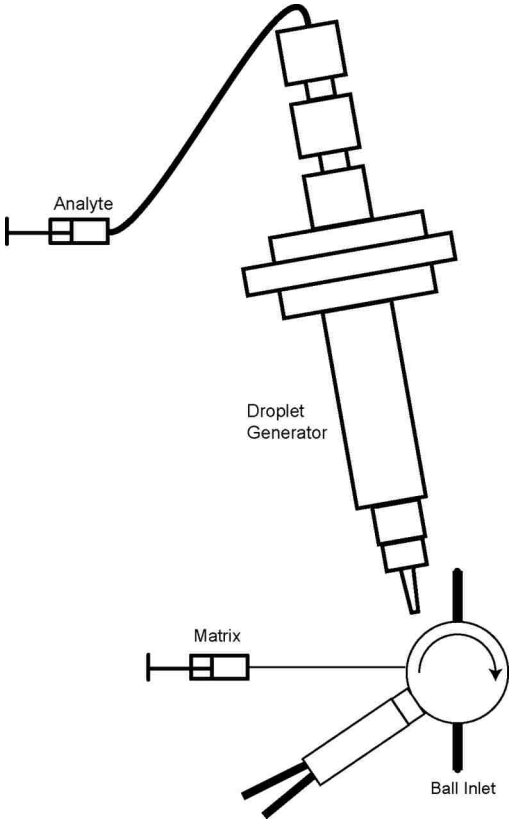

Figure 2-7. On-line single droplet deposition using the ball inlet interface.

ball inlet at a 10° angle from vertical. Sample droplets were ejected onto the matrix as the ball rotated below at 0.12 rpm while the generator was at a distance of 2.5 mm away from the ball. Once deposited, samples were rotated into the vacuum of the mass spectrometer ion source and ionized by a 10 Hz pulsed 355 nm Nd:YAG laser (Continuum Minilite 10, Santa Clara, CA). Single-stage extraction potentials were between 16 and 20 kV and mass spectra are an average of five laser shots.

Off-line single droplet studies were performed using a commercial MALDI-TOF mass spectrometer (Omniflex, Bruker, Billerica, MA). Matrix solution was first placed onto the sample target by depositing 0.27 μL of a 50 mM matrix solution using a 50 μm i.d. capillary. The distance between the droplet generator tip and the sample target was 2.5 mm. The matrix spot was allowed to air dry prior to analyte deposition. A single droplet of analyte at 100 μM concentration was then deposited onto the matrix-coated target. Mass spectra were a sum of 20 laser shots and acquired with a 337 nm nitrogen laser operating at a repetition rate of 2 Hz.

Figure 2-8. A photograph of the piezoelectric-actuated droplet generator mounted in XYZ stage with droplet ejection onto a commercial MALDI target.

2.4.4. Direct Contact Mass Spectrometry

Direct contact studies were carried out using the ball inlet interface. Samples were brought into contact with the rotating ball and detected on-line with and without the addition of MALDI matrix. Figure 2-9 illustrates the direct contact concept. On-line analysis is achieved by

manually bringing samples into contact with the ball inlet surface. The direct contact allows analyte to be smeared onto the ball surface where rotation and, later, ionization can take place. The solid or liquid phase material was then coated with a MALDI matrix layer and continuously delivered with a 50 μm fused silica capillary at a rate of 2 to 3 μl/min. To minimize sample

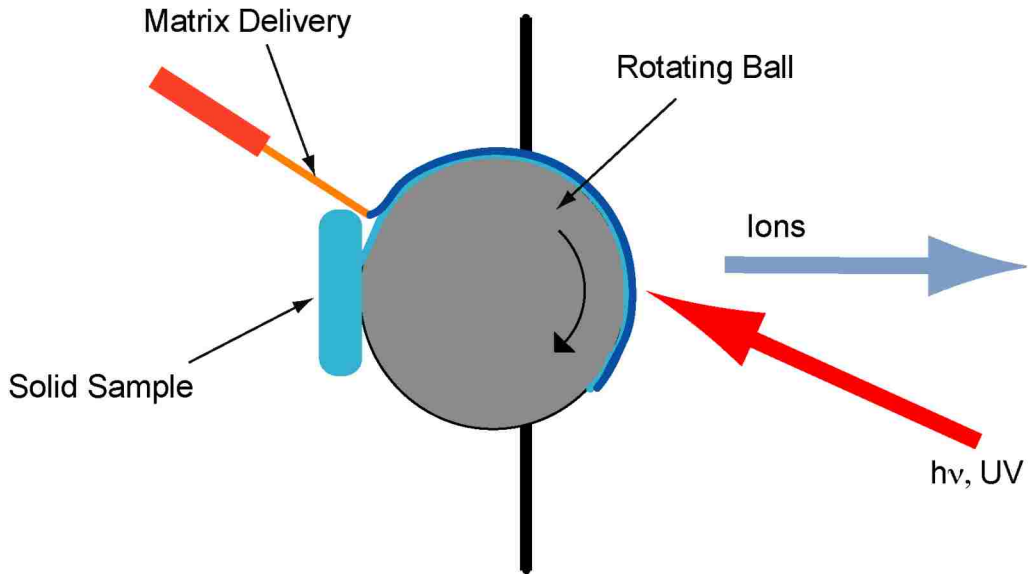

Figure 2-9. Direct contact ball inlet.

scraping from the interface gasket a specially designed ball inlet was constructed with a single track of wells spanning the circumference of the ball and is shown in Figure 2-10. Each well diameter was approximately 350 μm and 200 μm depth. The wells were spaced approximately 100 μm apart.

Acetaminophen tablets were broken and rubbed onto the ball surface without matrix addition or any further preparation. Biological fluids were obtained from the researcher. The MALDI matrix CHCA was flowed onto the ball surface at a flow rate of 3 μl/min with a 50 μm

fused silica capillary and syringe pump. Ionization was achieved at 355 nm, with ion extraction potential at 15 kV.

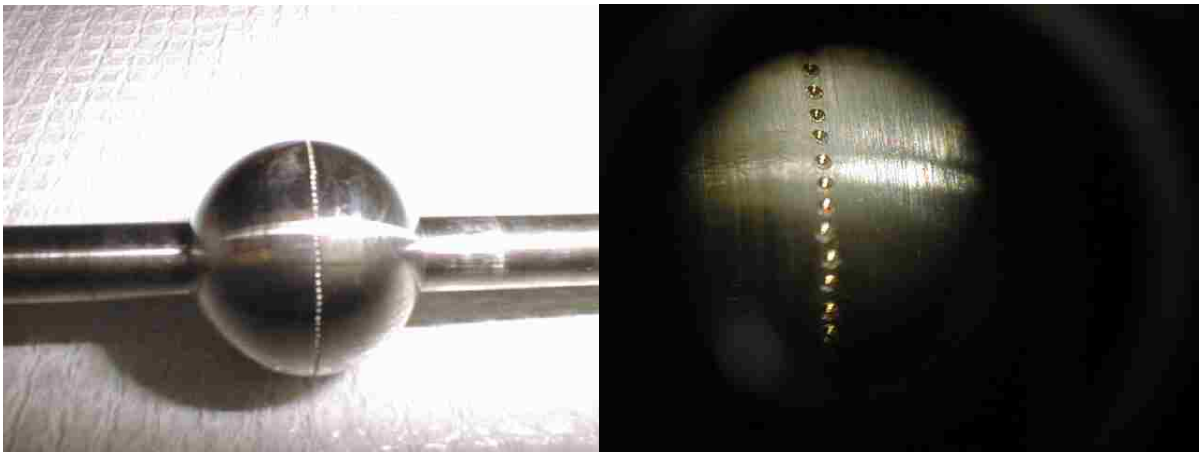

Figure 2-10. Ball and shaft configuration for direct contact solid sampling interface. The ball was machined with a trace of small wells seen in the lower image.

2.5. Direct Desorption/Ionization Interface

2.5.1. Interface

The direct desorption interface allows for multiple samples to be press mounted against the atmospheric side of the ionization source and laser ablated on the reverse (vacuum region) side. This allows for rapid sampling of successive samples by eliminating analyte insertion into the vacuum region of the mass spectrometer ion source. A side view illustration of the direct desorption interface is shown in Figure 2-11; the ion source configuration is as previously described. The direct desorption interface was constructed from stainless steel shim stock. The 0.559 mm thick shim stock was machined with a 1.5 in. circular diameter and a 1 mm centered hole. The hole functioned as an orifice for analyte exposure to laser irradiation, and a piece of clear adhesive tape was used to seal the hole when no analysis was being performed.

2.5.2. Direct Desorption/Ionization Analysis

The direct desorption interface lens was press mounted and centered onto a modified ISO 100 stainless steel flange (MDC). Figure 2-12 is a front view photograph of the direct desorption interface with lens and flange. The flange was machined with a 0.5 in. diameter centered hole. High vacuum grease (Dow Corning) and a thin nitrile o-ring was sandwiched in between the lens and flange. The vacuum grease provided adhesion for the lens to flange, and along with o-ring, provided a vacuum seal.

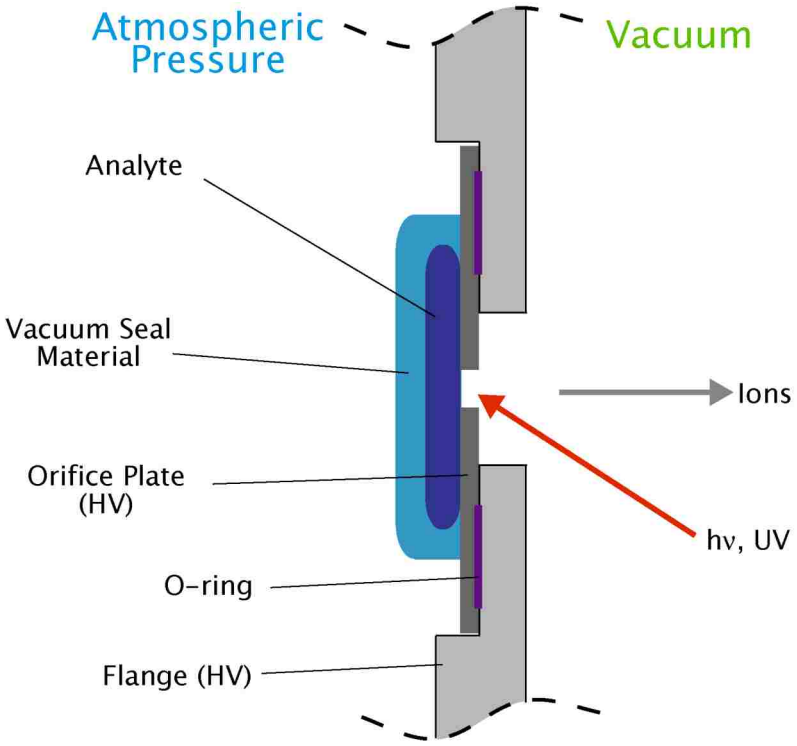

Figure 2-11. Side view illustration of the direct desorption/ionization interface and process. Analyte is pressed against the atmospheric side of the interface while laser ablation occurs on the vacuum side.

Direct desorption experiments were performed by affixing analyte to the sticky side of clear adhesive tape for analysis. Crystalline peptide samples and matrix were pipetted directly

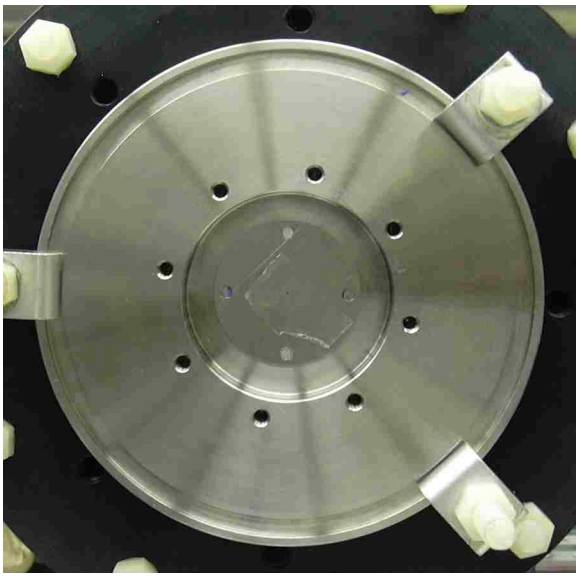

Figure 2-12. Direct desorption/ionization interface.

onto the tape using the dried droplet method, unless otherwise noted. Analytes were either placed directly onto the sticky side of clear adhesive tape or tape-lifted directly from a surface. Ionization was achieved with a pulsed 337 nm nitrogen laser.

CHAPTER 3. ON-LINE MALDI AS A DETECTION METHOD FOR MICROFLUIDIC CHROMATOGRAPHY*

3.1. Overview

The purpose of this research was to develop a ball inlet interface for on-line UV-MALDI detection for microfluidic chips. This chapter describes the application of the ball inlet interface as a continuous sampling apparatus for the mass spectrometry analysis of various electrokinetic separations. Capillary electrophoresis (CE) experiments were performed to study the capabilities of the ball inlet as an interface for on-line MALDI detection. A known peptide mixture was electrokinetically separated with capillary electrophoresis and detected, on-line, with MALDI-MS. Microchip electrophoresis experiments were performed with specially designed prefabricated polymer-based microfluidic chips. The ball inlet was used to directly couple a microfluidic chip to MALDI-MS for on-line detection of various microchip experiments. Peptide mixtures and protein digest were separated and detected with on-line MALDI-MS.

3.2. Introduction

The field of bioanalytical analysis has employed the merging of analytical techniques with chip-based, or micro total analysis systems (μTAS). The advantages of micro analytical devices are low sample consumption volumes, fast analysis and low fabrication cost. Generally, biological samples are available in small quantities, often in complex mixtures, making the merger of biological MS and microfluidic devices a logical option for proteomic analysis. Typically, ESI has been the desired ionization method when coupling microfluidic chips to mass spectrometry, but the advantages of MALDI make it a valuable alternative.

Off-line MALDI chip analysis are commonly employed, but due to the high vacuum of a typical MALDI ion source, transfer of samples from separation platforms into a mass

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spectrometer ion source is slow. The off-line approach is performed by mechanical insertion into the vacuum of the ion source making it less desirable for high-throughput applications. Alternately, chips employing rapid open access channels, multiple sample wells, chips placed into the vacuum of the mass spectrometer and chips containing microarrays have been reported for off-line anaylsis.[34, 35, 41, 59-62]

On-line approaches are defined as real-time where the sample is continuously delivered to the vacuum.[19] On-line MALDI analysis can be achieved with continuous sample delivery onto a ball inlet and, subsequently into the vacuum region of the mass spectrometer. Successful coupling of microfluidic chips to the ball inlet interface requires a chip designed with an open fluidic channel for the transfer of fluid onto the ball surface by direct contact.[57] The direct contact of capillary electrophoresis to ball inlet was previously demonstrated.[50] This interface can maintain an electrical connection necessary for electroosmotic flow through the chip when excess buffers are added when needed.

In this work, microfluidic chips, traditionally fabricated with silicon or glass, were fabricated with poly(methylmethacrylate) or PMMA polymer.[63] Polymer chips are advantageous due to the ease and low cost of fabrication, generally by injection molding or hot embossing, and they are easily replicated. Detection methods for microchips are traditionally optical methods such as fluorescence or absorption spectroscopy where optical clarity of the chip is a requirement, but in this case a mass spectrometer provides detection making chip optical properties insignificant.

3.3. Experimental

On-line capillary electrophoresis mass spectrometry was performed with a 20 cm long fused silica capillary in contact with the ball inlet at one end, and submerged into a buffer solution at the opposite end. The potential applied to the ball inlet for MS ion extraction, positive

10 kV, also operated as the cathodic potential for CE. A platinum electrode submerged in the buffer vial provided the anodic potential of 18-22 kV and was manually switched, with corresponding capillary end, to a sample vial containing analyte/buffer solution for a 10 s plug injection. Microfluidic chips were fabricated with a poly(methylmethacrylate) (PMMA) polymer and specially designed for direct coupling to the ball inlet. The fluidic channel exit was scored in a way as to allow eluant to electroosmotically flow out of the chip, directly onto the ball inlet surface, when chip/ball contact was established. Ball inlet speed was held constant at 0.2 rpm and matrix flow rate was 3 μl/min.

Thickness of the sample deposit on the ball surface was measured with a surface profiler (P-11, Tencor, Santa Clara, CA). After a sample trace was deposited onto the ball, on-line, the ball assembly was removed and the different areas of the ball surface were measured. Ten replicates at a scan length of 2 mm at 20 μm/s with a stylus force of 2 mg were taken of the blank ball surface and of the sample deposition thickness before and after passing the gasket.

Insulin (I-5500), bradykinin (B-3259), substance p (S-2136), neurotensin (N-6383), vasopressin (V-6879), bombesin (B-4272), adrenocorticotropic hormones (ACTH) fragment 18-39 (A-0673), cytochrome *c* (C-2506), ammonium bicarbonate (A-6141), trypsin digest buffer, trypsin (T-6567), peptide separation buffer (P-2188), and the matrix, α-cyano-4-hydroxycinamic acid (C-2020), were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. The peptide sample solutions were prepared in the separation buffer while the matrix was prepared in methanol. PMMA sheets were obtained from MSC industrial supply company (Melville, NY).

3.4. Results

The ball inlet interface allows for samples deposited at atmospheric pressure to be rotated into the vacuum region of the mass spectrometer for ionization. Surface profile measurements of

the ball inlet are shown in Figure 3-1. A 50 mM CHCA matrix solution dissolved in methanol was delivered by capillary onto the ball surface at a flow rate of 2 μl/min. The deposit thickness of the crystallized sample was 15 μm prior to rotation past the gasket, scanned perpendicular to the track (Figure 3-1a). The blank ball surface is shown in Figure 3-1c. After rotation past the gasket, the deposit thickness was 10 μm (Figure 3-1b). These results suggest there is a loss of, approximately, one-third of the sample deposit when rotated past the gasket.

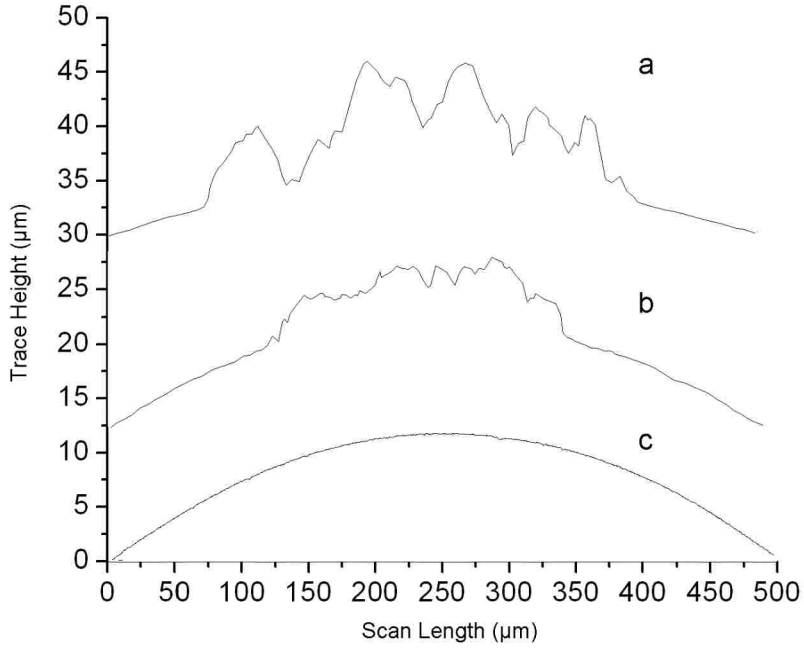

Figure 3-1. Surface profile of interface ball a) coated with a crystalline CHCA trace, b) after the sample trace has passed under the interface gasket, and c) the uncoated ball surface.

A comparison of static and on-line performance of the ball inlet interface is shown in Figure 3-2. A 20 μM solution of insulin, and a separate matrix solution were deposited at a flow rate of 2 μl/min for both experiments. Static mode operation was achieved by stopping the ball rotation after sample delivery, manually rotating the ball by breaking vacuum, then realigning

the sample track with the laser and restoring vacuum prior to mass analysis (Figure 3-2a). On-line studies were performed while the ball rotated continuously at a rate of 0.3 rpm (Figure 3-2b). The analyte and matrix deposit crystallized before passing under the interface gasket. Mass spectra were obtained at 10 kV extraction potential and were an average of 20 laser shots. Similarities in peak intensities and mass resolution in the resulting mass spectra indicate that scraping from the interface gasket leaves behind sufficient material for mass spectrometry analysis. However, on-line operation yields a higher ion signal in the low mass region.

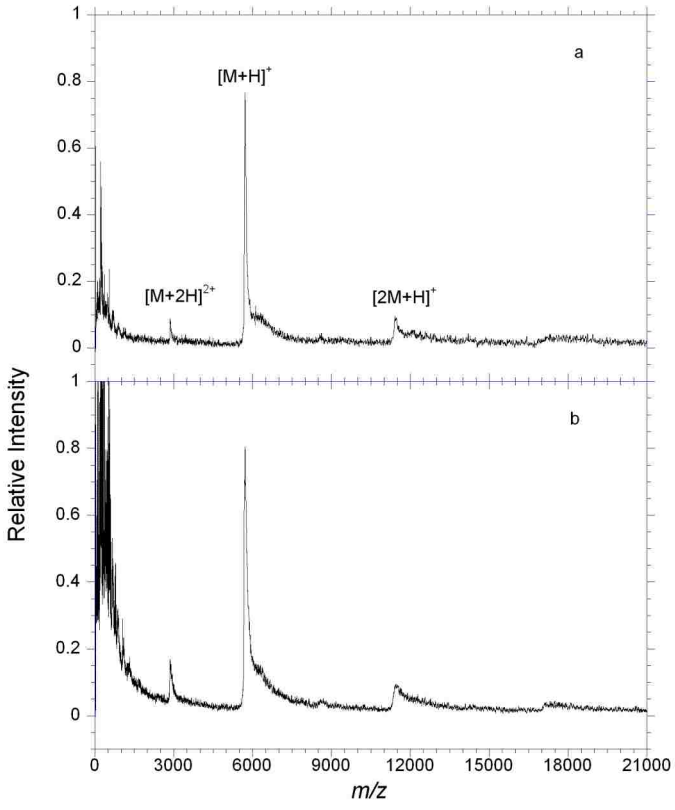

Figure 3-2. Ball inlet mass spectra of 20 μM Bovine insulin with CHCA matrix in a) static mode and b) on-line mode at 0.3 rpm.

Capillary electrophoresis (CE) was performed on-line with the ball inlet interface operating at 0.04 rpm. The slower rotation speed was chosen due to the lower flow rates under

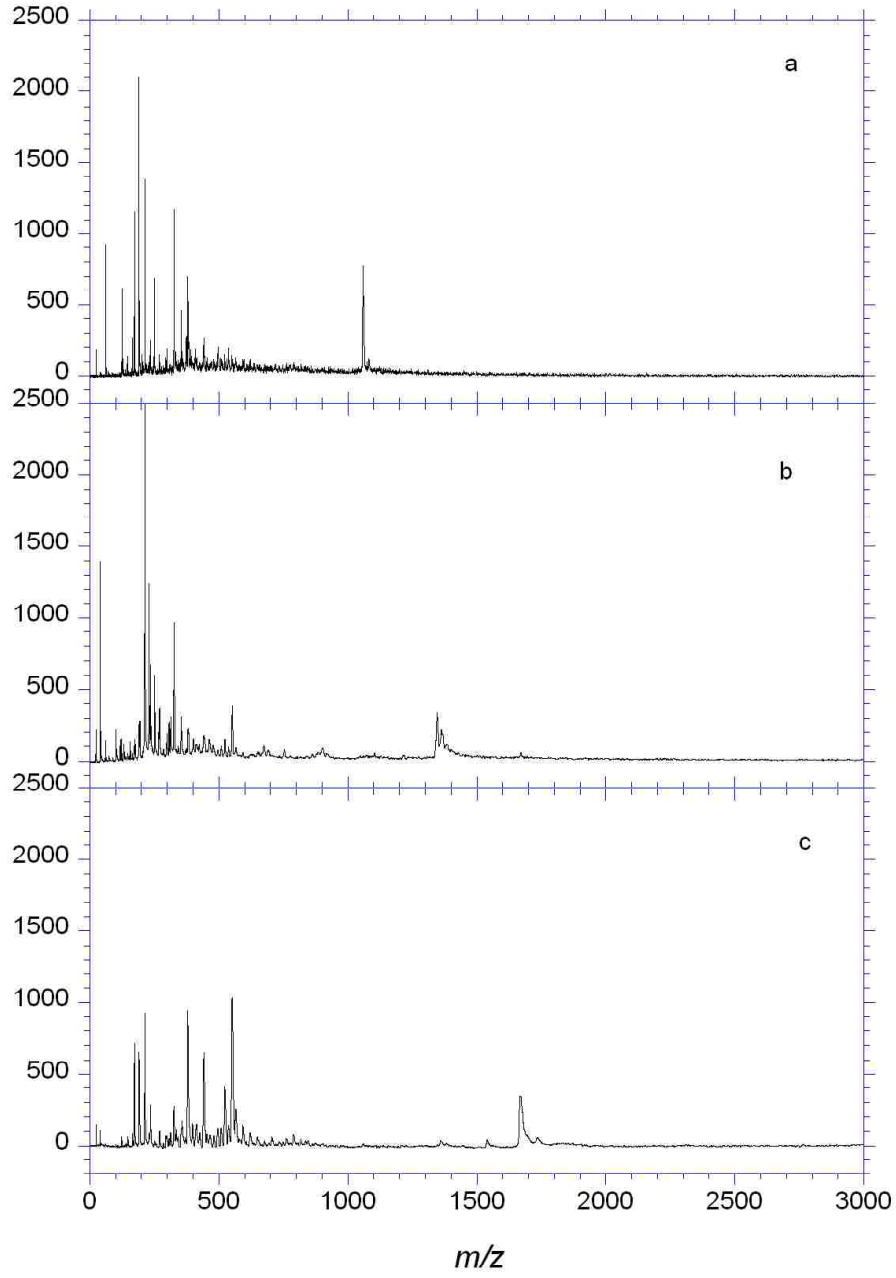

Figure 3-3. On-line capillary electrophoresis detection with the ball inlet interface. The peptides a) bradykinin, b) substance p, and c) neurotensin were electrokinetically injected.

CE operation. The electrokinetic injection and on-line MALDI-MS detection of peptides bradykinin, substance p, and neurotensin are shown in Figure 3-3. Peptides were prepared in

20 μM discrete sample solutions, and injection parameters yielded plug injection volumes of approximately 5 nL, resulting in 100 fmol quantities of each peptide. Mass spectra were obtained continuously after peptide migration through the capillary and onto the ball surface. Low mass interference below 700 *m/z* is mostly attributed to impurities from CE buffer salts. Protonated molecule peaks of each peptide were clearly observed with a mass resolution of approximately 250.

The separation of three peptides, vasopressin, substance p, and neurotensin, is shown in Figure 3-4. A 2-D grayscale contour plot was generated from multiple mass spectra obtained during the experiment. Ionization was achieved with a 355 nm UV laser at 2 Hz. The x-axis

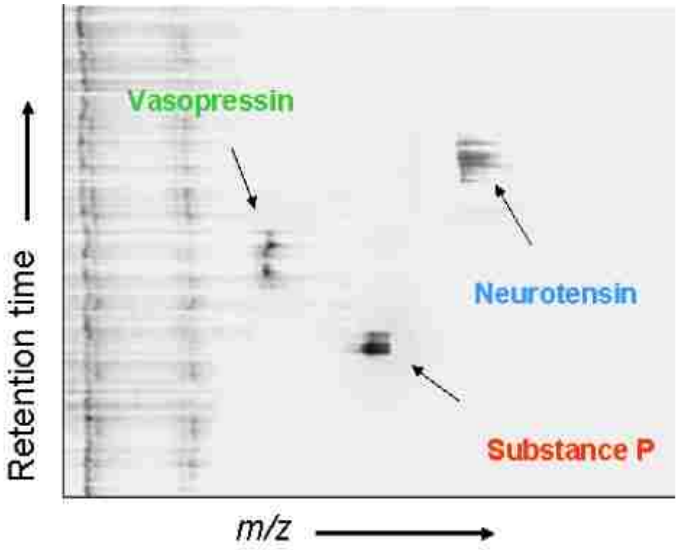

Figure 3-4. A grayscale 2-D contour plot of the on-line separation and detection of a three-peptide mixture.

corresponds to *m/z*, increasing from left to right, and the y-axis corresponds to retention time during CE, increasing from bottom to top. Substance p, vasopressin and neurotensin were detected at 14.80 min, 15.75 min and 16.80 min respectively. Low mass interference peaks from MALDI matrix and CE buffer are seen as the dark stripes along the y-axis.

3.4.1. On-line Microfluidic Chip Detection

Specially designed microfluidic chips were used for on-line chip electrophoresis and MALDI-MS detection with the ball inlet interface. A photograph of on-line chip analysis setup is shown in Figure 3-5. A specially designed power source supplied the chip with potentials for on-chip separation and plug injections. The PMMA chip was designed to allow fluids to exit the separation channel and flow directly onto the ball inlet surface. The ion extraction voltage applied to the ball inlet also acts as the cathodic potential for chip electrokinetics. The microfluidic chip was fabricated with poly(methylmethacrylate) polymer. Ball inlet rotation was 0.2 rpm and field strengths were 200 V/cm during analysis. Cleaning was achieved with a solvent saturated felt pad.

Figure 3-5. A photograph of on-line microfluidic chip detection with ball inlet MALDI-MS.

The PMMA chip was designed to allow fluids to exit the separation channel for deposit onto the ball inlet surface. Three electrodes from a home-built power source supply injection and separation potentials. The ion extraction potential applied to the ball inlet also acts as the

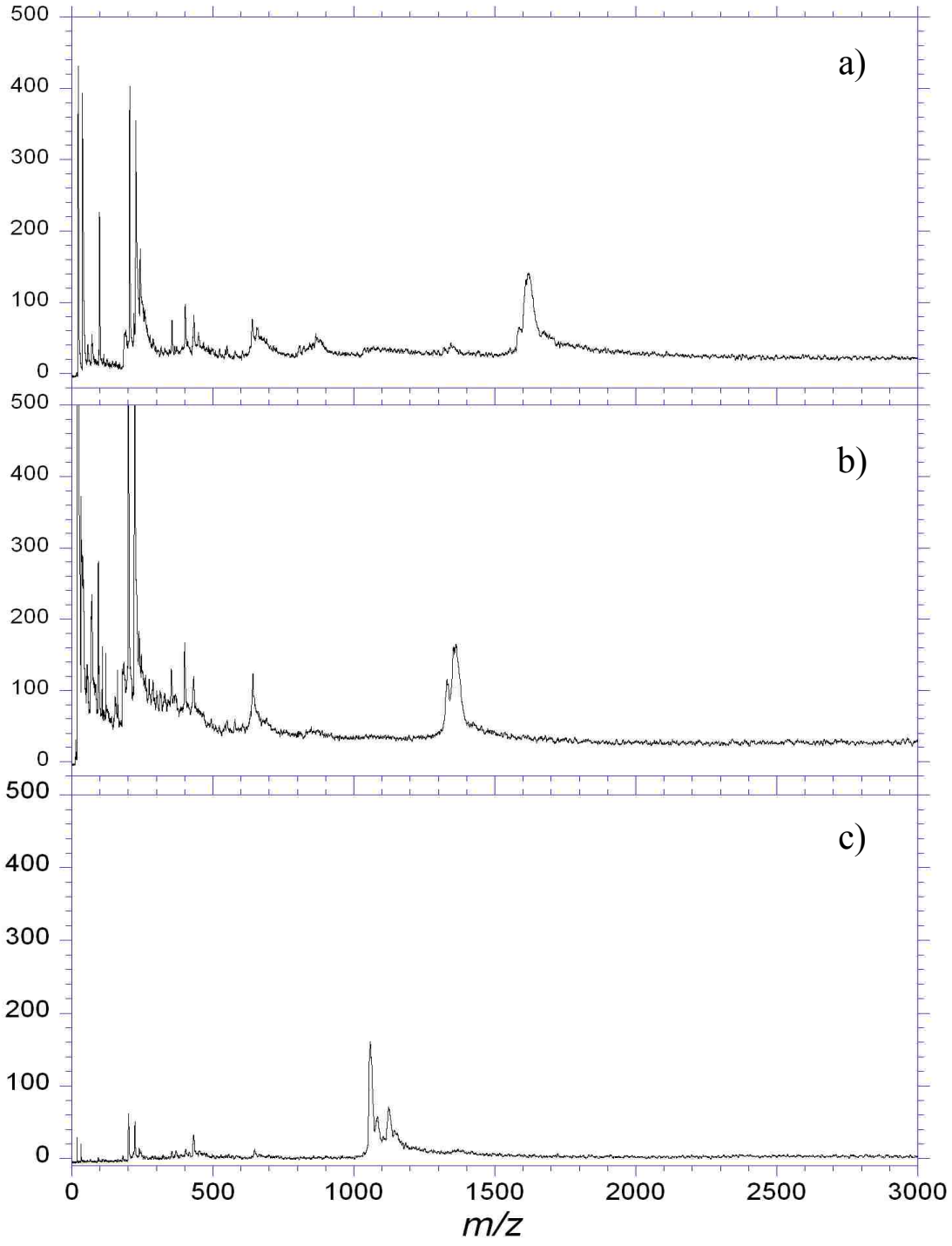

Figure 3-6. Averaged mass spectra acquired from on-line microfluidic chip detection with ball inlet MALDI-MS. Electrokinetic injections of 10 fmol each of a) bombesin, b) substance p, and c) bradykinin.

cathodic potential for chip electrophoresis. The chip was mounted on an electrically isolated holder and attached to an XYZ stage. MALDI matrix and supporting buffer were delivered with fused silica capillaries.

On-line chip electrophoresis was studied by injecting single peptides into the chip and observing their migration with MS detection. The on-line chip detection of peptides, bombesin,

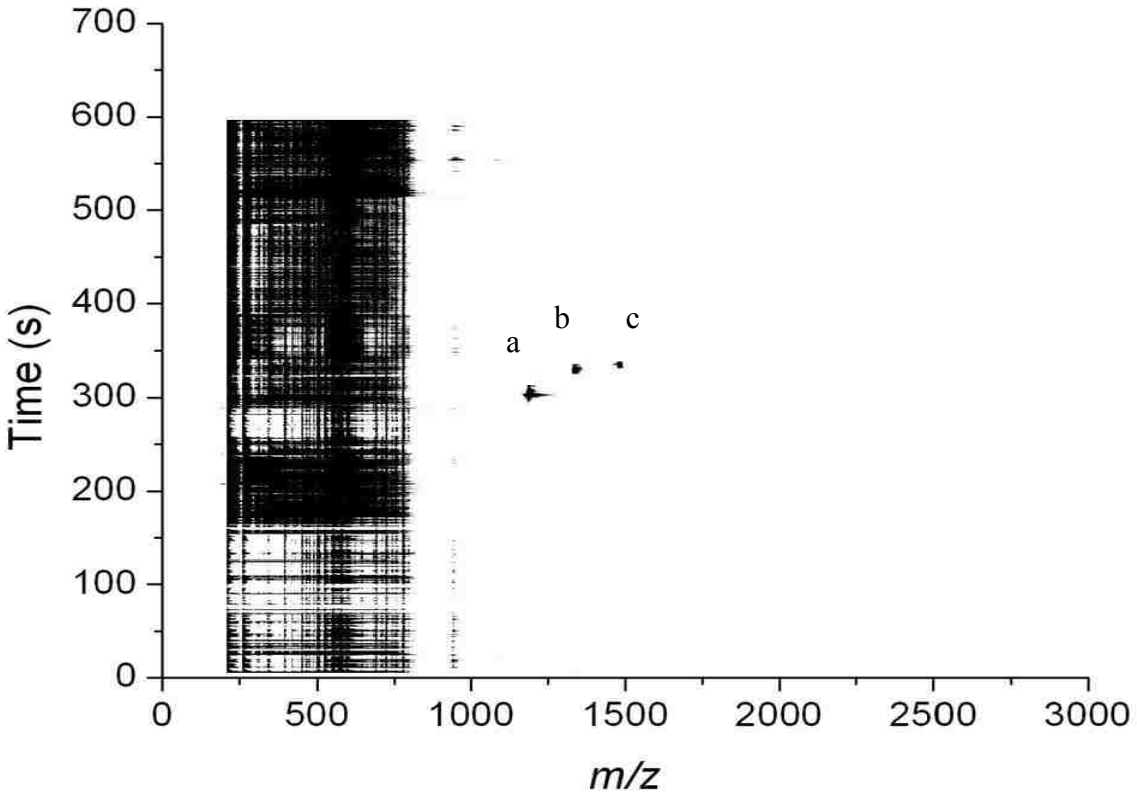

Figure 3-7. 2-D contour plot of a peptide separation with on-line ball inlet MALDI detection. The peptides a) bradykinin, b) substance p, and c) bombesin were separated in a microfluidic chip.

substance p, and bradykinin are shown in Figure 3-6. Each spectrum resulted from an electrokinetic injection of 10 fmol of each peptide. Mass spectra were an average of 5 laser shots using the experimental conditions described previously. This result demonstrates the capability of this chip design to perform microfluidic electrokinetics and transport fluids to the surface of

the ball inlet. The mass resolution of analyte ion signals was, on average, 150 fwhm. The decrease in analyte concentration and volume for chip analysis, in comparison to capillary, did not prevent the acquisition of a usable mass spectrum.

An on-chip separation was performed with a three-peptide mixture and detected on-line with ball inlet MALDI-MS. A 2-D contour plot of the separated peptides is shown in Figure 3-7. The peptides bradykinin, substance p, and bombesin were premixed and manually injected into the microfluidic chip. The three peptides, 10 fmol of each, were separated and detected on-line. The x-axis on the grayscale plot represents *m/z* and the y-axis represents the elapsed time before the peptide ion signal was detected. Each peptide can be clearly identified with its spot location in the appropriate mass range on the plot (bradykinin *m/z* 1061, substance p *m/z* 1349, and bombesin *m/z* 1619). The dark area in the low mass region represents MALDI matrix and buffer salts interference. This on-line separation with MALDI detection demonstrates the potential of a good tool for bioanalytical analysis.

3.5. Summary

In the work described in this Chapter, a new rotating ball interface for on-line MALDI was demonstrated and used for the on-line detection of microfluidic separations. The ball inlet design allows sample deposition at atmospheric pressure where the sample is allowed to dry then delivered into the vacuum region of the mass spectrometer for ionization. A specially designed polymer gasket maintained a vacuum seal for the ball inlet interface. After MALDI analysis, the sample trace exited the vacuum region and the ball surface was cleaned for subsequent deposits. On-line capillary electrophoresis separation was demonstrated with the MALDI-MS detection of a mixture of three peptides.

The coupling of a PMMA microfluidic chip on-line with MALDI-MS using the ball inlet interface was described. Specially designed chips eliminate the need for a sample transfer

capillary connection from the fluidic channel to the mass spectrometer. Additional transfer capillaries can reduce separation efficiencies in chromatography analysis. A peptide mixture was injected, separated, and detected on-line demonstrating the interface robustness, high separation efficiency and femtomole sensitivity.

CHAPTER 4. SINGLE DROPLET DEPOSITION*

4.1. Overview

In this chapter the use of a piezoelectric single droplet generator with the ball inlet for non-contact sample deposition is described. An increase in sensitivity of a MALDI analysis can be achieved by reducing the sample spot size to a value at or below the laser spot size. Individual picoliter volume droplets of analyte were deposited onto matrix spots and analyzed by on-line MALDI-MS. Protonated molecule signal from peptide and protein containing single droplets was obtained. The ball interface design allows for droplet deposition at ambient pressure. The non-contact approach prevents sample mixing when samples are transferred to the ball surface by eliminating the dead volume at the contact point.

4.2. Introduction

A typical MALDI sample preparation involves the deposition of microliter sample volumes on a metal target to produce a spot several millimeters in diameter.[64] Sample preparation is one of the most important aspects of MALDI-MS, directly affecting the sensitivity, sample consumption, and tolerance to impurities. Generally, a focused laser spot is a few tenths of a millimeter in diameter making it possible to improve sensitivity by reducing the sample spot size. Hydrophobic surfaces on MALDI targets have been shown to reduce sample spot size, for example, employing a hydrophobic coating with an array of 200 μm uncoated gold spots.[65] The final sample spot was minimized due to the contrasting surface properties which cause a polar solvent to bead on the surface during evaporation.

Single droplet production and deposition can further improve sensitivity and speed of analysis and this benefit has been widely displayed.[66-68] Small droplets can be generated by

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thermal, piezoelectric, electrostatic, or acoustic methods with piezoelectric being the most common.[69, 70] In these devices, a radially contracting piezoelectric cylinder or flexing diaphragm causes a rapid pressure pulse that releases a single droplet through an orifice upon the application of a voltage pulse. Spot sizes from 100 to 500 μm can be created from droplets of tens to hundreds of picoliters in volume, and multiple droplets can be deposited on the same spot to yield a different quantity of analyte.

Sample treatment on a nanoliter scale is contingent on the ability to manipulate small sample volumes in the form of droplets. Off-line coupling of MALDI to liquid separations has been described using single droplet deposition.[71-73] An integrated microanalytical platform for automated protein identification has been developed for enzymatic digestion of protein samples using a piezoelectric microdispenser for MALDI-TOF-MS.[69, 73] Also, biomolecules were identified using a piezoelectric flow-through microdispenser for interfacing capillary liquid chromatography with MALDI-TOF-MS.[74]

Single droplet deposition is available for off-line MALDI analysis but on-line MALDI analysis is achievable. The modified ball inlet allows for a non-contact deposition approach for on-line coupling. Matrix spots were formed on the ball using a 50 μm capillary and single droplets were deposited on top of the matrix spots. Results comparing on-line and off-line single droplet deposition MALDI analysis are described.

4.3. Experimental

A syringe pump delivered a 100 μM analyte solution to the droplet generator mounted on an XYZ translation stage pointing downward 2.5 mm away from the ball surface. Analyte droplets were ejected onto matrix spots deposited with a 50 μm i.d. fused silica capillary. Ball rotation was held constant at 0.12 rpm during online analysis, and 100 μM analyte was delivered at a flow rate of 1.5 μL/min. A 355 nm Nd:YAG laser at 10 Hz was focused to a spot size of 100

μm × 300 μm. Ion extraction was achieved with 16 to 20 kV positive potential applied to the ball and, upon rotation, sample spot cleaning was performed with a solvent saturated felt pad. Off-line comparison of single droplet deposits was accomplished with a commercial mass spectrometer equipped with a 337 nm nitrogen laser.

The analytes angiotensin I (A-9650, Sigma, St. Louis, MO), bradykinin (B-3259, Sigma), substance p (S-6883, Sigma), bovine insulin (I-5500, Sigma), cytochrome *c* (C-2506, Sigma), and horse myoglobin (M-1882, Sigma) were used without further purification. The MALDI matrices α-cyano-4-hydroxycinnamic acid (CHCA, Sigma), 3,5-dimethoxy-4-hydroxy cinnamic acid (sinapic acid, Fluka, Ronkonkoma, NY), 2-(4-hydroxyphenylazo)-benzonic acid (HABA, Aldrich, Milwaukee, WI), 2,5-dihydroxy benzoic acid (DHB, Aldrich) and 3,4-dihydroxy-cinnamic acid (caffeic acid, Aldrich) were prepared in methanol (200 Proof, AAPER Alcohol, Shelbyville, KY) with 1% trifluoroacetic acid (TFA, J. T. Baker, Pittsburgh, NJ) at concentrations of 50 mM. Analyte solutions were made in distilled water (house supply) with 1% TFA at concentrations of 100 μM. Droplet size was measured using a solution of 10% glycerol (99.7%, Fisher, Fairlawn, NJ) in distilled water. Details of the droplet generator are discussed in Chapter 2.

4.4. Results

Droplet studies were performed with a solution of 10% glycerol in water to reduce the evaporation rate of the droplets at ambient temperature and pressure. Droplet size measurements were performed with a series of concentric circles, printed on white paper, and covered with a layer of Parafilm. The droplets were deposited onto the hydrophobic Parafilm surface resulting in nearly spherical droplets. The concentric circles were used as rulers and were visible through the Parafilm as shown in Figure 4-1. The diameter of single ejected droplets was 57 ± 6 μm, yielding a volume of 97 ± 3 pL, obtained from 15 measurements. Droplet placement accuracy

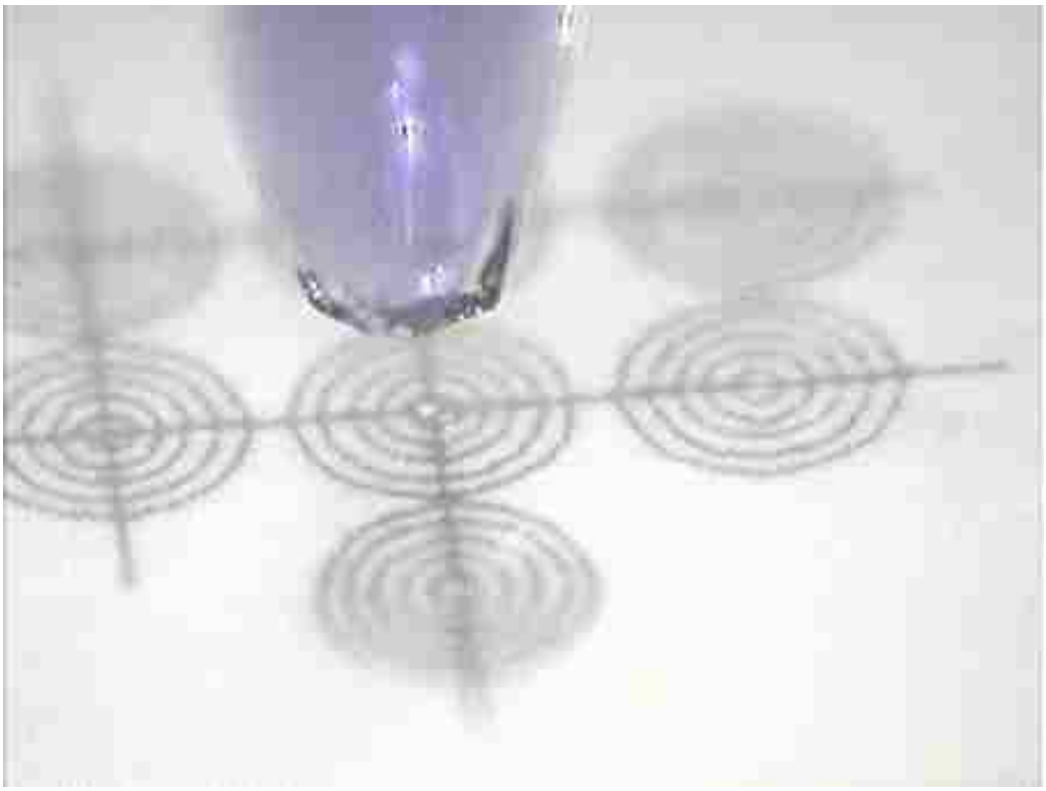

Figure 4-1. Photograph of droplet size analysis, the piezoelectric droplet generator glass tip was 40 μm i.d. producing droplets approximately 0.1 nL.

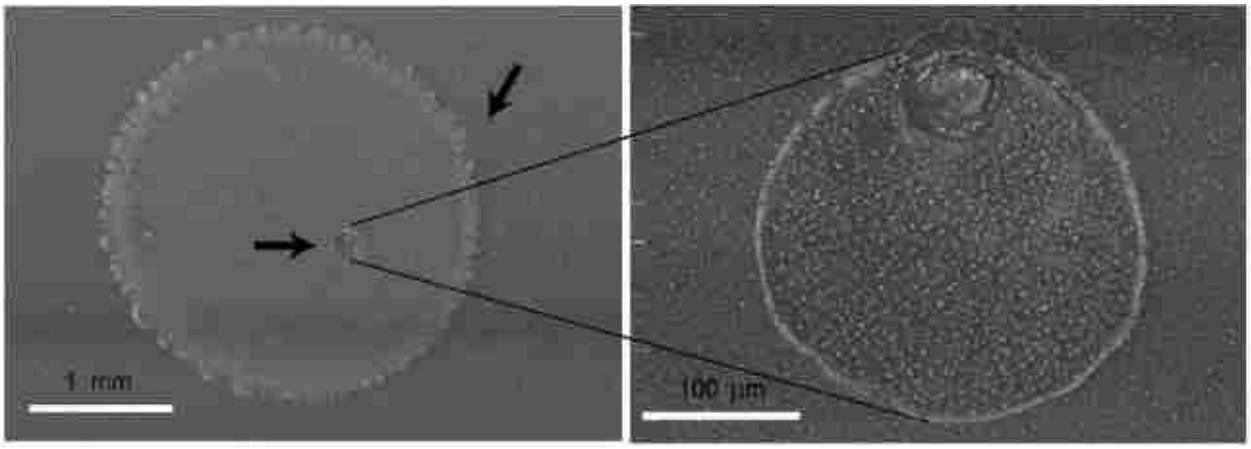

Figure 4-2. Scanning electron micrograph of matrix with interior analyte spot (left) and a close up of the analyte spot (right).

was measured without Parafilm by depositing 100 single droplets directly onto the printer paper. A solution of 100 μM angiotensin I in distilled water was deposited at a rate of 1 Hz and 1.5 μL/min, resulting in 94% ± 2% of the droplets falling within a 400 μm diameter circular spot.

4.4.1. Off-line Droplet Deposition

Matrix and analyte sample spots were studied using scanning electron microscopy and are shown in Figure 4-2. Approximately 0.27 μL of a 50 mM matrix solution was deposited with a 50 μm i.d. capillary and allowed to air dry resulting in a spot approximately 2.5 mm in diameter. An 80 pL droplet of 100 μM angiotensin I solution, or 8 fmol, was deposited on top of the matrix spot (Figure 4-2 left). The dried analyte spot was between 200 and 250 μm diameter (Figure 4-2 right). A slight deformity around the edge of the analyte spot may be due to uneven solvent evaporation, otherwise, the spot is consistent throughout.

Off-line MALDI-MS of single droplets was carried out on a commercial MALDI-TOF mass spectrometer. Mass spectra of proteins are shown in Figure 4-3. Deposits were made onto MALDI targets and spectra were obtained from single droplets. A mass spectrum from a single droplet of 8 fmol bovine insulin (M_r = 5733.5) is shown in Figure 4-3a. The $[M + H]^+$ peak was obtained at a mass resolution of 1500 fwhm. A mass spectrum from a single droplet of 8 fmol cytochrome *c* (M_r = 12,384) is shown in Figure 4-3b. The $[M + H]^+$ peak and the $[2M + H]^{2+}$ peak, corresponding to the doubly protonated species, are shown with a mass resolution of approximately 50 fwhm. Both proteins were analyzed with CHCA matrix and were detected at limits lower than the reported detection limit of the mass spectrometer with a standard sample preparation.

4.4.2. On-line Droplet Deposition

On-line droplet analysis was performed with ball inlet. Single droplets were deposited onto the ball inlet at atmospheric pressure and rotated into the vacuum region for ionization. The

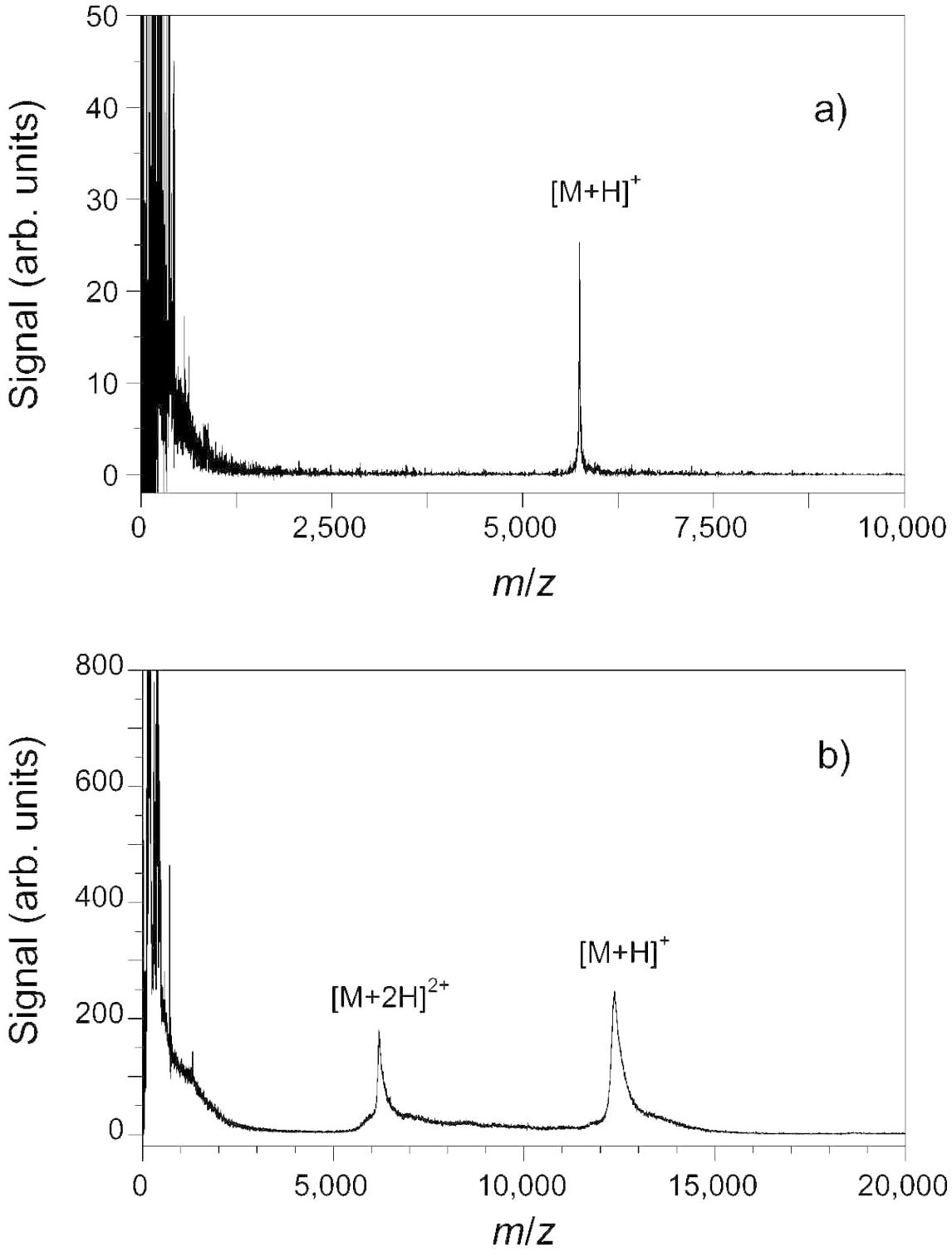

Figure 4-3. Off-Line MALDI mass spectra of single droplet deposition of a) 8 fmol bovine insulin and b) 8 fmol cytochrome *c* with CHCA matrix.

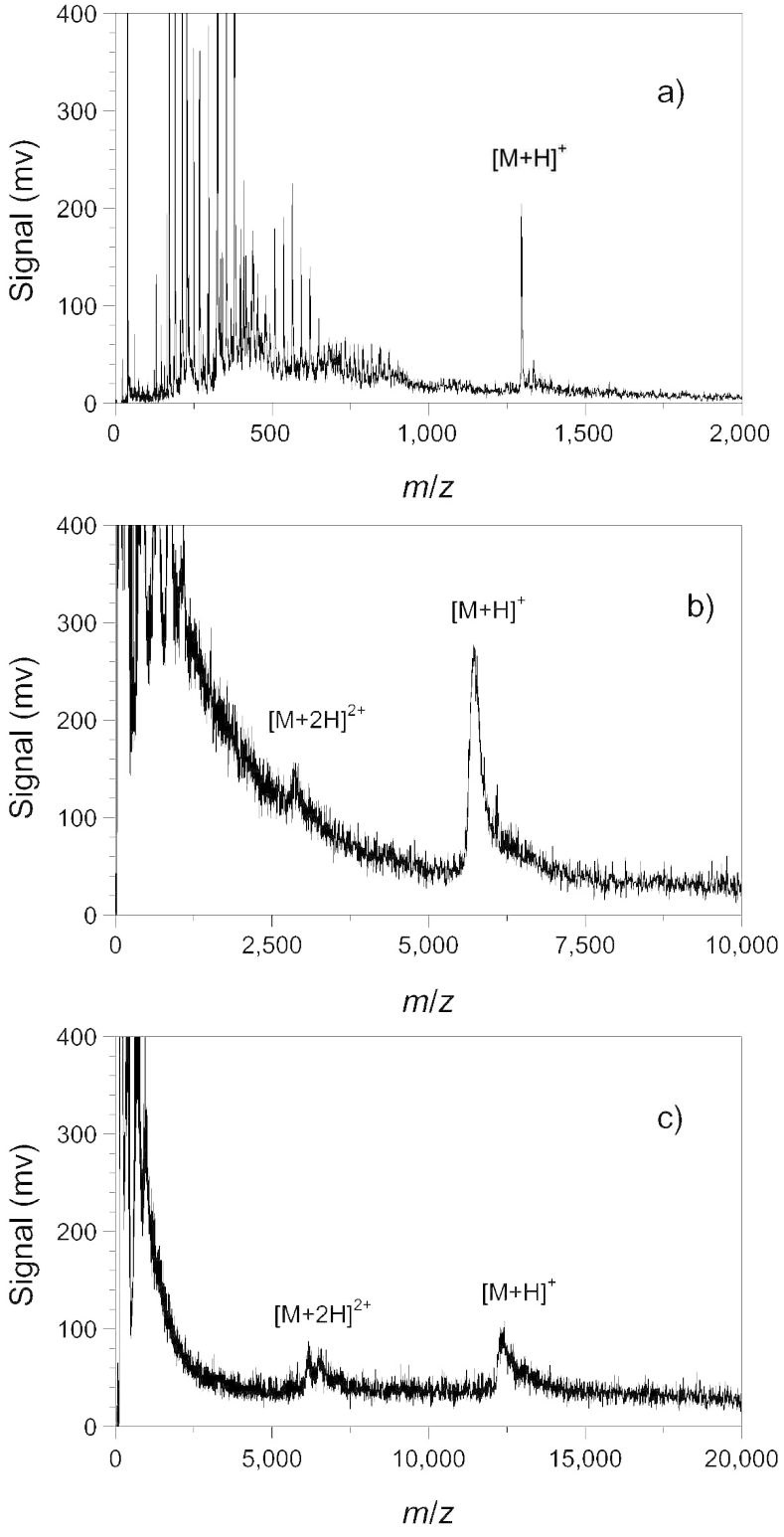

Figure 4-4. On-line MALDI single droplet deposition mass spectra of a) angiotensin I, b) bovine insulin, and c) cytochrome *c*.

droplet generator tip and matrix capillary were aligned at the center of the ball with translation stages. The alignment was necessary to preserve the laser alignment during analysis. Bringing the capillary into contact with the ball, intermittingly, made matrix spots prior to analyte droplet deposition. A mass spectrum of angiotensin I obtained in this manner is shown in Figure 4-4a. The protonated molecule peak is observed with a mass resolution of 400.

An on-line droplet deposition mass spectrum of 8 fmol bovine insulin is shown in Figure 4-4b. The analyte spot was deposited on a CHCA matrix spot coated on the ball surface. The spectrum contains the singly protonated $[M + H]^+$ peak and the doubly protonated $[2M + H]^{2+}$ peak. A mass spectrum of cytochrome *c* with on-line droplet deposition is shown in Figure 4-4c. The spectrum was obtained from 8 fmol of analyte with CHCA matrix. The singly protonated peak $[M + H]^+$ and the doubly protonated peak $[2M + H]^{2+}$ were observed. The resolution for both mass spectra is 40 fwhm, and is lower than that obtained from the off-line analysis.

4.5. Summary

The rotating ball inlet coupled with a piezoelectric single droplet generator for picoliter volume detection and femtomole sample detection with MALDI-TOF MS was demonstrated. Single droplets of 100 pL volume were deposited on the rotating ball with a placement precision of 400 μm. The easily accessible atmospheric pressure sample deposition region and vacuum detection are distinct characteristics of this interface. Peptides and small proteins were readily detected in low femtomole quantities; however, an increase in mass resolution and a reduction of analyte concentration will improve overall performance.

The ball deposition provided mass resolution significantly lower than the off-line single droplet deposit approach. The lower resolution may be attributed to the round surface of the ball having an adverse affect on the spatial focusing of the ions during desorption due to field inhomogenieties in the ion extraction region. This can result in a broad distribution of ion flight

times in the accelerated ion packet. Another possible reason for the lower mass resolution is that when the sample spot passes through the gasket, the mechanical force between the gasket and the ball change the surface morphology or adds contaminants to the deposit. Furthermore, the off-line instrument utilizes delayed ion extraction to improve mass resolution, which is not used on the rotating ball instrument.

The addition of delayed ion extraction, multiple stages of ion acceleration or a reflectron flight tube may improve mass resolution but the curvature of the ball surface may ultimately limit the obtainable mass resolution. Resolution loss due to the ball surface curvature may be overcome through electrostatic lensing and by limiting the exposed portion of the ball surface. Ultimately, orthogonal extraction may be the ideal configuration for the rotating ball MALDI interface where the decoupling of the ion formation and acceleration regions will allow the ball curvature to have little effect on mass resolution.[75]

The 100 μm concentrations used in this study are one to two orders of magnitude higher than common MALDI samples. Better droplet deposition methods can improve the detection limit performance. Here, small droplets with high organic solvent content are deposited onto a dry matrix layer. This method may not promote good mixing and co-crystallization of matrix and analyte prior to solvent evaporation. Some of the analyte may deposit on top of the matrix and consequently sample loss may occur due to mechanical forces when the sample passes under the gasket. Detection limit and mass range may be improved with a modified ball surface that promotes co-crystallization and limits sample loss. Methods such as separate single drop deposition of both matrix and analyte onto the ball surface or on-target sample concentration can improve the concentration detection limit.

Future studies for the single droplet deposition include improvements in detection limit and mass resolution, and developing single droplet deposition with microfluidic devices.

Implementation of on-line single droplet deposition for MALDI can be readily adapted to microfluidic devices using either piezoelectric or pneumatic ejection of droplets. A single droplet microfluidic system will allow a non-contact interface with on-line MALDI detection. Potentially, multiplexing can be achieved with the ejection of droplets onto adjacent tracks on the ball inlet or cylinder inlet. The addition of a stepper motor controlled rotation system will also improve the high-throughput capability by increasing the speed of analysis and allowing rapid and precise placement of the spots.

CHAPTER 5. DIRECT CONTACT BALL INLET

5.1. Overview

The work described in this chapter focuses on the ball inlet system as an interface for non-traditional MALDI samples. The previously described work demonstrates the ball inlet interface as a method for continuously sampling crystallized liquid solution analytes. Here, the ball inlet system displays the capability to analyze samples in solid and powdered forms. Analytes were manually brought into direct contact with the interface ball at atmospheric pressure for laser desorption/ionization analysis under vacuum. Target analytes ranging from over-the-counter medication to whole human blood demonstrated the low to high mass range detection of the direct contact approach. Compounds were detected from crude samples. The direct contact ball inlet mass spectrometry demonstrates a minimal sample preparation, and fast analysis approach to the ball inlet interface.

5.2. Introduction

Mass spectrometry is often employed in clinical, environmental and bioanalytical situations where rapid analysis is desired and expected.[76-79] Optimum analysis speed in mass spectrometry not only requires rapid data acquisition time common of mass spectrometers, but also fast sample preparation time and instrument accessibility. In recent years, laser desorption ionization and MALDI has expanded into broader areas of study due to the applicability to analytes over a wide mass range. Traditional MALDI sample preparation requires the analyte and MALDI matrix mixture to be co-crystallized on MALDI targets prior to analysis.[80] The targets are placed into the ionization source by breaking ion source vacuum and restoring it prior to analysis. These processes add to the complexity of MALDI sample preparation and make it difficult for applications where real-time sampling is needed.

The ball inlet interface has been previously described as a method for on-line MALDI analysis of crystalline MALDI samples.[50, 57, 58] On-line capillary electrophoresis, microfluidic chip detection, and single-droplet deposition analysis have been demonstrated using the ball inlet interface. Atmospheric pressure sample deposition and rotation give this configuration utility where a traditional MALDI sampling system is not ideal. The atmospheric pressure side of the ball is exposed, allowing unimpeded access to its surface for sample deposition. With rotation, sample is introduced into the ion source without breaking and reestablishing source vacuum. This feature makes the ball inlet a desirable approach for real-time sampling applications using direct sample contact. Sample deposition without breaking vacuum naturally increases the speed of analysis by eliminating time for vacuum reestablishment; however, the ball inlet was initially designed for crystalline sample analysis limiting it to samples prepared in this manner.

A real-time sampling system is characterized not only by analysis time but also by its ability to handle different sample types, which improves the overall utility of the instrument. Real-time monitoring for environmental analysis or biological agent detection may involve analytes in solid or powdered forms. The ball inlet design allows crystalline samples to rotate pass the interface vacuum gasket with minimal sample removal. Non-crystalline, solid or powdered, samples are scraped off a smooth ball surface due to gasket scraping. This interface issue was overcome with the design and construction of a ball inlet with micromachined wells on the surface. Sample brought into contact with the ball surface settles into the wells, preventing sample removal when rotated pass the vacuum gasket.

In this chapter, the capabilities of the ball inlet system with non-traditional MALDI samples are explored using a contact approach. Samples brought into contact with the interface are quickly analyzed using this method. The usefulness of this technique was displayed with a wide range of analytes varying in molecular mass. The applicability of the ball inlet for clinical,

pharmaceutical and environmental applications was demonstrated, and protein in whole human blood was identified with minimal sample preparation.

5.3. Experimental

Solid sampling was performed by manually bringing analytes into direct contact with the ball inlet interface with little or no sample pretreatment. A specially designed interface ball containing a single track of sample wells prevented sample removal when the analyte was rotated pass the interface gasket. Each well was approximately 350 μm in diameter and traversed the circumference of the interface ball at a spacing of approximately 100 μm. Crude mixtures in solid, powdered, and liquid forms were brought into contact with the ball, filling the multiple wells. For MALDI, A 50 mM matrix solution was delivered with a syringe pump and capillary or manually pipetted. No matrix was added for the LDI experiments. Ionization was achieved with a 355 nm Nd:YAG laser at 10 Hz with a focused spot of 100 × 300 μm. The ball rotation rate allowed for multiple laser shots on each sample well during analysis.

Analytes such as cold medicine tablets, non-dairy creamer, American cheese and cough drop were smeared directly onto the ball inlet surface and used without further purification. The MALDI matrix α-cyano-4-hydroxycinnamic acid (CHCA, Sigma) was prepared in 70:30 acetonitrile/dI H_2O with 0.1% trifluoroacetic acid (TFA, J. T. Baker, Pittsburgh, NJ) at concentrations of 50 mM. Analyte solutions were made in distilled water (house supply) with 1% TFA at concentrations of 100 μM. Acetaminophen tablets (Tylenol Cold, McNeil Consumer Healthcare) were broken prior to direct contact with the ball inlet. Cough drops (Halls, Cadbury Adams) were moistened with distilled water prior to direct contact with the ball inlet. For saliva studies, cough drops were administered orally for approximately 1 minute then saliva collected into vials after the cough drop was removed from mouth. Whole human blood from a volunteer was smeared directly onto the ball inlet directly from free-flowing thumb prick wound. For

MALDI analysis of American cheese and whole human blood, matrix was pipetted on top of the deposited analyte. The high voltage applied to the ball inlet for ion extraction was turned off for analyte contact then turned on during the ionization process.

5.4. Results

Direct contact studies with the ball inlet interface were successful at identifying many compounds from various analytes. Samples were brought into contact with the ball surface for analysis. Target analytes included small molecules, such as pharmaceuticals, as well as larger molecules such as proteins. The analytes encompassed a wide molecular mass range and, in some cases, were complex mixtures. Laser desorption/ionization with and without the addition of matrix was performed to investigate the usefulness of the direct contact ball inlet. Experiments that mimicked potential real-world situations were chosen to assess the overall capability of the ball inlet interface as a tool for universal mass spectrometric analysis.

The pharmaceutical industry is consistently on the forefront of scientific research and development incorporating drug discovery, clinical application and synthesis studies. Some pharmaceutical studies involve mass spectrometry of biomolecules due to implications in life processes but much of the research focuses on molecules such as small organics and metabolites. Over-the-counter medicine tablets are composed of active and inactive pharmaceutical compounds. The mixture of components in a single tablet can make the identification of specific compounds difficult without an initial separation and extraction. A direct contact ball inlet mass spectrum of an acetaminophen tablet is shown in Figure 5-1. The inner contents of a broken cold medicine tablet were rubbed onto the ball inlet surface. Active compounds pseudophedrine (165.23 g/mol), a nasal decongestant, and dextromethorphan (271.4 g/mol), a cough suppressant, were detected and identified from the crude tablet with no sample pretreatment, other than breaking the tablet, and no matrix addition.

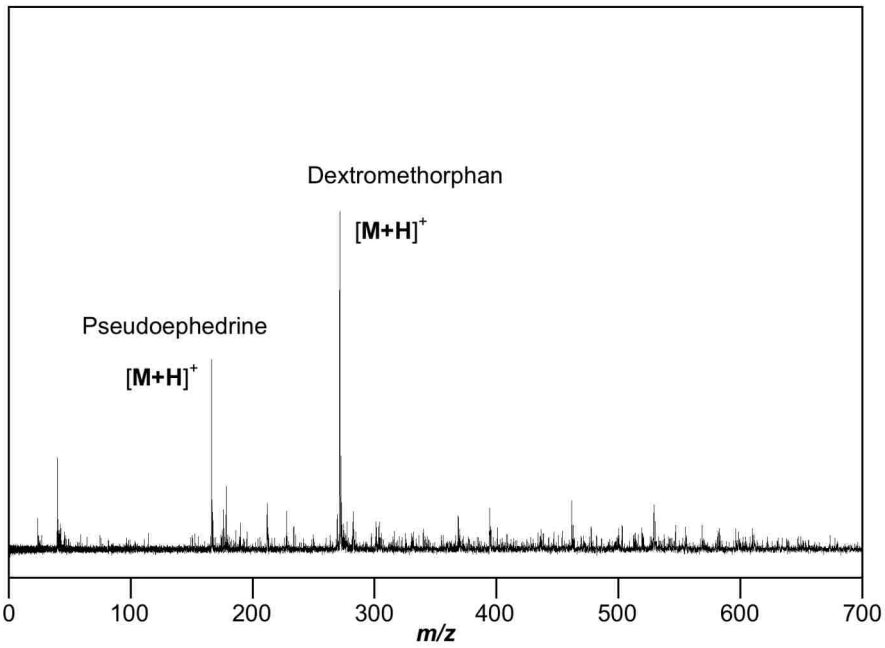

Figure 5-1. A direct contact mass spectrum of an acetaminophen tablet containing pseudophedrine and dextromethorphan.

When developing a system for diverse analysis the ability to distinguish different substances from one another is critical. Recently, powders have been of particular interest because of the fear of biological terrorism or contamination, although many powdered substances are harmless and are ubiquitous in our society. The utility of this system for powdered samples is demonstrated in Figure 5-2. A non-dairy creamer powder was deposited onto the surface of the ball and mass analyzed without a MALDI matrix. The ball inlet surface is not ideal for powdered samples; however, a small amount of the powdered creamer was statically attracted to the ball surface, permitting enough granules to be deposited into the sample wells for analysis. Non-dairy creamer consist of a mixture of many organic compounds. Notable mass peaks were located near 400 *m/z* and 900 *m/z*. The observed mass spectrum peaks were not identified, but

with advances in mass fingerprinting a distinction between compounds with similar appearances can be made.[81-83]

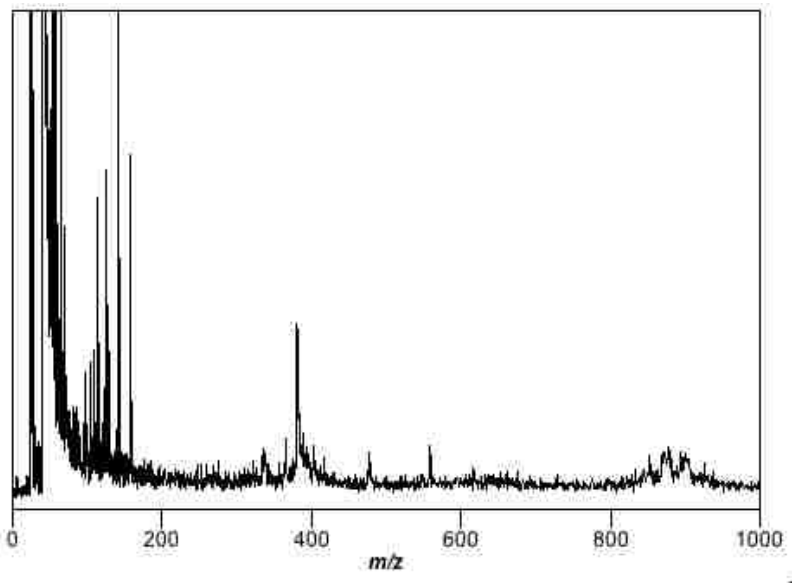

Figure 5-2. Direct contact mass spectrum of non-dairy creamer. The powdered sample was manually deposited directly onto the surface of the ball inlet and mass analyzed without matrix addition.

Ionization without a MALDI matrix is ideal for direct and rapid mass spectrometry detection, but a matrix is necessary to ionize compounds larger than 1000 Da. In some real-world cases, an unknown compound might initially be subjected to MALDI analysis revealing high mass molecules but analysis without a matrix may reveal some important low mass molecules that are suppressed by matrix ion peaks.[84] Mass spectra of American cheese analyzed by direct contact with and without the addition of MALDI matrix is shown in Figure 5-3. The constitution of cheese allows for relatively simple sticking onto the ball inlet surface. Without the addition of matrix (Figure 5-3a) peaks were detected near 380 *m/z* and a dominant peak near 490 *m/z*. Other less intense peaks were observed in the region between 600 and 800 *m/z*. The addition of CHCA

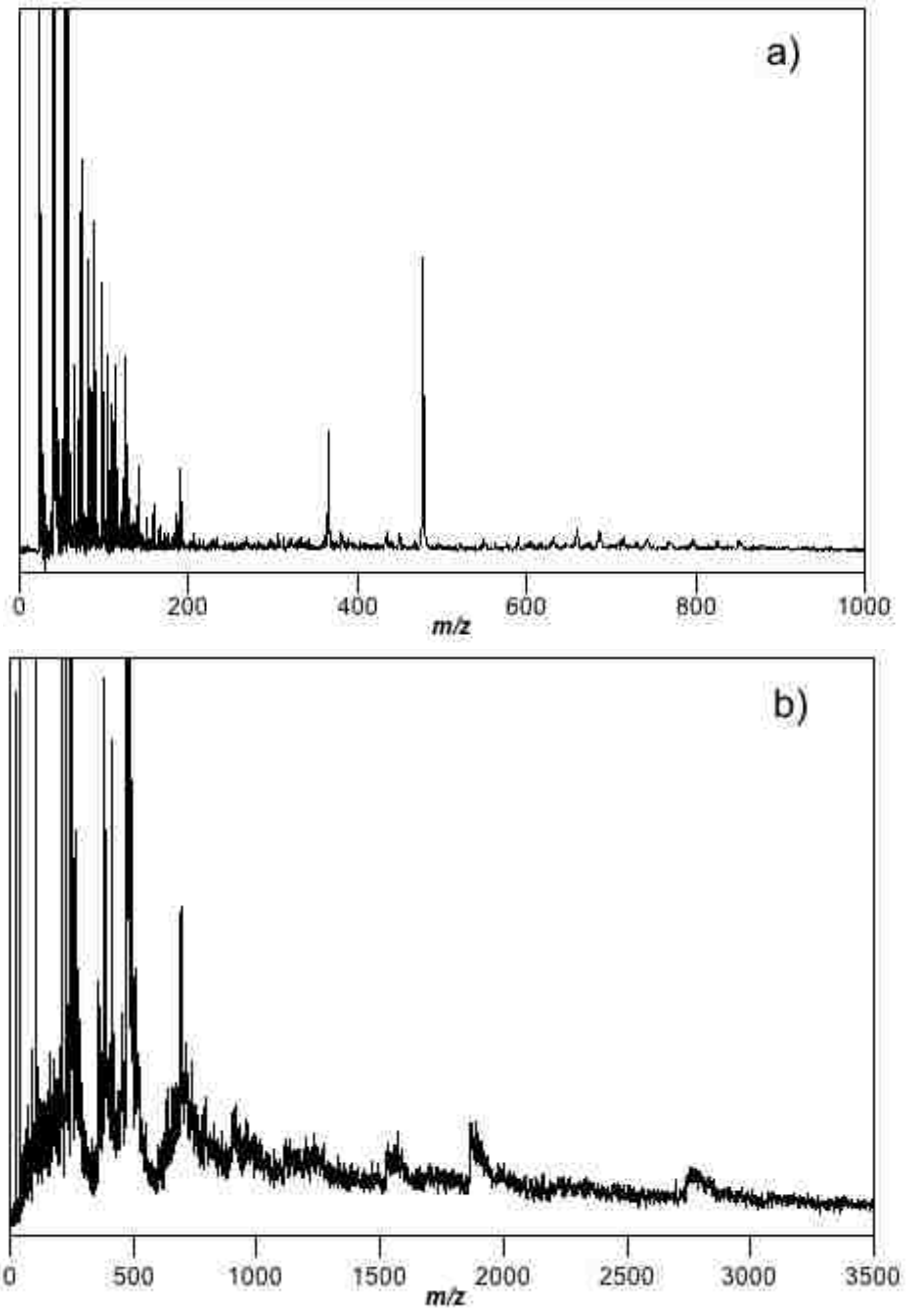

Figure 5-3. Direct contact mass spectrometry of American cheese a) without MALDI matrix and b) with CHCA matrix.

matrix (Figure 5-3b) allows the detection of higher mass ions. Peaks are observed above 1000 *m/z* and peaks in the 600 to 800 *m/z* region are intensified. Peak assignment in the 600 to 800 *m/z* region is more difficult in the MALDI spectrum. The low mass resolution may be due to the

crude sample and insufficient analyte and matrix incorporation because the MALDI matrix solution was deposited on top of the analyte.

Biological fluids such as urine, blood, and perspiration, and other biological material such as tissues and hair are media used to obtain physiological information when analyzed with mass spectrometry.[85] Saliva is a medium gaining interest for analyte detection because chemical exposure information can be obtained and it can be clinically acquired by noninvasive means. Human saliva as a biological matrix for target analyte detection is displayed in Figure 5-4. An over-the-counter cough drop was used to provide target analytes in saliva. A mass spectrum of the blank ball inlet was recorded for comparison (Figure 5-4a). Prior to saliva analysis, the cough drop was analyzed by direct contact to observe mass spectrometry peaks (Figure 5-4b). The cough drop was slightly moistened with deionized water prior to direct contact analysis, yielding many peaks in comparison to the blank ball inlet. Human saliva was analyzed by direct contact mass spectrometry immediately after cough drop consumption (Figure 5-4c). Many of the peaks present in the direct contact of the cough drop are also present when detected from saliva. Peaks not present in the saliva mass spectrum may be due to analyte dilution to concentrations below the detection limit of the mass spectrometer or ion suppression from compounds in the crude saliva medium.

The utility of the interface in analyzing crude biological mixtures was studied using blood. Whole human blood was analyzed and the resulting mass spectrum is shown in Figure 5-5. Whole blood was obtained from a volunteer and analyzed by direct contact mass spectrometry. Blood was smeared onto the ball surface directly from a wound resulting from a finger prick. With the addition of CHCA matrix, the protein hemoglobin was detected and identified from the crude mixture. Typically, multiple separation steps are needed to identify

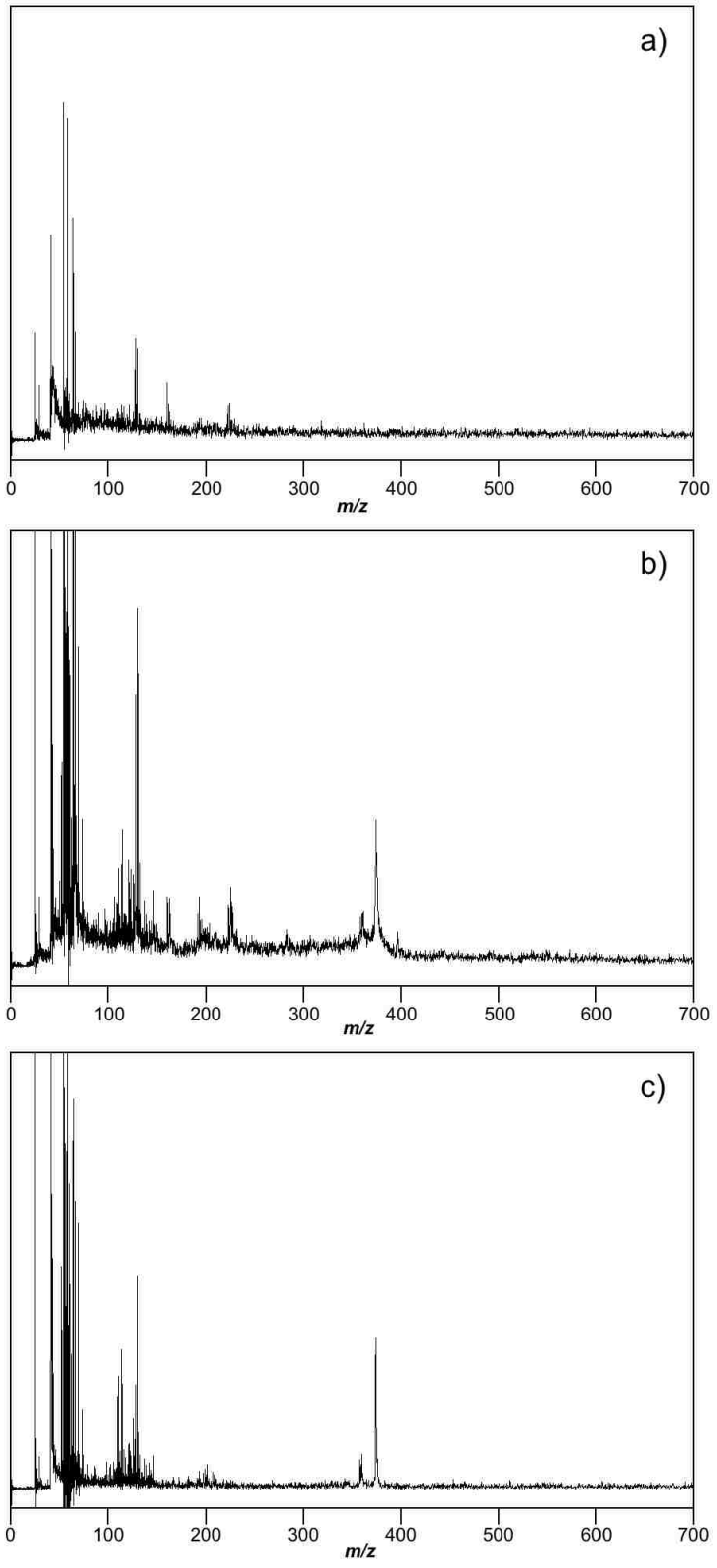

Figure 5-4. Direct contact mass spectrometry analysis of over-the-counter cough drop components from human saliva. Mass spectra from a) the blank ball inlet, b) direct contact of cough drop, and c) human saliva immediately after cough drop consumption.

proteins in blood but MALDI matrix addition was the only sample pretreatment performed here. Hemoglobin peaks appear near the upper mass limit of the time-of-flight analyzer contributing to the poor mass resolution.

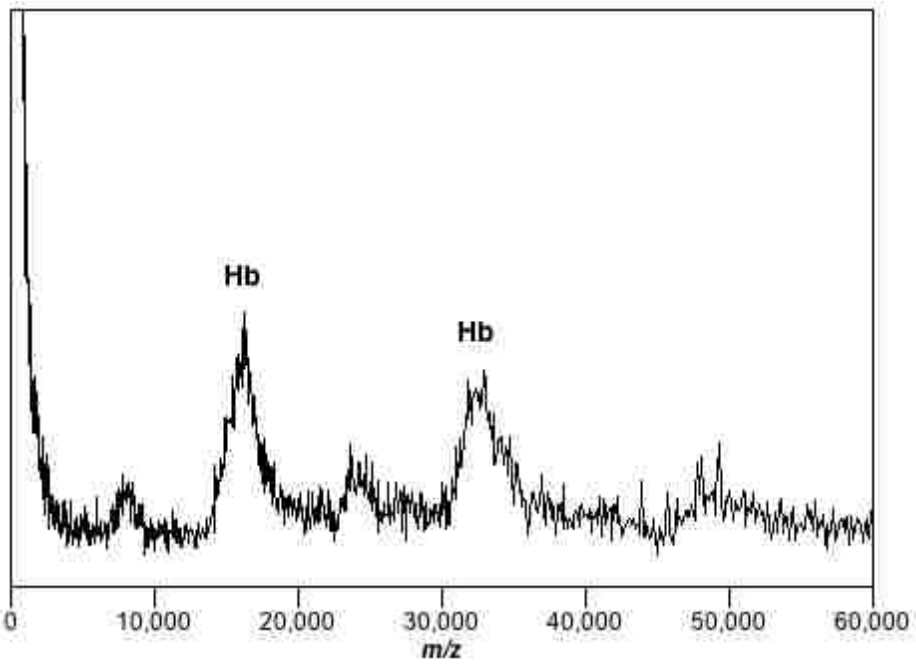

Figure 5-5. Direct contact mass spectrum of whole human blood with CHCA matrix.

5.5. Summary

Direct contact laser desorption interface that allows analytes to be brought into direct contact with the ball inlet for MS analysis was demonstrated. Solid and solution based samples were rubbed onto the surface of a ball which contained micromachined wells that allowed solid samples to rotate pass the gasket without being scraped off. The easily accessible atmospheric pressure sample deposition region and a ball inlet with micromachined wells is unique for sample pickup and delivery into the vacuum region of the ion source. Crude mixtures such as whole human blood and cold medicine tables were analyzed with direct contact ball inlet mass

spectrometry and dominant spectral peaks were identified. American cheese was analyzed with and without the addition of a MALDI matrix and both experiments produced observable analyte peaks in the mass spectrum. The addition of a functional cleaning system would allow for rapid sequential analysis of direct contact samples. Increasing the ball rotation speed and laser repetition rate can increase analysis speed for continuous sampling applications.

Non-dairy creamer was analyzed by direct contact ball inlet and analyte peaks were observed in the mass spectrum but were not identified. The identification of the peaks isn't necessary if the mass spectrum can be compared against a mass spectrum database of known compounds. A mass spectrum of unknown analytes can be searched and compared to mass spectra of known compounds. The results of a database search can determine the identity of unknown compounds and depending on a spectral match the compound can be identified as biologically harmful or harmless. Therefore, unassigned mass peaks can be useful if compared to the mass spectrum of a known compound of interest.

Whole human blood and saliva were studied with direct contact mass spectrometry. Contents of blood, saliva and other biological fluids contain chemical exposure information for living organisms. Hemoglobin was detected. Saliva may contain information of internal or external chemical exposure. Cough drop studies show that some chemical exposure information can be obtained from saliva without prior sample treatment.

Future work includes studying other biological media such as skin, tears or urine for chemical exposure relevance to develop noninvasive approaches in clinical analysis. Low target analyte concentrations in selected biological media will require low limits of detection. Some sample pretreatment, such as pre-concentration, may be needed to increase the sensitivity of this technique. Other future studies include using standards to build a concentration curve for target analytes to determine the frequency of chemical exposure in a clinical setting.

CHAPTER 6. DIRECT DESORPTION/IONIZATION MASS SPECTROMETRY

6.1. Overview

The goal of the research described in this chapter was to develop a real-time laser desorption technique for fast sampling of samples under ambient conditions. The direct desorption/ionization interface eliminates sample insertion into the high vacuum of the ion source. Direct desorption/ionization was demonstrated by bringing analyte into direct contact with the atmospheric side of the ion source target. The target, located at the atmosphere-to-vacuum interface of the ion source, contained a 1 mm hole that acted as an orifice for ultraviolet laser irradiation. Analytes were pressed onto the atmospheric side of the target orifice. The contact provided a partial vacuum seal of the orifice and additional sealing was provided with clear adhesive tape in some cases.

6.2. Introduction

The past decade has seen a broadening of the scope of mass spectrometry into areas where it has not been widely used, such as environmental analysis, forensics, and biological agent detection.[24, 86-88] Mass spectrometry has played a large role in the qualitative detection of target analytes in various media with broad applicability when the proper equipment is chosen. The inherent disadvantage of mass spectrometry has been the lack of portability due to the large size and weight of most instruments in use today. Portable mass spectrometers have been studied for many years and are a desirable approach to field analytical chemistry.[89-92] The miniaturization of mass analyzers and detectors demonstrated to date suggests that portable instrumentation, completely independent of laboratory constraints, will be available in the near future.

Laser desorption ionization has shown versatility in analyzing molecules over a broad mass range.[93] Desorption techniques have mostly been associated with high mass biomolecule analysis, but recently the utility of ionizing low mass analytes, such as small organic molecules, pharmaceutical compounds and metabolites has been reinvigorated.[94] The inherent advantages of sensitivity and speed of analysis make it ideal for these analytes in many applications. Laser desorption with a matrix (MALDI) can also be applied to analysis of low mass compounds.[95] Difficulties from low mass matrix interferences can be overcome in some cases with the correct matrix selection.

A highly versatile instrument must have an interface that is equally versatile and robust. Laser desorption ionization is not greatly affected by sample impurities and laser spots can be easily moved and focused. A major drawback of laser desorption is the general need to place analytes into the vacuum region of the mass spectrometer which requires waiting for an acceptable vacuum pressure to be restored prior to analysis. Several ambient ionization methods have been reported and ion transmission from the atmospheric region to the vacuum of the mass analyzer results in some ion loss.[96-98] Ionization methods such as AP-MALDI, DESI and DART generate ions at atmospheric pressure prior to transporting them into the vacuum of the mass analyzer.

We demonstrate an ion source that eliminates the need to place samples into the vacuum region of the ion source. This novel interface employs direct contact of the analyte with the interface on the atmospheric pressure side of the ion source. The direct desorption/ionization interface allows for laser ablation under vacuum while the sample is on the outside of the ion source. The direct desorption interface is a semi-ambient technique because ions are formed under vacuum inside the ion source, where a UV laser is focused onto the sample. The laser is focused at the center of a small orifice in the interface, when a sample is brought into contact

with the interface it covers the orifice, allowing for laser ablation and ionization. A vacuum seal is created when the sample is covered, or backed, with clear packaging tape during analysis. In between experiments, tape alone will vacuum seal the interface orifice. We have demonstrated the applicability of the direct desorption interface to a variety of analytes. Samples were analyzed with minimal sample preparation both with and without MALDI matrices. Whole plant tissue was analyzed and some compounds were identified. Whole human blood was analyzed directly with the tape-lift method to demonstrate the applicability to forensic analysis.

6.3. Experimental

Analysis was performed by bringing samples into contact with the ionization source of a home-built linear TOF by directly pressing analyte materials against the interface. Samples were pipetted or mounted on the sticky side of clear tape (Scotch Clear Packaging, 3M, St. Paul, MN) then brought into contact with the interface, at an orifice with a 1 mm diameter and .559 mm depth. A 337 nm nitrogen laser irradiated the analyte at the orifice, and the resulting ions were extracted with 15 kV positive potential under the vacuum of the ionization source. The MALDI matrix α-cyano-4-hydroxycinnamic acid (CHCA, Sigma) was prepared in 70:30 acetonitrile/dI H_2O with 0.1% trifluoroacetic acid (TFA, J. T. Baker, Pittsburgh, NJ) at concentrations of 50 mM. Saturated dithranol (DIT, Sigma) MALDI matrix solution was prepared in dichloromethane. Analyte solutions were made in distilled water (house supply) with 1% TFA at concentrations of 100 μM. The analyte bradykinin (B-3259, Sigma) was used without further purification.

Peptide mass spectra were obtained when bradykinin solution (4 mg/ml) and CHCA matrix solution was mixed at 1:1 (v/v) ratio, then 2 μl of the pre-mixed analyte and matrix solution was pipetted directly onto the clear tape. Plant tissue sections were spotted with 5 μl dithranol matrix and mounted onto clear tape. Blood samples were prepared by 1) pre-mixing

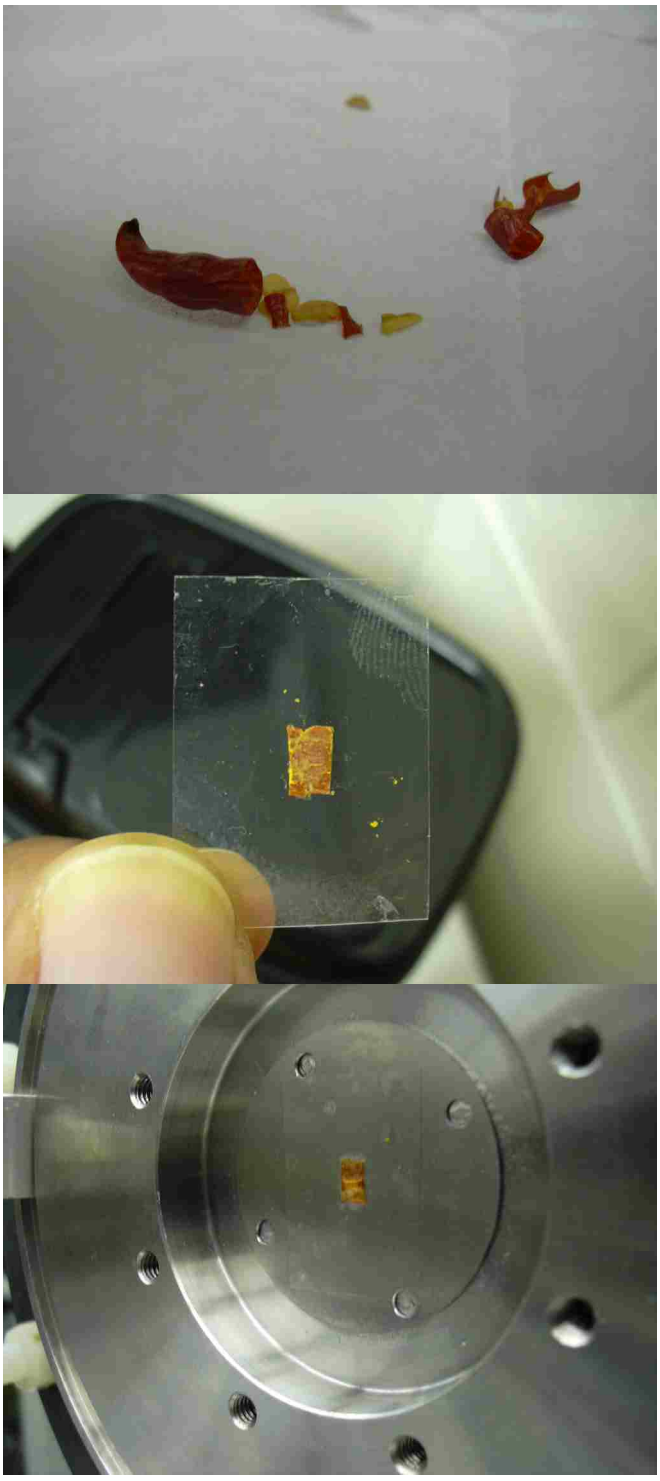

Figure 6-1. Preparation of dehydrated chili pepper tissue for direct desorption/ionization analysis. Tissue sections were spotted with matrix and affixed to adhesive tape with the membrane side exposed. The tape was then brought into contact with the direct desorption interface with the tissue section directly over the interface orifice, creating a vacuum seal.

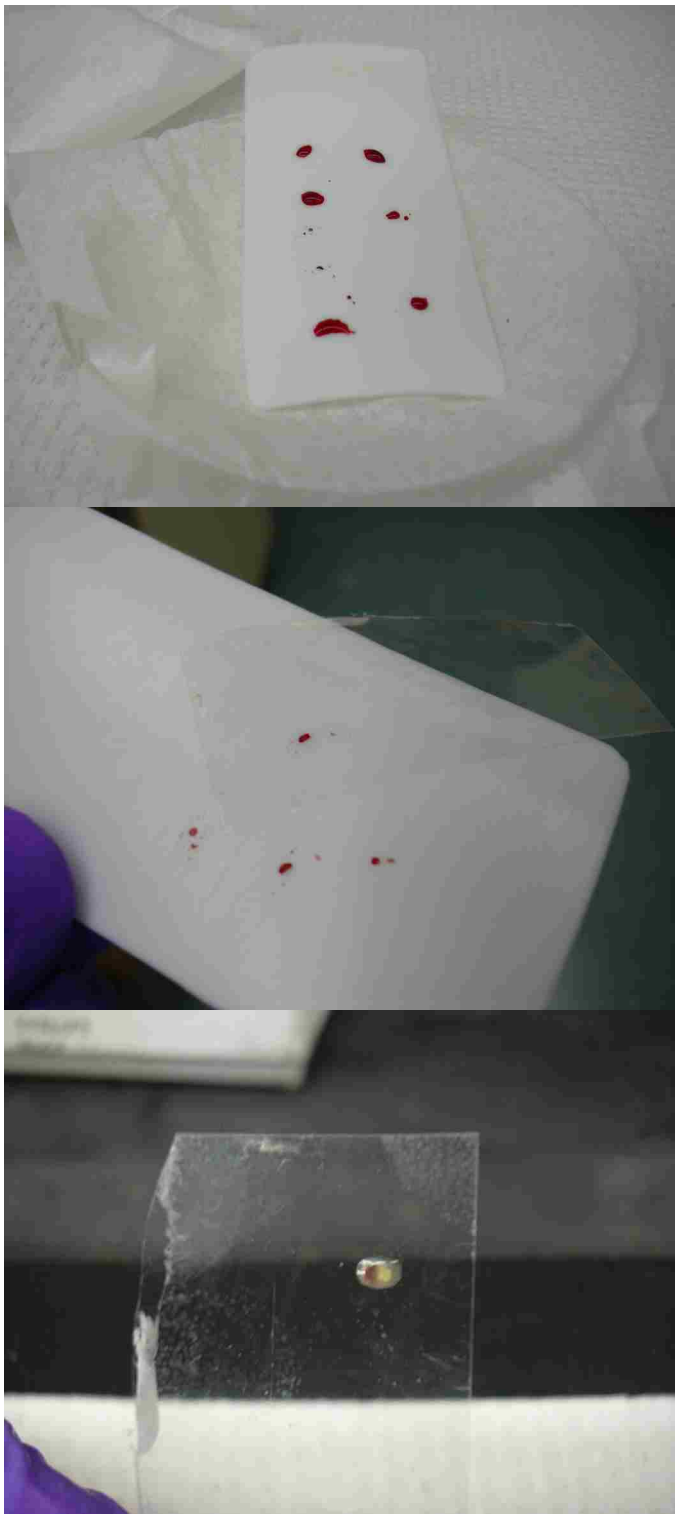

Figure 6-2. Tape-lift method for direct desorption/ionization. Blood was spotted onto a Teflon surface and allowed to dry before being lifted from the surface with clear adhesive tape. Matrix was added to the dried blood spot on the adhesive tape.

wet blood with CHCA matrix solution and 2) tape-lifting dried blood spots from a Teflon surface followed by pipetting of 2 μl CHCA matrix solution. Once on the tape, samples were brought into contact with the interface with no further sample preparation. The tape was also responsible for maintaining a vacuum seal during analysis.

Chili peppers were prepared for direct desorption analysis by cutting them into section sizes that would enable a firm vacuum seal when tape-mounted during analysis. Pepper tissue was cut into approximately 3 × 5 mm sections and the tape was cut into 18 × 24 mm pieces for mounting. Chili pepper preparation for direct desorption analysis is shown in Figure 6-1. Sections of dehydrated chili pepper pods were sliced with a razor blade to expose the membrane. After dithranol matrix addition and crystallization, tissue sections were affixed at the center of the adhesive tape, keeping at least a 7 mm adhesive clean perimeter around the tissue section for vacuum sealing when mounted onto the direct desorption interface. Sections were mounted with the inner membrane side exposed and matrix-coated for analysis. Once mounted, a vacuum seal was created at the tape adhesion point.

For tape-lifting studies, human blood retrieved from a volunteer was spotted onto a Teflon surface, Figure 6-2. This surface allowed for simple retrieval of blood spots with tape lifting. Once dried, blood spots were lifted from the surface with clear adhesive tape. A 50 mM CHCA matrix solution was pipetted (2 μl) onto affixed blood spots and allowed to crystallize. The sample spots were analyzed by direct desorption from the adhesive tape.

6.4. Results

The direct desorption/ionization interface was designed with a concept similar to that of the direct contact ball inlet interface: bring analyte into contact with the interface. However, the direct contact ball inlet is limited to samples that can be deposited onto the ball surface and rotated pass the interface gasket without being scraped off the ball surface. This mechanism is

not feasible for analytes such as tissue sections that cannot be deposited onto the ball surface or rotated pass the interface gasket without being scraped off the ball surface. Tissue represents a medium that is relevant for point detection of biological compounds.[99] Often, tissue insertion into the vacuum of an ionization chamber can cause analyte spreading onto different areas of the tissue, spoiling spatial resolution.[100] The direct desorption/ionization interface is able to detect analytes directly from tissue and other surfaces without placing the sample into the ionization source of the mass spectrometer. Peptide standards, organic compounds in plant tissue, and whole human blood was analyzed using this method.

Figure 6-3. Direct desorption/ionization interface. The plate (left) contains a 1 mm orifice for sample irradiation with laser light and sits in the center of an ISO 100 flange (right).

The direct desorption/ionization interface is shown in Figure 6-3. The orifice plate rests in center of an ISO 100 interface flange so that the front side is at ambient pressure and the opposite, back, side is under the vacuum of the ionization source. The ionization laser beam travels through the vacuum region of the ion source and is focused at the back side of the orifice at the center. Typically, desorption laser light is focused at a MALDI target made of stainless steel. Here, laser light is focused at the center of the interface orifice, where there is no surface until an analyte is present. For analysis, samples are brought into contact with the front side of the orifice plate, where laser irradiation and ionization occurs. The resulting ions are extracted

with a 15 kV potential applied directly to the orifice plate and flange. This unique system enables desorption and ionization to occur under vacuum without placing the sample inside the vacuum chamber of the ion source.

A MALDI peptide standard, bradykinin, was used to test the performance of the direct desorption/ionization interface. A direct desorption/ionization spectrum of bradykinin using CHCA matrix is shown in Figure 6-4. Matrix and analyte (4 mg/ml) solutions were pre-mixed and pipetted onto the sticky side of the adhesive tape. After crystallization, the tape was brought into contact with the direct desorption interface with the sample spot resting over the orifice. Sample spots were placed in the center of the tape so that a 7 mm adhesive surface surrounded the sample. The adhesive properties of the clear tape, along with the vacuum of the ion source, enabled a strong vacuum seal when properly affixed to the interface during experiments. A blank piece of adhesive tape was used to cover the orifice and provide a vacuum seal between experiments. A brief period of vacuum exposure occurred when the blank tape was removed and a new sample tape was placed on the interface. This period caused an increase in ion source pressure to 1×10^{-3} torr, but a pressure of 1×10^{-6} torr was restored in approximately 2 min once a vacuum seal was created at the orifice. The mass resolution obtained is not optimum; however, this instrument operates using static ion extraction. The addition of delayed ion extraction or a reflectron TOF mass analyzer will improve mass resolution.

During analysis, the tape-mounted sample is under vacuum without physically being inside of the ionization source chamber. The 1 mm orifice allows for sample irradiation with UV laser light. The presence of an analyte during analysis was the only factor preventing laser light from passing through the interface orifice unimpeded. The focused laser spot was approximately 200×300 μm which avoided any clipping of the laser beam by the interface orifice and

prevented any sample memory effects from analyte carryover. The polymer tape did not show any inherent low mass interference in the MALDI mass spectra.

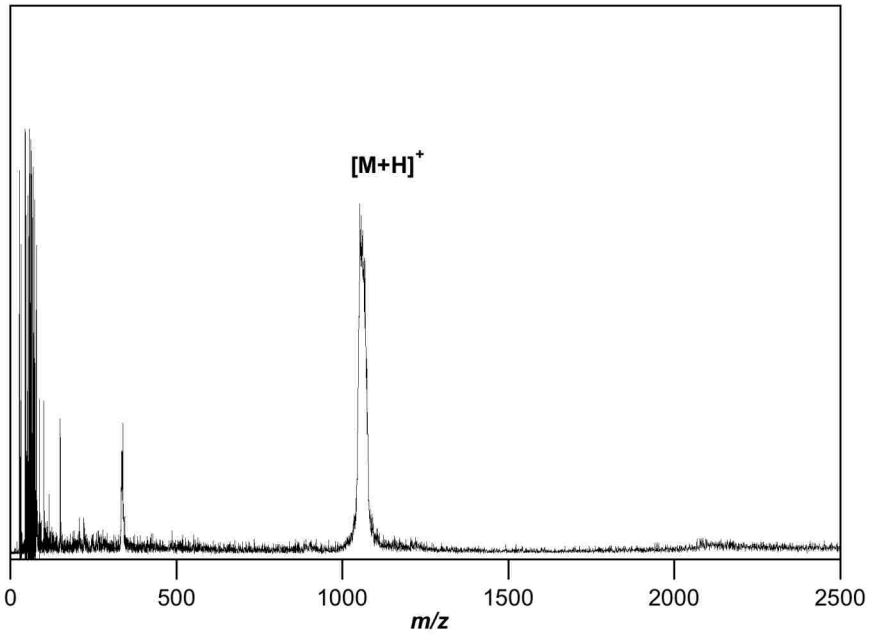

Figure 6-4. Direct desorption mass spectrum of bradykinin with CHCA matrix. Analyte and matrix was premixed and spotted onto clear tape.

Laser desorption/ionization analysis of animal and plant tissue has gained interest due to the spatial resolution afforded by a well-focused laser. Imaging with MALDI is useful for spatially resolved detection of drugs and metabolites for clinical diagnostics.[101, 102] The utility of the direct desorption/ionization interface for target analyte detection in tissue was demonstrated. Direct desorption/ionization was performed on plant tissue to identify compounds with minimal sample preparation. Chili pepper tissue was analyzed for the compound capsaicin (305.41 g/mol) which gives peppers their hot flavor.[103] Direct desorption/ionization mass spectra of chili pepper with dithranol matrix is shown in Figure 6-5. Capsaicin is known to be distributed throughout pepper tissue with the highest concentration found in the membrane, rather than seeds or flesh.[25]

Peaks observed near the 300 *m/z* region were assigned to capsaicin and dihydrocapsaicin (307.43 g/mol), with the doubly charged species and dimer being observed near the 150 *m/z* and 600 *m/z* regions respectively. Peaks below 100 *m/z* are attributed to sodium and potassium ions, the potassium peak, with matrix adducts, being the largest. This is due to the high potassium content in fruits and vegetables. The detection of capsaicin in pepper pods with minimal sample preparation displays the potential of this ion source as a method for chemical analysis when traditional ionization techniques may not be viable.

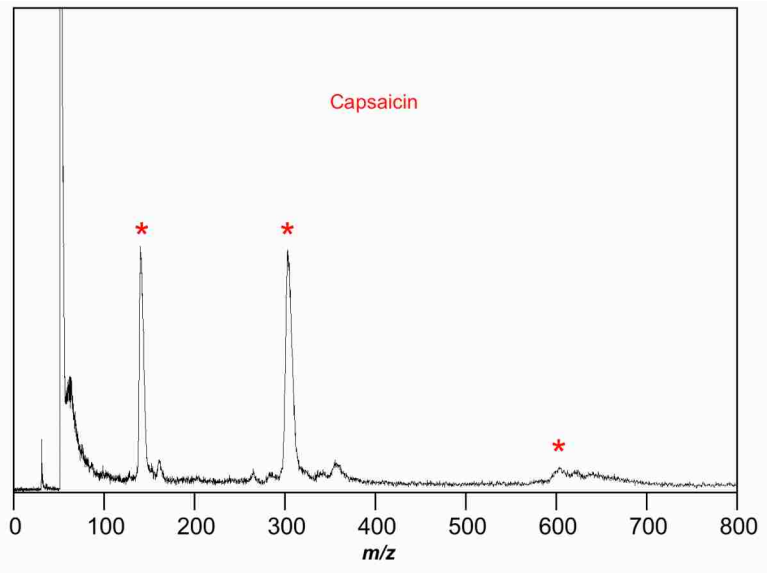

Figure 6-5. Direct desorption/ionization analysis of a chili pepper pod membrane. Capsaicin and dihydrocapsaicin are identified near the 300 *m/z* region, with the doubly charged and dimer species of these compounds near 150 *m/z* and 600 *m/z*, respectively.

A crude biological mixture, blood, was analyzed with the direct desorption/ionization interface and compared with the results from the direct contact ball inlet. Whole human blood with CHCA matrix was pre-mixed and analyzed with direct desorption/ionization mass spectrometry. The analysis of human blood was performed without any sample preparation such as cell lysis or chromatographic separation. Peaks observed in 1500 *m/z* region are shown in

Figure 6-6. Droplets of fresh blood, 3 – 5 μl, was taken from a volunteer and pipetted, after matrix pre-mixing, onto adhesive tape. The mass resolution was not high enough to identify the analyte peaks. Although no peak assignments were made, the peaks were confirmed with a subsequent experiment on a commercial MALDI-TOF. Commercial MALDI analysis also revealed analyte peaks near 1500 *m/z*. Therefore, the direct desorption mass spectrum results from a cluster of peaks with mass-to-charge ratios around 1466 *m/z* and 1537 *m/z*. The identity of the analyte peak present is difficult to obtain with database or literature searching due to the complexity of the sample.

When comparing the mass spectrum obtained from direct desorption/ionization of whole blood with direct contact ball inlet of whole blood many differences are noticed. High mass hemoglobin protein peaks were observed with the direct contact ball inlet interface but not with the direct desorption/ionization interface. This is possibly due to sample deposition on the ball inlet surface allowing for better MALDI matrix incorporation into the blood. The ball surface causes a thinner layer of blood to be deposited whereas the direct desorption/ionization was performed on thicker blood spots where MALDI matrix incorporation can be more difficult. Also, the blood cells may have been lysed when passing the gasket.

The success of clear adhesive tape as a sample target for laser desorption applications led to a test of possible forensic applications of the direct desorption/ionization approach. Tape-lift methods are popular throughout forensics for the collection of particles.[104, 105] Fingerprints, which are readily attained using the tape-lift approach, contain person-specific information due to the physical pattern of the print, but also contain biomolecules, like DNA, in the oils that make up the fingerprint.[106] A tape-lift procedure that allows mass spectrometry to be performed directly from a tape with the fingerprint would be a powerful forensic tool. The potential of a tape-lift approach for direct desorption/ionization was studied using human blood. A direct

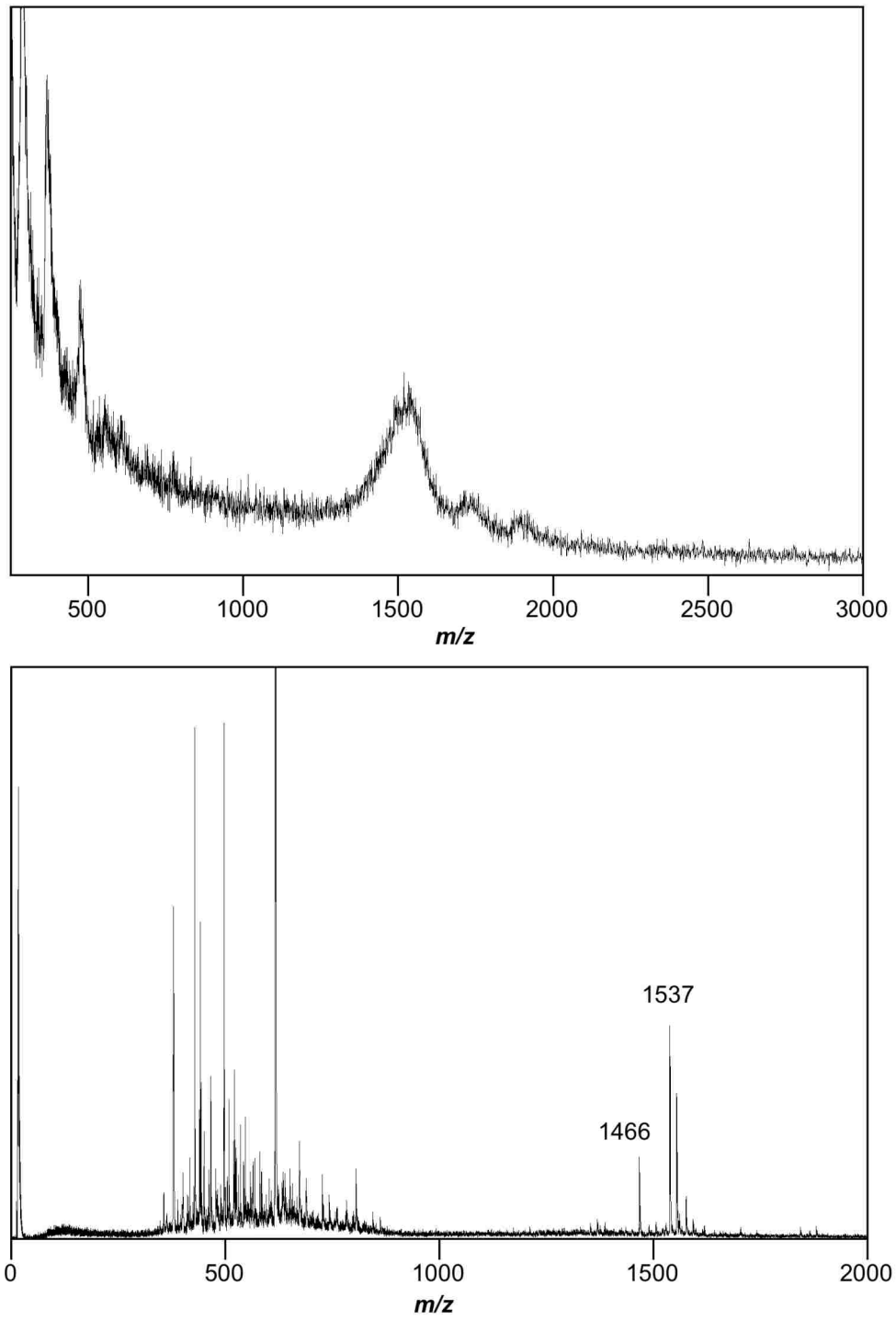

Figure 6-6. Mass spectrum of whole human blood with CHCA matrix using direct desorption/ionization (top) and commercial MALDI-TOF (bottom). Matrix and analyte were pre-mixed and pipetted onto clear adhesive tape with no further sample preparation.

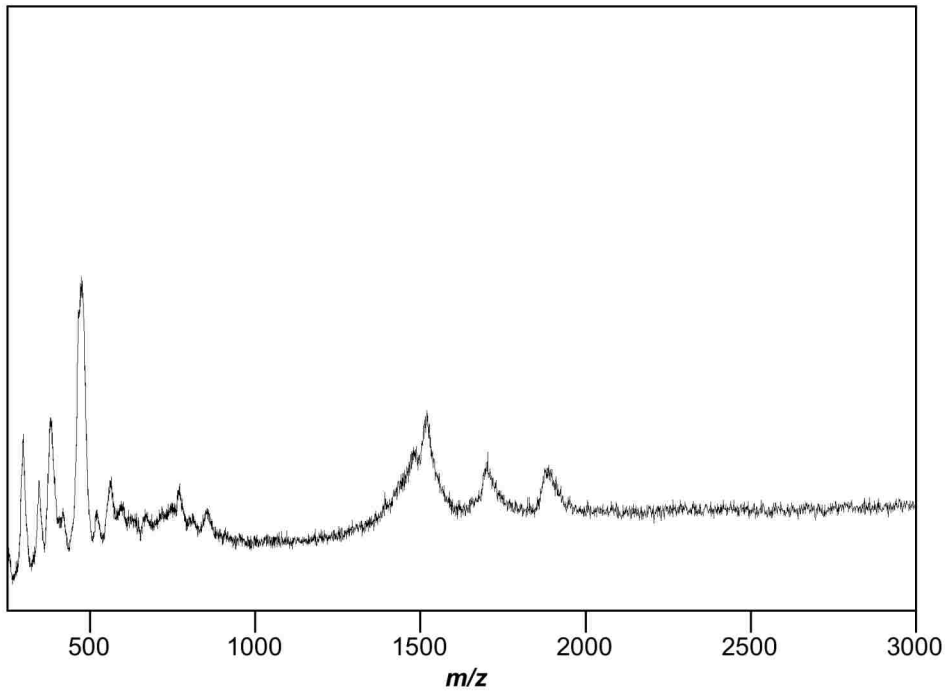

Figure 6-7. A direct desorption/ionization mass spectrum of human blood, tape-lifted from a surface. Dried blood was lifted from a Teflon surface with clear adhesive tape, spotted with CHCA matrix, and analyzed *in situ*.

desorption/ionization mass spectrum of blood tape-lifted from a surface is shown in Figure 6-7. After lifting, dried blood spots were coated with CHCA matrix and allowed to air dry. The unknown peaks in the region near 1500 *m/z* correspond to the peaks found in the mass spectrum of the blood pipetted onto clear tape (Figure 6-6). The lack of a large baseline rise in the region below 500 *m/z* may be due to the differences in sample preparation. The analyte/matrix incorporation is not as uniform when using the tape-lift method because no pre-mixing is involved. Both techniques for analyzing blood are absent of any traditional bioanalytical sample preparation.

6.5. Summary

A direct desorption/ionization interface that allows for laser irradiation and ionization of analyte without placing it inside of the ion source was demonstrated. Whole human blood and

plant tissue was analyzed with this novel interface. The unique aspect of this interface is the ability of samples to be laser interrogated without being placed inside of the vacuum region of the ionization source. This characteristic can allow for rapid, serial analysis of multiple samples. Chili pepper membrane was analyzed and the compound capsaicin was identified. Unidentified analyte mass peaks were obtained from whole human blood with minimal sample pretreatment. Clear adhesive tape was used during analysis as a sample stage and to provide a vacuum seal for the interface.

Spatially resolved detection of target analytes in tissues would be difficult with the current interface due to the small size of the interface orifice. Tissue samples need to be moved in order to expose the entire tissue section to the MALDI laser. It is also unknown if the location of target analytes in tissue is maintained during direct desorption/ionization analysis. The sample is not completely exposed to the vacuum of the ion source. Further studies can determine if spatial location is maintained.

The development of this interface could lead to advances in portable and high-throughput mass spectrometry analysis. Desirable characteristics of portable mass spectrometers include sample analysis with minimal sample handling by the operator. This includes analyzing compounds in their native environments or without the addition of a matrix. Eliminating sample carryover from previous analysis will allow for rapid serial analysis. The mechanism of the direct desorption/ionization interface naturally reduces or eliminates memory effects from previous samples. Portable instruments with a direct desorption/ionization interface will have the advantage of ambient mass spectrometry where the analyzer can be brought into contact with the analyte in most environments. Matrix addition is the only sample pretreatment needed if MALDI analysis is desired. Advances in mass spectrometry miniaturization will aid in the field of portable instrumentation.

The adaptation of clear adhesive tape for sample analysis highlights the possible forensic applications for direct desorption/ionization. In forensic analysis where the tape-lift method is employed, laser desorption mass spectrometry information can be obtained from the lifted material without removing the material from the tape surface. Improvements in mass resolution will improve the sensitivity and overall instrument performance needed for a forensic analytical tool.

CHAPTER 7. CONCLUSIONS AND FUTURE DIRECTIONS

New methods for on-line laser desorption/ionization mass spectrometry were performed on a specially designed and constructed linear time-of-flight mass spectrometer and are described in this dissertation. The ionization chamber allowed for novel interfaces based on laser desorption/ionization to be easily adapted. The interfaces described are an improved ball inlet interface for continuous mechanical sampling and a direct desorption interface for direct contact sampling. Both interfaced were designed to bridge the atmosphere and vacuum barrier of the mass spectrometer with minimal sample handling by the operator, and allowing analyte deposition at atmospheric pressure. Sample pretreatment prior to laser desorption/ionization is an important procedural step; therefore reducing pretreatment handling can prevent ambiguities in mass spectrometry data. The interfaces presented also address and inherent desire for gathering information as rapidly and efficiently as possible by reducing the sampling time it takes to obtain a useful mass spectrum.

In Chapter 3, the ball inlet interface was used for on-line MALDI detection of microfluidic chip analytes. Here, the ball inlet interface demonstrated the ability to continuously sample new analyte over time frames long enough for the detection of an entire microfluidic separation. An on-line cleaning system and specially designed microfluidic chips were utilized for these studies. The continuous sampling allowed for a multi-peptide separation to be visualized with a 2-dimensional contour plot of time versus *m/z* formed from many mass spectra. Generally, a separate chromatographic detector would be necessary to obtain elution time for a chromatography with mass spectrometry detection experiment.[107] A protein digestion and separation with MALDI-TOF detection was also performed on-line with a microfluidic chip and ball inlet interface.

Instrument performance for on-line microfluidic chip detection was adequate but there were some drawbacks with the interface. Some analyte scraping due to mechanical forces between the ball inlet and the gasket that provided the vacuum seal were observed. Also, when a trail of wet analyte and matrix solutions are deposited onto the ball surface there is the potential for sample mixing, which can spoil the previous separation. These issues can also result in memory effects over the course of an analysis and a loss of sensitivity. Methods for improving the interface were presented in Chapter 3 and Chapter 4.

A piezoelectric droplet dispenser was used to investigate a ball inlet interface with non-contact sample deposition. Single, picoliter analyte droplets deposited onto the ball surface, rather than a trail, prevents analyte mixing on the ball surface. Also, small analyte deposits improve instrument sensitivity due to an increase of analyte concentration when the deposit size is reduced. When analyte deposits are reduced to diameters equal to or smaller than the ionization laser spot the operator does not have to search for "sweet spots" in the sample. Microfluidic chips have been fabricated with piezoelectric and other types of micro-emitters for off-line MALDI detection.[108, 109] However, an on-line approach incorporating a micro-emitting chip and the ball inlet interface would benefit high-throughput capabilities in a bioanalytical setting.

Direct contact approaches were studied using the ball inlet interface and a direct desorption interface. These approaches were developed to answer the question of portable laser desorption from a sample interface standpoint. The direct contact interfaces promote minimal sample handling or pretreatment for non-traditional laser desorption/ionization samples, ideal for a portable instrument. In Chapter 5, solid samples were brought into direct contact with the ball inlet utilizing a modified ball containing a single line of micromachined wells spanning the circumference. Solid analytes were able to settle into the wells when deposited, preventing

gasket scraping of the analyte during ball inlet rotation. Continuous on-line analysis with the indented ball would require a more advanced cleaning system that would ensure analyte removal from the wells; however, the success of the solid sampling approach affords the capability of numbering the wells for indexed sampling. Chapter 6 presents a direct desorption/ionization interface for a contact approach for samples that would not be ideal with direct contact ball inlet, such as plant tissue. A portable instrument with this interface can be brought into contact with an analyte-laced surface under ambient conditions for MS detection.

Other advances in the ball inlet include a migration from a spherical, ball shape inlet to a cylinder inlet. The ball inlet allowed for a single analyte trace at the center of the ball, which requires cleaning of old sample to allow fresh sample to be deposited during on-line analysis. A cylinder inlet provides more usable surface area for a continuous single spiraling analyte trace or multiple simultaneous analyte traces. A cylinder inlet interface has been designed and constructed and is shown in Figure 7-1. Preliminary data suggest some design changes, such as reducing the cylinder recess, can increase the mass resolution. An improved design along with surface indexing and fully automated motion control could be useful as a high-throughput interface for MALDI-MS.

The size and weight of most mass spectrometers limits the usefulness of this analytical tool outside of a laboratory environment. Decreasing the size of mass spectrometers will increase the portability, giving rise to more in-field applications. Miniaturization of these instruments entails advances in the main components of a mass spectrometer; *i.e.* ionization source, mass analyzer, and detector; and advances in subsequent components; mainly vacuum systems. Some miniaturized mass spectrometers have been reported.[90, 92, 110]

Overall, the performance of the linear TOF constructed for this research lacked good mass resolution, which also effects detection limit and sensitivity. The presented work described

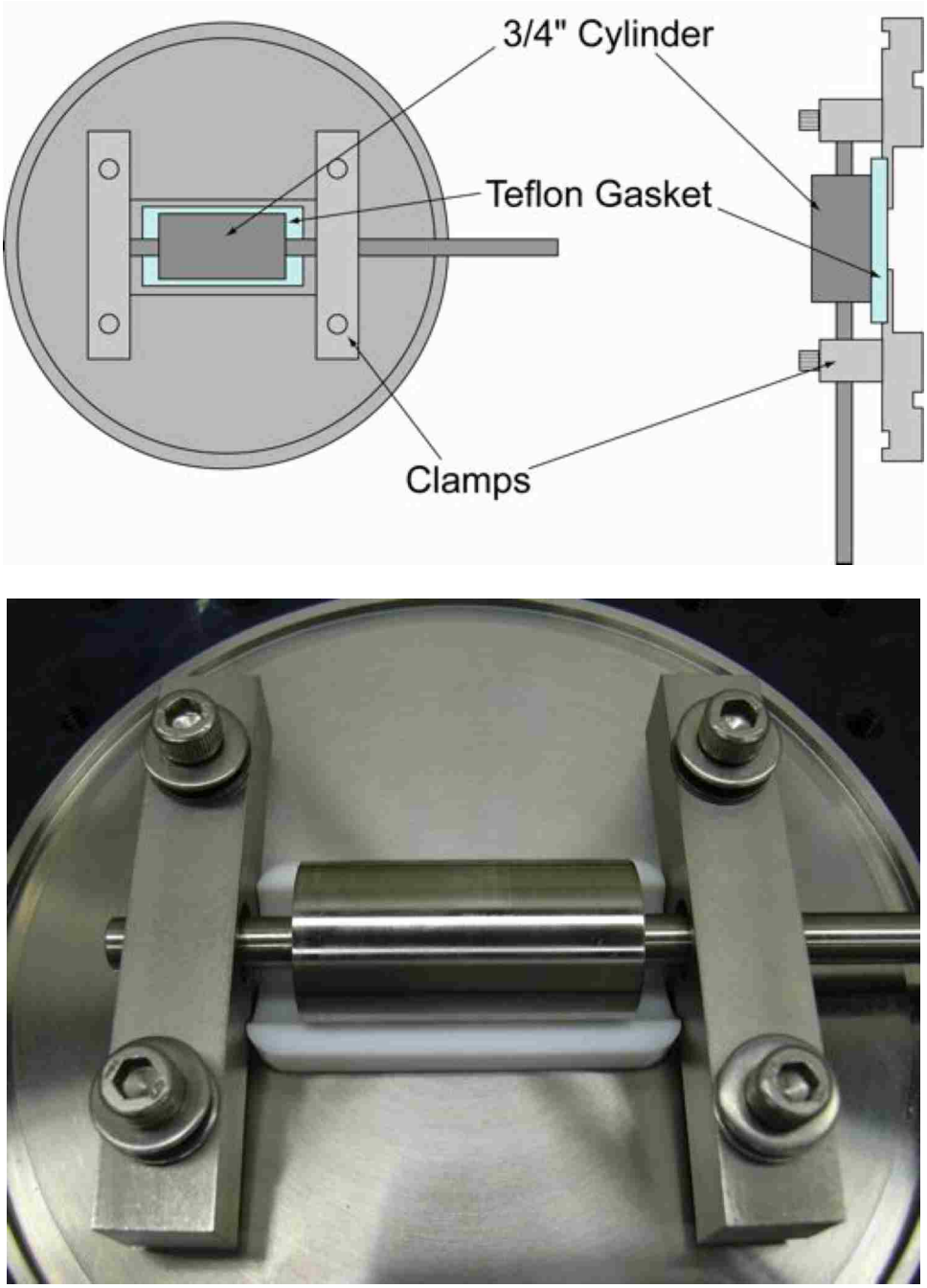

Figure 7-1. Cylinder inlet interface.

novel interfacing and ion source for on-line laser desorption in which most cases standards and known compounds were tested. Typically, higher mass resolution is necessary to characterize

new instrumentation but resolution provided by this instrument was enough to prove the principle and theory of the new interfaces.

Ultimately, the potential of the interfaces presented in this dissertation may be limited due to vacuum requirements. Adaptation to ambient techniques, such as MALDESI, will eliminate interface complexity due to ion source vacuum and could allow the ball inlet interface to be utilized to its full potential. Removing the constraints of vacuum ionization would make the ball inlet interface fully suited for continuous, real-time analysis.

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VITA

Damien Anthony Narcisse was born in October 1978 in Baton Rouge, Louisiana. After a brief period growing up in Baton Rouge and Clear Lake, Texas, he spent most of his childhood life in Midlothian, Virginia. During high school, his family relocated to Baton Rouge and he graduated from University Laboratory School where he excelled in math and science, and participated in the State Rally in Physics. Following his high school graduation he enrolled at Xavier University located in New Orleans, Louisiana, where he majored in chemistry and minored in mathematics. At Xavier, his senior research project was Natural Product Isolation for P450 Enzyme Inhibition allowing him to study exotic plant material. Damien graduated from Xavier University in the summer of 2000 and moved home to Baton Rouge. That fall, he worked in the Louisiana State University Agricultural Chemistry laboratory where he tested pesticide runoff from various Louisiana farms using gas chromatography mass spectrometry. In January 2001 he enrolled in the chemistry graduate program at Louisiana State University. There, he chose to work in Dr. Kermit Murray's research group where he immediately began constructing mass spectrometers. He later would design and construct a mass spectrometer for his doctoral research. While in graduate school, Damien was an active member in the National Organization for the Professional Advancement of Black Chemist and Chemical Engineers (NOBCChE), the American Chemical Society (ACS), and the American Society for Mass Spectrometry (ASMS). During his doctoral program, he has co-authored three publications and has presented his research at many national conferences. In 2003, he received a Pfizer travel award to present at the ACS fall meeting in New York City. He received fellowships from Graduate Alliance for Education in Louisiana (GAELA) and the National Science Foundation GK-12 Program that allowed him to teach at local public schools during his studies. In April 2008 his daughter was

born in Ft. Worth, Texas. Damien is currently a candidate for the degree of Doctor of Philosophy in chemistry, which will be awarded at the 2009 fall commencement.