Electrification Ionization: Fundamentals and Applications

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ELECTRIFICATION IONIZATION: FUNDAMENTALS AND APPLICATIONS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Chemistry

by

Bijay Kumar Banstola
B. Sc., Northwestern State University of Louisiana, 2011
December 2019
This dissertation is dedicated to
my parents: Tikaram and Shova Banstola
my wife: Laxmi Kandel
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<th>Definition</th>
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<tbody>
<tr>
<td>2,5-DHAP</td>
<td>2,5-dihydroxyacetophenone</td>
</tr>
<tr>
<td>2-NBN</td>
<td>2-nitrobenzonitrile</td>
</tr>
<tr>
<td>2-NPG</td>
<td>2-nitrophloroglucinol</td>
</tr>
<tr>
<td>3-NBN</td>
<td>3-nitrobenzonitrile</td>
</tr>
<tr>
<td>ABC</td>
<td>Ammonium bicarbonate</td>
</tr>
<tr>
<td>APCI</td>
<td>Atmospheric pressure chemical ionization</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>DESI</td>
<td>Desorption electrospray ionization</td>
</tr>
<tr>
<td>DLAM</td>
<td>Division of Laboratory and Animal Medicine</td>
</tr>
<tr>
<td>EI</td>
<td>Electron ionization</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>ETD</td>
<td>Electron transfer dissociation</td>
</tr>
<tr>
<td>FA</td>
<td>Formic acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>ICP</td>
<td>Inductively coupled plasma</td>
</tr>
<tr>
<td>IMS</td>
<td>Imaging mass spectrometry</td>
</tr>
<tr>
<td>I.D</td>
<td>Internal diameter</td>
</tr>
<tr>
<td>ITO</td>
<td>Indium-tin-oxide</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>kPa</td>
<td>Kilopascal</td>
</tr>
<tr>
<td>LIAD</td>
<td>Laser induced acoustic desorption</td>
</tr>
<tr>
<td>LSI</td>
<td>Laser spray ionization</td>
</tr>
<tr>
<td>m/z</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>MAI</td>
<td>Matrix-assisted ionization</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption ionization</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MS/MS</td>
<td>Tandem mass spectrometry</td>
</tr>
<tr>
<td>MSI</td>
<td>Mass spectrometry imaging</td>
</tr>
<tr>
<td>NP</td>
<td>Nanoparticle</td>
</tr>
<tr>
<td>O. D</td>
<td>Outer diameter</td>
</tr>
<tr>
<td>OCT</td>
<td>Optimal cutting temperature</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PSI</td>
<td>Pounds per square inch</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
</tr>
<tr>
<td>SA</td>
<td>Sinapic acid</td>
</tr>
<tr>
<td>SAI</td>
<td>Solvent assisted ionization</td>
</tr>
<tr>
<td>SAWN</td>
<td>Surface acoustic wave nebulization</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TIC</td>
<td>Total ion current</td>
</tr>
<tr>
<td>TOF</td>
<td>Time of flight</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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ABSTRACT

Electrification ionization is a method to produce ions from solid samples by charge separation. This separation of charge can be enhanced by organic molecules known as matrix compounds to produce highly charged molecules. In this research, new methods were developed for the application of electrification ionization for surface analysis and the ionization mechanism was investigated. First, an electrification ionization matrix was used for tissue imaging using laser ablation. A two-component matrix of 2-nitrophloroglucinol and silica nanoparticles was found to increase the number of multiply charged ions from the tissue. Images of mouse brain with multiply charged ions were obtained. In a second study, a method for electrification ionization was developed using a pulsed valve for material removal. The pulsed valve was directed at a thin metal foil with sample and matrix deposited on the opposite side. The resulting ions were sampled into a mass spectrometer. The method had an ion production time of 4 seconds but had limited spatial resolution. To improve spatial resolution, a piezoelectric cantilever striker with a needle tip attached to the arm was used. A lateral resolution of around 1 mm was obtained with the piezoelectric cantilever and the temporal resolution was comparable to the pulsed valve. Highly charged ions of peptides and proteins deposited on foil were obtained. With an improved nanoparticle co-matrix, lipid and gangliosides were detected from tissue. The addition of nanoparticles improved the ionization of molecules from tissue under both atmospheric and high vacuum conditions. To understand the ionization mechanism, sublimation electrification of matrix was studied. Electrification produces a current when the matrices are sublimed. The sign of the current depended on the compound sublimed. The polarity of the current could be altered by the application of an electric field or by changing the pH. The results are interpreted as separation of charge in particles ejected from stress cracking crystals.
CHAPTER 1. INTRODUCTION

Identification and quantification of biomolecules such as proteins, peptides, lipids, and metabolites in tissue is important for disease diagnosis and drug development.\textsuperscript{1-5} Every day, thousands of human samples including urine, blood, breath, and tissue are analyzed\textsuperscript{6-8} and these numbers are increasing. With the increasing number of samples, analytical techniques that can provide rapid and accurate chemical information is necessary.

Several analytical methods have been developed to study biological samples. These include fluorescence spectroscopy,\textsuperscript{9, 10} nuclear magnetic resonance spectroscopy,\textsuperscript{11, 12} ultraviolet/visible absorption spectroscopy,\textsuperscript{13, 14} electron microscopy,\textsuperscript{15, 16} and electrochemical biosensors.\textsuperscript{17, 18} Mass spectrometry (MS) is one of the most successful and popular techniques for the analysis of chemical compounds due to high sensitivity and specificity.\textsuperscript{19-21} Mass spectrometry has been used in different areas such as pharmaceutical science, petroleum industry, forensic science, and biological science.\textsuperscript{21-24}

Mass spectrometry can identify and quantify chemical species from small species such as metal ions and metabolites to larger molecules such as peptides and proteins. The wide popularity of mass spectrometry for analysis of large molecules has increased with the development of two ionization techniques called electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI).\textsuperscript{25, 26} The developers of these two ionization techniques received the 2002 Nobel Prize in chemistry. ESI produces high charge state ions that help in the identification of molecules and can be interfaced with different separation techniques.\textsuperscript{27, 28} On the other hand, MALDI can directly analyze samples from surfaces and can perform imaging.\textsuperscript{29} These two ionization methods are used complementary to one another.
Transfer of charge between two materials when contacted or rubbed is a well-known physical process dating back to Thales of Miletus with amber charging against wood.\textsuperscript{30, 31} Contact electrification occurs spontaneously in nature as in rubbing of fur or sand storms.\textsuperscript{32} Recently, an ionization method was developed which ionizes molecules directly from surfaces through electrification.\textsuperscript{33} The method is called matrix-assisted ionization (MAI) and has characteristics of both MALDI and ESI. MAI can produce highly charged ions similar to ESI and can produce ions directly from surfaces similar to MALDI.\textsuperscript{34} Imaging of surfaces with MAI has the potential to provide benefits of both ESI and MALDI. The development of more efficient ionization procedures and obtaining a deeper understanding of the ionization mechanism of MAI could open new aspects for the analysis of biological samples.

In this chapter, a brief overview of mass spectrometry is presented. Various ionization techniques used with MS are introduced and their application in biological analysis and imaging is discussed. Details of MAI along with the different techniques used for ionization are presented.

1.1 Mass spectrometry

Mass spectrometry (MS) is an analytical technique that transforms atoms and molecules into ions and measures the mass-to-charge ($m/z$) ratio of the ions.\textsuperscript{35, 36} A mass spectrometer consists of three components: an ion source, a mass analyzer, and a detector.

The first step in mass spectrometry is the conversion of neutral atoms or molecules into gas-phase ions. Conversion of neutral species into ions helps in the manipulation of motion and direction of ions using electric and magnetic fields. Various ion sources have been developed that can ionize molecules in atmospheric pressure and low-pressure conditions.\textsuperscript{37, 38} Selection of the correct ionization process determines the efficiency of converting neutrals to ions without destroying the properties of the sample of interest.\textsuperscript{39} Various ionization methods used in molecular
MS analysis are electron ionization, chemical ionization, spray ionization, and desorption ionization. Electron ionization is one of the oldest ionization techniques that use an energetic beam of electrons to produce ions. Electron ionization induces the breaking of chemical bonds and induces fragmentation of molecules, thus it is defined as “hard” ionization. Fragmentation helps in the structural determination and identification of smaller molecules. However, electron ionization is not possible for compounds with no vapor pressure. The development of “soft” ionization techniques such as electrospray ionization, matrix-assisted laser desorption ionization, and matrix-assisted ionization that ionize low vapor pressure large molecules without fragmentation helped overcome the limits of hard ionization. Each of these ionization techniques is discussed further.

The second step is the separation of these charged species based on their mass-to-charge ratio ($m/z$), where $m$ is the mass of the ion and $z$ is the number of charges carried by the ion. There are various mass analyzers used for the separation of ions such as the magnetic sector, quadrupole mass analyzer, time of flight (TOF), and ion traps. In the magnetic sector, ions are accelerated into a focused beam through an electric potential difference. Then, using the magnetic fields, ions are deflected according to their mass and charge. Lighter ions with more charges are deflected the most. Quadrupole mass analyzers use four parallel metal rods with the opposite rods connected electrically. A combined direct current (DC) and radio frequency (RF) voltage is applied to the rods to produce a dynamic saddle point potential. The RF voltage is tuned to allow $m/z$ of choice to pass to the detector. Only ions of particular $m/z$ values are transmitted to the detector while others do not. Quadrupole and magnetic sector analyzers are good separation techniques for continuous ion sources. A TOF analyzer is good for pulsed ion sources. An electric field is used to accelerate the ions and the time required by these ions to drift through a field-free region and
reach the detector is measured. Ions can also be stored and analyzed using ion trap analyzers such as 3D quadrupole ion trap, linear quadrupole ion trap, Orbitrap or Fourier transform ion cyclotron resonance ion trap. Multiple mass analyzers can be used in sequence or ion traps can be used for sequential separation for tandem mass spectrometry (MS/MS) for better resolution, separation, and identification.

After m/z selection, the ions are detected using a particle multiplier detector or with a Faraday cup detector or ion-to-photon detector or by image charge detection. The signal of the ion current is amplified and recorded and the results are displayed in the form of a mass spectrum with the relative abundance of ions as a function of m/z.

1.2 Ionization in mass spectrometry

There are various ways of producing ions for a mass spectrometer. A particular ionization method is selected based on the nature of the sample investigated and the type of information required. Some ionization methods such as thermal ionization, inductively coupled plasma (ICP) ionization, and resonance ionization can provide precise and accurate isotopic ratios and are used for elemental analysis. Chemical ionization, electron ionization, glow discharge ionization, and spark ionization are efficient for ionization of small molecules. For ionization of large molecules, ESI or MALDI are used.

1.2.1 Ionization of atoms and small molecules

Thermal ionization

Thermal ionization provides isotopic ratios and is used for elemental analysis. In thermal ionization, samples are deposited on a metal filament and are allowed to dry. The filament is then heated during which the atoms are desorbed from the hot filaments and are ionized in the process. Sometimes, a second filament can also be used for ionization. The filaments are made
from pure metals such as tungsten, platinum, tantalum, palladium, rhodium, or rhenium.\textsuperscript{53} The ionization efficiency of thermal ionization depends on the temperature (T) and work function of the filament (\(\phi\)), and the ionization potential of the sample species which is given by the Saha-Langmuir equation.\textsuperscript{36, 53}

**Electron ionization**

Electron ionization (EI) is used to produce ions from volatile and thermally labile compounds with molecular weight less than a few hundred Daltons.\textsuperscript{35, 36} Ionization occurs through the interaction of molecules with typically 70 eV energy electrons.\textsuperscript{36} The excess energy of the electrons results in fragmentation of the molecules. The fragmentation observed with EI helps in structural determination and identification of unknown compounds; however, due to fragmentation, molecular ions are often not observed for large molecules.\textsuperscript{35, 54}

In EI, a metal filament is heated to produce electrons which are then accelerated into the sample chamber kept at low pressure. The accelerated beam of the electron bombards the gas phase samples inside the sample chamber. When an energetic electron collides with a neutral molecule (M), an electron (e) from the neutral molecule is expelled during the collision. A positively charged ion (M\(^{+}\)) typically with one unpaired electron is formed which is accelerated into the mass analyzer using a repeller. A voltage is applied to a repeller electrode which causes the ion to accelerate. EI is used to determine the molecular weight and structure of molecules that are thermally stable and volatile.\textsuperscript{39} EI has library-searchable spectra for hundreds of thousands of organic compounds.\textsuperscript{55, 56} The fragmentation pattern of each molecular species is characteristic of that molecule and is highly reproducible. A direct comparison of the fragmentation pattern with the library helps in the identification of a compound.\textsuperscript{54}
Chemical ionization

In chemical ionization, the ionization occurs through gas-phase ion-molecule interaction. Chemical ionization is less energetic than electron ionization which makes it suitable for ionization of molecules that fragment with electron ionization. The ion source for chemical ionization uses reagent gases such as methane, isobutane, or ammonia in excess. The interaction of electrons with reagent gases produces reagent ions which then interact with analyte molecules to form ions.

Inductively coupled plasma ionization

Inductively coupled plasma (ICP) ionization is commonly used for elemental and isotopic analysis. The ICP uses induction coils to create a discharge in argon gas. Three concentric quartz tubes are used for the plasma torch. Aerosols of liquid samples or from laser ablation are carried by the center tube while the middle and the outer tube have a large flow of argon which is used to generate a plasma. Radio frequency is transmitted through a coil which creates an oscillating electromagnetic field. Once the ionization is initiated, the collisions between charged species and neutral species increase which causes heating. The temperature at the center of the plasma can reach more than 8000 K which atomizes and ionizes sample species.

1.2.2 Ionization of large molecules

For large molecules such as biological compounds and polymers, the above-mentioned ionization methods are not possible. Various ionization techniques have been developed for nonvolatile and thermally labile samples. Spray ionization and desorption ionization are used for the analysis of nonvolatile large molecules as well as other polar and nonvolatile compounds. Few of the ionization techniques that are used commonly are discussed below.
**Electrospray ionization**

Electrospray ionization (ESI) is an ionization technique that is used for large molecules such as lipids, peptides, proteins, and other biomolecules. Molecules do not have to be volatile or thermally stable and the ions generated are intact. However, samples must be in a solution.

A schematic of the ESI is shown in Figure 1.1. ESI is an ionization process where a fine spray of highly charged droplets is produced under the influence of a high electric field. Samples are usually dissolved in some mixture of water with volatile organic solvents. In some cases, a small amount of organic acid is added to the electrospray solution which provides protons to facilitate ionization. As these droplets pass through the atmospheric pressure towards an analyzer, solvent evaporation leads to the shrinkage of the droplets. The charge density on the droplet builds up until it reaches the Rayleigh limit, where surface tension is exceeded by Coulombic repulsion and the ions are ejected. Solvent evaporation is assisted with a flow of heated gas.

Subsequent solvent evaporation results in the production of highly charged molecules. There are two major theories for the production of ions from the highly charged droplets. The ion evaporation model suggests that the high electric field on the droplet causes the ejection of ions. The charge residue model suggests that the droplets eject ions at the Rayleigh limit. The multiply charged ions enter the mass spectrometer where they are separated based on $m/z$ value and detected.

One of the advantages of ESI is that this ionization technique can be coupled to liquid separation techniques such as liquid chromatography and capillary electrophoresis. The combination of separation techniques with mass spectrometry helps in the identification of components of complex mixtures. ESI produces high charge state ions which makes it suitable for
use with the instruments with limited mass range. The production of highly charged ions can also result in more efficient fragmentation of molecules in tandem mass spectrometry.\textsuperscript{64,65}

Figure 1.1. Schematic of ESI

Tandem mass spectrometry (MS/MS) is a technique that helps in the identification and determination of peptides and proteins by fragmenting precursor ions into fragment product ions. The product ions are used for the determination of the chemical structure of the precursor molecule. In MS/MS, ions are first separated by $m/z$ in the first stage of MS. Ions of specific $m/z$ are selected and fragmented. Those fragment ions are then separated according to their $m/z$ in the second stage of mass spectrometry. The product ions from the first MS can be sent to the detector for identification or can be used for further fragmentation. There are several fragmentation methods, such as collision-induced dissociation (CID), electron capture dissociation (ECD), and electron transfer dissociation (ETD).\textsuperscript{66-68}

**Matrix-assisted laser desorption ionization**

Matrix-assisted laser desorption ionization (MALDI) has been widely used for the analysis of synthetic and biopolymers. MALDI along with ESI has revolutionized the use of mass
spectrometry for biomolecule analysis. MALDI instruments are now available in various laboratories where they are used for clinical, biological, and pharmaceutical studies.\textsuperscript{22, 69, 70}

In MALDI, a laser is used to desorb and ionize biomolecules directly from surfaces.\textsuperscript{26, 71} A small quantity of material is mixed in a solution with an excess of UV absorbing molecules called matrix and are co-crystallized on a metal target. The sample-matrix crystals are then irradiated with a laser beam (Figure 1.2). Lasers of various wavelengths have been used but the most commonly used wavelength is 335 nm. The matrix absorbs the pulsed laser energy which facilitates desorption and ionization.\textsuperscript{36} Analyte and matrix ions are then formed in the expanding plume and detected in the mass spectrometer.

There are two major theories to explain ionization with MALDI.\textsuperscript{72} Gas-phase protonation proposes that the production of ions involves two steps. The first step is the ionization of matrix molecules with the laser. The second step is the transfer of the charge from matrix ions to analyte molecules.\textsuperscript{73, 74} The lucky survivor model suggests that the matrix molecules are incorporated in the matrix as precharged species. The clusters of analyte and matrix are desorbed during laser ablation. These clusters fragment and produce ions through charge separation. Ions that survive the neutralization are called the lucky survivors.\textsuperscript{75, 76}

One of the advantages of MALDI is that it can be used to ionize samples directly from surfaces which enables mass spectrometry imaging. MS imaging provides both chemical and spatial information of surface.\textsuperscript{29, 70, 77} Various matrix application methods have been developed to obtain images of lipids, peptides, and proteins from tissue samples. In addition to imaging, the production of singly charged ions makes MALDI mass spectra simple even for a complex mixture. However, the production of singly charged ions limits fragmentation efficiency compared to multiply charged ions\textsuperscript{78} and limits the use of fragmentation methods such as electron transfer
dissociation and collision-induced dissociation in tandem mass spectrometry for protein identification.\textsuperscript{66, 67}

Figure 1.2. Schematic of MALDI

Matrix-assisted Ionization

Several compounds were recently discovered that generate charges when cracked resulting in the production of highly charged ions from large molecules.\textsuperscript{34, 79} The method of ion production is called matrix-assisted ionization (MAI) MAI has characteristics of both MALDI and ESI.\textsuperscript{33, 79, 80} Similar to MALDI, ions are generated in MAI by interaction of an analyte molecule with special matrix compounds. However, the ions produced are highly charged as observed with ESI.
The sample preparation method for MAI is similar to MALDI. A small amount of analyte is mixed in a solution with a larger concentration of matrix. Ions are generated as these samples are introduced into the mass spectrometer inlet in solution or dried crystals. Various methods are used for the introduction of the sample into the mass spectrometer inlet. A laser can be used as in MALDI. \(^{79, 81}\) Ions can be generated by tapping the matrix-analyte crystals on the mass spectrometer inlet \(^{82}\) or using an external force with mechanical striker, or by evaporation or sublimation. \(^{33, 82-84}\) Regardless of the method used, the ions generated can be highly charged as observed with ESI. Different methods used for the introduction of samples into the mass spectrometer are shown in Figure 1.3, and each method is briefly described below.

Laser spray ionization (LSI) is a type of MAI where a laser is used either in transmission (back side) mode or in reflection (front side) mode to create ions. Laser spray ionization was initially obtained in transmission geometry with high laser fluence. \(^{81, 85}\) However, multiply charged ions have been produced in a reflective mode as well, with various MALDI targets and laser fluences. \(^{86}\) Ions from drugs, carbohydrates, lipids, peptides, proteins, and polymers have been obtained with laser spray ionization. \(^{81, 85-87}\) Using LSI, highly charged ions of molecules of masses up to 20 kDa were detected from mouse brain tissue under atmospheric pressure condition. \(^{85}\) Electron transfer dissociation (ETD) fragmentation of peptides form the tissue has also been obtained for accurate mass analysis. \(^{81}\)

Another method of producing ions with MAI is using physical force to dislodge the matrix-analyte mixture deposited on a sample target. Various methods can be used to dislodge samples from the sample target. Tap against the capillary, a pellet gun, or a mouse trap can be used to dislodge the samples. \(^{82, 88}\) Multiply charged ions of bovine serum albumin with up to 67 charges have been produced with tapping. \(^{80}\) Multiply charged ions of peptides and proteins can be
produced using only the heated inlet capillary of the mass spectrometer.\textsuperscript{79} Highly charged mass spectra of peptides and proteins can be obtained when analyte solutions are introduced into the heated inlet capillary of the mass spectrometer.\textsuperscript{84, 89} This method of producing ions is called solvent assisted ionization (SAI).

The methods discussed above used heated inlet capillary. However, with volatile matrices such as 3-nitrobenzonitrile (3-NBN) and 2-nitrobenzonitrile (2-NBN), it was found that heated capillary was also not necessary.\textsuperscript{33, 34} Ions can be produced when the matrix-analyte mixture is simply exposed to the vacuum. The method is called matrix-assisted ionization vacuum (MAIV). Ions from small molecules and proteins have been obtained using MAIV.\textsuperscript{33} Drugs have been detected and quantified directly from tissue sections of a mouse treated with drugs.\textsuperscript{90}

Figure 1.3. Schematic of MAI
1.3 Imaging mass spectrometry

Imaging mass spectrometry (IMS) helps to provide both chemical and spatial information of chemicals and biomolecules on surfaces and tissue. IMS can be performed using ionization sources methods such as secondary ion mass spectrometry (SIMS), desorption electrospray ionization (DESI), matrix-assisted laser desorption ionization (MALDI), and others.91-93 Typically, mass spectra are acquired sequentially at regular spatial intervals and the signal intensity for ions of a particular mass is used to generate heat map that provides the location and the concentration of the molecules within a section.92, 94 Acquisition can be done in microprobe mode where a laser, ion beam or a spray is rastered across a surface moving in x-y direction, or in a microscope mode where the entire surface is sampled simultaneously and ions are detected using a position-sensitive detection system.95, 96

IMS is becoming a valuable analytical tool for biological research since its introduction at the end of the 20th century.91 Images of biological samples can be obtained at high resolution with chemical information without staining or chemical tagging.92, 95 A single IMS experiment can provide information for various distinct ions, which can range from tens to thousands of Da in mass. IMS has been applied extensively in various fields of research such as biomedical, pharmaceutical, and plant biology.20, 77, 97, 98

Mass spectrometry techniques commonly used for imaging are SIMS, DESI, and MALDI. SIMS uses a focused ion beam to sputter surfaces and provide high lateral resolution.23, 92, 99, 100 DESI imaging mass spectrometry (DESI-IMS) uses a continuous spray of charged droplets while rastering the sample surface and has been used for imaging of metabolites, lipids, and peptides from surfaces.101-103 101, 102 Though SIMS and DESI imaging are efficient for small molecule
imaging; imaging of large molecules is performed with MALDI. MALDI imaging is commonly used to image large molecules such as peptides and proteins.

1.3.1 MALDI imaging

MALDI imaging mass spectrometry (MALDI-IMS) allows visualization of the spatial distribution of various molecules on a surface. MALDI-IMS has been used for the identification and determination of the spatial distribution of proteins, peptides, lipids, metabolites, and other biomolecules in tissue.\textsuperscript{29, 64, 70, 94, 104-108} For imaging of a sample surface with MALDI, the tissue is cut into thin sections using a microtome and mounted on a conductive microscope slide. The tissue sections are used as prepared or are washed with solvents such as ethanol and chloroform depending on the molecule of interest. Matrix solution is applied to these tissue sections and is loaded into the mass spectrometer for analysis. Mass spectra are acquired sequentially at regular spatial intervals depending on the resolution needed. The signal intensity for an ion of a particular mass is used to generate a heat map that indicates the molecule's location and concentration within the section.\textsuperscript{109}

1.4 Research objectives

The objective of this research was to develop a spatially and temporally precise methods for ionization from tissue using electrification ionization. In Chapter 2, a method was developed to produce dry crystals on tissue to enhance multiply charged ion production. In Chapters 3 and 4, a method was developed for precise temporal and spatial ionization of molecules from surfaces. In Chapter 3, an electromagnetic pulsed valve was used for sample target striking. The pulsed valve provided similar signals compared to manual tapping but with temporal selectivity. In Chapter 4, a piezoelectric cantilever was used as a target striker. Spatial resolution of around 1 mm was obtained for analytes deposited on thin foils. In Chapter 5, the ionization mechanism involved
in sublimation electrification was studied. The charge produced from the sublimation of organic compounds was measured to investigate the ionization mechanism.
CHAPTER 2. A NANOPARTICLE CO-MATRIX FOR MULTIPLY CHARGING IN MATRIX-ASSISTED LASER DESORPTION IONIZATION IMAGING OF TISSUE

This chapter describes MALDI mass spectrometry imaging of high-charge-state biomolecules in tissue using a two-component matrix of 2-NPG and silica nanoparticles. The combined matrix produced highly charged ions from tissue with high-vacuum MALDI. Images of mouse brain from multiply charged ions were obtained which showed similar spatial localization. The mechanism for the production of highly charged ions may rely on the large surface area of the particles which can dry the tissue and their ability to bind analyte molecules that assist in crystal formation and production of multiply charged ions on laser irradiation.

2.1 Introduction

Matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) is an important tool for the identification and determination of the spatial distribution of proteins, peptides, lipids, metabolites, and other biomolecules and xenobiotics in tissue.\textsuperscript{29, 93, 107, 110} MALDI-IMS does not require target-specific reagents such as antibodies, can be used to perform direct analysis of tissue sections without homogenization, and allows measurement of multiple compounds in parallel.\textsuperscript{64, 108} In a typical MALDI-IMS experiment, the tissue is cut into micrometer-thick sections that are mounted on a conductive microscope slide. Matrix is typically applied by droplet for profiling and spray, sublimation, or sieve for imaging. After the slide is loaded into the mass spectrometer, mass spectra are obtained at regular spatial intervals. Peak

intensities for a particular \( m/z \) range is then used to generate a heat map that provides information regarding location and quantity of biomolecules in the tissue.

MALDI imaging has been combined with tandem mass spectrometry for improved selectivity in compound identification.\(^{64,111,112}\) MALDI TOF/TOF instruments have the advantage of high data acquisition rate and large mass range,\(^{113-116}\) but other instruments such as triple quadrupole\(^{117}\) and hybrid quadrupole time of flight,\(^ {118,119}\) and quadrupole ion trap instruments have been used.\(^ {120}\) High-resolution Fourier transform ion cyclotron resonance\(^ {121}\) and Orbitrap sacrifice some scan speed but have high value in compound identification.\(^ {122,123}\) Tandem mass spectrometry with MALDI imaging is most often carried out with singly charged ions, which limits fragmentation efficiency compared with multiply charged ions\(^ {78}\) and precludes the use of electron-based fragmentation methods such as electron capture and electron transfer dissociation.\(^ {66,67}\)

Although MALDI tends to produce singly charged ions, there are some notable exceptions. One example is infrared MALDI, which tends to produce multiply charged analyte molecules at high vacuum,\(^ {124,125}\) or atmospheric pressure.\(^ {126}\) Liquid matrices have been used for multiple charging in atmospheric pressure MALDI with infrared, visible, and UV lasers.\(^ {126-130}\) Some degree of multiple charging can be produced using conventional matrix materials, UV laser wavelengths, and specific sample preparation protocols.\(^ {131,132}\) Recently, several compounds were discovered that produce highly charged ions not only under atmospheric pressure MALDI conditions, but also under conditions that do not require a laser at all, a method known generally as matrix-assisted ionization (MAI).\(^ {133-135}\) The MAI technique has been used to image tissue under intermediate pressure conditions, but not with a vacuum MALDI imaging mass spectrometer.\(^ {136,137}\)

In this work, we have used a MAI matrix and nanoparticle (NP) co-matrix to produce multiply charged ions from tissue in a commercial high-vacuum MALDI TOF mass spectrometer.
The MAI matrix 2-nitrophloroglucinol (2-NPG) was used in conjunction with silica NPs to obtain tissue images with highly charged analyte ions. Sections of mouse brain tissue of 10 μm in thickness were mounted on conductive microscope slides. Solutions of matrix and NP suspension were either dropped or sprayed onto the tissue which was subsequently profiled or imaged in the MALDI mass spectrometer.

2.2 Experimental

A Bruker Ultraflextreme MALDI TOF/TOF mass spectrometer was used in this research for tissue profiling and imaging. The instrument is equipped with a frequency tripled Nd: YAG 355 nm solid-state laser with a homogenized modulated beam (Smartbeam II) laser operating at 1 to 1000 Hz. The laser spot size is controlled with a computer at diameter from 10-100 μm. The MALDI-TOF mass spectrometer can be operated in both linear and reflectron mode. The resolution of this system is 40,000 in the m/z range from 700 to 5000, and the mass accuracy is 1 ppm and 5 ppm for internal and external calibration, respectively.

Tissue samples were obtained from 4 to 6-week old breeding rats at the LSU School of Veterinary Medicine Division of Laboratory Animal Medicine (DLAM). The animals were sacrificed by CO₂ (5psi) exposure and brain samples were collected, washed in 50 mM ammonium bicarbonate buffer for 30 s, and frozen in dry ice. Frozen samples were stored at 80 °C. Thin sections were prepared with a Leica CM1850 cryostat directly from the frozen tissue. The tissue was fixed on cryostat support with optimal cutting temperature compound (OCT), avoiding contact of the OCT solution with the exposed side of the tissue. Coronal or horizontal rat brain sections were cut at a thickness of 10 μm, thaw-mounted on indium tin oxide (ITO) coated microscope slides and stored at 80 °C until use. Horizontal sections were used for MALDI profiling whereas coronal sections were used for the imaging experiments. The tissue sections were vacuum dried.
for 10 min and washed with 70% ethanol and 95% ethanol for 30 s each followed by a chloroform wash for 15 s. The sections were vacuum dried for 10 min before matrix application.

Matrix was applied to tissue sections by manual spotting for profiling mode and by spraying for tissue imaging. Figure 2.1. shows the workflow for application of matrix on a tissue. Alignment of the spots from one section to another was achieved by visual inspection. Using a micropipette, an aliquot of matrix solution was deposited onto the slide and allowed to dry. For tissue imaging experiments, the matrix was spray deposited using a pneumatic nebulizer that was constructed using a 200 μL micropipette tip, a quarter-inch stainless steel compression fitting, and a 75 μm I.D. and 360 μm O.D. silica capillary. The flow rate of the matrix solution was 100 μL/min and the nitrogen gas pressure was 10 PSI. The matrix was sprayed from a fixed distance of 7 cm for several cycles. Matrix was applied in 14 cycles at 4 s per cycle and 8 s drying between cycles.

MALDI mass spectra were recorded in positive ion linear mode. For profiling mode data acquisition, the laser was set to randomly irradiate a 250 μm diameter region around the selected position and 500 shots were summed to produce a mass spectrum over the range 1000–20 000 m/z. For pure analytes, a 1 μL volume of analyte solution was deposited on a stainless steel MALDI target followed by a 1 μL volume of matrix solution. The quantity of protein deposited on the target was 100 pmol. For the two-component matrix, NPs were added to the matrix solution before deposition with the goal of improved crystal formation on the tissue. For tissue profiling, six mass spectra were obtained from an array of spots spaced by 1 mm on an approximately 5 mm × 5 mm tissue section and the spectra were summed to produce a single mass spectrum representative of that tissue section. For imaging, mass spectra were obtained using a 150 μm center-to-center spacing and 500 laser shots per mass spectrum.
For studies of the effects of NP size, solutions of 10 mg/mL 2-NPG matrix with a 12 mg/mL particle suspension were deposited in $2 \times 3$ arrays on consecutive tissue sections with each of the 20, 70, 400, or 1000 nm 2-NPG/NP suspensions applied to one of the tissue sections. Two additional sections, one sectioned preceding and one following, were used as control with 2-NPG only. The effect of particle concentration was studied with 20 and 70 nm particles at concentrations of 3, 6, 12, and 25 mg/mL. For each of three replicate experiments, six consecutive sections were used: four 2-NPG/NP at the different concentrations flanked by two control sections as above.

Profiling mode mass spectra were baseline-subtracted and smoothed using the Savitsky–Golay smoothing filter set at 10 Da width over three cycles. Peak identification was performed in centroid mode and peaks with signal-to-noise ratio above 3 were used for analysis. Spectrum deconvolution was used for multiple charge detection. It was manually confirmed that the peaks were within 0.1% of the calculated $m/z$ value for a charge state. Images were reconstructed and normalized using FlexImaging 2.1.

A sinapic acid matrix solution was prepared by dissolving 10 mg in 1 mL of 70:30 HPLC grade methanol and water with 0.5% trifluoroacetic acid. The 2-NPG matrix solution was prepared
by dissolving 5 mg in 100 μL of 50:50 acetonitrile (ACN)/water with 0.5% TFA. Stock solutions
of cytochrome C and myoglobin were prepared in water and diluted with a 50:50 ACN/water
mixture to make 100 μM solutions. Insulin was prepared in water with 0.1% TFA. Suspensions of
NPs with diameters 20, 70, 300, and 1000 nm were prepared in 70:30 methanol and water. NP
suspensions were added to matrix solution and the NP concentration ranged from 3 to 25 mg/mL.

2.3 Results and discussion

The MAI matrix 2-NPG was used because it produces high-charge-state ions and does not
sublime rapidly under vacuum. The common MALDI imaging matrix SA was used for
comparison. Figure 2.2 shows representative mass spectra obtained for cytochrome C using 2-
NPG/SA matrices with and without NPs of 20 nm in diameter. Figure 2.2a shows the cytochrome
C mass spectrum with SA matrix without NPs and Figure 2.2b shows the corresponding mass
spectrum with the NP co-matrix. The signal is slightly lower in all three trials with the NPs but the
spectra are otherwise similar. Figures 2.2c and 2.2d show representative mass spectra of
cytochrome C with 2-NPG matrix, without and with NPs. For 2-NPG, the NP co-matrix results in
a signal that is three times lower than without NPs and the matrix adduct peaks are larger by a
factor of three. Furthermore, there are several matrix and matrix fragment adduct peaks of
relatively high intensity that are observed. The extent of multiple charging is similar in both 2-
NPG mass spectra. Similar results were obtained with insulin and myoglobin as shown in
Figure 2.3.

Although silica NPs do not enhance high-charge-state ions for pure proteins, it was found
that they have a significant effect on MALDI mass spectra obtained from tissue. The initial
motivation for their use was an attempt to improve tissue drying, separate analyte molecules from
tissue lipids and facilitate crystal formation. Mouse tissue sections were analyzed in profiling mode for comparison of SA and 2-NPG matrix and NP co-matrix in terms of the number of multiply charged ion peaks observed. Mass spectra obtained in profiling mode from mouse brain tissue sections are shown in 2.4a and 2.4b, respectively. Mass spectra obtained from 2-NPG and 2-NPG/NP are shown in Figures 2.4c and 2.4d, respectively. The NPs had little effect on the tissue profiling mass spectra obtained using SA matrix. The mass spectra in Figures 2.4a and 2.4b are nearly identical and no peaks associated with multiply charged analyte molecules were observed. The mass spectrum obtained with 2-NPG matrix without NPs shown in Figure 2.4c is similar to the mass spectra obtained with SA in terms of the total number of peaks and the peak pattern, with few peaks observed below 5000 m/z. The mass spectrum obtained with 2-NPG/NP is shown in
Figure 2.3. Mass spectra of insulin with a) SA, b) SA/NP, c) 2-NPG, and d) 2-NPG/NP, and myoglobin with e) SA, f) SA/NP, g) 2-NPG, and h) 2-NPG/NP.
Figure 2.4d and is qualitatively different compared with the other three mass spectra: there was a notable decrease in the intensity of peaks above 10 000 $m/z$ and increase in the number of peaks below 10 000 $m/z$. This difference is due to multiple charging: mass spectra obtained with 2-NPG/NP had an average of 32 peaks corresponding to multiply charged ions whereas the 2-NPG mass spectra had an average of 7 multiply charged ion peaks. Multiply charged ion peaks were determined manually. For each peak detected, $m/z$ values of higher charge states were calculated, and peaks were assigned a charge state if they were within 0.1% of the calculated value. With the 2-NPG/NP matrix, one-third of the assigned masses had higher charge state ions than without NPs.

![Figure 2.4. MALDI mass spectra of mouse brain tissue obtained with a) SA, b) SA/NP, c) 2-NPG, and d) 2-NPG/NP](image)

The influence of NP size was tested using NPs with diameters of 20, 70, 400, and 1000 nm. An average mass spectrum was obtained for each section using the procedure described above. Figure 2.5 shows representative mass spectra obtained for each NP diameter. The peak intensity
in the spectra was similar for all NPs tested. The average number of peaks observed \((n = 3)\) with signal-to-noise ratio greater than 3 for the 20, 70, 400, and 1000 nm NPs was \(60 \pm 4, 53 \pm 6, 49 \pm 3,\) and \(48 \pm 3,\) respectively. The 20 and 70 nm NPs produced more peaks, a third of which were below 5000 \(m/z\). Further experiments were performed with NPs of 20 and 70 nm in diameter.

The effect of particle concentration was studied with 20 and 70 nm particles at concentrations of 3, 6, 12, and 25 mg/mL. Average mass spectra \((n = 3)\) were obtained for each section. The average number of peaks detected using 20 nm NPs at 3, 6, 12, and 25 mg/mL was \(50 \pm 8, 53 \pm 4, 55 \pm 4,\) and \(48 \pm 7,\) respectively, whereas the average number of peaks detected using 70 nm NPs at 3, 6, 12, and 25 mg/mL was \(43 \pm 3, 45 \pm 3, 50 \pm 4,\) and \(39 \pm 5,\) respectively. Although there is not a wide variation in the number of peaks obtained, the remainder of the experiments were performed with 20 nm NPs at 12 mg/mL.

Mass spectra obtained using 2-NPG with and without NPs were compared to ascertain the extent of multiple charging. Three tissue sections, each with a \(2 \times 3\) array of matrix spots, were prepared for 2-NPG and 2-NPG/NP with profiling mode deposits. A peak list was assembled from six spots for 2-NPG and 2-NPG/NP for each tissue section. From three tissue sections each, an average of 62 \(\pm 3\) peaks was observed in the 2-NPG mass spectra and average of 88 \(\pm 4\) peaks was observed in the 2-NPG/NP mass spectra. The charge for each peak was assigned using the assignment procedure described above. The \(m/z\) values that were common to both or had a difference in charge state were selected for comparison.
Figure 2.5. Mass spectra obtained from mouse brain tissue with 2-NPG/NP with diameters a) 20 nm, b) 70 nm, c) 400 nm, and d) 1000 nm.

A pie chart representation of the fraction of multiply charged peaks with their charge states is shown in Figure 2.6. A table of assigned mass, charge state, and peak intensity is provided in Table 2.1. With 2-NPG, a total of 71 peaks were identified and 8 of these were multiply charged ions and associated with 18 individual species. Approximately one-fifth of the identified peaks corresponded to ions with 3 or more charges.
2-NPG/NP was used to generate MALDI images from multiply charged ions. During initial imaging experiments, it was found that the signal was cut in half within 40 min due to sublimation of 2-NPG under high vacuum. Addition of NPs did not improve the signal loss. To limit the MALDI acquisition time, imaging experiments were performed on half of each brain section. The tissue was washed to remove salts and lipids and a solution of 2-NPG or a suspension of NPs and matrix solution was deposited using a pneumatic sprayer. Figure 2.7 shows MALDI images of ubiquitin (+1 m/z 8565) charge states +1 through +5 for 2-NPG and 2-NPG/NP. The 2-NPG matrix produced good images from the +1 and +2 charge states, but not with the lower intensity higher charge states. On the other hand, the 2-NPG/NP matrix produced mass spectra with localized signal at all charge states. Additional MALDI images are shown in Figure 2.8. for multiply charged ions of +1 m/z 7573. In this case as well, the 2-NPG/NP could be used to obtain images of the higher charge states.
Table 2.1. List of multiply charged peaks (m/z) from mouse brain with their intensity and charge obtained with a) 2-NPG, and b) 2-NPG/NP

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Figure 2.7. Images of consecutive mouse brain tissue sections obtained from 8565 $m/z$ and its multiply charged ions with A, 2-NPG and B, 2-NPG/NP matrix
Enhanced multiple charging with the MAI matrix combined with NPs deposited on tissue may result from the high surface area of the particles, the desiccation effect of the NPs, or selective interaction of the NPs with analyte molecules. We hypothesize that this promotes the formation of analyte-containing crystals on the tissue that fracture on laser irradiation to produce highly charged ions through the MAI effect. The surface area of the NPs is approximately one square centimeter per microliter of suspended particles. It is unlikely that all of this surface area is available for analyte crystal formation, but it can potentially provide greater surface area than bare tissue or function as nucleation sites for small matrix and analyte crystals. Recent studies suggest that the formation of highly charged analytes may be enhanced by high-surface-area matrix deposits. The importance of desolvation in the formation of multiply charged ions has also been reported. The silica NPs may bind with the water in the tissue and produce a dry surface conducive to crystal formation. An additional factor may be the concentration of the analyte through interaction with the NPs. It has been reported that an NP co-matrix will interact with and concentrate analyte thereby improving signal and reducing spot-to-spot variation in the signal. Bare silica NPs are believed to bind electrostatically to peptides and proteins thereby improving detection sensitivity.

2.4 Summary

The addition of NPs to SA and 2-NPG matrix combined with NPs has little effect on high-vacuum MALDI of pure peptides and proteins other than to decrease the observed signal. However, the 2-NPG/NP matrix was effective at producing highly charged ions from tissue, potentially due to its ability to bind analyte molecules and promote the formation of crystals. The number of multiply charged peaks observed with 2-NPG/NP on tissue was four times higher than with 2-NPG alone. Silica NPs with diameters of 20, 70, 400, and 1000 nm were tested, and those
Figure 2.8. Images of consecutive mouse brain tissue sections formed by 7573 \textit{m/z} and multiply charged ions with a) 2-NPG, and b) 2-NPG/np of 20 nm in diameter produced the highest charge state at a concentration of 12 mg/mL. MALDI images were also obtained of mouse brain with multiply charged ions; however, sublimation of 2-NPG under high vacuum limited the time for analysis. Future studies will be focused on further study of these multiply charged ions using high-resolution instruments such as Orbitrap or Fourier
transform ion cyclotron resonance and identification of the proteins with tandem mass spectrometry using collision-induced dissociation and electron-transfer dissociation.
CHAPTER 3. PULSED VALVE MATRIX-ASSISTED IONIZATION

This chapter is a description of the development of a new approach for MAI with high temporal resolution. An electrically actuated pulsed valve was used to produce ions. Dried droplet deposits of matrix and analyte prepared on a thin metal foil was placed at the inlet of a mass spectrometer. With the actuation of the pulsed valve, a short puff of higher-pressure gas strikes the foil and ejects material on the opposite side. Multiply charged ions were generated using a pulsed valve within a 4-second window.

3.1 Introduction

MAI is an ionization method in which ions are formed by the interaction of an analyte molecule with specific matrix compounds that promote the formation of ions.\textsuperscript{82, 135, 138} As with MALDI,\textsuperscript{128} the matrix is mixed with the analyte and deposited and dried on a sample target. Ion formation is associated with the production of particles by laser ablation, mechanical shock, solvent boiling, or sublimation.\textsuperscript{88, 139} Some matrix compounds developed for MALDI can also be used for MAI, many compounds are unique to MAI.\textsuperscript{140} Unlike MALDI, MAI tends to produce ions that are highly charged.\textsuperscript{80}

MAI has some potential advantages for mass spectrometry imaging due to its simplicity, low fragmentation, and tandem mass spectrometry facilitated by highly charged ion formation. For imaging in laser spray mode, a pulsed laser is directed at a thin tissue section in transmission mode (back side irradiation) to create ions by MAI.\textsuperscript{84, 141}

assisted ionization vacuum (MAIV) can be used for the analysis of tissue by spotting matrix on selected areas and applying vacuum to the entire tissue section. Precision spotting can limit the exposed tissue area to several hundred µm. An alternative approach uses a glass melting point tube to sample from tissue under ambient conditions for MAI. Precise temporal and spatial control of ion formation could add significant utility to these imaging approaches.

Precise control of material removal from metal sample surface for mass spectrometry analysis can be achieved using a locally directed shock pulse. For example, laser induced acoustic desorption (LIAD) uses a pulsed nanosecond laser directed in transmission geometry at a thin metal foil, which ejects material from the opposite side. Post-ionization can be accomplished using electron ionization, electrospray ionization, and photionization. A similar approach that does not require laser nebulizes liquid samples from piezoelectrically driven targets using surface acoustic wave nebulization. Here a high frequency piezoelectric device is used to nebulize a thin film of liquid from a surface and bare ions are formed upon solvent evaporation and sampled into a mass spectrometer ion source.

In this chapter, a method for temporally and spatially localized sampling for matrix-assisted ionization using a solenoid pulsed valve is presented. A high-speed pulsed valve is directed at the back side of a thin foil with a MAI sample deposited on the opposite side facing the inlet of a mass spectrometer. When the valve is actuated, the gas pulse creates impinges the foil and a plume of ions are detected in the mass spectrometer. The pulsed valve matrix-assisted ionization source was demonstrated for ionization of peptide and protein molecules under ambient conditions.
3.2 Experimental

Pulsed valves are used to inject a very small amount of gas in a very short period. Most of the pulsed valves are electromagnetically driven. Electromagnetic pulsed valves are composed of an electromagnetic operator, a diaphragm and a valve body. The diaphragm pressed closely against the outlet valve with a spring acts as a switch. When the current flows in the solenoid coil the electromagnetic field pulls the armature and poppet inward opening the orifice for a short period. Within those few milliseconds of opening, a puff of gas comes out of the orifice. The pressure of the gas coming out is the same as the backing pressure supplied to the valve.

The pulsed valve used in this research was a Parker Series 9 solenoid valve with an orifice diameter of 0.51 mm and a nitrogen gas backing pressure of 90 psi (600 kPa gauge pressure) (Figure 3.1). The valve was actuated with a 280 V high voltage pulse of 500 µs duration provided by a high voltage switch and high voltage power supply driven by a pulse and delay generator.

Figure 3.1. Pulsed valve
The mass spectrometers used in this research is an ion trap mass spectrometer. Figure 3.2 shows the schematic of the mass spectrometer. This mass spectrometer is equipped with both ESI and atmospheric pressure chemical ionization (APCI) source and could be operated in both positive and negative ions mode. A heated capillary is used to transfer ions into the ion funnel. The dual ion funnel transfer provides higher sensitivity and the scan speed of the instrument is 52,000 u/sec. The resolving power of the instrument is 20,000 fixed-width at half maxima. The instrument also can do tandem MS for protein identification and structural determination.

![Diagram of mass spectrometer](image)

Figure 3.2. Schematic of Bruker Amazon ETD mass spectrometer

The modified mass spectrometer ion source was comprised a pulsed valve aimed at the back side of a metal foil with matrix and analyte mixture deposited on the front. The ESI ion source was removed and the glass inlet capillary was heated from 200-350 °C. Ions created at ambient
pressure were sampled by the inlet of the mass spectrometer. Fig 3.3 shows a schematic of the ion source.

The sample target was a 250 µm thick sheet of aluminum foil that was mounted between two 0.64 mm thick 5 cm square stainless steel plates with a central 25 mm hole. The foil was held 1 mm from the pulsed valve orifice with the opposite side 9 mm from the inlet of an ion trap mass spectrometer. Both the valve and the sample holder were placed on a translation stage to adjust the distance from the mass spectrometer inlet. The electrospray interface was removed for inlet ionization operation and the inlet was heated to 350 °C. Samples were analyzed in Ultrascan mode between 100 m/z and 3000 m/z at 32,500 m/z per second in positive ion mode.

A solution of 10 μM bovine insulin was prepared in 1:1 acetonitrile: water with 0.1% FA and ubiquitin in HPCL grade water. Saturated solutions of 2,5-DHAP, 2-NPG, matrix solutions were prepared in 1:1 acetonitrile: water and 3-NBN was prepared in pure ACN. To create a sample deposit, 1 µL of analyte was deposited on the aluminum foil followed immediately by 2 µL of matrix solution and air dried.

3.3 Results and discussion

The pulsed valve matrix-assisted ionization configuration was installed at the inlet of the ion trap mass spectrometer in nanospray configuration with the commercial spray source removed and the interlock defeated. The foil was held vertically and placed as close as practical to the mass spectrometer with the MAI deposit facing the inlet. The pulsed valve was placed just behind the foil and backed with high pressure nitrogen gas. It was found that the highest gas pressure gave the highest signal. Conventional MAI was accomplished by removing the pulsed valve and foil and tapping a microscope slide with a matrix and analyte deposit against the side of the inlet.
MAI mass spectra of the proteins insulin and ubiquitin are shown in Figure 3.4. A 1 µL volume of 10 µM protein was droplet dried on the foil target with 2 µL of 3-NPG matrix and allowed to dry. The resulting spot was approximately 3 mm in diameter. The mass spectrum shown in Figure 3.4a results from insulin deposited on a glass slide and tapped against the inlet of the mass spectrometer. The pulsed valve matrix-assisted ionization of the same solution is shown in Figure 3.4b. A total of 5 pulses at 1 Hz repetition rate were used. Figure 3.4c is the mass spectrum of the protein ubiquitin from microscope slide tapping and Figure 3.4d is the corresponding pulsed valve matrix-assisted ionization mass spectrum of ubiquitin. The mass spectra are comparable, although the pulsed valve matrix-assisted ionization spectra are approximately a factor of two
larger than the mass spectra obtained by tapping. Mass spectra obtained using other inlet ionization matrix compounds produced similar results: pulsed valve matrix-assisted ionization mass spectra of insulin showing a comparison of 2-NPG, 3-NBN and 2,5-DHAP matrix compounds is shown in Figure 3.5. The 3-NBN produced the largest signal and the analyte signal intensity was more than 150 times as intense as with 2-NPG and 300 times more intense than 2,5-DHAP, consistent with previously reported results.\textsuperscript{140} The 2-NPG produced analyte with the highest charge state.

To assess the number of pulses required to deplete the sample was performed using 2-NPG matrix. Figure 3.6 shows the total ion current (TIC) as a function of time for MAI of insulin. Figure 3.6a shows results from tapping of a glass slide with sample deposit on the mass spectrometer at 30 s elapsed time. Figure 3.6b shows the TIC as a function of time for 1 valve pulse, Figure 3.6c for 2 pulses at 1 Hz, and Figure 3.6d for 5 pulses at 1 Hz. In all cases, the maximum signal is achieved after approximately 2 s and decayed rapidly with an approximately 2 s time constant.

The integrated ion signal for 2 and 5 pulses (and for 10, 20 and continuous pulses not shown) was similar and approximately twice the total intensity of a single pulse. This suggests that approximately half of the available particulate was removed with the initial pulse and nearly all of the remainder with the second pulse. A second broad ion signal maximum is observed between 40 and 60 s in the Figure 3.6 plots, which suggests two modes or regions of ionization and may be related to the bimodal particle size distribution for inlet ionization matrices that has been observed previously.\textsuperscript{150}

A comparison of the time response of the pulsed valve MAI signal for 2-NPG, 3-NBN and 2,5-DHAP is shown in Figure 3.7. In these plots, the pulsed valve was fired five times at a repetition rate of 1 Hz at a time of 30 s. For all of the matrix compounds, maximum signal was
Figure 3.4. Mass spectra of 2-NPG matrix-assisted ionization of a) insulin using tapping, b) insulin using pulsed valve and c) ubiquitin using tapping, and d) ubiquitin with pulsed valve
Figure 3.5. Pulsed valve matrix-assisted ionization mass spectra of insulin obtained with matrices a) 2-NPG, b) 3-NBN and c) 2,5-DHAP using 5 pulses at 1 Hz repetition.
Figure 3.6. Total ion current as a function of time for 2-NPG matrix-assisted ionization of insulin using (a) tapping, (b) 1 valve pulse, (c) 2 pulses and (d) 5 pulses at 1 Hz repetition starting at 30s.
observed about 2 s after the valve was fired, decreased rapidly with a 2 s time constant and a lower intensity tail returning to baseline within 30 s.

Figure 3.7. Total ion current as a function of time for pulsed valve matrix-assisted ionization of a) 2-NPG, b) 3-NBN and c) 2,5- DHAP using 5 pulses at 1 Hz repetition rate.
3.4 Summary

A new ion source was developed for matrix-assisted ionization with high temporal resolution. A high-pressure electric solenoid pulsed valve directed at a thin metal foil was capable of ionizing the available material in the sample within 5 seconds of valve actuation. This source has potential applications in matrix-assisted ionization imaging both at ambient pressure and under vacuum and shock wave technology that has been developed for biomedical applications.\textsuperscript{151-153} has potential applications for precision MAI imaging. A temporally and spatially focused system capable of selectively producing ions from an array of on tissue samples is being developed. Additional studies will be aimed at investigating the role of high voltage applied to the sample foil in determining the analyte charge distribution and measuring the size distribution of the ejected particulate.
CHAPTER 4. PIEZOELECTRIC MATRIX-ASSISTED IONIZATION

This chapter represents a description of development of a new actuation method for MAI with good temporal and spatial resolution using piezoelectric cantilever. A strike from the piezoelectric bimorph cantilever on a thin metal foil was used to remove materials deposited on the opposite side facing the mass spectrometer inlet. Highly charged ions of peptides and proteins were generated from dried droplet deposits and sampled into the inlet of the mass spectrometer. A lateral resolution of 1 mm was obtained with the piezoelectric sampling configuration. Singly charged lipids and gangliosides were detected from tissue with piezoelectric MAI using a silica nanoparticle co-matrix.

4.1 Introduction

Shock-generation of ions for MAI can be implemented in a number of ways. The simplest is to strike a target near the inlet of the mass spectrometer.\textsuperscript{79, 80} Other methods for ionizations include devices such as a pellet gun\textsuperscript{79} or mouse trap\textsuperscript{88} to produce a mechanical shock. We recently developed a pulsed valve method for precise temporal and spatial control of MAI in which a high-speed pulsed valve was used to direct a high-pressure gas pulse at the back side of a thin foil with a MAI sample on the opposite side facing the MS inlet.\textsuperscript{83} The shock from the gas pulse creates a plume of ions that are sampled into the mass spectrometer. The approach of gas-pulse-driven MAI was demonstrated for the ionization of peptide and protein molecules from ambient conditions.

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Some portions of the work reported in this chapter previously appeared as Banstola, B., Szot, C. W., Deenamulla Kankanamalage, A. P., & Murray, K. K., Piezoelectric matrix-assisted ionization, European Journal of Mass Spectrometry, 25(2), 202-207. Copyright © 2019, SAGE Publications. DOI: 10.1177/1469066718816696
Compared to tapping methods, the pulsed valve provides better temporal resolution; however, the spatial resolution achieved with the pulsed valve is limited.

In this chapter, a piezoelectric cantilever-based method was developed for temporally and spatially localized ion formation for MAI that uses a voltage pulse and does not require a high-pressure gas. Here, a piezoelectric bimorph cantilever with a sharp tip attached to the arm was used as an electrically-driven striker on a thin metal foil with an MAI sample on the opposite side. When the cantilever is actuated, the needle strikes the foil and the material ejected from the other side forms ions when introduced into the mass spectrometer inlet. The piezoelectric cantilever configuration was used for ionization of peptides and protein standards as well as lipids and gangliosides from thin tissue sections at atmospheric pressure.

4.2 Experimental

Piezoelectric bimorph cantilevers are precision ceramic actuators which convert electrical energy into mechanical motion with high speed and accuracy. Piezoelectric cantilevers are constructed of multiple piezoceramic layers. Figure 4.1 shows the photograph of the piezoelectric cantilever. The voltage on these piezoceramic layers are controlled independently. The length of the layers increases as the voltage applied across them increases. Tip deflection occurs when the top and the bottom layers elongate by a different amount.

A piezoelectric bimorph cantilever was used to remove samples. The piezoelectric cantilever has a length of 32 mm, width of 7.8 mm, and a 28 mm free length with maximum displacement of 0.45 mm. The resonant frequency of the bare cantilever is 370 Hz. A 4-mm section from the tip of a 100-mm diameter sewing needle was attached to the end of the arm of a cantilever with cyanoacrylate glue which added a mass of approximately 40 mg to the cantilever. The
modified cantilever was operated at 300 Hz, which was the highest frequency possible with the added mass; higher frequencies caused overheating and damaged the cantilever.

Figure 4.1. Piezoelectric cantilever

The mass spectrometers used in this research is an ion trap mass spectrometer as described previously (Figure 3.2). The ESI source was removed as with the pulsed valve and the glass inlet capillary was heated to various temperatures. The modified mass spectrometer ion source comprised of piezoelectric cantilever with a needle aimed at the back side of a metal foil with matrix and analyte mixture deposited on the front. The ESI ion source was removed for the studies in this research and the glass inlet capillary was heated from 200-350 °C.
Aluminum foil (13, 25, and 50 mm), tungsten foil (50 mm), and titanium foil (13 and 25 mm) were used for the experiments described below. The foil containing the sample was mounted between two 0.64-mm thick, 5-cm square stainless plates with a central 25-mm hole, similar to that previously described. The foil was held approximately 250 mm from the tip of the needle with the sample side 4 mm from the inlet of the mass spectrometer. Figure 4.2 shows the schematic of the ion source and the modified cantilever.

The mass spectrometer used for this experiment is an ion trap mass spectrometer. The captive spray interface was removed for inlet ionization operation and the glass capillary inlet was heated to maximum of 350°C. Before removing the interface, the voltage was turned off and the gas was disconnected. Samples were analyzed in Ultrascan mode between 100 m/z and 3000 m/z at 32,500 m/z per second in positive ion mode. Data collected were analyzed using the instrument control software.

Stock solutions of protein standards were prepared in HPLC grade water and diluted to 10 mM. The three matrices 3-NBN, 2-NBN and 2-NPG were selected because of their ability to produce high-intensity ions with ESI-like charge states. Stock solutions at 1 mM concentration were prepared for all proteins. Ubiquitin, cytochrome C, and myoglobin were prepared in HPLC grade water whereas insulin had 0.1% TFA added. Matrix solutions were prepared by dissolving 10 mg of 2-NPG in 200 mL of 1:1 ACN: water with 0.1% TFA, and 10 mg of 2-NBN or 3-NBN in 100 mL of ACN with 0.1% TFA. The sample target was a thin metal foil and sample deposits were formed by depositing 0.5 mL analyte on the foil followed by 0.5 mL matrix solution and mixing on the foil using the pipette tip. An additional 0.5 mL of matrix was deposited on top of the spot and left to dry. The resulting sample spots approximately 1 mm in diameter were used for the experiments described below.
Mouse brain tissue was obtained from the LSU School of Veterinary Medicine Division of Laboratory Animal Medicine (DLAM) as described previously using procedures approved by the LSU Institutional Animal Care and Use Committee (IACUC). Sections 10-μm thick were prepared from frozen tissue with a cryostat (CM1850, Leica Microsystem, Wetzlar, Germany),
thaw-mounted on foil, and stored at -80°C. Prior to analysis, the sections were thawed and dried under rough vacuum for 10 min to remove moisture from the tissue. After drying the tissue, silica nanoparticles 20 nm in diameter were sprinkled from a spatula onto the tissue to form a distributed layer. The matrix solution was then deposited using a micropipette onto nanoparticle-treated tissue and allowed to dry.

4.3 Results and discussion

The cantilever and foil were mounted at the inlet of the mass spectrometer. Initially, a bare cantilever was used to strike the surface either parallel to the surface or at a 45 angle. However, the flat edge of the cantilever distributed the force over a large area and was not efficient at material removal. To concentrate the force of the strike, approximately 4 mm of a sewing needle tip was attached to the end of the arm. The distance between the foil and the MS inlet was optimized for the highest signal intensity at 4 mm. Driving the cantilever with the added mass at frequencies above 300 Hz of the needle tip resulted in overheating and failure of the bimorph. Tungsten, aluminum, and titanium were tested, and it was found that it was difficult to remove material from the relatively thick and inflexible 50-mm tungsten foil and ionization was not observed. Aluminum (13, 25, and 50 mm) and titanium (13 and 25 mm) foils produced ions. Material removal from the thinner foils was more efficient; however, the 13-mm-thick foils were susceptible to damage by tearing. Though the goal was to remove material efficiently with each strike, care was taken such that the striker did not penetrate the foil. All the experiments described below were performed with 25-mm-thick aluminum foil.

A comparison of piezoelectric-driven MAI and manual tapping is shown in Figure 4.3. A 0.5-mL volume of a 10-mM ubiquitin solution was deposited on the aluminum foil followed by two deposits of 0.5 mL of 2-NPG matrix which was mixed and allowed to dry. To create ions by
manual tapping, the foil target was tapped once on the inlet capillary. For the piezoelectric configuration, 20 pulses at 300 Hz were used to remove material from the foil. Figure 4.3a and b shows the ion signal obtained by manual tapping and piezoelectric cantilever, respectively, as a function of time (strike at 20 s). The signal obtained from tapping (Figure 4.3a) was slightly higher than from the cantilever (Figure 4.3b) in a triplicate measurement. In the corresponding mass spectra, the charge distribution of peaks in the mass spectra from tapping (Figure 4.3c) is similar to that from piezoelectric strike (Figure 4.3d), with the maximum peak intensity observed for the +8 charge state in both cases. Mass spectra of cytochrome C and myoglobin also contained peaks from highly charged ions.

Figure 4.3. Matrix-assisted ionization mass spectra of ubiquitin protein using 2-NPG matrix: (a) total ion current tapping, (b) total ion current cantilever, (c) mass spectrum tapping, and (d) mass spectrum cantilever.
The MAI matrices 2-NPG, 2-NBN, and 3-NBN were tested and compared using the cantilever striker. A capillary inlet temperature of 350°C was used for 2-NPG whereas 200°C was used for 2-NBN and 3-NBN. The total ion signal and mass spectra obtained using 20 pulses at 300 Hz are shown in Figure 4.4 for each matrix. Of the three matrices, 2-NPG had the lowest peak signal intensity and longest signal duration with a decay time of 25 s obtained by fitting a single exponential to the data. The 2-NBN and 3-NBN had comparable peak signal intensity and had decay times of 9 and 6 s, respectively. The 2-NBN had a larger integrated signal intensity that was approximately four times larger than 3-NBN and 250 times larger than 2-NPG.

The number and frequency of cantilever strikes for efficient removal of sample material was assessed using 2-NBN and insulin. To determine the number of pulses required for complete removal of material, the cantilever was operated at a frequency of 1 Hz and number of strikes was varied. Figure 4.5a shows the total ion current for all insulin charge states as a function of the number of strikes. Three trials were done for each experiment and the error bars represent one standard deviation from three replicate experiments. The signal reached its maximum after approximately ten strikes, suggesting that this number is required to completely remove the deposit from the foil. Similar experiments were performed for 3-NBN and 2-NPG and it was found that ten strikes were required for the former and five for the latter to completely remove the deposit from the foil.

To assess the effect of the cantilever driving frequency, a burst of ten pulses was applied to the foil at a range of frequencies. A new spot was analyzed for each strike and the total ion intensity was recorded. Results for 2-NBN and insulin are shown in Figure 4.5b. The observed
Figure 4.4. Total ion current for piezoelectric matrix-assisted ionization of insulin using matrices (a) 2-NPG, (b) 2-NBN, and (c) 3-NBN; the insets show the mass spectra for each matrix.
Figure 4.5. Piezoelectric matrix-assisted ionization ion signal for insulin with 2-NBN matrix as a function of (a) cantilever strikes at 1 Hz and (b) cantilever frequency.
signal increases up to a frequency of 300 Hz; the cantilever could not be operated at higher frequencies without damage. Similar results were obtained for 3-NBN and 2-NPG. For the remaining experiments described below, the cantilever was operated with ten strikes at 300 Hz frequency for optimum removal of material from the foil.

An assessment of lateral resolution of the system was performed using pairs of deposited sample spots of proteins ubiquitin and insulin. Individual deposits of ubiquitin and insulin that were separated by 0 (overlapping), 0.5, 1, 2, or 3 mm were created on the aluminum foil. The goal was to strike one spot and determine how close the second spot could be without producing signal from the second protein. The cantilever was set to strike the center of either the ubiquitin spot or the insulin spot with ten strikes at 300 Hz. Figure 4.6 shows the signal intensity for the insulin +5 peak and the ubiquitin +8 peak for striking either the ubiquitin spot or the insulin spot plotted as a function of the center-to-center distance between the spots. When the distance from the strike point (at spot center) to the center of the adjacent spot is 1 mm or more, primarily the targeted protein is observed. At 1 mm center-to-center distance between spots, more than 75% of the signal corresponds to ubiquitin when the ubiquitin spot is targeted and struck and close to 100% of the signal corresponds to the insulin when the insulin spot is targeted and struck. This suggests that the piezoelectrically driven tip can remove material from a region localized to approximately 1 mm.

The piezoelectric cantilever striker was tested for ionization of biomolecules from tissue using a 10-mm mouse brain tissue section mounted on aluminum foil. After sectioning, the tissue was stored at –80°C and was thawed, dried under vacuum, washed with 70% ethanol followed by 90% ethanol, and dried again under vacuum. Matrix was deposited on the tissue as a 1 μL spot and allowed to dry. No signal from the tissue could be observed using the above sample preparation
either with or without washing. To create a more easily displaced sample deposit, silica nanoparticles were deposited on the tissue after washing and prior to matrix addition. It was found that these particles produced a deposit at the surface of the tissue that could be removed by the piezoelectric striker. Approximately 0.5 mg of 20-nm silica NPs were sprinkled over an area of approximately 3 mm² on the tissue followed by a 1 mL volume of matrix solution. A mass spectrum resulting from 10 strikes on tissue with a matrix and nanoparticle co-matrix deposit is shown in Figure 4.7. Phospholipids and gangliosides were detected from the tissue; no signals from peptides and proteins were observed. The molecules detected were identified as phosphocholine head group at 184 m/z, PC (34:1) at 760.6 m/z, and ganglioside (GM1a) at 1548

Figure 4.6. Fractional signal for targeted protein from spots separated by the indicated center-to-center distance for insulin (●) and ubiquitin (♦). Peaks of +5 and +8 charges were used for insulin and ubiquitin, respectively.
by comparison with results from previous studies. No multiply charged ions of these species were observed.

The mass spectra obtained with the piezoelectric cantilever are similar to those obtained previously with a high-pressure pulsed valve. The decay time constant obtained by fitting to a single exponential curve was less than 5 s for all three matrices using the pulsed valve, whereas it ranged from 6 to 25 s with the piezoelectric striker. This may be due to the relatively localized piezoelectric strike which may not remove the entire sample and could result in delayed emission from the surrounding area. Contrastingly, the pulsed valve rapidly ejects all of the material in a short period of time leaving no residual.

Figure 4.7. Mass spectrum obtained from mouse brain tissue using 2-NBN matrix.

Mass spectra of mouse brain tissue obtained using 337 nm laser spray MAI in negative ion mode reported the observation of both singly and doubly charged gangliosides. Proteins have
been observed from tissue samples using MAI in vacuum in which the tissue sample is subjected to a vacuum source and ions are formed during the sublimation process. In the results reported above for the piezoelectric striker at atmospheric pressure, singly charged gangliosides were observed in positive ion mode. Although widely different ionization configurations and mass spectrometers, the MAI matrixes have the ability to produce ions from tissue without fragmentation.

4.4 Summary

A new method for sample introduction for MAI has been developed that uses a piezoelectric cantilever striker. It was found that 25 mm thick aluminum foil provided an excellent target surface for the striker: thicker foils did not produce ions and thinner foils tended to tear. A needle tip attached to the cantilever allowed localization of the striking force to a zone of approximately 1 mm in diameter. The duration of the ion signal following the strike ranged from 10 to 40 seconds. The matrix 2-NBN was found to give the best overall performance for the system under present conditions. It was found that a silica nanoparticle co-matrix assisted in producing singly-charged ions from thin tissue sections using the striker. The piezoelectric cantilever system has potential applications for imaging using MAI. The piezoelectric device used for this study is relatively large with a low-resonant frequency yet was able to achieve relatively good spatial precision. Future work will aim at improving quantification using internal standards and on decreasing the spatial extent and duration of ion signal using smaller actuators. The use of one or more small high-frequency piezoelectric devices could potentially improve both the spatial precision as well as the speed of data acquisition for this approach.
CHAPTER 5. SUBLIMATION ELECTRIFICATION OF ORGANIC COMPOUNDS

The charge generated from sublimation of solid organic compounds was measured using a device comprising of a metal plate and vacuum chamber. A group of compounds that produce multiply charged ions in matrix-assisted ionization were sublimed from a thin film on a metal target and the current produced from the sublimation was used to calculate the net charge for each compound. Charge of both polarities was observed depending on the compound sublimed. The sign of the net charge could be altered with an applied electric field suggesting emission of both positive and negative charge. The quantity of charge generated also varied with amount of acid added to the solution deposited on the target. Addition of acid increased positive charge and decreased negative charge coming off the plate. This suggests that the charge detected is an overall net charge and the charge separation occurs through combined effects of ion and material transfer. The probable charge carriers are H\(^+\) and OH\(^-\) ions.

5.1 Introduction

There are four ways ions can be generated from solid surfaces: electric field, particle bombardment, photon or mechanical electrification. The first technique uses a high electrical field gradient of around 10\(^8\) V/cm\(^1\). The second category of technique includes fast atom bombardment,\(^2\) plasma ionization,\(^3\) and secondary ionization\(^4\), where a beam of ions or neutrals with kinetic energy ranging from few keV to 100 MeV is used to generate ions.\(^5\) The third category involves use of lasers as in laser desorption ionization\(^6\) and MALDI\(^7\), where photons are used to produce ions from surfaces. In addition to the above-mentioned techniques, ions can also be generated mechanically by application of force or phase change as in matrix-assisted ionization (MAI)\(^8\), contact electrification.
Ionization with MAI has been shown to occur with mechanical shock provided in various ways and the charge states of ions produced are large and similar to the charge states obtained with electrospray ionization.\textsuperscript{34, 79, 84, 138, 167} The ability to produce ions with vacuum suggests that charge separation is occurring on the surfaces due to crystal cracking during sublimation that ejects charged particles. To comprehend the ionization mechanism of MAI, understanding the role of contact electrification in surface charge separation during sublimation is important.

Contact electrification is the transfer of charge between surfaces that are brought into contact and then separated.\textsuperscript{30, 168-170} Charge separation can occur due to electron transfer,\textsuperscript{169, 171} ion transfer,\textsuperscript{172, 173} or material transfer.\textsuperscript{30, 174} Figure 5.1 shows various methods for charge separation.

![Electrification charge transfer](image)

Figure 5.1. Electrification charge transfer
It has been well established that contact electrification in conductors and semiconductors occurs due to the transfer of electrons.\textsuperscript{168} However, for the insulators, experimental observations and theoretical considerations do not support electron transfer.\textsuperscript{31, 168} CE through electron transfer is possible only for metals and semiconductors which have no gaps or small band gaps.\textsuperscript{168}

Another mechanism of charge separation occurs through the transfer of ions. Carrier ions could be protons, hydroxide ions, alkali metal cations or halide ions.\textsuperscript{168, 175} Several studies have proposed ions transfer as a mechanism for CE, and the study of surfaces that had covalently bound ions and mobile counterions provided the best evidence.\textsuperscript{168, 170} In the study, surfaces with covalently bound cations and mobile anions were used. Upon contact with the other surface, it was found that the charge separation was due to the transfer of mobile ions.\textsuperscript{176} For the surfaces without the mobile ions, adsorbed water on the surfaces can provide mobile ions. The segregation of hydroxide ions at the interface between the solid and thin layer of adsorbed water results in charge separation.\textsuperscript{168}

Along with ions and electrons, the transfer of materials can result in charge separation.\textsuperscript{30, 174} Charge separation due to the transfer of material between surfaces has been observed using x-ray photoelectron spectroscopy and Raman spectroscopy.\textsuperscript{30} Charged particle ejection has been proposed as precursors of highly charged ions observed in MAI.\textsuperscript{177} The pressure within the defects in matrix crystal when expands inside the vacuum can cause the ejection of particles. Figure 5.2 shows schematic for ejection of materials from a surface.

Charge transfer in contact electrification has been measured using various methods.\textsuperscript{169, 178-181} One of the simplest techniques of measuring charge is placing samples directly on a metal plate connected to an electrometer.\textsuperscript{169} The charge recorded on the metal is equal to charge carried away with the sample when sublimated or removed. Charge from the sublimation of organic compounds
has been measured previously using a modified mass spectrometer inlet source with detector plates. In the study, various compounds were sublimed by inserting them into the modified mass spectrometer inlet and charge produced was measured separately from the inlet tube and the detector plate placed downstream. Current in the range of 10-100 pA was recorded from sublimation electrification.

In this chapter, the current from the sublimation of organic compounds was measured using a device comprising a metal plate and a current amplifier. A solution of the compound was deposited on the metal plate, allowed to dry, and then sublimed. The current generated was integrated to obtain the charge produced for each compound. The charge produced by these compounds was measured at various pH, electric field, and temperature.

5.2 Experimental

A vacuum chamber with two compartments separated by a pneumatically actuated gate valve is shown in Figure 5.3 and was used for sublimation. Both compartments were stainless steel..
8" inch conflat flange six-way crosses. The first compartment, from now on referred to as the sample chamber, was attached to a mechanical pump to provide rough vacuum. The second compartment was evacuated with a turbomolecular pump with a pumping speed of 290 L/s of nitrogen. The pressure of the second compartment was maintained at $10^{-5}$ torr vacuum. Use of a second compartment helped apply intermediate vacuum to the sample chamber immediately, with the opening of the gate valve. The charge measurement plate was placed inside the sample chamber and was made of a 6.25 cm square brass plate with a thickness of 0.5 mm attached to a coaxial feedthrough connection as shown in Figure 5.4. Figure 5.5 shows a schematic of the sublimation configuration.

The sample plate was connected to a low noise current to voltage preamplifier and the signal was recorded using an analog to digital converter. One hundred data points were recorded per second. Data recording was initiated once the plate was placed inside the sample chamber and continued until the signal returned to baseline, which varied from 3 to 20 minutes. The gate valve was opened electrically with a switch only when the pressure on the sample chamber was $10^{-1}$ torr. The charge produced was obtained by integrating the signal from the time of application of high vacuum to the point at which the signal decayed to 5% of the maximum. Vacuum readings from both chambers were recorded along with the signal from the plate.

A group of compounds used in MAI was selected for measurement of sublimation electrification as shown in Table 5-1. Solutions were prepared by dissolving 10 mg of each compound in 100 μL of acetonitrile (ACN) except carbazole which was dissolved in acetone and 2,5-dihydroxyacetophenone (2,5-DHAP) which was only partially soluble in ACN. Saturated solution of 2,5-DHAP in ACN and ethanol was used. A 5 μL aliquot of the prepared solution was deposited on the brass plate and allowed to dry. The plate was then placed inside the sample
Figure 5.3. Photograph of sublimation chamber with A) sample chamber, B) high vacuum chamber and C) gate valve.

Figure 5.4. Photograph of sublimation plate

chamber. For studies that required heating the sublimation plate, a Kapton flexible heater connected to a thermostat was attached to the bottom of the plate for heating the plate. For acid studies, trifluoroacetic acid, formic acid, or hydrochloric acid were added to the solutions at different concentrations prior to the deposition. Particle capture for imaging studies was done using conductive double-sided adhesive carbon tabs and Formvar film-coated plates. Surfaces were imaged using a scanning electron microscope.
5.3 Results and discussion

A group of nine compounds that produce multiply charged ions with MAI was selected for the study of sublimation electrification. A solution of each of these compounds was deposited on sample plates, dried and placed in the vacuum chamber. The current produced from the sublimation of these compounds were measured along with the pressure at various temperatures, pH, voltages, and sample morphology.

Sublimation of 3-NBN produced negative current. Figure 5.6 shows current from the sublimation of 3-NBN and pressure of the sample chamber. The pressure of the chamber was around $10^{-1}$ torr with only rough vacuum and the current was approximately 4 pA. Once the gate valve was opened, the pressure decreased rapidly to $10^{-4}$ torr and remained constant. The current increased rapidly with decreasing pressure and reached a maximum of 180 pA within first 10 seconds and then started to decrease. The current returned to 5% of the baseline within two minutes.
Current generated from 3-NBN was integrated over time to obtain the net charge produced. Integration was done for two separate time intervals. To measure charge produced with low vacuum, the current was integrated from the time of application of rough vacuum to opening of the gate valve. To measure charge produced with high vacuum, the current was integrated from the time of opening of the gate valve to the time at which the signal returned to 5% of the baseline. Charge produced from the sublimation of 0.5 mg of 3-NBN was calculated to be -3.4 nC which corresponds to $10^{10}$ charges for $10^{18}$ molecules.

The current produced from the sublimation of 2-NBN was opposite in sign to the current produced from 3-NBN and the maximum current was three times larger. Figure 5.7 shows the current produced from 2-NBN. In contrast to 3-NBN, no current was detected for 2-NBN with the rough vacuum. However, upon opening the gate valve, the current increased rapidly. 2-NBN required around 20 seconds to reach maximum which was four times larger than 3-NBN. The current decreased to 5% of the baseline within two minutes, similar to 3-NBN.

The current produced from the sublimation of 2,5-DHAP, coumarin, 2-NPG, carbazole, 2-naphthol, anthracene, and 1,2-dicyanobenzene were also recorded. Figure 5.8 shows current generated from 1,2-dicyanobenzene, coumarin, and 2-NPG. The charge produced from the sublimation of 0.5 mg of 3-NBN, 2-NBN and 1,2-dicyanobenzene was -3.4 nC, 9.1 nC, and 16 nC, respectively. Coumarin and 2-NPG produced 1.4 nC and 0.45 nC, respectively, when 5 mg of each matrix was sublimed. The charge produced from 2,5-DHAP, carbazole, 2-naphthol, and anthracene was below the detection limit. Table 5-1 shows charge and current generated from the sublimation of each compound.
Figure 5.6. Current from sublimation of 3-NBN (solid line) and pressure inside the chamber (dotted line)

Figure 5.7. Current from sublimation of 2-NBN
The charge produced from 3-NBN was calculated for different spot sizes but the same quantity of matrix. The charge produced spots with diameter 0.5, 1 and 2 cm were obtained. The spot of diameter 2 cm produced the largest charge of around -11 nC (n=3) which was 2 and 3 times the charge produced from the spot of diameter 1 and 0.5 cm, respectively. 3-NBN crystals were deposited on the sublimation plate directly from the sample bottle and the charge produced was measured. The charge produced from dried droplet deposits were nearly twice as large as the charge produced from crystals directly deposited from the bottle. To investigate if the crystal size affects the charge, the matrix from the bottle as well as matrix recrystallized in lab was ground and the charge was measured. Measurement of charge from ground crystals were inconsistent and gave inconclusive results.

The sublimation plate was heated and the current produced was measured at various temperatures. For matrices 3-NBN and 2-NBN, the current got sharper with an increase in the temperature; however, the magnitude of charge was lower. The charge produced from 3-NBN decreased from -3.7 nC to -1.2 nC when the plate temperature was increased to 30 °C from the ambient condition. With further increase in temperature, no signal was detected. Circulating water bath heater and a homemade oven made with tungsten wire were also used for heating. Though the heating was rapid with the oven, temperature regulation was difficult. Higher temperatures were applied to matrices which did not previously yield any charge at ambient condition, but no current was detected. Current produced from 2-naphthol, anthracene, carbazole and 2,5-DHAP was still below detection limit of 1 pA.

Three different acids TFA, FA, or HCl were added to the matrix solution before deposition and the current produced was measured. Figure 5.9 shows the charge from 2-NBN and 3-NBN as a function of TFA mass percentage. 2-NBN produced a positive charge on the plate without any
Figure 5.8. Current from sublimation of a) 1,2-dicyanobenzene, b) coumarin and c) 2-NPG
Table 5.1. List of compounds with current and charge produced from sublimation with time required for each to reach maximum signal and to decay to 5% of the maximum

<table>
<thead>
<tr>
<th>Compound</th>
<th>Max. Current (pA)</th>
<th>Time (s)* Max. Current</th>
<th>Charge (nC) Low Vacuum</th>
<th>Charge (nC) High Vacuum</th>
<th>Decay Time (s)**</th>
</tr>
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<tr>
<td>3-NBN</td>
<td>-180</td>
<td>5</td>
<td>0.03</td>
<td>-3.4</td>
<td>110</td>
</tr>
<tr>
<td>2-NBN</td>
<td>620</td>
<td>20</td>
<td>&lt; 0.01</td>
<td>16</td>
<td>120</td>
</tr>
<tr>
<td>1,2-dicyanobenzene</td>
<td>160</td>
<td>20</td>
<td>&lt; 0.01</td>
<td>9.1</td>
<td>170</td>
</tr>
<tr>
<td>Coumarin</td>
<td>240</td>
<td>5</td>
<td>0.2</td>
<td>1.4</td>
<td>10</td>
</tr>
<tr>
<td>2-NPG</td>
<td>10</td>
<td>5</td>
<td>&lt; 0.01</td>
<td>0.45</td>
<td>180</td>
</tr>
<tr>
<td>2-Naphthol</td>
<td>&lt; 1</td>
<td>NA</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>NA</td>
</tr>
<tr>
<td>Carbazole</td>
<td>&lt; 1</td>
<td>NA</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>NA</td>
</tr>
<tr>
<td>Anthracene</td>
<td>&lt; 1</td>
<td>NA</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>NA</td>
</tr>
<tr>
<td>2,5-DHAP</td>
<td>&lt; 1</td>
<td>NA</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>NA</td>
</tr>
</tbody>
</table>

* time taken for current to reach maximum after opening of the gate valve
** time taken to decay to 5% of the maximum

acid, implying negative charge was leaving the plate. The charge decreased with the addition of TFA and was below 0.01 nC when the mass percentage of acid was greater than 1%. FA and HCl showed similar trend as obtained with TFA. However, for 3-NBN which had positive charge, sublimation charge increased with the addition of TFA. The maximum charge was observed when the mass percentage of TFA was around 0.5% and was four times higher than without any acid. Mass percentage of TFA above 0.5% resulted in a decrease of charge. Similar to TFA, maximum charge with HCl addition was observed at 0.5%; however, for formic acid the maximum charge was observed when mass percentage of acid was 5%.

Charge from the sublimation of 2-NBN and 3-NBN matrices was measured at different electric potentials. A second plate identical to sublimation plate was connected to a power supply and placed 3 mm above the sample plate as shown in Figure 5.10. The charge produced for 2-NBN and 3-NBN at five different potentials; 0, ±50, and ±100 volts were measured and are shown in Figure 5.11. For 2-NBN, which produces negative charge, sublimation charge should increase with the application of negative voltage and decrease with positive voltage. Similarly, for 3-NBN
which produces positive charge, sublimation charge should increase with application of negative voltage and decrease with application of positive voltage. As estimated, charge for 2-NBN was larger with application of positive voltage and charge for 3-NBN was larger with application of negative voltage. However, when negative 50 volt was applied, sign of charge changed for 2-NBN. Similarly, when positive 50 volt was applied sign of charge changed for 3-NBN. The detection of both positive and negative charge for a matrix at different voltages implies materials carrying both positive and negative charge are being ejected off the target and the charge measured is the net charge.

Charge produced from materials leaving the plate was measured using a second plate connected to an amplifier as shown in Figure 5.12. The charge measured off the ejected materials
Figure 5.10. Schematic with second plate for application of electric field

were approximately half of the charge measured from the plate. In addition to measuring the charge, an attempt was made to capture and image the materials. Conductive double-sided tape and Formvar surfaces attached to the surface of the second plate were used for capturing the particles. Though the charge could be measured from the plates, no particles were observed with SEM. From the absence of particles on the plate, two deductions can be made. First, the charge carriers are ions and not particles, because of which particles are not detected with SEM. Second, the particles ejected quickly sublime from the capturing plate. A cryogenic capture system that prevents sublimation of ejected particles can help determine the charge carriers.

In this chapter, it has been shown that the charge produced from the sublimation of organic compounds can be both positive or negative based on compounds sublimed, and the sign of the net charge measured depends on the electric field. The dependence of the charge polarity on the electric field suggests that both positive and negative charge is leaving the plate and the detected
charge is the net charge. The idea of net charge was suggested by Baytekin et al. who showed both positively and negatively charged regions on surfaces of electrified materials.\textsuperscript{30}

![Charge from sublimation of 2-NBN and 3-NBN at various electric field potentials](image)

Figure 5.11. Charge from sublimation of 2-NBN and 3-NBN at various electric field potentials

Charge separation could occur due to electron, ion or material transfer.\textsuperscript{168} In this chapter, it is shown that the addition of acid affected the charge produced. Charge was larger for 3-NBN while was smaller for 2-NBN with the addition of acid. This suggests that the probable charge carriers are protons and hydroxide ions. Partition of hydroxide ions resulting from adsorbed water has been proposed as charge carriers previously for contact electrification\textsuperscript{31, 168}. However, in addition to ion transfer, charge separation could also occur from the transfer of charged materials\textsuperscript{30, 31}. Charged particles expelled from the surface have been proposed as precursors to bare ions previously\textsuperscript{177}. However, the inability to directly image these particles limits the ability to elucidate the role of particles in charge transfer.
Figure 5.12. Schematic for measurement of charge from ejected particles

5.4 Summary

A method to measure charge produced from the sublimation of organic compounds was developed. Charge of both polarities was observed from the compounds sublimed. The polarity of the charge measured depended on the applied electric field suggesting that charges of both polarities are leaving the surface and the charge detected for each compound was an overall net charge. The charge measured was also affected by the spot size of the deposit, sample morphology, and temperature of the plate. The addition of acid increased positive charge leaving the surface while decreased negative charge coming from the surface. The charge separation is postulated to be the result of ion transfer and bulk material transfer and the probable charge carriers are H\(^+\) and OH\(^-\) ions.
CHAPTER 6. CONCLUSIONS AND FUTURE DIRECTIONS

In this dissertation, a technique is developed for ionization of large molecules from surfaces using contact electrification. The provides controlled removal of the sample from surfaces. Highly charged ions of analytes are obtained with a spatial resolution of 1 mm and a temporal resolution of around 5 seconds. A deeper understanding of the production of highly charged ions was obtained through the measurement of charge produced from the sublimation of the matrix compounds. The significance of this work lies in the ability to introduce samples for MAI with better temporal and spatial resolution and in determining the charge separation process occurring on the surfaces for the production of highly charged ions.

In the work described in Chapter 2, a two-component matrix of 2-NPG and silica nanoparticles was used for MALDI mass spectrometry imaging of high-charge-state biomolecules in tissue. MAI produces highly charged ions under atmospheric and intermediate vacuum conditions. However, only one of the matrices, 2-NPG generated ions under a high vacuum conditions. A mixture of 2-NPG matrix and silica nanoparticles is applied to mouse brain tissue and MALDI images are obtained under a high vacuum in a commercial TOF mass spectrometer. The mechanism for the production of highly charged ions from tissue seems to rely on the high surface area of the particles which dried the tissue, and their ability to bind analyte molecules in crystal formation and production of multiply charged ions on laser irradiation.

In Chapter 3, a new sample removal technique was developed for matrix-assisted ionization with high temporal resolution using an electrically actuated pulsed valve. The high-pressure electric solenoid pulsed valve was used to ionize pure analytes deposited on thin aluminum foils. Matrix and analyte samples were deposited on a thin metal foil and placed at the inlet of a mass spectrometer. When the pulsed valve is actuated, a short puff of high-pressure gas strikes the foil
and ejects particulates from the sample deposited on the opposite side. Highly charged ions of proteins were produced within a 4-second time window. The pulsed valve was temporally precise as it could be operated electrically, and sample removal was more controlled compared to tapping.

Chapter 4 describes the use of piezoelectric actuators for sample ionization. The new actuation method using a piezoelectric cantilever provides good spatial resolution along with good temporal resolution. Similar to the pulsed valve, the strike from the piezoelectric cantilever on a thin metal foil removes material deposited on the opposite side that faces the mass spectrometer inlet. Highly charged ions of peptides and proteins were generated from dried droplet deposits and samples into the mass spectrometer inlet. A lateral resolution of around 1 mm was obtained with the piezoelectric sampling configuration. Singly charged lipids and gangliosides were detected from tissue using silica nanoparticle co-matrix.

In Chapter 5, organic compounds that generate highly charged ions were sublimed and the mechanism for charge separation is studied. The charge generated from the sublimation of these compounds was measured using a metal plate and a current amplifier. The current produced from sublimation was measured to calculate the charge for each compound. Charge of both polarities was observed depending on the compound sublimed. The polarity of the detected depended on the applied electric field suggesting both positive and negative charge are produced. The intensity of charge depended on the acidity of the sample. The addition of acid increased positive charge and decreased negative charge. The charge detected is postulated to be an overall net charge and the charge separation occurs through a combined effect of ion and material transfer. The probable charge carriers are H⁺ and OH⁻ ions.

The nature of charge carriers for matrix-assisted ionization was determined and it was also shown that MAI matrices could generate highly charged ions from surfaces at different
atmospheric conditions with the correct sample preparation method. MAI has potential advantages for mass spectrometry imaging due to its simplicity, low fragmentation, and tandem mass spectrometry facilitated by highly charged ion production. However, for efficient utilization of this method deeper understanding of the ionization mechanism is required.

One of the future directions is to further improve the spatial resolution of the ionization technique. The piezoelectric striker used in this dissertation was relatively large with low-resonant frequency. Smaller piezo actuators with higher precision and higher frequency could potentially improve spatial precision and speed in data acquisition. Along with that, a device that can measure small changes in charge produced and mass loss from a single crystal will help to gain more insight into the charge separation mechanism.
APPENDIX A. CHEMICALS AND MATERIALS

The information on manufacturers/suppliers of the instruments and chemicals used in this dissertation are provided below.

Sinapic acid, HPLC grade water, HPLC grade acetonitrile, 2,5-dihydroxyacetophenone, 2-nitrophloroglucinol, 3-nitrobenzonitrile, 2-nitrobenzonitrile, coumarin, 2-naphthol, carbazole, anthracene, 1,2-dicyanobenzene, formic acid, cytochrome C, bovine erythrocytes ubiquitin, myoglobin, and bovine insulin were purchased from Sigma Aldrich (St Louis, MO, USA). HPLC grade methanol, trifluoroacetic acid and double-sided carbon adhesive tapes were purchased from Thermo Fischer Scientific (Waltham, MD, USA). Silica nanoparticles were purchased from US Research Nanomaterials (Houston, TX, USA). Aluminum foil was purchased from Reynolds Wrap (Pittsburgh, PA, USA). Tungsten and titanium foil were purchased from Alfa Aesar (Ward Hill, MA, USA). Stainless steel plates used for sandwiching the foils were purchased from Kimball Physics (Wilton, NH, USA). Kapton flexible heaters were purchased from Omega (Stamford, CT, USA). Brass plate used for sublimation electrification was purchased from Online Metals (Seattle, WA, USA). Formvar resin was purchased from SPI Supplies (West Chester, PA, USA).

Mass spectrometers used in this dissertation were UltrafleXtreme and Amazon Speed ETD manufactured by Bruker (Bremen, Germany). Tissue sections were prepared using Leica CM1850 cryostat (Leica Microsystems, Wetzlar, Germany). Indium tin oxide (ITO) coated microscope slides were purchased from Bruker. Mass spectrum were analyzed using Flex Analysis 3. And Compass Data Analysis 4.1 which were provided with Bruker mass spectrometers. Pulsed valve was purchased from Parker (Cleveland, OH, USA) and high voltage was supplied using MODEL GRX-R high voltage switch (Directed Energy, Fort Collins, CO, USA) and RR3-15R high voltage power supply (Gamma, Ormond Beach, FL, USA) driven by DG 535 pulse and delay generator.
(Stanford Research Systems, Sunnyvale, CA). Piezoelectric cantilever (PB4NB2S) and piezo controller were purchased from Thorlabs (Newton, NJ, USA). The low noise current to voltage preamplifier used was Oriel 70710 (Newport, Irvine, CA, USA) and the analog to digital converter used was USB-6001 (National Instruments, Austin, TX, USA). Scanning electron microscope used was FEI Quanta 3D (Thermo Fisher Scientific).
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VITA

Bijay Banstola was born in Pokhara, Nepal. After finishing high school in Nepal, Bijay moved to the USA in pursuit of higher education. He obtained his bachelor’s degree in chemistry from Northwestern State University in Natchitoches, with minor in biology in 2011. Following his graduation, he joined Airgas as lab analyst in 2012. His primary responsibility in Airgas was the analysis of specialty gases. During his stay in Airgas, Bijay gained skills to run and maintain instruments such as gas chromatography, mass spectrometry, and FTIR and was promoted to lab leader within a year. In the fall of 2014, Bijay enrolled to pursue a Ph.D. in the department of chemistry at Louisiana State University. Driven by an interest in analytical chemistry and mass spectrometry he joined Dr. Kermit K. Murray’s group. During his Ph.D. program, he has published four first-author papers and one second-author paper. He has one first-author manuscript in preparation for publishing. He also attended various conferences where he gave oral and poster presentation on his research. He has also received Coates travel award and teaching award at LSU. Bijay is currently a candidate for Ph.D. in chemistry, which is planned to be awarded at fall 2019 commencement.