Enhanced Electrospray Ionization for Mass Spectrometry and Ion Mobility Spectrometry

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ENHANCED ELECTROSPRAY IONIZATION FOR MASS SPECTROMETRY AND
ION MOBILITY SPECTROMETRY

by

Li Zhou

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry and Biochemistry
Brigham Young University
August 2006
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GRADUATE COMMITTEE APPROVAL

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This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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As chair of the candidate’s graduate committee, I have read the dissertation of Li Zhou in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrated materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

ENHANCED ELECTROSPRAY IONIZATION FOR MASS SPECTROMETRY AND ION MOBILITY SPECTROMETRY

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Doctor of Philosophy

Electrospray ionization (ESI) has become one of the most commonly used ionization techniques for mass spectrometry (MS) and ion mobility spectrometry (IMS), and efforts continue to improve its performance. ESI-MS is most recognized for its wide application to biomacromolecules where high sensitivity is of paramount importance. However, the major limitation in sensitivity with ESI-MS is due to its low ion transmission efficiency from the ESI source into the sampling orifice and through any stages utilized for transfer of ions from atmosphere to vacuum in the MS.

A series of atmospheric pressure ion focusing interfaces were designed and implemented to enhance the performance of ESI-MS. The technical objective of this work was to improve sensitivity and detection limits of ESI-MS using a combination of concentric high velocity converging gas flow (aerodynamic focusing) and regulated
external electric field (electrostatic focusing) to assist in focusing and transporting ions from the ESI sprayer tip into the sampling nozzle of the MS.

The separation time in IMS, based on differing gas phase ion mobilities, ranges from several hundred microseconds to milliseconds. This allows faster analysis than most other conventional separation techniques, such as gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE). However, the major limitation in ESI-IMS is its low resolution. It is believed that one of the most important contributions to low resolution in ESI-IMS is unwanted ion penetration through the ion gate.

In order to solve this ion penetration problem, two mechanical ion gates were designed and optimized to assist in gating sprayed ions from the ESI source into the drift region of the IMS with improved sensitivity and resolution at atmospheric pressure. Applying a voltage to the ion gate and using a high flow drift gas helped to further improve the performance of ESI-IMS.

Reduced pressure IMS should help to eliminate clustering and multiple peaks and, hence, improve experimental resolution when using ESI. Therefore, I report the design, construction and evaluation of new IMS systems for reduced pressures. However, the performance of the reduced pressure IMS was not as good as when using atmospheric pressure IMS.
I would like to acknowledge my advisor, Dr. Milton L. Lee, for his instruction, support, trust, kindness, encouragement and friendship through the years. I am very grateful for the opportunity to work on the projects he made possible, the opportunity to study under his supervision, and the opportunity to learn from a great tutor and scientist. I am very confident that I will be successful in my future professional career as well as my personal life.

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LIST OF SYMBOLS, ACRONYMS AND ABBREVIATIONS

Symbols

atm, atmosphere

$B_0$, magnetic strength

c, concentration

$D$, diffusion coefficient

$Da$, Dalton

d, distance

$E_c$, electric field at an ESI sprayer tip

$E_{on}$, onset ESI electric field at an ESI sprayer tip

$K$, conductivity

$k$, retention factor

$L$, length of the drift region

$I$, ESI charged droplet ion current

$I_{ms}$, mass spectrometrically observed total ion current

$m$, mass

$m/z$, mass to charge ratio

$P$, pressure

psi, pound per square inch
q, ion charge

$q_{Ry}$, charge of droplets at Rayleigh limit

$R$, average radius of ESI produced droplets

$R$, resolving power

$R_e$, experimental resolving power

$R_t$, theoretical resolving power

$R_{\text{FWHM}}$, theoretical mass resolving power in FTICRMS at the zero-pressure limit

$R_{pp}$, Peak-to-peak resolution

$r_e$, radius of the emission region for produced droplets at the tip of the “Taylor cone”

$r_c$, outside diameter of ESI capillary

$T$, transient length

$T$, temperature

$t_0$, dead drift time

$t_d$, drift time

$t_g$, FWHH of the initial Gaussian shape pulse gated into the drift region

$t_{\text{diff}}$, FWHH of the Gaussian peak produced by diffusional broadening of an infinitely narrow initial pulse

$U$, electrical potential

$V_c$, voltage applied to the ESI source

$V_f$, volume flow rate

$V_i$, infusion flow rate

$V_{\text{on}}$, onset ESI voltage
\( v_0 \), initial velocity of the ion
\( v_d \), drift velocity
\( z \), number of charges
\( \alpha \), separation factor
\( \lambda_{0,m} \), molar conductivity
\( \theta \), half angle of Taylor cone
\( \gamma \), surface tension
\( \sigma_{\text{space}} \), spatial spreading
\( \sigma_{\text{time}} \), time spreading
\( \varepsilon_0 \), permittivity of vacuum
\( \varepsilon \), relative permittivity
\( \eta \), Townsend energy factor
\( \omega_b \), full peak width at baseline
\( \omega_h \), full width at half height
Acronyms and Abbreviations

CE, capillary electrophoresis
CRM, charged residue method
ESI, electrospray ionization
FTICRMS, fourier transform ion cyclotron resonance mass spectrometry/ fourier transform ion cyclotron resonance mass spectrometer
GC, gas chromatography
i.d, inside diameter
IEM, ion evaporation model
ITMS, ion trap mass spectrometry/ ion trap mass spectrometer
IMS, ion mobility spectrometry/ion mobility spectrometer
LC, liquid chromatography
MS, mass spectrometry/mass spectrometer
nESI, nano electrospray ionization/nanospray ionization
o.d., outside diameter
PCR, polymeric chain reaction
RSD, relative standard deviation
TOFMS, time-of-flight mass spectrometry/time-of-flight mass spectrometer
v/v, volume ratio
µESI, micro electrospray ionization/microspray ionization
INTRODUCTION

1.1 Electrospray Ionization

1.1.1 Background

The phenomenon of electrospray has been known for approximately a hundred years.\(^1\) However, it was not until the late 1930’s that Chapman and co-workers carried out the first experiments with electrospray ionization (ESI). Using an Erikson mobility tube, a series of inorganic salt solutions were electrosprayed, and their mobility spectra were collected and recorded using a home-made electrometer.\(^2\) Other observations were demonstrated by Vonnegut,\(^3,4\) Drozin,\(^5\) and Kelly.\(^6\)

In the late 1960’s, Dole and coworkers were the first investigators to use electrospray ionization-mass spectrometry (ESI-MS) to analyze several polymeric macromolecules (e.g., polystyrenes). Although not completely successful, their pioneering work drove ESI to a promising practical method of sample ionization for modern mass spectrometry.\(^7,8\)

A further two decades elapsed until Fenn and Yamashita demonstrated for the first time the successful application of ESI for “soft” ionization of nonvolatile and thermally labile biomolecules, e.g., polypeptides and proteins, and subsequent analysis using mass spectrometry.\(^9-11\) It was called “soft” because the molecules being ionized had less chance to fall apart or break up during the process. Another similar, independent work was reported by Aleksandrov and coworkers almost at the same time.\(^12\) The significance of
electrospray ionization for mass spectrometry has been emphasized by the awarding of the Noble Prize to Fenn and Yamashita for their original work in this area - the 4th time a Nobel prize was awarded to mass spectrometrists.\textsuperscript{13}

Today, electrospray ionization has grown to be one of the most commonly used ionization techniques for mass spectrometry. The development of ESI has had a major impact on the mass spectrometric analyses of a broad range of compounds, in particular for biomacromolecules (e.g., polypeptides and proteins). ESI-MS has become a basic tool for biochemistry laboratories around the world.\textsuperscript{14,15}

1.1.2 Mechanism of Electrospray Ionization

Electrospray ionization sources have undergone continuous development since the earliest designs; however, the general arrangements remain basically the same. An analyte solution ($C > 1 \, \mu M$) is introduced into an ESI source either from a syringe/syringe pump or as an eluent from liquid chromatography (LC) or capillary electrophoresis (CE). An electrical potential ($U$) is applied to the ESI source by (1) connecting the ESI source to a high voltage power supply and the interface plate of the MS to ground, (2) connecting the interface plate of the MS to a high voltage power supply and the ESI source to ground, or (3) connecting both the ESI source and the interface plate of the MS to separate power supplies set to different voltages.\textsuperscript{16} Selection of these options depends on ion transmission with different ESI sources. The electrical potential is applied to the sprayer through the following methods: (a) using two or three sections of a capillary (ESI voltage on one section) whereby the separation column is connected to the sprayer tip via a non-conductive sleeve (e.g., plastic, Teflon);\textsuperscript{16-20} (b) splitting the effluent near the sprayer tip to fill the gap between the sprayer capillary and
an outer metallic sleeve;\textsuperscript{21-23} (c) inserting a conductive wire into the sprayer tip\textsuperscript{24} or through a pinhole in the wall of the sprayer capillary;\textsuperscript{25,26} (d) adjusting the sprayer tip position so that the electrical contact is formed from the ESI source through air to the grounded interface plate of the MS;\textsuperscript{27} and (e) coating the sprayer tip with a conductive material.\textsuperscript{28-36}

For simplicity, only positive ion mode ESI is considered in the following discussion. The application of the electrical potential to the ESI source forms a dipolar layer at the liquid meniscus attached to the capillary tip. This double layer is produced by the spatial separation of the ions in the capillary effluent. When a high positive voltage (e.g., $V_c > +2.5$ kV) relative to the counter-electrode is applied to the solution, the positive ions from the double layer are dragged toward the liquid meniscus surface which destabilizes it. A cone then forms due to the opposing effects of the electrostatic force and the surface tension at the surface. Taylor found that these two factors balanced when the meniscus had straight sides with a half angle $\theta \approx 49.3^\circ$ at the apex of the cone for all electrospray-able solutions, which is the so called “Taylor cone”.\textsuperscript{37} The reason that this special angle is suitable for all electrospray-able solutions is still unknown. Immediately after the “Taylor cone”, a liquid jet emerges from the cone tip and emits positively charged fine droplets. These fine droplets are charged due to an excess of positive ions. The excess of unipolar ions is provided by an electrolysis process at the capillary that either adds positive ions to the solution or removes negative ions from the solution. For a concentration of the solution ($C > 10$ $\mu$M), the number of ions added or removed by electrolysis is negligible compared to the number of original ions present in the solution.\textsuperscript{38-40} This is the so-called cone-jet mode for ESI-MS.\textsuperscript{14,41-44,139}
If the electrical potential applied to the ESI source is not high enough, a stable Taylor cone cannot be obtained. However, if the applied voltage is too high, the Taylor cone switches to another unstable state, i.e., a multi-jet mode.\textsuperscript{41} For both situations, the sensitivity is not satisfactory.

Ideally, the electric field at the capillary tip, $E_c$, when the counter-electrode is planar, uniform, and large compared to the cross-section of the capillary tip, can be described as\textsuperscript{45}

\[
E_c = \frac{2V_c}{r_c \ln \left( \frac{4d}{r_c} \right)} \quad (1.1)
\]

where $V_c$ is the electrical potential applied to the ESI source, $r_c$ is the outside diameter of the capillary, and $d$ is the distance between the capillary tip and the counter-electrode.

The onset electric field strength for ESI at the capillary tip, $E_{on}$, which leads to instability of the cone-jet, can be defined as

\[
E_{on} = \left( \frac{2\gamma \cos \theta}{\varepsilon_0 r_c} \right)^{\frac{1}{2}} \quad (1.2)
\]

where $\gamma$ is the surface tension of the solution, $\varepsilon_0$ is the permittivity of vacuum, and $\theta$ is the half angle at the apex of the Taylor cone. Equation (1.2), when combined with equation (1.1), leads to equation (1.3) for the onset electrical potential for ESI ($V_{on}$)

\[
V_{on} = \left( \frac{r_c \gamma \cos \theta}{2\varepsilon_0} \right)^{\frac{1}{2}} \ln \left( \frac{4d}{r_c} \right) \quad (1.3)
\]

Substituting $\varepsilon_0 = 8.8 \times 10^{-12} \text{ J}^{-1} \text{ C}^2 \text{ m}^{-1}$ and $\theta = 49.3^\circ$, we have

\[
V_{on} = 2 \times 10^5 \left( \gamma r_c \right)^{\frac{1}{2}} \ln \left( \frac{4d}{r_c} \right) \quad (1.4)
\]
where \( \gamma \) must be substituted in newtons per meter (N/m) and \( r_c \) in meters to acquire \( V_{on} \) in volts (V).

According to equation (1.2), the onset electric field strength for ESI increases with the surface tension of the solution. A solution with high surface tension requires a high onset electric field. However, the high field can lead to electrical discharge (i.e., the sprayed droplets drifting from the ESI source to the grounded interface plate produce an electrical current \( I > 1 \mu\text{A} \)) that partially suppresses the ESI process. The appearance of electrical discharge on this occasion also depends on the ambient pressure and the nature of the ambient gas.\(^{14,46,47}\)

The electrical current \( I \) due to the sprayed droplet stream can be described as\(^{44,45,48}\)

\[
I = \left( \frac{4\pi}{\varepsilon} \right)^3 \left( 9\gamma \right)^2 \varepsilon_0^3 \left( K E \right)^{\frac{1}{7}} \left( V_f \right)^{\frac{4}{7}} \tag{1.5}
\]

\[
R = \left( \frac{3\varepsilon \gamma^2 V_f}{4\pi \varepsilon_0^2 KE} \right)^{\frac{2}{7}} \tag{1.6}
\]

\[
q = 0.5 \left( 8 \varepsilon_0 \gamma R^3 \right)^{\frac{1}{2}} \tag{1.7}
\]

or\(^{49,50}\)

\[
I = f \left( \frac{\varepsilon}{\varepsilon_0} \right) \left( \gamma K V_f \frac{\varepsilon}{\varepsilon_0} \right)^{\frac{1}{2}} \tag{1.8}
\]

\[
R \approx \left( \frac{V_f \varepsilon}{K} \right)^{\frac{1}{7}} \tag{1.9}
\]

\[
q = 0.7 \left( 8\pi (\varepsilon_0 \gamma R^3)^{\frac{1}{2}} \right) \tag{1.10}
\]
where \( \varepsilon \) is the permittivity of the solution, \( K \) is the conductivity of the solution, \( R \) is the average radius of the electrosprayed droplets, \( q \) is the charge of the droplets, \( V_f \) is the volume flow rate, and \( f(\varepsilon/\varepsilon_0) \) is a semi-empirical numerical function. The electrical current, \( I \), increases with the conductivity, \( K \), of the solution and the volume flow rate, \( V_f \).

The conductivity, \( K \), is proportional to the concentration \((C < 0.1 \text{ M})\) of the strong electrolyte analyte in the solution\(^{14}\)

\[
K = \lambda_{0,m} C \tag{1.11}
\]

where \( \lambda_{0,m} \) is the molar conductivity of the solution. Combining equation (1.11) with equations (1.5) and (1.8), one obtains

\[
I \propto K^n = \left( \lambda_{0,m} C \right)^n \tag{1.12}
\]

where \( n \) is a semi-empirical constant between 0 and 1. This model is consistent with experimental results. In addition, the mass spectrometrically observed total gas phase ion current, \( I_{ms} \), is not closely coupled to the ion current due to the charged fine droplet stream \( I \). Although there is no simple equation for description, \( I_{ms} \) experimentally depends on the infusion flow rate \( V_i \) and average droplet radius \( R \). Therefore, smaller droplets obtained at lower flow rates can lead to higher \( I_{ms} \).\(^{49,50}\)

Solvent evaporation from the charged droplets induces droplet shrinkage, increases coulombic repulsion, and releases coulombic strain by droplet fission when the charge of the droplets over-reaches the Rayleigh stability limit \( q_{Ry} \)

\[
q_{Ry} = 8\pi \left( \varepsilon_0 \gamma R^3 \right)^{1/2} \tag{1.13}
\]

This equation offers the conditions under which the coulombic repulsion becomes equal to the surface tension force. The charged droplets become unstable when their radius, \( R \),
and charge, \( q \), satisfies equation (1.13). Gomez and Tang reported that each parent droplet emits approximately 20 offspring droplets with an average radius of one-tenth of the parent droplet. However, the offspring droplets carry off only 2% of the parent droplet’s mass, and 15% of its charge. Continuous solvent evaporation and droplet fission generates very small droplets (e.g., down to 10 nm). The duration of this process ranges from 100 to 500 \( \mu \text{s} \), which is close to the residence time for the droplets drifting from the ESI source into the sampling orifice of the MS. This is the so-called droplet-jet mode for ESI-MS.\(^{14,51}\)

Since the earliest utilization of ESI-MS, two working mechanisms have been most widely accepted to explain the generation of gas phase ions from highly charged droplets after the droplet-jet. Dole\(^7\) and Röllgen\(^{52}\) assumed that coulomb fission occurs continuously and consecutively until droplets containing only one “excess” ion are finally formed. This model is called the “charged residue model” (CRM). Another theory derived by Iribarne and Thomson,\(^{53,54}\) the “ion evaporation model” (IEM), provided detailed predictions that gas phase ions are directly emitted from very small droplets (\( R < 10 \text{ nm} \)). Several experiments revealed that many of the ESI-MS spectra could be qualitatively, even sometimes quantitatively, explained using either model. The distinction between the two theories only exists in the range 1 nm < \( R < 10 \text{ nm} \). For \( R < 1 \text{ nm} \), CRM and IEM are essentially indistinguishable.\(^{11,14,55-58}\)

1.1.3 Electrospray Ionization Sources for Mass Spectrometry

Electrospray ionization sources are currently most popular for liquid introduction into a mass spectrometer. ESI is a powerful technique that effectively forms intact multi-charged gas-phase ions of involatile and thermally labile compounds, especially
biomacromolecules (e.g., polypeptides and proteins). Other advantages of ESI-MS include high sensitivity, reliability and robustness, ease of operation and maintenance, and compatibility with high resolution separation techniques, such as liquid chromatography (LC) and capillary electrophoresis (CE).

Since the first experiments using ESI to produce gas phase ions of polymers using a “Faraday cage” mass spectrometer, ESI-MS has been widely applied in the analysis of various biomacromolecules with different types of mass analyzers, such as magnetic sectors, quadrupoles, time-of-flight instruments (TOFMS), and trapping devices–ion traps (ITMS) and fourier transform ion cyclotron resonance instruments (FTICRMS).

1.1.4 Electrospray Ionization Sources for Ion Mobility Spectrometry

Ion mobility spectrometry (IMS) appeared as an analytical technique in the early 1970’s (Figure 1.1). In IMS, a sample can be ionized and separated based on differences in ion mobility under the influence of an electric field in the drift region. The equation of ion mobility can be written as

$$v_d = KE$$

where $v_d$ is the drift velocity of the ion (cm s$^{-1}$), $K$ is the ion mobility (cm$^2$V$^{-1}$s$^{-1}$), and $E$ is the electric field strength in the drift region (V cm$^{-1}$). The ion mobility is determined by temperature, pressure, gas properties of the support atmosphere, and most importantly, size and shape (i.e., collisional cross-section) of the analyte ions. The advantages of IMS include high sensitivity (i.e., sub ppb or pg), real-time monitoring, minimal calibration and maintenance, low cost, etc.

However, using prime ionization sources, this technique requires that analytes,
Figure 1.1 Schematic diagram of an ion mobility spectrometer.
especially in solid or liquid samples, be volatilized before introduction into the spectrometer.

The first successful marriage of IMS with ESI was reported in the late 1970’s by Dole and coworkers.\textsuperscript{117,118} Using this technique, a liquid sample was directly introduced into the IMS, separated, and detected, allowing high-mass-molecule determination.\textsuperscript{119} More recently, Dion et al.\textsuperscript{120} and Dwivedi et al.\textsuperscript{121} reported ESI-IMS detection of aqueous nitrates and nitrites of importance to human health and disease. A number of groups\textsuperscript{122-128} have also applied ESI-IMS to determine polar non-volatile organic compounds, such as amino acids, drugs, explosives and chemical warfare agents (CWAs). Additionally, several groups have demonstrated that polypeptides and proteins can be electrosprayed and their multiply charged states separated and detected by IMS.\textsuperscript{129-131} However, the major limitation in ESI-IMS is its low resolution. Details of this problem will be discussed in Chapter 3.

1.1.5 Limitations in Electrospray Ionization

The major limitation in sensitivity in µESI-MS is due to low ion transmission efficiency from the ESI source into the MS (Figure 1.2).\textsuperscript{51,132-136} Ion transmission efficiency is the percentage of total gas phase ions produced by the ESI source that reaches the mass analyzer, and is proportional to the ion current density (\( J \)) and gas flow rate (\( v \)) at the sampling orifice.\textsuperscript{14,137} In µESI, a low infusion flow rate leads to small droplet size and high excess charge density, thus, providing high ionization efficiency approximating 100\%.\textsuperscript{138-141} However, the typical ion transmission efficiency from the ESI source to the vacuum region of the MS is only 0.01-0.1\%.\textsuperscript{23,142-145} Most sprayed ions are lost between the ESI source and the sampling orifice of the MS. This is due to the fact
Figure 1.2 Schematic diagram of an example of ion loss in electrospray ionization–mass spectrometry.
that gas-phase collisions and coulombic repulsion cause expansion of the ESI plume, hence, directing ions away from the extraction region of the MS.\textsuperscript{51,59,142,146}

### 1.2 Objectives

The main objective of this research was to develop high performance ESI sources for MS and IMS. This dissertation reports on the design, construction and evaluation of a series of atmospheric pressure ion focusing interfaces to enhance sensitivity in ESI-MS. It also reports on the design, construction and evaluation of a novel ESI source with mechanical ion gates to improve sensitivity and resolution in ESI-IMS.

### 1.3 References

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5449.


CHAPTER 2

ELECTROSPRAY IONIZATION INTERFACING FOR MASS SPECTROMETRY

2.1 Introduction

Electrospray ionization (ESI) has grown to be one of the most commonly used ionization techniques for mass spectrometry. Approximately ten years ago, Wilm and Mann experimentally verified that the radius of the emission region for produced droplets \( r_e \) at the tip of the so-called “Taylor cone”\(^1\) was proportional to two-thirds power of the sample infusion flow rate \( v^{2/3} \) in an ESI source. Therefore, small droplets with high surface-to-volume ratios, important for desolvation, could be obtained using a low infusion flow rate. As a result, “micro electrospray ionization” or “microspray ionization” (µESI) with 1-50 µm i.d. sprayer tip and 0.1-20 µL min\(^{-1}\) infusion flow rate was introduced.\(^2,3\) µESI is typically used for very small sample volumes or for interfacing to microseparation systems, and it produces excellent sensitivity compared to conventional ESI (50-200 µm i.d. sprayer tip and 10-1000 µL min\(^{-1}\) infusion flow rate).\(^4-10\)

The major limitation in sensitivity with ESI-MS is due to low ion transmission from the ESI source to the sampling orifice and through any stages utilized for transfer of ions from atmosphere to vacuum in the MS.\(^11-15\) Although the ionization efficiency approaches 100\%,\(^2,16-20\) the typical ion transmission efficiency from the ESI source to the extraction region of the MS is only 0.01-0.1%.\(^3,21-24\) During the process of electrospray ionization,
analyte ions are generated at atmospheric pressure and transferred into the low-pressure extraction region of the MS via a conductance-limiting aperture located in the high pressure region. Gas phase collisions and coulombic repulsion that are inevitably involved result in expansion of the ion cloud, directing ions away from the extraction region of the MS, thus, decreasing the sensitivity. Although conventional ion optic devices based on coulombic effects can effectively focus ions in a vacuum, they are largely ineffective in avoiding or reversing ion-cloud expansion generated by gas-phase collisions and coulombic repulsion at high pressures.\textsuperscript{11,21,25,26}

One approach to improve ion transmission from the ESI source into the MS is to increase the diameter of the sampling orifice and use a high speed pump to maintain low pressure in the spectrometer. The elevated gas flow transports more ions into the MS. However, this requires a substantial vacuum system.\textsuperscript{16,27,28} Other methods include positioning one or a series of ion lenses between the sprayer and sampling orifice.\textsuperscript{29-33} The authors of these papers reported that ion lenses were helpful for ion focusing, but none of them quantified improvements in ion intensities. Schneider et al. mentioned an oblong ion lens placed close to the sprayer which provided a 2-fold increase in ion intensities with a 2-fold reduction in ion signal relative standard deviations (RSD).\textsuperscript{34-36} Jorgenson and coworkers selected a hemispherical lens and profiled the 3-D current density of an ESI plume. They reported a 3 times improvement in the average current density with the ion lens.\textsuperscript{16} Smith and coworkers developed a so called “ion funnel” in the first stage of the mass spectrometer between the sampling orifice and nozzle of the mass spectrometer. With the combination of multi-capillary inlet and ion funnel, they observed an increase in ion transmission efficiency over one order of magnitude.\textsuperscript{23,28,37-43} However,
this device was only functional under reduced pressure.

In this chapter, I report the design and implementation of a series of atmospheric pressure ion focusing interfaces to enhance the performance of electrospray ionization-mass spectrometry (ESI-MS). The technical objective of this work was to improve sensitivity and detection limits of electrospray ionization-mass spectrometry using a combination of concentric high velocity converging gas flow (aerodynamic focusing) and regulated external electrostatic field (electrostatic focusing) to assist in focusing and transporting ions from the electrospray ionization sprayer tip to the sampling nozzle of the mass spectrometer.

2.2 Experimental Section

2.2.1 Safety Considerations

In order to test the new atmospheric pressure ion focusing interfaces, high voltages were applied to the ESI source and interface plates using high voltage power supplies. Safety precautions included electrically shielding the high voltage power supplies by enclosing them in insulating plastic boxes, and placing them as far as possible away from metal parts. All high voltage cables were checked for electrical leakage at 1.2 times the highest operating voltage.44

2.2.2 Chemicals and Materials

HPLC grade water and methanol were purchased from Mallinckrodt Baker (Paris, KY, USA). Glacial acetic acid was obtained from EM Science (Gibbstown, NJ, USA). Reserpine and cytochrome c (from horse heart) were acquired from Sigma (St. Louis, MO, USA). Reserpine standard solutions were prepared by dissolving the solid salt in a mixture of methanol/water/acetic acid (69.5:29.5:1.0 v/v/v) at concentrations of 0.5 μM,
1.0 μM, 5.0 μM and 10.0 μM, respectively. A prazepam solution was prepared by
dissolving the solid salt in a mixture of methanol/water/acetic acid (69.5:29.5:1.0 v/v/v)
at a concentration of 35 μM. A cytochrome c solution was prepared at a concentration of
17 μM in a solution of methanol/water/acetic acid (9.5:89.5:1.0 v/v/v). An ES tuning mix
in acetonitrile was obtained from Agilent (G2421A, Palo Alto, CA, USA).

2.2.3 Modeling and Simulation

SIMION 3D (Version 6.0) from Scientific Instrument Services (Ringoes, NJ, USA)
was used to model electrostatic potential arrays between the ESI source and sampling
nozzle of the MS.

2.3 Incorporation of an Electrode Plate Ion Lens

2.3.1 Introduction

In this section, I describe the design and implementation of a new electrode plate ion
lens close to the ESI sprayer tip to improve the performance of the ESI-MS at
atmospheric pressure. The equipotential lines near the sprayer tip were flattened, and
even reversed, to electrostatically reduce defocusing effects and to improve ion
transmission.

2.3.2 Instrumentation

A μESI configuration was utilized at an infusion flow rate of 1.0-1.5 μL min⁻¹ using
a syringe pump (Model 55-2222, Harvard Apparatus, Holliston, MA, USA) and a 250 μL
syringe with a 21 gauge needle (Gastight®, Hamilton, Reno, NV, USA). Tapered fused
silica μESI tips were purchased from LECO (Part 711-955, ~ 2.0 cm length, 90 μm o.d.,
20 μm i.d., St. Joseph, MI, USA). The MS used in this study was a LECO Jaguar
TOFMS equipped with an ESI source. Electrical potentials were directly applied to the sample solution through a stainless steel sleeve on the ESI source and to the interface plates. Compressed nitrogen (purity 99.9% to 99.99%, Airgas, Salt Lake City, UT, USA) was used as curtain gas at a flow rate of 100-500 mL min\(^{-1}\).

A schematic diagram of the ESI-MS without the electrode plate ion lens is shown in Figure 2.1 A. The interface plate (4.0 mm thick, 42.0 mm o.d.) had a conical hole in the center. The diameters of the hole on each side of the plate were 13.0 mm (entrance, i.e., close to the sprayer) and 2.5 mm (exit, i.e., close to the nozzle). The ESI sprayer capillary was axially inserted through the hole in the ion lens and positioned 1.0-20.0 mm in front of the sampling nozzle. The distance between the interface plate and sampling nozzle was 2.0 mm (default value). An ES tuning mix, a 17 \( \mu \)M cytochrome c solution and a 0.5 \( \mu \)M reserpine solution were electrosprayed at +2.8 kV when the infusion flow rate was 1.0-1.5 \( \mu \)L min\(^{-1}\). Voltages of +450 V, +350 V and +65 V were applied to the interface plate, sampling nozzle and skimmer, respectively, and the interface plate was heated to 70 °C.

A schematic diagram of the ESI source with the electrode plate ion lens is shown in Figure 2.1 B. The ion lens (4.0 mm thick, 42.0 mm o.d.) had a reversed conical hole in the center. The diameters of the hole on each side of the plate were 2.5 mm (entrance, i.e., close to the sprayer) and 13.0 mm (exit, i.e., close to the nozzle). The ES tuning mix, 17 \( \mu \)M cytochrome c solution and 0.5 \( \mu \)M reserpine solution formed a good electrospray at +4.5 kV with +2.0 kV applied to the ion lens. The infusion flow rate was 1.0-1.5 \( \mu \)L min\(^{-1}\) when the plate was heated to 70 °C. The sprayer capillary was axially inserted through the hole in the plate and positioned 1.0-20.0 mm in front of the sampling nozzle of the MS. Voltages of +400 V, +300V and +65 V were applied to the interface plate,
Figure 2.1 Schematic diagrams of the µESI source (A) without and (B) with the electrode plate ion lens. (1) µESI source, (2) interface plate, (3) sampling nozzle, (4) curtain gas, (5) vacuum pump, (6) quadrupole, (7) skimmer, (8) ion lens.
sampling nozzle and skimmer, respectively.

2.3.3 Results and Discussion

In order to determine the optimum positions of the ESI tip and ion lens relative to the sampling nozzle, the ESI tip and ion lens were axially moved relative to the sampling nozzle until the ion intensity reached its maximum for a 0.5 μM reserpine solution which was electrosprayed at an infusion flow rate of 1.5 μL min⁻¹. The ESI tip was moved from 15.0 mm behind the entrance side of the hole, through the hole, to 10.0 mm in front of the exit side of the hole (if possible) at 1.0 mm increments and, at each increment, the ESI tip and ion lens were axially moved together so that the ion lens was placed from 8.0 to 23.0 mm in front of the sampling nozzle at 1.0 mm increments. Using the ion lens, I observed that the optimum distance between the nearest surface of the ion lens and sampling nozzle was 7.0 mm with the ESI tip positioned through the hole and exactly in the middle of the exit of the electrode plate. Therefore, the optimum distance between the ESI tip and sampling nozzle was 7.0 mm. The interface plate was positioned 1.0 mm in front of the sampling nozzle. The curtain gas flow rate was 100 mL min⁻¹.

Using the ion lens, plots of the MS ion intensity, as a function of (1) distance between the ESI tip and exit side of the ion lens hole when the ion lens was 7.0 mm in front of the sampling nozzle, and (2) distance between the exit side of the ion lens hole and sampling nozzle when the ESI tip was positioned in the middle of the exit side of the ion lens hole, are given in Figures 2.2 B and 2.2 C, respectively. For the conventional ESI source configuration, moving the sprayer tip a few millimeters axially away (Figure 2.2 D) or off-axis (Table 2.1) from the optimum position resulted in a significant attenuation in ion signal intensity. The incorporation of the electrode plate ion lens lessened this
Table 2.1 Ion signal attenuation obtained using µESI-TOFMS with and without an electrode plate ion lens\(^a\) when the sprayer tip was 1.0 mm off-axis.

<table>
<thead>
<tr>
<th>Off-axis direction</th>
<th>Percent of optimum ion signal Intensity (%)</th>
<th>Without ion lens(^b)</th>
<th>With ion lens(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>77.9</td>
<td>95.0</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>79.3</td>
<td>96.5</td>
<td></td>
</tr>
<tr>
<td>Up</td>
<td>78.5</td>
<td>93.4</td>
<td></td>
</tr>
<tr>
<td>Down</td>
<td>74.8</td>
<td>89.7</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) 0.5 \(\mu\)M reserpine (m/z 609.3).

\(^b\) Ion signal intensity RSD = ± 20%.

\(^c\) Ion signal intensity RSD = ± 11%.
**Figure 2.2** Reserpine mass spectral base peak intensity using the electrode plate ion lens versus on-axis distance (RSD = ± 13%) (A) between the ESI tip and sampling nozzle, (B) between the ESI tip and ion lens (the ion lens was fixed at 7.0 mm in front of the sampling nozzle, and the ESI tip position was axially changed), (C) between the ion lens and sampling nozzle (the ESI tip was positioned through the hole and exactly in the middle of the exit of the ion lens), and (D) reserpine mass spectral base peak intensity versus on-axis distance between the ESI tip and sampling nozzle without the electrode plate ion lens (RSD = ± 17%). Zero point: (A), (C) and (D) sampling nozzle, (B) exit side of the ion lens hole. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.5 µL min\(^{-1}\), curtain gas flow rate at 100 mL min\(^{-1}\).
effect. Relatively strong ion intensities were maintained over a wider range of sprayer tip axial positions. Even when the distance between the ESI tip and sampling nozzle was 15.0 mm, ion signals were still stronger than when the ESI tip was optimally positioned 3.0 mm in front of the sampling nozzle without the lens (Figures 2.2 A and D).

Under the optimum conditions, Figures 2.3 and 2.4 show mass spectra of the ES tuning mix and cytochrome c with and without the electrode plate ion lens, respectively. The infusion flow rate was 1.0 μL min⁻¹. Comparing the figures, ion signal enhancements of approximately 3 times (RSD = ±24%) for the various ions were observed (Tables 2.2 and 2.3).

Similar testing was performed using a 0.5 μM reserpine solution at an increased infusion flow rate of 1.5 μL min⁻¹. Comparing the two mass spectra obtained (Figures 2.5 A and B), an ion signal enhancement of 3 times (RSD = ±19%) for m/z 609.3 was observed. Also, an approximate 4-fold reduction (RSD = ±23%) in method detection limit (based on 3 times signal-to-noise ratio) was obtained (Table 2.4).

In the absence of gas flow, the ion drift velocity (v) at atmospheric pressure can be represented as a product of the ion mobility and the electric field strength in the drift region [equation (1.14)]. Ions and, to some extent, small charged droplets have trajectories orthogonal to the electric equipotential lines. Figure 2.6 A shows the equipotential lines for the conventional ESI-MS interface when voltages of +2.8 kV, +450 V and +350 V were applied to the ESI source, interface plate and nozzle, respectively. Figure 2.6 B shows the equipotential lines for the ESI-MS configuration with the electrode plate ion lens. Voltages of +4.5 kV, +2.0 kV, +400 V and +300 V were applied to the ESI source, electrode plate ion lens, interface plate and nozzle,
Table 2.2 Amplification factors obtained using \( \mu \text{ESI-TOFMS} \) with an electrode plate ion lens.

<table>
<thead>
<tr>
<th>m/z (^a)</th>
<th>Base peak intensity</th>
<th>Amplification factor ( (I_c/I_b) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without ion lens ( I_b )</td>
<td>With ion lens ( I_c )</td>
</tr>
<tr>
<td>118.1</td>
<td>90</td>
<td>154</td>
</tr>
<tr>
<td>322.0</td>
<td>391</td>
<td>815</td>
</tr>
<tr>
<td>622.0</td>
<td>19990</td>
<td>52152</td>
</tr>
<tr>
<td>922.0</td>
<td>20956</td>
<td>52382</td>
</tr>
<tr>
<td>1523.0</td>
<td>1598</td>
<td>5331</td>
</tr>
<tr>
<td>2122.0</td>
<td>829</td>
<td>2302</td>
</tr>
<tr>
<td>2721.9</td>
<td>162</td>
<td>535</td>
</tr>
</tbody>
</table>

\(^a\) Ions observed from an Agilent ES tuning mix.

\(^b\) Signal obtained when the electrospray tip was positioned 3.0 mm from the sampling nozzle of the TOFMS, RSD = ± 18%.

\(^c\) Signal obtained when the electrospray tip was positioned 7.0 mm from the sampling nozzle of the TOFMS, RSD = ± 16%.
Table 2.3. Ion signal intensities for 17 µM cytochrome c obtained using µESI-TOFMS with and without an electrode plate ion lens.

<table>
<thead>
<tr>
<th>m/z</th>
<th>Charge state</th>
<th>Base peak intensity</th>
<th>Amplification factor (I_c/I_b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without ion lens</td>
<td>With ion lens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(I_b)</td>
<td>(I_c)</td>
</tr>
<tr>
<td>688</td>
<td>+18</td>
<td>214</td>
<td>527</td>
</tr>
<tr>
<td>728</td>
<td>+17</td>
<td>409</td>
<td>1118</td>
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<tr>
<td>774</td>
<td>+16</td>
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<tr>
<td>825</td>
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</tr>
<tr>
<td>952</td>
<td>+13</td>
<td>657</td>
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</tr>
<tr>
<td>1031</td>
<td>+12</td>
<td>304</td>
<td>1018</td>
</tr>
<tr>
<td>1125</td>
<td>+11</td>
<td>219</td>
<td>560</td>
</tr>
<tr>
<td>1237</td>
<td>+10</td>
<td>209</td>
<td>574</td>
</tr>
</tbody>
</table>

*a* Ions observed from a 17 µM cytochrome c solution.

*b* Signal obtained when the electrospray tip was positioned 3.0 mm from the sampling nozzle of the TOFMS, RSD = ± 23%.

*c* Signal obtained when the electrospray tip was positioned 7.0 mm from the sampling nozzle of the TOFMS, RSD = ± 11%.
Table 2.4 Ion signal intensities and detection limits for 0.5 µM reserpine (m/z 609.3) obtained using µESI-TOFMS with and without an electrode plate ion lens.

<table>
<thead>
<tr>
<th></th>
<th>Without ion lens&lt;sup&gt;a&lt;/sup&gt;</th>
<th>With ion lens&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base peak intensity</td>
<td>107</td>
<td>400</td>
</tr>
<tr>
<td>Detection limit (nM)</td>
<td>9.4</td>
<td>2.5</td>
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<tr>
<td>Detection limit (fmol)</td>
<td>14</td>
<td>3.7</td>
</tr>
<tr>
<td>Reduction (fold)</td>
<td>-</td>
<td>3.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Signal obtained when the electrospray tip was positioned 3.0 mm from the sampling nozzle of the TOFMS, RSD = ± 14%.

<sup>b</sup> Signal obtained when the electrospray tip was positioned 7.0 mm from the sampling nozzle of the TOFMS, RSD = ± 13%.
Figure 2.3 Mass spectra of the Agilent ES tuning mix obtained using µESI-TOFMS (A) without and (B) with the electrode plate ion lens. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.0 µL min⁻¹.
**Figure 2.4** Mass spectra of 17 μM cytochrome c obtained using μESI-TOFMS (A) without and (B) with the electrode plate ion lens. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.0 μL min⁻¹.
Figure 2.5 Mass spectra of 0.5 μM reserpine (m/z 609.3) obtained using μESI-TOFMS (A) without and (B) with the electrode plate ion lens. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.5 μL min⁻¹.
Figure 2.6 Schematic diagram of the equipotential lines for (A) conventional µESI source and (B) µESI source with the electrode plate ion lens. (A) +2.8 kV, +450 V and +350 V applied to the sprayer, interface plate and nozzle, respectively, and (B) +4.5 kV, +2.0 kV, +400 V and +300 V applied to the sprayer, electrode plate, interface plate and nozzle, respectively. (1) µESI source, (2) electrode plate, (3) interface plate, (4) nozzle.
respectively. Compared to Figure 2.6 A, the equipotential lines near the sprayer tip in Figure 2.6 B are flattened, and even reversed, to reduce defocusing. Such an effect should produce higher ion transmission and enhanced sensitivity.

2.3.4 Summary

An electrode plate ion lens assisted in focusing electrosprayed ions toward the sampling nozzle of a mass spectrometer. Using an ESI-TOFMS, a signal enhancement of 3 times and a detection limit reduction of 4 times were observed with the ion lens, as compared to a conventional ESI source. The enhancement in performance of the µESI source with the electrode plate ion lens can be attributed to an improvement in the shape of the equipotential lines near the sprayer tip. Furthermore, the longer distance from the sprayer tip to the sampling nozzle of the MS results in better desolvation of the ions.

2.4 Incorporation of a New Interface Plate

2.4.1 Introduction

According to the design discussed in section 2.3, I simplified, designed and tested a new interface plate to improve the sensitivity of µESI-MS. Details are presented on the construction and evaluation of this interface, as well as on computer simulation that supports the improved performance.

2.4.2 Instrumentation

A µESI configuration was utilized at an infusion flow rate of 1.0 - 3.0 µL min\(^{-1}\) using a syringe pump (Model 55-2222, Harvard Apparatus, Holliston, MA, USA) and a 250 µL syringe with a 21 gauge needle (Gastight\(^\circledR\), Hamilton, Reno, NV, USA). Tapered fused silica µESI tips were purchased from LECO (Part 711-955, ~ 2.0 cm length, 90 µm o.d., 20 µm i.d., St. Joseph, MI, USA). The MS used in this study was a LECO Jaguar
TOFMS equipped with an ESI source. Electrical potentials were directly applied to the sample solution through a stainless steel sleeve on the ESI source and to the interface plates. Compressed nitrogen (purity 99.9% to 99.99%, Airgas, Salt Lake City, UT, USA) was used as curtain gas at a flow rate of 100-300 mL min\(^{-1}\).

A schematic diagram of the ESI-MS with the original stainless steel interface plate (Figure 2.8) is shown in Figure 2.7 A as described in section 2.3.2. This plate (4.0 mm thick, 42.0 mm o.d.) had a conical hole in the center. The diameters of the hole on each side of the plate were 13.0 mm (entrance, i.e., close to the sprayer) and 2.5 mm (exit, i.e., close to the nozzle). The sprayer capillary was axially inserted through the hole in the plate and positioned 1.0-20.0 mm in front of the sampling nozzle. An ES tuning mix, a 17 \(\mu\)M cytochrome c solution and a 0.5 \(\mu\)M reserpine solution were electrosprayed at +2.8 kV when the infusion flow rate was 1.0-3.0 \(\mu\)L min\(^{-1}\). Voltages of +450 V, +350 V and +65 V were applied to the interface plate, sampling nozzle and skimmer, respectively, and the interface plate was heated to 70 °C. A schematic diagram of the ESI source with the new stainless steel interface plate (Figure 2.8) is shown in Figure 2.7 B. The plate (4.0 mm thick, 42.0 mm o.d.) had a reversed conical hole in the center. The diameters of the hole on each side of the plate were 2.5 mm (entrance, i.e., close to the sprayer) and 13.0 mm (exit, i.e., close to the nozzle). The ES tuning mix, 17 \(\mu\)M cytochrome c solution and 0.5 \(\mu\)M reserpine solution formed a good electrospray at +5.5 kV with +2.0 kV applied to the plate. The infusion flow rate was 1.0-3.0 \(\mu\)L min\(^{-1}\) when the plate was heated to 70 °C. The sprayer capillary was axially inserted through the hole in the plate and positioned 1.0-20.0 mm in front of the sampling nozzle of the MS. Voltages of +500 V and +65 V were applied to the sampling nozzle and skimmer, respectively. All experimental
Figure 2.7 Schematic diagrams of the µESI source with (A) original and (B) new interface plates. (1) µESI source, (2) interface plate, (3) sampling nozzle, (4) curtain gas, (5) vacuum pump, (6) quadrupole, (7) skimmer.
Figure 2.8 Photographs of (A) front and (B) back of (1) original and (2) new interface plates.
conditions were optimized as described below.

2.4.3 Results and Discussion

In order to determine the optimum positions of the ESI tip and interface plates relative to the sampling nozzle, the ESI tip and interface plates were axially moved relative to the sampling nozzle until the ion intensity reached its maximum for a 0.5 μM reserpine solution which was electrosprayed at an infusion flow rate of 1.5 μL min\(^{-1}\). The ESI tip was moved from 15.0 mm behind the entrance side of the hole, through the hole, to 10.0 mm in front of the exit side of the hole (if possible) at 1.0 mm increments and, at each increment, the ESI tip and interface plates were axially moved together so that the interface plate was placed from 1.0 to 15.0 mm in front of the sampling nozzle. Using the original interface plate, I found that the optimum distance between the plate and sampling nozzle was 3.0 mm with the ESI tip positioned exactly in the middle of the exit side of the hole. Therefore, the optimum distance between the ESI tip and sampling nozzle was 3.0 mm. Using the new interface plate, I observed that the optimum distance between the plate and sampling nozzle was 6.0 mm with the ESI tip positioned exactly in the middle and half-way through the hole. Therefore, the optimum distance between the ESI tip and sampling nozzle was 8.0 mm. The curtain gas flow rate was 100 mL min\(^{-1}\).

The results of two of these experiments are demonstrated in Figures 2.9 and 2.10. Using the original interface plate, plots of the MS ion intensity as a function of distance between the ESI tip and exit side of the hole when the plate was 3.0 mm in front of the sampling nozzle, and distance between the exit side of the hole and sampling nozzle when the ESI tip was positioned exactly in the middle of the exit side of the hole are given in Figures 2.9 B and 2.9 C, respectively. Similarly for the new interface plate,
Figure 2.9 Reserpine mass spectral base peak intensity using the original interface plate versus on-axis distance (A) between the ESI tip and sampling nozzle, (B) between the ESI tip and interface plate (the interface plate was fixed at 3.0 mm in front of the sampling nozzle, and the ESI tip position was axially changed), and (C) between the interface plate and sampling nozzle (the ESI tip was fixed in the middle of the exit side of the interface plate hole, and the interface plate position was axially changed). Zero point: (A) sampling nozzle, (B) exit side of the interface plate hole, and (C) sampling nozzle. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.5 µL min⁻¹, curtain gas flow rate at 100 mL min⁻¹, RSD = ± 15%.
Figure 2.10 Reserpine mass spectral base peak intensity using the new interface plate versus on-axis distance (A) between the ESI tip and sampling nozzle, (B) between the ESI tip and interface plate (the interface plate was fixed at 6.0 mm in front of the sampling nozzle, and ESI tip position was axially changed), and (C) between the interface plate and sampling nozzle (the ESI tip was fixed in the middle and halfway through the interface plate hole, and interface plate position was axially changed). Zero point: (A) sampling nozzle, (B) exit side of the interface plate hole, and (C) sampling nozzle. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.5 µL min⁻¹, curtain gas flow rate at 100 mL min⁻¹, RSD = ± 13%.
Figures 2.10 B and 2.10 C show the ion intensity as a function of distance between the ESI tip and exit side of the hole when the plate was 6.0 mm in front of the sampling nozzle, and distance between the exit side of the hole and sampling nozzle when the ESI tip was exactly positioned in the middle and half-way through the hole, respectively. Comparing Figures 2.10 A to 2.9 A, it can be seen that, even when the distance between the ESI tip and sampling nozzle was 15.0 mm, ion signal intensity was still stronger than when the ESI tip was positioned 3.0 mm in front of the sampling nozzle with the original interface plate. In addition, for the μESI source configuration with the original interface plate, moving the sprayer tip a few mm off-axis from the optimum positions resulted in a significant attenuation (22% for ±1.0 mm) in ion intensity. The incorporation of the new interface plate lessened this effect (6% for ±1.0 mm).

Comparing mass spectra (Figures 2.11 A and B) for reserpine under the optimum conditions described above, an ion signal enhancement of 5 times (RSD = ±13%) was observed with the new interface plate. Also, based on 3 times signal-to-noise, a 7-fold reduction (RSD = ±18%) in method detection limit was obtained (Table 2.5). Similar experiments were performed using the ES tuning mix and cytochrome c solution to compare the new interface plate to the original one. The infusion flow rate was 1.0 μL min⁻¹ and the curtain gas flow rate was 150 mL min⁻¹. Comparing Figures 2.12 A and B with 2.13 A and B, ion signal enhancements of 2-5 times (RSD = ±11%) for the various ions were observed. This new interface plate was shown to be more effective than any other ion lenses studied under atmospheric pressure.²⁰,³³⁻⁴⁰

In a gas-flow-free drift region, ions or even charged droplets can move in trajectories orthogonal to the electric equipotential lines.³⁴ Figure 2.14 A shows the
Table 2.5 Ion signal intensities and method detection limits for 0.5 µM reserpine (m/z 609.3) obtained using μESI-TOFMS with the original and new interface plates.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Original&lt;sup&gt;a&lt;/sup&gt;</th>
<th>New&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base peak intensity</td>
<td>107</td>
<td>567</td>
</tr>
<tr>
<td>Detection limit (nM)</td>
<td>9.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Detection limit (fmol)</td>
<td>14</td>
<td>2.0</td>
</tr>
<tr>
<td>Reduction (fold)</td>
<td>-</td>
<td>7.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ion signal intensity RSD = ± 14%.

<sup>b</sup> Ion signal intensity RSD = ± 11%.
**Figure 2.11** Mass spectra of reserpine (m/z 609.3) obtained using µESI-TOFMS with (A) original and (B) new interface plates. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion of 0.5 µM reserpine at 1.5 µL min⁻¹, curtain gas flow rate at 100 mL min⁻¹.
**Figure 2.12** Mass spectra of Agilent ES tuning mix obtained using µESI-TOFMS with (A) original and (B) new interface plates. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.0 µL min⁻¹, curtain gas flow rate at 150 mL min⁻¹.
Figure 2.13  Mass spectra of cytochrome c obtained using µESI-TOFMS with (A) original and (B) new interface plates. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion of 17 μM cytochrome c at 1.0 μL min⁻¹, curtain gas flow rate at 150 mL min⁻¹.
Figure 2.14 Schematic diagrams of the equipotential lines for the µESI source with (A) original and (B) new interface plates. (A) +2.8 kV, +450 V and +350 V applied to the sprayer, interface plate and nozzle, respectively; (B) +5.5 kV, +2.0 kV, and +500 V applied to the sprayer, interface plate and nozzle, respectively. (1) µESI source, (2) interface plate, (3) nozzle.
equipotential lines for the µESI-MS configuration with the original interface plate when voltages of +2.8 kV, +450 V and +350 V were applied to the ESI source, interface plate and nozzle, respectively. Figure 2.14 B shows the equipotential lines for the µESI-MS configuration with the new interface plate. Voltages of +5.5 kV, +2.0 kV, and +500 V were applied to the ESI source, interface plate and nozzle, respectively. In comparison to Figure 2.14 A, the equipotential lines near the sprayer tip in Figure 2.14 B are flattened, and even reversed, to reduce defocusing and improve ion transmission. Furthermore, the relatively long desolvation distance using the new interface plate could be another contribution to improved sensitivity.21

2.4.4 Summary

A new interface plate assisted in focusing electrosprayed ions toward the sampling nozzle of an MS. Using a µESI-TOFMS, a signal enhancement of 5 times and a method detection limit reduction of 7 times were observed, as compared to the original interface plate. The enhancement in performance of the µESI-MS with the new interface plate can be attributed to an improvement in the shape of the equipotential lines near the sprayer tip. Furthermore, the relatively long distance from the sprayer tip to the sampling nozzle of the MS resulted in better desolvation of ions.

2.5 Incorporation of an Air Amplifier

2.5.1 Introduction

In this section, I report the use of a modified air amplifier to assist in focusing and desolvating ions in ESI. This device generates a concentric high velocity converging gas flow around the electrospray tip to reduce spreading of the electrospray plume, to assist in desolvation, and to aerodynamically improve conduction of ions to the sampling
orifice of the MS. Applying a voltage to the air amplifier electrostatically assists in focusing and conduction of ions.\textsuperscript{46}

2.5.2 Faraday Plate Detection

\textit{Instrumentation.} Microspray configurations were utilized for low flow ESI (1.0 to 1.5 $\mu$L min$^{-1}$). Microspray tips were prepared by cutting 90 $\mu$m o.d., 20 $\mu$m i.d., fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) into ~1.0 inch lengths, which promoted the onset of electrospray at relatively low voltages. Electrospray ion currents were generated and measured using homemade instrumentation (Figure 2.15). An aluminum industrial air amplifier (HMC-Brauer, Mount Farm, Milton Keynes, UK) was re-machined out of stainless steel (Figure 2.16) and placed between the sprayer tip and a specially designed Faraday detector. Two high voltage power supplies (Series 230, Bertan, Hicksville, NY, USA) were connected to the ESI source and air amplifier, respectively. The ESI tip voltage was +4.0 kV. The air amplifier was grounded except when a voltage was applied. The Faraday detector was made using a stainless steel rod (0.41 mm o.d.). The rod was inserted through a Teflon tube (1.12 mm o.d., 0.41 mm i.d.) and then through a stainless steel tube (1.63 mm o.d., 1.12 mm i.d.). The stainless steel tube was grounded.

The prazepam solution formed an electrospray when the infusion flow rate was 1.5 $\mu$L min$^{-1}$. The spray was directed through the air amplifier toward the Faraday detector. The detector was placed at the exit of the air amplifier to collect the ion current along the spray axis. The current was amplified using a current amplifier (Model 428, Keithley, Cleveland, OH, USA) and transformed into a voltage signal. The intensity of the signal was monitored using an oscilloscope (Model 2465B, Tektronix, Beaverton, OR, USA).
Figure 2.15 Schematic diagram of electrospray ionization/air amplifier/Faraday detector. (1) ESI tip, (2) air amplifier entrance, (3) air amplifier exit, (4) Faraday detector, (5) current amplifier, (6) oscilloscope.
Figure 2.16 Photographs of (A) air amplifier, (B) chamber, and (C) nozzle. (1) nitrogen supply inlet, (2) entrance, (3) exit.
For increasing ion signal intensities further, a voltage in the range of approximately 0.0 to 3.0 kV was applied to the air amplifier using one of the high voltage power supplies.

**Results and Discussion.** A schematic diagram of the air amplifier is shown in Figure 2.17. High velocity compressed nitrogen gas at 4 to 6 L min\(^{-1}\) flows from the supply inlet (1) and into the annular chamber (2). The flow is throttled by the annular gap (3), and the resultant thin layer of high velocity nitrogen gas adheres to the wall profile, which turns the nitrogen gas through 90 degrees to pass down the bore (Coanda effect).\(^{47,48}\) The action of the high velocity nitrogen gas streaming down the bore of the air amplifier causes a pressure drop, which induces a large flow of ambient air into the device (4) (Venturi effect).\(^{49}\) The net effect is that the venturi device uses the energy from a small volume of compressed nitrogen gas to produce a large volume, large velocity, and low-pressure outlet air flow (5). The outlet flow volume can be as high as 100 times the supply flow, i.e., 400 to 600 L min\(^{-1}\).

Using the instrumental set-up shown in Figure 2.15, the electrospray tip and detector were moved along the axial direction of the air amplifier to determine the optimum positions where the measured voltage was the highest. As expected, the ion current from electrospray of the prepared prazepam solution increased as the distance between the spray tip and the detector decreased in the testing region (Table 2.6). It was found that, when the electrospray tip was positioned 6.0 mm inside the entrance of the air amplifier, and the detector was positioned 22.5 mm inside the exit of the air amplifier (i.e., the ESI tip was 14.0 mm from the detector along the axial direction), a 70% increase (RSD = ±19%) in the current intensity was measured at the detector compared to when the electrospray tip was positioned 1.0 mm in front of the detector without the amplifier.
Table 2.6 Ion signal intensities obtained using the air amplifier for various distances between the ESI tip and the Faraday detector.

<table>
<thead>
<tr>
<th>Distance 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Current&lt;sup&gt;c&lt;/sup&gt; (nA)</td>
<td>Distance 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Current&lt;sup&gt;c&lt;/sup&gt; (nA)</td>
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<td>0.0 mm</td>
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<tr>
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<tr>
<td>22</td>
<td>3.02</td>
<td>19</td>
<td>8.03</td>
</tr>
<tr>
<td>21</td>
<td>3.99</td>
<td>18</td>
<td>8.43</td>
</tr>
<tr>
<td>20</td>
<td>4.22</td>
<td>17</td>
<td>9.22</td>
</tr>
</tbody>
</table>

<sup>a</sup> distance of the ESI tip inside the air amplifier.

<sup>b</sup> distance between the ESI tip and the Faraday detector.

<sup>c</sup> RSD = ± 13%.
Figure 2.17 Schematic diagram of the internal configuration of the air amplifier. (1) nitrogen supply inlet, (2) annular chamber, (3) annular gap, (4) induced airflow, (5) output air flow.
Figure 2.18 Detector response versus distance between the electrospray tip and the Faraday detector. The response curves represent operation with (○, RSD = ±13%) and without (△, RSD = ±15%) the air amplifier. Conditions: direct infusion at 1.5 µL min⁻¹.
When the distance between the electrospray tip and detector was fixed at 14.0 mm, ion signal intensity was 340-fold (RSD = ±16%) greater than without the air amplifier at the same distance between the ESI sprayer tip and the Faraday detector. If the distance was much closer than the optimum position shown in Figure 2.18, the stability of the electrospray was compromised due to the high velocity gas flow around the electrospray needle. Based on the observed improvement in ion signal intensity, the air amplifier obviously assists in desolvation and focusing electrosprayed ions along the axial direction.

At the optimum axial positions of the ESI sprayer tip, air amplifier and Faraday detector, the detector was moved off-axis to determine the radial distribution of the ion current. When the detector was moved off-axis by ±1.0 mm, the current measured decreased by approximately 12% (Figure 2.19). The rate of signal attenuation in the direction perpendicular to the operational axis was the same order of magnitude as the standard deviation of the measurement.

For further increasing ion signal intensity, an electric potential in the range of approximately +0.0 to 3.0 kV was applied to the air amplifier as shown in Figure 2.15. At +2.0 kV, I obtained a 3-fold (RSD = ±18%) increase in total ion signal intensity with the Faraday detector compared to when the electrospray tip was positioned 1.0 mm in front of the detector without the amplifier (Figure 2.20). Again, when the distance between the electrospray tip and detector was fixed at 14.0 mm, the signal intensity was 840-fold (RSD = ±17%) greater than without the air amplifier at the same distance between the ESI sprayer tip and Faraday detector.
**Figure 2.19** Detector response versus distance off-axis (i.e., perpendicular to the center axis of the air amplifier). Conditions: direct infusion at 1.5 µL min⁻¹, RSD = ± 13%.
Figure 2.20 Detector response versus distance between the electrospray tip and the Faraday detector when 2.0 kV was applied to the air amplifier (Δ, RSD = ± 12%) and without the air amplifier (◊, RSD = ± 14%). Conditions: direct infusion at 1.5 µL min\(^{-1}\).
2.5.3 Time-of-flight Mass Spectrometry Detection

*Instrumentation.* Microspray configurations were utilized for low flow ESI (1.0 to 1.5 μL min⁻¹). Microspray tips were prepared by cutting 90-μm-o.d., 20-μm-i.d. fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) into ~1.0 inch lengths, which promoted the onset of electrospray at relatively low voltages.⁵⁰

A Jaguar time-of-flight mass spectrometer (TOFMS, LECO, St. Joseph, MI, USA) with a homemade heated capillary interface (Figure 2.21) was used to test the air amplifier focusing system. An aluminum industrial air amplifier (HMC-Brauer, Mount Farm, Milton Keynes, UK) was re-machined out of stainless steel and placed between the sprayer tip and interface capillary.⁵¹ Two high voltage power supplies (Series 230, Bertan, Hicksville, NY, USA) were connected to the ESI source and air amplifier, respectively. The voltages on the ESI source, air amplifier, interface capillary and skimmer were set at +2.8 to +4.0 kV, 0.0 to +3.0 kV, +300 V and +65 V, respectively. The air amplifier was grounded except when a voltage was applied. The various reserpine solutions were introduced at an infusion flow rate of 1.5 μL min⁻¹.

*Results and Discussion.* In order to test the air amplifier focusing system, ion signal enhancements were studied using the instrumental set-up shown in Figure 2.21. A series of reserpine concentrations were analyzed under the conditions of (1) no air amplifier; (2) with air amplifier and applied voltage (1.9-2.0 kV), but no venturi-induced gas flow; (3) with air amplifier and venturi-induced gas flow, but no applied voltage; and (4) with air amplifier, venturi-induced gas flow, and applied voltage. The peak intensities for m/z 609.3 were monitored. Ten determinations of each measurement were made for statistical considerations. The capillary interface was heated to 75 °C. Figure 2.22 shows examples
Figure 2.21 Schematic diagram of the ESI/air amplifier/TOFMS system. (1) ESI source, (2) air amplifier entrance, (3) air amplifier exit, (4) metallic heated capillary (capillary inlet), (5) nozzle.
Figure 2.22 Mass spectra of a 1.0 µM reserpine solution (m/z 609.3) obtained using an ESI-TOFMS and a separation distance of 14 mm between the ESI tip and capillary inlet: (A) without air amplifier; (B) with air amplifier and applied voltage, but no gas flow; (C) with air amplifier and gas flow, but no applied voltage; and (D) with air amplifier, gas flow, and applied voltage. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.5 µL min⁻¹.
of mass spectra obtained for 1.0 μM reserpine solution using the ESI-TOFMS without air amplifier (Figure 2.22 A); ESI/air amplifier without gas flow, but with applied voltage (Figure 2.22 B); ESI/air amplifier without applied voltage, but with gas flow (Figure 2.22 C); and ESI/air amplifier with both gas flow and applied voltage (Figure 2.22 D). These experiments were performed with the ESI tip axially positioned 6.0 mm inside the entrance of the air amplifier and the capillary inlet axially positioned 22.5 mm inside the exit of the air amplifier (i.e., the ESI tip was positioned 14.0 mm from the capillary inlet along the axial direction).

The greatest enhancement in ion signal intensity was observed when gas flow and voltage were used together with the air amplifier. With no air flow and 1.9 to 2.0 kV applied to the air amplifier (4.0 kV ESI voltage), the m/z 609.3 ion signal intensity increased by over 50% (RSD = ±21%), as compared to when the electrospray tip was positioned 1.0 mm in front of the capillary inlet (i.e., no air amplifier) and 2.8 kV was applied to the ESI source (Figures 2.22 A and B). With gas flow through the air amplifier and no voltage applied, over 5-fold increase (RSD = ±20%) was obtained (Figure 2.22 C). When 4.0 kV was applied to the ESI source and 1.9 to 2.0 kV was applied to the air amplifier with venturi-induced gas flow, I obtained an ~18-fold increase (RSD = ±24%) in ion signal intensity (Figure 2.22 D; Table 2.7).

To find the optimum positions of the ESI tip and capillary inlet as described above, the ESI tip, air amplifier and capillary inlet positions were axially changed relative to each other until the measured ion intensity was at a maximum. This was accomplished by moving the ESI tip from 12.0 mm inside the entrance of the air amplifier to 20.0 mm outside the entrance of the air amplifier at 1.0 mm increments and, at each increment,
Table 2.7 Amplification factors for reserpine m/z 609.3 ion intensity obtained using an ESI/air amplifier/TOFMS.

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>Without air amplifier&lt;sup&gt;a&lt;/sup&gt; (I&lt;sub&gt;A&lt;/sub&gt;)</th>
<th>With air amplifier&lt;sup&gt;b&lt;/sup&gt; (I&lt;sub&gt;B&lt;/sub&gt;)</th>
<th>Applied voltage but no gas flow&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Gas flow but no applied voltage&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Applied voltage and gas flow&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>134/-</td>
<td>228/0.7</td>
<td>1131/7.4</td>
<td>2809/20.0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>223/-</td>
<td>357/0.6</td>
<td>1727/6.7</td>
<td>4311/18.3</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>1111/-</td>
<td>1778/0.6</td>
<td>7812/6.0</td>
<td>26592/22.9</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>3394/-</td>
<td>5091/0.5</td>
<td>21689/5.4</td>
<td>63480/17.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> signal obtained when the electrospray tip was positioned 1.0 mm from the sampling orifice of the TOFMS, RSD = ± 16%.

<sup>b</sup> signal obtained when the electrospray tip was positioned 14.0 mm from the sampling orifice of the TOFMS.

<sup>c</sup> RSD = ± 19%.

<sup>d</sup> RSD = ± 16%.

<sup>e</sup> RSD = ± 20%.
moving the ESI tip and air amplifier axially together so that the capillary inlet was axially positioned from 25.5 mm inside the exit of the air amplifier to 8.5 mm outside the exit of the air amplifier. It was found, when the electrospray tip was axially positioned 6.0 mm inside the entrance of the air amplifier, and the capillary inlet was axially positioned 22.5 mm inside the exit of the air amplifier (i.e., the ESI tip was positioned 14.0 mm from the capillary inlet along the axial direction), the ion intensity reached its peak value.

The results of two of these experiments are shown in Figures 2.23 A and B. Figure 2.23 A shows the base peak intensity as a function of distance between the ESI tip and capillary inlet when the capillary inlet was axially fixed 22.5 mm inside the exit of the air amplifier. Figure 2.23 B shows the base peak intensity as a function of distance between the ESI tip and the capillary inlet when the ESI tip was axially fixed 6 mm inside the entrance of the air amplifier. The highest ion intensity was observed when the capillary inlet was positioned 22.5 mm inside the exit of the air amplifier and the ESI tip was positioned 6.0 mm inside the entrance of the air amplifier, i.e., the ESI tip was positioned 14.0 mm from the capillary inlet along the axial direction. Furthermore, a relatively broad range of ESI tip and capillary inlet positions was found for maintaining strong ion signal intensities. Even when the distance between the ESI tip and capillary inlet was 20.0 mm, the ion intensity was still higher than when the ESI tip was positioned 1.0 mm in front of the sampling orifice without the air amplifier (Figure 2.23 C).

In order to evaluate the relationship between ion intensity and off-axis distance of the ESI tip or the capillary inlet from their optimum positions, each was moved off-axis while the other was axially fixed in its optimum position. When the ESI tip was moved off-axis by ± 2.0 mm while the capillary inlet was axially fixed, the ion intensity
Figure 2.23 Reserpine mass spectral base peak intensity (m/z 609.3) versus on-axis distance between the ESI tip and capillary inlet (A) with air amplifier, capillary inlet fixed at 25.5 mm inside the exit of the air amplifier, and the ESI tip moved along the axis; (B) with air amplifier, ESI tip fixed at 6 mm inside the entrance of the air amplifier, and the capillary inlet position changed along the axis, and (C) without the air amplifier. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.5 µL min\(^{-1}\), RSD = ± 17%, 14% and 21% for A, B, and C, respectively.
decreased by 40%. When the capillary inlet was positioned off-axis by ±1.0 mm while the ESI tip was axially fixed, the ion intensity decreased by 19%. Very little loss in ion signal intensity was observed when the electrospray tip or the capillary inlet was moved ±1.0 mm off-axis (Figure 2.24).

Finally, the base peak intensity (m/z 609.3) was plotted against concentration with the air amplifier in its optimum position (Figure 2.25). After linear regression, the method detection limits were calculated based on concentrations corresponding to three times the signal-to-noise ratio. A 34-fold reduction (RSD = ±26%) in method detection limit was obtained (Table 2.8). In addition to enhancing analyte ion intensity, the air amplifier also suppresses background chemical noise.

2.5.4 Fourier Transform Ion Cyclotron Mass Spectrometry Detection

Instrumentation. A commercial ESI-FTICRMS (Model APEX47e), manufactured by Bruker Daltonics (Billerica, MA, USA) and coupled with a commercial electrospray ionization source (Model 102509, Analytica, Branford, CN, USA) was used to test the air amplifier focusing system. The electrospray tip voltage was +1707-1900 V. The interface tip voltage was +300-350 V. However, in these experiments, no voltage was applied to the air amplifier. The pressure on the gas supply inlet was set at 5 atm (i.e., 73.5 psi).

Results and Discussion. In order to verify the experimental results obtained using the Faraday plate and time-of-flight mass spectrometer, the ES tuning mix was electrosprayed and detected using a commercial FTICRMS. The optimum positions of the ESI tip, air amplifier and heated capillary inlet were obtained as discussed in section 2.5.3. The relative standard deviations of the measurements with and without the air amplifier were ±13% and ±17%, respectively. The ion current was increased by over
**Table 2.8** Method detection limits using an ESI/air amplifier/TOFMS.

<table>
<thead>
<tr>
<th>Condition(s)</th>
<th>Detection Limits (fmol)</th>
<th>Detection Limits (nmol/L)</th>
<th>Reduction (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No air amplifier</td>
<td>5.30</td>
<td>3.53</td>
<td>-</td>
</tr>
<tr>
<td>Air amplifier with gas flow</td>
<td>0.64</td>
<td>0.43</td>
<td>8.3</td>
</tr>
<tr>
<td>Air amplifier with gas flow and 1.9-2.0 kV applied</td>
<td>0.16</td>
<td>0.11</td>
<td>33.9</td>
</tr>
</tbody>
</table>
Figure 2.24 Reserpine mass spectral base peak intensity (m/z 609.3) versus off-axis distance for (A) ESI tip, with air amplifier, and the capillary inlet axially fixed, (B) capillary inlet, with air amplifier, and the ESI tip axially fixed. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.5 µL min\(^{-1}\), RSD = ± 21% and 14% for A and B, respectively.
Figure 2.25 Reserpine mass spectral base peak intensity (m/z 609.3) versus reserpine concentration (logarithmic scale) for (A) with air amplifier, gas flow, and applied voltage; (B) with air amplifier and gas flow, but no applied voltage; and (C) without the air amplifier. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion at 1.5 µL min⁻¹, RSD = ± 20%. The dotted line represents the experimental noise level.
50% (RSD = ±20%) for all component ions at the optimum positions, as compared to the results without the air amplifier. When the ESI tip was moved 14.0-19.0 mm from the interface tip, the ion current was relatively increased by 3 times (RSD = ±17%) as compared to measurements without the air amplifier. Moreover, when the distance between the ESI tip and the heated capillary inlet was over 19.0 mm, the m/z 622.0, 922.0 and 1522.3 ions still showed high signal improvement when using the air amplifier (Table 2.9). One technical difficulty in these experiments was that the voltage applied to the ESI source could not be set over +2.0 kV due to instrumental discharge limitations. In addition, no voltage was applied to the air amplifier. Therefore, the electric field for ion transmission was not as effective as described in section 2.5.3. This is probably the major problem why the FTICRMS in these experiments showed less signal enhancement than the TOFMS.

2.5.5 Summary

A modified air amplifier assisted in focusing electrospayed ions along the axial direction toward the sampling orifice of a mass spectrometer. Using an ESI-TOFMS and an ESI-FTICRMS, significant signal enhancements and detection limit reductions were observed when using the air amplifier, as compared to conventional µESI. The gain in ion signal intensity is attributed to the ability of the air amplifier to improve conduction of ions into the MS. The ESI tip can be located farther from the sampling orifice than for conventional ESI to produce better desolvation and less possibility of discharge. Another advantage of the device is that the sprayer can be positioned along the axial direction straight toward the nozzle. Complex devices with off-axis orientation of the ESI needle with respect to the nozzle for separating ions from neutrals and improving desolvation
Table 2.9 Amplification factors for ion signal intensities for m/z 622.0, 922.0 and 1522.0 ions obtained using FTICR MS when the distance between the ESI tip and interface inlet was 14.0 to 19.0 mm and 19.0 to 22.0 mm.

<table>
<thead>
<tr>
<th>m/z</th>
<th>Amplification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.0-19.0 mm</td>
</tr>
<tr>
<td>622.0</td>
<td>1.7</td>
</tr>
<tr>
<td>922.0</td>
<td>3.4</td>
</tr>
<tr>
<td>1522.0</td>
<td>2.6</td>
</tr>
</tbody>
</table>
are not necessary.52

2.6 Incorporation of a New Ion Focusing Device

2.6.1 Introduction

According to simulation results of gas flow profiles inside the air amplifier, much turbulence exists in the entrance of the chamber which compromises ion transmission. Therefore, a new ion focusing device (Figures 2.26 and 2.27) based on the air amplifier was designed to overcome this problem. In this design, a high-resistance conductive-plastic rod (ESD 420) was machined into a conical funnel and added to the entrance of the chamber of the new ion focusing device.

This design also optimally changed the internal wall configuration of the air amplifier to decrease the turbulence. When an optimized electric field gradient was applied through the body of the air amplifier, the electrosprayed ions were more effectively focused along the center axis, and directed toward the sampling orifice of the mass spectrometer, thus, increasing the ion transmission from the ESI source into the sampling orifice of the MS with enhanced sensitivity.

2.6.2 Mass Spectrometry Detection

*Instrumentation.* A µESI configuration was utilized at an infusion flow rate of 1.0-3.0 µL min⁻¹ using a syringe pump (Model 55-2222, Harvard Apparatus, Holliston, MA, USA) and a 250 µL syringe with a 21 gauge needle (Gastight®, Hamilton, Reno, NV, USA). Tapered fused silica µESI tips were purchased from LECO (Part 711-955, ~ 2.0 cm length, 90 µm o.d., 20 µm i.d., St. Joseph, MI, USA). The MS used in this study was a LECO Jaguar TOFMS equipped with an ESI source. Electrical potentials were directly applied to the sample solution through a stainless steel sleeve on the ESI source and to
Figure 2.26 Photographs of (A) new ion focusing device, (B) chamber, and (C) nozzle. (1) front lead, (2) entrance, (3) supply inlet, (4) back lead, (5) exit, (6) high resistance conductive plastic funnel.
**Figure 2.27** Schematic diagram of new ion focusing device internal configuration. (1) entrance, (2) exit, (3) high resistance conductive plastic funnel.
the interface plates (similar to Figure 2.21). Three high voltage power supplies (Series 230, Bertan, Hicksville, NY, USA) were connected to the ESI source and two metal leads on the ion focusing device, respectively. The voltages on the ESI source, two metal leads, interface capillary and skimmer were set at +4.5 kV, +2.5 kV, +1.5 kV, +400 V and +65V, respectively. The cytochrome c solution and ES tuning mix were introduced at an infusion flow rate of 1.5 μL min⁻¹.

Results and Discussion. The optimization process was similar to that described in section 2.5.3. Under the optimum conditions, Figure 2.28 shows mass spectra of 17 μM cytochrome c with and without the new ion focusing device. The infusion flow rate was 1.0 μL min⁻¹. Comparing the figures, ion signal enhancements of 12 to 24 times (RSD = ± 20%) for the various ions were observed. In addition, we found that the average charge states moved to a higher value (~1 unit). Similar testing was performed using a 0.5 μM reserpine solution at an increased infusion flow rate of 1.5 μL min⁻¹. Comparing the ion intensities obtained, an ion signal enhancement by 27 times (RSD = ± 15%) for m/z 609.3 was observed (not shown here). Also, an approximate 40-fold reduction (RSD = ± 18%) in method detection limit (based on 3 times signal-to-noise ratio) was obtained (Table 2.10). Similar results were reported using a 7 T FTICRMS (Ionspec, Irvine, CA, USA) to analyze a 16.2 kDa oligonucleotide and a 53-mer PCR product. Figure 2.29 shows the experimental set-up.

First, one order of magnitude total ion abundance improvement (RSD = ± 16%) was observed for most ions from the two compounds. Second, the average charge states of the oligonucleotide and 53-mer PCR product shifted from +12.5 to +14.5 and +10.9 to +12.6 using the new ion focusing device. The theoretical mass resolving power in FTICRMS at
Table 2.10. Method detection limits using an ESI/new ion focusing device/TOFMS.

<table>
<thead>
<tr>
<th>Condition(s)</th>
<th>Detection Limit (fmol)</th>
<th>Detection Limit (nmol/L)</th>
<th>Reduction (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No air amplifier</td>
<td>5.30</td>
<td>3.53</td>
<td>-</td>
</tr>
<tr>
<td>Air amplifier with gas flow</td>
<td>0.47</td>
<td>0.32</td>
<td>11.2</td>
</tr>
<tr>
<td>Air amplifier with gas flow and 1.9-2.0 kV</td>
<td>0.13</td>
<td>0.09</td>
<td>39.8</td>
</tr>
<tr>
<td>applied</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.28 Mass spectra of cytochrome c obtained using µESI-TOFMS (A) without and (B) with the new ion focusing device. Conditions: 5 kHz pulser frequency, 1 min run time, direct infusion of 17 µM cytochrome c at 1.0 µL min⁻¹, curtain gas flow rate at 150 mL min⁻¹.
Figure 2.29 Photographs of the experimental set-up observed from (A) above and (B) behind, and (C) schematic diagram of the cross-section.
the zero-pressure limit is defined as

\[ R_{FWHM} = \frac{(1.27 \times 10^7)ZB_0T}{M} \]  

(2.1)

where \( Z \) is the number of charges, \( B_0 \) is the magnetic field strength (Tesla), \( T \) is the transient length (seconds), and \( M \) is the molecular mass (Dalton). Therefore, increasing the average charge state improves the mass resolving power of the FTICRMS. This is probably helpful for top-down sequencing of biomacromolecules.

**Summary.** A new ion focusing device with aerodynamic and electrostatic focusing effects assisted in focusing electrosprayed ions along the axial direction toward the sampling orifice of a mass spectrometer. A signal enhancement of ~20 times and a detection limit reduction of ~40 times were observed when using the air amplifier, as compared to conventional μESI.

### 2.7 Conclusions

New atmospheric pressure ion focusing interfaces using a combination of aerodynamic and electrostatic focusing effects were designed and optimized to assist in focusing electrosprayed ions toward the sampling nozzle of an MS, thus, improving the sensitivity. Using a Faraday plate, a TOFMS and two FTICRMS systems, a signal enhancement of 3-20 times and a method detection limit reduction of 5-40 times were observed, as compared to conventional ESI.

### 2.8 References


47. [http://romania-on-line.net/halloffame/CoandaHenri.htm](http://romania-on-line.net/halloffame/CoandaHenri.htm)


3.1 Introduction

Recently, electrospray ionization has become attractive as an ion source for ion mobility spectrometry determination of a wide range of sample molecules based on their differing gas phase ion mobilities.\textsuperscript{1-12} The separation time in IMS ranges from several hundred microseconds to milliseconds. This allows faster analysis than most other conventional separation techniques, such as gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE). However, the major limitation in ESI-IMS is its low resolution. This is attributed to several factors. First, a relatively large volume of sample is typically introduced for trace analysis. Therefore, solvent evaporation from the electrosprayed fine droplets in the drift region can create broadening of analyte bands. Second, heat transfer from the atmospheric gas to the electrosprayed droplets is so low that desolvation cannot be effectively completed before the droplets enter the drift region. Third, the electrical potential of the ESI source perturbs the homogeneity of the electric field in the drift region.\textsuperscript{2,3,13,14} Finally, unwanted ions penetrate through the ion gate.

Using a Bradbury-Nielsen design,\textsuperscript{15} it is known that a percentage of electrosprayed ions can drift through the ion gate under the influence of the electric field even when the
ion gate is closed. To decrease the amount of ion penetration, the orthogonal electric field of the “closed” gate must be increased. Ion penetration results in both elevated background and band broadening, thus, compromising both sensitivity and resolution.\textsuperscript{16}

The separation performance of ESI-IMS can be quantified as either peak-to-peak resolution or resolving power.\textsuperscript{16} Peak-to-peak resolution, \(R_{pp}\), is defined similarly to the resolution used in chromatography as

\[
R_{pp} = 2 \left( \frac{t_{d2} - t_{d1}}{\omega_{b1} + \omega_{b2}} \right)
\]

(3.1)

where \(t_{d1}\) and \(t_{d2}\) are the drift times for two adjacent peaks, and \(\omega_{b1}\) and \(\omega_{b2}\) are their full widths at baseline. Since full-width-at-half-height (FWHH) is much easier and more accurate to measure, Equation (3.1) can be rewritten as

\[
R_{pp} = \frac{2(t_{d2} - t_{d1})}{1.7(\omega_{h1} + \omega_{h2})}
\]

(3.2)

where the peak shapes are assumed to be Gaussian and \(\omega_{h1}\) and \(\omega_{h2}\) are their FWHH values. The separation factor (\(\alpha\)) can be defined as

\[
\alpha = \frac{t_{d2}}{t_{d1}}
\]

(3.3)

and the retention factor (\(k\)) as

\[
k = \frac{t_{d} - t_{0}}{t_{0}}
\]

(3.4)

where \(t_{0}\) is the time that an ion drifts through the drift region without any interaction with the drift gas. Therefore, the fundamental resolution equation can be given as

\[
R_{pp} = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k}{1 + k} \right)
\]

(3.5)
where $N$ is the theoretical plate number.

In IMS, since $t_d$ is always over three orders of magnitude higher than $t_0$, the last term in equation (3.5) can be approximated as 1. Then, equation (3.5) can be simplified to

$$R_{pp} = \frac{\sqrt{N}}{4} \left( \frac{\alpha-1}{\alpha} \right)$$

(3.6)

The drift time of a single ion can be defined as

$$t_d = \frac{L}{v_d}$$

(3.7)

where $L$ is the length of the drift region and $v_d$ is the drift velocity of the ion. Using equation (1.14) in Chapter 1, one can obtain

$$t_d = \frac{L^2}{KV}$$

(3.8)

IMS peak shape is mainly determined by the initial shape of the ion packet admitted to the drift region and the diffusional broadening of the ion packet as it travels toward the detector

$$\omega^2 = t_g^2 + t_{diff}^2$$

(3.9)

where $t_g$ is the FWHH of the initial Gaussian shape pulse gated into the drift region and $t_{diff}$ is the FWHH of the Gaussian peak produced by diffusional broadening of an infinitely narrow initial pulse.

However, more strictly, the initial pulse is never Gaussian. First, the control of gating is usually operated by a step function. Spangler and Collins first simulated the experimental IMS peak shape by convoluting a step function as the initial pulse shape with a Gaussian function to represent diffusional broadening. Although the electrical potential applied to the ion gate could be approximated by a step function, Aronson’s
gating model has shown that ion intensity across a closed gate drops to zero considerably less rapidly than a step function, and this profile is retained by the leading edge of an ion packet admitted to the drift region when the gate is opened. It also shows that the trailing edge, created as the open gate suddenly closes, slopes less abruptly than a step function. In addition, the position and slope of the leading and trailing edges were found to depend on the details of the gating (i.e., gate closure field, wire diameter, spacing, etc.).

Second, Aronson’s model neglects diffusion while a pulse is being collected in the detection region, which causes the trailing edge to be more diffusive than the leading edge. Although these problems exist, equation (3.9) is still the best approximation of IMS peak width.

The spatial spreading of an ion peak, $\sigma_{\text{space}}$, can be defined as

$$\sigma_{\text{space}} = \left(2Dt_d\right)^{\frac{1}{2}}$$  \hspace{1cm} (3.10)

where $D$ is the diffusion coefficient (cm$^2$s$^{-1}$). In a low electric field, the diffusion coefficient is satisfied by the Einstein relationship

$$D = \frac{\eta kT K}{q}$$  \hspace{1cm} (3.11)

where $\eta$ is the Townsend energy factor, $k$ is the Boltzmann constant, $T$ is the temperature (Kelvin) in the drift region, and $q$ is the ion charge (Coulomb). Theoretically, $\eta$ is in the range of 1 to 3. However, under conditions common in IMS, $\eta$ is known to be close to 1. Then, equation (3.11) can be simplified as

$$D = \frac{kT K}{q}$$  \hspace{1cm} (3.12)

Combining equations (3.8), (3.10) and (3.12) gives
\[
\sigma_{\text{space}} = \left(\frac{2kT}{qV}\right)^{\frac{1}{2}} L
\]

(3.13)

The spatial spreading of the ion pulse is related to the temporal duration measured at the collector, \(\sigma_{\text{time}}\), by \(^\text{(3.14)}\)

\[
\frac{\sigma_{\text{space}}}{L} = \frac{\sigma_{\text{time}}}{t_d}
\]

(3.14)

Since the standard deviation and the FWHH have the relationship

\[
t_{\text{diff}} = \left(\frac{16\ln 2kT}{qV}\right)^{\frac{1}{2}} t_d
\]

(3.15)

equations (3.14) and (3.15) can be combined to give

\[
\omega^2 = t_g^2 + \frac{16\ln 2k}{q} \frac{T_t^2}{V}
\]

(3.16)

Again, for simplicity, equation (3.16) can be modified as

\[
\omega^2 = \gamma^2 + \beta t_g^2 + \alpha \frac{T_t^2}{V}
\]

(3.17)

where \(\alpha, \beta\) and \(\gamma\) are parameters that can be mathematically adjusted to give the best fit to \(\omega, t_g, T, t_d\) and \(V\). Combining equation (3.16) with the description of resolving power for IMS

\[
R = \frac{t_d}{\omega}
\]

(3.18)

the theoretical resolving power in IMS, \(R_t\), can be represented by

\[
R_t = \frac{t_d}{\sqrt{t_g^2 + \frac{16(\ln 2)kT_t^2}{qEL}}}
\]

(3.19)
However, as described before, this model also does not consider the electric field and gas flow inhomogeneities, coulombic repulsion, and ion-ion and ion-molecule interactions. Therefore, it is not suitable for routine use, but only for theoretical considerations. The experimental resolving power, $R_e$, can be defined as

$$R_e = \frac{t_d}{\alpha_b}$$

(3.20)

An obvious difference between $R_t \sim R_e$ and $R_{pp}$ is that only one peak is required to calculate $R_t \sim R_e$, whereas two are required to calculate $R_{pp}$.

To obtain an expression for the relationship of pressure in the drift region to resolving power, one divides equation (3.17) by $t_d^2$

$$R^{-2} = \frac{\gamma + \beta t_g^2}{t_d^2} + \frac{\alpha T}{V} = R_p^{-2} + R_d^{-2}$$

(3.21)

where

$$R_p = \frac{t_d}{\left(\gamma + \beta t_g^2\right)^{\frac{1}{2}}} = \frac{L^2}{\left(\gamma + \beta t_g^2\right)^{\frac{1}{2}} K V}$$

(3.22)

is the pulse-width-only resolving power, the resolving power that would be observed if diffusion were insignificant, and

$$R_d = \left(\frac{V}{\alpha T}\right)^{\frac{1}{2}}$$

(3.23)

is the diffusion-only resolving power, the resolving power that would be obtained with zero initial pulse width and $\gamma = 0$. The overall resolving power is dominated by the smaller of $R_p$ and $R_d$.\textsuperscript{14}

The reduced mobility of an ion, $K_0$, can be described as
\[ K_0 = K \left( \frac{273}{T} \right) \left( \frac{P}{760} \right) \]  

(3.24)

where \( T \) (Kelvin) and \( P \) (Torr) are the temperature and pressure in the drift region, respectively. Combining equation (3.21) with (3.24) gives

\[ R^{-2} = \kappa P^{-2} + \lambda \]  

(3.25)

where \( \kappa \) and \( \lambda \) are linear coefficients.

In this chapter, I report two newly designed mechanical ion gates that more effectively assist in gating sprayed ions from the ESI source into the drift region of the IMS with improved sensitivity and resolution at atmospheric pressure. Applying a voltage to the ion gate and using a high flow drift gas helped to further improve the performance of the ESI-IMS. In addition, several groups have reported the incorporation of reduced pressure electrospray ionization sources with a reduced pressure ion mobility spectrometer.\(^8\)\(^{22-31}\) Theoretically, this technique should help to improve IMS resolution by eliminating clustering and multiple peaks. Therefore, in this chapter, I also report on the design, construction and evaluation of IMS systems that can operate under reduced pressure conditions with atmospheric pressure ESI.

### 3.2 Electro spray Ionization for Atmospheric Pressure Ion Mobility Spectrometry

#### 3.2.1 Experimental Section

*Chemicals and materials.* Since high voltages were used for the ESI-IMS, safety precautions were taken to protect researchers from electrical shock. The high voltage power supplies and ESI source were electrically shielded using insulated plastic boxes, and they were moved as far as possible away from any metal parts. High voltage cables were tested for electrical leakage at 1.2 times the highest operating voltage.\(^{32}\)
HPLC grade methanol and water were purchased from Mallinckrodt Baker (Paris, KY, USA). Glacial acetic acid was obtained from EM Science (Gibbstown, NJ, USA). Benzodiazepines (i.e., diazepam and prazepam), antidepressants (i.e., nortriptyline and imipramine), antibiotics (i.e., ampicillin and cloxacillin) and gramicidin s were acquired from Sigma (St. Louis, MO, USA). All solutions were prepared by dissolving the solid analyte compounds in a mixture of methanol/water/acetic acid (49.5:49.5:1.0 or 69.5:29.5:1.0 v/v/v). Compressed nitrogen (purity 99.9% to 99.99%) was purchased from Airgas (Salt Lake City, UT, USA) and used as drift gas to assist in desolvation.

**Instrumentation.** A schematic diagram of the new ESI-IMS system is shown in Figures 3.1 and 3.2. It consists of six regions: (i) microspray (μESI) ion source, (ii) chopper wheel, (iii) optical sensor, (iv) drift tube, (v) aperture grid, and (vi) detection region.

The μESI ion source was operated with a continuous infusion flow rate of 1.0 to 4.0 μL min⁻¹ using a syringe pump (Model 55-2222, Harvard Apparatus, Holliston, MA, USA) and a 250 μL syringe with a 21 gauge needle (Gastight®, Hamilton, Reno, NV, USA). Tapered fused silica electrospray tips (~ 2.5 cm long, 90 μm o.d., 20 μm i.d.) were obtained from LECO (Part 711-955, St. Joseph, MI, USA). A voltage of +5.0 kV was applied to the μESI ion source using a high voltage power supply (Series 230, Bertan, Hicksville, NY, USA).

Figure 3.3 shows a diagram of the chopper wheel used in the ESI-IMS system. The chopper wheel (3.5 mm thick, 19.2 cm o.d.), similar to that reported by Katta et al., was machined out of aluminum. It contained two oval windows: one was the sensor window (2.0 mm wide, 7.0 mm high) at the edge of the chopper wheel and the other was the
Figure 3.1 Photographs of the new ESI-IMS instrument observed from (A) left side and (B) behind.
Figure 3.2 Schematic diagram of the construction of the new ESI-IMS system. (1) microspray (μESI) ion source, (2) optical sensor emitter, (3) optical sensor collector, (4) aluminum adaptor, (5) chopper wheel, (6) inlet window, (7) heating bands, (8) interface plate sampling inlet, (9) drift tube, (10) Teflon washer, (11) DC motor, (12) housing, (13) aperture grid, (14) Faraday plate, (15) back plate.
Figure 3.3 Schematic diagram of the chopper wheel. (1) Connection holes, (2) sample inlet window, (3) sensor window.
sample inlet window (8.0 mm wide, 3.0 mm high) positioned between the connection holes and the edge of the chopper wheel. A voltage of +500 V was applied to the rotating chopper wheel with a wire brush to help draw electrosprayed ions into the drift region using a separate Bertan Series 230 high voltage power supply. A +12 V DC motor (Miniature series, PITTMAN, Harleysville, PA, USA) was mounted on the interface plate and connected to the chopper wheel through an aluminum adaptor and the connection holes. Using a low voltage power supply (Model 2762, Heath, Taiwan, ROC), the DC motor rotated the chopper wheel parallel to the interface plate.

During most of the time, the ion gate was closed since the chopper wheel blocked ion transmission from the ESI source into the drift region, and it also blocked transmission of IR light from the optical sensor emitter (EE-SV3, Omron Electronic Components, Schaumburg, IL, USA) to the collector, synchronously. When the sample inlet window swept across the sampling inlet of the IMS, sample from the ESI tip entered the drift region (i.e., the ion gate was open). The distances between the ESI tip and sample inlet window and between the sample inlet window and sampling inlet were 2.0 mm and 5.0 mm, respectively. At the same time, the sensor window swept across the window of the optical sensor. The light signal from the emitter passed through the window to the collector and was detected by the optical sensor. Periodic pulses (pulse rate: 5-200 Hz, pulse width: 200-500 µs) could be generated for initiation and synchronization by simply adjusting the speed of the rotating chopper wheel. In my experiments, for every 100 to 200 ms, the “chopper” ion gate stayed open for 200 to 500 µs to allow electrosprayed ions to enter the drift region. The pulse width could also be adjusted without affecting the period by decreasing the area of the sample inlet window.
with copper tape (Stewart-MacDonald, Athens, OH, USA).

The drift region was operated under atmospheric pressure conditions (650 Torr in Provo, UT, USA). The total length of the drift tube was 45.0 cm. It consisted of 78 stainless steel rings (0.12 cm thick, 4.90 cm o.d., 2.55 cm i.d.). Each stainless steel ring was welded to a 1.0 cm long stainless steel lead. Between each lead was attached a high voltage resistor (3.3 MΩ, YAGEO, Taiwan, ROC). All of the stainless steel rings were 4.6 mm apart and connected together in series with a high voltage resistor between each. The interface plate was electrically grounded with a high voltage resistor between the interface plate and the first ring of the drift tube. It was heated up to 50 °C by two rectangular heating bands (Flexible Series, Watlow, St. Louis, MO, USA) to assist in desolvation of the electrosprayed ions, and electrically insulated from the chopper wheel using a Teflon washer. The temperature in the drift region was 20.0 to 22.0 °C. The round interface plate sampling inlet (2.5 mm i.d.) was centered on the interface plate. The back plate was electrically connected to a high voltage power supply (Series 225, Bertan, Hicksville, NY, USA) operated at -20.0 kV with a high voltage resistor between the back plate and the last ring of the drift tube. The drift tube was maintained at an electric field strength of ~ 400 V cm⁻¹. Nitrogen was used as the drift gas, which was introduced into the drift region from two 2.8 mm i.d. holes drilled in the back plate on each side of a mounted and electrically isolated Faraday plate detector. The drift gas was adjusted to a flow rate of 100 to 1500 mL min⁻¹ using a flowmeter obtained from Jaco (Berea, OH, USA).

The detector was a small round copper Faraday plate (5.5 mm o.d.) positioned at the end, and in the center, of the drift tube. Attached to the last ring was a stainless steel
screen operated as an aperture grid centered with the Faraday plate. The aperture grid consisted of parallel stainless steel wires (150 μm o.d.) placed 500 μm apart. When the gated ion packet approached the detector, the electrostatic field generated by the ion cloud induced an electrical current in the detection circuit with an image charge (δ) on the ion collector. Therefore, the collector sensed the ion cloud, but not the direct ion current. When the ion packet actually hit the ion collector, it also caused an electrical current in the detection circuit. The directions of the induced and direct current flows were the same. Thus, the two current flows were additive as the ion packet approached and hit the ion collector. When an aperture grid was placed close to the ion collector, it intercepted the electrostatic field generated by the approaching ion cloud. Theoretically, there was no induced electrical current in the detection circuit. In order to avoid the capacitance effect, the aperture grid was connected with a grounded “ballast” capacitor, a high pass filter, to drain the induced current away from the ion collector. Using the aperture grid, induced current, which was generated within the Faraday plate prior to ions striking the plate, could be effectively eliminated, thus, reducing peak fronting and improving resolution. Upon ions striking the Faraday plate, a very weak ion current was produced. Using a newly designed in-house current amplifier capable of operating up to –20.0 kV, the ion current was amplified, optically transferred, converted to a voltage signal, and finally displayed and recorded using an oscilloscope (9410, Dual 150 MHz, LeCroy, Chestnut Ridge, NY, USA). Data were averaged during acquisition. According to the abundance of analyte ions, 8 to 256 spectra were averaged.

When employing a Bradbury-Nielsen ion gate for data comparison, it was placed in front of the first ring of the drift tube (400 V cm⁻¹ electric field strength) without the
interface plate. The ion gate contained two electrically isolated sets of wires. During most of the time in the positive ion mode, the gate was closed when +200 V was applied to one set of wires, and +240 V to the other, thus, creating an electric field stronger than, and orthogonal to, the electric field in the drift region. Theoretically, electrosprayed ions deflected to, and collided with the +200 V wire set rather than continuously entering the drift region. When the gate was open, a voltage of +200 V was applied to both sets of wires. The drift region was heated through two rectangular heating bands attached to the metal housing. When comparing data, the experimental conditions, especially temperature in the drift region as well as pulse rate and width, were exactly the same as using the mechanical ion gate.

3.2.2 Results and Discussion

The new ESI-IMS instrument was evaluated and optimized. Figures 3.4A-C show IMS spectra obtained for diazepam (200 µM) and prazepam (220 µM) at different drift gas flow rates. At a high flow rate of 1500 mL min$^{-1}$, the separation of these two compounds ($R_{pp} = 2.88$, RSD = ±14%) was significantly better than at low flow rates of 100 mL min$^{-1}$ ($R_{pp} = 0.67$, RSD = ±16%) and 900 mL min$^{-1}$ ($R_{pp} = 1.26$, RSD = ±15%). At high flow rate, both peaks shifted to faster drift times due to improved declustering under the influence of the higher counter gas flow. Diazepam moved more than prazepam so that these two peaks, which overlapped at 100 mL min$^{-1}$, were completely separated at 1500 mL min$^{-1}$. The adjustment of selectivity through tuning the drift gas flow rate was consistent with an earlier report. Figure 3.4 D shows the IMS spectrum obtained for diazepam (200 µM) and prazepam (220 µM) at a drift gas flow rate of 1500 mL min$^{-1}$ without voltage application to the chopper wheel. As compared to Figure 3.4 A, applying
a voltage to the chopper wheel helped to draw ions through the sample inlet window into the drift region of the IMS when the ion gate was open. We observed that when a voltage of +500 V was applied to the chopper wheel, the ion peak intensities ($S/N_{\text{diazepam}} = 130$, $\text{RSD} = \pm 11\%$; $S/N_{\text{prazepam}} = 140$, $\text{RSD} = \pm 9\%$) were stronger than without voltage application ($S/N_{\text{diazepam}} = 18.3$, $\text{RSD} = \pm 12\%$; $S/N_{\text{prazepam}} = 22.1$, $\text{RSD} = \pm 13\%$).

Under the optimum conditions, a variety of compounds ranging from small molecules to macromolecules were dissolved in buffer solution and analyzed using the ESI-IMS system. Figure 3.5 shows an IMS spectrum of a mixture of histidine (150 μM), diazepam (200 μM), prazepam (220 μM) and gramicidin s (100 μM). Figure 3.6 shows an IMS spectrum of a mixture of drugs, i.e., nordoxepine (100 μM), imprimine (100 μM), ampicillin (120 μM) and cloxacillin (120 μM). For both spectra, baseline resolution was observed for all analyte ions. However, multiple peaks for single compounds could sometimes be observed in the IMS spectra (i.e., dimers, trimers, multiply-charged peaks, etc.). In Figure 3.6, the two small peaks adjacent to nordoxepine and imprimine are probably peaks related to these two compounds. When analyzing samples of the two compounds at different concentrations, the two small peaks always appeared in approximately the same relative intensities to nordoxepine and imprimine (data not shown). By heating the interface plate and using a long drift tube at a low electrospray flow rate, desolvation was usually quite successful, and the appearance of multiple peaks was not a serious problem.

Using the new ESI-IMS instrument, $R_t$ and $R_c$ were calculated to be 120-200 and 55-90 ($\text{RSD} = \pm 14\%$), respectively, with plate numbers of 120,000-160,000 (e.g., Figures 3.4 A, 3.5 and 3.6, $\text{RSD} = \pm 16\%$). Prazepam (70 μM), shown in Figure 3.7, demonstrated an
Figure 3.4 IMS spectra of diazepam (200 μM) and prazepam (220 μM) at different gas flow rates and with/without voltage applied to the “chopper” ion gate. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 3.0 μL min⁻¹ infusion flow rate (methanol/water/acetic acid, 49.5:49.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, (A) N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate, (B) N₂ drift gas at 100 mL min⁻¹ and +500 V applied to the ion gate, (C) N₂ drift gas at 900 mL min⁻¹ and +500 V applied to the ion gate, (D) N₂ drift gas at 1500 mL min⁻¹ and “floating” ion gate. Peak identifications: (1) solvent, (2) diazepam, (3) prazepam.
Figure 3.5 IMS spectrum of a test mixture using the “chopper” ion gate. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 3.0 μL min⁻¹ infusion flow rate (methanol/water/acetic acid, 49.5:49.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate. Peak identifications: (1) solvent, (2) histidine (150 μM), (3) diazepam (200 μM), (4) prazepam (220 μM), (5) gramicidin s (100 μM).
Figure 3.6 IMS spectrum of a mixture of antibiotics using the “chopper” ion gate. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 3.0 μL min⁻¹ infusion flow rate (methanol/water/acetic acid, 49.5:49.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate. Peak identifications: (1) solvent, (2) nordoxepine (100 μM), (3) imprimine (100 μM), (4) ampicillin (120 μM), (5) cloxacillin (120 μM).
Figure 3.7 IMS spectrum showing moderate resolving power for prazepam (70 μM) using the “chopper” ion gate. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 2.0 μL min⁻¹ infusion flow rate (methanol/water/acetic acid, 69.5:29.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate. Peak identifications: (1) solvent, (2) prazepam.
experimental resolving power of 74 (RSD = ±12%). Compared to other reports of IMS resolution,\textsuperscript{34-37} this value is moderately high.

Figures 3.8 A and B show IMS spectra of prazepam (220 μM) obtained with the new “chopper” ion gate and a Bradbury-Nielsen ion gate, respectively. After optimization, we observed that the “chopper” ion gate provided higher sensitivity (S/N = 143, RSD = ±13%) and resolution ($R_e = 76.5$, RSD = ±15%), as compared to the Bradbury-Nielsen ion gate (S/N = 115, RSD = ±15%; $R_e = 66.0$, RSD = ±17%). Under the influence of the electric field, ions with high kinetic energy may penetrate through the ion gate even when it is theoretically closed. This ion penetration would cause an increase in background and peak width. The new ion gate eliminated this problem. Other advantages of the new ion gate include excellent day-to-day stability and easy fabrication, operation and maintenance.

Another design investigated was a rounded rectangular (12.3 cm long, 2.9 cm wide) mechanical stainless steel “slider” ion gate (Figure 3.9) that was tested under experimental conditions similar to those described in section 3.2.1 (Figure 3.10). The ion gate consisted of two windows: the square one was the sensor window (2.0 mm wide, 2.5 mm high) at the bottom of the gate, and the round one was the sample inlet window (2.0 mm i.d.) positioned approximately on the center of the gate. A voltage of +5.0 kV was applied to the μESI ion source while +500 V was applied to the “slider” ion gate. A +12 V DC motor was mounted on a frame attached to the interface plate, and the motor shaft was connected to the “slider” ion gate through a stainless steel adaptor, a stainless steel lever and the connection slot. Using a low voltage power supply, a DC motor moved the ion gate horizontally parallel to the interface plate. When the sample inlet window swept
Figure 3.8 IMS spectra for prazepam (220 μM) showing comparison of (A) the novel “chopper” ion gate, and (B) a Bradbury-Nielsen ion gate. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 3.0 μL min⁻¹ infusion flow rate (methanol/water/acetic acid, 69.5:29.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate. Peak identifications: (1) solvent, (2) prazepam.
Figure 3.9 Schematic diagram of the “slider” plate. (1) Connection slot, (2) sample inlet window, (3) sensor window.
Figure 3.10 Photographs of the “slider” ion gate observed from (A) left and (B) right sides.
across the sampling inlet of the IMS, sample from the ESI tip entered the drift region (i.e., the ion gate was open). The distances between the ESI tip and sample inlet window and between the sample inlet window and sampling inlet were 3.0 mm and 6.0 mm, respectively. At the same time, the sensor window swept across the window of the optical sensor. The light signal from the emitter passed through the window to the collector and was detected by the optical sensor. Periodic pulses (pulse rate: 5-50 Hz, pulse width: 200-500 µs) could be generated for initiation and synchronization by adjusting the speed of the ion gate and/or using the ion gates with different sample inlet window diameters. The experimental results were very similar to those obtained using the “chopper” ion gate (Figure 3.11: $R_{pp} = 1.61$, RSD = ±15%; Figure 3.12: $R_e = 71.4$, RSD = ±12%; Figure 3.13). However, because the surface area of the “slider” ion gate across which the electrospray plume swept was much smaller than the “chopper” ion gate surface area, charge build-up from contamination was so fast that the sample inlet window and the surrounding regions needed to be cleaned approximately every 30 minutes. Therefore, this design was not suitable for routine use.

3.2.3 Summary

A novel ion gate for IMS was designed and tested under atmospheric pressure conditions. The ion gate effectively gated electrosprayed ions from the ESI source into the drift region of the IMS system. Both application of a voltage to the chopper wheel and utilization of a high drift gas flow further improved the performance of the new ESI-IMS system. Baseline separations were observed for mixtures of selected compounds with a moderate experimental resolving power of 55 to 90 and plate numbers of 120,000 to 160,000. Finally, under optimum experimental conditions, the new ion gate showed a
Figure 3.11 IMS spectrum of diazepam (200 µM) and prazepam (220 µM) using the “slider” ion gate. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 2.0 µL min⁻¹ infusion flow rate (methanol/water/acetic acid, 69.5:29.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate. Peak identifications: (1) solvent, (2) diazepam, (3) prazepam.
**Figure 3.12** IMS spectrum showing moderate resolving power for prazepam (70 μM) using the “slider” ion gate. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 2.0 μL min⁻¹ infusion flow rate (methanol/water/acetic acid, 69.5:29.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate. Peak identifications: (1) solvent, (2) prazepam.
Figure 3.13 IMS spectrum of a test mixture using the “slider” ion gate. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 3.0 μL min⁻¹ infusion flow rate (methanol/water/acetic acid, 49.5:49.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate. Peak identifications: (1) solvent, (2) histidine (150 μM), (3) diazepam (200 μM), (4) prazepam (220 μM), (5) gramicidin s (100 μM).
moderate increase in sensitivity and resolution, as compared to a Bradbury-Nielsen ion gate.

3.3 Electrospray Ionization for Reduced Pressure Ion Mobility Spectrometry

3.3.1 Experimental Section

Chemicals and materials. HPLC grade methanol and water were purchased from Mallinckrodt Baker (Paris, KY, USA). Glacial acetic acid was obtained from EM Science (Gibbstown, NJ, USA). Benzodiazepines (i.e., diazepam and prazepam), antidepressants (i.e., nordoxepine and imprimine), antibiotics (i.e., ampicillin and cloxacillin) and gramicidin s were acquired from Sigma (St. Louis, MO, USA). All solutions were prepared by dissolving the solid analyte compounds in a mixture of methanol/water/acetic acid (49.5:49.5:1.0 or 69.5:29.5:1.0 v/v/v).

Instrumentation. A schematic diagram of the ESI-reduced pressure IMS system is shown in Figure 3.14. The experimental conditions were similar to those described in section 3.2.1. The μESI ion source was operated with a continuous infusion flow rate of 0.3 to 1.5 μL min⁻¹. A voltage of +4.5 kV was applied to the μESI ion source and +500 V was applied to the chopper wheel. A copper ball valve was inserted between the mechanical pump and the corresponding outlet on the stainless steel housing behind the back plate. The handle position of the valve could be changed to adjust the pressure in the drift region.

Periodic pulses (pulse rate: 5-200 Hz, pulse width: 200-500 μs) were formed for initiation and synchronization using the rotating chopper wheel. In my experiments, for every 100 to 200 ms, the “chopper” ion gate stayed open for 0.2 to 1.0 ms to allow electrosprayed ions to enter the drift region.
Figure 3.14 Schematic diagram of the ESI-reduced pressure IMS instrument. (1) microspray (µESI) ion source, (2) optical sensor emitter, (3) optical sensor collector, (4) aluminum adaptor, (5) chopper wheel, (6) inlet window, (7) heating bands, (8) interface plate sampling inlet, (9) drift tube, (10) Teflon washer, (11) DC motor, (12) housing, (13) aperture grid, (14) Faraday plate, (15) back plate, (16) mechanical pump 1, (17) vacuum ion gauge, (18) mechanical pump 2, (19) ball valve.
The drift region was operated under reduced pressure from 20 to 550 Torr (local pressure: 650 Torr in Provo, UT, USA). The interface plate was electrically grounded with a high voltage resistor between the interface plate and the first ring of the drift tube. The back plate was electrically connected to a high voltage power supply operated at -5.0 kV with a high voltage resistor between the back plate and the last ring of the drift tube. The drift tube was maintained at an electric field strength of 400 V cm\(^{-1}\).

### 3.3.2 Results and Discussion

Reduced pressure in the drift region of the IMS could help to both maintain high resolution and eliminate clustering and multiple peaks. The pressure drop from the ESI source to the drift region (> 100 Torr) formed a high velocity gas flow to direct the electrosprayed ions through the interface orifice into the drift region toward the detector. Two technical problems existed at a reduced pressure of several tens of Torr. First, the relation between the electrical potential, \(U\), and the drift velocity of the ion, \(v_d\), can be described as

\[
Uq = \frac{1}{2}mv_d^2 - \frac{1}{2}mv_0^2
\]

where \(m\) is the mass of the ion and \(v_0\) is the initial velocity of the ion after leaving the ESI tip.

Differentiating both sides of equation (26) and assuming that the initial velocity of the ion was constant gives

\[
\frac{\Box U}{\Box v_d} = \frac{dU}{dv_d} = \frac{m}{q}v_d
\]
The electrosprayed ions migrated much more rapidly in the drift region when using atmospheric pressure ESI-reduced pressure IMS.\(^\text{8,22-31}\) Due to equation (3.27), the velocity differences in analyte ions were too small to be effectively discriminated by the IMS. Second, the breakdown voltage of air according to Paschen’s Law\(^\text{38}\) was critical under these pressure conditions. If the pressure temporarily became ~10 Torr due to the fluctuation of the pumping system, serious arcing would form with only several hundreds of volts through the drift region. This would likely destroy the detector. Theoretically and experimentally, increasing the pressure in the drift region decreased the drift velocity of the electrosprayed ions and enhanced the resolution of the IMS. However, the mechanical pumps used could not hold the high pressures (e.g. > 550 Torr). The practical optimum pressure was set at 450 Torr to provide the best resolution at normal pumping conditions.

Under this optimum condition, a variety of compounds ranging from small molecules to macromolecules were dissolved in buffer solution and analyzed using the ESI-reduced pressure IMS system. Figure 3.15 shows an IMS spectrum of a mixture of diazepam (200 \(\mu\)M), prazepam (220 \(\mu\)M) and gramicidin s (100 \(\mu\)M). The separation of these three compounds was not as good as when using atmospheric pressure IMS. From the spectrum, diazepam and prazepam were not separated, and gramicidin s was only partially separated from the other two compounds.

### 3.4 Conclusions

Two novel ion gates for IMS were designed and tested under atmospheric pressure. The ion gates effectively gated electrosprayed ions from the ESI source into the drift region of the IMS system. Both application of a voltage to the chopper wheel and utilization of a high drift gas flow further improved the performance of the new ESI-IMS
Figure 3.15 IMS spectrum of a test mixture using the reduced pressure IMS. Conditions: 45.0 cm drift tube, 20.0 kV drift voltage, +5.0 kV electrospray, 20.0 to 22.0 °C, 650 Torr, 3.0 μL min⁻¹ infusion flow rate (methanol/water/acetic acid, 49.5:49.5:1.0 v/v/v), 64 averages, 0.5 ms gate pulse width, N₂ drift gas at 1500 mL min⁻¹ and +500 V applied to the ion gate. Peak identifications: (1) solvent, (2) diazepam (200 μM), (3) prazepam (220 μM), (4) gramicidin s (100 μM).
system. Baseline separations were observed for mixtures of selected compounds with a
moderate experimental resolving power of 55 to 90 and plate numbers of 120,000 to
160,000. In addition, the performance of atmospheric pressure ESI-atmospheric pressure
IMS was better than that of atmospheric pressure ESI-reduced pressure IMS.

3.5 References

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CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

4.1 Conclusions

Several atmospheric pressure ion focusing devices were successfully designed and tested with mass spectrometry and ion mobility spectrometry. The major limitation in sensitivity with electrospray ionization-mass spectrometry is due to low ion transmission efficiency from the ESI source to the sampling orifice of the MS.\(^{1-5}\) In order to improve focusing and transporting ions from the electrospray ionization sprayer tip to the sampling nozzle of the mass spectrometer, I designed several ion focusing devices employing aerodynamic focusing (e.g., a concentric high velocity converging gas flow), electrostatic focusing (e.g., a regulated external electrostatic field), or both.

First, a new electrode plate ion lens was designed to electrostatically assist in ion focusing and transmission. A signal enhancement of 2 to 3 times and a method detection limit reduction of 4 times were observed with the ion lens. This enhancement can be attributed to an improvement in the shape of the equipotential lines near the electrospray tip. Furthermore, the longer distance from the sprayer tip to the sampling nozzle of the MS resulted in better desolvation of the ions.

Second, with a similar distribution of equipotential lines near the electrospray tip, we simplified the design of the electrode plate ion lens and modified a new interface plate
to assist in focusing electrosprayed ions toward the sampling nozzle of the MS. A signal enhancement of 5 times and a method detection limit reduction of 7 times were observed.\textsuperscript{6}

Third, we modified a commercial air amplifier that was designed to form a concentric high velocity converging gas flow to aerodynamically improve conduction of ions from the electrospray ion source to the sampling orifice of the mass spectrometer, and applied a voltage to the air amplifier electrostatically to assist in focusing and conduction of ions. Significant signal enhancement and method detection limit reduction were observed when using the air amplifier.\textsuperscript{7}

Fourth, in order to overcome the turbulence in the entrance of the air amplifier which compromised ion transmission, a new ion focusing device based on the air amplifier was designed. When an optimized electric field gradient was applied along the body of the air amplifier, the electrosprayed ions were more effectively focused along the center axis, and directed toward the sampling orifice of the mass spectrometer, thus, increasing the ion transmission from the ESI source into the sampling orifice of the mass spectrometer.\textsuperscript{8} A signal enhancement of 27 times and a method detection limit reduction of 40 times were observed, as compared to conventional ESI.

Electrospray ionization has become one of the most important ionization techniques for ion mobility spectrometry. The major limitation in electrospray ionization-ion mobility spectrometry is its low resolution,\textsuperscript{9-12} and the technical objective of this work was to overcome this problem and improve the resolution of the electrospray ionization-ion mobility spectrometer for high speed separations. First, we designed a couple of mechanical ion gates that were helpful in gating electrosprayed ions from the electrospray
ionization source into the drift region of the ion mobility spectrometer at atmospheric pressure. Applying a voltage to the ion gate and using a high flow drift gas led to further improvement of the performance of the electrospray ionization-ion mobility spectrometer. Baseline separations were obtained for several mixtures of selected compounds with a moderate experimental resolving power of 55 to 90 and plate numbers of 120,000 to 160,000. In addition, under optimum experimental conditions, the new ion gate showed a moderate increase in sensitivity and resolution, as compared to a Bradbury-Nielsen ion gate. Second, reduced pressure ion mobility spectrometry was reported to maintain high IMS resolution and eliminate clustering and multiple peaks. However, from equation (3.25) in chapter 3, increasing the pressure in the drift region should enhance the resolving power in the IMS. Therefore, we carried out the design, construction and evaluation of IMS systems that could operate under reduced or elevated pressures.

4.2 Recommendations for Future Research

4.2.1 Investigation of Biomacromolecules Using the New Ion Focusing Device in Electrospray Ionization-Mass Spectrometry

Although this dissertation discusses the successful incorporation of a series of atmospheric pressure ESI interfaces with mass spectrometry that yielded enhanced sensitivity (Chapter 2), there are still improvements that could be made. Using a new interface plate to assist in ion focusing and transmission instead of the commercial one, significant signal enhancement and method detection limit reduction (i.e., 7 times) were observed. The equipotential lines near the sprayer tip were flattened, and even reversed, to electrostatically reduce defocusing effects and to improve ion transmission. However,
the interface plate was specifically designed for and tested with the Jaguar time-of-flight mass spectrometer. Electrostatic focusing by an electrostatic field from the ESI sprayer tip to the sampling nozzle of the mass spectrometer, and proper adjustment of the gas flow properties, such as the amount of curtain gas flow and the aerodynamics at the holes of the ion lens and/or the interface plate, were also very important. Therefore, interface plates or sampling interfaces (e.g., heated capillary interface) suitable for other mass spectrometers should be possible.

A new ion focusing device that provided aerodynamic and electrostatic focusing effects, based on a commercial air amplifier, assisted in focusing electrosprayed ions along the axial direction toward the sampling orifice of a mass spectrometer. A significant sensitivity enhancement was observed using different mass spectrometers.\textsuperscript{8,14,15} The optimum conditions were very similar, however, not the same. Therefore, in order to obtain the best amplification, careful optimization of each instrument must be done. In addition, significantly improved mass spectrometer sensitivity should be capable of solving difficult problems involving trace components.

Due to the recent trend of analysis in biomacromolecules (e.g., peptide mapping and sequencing) using mass spectrometry, more applications of the new ion focusing device to real samples, e.g., blood and urine, should be carried out.

4.2.2 Investigation of Pharmaceutical Small Molecules Using Electrospray Ionization-Ion Mobility Spectrometry

Today, demands for greater sample throughput and faster results in urgent situations have driven the creation of fast or ultra-fast analytical methods, especially in rapid drug discovery in the pharmaceutical industry. This trend requires modification of
conventional separation techniques, e.g., gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE), and development of new techniques for high throughput screening (i.e., <1 min analysis time per sample). A mechanical ion gate was used to gate electrosprayed ions from the ESI source into the drift region of the IMS system, and showed a moderate increase in sensitivity and resolution, as compared to a Bradbury-Nielsen ion gate, the most commonly used ion gating technique in ion mobility spectrometry. Since the normal separation time in IMS ranges from several hundred microseconds to milliseconds, this technique offers the greatest analysis speed compared to other conventional separation techniques. Applications using the new ion gate for high through-put analysis of pharmaceutical compounds should be conducted.

4.2.3 Design, Construction and Evaluation of Elevated Pressure Ion Mobility Spectrometry

Another method to improve the resolution of IMS is to pressurize the drift region (i.e., > 20 psi). From equation (3.25), it is obvious that increasing the pressure in the drift region will enhance the resolving power. This is due to more efficient heat transfer and collisions between ion clusters and neutrals. Moreover, increasing the pressure in the drift region should allow a high electric field to further improve the resolution.

4.3 References


