Nanopore Mass Spectrometry

William Maulbetsch

Advisor: Dr. Derek Stein

Department of Physics
Brown University

This dissertation is submitted for the degree of

Doctor of Philosophy

Brown University May 2018
I would like to acknowledge all of my friends and family who have helped me throughout the years to achieve my goal of producing this very long and tedious document. In particular I thank my parents for always encouraging, or at least putting up with, my curiosity growing up. I thank the FP, and the greater FP community, for all of the goofs and guidance. I take everyone and everything I’ve learned with me as I venture forth into the great beyond academia. Special thanks goes to Ben Wiener whose support as a friend in life and fellow scientist in the lab cannot be overstated and will forever be cherished by me. Of course, part of this achievement belongs to all of the current and former members of the Stein lab. In particular I thank post docs Joe Bush and Mathilde Lepoitevin, as well as Professor Derek Stein without whom any of this research would have been possible. Derek has been a tremendous advisor who has always encouraged development of my own personal interests in what graduate school has to offer. Finally, I dedicate this thesis to one of my greatest cheerleaders throughout the experience, Marianna Curtin. Although she may not be able to see me graduate in a physical form, I know that the parts of her that continue to live inside my friends and family are proud to see me finally complete my degree.
# Table of contents

1 Introduction 1

1.1 Single molecule biopolymer sequencing with nanopore mass spectrometry 1

1.1.1 Challenges that must be overcome for this sequencing method to succeed 2

1.2 Background on early studies of electrospray 2

1.3 Electrospray as a source of ions for mass spectrometry 8

1.3.1 Electrohydrodynamic ionization mass spectrometry 8

1.3.2 Electrospray ionization mass spectrometry 9

1.3.2.1 From charged drops to bare ions with the help of a background gas 10

1.3.2.2 Conventional electrospray ionization mass spectrometry is incompatible with biopolymer sequencing 12

1.3.3 Ion evaporation: the emission of ions directly from charged liquid surfaces 12

1.3.4 Ion evaporation competes with the release of charged droplets 14

1.3.5 Insights into ion evaporation from the fields of nanoelectrospray and of space propulsion 14

1.3.6 Clearing up some terminology 17

1.4 Preserving the sequence of monomers before they are emitted into vacuum 18

1.5 Thesis Outline 19

1.5.1 Chapter 2: The nanopore mass spectrometer 19

1.5.2 Chapter 3: Mass spectra from ions emitted directly into high vacuum from both formamide and water solutions 20

1.5.3 Chapter 4: Studies on the mechanisms of charge emission from nanoscale capillary tips 20

1.5.4 Chapter 5: Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer 21
# Table of contents

1.5.5 Chapter 6: Conclusions and recommendations ................................ 22

2 The nanopore mass spectrometer .................................................. 23
   2.1 Introduction ............................................................................ 23
   2.2 Instrument overview .............................................................. 23
   2.3 Nanopore ion source ............................................................... 25
      2.3.1 Capillary nanotips ............................................................ 28
      2.3.2 Chip-based nanopores ...................................................... 29
      2.3.3 Tube-in-tube fluid delivery system .................................... 31
      2.3.4 Custom software ............................................................. 31
   2.4 Mass filter .............................................................................. 33
   2.5 Vacuum system ...................................................................... 35

3 Mass spectra from ions emitted directly into high vacuum from both formamide and water solutions ......................................................... 37
   3.1 Electrospray directly into high vacuum ...................................... 37
   3.2 Mass Spectra ........................................................................... 39
      3.2.1 Simple salts ................................................................. 39
      3.2.2 Mass spectra of organic molecules ................................... 42

4 Studies on the mechanisms of charge emission from nanoscale capillary tips .............................................................. 45
   4.1 Electrospray current and onset voltage measurements ................. 45
      4.1.1 Experimental setup for measuring electrospray onset voltages ... 46
      4.1.1.1 Applying voltages much greater than the onset voltage induces a mostly irreversible decrease in electrospray current 46
      4.1.1.2 Programmatic determination of onset voltage ................. 50
      4.1.2 Inner versus outer diameter .............................................. 54
      4.1.3 Onset voltages do not depend on solution conductivity .......... 55
      4.1.4 Theoretical model of the electrospray onset voltage ............. 58
         4.1.4.1 Semi-infinite line charge approximation ....................... 58
         4.1.4.2 Solving Laplace’s equation around a spherical meniscus in the sphere-on-cone (SOC) model .................. 62
         4.1.4.3 Comparison of semi-infinite line charge theory with the SOC model ................................................. 65
   4.2 Flow rate versus tip size .......................................................... 67
      4.2.1 Experimental methods ...................................................... 67
      4.2.2 Poiseuille flow through an infinite truncated cone ............... 70
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Section</th>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.3</td>
<td>Fluid conductance as a function of capillary tip size</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>Maximizing ion emission over drop production using nanocapillaries</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>4.3.1</td>
<td>Experimental methods</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>4.3.2</td>
<td>Varying tip size and solution conductivity</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>4.3.3</td>
<td>Increasing flow rates through applied pressure decreases ion transmission efficiencies</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>Theoretical model</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>5.1.1</td>
<td>The probability of particles crossing</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>5.2</td>
<td>Preserving the order of DNA mononucleotides entering a mass spectrometer</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>5.2.1</td>
<td>Elongational force gradients in an electrospray ion source</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>5.2.2</td>
<td>Cleaving the monomers of a stretched polymer by photofragmentation</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>5.2.3</td>
<td>Cleaving DNA with an exonuclease</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>5.2.4</td>
<td>The influence of circulating flows in the Taylor cone</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>5.2.5</td>
<td>The implications for sequencing single biopolymers</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>Conclusion</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Conclusions and recommendations</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

1.1 Single molecule biopolymer sequencing with nanopore mass spectrometry

The research presented here is motivated by an idea for a new method of sequencing single biopolymers, including proteins and nucleic acids, that combines mass spectrometry with nanopores. The basic idea is to take advantage of a mass spectrometer’s ability to identify monomers by their mass to charge ratios, paired with a nanopore’s ability to force biopolymers into a linear configuration so that their monomers are delivered into the mass spectrometer in sequence [17]. This concept is shown schematically in Fig. 1.1.

Fig. 1.1 Schematic of new protein sequencing concept.
A polymer in solution inside a needle-like capillary narrow enough to force it into a linear conformation is pulled through by an applied electric field. As it translocates the nanopore at the tip of the capillary, monomers are processively cleaved from the polymer’s leading end. These monomers are transferred from liquid into vacuum as ions, and sent through a mass spectrometer to determine their charge-to-mass ratios with the sequence intact.

1.1.1 Challenges that must be overcome for this sequencing method to succeed

We perceive three major technical challenges that must be overcome for our sequencing technology to work. First, a cleaving mechanism must be found to reliably break polymers into their constituent monomers as the polymer translocates the nanopore. Second, the monomers must be transferred from a liquid to a charged gas phase as ions for the mass spectrometer to analyze them, and this transfer must be done in such a way that all monomer ions can be collected in order at the mass spectrometer. Third, order between neighboring monomers must be preserved between when they are cleaved, and when they exit into this charged gas phase. This is because before monomers are transferred into vacuum, but after they are cleaved in solution from their parent polymer, they undergo random thermal motion which tends to randomize their sequential order. This thesis is based on work done to overcome the second and third challenges: how to deliver monomers as ions directly from the liquid surface into vacuum, and what conditions will preserve sequence information against the randomizing forces associated with Brownian motion.

1.2 Background on early studies of electrospray

If a charged object is brought near a liquid surface, ions in the liquid attracted to the object will bunch up at the surface and cause it to bulge. If the electric field caused by the object is strong enough, the charged liquid protrusion will take the shape of a cone and emit a spray of ions. The emitted ions in this spray can leave the liquid surface in two main ways. They can leave inside large multiply charged liquid drops. Experiments combining current and flow rate measurements with Doppler anemometry and flash shadowgraphs have found the typical size of these drops to be 10’s of micrometers in diameter containing many containing millions of ions [30], although drops as small as 10 nm in diameter have been observed with ion mobility mass spectrometers [3]. Alternatively, it is possible for them to leave the surface individually as bare, or partially solvated, ions. A partially solvated ion
simply refers to an ion with a handful of solvent molecules stuck to it, and often goes by the name ion cluster.

The earliest documented demonstration of this phenomenon dates back to the year 1600, when William Gilbert published his treatise on electricity and magnetism, *De Magnete*. Gilbert observed, "Indeed it plainly does draw the body itself in the case of a spherical drop of water standing on a dry surface; for a piece of amber applied to it at a suitable distance pulls the nearest parts out of their position and draws it up into a cone...". Charged liquid surfaces were addressed next in 1882 by Lord Rayleigh, who calculated a theoretical limit on the number of charges a liquid drop of a given size can stably hold, now called the Rayleigh limit [55]. Rayleigh assumed that in a stable charged drop the Coulomb forces pushing charges at the liquid surface away from each other must be balanced by surface tension forces pulling them together. These surface tension forces create what is known as a Laplace pressure in the drop that scales inversely with the drop’s size. By equating the electrostatic pressure pushing outwards on the drop’s surface with the Laplace pressure pulling inwards, Rayleigh was able to find the maximum number of charges that the surface tension of a drop of a given size could stably keep inside.

The phenomenon of spray extracted from liquid surfaces with electric fields was explored more systematically in 1914 with experiments conducted by John Zeleny. Zeleny photographed the surfaces of liquid drops at the tips of glass capillaries 1 mm in diameter filled with ethyl alcohol and dilute hydrochloric acid, and exposed to various applied electric field strengths and fluid pressures [86, 87]. He observed the formation of liquid jets at the tips of the glass capillaries, that ultimately broke up into many small charged drops. He also measured the current leaving the glass capillaries, noting spray modes in which intermittent current correlated with oscillations of the liquid meniscus. In 1964, G. I. Taylor, interested in how large drops disintegrate into smaller ones in strong electric fields, picked up where Zeleny left off. He predicted theoretically the shape a liquid surface forms at the critical voltage where a spray of ions is released, and compared this with liquid interfaces between water and oil, and soap films in air. He was able to show that a conical interface between two liquids in an electric field can exist in equilibrium, but only when the cone half angle is 49.3° [73]. That angle is now known as the Taylor angle, and the cone shape liquids take at this critical voltage is called a Taylor cone. Fig. 1.2 shows the shape and angle of such a Taylor cone at the interface between water and oil taken from Taylor’s original paper. It is water that takes the cone shape embedded in oil.

The onset of the formation of a Taylor cone is sudden. An example of this shape change can be seen in Fig. 1.3 for a glass capillary with a 150 µm inner diameter and a 360 µm outer diameter at its tip. As the applied voltage between the liquid and an electrode near the
Fig. 1.2 Image of a Taylor cone formed from a water drop embedded in oil showing the Taylor angle from G.I. Taylor’s original paper [73].

Liquid’s surface increases, the liquid meniscus begins to deform and protrude. Eventually a critical voltage is reached, called the onset voltage, and the meniscus pops into a Taylor cone. The release of current from the liquid surface happens concurrently with the formation of a Taylor cone. Just as with the sudden shape change, a sudden rise in current is also measured as the voltage applied to the liquid surface reaches the critical onset voltage. The technique by which current is extracted from liquid surfaces through the application of strong electric fields is now known as electrospray.

Taylor found the electric field strength and angle of the stable equilibrium cone shape of electrified liquid surfaces through a balance between the Laplace pressure from surface tension and the electrostatic pressure from the applied voltage at the onset of electrospray. The Laplace pressure from surface tension is related to the surface curvature so that:

$$\Delta P = \gamma \left( \frac{1}{r_1} + \frac{1}{r_2} \right)$$  \hspace{1cm} (1.1)

where $r_1$ and $r_2$ are the principal radii of curvature which denote the maximum and minimum curvature of the surface, and which are orthogonal to one another. In the case of a cone, one of the principal radii is the straight line running the radial length along the cone, so that $r_1 = \infty$, which results in zero curvature. The other principal radius forms an ellipse wrapping around the cone surface perpendicular to the line with zero curvature as shown in Fig. 1.4.
1.2 Background on early studies of electrospray

Fig. 1.3 Images of the tip of a capillary with a 150 µm inner diameter and a 360 µm outer diameter. The meniscus starts to bulge out before ultimately taking on the Taylor cone shape as spray emission occurs. Taken from the thesis of Ranato Krpoun, "Micromachined Electrospray Thrusters for Spacecraft Propulsion", Ecole Polytechnique Federale De Lausanne [40].

The radius of curvature of the ellipse at this point on the cone is

$$ r_2 = \frac{b^2}{a} $$

(1.2)

where $a$ is the semi-major axis and $b$ is the semi-minor axis. We can relate the semi-major and semi-minor axes through the eccentricity, $e$, of the ellipse as follows:

$$ b = a\sqrt{1-e^2}. $$

(1.3)

So that

$$ r_2 = \frac{a^2(1-e^2)}{a} = a(1-e^2) $$

(1.4)

Now the eccentricity of an elliptical conic section can be found to be:

$$ e = \frac{\cos \beta}{\cos \alpha}. $$

(1.5)

where $\beta$ is the angle between the plane creating the ellipse and the central axis and $\alpha$ is the cone half angle, measured between the surface of the cone and the central axis [75]. Since this ellipse is confined to be along one of the principle axis of the point on the cone surface,
Fig. 1.4 Laplace pressure from surface tension on a surface depends on the principle axes of curvature, \( r_1 \) and \( r_2 \), at each point \( \vec{R} \) on the surface, which is the distance away from the cone’s apex. \( r_1 \) is the flat curvature along the length of the cone surface, and \( r_2 \) defines the curvature on the ellipse, duplicated next to the cone, with semi-major axis, \( a \), and semi-minor axis, \( b \).
1.2 Background on early studies of electrospray

\[ \beta = \frac{
\pi}{2} - \alpha, \text{ so that } \cos \beta = \sin \alpha \text{ and } e = \tan \alpha. \text{ Thus} \]

\[ r_2 = a(1 - \tan^2 \alpha). \quad (1.6) \]

A relationship can be made using the right triangle with sides \( R, 2a, \) and angle \( 2\alpha, \) seen in Fig. 1.4, where \( R \) is the radial distance along the cone surface away from the cone’s apex, \( a \) is the semi-major axis and \( \alpha \) is the cone half angle:

\[ \tan 2\alpha = \frac{2a}{R} \quad \text{or} \quad a = \frac{R \tan \alpha}{1 - \tan^2 \alpha} \quad (1.7) \]

By combining Eq. 1.6 with Eq. 1.7 we find that

\[ r_2 = R \tan \alpha \quad (1.8) \]

and finally plugging \( r_1 = \infty \) and Eq. 1.8 into 1.1:

\[ \Delta P = \frac{\gamma \cot \alpha}{R} \quad (1.9) \]

This pressure in Eq. 1.9 can be compared with the electrostatic pressure on the Taylor cone, where electrostatic pressure can be stated in terms of the local electric field as \( \frac{1}{2} \varepsilon_0 E^2. \) Equating the two pressures gives

\[ \frac{1}{2} \varepsilon_0 E^2 = \frac{\gamma \cot \alpha}{R}, \quad (1.10) \]

which can be rearranged to obtain the field

\[ E = \sqrt{\frac{2\gamma \cot \alpha}{\varepsilon_0 R}}. \quad (1.11) \]

The electric field at the Taylor cone’s surface increases in strength as \( E \propto \frac{1}{r^2} \) towards its pointed tip and theoretically approaches infinity at the cone’s apex. Experimentally the electric field becomes strong enough close to the cone’s apex to form a jet of charged fluid as can be seen in Fig. 1.5. This jet of charged fluid ultimately becomes unstable downstream and breaks up into the charged drops that comprise the spray seen by Zeleny, and also explain the disintegration of large drops in an electric field into smaller drops for Taylor. Although in Zeleny’s experiments this cone shape would generally collapse the moment after it formed, it is possible to set up conditions under which the Taylor cone is stable, known as the stable cone-jet mode. It is worth noting here that the diameter of this jet has been
found experimentally to scale inversely with the conductivity of the liquid [15], and in the case of liquid metals in vacuum, the jet at the apex of the Taylor cone is found to disappear completely [54, 3]. Drop size from electrosprays scale with this jet diameter, however drop production is completely suppressed in electrospraying liquid metals and correspondingly this jet does not form.

Fig. 1.5 Detail of liquid jet that forms at the sharp tip of a Taylor cone. Image taken from M. Gamero Castano and J. de la Mora [27].

1.3 Electrospray as a source of ions for mass spectrometry

Two techniques were developed more or less at the same time in the late 1960’s and early 1970’s for producing mass spectra from a source of ions originating out of electrospray. Groups using the first technique, called electrohydrodynamic ionization mass spectrometry (EHI-MS), produced mass spectra from bare ions and ion clusters emitted directly from their electrospraying liquid surfaces [12]. Groups using the second technique, called electrospray ionization mass spectrometry (ESI-MS), instead sent the large multiply charged drops produced in their electrosprays through a background gas, which dried off the solvent to ultimately produce bare ions and ion clusters [25].

1.3.1 Electrohydrodynamic ionization mass spectrometry

The analysis of ions emitted directly into vacuum began with the work of Hendricks and Swatik [72], who in 1968 identified conditions which optimized ion emission to the almost
complete exclusion of droplets using a gallium-indium liquid alloy. Electrospray sources have previously been observed to emit ions directly from the charged surfaces of molten metals [72, 32, 53, 47], concentrated salt solutions in glycerol [68, 69], and more recently ionic liquids [51].

Liquids (in particular those with high vapor pressures) tend to freeze when exposed to vacuum since, as the liquid evaporates, heat is drawn from the liquid surface. This issue with freezing posed a practical problem for early EHI, and so studies of liquids other than molten metals focused on those liquids with low vapor pressures and high viscosities, such as glycerol [12], [72]. Mass spectra were obtained showing sodium ions clustered with several glycerol molecules, as well as sodium ions complexed with neutral peptides and clustered with glycerol molecules.

Several groups attempted to extend EHI mass spectrometry to aqueous solutions; however, electrical arcing and freezing of the water at the ion source prevented them from obtaining spectra [12]. Zolotoi and coworkers in 1980 and 1982 reported some success with aqueous solutions of salts, sucrose, and nucleic acids [89, 88], however these studies involved complicated spectra indicating a variety of possible ionization and emission modes as well as unstable spray conditions and evidence of electrical discharge.

In addition to freezing issues, the high voltages in the kV and 10’s of kV range required to produce spray would often result in discharge products which complicated the mass spectra. Generally the difficulty to obtain spectra combined with freezing and electrical arcing issues resulted in EHI-MS never becoming widely used, and the strongly preferred method became ESI-MS.

### 1.3.2 Electrospray ionization mass spectrometry

In 1968 Malcolm Dole realized that if charged drops produced in electrospray were sent through "air or other suitable gas" then, "on evaporation of the solvent the charged droplets should become electrically unstable and break down into smaller drops," [19]. Using a background gas, Dole was able to observe polystyrene dissolved in benzene and acetone as individual charged macromolecules. Building on the work of Dole, in 1989, John Fenn used the technique of combining electrospray with a background gas to produce mass spectra of large protein molecules [24]. This version of producing mass spectra using electrospray proved to be so valuable that Fenn’s work would ultimately earn him part of the 2002 Nobel prize in chemistry for contributing to techniques capable of analyzing biological macromolecules. The other parts of that prize went to Koichi Tanaka, who also used mass spectrometry, but with a different source of ions called soft laser desorption, and Kurt Wuthrich, who used the altogether different technique of nuclear magnetic resonance
spectroscopy. Electrospray ionization with a background gas is one of the most common ion sources used for mass analysis today.

1.3.2.1 From charged drops to bare ions with the help of a background gas

The background gas technique works to convert charged drops into bare ions through their collisions with neutral background gas molecules. When a charged drop collides with a neutral gas molecule, solvent is preferentially knocked off the drop. This is because unlike solvent molecules, ions leaving a charged drop feel an attractive force associated with their image charge just outside the drops surface. This attractive force creates an energy barrier that ions must have enough energy to overcome. Thus as these drops travel through the background gas, these collisions dry them of their solvent and they begin to shrink in size while maintaining their charge. Eventually these drops shrink to a size below their Rayleigh limits and become unstable.

As a drop shrinks below this point of instability it ejects a jet (similar to the jet at the tip of the electrospray source) of smaller, charged daughter droplets in a process known as a Coulomb explosion, or Coulomb fission. A drawing of this jet breakup can be seen in the inset to Fig. 1.6. These daughter droplets also dry off in the background gas until they too undergo Coulomb explosions resulting in even smaller droplets. Thus, through a cascade chain of such events, charged daughter droplets are sent flying in a widening plume before becoming small enough for single ions to emerge, shown in Fig. 1.6. It should be noted here that the final moments of ion production between small charged daughter droplets and actually bare ions are not well understood, and remain an active area of research. There is evidence that points to Coulomb explosion events and desolvation from collisions with the background gas explaining the full process for large macromolecules, such as those sprayed by Dole and Fenn, and is called the charged residue model (CRM). However, it is proposed that a competing mechanism exists in which thermal energy causes ions and ion clusters to evaporate from the surfaces of the drops and goes by the name ion evaporation model (IEM). The ion evaporation mechanism is also used to explain the origin of the ions that produced the mass spectra in the early EHI-MS experiments. There is even discussion in the literature of a third mechanism used to explain the ejection of long polymer chains from drops called the chain ejection model (CEM) [39].
Fig. 1.6 As drops dry off due to collisions with the background gas they reduce in size below the Rayleigh limit and undergo a Coulomb explosion. Through a series of these processes drops eventually become bare ions. The inset is an artist’s depiction of what the drop looks like as it ejects several charged daughter droplets. Image taken from Kebarle [37].
1.3.2.2 Conventional electrospray ionization mass spectrometry is incompatible with biopolymer sequencing

The cascade chain of Coulomb explosions used by conventional ESI to produce bare ions would scramble the sequential ordering of monomers emitted in those drops. Aside from the chaotic drying process, this method for creating ions is also problematic because it disperses ions widely, which makes focusing all emitted ions into the detector of the mass spectrometer difficult. Thus, the use of a background gas must be abandoned for our sequencing concept to work.

1.3.3 Ion evaporation: the emission of ions directly from charged liquid surfaces

The leading theory for the process by which ions are emitted from the Taylor cone of electrospraying fluids is called ion evaporation. First theorized in 1976 by Iribarne and Thomson, ion evaporation is a kinetic theory in which ions overcome an energy barrier through a thermally activated process. The stages of ion emission from a charged drop through evaporation can be seen in the energy landscape plot in Fig. 1.7. The ion starts solvated inside a charged drop. It must have enough energy to overcome an energy barrier

Fig. 1.7 Energy landscape of an evaporating ion from a drop. Taken from Labowsky, Fenn, and de la Mora [41]
associated with becoming unsolvated, distorting the liquid surface, and the attraction to its own image charge near the drop’s surface. Once the ion is far enough away from the charged drop, Coulomb repulsion ensures the ion and drop stay apart.

The lower this barrier is, the more likely an ion is to escape the surface of the drop for a given temperature. If this barrier is low enough, the rate of ions evaporating from the surface can lead to appreciable currents. Iribarne and Thomson modeled the rate, K, at which ions would leave the surface of a drop as a function of the energy barrier height, $\Delta G^\dagger$, and the temperature, T, shown in Eq. 1.12, where $k$ is Boltzmann’s constant and $h$ is Planck’s constant.

$$K = \left(\frac{kT}{h}\right) \exp\left(-\frac{\Delta G^\dagger}{kT}\right)$$  \hspace{1cm} (1.12)

They required this rate to be at least 100 Mhz for the ion evaporation to account for the ion emission they observed in their experiments [33].

They modeled the energy of the ion, $\Delta G(x)$, as a function of distance, $x$, away from the surface of the drop, ignoring the effects of distorting the surface, and including an attractive term from the ion’s image charge and a repulsive term from the charges remaining in the drop.

$$\Delta G(x) = e^2 \left[\frac{N}{R+x} - \frac{1}{4x}\right]$$  \hspace{1cm} (1.13)

where $N$ is the number of charges in the drop, $e$ is the charge of an electron, and $R$ is the drop’s radius. $\Delta G$ reaches its maximum height at $x_m$, which can be found by differentiating Eq. 1.13 and equating to zero,

$$x_m = \frac{R}{2\sqrt{N} - 1}$$  \hspace{1cm} (1.14)

To obtain the height of the barrier, they compared the energy of the ion at $x_m$, with its energy at some solvated position inside the drop, $d$. This solvated equilibrium position, $d$, results from a balance between Coulomb repulsion driving the ion out to the surface, with solvation effects pulling it into bulk solution, and so the barrier is

$$\Delta G^\dagger = \Delta G(x_m) - \left(\Delta G_s + \frac{Ne^2}{R-d}\right)$$  \hspace{1cm} (1.15)

where $\Delta G_s$ is the experimentally derived solvation energy of the ion that is evaporating. Combining Eqs. 1.14 with 1.15 results in the scaling relationship, $N \propto R^2$ [33]. This relationship defines drops that either have enough charges or are small enough for the ion evaporation rate, $K$, in Eq. 1.12 to reach Iribarne and Thomson’s experimentally relevant rate.
1.3.4 **Ion evaporation competes with the release of charged droplets**

Either ions are emitted from a charged drop through this evaporation mechanism, or the drop undergoes Coulomb explosion from crossing the Rayleigh limit. These two mechanisms are always in competition for ion emission, and Iribarne and Thomson’s plot of their $N$ versus $R$ scaling relationships can be seen in Fig. 1.8, where the scaling relationship for the Rayleigh limit can be found to be $N \propto R^{2.5}$ [30]. The most stable drops are large and uncharged and show up in the lower right side of the plot. However, as these drops have charge added to them they move vertically up the plot, and eventually for drops with radii larger than 134 Angstroms they cross the Rayleigh limit, shown as a solid black line, and undergo Coulomb explosion. However for small enough drops, as charge is added they cross this ion evaporation limit first, shown as dashed lines. The reason there are two dashed lines for ion evaporation relates to the dependence of the evaporation rate on the solvation energy, which is different for the positive ions versus the negative ions Iribarne and Thomson were interested in. Ions with different solvation energies would be expected to have different slopes in this plot. Thus, for enough charge on small enough liquid surfaces, ion emission through this evaporative process begins to dominate over ion emission due to this Rayleigh instability. Many argue that this evaporative process explains the final moments of bare ion formation at the end of the Coulomb explosion cascade chain [57, 39, 23].

1.3.5 **Insights into ion evaporation from the fields of nanoelectrospray and of space propulsion**

A version of ESI exists called nanoelectrospray, or nanospray, which is basically just ESI but with lower flow rates. Nanospray involves the use of electrospray tips with diameters on the order of 1-5 microns in place of the standard 10 microns and above, and has been shown to produce smaller initial drops which undergo fewer Coulomb explosions. This results in greatly enhanced fractions of ions that are ultimately collected and measured and is attributed to the much lower flow rates, on the order of nL/min [14]. Nanospray demonstrates the importance of smaller electrospray capillary tip diameters, and specifically their correspondingly lower flow rates, on what type of resulting electrospray is produced.

There has been renewed interest in electrospray directly into vacuum through ion evaporation from groups developing new methods for ion thrusters. Researchers, in particular J. Fernandez de la Mora’s group, interested in developing new methods of space propulsion using electrospray directly into vacuum, picked up where EHI left off. Their goals do not align perfectly with our own, since ultimately they are after thrust and fuel efficiency, not clear mass spectra [3, 27]. However, their desires to reduce the size and charge on drops
Fig. 1.8 For small enough drops and high enough electrostatic energy, ion evaporation becomes the dominant mode of charge emission. The solid curve represents the Rayleigh limit for the stable number of charges for a drop with some given radius. Above this curve in $N$, the number of charges on the drop, results in Coulomb explosion for the drop. The two dashed curves similarly represent ion evaporation for the drop (one for positive ions, one for negative ions), and for small enough drops ion evaporation becomes dominant before the drop reaches the Rayleigh instability limit. Taken from Iribarne and Thomson [33].
leaving the surface of electrospray sources in high vacuum still illuminate many of the same struggles.

Measurements of ion emission directly from the Taylor cone were made and the conditions under which electrospray would emit only ions and no drops, called the purely ionic regime (PIR), were explored [27, 26, 8]. Ion evaporation theory implies that ions are preferentially emitted over drop formation from the surfaces of electrified fluids at high electric field strengths. The electric field strength at the Taylor cone apex was calculated by de la Mora assuming that the fluid is conductive enough and the capillary is large enough for the jet diameter to be much smaller than the capillary diameter. Since the jet formation is so far away from the edges of the capillary, the effects on the jet only depend on the solution parameters of surface tension, fluid flow rate, and conductivity. The strength of the electric field was found by comparing the Taylor cone electric field with the electric field of the jet. The jet can be approximated as a charged cylinder and using Gauss’ law around some line charge \( \lambda \) gives

\[
E_{\text{jet}} = \frac{\lambda}{2\pi \varepsilon_0 R}. \tag{1.16}
\]

\( \lambda \) can be related to the velocity of the fluid, \( U \), and the current, \( I \), being emitted in the jet as

\[
\lambda = \frac{dq}{dx} = \frac{I}{U} \tag{1.17}
\]

The flow rate, \( Q \), is simply related to the velocity through the jet’s cross section with a radius, \( R \),

\[
Q = U \pi R^2 \tag{1.18}
\]

Combing the result from Gauss’ law with \( \lambda \) and \( Q \) gives

\[
E_{\text{jet}} = \frac{R I}{2 \varepsilon_0 Q} \tag{1.19}
\]

De la mora found empirically from experiments [26] that the current is related to the conductivity and flow rate as

\[
I = (\gamma K Q)^{1/2}. \tag{1.20}
\]

Plugging 1.20 into 1.19 results in

\[
E_{\text{jet}} = \frac{\gamma^{1/2} K^{1/2} R}{2 \varepsilon_0 Q^{1/2}} \tag{1.21}
\]
By equating 1.21 and 1.11 at the same radius $R$, a relationship between the surface tension, flow rate, and conductivity is found

$$E = \frac{\lambda^{\frac{1}{2}} K^{\frac{1}{2}}}{\varepsilon_0^{\frac{1}{2}} Q^{\frac{3}{2}}}.$$  \hspace{1cm} (1.22)

Intuitively, the field at the Taylor cone scales with the amount of charge concentrated at the tip of electrospraying capillary. Higher conductivity solutions have more, and more mobile, charges which leads to greater charge build up at the Taylor cone’s apex. Meanwhile, flow rate is a measure of how quickly that charge buildup is taken away, so higher flow rates lead to less charge build up. Thus higher conductivities and lower flow rates would tend to increase this electric field strength.

PIR is distinct from the general trends associated with reducing the size and rate of drop production, and is instead discussed in terms of a critical electric field strength [8]. This critical field strength represents the field at which ion evaporation begins to dominate over droplet formation. This regime has been achieved in liquid metals, some acids, and molten salts (which are often referred to as ionic liquids when they are molten at room temperatures). So far this regime has yet to be demonstrated in either solutions of formamide or water [8]. It turns out that the PIR is actually undesirable for thrust, since a lot of electrical power is wasted on accelerating ions with very little mass [3]. These ion thruster groups merely attempt to approach the PIR without actually entering it. However, this regime is precisely what we would like to reach with solutions suitable for biopolymers. We reason that by making an electrospray source with a small enough tip, it should be possible to restrict the flow of our electrospray emitters enough to bypass the Coulomb explosions altogether, and instead release single ions directly from the liquid inside the tip.

1.3.6 Clearing up some terminology

Electrospray ionization (abbreviated ESI) most often refers to the standard method of pulling charged drops out from a needle-like capillary and sending them through a background gas to produce bare ions for mass spectrometry. Since pairing electrospray ionization with a background gas for mass spectrometry (often abbreviated ESI-MS) is so ubiquitous a technique, the use of background gas often goes without saying. However, ESI also gets used as an umbrella term, referring abstractly to extracting current from liquid surfaces with the application of strong electric fields, agnostic of whether that current is composed of ions trapped in drops or emitted directly from the ESI source’s liquid surface. As mentioned earlier, though the term electrohydrodynamic ionization (EHI) was originally invented to refer
Introduction

specifically to early experiments that obtained mass spectra without a background gas, EHI is also often discussed under the umbrella term electrospray ionization, or ESI. Additionally, though electrohydrodynamic ionization is often abbreviated EHI, it is also abbreviated EHD at times. Finally, EHI often gets discussed in terms of ion evaporation (or ion field evaporation), which is the proposed mechanism that explains how ions are emitted directly from the charged liquid surface of the Taylor cone. There is even a version of ESI which goes by the name of nanoelectrospray ionization, and sometimes just nanospray. However, the technique uses a background gas and is really only different in that the capillaries used have small tip diameters on the order of 1-5 µm and low flow rates as low as 10’s nL/min [36, 21].

For the purposes of this thesis, I have chosen to use the term ‘electrospray’ as a blanket and abstract term referring simply to extracting current from liquid surfaces with the application of strong electric fields, agnostic of whether that current is composed of charged drops, ions clustered with a few solvent molecules, or bare ions. Electrospray source will refer generically to the device housing solution that electrospray current is being pulled out of. Most electrospray sources are needle-like capillaries, either made of metal or glass, and ours are always glass. The Taylor cone will refer to the liquid surface of an electrospray source while it is electrospraying. Ions emitted directly from the Taylor cone through ion evaporation often come out with a handful of solvent molecules stuck to them, which we refer to as ion clusters (as in, ions clustered with a few solvent molecules). Ultimately the distinction between completely bare ions and ion clusters is not important, since the scales involved in drop creation versus direct ion emission from the Taylor cone are wildly different in scale. Thus, unless specifically stated, the discussion of ion emission often refers to the emission of both bare ions and ion clusters. Also unless stated otherwise, ion emission refers to ions emitted from the Taylor cones of our electrospray capillaries. Electrospray ionization (ESI) is reserved for situations in which electrospray produces predominantly charged drops and is paired with a background gas. Nanoelectrospray (or nanospray) will be considered a version of ESI. Ion evaporation is reserved for situations in which electrospray produces significant amounts of ions directly from the Taylor cone, and is sprayed directly into vacuum without the use of a background gas.

1.4 Preserving the sequence of monomers before they are emitted into vacuum

Our sequencing concept also compels us to address the challenge of cleaving monomers such that they retain their sequential order upon entering the mass spectrometer for iden-
Monomers systematically cleaved from the end of a polymer stretched into a linear conformation in solution undergo Brownian motion [77, 78]. Before monomers are transferred into vacuum, but after they are cleaved in solution from their parent biopolymer, these random thermal forces that produce Brownian motion can result in a reversal of the original positions of two recently cleaved monomers and scramble the sequential ordering of the linearized polymer.

This sequence can be preserved either by giving cleaved monomers enough time to exit into the vacuum chamber before the next cleaving event occurs, or neighboring cleaved monomers can be exposed to a strong enough separating force to keep them from diffusing past one another before they exit into vacuum. Such a force would have to be an ‘elongational’ force gradient that increases in strength downstream. Both monomers move together downstream in the force gradient, however the monomer that is upstream would feel a weaker force. This difference in force strength results in the downstream monomer moving further and further away from the upstream monomer. Thus this field gradient tends to ‘elongate’ the separation distances between monomers. The electric field of a Taylor cone presents such a force gradient.

### 1.5 Thesis Outline

#### 1.5.1 Chapter 2: The nanopore mass spectrometer

Chapter 2 describes in detail the machine that was built to explore the feasibility of this single molecule biopolymer sequencing idea. The machine is a high vacuum chamber that houses an electrospray source made from a nanoscale glass capillary (nanocapillary), lenses to focus ions emitted from the nanocapillary into a mass spectrometer, and of course a mass spectrometer. There is an antechamber for transferring new nanocapillary electrospray sources into and out of the machine, and fluidic tubing to deliver new solutions to the tip of the nanocapillary. Since our machine has no background gas, any large multiply charged drops that are produced in the electrospray do not have a chance to dry off into ions through the Coulomb explosion cascade chain mentioned earlier in the introduction. The ions we detect in our machine must originate directly from the surface of the nanocapillary’s Taylor cone. The nanopore mass spectrometer is capable of measuring ions with mass-to-charge ratios (m/z) in the range of 0-4000 m/z.

The design of the machine was developed by my advisor Prof. Derek Stein and a previous post doc in the lab, Joe Bush. I helped to construct the machine, extend its mass range of sensitivity, and with the help of Ben Wiener, develop a way to exchange solutions inside tip
of the capillary while it is spraying in the chamber. I additionally developed data collection software, and with Ben Wiener, data visualization software.

1.5.2 Chapter 3: Mass spectra from ions emitted directly into high vacuum from both formamide and water solutions

Chapter 3 demonstrates the ability of the nanopore mass spectrometer to collect mass spectra from nanocapillary electrospray sources sprayed directly into high vacuum. The size of our nanocapillaries allow for electrospray from solvents with high vapor pressures without resulting in tip freezing issues. We report observations of both simple salt ions as well as amino acid ions, dissolved in solutions of both formamide and water. Notably, our mass spectra from aqueous solutions represent the first clear mass spectra taken from solutions of water.

Mass spectra created by myself, and post doc Mathilde Lepoitevin, are presented demonstrating the functioning of the machine, and its ability to produce ions, and ions clustered with a handful of molecules from solutions of formamide and water.

1.5.3 Chapter 4: Studies on the mechanisms of charge emission from nanoscale capillary tips

Chapter 4 explores the electric fields of electrospray sources at the 100’s of nanometers scale, and the mechanisms of ion emission directly from the Taylor cones at the tips of our nanocapillaries. We probed the electric fields through measurements of the electrospray onset voltages of our nanocapillaries as a function of their tip size. We introduce two theories from the literature for the electric field at the onset of electrospray, and use them to predict the dependence of onset voltages on the tip sizes of electrospray capillaries. We find our measurements better fit the theory that is based on modeling the capillary as a semi-infinite line charge. Our data, and its fit to a semi-infinite line charge theory, are in line with previous measurements by another group, who used capillaries with tip sizes between 200 microns and 1 mm.

We also probed the mechanisms of ion emission directly from the Taylor cones of our nanocapillaries through flow rate measurements, and the number of ions hitting our mass spectrometer’s single ion detector. As mentioned above in the ’Insights from the field of space propulsion’ section, the two most important electrospray parameters that contribute to the emission of ions as opposed to charged drops are flow rate and conductivity. Although we cannot track the flow rates of our electrospraying sources in situ, we present a technique
outside of the vacuum chamber that involves tracking the growth rate of inflating bubbles at the ends of capillaries dipped in a gel-like medium under an applied pressure. We measure the fluidic conductance of our capillaries as a function of their size and fit it to an infinite truncated cone Poiseuille flow model. We use a ratio of the rate of individual ions arriving at our mass spectrometer’s detector relative to the rate of charges leaving the electrospray nanocapillary as a measure of the relative abundance of ions versus charged drops emitted directly from the Taylor cones of our nanocapillaries. We measure how this ratio depends on the size of the tips of the nanocapillaries, the conductivity of the solutions being sprayed, and the applied pressure and find that the fraction of ions leaving directly from the Taylor cone increases with decreasing capillary size, with increasing solution conductivity, and lower applied pressures. These findings are in line with those of groups involved in space propulsion for much larger capillaries with tip sizes on the order of 10’s and 100’s of microns.

I collected the onset voltage measurements and ion collection measurements, and along with fellow grad student Ben Wiener, the flow rate measurements.

1.5.4 Chapter 5: Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer

Chapter 5 addresses the challenge associated with preserving the sequential order of recently cleaved monomers from a polymer stretched into a linear configuration. We develop a theoretical framework for evaluating the preservation of sequence information between monomers recently cleaved in solution from their parent strand. We use a simplified one-dimensional model of the dynamics of Brownian particles in the Taylor cone of an electrospray source, where two monomers drift towards the apex in an elongational force gradient along the central cone axis. Since the relevant processes are statistical in nature, we establish a metric for successful order preservation in which two monomers would retain their original order in 95% of cases. The likelihood that neighboring particles will invert their order decreases near the apex because the strength of the force gradient increases.

Neighboring monomers on a stretched biopolymer should be cleaved by photofragmentation within about 3 nm of the apex if they are to enter the mass spectrometer in sequence with 95% probability under typical experimental conditions. Alternatively, if the monomers are cleaved processively at milliseconds-long intervals by an enzyme, their sequence will be faithfully reported with 95% confidence if the enzyme is within about 117 nm of the apex.

I led the effort with help from then undergrad William Poole to analytically model and calculate the probability that the order of two monomers will invert as a function of time, their initial separation distance, and the strength of the force gradient. I estimated the conditions
under which the sequential information of these monomers remains intact as they are cleaved and emitted into the vacuum chamber, and ultimately the mass spectrometer for identification.

1.5.5 **Chapter 6: Conclusions and recommendations**

In chapter 6 I present possible improvements of the nanopore mass spectrometer for the purposes of sequencing and further studying the underlying ion emission mechanism. Decreasing the sizes of our nanocapillary ion sources, either through the introduction of a boron nitride nanotube, or by coating the tips with metal to reduce their inner diameters, could help both in increasing the ratio of ions emitted directly from the Taylor cone versus charged drops, and in creating a pore small enough to linearize biopolymers. The type of mass spectrometer we use is a mass filter which scans a narrow window of $m/z$ values over its full range of $m/z$ values to obtain a full mass spectrum. This scanning method is fundamentally incapable of collecting sequence information, since it can’t measure two ions with different $m/z$ arriving in rapid succession. Building a mass spectrometer capable of measuring ions of different masses simultaneously would allow for the exploration of biopolymer cleaving mechanisms, and a proof of concept for this sequencing strategy. Introducing the ability to record flow rates from actively electrospaying sources, as well as measuring the currents emitted from our EHI sources at higher frequencies, could help further illuminate the pathway towards achieving the full suppression of drop formation at the surfaces of our electrospray sources. Finally, our setup cannot directly measure drops emitted from our electrospray sources since they are far outside the range of $m/z$ values our mass spectrometer is sensitive to. A setup in which gated bunches of the spray are sent in what essentially amounts to a race, with the smallest ions arriving first in the bunch, and the large charged drops arriving last, would allow use to directly measure the drop versus ion cluster content of the electrosprays we produce. This would allow us further explore the feasibility of obtaining the purely ionic regime in solutions of water and formamide.
Chapter 2

The nanopore mass spectrometer

2.1 Introduction

In order to explore the feasibility of this new approach to single molecule biopolymer sequencing, we built the nanopore mass spectrometer. The key features that distinguish this instrument from a conventional electrospray ionization mass spectrometer is the absence of a background gas, and the use of a nanoscale electrospray source. We designed our machine, shown in Fig. 2.1, to deliver ions directly from liquid into high vacuum, where they are collected by electrostatic lenses and analyzed by a mass spectrometer.

2.2 Instrument overview

A schematic of the nanopore mass spectrometer illustrating the important components and two hypothetical ion trajectories — one reaching the detector and the other being filtered out — is shown in Fig. 2.2.

Ions start in solution inside a needle-like glass capillary which features a nanometer scale opening that allows for a stable interface with vacuum. Our machine was additionally developed to incorporate nanopores in 2D chips instead of needle-like glass capillaries, however all of the experimental results presented in Chapter 3 are taken using nanoscale glass capillaries. Ions are drawn from the liquid into vacuum when the liquid is voltage-biased about 200 – 500 V relative to an extraction electrode located about 0.5 cm away. The ions travel through a circular hole in the center of the extractor electrode and are sent to the quadrupole for mass analysis. Along their way they first pass through an electrostatic lens (Einzel lens) that focuses their trajectories into a second lens which collimates the ions, before they finally enter the mass spectrometer (Extrel MAX-500, Pittsburgh, PA).
The type of mass spectrometer we use is a quadrupole mass filter paired with a single ion detector. It is made of four long metal rods which have oscillating electric and magnetic fields that set up stable trajectories (shown in blue in Fig. 2.2) for a narrow window of mass to charge ratio ($m/z$) values. Ions within this narrow window will be transmitted and thus counted at the detector, whereas ions with either higher or lower $m/z$ ratios than this window will end up running into one of the four metal rods and not be transmitted through. In other words, a quadrupole can answer the question “Is the $m/z$ of this ion $X$?”, but not “What is the $m/z$ of this ion?” Since the $m/z$ transmission window is specified at each moment, the mass filter cannot simultaneously determine both the $m/z$ and timing of multiple unknown ions.

In order to filter out neutral particles that make their way through our quadrupole mass filter the transmitted ions go through one final step, where they are deflected $90^\circ$ by an energy-selective, electrostatic bender toward the detector.
The detector (DeTech 413, Palmer, MA) comprises a conversion dynode and an electron multiplier, and is sensitive to single ions. The instrument builds a mass spectrum by scanning this transmission window along a range of $m/z$ values and counting the ions the transmitted ions at each moment along the scan.

The instrument is housed in a vacuum chamber evacuated by a turbomolecular pump (Pfeiffer TMU-521-P) backed by a two-stage rotary vane pump (Pfeiffer Duo-20M). The mass spectrometer operates at a pressure of about $10^{-6}$ mbar. At that pressure, the mean free path of a molecule 5 Å in diameter is about 6 m, which is much longer than the 0.6 m path length between the ion source and the detector. A window in the vacuum chamber grants optical access to the nanopore and a section of the ion flight path.

### 2.3 Nanopore ion source

The heart of the mass spectrometer is the nanopore ion source, which transfers ions directly from solution into high vacuum and then corrals them into the mass filter. Our custom-made ion source, shown in Fig. 2.3, comprises the nanopore, the extraction electrode, and the first Einzel lens, all mounted on a single 8” CF flange at the “front” of the vacuum chamber. Four parallel rods bolted to the flange hold and align the electrostatic elements as well as a register for the nanopore mount. The nanopore, mounted on a Vespel block at the end of a hollow stainless steel rod, locks kinematically into position when three legs in the block engage slots in the register. The ion source can host two types of nanopore, ones at the tips of slender capillaries and ones in planar chips. We describe the two alternative configurations in the following subsections.

The liquid in the nanopore is usually biased to a constant potential of 201 V relative to ground by a voltage supply (Keithley 2657A) that also monitors the current of ions leaving the nanopore. The potential of the extractor electrode is adjusted between $-300$ V and $+300$ V to achieve ion emission. Electrical feedthroughs on the same flange establish connections to each element of the ion optics. A metal wire passes through two layers of PEEK tubing and the hollow rod to establish electrical contact with the liquid. The wire is usually made of silver, and plated with silver-chloride. The inner PEEK tube has a 1/16” outer diameter, is filled with liquid, and is open to the atmosphere at the external end. The outer PEEK tube has a 1/8” outer diameter and is used to further isolate the charged liquid from the grounded rod.

Because the nanopore is effectively a point source of ions and because charged droplets cannot undergo Coulomb explosions under high vacuum, Liouville’s Theorem predicts that excellent focusing properties are possible in our instrument [83]. The extractor and Einzel lens make up a four-element system designed to efficiently collect ions from a wide range of
Fig. 2.2 Schematic of the nanopore mass spectrometer. Electric fields draw ions from liquid into vacuum at a nano-scale aperture in the ion source. The ion-optic system, which includes two Einzel lenses, a quadrupole mass filter, an exit lens, and an electrostatic bender, gathers ions and transmits them selectively on the basis of their $m/z$. A single-ion detector registers the transmitted ions. The sketch illustrates the hypothetical trajectories of ions transmitted (solid blue) and rejected (dashed red) by the mass filter.
initial directions and kinetic energies. The electrostatic elements are made of stainless steel. Their design is notable for its use of unequal widths, which was guided by simulations of ion trajectories performed using the SIMION modeling program. The electrostatic elements are isolated from one another by ruby ball spacers, and held together in a single unit by way of threaded rods that are isolated from the metal elements. To prevent charging issues, each element has a lip in its cross section that nests inside a recess in its neighboring element, such that no line-of-sight exists between the inside of the ion optics and any insulating materials.

In highly optimized nanospray systems it is estimated that approximately 60-70% of the ions in solution will make it into the vacuum chamber, while the overall transmission efficiency from solution to detection is closer to 1-10% [21, 7]. This includes losses at ion optic interfaces, during mass separation, and at the detector. In such a situation, the ions emerge from what amounts to a point source without the possibility of undergoing Coulomb explosions and thus opening up the possibility of designing ion optics to collect and detect them with near 100% efficiency while preserving their order. Thus an important element

Fig. 2.3 The nanopore ion source. The configuration shown here features a pulled capillary with a nanoscale tip. The image also shows the register holding the nanopore mount in position, the extractor, the Einzel lens, and the gate valve of the vacuum load lock.
of our machine are our particular electrospray sources, composed of pulled glass capillary needles with inner diameters as narrow as 20-30 nm.

We designed the ion source to allow laser light to reach the nanopore or to intercept ions along their flight path. Our motivation was to enable photons to be used to stimulate photofragmentation, the desolvation of ionic clusters, or other processes. We introduced a bore in the Einzel lens and extractor, oriented at 45° from the ion beam axis, that leads to the location of the nanopore. A laser beam can be steered through the bore by a mirror (ThorLabs, PF05-03-F01) mounted on the ion source assembly. Light that is reflected by a planar chip can pass through a second bore, reflect off a second mirror, and exit the vacuum chamber. The reflected light can be used to image the chip or align the laser beam. Pictures indicating the bores, the mirrors, and the light path are presented in Fig. 2.4. Light enters and exits the vacuum chamber through a fused silica window (CVI PW1-2050-UV) mounted at Brewster’s angle on a custom flange that offers excellent transmission of light, including the ultraviolet part of the spectrum.

2.3.1 Capillary nanotips

A conventional electrospray ion source delivers ions into a mass spectrometer from the tip of a slender capillary. The high aspect ratio of the structure enhances the electric fields at the tip, thereby lowering the voltage required to generate an electrospray. Glass capillaries can be heated and pulled so as to produce nozzles whose diameters can be controlled at the level of single nanometers; diameters as small as 14 nm have been reported [9]. Our ion source accommodates nanotips at the end of 1 mm-diameter glass capillaries. A capillary is mounted in a Vespel block at the end of the hollow sample introduction rod, as shown in Figs. 2.5(a) and (b). A Viton O-ring surrounding the capillary, compressed between the threaded block and a threaded HPLC fitting, creates a vacuum-tight seal.

Our nanotips are made from borosilicate glass capillaries (Sutter Instruments, BF100-50-7.5, 1.0mm OD x 0.5mm ID) pulled using a pipette puller with a 2.5 mm box filament (Sutter Instruments P-97). Figs 2.5(c) and (d) show scanning electron micrographs of a nanotip with an inner diameter of 60 nm. A four-step pulling procedure created the steep taper, followed by the shallow taper extending out to the tip. The ratio of the outer diameter to the inner diameter of the nanotip was approximately 2:1, the same as that of the capillary. We have found that glass capillaries with a filament inside are much easier to fill with fluid than capillaries without a filament.
2.3 Nanopore ion source

Fig. 2.4 Laser access to the nanopore ion source. a) The blue arrows indicate the path of a laser beam to and from a chip-based nanopore. The locations of the mirrors and the chip are indicated. b) A part of the chip can be seen in the mirror.

2.3.2 Chip-based nanopores

Chip-based nanopores are appealing for several reasons, chief among them being that microfabrication and nanofabrication techniques are available for reproducibly creating large numbers of structures with nanopores whose dimensions can be controlled at the scale of single nanometers [70]. A chip-based nanopore can also be shaped to have a short fluid path.
Fig. 2.5 A capillary nanotip ion source. a) Mounting a nanotip. The picture shows the O-ring between the threaded HPLC fitting and the mounting block. b) The nanotip assembly, also showing the three legs that engage the register. c) Scanning electron micrograph of a pulled capillary nanotip. d) Detail showing the 61 nm inside and 170 nm outside diameters of the orifice. The nanotip was coated with $\sim 25$ nm of carbon for imaging.

to the orifice, allowing liquid samples to be more easily introduced or replaced than inside a capillary.

Fig. 2.6 shows the assembly of a second mounting block that accommodates nanopores in planar chips. The Vespel block has a recessed seat that holds a thin cylindrical Viton gasket
and a 5 mm × 5 mm chip. Above the chip sits a keyed Vespel cover-piece that does not rotate. A threaded cylindrical Vespel cap attaches to the mounting block and compresses the gasket, the chip, and the cover-piece without shearing them. This approach creates a vacuum-tight seal between the mounting block and the chip while distributing compression forces evenly so that brittle chips made of silicon or glass do not break. It is also possible to cover the chip holder with a stainless steel cap that can be voltage-biased using a second insulated wire threaded through the hollow rod, as shown in Fig. 2.6(e).

It is important to note that although we report our design for integrating a planar chip based nanopore into the nanopore mass spectrometer here, we were never able to obtain electrosprays from such planar devices. We found that strong electric fields would often develop at the corners and edges of the silicon chips which caused arcing to occur and the chip to crack before an electrospray from the nanopore could be observed. As a result we chose to focus on nanopores made from the aforementioned pulled glass capillaries. In the future one would revisit the idea of chip-based ion sources with a redesigned architecture that takes advantage of electric field enhancement at a sharp feature containing the nanopore.

### 2.3.3 Tube-in-tube fluid delivery system

A tubing system comprising a thin, inner tube (150 μm inner diameter, 360 μm outer diameter) that passes through a wide, outer tube (0.04” inner diameter, 0.06” outer diameter), as shown in Fig. 2.7, allows us to flush the solution inside a capillary sources while it is spraying. The thin inner tube delivers fresh liquid into the capillary while the wider outer tube provides a path for the waste solution to exit; the waste flows in the space between the outer surface of the inner tube and inner surface of the outer tube. The inner tube comes to within about 1 mm of the apex of the nanoscale capillary. The complete exchange of solutions at the tip relies on a combination of diffusion and electrospray emission and takes about 30-60 minutes for most aqueous and formamide solutions. This ‘tube-in-tube’ system has several advantages: First, it greatly increases the throughput in measurements, because one can test many different solutions without having to break vacuum and then pump down again with a new tip. Second, it enables us to eliminate the particular tip being used as a variable affecting different measurements; one can change the solution being measured on the fly while ions are being sprayed and measured.

### 2.3.4 Custom software

Custom-developed software written in MATLAB simultaneously controls the potentials applied to the ion source and ion lenses while recording those potentials, the electrical current
Fig. 2.6 A chip-based nanopore ion source. a) A Viton gasket sits in the square recess of the mounting block. b) A $5 \times 5$ mm silicon chip with a nanopore in a silicon nitride membrane sits on the gasket. c) A keyed cover-piece rests on top of the chip. d) A threaded cap compresses the gasket, the chip, and the cover-piece. e) Side view of the chip mount, assembled with a stainless steel cap. The wire that is visible controls the voltage of the cap. That wire enters through the hollow sample introduction rod.
from the ion source, and the pressure inside the chamber as functions of time. The interface between the software and the hardware is a National Instruments data acquisition device with an RS-232 serial link.

We also wrote a different program in Mathematica to display and analyze data as it evolves in time. The data from our experiments are inherently time-dependent. The spectrum we measure evolves as we change the chemical species inside the source, as we adjust applied voltages, and as the capillary ages. The software we wrote creates an animated plot of the mass spectrum with optional correlated plots of other data from the experiment, such as the ion source current, the ion source voltage, the detector current, and the pressure. This allows us to vary experimental parameters and observe the effects. For example, we can add a chemical species to the solution inside an ion source and watch new peaks emerge in the mass spectrum.

### 2.4 Mass filter

We opted for a quadrupole mass filter for several reasons. First, the quadrupole we initially selected offered a wide $m/z$ range of 10-500 amu and high resolution ($m/\Delta m = 2000$ for FWHM) when combined with a 1.2 MHz RF oscillator; we have since installed a 440 kHz RF oscillator that gives an even wider $m/z$ range of 10-4000 amu. Second, the width and the scanning rate of the $m/z$ acceptance window can be adjusted. Third, the approximately
1.5 cm-diameter entrance and exit apertures of the quadrupole are wide enough to achieve a relatively high transmission efficiency.

Since we developed the first version of the nanopore mass spectrometer to explore the feasibility of our single molecule biopolymer sequencing technique, it was not important that the mass analyzer was not capable of measuring ions of different $m/z$ ratios simultaneously. It should also be noted that essentially no commercially available mass analyzers are capable of simultaneous measurements of different $m/z$ ratios since it is much easier to focus all of your ions onto a single detector as opposed to setting up a full array of detectors.

The quadrupole is a set of four parallel, 19 mm-diameter metal rods, whose axes are arranged in a square. Diagonally opposing rods are electrically connected so their surfaces are equipotentials. The quadrupole works as a mass filter when a radio-frequency (RF) alternating voltage signal and a constant (DC) voltage offset are applied between the two independent pairs of rods. The time-dependent fields create a stable trajectory for ions within a specific range of $m/z$ and eject ions outside that range [48]. The acceptance window is specified in practice by controlling the amplitudes of the DC offset and the RF signal at a constant operating frequency.

The resolution of the quadrupole improves with the number of RF cycles the ions experience inside the quadrupole, which depends on the velocity of the ions and the frequency of the oscillator. The kinetic energy of ions entering the quadrupole can be controlled by offsetting the voltage of all four rods and the sheath that houses them. The voltages of the rods and the sheath can be offset independently by as much as $\pm 200$ V. In practice, however, we offset the voltage of the rods and the sheath by the same amount to maintain a consistent electric field distribution. That offset voltage is called the ‘probe bias’ and is usually close to the nanopore voltage so that ions travel slowly through the quadrupole. The optimal kinetic energy of ions traversing our mass filter is between 1-10 eV, however we have collected well-resolved spectra from ions with $\sim 100$ eV of kinetic energy.

The four-element electrostatic ion bender (Extrel) deflects the ions leaving the quadrupole into the detector, while filtering out neutral species. Alternatively, the bender can be turned off to allow the ions to travel straight through it into a Faraday cup, or its voltages can be flipped to steer the ion beam in the opposite direction onto a microchannel plate detector that we use to adjust the ion optics while imaging the beam.

The ion detector sits in a grounded metal housing with a small opening to admit incoming ions. Ions entering the detector housing are accelerated to $\sim 5$ keV before they impinge on a conversion dynode. When positive ions impinge the conversion dynode, they produce secondary electrons, which are amplified by a continuous dynode electron multiplier. Negative ions produce secondary positive ions, which are detected by the electron multiplier. In both
cases, a voltage bias of 2000 V is applied across the electron multiplier, yielding a gain of about $10^6$.

Although this version of the nanopore mass spectrometer uses a mass filter incompatible with sequencing, the types of detectors currently available are both sensitive enough and fast enough to detect the expected sequence of monomer ions from a single polymer. Continuous dynode detectors can detect single ions with near unit quantum efficiency with bandwidths able to resolve the arrival order of monomers delivered through the nanopore moving at speeds of centimeters per second, similar to the speeds observed in solution-based nanopore translocation experiments [17].

2.5 Vacuum system

The nanopore mass spectrometer is housed in a vacuum chamber evacuated by a single high vacuum pump. This simple configuration is possible thanks to the nanopore ion source, which transfers ions directly into vacuum from liquid with an extremely low gas load. Conventional electrospray mass spectrometers, by contrast, require differentially pumped zones because the ion source emits charged droplets that must evaporate in a zone containing background gas before individual ions can enter a separate, high vacuum zone where the mass selection occurs.

The main body of the vacuum chamber is an 8”, 6-way cross attached to an 8”, 6-way cube (MDC). The nanopore ion source is mounted to the “front” of the cube. The quadrupole mass filter, ion bender, and Faraday cup are all housed in the cross and mounted to its “back” flange. The ion detector is mounted to a separate flange on a side of the cross. The turbomolecular pump attaches to the cross from below. A cold cathode gauge and a thermocouple gauge monitor the pressure in the main chamber. The base pressure of the vacuum chamber is typically about $10^{-7}$ mbar.

A load lock on the same flange as the ion source permits the introduction of a nanopore filled with liquid into the high vacuum. The load lock is first evacuated using the rotary vane pump that also backs the turbomolecular pump. When the pressure inside the load lock drops below about 12 mbar, as indicated by a second thermocouple vacuum gauge, the valve to the rotary vane pump can be closed and the gate valve to the main chamber opened. The nanopore is loaded into position by sliding the hollow rod to which it is attached through a Cajon fitting. A Viton O-ring inside that fitting allows such extended linear motion without breaking the vacuum. The pressure inside the chamber rises to about $10^{-6}$ mbar with the introduction of a nanopore containing liquid sample. The safe operation of the mass filter and the ion detector requires a chamber pressure in the lower part of the $10^{-6}$ mbar range.
Chapter 3

Mass spectra from ions emitted directly into high vacuum from both formamide and water solutions

3.1 Electrospray directly into high vacuum

The use of an electrospray source with a sub-micrometer-diameter tip makes it possible to perform mass spectrometry on ions emitted directly into high vacuum. One of the important reasons for this is that nanoscale tips contribute a negligible gas load, even during electrospray, whereas tips with diameters of a micrometer or more tend to result in spikes in the chamber pressure that go beyond the safe operating range of the mass spectrometer. We will illustrate this point by comparing the behavior of two glass capillaries with very different tips diameters, shown in Figs. 3.1(a) and (b).

Fig. 3.1(a) shows a scanning electron micrograph of a pulled glass capillary whose tip has an inner diameter of 2.9 $\mu$m and an outer diameter of 4.3 $\mu$m. The capillary was cleaned by briefly exposing it to air plasma, then filled with a 1 M solution of sodium iodide (NaI) in formamide and then introduced into the nanopore mass spectrometer. Fig. 3.1(c) shows the time dependence of the chamber pressure and the ion emission current with the micron-scale tip inside the mass spectrometer. The liquid formed a stable interface with the high vacuum in the absence of an applied extraction voltage, and the pressure inside the chamber was about 3.5 $\times$ 10$^{-6}$ mbar. The application of an extraction voltage of 1.15 kV between the nanopore and the extraction electrode caused about 0.5 $\mu$A of current to flow from the tip. At the same time, the chamber pressure was observed to rise to about 6 $\times$ 10$^{-6}$ mbar. The current and the pressure varied in time in a correlated way. The pressure occasionally rose above a value
Mass spectra from ions emitted directly into high vacuum from both formamide and water solutions

Fig. 3.1 Influence of the ion source nozzle size on the vacuum chamber pressure during electrospray. (a) Scanning electron micrographs of capillary tips with (a) 4.3 µm outer diameter and 2.9 µm inner diameter, and (b) 350 nm outer diameter and 190 nm inner diameter. (c) and (d) show traces of the chamber pressure (black, left axis) and the electrospray current from the tip (grey, right axis) as functions of time for the tips in panels (a) and (b), respectively. Both tips contained 1 M NaI in formamide. The total applied extraction voltages were 1.15 kV in (c) and 0.248 kV in (d). The shaded regions indicate times when the safe operating pressure was exceeded and the mass filter and detector turned off.

(between 6–7×10⁻⁶ mbar) that triggered the automatic shutdown of the quadrupole mass filter and ion detector.

Fig. 3.1(b) shows a pulled glass capillary whose tip has an inner diameter of 190 nm and an outer diameter of 350 nm. That tip was prepared and introduced into the instrument as before. Fig. 3.1(d) shows the time dependence of the chamber pressure and the ion emission
current with the nanotip inside the mass spectrometer. In this case, the pressure inside the chamber was about $1.5 \times 10^{-6}$ mbar with the unbiased tip inside, and it took a total extraction voltage of only 248 V to induce current to flow. The current was about 10 nA, but it varied significantly over time; this particular tip showed much larger variations, including spikes, in the current than we typically observe. Despite that, the chamber pressure showed no hint of rising together with the current. The pressure remained low, decreasing slowly over time to a value below $1.0 \times 10^{-6}$ mbar. In our experience, capillary tips with outer diameters below about 500 nm consistently give ion emission without significantly affecting the chamber pressure. We find similar results with formamide and aqueous solutions.

There are two properties of small tips that help to explain why they do not contribute a significant gas load during electrospray: first, the pressure-driven fluid flow rate decreases rapidly as the diameter of a cylindrical channel constricts. Second, the high geometric field enhancement factor of narrow tips lowers the voltage required for ion emission to occur, which results in a lower emission current and in turn less Joule heating and less entrained fluid flow.

### 3.2 Mass Spectra

#### 3.2.1 Simple salts

The first mass spectrometry measurements we performed with our new ion source made use of sodium iodide solutions in formamide rather than water or glycerol. The choice to use formamide was inspired by the work of de la Mora and colleagues [26, 16], who worked to produce electrosprays in the pure ion evaporation regime. Like glycerol, formamide has a much lower vapor pressure than water, which leads to more stable electrosprays. However, formamide can also dissolve salt and organic molecules at high concentrations that are comparable to those achievable in water.

Fig. 3.2 shows mass spectra of both negative and positive ions obtained from the same 2 M solution of NaI in formamide using a glass capillary with an inner diameter of 295 nm and an outer diameter of 412 nm. The negative ion spectrum was obtained by biasing the tip voltage to -300 V while keeping the extractor electrode grounded. To obtain the positive ion spectrum, the tip was biased to a voltage of 290 V. In both cases, data were collected for 10 minutes. A 1.2 MHz RF oscillator with a mass range of 500 drove the mass filter.

The mass spectra presented in Fig. 3.2 reproduce the main features of previous measurements of NaI in formamide by Lozano and Martinez-Sánchez et al. [11] and Luedtke et al. [46]. The spectra show sequences of masses separated by 45, which is the molecular mass of
Fig. 3.2 Mass spectra acquired during successive experiments in (a) positive and then (b) negative ion mode using the same tip filled with a 1 M NaI solution in formamide. The total extraction voltage applied to the nanotip relative to the extractor L1 and the \( m/z \) values of the major peaks are indicated.

The most prominent peaks in the spectra we measured correspond to the most stable ion clusters. Luedtke et al. previously used density functional theory calculations of ion-solvent cluster energetics to support their interpretation of similar mass spectra [46]. The distribution of peak sizes in the mass spectra indicate a thermally activated ion emission process because the ion cluster abundances reflect their relative energetic stabilities. The mass spectra we measured are in good agreement with those of Luedtke et al., with similar distributions of
ion clusters observed in both cases. The most abundant ion clusters in our measurements corresponded to one sodium ion clustered with 5 formamide molecules in positive mode, to one iodine ion clustered with 3 formamides in negative mode. By comparison, the most abundant sodium and iodine ion clusters in the measurements of Luedtke et al. had 4 and 2 formamide molecules, respectively. This close agreement indicates that the ions measured in our mass spectrometer were also produced by an ion evaporation processes. We cannot exclude the possibility that charged droplets also emerged from our nanopore ion source, because the instrument is insensitive to species with such large masses. Previous work with low volatility solvents including formamide [3], sulfolane [3], and propylene carbonate [31] showed that nanodrops are produced together with solvated ions.

The narrow peaks in our spectra are indicative of a narrow ion energy distribution. We infer this because ions with large kinetic energies relative to the mean can pass through the quadrupole rather than being filtered out for a given mass acceptance window. Thus wide energy distributions tend to broaden peaks and can possibly create double peak artifacts [49], but we do not see these effects in our spectra. We also surmise that the distribution of ion energies was already narrow upon the ions’ emergence from the source, because the high vacuum conditions guarantee that they travel ballistically from the source to the detector.

These findings echo the literature on liquid metal and ionic liquid ion sources, where the ion energy has been well characterized and shown to be close to the emitter voltage [58]. These similarities are remarkable, firstly because ionizing a minor species in a neutral solvent is a different physical process from emitting ions from a liquid metal or an ionic liquid, and secondly because of the vastly different regimes of conductivity of those situations.

Notably, we have been able to obtain mass spectra from salt solutions in water. Fig. 3.3 shows mass spectra obtained from 1 M solutions of NaCl in DI water in negative and positive modes. A 440 kHz RF oscillator with a mass range of 4000 drove the mass filter in this experiment. The current emitted from the tip was less than 100 pA for the 1.5 hours over which the mass data were recorded in positive ion mode, and about 100 nA for the 0.5 hours during which data were recorded in negative ion mode. The spectra show series of peaks corresponding to the masses of chloride and sodium ions clustered with a small number of water molecules.

There have been no previous reports of clean mass spectra obtained after evaporating ions directly from the surface of a volatile liquid like water. Previous attempts have mostly been met with problems like electrical arcing at the source and freezing of the liquid [12]. The closest comparison to our measurements was from the Zolotoi group, who in 1980 reported EHD spectra from water. However, they reported only intermittent electrospray conditions and very complicated mass spectra [88, 89].
Mass spectra from ions emitted directly into high vacuum from both formamide and water solutions

Fig. 3.3 Mass spectra acquired during successive experiments in (a) positive and then (b) negative ion mode using a single tip filled with a 1 M solution of NaCl in water. The total extraction voltage applied to the nanotip relative to the extractor L1 and the $m/z$ values of the major peaks are indicated.

The mass peaks in Fig. 3.3 are broader than the ones measured in formamide, and there is a hint of a shoulder to the left of the peaks. These features are probably consequences of the lower oscillator frequency used in this experiment, possibly in combination with a higher kinetic energy of the ions. Peak broadening and peak splitting are known to occur when ions pass through the quadrupole before the oscillator can complete a sufficient number of cycles [50].

3.2.2 Mass spectra of organic molecules

The nanopore mass spectrometer is capable of analyzing DNA bases, and amino acids. Fig. 3.4 shows a mass spectrum from 100 mM cytosine and 50 mM acetic acid in formamide. The acetic acid was added in order to turn more cytosine bases into cations in solution. The capillary used in this experiment had an outer diameter of 256 nm and an inner diameter
Fig. 3.4 A mass spectrum acquired from 100 mM cytosine in formamide with 50 mM acetic acid. The total extraction voltage applied to the nanotip relative to the extractor L1 and the m/z values of the major peaks are indicated.

of 100 nm. A 440 kHz RF oscillator with a mass range of 4000 drove the mass filter in this experiment. We held the tip at 201 V and the total extraction voltage between the tip and the extractor at 216 V. The ion current from the source was about 0.8 nA. The mass data presented in Fig. 3.4 were collected over 3 hours, but the spectrum was well resolved after only 10 minutes. The mass spectrum clearly shows cytosine ions clustered with a varying number of formamide molecules; the most abundant cluster has 14 formamides. A minor sequence of peaks seen in between the cytosine sequence is consistent with singly ionized formamide clusters. Similarly it is also capable of analyzing amino acids in both formamide and water. Fig. 3.5, shows a spectrum for histidine with a molar mass of 155 g/mol in formamide, and in Fig. 3.6 in water.

As a result of our nanocapillaries size, we are able to avoid the freezing issues that prevented previous groups from obtaining EHI mass spectra from such volatile solutions. In the past Loscertales et al., and Castro and Bocanegro, attempted to avoid this issue by encasing the jet of water with an outer sheath of oil [44, 10]. While this sheath of oil allows water to be electrosprayed into high vacuum, it is likely that either no mass spectra could be obtained, or the spectra would be very difficult to interpret. Either way, we are able to avoid this unnecessary complication completely with the use of such small tipped nanocapillaries. Additionally, traditional EHI and ESI, including nanospray, use very high voltages in the kV and even 10’s of kVs range. These voltages require complicated setups and at times specific background gases that suppress coronal discharge, complications which the use of these nanocapillaries allows us to avoid.
Mass spectra from ions emitted directly into high vacuum from both formamide and water solutions.

Fig. 3.5 A mass spectrum acquired from 35 mM Histidine in formamide. The total extraction voltage applied to the nanotip relative to the extractor L1 was 272V.

Fig. 3.6 A mass spectrum acquired from 100 mM Histidine with 0.3% acetic acid at pH 6.2 in water.
Chapter 4

Studies on the mechanisms of charge emission from nanoscale capillary tips

In our efforts to create an ion source compatible with a single biopolymer sequencing technique we would like to be able to completely suppress the creation of charged drops, since drops obfuscate and randomize sequence information held in biopolymers. As was mentioned in subsection 1.3.5 conditions have been found for electrosprays in which only ions are emitted directly from the Taylor cone. We have already demonstrated an ability to observe individual ions and ion clusters, emitted directly from the Taylor cones of our electrospray sources. Ultimately, we would like to develop techniques to achieve a purely ionic regime of emission suitable for biopolymers. A deeper understanding of the ion emission mechanisms could guide the way. Accordingly we have focused our studies on the electric fields that develop on electrospray sources as they begin to emit current, and conditions that favor ion emission over charged drop creation.

4.1 Electrospray current and onset voltage measurements

The effects of voltage have been established to depend strongly on the size regime of the electrospray sources being used. De la Mora’s group made current measurements while varying the flow rate of electrosprays on the order of 1mm in diameter. They made these same current versus flow rate measurements at various applied voltages and did not notice a difference [15]. Subsequently, Stark et al have found that voltage has a strong impact on flow rate, for capillaries on the order of 1µm in diameter [60, 2]. Additionally, many studies have found evidence for what is called a multi-jet mode at voltages much higher than the electrospray onset voltage; as the name multi-jet implies, more than one jet of
charged liquid forms from the electrospray surface [1]. The kV range of voltages necessary to produce electrospray in traditional sources results in many complications which could be alleviated by ion sources which have more moderate onset voltages. Lower operating voltages could also potentially open the door for the miniaturization of ESI-MS technology. Previous measurements of how electrospray onset voltages are affected by the size of the capillary have only been done for capillaries down to the single micron scale of what is called nanospray. We show that electrospray follows a previously predicted theoretical model down to 100’s of nanometers.

4.1.1 Experimental setup for measuring electrospray onset voltages

The experimental setup shown in Fig. 2.2 consists of a silver wire placed inside a solution filled glass capillary tip and a toroidal extractor electrode held at a fixed distance of approximately 0.5 cm away from the tip’s apex. Variations in both the capillary tube length as well as the taper length of the melted and pulled sections of the capillary adjust this ultimate separation distance between needle tip and extractor electrode by ± 0.1 cm. Current leaving the capillary is measured by an ammeter (Keithley 2657a) connected to the silver wire that runs up to the base of the capillary tip. To reduce noise in the current measurement each current data point is an average of 10 measurements collected at 60 Hz. In addition to measuring the current, the ammeter (Keithley 2657a) is also a voltage source and applied controllable voltage to the silver wire in the capillary while the extractor electrode was held at ground. The applied voltage was ramped up in increments of 3.3 volts, pausing for 0.1 seconds between increments. At each voltage increment the current leaving the capillary was measured until a threshold current of 1 nA had been reached, at which point the voltage was ramped back down. As a consistency check the voltage was increased above and below the onset voltage five times. Unless otherwise stated the solution being sprayed is 1M NaI dissolved in formamide.

4.1.1.1 Applying voltages much greater than the onset voltage induces a mostly irreversible decrease in electrospray current

We noticed a reproducible change in the qualitative nature of the electrospray from capillaries subjected to applied voltages significantly above their onset voltages. An example of this change is shown for a capillary with an inner diameter of 85 nm and and outer diameter of 224 nm in Fig. 4.1. Note that for the first voltage ramp the current increases with voltage until around 400V, where the tip current actually begins to decrease with additional increases in voltage. Additionally, the current from the first ramp is significantly greater than the
4.1 Electrospray current and onset voltage measurements

Fig. 4.1 Each voltage ramp from 100V to 600V was increased several times higher than the onset voltage for this capillary. It is clear that the first ramp, which peaks in current around 400V, exhibits very different electrospray behavior from all subsequent ramps. Generally higher voltages and multiple ramps decrease electrospray current for a given voltage.

subsequent voltage ramps. Subsequent ramps exhibit remarkably different current-voltage behaviors with significantly reduced electrospray current outputs.

This phenomenon might be explained by the electrosprays produced at higher voltages no longer spraying in a stable cone-jet mode, but instead chaotically turning on and off at frequencies our setup is insensitive to resulting in solution wetting the outer surface of the tips. As will be demonstrated later in this chapter, we know that the meniscus wets the capillary tip to its outer diameter as can be seen Fig. 4.10b. It is possible in this chaotic high voltage electrospray mode that the pinned liquid surface is pushed further along the outside and up the sides of the capillary tip’s outer surface. Thus with a wider meniscus from a wetted capillary tip we would expect the onset voltage to increase after reaching high voltages. However we generally observe two current regimes, one starting at voltages actually lower than the original onset voltage, and a second regime of current rise distinguished by a faster rate at voltages higher than this onset. Since we would expect the salty liquid which wets the outer surface to leave salt deposits after the voltages are turned off and the liquid on the tip dries off, we would also expect that when the voltages are turned back on, the liquid would be drawn into the salt deposit through osmosis. This could explain the two regimes of current. The lower voltage current rise is explained by liquid wetting the tip’s outer surface. The higher voltage, and steeper rise, in current is explained by a higher onset voltage required by a bigger and wider liquid meniscus from this same wetting process.
This idea was explored by imaging tips after they have electrosprayed at much higher voltages than their onset voltages. Fig. 4.2 shows representative SEM images of capillary tips after having been sprayed with voltages ramped up to approximately 3 times their onset voltages. Noticeable blobs of salt residue can be found on tips subjected to high voltages.

It is possible to take a tip that has undergone this change in emission behavior and dip it in pure formamide in hopes of dissolving the salt buildup at the end. Shown in Fig. 4.3 are the first five voltage ramps that extended to voltages well above the onset voltage for that particular tip. A qualitative change in electrospray behavior is observed between ramp 1 and all subsequent ramps. This tip was then submerged in a beaker of pure formamide for 1 minute, and five additional voltage ramps were measured, shown in Fig. 4.4. After dipping the tip in pure formamide, the first voltage ramp of the new measurement series appears remarkably similar to the first ramp of the original series. The first ramp again stands out with significantly higher electrospray currents compared with subsequent ramps, which show much lower currents at similar voltages. The pattern of these two current behaviors is more apparent in the tip after dipping. The lower current increase potentially explained by the wetting of the capillary’s outer surface starts around 250V, until around 800V where the second higher voltage current behavior occurs distinguished by a marked increase in slope. This would be considered the true onset voltage and is correspondingly higher than the first voltage ramp. It should additionally be noted that while the general pattern of the original five ramps is replicated, the magnitude of the current produced after dipping the tip in formamide never reached a level as high as the original voltage ramps.

However these strange high voltage electrospray behaviors can be explained, they complicate these onset voltage measurements, and have also been seen to not produce spectra in our mass spectrometer. Thus measuring onset voltages required a method for keeping
4.1 Electrospray current and onset voltage measurements

Fig. 4.3 Initial five voltage ramps before the tip has been dipped in pure formamide. Sprayed solution is 1M NaI in formamide. The first ramp exhibits clearly different behavior, decreasing with increasing voltage around 700 V and at substantially higher currents than subsequent ramps.

Fig. 4.4 Second round of voltage ramps after the same tip as in Fig. [?] has been dipped in pure formamide for 1 minute. Much of the previous electrospray behavior returns, with the first ramp decreasing with increasing voltage around 700V and at substantially higher currents than subsequent ramps. However the magnitudes of the currents are overall lower.
the voltages near the voltage at which the onset of electrospray current appeared. This was achieved by setting a current threshold at which the voltage ramp would increment back down. Shown in Fig. 4.5a is a typical example of the electrospray currents versus applied voltage for five ramps that stop and ramp back down after a 1 nA threshold current has been crossed. When the applied voltage was kept from greatly exceeding the capillary’s onset voltage using this threshold, the current versus voltage ramps remained consistent past the first voltage ramp. This current threshold on the voltage ramp prevents the electrospray tips from undergoing the transition seen after voltages well beyond their onset voltage were applied.

Most voltage ramps consistently reproduced similar onset voltages as in the case of Fig. 4.5a, however, there were several examples in which the first voltage ramp was observed to produce anomalously high onset voltages as compared with subsequent voltage ramps as shown in Fig. 4.5b. The first voltage ramp reaches about 205V before the electrospray current begins to increase, whereas the onset of current in four subsequent ramps all cluster around 185V. Additionally, onset voltages tended towards higher and higher voltages as the number of voltage ramps a capillary was subjected to increased, as shown in Fig. 4.5c. Note that the electrospray current for voltage ramps 4 and 5 begin at 410V and 400V, respectively, increase, and then begin to ramp back down before electrospray current turns off at voltages similar to the previous ramps. Onset voltage data was therefore most consistent for data taken during the second voltage ramp, and therefore only the second voltage ramp was used in the onset voltage versus tip diameter analysis.

4.1.1.2 Programmatic determination of onset voltage

The voltage at which the onset of electrospray current occurred was programmatically determined. We wrote software that would detect the sudden rise in current from the appearance of electrospray by finding a sudden increase in the slope of the recorded current measurements. The automation program works by taking the derivative of the recorded current measurement data and comparing each point for a given voltage step against a moving average of prior data points. The point under consideration is determined to be the onset voltage if it is larger than some predefined multiple of standard deviations from the mean of that moving average. In fact not only the point under consideration, but that point and the next point as well, must both be above this moving average to count as electrospray onset.

Shown in Fig. 4.6 is some example data and in Fig. 4.7 the corresponding derivative of the current data with the point that was selected by the program as the onset voltage highlighted at 297.1V. The software compared the point at 297.1V against an average of 10 consecutive current measurements, which occurred 7 measurements prior. This is a moving average
Fig. 4.5 Voltage ramps with a 1nA threshold at which the voltage begins to ramp back down. Plot a shows a representative example of a standard voltage run. Plot b shows an example of an anomalously higher onset voltage for the first ramp, however consistent subsequent ramps. Plot c shows an example of ramps where the last two ramps went to much higher voltages before electrospray onset than the rest.
Fig. 4.6 Current measurements around the onset of electrospray for a tip with an inner diameter of 60 nm and outer diameter of 150 nm.

Fig. 4.7 The derivative of current measurements for the same tip as in Fig. 4.6 with the programmatically determined onset voltage, 297.1V, highlighted.
window that essentially lags behind the point being examined as illustrated in Fig. 4.8. This

Fig. 4.8 The moving average window of 10 points is shown as a black rectangular box on the same derivative of current data from Fig. 4.6. The box lags behind the point at 297.1V by 7 measurements, and the height of the point at 297.1V is shown to be 7 standard deviations above that average.

is the same derivative plot from Fig. 4.7 overlaid with a box around the points in the moving average, and a line indicating that the point being considered is above some number of standard deviations of that moving average box. When both this point and the one after rise above this moving average by 7 standard deviations, the first point is considered to have been the onset voltage. The number of previous data points that compose the moving average is referred to as the Range, the number of standard deviations that the point being considered must be greater than is referred to as the Sigma, and the separation between the points that compose the moving average and the point being considered is referred to as the Lead.

If the parameters Range, Lead, and Sigma are set to values that result in meaningful onset voltage determination, the program’s results should be fairly insensitive to slight variations in the particular parameter settings. Figs. 4.9a-c show how the determined onset voltages change when one of the three parameters is varied, while holding the remaining two constant. The values at which the remaining parameters not being varied were held constant were Range = 10, Lead = 7, and Sigma = 7. Each line is a different capillary for a total of 6 tips and only data from the second ramp of voltage of each of these tips was used, as was mentioned earlier in this section. No data point is plotted for situations when the program ran through to the end of a voltage ramp without having found a point that meets its onset criterion. A flat line means that no matter what the parameter was changed to, within reasonable values, the
onset voltage was determined to be the same value each time. And finally, all of these tests can be seen stacked on top of one another so that their consistency between one another in Figs. 4.9d. The voltages determined by the program only change for significant deviations of the lead, range and sigma parameters from the chosen values of lead=7, range=10, sigma=7. This indicates that the program chooses onset voltages in a way that is relatively insensitive to small adjustments in the specific parameters chosen, and thus that it provides a reliable determination of the voltage at which electrospray current appears.

Fig. 4.9 Analysis of the sensitivity of the programmatically determined onset voltage to the parameters used to determine onset voltages for 6 different tips. Plots a-c show the voltages determined as one of those parameters was adjusted. Since the determined voltages do not change significantly as functions of these parameters, the determination is relatively robust. Plot d shows a-c overlaid on top of one another.

### 4.1.2 Inner versus outer diameter

It is not obvious where the base of the Taylor cone attaches to the tip of the glass capillary during electrospray. This is important because we have observed that the electrospray source’s diameter affects the type of mass spectra it produces, and we want to develop
a quantitative understanding of the diameter’s effects. It is easy to imagine two possible scenarios shown in Fig. 4.10. Fig. 4.10a shows the electrospray meniscus pinned to the inner walls of the nanocapillary tip, and in Fig. 4.10b the whole tip surface out to the outer diameter is wetted with solution. To examine this we have prepared nanocapillaries with different capillary wall thickness and overlaid their onset voltages with respect to either their inner diameters shown in Fig. 4.11, or outer diameters shown in Fig. 4.12. If the correct scenario is Fig. 4.10a, then the voltages at which onset occurs should not be affected by how thick or thin the capillaries are. However Fig. 4.11 shows that the data for the thicker walled capillaries have systematically higher onset voltages than the thinner walled capillaries. Meanwhile the data plotted against outer diameters Fig. 4.11 shows an insensitivity to these different wall thicknesses. The data show that the same onset voltage is measured for tips with the same outer diameter, independent of their inner diameter. This indicates that the meniscus has wetted the surface of the capillary tips out to their outer diameter as is the case for Fig. 4.10b.

![Fig. 4.10 Two possible scenarios for the how the liquid meniscus pins to the capillary tips during electrospray. a on the left shows the scenario where the liquid is pinned to the inner diameter of the capillary, while b on the right shows the whole surface of the capillary tip to be wetted.](image)

### 4.1.3 Onset voltages do not depend on solution conductivity

Onset voltages do not depend on the conductivity of the solution that is electrosprayed. Fig. 4.13 shows onset voltages versus tip outer diameters for solutions from 1M NaI in formamide down to pure formamide with no added NaI. These data all line up along the same outer diameter dependency for onset voltages. A similar finding of the independence
Fig. 4.11 Onset voltages versus inner diameter of the capillary tip. Blue data points have thicker capillary walls than red data points. The thicker walled capillaries have systematically higher onset voltages indicating that these electrospray onset voltages cannot be predicted by the inner diameter.
Fig. 4.12 Onset voltages versus outer diameter of the capillary tip. Blue data points have thicker capillary walls than red data points, however the electrospray onset voltages for both data sets can be explained by the same functional dependence on outer diameter alone. This indicates that electrospray is insensitive to the tip’s inner diameter and the liquid meniscus of the Taylor cone wets out to the outer diameter as in Fig. 4.10b.
Studies on the mechanisms of charge emission from nanoscale capillary tips

of electrospray onset voltages on solution conductivity was found for capillaries with tip diameters ranging in size from 450 $\mu$m to 1.6 mm by Smith [64].

![Fig. 4.13 Onset voltages versus salt concentration](image)

4.1.4 Theoretical model of the electrospray onset voltage

We compare our measurements of the sudden rise in current, which is indicative of the onset of electrospray, and its dependence on the size of the capillary against two theories found in the literature. The first theory approximates the electric fields of a capillary electrospray source as a semi-infinite line charge, and the second solves Laplace’s equation for the region outside an approximation of the electrospray meniscus subject to boundary conditions. It should be noted that this approximation involves a capillary with an infinite wall thickness and a liquid surface composed of a sphere as the meniscus, and a infinitely long cone as the rest of the capillary length. We find that the semi-infinite line charge theory fits our data better.

4.1.4.1 Semi-infinite line charge approximation

The first theoretical model we compare our data against is based on work by Loeb [43], Jones and Thong [35], and Smith [64], in which an electrospray capillary tip is approximated
by a semi-infinite line charge near an infinite grounded plate. The radius of the tip is assumed to be much smaller than the distance between the tip and the grounded plate. Fig. 4.14 sets up the geometry in cylindrical coordinates \((r, z)\). A semi-infinite line with a charge per unit length of \(\sigma\) sits a distance \(z_0\) away from a grounded infinite plane at the origin. The electric field is solved using the method of images, which is then compared with the electric field on the cone shape of the electrified liquid surface in stable equilibrium found through the balance of electrostatic pressure and Laplace pressure by G. I. Taylor.

**Fig. 4.14** A semi-infinite line charge and its image charge are shown as thick black lines along the radial axis in cylindrical coordinates. \(Z_0\) represents the distance of the capillary tip away from the extractor electrode.

Coulomb’s law gives us the electric field, \(\vec{E}\), in cylindrical coordinates as an integral over all charge contributions, \(dq\):

\[
\vec{E} = \frac{1}{4\pi\varepsilon_0} \int \frac{dq\hat{R}}{|\hat{R}|^2} \quad \text{and} \quad dq = \sigma dz'
\]

where \(\hat{R} = \frac{r\hat{r} + (z - z')\hat{z}}{|\hat{R}|}\) (4.1)

and \(\varepsilon_0\) is the permittivity of free space, \(\hat{z}\) is the axial direction, and \(\hat{r}\) is the radial direction. The method of images is based on the semi-infinite line charge of equal and opposite charge distribution induced by bringing the original semi-infinite line charge near a conducting surface, which is the grounded electrode in our electrospray setup. The electric field is the sum of the contributions from the semi-infinite line charge and its induced image charge with...
Studies on the mechanisms of charge emission from nanoscale capillary tips

\(-\sigma\) at \(-z_0\).

\[
E_r = \frac{1}{4\pi\varepsilon_0} \int_{z_0}^{\infty} \frac{\sigma}{|\vec{R}|^2} \left( \frac{r\hat{r}}{|\vec{R}|} \right) dz' + \frac{1}{4\pi\varepsilon_0} \int_{-\infty}^{-z_0} \frac{-\sigma}{|\vec{R}|^2} \left( \frac{r\hat{r}}{|\vec{R}|} \right) dz'
\]

(4.2)

Plugging in for \(R\) and integrating gives:

\[
E_r = \frac{\sigma}{4\pi\varepsilon_0 r} \left( \frac{z - z_0}{\sqrt{r^2 + (z - z_0)^2}} + \frac{z + z_0}{\sqrt{r^2 + (z + z_0)^2}} \right)
\]

(4.3)

We can then solve for the voltage at the capillary tip radius by integrating with respect to \(r\) and setting \(r = r_c\), where \(r_c\) is the capillary tip radius, and \(z = z_0\). This will give us a relationship between how the charge density of the line charge affects the voltage at the capillary tip’s surface, which we will then use to relate the strength of the electric field at the capillary tip with the corresponding voltage at the capillary tip.

\[
V = -\frac{\sigma}{4\pi\varepsilon_0} \int \frac{z - z_0}{r\sqrt{r^2 + (z - z_0)^2}} + \frac{z + z_0}{r\sqrt{r^2 + (z + z_0)^2}} dr
\]

(4.4)

\[
V = -\frac{\sigma}{4\pi\varepsilon_0} \ln \left( \frac{z_0 - z + \sqrt{r^2 + (z - z_0)^2}}{z_0 + z + \sqrt{r^2 + (z + z_0)^2}} \right)
\]

(4.5)

Setting \(r = r_c\) and \(z = z_0\) gives us an equation relating the voltage at the capillary tip to the charge density of the semi-infinite line charge.

\[
V = -\frac{\sigma}{4\pi\varepsilon_0} \ln \left( \frac{r_c}{2z_0 + \sqrt{r_c^2 + 4z_0^2}} \right)
\]

(4.6)

We assume that the capillary is much smaller than the distance to the grounded plane, i.e. \(r_c \ll z_0\), so that

\[
V = -\frac{\sigma}{4\pi\varepsilon_0} \ln \left( \frac{r_c}{4z_0} \right)
\]

(4.7)

and rearranging gives

\[
\sigma = \frac{4\pi\varepsilon_0 V}{\ln \left( \frac{4z_0}{r_c} \right)}.
\]

(4.8)
4.1 Electrospray current and onset voltage measurements

To find the critical voltage at the onset of electrospray, we now can incorporate Eq. 4.8 into Eq. 4.3 and, using the same assumption of $r_c \ll z_0$ and setting $r = r_c$ and $z = z_0$, obtain

$$E_r(r_c) = \frac{V}{r_c \ln \left( \frac{4z_0}{r_c} \right)}$$  \hspace{1cm} (4.9)

It turns out that $|E_r| = |E_z|$ so that,

$$E_0 = \left| \vec{E} \right| = \frac{\sqrt{2V}}{r_c \ln \left( \frac{4z_0}{r_c} \right)}$$  \hspace{1cm} (4.10)

We then relate this equation with the electric field on the Taylor cone, Eq. 1.11, derived from the equilibrium condition between the Laplace pressure from surface tension and the electrostatic pressure from the applied voltage at the onset of electrospray, as was originally derived by G. I. Taylor [74] in the introduction

$$E_0 = \sqrt{\frac{2\gamma \cot \alpha}{\varepsilon_0 R}}.$$  \hspace{1cm} (4.11)

Since Eq. 4.11 is derived in spherical coordinates, whereas Eq. 4.10 is in cylindrical coordinates, we must convert $R$, through $R = \frac{r}{\sin \alpha}$. We then set $r = r_c$ to equate the two fields on the same point at the capillary tip. We are interested in the pressures and electric fields at this point, because we are comparing them with the electric fields at this point from the semi-infinite line charge approximation in Eq. 4.9. Thus Eq. 4.11 becomes

$$E_0 = \sqrt{\frac{2\gamma \cos \alpha}{\varepsilon_0 r_c}}$$  \hspace{1cm} (4.12)

And finally combining Eq. 4.12 with Eq. 4.10 and solving for $V_{\text{onset}}$ results in:

$$V_{\text{onset}} = \sqrt{\frac{r_c \gamma \cos \alpha}{\varepsilon_0}} \ln \left( \frac{4z_0}{r_c} \right).$$  \hspace{1cm} (4.13)

Smith and others in the literature additionally include what is described as an "empirical constant", $A_1$

$$V_{\text{onset}} = A_1 \sqrt{\frac{r_c \gamma \cos \alpha}{\varepsilon_0}} \ln \left( \frac{4z_0}{r_c} \right)$$  \hspace{1cm} (4.14)
4.1.4.2 Solving Laplace’s equation around a spherical meniscus in the sphere-on-cone (SOC) model

An alternative approach to deriving the voltage at electrospray onset, based on the work of Ward and Seliger [80], as well as Lozano, Martinez-Sanchez, and Lopez-Urdiales [45], involves solving Laplace’s equation. The electrified liquid in an electrospray capillary is here approximated using what is referred to as a sphere-on-cone (SOC) model, shown in Fig. 4.15. As the name of the model implies, the meniscus at the end of a capillary is modeled as a conducting surface composed of a sphere and a cone positioned with its apex at the center of the sphere. The electric field is calculated by solving Laplace’s equation, subject to boundary conditions for the space outside of the surface. It should be noted here that the cone part of this sphere-on-cone model should not be confused with the Taylor cone. In this case it is the electric fields before electrospray has yet to begin that are being modeled, and the cone here refers instead to the upstream liquid in the capillary tube. Basically the cone is approximating a long cylinder, but since the math is spherically symmetric the cylinder becomes a really stretched out cone.

![Fig. 4.15 SOC model](image)

The theory assumes that the electric potential can be written \( \Phi = R(r)\Theta(\theta) \) where \( R(r) \) is the radial dependence on the potential, and \( \Theta(\theta) \) represents the dependence on the angle away from the capillary’s central axis. Since the geometry is spherically symmetric, there
4.1 Electrospray current and onset voltage measurements

is no $\Phi$ component. General solutions for the radial part $R(r)$ are of form $r^\nu$, and $r^{-\nu-1}$, while general solutions for the $\Theta(\theta)$ are Legendre functions of the first and second kind, $P_\nu(\cos \theta)$ and $Q_\nu(\cos \theta)$. $Q_\nu(\cos \theta)$ does not show up very often as it blows up to infinity at $\cos \theta = \pm 1$, however since the geometry is a cone, there are parts of the solution where $\theta$ stays between $\frac{\pi}{2}$ and the cone angle, $\theta_c = \pi - \beta$, and so $\cos \theta$ never approaches 1. The surface of the combined sphere and cone is assumed to be an equipotential surface at one constant voltage, and the solutions to the potential are split into the vacuum $\Phi_0(\theta < \frac{\pi}{2})$, and inside the dielectric representing the capillary $\Phi_1(\frac{\pi}{2} < \theta < \theta_c)$. For simplicity the capillary is assumed to be grounded and so the equipotential surface must be at zero for $r = a$. This implies that the coefficients for the radial part, $R(r)$ end up being 1 and -1 such that,

$$\Phi_0 = \left[ \left( \frac{L}{a} \right)^\nu - \left( \frac{a}{r} \right)^{\nu+1} \right] A P_\nu(\cos \theta) \quad (Q_\nu(\cos 0) \text{ is undefined at } \theta = 0). \quad (4.15)$$

$$\Phi_1 = \left[ \left( \frac{r}{a} \right)^\nu - \left( \frac{a}{r} \right)^{\nu+1} \right] [B P_\nu(\cos \theta) + C Q_\nu(\cos \theta)] \quad (4.16)$$

The potential must be continuous at the boundary ($\theta = \frac{\pi}{2}$) or

$$A P_\nu^0 = B P_\nu^0 + C Q_\nu^0 \quad (4.17)$$

where $P_\nu^0 = P_\nu(0)$ and $Q_\nu^0 = Q_\nu(0)$. And Gauss’ law, $\nabla \cdot \vec{D} = 0$ implies the $\vec{E}$ field normal to the surface are equal or

$$\epsilon \frac{\delta \Phi_1}{\delta \theta} = \frac{\delta \Phi_0}{\delta \theta} \quad (4.18)$$

Thus

$$\frac{A}{\epsilon} P_\nu^0 = B P_\nu^0 + C Q_\nu^0 \quad (4.19)$$

where $P_\nu^0 = \frac{\delta P_\nu}{\delta \theta}(0)$ and $Q_\nu^0 = \frac{\delta Q_\nu}{\delta \theta}(0)$.

Finally the potential on the surface of the cone ($\theta = \theta_c$) is defined to be zero

$$B P_\nu(\cos \theta_c) + C Q_\nu(\cos \theta_c) = 0 \quad (4.20)$$

Combining Eqs. 4.17, 4.19, and 4.20 gives an equation from which $v(\beta, \epsilon)$ can be found

$$\left( \frac{Q_\nu^0}{P_\nu^0} - \frac{Q_\nu^0}{\epsilon P_\nu^0} \right) P_\nu(\cos \theta_c) - \left( \frac{\epsilon - 1}{\epsilon} \right) Q_\nu(\cos \theta_c) = 0 \quad (4.21)$$
Assume the extractor to be fixed at an axial distance $R$ away at potential $-V$, then $\phi_0(R, 0) = -V$ and $P_V(1) = 1$

$$A = -V \left[ \left( \frac{R}{a} \right)^v - \left( \frac{R}{a} \right)^{v+1} \right]^{-1} \quad (4.22)$$

$$\Phi_0 = -V \left( \frac{\rho}{a} \right)^v \left( \frac{R}{a} \right)^v \left( \frac{R}{R} \right)^{v+1} P_V(\cos \theta) \quad (4.23)$$

and

$$\Phi_1 = \frac{\Phi_0}{\left( Q_V^0 \frac{P_V^0}{P_V^0} \frac{Q_V^0}{P_V^0} \right)} \left[ \left( \frac{Q_V^0}{P_V^0} - \frac{Q_V^0}{P_V^0} \right) \frac{\epsilon - 1}{\epsilon} \frac{Q_V(\cos \theta)}{P_V(\cos \theta)} \right] \quad (4.24)$$

Assuming the meniscus is spherical and the pressure inside the liquid is negligible, then the electric forces will overcome surface tension when

$$\frac{1}{2} \epsilon_0 E_n^2 > \frac{2\gamma}{r_m}, \quad (4.25)$$

where $E_n$ is the field normal to the surface or $-\delta \phi_0 / \delta r$, and $r_m$ is the radius of the meniscus. We set $r_m = fa$ for $f > 1$ where $f$ is a parameter used to set the equipotential surface to more closely match the geometry of the actual liquid surface and was set to $f = 1.15$ by Lozano et al. This has the effect of pushing the surface out to a smoother equipotential as shown in Fig. 4.15 as the curvy line just outside of the sphere on cone.

$$E_n = -\frac{\delta \phi_0}{\delta r} \mid_{r_m} = C_1 \frac{\delta}{\delta r} \mid_{r_m} \left( \frac{R}{a} \right)^v \left( \frac{a}{r} \right)^{v+1}, \quad C_1 = \frac{VP_V(\cos \theta)}{(\frac{R}{a})^v - (\frac{a}{R})^{v+1}} \quad (4.26)$$

$$= C_1 \left[ \frac{v}{a^v} r_m^{v-1} + (v + 1) \frac{a^{v+1}}{r_m^{v+2}} \right], \quad \text{where} \quad a = \frac{r_m}{f} \quad (4.27)$$

so that

$$= C_1 \left[ \frac{vf^v}{r_m^v} r_m^{v-1} + (v + 1) \frac{r_m^{v+1}}{f^{v+1} r_m^{v+2}} \right] \quad (4.28)$$

$$= \frac{C_1}{r_m} \left[ vf^v + (v + 1) f^{-v-1} \right] \quad (4.29)$$

Assuming $R \gg a$ then

$$C_1 = \frac{VP_V(\cos \theta)}{(\frac{R}{a})^v} \quad (4.30)$$

and plugging in for $a$

$$C_1 = \frac{Vr_m^v}{(Rf)^v P_V(\cos \theta)} \quad (4.31)$$
and plugging in $C_1$

$$E_n = \frac{V}{r_m} \left( \frac{r_m}{R} \right)^v \left( v + (v + 1) f^{-2v-1} \right) P_v(\cos \theta) \quad (4.32)$$

Plugging Eq. 4.32 into Eq. 4.25

$$\frac{V}{r_m} \left( \frac{r_m}{R} \right)^v \left( v + (v + 1) f^{-2v-1} \right) P_v(\cos \theta) > 2 \sqrt{\frac{\gamma}{\varepsilon_0 r_m}} \quad (4.33)$$

Assuming that if the electrostatic pressure overcomes surface tension anywhere along the surface it will lead to the formation of a Taylor cone, we can take the maximum $P_v$ (corresponding to a min $V$) of $P_v(1) = 1$, and to be consistent with our previous semi-infinite line charge theory set $R$ to $z_0$, thus

$$V_{onset} = 2BI \sqrt{\frac{\gamma r_m}{\varepsilon_0}} \left( \frac{z_0}{r_m} \right)^v \frac{1}{v + (v + 1) f^{-2v-1}} \quad (4.34)$$

The sphere on cone model is also given a scalar fitting parameter similar to $A_1$ for the semi-infinite line charge model.

4.1.4.3 Comparison of semi-infinite line charge theory with the SOC model

The theories developed in section 4.1.4 are compared with data collected for capillaries at two different distances away from the electrospray extractor electrode, 0.5 mm and 13 mm ±0.5 mm. The dielectric constant of our glass is around $\varepsilon \approx 4.6$, which according to Eq. 4.21 means that $v = 0.206$. The capillary angle in the SOC model is set to 2 degrees, based on SEM images of an average capillary, and $f=1.15$ as was done by Lozano et al [45].

The SOC model was fit to the data and the scalar constant $B_1$ was set to 0.8. The scalar constant, $A_1$, in the semi-infinite line charge theory was set to the same value, 0.667, used by Smith [64]. Clearly our data line up much better with the semi-infinite line charge theory represented by the light blue and dark blue lines. Previous data from Smith was limited to capillaries between 200 microns and 1 mm [64]. We have demonstrated that it is possible to produce electrospray from capillaries with nanoscale openings, and that the voltages required are in the hundreds of volts, as opposed to the standard operating voltages of kilovolts with the lowest requiring only 150 V to produce electrospray current. Previous data from Smith was limited to capillaries between 200 microns and 1 mm [64]. Our data shows that the semi-infinite line charge model works well with the fitting parameter $A_1=0.667$ both in the 100s of microns range as well as in the 100’s of nanometers range [64].
Fig. 4.16 Dependence of the onset voltage on the tip outer diameter for two different tip-extractor separations, $z_0$. The light green data was measured when $z_0 = 13$ mm and the dark green data was measured when $z_0 = 0.5$ mm. The light blue and dark blue lines represent the semi-infinite line charge theory when $z_0$ is set to 13 mm and 0.5 mm respectively. The light red and dark red lines represent the SOC model for $z_0$ at 13 mm and 0.5 mm respectively. Clearly our data is more in line with the semi-infinite line charge theory.
4.2 Flow rate versus tip size

Flow rate measurements at the sub nL/s regime are difficult to measure directly. We have developed a method capable of measuring these incredibly low flow rates. The basic flow rate measurement involves submerging a capillary’s tip in silicone grease, inflating a liquid water bubble, and then tracking the bubble’s growth over time with a microscope. Assuming the bubble remains affixed to the tip of the capillary, maintains a mostly spherical shape, and grows at a reasonable rate, the flow rate can then be deduced from its size. To create a bubble requires overcoming the pressure associated with the bubble’s surface tension, or the Laplace pressure. The pressure scales with surface curvature, and is at its maximum in the bubbles at the beginning of the flow rate measurement, when they are just the size of the capillary opening. This means that for flow to begin the Laplace pressure specific to the capillary’s size must be overcome. Thus our measurement is limited to tips with \( \approx 250 \text{ nm} \) diameter openings or greater, since that corresponds to around 12 atmospheres of pressure, the maximum our setup can deliver. Since the pressure required to make one of these flow rate measurements changes for different tip sizes, ultimately we report the fluidic conductance of our capillaries, which is pressure independent.

4.2.1 Experimental methods

Capillary tips were held with the same tip holder mentioned previously, however the fluidic tubing was instead connected to a nitrogen cylinder capable of applying up to 12 atm of pressure. The tip holder was affixed to three micrometer positioning stages above a small dish of silicone grease placed on top of a microscope with a 10x objective and 7 mm working distance. Tips were initially submerged in mineral oil, however bubbles formed in oil often dislodged from the tip ends before growing to an appreciable size for reasonable growth rate measurements. Mineral oil was replaced by silicone grease to make sure the bubbles stayed fixed to the ends of our capillary tips long enough to measure a bubble growth rate. Greases are rated for their relative hardness by the National Lubricating Grease Institute based on penetration tests that measure how far specifically weighted cones sink into the grease. They rate grease consistencies starting at 000, which has the consistency of cooking oil, all the way to 6, which is apparently similar to cheddar cheese. We used silicone grease with an NLGI consistency number of 1, which is a bit like tomato paste. Tips were submerged in the grease, and the pressure was ramped up until a bubble began to form at which point the pressure was kept constant and images were collected at 10 Hz. Typical bubble growth can be seen in Fig. 4.17 for one particular capillary with an inner diameter of 185 nm. Bubble growth rates were derived from measuring a bubble’s diameter in pixels at various times.
Studies on the mechanisms of charge emission from nanoscale capillary tips

along the growth, converting to volumes assuming spherical bubbles, and fitting the growth to a linear function. Shown in Fig. 4.18 is a representative example of the growth rate fit for the same capillary from Fig. 4.17. Eventually all bubbles would begin to distort and inflate into oblong shapes, and so growth rates were only considered during the early growth of the bubble for which it maintained an approximately spherical shape. The camera and objective lens we used had a pixel size of 16 microns, with a 10x magnification objective, and so each pixel is 1.6 microns wide.

Fig. 4.17 Several example images of a water bubble grown on a tip with an inner diameter of 185 nm and an outer diameter of 369 nm in silicone grease.
Fig. 4.18 Flow rate measurements with a linear fit line for a single capillary with an inner diameter of 185nm. The bubble grows to a diameter of \( \approx 100\mu \text{m} \) over the course of \( \approx 20 \) seconds with an applied pressure of 550 kPa. This results in a fluidic conductance of 0.05 pL/(s kPa).
4.2.2 Poiseuille flow through an infinite truncated cone

We approximate the flow in these capillary tips as Poiseuille flow, or laminar flow of an incompressible fluid, through a truncated cone with some opening radius, \( R \), and cone angle, \( \theta \), for fluid with viscosity \( \eta \). Starting with the equation for Poiseuille Flow in a pipe:

\[
Q = -\frac{dP}{dx} \frac{\pi R^4}{8 \eta},
\]

where \( Q = \frac{dV}{dt} \) or the volumetric flow rate. Integrating the pressure drop \( dP \) over the length of the capillary \( dx \) and accounting for the changing radius of the capillary over its length will result in a flow rate for a given pressure.

\[
\int_0^\infty dP = -\int_0^\infty \frac{8\eta \Phi}{\pi R^4} dx
\]

(4.36)

For a uniform cylindrical pipe the radius \( R \) would just be a constant, however we integrate over a cone that begins at some minimum radius, \( R_{\text{min}} \) at \( x=0 \) and has some small cone angle, \( \theta \), so that \( R = R_{\text{min}} + x \sin(\theta) \).

\[
\Delta P = -\frac{8\eta Q}{\pi R_{\text{min}}^4} \int_0^\infty \frac{1}{\left(1 + x \frac{\sin(\theta)}{R_{\text{min}}} \right)^4} dx
\]

(4.37)

\[
Q = \frac{8\eta \Delta P}{3\pi R_{\text{min}}^3 \sin(\theta)}
\]

(4.38)

4.2.3 Fluid conductance as a function of capillary tip size

Before an initial bubble is created and grown at the capillary tip the curvature at the meniscus produces a Laplace pressure that varies in magnitude with the capillary tip’s inner diameter. As the bubble inflates the radius of the drop increases and the Laplace pressure decreases, which means that the Laplace pressure is at it’s maximum at the beginning of bubble creation. This pressure must be overcome for there to be flow through the capillaries and for the bubbles to inflate as a result. Since different sized tips have different maximum Laplace pressures from the surface tension at the water/grease interface, different pressures were needed to initiate bubble creation and growth. This means that the flow rates of different tips will be measured with different applied pressures. Thus in order to compare tips, we are interested in what their flow rates would be for any given flow rate, or the fluid conductance. Fluid conductance is measured in flow rate per unit applied pressure, and so each flow rate measurement is essentially normalized by dividing by the applied pressure used to create and
grow each bubble. The resulting fluid conductance measurements are then plotted versus nanocapillary tip size in Fig. 4.19 for twelve capillaries spanning a range of sizes with inner diameters from $\sim 1\, \mu\text{m}$ down to $\sim 120\, \text{nm}$.

Generally, data was only taken from the first bubble blown; however, the tips with inner diameters of 426nm and 608nm were pulled away from their first bubbles, as well as second bubble in the case of the tip with 426nm, and subsequent bubble growth rate measurements were taken. These subsequent measurements were fairly consistent with the original, however it was clear that if enough subsequent measurements were taken on the same capillary, eventually a change resulting in orders of magnitude slower flow rates would occur. Glass is very hydrophilic, so the ultimate loss of bubble growth could be caused by the outer surface of the drop becoming wetted. Instead of flow inflating a bubble, the flow leaks up the sides of the capillary, potentially even accumulating out of the field of view of the microscope. Shown plotted with the data are fits to the truncated infinite cone model as well as for a simple finite length tube. The infinite truncated cone model works fairly well at approximating the expected fluid conductance given a capillary’s inner diameter.

We have thus developed a way to directly measure flow rates among the lowest reported values used for electrospray. This is a difficult regime to measure flow rates accurately, however the bubble inflation method proves to be fairly robust with highly linear fits to bubble volume growth. This fit can be used to estimate the flow rates of capillaries in subsequent experiments with formamide by accounting for the different surface tension of formamide relative to water. These flow rates have been shown by de la Mora and others to be very important to the preferential emission of ions over droplet formation. We will use Eq. 4.38 to calculate the flow rate in our estimates of the electric field strength at the apex of the Taylor cone’s of nanocapillaries using the equation derived in the introduction, $\frac{\lambda^\frac{3}{2} K^{1}}{\varepsilon_0^\frac{3}{2} Q^6}$.

(4.39)

4.3 Maximizing ion emission over drop production using nanocapillaries

It is important to the single biopolymer sequencing strategy that all monomers in solution be transferred into the gas phase as ions. This requires the complete suppression of drop production and for all current leaving the nanocapillary electrospray source to leave as evaporated ions. We use a comparison between the total measured current leaving the nanocapillary
Studies on the mechanisms of charge emission from nanoscale capillary tips

Fig. 4.19 Fluid conductance versus capillary size

with the ion count rate output from the single ion detector in our mass spectrometer, to directly explore the competition between the emission of ions versus the production of drops from our electrospray sources. Drops have mass to charge ratios order of magnitude greater than what the range our mass spectrometer is sensitive to. This comparison of ions versus drops takes the form of an efficiency ratio which would be 1 if all current leaving the nanocapillary is emitted through ion evaporation as bare and partially solvated ions, while a ratio of 0 means that all current leaves as drops. We expect the low flow rates that result from the nanoscale openings of our capillaries to encourage ion evaporation by increasing the field strength at the apex of the Taylor cone. Additionally we expect a higher efficiency when the solution conductivity is higher. We use flow rate measurements from our previous bubble inflating experiments combined with bulk solution conductivity measurements to estimate the electric field strength for these nanocapillaries. The electric field strength at which the proportion of ions versus drops increases is expected to be the critical electric field at which ion evaporation begins to dominate, however we do not find our data to align with existing critical electric field theories.

4.3.1 Experimental methods

As mentioned in the introduction, our mass spectrometer is a filter and produces mass spectra through a scanning technique. This means that, aside from being insensitive to drops,
our mass spectrometer is throwing out some large fraction of the ions within its range of sensitivity at any given moment during a mass scan. That fraction is the size of the scanning window divided by the total mass to charge ratio range of sensitivity. Assuming that the emission of ions is constant, we can account for this loss and obtain an ion count rate for the full mass range by multiplying the ion count at our detector by the fraction of the full mass range divided by this mass window. We must additionally keep in mind that mass spectra specific to some particular electrospray output will have some parts of the scan with many ions and some parts with very few. If we compare this ion count rate with the current leaving the electrospray source scan by scan, while accounting for the losses inherent to the scanning mass window, we should get a fairly accurate ratio of the number of ions arriving at the detector versus the number leaving the electrospray source. Thus we make a transmission fraction, or efficiency measurement, which is the number of ions that hit the detector summed per scan, divided by the number that left the electrospray source over the time that scan took and multiplied by the mass range to mass window ratio.

\[ \Sigma = \frac{\text{# ions per scan} \times \frac{\text{mass range}}{\text{mass window}}}{\text{source current} \times \text{scan duration}} \] (4.40)

The ion count rate we observe in our mass spectrometer is a lower bound measurement of the proportion of ions versus drops leaving our electrospray sources. Our setup does not have perfect alignment of the electrospray source and electrostatic lenses. This means that ions emitted off axis may be thrown off to the sides of the vacuum chamber or collide with one of the Einzel lenses, never to be counted by the detector of the mass spectrometer. Also the cutoff between what constitutes an ion cluster and what counts as a drop is not well defined, although drops are many orders of magnitude larger in m/z. All mass spectra were collected in positive mode over a range of 150 to 1000 m/z electrospaying positive sodium ions, and the detector collected ions over this mass range every 0.61 seconds. This drop versus ion cutoff is certainly much greater than the upper limit of 1000 m/z to the sensitivity range of our mass spectrometer, since even at 2000 m/z the particles that would hit the detector would still be singly charged and with fewer than a hundred solvent molecules. Thus this efficiency measurement as a proxy for counting all ions leaving our electrospray sources is certainly under-counting the actual ion emittance rate.

Mass spectra were collected from capillaries with inner diameters ranging from \( \sim 2.9 \mu m \) down to \( \sim 82 \) nm and conductivity of the solution being electrosprayed was also varied. Solutions were made from NaI dissolved in formamide, with various conductivities achieved by adjusting the NaI molar concentration. Even though we are changing the abundance of ions in solution by changing the concentration of salt in formamide, this efficiency fraction
Studies on the mechanisms of charge emission from nanoscale capillary tips allows us to track how the proportion of ions versus drops changes with conductivity, without also being sensitive to those corresponding ion abundance changes. We expect ion abundance to increase both the number of ions leaving the electrospray source, as well as the number hitting the mass spectrometer’s detector, so this fraction should stay the same. Solution conductivities ranged from \(\sim 13 \text{ mS/cm} \) down to \(\sim 0.3 \text{ mS/cm} \) were measured using a Hach sensION+ EC71 with a 50-70 three pole platinum probe.

The focusing of ions is affected by the total voltage drop the ions experience before entering the mass spectrometer, as well as the voltages applied to the electrospray source relative to the lens elements. The voltage drop between the tip voltage and the mass spectrometer was held constant among all tips and salt concentrations as well as the voltages applied to the lens focusing elements. The extracting voltages are necessarily different for different capillaries since larger capillary diameters require higher electrospray onset voltages. Electrospray onset was achieved with a variable negative extracting voltage was applied to the first lens element \(\approx 1\text{mm} \) away from the capillary tip. Differences in the losses of ions due to various capillaries having better or worse focusing were minimized by holding the positive voltages applied to the capillary and lens elements constant among all tips and salt concentrations. Additionally, capillaries exposed to extraction voltages much greater than their onset voltage stop emitting detectable ions. This is likely related to the salt deposits observed earlier in the onset voltage measurements section. Thus voltages used to produce these electrosprays were held close to the electrospray onset voltages for each capillary to give more consistent and stable electrosprays. Changes in solution were introduced with a syringe pump set to a flow rate of 0.7 mL/hr. As mentioned in more detail previously, the fluid setup consists of an inlet tubing that extends into the capillary tube up to its taper point. Solutions were given on the order of an hour to change at the tip via diffusion.

### 4.3.2 Varying tip size and solution conductivity

Mass spectra were collected on each capillary for various solution conductivities, limited by the time it takes to change solutions and the continued presence of electrospray current, which for most capillaries would eventually cease. Fig. 4.20 shows some example data from two capillaries in which the efficiency is plotted in black while the current leaving the electrospray source is plotted in blue. Changes in solution involved the injection of new solution about 1 mm away from the tip of the capillary. On average the new solution takes an hour to diffuse to the liquid/vacuum interface, and so efficiency measurements were recorded for new solutions after this hour had passed. Transmission efficiency measurements were collected for many tips at various solution conductivities, and the electric field at the apex of
Fig. 4.20 Dependence of the ion detection efficiency, $\Sigma$, and the ion emission current (right axis) on time. The top plot shows a 111 nm inner diameter capillary starting with 4 mS/cm solution (200mM NaI in formamide) and at around 2300 seconds and 8 mS/cm solution (500mM NaI in formamide) is injected. Efficiency measurements are taken from the beginning and end of this plot for the two conductivities. Similarly the bottom plot shows a 1406nm inner diameter capillary electrospraying a 4 mS/cm solution, which is then changed to a 2 mS/cm solution, followed by a 8 mS/cm.
their Taylor cone was estimated by

\[
E = \frac{\lambda^{1/2} K^{1/6}}{\varepsilon_0^{3/2} Q^{1/6}},
\]

as derived in the introduction. As the conductivity of the solution being electrosprayed increases, we expect this electric field strength to eventually increase past the critical electric field, which previous experiments have calculated to be between 1 and 3 V/nm [26]. This should manifest itself in the transmission efficiency, where the amount of ions versus drops produced in the electrospray should begin to increase around this critical electric field. Thus, even though various tips will have different flow rates given their different inner diameters according to our previously demonstrated flow rate measurements, we expect the increase in the transmission efficiencies of the various tips to all line up around a single critical electric field. Each line plotted in Fig. 4.21 represents transmission efficiency measurements for a different capillary, as the solution conductivity was adjusted. The transmission efficiencies of the capillaries increases as the conductivity of the solution, and thus the strength of the electric field, increases. However, this increase in transmission efficiency does not line up at any one critical electric field using Eq. 1.22 amongst the capillaries.

Work done by Ryan, Smith, and Stark found that for tips in the micron size range electrospray parameters like voltage and the shape and size of the capillary producing electrospray begin to become important[59, 2, 60]. Unlike previous experiments by de la Mora and others in which the capillaries were large enough and the solutions electrosprayed conductive enough for geometry specific parameters to matter, Ryan et al found that the flow rate of their micron sized tips would be affected by the applied voltage. They based their model on this additional flow on the same semi-infinite line charge we used to model the electric fields at electrospray onset. Plugging in the electric field in Eq. 4.10 into electrostatic pressure \( E = \frac{1}{2} \varepsilon_0 E^2 \) and adding this pressure into the electric field equation gives a new calculation of the electric field strength of the Taylor cone. The same data as in Fig. 4.21 is then plotted with this new electric field strength calculation and shown in Fig. 4.22. However the capillaries do not line up along a single critical electric field accounting for this additional flow rate either. Thus existing theories do not explain our data in the context of a critical electric field strength.

However these transmission efficiency measurements do follow many of the same trends associated with flow rate and solution conductivity. Efficiency measurements were collected from many tips for different solutions and combined into a log-log plot of efficiency versus tip size for various conductivities. Fig. 4.23 shows such a log-log plot for tips spraying 8 mS/cm
4.3 Maximizing ion emission over drop production using nanocapillaries

Fig. 4.21 Transmission efficiencies versus calculated electric fields. Each plotted line represents a different capillary measured at different solution conductivities. The increases in transmission efficiency as the conductivities are increased do not occur for a single critical electric field strength.
Fig. 4.22 Transmission efficiencies versus calculated electric fields. Each plotted line represents a different capillary measured at different solution conductivities. The increases in transmission efficiency as the conductivities are increased do not occur for a single critical electric field strength.
solution (500mM NaI in formamide). There is a clear trend towards higher transmission efficiencies with smaller tip sizes.

There are many ways for these transmission efficiencies to be lower than what is possible for a given tip size and solution conductivity. Spray behavior can change from voltages straying too far away from onset, from having been electrosprayed for too long, other spray modes were active at various times with lower ratios, or possibly salt residue building up outside the tip opening. As a result, these efficiencies serve as a lower bound for each tip size, and the upper envelopes of the data should reveal more general trends for electrospray with nanoscale capillaries. We have used the convex hull algorithm to define this envelope. Simpler than it sounds, this algorithm basically tries to form the smallest closed convex shape around data points it’s given. This shape can be seen for the same data as Fig. 4.23 in Fig. 4.24. After that we just take the upper curve as the envelope, as seen in Fig. 4.25. We have done this for a few other solution conductivities and compiled them into Fig. 4.26. Conductivity increases this transmission efficiency, and each solution conductivity separately also exhibits the trend of smaller capillary openings resulting in higher transmission efficiencies.

We find that when we use existing theories of the electric field strength at the Taylor cone apex, our measurements do not converge on a particular critical field strength. These critical field theories have previously only been tested with large capillaries in the mm range.
Fig. 4.24 Data from Fig. 4.23 with a the convex shape found using the convex hull algorithm plotted on top. The algorithm finds the smallest closed convex shape for a given set of points.

Fig. 4.25 The upper envelope of data from Fig. 4.23 represents a lower bound for transmission efficiency as a function of tip diameter for this solution conductivity of 8 mS/cm.
4.3 Maximizing ion emission over drop production using nanocapillaries

Fig. 4.26 Log-log plot of the lower transmission efficiency bound versus nanocapillary tip inner diameters with a compilation of data envelopes for four different solution conductivities. The conductivities for these curves from top to bottom are: 13 mS/cm in blue, 8 mS/cm in orange, 4 mS/cm in green, and 2 mS/cm in red. Increasing conductivity and decreasing tip openings increase the transmission efficiency.
in which flow rates can be carefully controlled with applied pressures, and using solution conditions that intentionally minimize the effects of geometry. These conditions have the effect of electrically isolating the location of jet formation on the Taylor cone apex from the edges of the capillary, and so parameters such as the applied voltage are not factored in [15]. We believe our capillaries are small enough for the effects of geometry to be important. Although theories from Ryan et al were used to calculate additional flow rate, it should be noted that these theories have only been successful in calculating the slope of their flow rate versus voltage measurements and not the absolute flow rates. Additionally they did not conduct measurements for capillaries in the 100’s of nanometers range. And so current theories of this critical field strength do not predict our observations of ions in our mass spectrometer. However, we do find that our nanoscale capillaries follow similar trends of increased ion production relative to drop production for decreasing capillary size, which is related to flow rate, and increasing solution conductivities.

4.3.3 Increasing flow rates through applied pressure decreases ion transmission efficiencies

Since increasing flow rate is expected to reduce the electric field strength at the Taylor cone’s apex, we expect the transmission efficiency to decrease when the flow rate is increased through an applied pressure. We also expect that increasing the applied pressure should result in greater measured current leaving the electrospray source since a higher flow rate of charged fluid means a higher flow rate of charge. Indeed, increasing the applied pressure to our capillaries has the effect of increasing the emitted current from the nanocapillary electrospray sources as expected. Although the total number of ion counts hitting our detector increase with applied pressure as well, there is a small but noticeable as can be seen in Fig. 4.27. Eventually the pressure is increased beyond atmospheric pressure enough that the emitted current suddenly increases and the counts hitting the detector decreases almost to zero seen in the plot around 310 seconds. If the pressure is brought back down to atmospheric pressure, the current emitted and detected return roughly to their original levels at around 350 seconds. This effect was repeatable on two different capillaries, assuming the pressure was not further increased beyond this sudden change. This sudden drop in ion counts may result from the activation of a different spray mode in which much more current is emitted through charged drops as opposed to through the ion evaporation process. The decrease in transmission efficiency with increasing pressure is consistent with our expectations that higher flow rates result in less direct ion emission from the Taylor cone.
4.3 Maximizing ion emission over drop production using nanocapillaries

Fig. 4.27 Ion efficiency versus applied pressure. The applied pressure was increased from 100 kPa at around 125 seconds to 210 kPa at around 320 seconds, then held constant and subsequently reduced back to atmospheric pressure. The capillary’s inner diameter was 262nm.
Chapter 5

Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer

We analytically model the dynamics of a pair of Brownian particles in a one-dimensional linear force gradient and calculate the probability that their order will invert as a function of time, the initial separation distance, and the strength of the force gradient.

5.1 Theoretical model

We consider the dynamics of two distinguishable, non-interacting Brownian particles in one dimension. The particles move in a convex potential which generates a linear force gradient. A particle at position $x$ feels a force $kx$, where $k$ is the strength of the force gradient. The system is illustrated in Figure 5.1.

In addition to the gradient force, each particle experiences a fluctuating thermal force, $f(t)$, which is the cause of Brownian motion [77]. The exact course of $f(t)$ in time $t$ cannot be known, so $f(t)$ is treated as a stochastic variable. We assume, as is conventional, that the distribution of $f(t)$ is Gaussian, its average is $\langle f(t) \rangle = 0$, and its autocorrelation function is $\langle f(t)f(t') \rangle = 2\xi k_B T \delta(t-t')$, where $\xi$ is the drag coefficient of a particle, $k_B T$ is the thermal energy, and $\delta(t)$ is Dirac’s delta function.

Finally, a moving particle experiences a viscous drag force $-\xi \frac{dx(t)}{dt}$. We assume the system to be in the overdamped, low Reynolds number regime, therefore inertia can be ignored. The balance of the applied, thermal, and viscous drag forces gives the equation of motion for a single particle, $\xi \frac{dx(t)}{dt} = kx(t) + f(t)$.
Fig. 5.1 Basic model. Two Brownian particles are at positions \( x_1 \) and \( x_2 \) in a repulsive force gradient which results from a convex potential energy landscape. The net force driving the two particles apart is proportional to \( |X| \), their separation distance.

We are interested in the ordering of the particles, therefore we consider the separation between them, \( X \equiv x_2 - x_1 \), where \( x_1 \) and \( x_2 \) are the positions of particles 1 and 2, respectively. Note that when \( X \) changes sign, the original order of the particles reverses.

If \( \xi \) is the same for both particles, we can subtract the equation of motion for particle 1 from that for particle 2 to obtain the equation describing the dynamics of the separation,

\[
\xi \frac{dX(t)}{dt} = kX(t) + F(t),
\]

where \( F(t) \equiv f_2(t) - f_1(t) \) is a new stochastic force corresponding to the difference between the thermal forces acting on each particle. \( f_1(t) \) and \( f_2(t) \) are uncorrelated, and it can be shown that the average and the autocorrelation function of \( F(t) \) are, respectively,

\[
\langle F(t) \rangle = 0; \quad (5.2)
\]

\[
\langle F(t)F(t') \rangle = 4\xi k_BT \delta(t-t'). \quad (5.3)
\]

According to Eq. (5.1), the force gradient drives particles apart at a rate proportional to \( X \). The force gradient is “elongational” because the farther apart the particles are, the faster they are driven apart. This is true even when both particles move in the same direction; the force
on the leading particle always exceeds that on the trailing one by an amount proportional to $X$.

This situation is similar to an Ornstein-Uhlenbeck process or to a small bead held in an optical trap [71]. The crucial difference is the sign of the applied force; whereas a particle undergoing an Ornstein-Uhlenbeck process or held in an optical trap feels a restoring force proportional to its displacement, particles in an elongational force gradient are pulled apart by a force proportional to their displacement. Despite this important difference, we can solve Eq. (5.1) as we would the standard Ornstein-Uhlenbeck problem [77]. Our approach follows a familiar one for the motion of a Brownian particle in a harmonic trap [18].

The dynamics of the inter-particle separation distance are driven in part by the unknowable random thermal force, $F(t)$, so we must treat the problem probabilistically. Thus we solve for the Green function $G(X,X_0;t)$, which gives the likelihood of finding two particles, initially a distance $X_0$ apart, separated by a distance between $X$ and $X + dX$ after a time $t$. The Green function for this system is [77, 18]

$$G(X,X_0;t) = \frac{1}{\sqrt{\frac{4\pi k_B T}{k}}} \exp \left[ -\frac{(X - X_0 e^{\frac{k}{2} t})^2}{\frac{4 k_B T}{k} \left( e^{\frac{k}{2} t} - 1 \right)} \right]. \quad (5.4)$$

Eq. (5.4) differs from the standard Ornstein-Uhlenbeck solution in that the sign of the force gradient is flipped and factors of 2 appear because Eq. (5.4) describes the separation between two diffusing particles.

### 5.1.1 The probability of particles crossing

When the two particles cross, the sign of $X$ flips. The probability that particles initially separated by $X_0$ will have crossed after a time $t$, $P(X_0;t)$, is obtained from Eq. (5.4) by integrating $G(X,X_0;t)$ over all negative values of $X$,

$$P(X_0;t) = \int_{-\infty}^{0} G(X,X_0;t) dX. \quad (5.5)$$

Eq. (5.5) can be rewritten in terms of the error function, $\text{erf}[q] \equiv \int_{0}^{q} e^{-t^2} dt$, as

$$P(X_0;t) = \frac{1}{2} \left( 1 - \text{erf} \left[ \frac{1}{\sqrt{1 - e^{-\frac{k X_0^2}{2 k_B T}}}} \right] \right). \quad (5.6)$$
Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer

Fig. 5.2 Dependence of the crossing probability on $\tau$ for various fixed values of $\lambda$ ranging from vanishingly small to 2.

We can gain physical insight and simplify Eq. (5.6) by introducing two dimensionless variables. The first, $\lambda \equiv \sqrt{\frac{k_b T}{4k_B T}}$, parameterizes the strength of the force gradient driving the particles apart. $\lambda$ is the work required to bring the particles from their initial positions to their midpoint against the force gradient, divided by the thermal energy $k_B T$. The second variable, $\tau \equiv \frac{4k_B T t}{\tau X_0^2}$, is the time nondimensionalized by the mean time a particle takes to diffuse across half the initial separation distance in the absence of a force gradient. The probability $P(X_0; t)$ is expressed in terms of these natural variables as

$$P(\lambda; \tau) = \frac{1}{2} \left( 1 - \text{erf} \left( \frac{\lambda}{\sqrt{1 - e^{-2\lambda^2\tau}}} \right) \right). \quad (5.7)$$

Figure 5.2 shows the dependence of $P(\lambda, \tau)$ on $\tau$ for various fixed values of $\lambda$. Note that in the absence of a force gradient ($\lambda \to 0$), $P(\lambda, \tau)$ eventually reaches a plateau at 0.5. This means that freely diffusing particles have an equal chance of crossing one another or not, corresponding to the complete randomization of order. Conversely, for a sufficiently strong force gradient ($\lambda = 2$), the particles rarely reverse their order, even after an arbitrarily long time. The rapid rise in monomer crossing probability in Figure 2 results from particles needing sufficient time to diffuse together from their initial positions. The subsequent probability saturation occurs because as particles move apart, the growing repulsive force between them makes a future crossing ever more unlikely. In Figure 5.2, the crossing probability approaches a plateau beyond $\tau = 1$ because after a long time, the particles have either crossed already or they never will. The strength of the force gradient is what determines
the likelihood of having crossed in the limit of long times. The ordering of particles can be preserved to any desired level of confidence by applying a sufficiently strong force gradient.

5.2 Preserving the order of DNA mononucleotides entering a mass spectrometer

In this section, we apply our model to the motion of the ordered monomers of a biopolymer, such as a DNA, RNA, or protein, near the tip of an electrospray ion source. We envision sequencing a single molecule by cleaving its monomers from one end, delivering them one by one and in order into a mass spectrometer, and identifying them by their unique charge-to-mass ratios. For this strategy to succeed, the order of the monomers must be preserved to a high degree of confidence until they enter the mass spectrometer for identification. The calculations that follow focus on DNA nucleotides, which are the monomers of DNA. They also suppose that the required confidence level is 95%, which corresponds to Q13 bases in the standard measure of quality factor [22]. It is easy to repeat these calculations for any desired confidence level or for a different type of biopolymer. The implications of our results for biopolymer sequencing will be discussed later.

We consider two distinct methods for cleaving nucleotides from a DNA strand. The first is to use laser light to photo-fragment a stretched DNA strand. The second is to have an exonuclease cleave the DNA enzymatically and processively, as was first proposed by Keller et al. [34]. We will show that in both cases the nucleotides must be cleaved within less than a micrometer of the electrospray tip in order for their order to be preserved; in the case of photofragmentation, the critical distance is only a few nanometers. The precise distance depends significantly on the initial separation between liberated monomers, which depends in turn on the mechanism by which the polymer is cleaved.

5.2.1 Elongational force gradients in an electrospray ion source

The electrospray ionization technique transfers ions into a mass spectrometer from the liquid inside a capillary tube that tapers to a needle-like tip. A voltage is applied between the liquid and an electrode located a short distance in front of the tip. When the voltage is large enough, the electric fields it generates deform the liquid meniscus into a pointed shape called a Taylor cone [74]. Ions escape the Taylor cone from the apex in a charged fluid jet or by the mechanism of ion evaporation [25]. Figure 5.3 sketches an electrospray ion source with two nucleotides in it.
Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer

Fig. 5.3 Schematic of an electrospray ion source. A Taylor cone forms at the tip of a capillary needle when a high voltage is applied between the conducting fluid inside and a nearby electrode, held in vacuum. A pair of DNA mononucleotides approach the apex. The sketch shows $r$ and $\alpha$.

Inside the Taylor cone, a nucleotide experiences a combination of electric and viscous forces. We will show that the electric force increases in a straightforward manner as the nucleotide approaches the apex. The *average* viscous force, which is linked to the flow rate in the jet, also grows in a straightforward manner as a nucleotide approaches the apex. But the action of the electric fields on the induced charge at the Taylor cone surface also generates circulating flow patterns that complicate the situation substantially [6]. We will first estimate the conditions needed for preserving the order of nucleotides by making simplifying assumptions about the electrospray and by considering only the average electrical and viscous forces. We will later discuss the effect of the circulating flow component.

To find the strength of the electric force, we take the Taylor cone to be an ohmic liquid whose resistivity, $\rho$, is constant and uniform. The ions emitted from the apex carry a total current $I$ that must be supplied by a current density $J$ inside the Taylor cone. Far away from the jet, the spherically symmetric current density is $J(r) = \frac{I}{2\pi(1-\cos(\alpha))r^2}$ [15], with $r$ the distance from the apex of the Taylor cone along its axis and $\alpha = 49.3^\circ$ the characteristic half angle of a Taylor cone [74]. The electric field $E(r)$ is related to $J(r)$ and $\rho$ by Ohm’s law, $E(r) = \rho J(r)$. Each nucleotide in solution carries the charge of an electron, $-e$, so it
experiences an electrical force

\[ f_e = -eE(r) = -\frac{e\rho I}{2\pi (1 - \cos(\alpha)) r^2}. \] (5.8)

Fluid flows also arise in electrosprays due to the flow of ions in charged interfacial layers. A narrow jet typically shoots from the apex of the Taylor cone. The volume flow rate \( Q \) must be supplied by a sink flow \( U(r) = \frac{Q}{2\pi (1 - \cos(\alpha)) r^2} \), where \( U(r) \) is the flow velocity averaged over the section of the Taylor cone at radius \( r \); note that it has the same form as the electric current density. The average viscous force on a nucleotide is \( f_v = \xi U(r) \), where \( \xi \) is the nucleotide’s viscous drag coefficient. De la Mora and coworkers [15] have shown theoretically and experimentally that under generic electrospray conditions, \( I \) and \( Q \) are related by

\[ |I| = f(\varepsilon) \sqrt{\gamma Q/\rho \varepsilon}, \] (5.9)

where \( \gamma \) is the surface tension of the liquid interface, \( \varepsilon \) is the dielectric constant, and \( f(\varepsilon) \approx 18 \) for high-\( \varepsilon \) fluids like water (\( \varepsilon = 80 \)) and formamide (\( \varepsilon = 111 \)). Using the above relations and Eq. 5.8, we find an expression for the total force

\[ f = -\frac{e\rho I}{2\pi (1 - \cos(\alpha)) r^2} \left( 1 + \frac{I}{I_0} \right). \] (5.10)

The first term in Eq. 5.10 corresponds to the electric force, which grows stronger in proportion with \( I \). The second term corresponds to the viscous force, which grows in proportion with \( I^2 \); \( I_0 = f(\varepsilon)^2 e\gamma/\xi \varepsilon \) is the characteristic current at which the viscous force reaches the same strength as the electric force, and is typically on the order of a nanoampere.

As a nucleotide approaches the apex, the force on it increases as the inverse square of \( r \), rather than depending linearly on \( r \), as our theoretical model assumes. To apply the model, therefore, we Taylor expand (different Taylor) \( f \) and find the strength of the linearized elongational force gradient at \( r \),

\[ k \approx (\nabla \cdot f)|_r = \frac{2e\rho I}{2\pi (1 - \cos(\alpha)) r^3} \left( 1 + \frac{I}{I_0} \right). \] (5.11)

The force gradient diverges at the apex of the Taylor cone according to Eq. (5.11), so the order of two particles can be preserved to an arbitrary confidence level by releasing them close enough to the tip. Here we estimate \( R_{95} \), the maximum distance from the apex at which two ordered nucleotides can be released and expected to enter the mass spectrometer in their original order with 95% confidence. Recall that the likelihood two particles will invert their order is greatest after long times. Thus, if we take the limit \( t \to \infty \) of Eq. (5.7), we obtain
Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer

an expression for the upper bound on the expected disorder, \( P(\lambda) = \frac{1}{2} (1 - \text{erf}[\lambda]) \). Setting \( P = 0.05 \) and numerically solving for \( \lambda \) leads to

\[
k_{95} \approx 5.4 \times \frac{k_B T}{X_0^2}. \tag{5.12}
\]

We will combine Eq. (5.11) and Eq. (5.12) to obtain the value of \( R_{95} \) from estimates of \( X_0 \). The value of \( X_0 \) depends on the particular technique used to cleave monomers from a DNA strand, as we will show. Also note that the linearization in Eq. 5.11 systematically underestimates the difference in forces acting on a pair of particles. Consequently, the value of \( R_{95} \) we obtain will be a conservative (short) estimate of the actual safe distance. The ratio of the leading nonlinear correction to the linearized gradient force is \( 3X_0/2R_{95} \).

5.2.2 Cleaving the monomers of a stretched polymer by photofragmentation

It may be possible to cleave a DNA polymer into monomer-sized pieces by irradiating it with ultraviolet light as it approaches the apex of the Taylor cone. DNA tends to break into fragments when a molecule absorbs more than a few eV of energy; mass spectrometric studies on nucleic acids in vacuum have found that DNA fragments primarily at the glycosidic bond which holds a base to the sugar-phosphate backbone, and secondarily along the backbone \[84\]. Furthermore, DNA has strong optical absorption bands near 200 nm and 280 nm in wavelength.

We suppose that a single strand of DNA approaches the apex of the Taylor cone and becomes stretched out by the force gradient before incident light causes the molecule to fragment, releasing neighboring bases essentially simultaneously. The initial separation distance of the bases is \( X_0 = 5.9 \) Å, the linear distance between them along the backbone of a single stranded DNA molecule \[66\]. Plugging this initial separation distance into Eq. (5.12), we find the critical force gradient to be \( k_{95} = 0.068 \text{ Nm}^{-1} \).

Let us estimate a typical \( R_{95} \) from Eq. (5.11), given \( k_{95} = 0.068 \text{ Nm}^{-1} \). In our laboratory, we routinely generate electrosprays from 2M solutions of sodium iodide (NaI) in formamide, similar to what the De la Mora group has reported \[28\]. The solution resistivity is \( \rho = 0.45 \Omega\cdot\text{m} \) and the surface tension is 58 mN m\(^{-1}\). The viscous drag coefficient for the DNA monomer adenosine monophosphate has been measured experimentally in water and found to be \( \xi \approx 9.1 \times 10^{-12} \text{ Ns m}^{-1} \) \[20\]. Since \( \xi \) should be proportional to the viscosity of the fluid, we expect \( \xi \) to be 3.3 times greater in formamide than in water, giving \( \xi \approx 3.0 \times 10^{-11} \text{ Ns m}^{-1} \). With these parameters, Eq. 5.10 predicts \( I_0 = 0.91 \text{ nA} \) for the electrospray...
current at which the electric and viscous contributions to the total force on a monomer are equal. Electrospray currents are typically higher than $I_0$, so the viscous force from the sink flow is the larger effect. Here we consider a typical current of $I = 5 \text{nA} \ (I/I_0 \approx 5.5)$. The critical force gradient is achieved at $R_{95} \approx 3.2 \text{ nm}$, the greatest distance from the apex at which photofragmentation can occur while preserving the monomer order with 95% confidence. We also note that the nonlinearity in the force gradient is significant at that location – the leading correction to the force difference between neighboring monomers is 0.35 times the linearized term.

It is in principle possible to control the critical distance by changing the force gradient through $I$ or $\rho$; in practice, however, these approaches can have at best a modest impact on $R_{95}$. As the electrospray current is increased from $I = 1 \text{nA}$ to $I = 10 \text{nA}$, for example, $R_{95}$ only rises from $1.3 \text{ nm}$ to $4.9 \text{ nm}$. If the resistivity is additionally increased tenfold to $\rho = 4.5 \Omega \text{m}$, $R_{95}$ rises to $2.7 \text{ nm}$ for $I = 1 \text{nA}$ and to $10.5 \text{ nm}$ for $I = 10 \text{nA}$. Clearly, neighboring monomers must be cleaved within a few nanometers of the electrospray apex if their order is to be preserved.

We note that at such short distances, an important assumption underlying our model of the Taylor cone no longer holds. Equation 5.8 is a good description of the electric fields inside the Taylor cone far from the apex, where charge relaxation by conduction is fast compared with convective transport [25, 6]. However, convective charge transport becomes comparable with conduction close to the apex, on length scales comparable to or smaller than [25, 15]

$$r^* \approx (\rho Q \epsilon \epsilon_0).$$  

Using Eqs. 5.9 and 5.13, we find $r^* \approx 2.9 \text{ nm}$. The fact that $R_{95} \approx r^*$ suggests that the corrections to Eq. 5.11 are non-negligible, and therefore the values of $R_{95}$ we found are only approximate.

### 5.2.3 Cleaving DNA with an exonuclease

An alternative to photofragmentation is to allow an enzyme to processively cleave the nucleotides of a single-stranded DNA molecule. This idea comes from Keller and co-workers, who first proposed a single molecule DNA sequencing technique in 1989 [34, 4, 13, 81]. In their scheme, exonuclease I would be held in a fluid flow so that the released monomers would drift downstream through an optical focal volume where each one would be identified by fluorescence spectroscopy. The main problem this approach faced was the low signal-to-noise ratio obtained using optical spectroscopy. That problem might be solved by instead using mass spectrometry, which can easily detect and analyze a single ion.
Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer

Following this sequencing strategy, an exonuclease I molecule is immobilized a distance $R_{\text{exo}}$ from the apex of the Taylor cone and made to processively cleave the nucleotides from a single DNA strand [34]. The kinetics are stochastic, giving rise to a distribution of intervals $\tau$ between subsequent nucleotide cleavages. Werner et al. measured the distribution of cleaving rates and fit the data to a truncated Gaussian with a mean duration of $\langle \tau \rangle = 6.7$ ms and cutoffs for cleaving rates slower than 10 nucleotides/sec [82]. Through a simple change of variables, we converted the distribution of cleavage rates to a distribution of cleavage times

$$P_{\text{exo}}(\tau) = \frac{1}{\sqrt{2\pi \sigma_k^2 \tau^2}} \exp \left[ -\frac{(\frac{1}{\tau} - k_0)^2}{2\sigma_k^2} \right],$$

where $\sigma_k = 63$ nucleotides/sec is the measured rate variance and $k_0 = 97$ nucleotides/sec is the mean rate.

It might be tempting here to apply our model for order preservation in an elongational force gradient, but Eq. (5.7) does not apply well to this situation. To understand why, consider the average distance a monomer diffuses in a time $\langle \tau \rangle$, $\sqrt{2D \langle \tau \rangle} \approx 1.4 \mu m$ [18]. Compare this with the distance $X_{\text{drift}}$ that a monomer drifts in the electric fields inside the Taylor cone in $\langle \tau \rangle$ when released 1.4 $\mu m$ away from the apex. We find $X_{\text{drift}}$ by integrating Eq. (5.10)

$$X_{\text{drift}} = \sqrt{2D \langle \tau \rangle} - \left( \sqrt{2D \langle \tau \rangle} \right)^3 - \frac{3e\rho I \langle \tau \rangle (1 + I/I_0)}{2\pi \xi (1 - \cos(\alpha))} \right)^{\frac{1}{3}}$$

$$\approx 135 \text{ nm}.$$ 

(5.15)

Thus diffusive motion typically overwhelms drift in the time between cleavages. Drift only becomes important very close to the apex of the Taylor cone. The location where drift and diffusion are comparable effects depends on $I$; that location is only about 24 nm from the apex when $I = 1$ nA, but grows to about 1.36 $\mu m$ when $I = 10$ nA.

The arrival of monomers at the apex is better thought of as a first passage problem where only diffusion matters. When a free monomer diffuses to the apex of the Taylor cone, it is ejected into the mass spectrometer, never to return. If that happens before the next monomer is cleaved, then their relative order has been preserved. Alternatively, if the first monomer does not reach the apex before the next monomer is cleaved, the former could have diffused upstream and flipped the ordering. We will compute the distance from the apex where a cleaved monomer stands a 95% chance of reaching the apex within $\tau$. This will establish a lower bound on $R_{95}$. It is only a lower bound because we neglect the fluid flow and the electrophoretic drift which both help to bring the monomers towards the apex and preserve
5.2 Preserving the order of DNA mononucleotides entering a mass spectrometer

their order, and because some fraction of monomers that do not reach the apex within \( \tau \) will nevertheless reach it before the next monomer does.

The probability that a Brownian particle will not have diffused passed some point \( x_c \) after time \( \tau \) is called the survival probability, \( P_s(x_c, \tau) \), and is given by [56]

\[
P_s(x_c, \tau) = \text{erf} \left( \frac{x_c}{2\sqrt{D\tau}} \right) .
\]

In our sequencing strategy \( x_c = R_{\text{exo}} \), the distance from the exonuclease to the Taylor cone apex. We find the total survival probability \( P_s(R_{\text{exo}}) \) and also account for the stochastic exonuclease cleavage kinetics by multiplying the survival probability in Eq. (5.16) with the distribution of \( \tau \) in Eq. (5.14) and then integrating over all possible \( \tau \),

\[
P_s(R_{\text{exo}}) = \int_0^{\infty} P_s(R_{\text{exo}}, \tau) P_{\text{exo}}(\tau) d\tau .
\]

We evaluated Eq. (5.17) numerically and obtained the value of \( R_{\text{exo}} \) for which \( P_s(R_{\text{exo}}) = 0.05 \), in accordance with our 95 \% confidence criterion. We find that the monomer order will be preserved if the exonuclease I is located within \( R_{95} = 117 \) nm of the apex.

5.2.4 The influence of circulating flows in the Taylor cone

Barrero et al. investigated circulating fluid flows inside the Taylor cone and showed that these are a fundamental feature of electrosprays at low and high Reynolds number [6]. The electrospray voltage draws ions to the liquid interface, which obtains a net charge within a thin interfacial layer. The tangential component of the electric field exerts an electric stress on the fluid at the surface of the Taylor cone, pulling it toward the apex. The fluid returns up the axis of the Taylor cone, creating a meridional circulation. At low Reynolds number, the characteristic velocity of the circulating flow is

\[
U_c(r) \sim \left( \frac{\gamma \varepsilon_0 \rho^2 I^2}{r^3 \eta^2} \right)^{\frac{1}{2}},
\]

where \( \gamma \) is the surface tension, \( \varepsilon_0 \) is the permittivity of vacuum, and \( \eta \) is the fluid viscosity. Eq. 5.18 is obtained by balancing the electric stress with the fluid shear across the Taylor cone at \( r \). \( U_c \) can be large compared with other components of a monomer’s velocity, and circulating flows have the potential to shuffle the order of nearby monomers. Let us consider the two sequencing strategies separately.

In the first case, we found that monomers needed to be cleaved by photofragmentation within 3.2 nm of the apex for \( I = 5 \) nA. At such short distances from the apex, the velocity
Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer of a monomer from the combined effects of electrophoresis and the sink flow exceeds the characteristic velocity of the circulating flow; the net motion toward the apex, driven by the total force in Eq. 5.10, exceeds $U_c$ at distances shorter than $r = 4.4 \text{ nm}$. Furthermore, as we have already discussed, the assumption that charge relaxation by conduction is fast compared with convective transport does not hold at distances comparable to $r^* \approx 2.9 \text{ nm}$; the development of the circulating flow rests on that assumption \cite{25, 6}. Given these considerations, we speculate that sink flow and electrophoretic motion are the dominant effects at the short distances required to preserve the order of photofragmented monomers. Numerical studies are likely required to understand the dynamics in detail, but such studies are beyond the scope of this paper.

In the case of exonuclease sequencing, the circulating flow at $R_{95}$ is fully developed and fast compared with the other velocity components; however, its influence is somewhat counterintuitive. The circulating flow will tend to enhance order preservation by increasing the effective diffusivity of monomers and thereby decreasing their first passage time at the apex. The underlying mechanism is similar to Taylor-Aris diffusion: When a free monomer can diffuse laterally, it will randomly sample the different streamlines of a circulating flow. The randomness in its axial motion is magnified as it rides the various streamlines. The effective diffusion coefficient, $D_{\text{eff}}$, is related to the thermal diffusion coefficient, $D_0 = k_b T / \xi$ and the Péclet number, $Pe \sim r U_c / D_0$, as \cite{38}

$$D_{\text{eff}} = D_0 \left( 1 + \beta Pe^2 \right),$$  

(5.19)

where $\beta$ is a factor that depends on the geometry and the flow ($\beta = \frac{1}{48}$ for Poiseuille flow in a cylinder). Under typical experimental conditions, $Pe \approx 50$ at $R_{95}$, so the enhancement in the effective diffusion coefficient should be substantial. We therefore conclude that our earlier finding was likely an underestimate of $R_{95}$. We do not attempt to make a more accurate estimate here.

Note that Eq. 5.19 is only valid in the limit where the monomer has enough time to fully explore the flow in the transverse direction. Since a monomer will diffuse about 2 $\mu$m perpendicular to the Taylor cone axis in between subsequent cleavages, it is reasonable to expect it to fully sample the circulating flow from a starting distance of 117 nm from the apex.

### 5.2.5 The implications for sequencing single biopolymers

Our analysis sheds light on the feasibility of sequencing single biopolymers using electrospray ionization mass spectrometry. To implement Keller’s idea of sequencing DNA with
the help of exonuclease I, the enzyme would need to be placed within about 100 nm of
the apex of the ion source’s Taylor cone, otherwise the sequence of bases would become
significantly scrambled by Brownian motion. Although to our knowledge this has not yet
been demonstrated, it seems technically feasible; it is straightforward to make electrospray
sources with tip diameters on the 100 nm scale by pulling glass capillaries [85], and one
could imagine fixing exonuclease I enzymes to the tip’s surface. Those enzymes would be
within about a tip diameter of the apex of the Taylor cone, which is close enough to ensure
that the sequence of bases is reliably preserved. A similar approach to single-molecule
protein sequencing is possible by replacing the exonuclease I with a AAA+ family protease
that processively degrades proteins and releases their constituent amino acids one by one [61].
Since the rate at which such proteases typically operates is slower than that of exonuclease I,
we expect better than 95 % sequence preservation when operating 100 nm from the apex.

Using photofragmentation instead of an enzyme to cleave the monomers of a biopolymer
presents different challenges. Most significantly, the photofragmentation must be carried
out within a few nanometers of the apex of the Taylor cone. An electrospray source with
a diameter that small has yet to be demonstrated. We further note that our model may not
accurately describe such a small electrospray source, whose structure is expected to deviate
from a perfect Taylor cone at such short distances from the apex; that is the scale at which
the cone typically transitions into a thin jet of fluid [15, 29], which ultimately breaks up into
charged droplets.

New fabrication techniques offer a possible solution to the challenge of preserving the
monomer order. One can create an electrospray source featuring a nanotube made of carbon
or boron nitride at the tip, whose aperture has a diameter in the single nanometer range. It
has been shown that nanotubes with diameters less than 10 nm can be incorporated into a
chip-based nanofluidic device [63]; the same technique has been used to insert nanotubes
into pulled glass capillary tips [62]. Such a nanotube electrospray source could additionally
prevent bases from swapping their order by allowing too little room inside the tube for
monomers to diffuse past one another.

Finally, we remark that other technical challenges must be overcome in order for the
biopolymer sequencing strategy to be viable. Immobilizing a single enzyme within tens of
nanometers of a desired location is not trivial, though solutions to similar problems have been
developed in the context of zero-mode-waveguide DNA sequencing [42]. Another challenge
for DNA sequencing with an exonuclease is that the technique would need to be massively
parallelized in order to process the vast number of nucleotides in a genome in a reasonable
amount of time. This challenge is somewhat daunting because while there has been progress
Preserving the sequence of a biopolymer’s monomers as they enter an electrospray mass spectrometer toward miniaturizing mass spectrometry [5, 79], there remains considerable distance to go before it can be parallelized.

We believe that the biopolymer sequencing strategy considered here is best suited for proteins. Proteins, which are typically a few hundred to a few thousand amino acids long, are 6-7 orders of magnitude shorter than DNA genomes, therefore parallelization is not required for high throughput. Furthermore, de novo sequencing of proteins, which is the direct determination of a protein’s sequence in the absence of a reference sequence, remains difficult even by state-of-the-art methods. At present, there two different methods that are commonly employed. The first is Edman degradation [52], which provides the most reliable protein sequences, but requires short protein fragments and slow, costly chemical cycles. The typical sequence read length is 10 to 20 amino acids, and the process can take around 20 minutes per amino acid and cost around $70 per amino acid. The second is based on mass spectrometry and algorithmic reconstructions of multiply fragmented protein sections [67]. This method can sequence polypeptides that are tens to thousands of amino acids long. Analyses are still slow and expensive, however, taking weeks to complete at a cost of around $10 per amino acid. Overall, protein sequencing is still orders of magnitude slower and more costly than DNA sequencing, which is presently nearing the $1000 per genome milestone. This is why de novo protein sequencing is less common an objective than protein identification, whereby parts of a molecule’s sequence are compared against a database.

The challenge of protein sequencing stems partly from the fact that there are twenty amino acids to discriminate, as opposed to only four DNA bases. This places a heavier burden on the resolution of the sensor. Mass spectrometry arguably offers the best hope for a single-molecule protein sequencing technology, as it is uniquely capable of identifying all twenty amino acids. The strategy considered here therefore has the potential to greatly improve the state-of-the-art. We envision an entire, denatured protein passing through a nanoscale aperture leading into a mass spectrometer, which sidesteps the need to prepare purified small protein fragments or to run slow and expensive chemical cycles. Long read lengths would further reduce the computational post-processing needed to stitch together small fragments. Finally, the speed of sequencing by mass spectrometry with photofragmentation is in principle limited by the count rate of the single ion detector, which can exceed $5 \times 10^8$ Hz [76].

5.3 Conclusion

We have analyzed the use of elongational force gradients for preserving the linear order of particles against the randomizing effects of Brownian motion. The analytic expression we derived for the probability that two particles will invert their order after being released from
a known initial separation depends on two re-scaled parameters, one related to the strength of the force gradient and the other to the time interval following the release of the particles. The model can be easily applied to a variety of micro- and nanoscale situations where Brownian motion competes with a spatially varying force. We have applied our model to the case of DNA nucleotides being released within the Taylor cone of an electrospray ion source in order to evaluate the feasibility of a sequencing strategy for single biopolymers. The model makes simplifying assumptions about the dynamics inside the Taylor cone.

The force gradient inside the electrospray ion source can preserve the ordering of DNA monomers cleaved from a parent strand of DNA. If the exonuclease I enzyme sequentially cleaves the monomers of DNA from a parent strand within about 100 nm of the tip of the electrospray source, the sequence of monomers entering the mass spectrometer would be 95% preserved. If the monomers are cleaved from a stretched parent DNA strand by photofragmentation, the corresponding distance from the tip is between 1 nm and 10 nm, depending on the experimental conditions. The sequencing strategy is best suited for proteins because they are composed of twenty different monomers and relatively short, which takes advantage of the resolution of mass spectrometry while minimizing the importance of parallelization. Our theoretical model could be improved in the future by accounting for finite-size objects, interactions between them, and the detailed motion of the fluid and particles in three dimensions.
Chapter 6

Conclusions and recommendations

In our efforts to develop a new method for single biopolymer sequencing, we have demonstrated the feasibility of analyzing individual amino acids and DNA bases that have been emitted directly from a nanocapillary. This was possible as a result of our ability to produce electrospray from nanocapillary tips with diameters in the 10’s and 100’s of nanometers as opposed to the traditional 10’s and 100’s of micrometers.

We have collected mass spectra from capillaries electrospraying directly into high vacuum from solutions of both formamide and water. Only singly charged ions are observed in our mass spectrometer indicated by mass spectrum peak separations of whole solvent molecule masses, never fractions. Additionally these peaks have narrow energy distributions and solvation state distributions. Since our setup lacks a desolvating background gas, these mass spectra serve as evidence that these ions are being emitted from the electrospray source individually through a thermally activated process known as ion evaporation.

Currently, our mass spectrometer works by filtering out all ions outside a small window of \( m/z \) and creates mass spectra by scanning that window over a full \( m/z \) range. This means that it cannot measure two ions with different masses simultaneously. It turns out that basically all commercial mass spectrometry creates mass spectra with methods fundamentally incompatible with sequencing. Either the mass spectrometer focuses a filtered beam of ions onto a single detector and scans over a range of masses, or periodically a gate releases a small bunch of ions in a race to the detector with the least massive and most charged ions arriving first. Either way, only one ion detector is involved. To further test the feasibility of this sequencing technique and to probe possible cleaving mechanisms, we would want a setup capable of simultaneous measurements of ions with different masses. Building an array of detectors with a magnetic sector type of mass spectrometer would make this possible. A single strong magnetic field separates ions into trajectories that reflect their masses with less massive ions bending more tightly than heavier ions. If we sent polymers composed of three
monomers with different masses that were successfully cleaved apart, we would see a time resolved sequence register in three different detectors, each one tuned to a specific monomer. This would exhibit a true proof of concept.

We have developed a new method of directly measuring the very low flow rates of nanocapillaries and fit these measurements to an infinite truncated cone Poiseuille flow model. Recent studies from Stark et al into the voltage dependence on the flow rates of electrospray indicate that there is a complicated interplay between voltage, flow rate, pressure, and current involved in electrospray. Additionally, these studies indicate that the current from these electrospray sources in more cases than not pulsate with some frequency in the kHz range [60, 2, 65]. We are not currently set up to record flow rates as our sources are electrospraying, or these frequency resolved current measurements. Adjusting our setup to include the ability to make these measurements in situ may help further illuminate a pathway towards achieving the full suppression of drop formation at the surfaces of our electrospray sources.

We have concluded that the liquid meniscus of the Taylor cone pins to the outer diameter of our nanocapillaries. The voltages at which electrospray onset have been measured in a new nanoscale regime, never explored before. These measurements agree with a semi-infinite line charge model for the electric fields on the liquid menisci of nanocapillaries at electrospray onset.

We have used the ion count rate from our mass spectrometer and compared it with the amount of current leaving nanocapillaries as a proxy for the amount of ions versus drops created in the electrosprays. This ratio creates a transmission efficiency and we have compared the rise in this efficiency with theories for the electric field strength at the apex of the Taylor cones on these nanocapillaries. We have found existing theories of the critical electric field strength at which ion evaporation should be expected to dominate over drop production to not accurately explain our experiments. However we have observed that smaller tips increase the proportion of ions relative to drops emitted from our electrospray sources which we attribute to their higher fluidic impedance and correspondingly low flow rates.

A next big step would be to create electrospray sources with even smaller diameters in the single nm range capable of demonstrating electrospray. Tips this small would be capable of linearizing biopolymers and allow for the possibility of maintaining the sequential order of the monomers. We have already collaborated with a group in France who are able to insert, and glue into place, boron nitride nanotubes into the already small openings of pulled glass capillary needles [62]. Aside from confining biopolymers to linear configurations, these nanotube-tipped capillaries might also provide low enough fluid flow rates to encourage more complete ion evaporation, as opposed to drop formation. An alternative to this could
simply be to modify the tips of the nanocapillaries with the addition of some metal, which could further increase their fluidic impedance. Though this would do nothing to decrease the outer diameter of the nanocapillaries, it is possible that the higher fluidic impedance the subsequently smaller inner diameters would create is ultimately more important to direct ion emission from the Taylor cone.

Finally, the mass spectra we have obtained clearly indicate ion emission directly from the Taylor cones of our nanoscale capillaries; however, we do not currently have a way of directly measuring the drop content of the electrosprays we produce. A gated time-of-flight (TOF) setup would allow us to measure the relative ion versus drop abundances as functions of the tip’s inner and outer diameters, as well as solution and electric field parameters. Since these work by releasing a small bunch of ions during a very short pulse of time and accelerating all of them in the same electric field. Since all ions gain the same amount of energy, but have different mass, they will be accelerated to different velocities. This creates a race in which the lightest ions appear earliest, followed by the largest drops arriving last. The purely ionic regime has not yet been achieved in solutions of water or formamide. Using this TOF technique, which is sensitive to ions and drops of all sizes, would help further explore the feasibility of obtaining this purely ionic regime for our solutions using nanocapillaries.
References


