



Ion transport by viscous gas flow through capillaries and electrospray mass spectrometry of proteins
by Baiwei Lin

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Chemistry

Montana State University

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Abstract:

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A Monte Carlo method was used to simulate the radial diffusion of ions in the laminar gas flow. These simulations showed that the effect of the parabolic flow profile is to greatly increase the transmitted current through long capillaries. Indeed, all the data is consistent with ion loss by radial diffusion to the walls. In very long capillaries, higher mass ions can be effectively separated from lower mass ions due to the difference in diffusion constants.

A home-built electrospray ion source was installed on a VG-TRI02 quadrupole and the experimental conditions were optimized for good signals. The performance of this source, particularly with regard to protein spectra, was evaluated.

Electrospray mass spectrometry was used to probe conformational changes of proteins. The effects of acids and organic solvents in the electrospray solution and in the gas phase of the ion source as well as the effect of temperature on the protein mass spectra and conformations were investigated. Acids or methanol in the electrospray solution strongly promotes the unfolding of the native myoglobin, while the addition of acids or methanol to the gas phase can well retain the native conformation of myoglobin and greatly increase the native ion intensities.

Electrospray mass spectrometry was used as an off-line HPLC detector for tryptic digests of cytochrome c, unlabeled and labeled with a fluorescent Cascade Blue ligand. Almost all of the tryptic peptides were observed in the ESI mass spectra. The additional dimension provided by ESI mass spectrometry makes the combined HPLC/ESI/MS an extremely powerful analytical method, particularly for modified proteins.

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CAPILLARIES AND ELECTROSPRAY MASS
SPECTROMETRY OF PROTEINS

by

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APPROVAL
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Baiwei Lin

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The efficiency of ion transport by viscous gas flow through narrow capillaries has been studied. The currents to the front end and to the walls of the capillary, as well as the currents passing through, were measured. A number of experimental parameters were varied, such as the dimensions and the material of the capillaries, the gas throughput, and the temperature. Both electrospray and corona ion sources were used. A transmitted current was measured even through the longest capillaries used, 50 ft (I.D. 2.1 mm).

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INTRODUCTION

Electrospray Ionization

Electrospray is a new mass spectrometric technique that constitutes a major advance in the production of gas phase analyte ions from liquid solutions. This technique depends on dispersing analyte solution as a cloud or mist of small charged droplets, from which ions desorb into a bath gas at atmospheric pressure [1-4]. With electrospray, intact gas phase ions, with multiple charges, can be produced from remarkably large, complex, and fragile parent species. The pioneering efforts of Malcolm Dole [5-7] demonstrated the feasibility of electrospray ionization of non-volatile, liquid analytes. Later John B. Fenn [8-10] successfully interfaced an atmospheric pressure electrospray ion source to a quadrupole mass analyzer which revealed the remarkable ability of the technique.

Ionization methods for non-volatile species include laser desorption (LD), fast atom bombardment (FAB), plasma desorption (PD), and secondary ion mass spectrometry (SIMS). These are referred to as "desorption ionization" methods where sufficiently rapid heating vaporizes complex molecules before decomposition [11]. LD, FAB, PD, and SIMS are "soft" in the

sense that they can produce intact ions of species having molecular weights up to several tens of thousands [12-14]. These methods typically give only singly charged ions from parent species having molecular weights below ca 10,000 Da. In spite of this problem, these "energy-sudden" techniques have been very important in mass spectrometry for the analysis of large and complex molecules. However, electrospray ionization has become an effective complement to LD, FAB, PD, and SIMS because it overcomes some of the problems encountered in their use, such as limited mass range, large m/z values and extensive background noise.

The production of multiply charged ions by electrospray ionization (ESI) at atmospheric pressure presents an opportunity to study very large biomolecules [15-18]. Multiply charged molecular ions, which can be formed from proteins or oligonucleotides, can be efficiently mass analyzed and detected using magnetic and quadrupole MS due to the relatively low m/z values of these ions. With ESI, 100 or more charges can be placed on a large protein, bringing m/z down to below 2000 [20]. This is within the mass range of a quadrupole MS which are now extensively used for molecular weight measurements of large biomolecules [4,17,19]. Molecular weights of large biomolecules can be determined within 0.01%. There is, as yet, no evidence of an upper limit for the production of ions by ESI. However, the practical upper mass limit is ca. 100,000 Da due to increasing

congestion in the spectra coupled with the width of the peaks due to statistical variations in isotopic composition [10,17]. ESI is a near-ideal interface for liquid chromatography/MS because of its low m/z for large molecules [21]. The potential of this methods is further enhanced by tandem mass spectrometry (MS/MS), in which structural information is obtained by dissociating precursor molecular ion into product ions in a collision chamber [22].

Spray Ionization Process

Electrospray is one of several spray desorption techniques. The other two are aerospray (AS) and thermospray (TS). All three techniques are thought to share the same basic mechanism of ion production. Electrospray ion production requires two steps: (1) dispersal of highly charged droplets at near atmospheric pressure, and (2) subsequent droplet evaporation. An electrospray is produced by applying a high electric potential to a hypodermic needle through which 1-10 $\mu\text{L}/\text{min}$ of liquid flows. A few kV potential difference between a spray needle and a counter electrode located 3 - 40 mm away is sufficient [23]. The electrical field at the needle tip results in charge accumulation on the surface of the liquid emerging from the needle terminus. The high electric field results in disruption of the liquid surface and formation of highly charged droplets. The liquid flow rate, resistivity, and surface tension are important parameters for droplet production. Positively and negatively charged

droplets are produced depending upon the needle voltage polarity. The negative ion mode requires the presence of SF_6 to inhibit electrical discharge [24,36]. Ions, charged clusters, and even charged droplets, depending on the extent of desolvation, are sampled by the mass spectrometer through either a small orifice or a narrow transport capillary.

The effects of experimental parameters on ESI voltage and current have been explored by several researchers. It is found that fluids with higher surface tension require a higher threshold voltage (onset voltage) for stable electrospray production. Thus about 8 kV is needed for water and 4 kV for methanol at a 10 mm spray needle to counter electrode distance [25]. The use of higher voltages does not substantially increase the electrospray ion current until the onset of corona discharge. High dielectric liquids produce higher currents [26]. ESI currents for a typical water/methanol/5% acetic acid solution are in the range of 0.1-0.5 μA . Conducting liquids can produce much higher ESI currents (up to 500 μA for liquid metals) [27]. The total electrospray ion current increases only slightly with increasing liquid flow rate. Increasing the flow rate results in the formation of large droplets and so decreases the evaporation of charged droplets [28].

The formation of molecular ions from charged droplets requires effective evaporation of the initial droplets. In some instruments, this is accomplished at atmospheric pressure

by forcing the ions to drift through dry nitrogen gas at a temperature of ca. 60 °C. The dry bath gas provides the enthalpy for vaporizing the solvent from the droplets. Both the internal and translational temperatures of the desorbed ions are maintained at the bath gas temperature. Alternatively, desolvation can be promoted by heating during ion transport through a interface capillary.

Spray Ionization Mechanisms

Two mechanisms are commonly cited: droplet fission at the Rayleigh limit [29] and direct field evaporation of ions [30]. The mechanism of ion formation from charged droplets is, however, still unclear and this is a very active area of research [31-33].

One electrospray ionization mechanism, described by Dole over 20 years ago, is known as the "charge residue" mechanism [5-7]. As the solvent evaporates from charged droplets, the surface charge density increases until the Rayleigh limit is reached. At this point the forces due to electrostatic repulsion are large enough to overcome the surface tension. The resulting instability, sometimes called "Coulomb explosion", tears the droplet apart and produces charged daughter droplets that evaporate further. Dole's idea was that the "Coulomb explosion" repeats until the ultimate droplets contain not more than one solute molecule each, provided that the original solution was sufficiently dilute. As the last solvent molecule evaporate from these ultimate

droplets, the charge would be retained by the solute molecule to produce a free ion.

Since the experimental data show that there are large number of multiple charged ions in electrospray ionization, Dole's mechanism to produce singly charged free ions seems to be a remote possibility.

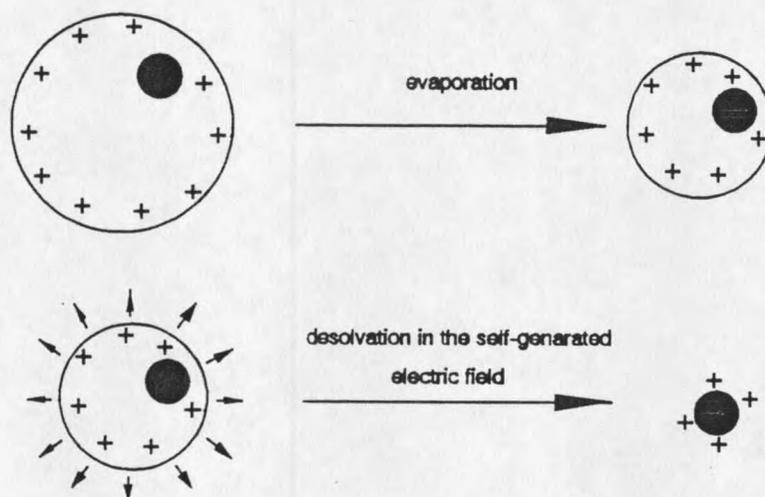


Figure 1. Sketch of the ion desolvation process [10].

An alternative to the "charge residue" mechanism was proposed in 1976 by Iribarne and Thomson, known as "field ion evaporation" [34,35]. According to this model, the Rayleigh equation (eq. 1) predicts that the electric field at the droplet surface increases as the droplet radius decreases during evaporation. Charged droplets are torn up by "Coulomb explosions" into smaller droplets. This sequence repeats

until the daughter droplets become so small that the electric field due to the surface charge density is strong enough to desorb ions directly from the droplet into the ambient gas. This process is often referred to as "ion evaporation" [1,34]. Figure 1 shows an artist's conception of the sequence of events.

When a droplet radius reaches the Rayleigh radius R_R as a result of evaporation, the droplet becomes disrupted (for a given charge) due to the Rayleigh instability [20]. The Rayleigh radius, R_R , for a charged droplet is given by

$$\frac{N^2 e^2}{R_R} = 16 \pi \sigma R_R^2 \quad (1)$$

where N is the number of elementary charges in the droplet and σ is surface tension of the droplet.

Iribarne and Thomson analyzed the energy relations and kinetic aspects controlling the possible separation of small ions from the bulk of an evaporating charged droplet. The droplet radius $R=R_E$ at which ion evaporation takes place can be related to charge number N and an energy barrier ΔG . The resulting formula is

$$R_E = A + \sqrt{A^2 + B} \quad (2)$$

As evaporation proceeds, N remains constant while R decreases until reaching either the curve R_R or curve R_E in Figure 2. The curve R_E will be reached first (path A in Figure 2) resulting in ion evaporation if $N < 192$ for negative ions and if $N < 96$ for positive ions (the critical radii are 134 and 84 Å, respectively). If instead N is larger than these critical values, the curve R_R will be reached first (path B in Figure 2), and at this point the droplet will lose a considerable fraction of its charge. After one or more steps, the charge on the droplet falls below the critical limit, and ion evaporation takes place.

Ion Transport Through Capillaries

In contrast to EI, CI, FAB, and other common MS ionization methods, the electrospray source operates at atmospheric pressure. This means that the ions must be transferred from the high pressure environment into the vacuum of the mass spectrometer, i.e. the ions must be separated from the neutrals. This is not a new problem in mass spectrometry. Atmospheric pressure ionization (API), usually using a corona discharge or a β -emitter such as ^{63}Ni , has a long history. There are two basic designs for the interfacing of an API source with a mass spectrometer. In one, the ions pass directly from atmospheric pressure into the vacuum chamber through a single, pin-hole orifice [81]. Here, the gas flow

through a ca. 100 μm diameter orifice is pumped by a very efficient cryopump on the vacuum side. DC or RF electrical fields are used to extract the ions from the gas jet. Extensive cooling in the super-sonic jet may result in unwanted cluster ions, particularly with water molecules. Therefore, a dry, nitrogen interface gas is covering the high pressure side of the orifice [81]. This API design is presently used in the Sciex electrospray sources [82].

In a second API design, the pump-down is achieved in two stages. The ions pass through a first orifice and enter a chamber with a pressure in the torr range. This chamber is usually pumped by a mechanical rough pump. The ions are then sampled into the mass spectrometer through a conical skimmer. By applying an electrical field in the intermediate pressure chamber, the ions can be declustering and even fragmented. This API design is very common for electrospray interfaces. Also in this design, it may be beneficial to force the ions to drift through a layer of dry gas on the atmospheric pressure side of the first orifice.

Capillary ESI/MS Interface

A remarkable new atmospheric pressure/MS interface was designed by Fenn and his co-workers [1,3,9]. In this interface the ions were sampled through a $\phi 0.2 \times 60$ mm glass capillary that connected the atmospheric ESI source with the intermediate pressure chamber. Ions are entrained in the flow of high pressure gas that enters the transport capillary. The

gas emerges at the exit as a supersonic free jet. The gas throughput was close to that of a 100 μm orifice and equally good mass spectra were obtained. It is particularly noteworthy that the capillary gas flow can drag positive ions against a potential difference between the inlet and the exit ends of the capillary. Thus, positive ions were transported 4.5 kV "uphill" in Fenn's experiment [3,9] and 15 kV "uphill" in another experiment reported in the literature [9].

Figure 3 shows a schematic drawing of Fenn's electrospray mass spectrometer [1,9,10]. Sample solution flows, typically at 5-20 $\mu\text{L}/\text{min}$, through a stainless steel hypodermic needle. Voltages were applied to each of the following components: needle (ground), cylindrical electrode (-3500 V), metalized inlet and exit ends of the glass transport capillary (-4500 V and +40 V, respectively), skimmer (-20 V).

In Fenn's early instrument, Figure 3, desolvation of the electrospray ions occurred in a drying gas in front of the capillary. However, in later designs by Chait et al., no curtain gas is required [38]. Instead ion desolvation is promoted by heating the ion transport capillary. Desolvation of the electrospray ions is carried out by heat transfer to the charged droplets during their transport through the capillary, and additional desolvation is induced by collisional activation in the region of reduced pressure between transport capillary and skimmer [37]. By controlling the temperature of the transport capillary and the potential

between capillary exit and skimmer, the degree of desolvation can be gradually increased to obtain either a desired solvated ion or a completely desolvated ion [15,22].

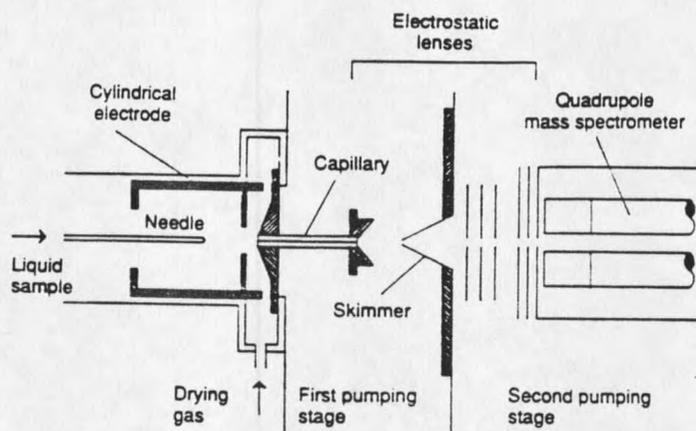


Figure 3. Schematic diagram of an ESI/MS apparatus [1].

For mass spectrometrists used to work at low pressures, ion transport through a narrow capillary seemed a surprising and strange arrangement. One might have guessed that only very few ions could have avoided collisions with the walls. Furthermore, the lore among ESI practitioners has been that ions would pass through very long capillaries. There has, therefore, been some doubts as to the mechanism of ion transport through the capillary tubes. There are several possibilities. For example, with electrospray, it is possible that it is mainly charged droplets and not gas-phase ions that

