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**Studies of Electrospray / Ion Mobility
Spectrometry / Time-of-Flight Mass
Spectrometry**

By

Luyi Ding, M.Sc.

A Thesis Submitted to
the Faculty of Graduate Studies and Research
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

Ottawa-Carleton Chemistry Institute
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October 1999

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The Undersigned Recommend to the Faculty of Graduate Studies and
Research Acceptance of the Thesis

**Studies of Electrospray / Ion Mobility
Spectrometry / Time-of-Flight Mass
Spectrometry**

Submitted by Luyi Ding, M.Sc.

in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

Chairman, Department of Chemistry

Thesis Co-Supervisor

Thesis Co-Supervisor

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Carleton University

1999

Abstract

This thesis describes the use of ion mobility spectrometry (IMS) coupled to time-of-flight mass spectrometry (TOF-MS) for examining electrospray-generated (ESI) ions. Two versions of ion mobility spectrometers were used, a conventional low field drift tube IMS and the new technology called high-field asymmetric-waveform ion mobility spectrometry (FAIMS).

A commercially-available drift tube ion mobility spectrometer was interfaced to a laboratory-built linear TOF mass spectrometer. The ions were generated by a laboratory-made electrospray ionization source. The ion mobility spectra were recorded after ions were transferred from the ESI ion source to the low field drift tube of the ion mobility spectrometer (atmospheric pressure). Ionic species that were separated in the ion mobility spectrometer could be selectively detected with the TOF mass spectrometer. The system was evaluated with small molecules including tetraalkylammonium ions, and with larger ions including the multiply-charged ions of proteins. Several proteins were examined. Their low resolution ion mobility spectra typically comprised two peaks; one peak yielded characteristic electrospray ions while another of lower mobility did not. The TOF mass spectra of protein at the lower IMS cell temperature (30°C) showed signs that were characteristic of protein-solvent clustering. The degree of solvation decreased with increasing IMS temperature. The mobility observed in IMS spectra and the charge state distributions seen in TOF mass spectra were somewhat dependent on the solution composition, such as the concentrations of acetic acid and methanol, which can affect the conformation of protein. This system could also be used for determination of cross-

sections of gas phase protein ions generated by electrospray ionization. Identification of charge states for each peak in the high resolution IMS spectra was carried out using TOF-MS. The dependence of ion mobility on ion cross-section (described by Mason and McDaniel) was used to calculate cross-sections for ubiquitin, cytochrome c, myoglobin, lysozyme and α -lactalbumin ions. Disulfide-intact proteins, lysozyme and α -lactalbumin, were studied in the disulphide-intact and the disulphide-reduced states. The cross-sections ranged from approximately $1609 \pm 80 \text{ \AA}^2$ for native lysozyme to approximately $4768 \pm 238 \text{ \AA}^2$ for denatured myoglobin and generally increased with the number of charges on the ion. The results were compared with literature cross-sections measured by ion mobility techniques at reduced pressure.

A newly-developed device called high-field asymmetric-waveform ion mobility spectrometer (FAIMS) with a cylindrical geometric configuration that utilized an ion separation technique based on the change in ion mobility at high electric fields, and which focused ions in two dimensions, has been revealed recently. This thesis describes a version of the FAIMS device in which this focusing was extended to 3-dimensions (i.e., ion trap). A method for the 3-dimensional confinement of ions with mass spectrometric interesting at 760 Torr and room temperature has not been described previously. Several prototypes of FAIMS which were designed to evaluate the ion focusing capability of FAIMS have been studied. The ions were pulsed out of FAIMS into a laboratory-built TOF system which included an octopole ion guide followed by a linear TOF-MS with spatial focusing. With this system, several experiments have been conducted showing the trapping of low-mass water cluster ions, high mass ions generated by corona discharge and multiply-charged protein ions generated by electrospray ionization technique. The

experimental results reported in this thesis indicate that FAIMS is a new generation of simplified, high sensitivity technology, which one day may replace the measurements made with a conventional drift tube ion mobility spectrometers.

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CHAPTER I

INTRODUCTION

1-1 Atmospheric Pressure and Electrospray Ionization

Atmospheric pressure ionization (API) techniques include corona discharge [1] radioactive foil [2], electrospray ionization [3], ion-spray [4], and thermospray [5]. Due to its high sensitivity for trace components, and being a softer ionization method which produces mainly pseudo-molecular ions, API has become an important ionization method for mass spectrometry (MS) [6] and ion mobility spectrometry (IMS) [7, 8, 9].

Some API sources produce ions through ion/molecule reactions [10-12]. These reactions are initiated by reactant ions formed in the source region from a primary ionization source such as a radioactive Nickel-63 source or a corona discharge. These reactant ions ultimately transfer a proton or charge to a trace analyte yielding molecular ions (product ions). The large number of collisions at atmospheric pressure make this method a very efficient ionization source for high proton affinity or high electron affinity molecules. A further significant property of this atmospheric pressure ionization method is that it results in soft ionization, i.e. the product ions rarely dissociate or fragment since the energetics of API processes are weak.

Electrospray ionization (ESI) [3, 13-15] is an atmospheric pressure ionization method which allows direct sampling of ions from solution into the gas phase. Ionization occurs usually by multiple protonation, and sometimes cationization (alkali cations or ammonium ions). In negative ionization conditions, multiple deprotonation or anion attachment occurs. This phenomenon of multiple charging makes ESI a powerful means of ionizing macromolecules of biological interest and is probably the most significant development in mass spectrometry in the past ten years [3, 6]. The extent of this multiple charging can be great enough to reduce mass-to-charge (m/z) ratio to values below 3000

units, even for very high molecular weight species, such as proteins. This reduction allows mass spectrometers with relatively low m/z ranges to be used for bio-polymer analysis.

1-1-1 The Apparatus for Electrospray Ionization

A simplified schematic diagram of the ESI process for production of positive ions is illustrated in Figure 1-1. A metallic, narrow-bore capillary tube, continuously filled with a solution consisting of a polar solvent in which electrolytes (the analyte) are soluble, is connected to the positive terminal of a high-voltage dc power supply (applied voltage of 3-5 kV). The negative terminal of this supply is connected to a metal plate located a few millimeters away from the capillary tip. The intense electric field at the capillary tip draws some of the positive electrolyte ions in the exposed solution to the liquid surface. The negative ions that formerly neutralized the charge on these positive ions are driven back toward the tip of the capillary. The electrohydrodynamics of the charged liquid surface cause it to form the shape of a cone (called the *Taylor cone*) from which a thin filament of solution extends until it breaks up into droplets [13, 16-18]. The droplets are positively charged due to the excess of positive electrolyte ions at the surface of the cone.

These positively-charged droplets produced by the cone move downfield toward the metal plate. On their trip to the plate, much of the solvent evaporates from the droplets and, by one or more of several proposed mechanisms, some solvent-free positive gas-phase ions are formed. When the gas-phase ions and the remaining charged droplets hit the metal plate, electrons from the metal plate neutralize their charge. The net motion of positive ions from the capillary tip to the metal plate conducts an electrical current

Figure 1-1 Schematic of major processes occurring in electrospray ionization [13].

(usually 0.1 - 0.2 μA) passing clockwise around the circuit through the power supply, up to the capillary metal, into the solution, out into droplets, and so on. An electrochemical oxidation process must occur where the current passes into the solution [13, 17]. In practical mass spectrometry employing ESI, the metal plate is part of an assembly that separates the atmospheric pressure electrospray area from the high vacuum of the mass spectrometer. An orifice exists in this metal plate through which some of the gas-phase ions pass into the mass analyzer.

The description given above is for the production of positive ions. In an analogous way, reversing the polarity of the power supply will result in generation of negatively-charged ions. In this case, the positive electrolytes are driven back away from the tip of the Taylor cone and the electrochemical process is reduction.

The diameter of the droplets formed during ESI is influenced by a number of parameters, including the applied potential, the solution flow rate and the solvent properties [19].

1-1-2 Mechanism of Ion Desolvation

There are two principal mechanisms proposed for the desolvation of the observed ions. One is '*Coulomb fission*' [20] in which the repulsive effect of the increasing surface charge density eventually completely overcomes the surface tension holding the droplet together. At this point (the '*Rayleigh limit*'), the fission ('*Coulomb explosion*') will occur, causing the droplet to split, temporarily increasing the overall surface-to-volume ratio. The fission process may result in product droplets of roughly equal size, or more often, it may cause the ejection of a series of much smaller droplets [21] in a process now called

'droplet jet fission' [6]. Dole [20] proposed the fission process continuing (by solvent evaporation and fission, respectively) until the particles contain only a single charged species. The Rayleigh stability limit is represented mathematically in the following equation [6],

$$q_{Ry} > 8\pi(\epsilon_0\gamma R^3)^{1/2} \quad (1-1)$$

This Rayleigh equation gives the condition at which the charged droplet becomes unstable when the electrostatic repulsion (due to charge q) greater than the force due to the surface tension γ , which holds the droplet of radius R . Here, ϵ_0 is permittivity of vacuum (8.8×10^{-12} coul²N⁻¹m⁻²).

The second proposed mechanism is '*ion evaporation*' [22, 23] in which the increase in surface charge density as a result of solvent evaporation produces a Coulombic repulsion that exceeds the charged species' adhesion to the drop surface and thus some ions are expelled from the surface ('emission'). This process also continues as the droplet decreases in surface area due to solvent evaporation. The ion emission becomes the dominant process for droplets with radius $R < 10$ nm [6].

The relative importance of the Coulomb fission and the ion evaporation mechanisms remains the topic of discussion and research. Kebarle and Tang [13] noted that the ion separation in the electrospray capillary tip is essentially electrophoretic and that the interface may be considered as an electrolytic cell in which part of the charge transport occurs in the gas phase. The predicted electrochemical process at the liquid/metal interface of the electrospray capillary has been demonstrated experimentally by Blades *et al.* [18]. Electrochemical oxidation occurring in the electrospray capillary has analytical implications in that radical cations may be generated from analytes, thereby permitting

the use of electrospray as a true ionization method rather than a procedure for phase transfer of pre-formed ions [24, 25]. Guevremont *et al.* found that the distribution of charge states for ESI ions from protein solutions was very similar to the most probable distribution of charge states for protein ions within that solution as calculated from pK_a values for the basic groups of those proteins [26]. They concluded that the electrospray ions could be identified with the solution ions, not only in the case of proteins but also for all other solutes. Siu and co-workers have suggested that electrospray ions are not formed by the evaporation of charged droplets but are emitted directly from the tip of the Taylor cone at the exit of the electrospray needle [27, 28].

1-1-3 ESI Multiply-Charged Ions

Electrospray is unique among ionization methods because it can produce multiply-charged species in abundance, and hence open new possibilities for the analysis of large and labile biological molecules, such as proteins and oligonucleotides. Proteins of molecular masses exceeding 5,000,000 Da were successfully determined with the ESI technique [29]. The availability of multiply-charged ions artificially extends the effective mass range of the mass spectrometer, especially for quadrupole mass spectrometry, since a mass spectrometer measures ion intensities versus the mass-to-charge ratio of the sample ions.

Electrospray mass spectra of proteins are characterized by a peak distribution of various charged (protonated) states, due to ions of the same protein that are differently charged (Figure 1-2). This distribution depends on the sample and on experimental conditions. The multiplicity of protonation is certainly related to the number of basic

Figure 1-2 A typical electrospray mass spectrum of cytochrome c.

amino acid residues in the protein structure. It should be noted, however, that the maximum charge state may exceed or be less than the number of sites that are protonated in solution [30], reflecting the influence of gas-phase processes [31]. This will be discussed in more details in a later section (Section 1-5 Protein Structure, page 48). Several assumptions were made in order to relate each charge state to the mass of the protein [32, 33], (1) adjacent peaks are different by only one charge; (2) charging is due to proton attachment to the protein; and (3) the mass of the protein is the same for all peaks.

Assuming that the compound (i.e., electrically neutral) mass is M , the mass-to-charge ratio (m/z) of the ESI-generated ions is described by $(M + zc)/z$, where the charge on the ion is $z = 1, 2, 3, \dots$, etc., and c is the mass of the cations (e.g., proton $c = 1$) that impart the total charge z to the ion via cation addition [32]. The mass-to-charge separation between these ions is not constant and gives the ions an appearance of being more closely spaced at higher charge (thus lowering the mass-to-charge ratio) (Figure 1-2). From the mass-to-charge values of two neighboring ions, the charged state and the mass of the sample can be both calculated by simple arithmetic [34]. If a positive ion series is assumed to represent different protonation states, then the mass/charge ratio, x_z and x_{z+1} , of adjacent members of the ion series are given by:

$$x_z = (M + z) / z \quad (1-2)$$

and

$$x_{z+1} = (M + z + 1) / (z + 1) \quad (1-3)$$

where z is the charge state and M is the molecular mass. Solving these equations gives

$$z = (x_{z+1} - 1) / (x_z - x_{z-1}) \quad (1-4)$$

and allows the estimation of M .

1-2 Conventional Ion Mobility Spectrometry (IMS)

Ion mobility spectrometry (IMS) is an instrumental technique for the characterization of molecules based upon gas-phase mobilities of ions in a drift tube with a weak uniform electric field and a drift gas at atmospheric pressure [7, 8]. This technique was first introduced in 1970 under the name of '*plasma chromatography*' [7]. Interest in the technique was aroused by the excellent detection limits, the speed of response, and its applicability to numerous organic functionalities.

1-2-1 Conventional IMS Hardware

A typical ion mobility spectrometer consists of an ionization source, an ion/molecule reaction region, an ion injection shutter, an ion drift region, and an ion collector (*Faraday plate*). An illustration of the basic construction of an ion mobility spectrometer (Phemto-Chem 100, PCP Inc., West Palm Beach, FL) [35] is presented in Figure 1-3.

A uniform electrical field is maintained in the drift region between the ionizer and collector by means of a series of guard rings and resistor voltage dividers. A carrier gas, normally air or nitrogen, transports gases or vapors from sample material to be analyzed into the ion mobility spectrometer. Trace impurities, such as water and ammonia present in the carrier gas, are ionized by the energetic electrons released from the radioactive *Nickel-63* source and a number of positive and negative reactant ions are formed, such as $(\text{H}_2\text{O})_n\text{H}^+$ and $(\text{H}_2\text{O})_n\text{O}_2^-$. These reactant ions undergo a complex series of ion/molecule reactions with the analyte, and product ions are formed. Depending on the polarity of an applied electric field, either positive or negative ions are periodically pulsed into the ion drift region through the shutter grid. In the drift region, ions travel towards the collector

Figure 1-3 Schematic of an ion mobility spectrometer (Phemto-Chem 100) [35]

through a counter current of drift gas while under the influence of an electric field. Due to the linear electrical field, ions of different mobilities become spatially separated within the drift region. As each separated ion bunch approaches and strikes the collector, it generates a small electrical current in the collector circuit. The peak currents are on the order of 5×10^{-10} amperes. These low signal levels are amplified by a wide-band electrometer located as close as possible to the actual detector electrode. The resulting amplified signal is further amplified and recorded by a synchronous signal-averaging device. Thus it is possible to identify the different ionic species by monitoring the time between the introduction of the ions into the drift region at the electric shutter, and the arrival of the ions at the collector plate. The intensity of ions collected as a function of drift time is recorded as an ion mobility spectrum.

1-2-2 Ionic Properties Deduced from IMS Measurements

1-2-2-1 Ion Mobility

The *mobility*, K , of an ion is a function of the ion charge, mass and dimensions [7, 8]. Experimentally, the mobility is derived from the drift time, t_d , that the ion requires to travel the length of the drift tube. It is obtained from the peak maximum of the ion arrival time in the ion mobility spectrum measured relative to the time when the ion was introduced into the drift tube. Theoretically, the drift time is given by [8],

$$t_d = l_d / v_d \quad (1-5)$$

where l_d (cm) is the length of the drift tube, v_d (cm/s) is the drift velocity for the ion, and the mobility K ($\text{cm}^2\text{V}^{-1}\text{sec}^{-1}$) is given by

$$K = v_d / E \quad (1-6)$$

where E (V/cm) is the constant electric field applied across the drift tube, or

$$K = l_d^2 / (t_d V) \quad (1-7)$$

where V (volts) is the applied voltage.

The mobility, K , of very large ions can be related to the charge, q , and the radius of the ion, R , by Millikan's Equation [36],

$$K = \frac{q}{6\pi\eta R} \left[1 + \frac{\lambda}{R} \left(A + B e^{-\frac{cR}{\lambda}} \right) \right] \quad (1-8)$$

where η is the gas viscosity and λ is the mean free path; A , B and c are constants and equal 1.234, 0.414 and 0.876, respectively.

The ion mobility in a given gas is inversely proportional to the number density of the gas molecules but relatively insensitive to small changes in gas temperature if the number density is held constant [38]. To facilitate the comparison and use of data a measured mobility K is usually converted to a 'reduced' mobility, K_o , defined by the following equation,

$$K_o = K (273/T)(P/760) \quad (1-9)$$

where T is the gas temperature in Kelvin and P is the gas pressure in Torr at which the mobility K was obtained. Therefore, the reduced mobility K_o is K which has been corrected to standard gas number density, 2.69×10^{19} molecules/cm³ under standard conditions of pressure and temperature (760 Torr and 273 K).

1-2-2-2 Collision Cross-Section

The ion mobility, K , can be related to molecular structure (collision cross-section) through the Mason & McDaniel equation [37, 38],

$$K = \frac{3ze}{16N} \left[\frac{2\pi}{\mu k_b T_{eff}} \right]^{\frac{1}{2}} \frac{1 + \alpha}{\Omega_{avg}^{(1,1)}} \quad (1-10)$$

where z is the total number of elementary charges on the ion; e the electronic charge (1.602×10^{-19} C) and N the number density of neutral molecules. The reduced mass of a colliding ion-drift gas molecule pair is $\mu = mM / (m + M)$, where m is the mass of the ion and M is the mass of neutral gas molecule. k_b is the Boltzmann's constant (1.381×10^{-23} J/K). α is a small correction term whose magnitude is less than 0.02 if $m > M$. T_{eff} is the effective temperature of the ions and $\Omega_{avg}^{(1,1)}$ is the averaged collision integral which depends on the effective temperature.

Under the 'low-field' conditions of IMS measurements (i.e., E/N is below about 2 Td, here, Td, Townsend unit described as $1 \text{ Td} = 10^{17} \text{ V cm}^2$), the effective ion temperature is nearly equal to the neutral gas temperature, so that T_{eff} is equal to the drift tube temperature, T . From Equation (1-9) and use of N_0 , the standard number density of neutral gas molecules, Equation (1-10) can be rewritten as reduced mobility K_0 ,

$$K_0 = \frac{3ze}{16N_0} \left[\frac{2\pi}{\mu k_b T} \right]^{\frac{1}{2}} \frac{1}{\Omega_{avg}^{(1,1)}} \quad (1-11)$$

or,

$$\Omega_{avg}^{(1,1)} = \frac{3ze}{16N_0} \left[\frac{2\pi}{\mu k_b T} \right]^{\frac{1}{2}} \frac{1}{K} \frac{T}{273.2} \quad (1-12)$$

where $\Omega_{avg}^{(1,1)}$ is the orientationally-averaged collision integral. The collision integral is related to the scattering angle, the angle between the trajectory before and after a collision between the ion and a drift gas molecule. The orientationally-averaged collision integral is calculated by averaging the momentum transfer cross-section over the relative velocity and the collision geometry [39]. The collision integral has been approximated by [40],

$$\Omega_{avg}^{(1,1)} \approx \frac{1}{8\pi} \int_0^{2\pi} d\theta \int_0^\pi d\phi \sin\phi \int_0^{2\pi} d\gamma \pi b_{min}(\theta, \phi, \gamma)^2 \quad (1-13)$$

where b_{min} is the minimum impact parameter for a collision geometry defined by θ , ϕ and γ that avoids a hard-sphere contact with any atom in the molecule. For rigid-sphere collisions, integrating analytically yields collision cross-section, $\Omega (\text{\AA}^2)$,

$$\Omega = \pi d^2 \quad (1-14)$$

where d is the sum of the ion and drift gas molecule radii.

From Equation (1-10), it can be seen that the mobility, K , is essentially related to the $\pi \mu^{-1/2} \Omega^1$. For small ions (ion radius $\ll$ mean free path) in the same neutral drift gas, mobility is mainly controlled by the reduced mass μ . For a macro ion or charged droplet (radius $\gg$ mean free path), drag flow applies and the relevant dimension is the ion radius with the gas viscosity taken into consideration. The reduced mass is essentially equal to M , the mass of the neutral drift gas molecule, thus the distinguishing property upon which large ions are separated is ionic size through the Ω^1 term. For those ions falling between

these extremes, which includes most of polyatomic ions, mobility is a function of both mass and shape [37].

The mobility equation also reveals an inverse relationship between K and N , the number density of the drift gas. This is as expected since it is collisions with the drift gas that ultimately limits the ionic velocity. Increased number density, N , yields increased number of collisions, decreased ionic velocity, v_d , and decreased mobility, K .

In addition to the temperature dependence of the number density, N , the mobility equation shows a variation in K with $T^{1/2}$. The ion/neutral cross-section, Ω , for most systems varies as $T^{1/2}$.

Equation (1-6), $K = v_d/E$, is true (i.e., K is a constant) only at low field strengths [38]. For most IMS used in analytical applications, the electric fields are normally in the range of 100 to 300 V/cm, the suitable range for which the drift velocity, v_d , is directly proportional to the electric field, E , and the mobility, K , is independent of the electric field. However, at high field, mobility K is no longer a constant value [38]. This phenomenon will be discussed in Section 1-3 (page 23). High-field asymmetric-waveform ion mobility spectrometry (FAIMS) is a new technique based on the change in mobility at high electric field.

1-2-2-3 Resolution of an IMS Peak

Resolution, R , describes the ability of an instrument to resolve two neighboring peaks in a spectrum. A practical measure of resolution in IMS is,

$$R = t_d/t_{1,2} \quad (1-15)$$

where t_d is the drift time of the peak, and $t_{1/2}$ is the width of the peak at half its maximum height. Experimentally, a number of factors potentially contribute to $t_{1/2}$ and to peak shape [41]. These include (a) initial pulse width; (b) field gradients; (c) diffusional broadening as the ions drift down the electric field; (d) coulombic repulsion between ions (space charge effect); (e) temperature gradients; (f) pressure fluctuations, and (g) ion/molecule reactions with the drift gas and impurities in the drift tube.

1-2-3 IMS/MS

In one of the first publications of ion mobility spectrometry (referred to plasma chromatography) [42], the technique of GC/IMS/MS was described. In this system, a gas chromatography was used to separate the compounds of the sample, with the IMS used as its detector. The ions detected in the IMS could be further mass analyzed by a mass spectrometer. Subsequently, the GC was removed from the front end of the instrument, and IMS/MS remained as a very useful analytical tool.

The Phemto-Chem MMS-160 Atmospheric Ion Mobility Spectrometer/Quadrupole Mass Spectrometer (PCP Inc., West Palm Beach, FL) is a commercial IMS/MS system which has been widely used in many applications [43-59]. This instrument combines the functions of the ion mobility spectrometer and the quadrupole mass spectrometer. It generates and measures, in real time, the ion mobility and mass of both positive and negative ions in the gas phase at atmospheric pressure. Ions formed by ion/molecule reactions in the high pressure region are focused into the mass spectrometer vacuum region by a set of extracting and focusing ion lenses. This system is used for both chemical analysis and physical chemistry applications. In combination with a suitable

digital data acquisition and storage system, the MMS-160 will perform studies in (1) ion/molecule reaction kinetics; (2) ion mobility with mass identification; (3) mass analysis with precursor identification; (4) chemical analysis with low detection limits. With this instrument, studies can be performed at pressures ranging from well below to above atmospheric pressure (1 Torr to 1500 Torr) with ambient air, nitrogen, carbon dioxide, and all gas mixtures. Sub-ambient to 220°C operation is available [35].

Four types of data can be collected from the IMS/MS system, (1) ion mobility spectra (IMS) collected from the Faraday plate of the ion mobility spectrometer; (2) total ion mobility spectra (TIMS) collected from the electron multiplier of the mass spectrometer operated in the RF-only mode; (3) mass spectra (MS) of the total ion current (i.e., shutter grid open) of the IMS; and (4) mass identified mobility spectra (MIMS), where the shutter grid of the IMS is pulsed and the mass spectrometer is tuned to a specific mass.

1-2-4 Applications of IMS

IMS has several attractive features for investigation of ion/molecule chemistry. These include (1) simple instrumentation, comprised of only a few relatively inexpensive components; (2) sensitivity to a broad range of molecular functional groups; (3) little or no sample preparation; (4) measurement on the millisecond time scale; (5) low sample residence time and few memory effects; (6) simple ion mobility spectra; and (7) excellent detection limit (sub-ppt range) and speed of response. Additionally, IMS has some special advantages over other (high vacuum) techniques. For example, temperature of the IMS drift tube can be varied conveniently between 0 and 250°C to permit a study of

ion/molecule reactions as a function of temperature, while cluster ions such as $(\text{H}_2\text{O})_n\text{H}^+$ may form or dissociate during sampling into the mass analyzer.

IMS and IMS/MS systems have been used as an analytical tool in many real-time monitoring applications such as detection of drugs [43-47], explosives [48-51], chemical warfare agents [52], trace vapor constituents in gaseous mixtures [16, 54] and environmental pollutants [53, 54]. Additional applications include identification of wood species [55, 56], sensing of petrochemical fuels in the head-space of soil [57], detection and monitoring of a toxic perfluoroisobutene in the negative ion mode [58], and applications in the semiconductor industry [59]. IMS was also used as a versatile detector for gas and supercritical fluid chromatography [60-62]. Several groups have used mobility measurements to characterize the size distribution of aerosol particles and small metal particles [63].

1-2-5 Electrospray Ionization IMS

Several methods have been used as ionization sources for IMS. These include *Nickel-63* [7, 8], thermo-ionic ionization [64], corona-discharge ionization [1], photo-ionization [65], laser multi-photon ionization [66], electrospray ionization [1], and surface ionization [67].

The first example of an IMS interfaced to an electrospray ionization source was demonstrated in 1972 by Dole *et al.* who reported electrospraying lysozyme into an IMS spectrometer [68]. Smith and co-workers have also reported investigation of cytochrome c [8] and lysozyme [69] by ESI/IMS. Although this approach had originally showed promise for high molecular weight determinations, ion peaks resulting from these

experiments were very broad. These broad peaks were argued to be due to the excess solvent ions adsorbed to the surface of the macro-ion aggregates [70]. Improvement in both sensitivity and resolution was reported by Shumate and Hill [1] by incorporating an electrospray needle directly into a unidirectional gas flow ion mobility spectrometer, although this method was reported to be most useful for low molecular weight compounds. Subsequently, a water-cooled electrospray ionization source that could be interfaced directly to high-temperature IMS was developed [71]. The advantages of this cooled electrospray source included rapid desolvation of sprayed droplets, prevention of solute precipitation in the spray needle, efficient transmission of ion spraying into IMS, and elimination of corona discharge [72]. In addition, both signal to noise (S/N) ratio and resolution were improved for ESI/IMS by using the Fourier transform mode of operation [73].

Electrospray ionization / ion mobility spectrometry has been demonstrated as a detection method for liquid chromatography [74], capillary electrophoresis [75], on-line liquid process streams [76] and as a field monitoring method [77]. Using an electrospray ionization source and a downstream quadrupole mass spectrometer with electron multiplier as detector, the instrument demonstrates the potential of IMS for rapid analytical separations [78].

Another exciting recent application of electrospray ion mobility mass spectrometry is the study of the conformations of peptides and proteins in the gas phase. Measurements have now been reported for ESI of cytochrome c, ubiquitin, lysozyme, apomyoglobin and bovine pancreatic trypsin inhibitor (BPTI) [79] although, in all these studies, the ion

mobilities were measured in a drift tube at low pressure and the mass of ions was confirmed with a quadrupole mass spectrometer.

We will present, in Section 3-1-3 of Chapter III (page 123), a newly-designed tandem system composed of a conventional IMS coupled to a TOF mass spectrometer. With this system we examined the conformation of electrosprayed protein ions which have been separated by IMS at atmospheric pressure.

1-3 High-Field Asymmetric-Waveform Ion Mobility Spectrometry (FAIMS)

1-3-1 Ion Separation in FAIMS

High-field asymmetric-waveform ion mobility spectrometry (FAIMS) is a new technology capable of separation of gas-phase ions at atmospheric pressure (760 Torr) and at room temperature. FAIMS can be compared to conventional IMS since both techniques are based on 'ion mobility' which is defined as the motion of ions in an electric field through a buffer gas. However, in the conventional IMS, the external electric field strength is very low (typically, < 300 V/cm), and Equation (1-6), $K = v_d/E$, can be applied. This means the ion drift velocity, v_d , is directly proportional to electric field, E , and the mobility, K , is independent of the electric field. If, in contrast, the applied electric field is very high (e.g. 10,000 V/cm), K is no longer constant and the ion drift velocity is no longer directly proportional to the electric field. This dependence of ion mobility on electric field has been the basis for the development of FAIMS [80-83].

A plot of the dependence of ion mobility on electric field, i.e. the ratio of ion mobility at high electric field K_h relative to the mobility at low electric field K , K_h/K , versus electric field E , for three possible types of ions A, B and C, is shown in Figure 1-4. In this figure, the ratio of K_h/K of a type A ion increases with increasing electric field, a behaviour which is typical of low mass ions. The ratio of K_h/K of a type C ion decreases with increasing electric field strength, a behaviour which is typical of high mass ions and the ratio of K_h/K of a type B ion increases initially before decreasing at yet higher fields.

Figure 1-4 Dependence of ion mobility on electric field strength for three types of ions, A, B, and C [82].

Several examples are discussed by Mason and McDaniel [38].

The mobility of a given ion under the influence of a high electric field can be expressed by

$$K_h(E) = K [1 + f(E)] \quad (1-16)$$

where K is the ion mobility at low electric field and ' $f(E)$ ' describes the functional dependence of the ion mobility on the electric field at a constant number density of drift gas at atmospheric pressure and constant temperature. Mason and McDaniel [38] have stated that the function $f(E)$ is composed only of even polynomial terms,

$$K_h = K (1 + \alpha E^2 + \beta E^4 + \dots) \quad (1-17)$$

Since ion mobility is a function of gas pressure, the electric field is usually reported in terms of either E/N or E/P , where N is the gas number density and P is the gas pressure (Torr).

Figure 1-5 illustrates the motion of a type A ion (in Figure 1-4) carried by a gas stream between two parallel plates, as used in the first version of FAIMS described by Buryakov *et al.* [80]. An asymmetric period electric field $V(t)$ is placed perpendicular to the ion motion. Under the influence of this waveform (a simplified square waveform), the net motion of the ion between two plates is comprised of two components. One is horizontal (longitudinal) component due to the flowing stream of gas and the other is the transverse component due to the electric field between the plates. Usually, one of the plates is grounded and the other has the high voltage asymmetric waveform, $V(t)$, applied to it. The asymmetric waveform is composed of a positive high-voltage (V_1) component lasting for a short period of time t_1 and a negative lower-voltage (V_2) component lasting for a longer period of time t_2 (see Figure 1-5 lower trace).

Figure 1-5 Schematic of the ion motion between parallel plates during the application of an asymmetric waveform shown as $V(t)$, the ion is transported horizontally by a gas flow [82].

The waveform is synthesized such that the integrated voltage time product applied to the plate during a complete cycle of waveform is zero, (i.e., $V_1t_1 + V_2t_2 = 0$). During the high voltage portion of the waveform, the field will cause the ion to move with transverse velocity component $v_1 = K_h E_{high}$, the distance travelled will be $s_1 = v_1t_1 = K_h E_{high}t_1$. During the low-voltage portion of the waveform, the velocity component of the ion will be $v_2 = KE_{low}$, and the distance travelled is $s_2 = v_2t_2 = KE_{low}t_2$. Since the asymmetric waveform is synthesized so that $(V_1t_1 + V_2t_2) = 0$, the field-time products $E_{high}t_1$ and $E_{low}t_2$ are equal in magnitude. Thus, if K_h and K are identical, s_1 and s_2 are equal, and the ion will be returned to its original position relative to the plates during the negative cycle of the waveform. If at E_{high} the mobility $K_h > K$, the ion experiences a net displacement from its original position relative to the lower plate. If an ion is migrating away from the upper plate, a constant negative dc voltage can be applied to this plate to reverse or “compensate” for this offset drift. This dc voltage, called the ‘compensation voltage’ or CV , prevents the ion from migrating toward either plate. If the ions derived from two compounds respond differently to the applied high electric fields, the ratio of K_h to K is different for each compound. Consequently, the magnitude of the compensation voltage necessary to prevent the drift of the ion toward either plate will also be different for each compound. Under conditions in which the compensation voltage is suitable for one ion to pass between the two plates, the other will drift towards one of the plates and subsequently be lost. Experimentally, the value of CV is typically between -50 and +50 volts. To detect a mixture of ions, the compensation voltage can be scanned to yield a compensation voltage spectrum (CV spectrum). Since the mobility of an ion at high field is dependent on (unknown) properties of the compound, only those ions with a particular

ratio of K_H/K will successfully travel parallel to the plates at a given CV . FAIMS is therefore capable of ion separation [82].

1-3-2 Ion Focusing in FAIMS

Carnahan and Tarassov [81] designed a FAIMS system using cylindrical electrodes, rather than the plates described by Buryakov *et al.* [80]. The asymmetric waveform is applied to the inner tube of two concentric cylinders, and the annular space between the cylinders corresponds to the space between the parallel plates described by Buryakov *et al.*

The cylindrical geometry provided considerable improvement in sensitivity over the flat plate design because, in addition to the ion separation capability, an ion focusing region was formed in the annular space between the cylinders [83]. At a suitable combination of DV and CV , an ion will travel with the gas flow along the length of the FAIMS device. But because of the focusing effect, the ion will be unable to travel in either direction radially. This focusing decreases ion loss to the walls of the FAIMS and, therefore, increases ion transmission efficiency.

The ion focusing mechanism was realized from several unexpected experimental results using FAIMS [82, 83]. Figure 1-6 shows two asymmetric waveforms (#1 and #2) with different polarities that were used in FAIMS. These two asymmetric waveforms are synthesized by summation of a *sine* (or *cosine*) wave and a harmonic as shown in the following equation [84],

$$V(t) = \frac{2}{3} DV \sin(\omega t) + \frac{1}{3} DV \sin(2\omega t + \frac{\pi}{2}) \quad (1-18)$$

Figure 1-6 Asymmetric waveform applied to FAIMS

(a) waveform #1 (P1, N2 modes) and (b) waveform #2 (P2, N1 modes) [82].

where DV is the peak voltage of the waveform, also referred to as the 'dispersion voltage'. It was expected that a reversal of the polarity of the asymmetric waveform would result in the reversal of the polarity of the CV necessary to pass an ion through the FAIMS analyzer. Experimentally, much lower transmission of an ion, if any, was observed when the polarity of the CV was reversed [82]. Thus, the application of the two asymmetric waveforms is considered separately as mode 1 and mode 2. When considering positive ions, these are abbreviated to modes P1 and P2, and similarly, modes N1 and N2 refer to negative ions [85].

Another unexpected result was that the sensitivity of ion transmission increased substantially when DV was increased [82]. The FAIMS instrument was expected to act as an ion 'filter', with the capability of selectively transmitting one type of ion out of a mixture. With no voltages applied to the FAIMS analyzer, the optimum transmission for every ion was expected, albeit without any separation. The conditions designed to yield ion separation were expected to decrease ion transmission because of increased ion loss due to collisions with the walls during application of the high voltage periods of the waveform (i.e., effectively narrowing the width of the analyzer region). Contrary to this prediction, the ion current increased as DV was increased [82]. The unusual observations noted above suggested that atmospheric pressure ion focusing was occurring in the FAIMS analyzer [82, 83].

The ion focusing phenomenon in FAIMS results in a significant change in the motion of an ion when the waveform voltage polarity is reversed. If the polarity of $V(t)$ is inverted, the trajectory of an ion, which was focused in the FAIMS analyzer, will become unstable, and the ion will be lost from the analyzer. That is to say, upon application of the

mirror image of $V(t)$, the fields in the FAIMS analyzer also are mirrored, and the focusing fields are replaced by defocusing fields. In general, ions whose mobility increases with electric field appear in modes P1 and N1, and are usually ions with m/z below about 300 (type A, Figure 1-4). Ions that are focused in mode P2 and N2 tend to have ion mobilities that decrease with increasing electric field (type C, Figure 1-4), and have higher m/z ratio. These are only guidelines, because (a) there are exceptions and (b) an ion can (in principle) be detected in both modes [85].

This FAIMS, with cylindrical geometry, formed the basis of a new instrument called 'Field Ion Spectrometer' (FIS[®]), built by Mine Safety Appliances Company, Pittsburgh, PA, as shown in Figure 1-7. The FIS was designed for trace gas analysis [81]. Development of this device was cancelled in July 1999.

1-3-3 Two-Dimensional Ion Focusing in FAIMS

Detailed analysis of the motion of an ion in the annular space between the cylinders of FAIMS is required to study the ion focusing inside the FAIMS. The physics of the two-dimensional focusing of ions in FAIMS was considered previously [83], and will be briefly described here.

The ion motion in the FAIMS was modelled using a combination of experimental and theoretical considerations. First, consider the two cylinders used in FAIMS as displayed in Figure 1-8. When the inner electrode of the coaxial cylinders of FAIMS is held at a potential V_a relative to the ground potential of the outer cylinder, the voltage at any point between the two cylinders can be calculated using the following equation,

$$V_r = V_a [\ln(r/b)/\ln(a/b)] \quad (1-19)$$

Figure 1-7 Schematic of a high field asymmetric waveform ion mobility spectrometer with an electrometer ion detector (FAIMS-E)

(a) 3-dimensional view, (b) cross-sectional view [83].

Figure 1-8 Schematic view of the inner and outer electrodes of the cylindrical FAIMS analyzer [83].

where V_r is the electric potential at some radial distance r between the tubes; a (cm) is the outer radius of the inner cylinder and b (cm) is inner radius of the outer cylinder. The outer cylinder is electrically grounded (i.e., 0 V). The annular space (i.e., the FAIMS analyzer region) falls in the radial distance between a and b as shown in Figure 1-8. The voltage between the tubes does not vary linearly as a function of radius, and the electric field ($dV(r)/dr$) has a radial dependence. The electric field between the cylinders (at location r) can be expressed as,

$$E(r) = -V_a / [r \ln(a/b)] \quad (1-20)$$

Figure 1-9 shows the experimentally-determined change in ion mobility of one type of ion, $(H_2O)_nH^+$, as a function of electric field using FAIMS-E instrument [83]. Assuming that the change in the ion mobility of $(H_2O)_nH^+$ at high electric field is represented by the curve shown in Figure 1-9, the trajectory of this ion within the cylindrical geometry shown in Figure 1-8 can be calculated. As a first approximation [83],

$$R_{final} = [2 t K (V_a / \ln(a/b)) + R_{initial}^2]^{1/2} \quad (1-21)$$

where R_{final} is the radial location of the ion after a time period t , and $R_{initial}$ is the radial location before the time period t . However, this equation only gives useful values of final radial distance if the electric field does not vary too much between $R_{initial}$ and R_{final} . In Equation (1-21), K is assumed to be constant for the trajectory distance. However, K is not constant when the ions experience high electric fields; E varies with r , and K varies with E as shown in Figure 1-9. For example, if an ion is located at a distance r , and at some selected time (during application of the asymmetric waveform) such that the voltage applied to the inner electrode results in an electric field of about 10,000 V/cm, the

Figure 1-9 Measured variation in the ion mobility of $(\text{H}_2\text{O})_n\text{H}^+$ ion with electric field. The measurements are based on compensation voltage scans [83].

ion mobility is calculated to be $1.01K$, where the 1.01 value is taken from Figure 1-9. The value of K is about $2.3 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ for $(\text{H}_2\text{O})_n\text{H}^+$ at room temperature. This value can be found in the conventional ion mobility spectrometry literature [8].

The trajectory of ion motion in the space between two cylindrical electrodes can be calculated using numerical techniques, with a computer program called 'FAIMS-Traj' that divides the trajectory into very small steps. At each step, all of the variable parameters including electric field, ion mobility, ion velocity, and ion location can be estimated, as well as the new location of the ion after each step.

Figure 1-10 illustrates the trajectory of an ion with the high field properties given by the curve in Figure 1-9. Figure 1-10a displays 20 oscillations of this ion resulting from applying an asymmetric waveform #1 shown in Figure 1-6 (page 29). For this calculation, the cylindrical geometry shown in Figure 1-8 with $a = 0.1 \text{ cm}$, $b = 0.3 \text{ cm}$ was used. The applied voltage for the trajectory simulation was $CV = 0 \text{ V}$, $DV = 2500 \text{ V}$ at a frequency of 200 kHz and a high-field/low-field time period ratio (t_2 and t_1 in Figure 1-5, page 26) of 1:2. The ion trajectory shown in Figure 1-10a is calculated with the ion initially at the inner electrode, at a radial distance of 1 mm. The trajectory simulation shows that the ion does not travel exactly the same radial distances during the low-field and high-field portions of the waveform, and consequently, the ion experiences a 'net' drift. The simulation was repeated for some other conditions. Figure 1-10b was simulated in exactly the same manner as Figure 1-10a, except that in Figure 1-10b the number of waveform cycles was increased to 200, and thus the magnitude of the net ion deflection was significantly higher. Figure 1-10b shows that the ion will eventually move across the

Figure 1-10 Calculated ion trajectory of $(\text{H}_2\text{O})_n\text{H}^+$ in a cylindrical FAIMS device with $a = 0.1$ cm and $b = 0.3$ cm. The waveform has a frequency of 200 kHz, a high-field/low-field time period ratio of 1:2, and the $DV = 2500$ V.

(a) $CV=0$, waveform cycles=20; (b) $CV=0$, waveform cycles=200; (c) $CV=-15$ V, waveform cycles=1000; (d) same as (c) but starting point for the ion motion was selected to be near the outer electrode.

Figure 1-10 Continued.

FAIMS analyzer space and collide with the walls of the outer electrode that is located at the top of the figure, at a radial distance of 3 mm.

The DV and CV conditions that were used to simulate the motion of the $(H_2O)_nH^+$ ion in Figures 1-10a and 1-10b are not suitable for ion focusing in a FAIMS device since the ion will hit the electrode walls. A condition that is suitable for $(H_2O)_nH^+$ ion focusing is shown in Figure 1-10c. The conditions are $DV = 2500$ V, $CV = -15$ V, at a frequency of 200 kHz and a high-field/low-field time period ratio of 1:2 with 1000 waveform cycles. The only change in conditions compared to Figure 1-10b was the application of a negative CV to the inner electrode. Figure 1-10c shows that the ion will experience a net drift outwards from its starting position of 1 mm (radial distance), but the drift quickly stops. The ion oscillates because of the application of the asymmetric waveform, but after a short period of time there is no net radial motion inward or outward. This could have been predicted from Figure 1-10b, since the outward drift of the ion might be expected to be retarded by the application of a negative dc potential to the inner electrode. Figure 1-10d shows the calculated ion trajectory for the same condition as Figure 1-10c except that the original radial starting point for the ion motion was selected to be from outer electrode at 3 mm. The ion initially experiences a drift towards the inner electrode and stabilizes exactly the same radial distance as the ion shown in Figure 1-10c. This means that any ion, despite its starting position, will fall into the focusing region.

1-3-4 Comparison of Conventional IMS and FAIMS

Although FAIMS and conventional IMS are similar, i.e., both are based on 'ion mobility' and operate at atmospheric pressure, the two techniques are different from each

other in several ways. These include (1) in FAIMS, the ions travel in the axial direction carried by a stream of carrier gas and a high-frequency, high-voltage asymmetric-waveform is applied in a direction perpendicular to the gas flow, while in conventional IMS, ions are driven axially by a weak electric field; (2) the FAIMS device separates ions based on differences in their mobilities at a high electric field, relative to a low electric field. IMS separates ions that have different drift velocities in a weak constant electric field; (3) the FAIMS device is well-suited for operation at room temperature, whereas conventional IMS is usually operated at an elevated temperature to decrease the degree of solvation of the analyte ions. An elevated temperature is not required in FAIMS, because the 'heating' of the ions by the high electric fields appears to promote desolvation [83]; (4) FAIMS works in a continuous fashion in which the ions pass through the analyzer in a non-interrupted stream. IMS is usually operated in a gated, time-of-flight mode; (5) the cylindrical FAIMS focuses the ions that it transmits. Thus, it gives higher sensitivity than IMS, in which diffusion in the drift tube causes the ions to spread out, resulting in ion loss to the walls of the IMS.

1-3-5 Applications of FAIMS

The high ion transmission efficiency and separation capabilities of FAIMS make this new analyzer an ideal interface between an API or ESI ion source (at atmospheric pressure) and a mass spectrometer. Recently, the instrumentation and applications of FAIMS have been described [82-90].

FAIMS has been coupled to a mass spectrometer (PE-Sciex, API 300) to identify the (corona discharge) ions that were transmitted under various operating conditions [82]

and, more recently, has been used to detect electrospray-generated ions [85]. The ESI/FAIMS/MS has been used to investigate the cluster ions of leucine enkephalin, [86], to separate structural isomers of amino acids (leucine and isoleucine, both m/z -130) [87]. An ESI/FAIMS/MS instrument was also used to study the conformers of the protein bovine ubiquitin. Multiple conformers for some charge states of bovine ubiquitin were resolved by FAIMS. The number and abundance of the conformers of several charge states of bovine ubiquitin were dependent on solution pH and solvent composition. Mass spectra of individual conformers showed conformer-specific distributions of sodium and phosphate adduct ions [88].

Since the FAIMS is an ion 'filter', and possesses separation capabilities, detection of chlorinated and brominated by-products of drinking water disinfection using ESI/FAIMS/MS has been achieved [89]. FAIMS was shown to discriminate effectively against background ions resulting from the electrospray of tap water solutions containing the haloacetic acids. Consequently, mass spectra were simplified, the selectivity of the method was improved, and the limits of detection were lowered compared with conventional ESI/MS. The detection limits of ESI/FAIMS/MS for six haloacetic acids ranged between 0.5 and 4 ng/mL with no pre-concentration, derivatization, or chromatographic separation prior to analysis [89].

With the high ion transmission efficiency of FAIMS, determination of part-per-trillion levels of chlorate, bromate and iodate by electrospray ionization/FAIMS/MS was described [90]. The unique separation and focusing properties of the FAIMS analyzer improved signal to background for all these analytes by 3-4 orders of magnitude

compared with conventional ESI-MS and produced part-per-trillion detection limits for ClO_3^- , BrO_3^- and IO_3^- in methanolic solution.

ESI/FAIMS/MS has also been used to study the transport properties of chloride ion at high E/N [84], where E is the electric field and N is the number density of the neutral gas molecule.

The newly-developed technique of high-field asymmetric-waveform ion mobility spectrometry (FAIMS) is capable of ion separation and focusing in two dimensions at atmospheric pressure and room temperature. In the following chapters, we will describe the extension of the ion focusing capability of FAIMS, and the development of a 3-dimensional ion trap which operates at 760 Torr and room temperature based on the principle of FAIMS. We will discuss the performance of the 3-dimensional ion trap and consider experimental results with both low mass ions and high mass (protein) ions.

1-4 Time-of-Flight Mass Spectrometry (TOF-MS)

In most of the IMS/MS applications reported to date, the ion mass measurement and assignment were accomplished using a quadrupole mass spectrometer. In this thesis, we demonstrate the use of a time-of-flight mass spectrometer for ion characterization after IMS separation. There are several advantages for the use of the TOF-MS technique. These include a mass range limited only by the ion detector, high ion transmission, the ability to register ions of all masses at the same time which provide a sensitivity over the full spectrum equal to that in the single ion monitoring regime. Moreover, several approaches have been developed for improving the mass resolution of TOF-MS, including orthogonal instruments [91] and reflectron instruments [92]. Finally, TOF-MS is a natural choice for a pulsed ionization technique since it provides a complete spectrum for each pulsing event [93].

1-4-1 Linear TOF Mass Spectrometer

In a time-of-flight mass spectrometer, the ions are pulsed from the ion source through a control grid and accelerated by an electric field to give each ion a velocity dependent on its mass-to-charge ratio. During passage down the field-free drift tube, the ions separate and are recorded at the detector before the next pulse of ions is released from the source.

Ions are introduced into a short source region, defined generally by a backing plate and an extraction grid separated by distance s . A positive voltage (V) placed on the backing plate provides an electric field ($E = V/s$) across the source region, which accelerates all of the ions to the same kinetic energy,

$$\frac{mv^2}{2} = zeEs \quad (1-22)$$

where m is the mass of the ion, v is ion velocity, e is electron charge, and z is the number of charges. As ions pass through the extraction grid they will have velocities which depend inversely upon the square root of their mass,

$$v = \left(\frac{2zeEs}{m} \right)^{\frac{1}{2}} \quad (1-23)$$

the ions then pass through a much longer drift region (D). Because they spend most of their time in this region, their time-of-flight measured at the detector is approximately,

$$t = \left(\frac{m}{2zeEs} \right)^{\frac{1}{2}} D \quad (1-24)$$

thus, the arrival time of two different ions is proportional to the square root of their mass,

$$\frac{t_1}{t_2} = \frac{v_2}{v_1} = \left(\frac{m_1}{m_2} \right)^{\frac{1}{2}} \quad (1-25)$$

All ions of the same mass and kinetic energy arrive together at the end of the flight tube in a group. The flight time of each ion is used to establish the mass scale by a conversion equation,

$$\frac{m}{z} = 2eEs \left(\frac{t}{D} \right)^2 \quad (1-26)$$

In practice, however, one measures the flight times of ions of at least two known masses, and determines the constants a and b in the empirical equation,

$$\frac{m}{z} = at^2 + b \quad (1-27)$$

as a means for calibrating the mass spectrum and determining unknown masses [94].

1-4-2 Spatial Focusing

Typically, a mass resolution of 250 is obtained in a conventional TOF-MS system. The limitation in mass resolution of TOF-MS originates from the spatial differences and the initial kinetic energy spreads of the ions introduced from the source. The spatial distribution causes a distribution of potential energies in the electric field that transfers into a distribution of kinetic energies and velocities of the ions in the field-free region. Therefore, not all ions of the same mass reach the detector at the same time. This factor leads to a broadening of signals in the TOF mass spectrum and to a reduction of the resolution of the instrument.

The spatial distribution is usually compensated using a double-field acceleration region [95] established in the linear TOF instrument as shown in Figure 1-11. While the ions are being formed, the source backing plate voltage is the same as that of the first grid. At all times the accelerating region, d , has an electric field E_d and the region D is field free. Ions are accelerated out of the source toward the collector when a positive pulse is applied to the source backing plate to produce the electric field, E_s . This pulse lasts until all ions have left the first field region.

Consider two ions of the same mass created at the same time, but separated by a certain distance in the direction of the field E_s . Therefore, after leaving the acceleration region their kinetic energy will slightly differ. As a result, there is a point in the drift region where the high energy ion, which starts its flight farther away from the extraction grid, will pass the low energy one. This point is called the space focal point. It is possible to adjust the electric potential gradient of the acceleration grids so that an ion initially closer to the detector acquires less energy and is eventually overtaken by the ion formed

Figure 1-11 Schematic of TOF mass spectrometer with the spatial focusing [95].

closer to the ion source so that both ions with the same mass arrive at the detector at the same time.

1-5 Protein Structure

1-5-1 Structure

Proteins are large polymeric bio-molecules made of amino acids as the basic structural units. An amino acid consists of an amino group, a carboxyl group, a hydrogen atom, and a distinctive R group bonded to a carbon atom, which is called the α -carbon as shown in Figure 1-12a. An R group is referred to as a side chain. The side chain group R characterizes each amino acid. Twenty different R groups are considered to form the 'essential' amino acid series for living organisms, although variants exist [96].

Proteins have four levels of structure. *Primary structure* is simply the linear arrangement of amino acids (or amino acid sequence) and the locations of disulphide bridges (cys-cys), if there are any. The primary structure is thus a complete description of the covalent connections of a protein. *Secondary structure* refers to the steric relationship of amino acid residues that are close to one another in the linear sequence. The α -helix, the β -pleated sheet, and the collagen helix are examples of the secondary structure. *Tertiary structure* refers to the steric relationship of amino acid residues that are far apart in the linear sequence. Proteins that contain more than one polypeptide chain display an additional level of structural organization, namely *quaternary structure*, which refers to the way in which the chains are packed together. Although the primary structure does not change, unless chemically modified, the secondary and tertiary structures can change in solution as a result of changes in pH, temperature, solvent composition and/or ionic strength. When a protein retains all its physiologically active structure in solution, it is

Figure 1-12a Structure of Non-ionized and dipolar ion form of an amino acid [96].

Figure 1-12b Ionization states of an amino acid as a function of pH [96].

said to be in a *native state*. The native state of a protein is typically folded into a well-defined three-dimensional structure held together by relatively weak intramolecular forces (e.g., hydrogen bonding). Modification of the secondary and tertiary structures of a protein is called *denaturation*.

1-5-2 Charge States

Amino acids in solution at neutral pH are predominantly dipolar ions, in which the amino group is protonated ($-\text{NH}_3^+$) and the carboxyl group is deprotonated ($-\text{COO}^-$). The ionization state of an amino acid varies with pH (Figure 1-12b). In acid solution (e.g., $\text{pH} = 1$), the carboxyl group is neutralized ($-\text{COOH}$) and the amino group is protonated (NH_3^+). In alkaline solution (e.g., $\text{pH} = 11$), the carboxyl group is deprotonated ($-\text{COO}^-$) and the amino group is neutralized ($-\text{NH}_2$).

Some ionizable groups (i.e., $-\text{NH}_2$, $-\text{COOH}$, $-\text{SH}$ and $-\text{OH}$) of the side chains can also lose or gain a proton, resulting in a net negative or positive charge on the amino acid. Out of a total of twenty, only seven amino acids have side chain groups that are ionizable. In addition, the terminal amino and carboxylic groups in a protein may also be ionized. Thus, arginine, lysine, histidine and the N-terminus will contribute to the total number of positively-charged sites on a protein. On the other hand, tyrosine, aspartic acid, glutamic acid and the C-terminus will be responsible for the total number of negatively-charged sites. The overall charge on the protein is the difference between the positive and negative sites. For example, the protonation of lysine (pK_a 2.18) at low pH ($z = +1$ to $z = +2$) is illustrated as follows:

The two charged species of lysine will coexist in solution at a 1:1 ratio when the solution pH is equal to the pKa (2.18) of the carboxylic group. If the solution pH is lowered below 2.18, the species with two net positive charges will become predominant. The distribution of coexisting, multiply-charged ions of protein in aqueous solution is an extrapolation of this simple case.

The charge states of a protein in solution depend on the nature of the side chain groups as well as their immediate electrostatic and chemical environments. Several factors like protein conformation, the solution pH, the solvent polarity, the ionic strength or any combination of these factors will affect the side chain groups' environment. The charge distribution observed for proteins in the gas phase by ESI/MS may be related to the solution phase structure and chemistry of the protein.

1-5-3 Study of Protein Conformation

Knowledge of high-order protein structure is important for understanding and predicting protein function in biological systems.

Protein conformation in solution depends in a complex fashion upon the intramolecular forces dictated by amino acid sequence. Conformation information on biomolecules has relied almost exclusively on X-ray analysis of crystals of molecules of

known primary structure [97], and multiple-pulse NMR methods have been useful in obtaining conformational information on smaller bio-molecules in solution [98, 99].

In the last several years mass spectrometry has begun to address the question of molecular conformation of complex molecules, especially when ESI opened up the large molecule field to mass spectrometric analysis [100]. It has been demonstrated that the net charge on the multiply-protonated protein ions observed in the positive electrospray mass spectra has been shown to reflect, to some extent, the degree of protonation of proteins in solution [26, 101-107]. Therefore, electrospray ionization mass spectrometry can be used to probe changes in protein conformations. For protein molecules in solution, the net charge on a tightly-folded native conformer is generally smaller than that on the unfolded form because some of the basic groups are buried or involved in intra-molecular interactions and are not available for protonation. The higher-charged ions in ESI mass spectra can be observed for denatured proteins by changing the solution pH [103], addition of organic solvents [104, 105] and increasing temperature [106, 107].

Several other methods have been employed to study the conformations of gas-phase proteins, among them are the gas-phase hydrogen/deuterium exchange electrospray mass spectrometry [108-110], proton transfer kinetics exchange electrospray mass spectrometry [111-114], measurements of average collision cross sections [114-127] and scanning probe microscopy studies of the defects formed on surfaces after high-energy ion impact [128, 129].

Ion mobility measurements [114, 120-127] and ion energy loss experiments [115-119] have been used to determine collision cross-sections of protein ions with neutral gases in the absence of solvent. In energy-loss experiments, ions are injected into a

quadrupole mass filter and the loss of kinetic energy from collisions with a neutral gas is measured and related to the cross-section through kinetic theory. In ion mobility experiments, ions are injected into a low-field drift cell with He as a collision gas at a pressure of 0.1 - 10 Torr. The ions with different sizes, charges and geometries can be identified in terms of their mobilities; thus different conformations can be separated. Since collision cross-section can be determined from the mobility, information about the geometry can be obtained by comparing the experimental cross-sections to those calculated for trial geometries. Highly-charged ions appear to favour conformations that are elongated instead of compact because of lower Coulombic repulsion energies. Low-charge states can exist in more compact forms.

In the past several years, Bowers' group has developed mobility-based methods [130] (named 'ion chromatography' or 'IC') that allow the determination of conformations of gas phase ions. Recent reviews of the technique and its application to structures of atomic cluster and of transition metal ions have been written by Bowers *et al.* [131, 132] and by Jarrold [133].

More recently, Clemmer and Jarrold [134] have added an electrospray ion source to their double-quadrupole IC instrument and have shown that ion mobility measurements can resolve a number of conformations for bovine cytochrome c. They also described evidence showing that cytochrome c ions can spontaneously fold in the gas phase [121] and more detailed examination of cross section has been carried out for the bovine pancreatic trypsin inhibitor (BPTI) and cytochrome c [123]. Valentine *et al.* have studied the conformations of the disulphide-intact and disulphide-reduced lysozyme using IMS techniques [125]. They also used the technique to study the conformations of ubiquitin

ions before and after being exposed to proton transfer reagents [114]. Conformations, unfolding and refolding of apomyoglobin were also studied by Jarrold's group using ion mobility technique [124].

The measurement of the cross-sections of gas phase protein ions using the ion mobility method mentioned above was carried out in a drift tube at a reduced pressure. In the next chapter, we will describe the use of a conventional atmospheric pressure ion mobility spectrometer to examine the cross-sections of gas-phase ions of ubiquitin, cytochrome c, myoglobin, lysozyme and α -lactalbumin as a function of charge states.

1-6 Objectives

1-6-1 IMS Investigation of ESI-Generated Ions

Electrospray mass spectrometry has evolved from a scientific curiosity to one of the most powerful tools in the bioanalytical laboratory since Fenn and co-workers' pioneering work on applying this technique to the determination of molecular masses of proteins [135]. As in the development of many analytical techniques, the pace of our understanding of the electrospray process lags far behind the pace at which the technique is being employed in new applications. A difficulty in arriving at a better understanding of the electrospray process, based on the ions that one sees in the electrospray spectrum, is that the spectrum is an accurate picture of only those ions that enter the mass analyser. These are the ions that have travelled from a region of one atmosphere (the ion source) to another of 1×10^{-5} Torr or lower in pressure, during which their associated solvent molecules, and presumably molecular adducts, are stripped by means of a series of collisions with predominantly nitrogen and oxygen molecules that enter, along with the analyte ions, into the low pressure regions of the mass spectrometer. The energy of this collision-induced dissociation (CID) is typically optimized to result in maximal desolvation but minimal decomposition of analyte ions [15].

While a spectrum consisting of desolvated ions is desirable for an analysis, it is less useful for an investigation of the nature of the ionic species created originally in the electrospray process. In a few studies [136-138], some knowledge was gained by minimizing the energy of the CID that took place between the orifice and the mass analyser (in the so-called 'lens region'). For example, Mirza and Chait [136] reported

observation of trifluoroacetic acid adducts of melittin after lowering the potential difference between the transfer capillary and the skimmer of their mass spectrometer. Le Blanc *et al.* [137] observed ethylamine adducts of gramicidin S after lowering the potential difference between the orifice (OR) and the radio-frequency-only quadrupole (R0) to nominally zero in their mass spectrometer. Adducts between ubiquitin and butylamine were also observed under similar conditions [138]. While these studies provided some information about the ionic species created in the electrospray ion source, they were still limited because the composition of the ions passing through the lens region was likely to have changed significantly even under mild CID conditions. It is worth noting that none of these studies reported observation of solvent (water and/or methanol) adducts even for experiments in which the potential difference across the lens region was nominally zero [137].

In an effort to create an alternative method for observation of the electrospray process, we have elected to insert a separation step-'ion mobility spectrometry', based on differences in ion mobility, between electrospray generation of ions and mass spectrometric detection.

The impetus for our work stemmed from our interest in gaining a better understanding of the ionic species produced by electrospray. Therefore, quite apart from gathering mobility data, the primary reason for implementing IMS was to separate these species prior to mass spectrometric detection. Towards that end, we built a linear time-of-flight (TOF) mass spectrometer and coupled it to a commercially-available ion mobility spectrometer which had originally been equipped with a quadrupole mass spectrometric detection stage. This change enabled us to collect full-scan mass spectra of individual

peaks separated in the ion mobility spectrometer, an experiment which is not possible using a quadrupole mass spectrometric system.

Conceptually, our hardware design may be considered to be the reverse of the ‘ion chromatography’ design of Bowers and co-workers [131, 139] and the ion mobility apparatus design of Jarrold and co-workers [133, 134]. These instruments employ a mass analyzer front end to isolate the analyte ions of identical mass-to-charge ratios prior to reduced-pressure mobility separation. In contrast, our instrument employs ion mobility separation up front to resolve ionic species for mass spectrometric characterization. It should also be noted that our technique differs from that being developed by Bowers and co-workers, and Jarrold and co-workers, who conducted ion mobility analysis in a drift tube at a reduced pressure (1-5 Torr) for ions mass-to-charge-selected by a quadrupole mass spectrometer. We separated ions in a conventional ion mobility spectrometer at atmospheric pressure followed by mass analysis using a time-of-flight mass spectrometer.

1-6-2 Atmospheric Pressure Ion Trap

Ions at atmospheric pressure are routinely separated with ion mobility spectrometry. The ion cloud, which is drifting through the flight tube of an IMS instrument, is subject to spreading by diffusion, ion-ion repulsion, and gas turbulence. There is no known method to reduce the expansion of the ion cloud at atmospheric pressure. Consequently, the resolution and sensitivity of IMS are limited by the physics and kinetics of these ion-expansion processes.

Modern mass spectrometric instrumentation is routinely used to detect ions which are formed at atmospheric pressure (especially via electrospray ionization technique). The

ions formed at 760 Torr by API or ESI sources are transported, at atmospheric pressure, to the orifice of the mass spectrometer without the benefit of an instrumental method for ion focusing. Consequently, the ions produced by these sources will disperse in space due to a combination of divergent electric fields (ESI), gas turbulence, ion-ion repulsion, and diffusion.

There are several ion optical devices capable of confining, or trapping ions, including the radio-frequency (rf) quadrupole ion trap. The rf quadrupole ion trap (QIT) was invented by Paul and Steinwedel [140], and 1/3 of the Nobel Prize in Physics was awarded to Paul in 1989 [141]. QIT works by collecting and storing gas phase ions in a rotationally symmetric, oscillating quadrupolar electric field at low pressure. Ions are first transmitted to, or created in, the storage volume of an ion trap. There they are trapped by the pseudo-potential well created by a rf potential of constant frequency (~ 1.1 MHz) and variable amplitude (0–2200 V_{pp}) applied to the ring electrode. Varying the rf amplitude varies the mass-to-charge range of ions that are stable within the trap. Ions with appropriate mass-to-charge for a particular rf amplitude have a stable trajectory within the trap and, therefore, are trapped [146].

The QIT possesses the advantages of excellent sensitivity, the ability to manipulate ions during storage to effect ion dissociation or reaction, comparatively easy coupling to many different ion sources, high mass range and resolution, yet all at a relatively low cost and size compared to other mass spectrometers. The quadrupole ion traps have been reported with impressive capabilities including mass resolution exceeding 10^7 [142], mass ranges in excess of 70,000 Da [143], and up to nine consecutive collisional dissociation reactions starting with a single ion species [144].

The rf quadrupole ion trap, however, cannot operate at a pressure of 760 Torr for trapping ions of mass spectrometric interest because the ion motion in these devices requires a long mean-free path [145, 146], hence a reduced operating pressure. The efficiency, and thus usefulness of these traps, degrades extremely rapidly as the pressure increases, and there is no experimental or theoretical basis to suggest that any trapping exists at a pressure of 760 Torr for those ions of mass spectrometric interest. Therefore, a method for temporal or spatial concentration of ions at atmospheric pressure prior to their transfer into the vacuum would be a major breakthrough.

In this thesis, we describe the use of a high-field asymmetric-waveform ion mobility spectrometer (FAIMS) as a means of trapping ions generated by corona discharge or electrospray ionization at atmospheric pressure and room temperature. The goal was to use a FAIMS storage device as a front-end source for a TOF mass spectrometer or an ion mobility spectrometer. This device combines both the storage and separation properties inherent to FAIMS. The key feature is that this ion trap is used to store ions injected from a continuous ion source for an extended period of time at atmospheric pressure. The ions are subsequently ejected into the TOF for mass analysis via a dc pulse applied to the exit orifice.

In the following chapters, the details of design, construction and theoretical description of FAIMS as a potential atmospheric pressure ion trap will be described. Some preliminary results of the evaluation of FAIMS ion trap will be presented.

CHAPTER II

EXPERIMENTAL

2-1 Instrumentation for ESI/IMS/TOF-MS

2-1-1 *Electrospray Ion Source*

Electrospray ionization was performed at room temperature external to a conventional drift tube ion mobility spectrometer. The electrospray probe, fabricated from a 2 cm long stainless steel capillary needle (Hamilton, 33-gauge, 100 μm I.D.) attached to an approximately 5 cm x 1/16" O.D. stainless steel tube, was enclosed in a small Plexiglas chamber as shown in Figure 2-1. Polarization of the electrospray probe was achieved with a high-voltage power supply (Glassman High Voltage, Inc., PS/EH60R01.5, Whitehouse Station, NJ). For positive-ion detection, the applied potential to the probe was typically +4 to +5 kV, and the current 0.1 to 0.5 μA . The electrospray current was monitored with a laboratory-built micro-ammeter that could be floated above ground potential. The voltage required to provide a stable electrospray depended on the sample solution composition, and was optimized by gradually raising the voltage from ground, until a fine spray (and a stable current) was achieved.

Sample solution was continuously infused into the spray probe by means of a syringe pump (Harvard Apparatus, Model 22) at a typical flow rate of 1 to 10 $\mu\text{L}/\text{min}$. Electrospray-generated ions were swept into the ion mobility spectrometer by means of a stream of dry nitrogen via a stainless steel transfer tube (15 cm in length, 1/8 inch O. D.). The voltage applied to this transfer tube was typically optimized for efficient extraction of electrospray-generated ions from the transfer tube to the shutter grid of the ion mobility spectrometer using the ion mobility signal as the guide. The optimum voltage

Figure 2-1 Schematic diagram of the electrospray chamber used in this study

applied to the tube was approximately 2 kV with a usable range of ± 50 V. A nitrogen gas stream (5.5 L/min) was introduced into the electrospray chamber in a direction counter-current to that of the electrospray stream. Three-fifths of the nitrogen was vented; the remaining portion was transported into the ion mobility spectrometer. Splitting of the nitrogen flow ensured only ions and relatively small droplets were admitted into the ion mobility spectrometer.

2-1-2 Ion Mobility Spectrometer

A commercially-available ion mobility spectrometry / mass spectrometry (IMS/MS) instrument (PCP Inc., West Palm Beach, FL) was modified to accommodate time-of-flight mass spectrometric detection as shown in Figure 2-2. The IMS portion of the instrument consisted of three ion flight regions, including a 'reaction region' 5 cm in length and two 'drift regions' each 5 cm in length. The transfer tube from the electrospray source terminated within the reaction region, approximately 2 cm from the first shutter grid. In practice, the original *Nickel-63* ion source and the reaction region were deactivated. A voltage of 3 kV was applied to the ion mobility spectrometer. Since the voltage gradient applied was linear, the resulting bias of the first shutter grid was approximately 2 kV. Because the transfer tube from the electrospray chamber ended 2 cm from the first shutter grid, the gas turbulence due to the nitrogen flow in the region decreased the effectiveness of this shutter grid. Consequently, the first shutter grid was left in the open state for all of the work described here. Ions exiting from the transfer tube crossed the first shutter grid and traversed the first drift region without separation. The second shutter grid, controlled by a Digital Delay/Pulse Generator (Stanford Research

Figure 2-2 Schematic diagram of ion mobility spectrometer/time-of-flight mass spectrometer

System, Inc., DG535, Sunnyvale, CA), was closed most of the time but opened briefly to permit a sample of ions to pass into the second drift region. The electric field gradient in the drift region was typically 200 V/cm. A counter-current stream of nitrogen (the 'drift gas', 0.5 L/min) flowed from the detector end of the ion mobility spectrometer towards the entrance. This flow reduced the solvent load in the drift regions and ensured that ions traversed in a gas that was thermally equilibrated with the spectrometer. The carrier gas and drift gas were nitrogen which was purified by a molecular sieve filter.

The ion mobility spectrometer was operated in two modes: (1) low resolution mode, and (2) high resolution mode.

In low resolution mode, the period during which the second shutter grid was open (the width of the gating function) was extended from the typical 0.1 ms up to 5 ms to accommodate the low mobility ions of interest in this study. This resulted in good sensitivity for detection of ions with mobility as low as $0.2 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ (e.g., 'cluster ions'); however, ions with high mobility were recorded as square-top peaks (see, for instance, Figure 3-1 in Chapter III, page 85). This result was not a consequence of detector saturation but occurred because a steady flux of ions passing through the shutter grid during the relatively long time over which the grid was open; the sharp rising and falling edge signified the rapid opening and closing of the grid. The ion transit time across the drift region (from which the ion mobility was calculated) was measured from the time that the grid was open to the time of the half maximum of the rising edge.

In high resolution mode (the conventional operating manner), the period during which the second shutter grid was open was typically 0.1 ms to 0.5 ms. In this mode, the ion transit time was measured in the conventional manner; i.e., from the time at the middle of

the period during which the shutter grid was open to the time of the peak maximum. The ion current registered on the ion collector of the IMS was amplified using a current amplifier (Keithley 428, U.S.A.). The transient signals were processed with a LeCroy digital storage oscilloscope (Model 9350, Chestnut Ridge, NY). Signal averaging was used to enhance the signal-to-noise ratio of the reported IMS spectrum. The ion mobility spectrometer was typically operated at a temperature of 150-200°C, with the exception of 25–210°C in the temperature dependent experiments. Table 2-1 lists some instrumental operating conditions for IMS used in this study.

2-1-3 TOF Mass Spectrometer

A laboratory-built linear TOF mass spectrometer and the associated lens and grids replaced the quadrupole mass spectrometer of the original IMS/MS system. The TOF is shown to the right of the IMS system in Figure 2-2 on page 64. Ions exiting the ion mobility spectrometer (at one atmosphere) entered the vacuum system through an orifice of approximately 25 μm in diameter. These ions were steered into the flight area by means of four lens elements and accelerated using simultaneous pulses (Directed Energy Inc., GRX-3.0K-H pulse generator, Fort Collins, CO) applied to two grids approximately 5 cm apart. The lens elements were typically biased to -370, -370, -385, and -400 V starting from the orifice side. Typical pulse amplitudes were +1800 V to the first and +1710 V to the second grid; the pulse width was 50 μs . The ions then passed through a third grid held at ground potential located 1 cm downstream. The amplitudes of the pulses applied to the two acceleration grids were optimized to give a space focused signal at the mass spectrometric detector. Ions that were originally near the first acceleration

Table 2-1 Typical instrumental operating conditions for IMS

<i>Grid pulse width</i>	0.1-0.5 ms (for high resolution) 5 ms (for low resolution)
<i>Maximum continuous ion current</i>	$> 5 \times 10^{-10}$ amp.
<i>Drift region diameter</i>	4.25 cm
<i>Drift length</i>	5 cm or 10 cm
<i>Operating pressure</i>	atmospheric
<i>Operating temperature</i>	25 - 210°C
<i>Drift field</i>	200 V/cm
<i>High-voltage power supply</i>	3000 V
<i>Drift gas flow (nitrogen)</i>	500 mL/min

grid would receive the largest acceleration while those that were originally at the second grid would receive the smallest. The amplitudes of the pulses were adjusted such that these ions would arrive at the detector simultaneously. The individual IMS peaks could be gated for TOF-MS. This was achieved by acquiring data (i.e., pulsing the TOF acceleration grids) only when the IMS peak of interest reached the TOF mass spectrometer.

The TOF instrument was evacuated by means of two diffusion pumps, the first one, with a pumping speed of 2400 L/s (Varian, Model VHS-6, Massachusetts, U.S.A.), was located beneath the orifice, and the second one, 2500 L/s (Edwards High Vacuum International, Model 250, West Sussex, England) beneath the TOF flight tube (107 cm in length). The typical operating pressure in the flight tube was below 1×10^{-5} Torr.

An electron multiplier (ETP Electron Multipliers, AF850, Ermington Sydney, Australia) was used to detect the ions. Typically, the multiplier was biased to -1800 V while the conversion dynode voltage was maintained at -5000 V. The width of a TOF peak was about 0.2 μ s for this instrument.

The ion current detected by the electron multiplier was amplified using a fast current amplifier (LeCroy, VV100BTB). A LeCroy digital storage oscilloscope (Model 9350, Chestnut Ridge, NY) was used to process the TOF mass spectrum. Signal averaging was used to enhance the signal-to-noise ratio of the reported spectra (typically averages of 2000 spectra). The TOF mass spectra were transferred from the oscilloscope to a 486 IBM PC using a GPIB control interface established between the LeCroy and the computer. A user-written Visual Basic program (ElanData) was used to manipulate the spectra.

2-1-4 Identification of the Charge States of Protein Ions Observed in the IMS

Spectrum

The TOF mass spectra of ions observed in IMS were collected in two ways: (1) the ions were transferred through the IMS continuously by leaving the shutter grids open. The TOF-MS spectra were therefore representative of all of the ions transferred through the instrument; (2) the shutter grids of the IMS were opened and closed in the conventional manner. Selected windows of ion arrival times were analysed by collecting mass spectra only during selected periods of time relative to the gate pulse.

The charge state of each protein ion in a high resolution IMS spectrum cannot be established without mass spectrometry. The mobility of a protein ion of specific charge was determined by collecting TOF-MS spectra for two windows in the mobility spectrum. The time between these two windows was set equal to the IMS shutter grid open time (typical 3 ms, low resolution). All ions are expected to arrive at the detector of the mobility spectrometer in bands at least as wide as the shutter grid's open period with additional widening from diffusion and space charge repulsion. Comparison of the mass spectra collected from the two IMS windows with the composite TOF-MS spectrum in some cases revealed that a specific charged ion was missing from both window spectra. This missing ion arrived in a 3 ms wide band very close to the middle of the time between the two windows. The arrival time of this ion corresponded to the middle of the time gap between the two windows, and its mobility was therefore calculated. Having determined the mobility of one of the protein ions, this ion can be easily identified in the high resolution IMS spectrum and the mobility of the other charge states of the protein determined (see example in Section 3-1-3 of Chapter III, page 123).

2-2 The FAIMS Ion Trap Apparatus

2-2-1 The FAIMS Ion Trap

The FAIMS ion trap, illustrated in Figure 2-3 in a cross-sectional view, was composed of two concentric electrodes. The outer cylindrical electrode was approximately 7.5 cm in length, 0.6 cm inner diameter, and was mounted 0.5 mm from the front surface of the sampler cone. The inner electrode was a solid rod with a 0.2 cm diameter and the same length as the outer electrode, terminating in a spherical surface about 3 mm from the sampler cone. The inner electrode was held within the outer electrode by an insulating Torlon spacer (DSM Engineering Plastic Products Inc., Reading, PA). The FAIMS ion trap was surrounded by an ionization chamber, which has a volume of 300 cm³ and was about 10 cm in diameter. Compressed nitrogen gas was passed through a charcoal/molecular sieve gas purification cylinder and introduced to the FAIMS and the chamber. Gas connections to both FAIMS and ionization chamber are shown in Figure 2-3. A port in the outer electrode of the FAIMS permitted entry of a carrier gas (C_{in}) at about 1.2 L/min. This carrier gas flowed along the annular space between the outer and inner electrodes (FAIMS analyzer region), and exited through 3 vents which include: the gap between the outer electrode and the sampler cone, the ion entrance port (I_{in}) communicating with the ionization chamber, and the 100 μ m orifice in the sampler cone. The portion of C_{in} which flowed out through I_{in} served as a 'curtain gas' flow to ensure that the gas flowing along the FAIMS analyzer region was free of impurities. The ionization chamber was flushed with purified gas (purge gas in, P_{in}). This gas, combined with the carrier gas that enters the chamber through I_{in} , exited through a

Figure 2-3 Schematic of the atmospheric pressure FAIMS ion trap.

purge gas out (P_{out}) port. This purge gas prevented neutrals from entering the annular analyzer region of the FAIMS. Both C_{in} and P_{in} could be adjusted. A balance in gas flow rate between these two gas streams had to be reached in order to maximize ion transmission while minimizing the entry of the neutrals and impurities into the FAIMS analyzer region.

Both corona discharge and electrospray ionization methods were used in this study. A corona discharge needle was supported about 1 cm from I_{in} within the ionization chamber. The needle was held at approximately +2000 V (1.2 μ A). Electrospray ionization was carried out in exactly the same manner, replacing the corona discharge needle with a 100 μ m (I.D.) stainless-steel capillary. For generating positive ions, the electrospray needle was typically maintained at approximately +1500 V giving an electrospray current of 50 nA. Solutions were pumped to the electrospray needle tip by a Harvard Apparatus Model 22 syringe pump, at a flow rate of 1 μ L/min.

Since the FAIMS is very sensitive to moisture and contamination [82], the FAIMS device was thoroughly cleaned with detergent and then dried in an oven of 100°C over the weekend before it was mounted on the mass spectrometer.

2-2-2 FAIMS Ion Trap/ TOF Mass Spectrometer

The experimental set-up for the time-of-flight mass spectrometric evaluation of the FAIMS ion trap is shown in Figure 2-4. The FAIMS ion trap is operated at atmospheric pressure and the TOF-MS is operated at high vacuum. A vacuum interface was mounted between the FAIMS ion trap and the TOF-MS. The sampler cone was mounted on a flange insulated by a MACOR ceramic support (Figure 2-3). The sampler cone can be

Figure 2-4 Schematic of the combined atmospheric pressure FAIMS ion trap and a linear time-of-flight mass spectrometer

applied with a dc voltage and a dc pulse. The vacuum components of the low resolution, linear time-of-flight mass spectrometer are shown to the right of the sampler cone in Figure 2-4. These components included a differentially-pumped interface with a sampler cone, a skimmer cone, an octopole ion guide, ion acceleration grids, and an electron multiplier detector. This TOF mass spectrometer was essentially the same as the one described in Section 2-1-3 (page 66) of this chapter, except that the ion focusing lens had been replaced by a custom-made octopole ion guide (ABB Extrel, Pittsburgh PA). The pressure of interface was normally held at 0.7 Torr pumped by an Edwards E1M 80 rotary pump (Edwards High Vacuum International, West Sussex, England). The pressure of the octopole ion guide chamber was maintained at 9×10^{-5} Torr pumped by a Varian diffusion pump (Model VHS-6, Massachusetts, U.S.A), while that of the TOF flight tube at 2×10^{-6} Torr, pumped by another diffusion pump (Edwards High Vacuum International, Model 250, West Sussex, England).

The FAIMS ion trap was operated with three electrical power sources. First, the asymmetric waveform and CV were both applied to the inner electrode. The waveform was generated using a custom-made high voltage waveform generator (Mine Safety Appliances Company, Pittsburgh, PA). The waveform has approximately the same shape as the one shown in Figure 1-6 (page 29), but at a frequency of about 200 kHz and a high-field/low-field time period ratio of 1:2. The dispersion voltage (DV) could reach 4 kV. The compensation voltage (CV) was adjusted by a dc power supply (Kepco Inc., New York). Second, the FAIMS was held at a dc offset voltage (V_{FAIMS} , typically +10 V - +50 V), allowing a voltage difference to be established between the FAIMS unit and the sampler cone. Finally, a pulsed power supply (Kepco Inc., New York) was used to set the

sampler cone voltage (OR) in two possible states; one for storage of ions, and the other for extraction of ions from FAIMS. In a typical experiment, the timing control electronics were set as following: the OR was +40 V for 30 ms during trapping of the ions and at +1 V for 10 ms during ion extraction (i.e., with $V_{FAIMS} +20$ V, and $CV -3$ V). After initiation of the ion extraction by lowering OR, the cloud of ions, which was located near the terminus of the inner electrode, moved towards the orifice in the sampler cone.

A pulse of ions from FAIMS entered the TOF-MS through a differentially-pumped interface, and continued into the octopole ion guide. The octopole ion guide was operated at low pressure (9×10^{-5} Torr) so that the transit time, and thus broadening of the pulse of ions inside the octopole, was minimized. The ions passed from the octopole, through an exit lens, and into the acceleration grids of the TOF-MS. The acceleration region of the TOF-MS was composed of 3 fine mesh metal grids (Buckbee Mears, St. Paul, Minnesota). The grid which was located closest to the flight tube was held at constant ground potential. The other two grids (i.e., 'high grid' and 'low grid' in Figure 2-4) were each connected to a high voltage pulse generator (Directed Energy, Inc., GRX-6.0K-H, CO). The grids were set at low and high voltage states, controlled by an external digital logic pulse generator (Stanford Research System, Inc., DG535, Sunnyvale, CA). In the low voltage state, i.e., -5.5 V, the ions travelled from the exit lens of the octopole ion guide through the grids. The grids were then rapidly stepped (less than 0.1 μ s risetime) to high voltage, and held constant for about 50 μ s. The acceleration region was designed so that the ions have energy (in part) dictated by their starting location between the two pulsed acceleration grids. This difference in energy gives the device a capability for 'spatial' focusing, which means that all of the ions with a given mass-to-charge ratio,

regardless of their starting positions in this region, will reach the detector simultaneously. Even with spatial focusing, the linear TOF-MS has limited resolution because the ions (in this system) enter the acceleration region with a range of kinetic energies.

2-2-3 Operation of the FAIMS Ion Trap/TOF-MS

The FAIMS ion trap/TOF-MS included two pulsed components, the sampler cone and the TOF-MS acceleration grids. The TOF-MS acceleration region was pulsed at a series of selected delay times relative to the pulse applied to the sampler cone. At each delay time, a mass spectrum, which was the average of a minimum of 2000 repeated pulses, was collected. In this way, a series of TOF-MS spectra which monitored the arrival time vs. intensity profile of the ions relative to the application of the pulse applied to the sampler cone, were acquired. If an assembly of ions has been trapped at the spherical terminus of the FAIMS inner electrode, the pulse of ions should be characterized by the appearance of a strong, transient signal, at some delay time after the sampler cone was stepped to a low voltage. This signal would then decay to a constant value equal to the signal that would be detected if the sampler cone was held at the low voltage (e.g., +1 V) state continuously. The trapping phenomenon was further investigated by varying the period of time during which the sampler cone was held in the high voltage state, thus varying the 'trapping time'. The number of ions trapped should be a combination of an input flux of ions (constant) and an ion loss mechanism wherein (for example) the rate of loss is proportional to the density of ions in the FAIMS ion trap. This kinetic evaluation of the ion storage in FAIMS will be described in the next chapter.

The timing diagram for control of the FAIMS offset voltage (V_{FAIMS}), DV , CV , sampler cone voltage (OR), and the TOF-MS acceleration pulse appears in Figure 2-5. Typical operating parameters for the system are reported in Table 2-2.

Figure 2-5. The timing diagram for control of the FAIMS offset voltage (V_{FAIMS}),

DV, CV, sampler cone voltage (OR), and the TOF-MS acceleration pulse

**Table 2-2 Summary of typical instrumental operating conditions of
FAIMS/TOF-MS for ion trapping experiments**

FAIMS conditions:

<i>Ionization method</i>	corona discharge
<i>Discharge voltage</i>	+5 kV
<i>Discharge current</i>	3.1 μ A
<i>Inner electrode radius</i>	1 mm
<i>Outer electrode radius</i>	3 mm
<i>FAIMS dc offset: V_{FAIMS}</i>	+20 V
<i>Inner electrode dc</i>	+17 V
<i>Compensation voltage (CV)</i>	-3 V
<i>Dispersion voltage (DV)</i>	-3500 V (P2 mode)
<i>Asymmetric waveform</i>	210 kHz
<i>Asymmetric waveform</i>	$2/3DV\sin(\omega t) + 1/3DV\sin(2\omega t - \pi/2)$
<i>Bath gas</i>	nitrogen, purified
<i>Bath gas pressure</i>	770 Torr (approx.)
<i>Sample compounds</i>	trace impurities
<i>Carrier gas In</i>	1.2 L/min
<i>Purge gas In</i>	1.8 L/min

Interface conditions:

<i>Sampler cone (OR)</i>	pulsed
<i>Sampler cone (OR) high</i>	+40 V
<i>Sampler cone (OR) low</i>	+1 V
<i>Time OR high</i>	40 ms
<i>Time OR low</i>	10 ms
<i>Skimmer cone</i>	0 V
<i>Interface vacuum</i>	0.7 Torr

Octopole ion guide conditions:

<i>Frequency</i>	1.2 MHz
<i>Voltage</i>	± 700 V
<i>Octopole DC offset</i>	-4 V
<i>Octopole AC setting</i>	m/z 200
<i>Octopole exit lens</i>	-5.5 V
<i>Octopole vacuum chamber</i>	9×10^{-5} Torr

Table 2-2 Continued

TOFMS conditions:

<i>Acceleration high grid On</i>	+1500 V
<i>Acceleration low grid On</i>	+450 V
<i>Acceleration high grid Off</i>	-5.5 V
<i>Acceleration low grid Off</i>	-5.5 V
<i>Acceleration pulse width</i>	50 μ s
<i>Flight tube offset</i>	0 V
<i>Multiplier HV</i>	-1800 V
<i>Conversion dynode</i>	-5000 V
<i>Vacuum chamber</i>	2×10^{-6} Torr
<i>Flight tube length</i>	62 cm
<i>Acquisition average</i>	2000 cycles
<i>Ion peak width at 27 μs</i>	0.2 μ s

System timing:

<i>Repeat frequency</i>	20 Hz
<i>Cycle time</i>	50 ms
<i>Time A</i>	0 ms
<i>Time B</i>	40 ms
<i>Time C</i>	44.55 ms
<i>Time D</i>	44.60 ms
<i>Sampler cone control</i>	A-B time window
<i>TOF-MS acceleration control</i>	C-D time window

2-3 Samples and Chemicals

Samples used were solutions of a series of tetraalkylammonium bromides and peptides and proteins including gramicidin S, bovine ubiquitin, bovine insulin, equine cytochrome c, myoglobin, hen egg lysozyme and α -lactalbumin. Other chemicals used included methanol, glacial acetic acid, and 1, 4-dithiothreitol (DTT). All chemicals were obtained from Sigma Chemical Company (St. Louis, MO) and used without further purification. The sample solutions were prepared in a solvent mixture of water /methanol and were adjusted to a suitable pH with acetic acid for positive ion detection.

In some cases, proteins were treated with 1, 4-dithiothreitol (DTT) for the reduction of disulphide bridges according to the following procedure. A small volume of aqueous DTT solution was added to an aliquot of the native protein solution in 50/50 (v/v) methanol/distilled water such that the final DTT concentration was approximately 5 mM. After a period of 24 hours at room temperature, glacial acetic acid was added (~ 0.2%) to the protein sample containing DTT and the IMS and TOF-MS spectra obtained.

The concentrations of protein and the solution compositions used for this study are summarized in Table 2-3.

Table 2-3 Composition of sample solutions

Sample	Concentration (μM)	MeOH/H ₂ O (v/v)	Acetic acid (%)	DTT (mM)
<i>Cytochrome c</i>	10	50/50	0.5	0
<i>Ubiquitin</i>	20	50/50	0.5	0
<i>Myoglobin</i>	10	50/50	0.5	0
<i>Insulin</i>	20	50/50	0.5	0
<i>Gramicidin S</i>	20	50/50	0.5	0
<i>Lysozyme</i> [native]	20	50/50	0.2	0
<i>Lysozyme</i> [denatured]	20	50/50	0.2	5
α -Lactalbumin [native]	20	50/50	0.2	0
α -Lactalbumin [denatured]	20	50/50	0.2	5
<i>Tetraalkylammonium</i>				
ethyl	100	50/50	0.2	0
butyl	100	50/50	0.2	0
pentyl	50	50/50	0.2	0
heptyl	50	50/50	0.2	0
octyl	20	50/50	0.2	0

CHAPTER III

RESULTS & DISCUSSION

3-1 IMS/TOF-MS Study of ESI Generated Ions

3-1-1 Electrospray Ionization of Tetraalkylammonium Salts

The performance of the IMS/TOF-MS system was evaluated using electrospray ionization of tetraalkylammonium salts because they had been previously examined by means of both quadrupole mass spectrometry [147] and ion mobility spectrometry [71]. These compounds have been widely used as mass markers for calibration of analyzer scales in the mass-to-charge range below 500 since it has been demonstrated that these compounds produce a very clean peak for each species with very high signal/noise ratio, and no evidence of fragmentation [147]. They desolvate easily, do not readily form clusters, are always singly charged and show a linear dependence of free-ion abundance on initial solution concentration over orders of magnitude.

The ion mobility spectrometer was operated in the low resolution mode for all the experiments described below, that is, the width of the second grid gating was set to 5 ms in the IMS.

3-1-1-1 IMS/TOF-MS Spectra of Tetraalkylammonium Ions

Figure 3-1 shows the low resolution ion mobility spectra while Figure 3-2 shows the corresponding TOF-MS spectra of four tetraalkylammonium (ethyl, butyl, pentyl and heptyl) bromide samples. Note that square topped peaks in IMS spectra were due to a steady flux of ions passing through the shutter grid during the relatively long open time of the grid which had been extended from the conventional 0.1 ms to the present 5 ms.

Figure 3-1 Ion mobility spectra of tetraalkylammonium bromides: (a) ethyl; (b) butyl; (c) pentyl; and (d) heptyl. IMS temperature, 150°C, the mobility scales shows reduced mobility, K_o , in this and subsequent figures.

Figure 3-2 Time-of-flight mass spectra of tetraalkylammonium bromides whose ion mobility spectra are shown in Figure 3-1: (a) ethyl; (b) butyl; (c) pentyl; (d) heptyl.

Also note that the TOF spectra were obtained without 'gating' of the IMS peaks and that the TOF spectrum was a composite of all the ions that reached the mass spectrometer. The identity of the individual IMS peak was determined by collecting spectra only during the period when ions of that peak arrived at the orifice of the TOF mass spectrometer. An example is shown in Figure 3-3 for tetraoctylammonium. Figure 3-3a shows the ion mobility spectrum of tetraoctylammonium bromide at 150°C. Figure 3-3b shows the composite TOF mass spectrum of the IMS ions. The following ions are discernible: M^+ , $M(MBr)^+$, and $M(MBr)_2^+$. The TOF mass spectra obtained from gating the IMS peaks, 'A' through 'D', are shown in Figures 3-3c - 3-3f, respectively. Evidently, peak 'A' is M^+ , 'B' is $M(MBr)^+$, and peak 'C' is $M(MBr)_2^+$; the $M(MBr)^+$ ion seen in Figure 3-3e could originate from the tail of peak 'B', or likely from fragmentation of $M(MBr)_2^+$. Every attempt to collect mass spectra of relative low mobility ions, such as peak 'D', failed to produce characteristic ions. This means that our mass spectrometric measurements might be of insufficient sensitivity and/or range for ions of those mass-to-charge ratios (upper limit measured = 4000). In spite of this, some properties of these ions are discernible from the mobility spectrum in Figure 3-3a. The abundance of peak 'D' increased as the concentration of solute (tetraoctylammonium salt, in this case) increased (data not shown), which suggests this peak may be related to residual 'particles' that incorporate solute. Moreover, peak 'D' has a maximum intensity at 75 ms, and a range of 63-90 ms, corresponding to a reduced mobility of $0.22 \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$, and a range of $0.18\text{-}0.26 \text{ cm}^2/\text{V s}$. This peak is relatively broad (in comparison with peaks 'A' to 'C') suggesting that it is likely to be comprised of a mixture of ions (from both solvent and solute), and possibly

Figure 3-3 Tetraoctylammonium bromide: (a) ion mobility spectrum at 150°C; (b) composite TOF mass spectrum; (c) TOF mass spectrum of peak A; (d) TOF mass spectrum of peak B; (e) TOF mass spectrum of peak C; (f) TOF mass spectrum of peak D.

Figure 3-3 Continued.

of varying dimensions and charges (two key parameters upon which ion mobility is dependent). Although the exact nature of these ions is not known, it is tempting to assign these ions as 'clusters' or 'charge residues' from evaporating electrospray-generated droplets, which are believed to have a relatively narrow size distribution [105]. Table 3-1 summarizes the characteristics of ions observed in the electrospray of a tetraoctylammonium bromide solution (data from Figure 3-3. The ionic radii were calculated from Millikan's Equation, i.e., Equation 1-8 in Chapter I, page 14).

3-1-1-2 Comparison with Other IMS Studies

Table 3-2 is a comparison of the K_o of the M^+ ions of the tetraalkylammonium ions, most of which are shown in Figure 3-1 (page 85), with the corresponding K_o reported in ref. 71. It is apparent that the two sets of reduced mobilities are quite similar. A plot of the inverse reduced mobilities versus the ionic masses of this homologous series of ions yielded a linear plot (Figure 3-4); similar plots of other homologous series have previously been reported [8].

3-1-1-3 Mass Discrimination of the TOF-MS

Figure 3-5 illustrates the ratio of measured TOF-MS signal intensity to IMS peak height for ions derived from the electrospray of a series of tetraalkylammonium compounds ranging in size from tetraethylammonium to tetraoctylammonium. The apparent TOF-MS response decreases with mass relative to the detected IMS electric current for that ion delivered to the IMS/TOF-MS interface. This variation of the ratio of

Table 3-1 Characterization of tetraoctylammonium ions

<i>Ion type</i>	M⁺	M(MBr)⁺	M(MBr)₂⁺	Cluster Ions
<i>Drift time (ms)</i>	20	31	42	63 - 90
<i>Reduced mobility (cm²V⁻¹s⁻¹)</i>	0.8	0.5	0.4	0.26 – 0.18
<i>Ionic radius (nm)</i>	0.7	0.9	1.0	1.3 – 1.5 (+1) 64 – 77 (+50)
<i>Ion mass (m/z)</i>	466	1013	1560	N/A

Table 3-2 Reduced mobilities (K_o , $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$) previously reported [ref. 71] ($P=760$ Torr, $T=167^\circ\text{C}$) and measured in this study ($P=760$ Torr, $T=150^\circ\text{C}$)

Cation	Mass (Da)	K_o [ref. 71]	K_o [this work]
<i>Tetraethylammonium</i>	130.3		1.88
<i>Tetrabutylammonium</i>	242.5	1.19	1.31
<i>Tetrapentylammonium</i>	298.6	1.03	1.12
<i>Tetrahexylammonium</i>	354.7		0.99
<i>Tetraheptylammonium</i>	410.8	0.81	0.89
<i>Tetraoctylammonium</i>	466.9		0.80

Figure 3-4 Plot of inverse reduced mobilities ($1/K_o$) versus tetraalkylammonium ion mass (data from Table 3-2).

Figure 3-5 Ratio of measured TOF signal intensity to IMS peak height for a series of ions produced during ESI of tetraalkylammonium salts

TOF/IMS signals with ion mass illustrates the degree of possible mass discrimination in the IMS/TOF-MS interface.

3-1-1-4 IMS Drift Gas Flow Rate

The effect of IMS drift gas flow rate on IMS/TOF mass spectra was examined. In this experiment, IMS spectra and TOF mass spectra were collected for electrosprayed tetraoctylammonium bromide at various IMS drift gas flow rates at 150°C. Figure 3-6 shows the ion mobility spectra while Figure 3-7 shows the corresponding TOF mass spectra of tetraoctylammonium ions. Several trends can be seen from both figures. As the drift gas flow rate increases, the intensity of lighter ion (such as M^+) peaks increases as a result of decomposition of heavier ions (such as $M(MBr)^+$, $M(MBr)_2^+$). Furthermore, the decomposition of 'cluster ions' as a result of increasing gas flow rate also makes a possible contribution to the increasing signals of the lighter ions. Finally, the effect of gas flow rate on ion mobility is not significant.

3-1-1-5 IMS Drift Tube Temperature

A series of experiments were carried out at IMS drift tube temperatures of 25, 90, 150, and 190°C with gas flow at 2 L/min. Figure 3-8 shows the ion mobility spectra while Figure 3-9 shows the corresponding TOF mass spectra of the tetraoctylammonium ions with indicated temperatures. The Figure 3-10 plots the integration of signals from IMS (top) and IMS/TOF-MS (bottom) (data from Figures 3-8 and 3-9) as a function of time at various IMS drift tube temperatures.

Figure 3-6 IMS spectra of tetraoctylammonium ions at several drift gas flow rates at 150°C.

Figure 3-7 Composite TOF mass spectra of tetraoctylammonium ions at several drift gas flow rates at 150°C.

Figure 3-8 Ion mobility spectra of electro sprayed tetraoctylammonium bromide at various IMS drift tube temperatures with gas flow at 2 L/min.

Figure 3-9 Composite TOF mass spectra of electrosprayed tetraoctylammonium bromide at various IMS drift tube temperatures with gas flow at 2 L/min.

Figure 3-10 A plot of integrated signals from (a) IMS and (b) IMS/TOF-MS (data from Figures 3-8 and 3-9) as a function of time at various IMS drift tube temperatures.

The drift velocity of the tetraoctylammonium ion and its adduct ions increased with increasing temperature, corresponding to an increasing mobility. This is not surprising, since elevated temperature reduced the drift gas density in the cell, yielded decreased number of collisions between the ions and the drift gas, increased ionic velocity, and hence increased ion mobility. These relationship can be seen from the Equation (1-10) in Chapter I (page 15). In contrast however, the mobility of the 'cluster ion' decreased with increasing temperature, most likely due to the loss of charges from the 'cluster ion'. Moreover, the intensity of the tetraoctylammonium ion (M^+) peak remained constant with increasing temperature, while that of the small adduct ion ($M(MBr)^+$) increased and that of the 'cluster ion' decreased. Despite these changes, the total integrated electrical current detected by IMS remained constant (Figure 3-10a). On the other hand in the IMS/TOF-MS, the total integrated signal increased with increasing temperature (Figure 3-10b). The large 'cluster ions' (not detected by TOF-MS) appeared to be undergoing decomposition at elevated temperatures, via charge repulsion, to yield an increased flux of low mass ions (in this case, $M(MBr)^+$ ions, in Figure 3-10b).

3-1-2 Electrospray Ionization of Proteins

In this study, the ion mobility spectrometer was operated in the low resolution mode with the second gate of IMS open for 5 ms to allow the lowest mobility ions, i. e., 'cluster ions', to be detected in the ion mobility spectrum. All of the data were gathered with an indicated ion mobility spectrometer temperature in the range 150-200°C, except for the temperature dependent experiments.

*3-1-2-1 IMS/TOF-MS Spectra of Proteins**Gramicidin S*

Gramicidin S (MW 1140) is a cyclic peptide antibiotic made up of two identical pentapeptides joined head-to-tail. Figure 3-11 displays an ion mobility spectrum obtained for a solution of Gramicidin S (~20 μ M) in a 50/50 (v/v) water/methanol solution to which 0.5% acetic acid had been added. The IMS spectrum shown in Figure 3-11a comprises three peaks, 'A' to 'C', related to solvated gramicidin ions. The composite TOF mass spectrum in Figure 3-11b shows the characteristic $[M+H]^+$ and $[M+2H]^{2+}$ ions of gramicidin S. Gating of peak 'A' identified it as $[M+2H]^{2+}$ (Figure 3-11c); similarly, that of peak 'B' as $[M+H]^+$ (Figure 3-11d). Gating of peak 'C' produced no characteristic ions (Figure 3-11e). This ion (broad peak 'C') with very low mobility is similar to the one detected in the tetraalkylammonium salts experiments described before, and is likely from evaporating electrospray-generated droplets, and related to the protein ion and solvent cluster.

Ubiquitin

Bovine ubiquitin is a small protein (MW 8565) with a single polypeptide chain of 76 amino acid residues. The spectra of ubiquitin solution, with same solvent composition as that for gramicidin S, are comparatively simple. These are shown in Figure 3-12, where 3-12a is the IMS spectrum and 3-12b the composite TOF spectrum. The IMS spectrum shows three peaks, the two lowest mobility species 'B' and 'C' (at about 25 ms and 70 ms drift time) are associated with solvated ubiquitin. The highest mobility (shortest drift

Figure 3-11 Gramicidin S: (a) ion mobility spectrum at 200°C; (b) composite TOF mass spectrum; (c) TOF mass spectrum of peak A; (d) TOF mass spectrum of peak B; (e) TOF mass spectrum of peak C.

Figure 3-11 Gramicidin S: continued.

Figure 3-12 Ubiquitin: (a) ion mobility spectrum at 150°C; (b) composite TOF mass spectrum; (c) TOF mass spectrum of peak B.

time) peak 'A' is observed regardless of whether ubiquitin is present, and clearly arises from solvent and small ions. The precise mobility and relative abundance of this component is somewhat solution dependent. Figure 3-12c is the gated spectrum of peak 'B'. The protein ion envelope encompassed $[M+4H]^{4+}$ to $[M+11H]^{11+}$ with the centroid on approximately the $[M+9H]^{9+}$ ion. No characteristic gated spectrum was observed in TOF-MS for the peak 'C'.

Insulin

Bovine insulin (molecular weight of 5733) consists of two chains, an 'A' chain of 21 residues and a 'B' chain of 30 residues, which are covalently joined by two disulphide links. Figure 3-13 shows the IMS and TOF-MS results for 20 μ M insulin in a methanol/water/acetic acid solution. Three relatively broad peaks ('A' to 'C') were observed in the IMS spectrum (3-13a) of this protein. The composite TOF mass spectrum shows the $[M+3H]^{3+}$, $[M+4H]^{4+}$, and $[M+5H]^{5+}$ ions of insulin (3-13b). Gating of peak 'A' produced a similar TOF mass spectrum (3-13c). Gating of peak 'B' (3-13d) yields characteristic ions of the lower charged (+3, +4) insulin ions. No characteristic ions were identifiable in the mass spectrum of peak 'C' which was assigned to 'cluster ions'.

3-1-2-2 IMS Temperature and Solvent Composition

As mentioned in Section 1-5 of Chapter I, the changes in protein environment, such as the addition of acid or base (changes in pH), organic solvent (changes in composition), and changes in temperature, etc. will alter the solution structure and chemistry of the protein. That means the native protein structure (usually folded in a well-defined

Figure 3-13 Insulin: (a) ion mobility spectrum at 150°C; (b) composite TOF mass spectrum; (c) TOF mass spectrum of peak A; (d) TOF mass spectrum of peak B.

Figure 3-13 Insulin: continued.

3-dimensional structure) will change to a denatured state (linear, more open structure). Cytochrome c and lysozyme have been shown to denature from a compact, folded conformation to a random coil state upon lowering the pH from 3.3 to 1.6 [104, 105, 148]. It is also well established that the use of organic solvents, such as those common to ESI methods, can cause protein denaturation [149,150]. Increasing the alcohol content was shown to increase the α -helical form of ubiquitin [151]. For disulphide bridge-containing proteins, denaturation may further be achieved via the action of bridge-breaking reagents, such as 1,4-dithiothreitol (DTT) [152]. In addition to the effects of solvent and pH on protein structure, it has been observed that protein structure changes can be induced by heating (thermal denaturation), as evidenced by the shifting of the charge state distribution in the ESI mass spectrum [106, 107].

Evidence of the effect of temperature, pH and solvent composition on the protein structure may be obtained from IMS studies. Ion mobility analysis allows a direct examination of electrospray ions under conditions where mass spectrometric discrimination and possible changes due to the extent of solvation or reactions in the ESI-MS interface are avoided [68-71, 79].

IMS Drift Tube Temperature

ESI/IMS measurements of horse heart cytochrome c (MW 12,360) were carried out under a wide series of IMS temperatures: 30, 60, 90, 120, 150, 180, and 210°C. The IMS spectra obtained from a 10 μ M cytochrome c solution in 50/50 (v/v) water/methanol and 0.5% acetic acid, under the seven indicated temperatures are shown in Figure 3-14. IMS/TOF-MS measurements of the three IMS peaks revealed that peak 'A' contained

Figure 3-14 Ion mobility spectra of cytochrome c at various temperatures

solvent ions, peak 'B' contained cytochrome c ions and peak 'C' produced no observable ions. The corresponding TOF mass spectra are displayed in Figure 3-15. Increasing drift tube temperature resulted in a decrease in mass spectral peak width which was accompanied by a concomitant increase in ion abundance. This is shown more clearly in a direct comparison between the 30 and 200°C data (Figure 3-16). We interpret the decrease in mass spectrometric peak width and the increase in ion abundance with increasing temperature as an indication of thermal desolvation of cytochrome c ions in the ion mobility spectrometer. That is to say, the cytochrome c ions reaching the TOF mass spectrometer were in a lower degree of solvation for a higher IMS temperature than those for a lower temperature. Furthermore, our ESI/IMS and IMS/TOF-MS interface conditions must have been relatively inefficient in declustering protein ions since electrospraying the same cytochrome c sample under room temperature in a commercial mass spectrometer, e.g., the SCIEX TAGA 6000E, produced a mass spectrum of declustered multiply-charged cytochrome c ions.

The IMS spectra in Figure 3-14 on page 110 showed that ion drift times changed, and therefore their mobilities (K) changed, with changing temperature. Mobility is related to the drift time as in Equation (1-7) of Chapter I. Most of the changes were a consequence of the decrease in gas density with increasing temperature. To compare the K values measured under different temperatures, they may be converted to reduced mobility, K_o , as shown in Equation (1-9) of Chapter I. The K_o of peaks 'A' - 'C' (shown in Figure 3-14) are plotted against temperature in Figure 3-17. As discussed earlier, we interpreted the decrease in mass spectrometric peak widths in Figure 3-16 as an indication of thermal declustering. If this occurred, there would be a reduction in ion size and a concomitant

Figure 3-15 TOF mass spectra of cytochrome c at various temperatures

Figure 3-16 Comparison of TOF mass spectra at (a) 30°C with (b) 200°C.

**Figure 3-17 Temperature dependence of reduced mobilities of cytochrome c peaks:
peak A, B and C identified in Figure 3-14.**

increase in mobility provided the change was not masked by any intrinsic thermal dependence of K_o . This dependence reflects the nature of the ion-neutral interaction, and needs not be a monotonic function [38]. Furthermore, even for members of a homologous series of compounds (e.g., amines), measured reduced mobilities exhibit a temperature dependence that is mass-related [8, 153]. For amines, the temperature dependence is positive (i.e. reduced mobility increases with increasing temperature) for low molecular mass ions, is essentially invariant for intermediate mass (90-180 Da) ions, and is negative for heavy ions [153]. The data in Figure 3-17 showed that, for peak 'A' (solvent and small ions), its reduced mobility was insensitive to temperature changes while for peaks 'B' (protein ion) and 'C' (cluster ion), their reduced mobilities decreased with increasing temperature. This decrease in reduced mobilities with increasing temperature may also be due to the charge loss with increasing temperature as shown in Figure 3-18, a plot of average charge (data from Figure 3-15) of cytochrome c ions as a function of reduced mobility at various temperatures. The average charge for the protein was calculated by the addition of the product of each charge state and its peak height, and then division by the total peak height. With the increasing temperature, the average charge of cytochrome c decreased.

The temperature dependence experiments were also carried out for ubiquitin, myoglobin and lysozyme at various IMS drift tube temperatures. Similar trends of reduced mobility of protein ions and cluster ions changing with temperature were also observed for these proteins as shown in Figure 3-19. From Equation (1-11), cross section (Ω) is temperature dependent, i.e., $\Omega \propto T^{1/2}$. The cross-section of protein ion decreases

Figure 3-18 Average charge of cytochrome c (from Figure 3-15) as a function of reduced mobility at various temperatures.

Figure 3-19 Reduced mobility of the protein (cytochrome c, ubiquitin, myoglobin and lysozyme) and cluster ions as a function of the IMS drift tube temperature.

The dashed line is the calculated K_0 vs. temperature (from Equation. 1-11).

with increasing temperature provided the reduced mobility (K_o) is constant. Experimentally, the reduced mobility decreased with increasing drift tube temperature as shown in Figure 3-19. Equation (1-11) was derived under the assumption of rigid sphere interaction, i. e., Ω is constant [38]. The only variable with temperature in Equation (1-11) is K_o , i.e. K_o varies as $T^{-1/2}$. The dashed line in Figure 3-19 is the calculated reduced mobility as a function of temperature from Equation (1-11) (assuming Ω is constant), and it agrees well with the experimental data. That means the changes in the dimension of the protein ion with drift tube temperature in our IMS experiments could be assumed to be very small.

Acetic Acid

As mentioned before, the protein structure can be changed from a compact, folded conformation to a random coil arrangement upon lowering the pH of a protein solution, and this might have some effect on the ion mobility spectrum. Figure 3-20 illustrates the IMS spectra (left) and corresponding TOF-MS spectra (right) of cytochrome c at different concentrations of acetic acid. The reduced mobility, K_o , increased with increasing concentration of acetic acid in the protein solution. This was in agreement with the charge state distribution for cytochrome c that shifted to a higher charge as seen in the TOF mass spectra when the amount of acetic acid was increased. This changing in charge distribution was due to a conformational change of the protein species in solution from a more compact to a less compact state with increasing acid concentration.

Figure 3-20 IMS spectra (left) and corresponding TOF mass spectra (right) of cytochrome c at various concentrations of acetic acid.

The same trend can also be seen for other proteins, such as ubiquitin, myoglobin and lysozyme. Figure 3-21 shows the plots of reduced mobility as a function of concentration of acetic acid for the protein and cluster ions. The reduced mobility of both protein ions and cluster ions increased with increasing percentage of acid, but reached an equilibrium when the concentration of acid was higher than 1.5% in the solution. That means, at this pH (~ 2), the maximum number of basic amino acid residues in the protein structure could have been reached and the protein was fully denatured.

Methanol

Methanol is a relatively mild denaturant [154]. However, unlike acid denaturation at low ionic strength which yields extended and unfolded states, alcohol denaturation leads to a structure with increased helical content and six-fold heme Fe-protein coordination [155].

Figure 3-22 illustrates the IMS spectra (left) and corresponding TOF mass spectra (right) of cytochrome c at different concentrations of methanol. The reduced mobility, K_o , of cytochrome c increased with increasing concentration of methanol up to 60%, and then decreased as the concentration of methanol further increased, corresponding to the changes in charge distribution as seen in the TOF mass spectra. The addition of methanol to water induced significant changes in the protein-unfolding process. It has been observed that on increasing methanol concentration, the enthalpy and entropy of denaturation of protein first reached a maximum at an intermediate concentration and then decreased with increasing concentration of the alcohol [156].

Figure 3-21 Reduced mobility of the protein and cluster ions as a function of the concentration of acetic acid.

Figure 3-22 IMS spectra (left) and corresponding TOF mass spectra (right) of cytochrome c at various concentrations of methanol

The same experiments were carried out for other proteins, such as ubiquitin and myoglobin. Figure 3-23 shows the plots of reduced mobility as a function of concentration of methanol for the protein and cluster ions. Between 0% and 60% MeOH, the abundance of protein ions and their K_o increased with increasing amount of methanol but decreases as concentration of methanol further increased, while the reduced mobility of the 'cluster ions' decreased with increasing amount of methanol in the solution. The decrease in both K_o and ion abundance of protein ions when the concentration of MeOH exceeds 60% is probably due to the solvation or deprotonation of protein ions. It is also likely due to the 'hydrophobic clustering' of methanol molecules in the water-rich region of composition [156], and this may be the reason why that the reduced mobility of 'cluster ions' decreased with increased concentration of methanol.

3-1-3 Measurement of the Collision Cross Section of Proteins

In this study, we describe the use of a conventional atmospheric pressure ion mobility spectrometer to examine the cross-sections of gas phase ions of ubiquitin, cytochrome c, myoglobin, lysozyme and α -lactalbumin as a function of charge states. By use of this system, the cross-sections of protein ions were determined as soon as possible after ionization, and prior to transfer of the ions into a vacuum chamber. Cross-sections determined at atmospheric pressure were compared to those determined previously with reduced pressure instruments.

In this study, the ion mobility spectrometer was operated in a high resolution mode. That is, the period during which the second shutter grid was open was 0.1 ms to 0.5 ms.

Figure 3-23 Reduced mobility of the protein and cluster ions as a function of the concentration of methanol.

3-1-3-1 Cytochrome c

Cytochrome c has 24 basic amino acids and 15 carboxylic acid sites including the C-terminus and the two propionic acids on the heme group. Positively-charged protein ions are formed in ESI mainly by proton attachment to basic amino acid residues (arginine, lysine, histidine). The number of sites that can be protonated is related to the conformation of the protein in solution. An unfolded protein generally has more protonation sites available and, therefore, shows higher charge states in the ESI mass spectrum than the same protein in a folded state.

The low resolution IMS spectrum of cytochrome c (Figure 3-24a) was characterized by two wide distributions of ions at reduced mobility $K_o = 0.74$ and $K_o = 0.34 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$. The reduced mobility of the protein ion (peak at $K_o = 0.74$) varied with temperature from $K_o = 0.88$ (30°C) to $K_o = 0.74 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ (200°C). Since the K_o value was not constant with temperature, all mobility values reported hereafter will not be corrected for temperature, i.e. will not be 'reduced mobility'.

The mobility of an ion of specific charge of a protein in an IMS spectrum was determined using the method described in Section 2-1-4 of Chapter II. An example is given below for cytochrome c. The mobility for a charge state of cytochrome c ion was determined by collecting TOF-MS spectra for two windows in the IMS spectrum of cytochrome c (Figure 3-24b). The two windows were 4 ms apart, the first one from 17 to 20 ms and the second from 24 to 30 ms of the IMS spectrum (Figure 3-24a). The delay between the two windows was selected to be slightly wider than the gate 'open' time (3 ms) of the mobility spectrometer since all ions were expected to arrive at the detector of

Figure 3-24 (a) Low resolution IMS spectrum of cytochrome c

(b) TOF-MS spectra of regions of the ion mobility spectrum of cytochrome c

(c) High resolution ion mobility spectrum of cytochrome c.

Figure 3-24 (b).

Figure 3-24 (c).

the mobility spectrometer in bands of at least 3 ms wide with additional widening from diffusion and space charge repulsion. Comparison of the resulting mass spectra (Figure 3-24b) with composite TOF mass spectrum showed that cytochrome c ion with +12 charge was entirely missing from both window spectra. The +12 ion therefore arrived in a 3 ms wide band close to the middle of the time between the two windows noted above. The arrival time of the +12 ion was therefore estimated to be 22 ms, corresponding to a mobility of $1.22 \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ (see Figure 3-24a).

High resolution IMS spectra (gate open for 0.2 ms) of cytochrome c were collected under various conditions. A typical spectrum is shown in Figure 3-24c. The charge states were assigned beginning with +12 with a mobility of $1.22 \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ as determined above. Mobilities of the ions from +8 to +16 were also determined from a series of spectra collected using solutions containing different concentrations of acetic acid.

Collision cross-sections of cytochrome c ions were calculated using Equations (1-12) in Chapter I. Figure 3-25 shows the ion cross-sections measured for several charge states of cytochrome c. There is a gradual increase in collision cross-section with increasing charge. The dashed line in Figure 3-25 labelled 'F' shows the cross-section ($1339 \text{ \AA}^2$) predicted for the native conformation using the exact hard-spheres scattering model (with helium) [123]. The cross-section determined using the exact hard-spheres scattering model for cytochrome c in a completely α -helix conformation is $2351 \text{ \AA}^2$ (see dashed line in Figure 3-25 labelled 'H') and the cross-section is $3453 \text{ \AA}^2$ for a nearly linear string conformation of cytochrome c (see dashed line in Figure 3-25 labelled 'U') [123]. The conformation of all the cytochrome c ions determined here fall between the linear structure and α -helix conformation under our experimental conditions. These ions appear

Figure 3-25 The cross-sections of cytochrome c ions having charge states +8 to +16.

The dashed line labelled 'F' shows the calculated cross-section of 1339 Å^2 for the native conformation. The dashed line labelled 'H' is the calculated cross-section of 2351 Å^2 for cytochrome c in a completely α -helix conformation. The dashed line labelled 'U' shows the calculated cross-section of 3453 Å^2 for a nearly linear string conformation of cytochrome c [123].

to have extended geometries, incorporating helical regions. Elongated geometries are favoured for high charge-state ions because of their lower Coulombic repulsion energy. The cross-section values reported here are comparable both with cross-sections determined from energy loss experiments [115, 119], and from ion mobility experiments at low pressure [123, 134].

3-1-3-2 Ubiquitin

Ubiquitin is a relatively small and simple protein. It contains a total of 144 labile hydrogens with 72 on the side chains and 72 on the backbone but no disulphide bonds. Ubiquitin can be very easily denatured.

Figure 3-26 illustrates the high resolution IMS spectrum of ubiquitin. The sample was prepared in 50/50 MeOH/H₂O with 0.5% acetic acid. The ions were separated in the IMS at a cell temperature of 200°C. The mobility of a specifically charged ion of ubiquitin was determined in the same way as described above for cytochrome c.

Figure 3-27 shows the ion cross-sections measured for several charge states of ubiquitin. The dashed line marked 'F' represents the average hard-sphere projection cross-section of 897 Å² [114] for the crystal conformation of ubiquitin [157]. The cross-section of a near-linear form of the protein [158] calculated using hard-sphere projection model is 2140 Å² [114] as shown in a dashed line labelled 'U'. From the measured cross-sections it appears that, under our experiment conditions, the gas-phase ubiquitin ions have an elongated conformation.

As the charge state increases from +6 to +12, the experimental collision cross-sections of ubiquitin increase from 1690±85 Å² for the +6 charge state to a maximum of

Figure 3-26 High resolution ion mobility spectrum of ubiquitin.

Figure 3-27 The cross-sections of ubiquitin ions having charge states +6 to +12. The dashed line marked 'F' represents calculated cross-section 897 Å^2 of the crystal conformation of ubiquitin [114]. The dashed line labelled 'U' is the calculated cross-section of 2140 Å^2 for a near-linear form of ubiquitin [114].

$2240 \pm 112 \text{ \AA}^2$ for the +12 charge state. This increase is comparable to the increase observed in the most open forms of multiply-charged cytochrome c [115, 123, 134] and shows the effect of increasing Coulombic repulsion on conformation. As the Coulombic repulsion energy increases, the cross-sections approach the value calculated for a near-linear conformation (Figure 3-27 dashed line 'U'). The cross-sections reported here are comparable both with cross-sections determined from energy loss experiments [115], and from ion mobility experiments at low pressure [114].

3-1-3-3 Myoglobin

Like cytochrome c, myoglobin (Mb) is a relatively small protein (153 residues) (MW 16953) that does not have any disulphide bridges and is, therefore, free to adopt preferred gas-phase conformations.

Covey and Douglas [115] reported values of the cross-section of the +8, +9, +11, +13, +15, +17, +19, +21 charge states of Mb using an energy-loss method. The gas-phase cross-section for denatured Mb was $3795 \text{ \AA}^2$, averaged over charge states of +8 to +21. Denatured Mb at higher charge states showed a more unfolded structure.

Collings and Douglas [118] have recently reported a study of the conformations of gas-phase myoglobin and apomyoglobin (apoMb) using the kinetic energy-loss in ion beam scattering experiments. They found that the collision cross-sections for the +7 to +14 charge states systematically increased with charge and with the collision energy in the desolvation region. The average collision cross-section reported by Collings and Douglas was $1603 \text{ \AA}^2$ for native Mb and $2638 \text{ \AA}^2$ for native apoMb.

Javahery and Thomson [126] have reported mobility cross-sections for Mb ions in charge states of +8, +9, +11, +13, +15 and +17 using nitrogen collision gas. The cross-sections obtained from a specular scattering model were between 2400 Å² and 3500 Å².

Figure 3-28 illustrates the high resolution IMS spectrum of myoglobin from an electrospray of acidified Mb solution with an IMS cell temperature of 200°C. The mobility of a specific charged ion was determined in the same manner as described for cytochrome c above.

Figure 3-29 shows the ion cross-sections measured for several charge states of myoglobin. The dashed line labelled 'F' shows that the collision cross-section determined for the native conformation of myoglobin [159] with the exact hard-spheres scattering model is 1768 Å² [124]. The dashed line labelled 'H' is the average collision cross-section of 3258 Å² [124] calculated for a α -helical form. The average collision cross-section for a string-like conformation is 4944 Å² [124], plotted as the dashed line 'U'. The cross-sections that we have measured for the Mb ions show that it is extended. Two conformations of myoglobin can be detected in Figures 3-28 and 3-29. Figure 3-28 shows that the charge state envelope has two maxima, near $z = +18$ and near +14. The slopes S_1 and S_2 (Figure 3-29) for the lower and higher charge state ions are not equal.

3-1-3-4 Lysozyme

The maximum number of observed charges for peptides and smaller proteins (e.g. cytochrome c, ubiquitin and myoglobin), correlates well with the number of basic amino acid residues (Arg, Lys, His), except for disulphide-intact molecules such as hen egg lysozyme (MW 14,306) and bovine α -lactalbumin that have four disulphide bonds each.

Figure 3-28 High resolution ion mobility spectrum of myoglobin.

Figure 3-29 The cross-sections of myoglobin ions having charge states +13 to +22. The dashed line labelled 'F' shows the calculated collision cross-section [124] of 1768 Å² for the native conformation. The dashed line labelled 'H' is the average collision cross-section 3258 Å² calculated for a α -helical form [124]. The dashed line 'U' is the average collision cross-section of 4944 Å² for a string-like conformation [124].

However, reduction of disulphide linkages with 1,4-dithiothreitol (Cleland's reagent) may allow the protein to be in an extended conformation and make 'buried' basic residues available for protonation to yield more highly-charged ions in the ESI process [103].

ESI/IMS and TOF-MS spectra were obtained for native proteins. Reduction of disulphide bonds was first carried out with 1, 4-dithiothreitol (DTT). A small amount of aqueous DTT solution was added to an aliquot of 20 μ M native protein in 50/50 methanol/distilled water such that the final DTT concentration was approximately 5 mM. After a period of over 24h at room temperature, glacial acetic acid was added (0.2%) to the protein solution containing DTT and its IMS and TOF-MS spectra were immediately obtained.

A typical high resolution IMS spectrum for lysozyme is shown in Figure 3-30a with multiple charging clearly evident from +7 to +10. However, egg lysozyme has a total of 19 basic amino acids. Breaking cysteine-cysteine linkages with the addition of DTT allowed additional protonation and yielded the +12 to +19 states, as demonstrated in Figure 3-30b. The charge distributions are consistent with a more open conformation in the DTT solution.

Figure 3-31 shows the ion cross-sections measured for +7 to +19 charge states of lysozyme. The cross-sections fall between two structures: the compact structure and a near-linear extended conformation. They were estimated at 1180 $\text{\AA}^2$ (dashed line labelled 'F') and 3750 $\text{\AA}^2$ (dashed line labelled 'U'), respectively, by Valentine *et al.* [125] who examined the conformations of disulphide-intact and disulphide-reduced lysozyme ions in the gas phase using ion mobility techniques at low pressure. The gas-phase ions that are produced by ESI from disulphide-intact and disulphide-reduced solutions have

Figure 3-30 (a) High resolution ion mobility spectrum of the disulphide-intact lysozyme, (b) High resolution ion mobility spectrum of the disulphide-reduced lysozyme.

Figure 3-31 The cross-sections of lysozyme ions having charge states +7 to +19. The dashed line labelled 'F' is an estimated cross-section of 1180 Å^2 of compact conformation. The dashed line labelled 'U' is the calculated cross-section of 3750 Å^2 for a near-linear extended conformation [125].

distinctly different collision cross-sections. Disulphide-intact ions favour a highly (or partially) folded conformer with a cross-section near that calculated for the crystal structure. Ions formed from the disulphide-reduced solution have collision cross-sections that are much larger than those observed for the disulphide-intact protein, showing that these ions are largely unfolded.

The compact conformations observed for the disulphide-intact lysozyme ions appeared to be slightly more open than the folded crystal conformation (dashed line 'F'). Since disulphide-intact lysozyme solution was acidified, it might be partly denatured even without the disulphide bonds broken by addition of DTT. Thus the observation of conformations slightly more open than the folded conformation is expected.

3-1-3-5 α -Lactalbumin

The native structure of bovine milk α -lactalbumin (MW=14,174), a protein with a covalent structure quite similar to egg lysozyme, is composed of 17 basic amino acid residues and four cysteine-cysteine disulphide bridges. Yet a maximum of only +11 charges was observed in the TOF-MS spectrum (Figure 3-32a). Reduction of the disulphide bonds with DTT resulted in ions with as many as +16 positive charges (Figure 3-32b).

High resolution IMS spectra of the disulphide-intact and disulphide-reduced α -lactalbumin solutions are shown in Figure 3-33. ESI of the disulphide-intact solution yielded ions with charge states from +8 to +10 (Figure 3-33a), while the disulphide-reduced solution yielded ions with higher charge states, +10 to +16 (Figure 3-33b).

Figure 3-32 (a) TOF mass spectrum of the disulphide-intact α -lactalbumin; (b) TOF mass spectrum of the disulphide-reduced α -lactalbumin.

Figure 3-33 (a) High resolution ion mobility spectrum of the disulphide-intact α -lactalbumin, (b) High resolution ion mobility spectrum of the disulphide-reduced α -lactalbumin.

The mobility of each charge state of the α -lactalbumin ion was determined in the same manner as described for cytochrome c above. Figure 3-34 shows the ion cross-sections measured for several charge states (+8 to +16) of α -lactalbumin. The disulphide-intact ions favoured a highly-folded conformation while disulphide-reduced ions were largely unfolded. The cross-section increased with increasing charge states.

The data presented here are the first measurements of collision cross-sections for disulphide-intact and disulphide-reduced α -lactalbumin. The results of cross-sections for all the protein ions studied here are summarized in Table 3-3. Table 3-3 also includes literature values for cross-sections determined in low-pressure ion mobility instruments.

Figure 3-34 The cross-sections of α -lactalbumin ions with charge states +8 to +16.

Table 3-3 Gas-phase cross sections of protein ions

Protein	Charge state	Mobility K (cm ² /Vs)	Cross-section (Å ²)				
			this work	ref 114	ref 123/134	ref 125	ref 115
Ubiquitin (MW 8564)	6	0.94	1690	1560			
	7	1.04	1790	1600			1460
	8	1.13	1880	1650			
	9	1.21	1990	1680			1900
	10	1.28	2080	1760			
	11	1.34	2180	1830			2220
	12	1.43	2240	1870			
Cytochrome c (MW 12231)	13			1930			
	7				2000		
	8	0.88	2520		2050	1300	
	9	0.97	2560		2200	1450	
	10	1.05	2620		2220	1610	2370
	11	1.14	2670		2300	1720	2430
	12	1.22	2730		2350	1850	3230
	13	1.28	2820		2400	1900	3230
	14	1.33	2930		2500	2000	
	15	1.38	3030		2600	2050	3830
	16	1.42	3130		2750	2100	
	17				2800	2150	3450
α -Lactalbumin (no DTT) (MW 14175)	8	1.09	2040			2180	
	9	1.18	2130			2200	4120
	10	1.26	2200			2220	
α -Lactalbumin (with DTT) (MW 14175)			2280		2900	2300	4310
	10	0.94	2970				
	11	1	3060				
	12	1.07	3120				
	13	1.13	3220				
	14	1.18	3320				
	15	1.23	3410				
	16	1.26	3530				
			*ref 134, 126, 125, 124, 123, 114				
			--from ion mobility experiments				
			*ref 119, 115				
			--from energy loss experiments				

Table 3-3 Continued

Lysozyme (no DTT) (MW 14300)	7	1.09	1790			1450		
	8	1.16	1920			1750		
	9	1.21	2070			1900		
	10	1.3	2150			1970		
	11					2000		
Lysozyme (with DTT) (MW 14300)	10					2420		
	11					2480		
	12	1.05	3170			2500		
	13	1.11	3270			2600		
	14	1.16	3350			2650		
	15	1.21	3450			2750		
	16	1.24	3580			2850		
	17	1.28	3690			2900		
	18	1.33	3760					
	19	1.37	3880					
Myoglobin (MW 16950)	7							2250
	8				2410			2350
	9				2550		2520	2570
	10					2400		
	11				2700		2600	3020
	12							2950
	13	0.99	3690		3100		2720	3050
	14	1.04	3770					3150
	15	1.09	3840		3320		2800	3210
	16	1.14	3930					3300
	17	1.18	4040		3500		2830	3400
	18	1.21	4150					3500
	19	1.25	4270					3600
	20	1.28	4340					3700
	21	1.31	4460					3710
	22	1.35	4550					3810

3-2 FAIMS Ion Trap

In this section, the theoretical description of FAIMS as a potential atmospheric pressure ion trap will be described. Some preliminary results showing ions generated by corona discharge or electrospray trapped in the 3-dimensional space of FAIMS at atmospheric pressure and at room temperature will be discussed.

3-2-1 Three-Dimensional Ion Trajectory in the FAIMS

The FAIMS was a cylindrical structure containing three electrodes: the inner and outer FAIMS cylinders, and a sampler cone as shown in Figure 2-3 (page 71) and Figure 3-35. Since the geometry of FAIMS was not a simple cylinder, the ion trajectory calculation was more complex and was carried out in two steps.

The first step established the electric fields within the three electrodes, the inner and outer FAIMS electrodes, and a sampler cone. The electric field calculation was performed using the 'relaxation' technique often used in fluid dynamics, involving a repetitive series of approximations of the voltage at every point in the physical space. The voltage at a given point was estimated to be the 'average' of the points around it. This procedure was repeated for every point in the space, and the calculation was iterated using the estimations from the previous calculation. This process was repeated until changes in the data array were within specified error limits. The 'relaxation' and 'successive over relaxation' methods for numerically solving Laplace and Poisson equations are described in most textbooks of fluid dynamics [160]. In the second step of the calculation, the trajectories within the arbitrary geometry were calculated based on the electric fields that were established by the 'relaxation' calculation discussed above.

Figure 3-35 Calculated ion trajectories beginning at several starting locations in the FAIMS. The ions are carried by a ‘simulated’ gas flow from right to left. The calculation parameters are given in Table 3-4.

(a) starting location I ($X = 0.49$, $Y = 0.28 \text{ cm}$); (b) starting locations II to IV.

Figure 3-35 (b).

The calculation of ion trajectories was achieved by breaking down the ion motion into a series of small translational steps based on the local electric fields due to the asymmetric waveform and dc voltages on the electrodes and the sampler cone. From the electric field strength at each point in space/time, the ion mobility was calculated (e.g., by using Figure 1-9 for $(\text{H}_2\text{O})_n\text{H}^+$, page 35). The ion velocity was estimated to be $v = KE$ (or $v = K_h E$) and the distance travelled in each time step was thus determined.

In Figure 3-35a, the ion trajectory was initiated near the outer electrode. A gas flow was simulated as a constant translation of the ion from right to left in the figures, and did not include turbulence, or changes in gas velocity near surfaces. The ion oscillated because of the applied asymmetric waveform and two types of net motion are illustrated in Figure 3-35a. Initially, the ion moves away from the outer electrode and after a short time the distance from the outer electrode becomes constant. This is the condition shown in Figure 1-10d (page 38) where the net motion in the radial direction quickly became zero. The ion does not leave the location near the end of the electrode even with the artificial axial direction of 'gas flow'. That is, the ion has entered a 3-dimensional trap near the spherical terminus of the inner electrode.

Figure 3-35b includes calculated ion trajectories starting at three other locations (marked II to IV).

The motion of the ions in FAIMS, shown in Figure 3-35, can be summarized as the superposition of several independent motions. First, the ion oscillates back and forth along a direction approximately perpendicular to the nearest surface of the inner electrode due to the ~200 kHz applied asymmetric waveform. These oscillations are 'blurred' into what appears to be a wide band in the figure. Secondly, the ion falls into the two-

dimensional trapping region along the length of the inner electrode. Finally, the ion is carried along the electrode by the artificial application of gas flow. In all cases, the ion travels to the spherical terminus of the FAIMS inner electrode, and is unable to proceed further even with the flow of gas. As shown in Figures 3-35a and 3-35b, the final location of the ion is independent of the starting location.

Figure 3-36 illustrates the motion of ions from the trapping region when the voltage of the sampler cone (orifice voltage, OR) was decreased stepwise from 0 V to -20 V. The ions, which were captured in the trap when using the conditions shown for Figure 3-35, were extracted from the trap and travelled toward the sampler cone as shown in Figure 3-36. A 100 μm orifice leading into the vacuum of a mass spectrometer was located in the center of the sampler cone. The details of the parameters used for the above calculations are summarized in Table 3-4.

There exists an optimum condition of the sampler cone (OR) at which the ion signal intensity will be maximal. In Figure 3-37a, the sampler cone was held at 0 V, i.e., OR = 0 V. The other conditions used for the ion trajectory simulation are given in Figure 3-37a. Figures 3-37b through 3-37e were prepared in the same manner, except that the sampler cone voltage was changed to -2.5, -5, -7.5 and -15 V, respectively. These low applied OR voltages had the effect of drawing the ions out of the 3-dimensional trapping region. If this extraction occurred at voltages within 2 V to the normal trapping conditions (i.e., indefinite ion trapping), then the ions would tend to focus near the axis of the inner electrode in a region very close to the exit orifice. The ion signal intensity would be maximal at an OR voltage which confines the ions as closely as possible to the electrode axis.

Figure 3-36 Calculated ion trajectories after stepping the sampler cone from 0 V to -20 V. The ions were initially within the trapping region shown in Figures 3-35a and 3-35b.

Table 3-4 Parameters used in ion trajectory calculations

<i>Scale</i>	200	points/cm
<i>NX, number points</i>	100	points
<i>NY, number points</i>	62	points
<i>Inner electrode od</i>	2.0	mm
<i>Outer electrode id</i>	6.0	mm
<i>FAIMS analyzer</i>	2.0	mm
<i>Starting</i>	x	varied
<i>Starting</i>	y	varied
<i>DV</i>	2500	V
<i>CV</i>	-19	V
<i>Frequency</i>	200	kHz
<i>Asymmetric waveform</i>		
<i>(ratio of high/low voltage time period)</i>	1:2	
<i>Calc. steps/cycle</i>	200	steps
<i>Applied gas flow vel.</i>	-50	cm/s
<i>Assumed ion mobility at high field (K_h) for trajectory calculations</i>		
$K_h = 2.3(1 + 1.1 \times 10^{-7} E + 1.29 \times 10^{-10} E^2)$ where E is the field (V/cm) at 760 Torr

Figure 3-37 Calculated ion trajectories at (a) OR = 0 V; (b) OR = -2.5 V;
(c) OR = -5 V; (d) OR = -7.5 V; (e) OR = -15 V.

(b) OR = -2.5 V

(c) OR = -5.0 V

Figure 3-37 Continued.

(d) OR = -7.5 V

(e) OR = -15 V

Figure 3-37 Continued

3-2-2 TOF Mass Spectra and Compensation Voltage (CV) Plots

3-2-2-1 Water Cluster Ions - PI Mode

Protonated water cluster ions were generated using corona discharge ionization. Figure 3-38 is an example of TOF-MS spectrum of water cluster ions acquired with FAIMS conditions of $DV = 2900$ V and $CV = -19$ V. This spectrum only includes the ions that can be transmitted by this particular combination of DV and CV . The peak at $8.5 \mu\text{s}$ is the water cluster ion $(\text{H}_2\text{O})_2\text{H}^+$ with m/z 37. The peak at $10.3 \mu\text{s}$ is the water cluster ion $(\text{H}_2\text{O})_3\text{H}^+$ with m/z 55. The separation of ions in FAIMS is controlled by CV [82]. The optimal CV for the transmission of the two cluster ions at DV 2900 V was experimentally determined, as shown in Figure 3-39. The ion intensity at each experimental point was acquired by averaging the spectra recorded from 5000 TOF-MS acceleration pulses. The CV was adjusted manually. The traces appearing in Figure 3-39 correspond to two methods for the collection of CV scans including: (1) OR pulsed, i.e., the OR (sampler cone) was at +32 V for 20 ms during ion trapping and reduced to +1 V for 10 ms during ion extraction from the FAIMS ($V_{\text{FAIMS}} + 25$ V) with TOF detection at 2 ms after the reduction of OR voltage; (2) OR constant, i.e., the sampler cone was held constant at low OR of +1 V for continuous ion transport from the FAIMS to the TOF. Each ion has its own optimum CV , e.g., m/z 37 ion appears at CV of around -17 V while ion with m/z 55 requires an optimum CV of -11 V to pass through the FAIMS.

Figure 3-38 FAIMS/TOF mass spectrum for water cluster ions in P1 mode with $DV = 2900$ V and $CV = -19$ V.

Figure 3-39 CV plots of water cluster ion abundance in P1 mode with $DV = 2900$ V at OR pulsed (from +32 V to +1 V) and OR constant (+1 V).

3-2-2-2 Trace Impurity Ions - P2 Mode

Protonated water cluster ions are easily detected when using corona discharge ionization. However, these low mass ions are at a very high density, and would therefore be expected to travel very fast through the FAIMS analyzer and fill the ion trap rapidly. Moreover, since these water cluster ions would undergo many collisions with other water cluster ions or neutral gas molecules, they might change to another structure. This change in structure might cause ion loss to the walls of the FAIMS device and hence lifetimes too short for the present study. To avoid these problems, higher mass ions were investigated in P2 mode. The abundance of high mass ions was low since they were formed from trace impurities in the carrier gas. As previously reported [82], the P2 mode was much less susceptible to contaminants than the P1 mode, and the lifetimes of ions were expected to be maximal since loss to the walls of FAIMS via changes in chemical structure (e.g., hydration) would be minimized.

Figure 3-40 illustrates an example of FAIMS/TOF-MS spectrum for trace impurity ions acquired with $DV = 3500$ V and $CV = -3.0$ V in the P2 mode. The mass-to-charge ratio (m/z 380 (± 10)) of the trace contaminant with a flight time of 27.0 μ s was determined using a calibration based on the flight times of some lower mass ions including the protonated water cluster ions. Several trace impurity ions appear in the spectrum; however only the ion of highest abundance (m/z 380, the compound may be a silicone-based oil normally used in diffusion vacuum pump) was used in a further study. The mass spectrum is not a conventional API spectrum. Low mass ions, including protonated water cluster ions, are conspicuously absent. This spectrum was collected in the P2 mode, with the same waveform but different polarity from that used in the P1

Figure 3-40 FAIMS/TOF mass spectrum for trace impurity ions in P2 mode with $DV = 3500$ V and $CV = -3$ V.

mode. Thus all low mass ions would be filtered out by FAIMS. Moreover, recall that only those ions with approximately the correct ratio of K_H/K can be transmitted at a particular combination of DV and CV . Therefore the spectrum in Figure 3-40 displays only a small subset of the mixture of ions delivered from the ionization chamber into the FAIMS device. The optimal CV for transmission of the m/z 380 ion through FAIMS at a DV of 3500 V (in the P2 mode) was also experimentally determined, and the results are shown in Figure 3-41. The ion intensity at each experimental point was acquired in the same manner as that in the P1 mode for water cluster ions. The traces appearing in Figure 3-41 correspond to three methods for the collection of CV scans including: (1) pulsed OR with TOF detection at 4.5 ms after the reduction of OR (from +40 V to +1 V); continuous ion transport through the FAIMS to the TOF-MS with: (2) OR of +15 V and (3) OR of +1 V. The $CV = -3V$ corresponding to the maximum transmission of the m/z 380 ion was comparable for these three methods of data acquisition ($CV = -3$ V). For the same ion, the optimum CV value did not change between the OR pulsed mode and OR constant mode.

3-2-3 Ion Transport Delays within the Ion Optics of the FAIMS/TOF-MS

Figure 3-42 illustrates the results of an experiment designed to determine the response time of the combined FAIMS/TOF-MS system by examining trace impurity ions in the P2 mode. The OR was stepped between two values, one of which was suitable for ion transmission through the FAIMS into the vacuum system (+15 V), and the second voltage of which was unsuitable for either trapping or ion transmission (-10 V). Figure 3-42 shows the TOF-MS intensity of the m/z 380 ion at a series of times relative to the OR transitions from high to low voltage, and from low to high voltages.

Figure 3-41 CV plots of trace impurity ion (m/z 380) in P2 mode with $DV=3500$ V at (a) OR pulsed (+40 V to +1 V); (b) OR constant +15 V; and (c) OR constant +1 V.

Figure 3-42 Evaluation of the response time of the FAIMS/TOF-MS for trace impurity ion (m/z 380). At $t=0$, OR was pulsed to +15 V (start ion transmission), and at 30 ms, OR was stepped to -10 V (stop ion transmission).

The 'down' transition of OR occurs at 30 ms in Figure 3-42. After this transition, the electric field will prevent any (positively-charged) ions from passing between the sampler cone (OR = -10 V) and the skimmer cone (0 V). This 'down' transition should create an extremely abrupt decrease in ion flux to the octopole ion guide. Experimentally, the decrease in ion density required about 2 ms. This delay is attributed to: (1) the range of kinetic energies of the ions entering the octopole, and (2) reduction in the kinetic energies of some fraction of the ions through collisions with the residual gas within the octopole. Since the octopole is an ion guide, this longitudinal spreading will be accentuated because ions which have undergone collisions with the residual gas will remain contained within the octopole.

The 'up' voltage transition of OR from low to high voltage occurred at time 0 ms in Figure 3-42. The time required for ion abundance to reach a plateau was about 10 ms. At the low state of OR, relatively few ions were located near the end of the inner FAIMS electrode because an electric field pulled the ions to the sampler cone ($V_{FAIMS} + 20$ V, OR -10 V). When OR was raised to +15 V, the relatively low density of ion in front of the terminus of the inner electrode was augmented by newly-arriving ions that had been passing through the annular region of the FAIMS cylinders. The ion density increased until ions passed to the sampler cone and out of the orifice. From the above discussion of the down edge of the OR voltage pulse, it requires a minimum of 2 ms for changes in ion density to be transmitted through the octopole. The remaining 8 ms delay is attributed to the increase of ion density in the FAIMS trapping region.

3-2-4 Experimental Verification of Ion Trapping in FAIMS

Several experimental verifications of ion trapping in FAIMS have been carried out in P1 mode for water cluster ions and in P2 mode for trace impurity ions.

3-2-4-1 Water Cluster Ions - P1 Mode

Figure 3-43 shows a plot of the measured water cluster ion abundance (m/z 37, at 8.48 μ s) collected at various times after the low state ($t = 0$) of OR at $DV = 2900$ V and $CV = -18$ V. The sampler cone was pulsed from the high voltage state (OR +35 V, which was suitable for ion trapping) to the low voltage state (OR +1 V) for 10 ms, in order to extract ions from the FAIMS. The ions required about 2 ms to pass through the system to the TOF-MS acceleration region. The pulse of ions was widened during passage, to about 2 ms (half-height) when detected. This was most likely due to changes in kinetic energies of the ions and ion diffusion through collisions with the neutral gas and water molecules within the octopole. Figure 3-43 also includes one horizontal line that corresponds to the ion abundance collected in a continuous mode of OR = +1 V, which is equivalent to the 'low' state of OR when operated in pulsed mode.

3-2-4-2 Trace Impurity Ions - P2 Mode

A similar experiment was carried out using a trace impurity ion in the P2 mode. Figure 3-44 shows the measured intensity of the ion (m/z 380) collected at various times after the 'down' transition ($t = 0$) of OR with FAIMS operated at $DV = 3500$ V and $CV = -3$ V. The sampler cone was pulsed from the high voltage state (OR +40 V for ion trapping) to the low voltage state (OR +1 V for ion extraction) from the FAIMS.

Figure 3-43 TOF-MS detection of the pulse of water cluster ions (m/z 37) after 20 ms of ion storage in FAIMS.

Figure 3-44 TOF-MS detection of the pulse of ions (m/z 380) after 40 ms of ion storage in FAIMS.

The ions require about 5 ms to travel through the system to the TOF-MS acceleration region. The detected pulse of ions is about 3 ms wide (half-height). This peak delay time and peak width are consistent with the response times determined by the experiments described previously in Section 3-2-3. A more intense pulse of ions may be observed by using a detection system with faster response.

Figure 3-44 also shows two horizontal lines corresponding to the continuous collection of ions at two different OR settings. The lower line of data was collected with $OR = +1$ V, which corresponds to the low state of OR pulsed. The higher line of data was collected at an experimentally optimized setting of $OR = +15$ V. The enhanced signal intensity at $OR +15$ V has been determined to be the result of 'focusing' ions into the sampler cone orifice by the inner electrode of FAIMS. Ion trajectory modelling has shown that the ions passing around the terminus of the inner electrode will be focused towards the center channel (see Figure 3-35, page 149), with a concomitant increase in sensitivity.

For the purpose of establishing the existence of a cloud of trapped ions in a region near the spherical terminus of the inner FAIMS electrode, the results shown in Figures 3-43 and 3-44 are unambiguous. If the cloud did not exist, there could be no intense pulse of ion abundance extracted after an ion storage period.

3-2-5 Ion Storage Time

3-2-5-1 Water Cluster Ions - P1 Mode

An experiment was performed to determine the effect of the length of the ion storage period on the intensity of the pulse of ions extracted from the trapping zone near the

spherical end of the inner FAIMS electrode. The intensity of the TOF-MS peak for the water cluster ion at m/z 37 is plotted as a function of the storage period in Figure 3-45, at CV -17, -18, and -19 V with $DV = 2900$ V. The pulses applied to the sampler cone consisted of a variable period at $OR = +35$ V during which ions were trapped and stored in FAIMS, and a constant time period (10 ms) at $OR = +1$ V to extract ions from FAIMS. The ion storage time is shown on the x-axis of Figure 3-45. The signal intensity for the m/z 37 ion was measured by pulsing the TOF-MS acceleration grids at 2 ms after the OR voltage was lowered. For each of the CV s shown in Figure 3-45, the ion intensity rose over the first 20 ms. At trapping times in excess of 20 ms, the trap reached a steady state, wherein the influx of ions via the gas flow was balanced by ion losses to the walls of FAIMS. The loss of ions may be due to diffusion, ion-ion repulsion, ion-ion collision and exchange of some groups and outflux of ions via gas flows.

3-2-5-2 Trace Impurity Ions - P2 Mode

A similar ion storage time experiment was also performed for a trace impurity ion. The intensity of the TOF-MS peak for the impurity ion of m/z 380 was plotted as a function of the storage time period in Figure 3-46, at CV -3, -3.5 and -4 V with DV 3500 V in P2 mode. The pulses applied to the sampler cone consisted of $OR = +40$ V for a variable period during which ions were trapped in FAIMS, and $OR = +1$ V for 10 ms to extract ions from FAIMS. The signal intensity for the m/z 380 ion was measured by pulsing the TOF-MS acceleration grids at 4.5 ms after the OR voltage was lowered. For each of the CV s shown in Figure 3-46 the ion intensity rose over the first 25 ms. Beyond

Figure 3-45 Abundance of water cluster ion (m/z 37) extracted from the FAIMS after ion trapping periods of up to 60 ms.

Figure 3-46 Abundance of trace impurity ion (m/z 380) extracted from the FAIMS after ion trapping periods of up to 60 ms. The solid traces are least squares fits to the data based on a simple kinetic model of the trapping (from Equation 3-4, page 174).

25 ms of trapping time, the ion intensity reached a steady state, indicating a balance between the influx of ions via the gas flow to the FAIMS and ions losses to the walls of FAIMS through diffusion, ion-ion repulsion, ion-ion collision and outflux of ions via gas flows.

The trapping experiment can be considered to be a kinetics problem. Assuming that the influx of ions to the FAIMS is X ions/s, the loss of ions to the walls of the FAIMS, Y ions/s, is proportional to the number of ions in the trap. The number of ions in the trap, Z , will continue to increase until steady state is reached and

$$X = Y = kZ \quad (3-1)$$

where k is the rate constant for the function describing ion loss from the trap. For a short trapping time, Z is small and kZ or Y is small. It therefore can be assumed that

$$Z = Xt \quad (3-2)$$

where t is the trapping period. The differential expression of Equation (3-2) can be written as,

$$dZ/dt = X - kZ \quad (3-3)$$

and the solution to Equation (3-3) is:

$$Z(t) = (X/k)(1 - e^{-kt}) + Z_0 e^{-kt} \quad (3-4)$$

where Z_0 is the number of ions present in the trap at $t = 0$.

Using Equation (3-4), a curve which fits the experimental data can be calculated for the trapping experiments and the lifetime of the ions trapped in the FAIMS can be estimated. For example, the solid curves in Figure 3-46 (trapping experiment for impurity ion m/z 380 in P2 mode) are based on a fit to the data points. The k values were 0.13, 0.15 and 0.09 ms^{-1} for the CV curves -3, -3.5 and -4 V, respectively. The lifetimes (half-

life) of the impurity ions in the FAIMS ion trap are, therefore, about 5 ms at CV -3.5 V and about 8 ms at CV -4 V. The corresponding X values were 1.7, 1.6 and 0.35 (intensity/ms), respectively.

3-2-6 Optimization of Operating Conditions

Several experimental parameters could affect the ion trapping in the FAIMS device. These included the gas flow rate, the position between the spherical end of the inner electrode and the sampler cone, and the applied DV and CV . Several of these parameters were studied systematically.

3-2-6-1 Gas Flow Rate

The gas flow controlled at least two factors, the rate that ions flowing into the trapping region from the FAIMS annular space, and the turbulence at the spherical end of the inner electrode. The effectiveness of the focusing action required a gas flow that maximized the ion transport into the trapping region and simultaneously minimized ion loss through turbulence.

Experiments were carried out in the trapping mode, i.e., the sampler cone was pulsed. Recall from Figure 2-3 (page 71) that there were two ports for gas to enter the FAIMS. One was for sample carrier gas (C_{in}) entering to flow along the annular space between the outer and inner electrodes, and the other was purge gas in (P_{in}) for flushing the ionization chamber. Both gas flow rates could be adjusted.

Figure 3-47a shows the CV plots at different flow rates of purge gas and sample carrier gas (as indicated in Figure 3-47a) for the water cluster ion of m/z 37.

Figure 3-47 (a) CV plots of water cluster ion (m/z 37) at different gas flow rates in P1 mode with $DV = 2900$ V; (b) optimal peak height as a function of total ($P_{in}+C_{in}$) gas flow rate; (c) optimal CV as a function of total gas flow rate.

Figure 3-47 (b) **Figure 3-47 (c)**

Three trends can be revealed. First, the maximal peak height increases with increasing gas flow initially but decreases when the gas flow further increases. There is an optimal gas flow rate for a certain combination of experimental conditions (DV , CV and OR) to get a maximum ion abundance. Under the conditions of $DV = 2900$ V, $CV = -19$ V, the optimal gas flows were $P_{in}=1.2$ L/min and $C_{in}=0.8$ L/min as seen in Figure 3-47b. Secondly, the optimal CV value increased with increasing gas flow rate, as seen in Figure 3-47c. Thirdly, the CV plot became wider with increasing gas flow rate. That is, the ions became more dispersed in the FAIMS with increasing gas flow rate. At high gas flow rates, the changes of pressure at the spherical terminus of the inner electrode could be large, changing the trapping conditions of the ions.

Figure 3-48 shows the intensity of the pulse of water cluster ion (m/z 37) extracted from the FAIMS after ion trapping periods of up to 60 ms at various gas flow rates. It seems that the ions took a shorter time to reach a steady state at a higher gas flow rate since the balance between the ion loss to the wall of the FAIMS through diffusion, ion-ion repulsion, ion-ion collision and gas flows and the influx of ions via the gas flow to the FAIMS is rapidly reached. The best trapping condition is not at the highest gas flow rate but at the optimal gas flow rates, i.e., $P_{in}=1.2$ L/min and $C_{in}=0.8$ L/min, in this experiment.

The optimal gas flow rates were also tested for the trace impurity ion (m/z 380) in P2 mode as shown in Figure 3-49. The optimal gas flow rates for the conditions of $DV = 3500$ V and $CV = -3$ V in the trapping experiment for the trace impurity ion were at a combination of $P_{in} = 1$ L/min and $C_{in} = 0.9$ L/min. Therefore, it is very important that the

Figure 3-48 Abundance of water cluster ion (m/z 37) extracted from the FAIMS after ion trapping periods of up to 60 ms at three gas flow rates.

Figure 3-49 Abundance of trace impurity ion (m/z 380) as a function of total gas flow rate in a trapping experiment at $DV = 3500$ V and $CV = -3$ V in P2 mode.

gas flow rates be examined in order to determine the optimal conditions for the trapping experiment.

3-2-6-2 Dependence of Sensitivity on the Dispersion Voltage (DV)

The effect of the DV on CV plots in P1 mode for the water cluster ion (m/z 37) is illustrated in Figure 3-50, where CV plots of m/z 37 water cluster ion abundance are shown for several DV values (0 V and 2000 V). Without any applied DV , i.e., $DV = 0$ V, there was a very weak signal detected near $CV = 0$ V, which might result from the small fraction of ions carried through the FAIMS trap by the flow gas. Three trends are apparent with increasing DV value. First, the peak shifts to a more negative compensation voltage. Second, the sensitivity for ion detection increases substantially. Third, the peak width increases. The trend toward a more negative CV is a consequence of the change in high-field mobility (K_h) as shown in Figure 1-4 (page 24) trace A. The increase in sensitivity is a direct consequence of a decrease in the ion loss to the walls of the FAIMS analyzer due to better ion focusing in the FAIMS analyzer region (atmospheric pressure ion focusing mechanism) [73].

Although the sensitivity of the CV plot can be further improved at higher DV value (> 2000 V), it could not be investigated without increasing the FAIMS outer electrode voltage in the present FAIMS/TOF-MS configuration.

3-2-6-3 Horizontal Location of FAIMS

An experiment was designed to measure the radial distribution of ions in the annular

Figure 3-50 CV plots of water cluster ion (m/z 37) at several dispersion voltages in P1 mode.

region of the FAIMS ion trap. The FAIMS device was translated horizontally relative to the sampler cone step-by step with a micrometer. The experiment was first carried out for the water cluster ion (m/z 37) at $DV = 3500$ V in the P1 mode. The OR was constant +8 V. Figure 3-51 shows the plots of abundance of water cluster ion m/z 37 as a function of the FAIMS offset position. The ion abundance of each point was taken from a TOF mass spectrum. As expected, at CV -22 V, the ions were located in the annular space of FAIMS. The 'valley' of this plot corresponded to the terminus of the inner electrode. At more negative CV (-26 V), the ions seemed to be focused closer to the axis of inner electrode.

A similar experiment was carried out with the trace impurity ion (m/z 380) in the P2 mode. Figure 3-52 shows the ion abundance in the FAIMS with $DV = 3500$ V and $CV = -3.5$ V. The lower trace shows the ion distribution for OR constant (+5 V) which is similar to the ion profile seen in P1 mode of water cluster ion experiment. The upper trace is the ion distribution for OR pulsed (from +40 V to +1 V), which clearly shows that ions were trapped at the spherical terminus of inner electrode with disappearance of the 'valley' and a higher sensitivity than that for OR constant operation.

These radial ion distribution experiments support the results of the ion trajectory calculations and modelling described earlier in Section 3-2-1.

Figure 3-51 Abundance of water cluster ion of m/z 37 as a function of the FAIMS offset position relative to the sampler cone in P1 mode at $DV = 3500$ V with $CV = -22$ V and $CV = -26$ V.

Figure 3-52 Abundance of trace impurity ion m/z 380 as a function of the FAIMS offset position relative to the sampler cone (P2 mode at $DV = 3500$ V and $CV = -3.5$ V). The lower trace is for OR constant +5 V and the upper trace is for OR pulsed from +1 V to +40 V.

3-2-7 *Electrospray/FAIMS Ion Trap/TOF-MS*

The FAIMS ion trap was evaluated with electrospray ionization of proteins. A new FAIMS device with increased diameter was set up for this study. The outer electrode of this FAIMS was approximately 0.8 cm in inner diameter and 8 cm in length, while the inner electrode is 0.4 cm in outer diameter and 8.5 cm in length. The corona discharge needle was replaced with an electrospray needle. All electrospray samples were prepared in 50/50 (v/v) methanol/water solution with about 0.5% acetic acid.

3-2-7-1 *ESI/FAIMS/TOF Mass Spectra*

Figure 3-53 shows the ESI/FAIMS/TOF mass spectrum of (a) tetraoctylammonium ions (M^+) collected with FAIMS operating at $DV = 3500$ V and $CV = -3.8$ V in the P2 mode, and (b) corresponding solvent ions collected continuously in the P1 mode at $DV = 3500$ V and $CV = -30$ V. These mass spectra served for mass calibration and background identification for the protein experiments described below. The collection of spectra for protein ions requires FAIMS operating in P2 mode with negative polarity asymmetric waveform (waveform #2 in Figure 1-6, page 29). The ESI/FAIMS/TOF mass spectrum of 20 μ M ubiquitin is shown in Figure 3-54 with FAIMS operated at OR constant +47 V with $DV = 3500$ V and $CV = -7$ V. Similar conditions were used in collecting the 10 μ M cytochrome c spectrum shown in Figure 3-55 except for $CV = -7.5$ V. These mass spectra collected using FAIMS/TOF-MS for electrosprayed protein ions were similar to the ESI spectra collected using a conventional IMS coupled to TOF-MS for ubiquitin or cytochrome c ions, as described in Section 3-1, with some apparent differences as described below:

Figure 3-53 ESI/FAIMS/TOF mass spectra for electrosprayed (a) tetraoctylammonium ion and (b) solvent ions.

Figure 3-53 Continued.

Figure 3-54 TOF mass spectrum of electrosprayed ubiquitin ions with FAIMS operating in OR constant mode (+47 V).

Figure 3-55 TOF mass spectrum of electrosprayed cytochrome c ions with FAIMS operating in OR constant mode (+47 V).

- (1) The low-mass solvent cluster ions were entirely absent from the mass spectrum of ubiquitin or cytochrome c compared with Figure 3-24b (page 128). These ions, which were lost to the walls of the FAIMS analyzer in P2 mode, were detected by operating FAIMS in P1 mode (Figure 3-53b, page 188). This absence of solvent ions could simplify the interpretation of mass spectra for proteins.
- (2) At room temperature, the peaks in conventional IMS/TOF-MS spectra of proteins (such as ubiquitin and cytochrome c) cannot be well resolved (see Figure 3-16 in Section 3-1, page 113). With FAIMS ion trap, the TOF mass spectrum of ubiquitin (or cytochrome c) is already well resolved at room temperature. This improvement in resolution using electrospray FAIMS/TOF-MS is due to the desolvation capability of the FAIMS device.
- (3) The signal-to-noise ratio (S/N) of the mass spectra obtained using electrospray FAIMS/TOF-MS was also improved with respect to the conventional IMS/TOF-MS at room temperature. This improvement in S/N is the result of (a) the background solvent cluster ions were absent under P2 detection conditions and (b) the atmospheric pressure ion focusing mechanism in the FAIMS. The improvement in S/N was seen to increase further when CV was optimized for a particular charge state.

3-2-7-2 Compensation Voltage (CV) Plots

Figure 3-56 is the CV plot of TOF intensity of charged +11 ion of ubiquitin, while Figure 3-57 is the CV plot of total integration of ion intensity of ubiquitin. The total ion

Figure 3-56 CV plots for ubiquitin +11 ion at (a) OR constant +47 V and (b) OR pulsed from +60 V to +47 V.

Figure 3-57 CV plots of integration of all charge states of ubiquitin ion at (a) OR constant (+47 V) and (b) OR pulsed (from +60 V to +47 V).

abundance for each experimental point in Figure 3-57 was acquired by averaging the spectra recorded from 5000 repeat TOF-MS acceleration pulses, and integrating the intensities of all charge states. The lower trace appearing in both Figures 3-56 and 3-57 corresponds to the continuous ion transport through the FAIMS to the TOF-MS with OR constant +47 V. The upper trace corresponds to pulsed OR with TOF detection at 11 ms after the reduction of OR voltage from +60 V to +47 V. Two trends can be derived from the plots: (1) the abundance of charged protein ions is higher in the pulsed mode than that in the continuous mode, i.e. the protein ions were trapped in the FAIMS when OR was at the high voltage; (2) the optimal CV values were different between continuous mode and pulsed mode. For example, the optimal CV for the +11 ion in OR pulsed mode was -6 V while that in OR constant mode was -7 V (see Figure 3-56); (3) different charge states have slightly different optimal CV s, and the degree of the ion signal enhancement also differed for each charge state when OR was pulsed (see Figure 3-58). Similar trends were also found in the CV plots of cytochrome c, as shown in Figure 3-59. The difference in optimal CV between the OR pulsed mode and OR constant mode is probably due to the multiply-charged ions experiencing different focusing conditions in the annular space of the FAIMS and at the spherical end of the inner electrode.

3-2-7-3 Trapping of ESI Protein Ions in FAIMS

Figure 3-60 shows the ESI/FAIMS/TOF mass spectrum for ubiquitin with the FAIMS ion trap operated at OR pulsed. A similar trapping experiment was done for cytochrome c to yield the mass spectrum shown in Figure 3-61. These spectra can be compared with the mass spectra with FAIMS operating in non-trapping mode shown in Figures 3-54 (page

Figure 3-58 Ratio of ion abundance at OR pulsed to OR constant for several charge states of ubiquitin ions using different CVs.

Figure 3-59 CV plots of cytochrome c acquired by averaging the spectra recorded from 5000 repeat TOF-MS acceleration pulses, and integrating the intensities of all charge states at (a) OR constant (b) OR pulsed.

Figure 3-60 TOF mass spectrum of electrosprayed ubiquitin ions with FAIMS operating in OR pulsed mode (OR from +60 V, 80 ms for ion trapping to +47 V, 20 ms for ion extraction).

Figure 3-61 TOF mass spectrum of electrosprayed cytochrome c ions with FAIMS operating in OR pulsed mode (OR from +60 V, 80 ms for ion trapping to +47 V, 20 ms for ion extraction).

189) (for ubiquitin) and 3-55 (page 190) (for cytochrome c). For accurate comparison, the signal intensity on the y-axis in Figure 3-60 was scaled to 100 while the y-axis in Figures 3-54, 3-55 and 3-61 have been scaled relative to that in Figure 3-60. With OR pulsed, the signal intensity was about ten folds of that in OR constant mode for ubiquitin ions, and about 5 folds increase for cytochrome c ions.

Figure 3-62 shows the measured total intensity of all charge states of ubiquitin collected at various TOF detection times after the 'down' side ($t = 0$) of the OR pulse with $DV = 3500$ V and $CV = -7$ V. The sampler cone was pulsed from the high voltage state (OR +60 V for 80 ms) which was suitable for protein ion trapping, to the low voltage state (OR +47 V for 20 ms), thereby extracting ions from the FAIMS ion trap. The ions required an average of 11 ms to travel through the system to the TOF-MS acceleration region. The pulse of ions was widened during the passage, and appeared to be about 9 ms wide (half-height) when detected. A similar experiment was done for electrosprayed cytochrome c ions with $DV = 3500$ V and $CV = -6$ V as illustrated in Figure 3-63.

3-2-7-4 Protein Ion Storage Time

The effect of the length of the ion storage period on the intensity of the pulse of ions extracted from the trapping zone near the spherical end of the inner FAIMS electrode was also determined for electrosprayed protein ions. The experiment was designed similarly to the experiments carried out for ions generated using corona discharge. The pulses applied to the sampler cone at high voltage (OR = +60 V) for a variable period during which the protein ions were trapped in FAIMS, and at low voltage (OR = +47 V) for 20 ms to extract the protein ions from the FAIMS.

Figure 3-62 TOF-MS detection of the pulse of electrosprayed ubiquitin ions after 80 ms of ion storage in FAIMS.

Figure 3-63 TOF-MS detection of the pulse of electrosprayed cytochrome c ions after 80 ms of ion storage in FAIMS.

The total intensity of the TOF-MS peak of all charge states of ubiquitin ions was plotted as a function of the storage period (up to 100 ms) in Figure 3-64, at CV -6.5 , -7 and -7.5 V with DV 3500 V in P2 mode. The signal intensity for ubiquitin ion was measured by pulsing the TOF-MS acceleration grids 11 ms after the OR voltage was lowered. At each of the CV 's shown in Figure 3-64 the ion intensity rose rapidly from 10 ms-60 ms. After 60 ms of trapping time, the ion intensity reached an approximately steady state, indicating a balance between the influx of ions via the gas flow to the FAIMS and the ion loss to the walls of FAIMS through diffusion, ion-ion repulsion and gas flows.

A similar experiment was carried out for electrosprayed cytochrome c ions to yield very similar results (data not shown).

Figure 3-64 Abundance of ESI generated ubiquitin ions extracted from the FAIMS after ion trapping periods of up to 100 ms.

CHAPTER IV

SUMMARY & FUTURE WORK

4-1 ESI/IMS/TOF-MS

A conventional ion mobility spectrometer has been coupled to a linear time-of-flight mass spectrometer to investigate ESI-generated ions. The IMS was operated both in low resolution mode in order to study low mobility 'cluster ions' and in high resolution mode to study the collision cross-section of protein ions at atmospheric pressure. Ionic species that were separated in the ion mobility spectrometer could be selectively detected with the TOF mass spectrometer. The system was evaluated with small molecules including tetraalkylammonium ions, and with larger ions including the multiply charged ions of proteins, such as cytochrome c, ubiquitin, myoglobin, lysozyme and α -lactalbumin.

The low resolution ion mobility spectra typically comprised two kinds of peaks. One was the molecular ion and adduct ions (if there are any), which yielded conventional TOF-MS spectra. The second was 'cluster ions' of relatively lower mobility (possibly of varying dimensions and charges), incorporating solvent molecules as well as analyte, which did not produce TOF-MS peaks. These 'cluster ions' are probably never detected using a conventional electrospray mass spectrometer.

The drift velocity of the tetraoctylammonium ion, and its adduct ions increased with increasing temperature, since an elevated temperature reduces the drift gas density in the drift tube to yield a decreased number of collisions between the ions and the drift gas.

The large 'cluster ions' (not detected by TOF-MS) appeared to undergo conversion at elevated temperatures, via charge repulsion, to yield an increased flux of low mass ions while their mobility decreased due to loss of charges. Furthermore, the decomposition of heavier ions and the 'cluster ions' at higher drift gas flow rate were also observed.

The TOF mass spectra of protein ions at relatively low IMS drift tube temperatures showed that they were characteristic of protein-solvent clustering. The degree of solvation decreased with increasing IMS temperature, resulting in a decrease in mass spectral peak width and an increase in ion abundances. The loss of charge on protein ions with increasing temperature could contribute to the decrease in reduced mobilities.

The reduced mobility of peaks observed in IMS spectra and the charge state distributions seen in TOF mass spectra have been shown to be somewhat dependent on the solution composition, such as concentration of acetic acid and methanol, which can affect the conformation of proteins. The reduced mobility, K_o , increased with increasing concentration of acetic acid in the protein solution, in agreement with the increasing average charge of the protein. The reduced mobility of both protein ions and 'cluster ions' increased with increasing percentage of acid, but reached an equilibrium when the concentration of acid became higher than 1.5% in the solution. Moreover, the reduced mobility, K_o , of protein ions increased with increasing concentration of methanol up to 60 %, and then decreased as the concentration of methanol further increased, corresponding to changes in the charge distribution.

The cluster ions found in IMS for all the sample solutions investigated could not be mass-analyzed by our TOF-MS. These species had some similar behavior to the protein ion (such as temperature and concentration of acid dependences of their reduced mobilities). This suggests they were likely to comprise a mixture of solvent and solute, possibly of varying dimensions and charges. Apparently, our inability to observe the lower mobility 'cluster ions' appear to confirm the Smith group's assertion [69] that these species 'do not contribute to conventional (electrospray) mass spectra'. However, as we

previously discussed, our IMS/TOF interface appeared to be relatively inefficient in desolvating the protein ions, and therefore, by extension, would be relatively inefficient in performing declustering of protein 'particles' or multimers. It is also possible that these cluster species were undergoing decomposition at the IMS/TOF interface, and would never produce the characteristic mass spectrometric peaks. We have demonstrated that these low mobility species were readily dissociated under the influence of gas flow and IMS cell temperature.

The ESI/IMS/TOF-MS system was used for the determination of cross-sections of gas-phase protein ions produced by electrospray ionization. Identification of charge states for each peak in the high resolution IMS spectra was carried out using TOF-MS. By comparing the mass spectra collected for selected windows in the ion mobility spectrum with the composite TOF-MS spectrum, the charge states of specific ions seen in the ion mobility spectra were identified. The cross-sections of protein ions with several charge states were calculated from the ion mobility values determined by high resolution IMS.

The studies of disulphide-intact and disulphide-reduced lysozyme and α -lactalbumin ions showed that conformations observed in the gas phase were highly dependent on the presence of disulphide bonds. The measured cross-sections of the disulphide-intact protein ions showed a highly folded conformation while the cross-sections of the disulphide-reduced protein ions showed a highly-extended, unfolded conformation. In contrast to disulphide-intact proteins, cytochrome c, myoglobin and ubiquitin have no disulphide bridges, and are relatively free to be denatured in acid solution and therefore yielded highly unfolded conformations.

The ion cross-sections systematically increased with increasing charge, presumably because the protein expands to minimize Coulombic repulsion energy. The cross-sections obtained are larger than the values obtained by low pressure ion mobility methods in the literature for two reasons. First, the ion size was changed slightly from atmospheric pressure to vacuum because of water (solvent) molecule stripping. Secondly, the low pressure ion mobility experiments were performed in helium, whereas our atmospheric pressure IMS measurements were performed in nitrogen. The use of various drift gases would result in differences in the measured ion cross-sections, due to differences in the dimensions and properties (e.g., polarizability) between helium and nitrogen.

This is the first report that the conventional ion mobility spectrometry / time-of-flight mass spectrometry has been used to study the geometric conformations of several protein ions in the gas phase. The advantage of using the conventional ion mobility analysis to measure the cross-sections of gas-phase protein ions is that a direct examination of ions immediately after production by the ESI source can be achieved. By means of this method, the mass spectrometric discrimination and charge states discrimination due to desolvation/solvation or reactions in the ESI/MS interface are avoided and hence the secondary modifications of ESI generated ions are minimized. Furthermore, the operation of ion mobility experiment at atmospheric pressure is somewhat simpler than the corresponding low pressure experiment.

A newly-developed type of ion mobility measurement called FAIMS offers the potential for simpler and more sensitive measurements than the conventional IMS. One specific aspect of FAIMS development is considered in this thesis, namely the sensitivity enhancement of FAIMS measurements using the 3-dimensional trapping capability.

4-2 FAIMS Ion Trap

An atmospheric pressure ion trap based on the newly-developed FAIMS technique, with which ion separation can be achieved based on changes in ion mobility at high electric fields, has been constructed. With a cylindrical geometry, ion focusing occurred in the annular space between the outer electrode and the inner electrode of the FAIMS analyzer. By pulsing the sampler cone of the FAIMS, ions could be confined in a 3-dimensional space near the spherical terminus of the inner electrode. The ions could be extracted from the trap when the pulse was stepped to a low voltage state. Characterization of the ion trapping was carried out using a laboratory-constructed linear time-of-flight mass spectrometer.

Preliminary results of studies of a FAIMS ion trap that operates at atmospheric pressure and room temperature have been presented in this thesis. The system was evaluated using water cluster ions (in P1 mode, positive polarity of the asymmetric waveform) and trace impurity ions (in P2 mode, negative polarity of waveform) generated by corona discharge. The trapping system was also evaluated with electrospray-generated bio-molecular ions (ubiquitin and cytochrome c). The low molecular weight ions (e.g., water cluster) could be transmitted, with focusing, in P1 mode, while higher molecular weight ions (e.g., proteins) could be transmitted, with focusing, in P2 mode.

Several experimental parameters, which could affect the ion focusing in the FAIMS ion trap, have been investigated. These included the gas flow rate and the applied DV and CV . The flows of gas in the FAIMS ion trap had to be carefully adjusted for maximum

transmission of ions and minimum ion loss to the walls. The best trapping condition could be found when the combination of compensation voltage, dispersion voltage and OR voltage was optimized.

The optimized operating conditions were then used to conduct some experiments to demonstrate ion trapping in the FAIMS. These experiments included a comparison of CV plots and TOF mass spectra between the trapping mode (OR pulsed) and the continuous mode (OR constant); ion transport delays within the ion optics of the FAIMS/TOF-MS system; TOF-MS detection of the pulse of ions after some storage period in FAIMS ion trap; and the effects of ion storage time on trapping of ions in the FAIMS.

The two most important indicators of the three-dimensional trapping of ions in the FAIMS have been observed: first, the existence of a pulse of ions detected via extraction from the trap; and second, a predictable variation in pulse intensity of extracted ions as a function of ion storage time. For example, the half-life of the m/z 380 trace impurity ion in the trap was determined to be 6 ms.

The FAIMS device behaved like an ion filter, capable of transmitting some fraction of a mixture of ions entering the device, by adjusting the CV and the polarity of DV applied to the inner electrode. This ability to pre-separate a targeted analyte from a large excess of other ions may find application to electrospray mass spectrometry in which the mass spectrum could be simplified and the signal-to-noise ratio could be increased.

Our FAIMS ion trap differs from the conventional quadrupole ion trap since it is capable of focusing and increasing the concentration of ions at atmospheric pressure and room temperature. The ions stored in this manner could be released by an applied voltage pulse. FAIMS could concentrate corona discharge ions or electrosprayed ions at

atmospheric pressure before they enter the vacuum for mass analysis. The device would be an ideal storage/preconcentrator for systems that are based on transient detection, including ion mobility spectrometry and time-of-flight mass spectrometry.

Future work on the FAIMS ion trap will focus on the following aspects:

1. Modification of the existing FAIMS ion trap to provide improved focusing of ESI ions. It is possible that further improvements to ion beam focusing can be achieved by modification of the shape of the front of the sampler cone. This might involve addition of extra lenses. Additional improvement may also be achieved by 'shaping' the inside surface at the end of the outer cylinder of the FAIMS that is adjacent to the sampler cone so that it has a curved inner surface to maintain a constant distance between the outer cylinder and the inner electrode at the spherical end of the inner electrode.
2. The ionization chamber will also be modified to make more suitable for electrospray ionization and more efficient for ion desolvation.
3. The FAIMS ion trap is useful as ion storage device at atmospheric pressure. This feature may allow the enhancement of signal and the ability to analyze low amounts of analytes. By using a high resolving power TOF, such as an orthogonal instrument or a reflectron instrument, excellent mass accuracy and resolution should be obtainable.
4. A more thorough study of other parameters that may influence ion trap performance will be necessary, including (1) carrier gases other than nitrogen; (2) variation of the

FAIMS ion trap temperature; and (3) coupling a FAIMS ion trap to a conventional ion mobility spectrometer.

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