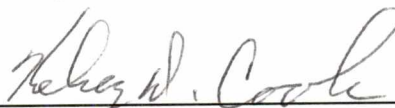


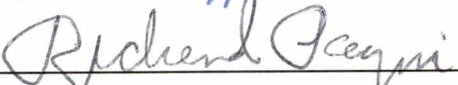
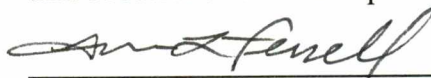
To the Graduate Council:

I am submitting herewith a dissertation written by Mona Shahgholi entitled "FUNDAMENTAL STUDIES OF ELECTROSPRAY MASS SPECTROMETRY." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.



Kelsey D. Cook, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor
and Dean of The Graduate School

FUNDAMENTAL STUDIES OF ELECTROSPRAY
MASS SPECTROMETRY

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Mona Shahgholi

December 1993

DEDICATION

This thesis is dedicated to the members of my family
for their support, love, and trust.

ACKNOWLEDGMENTS

The accomplishment of this work would not have been possible without the grateful assistance of many individuals and organizations. I am eternally grateful to my mother, Farah Shahgholi, for her guidance, support, encouragement, love, and trust. Similarly, I owe many thanks to my sisters for their love and much needed sense of humor. The list of friends and associates who contributed to this work is very long. I sincerely acknowledge all of the individuals at Oak Ridge National Laboratory and University of Tennessee who helped in one way or another to make my work easier.

My thesis committee deserves thanks for the interest shown in my work and for helpful suggestions and corrections for this dissertation. Their review and approval of this dissertation has meant a great deal.

Several organizations are gratefully acknowledged for providing financial support during the last few years. These are the National Science Foundation for the first year assistantship at University of Tennessee, the U.S. Department of Energy and Oak Ridge Associated Universities for the following two years at ORNL, and the Organic Chemistry Section, Analytical Chemistry Division of Oak Ridge National Laboratory for the remaining months.

ABSTRACT

FUNDAMENTAL STUDIES OF ELECTROSPRAY MASS SPECTROMETRY

Electrospray is a new soft ionization technique in which intact gas phase ions of large, nonvolatile molecules present in a solution are produced at atmospheric pressure. In this work, some of the parameters that control the electrospray response of analytes are examined. These are the surface tension of the solution, analyte surface activity, ion-solvation and ion-pairing interactions, acidity/basicity effects, and polarizabilities. The effect of surface tension is studied in samples where the surface tension is modified by addition of cationic, anionic, and nonionic surfactants. The results show an inverse correlation between the surface tension of the solution and the electrospray response of the analyte. Surface activity effects are assessed in solutions containing equimolar amounts of the cationic surfactants, *n*-alkyltrimethylammonium bromides, $C_nH_{2n+1}N^+(CH_3)_3Br^-$ ($n = 6, 10, 12, 14, 16, 18$). Equal intensities are observed for the various *n*-alkyltrimethylammonium salts present in the mixtures, when the concentration of each component is $\leq 2 \mu M$. At higher concentrations, a linear correlation is observed between the electrospray response of a given species and its surface activity presumably because of competition for surface sites on the electrosprayed droplets. To study the effects of ion-solvent interactions on the electrospray response of analytes, a set of tetraalkylammonium bromides (quats) with known solvation enthalpies is utilized. An inverse correlation between the observed intensities and solvation enthalpies suggests that minimizing electrostatic interactions with the solvent improves desorption efficiencies.

Ion pairing interactions are also observed in diquatery ammonium salts with counterions that show different ion association tendencies and in two tetraalkylammonium salts. Ion pairing interactions, primarily noted at high analyte concentrations ($\geq 5 \mu\text{M}$), reduce the observed response of singly charged species and slightly enhance the overall response of doubly charged diquats. The electrospray behavior of a group of amines and amino acids is studied in the final part of this work. The goal is to determine whether thermodynamic measures of acidity/basicity, solvation energies, and polarizabilities could be used to predict their electrospray response. The results of these studies suggest that the calculated polarizabilities of analytes can provide a rough estimate of their electrospray response (in positive mode electrospray) when their pK_a is greater than approximately 9.0.

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CHAPTER 1

INTRODUCTION

Mass spectrometry has long been a cornerstone of analytical chemistry because of its inherent strength, sensitivity, and specificity. Only recently have the advantages of mass spectrometry been applied to the domain of large, nonpolar, thermally labile, and fragile molecules. This was made possible by the advent of improved design in mass analyzers, and, more significantly, by the introduction of new ionization techniques that are "soft" in the sense that they cause little fragmentation of the large, fragile molecules.

These "soft" ionization techniques have been categorized suitably by Vestal¹ according to the state of the sample and the method of energy application. Thus liquid samples can be ionized in a strong electric field by electrospray³ (ES), or atmospheric pressure ionization⁴ (API), and in a weak electrical field by thermospray⁵ (TS). Field desorption⁶ (FD) refers to the ionization of solid samples in a strong electric field. Liquid and solid samples could also be ionized by particle bombardment as in fast atom bombardment⁷ (FAB) and plasma desorption⁸ (PD); or through use of lasers (e.g. in laser desorption⁹ and matrix-assisted laser desorption¹⁰).

This work deals with electrospray ionization mass spectrometry (ES-MS), one of the latest additions to the list of soft ionization techniques. The electrospray process refers to an electrical dispersion of liquids into a spray of charged droplets. For most of its history, electrospray has been noted for its application to a multitude of processes in

industry, such as paint spraying¹¹, crop dusting¹², and fuel atomization in combustion systems¹³; however, it has recently come to be known in the context of mass spectrometry as an ionization technique¹⁴ that is particularly suitable for analysis of large, fragile, non-volatile, and thermally labile molecules, and as a powerful interface for liquid chromatography to mass spectrometry (LC-MS)¹⁵.

Most reports of electrospray mass spectrometry focus on the many advantages of this ionization process and little is said about its limitations, constraints, and peculiarities, issues which are critical to those who routinely use and rely on ES-MS as an analytical technique. Indeed, ES-MS allows detection of very large molecules; however, this is not by any means simple or routine. There are problems associated with running multicomponent samples, samples with high salt concentrations, reproducibility, and so on. Thus, in spite of its conceptual simplicity, the processes that occur between feeding the liquid into the electrospray needle and ion detection are theoretically complex. These processes can be roughly categorized into four stages: spray formation, ion formation, ion sampling and detection. The major stages of ES-MS are depicted schematically in Figure 1.0. Region one shows the formation of electrospray which is a spray of highly charged droplets (stage 1). This is simply achieved by applying high voltage (3 - 4 kV) to a low flow (2 - 10 $\mu\text{L min}^{-1}$) of a moderately conductive liquid through a finely tapered needle. The magnitude of the applied voltage, the surface tension of the sample, its conductivity, and flow rate through the needle are among factors that are important in this stage because they control the actual electrospray separately from the way they affect the mass spectrometric response. These parameters will be discussed in Chapter 2 in a historical

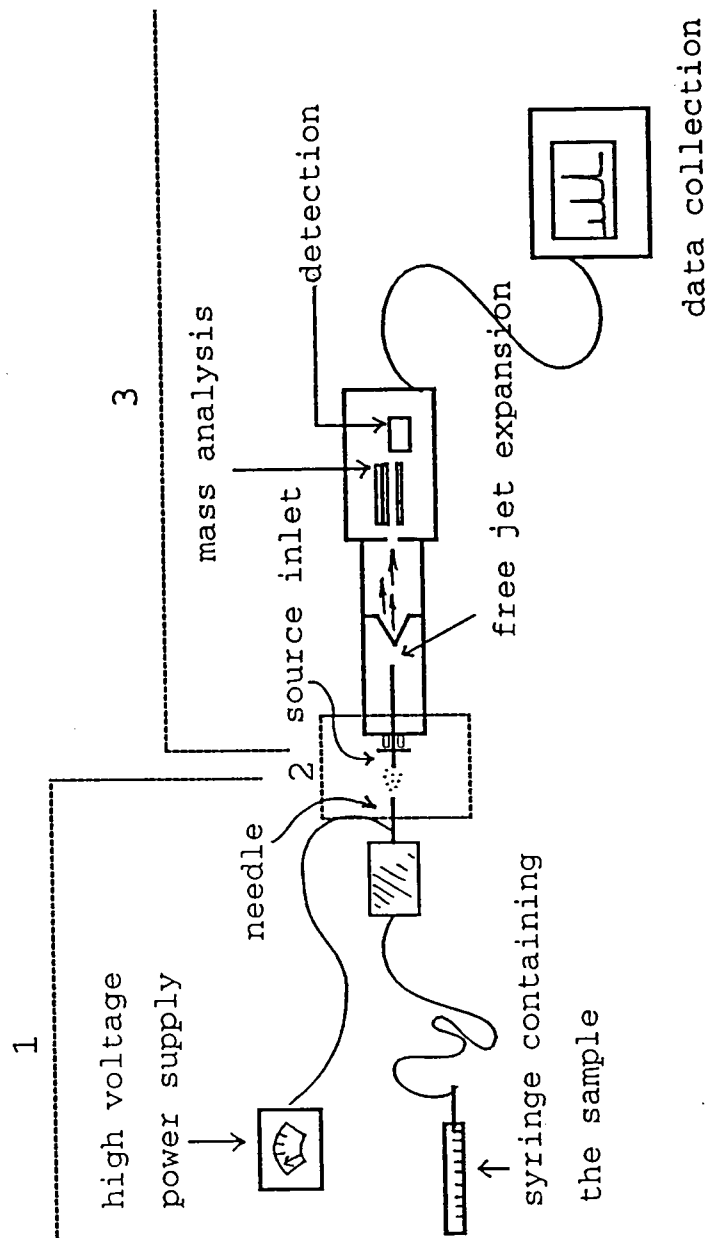


Figure 1.0 Schematic drawing of the processes involved in electrospray mass spectrometry.

and practical context.

Region two schematically depicts the second stage of ES-MS which is the ion formation process. This region is of particular importance because it determines what samples work and do not work in electrospray. Essentially two models of ion production have been proposed for this process. These are the "charge residue model" of Dole and coworkers¹⁶ and the "ion evaporation model" of Thomson and Iribarne¹⁷. Briefly, the charge residue model proposes that ions are the result of solvent evaporation from very small droplets that contain one analyte ion. The ion evaporation model, on the other hand, suggests an active desorption process where an analyte ion clustered with several solvent molecules leaves the charged droplet under the influence of the high surface-field of the electrosprayed droplet. Neither model is entirely adequate and consistent with experimental data; however, they provide a framework for examination of observations made in the laboratory. In this work, mechanistic discussions appear in Chapters 5 and 6, where they seem to be useful in explaining the results of various experiments.

Finally, the third stage of ES-MS (region three) shows the sampling of ions into vacuum to produce an ion beam for analysis by the mass spectrometer. Electrosprayed ions are transported in a free jet expansion into the first region of a differentially pumped mass spectrometer. A skimmer is shown to sample the jet at this point and transmit the ions into the second differentially pumped area where ion focusing is achieved with the aid of a lens assembly. Last, the ion beam enters the third region of the differentially pumped mass spectrometer (housing the mass analyzer) where it is detected. Chapter 3 will discuss the ion sampling and transport processes. A brief theoretical description of

the ES source, some design considerations, and specifics regarding ion transport and ion sampling biases will be considered.

Chapter 4 will describe the actual construction of the ES source that was utilized for the studies reported here. A list of materials used as well as miscellaneous auxiliary techniques will also be presented in Chapter 4. The fundamental studies of ES are the topics of Chapters 5, 6, and 7 where the roles of surface tension and surface activity, ion solvation and ion pairing, and acid/base phenomena are respectively addressed. In general, the studies presented in these chapters focus on the effects of these factors on the mass spectrometric response of electrosprayed analyte ions. The work will close in Chapter 8 with a brief summary and suggestions for future work.

CHAPTER 2

BASIC FEATURES OF THE ELECTROSPRAY PROCESS

Disruption of a liquid into a spray of charged droplets has been known for over two centuries since Bose¹⁸ recorded the phenomenon in 1745. Earlier in this century (1914), the first published experimental study on electrospray was conducted by Zeleny¹⁹, who showed that the disintegration of liquid droplets in an electric field is due to instability rather than the formation of ionic currents. Zeleny further derived an expression that defined the criterion of instability in terms of the applied voltage, the radius of the drop, and the surface tension of the liquid. Despite a good deal of interest in this area for some years thereafter^{20,21}, efforts made in this field were unconcerted until Vonnegut and Neubauer²² noticed that the aerosol produced by electrospraying a variety of liquids exhibited Higher Order Tyndall Spectra (HOTS). HOTS, which are indicative of monodispersity of droplets or particles in the 0.1 to 1 μm range, had been the subject of serious investigation a few years earlier²³. Observation of HOTS in electrospray spurred a new wave of studies of industrial and academic nature in this field. This was largely due to the promise of a way to exploit a charged aerosol of high charge to mass ratio in a variety of industrial applications (examples given in Chapter 1). Herein is presented a discussion on the formation of electrospray and the stages involved in this process, a few comments on the behavior of the spray, and a brief description of two of the more widely accepted studies that model the electrospray process.

DESCRIPTION OF THE VARIOUS SPRAY MODES

The various stages of electrospray have been previously described in detail^{24,25,26,27}. An overview of the various modes of electrospray (as described in literature) is provided in this section in order to create a more unified approach to electrospray terminology.

Formation of an electrospray proceeds through a number of stages as increasingly higher voltages are applied to a metallic needle through which a relatively low flow of a moderately conductive fluid is established. When no voltage is applied to the electrospray needle, liquid droplets detach from the needle at a critical volume which depends on their surface tension. This mode is appropriately called "the no-voltage mode"^{24,25}. A "dripping mode"²⁴ is established when small voltages are applied to the needle resulting in an increase in the dripping rate of the droplets from the needle. Further increase in the applied voltage will result in a "pulsating mode"^{24,25} characterized by the instability of the liquid meniscus which ejects a droplet or filament and subsequently relaxes back to the initial shape. The term "rain mode"^{26,27}, seen in literature, appears to encompass both dripping and pulsating modes. The next stage in the formation of the electrospray corresponding to even higher applied voltages is called the "stable jet"²⁴, or the "electrohydrodynamic mode"²⁵. The electrospray, in this mode, is a cone-shaped plume that emanates from a liquid stem formed at the apex of a cone-shaped liquid meniscus (Taylor cone) (Figure 2.1). It is further characterized by Higher Order Tyndall Spectra (HOTS) when inspected under bright white light. The term "fog mode"^{26,27}, often noted in electrospray mass spectrometric literature, refers to the stable

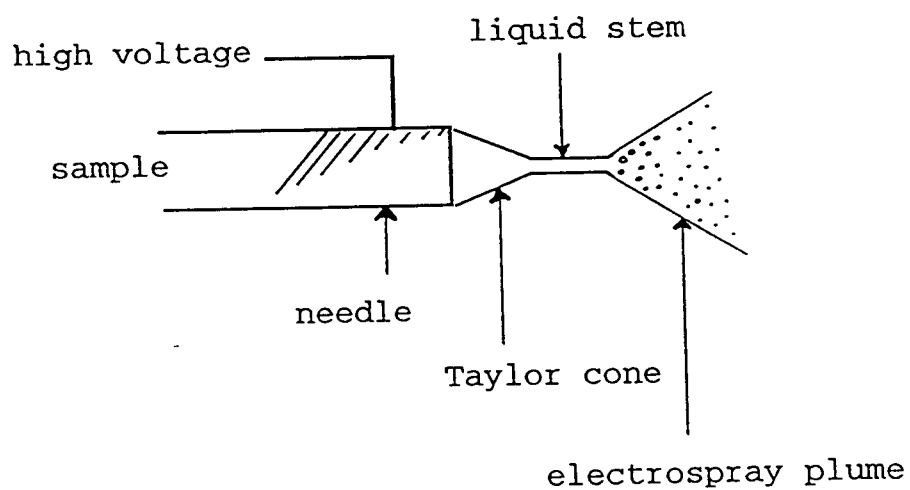


Figure 2.1 Schematic drawing of the Taylor cone.

jet mode described above with the further distinction that it is characterized by a sudden onset voltage, hysteresis (explained later), and "mode hopping" phenomena (explained later).

A subset of the stable jet mode is called the "rainbow mode"^{26,27} and it corresponds to the most stable spray (free from mode hopping annoyances) and the highest achievable electrospray currents. The HOTS are also observed in the "rainbow mode". The stable jet mode is followed by a "high-voltage mode"^{24,26}, also referred to as the "unstable mode"²⁶, where several jets are seen to form around the rim of the electrospray needle. The final mode observed in the formation of the electrospray is that of breakdown to corona discharge, which occurs close to the needle tip and results in a substantial corona current.

LABORATORY OBSERVATIONS OF THE ELECTROSPRAY PROCESS

The previous section provided a general description of the various stages of the electrospray to introduce electrospray terminology. Herein is presented a working description of the electrospray process as observed by the author in the laboratory.

The formation of the electrospray was observed by gradually increasing the potential applied to a needle through which a low flow rate (4 $\mu\text{L}/\text{min}$) of a moderately conductive fluid was maintained with the aid of a syringe pump (Figure 2.2 A - 2.2 G). When the solution flow is started in the absence of any applied voltage liquid drops form at the end of the needle. They detach and fall off when they become large enough so that the gravitational force due to their mass exceeds the surface tension forces that attach

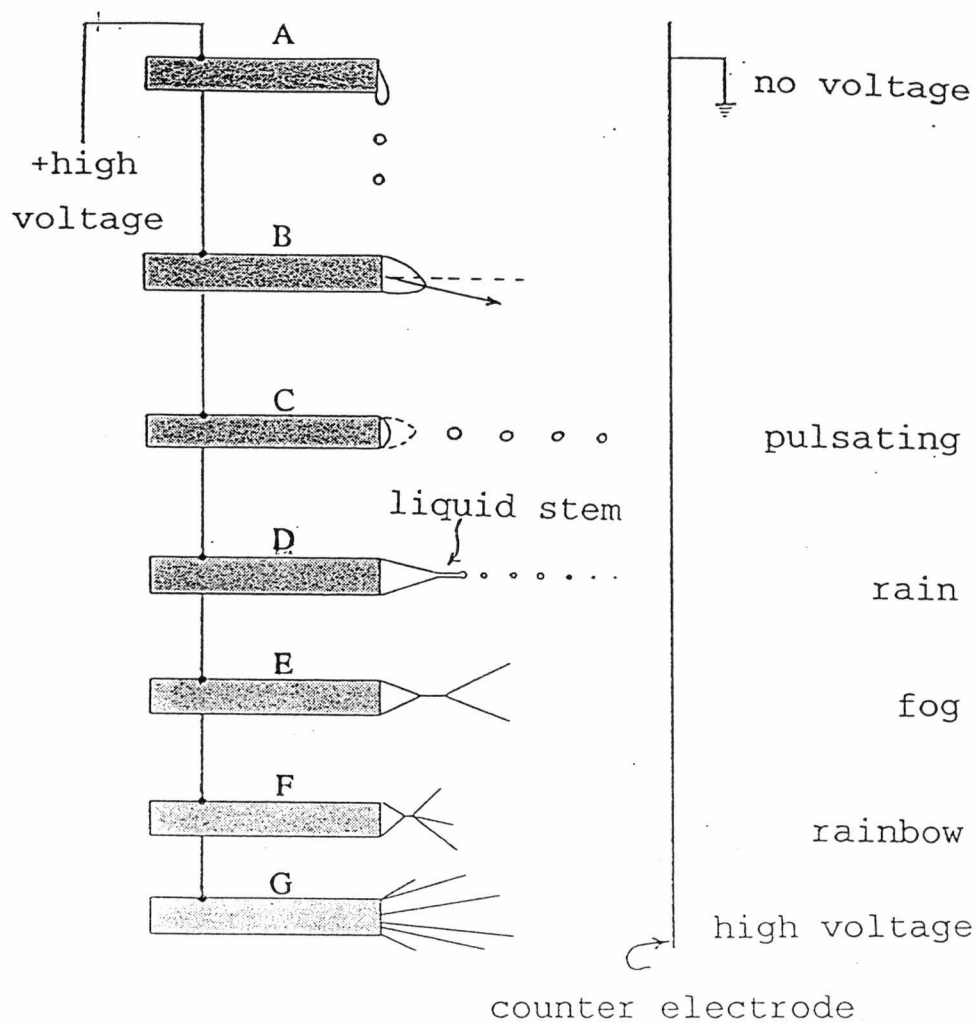


Figure 2.2 Schematic drawing of the various stages in the formation of an electrospray.

them to the liquid column in the needle (Figure 2.2 A). As a voltage is applied to the needle the trajectories of the falling drops begin to develop a horizontal component in the direction of the end plate, the counter electrode (Figure 2.2 B - shown as an arrow). With the liquid flow rate held constant, increases in the applied potential result in a decrease in the size of the droplets, a higher frequency of detachment, and a completely horizontal component to their trajectories (Figure 2.2 C). Further increase in the applied voltage results in the formation of a stationary liquid stem at the needle tip, a few millimeters downstream of which a cone-shaped fog can be seen (Figure 2.2 D & E). This visible spray gradually fades away as we move further downstream because the droplets have become too small to scatter light. Increasing the applied voltage beyond this point results in shortening of the column of liquid and a narrowing of its tip to a fairly sharp point (Figure 2.2 F). This is followed by a condition in which the liquid column has disappeared (meniscus is retracted into the needle) and the spray appears to start from multiple points scattered around the needle (Figure 2.2 G). At sufficiently high voltages breakdown to corona discharge occurs and the spray collapses.

It is important to identify the various modes of the electrospray because a mass spectrometric response is not observable under all modes, and further, the ions detected depend on the mode of operation. Indeed, it has been reported that a mass spectrometric response is not observable when the electrospray is in the "rain mode" because the droplets are too large to evaporate at a sufficiently fast rate that would allow the necessary fields for ion desorption to develop at the droplet surface²⁶. The "rainbow" mode has been suggested to be the most efficient, i.e., provide the highest yield of solute

ions²⁷. Different ions have also been detected when the same sample was electrosprayed in the stable jet and high-voltage modes^{28,29}.

GENERAL COMMENTS ON THE BEHAVIOR OF THE ELECTROSPRAY

Mode hopping phenomenon^{26,27}

This is an experimentally irritating aspect of the electrospray experiment since it results in irreproducible signals. As implied by the name, it refers to the electrospray hopping between two distinct stages of electrospray; usually, between the dripping mode and the stable jet mode. Mode hopping can be induced by relatively minor external disturbances; for example, blowing air across the spray, or changes in liquid flow rate (pulsations from an inferior quality syringe pump), or changes in ambient humidity levels. Indeed, at higher than normal flow rates, one might see a "mixed" mode where a fog is seen with a liquid thread in the middle. The mode hopping frequency depends upon the magnitude of applied voltage as well as the flow rate. Mixed-mode operation is undesirable for electrospray mass spectrometry (ES-MS) because it results in a fluctuating signal at the detector. It can generally be avoided by reducing the liquid flow rate, changing to a new, well-tapered needle, and controlling external disturbances (ambient humidity levels and air drafts).

Hysteresis^{26,27}

This refers to the phenomenon that once the electrospray is established in a

particular mode, for example, the stable jet mode, it can be sustained at voltage differences less than that required to start it. However, the spray is very sensitive in this condition and reverts back to a lower voltage mode upon any minor disturbance, such as blowing over the plume.

Droplet Size

Knowledge of the exact size of the electrosprayed droplets is of relevance in understanding the mechanism underlying electrospray ionization. Based on laser scattering measurements of ion spray plumes, the average droplet size has been estimated at $15\text{ }\mu\text{m}^{30}$. This value is not universally accepted because of the high flow rates that are typically used in the ion spray technique ($40\text{ }\mu\text{L}/\text{min}$). It is suggested that the reported value possibly reflects the size of the larger droplets in a spray consisting of both small and large droplets²⁶. Observation of HOTS^{27,31} in the spray under optimal conditions suggests a smaller droplet size. HOTS are seen under white light as an ordered display of colors from gold in the region close to the needle to purple and blue farther away. Existence of distinguishable HOTS suggests that the droplets are fairly monodisperse with diameters ranging between 0.1 to $1\text{ }\mu\text{m}$. If the droplets were outside this size range, they would not scatter light selectively by color, and if they were not monodisperse the selectively scattered components would cancel each other leading to a white reflection.

Electrospray Stability

It has been shown that a necessary condition for the production of stable sprays is the existence of electrical shear stresses on the surface of the Taylor cone^{32,33}. For an interface to be subjected to shear stresses, the surface must simultaneously support a surface charge and be subjected to a tangential electrical field. When high voltage is applied to a flowing liquid that contains sufficient free charge, the electric field within the liquid drives the free ions (same polarity as the applied voltage) to the surface of the cone of liquid at the tip of the needle; hence, the surface of the liquid is charged and subjected to a normal (perpendicular) electric field (E_n), $E_n = q/\epsilon_0$, where q is the surface charge density and ϵ_0 is the permittivity of free space. Additionally, charge separation and the semi-insulating nature of the liquid result in a potential drop between the base of the needle and the tip of the liquid cone. This potential drop ensures that the surface is also subjected to a tangential electrical field (E_t) in the direction of the flow ($E_t = \Delta\Phi/l$, where $\Delta\Phi$ is the potential difference and l is the distance along the cone interface) and, hence, to an electrical shear stress on the surface of the cone. This stress smoothes out the surface and keeps the shape of the liquid tip relatively undisturbed²⁴ (Figure 2.3).

From the above discussion on spray stability (in terms of forces that act on a liquid surface) it becomes apparent that, in practice, three parameters are significant in controlling the stability of the spray, namely, the conductivity of the solution, the flow rate of the sample, and the applied field. At a given flow rate and applied field, an optimum conductivity range is necessary to produce the most stable spray. With insulating liquids, no electrostatic force can build on the surface of the liquid because of

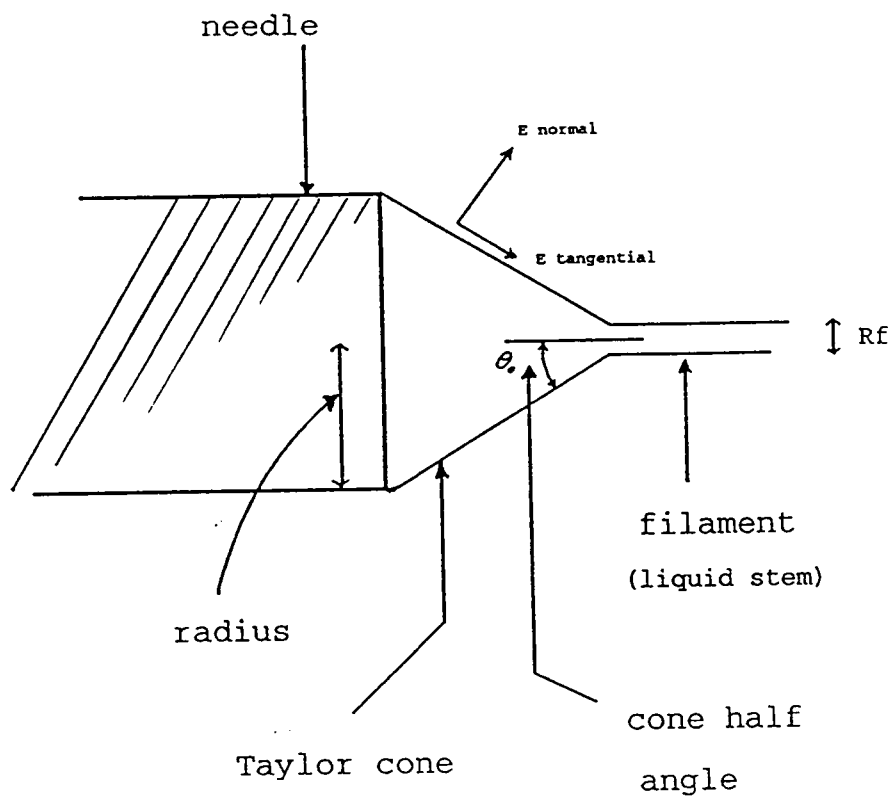


Figure 2.3 Schematic drawing of the Taylor cone showing the electrical forces acting on the cone, the cone half angle, and the filament.

a lack of a sufficient number of free charge carriers in the bulk of the liquid. In such instances, the droplet remains attached to the needle and continuously grows in size until its volume reaches a critical level beyond which it is expelled from the needle and the process is repeated. The relaxation time (the period of such charge/discharge cycles) is exceedingly long and the major force acting on the liquid is a dielectrophoretic force (polarization), which causes the liquid to migrate to the region of highest field intensity, namely, the tip of the needle. On the other hand, with highly conductive liquids, the major force acting on the liquid is the electrophoretic force which will induce the flow of ions to the surface resulting in a normal ($\perp$) electrical field at the surface. However, the spray remains unstable because there is no tangential component to the electrical field (it is not possible to develop a potential drop in a highly conductive environment) and the relaxation time is shorter than the time required for the formation of a drop. This means that surface charges may build up in excess of the Rayleigh limit (amount of charge that a liquid droplet can accommodate without disrupting) before a drop can completely form and break away from the surface.

The parameters that were suggested to control spray stability, namely the conductivity of the liquid, its flow rate, and the magnitude of the applied voltage, are briefly discussed below.

Solution conductivity

Conductivities of a number of samples were measured to become familiar with the range of conductivities of the ES samples in the matrices of interest (e.g., water,

Table 2.0 Conductivity measurements of a few representative electrospray solutions

Sample	Conductivity ($\Omega^{-1} \text{ cm}^{-1}$)
deionized water*	2.23×10^{-9}
100 μM arginine in water*	7.70×10^{-8}
100 μM arg in methanol	1.20×10^{-8}
100 μM arg in 50:50 (v/v) water:methanol	1.46×10^{-8}
100 μM arg in water + 1% (v/v) acetic acid*	6.38×10^{-8}
100 μM arg in methanol + 1% (v/v) acetic acid	1.56×10^{-8}
100 μM arginine in 50:50 (v/v) water:methanol + 1% acetic acid	7.22×10^{-8}
50:50 (v/v) water:methanol + 1% (v/v) acetic acid	7.22×10^{-8}

* These samples could not be electrosprayed.

methanol, water:methanol, etc.), and to verify that they were indeed in the prescribed range (10^{-8} - $10^{-10} \Omega^{-1} \text{ cm}^{-1}$)³². A few sample measurements are given in Table 2.0, demonstrating the range of conductivities of the samples utilized throughout this work. In practice, all of the prepared solutions (with the exception of the 100% aqueous samples) were within the acceptable conductivity range for the formation of the electrospray. Even neat samples of solvents (with the exception of deionized water) were found in the specified range because of the presence of a variety of ionic impurities such as Na^+ and K^+ ions, and others.

Liquid flow rate

Liquid flow rates are constrained to a range determined by the rate of droplet evaporation and the electrophoretic flow. This means that flow rates cannot be increased above an upper limit because of incomplete desolvation of the droplets, nor decreased beyond a lower limit due to the presence of an electrophoretic flow established by application of high voltage. In practice, electrospray is seen with flow rates in the range 0.5 to 40 $\mu\text{L}/\text{min}$ depending on the voltages applied, efficiency of the droplet evaporation process, and the use of a nebulizer (as seen in ion spray)³⁴. More recently, higher flow rates have been tolerated with new advances such as thermally assisted electrospray (500 $\mu\text{L}/\text{min}$)³⁵ and ultrasonic nebulization API (2 mL/min)³⁶.

Applied voltage

For a given needle-counter electrode separation distance, the magnitude of the

voltage applied to the needle depends primarily on the flow rate of the sample assuming that it is within the proper conductivity range. In practice, for a given sample flow rate, the voltage is increased until visual inspection of the spray shows that a stable spray is established. In the work presented here, the applied voltage was in the +3 to 4 kV range for a 4-mm needle-counter electrode separation distance and a flow rate of approximately 4 $\mu\text{L}/\text{min}$.

MODELS OF ELECTROSPRAY

To close this chapter, a description of the mechanism suggested for the formation of the charged droplets (first stage of the ES-MS process) will be presented. This stage is widely believed to be due to an "electrophoretic" charging mechanism; i.e., a result of the separation of positive and negative ions in solution upon application of an external electric field³⁷. Of the large number of studies on electrospray that have predated the modern mass spectrometric work^{38,39,40,41,42}, two models in the work of Pfeifer and Hendricks⁴⁰ and that of Smith²⁵ are found to be most successful in describing the above mechanism.

The model of Pfeifer and Hendricks gives an expression (Equation 2.1) for the specific charge of the droplets as a function of fluid conductivity, electric field at the needle tip, and the mass flow rate through the needle.

$$q_s \approx [(4\pi/\epsilon)^3 (9\gamma)^2 (\epsilon_0^5/\rho^4)]^{1/7} (\sigma E_0/M)^{3/7} \quad \text{Equation 2.1}$$

where q_s is the specific charge of the droplets, ϵ is the permittivity of fluid, γ is the surface tension of the working fluid, ϵ_0 is the permittivity of vacuum, ρ is the density of

the working fluid, σ is the conductivity of the solution, E_0 is the imposed electric field (in vacuum) at the needle tip, and M is the mass flow rate. Equation 2.1 can be rewritten into a more useful form in terms of the electrospray current as shown in Equation 2.2 below:

$$I = [(4\pi/\epsilon)^{3/7} (9\gamma)^{2/7} (\epsilon_0^{5/7})] V^{4/7} (\sigma E_0)^{3/7} \quad \text{Equation 2.2}$$

where V is the volume flow rate (volume/time). The expression for E_0 is^{25,40,43} (Equation 2.3):

$$E_0 = 2V_0/r \ln(4d/r) \quad \text{Equation 2.3}$$

where r is the outer radius of the needle, d is the distance from the needle tip to the planar counter electrode, and V_0 is the onset potential for the electrohydrodynamic mode.

This model accounts reasonably well for the dependence of the electrospray current on parameters such as the electric field, flow rate, and conductivities. However, quantitative agreement with experiment is not always obtained. This is partly a result of the assumptions that were used in developing this model. For example, the derivation of the Hendricks model only considers the Taylor cone⁴⁴ as the site where droplet formation occurs and assumes that the charge on the cone eventually all ends up on the emitted droplet. Furthermore, it assumes that the formed droplets are "Vonnegut" droplets which have one half of the charge of Rayleigh droplets. The assumption of sequential droplet charging and emission is not suitable for the general electrospray conditions where the Taylor cone is drawn out into a thin liquid filament^{24,25,45}. It is more applicable to electrospray operated at higher electric fields where droplet emission is very rapid and a filament is not formed.

Smith's model²⁵ is based on his results of experiments that characterized the electrohydrodynamic atomization of liquids in terms of the fluid delivery system and the liquid properties. The model provides an expression which predicts the droplet current for electrospray under the electrohydrodynamic mode of operation (Equation 2.4):

$$I \sim \gamma^{3/2}/(\rho\sigma R_f^{1/2}) \quad \text{Equation 2.4}$$

where γ is the surface tension, ρ is density, σ is the liquid conductivity, and R_f is the diameter of the filament. In Smith's model, a charged liquid sheath is assumed to travel down the cone surface and join at the tip to form a highly charged thin liquid filament. Instabilities set in, and the filament breaks up into a plume of charged droplets. The problem with Smith's model is that his expression for the electrospray current includes one parameter, R_f , the diameter of the filament, which must be obtained from experiment. Therefore, Smith's final equation is incomplete. However, a partial result obtained by Smith giving the required electric field for the onset of the electrohydrodynamic mode is of practical utility. This equation is (Equation 2.5):

$$E_0 = [2\gamma\cos\theta_0/\epsilon_0 r]^{1/2} \quad \text{Equation 2.5}$$

where γ is the surface tension of the liquid, θ_0 is the cone half angle, r is the radius of the needle, and ϵ_0 is the permittivity of free space (Figure 2.3). The actual electric field, E_0 , at the tip of a blunt needle of radius r and a distance d away from a disk-shaped counter electrode can be estimated from Equation 2.3 shown earlier. Combining the equations for E_0 , i.e., Equations 2.3 and 2.5, will give an expression that demonstrates the voltage dependence of the electrohydrodynamic mode (Equation 2.6):

$$V_0 = A_1 [2T r \cos\theta_0 / \epsilon_0]^{1/2} \ln(4d/r) \quad \text{Equation 2.6}$$

where A_1 is a geometric constant (for a point-to-plane field geometry).

Kebarle et al.³⁷ have indeed verified the utility of the above equation in predicting V_0 for electrospray. Thus Smith's treatment, although incomplete, provides a useful expression for electrospray.

SUMMARY & CONCLUSIONS

Prior to the flood of activity in the area of electrospray mass spectrometry, many studies, fundamental as well as industrial, were carried out in this area. These studies have in general examined the spray and the physical parameters that govern its formation and behavior. Furthermore, they have attempted to model the processes of liquid cone instability and droplet formation in high electric fields. Much of this chapter has dealt with examining the electrospray in that manner. Thus, the formation of the spray, the various modes that it can go through, and the parameters of significance in its behavior were described in sufficient detail. This is an important aspect of ES-MS because it allows the practitioner to decide if an observation is of chemical (related to analyte) or physical origin (from the perturbation of the spray). Additionally, since only certain modes of the spray are useful mass spectrometrically, familiarity with the spray is beneficial. Invaluable as they are, the studies referred to in this chapter generally ignore the origin of charge on the droplets, i.e., the presence of ions and their nature. The mass spectrometric approach attempts to address the electrospray puzzle from a more chemical angle, dealing with ions in solution and their behavior in electric fields. This aspect of electrospray will be discussed in the following chapters of the work presented here.

CHAPTER 3

ELECTROSPRAY MASS SPECTROMETRIC INSTRUMENTATION

TRANSPORT OF IONS INTO VACUUM

The previous chapter dealt with the formation of the electrospray which is a dispersion of analyte ions, generally desorbed as ion-solvent clusters, and small highly charged droplets. This chapter discusses the transport of electrosprayed ions into vacuum for mass analysis. In electrospray mass spectrometry (ES-MS), the ions generated by the electrospray process are transported into the mass spectrometer by means of a free jet expansion from atmosphere into the vacuum conditions of the MS system. This results from the expansion of a gas from a high-pressure region into a low-pressure region through an orifice with diameter D , where $D \gg \lambda_0$ (λ_0 is the mean free path of the gas in the high-pressure region). Under this condition, the species in the free jet expansion experience many collisions as they flow through the orifice as well as downstream of the orifice. The above process results in the conversion of the enthalpy of random motion into directed mass flow through the nozzle, and hence, the formation of an intense axial molecular beam. Another consequence of the above condition is rapid translational cooling of the molecules present in the beam. The above source is referred to as a supersonic jet because the classical speed of sound " a " decreases with cooling while " u ", the mass flow velocity, increases with each collision during the expansion⁴⁶. The schematic representation of a supersonic expansion is shown in Figure 3.1⁴⁷. As demonstrated, the gas flows from a high pressure region P_0 (at temperature T_0) through

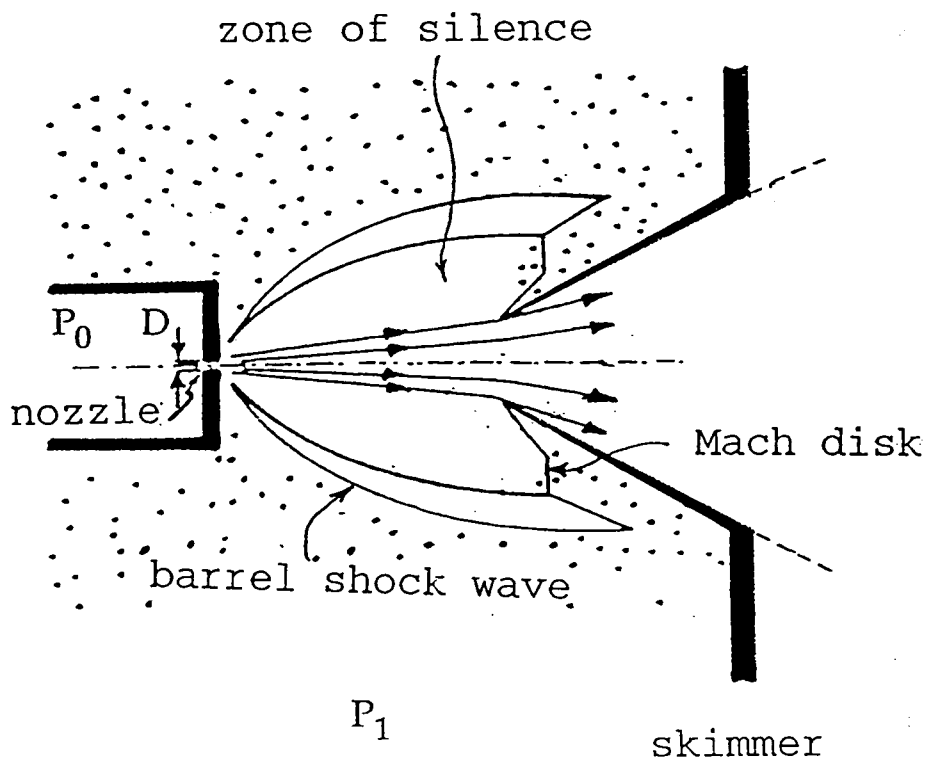


Figure 3.1 Schematic drawing of a beam source skimming in the region of overexpansion of a free jet zone of silence; reference 47.

a nozzle with an orifice diameter of D into a region of pressure P_1 . The interaction of the directed axial flow with the random flow of gas in the chamber is depicted as a well-defined free jet shock wave system called the barrel shock wave. The Mach disk is shown as a plane shock wave normal to the jet axis defining the lower boundary of the barrel shock wave. The region within this envelope is called the zone of silence, representing an area where the flow regime develops under isentropic conditions, finally reaching a state of "free flow" where collisions do not exist and cooling has been maximized. The skimmer is also shown in Figure 3.1 as a cone that samples the central portion of the beam. In the boundary between the barrel shock wave and the ambient gas, ions and gas molecules undergo many collisions, resulting in a scattering of the beam of ions.

The final ("coldest") temperature achievable in the supersonic expansion can be described by the isentropic equation for the state of an ideal gas as follows:

$$T/T_0 = (P/P_0)^{(\gamma-1)/\gamma} \quad \text{Equation 3.1}$$

where T and P are the translational temperature and pressure in the beam; T_0 and P_0 are the translational temperature and pressure in the high pressure region; γ is the heat capacity ratio C_p/C_v . In this equation, $\gamma \geq 1$ for polyatomic gases and $\gamma \leq 5/3$ for monoatomic gases. Equation 3.1 can be expressed in terms of a different parameter called the Mach number, M giving (Equation 3.2):

$$T = T_0 [1 + 1/2(\gamma - 1)M^2] \quad \text{Equation 3.2}$$

The Mach number is defined as the ratio of mass flow velocity to the velocity of sound. It is also expressed as a function of the distance away from the nozzle by Equation 3.3:

$$M=A(X/D)^{\gamma-1} \quad \text{Equation 3.3}$$

where γ is the heat capacity ratio C_p/C_v ; A, is a constant which depends on γ ($A=3.26$ for a monoatomic gas); X, is the distance downstream from the nozzle; D, is the diameter of the nozzle. Equation 3.3 shows that the Mach number, M, increases with distance away from the nozzle and that increasing the nozzle diameter enlarges the expansion field, i.e., molecules must travel farther to attain a given Mach number.

Several characteristics of supersonic expansions are given in Figure 3.2⁴⁸. For example, it can be seen that both temperatures and densities decrease with distance away from the nozzle while the Mach number increases. As shown by Anderson and Fenn⁴⁹, this process cannot continue indefinitely because the above equations were derived on the assumption that the expanding beam is a continuous medium. This assumption no longer holds in the final stages of jet expansion where densities are so low that all 2-body collisions essential to redistribution of velocity required by the hydrodynamic equations of flow cease to occur. In other words, since the total number of collisions is finite, the Mach number and also the temperature reach terminal values. The terminal Mach number M_T for this free flow regime is given by:

$$M_T=2.05\epsilon^{(1-\gamma)/\gamma}(\lambda_0/D)^{(1-\gamma)/\gamma} \quad \text{Equation 3.4}$$

where ϵ is called the collisional effectiveness constant. Evaluating Equation 3.4 for Argon gives:

$$M_T=133 (P_0 D)^{0.4} \quad \text{Equation 3.5}$$

with P_0 in atm and D in cm. The product $P_0 D$, represents the number of two-body collisions during the expansion and consequently, characterizes the degree of cooling.

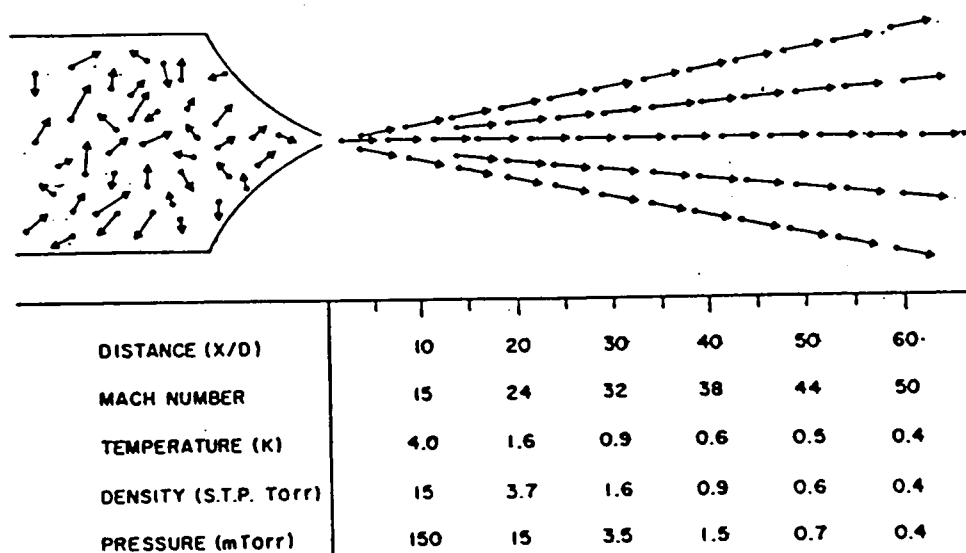


Figure 3.2 The supersonic (free jet) expansion. Several characteristics of the expansion are given as a function of X/D , where D is the nozzle diameter, and X is the distance downstream. The values given are based on a stagnation pressure of 10 atm of He at 300K. 1 S.T.P. Torr = 3.54×10^{16} molecules/cm³; reference 48.

Mach disk The distance from nozzle is given by:

$$x_M = 0.67 D (P_0/P_1)^{1/2} \quad \text{Equation 3.6}$$

where D is the diameter of the nozzle, P_0 is the upstream (atmospheric) pressure, and P_1 is the downstream pressure in the vacuum chamber⁴⁷. x_M is of significance in the design of the electrospray source for two reasons. Firstly, the Mach disk must be confined to the source boundaries! Secondly, it determines where the beam should be sampled for best results. The sample calculations shown below demonstrate how x_M determines the physical boundaries of the source:

example 1. D= 0.1 mm

$$P_1 = 10^{-5} \text{ Torr}$$

$$x_M = 670 \text{ mm (too far out)}$$

example 2. D= 0.3 mm

$$P_1 = 1.2 \text{ Torr}$$

$$x_M = 5 \text{ mm from the nozzle}$$

example 3. D=0.5 mm

$$P_1 = 2.8 \text{ Torr}$$

$$x_M = 5.6 \text{ mm (ES system built in the laboratory)}$$

Thus, x_M is determined by the pumping speed available in the area near the nozzle opening⁵⁰; for example, a low pumping speed in the nozzle area (high pressures) translates into a short x_M . With respect to the issue of sampling of the supersonic jet, there are two questions to consider: why is the beam sampled, and at what distance away from the nozzle is this usually done? The central portion of the beam is usually sampled into the next vacuum stage by means of a skimmer (Figure 3.1) in order to allow differential pumping of the mass analyzer. Sampling of the beam can be done from at

least two sites. This is pictorially described in Figure 3.3⁵¹. In sampling the beam from the zone of silence (Figure 3.3 A) the skimmer penetrates through the Mach disk into a region where the beam is highly directional. Collection of ions from this site is very efficient; however, the ions in this region are more prone to be clustered with neutral gas molecules because of the strong rapid cooling that occurs during the expansion. The beam could alternatively be sampled from behind the Mach disk (Figure 3.3 B). In this case, ion extraction and focusing is difficult because the ions and gas molecules have undergone extensive scattering; however, clustering is reduced due to heating of gas through collisions in the Mach disk. The choice of sampling site is generally made by the operator based on sensitivity and resolution requirements.

In practice, the location of the Mach disk can be determined by monitoring the downstream pressure, P_1 , as the distance between the nozzle and skimmer is increased. What is noticed in this process is a sudden decrease in pressure (or beam intensity) with increasing distance followed by a rise in pressure to a maximum value. This behavior is shown in Figure 3.4 and is attributed to the presence of the Mach disk (shock wave) at the skimmer inlet⁵². This shock wave is "swallowed" at very short nozzle-skimmer distances corresponding to high pressures and beam intensities. Under this condition, the skimmer essentially behaves as a nozzle. The abrupt decrease in pressure and intensity is seen when the shock wave "pops out" or forms in front of the skimmer inlet as the distance between the nozzle and skimmer is increased.

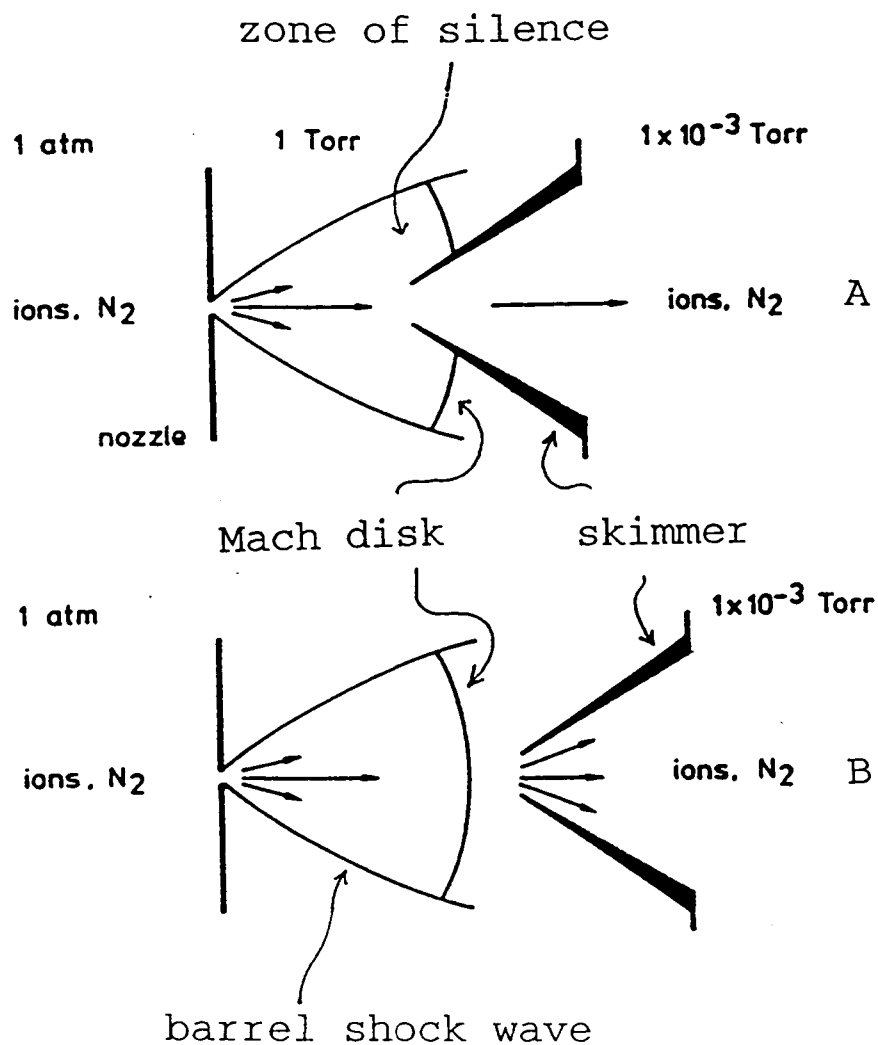


Figure 3.3 Expansion of gas and ions into vacuum. A: sampling from the silent zone; the skimmer penetrates through the Mach disk. B: sampling with a skimmer beyond the Mach disk; the beam is scattered by passage through the Mach disk; reference 51.

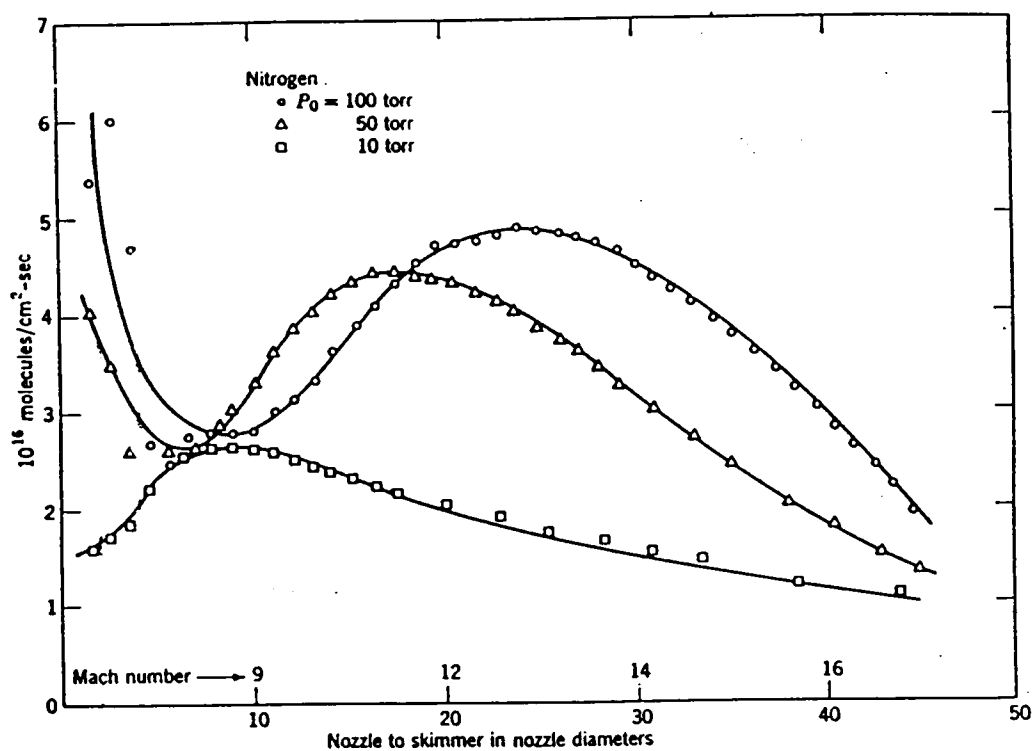


Figure 3.4 Measured beam intensities at various nozzle pressures as a function of nozzle-skimmer distance (Mach number). Data are from Deckers and Fenn for nitrogen at room temperature; reference 52.

Skimmer⁴⁹

A cone with a sharp point has been suggested as the practical skimmer shape (Figure 3.1). Immersion of the skimmer cone in the beam gives rise to the formation of a shock wave known as the bow shock wave. The angle of the skimmer cone is significant in "attachment" of this shock wave to the cone apex or tip during the sampling of the beam. If the cone angle is greater than a critical value for a particular Mach number the shock wave will be "detached", i.e., there will be a finite distance between the shock wave and the cone tip. In this region, the gas will be in a post-shock state of subsonic Mach number and high temperature where all the advantages of a supersonic beam are lost. Therefore, the cone angle must be such that the shock wave will be as close as possible to the cone apex, i.e., "attached". For cone angles of 60° or less, all bow shock waves at Mach numbers above 1.5 will be attached. Larger angles could be tolerated at higher Mach numbers.

The skimmer inlet diameter is also of concern in source design for a number of reasons. First, the diameter of the skimmer inlet must be small enough to avoid collisional perturbation of the beam when the bow shock wave is attached. An inlet diameter of the order of the mean free path in the beam generally meets the above size requirement. Second, the edge of the skimmer opening must also be very sharp, smooth, and burr-free to minimize possibility of collisional perturbation of the skimmer flow by molecules reflected from the skimmer surface after the inlet. And last, the inlet diameter influences the pressures that can be achieved in the skimmer exhaust.

Ion Sampling Orifice (Nozzle/ Capillary Inlet) and Ion Sampling Site

A number of approaches have been reported for sampling of ions from the atmospheric region into the source. These range from a simple orifice in a thin plate^{51,53,54} to glass capillaries with metallized ends^{15,55} or stainless steel capillaries⁵⁶. The choice of a particular approach depends on a number of factors. For example, stainless steel capillary inlets are more durable than glass capillary inlets. On the other hand, the ends of the glass capillary inlets can be biased to different voltages, if necessary, whereas the same cannot be done with metal plates or capillaries. The important point is that all of the named approaches have been utilized successfully and have not been problematic.

In addition to the type of sampling inlet to be used in the ES source (e.g., capillary versus a simple orifice), the geometry of the inlet also requires consideration. In general, the geometry of the sampling inlet determines the electric field distribution that is important in efficient nondiscriminate ion sampling. However, this process is more complicated in sources where ionization occurs at atmospheric pressure because the fields in the ion sampling region are determined primarily by build-up of space charge and only to a minor extent by the geometry of the source inlet and the potentials applied⁵⁷. Space charge effects result from mutual electrostatic repulsion of ions in a unipolar cloud as it moves along the electric field lines through a gas. The visible manifestation of space charge is the expansion or space-charge "blow-out"⁵⁸ seen in the ion drift space. The physical consequence of space charge which is of significance to the ion sampling process is that, for the point-to-plane geometry (e.g., the source in use), the electric fields are

enhanced in the vicinity of the point of ion formation (the ES needle) and the counter electrode (the sampling orifice), and slightly suppressed in the drift region (Figure 3.5)⁵⁹. An expected consequence of the distorted field lines is a greater extent of scattering for lighter ions, i.e., a bias in sampling ions of low mass. The above discussion on space charge effects in the ES source suggests that, in electrospray mass spectrometry, the mass spectrum may not be representative of the actual species present at atmospheric pressure. Thus, it becomes essential to assess the extent of ion sampling bias (mass discrimination) for the ES-MS instrument in use in order to be able to reliably interpret the acquired data. Two experiments were performed to examine the extent of ion sampling bias in the source that was constructed in the laboratory; the results of these are presented in Chapter 4 (Experimental Techniques).

Another significant consequence of space charge deals with how it affects sensitivity. The *unipolar charge drift formula*⁶⁰ describes the rate of change of charge density along the path of ions (of one sign and mobility) in an electric field. This formula is independent of source geometry and describes exactly the inexorable spreadout of charge density along the path of a cloud of unipolar ions drifting with constant mobility in an electrical field. The important consequence of this formula is that ion density becomes a function of ion drift times and independent of the initial ion density. Thus, for a given ion drift time, signal cannot be improved by increasing the initial charge density as might be accomplished, for example, by increasing a discharge current. Furthermore, it becomes impossible to concentrate ions in a space-charge-dominated source (SCD) by applying electric fields⁵⁷. Optimum sensitivity is only achievable by

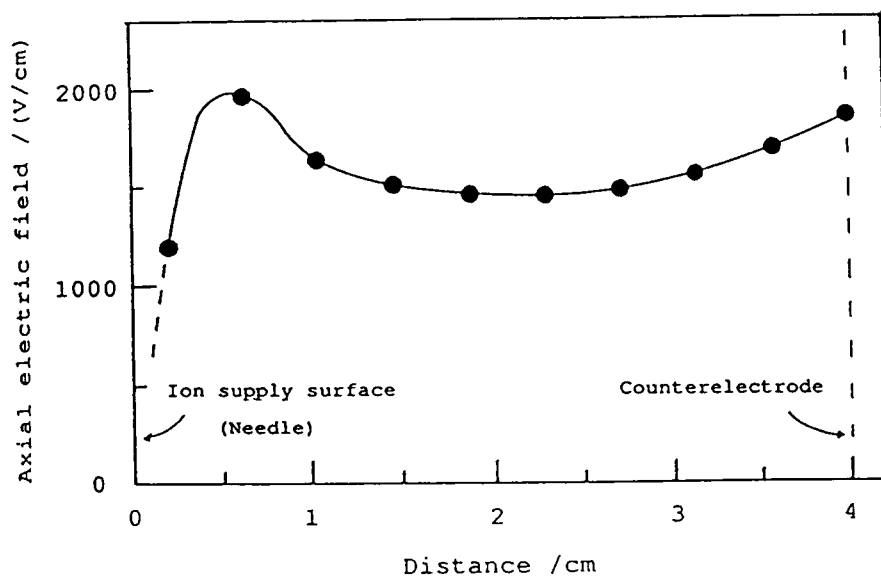


Figure 3.5 Simulated axial electric field distribution in a corona API with 4 cm gap.
Discharge current is 3 μ A; reference 59, 60.

reducing ion drift times thereby increasing charge densities at the counter electrode.

ELECTROSPRAY DESOLVATION AND CLUSTER ION REMOVAL

Earlier, the electrospray plume was described as a dispersion of analyte ions, generally desorbed as ion-solvent clusters, and small highly charged droplets in various stages of drying (desolvation). For the mass spectrometrists who are interested in mass analysis of the electrosprayed ions rather than examining cluster ions, efficient electrospray desolvation and cluster ion removal is a significant consideration in the design of the source. Furthermore, if water or solvent molecules are not excluded from the area around the sampling inlet, new clusters of ions with water or the solvent will form as a result of cooling during the adiabatic expansion of gas from the atmosphere to the vacuum in the source housing. Instrument designs thus attempt to efficiently desolvate the electrospray plume and to circumvent further formation of new clusters within the source during the jet expansion.

The above task is generally achieved by one of two methods. A curtain⁶¹ or countercurrent flow of a dry gas¹⁵, usually nitrogen, at the source inlet where ion sampling occurs (Figure 3.6 A & B)⁵¹ has been one approach to desolvate the ES plume and to exclude water vapor and other neutrals from entrance into the vacuum system. This approach also minimizes source contamination. Desolvation and cluster removal has also been achieved by heating the capillary inlet that samples the electrospray⁵⁶ or the nozzle-skimmer and the ionization regions⁶².

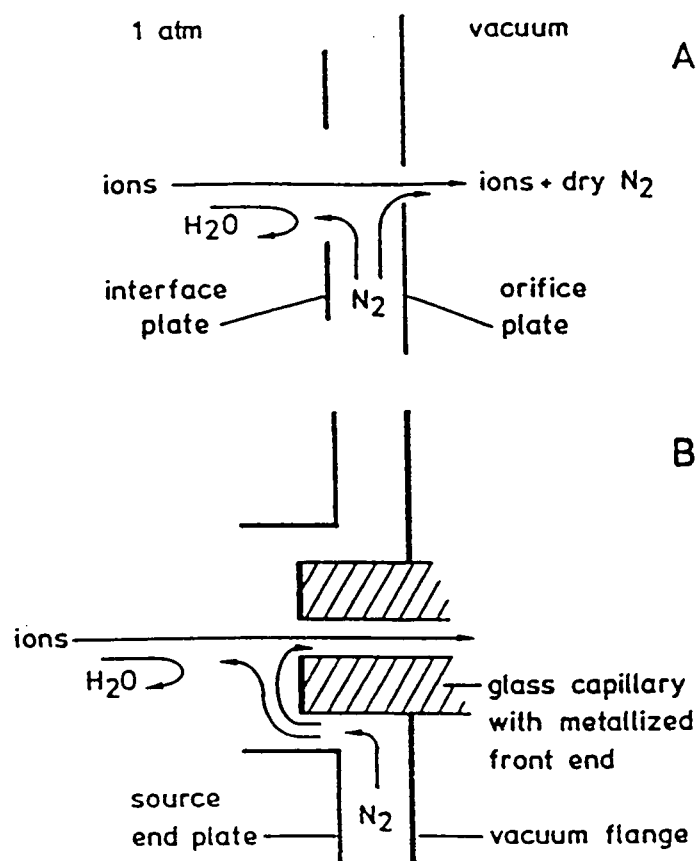


Figure 3.6 Desolvation of the electrospray plume with the aid of (A) a dry gas curtain or (B) a countercurrent flow of drying gas; reference 51.

Another method that is sometimes used for removal of clusters is called collision-induced dissociation (CID). In this approach ion-clusters are accelerated between the nozzle (capillary inlet) and the skimmer by applying appropriate voltages to those elements. Declustering by CID was first reported in 1966⁶³ in the context of studies on corona discharges in air. In 1977, it was adapted by Kambara⁶⁴ and later patented by Kambara⁶⁵ and French⁶⁶. Low CID energies decluster and high CID energies fragment molecules.

VACUUM CONSIDERATIONS

Both single-stage and multiple-stage vacuum systems have been used in conjunction with all the various ion sources that operate at atmospheric pressure⁵¹. Four parameters essentially determine the design of the vacuum system: the sizes of the sampling orifice (inlet) and the skimmer orifice, the pumping speed of the vacuum pumps, and the maximum pressure that can be tolerated by the mass analyzer. The general trend is to use a larger sampling orifice which offers the benefits of higher throughput and minimal blockage in conjunction with several stages of differential pumping. An ion-optics stage or an rf-only quadrupole is commonly used in the middle vacuum stage (in the skimmer exhaust region between the source and mass analyzer) to maintain a tight beam as it emerges from the skimmer. Figure 3.7 shows several vacuum system arrangements employed with API sources⁵¹.

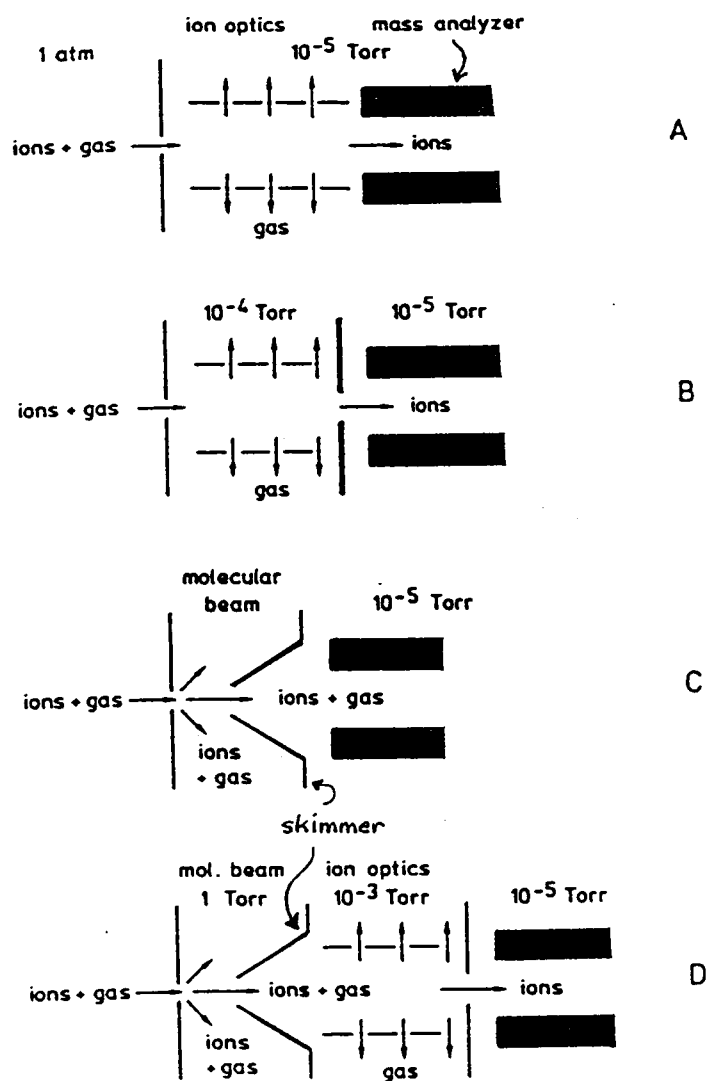


Figure 3.7 Vacuum systems for API systems; pressures shown are typical values; (A) single-stage; (B) & (C) two-stage differentially pumped; (D) three-stage differentially pumped; reference 51.

SUMMARY & CONCLUSION

Electrospray mass spectrometry is unique in that it has its operational roots in two very different technologies; that of electrospray and supersonic beams. This results in a mass spectrometric source that is significantly different from most other sources thus presenting special challenges to the person who decides to build one. For example, the electrospray source operates at ambient pressures where ion molecule reactions occur readily thereby resulting in neutralization losses. Furthermore, transport of ions from the ionization region (atmosphere) into the source (vacuum) poses new challenges because of space charge effects which distort electric field lines in the vicinity of the sampling orifice. With regards to the above two challenges, the goal would be to minimize neutralization losses and ion sampling biases. Decisions have to be made regarding the shape of the skimmer, the diameter of its orifice, and where it should sample the beam. Another consideration is the actual interface to the mass analyzer, a parameter that was not addressed in this chapter. Two common approaches to this task are the use of quadrupoles operating in the rf-only mode or of an ion optical assembly. Both approaches are used in practice with apparently equal success. The point of the above discussion is that constructing a mass spectrometric source is not simply a matter of hardware. The complexity of the task leads most practitioners to follow the few leaders in the field who have designed their own sources. The author opted for the same general approach (further explained in Chapter 4 - Experimental Techniques) while attempting to understand the significance of various components utilized and/or of measures taken in the design of the source. To the author's knowledge, a "flawless" ES source has still not

been made; however, several quality designs are now commercially available.

CHAPTER 4

EXPERIMENTAL TECHNIQUES

The stages involved in the formation of an electrospray and its overall behavior were the topics of discussion in Chapter 2. The considerations involved in the transport of electrosprayed ions from atmosphere into the mass spectrometer and a discussion of space charge effects and ion sampling bias were presented in Chapter 3. The present chapter describes the construction of the entire electrospray instrument, beginning with a description of the electrospray source and its interface to a quadrupole mass spectrometer. Some operational notes are then provided for others who may wish to use this apparatus, along with an assortment of "useful" notes and observations. A list of materials used, some examples of sample preparation schemes, and explanations of auxiliary techniques (e.g., surface tension measurements, conductivity measurements) that were required throughout this work are then given in detail. Finally, the remainder of the chapter presents the results of two experiments that were performed to assess the extent of ion sampling bias and ion scattering in the ES-MS source in use.

DESCRIPTION OF THE ELECTROSPRAY SOURCE AND MASS SPECTROMETER

Most ES sources currently in use fall into two categories on the basis of the method used for desolvation of the electrospray plume. Some utilize a flow of heated gas to dry the electrospray; e.g., the electrospray source designed by Fenn *et al.*³. Others

employ a heated drying capillary, without the auxiliary gas flow; e.g., the source designed by Chait *et al.*⁵⁶. The source built in this laboratory was of the second category and is schematically represented in Figures 4.1 through 4.6. Please note that these figures are not drawn to scale.

The electrospray needle is a 10 cm l. x 0.18 mm i.d. x 1.6 mm o.d. stainless steel tube that is machined and polished to a fine point at its tip (Figure 4.1). It is connected to a 1-mL syringe with a 75 cm long section of 0.192 mm i.d. fused silica capillary. The connections between the fused silica capillary, the syringe, and the electrospray needle are made with zero dead volume HPLC fittings (Valco #45836; Alltech Associates Inc., Deerfield, IL) and insulating Vespel[®] ferrules (RF-100/0-V; Alltech Associates, Inc., Deerfield, IL). A syringe pump (Sage Instruments, Model 355, Orion Research Inc., Cambridge, MA) maintains a flow rate of approximately 4 μ L/min through the needle.

The electrospray needle is fitted into an insulating cylinder of Delrin[®] (2" o.d. x 4" height) that is held between the outside portions of two 2.75" nominal diameter rotatable flanges (Varian, Palo Alto, CA) with three 5" long bolts and three nuts. This is mounted in front of the ES source on three equally-spaced 1/4" o.d. rods such that the distance between the needle tip and the capillary inlet of the source is approximately 4 mm (Figure 4.2). In this arrangement the needle is always coaxial with the capillary inlet which is actually a 1" long section of the drying capillary (#8, Figure 4.3) that protrudes from the source, approximately 0.5 cm beyond a 1.7 mm hole in a square stainless steel plate (1.5" x 1.5" x 0.0125" (thick)) used as the counter electrode (Figure 4.2). This plate is mounted on the exterior of the source with two threaded rods sleeved with insulating

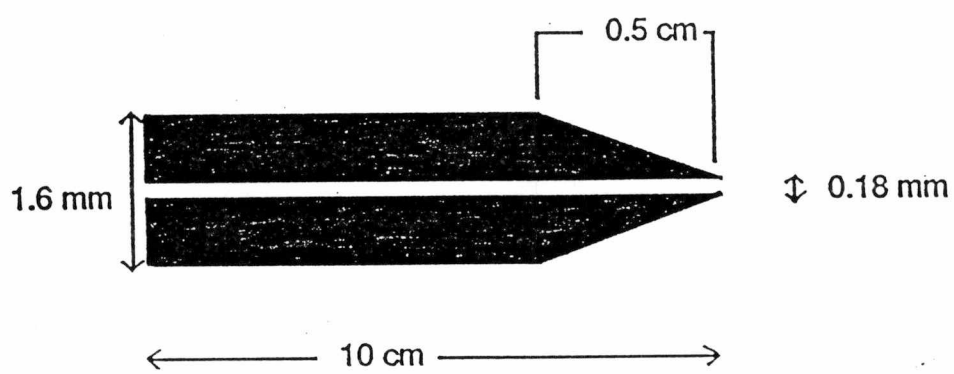


Figure 4.1 Schematic drawing of an electro spray needle.

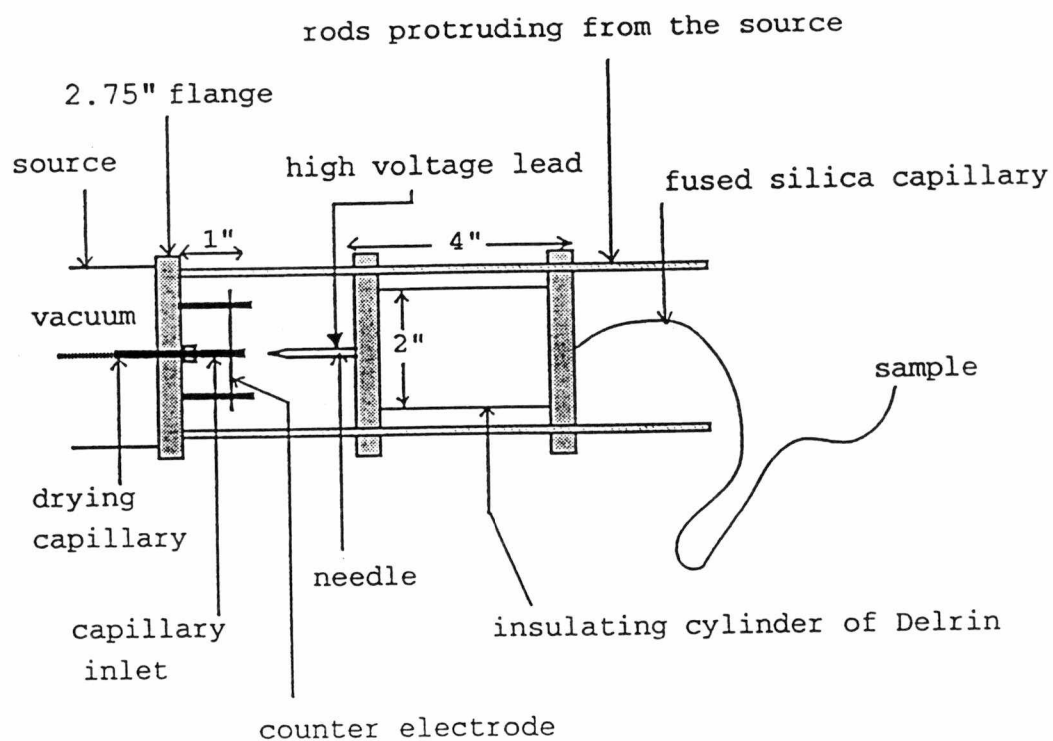


Figure 4.2 Schematic drawing of the electrospray needle assembly placed in front of the capillary inlet (ES source).

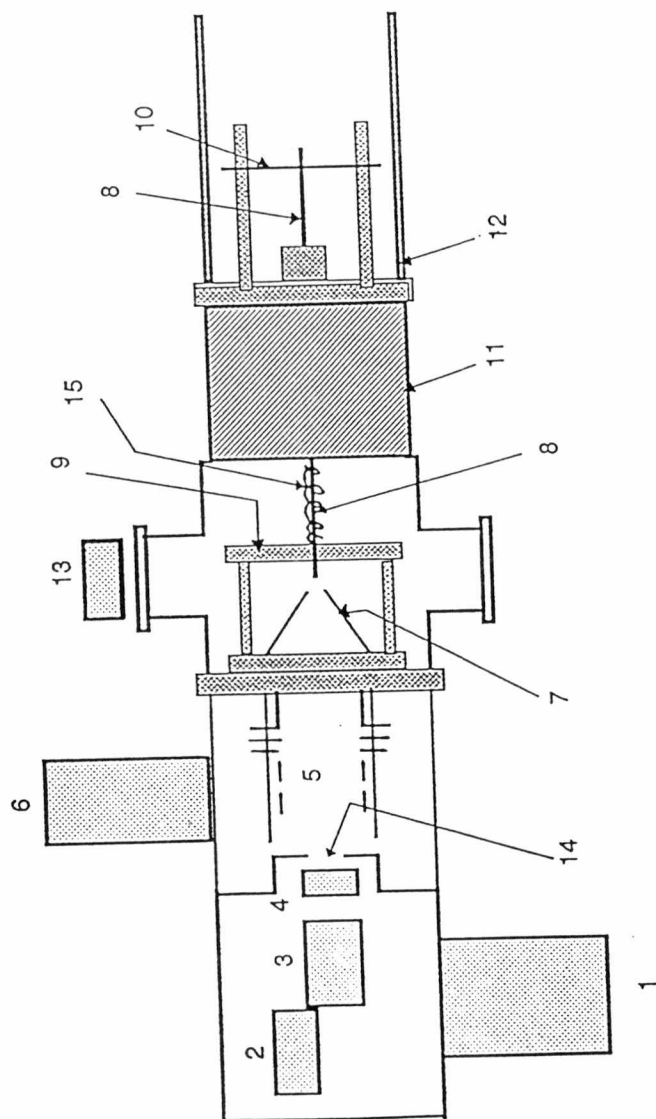


Figure 4.3 Schematic drawing of the electrospray instrument built in this laboratory. (1) diffusion pump; (2) electron multiplier; (3) quadrupole mass filter; (4) electron ionization source; (5) ion optical assembly (see Figure 4.6); (6) cryopump; (7) skimmer (see Figure 4.4); (8) drying capillary; (9) centering bracket (see Figure 4.5); (10) counter electrode; (11) linear motion mounting device; (12) rods protruding from the source to hold the needle assembly; (13) mechanical pump; (14) 5-mm i.d. differential pumping orifice; (15) capillary heater.

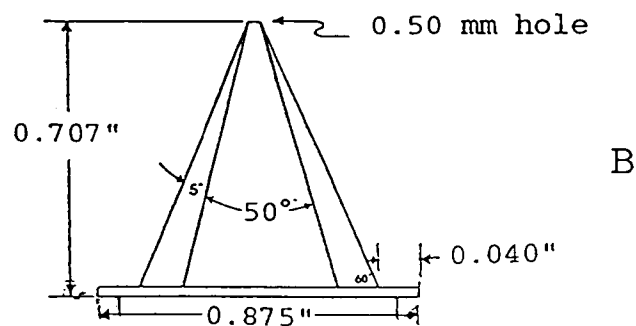
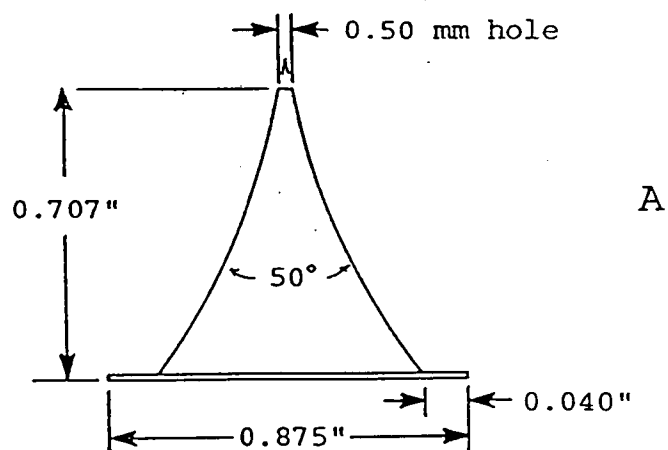


Figure 4.4 Schematic drawing of the skimmer showing various critical dimensions.

A: Cu or Ni skimmer (Beam Dynamics Inc., Minneapolis, MN); the thickness at the orifice edge is 10 μm . B: stainless steel skimmer (Spectron, Oxnard, CA).

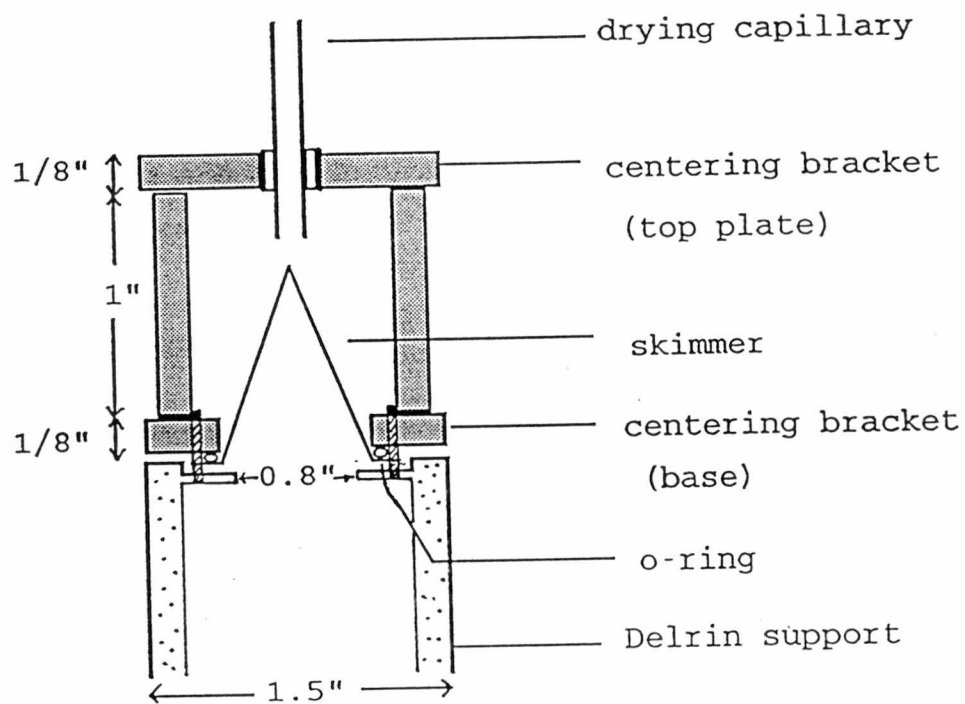


Figure 4.5 Schematic drawing of the centering bracket.

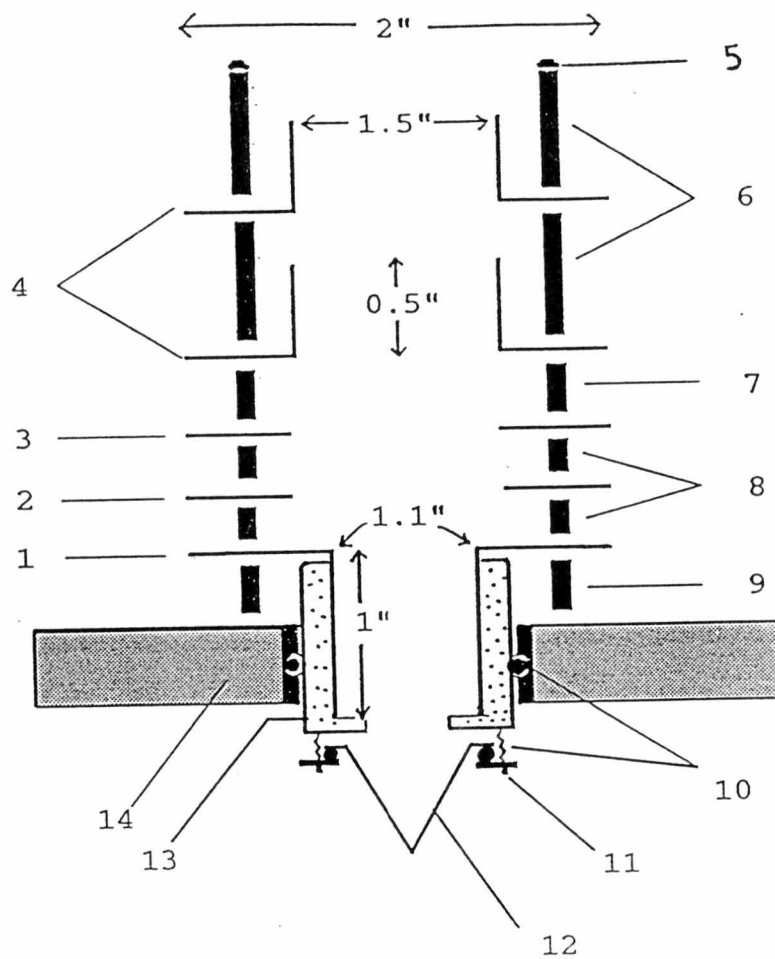


Figure 4.6 Schematic drawing of the ion optical assembly. (1) extractor; (2) focus electrode; (3) final electrode; (4) square lenses; (5) washer and nut; (6) 1.5" ceramic spacers; (7) & (9) 3/8" ceramic spacers; (8) 1/4" ceramic spacer; (10) o-ring; (11) screw to hold the skimmer in place; (12) skimmer; (13) Delrin support for the skimmer; (14) 6" adapter flange with 1.5" opening.

ceramic spacers and maintained at the same potential as the capillary inlet. Approximately +3.5 kV (High Voltage Power Supply, John Fluke Mfg. Inc., Seattle, WA) is applied to the needle by means of an alligator clip. A 20 M Ω (1 watt) resistor is placed between the power supply and the needle for safety precautions. The electrospray current is monitored by means of a microammeter placed in series between the needle and the power supply. The electrospray currents measured are typically 0.5 μ A except when breakdown to corona discharge occurs.

The drying capillary (#8, Figure 4.3) is a 20 cm long section of stainless steel tubing (0.51 mm i.d. x 1.6 mm o.d.) that is used for transporting the electrosprayed ions from the atmospheric pressure region into the first stage of the differentially-pumped mass spectrometer. This region was pumped by an Edwards (Wilmington, MA) E1M80 one stage mechanical pump (#13, Figure 4.3) resulting in a pressure of 550 mTorr measured with a thermocouple vacuum gauge tube and controller (Varian Vacuum Products, Lexington, MA) at the pump head. To facilitate desolvation of the electrospray, all but the first and last inch of the capillary was heated to approximately 100°C with a coil of 22 gauge Nichrome wire (#15, Figure 4.3) that was threaded through a section of Thermeez insulating sleeve of flexible ceramic (Cotronics Corp., Brooklyn, NJ) to electrically isolate it from the capillary. The portion of the capillary that remained outside the source housing toward the electrospray needle was heated to approximately 100° C by a BriskHeat (Columbus, OH) heating tape wrapped around the front end of the source (heating tape not shown). The capillary temperature was measured by a Type-J thermocouple (Omega Engineering Inc., Stamford, CT) that was attached to the midpoint

of the capillary and a digital thermometer (Ω E Omega Model 871, Omega Engineering Inc., Stamford, CT).

The free jet formed at the outlet of the capillary was initially sampled by a Cu or Ni skimmer (Beam Dynamics Inc., Minneapolis, MN) with 0.5 mm diameter orifice and 50° cone angle (#7, Figure 4.3 and Figure 4.4A). This was later replaced by a more durable stainless steel skimmer of similar dimensions (Spectron, Oxnard, CA) (Figure 4.4B). The skimmer and capillary were coaxially arranged in a 2 3/4" nominal diameter Conflat^R cross housing (Varian, Palo Alto, CA) by means of a copper centering bracket (#9, Figure 4.3). An enlarged view of the skimmer, centering bracket and the capillary is provided in Figure 4.5. As noted in Figure 4.5, the skimmer is electrically isolated from the base of the centering bracket by means of a Viton o-ring; the capillary is electrically isolated from the top plate of the centering bracket by means of a ceramic spacer (1/8" long x 1/8" o.d.) that tightly fits into a hole drilled in the center of that plate. A linear motion mounting device (Huntington Laboratories, Mountain View, CA) (#11, Figure 4.3) was used to adjust the distance between the capillary end (nozzle) and the skimmer. This distance was usually about 3 mm.

The nozzle and skimmer were empirically biased to maximize ion signal in the mass analyzer and to minimize clustering of analyte ions with solvent molecules by collision induced dissociation⁵¹. Typical voltages used were +90 - 130 V, applied to the capillary, and +45 - 65 V, to the skimmer. This was achieved with the aid of two separate power supplies (Model 456, EG&G ORTEC, Oak Ridge, TN).

Beyond the skimmer, in a second vacuum chamber, a 5-element ion-optical

assembly (#5, Figure 4.3, shown in more detail in Figure 4.6) was installed to focus ions into the mass analyzer chamber through a 5 mm differential pumping orifice (#14, Figure 4.3)^{1*}. The ion optical assembly (Figure 4.6) and the skimmer were mounted on the opposite sides of the same vacuum flange in order to ensure that the skimmer orifice (point source for ions) would always coincide with the ion optical axis. This arrangement would not allow any wobble in the source of ions with respect to the lens stack. The lens elements (Kimble Physics, Wilton, NH) consisted of an extractor (#1, Figure 4.6), a focus electrode (#2, Figure 4.6), a final electrode (#3, Figure 4.6), and two square lenses (#4, Figure 4.6). The extractor lens is a stainless steel cylinder, 1.100" i.d. x 1.0" height, that is spot welded to the 1.100" i.d. circular aperture of a 2.000" square stainless steel plate (0.025" thick). The focus and final lenses are 2.000" square stainless steel plates (0.025" thickness) with 1.500" i.d. circular apertures. Each square lens consists of two rectangular stainless steel plates, 0.500" h x 0.875" w, that are attached with L-brackets to the opposite sides of a 2.000" square plate with a 1.500" square opening. The square lenses are arranged orthogonally but represented as parallel plates in Figure 4.6. The lens elements were mounted on four threaded stainless steel rods sheathed in ceramic sleeves (Figure 4.6). The distance between the lens elements was set by ceramic spacers (#6, 7, 8, 9, Figure 4.6) that slid over the ceramic sleeves.

The ion optical assembly was first designed with the aid of SIMION⁶⁷, a computer program, originated by J. Morrison, which simulates ion trajectories from a set of parameters (such as ion energies, electrode geometries, etc.) specified by the user.

^{1*} The ion optical work is accredited to Peter Todd and Tim Short; Present Address: ORNL, Oak Ridge, TN 37831

Figure 4.7 demonstrates the ion trajectories predicted by SIMION for the ions exiting the skimmer. The recommended voltages were applied to the lens elements with two power supplies (Model OPS 500B, KEPCO, Flushing, NY) set at ± 200 V and parallel voltage dividers^{2*}.

The skimmer exhaust region was pumped to below 1 mTorr with a CT-100 closed cycle helium cryogenic pump (CTI-Cryogenics, Waltham, MA). The pressure of this region was measured with a thermocouple vacuum gauge tube and controller (Varian Vacuum Products, Lexington, MA). Approximately 5 mm beyond the differential pumping orifice (#14; Figure 4.3), ions passed through the electron ionization (EI) source (#4, Figure 4.3, shown in greater detail in Figure 4.8) of a Model 065-2 quadrupole mass filter (Extrel, Pittsburgh, PA; mass range 350) into the quadrupole region (#3, Figure 4.3) where they were mass analyzed. The EI source of the Extrel mass spectrometer (Figure 4.8) possesses 6 lens elements, all of which were required for the extraction, focusing, and transmission of the beam of electrosprayed ions into the quadrupole region.

The mass analyzer region was pumped by an Edwards Model 160/700P Diffstak diffusion pump (#1, Figure 4.3) which maintained a pressure of approximately 5×10^{-6} Torr. The pressure of this region was measured with a Bayard-Alpert type ionization gauge tube and controller (Granville-Phillips Co., Boulder, CO). The quadrupole mass spectrometer was equipped with a conversion dynode (biased to -3.95 kV) to post-accelerate the ions, and an off-axis electron multiplier (#2, Figure 4.3) operated at -1.8 kV for detection.

^{2*} The voltage dividers were designed and constructed by Eric Kerley, a former post-doctoral associate at ORNL.

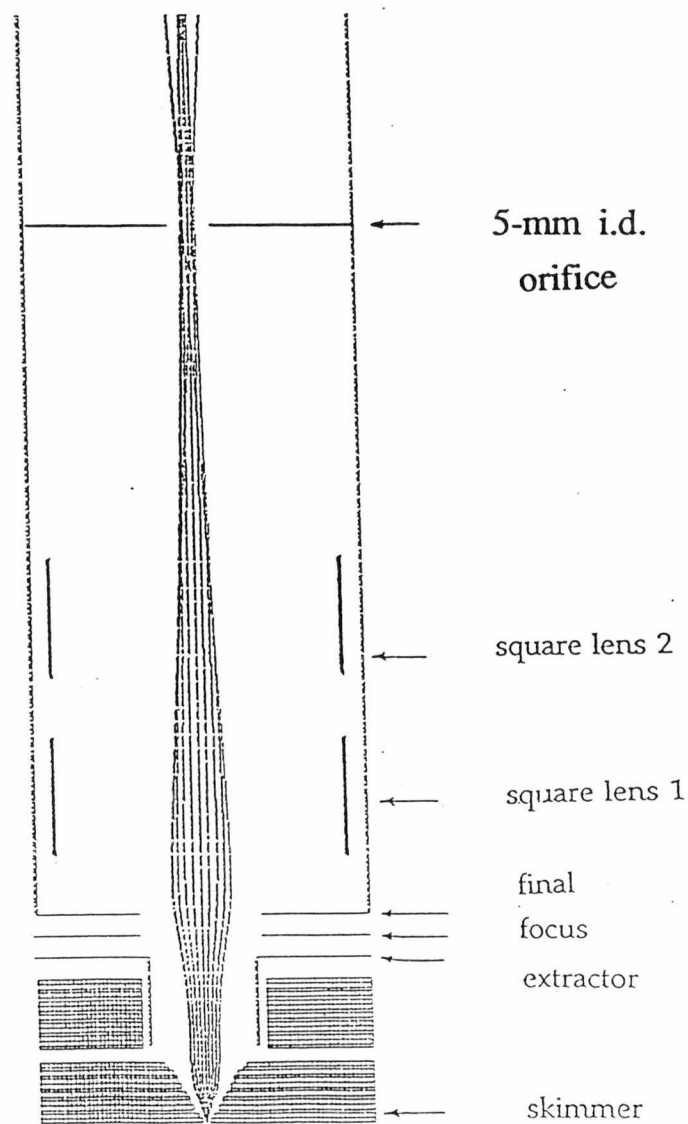


Figure 4.7 Ion trajectories generated by SIMION for the following lens settings:
 extractor at -172 V; focus electrode at +20 V; final electrode at 0 V;
 square lens 1 & 2 at -10 V; ion energy at +2 V.

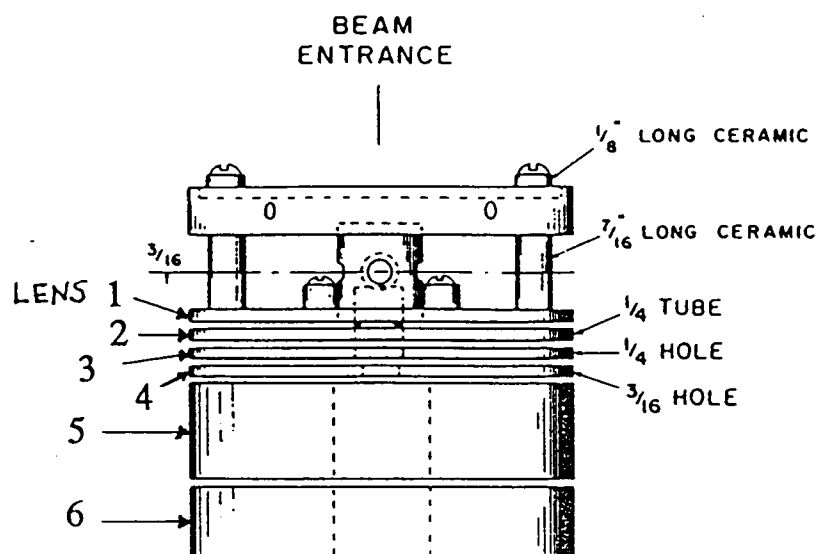


Figure 4.8 Schematic drawing of the electron ionization source of the Extrel quadrupole mass spectrometer.

OPERATION OF THE ES-MS INSTRUMENT

The ES-MS instrument was set up for sample runs as described below. First, the mass spectrometer was turned on to warm up the electron multiplier which was held at -0.1 to -0.3 kV for approximately 30 minutes and subsequently biased to -1.8 kV for ion detection. The dynode, off-axis relative to the electron multiplier, was set at -3.93 to -3.95 kV from the start. Next, the ion optical components of the EI source and those of the ES source were biased to their assigned voltage settings. Typical voltages applied to the ion optical components are shown in Table 4.1. The pressures of the mechanical pumps and in the analyzer area were monitored. The expected pressure readings for these were: 5 - 8 mTorr for the pump that backed the diffusion pump, and approximately 75 mTorr for the pump used in the nozzle exhaust region. The pressure in the analyzer area was approximately 4×10^{-8} Torr (no load). The cryopump temperature indicator was also checked for a green light that indicated a "cold and ready" state.

Prior to loading the sample, the syringe, the fused silica capillary, and the ES needle were rinsed with water and methanol. Next, the syringe was filled with the sample and loaded on the syringe pump. Typically, methanolic solutions of 100 μ M arginine ($[M+H]^+ = 174$) or 25 μ M *n*-hexyltrimethylammonium bromide ($[M^+] = 144$) were used at the beginning of each day for checking the performance of the electrospray apparatus or tuning of the source. Immediately prior to the ES runs, the heater for the drying capillary was turned on and its temperature monitored. When the capillary temperature reached approximately 100° C, the ES-MS instrument was ready to be opened to atmosphere. This was done by opening the gate valve that isolated the cryopump from

Table 4.1 Typical voltages applied to the ion optical components of the ES-MS instrument

ES lens stack ^a	Voltage	MS lens stack ^b	Voltage
extractor	-172	lens 1	+4.0
focus	+20	lens 2	-28
final	+106	lens 3	-28
square lens 1	-10	lens 4	-89
square lens 2	-10	lens 5	-30
nozzle	+90 -130	lens 6	+56
skimmer	+45 - 65	pole offset	-12

^a See Figure 4.6 for details of the ES source lens positions.

^b See Figure 4.8 for details of the EI source lens positions.

the housing and removal of the Swagelok fitting that was used to cap the ES-MS source inlet (drying capillary). The pressures in the analyzer area would generally rise to approximately 2×10^{-6} Torr and that in the nozzle exhaust to 550 mTorr (measured at the pump head). The ES-needle assembly (Figure 4.2) was then mounted in front of the source inlet on the rods that protruded from the source. As mentioned earlier, the distance between the needle and the capillary inlet was set at approximately 4 mm. With the solution flow established at approximately 4 $\mu\text{L}/\text{min}$, +3.2 to 3.5 kV was applied to the needle and the electrospray experiment was begun. The physical appearance of the electrospray was always monitored visually under bright white light: drips and flickers, etc., indicated a problem (e.g., poor electrical connection, clogged needle) that had to be addressed before data acquisition could begin.

A significant consideration in the operation of the ES source is determination of the nozzle-skimmer separation distance. It is desirable to sample the free jet at a distance that provides maximum ion intensity, minimal clustering, and minimal scattering. In the source described above, the distance between the skimmer and the nozzle was adjusted with the aid of a linear mounting device with a 2 cm drive range and 0.1 mm resolution. It was found that sampling the jet slightly upstream of the Mach disk resulted in a tighter beam that was easier to focus (discussed in Chapter 3, Figure 3.3). The main disadvantage in this approach was the presence of a larger number of solvent clusters which were eliminated by heating the entire source with the aid of heating tapes. Figure 4.9 shows the selected nozzle-skimmer separation distance (boxed area with arrow) in a plot of pressure in the mass analyzer versus the latter. The selected distance offered an

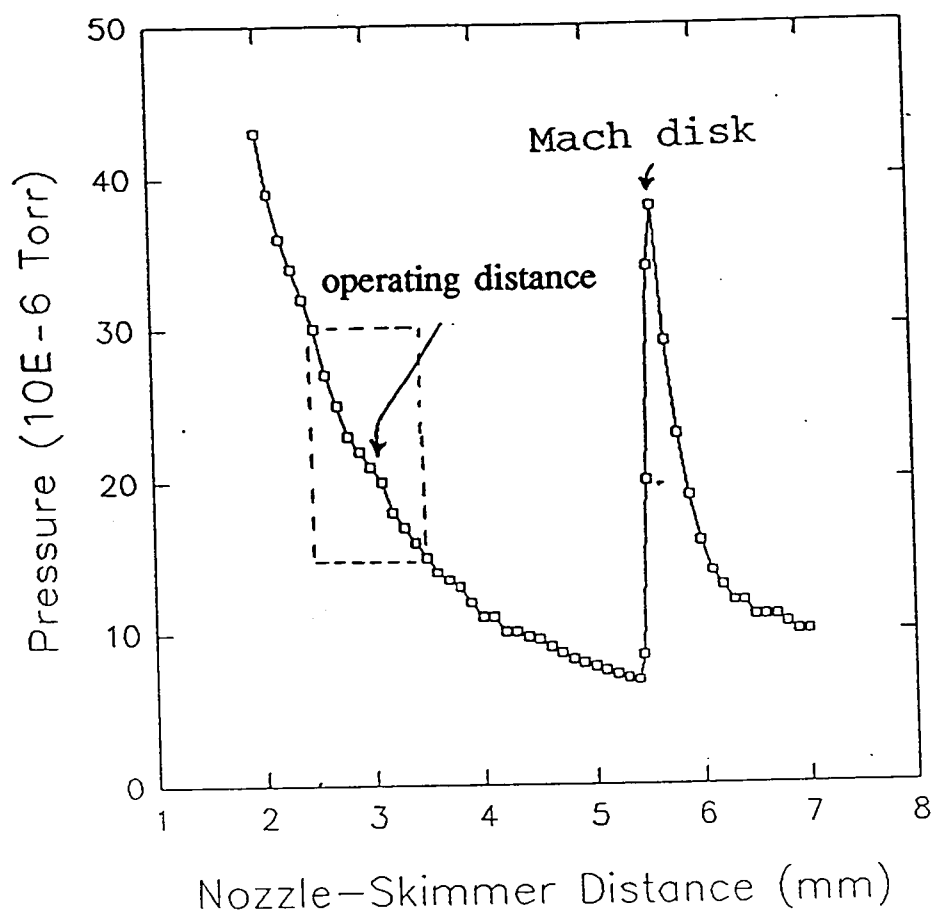


Figure 4.9 Measured pressures in the mass analyzer as a function of nozzle-skimmer distance.

optimal balance between the extent of ion-solvent clustering, the scattering of the ion beam, and the intensity of the signal. The location of the Mach disk is also indicated in that figure.

The approach used for finding ions and tuning of the ion beam was to start by grounding all 11 lenses (5 from the ES source and 6 from the EI source of the Extrel mass spectrometer), the nozzle and the skimmer. The quadrupole rods were then operated in the rf-only mode by turning off the applied dc voltages in order to pass all ions to the detector. At this point the nozzle and skimmer were biased to an arbitrary value, e.g., +100 and +30 V, respectively, and the lens voltages were adjusted to maximize ion currents at the detector. Once the ion currents were maximized in the rf-only mode, the dc voltages were reapplied to the rods and further re-optimization was carried out until a peak at m/z 174 (for protonated arginine) or at m/z 144 (for *n*-hexyltrimethylammonium ion) was detected. With the electron multiplier set at -1.8 kV, the signal from 100 μ M arginine prepared in methanol, was $\approx$ 300 mV and that from 25 μ M *n*-hexyltrimethylammonium bromide was $\approx$ 600 mV.

On occasions when ions could not be detected, the general approach was to systematically inspect the various regions where problems could arise. The electrospray plume was visually examined to ensure the presence of a stable jet. The pressures in the three vacuum regions were monitored. Unusually low pressures in the mass analyzer indicated the presence of an obstruction in the capillary inlet which had to be cleared out. It was not unusual to find the capillary inlet clogged due to freezing of solvent in the jet expansion, a problem that was easily remedied by increasing the temperature of the

capillary. A less obvious problem was the proper alignment of the nozzle with the skimmer. This could be verified by changing the nozzle-skimmer separation distance while monitoring pressures. Observation of a behavior similar to that shown in Figure 4.9 usually implied a well-aligned source. If this was not observed, the source had to be reassembled. The most elusive reason for not detecting ions was excessive solvation of the ions in the beam. Heavily solvated ions produce an intense response at the detector when the rods are operated in the rf-only mode; however, they cannot be detected when the dc voltages are applied because they exceed the mass range of this mass spectrometer. This problem was generally resolved by increasing temperatures and lowering the sample flow rate to approximately 3 $\mu\text{L}/\text{min}$.

Mass spectral data were acquired in part with an IBM-compatible personal computer that was interfaced to the Extrel mass spectrometer with a multifunction high-speed analog/digital I/O expansion board (Model DAS-20, MetraByte, Taunton, MA) and, in part with a digital oscilloscope (Model 4094; Nicolet Corp.; Madison, WI). The PC data system^{3*} (12-bit ADC; 12-bit DAC; 5 kHz sampling rate) controlled the mass spectrometric functions related to data acquisition, such as, setting the mass range and scan rate. The digital oscilloscope, on the other hand, simply acquired data and averaged the collected spectra. To perform this task, it had to be triggered from an appropriate signal that marked the beginning of the scan. A so-called "blanking signal" which activates the analog oscilloscope on the Extrel mass spectrometer was used to trigger the

^{3*} The PC data system had been previously developed by Marcus Wise and Cyril Thompson for use with a glow-discharge mass spectrometer. Present address: ORNL, Oak Ridge, TN 37831.

Nicolet digital oscilloscope. This blanking signal rose from a value of approximately zero volts to 10 volts when a scan was initiated; it remained at that level for the duration of the scan and then decreased to zero at the end of the scan. This sequence was repeated as long as the mass spectrometer scan function was enabled. The trigger level of the Nicolet oscilloscope was therefore set to a level of 4.0 volts on the rising portion of the signal to coordinate the latter with the Extrel mass spectrometer. The data acquired by the Nicolet oscilloscope (time domain) were converted to mass information after acquisition had ended. Each spectrum collected represents an average of ten spectral scans. In general, $N \geq 3$ spectra were acquired over the mass range of interest (typically m/z 50 - 350) at a rate of 20 amu per second. The intensities were reported as the average peak areas of the N (at least equal to 3) spectra with error bars depicting the standard deviation of the mean; i.e., $s/(N)^{1/2}$.

Quadrupole mass discrimination was assessed on a routine basis by acquiring a 70-eV EI spectrum of perfluorotributylamine, with the lens voltages normally used to focus electrosprayed ions. The relative intensities of the major ions in the EI spectrum of perfluorotributylamine (m/z 69, 100, 131, 219, 264) were checked against those cited in the literature for a magnetic sector instrument^{68,69}. Performance was regarded as acceptable if intensities were within 10% of literature values. Typical data for the EI spectrum of perfluorotributylamine with the lenses set for operation in the ES mode are shown in Table 4.2. In general, quadrupole discrimination over the relatively small mass range used (maximum 350 amu) was negligible, and data were therefore presented without modification.

Table 4.2 Relative intensities of the major ions in the EI spectrum of perfluorotributylamine using the lens settings for the ES mode of operation

m/z	suggested intensity*	observed intensity
69	100	100
100	17.7	17.9
131	47.9	41.1
219	28	31.1
264	13.4	13.0

* Suggested intensities were obtained from reference 68.

For this mass spectrometer, the mass axis calibration was also checked on a routine basis and always found to be satisfactory (i.e., ± 0.1 mass units of the correct mass). The resolution of the quadrupole could be set by the operator or the mass spectrometer "auto mode" which would set it to unit resolution. The latter was chosen for all data presented in this work.

In quantitative mass spectrometry, internal standards consisting of labeled analogs of the species of interest are typically used to account for sources of variation in the measured signal. However in ES-MS, an internal standard may not be the best choice because the absolute intensity of a given electrospray signal also depends on the concentration of all ionizable species in solution^{29,70}. External standards were therefore used for quantitation of results throughout this work. The initial choice of external standard was the basic amino acid arginine which gave a relatively strong signal at $m/z = 174$ corresponding to the protonated molecular ion, $[M+H]^+$. This was prepared as a 100 μM solution in the same solvent as the sample. Arginine was later replaced with *n*-hexyltrimethylammonium bromide (25 μM) which gave a signal at $m/z = 144$ corresponding to the molecular ion, M^+ ; the change was a result of experiments with sodium hexadecylsulfonate which contaminated the quadrupole region of the mass spectrometer with sodium ions and affected the mass spectrum of arginine. In fact all compounds that used to be seen as protonated ions were observed as ion-sodium adducts; i.e., as $[M+Na]^+$ after this contamination event. Sample measurements were bracketed by measurements of the external standard solution and intensities of analyte ions were generally reported relative to the $[M+H]^+$ of arginine at $m/z = 174$ or M^+ of *n*-

hexyltrimethylammonium bromide at $m/z = 144$.

MATERIALS

Ammonium salts were obtained from commercial suppliers as follows: *n*-hexyltrimethyl- (HTMA) and *n*-decyltrimethylammonium bromide from TCI (Portland, OR); *n*-dodecyltrimethyl-, *n*-cetyltrimethyl-, and *n*-hexadecyltrimethylammonium bromide from Sigma (St. Louis, MO); *n*-octadecyltrimethylammonium bromide (ODTMA) from Fluka Chemical Corp. (St. Louis, MO); and tetramethyl-, tetraethyl-, tetrapropyl-, tetrabutyl-, and tetrapentylammonium bromides from Eastman Kodak Co. (Rochester, NY). The diquatery ammonium diiodide, $(\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3 \cdot 2\text{I}^-$, was previously synthesized in the laboratory by standard methods and recrystallized⁷¹. The anionic surfactant, sodium hexadecyl sulfonate (SHS), was obtained from Aldrich Chemical Co. (Milwaukee, WI). The nonionic surfactant, Triton X-100, was obtained from Fluka Chemical Corp. (St. Louis, MO).

The salts used for the ion-exchange process were: potassium nitrate, potassium chloride, and potassium bromide from Mallinckrodt (St. Louis, MO); and potassium acetate from EM Science (Cherry Hill, N.J.). A strongly basic anion exchange resin, Amberlite IRA 900, was provided by Rohm & Haas (Cherry Hill, NJ). The amines were obtained from commercial suppliers as follows: perfluorotributylamine from Scientific Instrument Services, Inc. (Ringoes, NJ); butylamine*, nicotine, dipropylamine, tripropylamine*, propylamine, N,N-diethylethanolamine, N,N-dimethylbenzylamine*, benzylamine*, N,N-dimethylaniline, dipentylamine, N-ethylbenzylamine,

benzylmethamine and tripropylamine from Aldrich Chemical Co. (Milwaukee, WI); caffeine and piperazine from Sigma Chemical Co. (St. Louis, MO); and dibutylamine* and isopropylamine from MCB Matheson Coleman Bell (Cincinnati, OH). The amines labeled with an asterik * required purification. These were either fractionally distilled under a blanket of argon or distilled under vacuum. Amino acids were obtained from Sigma Chemical Co. (St. Louis, MO). Methanol was HPLC grade, J.T. Baker, Inc. (Phillipsburg, NJ). Water was distilled and deionized. Other reagents were used without further purification.

SAMPLE PREPARATIONS

To study the effects of surface tension, 50 μ M samples of HTMA containing varying amounts of cationic, anionic, and nonionic surfactants were prepared in 50:50 (v/v) water:methanol or methanol. A typical preparation scheme is shown in Table 4.3 for samples of HTMA and ODTMA, the cationic surfactant, in 50:50 (v/v) methanol:water.

To test the role of competition for surface sites, solutions containing equimolar amounts of six *n*-alkyltrimethylammonium bromides were prepared in 50:50 (v/v) water:methanol and methanol. For example, 10 μ L of each of six 1 mM *n*-alkyltrimethylammonium stock solutions was diluted to 1 mL by addition of 940 μ L of methanol (i.e., 6 x 10 μ L stock + 940 μ L methanol); this resulted in a sample that was 10 μ M in each component.

Ion solvation phenomena were examined in a homologous series of tetraalkyl-

Table 4.3 Typical preparation scheme for samples of HTMA and ODTMA in 50:50
(v/v) water:methanol

V_{HTMA} (μL) (1mM stock in methanol)	$V_{\text{surfactant}}$ (μL) (1 mM stock in methanol)	V_{methanol} (μL)	V_{water} (μL)	V_{total} (μL)
50	00	450	500	1000
50	10	440	500	1000
50	20	430	500	1000
50	30	420	500	1000
50	40	410	500	1000

V = volume

ammonium bromides (quat salts). The quats were oven dried overnight at approximately 100° C due to their hygroscopic nature prior to preparation of the stock solutions. Equimolar samples of these compounds were prepared in 50:50 (v/v) water:methanol and methanol at varying concentrations, similar to the procedure described for the surfactants.

The ion pairing phenomena were examined in a group of diquatery ammonium salts (diquats) and tetraalkylammonium salts (quats) containing different counterions. These were prepared by ion exchange chromatography from the initially synthesized diquat diiodide or quat bromide. A 50 mL buret was packed with an aqueous slurry of the strongly basic Amberlite IRA 900 anion exchange resin (20 grams of dry resin $\approx$ 25 mL of wet resin). The resin was then dosed with one of the anions of interest, e.g., chloride, by passing 250 mL of a 5% (w/v) aqueous solution of potassium chloride through the buret at approximately 2 mL/min. The resin was next washed with approximately 250 mL of deionized water at the same flow rate to remove the potassium ions. 100 mL of a 250 μ M aqueous solution of quat bromide or diquat diiodide was then passed over the resin at the same flow rate. The eluant was collected in a 250 mL volumetric flask and brought up to the 250 mL mark on the neck by passage of additional water over the resin, resulting in a solution of quat chloride or diquat chloride that was assumed to be 100 μ M in concentration. For the electrospray runs, samples of these salts were pipetted into small vials and lyophilized to dryness. These were next diluted to the desired concentration by addition of the appropriate volume of the mass spectrometric matrix of interest.

A number of amines and amino acids were used in the studies investigating the

role of basicity and solvation parameters in ES-MS. The amines were prepared in methanol at concentrations equal to 10 and 100 μM . Samples of the amino acids were prepared at a concentration of 100 μM in 50:50 (v/v) water:methanol because they showed greater solubility in this matrix than in pure methanol.

SURFACE TENSION MEASUREMENTS

Surface tension measurements were made with a Model 70535 Du Nuoy Tensiometer (Central Scientific Co., Chicago, IL) which is a sensitive torsion balance. The surface tension is measured by applying a slowly increasing force to an accurately constructed platinum-iridium ring (2 cm i.d.) in contact with the surface of the liquid under measurement. The amount of force is indicated upon a circular graduated scale which was calibrated with water (surface tension at 25° C = 73.05 dyne/cm⁷²). A 10 mL sample of each compound was prepared in water and left at room temperature for several hours before its surface tension was measured in triplicate. The platinum-iridium ring was rinsed in acetone and flame dried between each measurement.

CONDUCTIVITY MEASUREMENTS

Conductivities of a number of samples were measured with a YSI Model 3401 conductivity cell and a YSI Model 35 Conductance Meter (Yellow Springs, OH). The conductivity cell was calibrated with aqueous solutions of NaCl⁷³. The cell was immersed in a controlled-temperature water bath (VWR Model 1140 Circulating Bath, VWR, Pittsburgh, PA) maintained at 25 °C until a stable reading ($\pm 0.02 \mu\Omega^{-1}$) was

registered on the conductance meter. Blank samples of deionized water were run between the samples of analyte to monitor background drift and test for cross contamination.

EVALUATION OF ION SAMPLING BIAS DUE TO SPACE CHARGE

Earlier in Chapter 3 in the discussion of the electrospray source, space charge effects were suggested to be capable of introducing a bias in the sampling of ions from the atmospheric region into vacuum, to the extent that the acquired mass spectrum would not represent the relative abundances of the actual species present at atmospheric pressure. In general, the operational strategy for dealing with space charge was to minimize the ion residence time by operating at the smallest needle-capillary inlet separation distance that would not result in a corona discharge. This resulted in the maximum ion density at the sampling orifice and further ensured that a more uniform field was present throughout to the sampling orifice (capillary inlet).

Below are presented the results of two experiments used to assess the extent of ion sampling bias in the present electrospray source so that the data presented could be interpreted with greater confidence. It was thought that observation of different intensities for isobaric species should suggest that their ES response is determined predominantly by their chemical nature. As a test of this theory, two pairs of compounds, consisting of a quat and a surfactant with a 14 unit difference in mass, were selected. An equimolar (1 μ M) mixture of these compounds (surfactants: tetradecyltrimethyl- (m/z 256) and hexadecyltrimethylammonium bromides (m/z 284); quats: tetrabutyl- (m/z 242) and tetrapentylammonium bromides (m/z 298)) was electrosprayed and the following intensity

ratios were observed:

$$\text{quat at } m/z \text{ 242} : \text{surfactant at } m/z \text{ 256} = (1.1) : (1)$$

$$\text{surfactant at } m/z \text{ 284} : \text{quat at } m/z \text{ 298} = (1.3) : (1.7)$$

The intensities were essentially equal for the low mass ions (m/z 242 and 256) but differed for the high mass ions (m/z 284 and 298) despite the equimolar composition of the mixture. The intensity ratio observed for the high mass ions was assumed to reflect the chemical differences between the quats and the surfactants. If this assumption is true, the same chemical differences should be reflected in the ES intensity ratio of the other lower mass quat/surfactant pair present in the solution. However, the intensities of the quat at m/z 242 and the surfactant at m/z 256 are essentially equal suggesting that yet another parameter is interfering with the ES response.

A mass discriminating effect that is expected to affect lighter ions more significantly than heavier ions is ion scattering from the principal ion axis. This effect could be partially responsible for the equal intensities observed for the ions at m/z 242 and 256; i.e., the lower mass ions. To test for such scattering phenomena, the spatial distribution of ions electrosprayed from an equimolar (5 μM) solution of three quats, tetrapropyl-, tetrabutyl-, and tetrapentylammonium bromides, at m/z 186, 242, and 298 in methanol was assessed by comparing the spectrum obtained at four needle/inlet positions (needle moved laterally with respect to the capillary inlet): on-axis, 0.5 mm, 1 mm, and 2 mm off axis. As shown in Figure 4.10, the absolute intensities of ions decreased with increasing radial distance from the inlet. This is particularly noticeable for the ion of highest mass and insignificant for the lowest mass ion. This observation

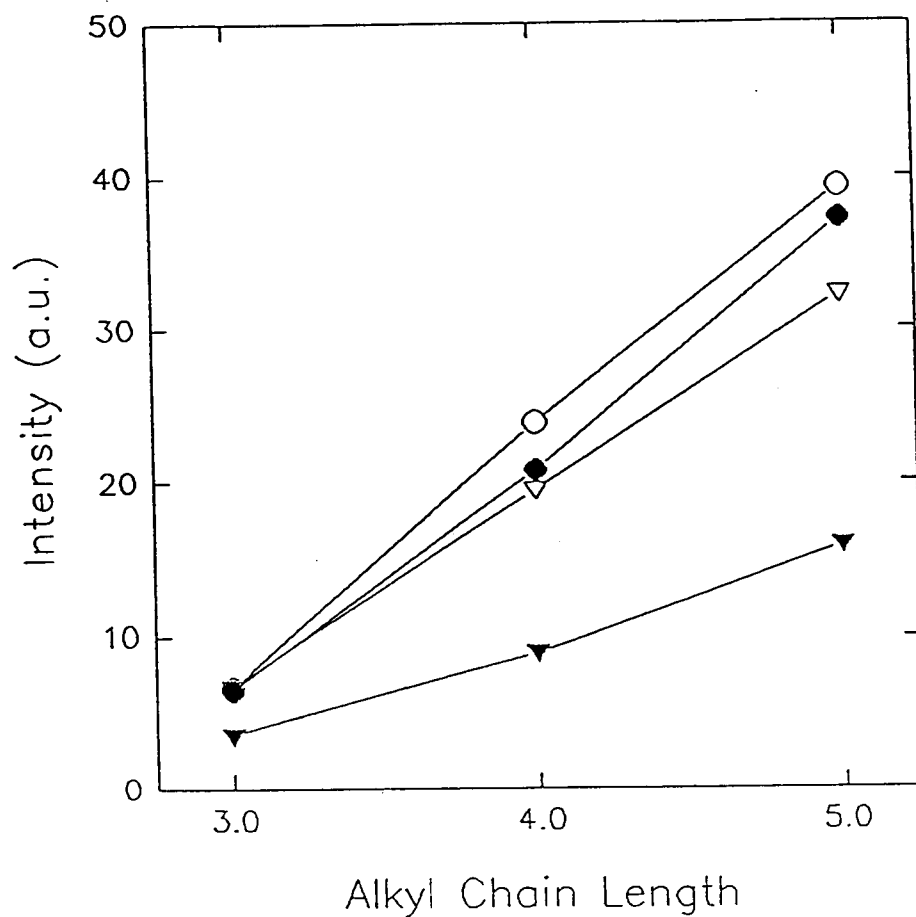


Figure 4.10 ES-MS ion intensities of an equimolar mixture (5 μM) of tetraalkylammonium bromides in methanol at four needle/capillary inlet positions: on-line (O); 0.5 mm off-axis (●); 1 mm off-axis (▽); 2 mm off-axis (▼). Ion intensities are reported in arbitrary units (a.u.).

is consistent with a scattering of low mass ions from the principal ion axis as a result of space charge but inconsistent with the reports of another study relating the inhomogeneous spatial distribution of the ions in the ES plume to differences in their desorption efficiencies⁷⁴. It is suggested that ions with higher desorption efficiencies ("volatilities"⁷⁴) are found in the periphery of the cone because they desorb faster and drift in the radial direction under the influence of the space charge field whereas those with lower desorption efficiencies remain in the center of the cone. If the latter suggestion is true, then compounds with higher surface activities, the higher mass ions, should be found in the periphery of the electrospray plume because they are believed to have higher desorption efficiencies⁷⁵. This clearly is not the case.

In summary, the above experiments may indicate a mechanism for bias in sampling of the low mass ions; however, the effect thereof does not entirely mask the apparent chemical trend. Further, they demonstrate that the events in this region are not well understood and require more investigation. Mass discrimination effects could also occur in the nozzle-skimmer region and in the quadrupole area. At the nozzle exhaust, ions of different mass acquire different translational energies in the gas expansion from atmosphere⁷⁶. Indeed, certain lens potentials can almost completely discriminate against lower energy (mass) species, as in retarding potential analysis⁷⁶. Mass discrimination effects resulting from energy differences of ions in the supersonic jet could not be readily assessed and were assumed to be negligible because of the limited mass range of the mass analyzer in use (350 amu). Mass discrimination effects related to transmission of ions through the quadrupole mass analyzer were assessed by standard methods as described

earlier in the chapter (page 62).

CHAPTER 5

EFFECTS OF SURFACE TENSION AND SURFACE ACTIVITY ON ELECTROSPRAY RESPONSE

In electrospray mass spectrometry (ES-MS), the choice of solvent is fundamentally constrained by the solubility of the analyte, but within this limit, other parameters can be taken into consideration (e.g., the dielectric constant and dipole moment of the solvent, surface tension, volatility, viscosity, redox potential, and the gas-phase ions that form from the charged droplets³⁷). Among these parameters, the effect of surface tension^{4*} on the formation of a stable electrospray (ES) has been previously studied³⁷ and shown to affect the onset voltage for electrospray. However, its effect on the mass spectrometric response has not been characterized. The first part of this chapter presents the results of experiments performed to assess the effect of surface tension on the mass spectrometric response of electrosprayed analytes.

One approach to the examination of surface tension effects would be through use of solvents with different surface tension values. However, other solvent parameters also vary, making it difficult to ascribe any changes in response to the surface tension parameter alone. The approach taken in this work was to modify the surface tension of a single solvent by addition of varying amounts of surfactants assuming that other solvent parameters remained unchanged.

The second part of the chapter attempts to assess the effect of analyte surface

^{4*} The surface tension of a liquid is the force per unit length acting in the liquid surface and is commonly expressed in units of dyne/cm.

activity on its ES-MS response (see page 99 for definition). The interest in exploring the latter parameter arose from the surface tension experiments with the surfactants. It was thought that analyte surface activity might influence its ES-MS response because the ES process involves a droplet evaporation stage that gives the analytes residing closer to the surface of the droplet a better chance for desorption. Thus, it was decided to examine the effect of analyte surface activity in a group of cationic surfactants (*n*-alkyltrimethylammonium bromides) that were previously used in the surface tension studies.

RESULTS & DISCUSSION

SURFACE TENSION STUDIES

The role of solution surface tension on the ES-MS response of analyte HTMA (*n*-hexyltrimethylammonium bromide) was first examined in a number of solutions where the surface tension was lowered by the addition of increasing amounts of a cationic surfactant, ODTMA (*n*-octadecyltrimethyl ammonium bromide). Figure 5.1 demonstrates the effect of ODTMA on the surface tension of the 50 μ M HTMA samples that were prepared in three different matrices: water (top), 50:50 (v/v) water:methanol (middle), and 100% methanol (bottom). The surface tension lowering property of ODTMA is most prominent in aqueous samples and yet, the mass spectrometric experiments were performed in 50:50 (v/v) water:methanol. The reason for the latter was that electrospraying 100% aqueous samples was not possible. However, it has been suggested that evaporating droplets can become enriched in water due to preferential evaporation

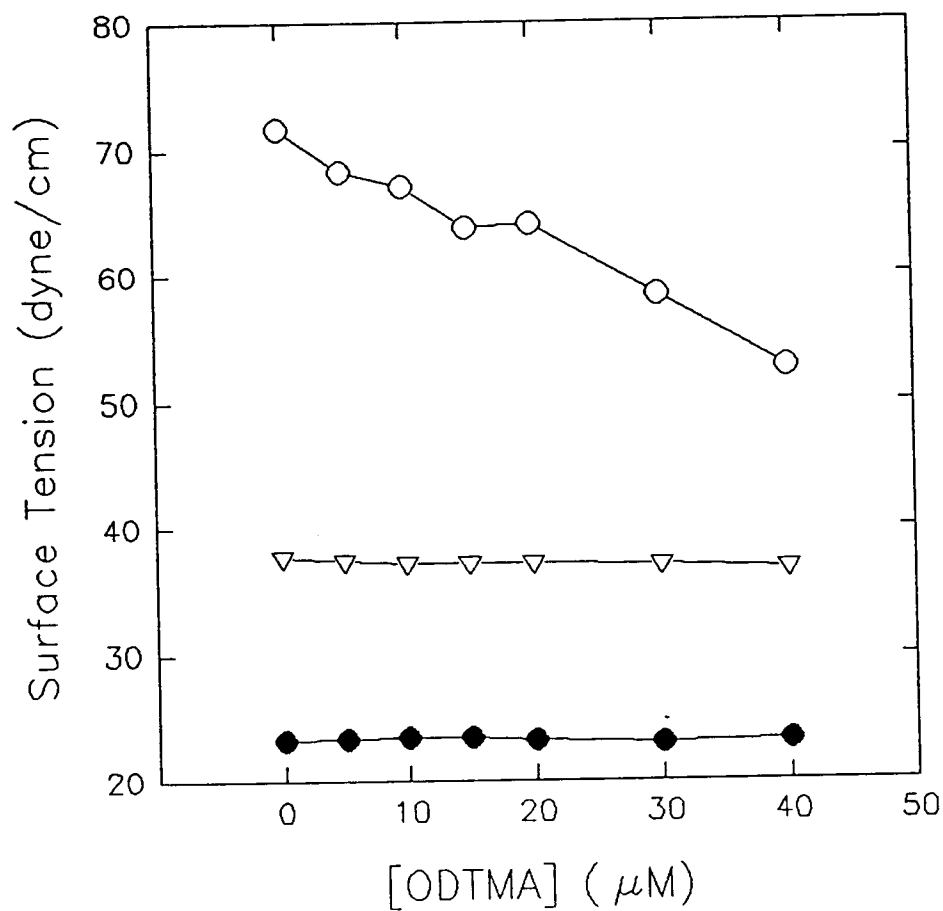


Figure 5.1 Dependence of the surface tension of 50 μM HTMA solutions on [ODTMA] in water (O); methanol (●); and 50:50 (v/v) water:methanol (▽).

of the more volatile component⁷⁷. Thus, although added methanol greatly reduces solution surface tension and diminishes the surfactant effect (Figure 5.1, middle), fractional distillation of methanol (higher volatility) is suggested to restore the surfactant effect as evaporation proceeds. Figure 5.2 shows the relative ion intensities from solutions containing 50 μM HTMA and varying amounts of ODTMA in 50:50 (v/v) water:methanol. The ion intensities at m/z 144 corresponding to HTMA and m/z 312 corresponding to ODTMA increase with the concentration of ODTMA, the more surface active quaternary ammonium salt, until the ODTMA concentration exceeds 30 μM . Thereafter the ion intensity at m/z 144 (HTMA) begins to decline while that at m/z 312 (ODTMA) does not.

The data of Figure 5.2 seem to reflect a complicated balance between several competing phenomena. The initial rise in the HTMA signal can perhaps be rationalized in light of what is known regarding the effect of surface tension on the size of sprayed droplets. The latter is reported to decrease as the surface tension of the solution is lowered^{78,79}. This is expected to result in smaller droplets that desolvate more efficiently because of their larger surface-to-volume ratio. Hence, the improved ES-MS response of HTMA can be attributed to a lowering of the surface tension of the solution, its effect on droplet size, and droplet desolvation.

Such an explanation is consistent with the "charge residue mechanism" of Dole¹⁶, one of the two major mechanisms proposed for the production of gas phase ions in ES-MS. Briefly, the charge residue mechanism suggests that gas phase ions are the result of solvent evaporation from very small droplets, approximately 1 nm in diameter, which

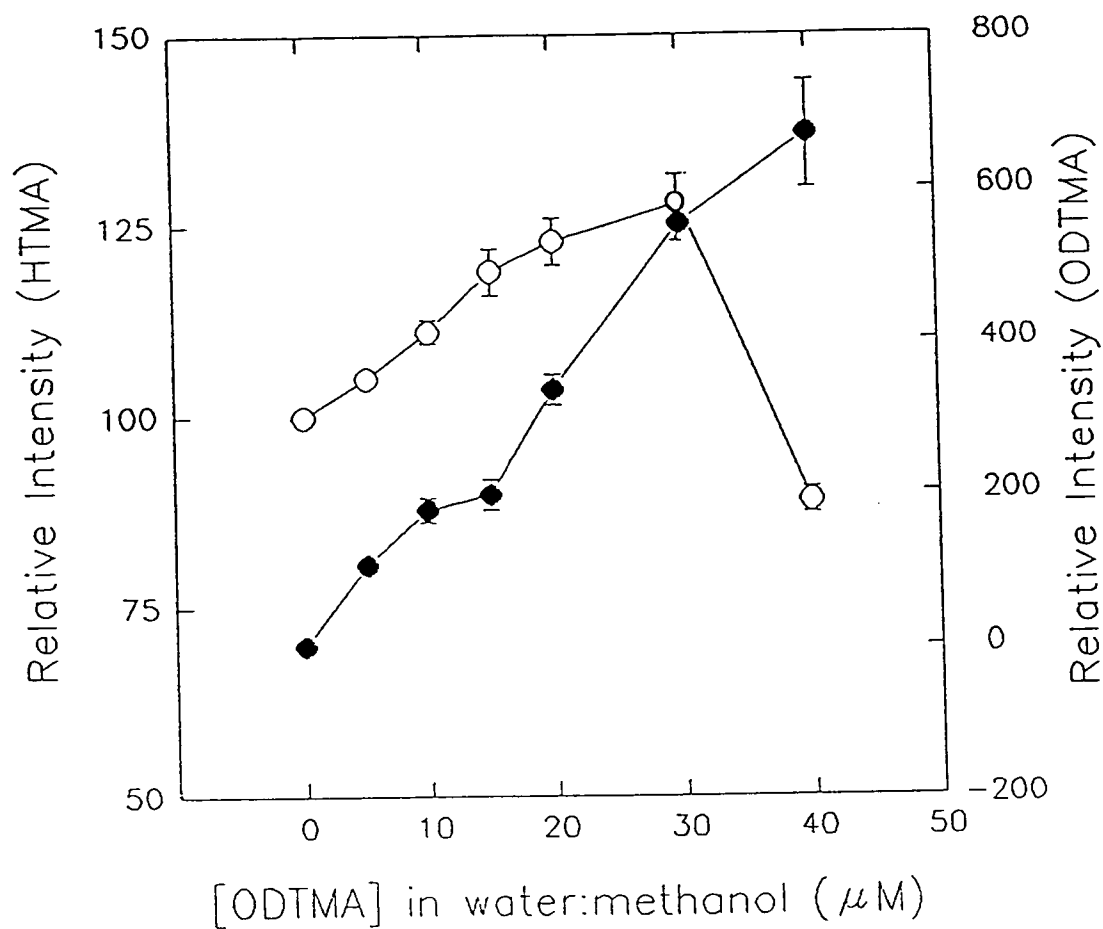


Figure 5.2 Dependence of the electro spray response of 50 μM HTMA (O) and [ODTMA] (●) on [ODTMA] in 50:50 (v/v) water:methanol. All intensities are relative to that of HTMA in the absence of ODTMA.

contain only one ion. The charge residue model is not entirely adequate and has been criticized on several of its experimental assumptions. In general, it is suggested to account reasonably well for the ES behavior of very large multiply charged species but cannot explain the multiple charging and selectivity that is seen in the ES behavior of small electrosprayed compounds. Nonetheless, the charge residue model represents a route by which gas phase ions could be generated in ES-MS. Hence, if we consider a situation where gas phase ions result from desolvation of small electrosprayed droplets, it becomes apparent that lowering of the surface tension of the solution would improve the observed response because it results in smaller droplets and a more efficient desolvation step.

The suggestion ascribing the enhanced ES response of HTMA to a lowering of surface tension is also consistent with the other major mechanism of ion formation in ES-MS which is the "ion evaporation model" of Iribarne and Thomson¹⁷. As briefly described below, the ion evaporation model provides a theoretical treatment for the desorption of singly charged ions from the surface of charged droplets. It derives a potential energy curve for the process by considering the solvation energy of the ion, the induced-image charge as the ion leaves the surface, and the coulombic potential between the ion and the charged droplet. It then expresses the height of the potential maximum in terms of the radius and charge of the droplet, and uses rate theory to determine the conditions under which desorption can occur. According to this model, direct desorption of single ions, clustered with several solvent molecules, can occur from very small droplets (radius $\leq 1 \mu\text{m}$) which are near the Rayleigh limit.

The ion evaporation model is also not fully adequate and has been criticized for the implicit assumption of the fast image charge in liquids⁸⁰. Further, it has been suggested that the fields necessary for ion desorption cannot be attained and that hydrodynamic instabilities will occur after an ion has left the droplet⁸¹. In spite of its shortcomings, the ion evaporation model appears to be useful in explaining the rise in HTMA signal observed in Figure 5.2. The term of the activation energy barrier (height of potential maximum) that should change if the surface tension of the solution is altered is the free energy of solvation of the ion in the charged droplet, $-\Delta G_s^0$. As shown below in Equation 5.0, $-\Delta G_s^0$ consists of a surface energy term that represents the work against surface tension to create a cavity for the ion to occupy in the solution (first term) and a "Born charging" term⁸² for the electrostatic interactions of the solvent with the ion (second term).

$$-\Delta G_s^0 = 4\pi\gamma r^2 + z^2e^2/4\pi\epsilon_0 2r \quad \text{Equation 5.0}$$

where γ is the surface tension, z is the charge of the ion, e is the charge of the electron, ϵ_0 is the permittivity of vacuum, and r is the radius of the ion cluster. From Equation 5.0, it can be seen that lowering of the surface tension should result in larger signals because it decreases the activation energy barrier to ion desorption.

One way to confirm that the improvement in the ES-MS response is related to lowering of the surface tension of the solution is to repeat the experiment shown in Figure 5.2 (with 50:50 (v/v) water:methanol) in a mass spectrometric matrix with a low surface tension value; for example, methanol. Sensitivity-enhancing surface tension effects resulting from the addition of ODTMA are expected to be less pronounced in methanol

because surface tensions of purely methanolic solutions are significantly lower than those employing mixed water/methanol solvent (Figure 5.1, bottom and middle curves) and are not apparently affected by changes in the ODTMA concentration. Thus, the HTMA response is not expected to improve with the addition of ODTMA in this medium.

The results of this experiment are shown in Figure 5.3. As predicted, ion intensities at m/z 144 (HTMA) do not increase with the addition of ODTMA to the methanolic solutions. Rather, the HTMA ion intensities decrease from the outset of ODTMA addition. A number of factors could result in the HTMA behavior noted in Figure 5.3. Among these are the onset of micellization (*i.e.*, the formation of surfactant aggregates in the bulk solution at concentrations above the "critical micelle concentration," or CMC)⁸³, solubility problems, increase in the total ionic strength of the solution at high concentrations of ODTMA²⁹, or surface activity effects (competition for surface sites). Incorporation of HTMA into micellar aggregates (*i.e.*, comicellization) could strip ionic analyte from the droplet surface into the droplet core. However, micellization should also affect the signal at m/z 312 (ODTMA). The response of ODTMA appears to be reaching a plateau rather than declining thereby precluding the presence of micellar effects in the range of concentrations tested. Solubility effects can also be ruled out as a reasonable explanation that is consistent with the data of Figure 5.3 because the solubility of HTMA in water⁸⁴ (Table 5.1) (and presumably in methanol) is higher than that of ODTMA; hence solubility-related sensitivity limitations should affect ODTMA more severely than HTMA.

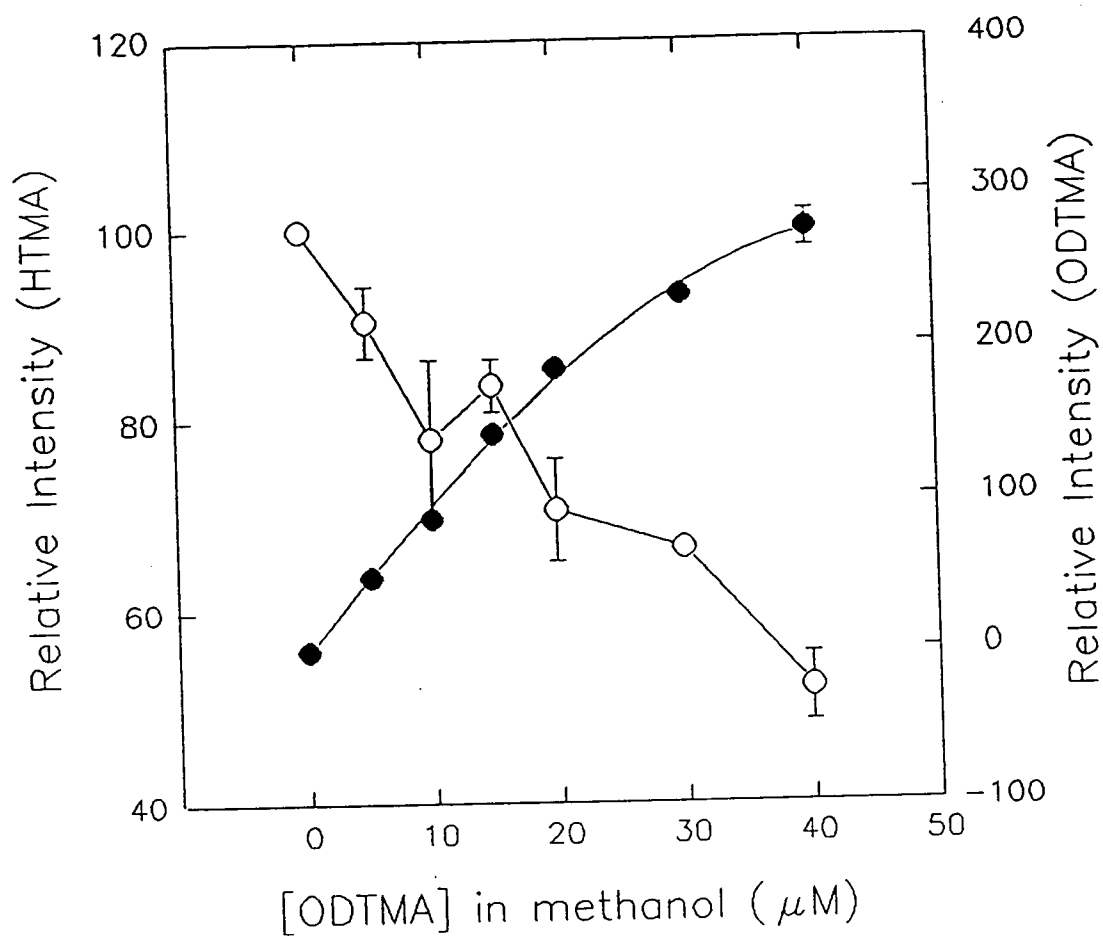


Figure 5.3 Dependence of the electrospray response of 50 μM HTMA (O) and [ODTMA] (●) on [ODTMA] in methanol. All intensities are relative to that of HTMA in the absence of ODTMA.

Table 5.1 Solubilities and Critical Micelle Concentrations, CMC, of *n*-alkyltrimethyl ammonium bromides, RN(CH₃)₃ Br, in water at room temperature

R	Formula	Solubility in water ^a	CMC (M) ^b
<i>n</i> -hexyl	C ₉ H ₂₂ NBr	1	- - -
<i>n</i> -octyl	C ₁₁ H ₂₆ NBr	4	1.4 x 10 ⁻¹
<i>n</i> -decyl	C ₁₃ H ₃₀ NBr	- -	6.8 x 10 ⁻²
<i>n</i> -dodecyl	C ₁₅ H ₃₄ NBr	20	1.6 x 10 ⁻²
<i>n</i> -tetradecyl	C ₁₇ H ₃₈ NBr	5	3.5 x 10 ⁻³
<i>n</i> -hexadecyl	C ₁₉ H ₄₂ NBr	10	9.2 x 10 ⁻⁴
<i>n</i> -octadecyl	C ₂₁ H ₄₆ NBr	1000	3 x 10 ⁻⁴

^a Solubility reported in parts by weight of water per part by weight of analyte. Solubility data were obtained from reference 84.

^b CMC data were obtained from reference 90.

The decreasing HTMA signal can be in part rationalized by a surface activity effect; i.e., by invoking competition between HTMA and ODTMA for the surface sites on the electrosprayed droplets, and alternatively by an increase in the total ionic strength of the solution²⁹. The former effect, that is the suppression of the signal of a less hydrophobic and therefore less surface active analyte by a more hydrophobic co-solute, has been observed in fast atom bombardment (FAB)^{85,86,87} and continuous flow FAB⁸⁸ and could presumably also occur in ES-MS. The latter effect, namely the increase in the total ionic strength of the solution (e.g., at high ODTMA concentrations), is reported to lower analyte ion intensities in ES-MS²⁹.

In summary, the decline in HTMA signal shown in Figure 5.3 can be ascribed to the convoluted effects of high ionic strength and competition for surface sites of the electrosprayed droplets which is not offset by an overall increase in sampling efficiency attributable to reduced surface tension. The above experiments have only demonstrated that lowering the surface tension of the solution can improve the mass spectrometric response of electrosprayed ions. However, it is not obvious why the ES-MS response reverses at even lower values of surface tension which correspond to higher concentrations of surfactants; i.e., why the HTMA signal decreases when the ODTMA concentration exceeds 30 μM (Figure 5.2). As stated earlier, the onset of micellization, solubility constraints, ionic strength effects, and competition for surface sites on the droplets comprise a list of factors that could possibly contribute to the behavior observed in Figure 5.2. Micellar and solubility effects can be ruled out as before, on the basis of the signal for ODTMA which appears to only reach a plateau rather than decline. The

factors that remain as reasonable candidates are the high ionic strength of the medium and competition for surface sites of the electrosprayed droplets.

In an attempt to deconvolute these factors, another experiment was performed where 50 μM solutions of ODTMA containing varying concentrations of HTMA were prepared in 50:50 (v/v) water:methanol and electrosprayed. This experiment is similar to the experiment shown in Figure 5.2 with the exception that now the ODTMA concentration is held constant at 50 μM and the HTMA concentration is allowed to vary. It was thought that surface activity interferences would be minimized in this experiment because ODTMA is more surface active than HTMA. The results of this experiment are shown in Figure 5.4 where ion intensities for ODTMA decline consistently with the addition of HTMA while the HTMA intensities increase at least up to 30 μM .

In this experiment, the decrease in the ODTMA signal from the outset of HTMA addition cannot be attributed to surface activity effects since ODTMA is more surface active than HTMA and also present at higher concentrations. Other possibilities are micellization, ionic strength effects, and solubility effects. The former two factors should be ruled out because they would be expected to influence ion intensities at m/z 144 (HTMA) as well as at m/z 312 (ODTMA). The remaining factor is the solubility difference between HTMA and ODTMA. As noted in Table 5.1, ODTMA is significantly less water soluble than HTMA. It is reasonable to assume that a 50 μM ODTMA bromide solution is at its solubility limit in the evaporating electrosprayed droplets and that further addition of HTMA bromide will precipitate it out of the solution because the bromide supplied by the HTMA bromide shifts the solubility equilibrium of ODTMA

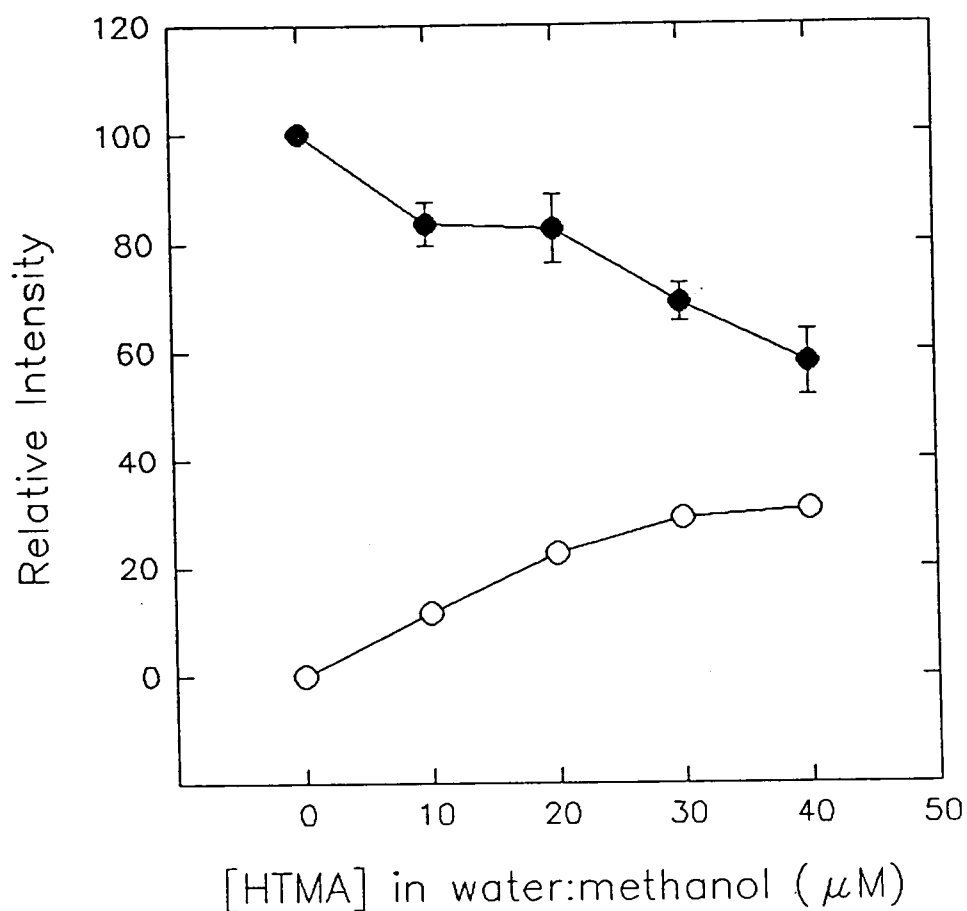


Figure 5.4 Dependence of the electrospray response of 50 μM [ODTMA] (●) and HTMA (O) on [HTMA] in 50:50 (v/v) water:methanol. All intensities are relative to that of ODTMA in the absence of HTMA.

bromide to the left (undissociated form). In contrast, a 50 μM HTMA solution is not at its solubility limit and should not precipitate out with the addition of ODTMA (experiment shown in Figure 5.2). The above argument indicates that, whereas the ODTMA decline in Figure 5.4 could be solubility-related, the HTMA decline in Figure 5.2 is probably not. In summary, competition effects were eliminated in the above experiment but solubility problems were introduced in its place.

Thus, ionic strength effects and competition for surface sites on the droplets still remain as reasonable candidates for the observed decline in HTMA signal when ODTMA concentration exceeds 30 μM in the experiment shown in Figure 5.2. It should be apparent that deconvoluting surface activity and ionic strength effects is difficult because high concentrations of the surfactants must be used before competition for surface sites sets in. Nonetheless both effects are reasonable and likely to contribute to the diminished response of HTMA observed in Figure 5.2.

The experiments with the cationic surfactants demonstrated that the surface tension-lowering property of these compounds could be exploited in ES-MS to improve the efficiency of the droplet evaporation process and lower the barrier to ion desorption. However, three factors limited the amount of a cationic surfactant that could be added to the matrix: its solubility, the total ionic strength of the solution, and the possibility of competition between the surfactant and the analyte. One possible way to reduce the extent of competition between the added surfactant and the analyte would be through the use of an anionic surfactant. It is suggested that negatively charged species are partially removed from the solution as a result of an oxidation process at the electrospray

electrode⁸⁹. If SHS is the anion that is removed through such an oxidation process, then it should not be transmitted into the spray as effectively as a cationic surfactant such as ODTMA would, and thus not compete with HTMA as severely. Furthermore, transmission of the anionic surfactant into the spray could even be considered beneficial, if it could enrich the droplet surface with the positively charged analyte through Coulombic attractive forces. Parameters that are expected to interfere in experiments utilizing anionic surfactants are the total ionic strength of the solution, solubility limitations, and the possibility of ion pair formation between the positively charged analyte and the anionic surfactant.

As a test of this proposition, 50 μM solutions of HTMA containing varying amounts of SHS (sodium hexadecyl sulfonate; aqueous $\text{CMC}=7\times 10^{-4}\text{ M}$ at 40° C^{90}) were prepared in 50:50 (v/v) water:methanol and electrosprayed. The results depicted in Figure 5.5 show that the HTMA signal decreases upon addition of the anionic surfactant. The mass spectra do not show any peaks corresponding to SHS or an SHS-adduct in the 0-350 amu range ($\text{MW} = 328$). Surface tension data are also provided in Figure 5.6, demonstrating that surface tension values of samples prepared with SHS are in the same range as those of equivalent solutions prepared with ODTMA (Figure 5.1). The lower signal cannot be attributed to an increase in the ionic strength of the medium (Na^+ counterions from SHS), at least not at the outset of SHS addition. The surface tension data of Figure 5.6 also do not indicate the onset of micellization of SHS in the solution. The latter effect is usually seen as a discontinuity (change in slope) in the plots of surface tension versus concentration and such a behavior is not observed for SHS. Competition

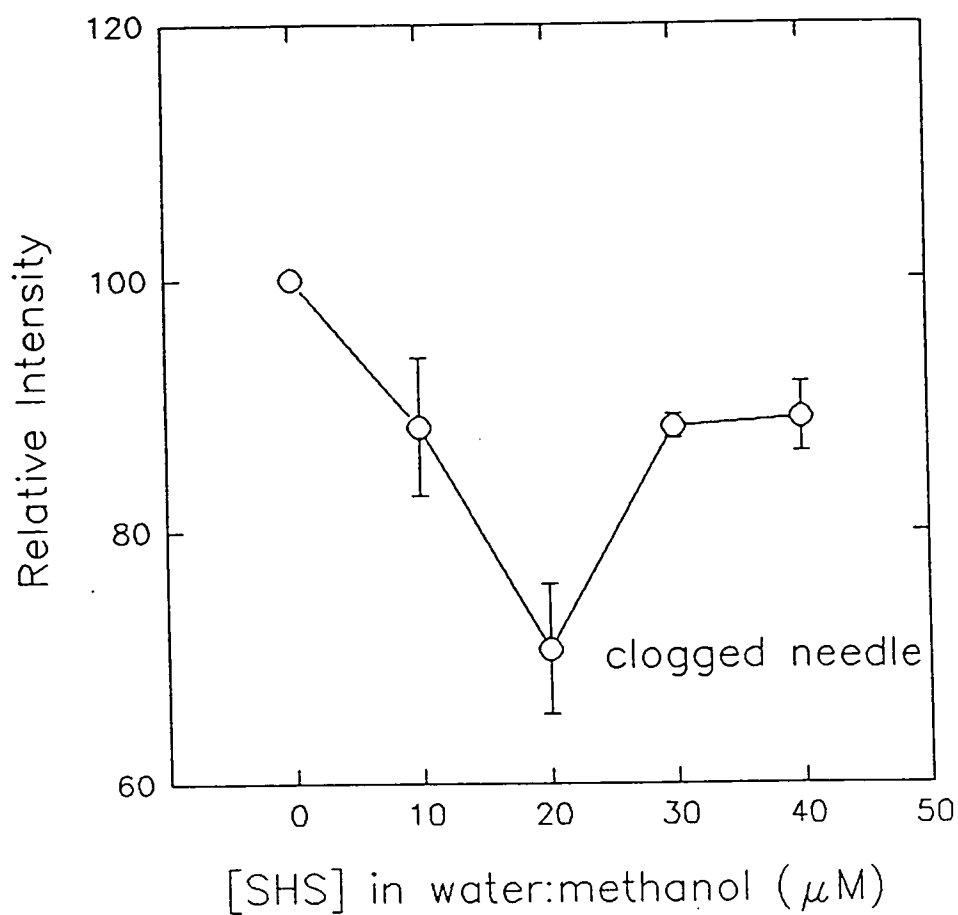


Figure 5.5 Dependence of the electro spray response of 50 μM HTMA (O) on [SHS] in 50:50 (v/v) water:methanol. All intensities are relative to that of HTMA in the absence of SHS.

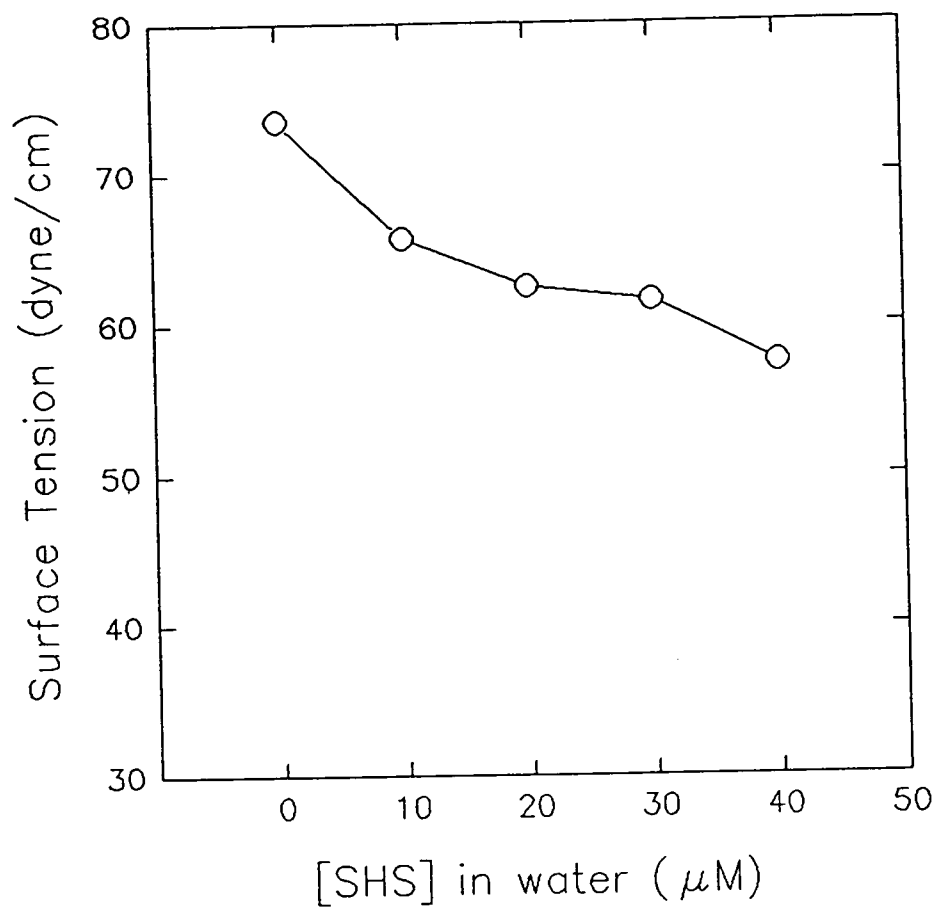


Figure 5.6 Dependence of the surface tension of 50 μM HTMA solutions on [SHS] in water (O).

effects between the surfactants (HTMA and SHS) can also be ruled out if we maintain the assumption that negatively charged species are less abundant in the electrospray when it is operated in the positive ion mode.

The major differences between ODTMA and SHS which could account for the data of Figure 5.5 are their solubilities and charge state. The charge state of the surfactant could have a negative role rather than the previously suggested positive effect. In this case, electrostatic interactions between the negatively charged SHS and the positively charged HTMA, i.e., ion pairing interactions, would decrease the HTMA response by decreasing its free concentration in the spray. Such interactions are very likely at the high concentrations used in these experiments. Solubility constraints provide a more plausible explanation for the observed decline in the HTMA signal. SHS is approximately five times less soluble in water than ODTMA⁹¹. It is suggested that SHS precipitates out of solution during the droplet evaporation stage as the droplet liquid becomes enriched in water. Indeed, persistent clogging of the ES needle observed in these experiments is consistent with this suggestion.

In summary, the use of this anionic surfactant did not offer any advantages over the previously tested positively charged surfactant, ODTMA. In fact, two new problems were encountered in the course of experimentation with SHS: continuous clogging of the electrospray needle, believed to be a consequence of its lower solubility, and contamination of the mass spectrometer with sodium, the counterion of the anionic surfactant. The sodium contamination mentioned above turned out to be a serious problem because it affected the mass spectra of the species that were normally detected

as protonated molecular ions. After the contamination episode, both $[M+H]^+$ and $[M+Na]^+$ ions were observed in the mass spectra of these species.

The final part of the study assessing the role of surface tension on the ES-MS response of analyte ions utilizes a nonionic surfactant for modifying the surface tension of the solution. Competition for surface sites is expected to be less severe if the analyte and surfactant are not of the same charge. Furthermore, use of a nonionic surfactant allows modification of solution surface tension without contributing to the total number of charge carriers in the solution. As noted above, this could be an important consideration because ion intensities are shown to decrease with the addition of a foreign electrolyte²⁹. Triton X-100 ($t\text{-Oct-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_x\text{OH}$, where $x = 9\text{-}10$) is a nonionic surfactant commonly used for biological membrane dissolution. 50 μM solutions of HTMA were prepared in 50:50 (v/v) methanol:water containing 0.016 - 1.6 mM Triton X-100. These relatively high surfactant concentrations were accessible because of the higher solubility of Triton X-100 (relative to ODTMA and SHS) in the mass spectrometric matrix. Over the range of concentrations used with ODTMA, there is a reasonably smooth increase in HTMA ion signal with increasing concentration of Triton X-100 (Figure 5.7). An ultimate decrease in signal strength is again observed, but at much higher concentrations than for ODTMA.

In this case, the observed effect is attributed to formation of micellar structures in the evaporating droplets. This suggestion is supported by the surface tension data of Figure 5.8 (line through the hollow circles) which show indications of micelle formation (a change in slope) in the aqueous solutions (the aqueous CMC of Triton X-100 is on the

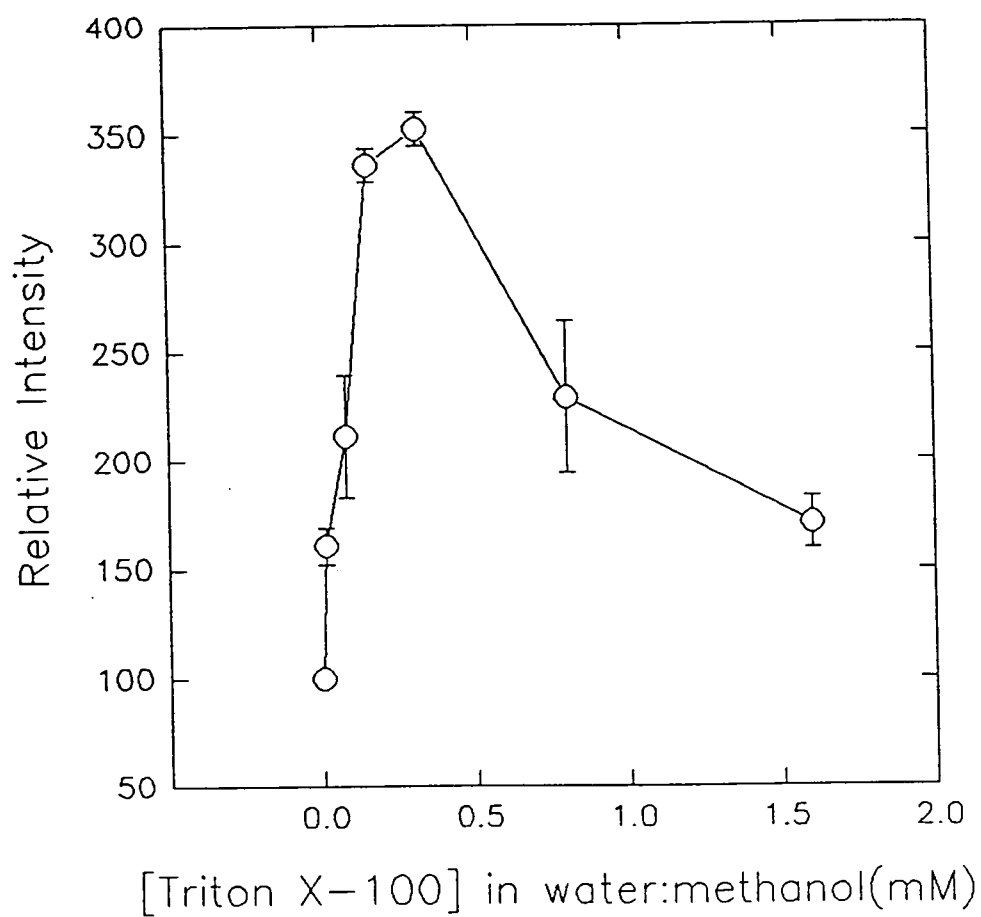


Figure 5.7 Dependence of the electrospray response of 50 μM HTMA on [Triton X-100] in 50:50 (v/v) water:methanol. Intensities are relative to that of HTMA in the absence of Triton X-100.

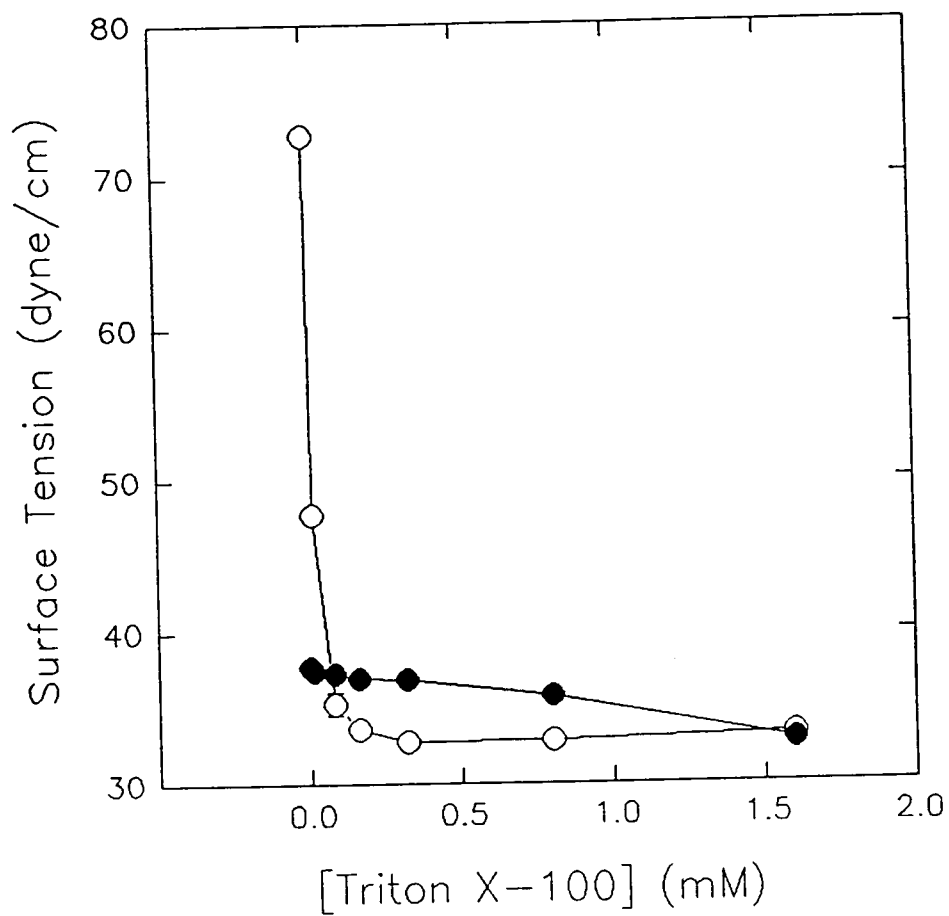


Figure 5.8 Dependence of the surface tension of 50 μ M HTMA solutions on [Triton X-100] in water (O) and in 50:50 (v/v) water:methanol (●).

order of $3 \times 10^{-4} \text{ M}^{90}$). As before, the mass spectrometric experiment was performed in 50:50 (v/v) water:methanol where micellization cannot be detected from inspection of surface tension measurements (Figure 5.8). However, selective evaporation of methanol in the drift space between the needle and the capillary inlet is believed to enrich the water content of droplets electrosprayed from mixed water/methanol solution⁷⁷ to the extent that would allow observation of micellar effects.

To invoke micellization for the behavior observed at high concentrations of Triton X-100 in Figure 5.7 further requires that it occurs on a timescale comparable to droplet lifetimes. From the model of Busman *et al.*⁵⁷, the ion drift time, t_{res} , for a point-to-plane geometry electrospray source with 4 mm separation and operating at 1 μA (in air; $\epsilon = 8.85 \times 10^{-12} \text{ C/V}\cdot\text{m}$) was estimated as $\approx 0.2 \text{ ms}$. Although droplet lifetimes may be somewhat shorter than this (due to Rayleigh subdivision), comparison with the micellization timescale (10^{-8} to $10^{-3} \text{ s}^{92,93}$) indicates that participation of micelles is not unreasonable. Alternatively, increased viscosity at high concentrations of Triton X-100 may contribute to the declining HTMA signal by interfering with droplet formation, subdivision, or evaporation processes.

A practical implication of examining the role of surface tension is in the electrospray of aqueous solutions. Chait *et al.* have electrosprayed aqueous solutions by utilizing a finely tapered needle⁹⁴. However, most electrospray work is generally done with some organic solvent, at least in part to lower the surface tension of the solution, reduce droplet size, and improve volatility. It would be of interest to see if the addition of surfactants would allow electrospraying purely aqueous solutions at levels which would

not interfere with the analyte. Triton X-100 was chosen for this experiment because of its high solubility and electroneutrality. The constraint in the use of Triton X-100 is the fact that it micellizes as previously demonstrated. The anionic and cationic surfactants were not utilized because of solubility and ionic strength constraints.

50 μM samples of HTMA were prepared in water containing varying amounts of Triton X-100. The results of this experiment are described in Table 5.2. Triton X-100 is shown to be effective in lowering the surface tension of aqueous solutions to values low enough for a stable electrospray to form. The aqueous surface tension data (second column in Table 5.2) demonstrate that this occurs at surface tension values approximating 33 dyne/cm, almost at the point of micellization. In fact, at concentrations exceeding the CMC of Triton (the 0.80 mM and 1.6 mM samples), the HTMA peak disappears suggesting that the analyte is stripped into the micelles. It is also noteworthy that surface tension values of 50:50 (v/v) water:methanol solution, a standard ES-MS matrix, are in the same range (30 - 35 dyne/cm). The signal for 50 μM HTMA was measured in the Triton-treated aqueous solutions and noted to be six times smaller than the response of 50 μM HTMA in methanol. This is presumably related to the higher volatility and lower surface tension value of purely methanolic samples compared to the aqueous samples that contain Triton X-100. The above observation does not detract from the overall utility of Triton X-100 in ES-MS because there are instances where the use of an organic co-solvent must be avoided. Furthermore, it is conceivable that an ES source with a more efficient desolvation mechanism would tolerate the lower volatility of the aqueous samples somewhat better.

Table 5.2 Electrospray of aqueous solutions of HTMA (50 μ M) with varying amounts of added Triton X-100.

Triton (mM)	Surface tension (dyne/cm)	Onset voltage (V)	Observations
0	72.7 ± 0.2	+4900	Spray hops between pulsating and spraying modes.
0.016 mM	47.8 ± 0.2	+4900	Unstable spray.
0.080 mM	35.3 ± 0.8	+4500	Form stable spray; see HTMA peak; spray relaxes back into unstable mode after a few minutes.
0.16 mM	33.6 ± 0.1	+4400-4500	Same as 0.080 mM solution.
0.32 mM	32.8 ± 0.0	+3900-4000	Reasonable spray. See HTMA peak.
0.80 mM	32.9 ± 0.2	+3700	Stable spray; no ions detected.
1.6 mM	33.3 ± 0.1	+3700	Same as above (0.80 mM solution).

SURFACE ACTIVITY STUDIES

In the experiments with the cationic surfactant, competition between the cationic surfactants, HTMA and ODTMA, was invoked as one of two effects that contributed to the declining signal of HTMA at high ODTMA concentrations. This observation initiated a study of surface activity effects in ES-MS.

Surface activity is a property of compounds possessing a hydrophilic functional group (ensuring in most cases at least moderate solubility in water), and a hydrophobic nonpolar group. To minimize solvent disruption by interaction with non-polar surfactant moieties, surfactants tend to concentrate at interfaces, including the solution surface. The resulting surface excess concentration gives rise to the reduction in surface tension for which these reagents are named⁹⁵. A homologous set of *n*-alkyltrimethylammonium bromides ($C_nH_{2n+1}N(CH_3)_3Br$ where $n = 6, 10, 12, 14, 16, 18$) was thus selected to study the role of surface activity in electrospray mass spectrometry. For the members of this homologous set, surface activity correlates with molecular weight; *i.e.*, the heaviest cations are known to have the highest surface excess concentrations in water⁹⁶ and presumably in other hydrophilic solvents like methanol. As a result, separate equimolar solutions of these salts would be expected to display surface tensions inversely proportional to analyte molecular weight. This in fact was observed (see Table 5.3). Furthermore, these homologous analytes should vary little in their solvation or gas-phase ion-molecule chemistry, making them suitable test compounds for isolation of surface activity effects.

To allow observation of surface activity effects without convolution of surface

Table 5.3 Surface tension of 50 μM aqueous solutions of *n*-alkyltrimethylammonium bromides.

Sample	Surface tension (dyne/cm)*
<i>n</i> -hexyltrimethylammonium bromide	71.5 ± 0.1
<i>n</i> -decyltrimethylammonium bromide	70.3 ± 0.1
<i>n</i> -dodecyltrimethylammonium bromide	69.6 ± 0.1
<i>n</i> -tetradecyltrimethylammonium bromide	68.6 ± 0.1
<i>n</i> -hexadecyltrimethylammonium bromide	65.8 ± 0.1
<i>n</i> -octadecyltrimethylammonium bromide	59.3 ± 0.03

* The error limits represent the standard deviation of the average of three surface tension measurements (See page 62).

tension-related variation in the efficiency of electrospray droplet formation, a number of solutions were prepared that were 1, 2, 5, 10, 15, 20, 33.3 and 50 μM in each of six *n*-alkyltrimethylammonium salts in 50:50 (v/v) water:methanol or methanol. For equimolar mixtures that were 1 and 2 μM in each component, intensities of each component were found to be virtually equal for all members of the homologous set. This is demonstrated in Figure 5.9 which shows a typical ES mass spectrum obtained for a solution that is 1 μM in each of the six listed *n*-alkyltrimethylammonium bromides. When the concentrations of the various *n*-alkyltrimethylammonium bromides in the solution are in the 2 - 10 μM range, signal intensities are seen to correlate strongly with the length of the alkyl chain, and thus with the surface activity of the analyte. This is shown in a mass spectrum of a 10 μM mixture presented in Figure 5.10. Finally, for concentrations exceeding 10 μM in each component, the trend observed is shown in Figure 5.11 which plots the observed intensities of 50 μM mixtures in both 50:50 (v/v) water:methanol and methanol, as a function of the length of the alkyl chain of the quaternary ammonium bromides. Here intensities rise for the $\text{C}_6\text{H}_{13}^-$ to $\text{C}_{16}\text{H}_{33}^-$ homologs and subsequently decline for the $\text{C}_{18}\text{H}_{37}^-$ homolog.

The observed behavior (i.e., intensity of the $\text{C}_{18}\text{H}_{37}^-$ homolog < intensity of $\text{C}_{16}\text{H}_{33}^-$ homolog) appears to reflect the much lower solubility of the $\text{C}_{18}\text{H}_{37}^-$ homolog compared to the rest of the members in this set. The solubility data of Table 5.1 and the mass spectrum of the 10 μM solution where the intensity of the $\text{C}_{18}\text{H}_{37}^-$ homolog is larger than that of the $\text{C}_6\text{H}_{13}^-$ homolog (Figure 5.9) support the above statement.

The ion intensities of the 50 μM samples of the quaternary ammonium salts are

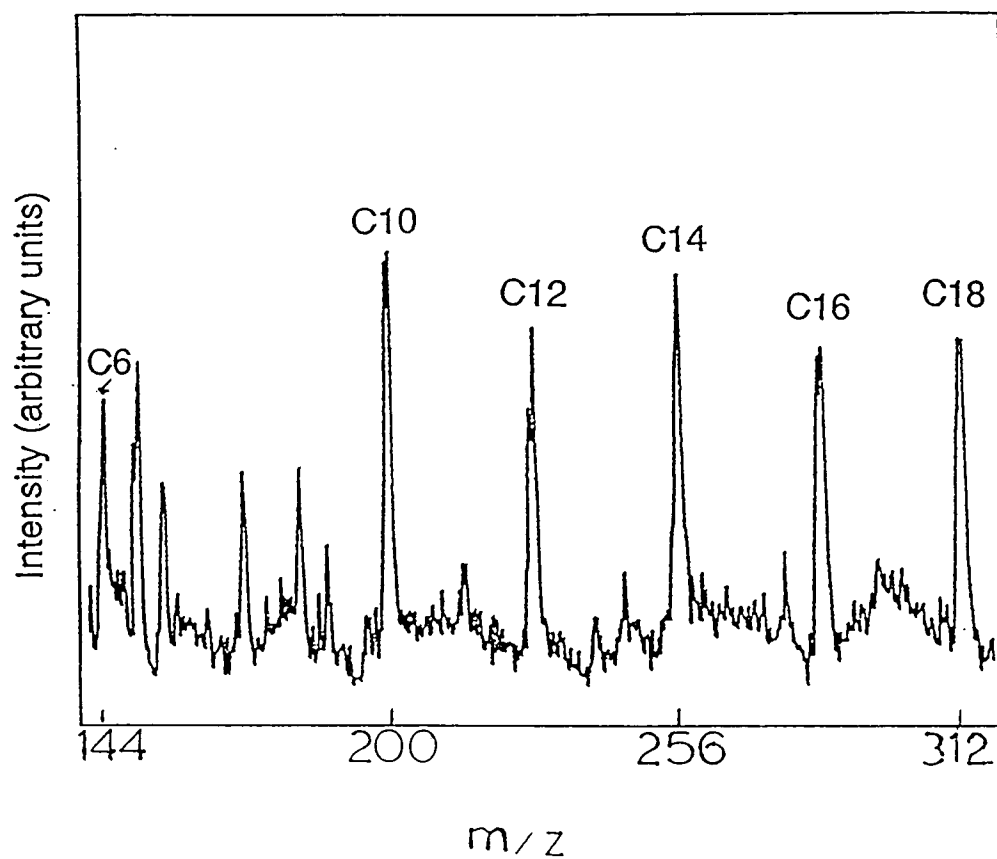


Figure 5.9 ES mass spectrum of a mixture of six *n*-alkyltrimethylammonium bromides, $[(C_nH_{2n+1})N(CH_3)_3Br]$, 1 μM in each component, in methanol. The label C6 designates $C_6H_{13}N^+(CH_3)_3$ and so on.

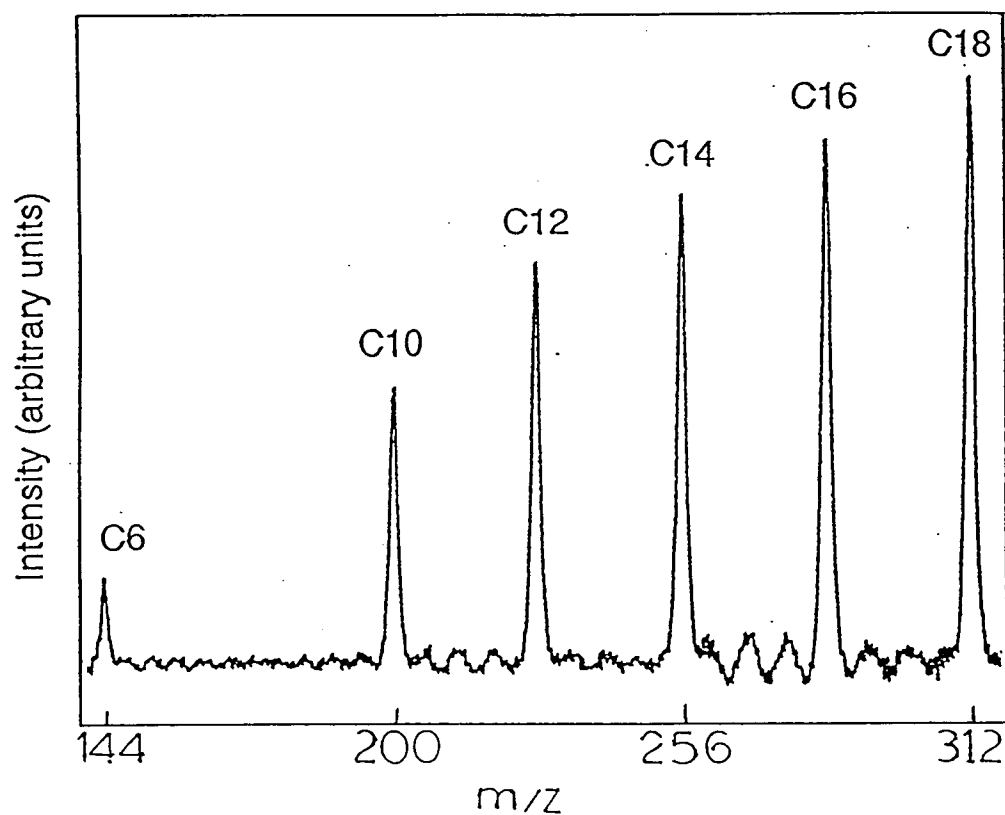


Figure 5.10 ES mass spectrum of a mixture of six *n*-alkyltrimethylammonium bromides, $[(C_nH_{2n+1})N(CH_3)_3Br]$, 10 μM in each component, in methanol. The label C6 designates $C_6H_{13}N^+(CH_3)_3$ and so on.

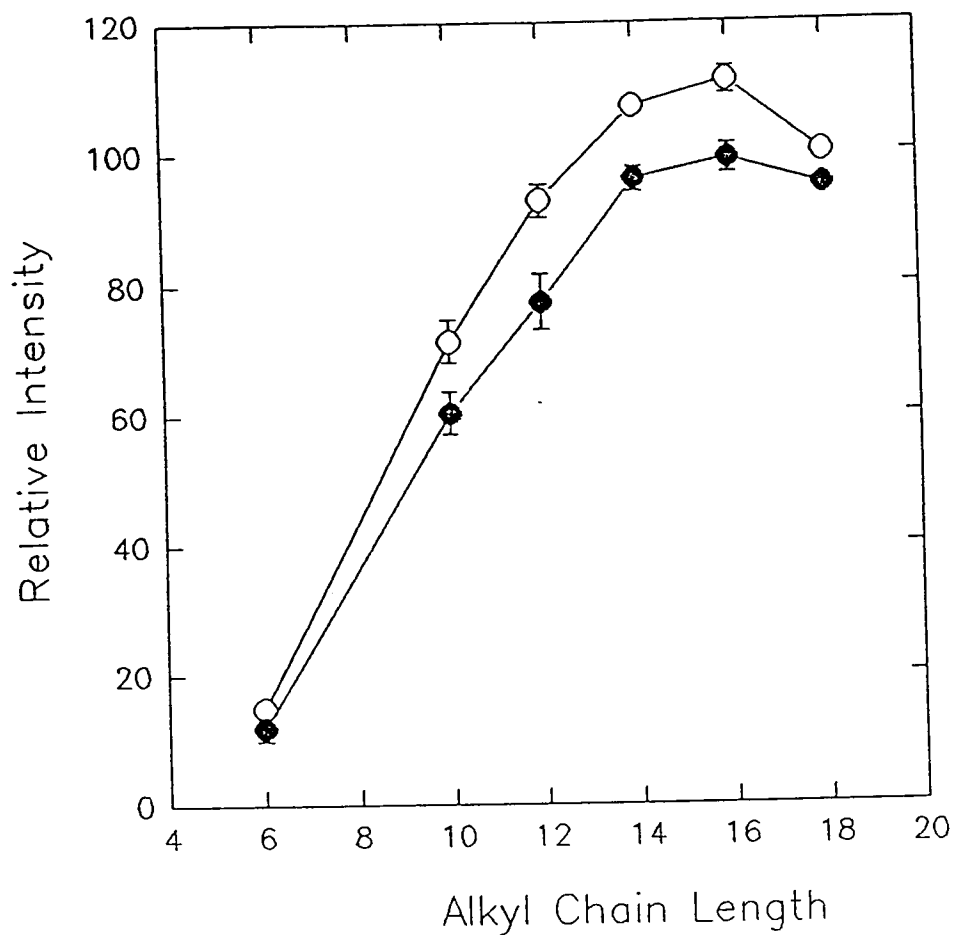


Figure 5.11 ES-MS ion intensities for an equimolar (50 μ M) mixture of *n*-alkyl trimethylammonium bromides $[(C_nH_{2n+1})N(CH_3)_3Br]$ in 50:50 (v/v) water:methanol (O) and in methanol (●). Data for methanol solutions are offset -10 intensity units for clarity. Intensities reported are relative to that of ODTMA.

also shown in two matrices (50:50 (v/v) water:methanol and 100% methanol) in Figure 5.11. This was done in order to confirm that surface activity trends would remain the same in both 50:50 (v/v) water:methanol and 100% methanol. The main difference observed between the two matrices was in the magnitude of the ion intensities which were higher in methanol relative to 50:50 (v/v) water:methanol. As before, this may be ascribed to the higher volatility and lower surface tension value of methanolic solutions. In summary, the above experiments suggest that competition between the surface active quaternary ammonium salts in the electrosprayed droplets may contribute to their mass spectrometric response. The latter effect is evident in equimolar mixtures of *n*-alkyltrimethylammonium bromides when the concentration of each component in the mixture exceeds 2 μM . At lower concentrations all components appear to be sampled with equal efficiency presumably because there are plenty of surface sites available on the electrosprayed droplets.

SUMMARY AND CONCLUSIONS

Of the two factors considered in this work, the effects of solution surface tension have been previously investigated. Surface tension has been shown to affect the onset voltage for electrospray^{25,37} and predicted to correlate directly with the electrospray current (although the correlation can be masked by changes in solution conductivity²⁵). These effects were not dominant in the experiments described here; the needle voltage was fixed well above onset, and there was no obvious correlation between the 0.5 - 1.0 μA electrospray current and surface tension. Nevertheless, at most surfactant concentrations

(cationic and nonionic) there was a noticeable inverse correlation between analyte ion intensity and solution surface tension; this was attributed to a more efficient desolvation process as well as an improved ion desorption step which could both result from the lowering of the surface tension. Increasing concentrations of surfactant eventually suppressed the analyte ion signal.

In the case of the cationic surfactant, the suppression apparently resulted from competition between the analyte and surfactant for surface sites as well as from the increased ionic strength of the medium. With Triton X-100, suppression occurred at much higher surfactant concentrations, suggesting a possible role for micelles within the electrosprayed droplets. In the case of the anionic surfactant, lowering of the surface tension did not improve ion intensity. This was attributed to the continuous clogging of the ES needle which presumably related to the inherent low solubility of the anionic surfactant. In summary, lowering of the surface tension was shown to improve the electrospray mass spectrometric response of analytes. A more significant outcome of the surface tension studies was the newly acquired ability to electrospray purely aqueous solutions by lowering their surface tension with the addition of surfactants. The issue of electrospraying aqueous solutions is of significance in biological mass spectrometry and not fully resolved; thus, the above finding should be of interest and utility to those who generally deal with such samples.

In contrast with surface tension, the role of surface activity in ES has not previously been considered in detail. FAB signals have long been known to correlate with surface activity⁹⁷. Derivatization techniques have been utilized to improve FAB

sensitivities of ions⁹⁸. In ES-MS, surface activity effects appear to become apparent only at concentrations where competition for surface sites on the electrosprayed droplets prevails. This suggests that one may not benefit significantly from introducing a derivatization step into the overall sample preparation scheme; however, it rationalizes the higher intensities observed upon denaturation of globular proteins that possess hydrophobic (and therefore surface active) amino acid residues in their core⁹⁹.

CHAPTER 6

ION SOLVATION AND ION PAIRING

The formation of gaseous ions in ES can be divided into two steps (Figure 6.1). The first involves setting-up of a charge at the surface of the sample, a process generally attributed to an "electrophoretic" charging mechanism³⁷ whereby preformed ions present in the solution migrate to the interface under the influence of the applied electric field. The second deals with the evaporation of ions under the influence of the electric field associated with this charged interface. A number of parameters, such as the surface tension of the solution, the extent of ion-solvent interactions, and ion-pairing processes are expected to be important in determining the barrier to this ion evaporation stage and hence, to ultimate detection. Among these, the effect of the surface tension of the solution was studied in Chapter 5. This chapter deals with the effects of ion-solvent and ion-ion interactions on the observed ES-MS response.

In testing for ion-solvent interactions, it is desirable to find a set of model compounds where solvation parameters are the only variables. This is not usually feasible since several parameters tend to vary simultaneously, even in the simplest of chemical systems. Nonetheless, some compounds better fit the requirements than others. It was thought that the tetraalkylammonium salts (quats) would provide a suitable set of homologous compounds for which solvation energies are determined and known to vary systematically. Indeed, for the symmetric quats, the changes in solvation enthalpies have been explained by the simple classical theory of Born¹⁰⁰, which suggests an inverse

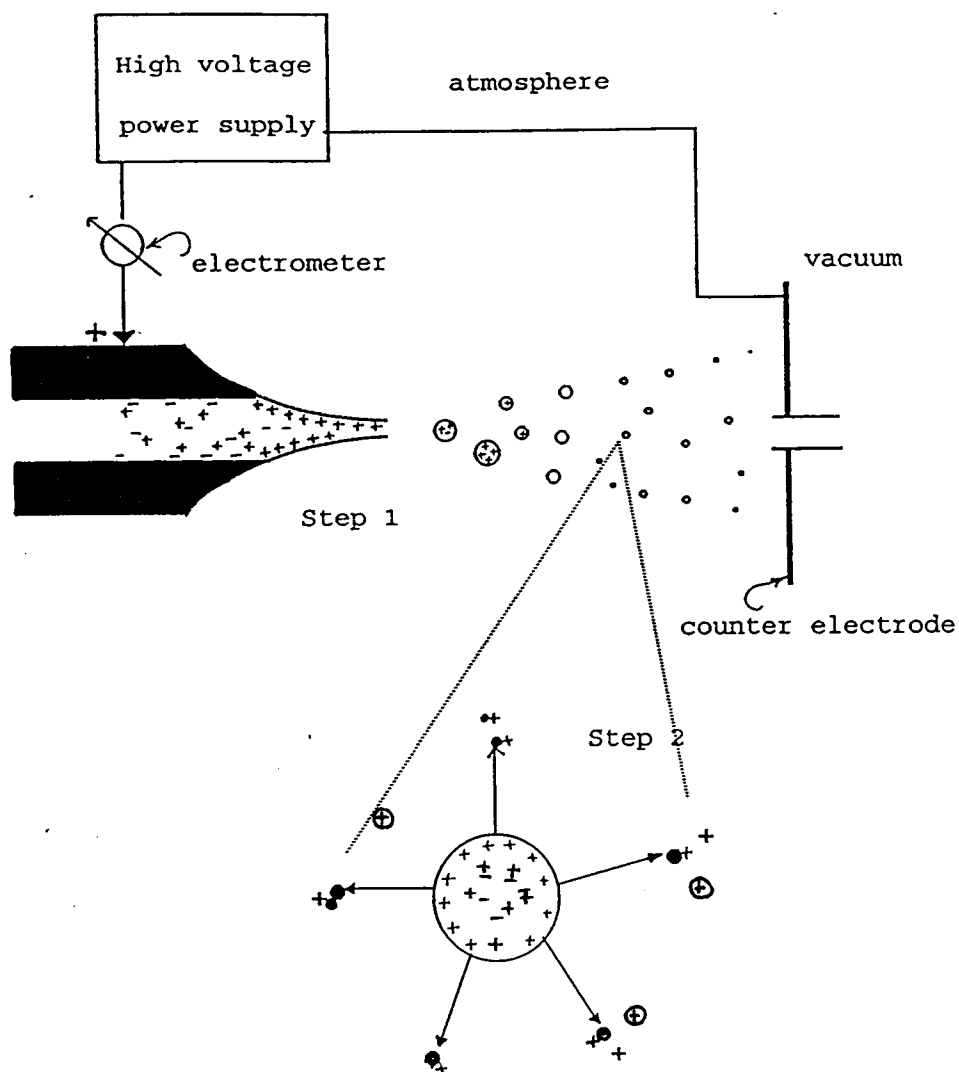


Figure 6.1 Suggested steps in the formation of gaseous ions in electrospray mass spectrometry.

relationship between the ionic radius and the free energy of solvation. Ions with large effective ionic radii, i.e., ions which already benefit from a high degree of internal charge delocalization, interact less strongly with the solvent because further delocalization of the charge is energetically less effective¹⁰¹. This is evident in the solvation enthalpies¹⁰² of quat solutions in water and methanol (Table 6.1). Furthermore, with quats as model compounds, concerns relating to acid-base effects can be excluded since these species are essentially electrolytes devoid of proton abstracting or donating properties.

The quats were therefore used for examination of ion-solvent interactions in ES-MS. For these studies, mixtures of a homologous set of these species, consisting of the tetramethyl-, tetraethyl-, tetrapropyl-, and tetrabutylammonium bromides, were prepared in two matrices (50:50 (v/v) water:methanol and 100% methanol) and electrosprayed.

To assess the occurrence of ion pairing phenomena and the counterion dependence of analyte response, diquatery ammonium salts $(\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3 \cdot 2\text{A}^-$ (where A^- is the counterion of the diquat salt), were utilized. These salts were prepared by ion exchange chromatography from $(\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3 \cdot 2\text{Br}^-$ as described in Chapter 4. The influence of ion pairing on ES-MS response was also tested by comparing the responses of two tetrabutylammonium salts possessing counterions with different ion association tendencies. These species were also prepared from tetrabutylammonium bromide by ion exchange chromatography (See Chapter 4 for details).

Table 6.1 Solvation Enthalpies* (kJ mol⁻¹) of tetraalkylammonium ions in water and methanol at 25° C.

Sample	Water	Methanol
tetramethylammonium	-523.7	-517.7
tetraethylammonium	-508.7	-493.5
tetrapropylammonium	-502.6	-482.1
tetrabutylammonium	-502.5	-476.5

* Solvation enthalpies were obtained from reference 102.

RESULTS & DISCUSSION

ION SOLVATION STUDIES

Due to the hydrophobic nature of their alkyl groups, quat salts larger than tetramethylammonium bromide accumulate at liquid-air interfaces and thereby lower the surface tension of solution¹⁰³. This effect is small at the concentration level of interest (50 μM) as demonstrated in the surface tension measurements of aqueous quat samples presented in Table 6.2; however, the concentration of the quats in the electrosprayed droplets is expected to increase as the droplets evaporate, resulting in a more significant decrease in the surface tension of the droplet liquid¹⁰³. Two measures were therefore taken to minimize contributions from changes in surface tension. Equimolar mixtures consisting of the four quat salts were prepared instead of individual samples containing only one quat. A mixture should possess a unique surface tension value which is presumably determined by all of the hydrophobic analytes present in that mixture. Further, the mixtures of quats were prepared in methanol in addition to the standard matrix consisting of 50:50 (v/v) water:methanol because the inherently low surface tension of methanol would not be lowered significantly by the addition of quats. This was previously observed with the surfactants in Chapter 5. The above measures were assumed to minimize surface tension contributions to the observed response for these species.

Figure 6.2 (top and center) shows the ES-MS intensities of the quats in equimolar mixtures that were 50 μM in each component. The top trace in Figure 6.2 shows the observed intensities of the 50 μM mixture in 50:50 (v/v) water:methanol, and the middle

Table 6.2 Surface tension of 50 μM aqueous solutions of tetraalkylammonium bromides.

Sample	Surface tension (dyne/cm)
water	73.0 ± 0.02
tetramethylammonium bromide	70.7 ± 0.03
tetraethylammonium bromide	70.5 ± 0.2
tetrapropylammonium bromide	70.5 ± 0.2
tetrabutylammonium bromide	69.9 ± 0.1
tetrapentylammonium bromide	69.7 ± 0.2

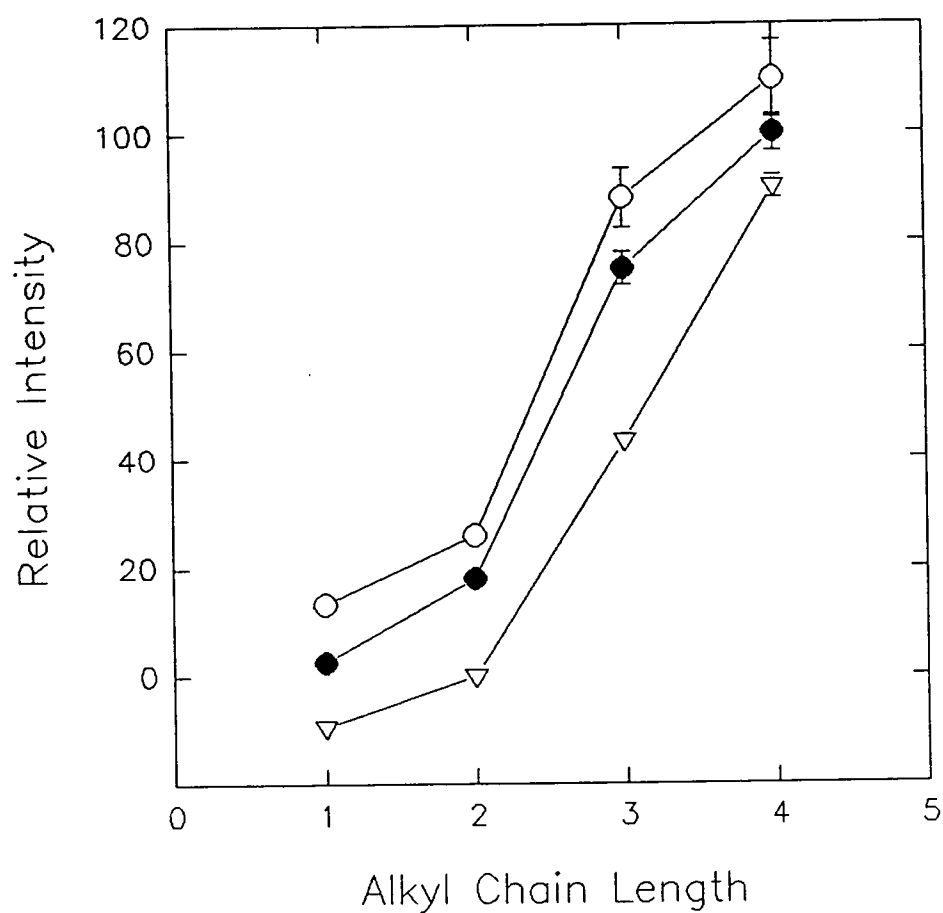


Figure 6.2 ES-MS ion intensities of an equimolar mixture of tetraalkylammonium bromides in 50:50 (v/v) water:methanol (50 μ M) (O); in methanol (50 μ M) (●); and in methanol (25 μ M) (▽). Data for methanol:water are offset +10 and those for methanol (25 μ M) are offset -10 intensity units for clarity.

trace shows the intensities of a similar mixture prepared in 100% methanol. For each mixture, correlation is observed between ion intensity and the presumed degree of solvation, with the least solvated species (largest quat) producing the most intense response. For both matrices, the intensities seem to level off from the tetrapropyl- to the tetrabutyl- homolog. This behavior was attributed to the lower solubility of the tetrabutyl- quat because lowering the concentration of the quats in the mixture to 25 μM in methanol (Figure 6.2, bottom) resulted in a signal that simply increased without any indication of reaching a plateau.

The results of the ion solvation experiment with the quats are consistent with the "ion evaporation theory" of Iribarne and Thomson¹⁷ simply because the latter proposes an activation energy barrier to ion desorption which contains a term for the standard free energy of solvation of the ion in solution (ΔG^0_s). As mentioned earlier, ΔG^0_s is estimated by the Born equation⁸² and is roughly proportional to z^2/r , where z is the charge of the quat ion and r is its radius (See Chapter 5). In other words, the standard free energy of solvation, and hence, the barrier to desorption decrease proportionally with increasing size of the ion. This results in the trend observed in Figure 6.2 where the ES-MS intensities follow the size of the quat ions.

Having demonstrated the correlation between the ES intensities of the quats and their solvation enthalpies, it is worthwhile to digress and comment on the hydrophobic nature of these species which was earlier suggested to lower the surface tension of the

solution¹⁰³. The term hydrophobic interactions describes the structure-making^{5*} effect of quats on water and other highly structured solvents. By this is meant that the solvent molecules become highly ordered around ions possessing nonpolar groups in an attempt to minimize disturbance to solvent structure. Hydrophobic interactions are more prominent for large organic ions of low charge state and are particularly important in media of high dielectric constant¹⁰⁴. This is in contrast to the electrostatic interactions that are significant for small ions that are highly charged¹⁰⁴. An outcome of the hydrophobic interactions is that large ions tend to be rejected by the solvent phase and accumulate at the surface of that phase. Surface tension measurements reported for aqueous quat solutions reflect this effect¹⁰³. Among the quats tested, the tetramethyl-homolog does not accumulate at the surface of the liquid and hence does not lower the surface tension. The larger quats, on the other hand, adsorb at the surface of the liquid in proportion to the size of their alkyl groups¹⁰³. This issue is of significance in ES-MS because the presence of a concentration gradient in the droplets could influence the desorption efficiencies of ions.

Another consequence of the varying hydrophobicities of the quats is that their interactions with solvent are not simply electrostatic in nature. Indeed the behavior of quats in water and other structured solvents, such as methanol, is generally discussed in terms of a competing balance between hydrophobic interactions due to the nonpolar groups and electrostatic interactions due to the charge of the ions¹⁰⁵. For tetramethyl-

^{5*} The terms "structure making" and "structure breaking" refer to the ability of electrolytes to increase or decrease the structure of solvent in their vicinity.

and tetraethylammonium salts, the interaction of the ionic parts have been shown to surpass the hydrophobic interactions due to the alkyl groups¹⁰⁶. The opposite situation has been reported for the tetrapropyl- and tetrabutylammonium salts where interactions of the alkyl groups override electrostatic interactions¹⁰⁶. This suggests that the trends shown in Figure 6.2 represent a balance between ionic and hydrophobic effects which are not weighted equally among the four quats tested.

ION PAIRING STUDIES

Another solution process that is expected to affect the ES-MS response is the ion pairing phenomenon. Ion pairing effects have been observed in other mass spectrometric techniques such as FAB (fast atom bombardment)⁸⁶ and EH (electrohydrodynamic ionization)¹⁰⁷ and, if present in ES, could potentially alter desorption efficiencies of ions and thus influence ES responses. It is therefore essential to assess their role in ES and learn if they can be used to improve ES sensitivities. Ion pairing phenomena have not been investigated extensively for ES presumably because their influence is considered to be minimal¹⁰⁸. For example, in positive mode ES, because the spray consists predominantly of positively charged species, little consideration is given to the whereabouts and fate of the negatively charged counterions that are naturally present in the solution. Even with the high reported efficiencies of the electrophoretic charging mechanism, some negative ions indeed float around in the electrosprayed droplets²⁹. The total ion current measured for a 15 $\mu\text{L}/\text{min}$ flow of 3×10^{-5} M NaCl solutions is reported to be approximately 3×10^{-7} A²⁹. This represents a 40% excess of positive ions in the

droplets with the remaining positive ions counterbalanced by negative ions. An implication of the presence of negative ions in the droplets is that, at least in certain circumstances, ion pairing phenomena could influence the ES response of analyte ions. This portion of the chapter describes the results of experiments performed to assess the role of ion pairing in ES-MS.

The extent of ion pairing was first assessed through examination of 25 and 50 μM samples of diquaternary ammonium salts (diquats), $(\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3\ 2\text{A}^-$ (where A^- = acetate, bromide, chloride, iodide, nitrite, and nitrate) prepared in 100% methanol. The diquats allow mass spectrometric observation of the ion pairing phenomenon since the singly paired species (only one A^- present) will be detected at an m/z different from that of the free unpaired species (no A^- present). Of course, no signal is expected for the fully paired-up diquat (both A^- present)! Figure 6.3 is a representative electrospray mass spectrum of a 50 μM solution of diquat diiodide in methanol showing the occurrence of ion pairing in ES. Up to two ions attributable to the analyte were detected: the doubly charged cation C^{2+} , $(\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3$, with m/z 87 and the singly ion paired species (CA^+ ; $\text{A}=\text{anion}$) with m/z equal to the sum of the masses of the diquat (174) and the counterion tested (i.e., m/z 301 for diquaternary ammonium iodide).

It is more convenient to use a numerical estimate of the extent of ion pairing in discussions of its influence on the measured ES-MS response, trends observed for various anions, and factors that govern ion pairing processes. A measure of the extent of ion pairing (%IP) can be calculated from the intensities of these ions using equation 6.2¹⁰⁷:

$$\%IP = I_{\text{CA}^+} / (I_{\text{CA}^+} + I_{\text{C}^{2+}}) \times 100 \quad \text{Equation 6.2}$$

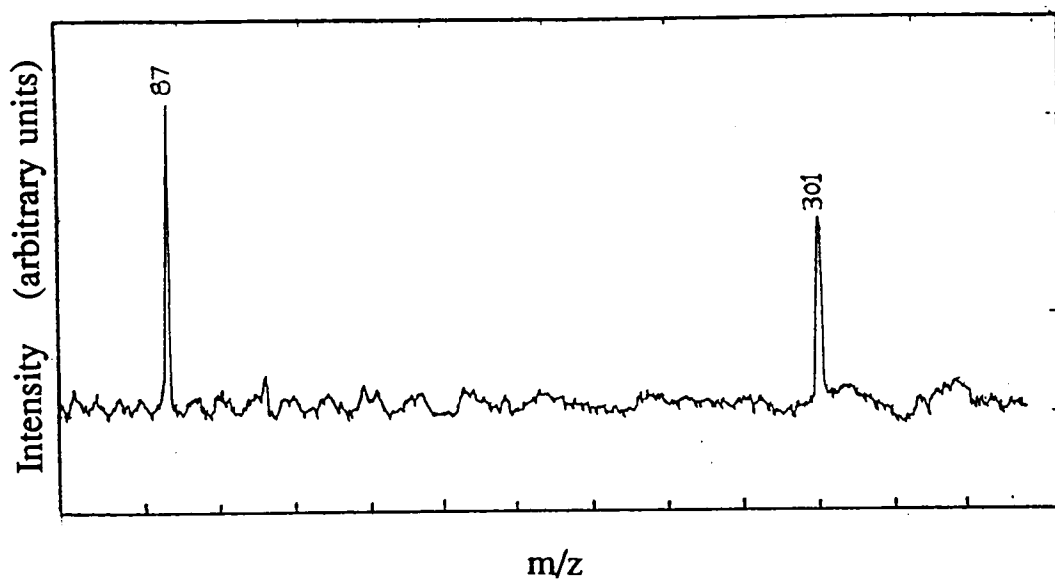


Figure 6.3 Electrospray mass spectrum of a 50 μM solution of $(\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3\ 2\text{I}^-$ in methanol.

where I is the average intensity of the subscripted ion, CA^+ is the singly ion paired diquat, and C^{2+} is the doubly charged, unpaired diquat. For the six anions tested, the calculated estimates of %IP are listed in Table 6.3 in the order of increasing ion pairing. The data of Table 6.3 also show that the extent of ion pairing appears to be concentration dependent, i.e., smaller in the more dilute solutions. This observation is consistent with the fact that ion-ion interactions predominate at high concentrations.

The trend observed for the ionic association (ion pairing) of the diquat with the various anions cannot be explained in terms of a single parameter. Factors such as ion size and shape, polarizability, dipole moment, heat of solvation, and entropy effects are all likely to contribute. An exhaustive analysis of all conceivable parameters is beyond the scope of this work; however, some of the more prominent factors (those from Table 6.4) will be discussed. In general, it is thought that small ions are strongly solvated by Coulombic type interactions and are therefore not readily available for interaction with the counterions in the solution. Conversely, large ions are more susceptible to ion pairing because their interactions with the solvent are smaller. Furthermore, for large organic ions, the ion pairing process is enhanced to a considerable extent by structural effects rather than by simple Coulombic interactions. This means that the structure-making property of the large organic electrolytes favors ionic association. Another parameter of significance is the polarizability (α) of the ions involved. It can be envisioned that "soft" polarizable ions more readily overlap than "hard" nonpolarizable ones.

The parameters mentioned above are listed in Table 6.4 for examination. The entropy term is taken as a measure of the solvent-structuring ability of the anions. The

Table 6.3 Percent ion pairing (%IP) for 25 and 50 μM diquat solutions

Anion (A)	%IP in methanol (50 μM) [*]	%IP in methanol (25 μM) [*]
acetate	11.4 $\pm$ 0.01	7.58 $\pm$ 0.01
nitrite	13.6 $\pm$ 0.01	7.96 $\pm$ 0.01
chloride	16.6 $\pm$ 0.01	9.77 $\pm$ 0.01
bromide	18.4 $\pm$ 0.01	10.5 $\pm$ 0.02
nitrate	25.1 $\pm$ 0.01	14.9 $\pm$ 0.01
iodide	47.1 $\pm$ 0.02	25.6 $\pm$ 0.02

* The error limits represent the standard deviation of the average of three spectral measurements (See page 62).

Table 6.4 Parameters examined for correlation with ion pairing

Anion	$\Delta H^0_{\text{hydration}}$ (kJ.mol ⁻¹)	V^0 (cm ³ .mol ⁻¹)	$\Delta S^0_{\text{hydration}}$ (JK ⁻¹ .mol ⁻¹)	α^d (Å ³)
acetate	-423	46.9	-170	≈4.8
nitrite	-366	32.6	-91	≈2.4
chloride	-376	24.2	-87	3.0
bromide	-345	30.8	-70	4.3
nitrate	-329	35.4	-77	4.5
iodide	-300	42.6	-47	6.5

The values for $\Delta H^0_{\text{hydration}}$ (enthalpy of hydration), V^0 (partial molar volume), $\Delta S^0_{\text{hydration}}$ (entropy of hydration) were obtained from reference 104. The values for α (polarizability) were obtained from reference 109.

anions listed in Table 6.4 are in the order of increasing ion pairing as before (Table 6.3). For the halides, the trends in hydration enthalpies¹⁰⁴ ($\Delta H_{\text{hydration}}^0$), partial molar volumes¹⁰⁴ (V^0) (used as a measure of ionic size), entropies¹⁰⁴ ($\Delta S_{\text{hydration}}^0$), and polarizabilities¹⁰⁹ (α) are consistent and reasonable; i.e., the smallest halide is the most hydrated, least polarizable, and most effective in structuring the solvent molecules in its vicinity. The iodide ion, on the other hand, shows the reverse behavior; i.e., it is weakly hydrated, highly polarizable, and ineffective in structuring solvent molecules. Thus the extent of ion pairing is expected to increase from chloride ion to the iodide ion, and that is in fact observed. For the oxygen-containing anions, the trends are not as clear and definite as they were with the halides. The nitrite and nitrate ions roughly fall into the overall pattern of the halides; i.e., some correlation exists between the hydration enthalpies, ion size, entropies, and polarizabilities. The acetate ion stands alone. The only reasonable correlation is with hydration enthalpies and entropies that are indicative of strong solvation and the presence of a structured shell of solvent molecules surrounding the anion, both of which preclude ion-ion interactions.

The results of the experiments on ion pairing appear to be consistent with parameters that influence ion pairing phenomena in solution thereby suggesting that solution processes are strongly represented in ES-MS. This is of significance because by controlling processes in solution one might be able to control the ES response of analytes. For example, if ion pairing is determined to result in a larger net signal for the analyte, it would make sense to adjust the experimental conditions such that they favor such processes. This could be done by addition of anions that show prominent ion pairing

tendencies. If, on the other hand, ion pairing is determined to decrease the observed response, conditions should be modified to preclude such a process; e.g., by using dilute solutions. The point of the argument is that to exploit this process in ES-MS, its influence on the sampling efficiency of analytes must be determined.

As a preliminary examination of this, the sampling efficiency of the six diquat salts was estimated by summing the signals from the doubly-charged diquat and the singly-charged ion paired species. The results, shown in Figure 6.4, reveal a slight enhancement in the total response only for the 50 μM samples. Hence, ion pairing does not appear to influence sensitivity when samples are sufficiently dilute. As a further test of the effect of ion pairing on the ES-MS response of analytes, two quaternary ammonium salts were prepared with anions that previously showed widely different ion pairing tendencies. These were the iodide and acetate ions. Individual samples of tetrapropylammonium iodide and tetrapropylammonium acetate were prepared in methanol at concentrations of 2, 5, 10, and 20 μM . Comparison of their electrospray intensities shown in Table 6.5 demonstrates that the observed response is dependent on the nature of the counterion at concentrations exceeding 5 μM . It is also noted that at the higher concentrations (10 and 20 μM), the signal is larger for tetrapropylammonium acetate, the quat salt with the anion that showed the least tendency for ion pairing.

This experiment demonstrates that ion pairing in solution affects the observed ES intensities of analyte ions. For example, for the quat salts tested above, the effect was detrimental, that is, ion pairing resulted in a smaller ES response. The data of this study are further consistent with the results of another study of ion pairing in ES-MS that

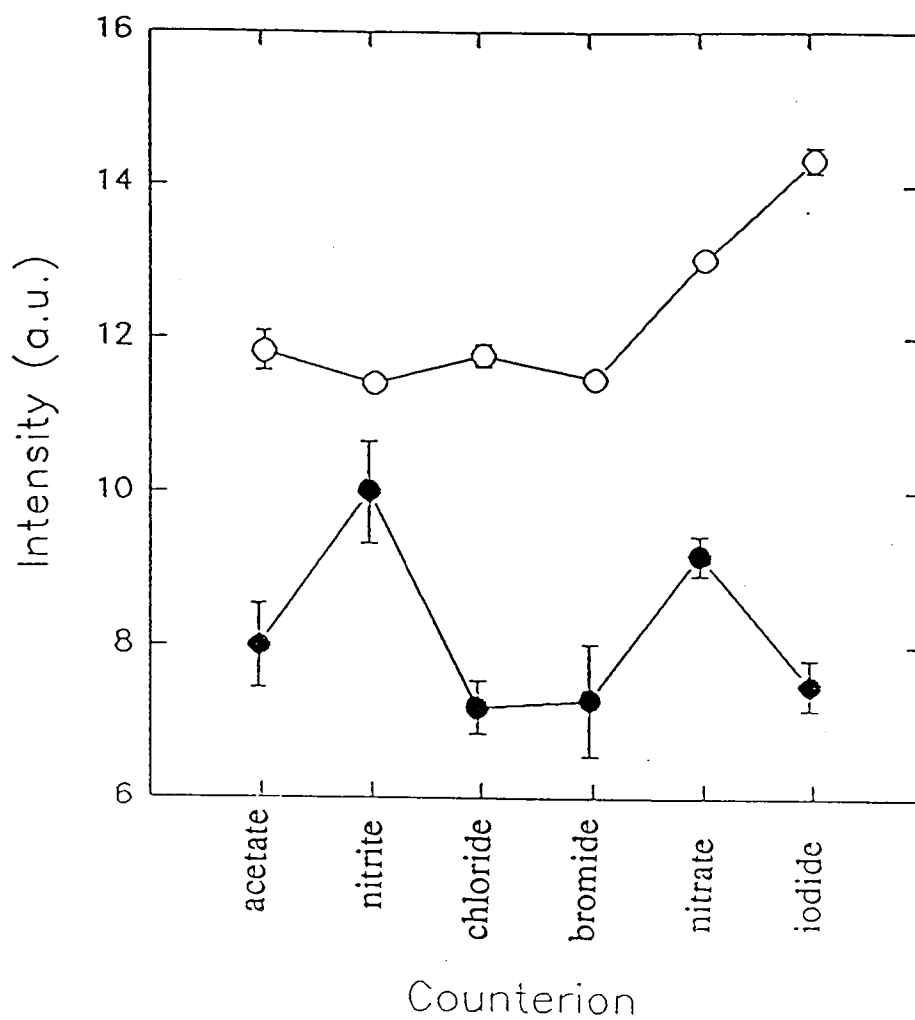


Figure 6.4 ES-MS intensities of $(\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3 + (\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3$
 A^- in methanol : 50 μM (O) and 25 μM (●); A^- is the counterion.

Table 6.5 Counterion dependence of ES-MS response

Concentration (μM)	$(\text{propyl})_4\text{N}^+\text{I}^-$	$(\text{propyl})_4\text{N}^+\text{CH}_3\text{CO}_2^-$
2	4.63 ± 0.2	4.64 ± 0.04
5	5.25 ± 0.3	5.76 ± 0.5
10	8.57 ± 0.4	9.83 ± 0.1
20	11.3 ± 0.03	12.9 ± 0.1

* The reported ion intensities are given in arbitrary units with error limits representing the standard deviation of the average of three spectral measurements (See page 62).

reported no effects from varying the counterions of the quat salts¹⁰⁸ in solutions that were 2 μ M in each quat. Indeed, this was also observed in the present work (Table 6.5).

The last ion pairing experiment reported here can be qualitatively described in terms of the first model that proposed the occurrence of ion pairing in solution. Formation of ion pairs in solution was first recognized by Bjerrum in 1926¹¹⁰. From purely electrostatic considerations, Bjerrum's theory of ion pairing defines a distance between ions of opposite charge below which they are considered to exist as ion pairs. This theory relates the association constant for an ion pair, K , with the dielectric constant ϵ , the absolute temperature T , and the ionic valencies z_+ and z_- (for cation and anion), by means of equation 6.2¹¹¹:

$$K = 4\pi N/1000 \int_a^q \exp \left[-z_+ z_- e^2 / \epsilon r k T \right] r^2 dr \quad \text{Equation 6.2}$$

where N is Avogadro's number, a is the distance of closest approach, and q is the smallest distance beyond which ions pair up, k is the Boltzmann constant, and r is the distance between the centers of two ions. Bjerrum's equation thus predicts greater ion pair formation for ions of higher charge and in media of lower dielectric constant. Bjerrum's treatment of ion pairing phenomena is primitive and has been replaced by more modern models¹¹². However, it is invaluable for qualitative interpretation of experimental results.

To assess the dependence of ion pairing on the dielectric constant of the medium, 25 μ M solutions of diquat diiodide were prepared in matrices of known dielectric constant¹¹³. The matrices were mixtures of water and methanol prepared by weight as shown in Table 6.6. The %IP was calculated for the various samples using Equation 6.1

Table 6.6 Dielectric constants at 25° C of mixtures of water and methanol*

Water (Wt. %)	Dielectric constant
0	31.5
10	35.7
20	40.1
30	45.0
40	50.1
50	54.9

* Dielectric constants were obtained from reference 113.

and plotted as a function of the dielectric constant of the medium. The results shown in Figure 6.5 are in qualitative agreement with Bjerrum's theory of ion pairing; i.e., the extent of ion pairing is seen to decrease with increasing dielectric constant of the medium.

The other prediction of Bjerrum's theory addressing the effect of charge on the extent of ion pairing would be interesting to explore and relevant to the ES-MS of large, multiply charged species. For example, it would be interesting to see if ion pairing indeed increases with the number of charges of the ion, whether a distribution of ions with varying number of attached anions is observed, and if the more heavily ion paired components show higher desorption efficiencies.

SUMMARY AND CONCLUSION

The roles of ion solvation and ion pairing were examined in electrospray mass spectrometry. Ion-solvent interactions affect the ES response of analytes by controlling the energy barrier to desorption. Although the exact mechanism of ion desorption in ES is still in debate, it is generally found that minimizing interactions with the solvent improves the observed ES intensities. This was demonstrated in a homologous series of quats which showed enhanced response with decreasing solvation enthalpies.

Ion pairing interactions were also observed in ES-MS and their influence on ES response was assessed. Such ion/counterion interactions were shown to predominate mainly at high analyte concentrations. Furthermore, the extent of ion pairing was found to be counterion dependent with iodide pairing the most extensively and acetate the least extensively of the six anions tested. A consequence of the ion pairing interactions was

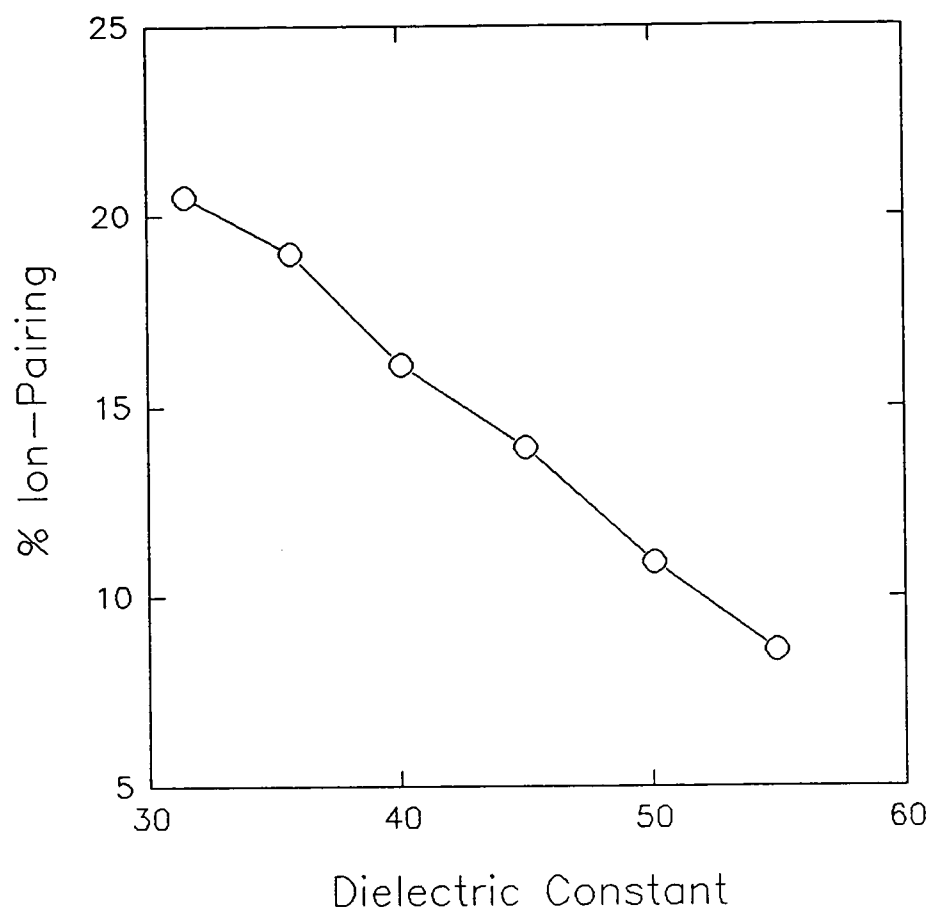


Figure 6.5 Plot of % ion pairing of $(\text{CH}_3)_3\text{N}^+(\text{CH}_2)_4\text{N}^+(\text{CH}_3)_3$ 2I^- in methanol (25 μM) as a function of the dielectric constant of the medium.

reduced response for singly charged species and slightly enhanced response for the doubly charged species.

The ion solvation and ion pairing studies demonstrate the significance of solution phase phenomena to ES-MS. However, it must be emphasized that for species of low charge state (e.g., the diquats studied herein), ion pairing effects are only noticeable at high analyte concentrations, and that they would not be encountered under standard ES operating conditions ($<10^{-5}$ M). Ion solvent interactions, on the other hand, predominate at very low analyte concentrations and are potentially more significant.

CHAPTER 7

BASICITY, SOLVATION, AND POLARIZABILITIES IN ELECTROSPRAY MASS SPECTROMETRY

The electrospray mass spectrometric response of analytes is controlled by a number of parameters, some of which were examined and discussed in Chapters 5 and 6. For example, lowering of surface tension was found to enhance the ES-MS response of analytes by improving the efficiency of the droplet evaporation stage and lowering the barrier to ion desorption. The surface activity of analytes appeared to play a role in ES-MS under conditions where the surface active analyte ions began to compete for the surface sites on the electrosprayed droplets. Ion-solvent and ion-counterion interactions were suggested to influence the ES-MS response by controlling the energy barrier to the ion desorption process. Another factor of significance in the ES-MS analysis of compounds is their acid/base behavior. For example, for positive mode electrospray, a correlation has been established between the pK_b of the analyte and its ES-MS response²⁹. Proton transfer reactions have also been observed in the drift region between the ES needle and capillary inlet²⁹ suggesting a role for the gas phase basicities of analytes. The polarizabilities of analytes have not been previously addressed in any study of ES-MS; however, it is conceivable that they would influence the observed response if the barrier for ion desorption is at least in part dependent on the polarizabilities of the ions in solution.

It is desirable to be able to predict the ES-MS behavior of analytes from

parameters such as those cited above (e.g., surface activity, acid-base behavior, solvation energies, and others). For example, in API-MS, analyte response has been predicted with the aid of two thermodynamic parameters: the gas phase basicity of the analyte and its solvation energy¹¹⁴. A similar understanding of the parameters governing analyte response has not been achieved in electrospray mass spectrometry.

The objective of this chapter is to assess whether thermodynamic measures of acidity/basicity, solvation, and polarizabilities can be utilized to predict the ES-MS response of small species of low charge state. Towards this end, a group of amines and amino acids will be tested for correlation of their ES response with solution phase acidity (pK_a), gas phase basicity (GB), heats of solvation (ΔH_s^0), and polarizability (α). Gas phase acidities (GA) and Bull & Breese indices (explained later) are additional parameters that will be addressed in the correlation studies of the amino acids. The amines selected (specifically the alkylamines) represent a fairly homogeneous class of compounds for which various chemical trends, such as basicity and solvation, are relatively well understood. Amino acids, on the other hand, represent a diverse group of compounds, primarily as a result of their side chain R ($NH_2CH(R)COOH$) which could be hydrophilic or hydrophobic, acidic or basic, charged or neutral. The amines and amino acids have very little in common and provide two substantially different groups of compounds where the validity of parameters believed to be important in ES-MS can be tested.

The chapter will begin by introducing definitions and terminology which are relevant to the topic of discussion. It will then present the results of correlation studies (with the aforementioned parameters) for the amines and amino acids. A short discussion

will follow each section to review and examine the acquired data.

DEFINITIONS

ACID/BASE AND SOLVATION BEHAVIOR

The thermodynamic cycle shown in Figure 7.1¹¹⁵, depicting the protonation of a base (B) in gas and in solution, is provided to introduce some of the parameters that are used in the ES-MS correlation studies. In this cycle $\Delta H_{\text{prot,g}}^0$ is the heat of protonation in the gas phase, ΔH_s^0 (B) is the heat of solvation of the neutral amine or the neutral solvation term, $\Delta H_s^0(\text{H}^+)$ is the heat of solvation of the proton, $\Delta H_s^0(\text{BH}^+)$ is the heat of solution of the alkylammonium ion, and $\Delta H_{\text{prot,s}}^0$ is the heat of protonation in solution. The subscripts g and s represent the gas and solution phase respectively.

For protonation in the gas phase (reaction 1), the negative enthalpy of the reaction ($-\Delta H_{\text{prot,g}}^0$) is defined as the proton affinity (PA) and the negative free energy of the protonation reaction ($-\Delta G_{\text{prot,g}}^0$) is called the gas phase basicity (GB). Analogously, the key index of basicity in aqueous solution is the free energy of protonation of the base in water (reaction 2), $\Delta G_{\text{prot,s}}^0$, or the pK_a of the conjugate acid ($\Delta G_{\text{prot,s}}^0 = -RT \ln K_a = 2.303 RT \text{pK}_a$). The factors which account for the discrepancies between the aforementioned parameters (GB and pK_a) are operationally defined as solvation¹¹⁵. The solvation enthalpy of the protonated amine ($\Delta H_s^0(\text{BH}^+)$) is comprised of two terms as shown in Equation 7.1¹¹⁵:

$$\Delta H_s^0(\text{BH}^+) \approx \Delta H_s^0(\text{B}) + \Delta H_s^0(\text{BH}^+)^{\text{el}} \quad \text{Equation 7.1}$$

In this equation, $\Delta H_s^0(\text{B})$, the neutral solvation term, accounts for the introduction of the

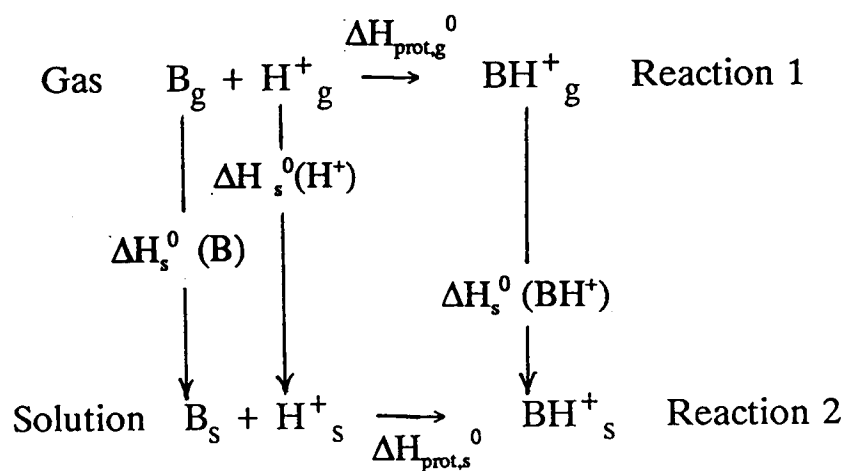


Figure 7.1 Thermodynamic cycle showing the protonation of base (B) in gas phase and liquid phase; reference 115.

neutral amine the same size as the ion into the solvent and the ensuing neutral amine-solvent interactions; $\Delta H_s^0(\text{BH}^+)^\text{el}$, the electrostatic solvation term, accounts for the interaction of the charge of the ion with the solvent; i.e., it reflects those solvation effects that result from addition of a proton and its positive charge to the neutral amine.

For alkylamines, aliphatic alcohols, amides, alkanes, and carboxylic acids, the neutral solvation term ($\Delta H_s^0(\text{B})$) has been suggested to be a measure of the hydrophobic interactions of the alkyl groups with the solvent and is loosely called the hydrophobic term^{116,117,118}. In the correlation studies of the amines, discussed later in this chapter, the above term is assumed to be a measure of the hydrophobicity of these species. The remaining parameter in discussing acid/base properties is the gas phase acidity of the analyte. Analogous to the description of gas phase basicity of a base B, the gas phase acidity or merely acidity $\Delta_\text{acid}G(\text{AH})$ ¹¹⁹ of a molecule AH is the Gibbs free energy change of the reaction shown below:



POLARIZABILITY

Polarizability¹²⁰ (α) is a measure of the "softness" of a charge cloud, that is, the ease with which it can distort in an electric field. The polarizability of a spherical molecule shows a cubic dependence on its radius r , thereby indicating a strong correlation with size. Polarizable bodies generally develop an induced dipole, μ_ind , as a result of their interactions with the electric field. The magnitude of this induced dipole is linearly proportional to the electric field, E_0 ($\mu_\text{ind} = \alpha \cdot E_0$). In high fields, anisotropic molecules

orient in such a way that their axis of highest polarizability coincides with the external field. Polarizabilities of molecules used in this study were determined with the aid of a computer program called "ADO" which stands for "average dipole orientation"¹²¹. This software performs a number of calculations one of which is the determination of the polarizabilities of ions and molecules from the number and state of hybridization of the different atoms that comprise these species. The polarizabilities calculated for the amines and amino acids are tabulated later in this chapter.

CORRELATION STUDIES OF THE AMINES

For the ES-MS studies of the amines, individual samples of each species were prepared at two concentrations, 10 and 100 μM in methanol solvent. The samples were electrosprayed at 4 $\mu\text{L}/\text{min}$ with a needle potential of +3.0 to 3.5 kV. The capillary and skimmer potentials were +90 - 130 V and +45 - 65 V respectively. The drying capillary was heated to approximately 100 $^{\circ}\text{C}$ for desolvation of the sprayed sample. Sample measurements were bracketed by measurements of the external standard (100 μM arginine in methanol). Intensities were measured and reported relative to the $[\text{M}+\text{H}^+]$ of arginine at $m/z = 174$.

A. ACID DISSOCIATION CONSTANT

As stated earlier, the ES-MS response of some thirty amines is reported to correlate with their solution pK_a in positive mode electrospray²⁹. To overlap with the latter study, individual samples of a number of amines were prepared in methanol and electrosprayed. The working concentration for this study was 100 μM because a signal

could not be detected for the species with low sensitivities at a lower concentration. Table 7.1 lists the amines tested, their aqueous pK_a values^{72,122}, calculated polarizabilities, and relative ES responses. It should be noted that the pK_a values listed in Table 7.1 and used in this correlation study refer to aqueous solutions while the mass spectrometric measurements were made in methanol. Since the main goal of this study was to demonstrate prevailing trends and to overlap with the earlier study on this topic, the methanolic and aqueous pK_a values were assumed to be equal. As demonstrated in Figure 7.2, the ES responses of the amines roughly correlate with their solution pK_a , that is, weak bases generate small signals (e.g., aniline) and strong bases produce large signals (e.g., dipropylamine). The scatter observed in the presented data is believed to be partially a result of the assumption of equality of pK_a values in methanol and water and, in part, due to the expected differences in the desorption efficiencies of these species. Thus, the results of the above experiment are consistent with the study reported earlier²⁹.

B. GAS PHASE BASICITIES & SOLVATION ENTHALPIES

To assess correlation with gas phase basicities and solvation enthalpies, a number of amines of known thermochemistry were selected from the list of amines in Table 7.1. This new set of amines consists only of alkylamines, including 1°, 2°, 3°. Please note that the classification of amines into 1°, 2°, 3° classes is based on the degree of substitution on nitrogen. The remaining amines in Table 7.1 could not be incorporated in these studies because their thermodynamic parameters were not all available. In this study, individual samples of the alkylamines were prepared in methanol at a concentration

Table 7.1 Electrospray response of amines (100 μ M in methanol) relative
to the external standard, arginine (100 μ M in methanol)

Amine (#) ^a	pK _a ^b	Polarizability ($\text{\AA}^3$) ^d	Relative Intensity ^c
aniline (1)	4.7	11.49	0.94 $\pm$ 0.1
N-ethylaniline (2)	5.11	15.19	3.3 $\pm$ 0.3
N,N-diethylaniline (3)	6.6	18.89	29.4 $\pm$ 0.7
benzylamine (4)	9.3	13.34	95.5 $\pm$ 2.0
ethanolamine (5)	9.5	6.26	33.0 $\pm$ 0.6
piperazine (6)	5.33/ <u>9.78</u> ^c	9.64	64.6 $\pm$ 2.5
nicotine (7)	3.15/ <u>7.87</u> ^c	18.71	86.6 $\pm$ 10
caffeine (8)	$\approx$ 0.1	17.70	12.7 $\pm$ 0.8
diethylamine (9)	11.02	10.02	64.0 $\pm$ 3.3
triethylamine (10)	10.72	12.93	90.4 $\pm$ 4.0
propylamine (11)	10.57	7.39	55.5 $\pm$ 5.6
dipropylamine (12)	11.00	12.93	101 $\pm$ 5.8
tripropylamine (13)	10.66	18.47	142 $\pm$ 14
butylamine (14)	10.64	9.24	70.4 $\pm$ 6.6

dibutylamine (15)	11.25	16.63	137 ± 8.4
tributylamine (16)	9.93	24.01	297 ± 33

- ^a The numbers assigned to the amines in this table correspond to numbers shown in Figure 7.2.
- ^b pK_a values listed were obtained from reference 72 for amines #1 - #8 and from reference 122 for amines #9 - #16.
- ^c For piperazine and nicotine (diprotic bases), the underlined value was used in plotting.
- ^d Polarizabilities were calculated with the program ADO.
- ^e The error limits represent the standard deviation of the average of three spectral measurements (See page 62).

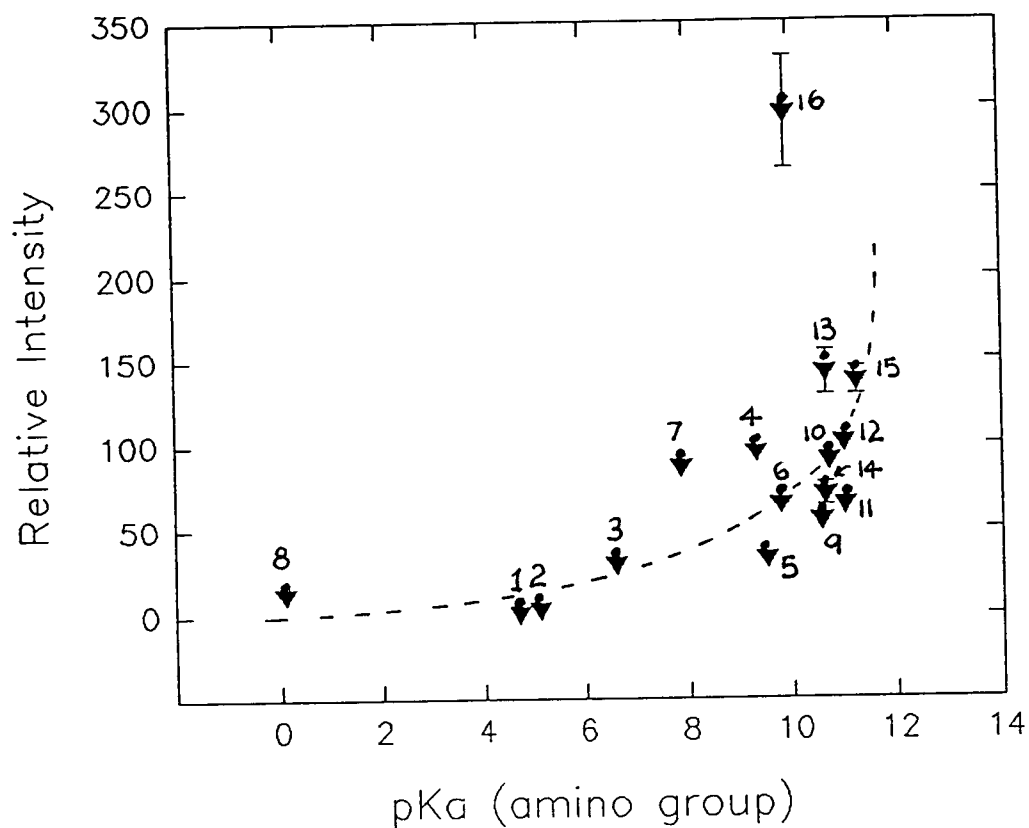


Figure 7.2 ES-MS intensities of amines (100 μ M in methanol) plotted as a function of their aqueous pK_a (Table 7.1). Intensities are reported relative to that of 100 μ M arginine in methanol.

of 10 μM and electrosprayed as before. The amine concentration was lowered in this correlation study because the ES response of alkylamines is very intense, making it feasible to go to the more conventional concentration range, $\leq 10^{-5}$ M, where space charge effects are reported to be less severe²⁹. The thermodynamic data¹²³ for the selected alkylamines and their experimentally measured ES-MS intensities (reported relative to 100 μM arginine) are summarized in Tables 7.2 and 7.3. As shown in these tables, solution basicities vary little among these species (Table 7.2); this is in contrast to their gas phase basicities (Table 7.2) and solvation energies (Table 7.3) that change noticeably from one class to another. The relative ES responses of the alkylamines are plotted versus their gas phase basicities in Figure 7.3. Three nearly parallel traces are observed corresponding to the three classes of alkylamines (1° , 2° , and 3°). Within each class, the observed ES-MS intensities correlate reasonably well with their corresponding gas phase basicities; however, the correlation cannot be extended between the three classes. This behavior will be addressed later in the discussion.

The ES-MS responses of the alkylamines were tested for correlation with their solvation enthalpies listed in Table 7.3. Correlations with $\Delta H_s^0(\text{BH}^+)$ and $\Delta H_s^0(\text{BH}^+)^{\text{el}}$ are shown in Figures 7.4 and 7.5 respectively. In both instances, three nearly parallel traces are obtained allowing a distinction to be made between the 1° , 2° , and 3° amines. As before (behavior shown in Figure 7.3), the correlation observed within a class, 1° , 2° , 3° , is reasonable; however, it cannot be extended between the classes (1° , 2° , 3°). Correlation of intensities with the neutral heat of solvation $\Delta H_s^0(\text{B})$, is significantly different (Figure 7.6) in that all classes fit on a smooth curve.

Table 7.2 Electrospray response of alkylamines (10 μ M in methanol) relative to arginine (100 μ M in methanol) and their pK_a values and gas phase basicities, $-\Delta G^\circ_{\text{prot},g}$

Label	Amine	Relative Intensity	pK_a^*	$-\Delta G^\circ_{\text{prot},g}^*$ (kcal/mol)
PR	propylamine	23.2 ± 1.4	10.56	210.1 ± 2.00
BU	butylamine	31.6 ± 3.4	10.64	210.6 ± 2.00
PN	pentylamine	68.7 ± 3.8	10.63	211.1 ± 2.00
DE	diethylamine	24.6 ± 1.9	11.02	217.7 ± 2.00
DP	dipropylamine	42.2 ± 1.0	11.00	219.7 ± 2.00
DB	dibutylamine	62.9 ± 0.7	11.25	220.3 ± 2.00
TE	triethylamine	34.3 ± 1.8	10.72	224.5 ± 2.00
TP	tripropylamine	67.8 ± 2.7	10.66	226.2 ± 2.00
TB	tributylamine	153 ± 9.0	9.93	227.0 ± 2.00

* Data obtained from reference 123.

Table 7.3 Electrospray response of alkylamines (10 μ M in methanol) relative to arginine (100 μ M in methanol) and their solvation enthalpies

Label	Amine	Relative Intensity	$\Delta H^\circ_s(\text{BH}^+)^*$	$\Delta H^\circ_s(\text{B})^*$	$\Delta H^\circ_s(\text{BH}^+)^{\text{el}*}$
PR	propylamine	23.2 ± 1.4	-79.1 ± 2.5	-13.4	-64.64
BU	butylamine	31.6 ± 3.4	-79.5 ± 2.4	-14.1	-64.28
PN	pentylamine	68.7 ± 3.8	-79.8 ± 2.5	-14.9	-63.78
DE	diethylamine	24.6 ± 1.9	-72.1 ± 2.5	-15.5	-55.53
DP	dipropylamine	42.2 ± 1.0	-72.7 ± 2.5	-17.3	-54.37
DB	dibutylamine	62.9 ± 0.7	-73.9 ± 2.5	-18.8	-53.96
TE	triethylamine	34.3 ± 1.8	-64.6 ± 2.5	-16.8	-46.72
TP	tripropylamine	67.8 ± 2.7	-65.2 ± 2.5	-18.9	-45.2
TB	tributylamine	153 ± 9.0	≈ -66	≈ -20	≈ -44

* Solvation enthalpies given in kcal/mol (Reference 123)

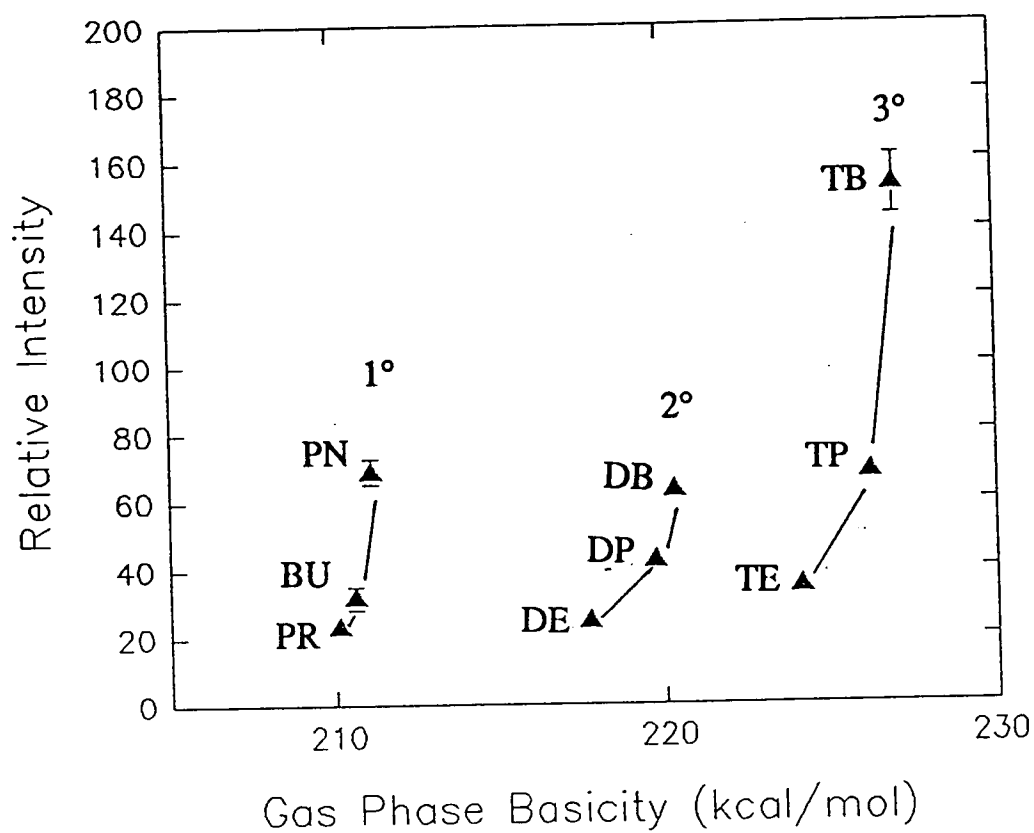


Figure 7.3 ES-MS intensities of alkylamines (10 μ M in methanol) plotted as a function of their gas phase basicities, $-\Delta G_{\text{prot,g}}^0$, (Table 7.2). Intensities are reported relative to that of 100 μ M arginine in methanol.

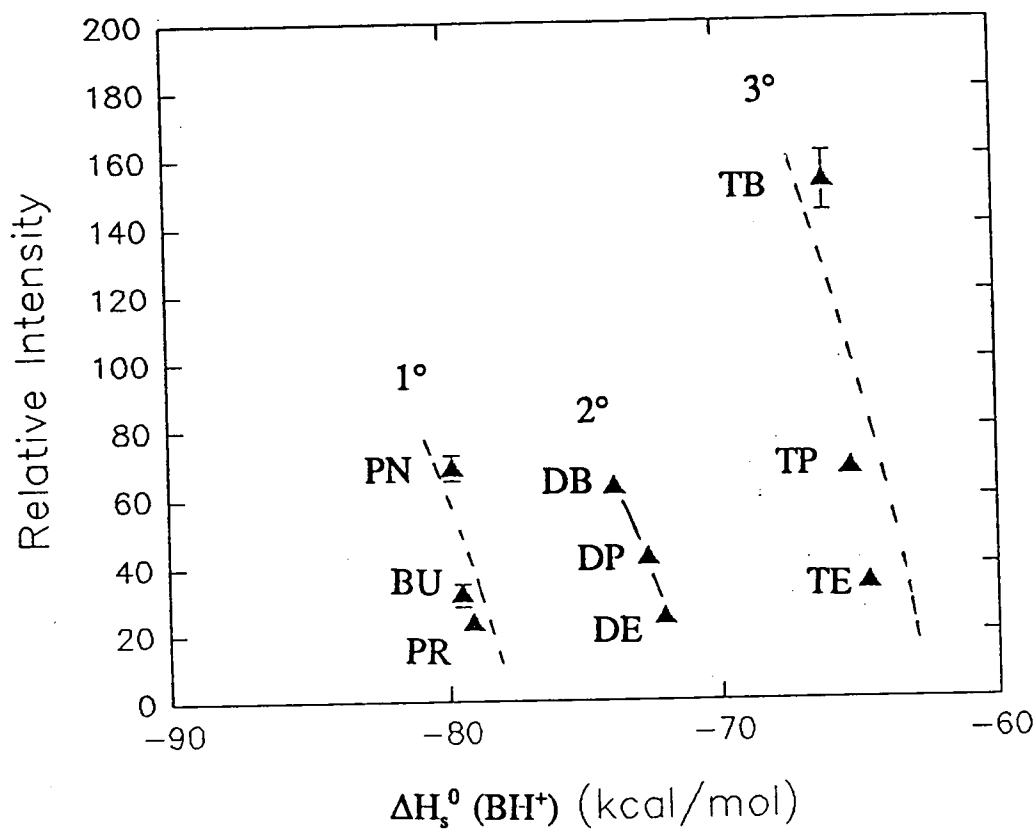


Figure 7.4 ES-MS intensities of alkylamines (10 μM in methanol) plotted as a function of their heat of solvation, $\Delta H_s^0(\text{BH}^+)$, (Table 7.3). Intensities are reported relative to that of 100 μM arginine in methanol.

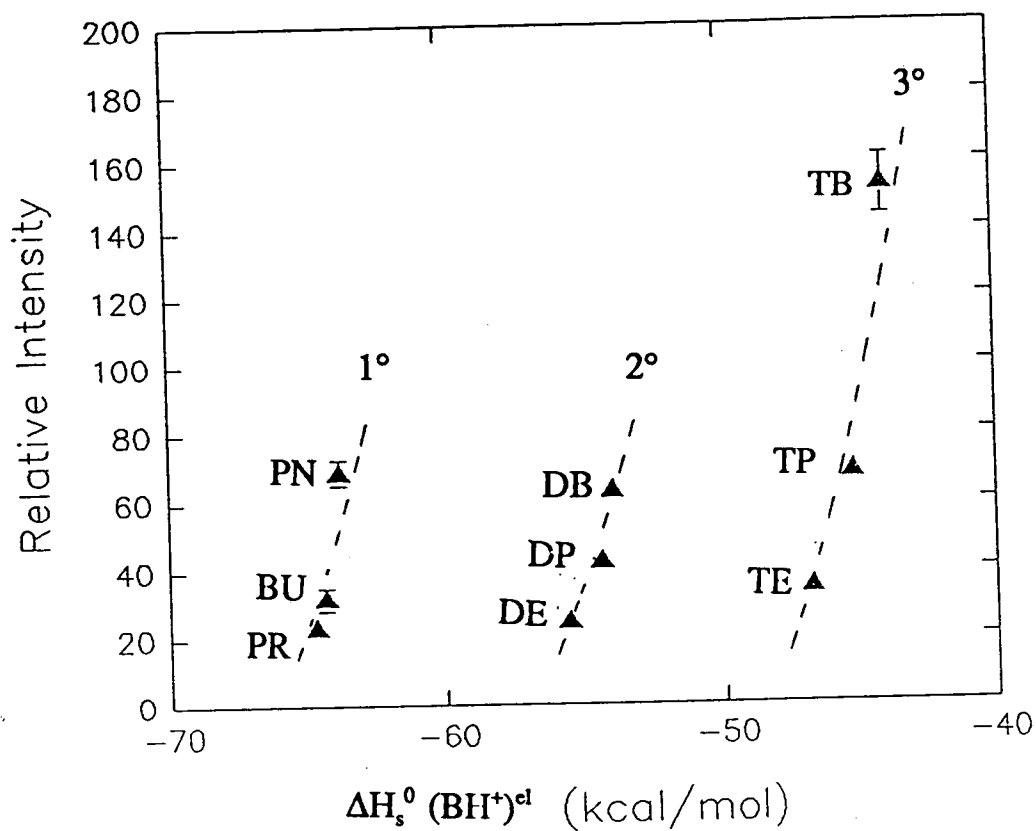


Figure 7.5 ES-MS intensities of alkylamines (10 μ M in methanol) plotted as a function of their electrostatic heat of solvation, $\Delta H_s^0 (BH^+)^{el}$, (Table 7.3). Intensities are reported relative to that of 100 μ M arginine in methanol.

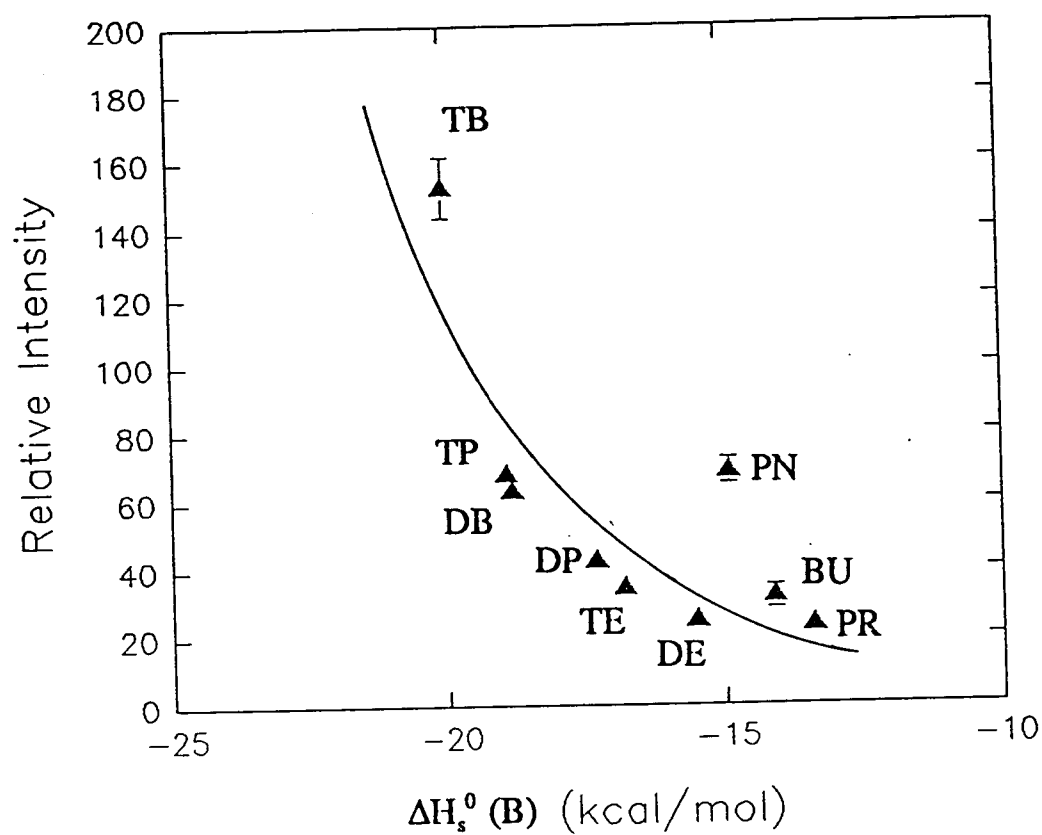


Figure 7.6 ES-MS intensities of alkylamines (10 μM in methanol) plotted as a function of their neutral heat of solvation, $\Delta H_s^0 (B)$, (Table 7.3). Intensities are reported relative to that of 100 μM arginine in methanol.

C. POLARIZABILITY

Table 7.4 lists the alkylamines, their ES responses relative to the external standard, 100 μ M arginine, and their calculated polarizabilities. The same information is presented graphically in Figure 7.7. The trend shown in this Figure is very similar to that observed with the neutral heat of solvation in Figure 7.6; i.e., a relatively smooth increase in signal is observed with increasing polarizability of the analyte.

D. RESULTS & DISCUSSION

The effects of the acid dissociation constant of the amines, their gas phase basicities, electrostatic solvation energies, hydrophobicities, and polarizabilities were studied. Among these factors, the role of polarizabilities has not been previously addressed; neither has the role of solvation energy been examined in terms of its two components, the electrostatic term and the hydrophobic term.

The data shown in Figure 7.2, indicate a moderate correlation between the acid dissociation constant of the amines and their ES-MS response. These results are consistent with the earlier work in this area²⁹ and with the widely held belief that in ES-MS, ions are "preformed" in solution and then extracted into the gas phase under the influence of high electric fields^{17,34,124}. These data also support the suggestion that ES spectra reflect condensed phase chemistry within the droplet as ion desorption proceeds^{77,125}, even though the validity of the latter suggestion has sometimes been questioned. For example, in the ionspray (pneumatically assisted electrospray) analysis of some aliphatic dicarboxylic and aromatic polycarboxylic acids, the intensities predicted

Table 7.4 Electrospray response of alkylamines (10 μM in methanol) relative to arginine (100 μM in methanol) and their polarizabilities.

Label	Amine	Relative Intensity	Polarizability* ($\text{\AA}^3$)
PR	propylamine	23.2 ± 1.4	7.39
BU	butylamine	31.6 ± 3.4	9.24
PN	pentylamine	68.7 ± 3.8	11.1
DE	diethylamine	24.6 ± 1.9	10.0
DP	dipropylamine	42.2 ± 1.0	12.9
DB	dibutylamine	62.9 ± 0.7	16.6
TE	triethylamine	34.3 ± 1.8	12.9
TP	tripropylamine	67.8 ± 2.7	18.5
TB	tributylamine	153 ± 9.0	24.0

* Polarizabilities calculated with the program ADO.

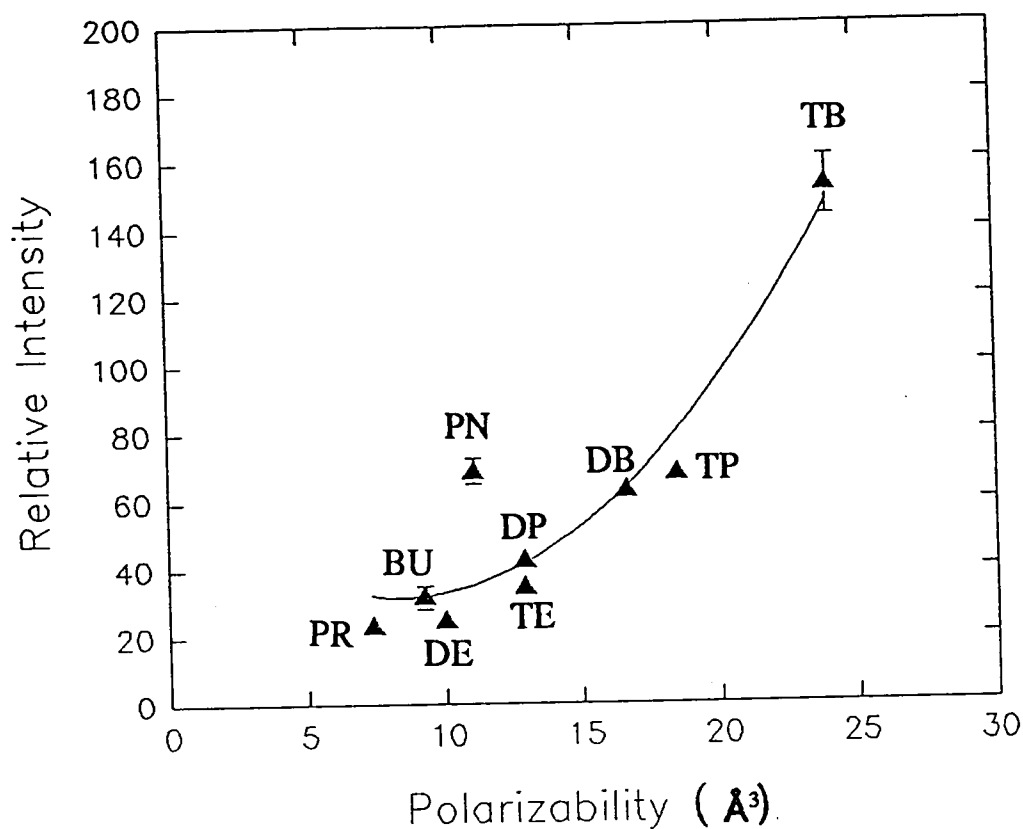
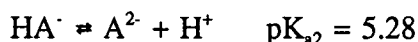


Figure 7.7 ES-MS intensities of alkylamines (10 μM in methanol) plotted as a function of their calculated polarizabilities, α , (Table 7.4). Intensities are reported relative to that of 100 μM arginine in methanol.

by the ionization constants were inconsistent with the observed mass spectrometric responses¹²⁶. Thus for adipic acid, H₂A, the ratio of the doubly charged species (A²⁻) to the singly charged species (HA⁻) at pH = 9 was obtained from equations describing equilibria in solution and compared to the observed ratio (see below).



$$\text{Expected } [\text{A}^{2-}]/[\text{HA}^-] \approx 3900$$

$$\text{Observed } [\text{A}^{2-}]/[\text{HA}^-] \approx 10.6$$

The observed discrepancy between these ratios was attributed either to preferential desorption of HA⁻ in ionspray or to differences between the ionic equilibria in the charged droplets and in the bulk solution¹²⁶. In either case, the point is that the ES mass spectra do not entirely reflect the processes that occur in the condensed phase. Processes in ES-MS are often more complicated than is generally realized and exceptions to the "rule" are usually more prevalent than is desirable.

In testing correlations with gas phase basicities (Figure 7.3) and solvation enthalpies of the alkylammonium ions (Figures 7.4 - 7.5), it was noted that correlations were reasonable within each class but they could not be extended consistently over the entire range of gas phase basicities and solvation energies, that is, between the three classes. The parameter that changes within each class is the hydrophobicity term or the neutral solvation term (see Table 7.3) which becomes more exothermic as the alkyl group of the amine becomes longer. Thus, it is suggested that the trends within each class also reflect changes in the hydrophobicities of these species.

With respect to correlation with the neutral solvation term, $\Delta H_s^0(B)$, shown in Figure 7.6, the measured ES intensities of the alkylamines increase smoothly with increasing exothermicity (increasing hydrophobicity) of these species, such that the three classes of amines cannot be distinguished one from the other. The observed trend is consistent with the studies of Chapters 5 and 6 where surface activities and hydrophobicities were suggested to contribute to (enhance) the observed ES-MS intensities of analytes.

ES-MS responses of the amines also increase smoothly with analyte polarizability (Figure 7.7), similar to the trend observed for the neutral heat of solvation (Figure 7.6). The observed correlation with polarizability is considered to be a result of ion-molecule reactions between the neutral amines and the protonated solvent molecules in the drift space between the needle and the capillary inlet. Collision rate constants for such reactions are a function of the polarizabilities of the molecules involved; hence, any correlation with these constants would also be seen as a correlation with polarizabilities. Such ion-molecule reactions require the presence of a sufficient concentration of the neutral amine in the gas phase, a source of ions, and finally, sufficient time for the suggested reactions to take place. In the experiments with the amines, 10 μM samples were prepared in methanol and electrosprayed. At this concentration, at least 60% of the analyte is expected to be in the neutral form²⁹. Considering the high vapor pressures of these species, it is reasonable to suggest that a high concentration of the neutral amine is present in the gas phase between the needle and the capillary inlet. The ions involved in the ion-molecule reactions are believed to be protonated methanol ions since they tend

to be prevalent in ES mass spectra when methanol is used as solvent⁷⁴. The ion-molecule reactions suggested are proton transfer reactions which are known to be very fast (rate constant = $2 \times 10^{-9} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$)¹²⁷. The ion residence time in the drift space between the needle and the capillary inlet was previously estimated to be on the order of 0.2 ms (see Chapter 5), allowing sufficient time for these reactions to occur. It thus appears that the conditions in the drift space between the needle and the capillary inlet strongly favor ion-molecule reactions in this region. Hence, it is reasonable to suggest that the observed correlation with polarizabilities stems from such ion-molecule reactions.

CORRELATION STUDIES OF THE AMINO ACIDS

As stated in the beginning of this chapter, the objective of this work is to determine if the ES-MS response of analytes can be expressed as a function of their thermodynamic measures of their acidity/basicity, solvation energy, and polarizability. The amine data presented in Figures 7.2 - 7.7 demonstrate reasonable correlation with all of the tested parameters; however, correlations with the neutral heat of solvation (hydrophobicity) and polarizability are noticeably stronger than correlations with the remaining factors. The correlation with polarizability was explained by invoking ion-molecule reactions that could occur in the drift space between the needle and the capillary inlet; the correlation with the hydrophobicity term was found consistent with earlier studies (Chapter 5 & 6) where compounds with higher surface activities and hydrophobicities showed a stronger ES-MS response. Thus, it is suggested that the hydrophobicity of the amines could be a useful parameter for predicting their ES-MS

response. In the following section, the validity of the above conclusion is tested by examining correlation between the ES response of amino acids and their various thermodynamic parameters.

For the studies with the amino acids, individual samples of these species were prepared at a concentration of 100 μ M in 50:50 (v/v) water:methanol. The solubility of amino acids is higher in this matrix than in 100% methanol which was previously used in the studies of the amines. The concentrations were changed back to 100 μ M for this study because some amino acids could not be detected at a lower concentration. The samples were electrosprayed under conditions similar to those stated earlier for the amines and their responses were normalized to that of the external standard, 100 μ M arginine in 50:50 (v/v) water:methanol. Tables 7.5 and 7.6 list the amino acids studied, their relative ES-MS responses, and thermochemical data of interest; Figure 7.8 gives the structures of these species. The pK_{a2}^{128} values (for the ionization of the carboxyl group) and the gas phase acidities¹²⁹ (GA) of the amino acids are included in the correlation studies because amino acids are zwitterionic in solution^{6*} and must become protonated on the carboxyl group (in the gas phase) in order to be detected mass spectrometrically (i.e., in positive ion ES). Hence, it is reasonable to expect the pK_{a2} values and/or gas phase acidities to contribute to the ES response of the amino acids. As before, correlations of the relative response with the listed parameters will be shown graphically.

Figures 7.9 - 7.16 show the dependence of the ES response of the amino acids on their pK_{a2} (carboxyl group) and pK_{a1} (amino group), gas phase basicities, gas phase

^{6*} Amino acids in solution at neutral pH are predominantly dipolar ions or "zwitterions". (Reference 126)

Table 7.5 Electrospray response of amino acids (100 μ M in 50:50 (v/v) water:methanol relative to arginine (100 μ M in the same matrix), their pKa values, gas phase basicities (GB) and gas phase acidities (GA).

Name	Relative Intensity	pK _{a2} ¹	pK _{a1} ¹	GB ² (kcal/mol)	GA ³ (kcal/mol)	B&B ⁴ (cal/mol)
Ala	10 $\pm$ 0.6	2.35	9.69	206.6	334.3	+610
Ile	84 $\pm$ 3.2	2.36	9.68	211.1	331.9	-1450
Phe	82 $\pm$ 2.3	1.83	9.13	208.7	329.6	-1520
Pro	21 $\pm$ 2.9	1.99	10.6	212.4	333.4	-170
Thr	22 $\pm$ 3.0	2.63	10.43	210.8	325.2	+290
Val	33 $\pm$ 2.1	2.32	9.62	209.2	332.5	-1560
Gly	5 $\pm$ 1.2	2.34	9.6	203.7	335.1	+810
Arg ⁵	100 $\pm$ 2.0	2.17	9.04	237	324.9	+690

¹ pK_a values were obtained from reference 128.

² Gas phase basicities (GB) were obtained from reference 123.

³ Gas phase acidities (GA) were obtained from reference 129.

⁴ B&B hydrophobicity indices were obtained from reference 130.

⁵ External standard: 100 μ M arginine in 50:50 (v/v) methanol:water.

Table 7.6 Electrospray response of amino acids (100 μ M in 50:50 (v/v) water:methanol relative to arginine (100 μ M in the same matrix), their solvation energies and polarizabilities.

Name	Relative Intensity	$\Delta H_s(BH^+)^a$ (kcal/mol)	$\Delta H_s(B)^a$ (kcal/mol)	$\Delta H_s(BH^+)^{el\ a}$ (kcal/mol)	Polarizability($\text{\AA}^3$) ^b
Ala	10 $\pm$ 0.6	-87.0	-31.2	-55.8	8.11
Ile	84 $\pm$ 3.2	-86.8	-35.9	-50.9	13.58
Phe	82 $\pm$ 2.3	-90.6	-35.0	-55.6	17.62
Pro	21 $\pm$ 2.9	-88.9	-39.0	-49.9	10.69
Thr	22 $\pm$ 3.0	-102.1	-49.6	-52.5	10.69
Val	33 $\pm$ 2.1	-91.1	-38.2	-53.7	11.75
Gly	5 $\pm$ 1.2	-88.9	-29.2	-59.7	6.66
Arg ^c	100 $\pm$ 2.0	- - -	- - -	- - -	16.58

^a Solvation energies were obtained from reference 123.

^b Polarizabilities calculated with the program ADO.

^c External standard: 100 μ M arginine in 50:50 (v/v) methanol:water.

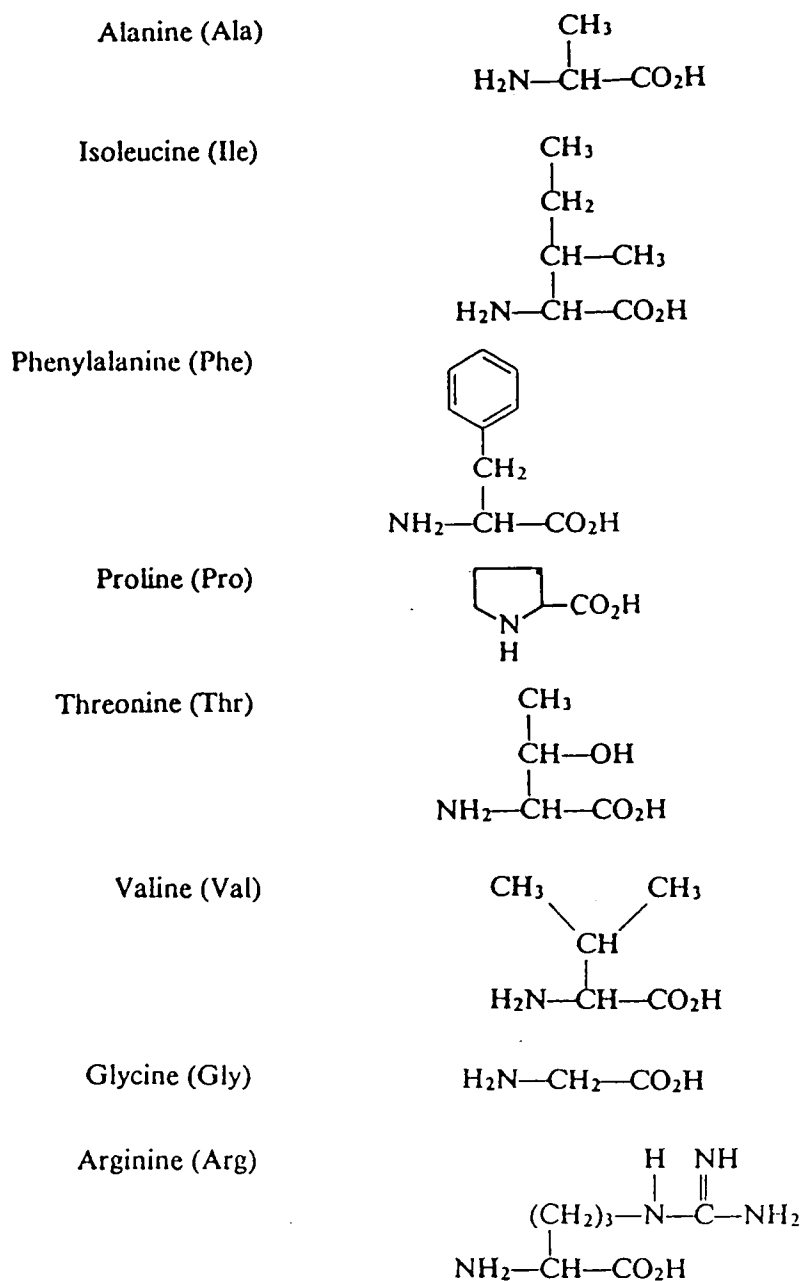


Figure 7.8 Structures of amino acids studied by ES-MS.

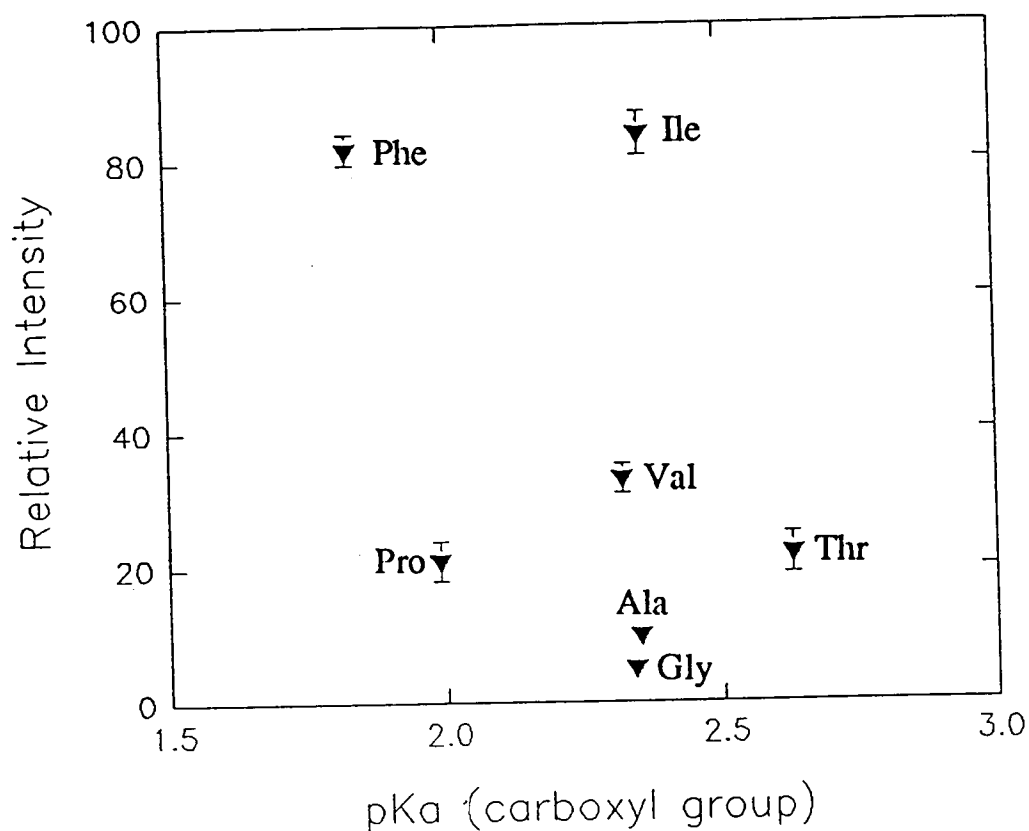


Figure 7.9 ES-MS intensities of amino acids (100 μ M in 50:50 (v/v) water:methanol) plotted as a function of their pK_{a2} (carboxyl group), (Table 7.5). Intensities are reported relative to that of 100 μ M arginine in 50:50 (v/v) water:methanol.

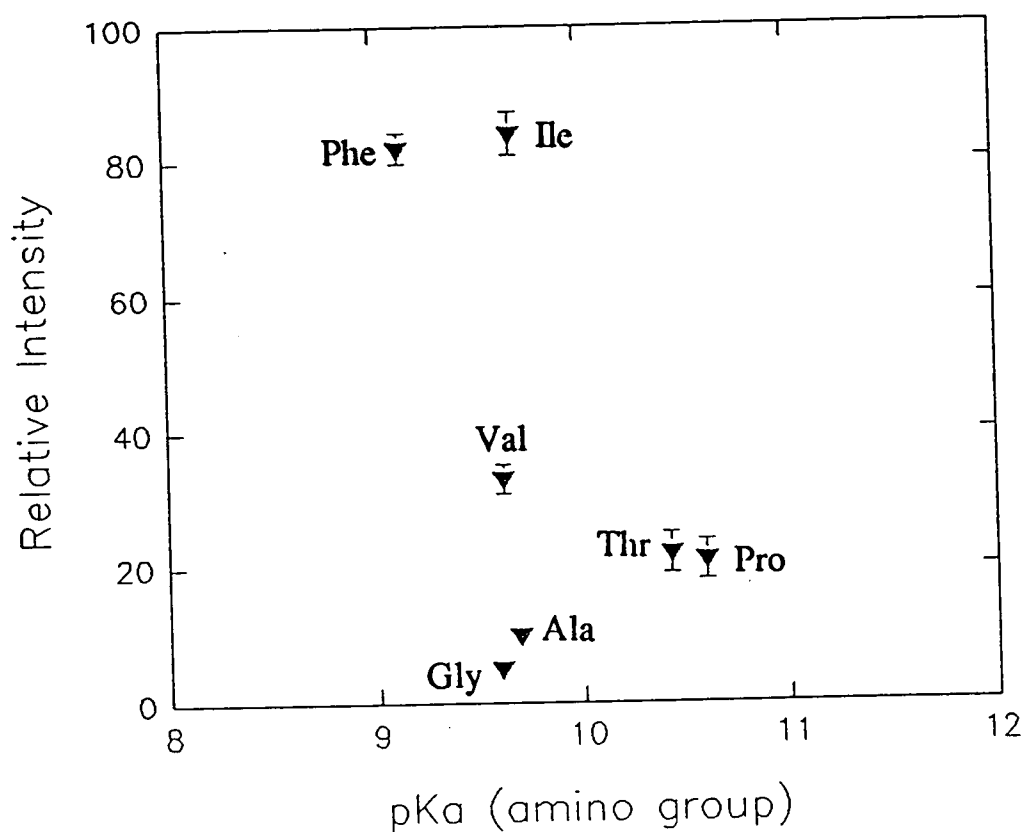


Figure 7.10 ES-MS intensities of amino acids (100 μ M in 50:50 (v/v) water:methanol) plotted as a function of their pK_{a1} (amino group), (Table 7.5). Intensities are reported relative to that of 100 μ M arginine in 50:50 (v/v) water:methanol.

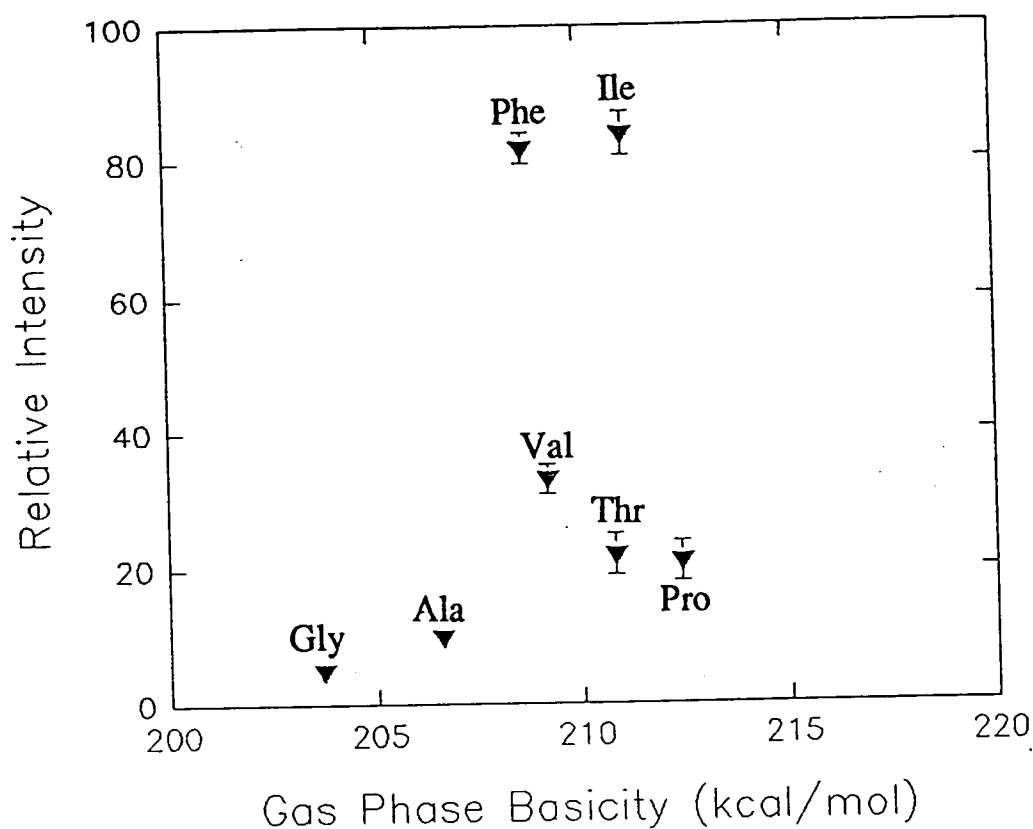


Figure 7.11 ES-MS intensities of amino acids (100 μ M in 50:50 (v/v) water:methanol) plotted as a function of their gas phase basicities (Table 7.5). Intensities are reported relative to that of 100 μ M arginine in 50:50 (v/v) water:methanol.

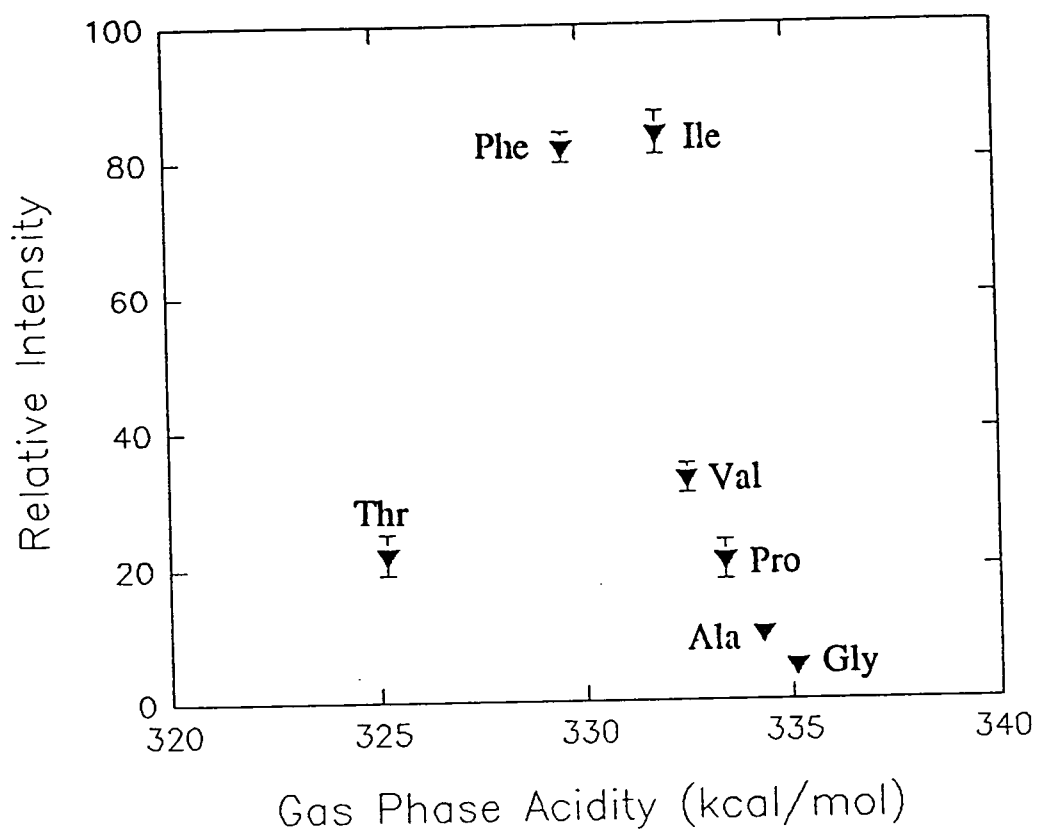


Figure 7.12 ES-MS intensities of amino acids (100 μ M in 50:50 (v/v) water:methanol) plotted as a function of their gas phase acidities (Table 7.5). Intensities are reported relative to that of 100 μ M arginine in 50:50 (v/v) water:methanol.

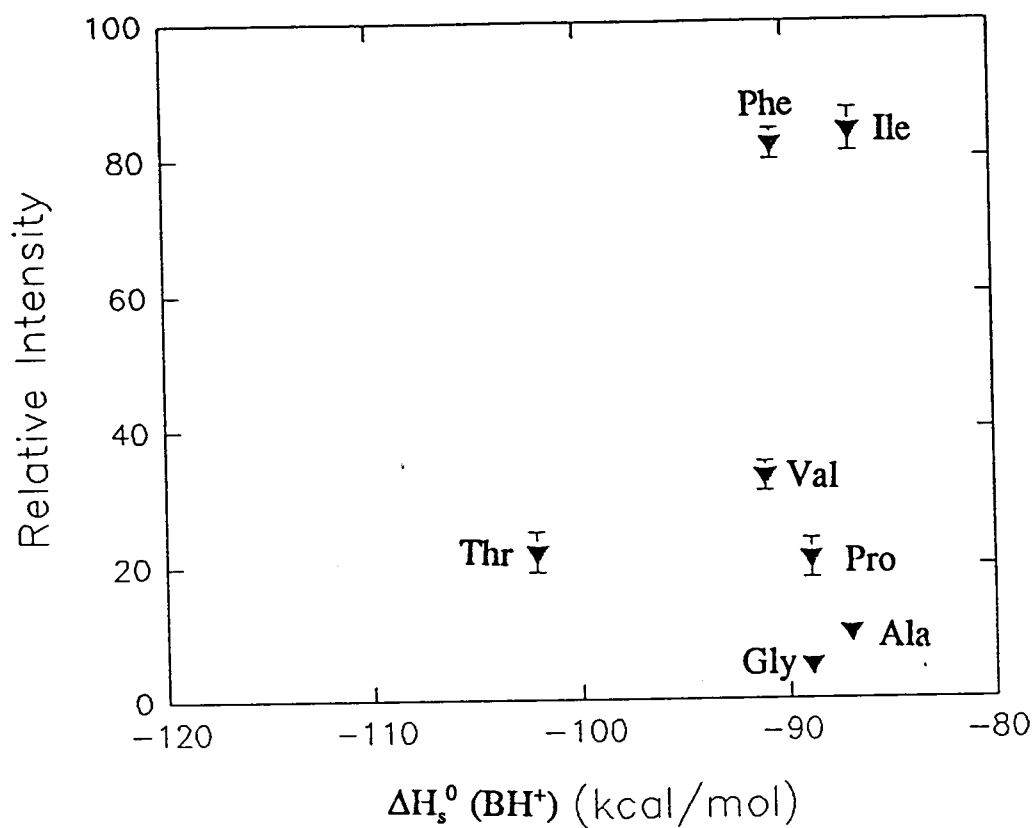


Figure 7.13 ES-MS intensities of amino acids (100 μM in 50:50 (v/v) water:methanol) plotted as a function of their heats of solvation, $\Delta H_s^0 (\text{BH}^+)$, (Table 7.6). Intensities are reported relative to that of 100 μM arginine in 50:50 (v/v) water:methanol.

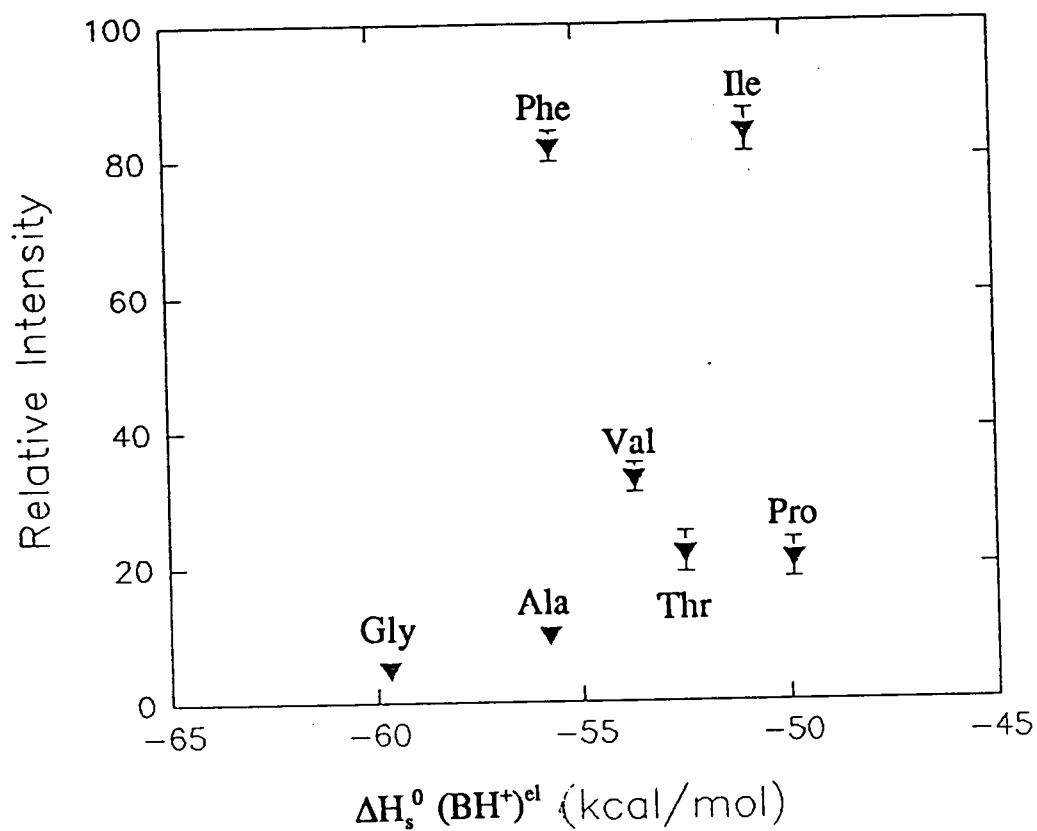


Figure 7.14 ES-MS intensities of amino acids (100 μM in 50:50 (v/v) water:methanol) plotted as a function of their electrostatic heats of solvation, $\Delta H_s^0 (\text{BH}^+)^{\text{el}}$, (Table 7.6). Intensities are reported relative to that of 100 μM arginine in 50:50 (v/v) water:methanol.

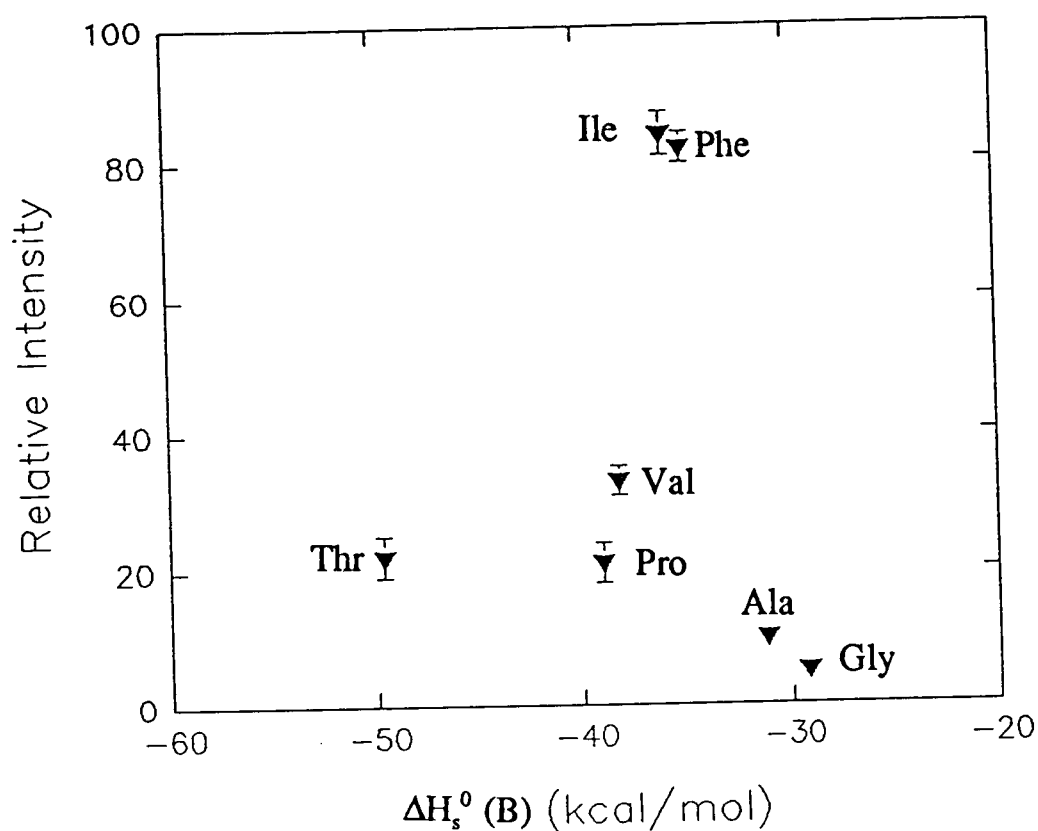


Figure 7.15 ES-MS intensities of amino acids (100 μ M in 50:50 (v/v) water:methanol) plotted as a function of their neutral heats of solvation, ΔH_s^0 (B), (Table 7.6). Intensities are reported relative to that of 100 μ M arginine in 50:50 (v/v) water:methanol.

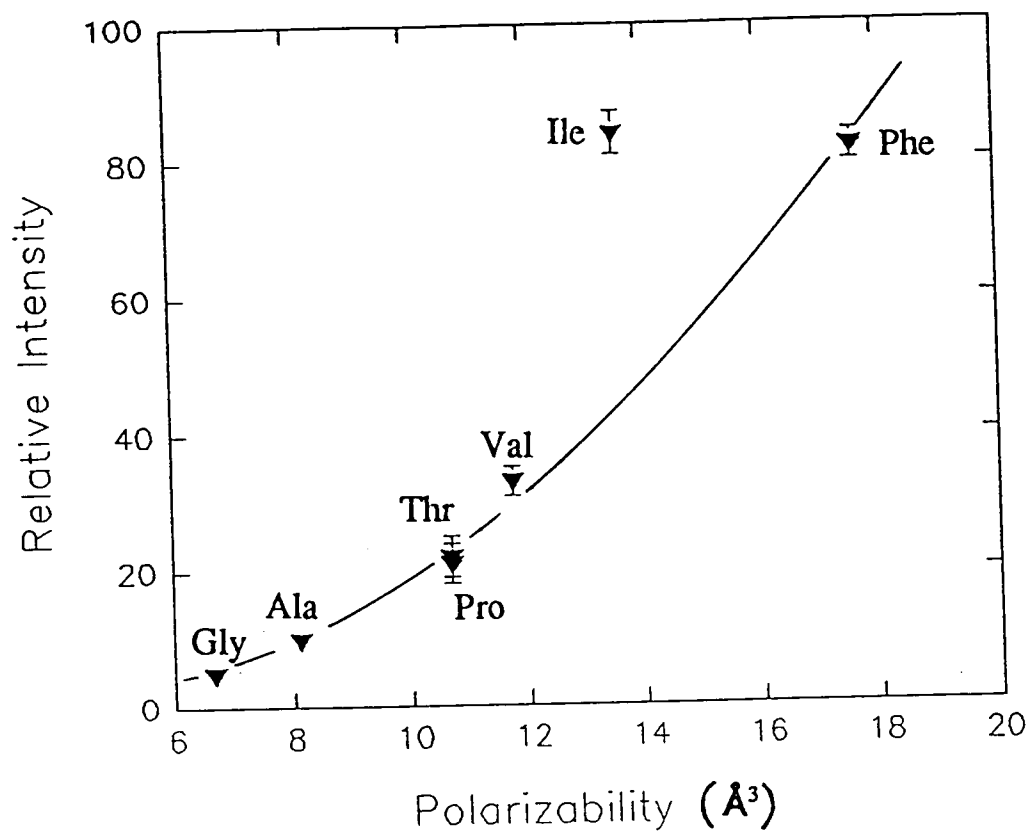


Figure 7.16 ES-MS intensities of amino acids (100 μM in 50:50 (v/v) water:methanol) plotted as a function of their calculated polarizabilities, α , (Table 7.6). Intensities are reported relative to that of 100 μM arginine in 50:50 (v/v) water:methanol.

acidities, solvation enthalpies, and polarizabilities. As may be seen from these figures, strong correlations are noted with gas phase acidities (Figure 7.12) and polarizabilities (Figures 7.16). The outliers in these Figures are Thr (Figure 7.12) and Ile (Figure 7.16); an explanation is presently not available for these data points.

The weak correlations observed with the remaining parameters are neither surprising nor interesting in view of the chemical diversity of these compounds (see Figure 7.8). The observation that is interesting is the strong correlation noted between the relative response of the amino acids and their polarizabilities. In contrast to the situation with the amines where ion-molecule reactions provided a satisfactory explanation for the observed correlation, herein, the same argument is not applicable. This is due to the absence of experimental conditions that normally favor such reactions; specifically, a sufficient concentration of the neutral amino acids cannot be present in the gas phase as a result of their low vapor pressures. Thus ion-molecule reactions have to be ruled out as an explanation for the observed correlation with polarizabilities. A different effect, such as ion-field interactions, might be responsible for the observed behavior; however, the exact mechanism of such interactions is not clear.

The poor correlation observed between the relative responses of the amino acids and the neutral heats of solvation was unexpected because the latter parameter was believed to reflect the hydrophobicity of the amino acid side chain. It was thought that a different measure of hydrophobicity, one that was obtained empirically, might be more suitable in testing correlation with hydrophobicities. An empirically derived parameter used to estimate the hydrophobicity of amino acids is the "Bull and Breese hydrophobicity

index"¹³⁰ (Table 7.5) which is based on the effect of amino acids on the surface tension of water. The Bull and Breese (B&B) indices estimate the energies (cal/mol) required for transferring the amino acids from the solution to the surface. Positive values denote hydrophilic amino acids and negative values, hydrophobic ones. The correlation between the relative response of the amino acids and the B&B indices is significant as shown in Figure 7.17. This indicates that for amino acids, the B&B hydrophobicity index is a better measure of hydrophobicity than the neutral heat of solvation.

The above studies demonstrate that strong correlations exist between the ES response of the amino acids and their gas phase acidities, polarizabilities, and the B&B hydrophobicity indices. Among these parameters, only analyte polarizability shows reasonable correlation with the ES response of both amines and amino acids. To verify the utility of this parameter, the ES-MS intensities of the amines and amino acids from the previous studies (data listed in Tables 7.1 and 7.6) were plotted as a function of their polarizabilities. As shown in Figure 7.18, a rough correlation exists between the relative ES responses of most of these species and their calculated polarizabilities. The outliers in this Figure are aniline (1), N-ethylaniline (2), N,N-diethylaniline (3), nicotine (7), and caffeine (7). From the earlier examination of the role of pK_a in ES-MS (Figure 7.2 and Table 7.1), it is known that the latter species have relatively low pK_a values compared to the rest of the members of the set. It is thus suggested that the low ES response of the outliers in Figure 7.18 is a result of their weakly basic nature which results in the presence of low concentrations of the protonated amines in solution. The correlation observed for the remaining compounds (those with pK_a values greater than approximately

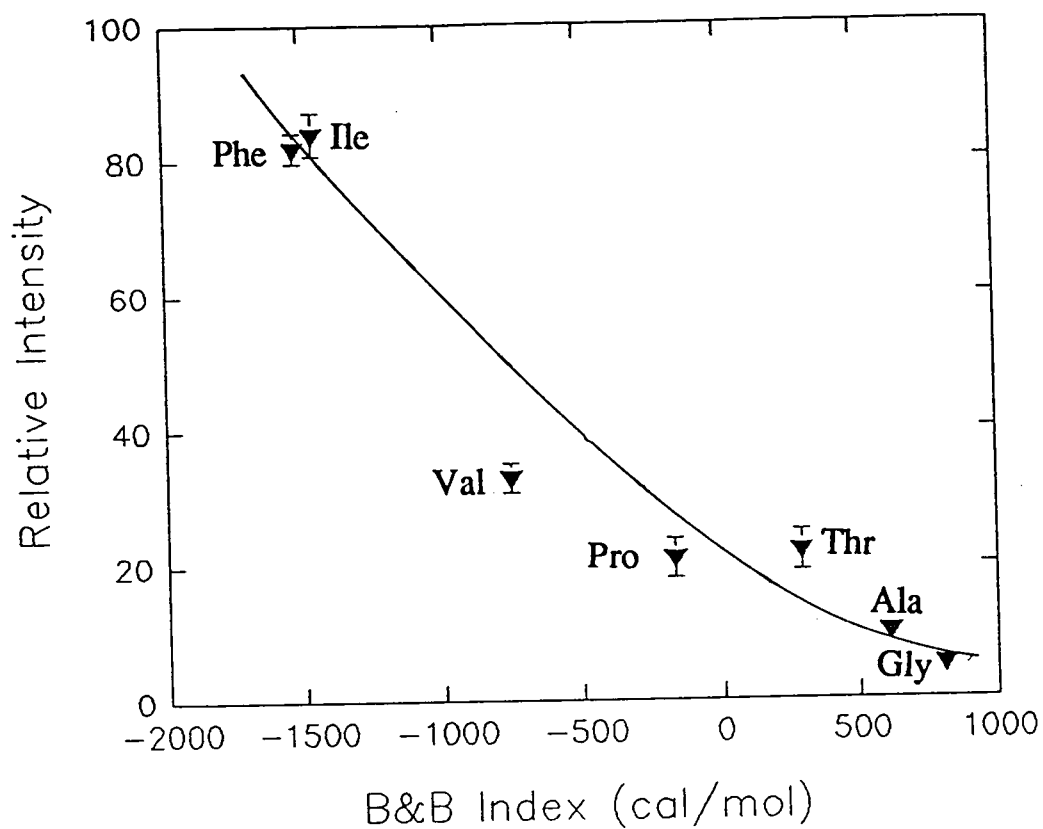


Figure 7.17 ES-MS intensities of amino acids (100 μ M in 50:50 (v/v) water:methanol) plotted as a function of their B&B hydrophobicity indices (Table 7.5). Intensities are reported relative to that of 100 μ M arginine in 50:50 (v/v) water:methanol.

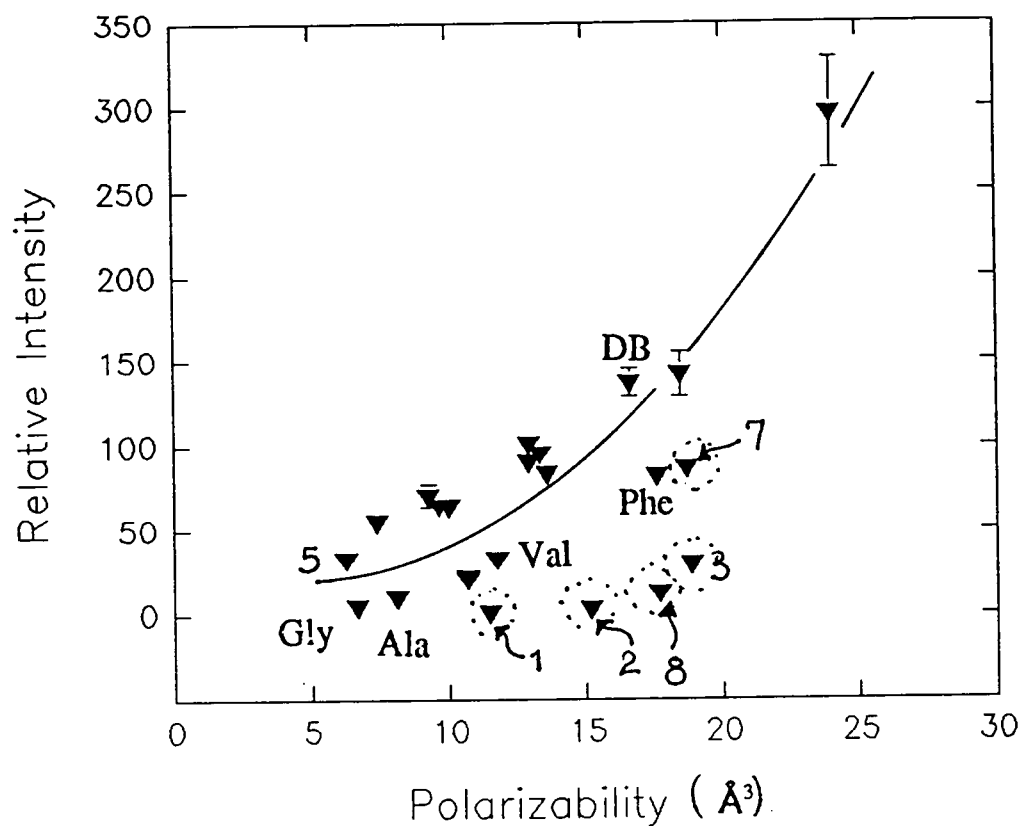


Figure 7.18 ES-MS intensities of amino acids (100 μM in 50:50 (v/v) water:methanol) and amines (100 μM in methanol) plotted as a function of their polarizabilities. Amino acid intensities are reported relative to that of 100 μM arginine in 50:50 (v/v) water:methanol; amine intensities are relative to 100 μM arginine in methanol.

9.0) suggests a role for analyte polarizability in determining their electrospray response, and the scatter in the data reflects the chemical diversity present among the members of the two groups and the multiplicity of parameters that operate in ES-MS.

SUMMARY & CONCLUSION

This chapter presented the results of a number of experiments which were performed to test the correlation of the observed ES-MS response of amines and amino acids with a number of parameters which were thought to influence their response. The goal was to assess whether the dominating parameters could be identified and subsequently used for predicting the ES-MS response of other analytes. The parameters evaluated were the acid dissociation constant of the analyte, gas phase acidities and basicities, solvation energies, polarizabilities, and hydrophobicities. The ES response of amines showed correlation (to varying extents) with all of the tested parameters; the amino acids, in contrast, only showed correlation with gas phase acidities, polarizabilities, and hydrophobicities (estimated by the B&B index). The main parameter that was shared by both amines and amino acids was their polarizabilities, a parameter that had not been previously considered. It was found that for relatively strong solution bases, the electrospray responses correlated with analyte polarizabilities. To suggest that polarizabilities can predict the ES response of small analytes is simplistic and premature; however, it is an important observation that requires further investigation.

CHAPTER 8

CONCLUSIONS & SUGGESTIONS FOR FUTURE WORK

In the beginning of this work, electrospray was introduced as a soft ionization technique in which intact gas phase ions of large, non-volatile, thermally labile, and fragile molecules are produced from application of high voltage to a solution of analyte at atmospheric pressure. A unique feature of electrospray is the production of multiply charged ions with high efficiency as a result of the attachment of protons or cations to "acceptor" sites on the molecule (positive mode electrospray). A useful consequence of multiple charging is that it allows mass analysis of large species with mass spectrometers of limited mass range. Finally, the electrospray source comprises an effective and practical LC-MS (liquid chromatography-mass spectrometry) interface. Recent developments in this area allow coupling of HPLC (high-performance liquid chromatography) to mass spectrometry³⁵. These properties make electrospray mass spectrometry (ES-MS) a tremendously valuable tool to structural biologists and others who deal with analysis of high molecular weight, nonvolatile samples in their work. As a result, many studies of ES-MS are applications-oriented; however, fundamental studies are increasing in number because widespread use of ES-MS has revealed its peculiarities, and limitations in addition to its strength and utility.

The work presented here essentially dealt with fundamental issues. After a relatively thorough discussion on the history of electrospray and the electrospray source,

the results of fundamental studies that addressed the role of surface tension, surface activity, ion solvation, ion pairing, and acid/base effects were discussed. Briefly, it was shown that lowering of the surface tension of the solution was beneficial because it enhanced the ES response of analytes. The use of Triton X-100 was suggested as a means for lowering the surface tension of the solution thereby allowing the electrospray of purely aqueous solutions.

ES-MS responses of quaternary ammonium salts were seen to correlate strongly with their solvation enthalpies. Ion pairing effects were noted in samples where analyte concentration was high (according to ES standards) and shown to decrease the ES response of singly charged quaternary ammonium salts. Finally, correlation studies of the ES response of amines and amino acids with their acid dissociation constant, gas phase acidity and basicity, solvation energy, and polarizability suggested that the latter parameter could be used to provide a rough estimate of their ES-MS response.

Two of the major mechanisms suggested for the production of gas phase ions in electrospray were also introduced. These are the "charge residue model" of Dole¹⁶ and the "ion evaporation model" of Iribarne and Thomson¹⁷. The charge residue model proposes that gas phase analyte ions result from evaporation of solvent from very small charged droplets each containing a single ion. The ion evaporation model, on the other hand, suggests that single ions clustered with one or more solvent molecules desorb from very small charged droplets. In general, the charge residue model appears to apply to the ES-MS of very large multiply charged species, whereas the ion evaporation model best describes the ES response of small singly charged molecules. These models represent the

opposite ends of a spectrum in their approach to explain the formation of gas phase ions in electrospray. Both models have been criticized on one or more issues and variations on both themes are available. In this work, mechanistic discussions were kept brief because the limited scope of the studies performed (only small singly or doubly charged species were examined) would not allow a balanced examination of both views. Nonetheless, in Chapters 5 and 6, the data were explained in light of the proposed models of ion formation. In summary, these studies addressed some important issues in electrospray mass spectrometry, using relatively simple chemical systems. They further pointed to areas that would be interesting and useful to investigate. These will be addressed in the remainder of this chapter.

An issue of concern conveyed to the readers early on was the subject of ion sampling in the electrospray source. The ES source was described as a source where the electric fields are determined by space-charge build-up and not by the voltages applied to the source components. The concern was that space charge would cause sampling bias resulting in mass spectra that would not faithfully represent the ions generated by the ES process. Thus, it is necessary to examine the spatial distribution of the ions in the electrospray under different conditions; e.g. at various concentrations of analyte ions, in multicomponent and single-component samples, at different needle-capillary inlet positions, and so on.

Another area that could benefit from further research deals with ion solvation and ion pairing phenomena. In the ion solvation studies (Chapter 6), a strong correlation was noted between the ES response of tetraalkylammonium salts (quats) and their solvation

enthalpies. It was also suggested that different hydrophobicities of the quats could result in differing desorption efficiencies and produce a similar trend. In other words, if ion transport processes in the condensed phase are at a rate that would allow distribution of the more hydrophobic quats to the surface, then it would be reasonable to expect that their field-assisted desorption efficiencies would be influenced by their distance away from the surface. It is thought that utilizing inorganic species should remove concerns about variations in desorption efficiencies stemming from varying hydrophobicities. The ion pairing experiments could also be expanded to include larger multiply charged species. The studies presented in Chapter 6 were limited to one diquatery ammonium salt (diquat) mainly because the limited mass range of the mass analyzer (≈ 350 amu) would not allow examining larger diquats. To be of practical utility, larger multiply charged species must be examined under standard ES conditions; i.e., at low concentrations.

Recent use of ES-MS in determining the configuration of proteins in solution is intriguing. Proteins are known to fold and unfold as a function of the pH of the medium and temperature. The charge state distribution of proteins has been demonstrated to change when the pH of the medium is changed or when the sample is heated¹³¹. This has been taken to indicate that ES mass spectra reflect the conditions in the solution. On the other hand, solvent oxidation during the electrospray process has been suggested³⁷. If the latter process is significant enough to affect the pH of the solution, it is reasonable to assume that the pH of the droplets is different from that of the bulk solution, in which case, the ES spectra of proteins reflect conditions in the droplets. Early in the author's electrospray work when a variety of samples were being attempted to find out what

compounds work and do not work in ES, a number of pH sensitive dyes were run. It was simplistically thought that visual observation of the ES plume with naked eye would reveal color changes indicative of changes in pH. This was not the case and the project was therefore not continued; however, it led to the idea that optical probing of the actual electrospray could demonstrate if the pH of the solution was changing as a result of solvent oxidation.

Another area of interest deals with collision induced dissociation (CID) of molecular ions in the region of free jet expansion. This is performed by simply increasing the potential difference between the nozzle (capillary) and the skimmer. In electrospray, CID serves two purposes. Large CID voltages (e.g., 300 - 400 V) are used to induce fragmentation of large ions in order to obtain fragments that would provide structural information; small CID voltages (e.g., 50 - 100 V) are used for destroying ion-solvent clusters. Collision induced fragmentation was mainly used for the latter purpose in this work; however, it was noticed that different CID voltages could produce different fragment ions which presumably correspond to products of different ion dissociation pathways. It would be interesting to examine CID processes in both small and large molecules, and compare the results to those obtained from other mass spectrometric techniques.

The last few pages described some of the experiments that were thought to be interesting as well as useful to practical ES-MS work. Much of what is learned about ES-MS has been initiated by those who use the technique and find its advantages, note its peculiarities, and tolerate its shortcomings. This technique has offered a great deal to the

area of biological sciences where most samples are large and nonvolatile; i.e., not amenable to routine mass spectrometric analysis. However, before it can be exploited to its full potential, a lot more remains to be investigated. Simple issues such as running multicomponent samples, the relatively low reproducibility, mass discrimination effects stemming from space charge build-up, and others, are among a few inconveniences and challenges of practical electrospray work.

These various problems become significantly magnified when samples consist of large multiply charged species present in complex matrices. Electrospray mass spectra of multiply charged ions consist of an array of peaks corresponding to the different charge states of the molecular ion. Overlapping mass spectra of such species are very complicated; hence, most practical ES-MS work of real-life samples requires some preliminary separation step to isolate the different components. Even with samples of small compounds, the presence of more than one species in the solution may create complications. For example, a hydrophobic analyte might suppress the response of a hydrophilic one; or an analyte of high gas phase basicity might attenuate the response of one that has lower gas phase basicity.

Complex matrices such as those in biological systems and environmental samples are further challenges for practical electrospray mass spectrometry. For example, high salt concentrations are not tolerated in ES-MS and other preparatory work (extraction) may be essential. In spite of the above difficulties, electrospray mass spectrometry remains as one of the most successful ionization techniques available for the analysis of macromolecules. The rapid expansion in electrospray research suggests that its

advantages outweigh its disadvantages, and that many of the problems and mysteries in the current understanding of electrospray mass spectrometry may well be solved in the foreseeable future.

REFERENCES

REFERENCES

1. Vestal, M.L. *Mass Spectrom. Rev.*, **1983**, 2, 447.
2. Evans, C.A., Jr.; Hendricks, C.D. *Rev. Sci. Instrum.*, **1972**, 43, 1527.
3. Yamashita, M.; Fenn, J.B. *J. Phys. Chem.*, **1984**, 88, 4451.
4. Huang, E.C.; Wachs, T.; Conboy, J.J.; Henion, J.D. *Anal. Chem.*, **1990**, 62(13), 713A.
5. Blakley, C.R.; McAdams, M.J.; Vestal, M.L. *J. Chromatogr.*, **1978**, 158, 264.
6. Beckey, H.D. *Int. J. Mass Spectrom. Ion Phys.*, **1969**, 2, 500.
7. Barber, M.; Bordoli, R.S.; Sedgwick, R.D.; Tyler, A.N.; Whalley, E.T. *Biomed. Mass Spectrom.*, **1981**, 8, 337.
8. Hakansson, I.; Kamensky, I.; Sundqvist, B.; Fohlman, J.; Peterson, P.; McNeal, C.J.; Macfarlane, R.D. *J. Am. Chem. Soc.*, **1982**, 104, 2948.
9. Karas, M.; Hillenkamp, F. *Anal. Chem.*, **1988**, 60, 2299.
10. Karas, M.; Hillenkamp, F. *Adv. Mass Spectrom.*, **1989**, 11A, 416.
11. Kleber, W. *Tech. Dig.*, **1967**, 9, 550.
12. Steiner, A.M.; Fuchs, H. *Seed Sci. Technol.*, **1987**, 15(3), 707.
13. Childs, R.E.; Mansout, N.N. *J. Propul. Power*, **1989**, 5, 641.
14. Meng, C.K.; Mann, M.; Fenn, J.B. *Z. Phys. D.*, **1988**, 10, 361.
15. Whitehouse, C.M.; Dreyer, R.N.; Yamashita, M.; Fenn, J.B. *Anal. Chem.*, **1985**, 57, 675.
16. (a) Dole, M.; Mack, L.L.; Hines, R.L.; Mobley, R.C.; Ferguson, L.D.; Alice, M.B. *J. Phys. Chem.*, **1968**, 49(1), 2240.
 (b) Mack, L.L.; Kralik, P.; Rheude, A.; Dole, M. *J. Phys. Chem.*, **1970**, 52(10), 4977.
 (c) Clegg, G.A.; Dole, M. *Biopolymers*, **1971**, 10, 821.
 (d) Teer, D.; Dole, M. *J. Polym. Sci.*, **1975**, 13, 985.
 (e) Dole, M.; Cox, Jr., H.L.; Gienièc, J. *Adv. Chem. Ser.*, **1973**, No.125, 73.

17. (a) Iribarne, J.V.; Thomson, B.A. *J. Phys. Chem.*, **1976**, *64*, 2287.
(b) Thomson, B.A.; Iribarne, J.V. *J. Chem. Phys.*, **1979**, *71*, 4451.
18. Bose, G. M., "Recherches sur la cause et sur la veritable theorie de l'electricite'." Wittenberg, 1745.
19. (a) Zeleny, J. *Phys. Rev.*, **1914**, *3*, 69.
(b) Zeleny, J. *Proc. Camb. Phil. Soc.*, **1915**, *18*, 17.
(c) Zeleny, J. *Phys. Rev.*, **1917**, *10*, 1.
20. Nolan, J.J. *Proc. Roy. Irish Acad.*, **1926**, *37A*, 28.
21. Macky, W.A. *Proc. Roy. Soc. A.*, **1931**, *133*, 565.
22. Vonnegut, B.; Neubauer, R.L. *J. Colloid Sci.*, **1952**, *7*, 616.
23. (a) Johnson, I; La Mer, V.K. *J. Amer. Chem. Soc.*, **1947**, *69*, 1184.
(b) La Mer, V.K. *J. Phys. Colloid Chem.*, **1948**, *52*, 95.
(c) La Mer, V.K.; Sinclair, D. *Chem. Rev.*, **1949**, *44*, 245.
24. Hayati, I; Bailey, A.I.; Tadros, Th.F. *J. Colloid Interface Sci.*, **1987**, *117*, 205; 222.
25. Smith, D.P.H. *IEEE Trans. Ind. Appl.*, **1986**, *IA-22*(3), 527.
26. Mann, M. *Organic Mass Spectrom.*, **1990**, *25*, 575.
27. Nohmi, T.; Fenn, J.B. *J. Am. Chem. Soc.*, **1992**, *114*, 3241.
28. Yamashita, M.; Fenn, J. B. *J. Phys. Chem.*, **1984**, *88*, 4451.
29. Ikonomou, M.G.; Blades, A.T.; Kebarle, P. *Anal. Chem.*, **1990**, *62*, 957.
30. Covey, T. R.; Bruins, A. P.; Henion, J. D. *Org. Mass Spectrom.*, **1988**, *23*, 178.
31. Vonnegut, B.; Neubauer, R.L. *J. Colloid Sci.*, **1953**, *8*, 551.
32. Hayati, I.; Bailey, A.; Tadros, Th. F. *Nature (London)*, **1986**, *319*, 41.
33. Melcher, J. R.; Taylor, G. I. *Ann. Rev. Fluid Mech.*, **1969**, *1*, 111.
34. Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C. *Mass Spectrom. Rev.*, **1990**, *9*, 37.
35. Lee, E.D.; Henion, J.D. *Rapid Commun. Mass Spectrom.*, **1992**, *10*, 727.

36. Shen, S.; Whitehouse, C.; Quinn, J. *41st ASMS Conference in Mass Spectrometry and Allied Topics*, San Francisco, 1993.
37. Ikonomou, M.G.; Blades, A.T.; Kebarle, P. *Anal. Chem.*, **1991**, *63*, 1989.
38. Drozin, V.G. *J. Colloid Sci.*, **1955**, *10*, 158.
39. Hendricks, C.D. in *"Electrostatics and its Applications"*, ed. A.D. Moore, Wiley-Interscience, New York (1973), Chapter 4.
40. Pfeifer, R.J.; Hendricks, C.D. *AIAA Journal*, **1968**, *6*, 496.
41. DeShon, W.E.; Carson, R.S. *J. Colloid Interface Sci.*, **1968**, *28*, 161.
42. Huberman, M.N. *J. Appl. Phys.*, **1970**, *41*, 578.
43. Loeb, L. B.; Kip, A. F.; Hudson, G. G.; Bennett, W. H. *Phys. Rev.*, **1941**, *60*, 714.
44. Taylor, G. I. *Proc. R. Soc. A*, **1964**, *A280*, 383.
45. Melcher, J. R.; Warren, E. P. *J. Fluid Mech.*, **1971**, *47*, 127.
46. Smalley, R. E.; Wharton, L.; Levy, D. H. *Acc. Chem. Res.*, **1977**, *10*, 139.
47. Campargue, R. *J. Chem. Phys.*, **1984**, *88*, 4466.
48. Campana, J.E. *Mass Spectrom. Rev.*, **1987**, *6*, 395.
49. Anderson, J.B., Fenn, J. B. *Phys. Fluids*, **1965**, *8*, 780.
50. Beijerinck, H.C.W.; van Gerwen, R.J.F.; Kerstel, E.R.T.; Marten, J.F.M.; van Vliembergen, E.J.W.; Smits, M.R.Th.; Kaashoek, G.H. *Chem. Phys.*, **1985**, *96*, 153.
51. Bruins, A.P. *Mass Spectrom. Rev.*, **1991**, *10*, 53.
52. Fenn, J. B., Deckers, J., in Laurmann, J. A., ed., *"Rarefied Gas Dynamics"*, Vol. I, Academic Press, New York, 1963, 497-515.
53. Horning, E.C.; Horning, M.G.; Carroll, D.I.; Dzidic, I.; Stillwell, R.N. *Anal. Chem.*, **1973**, *45*, 936.
54. Hasted, J. B. *Int. J. Mass Spectrom. Ion Phys.*, **1975**, *16*, 3.
55. Whitehouse, C.M.; Fenn, J.B.; Yamashita, Y. (1984) *European Patent 0,123,553*.

56. Chowdhury, S.K.; Katta, V.; Chait, B. *Rapid Commun. Mass Spectrom.*, **1990**, *4*, 81.
57. Busman, M.; Sunner, J.; Vogel, J.R. *J. Am. Soc. Mass Spectrom.*, **1991**, *2*, 1.
58. Gobby, P.L.; Grimsrud, E.P.; Warden, S.W. *Anal. Chem.*, **1980**, *52*, 473.
59. Busman, M.; Sunner, J. *Int. J. Mass Spectrom. Ion Phys.*, **1991**, *108*, 165.
60. Sigmond, R.S. in *Electrical Breakdown of Gases*; Meek, J.M.; Craggs, J.D., (Eds, Wiley: Chichester, 1978; Chapter 4.
61. (a) Lane, D.A.; Thomson, B.A.; Lovett, A.M.; Reid, N.M. *Adv. Mass Spectrom.*, **1980**, *8B*, 1480.
(b) French, J.B.; Reid, N.M.; Buckley, J.A. *United States Patent 4,137,750*, 1979.
62. Allen, M.A.; Field, F.H.; Vestal, M.L. Paper TOC 11:00, *38th ASMS Conference on Mass Spectrometry and Allied Topics*, Tucson, Arizona (1990).
63. Shahin, M. M, *J. Chem. Phys.*, **1966**, *45*, 2600.
64. Kambara, H.; Kanomata, I. *Anal. Chem.*, **1977**, *49*, 270.
65. Kambara, H. (1979) *United States Patent 4,144,451*.
66. French, J.B.; Reid, N.M.; Buckley, J.A. (1978) *United States Patent 4,121,099*.
67. SIMION User's Manual (1988), EG&G Idaho Inc., Idaho Falls, ID
68. Scientific Supplies Catalog (1991-1992) Scientific Instrument Services, Inc., p. 77.
69. Watson, J.T. *Introduction to Mass Spectrometry*; Raven Press: New York, 1985.
70. Tang, L.; Kebarle, P. *Anal. Chem.*, **1991**, *63*, 2709.
71. Ryan, T.M.; Day, R.J.; Cooks, R.G. *Anal. Chem.*, **1980**, *52*, 2054.
72. Dean, J.A. *Lange's Handbook of Chemistry*; McGraw-Hill, Inc.; New York, 1992.
73. YSI Model 35 Conductance Meter Instruction Manual, YSI Inc., Yellow Springs, OH
74. (a) Hiraoka, K. *Rapid Commun. Mass Spectrom.*, **1992**, *6*, 463.
(b) Hiraoka, K.; Murata, K.; Kudaka, I. *Rapid Commun. Mass Spectrom.*, **1993**, *7*, 363.
(c) Hiraoka, K. *Anal. Chem.*, **1992**, *64*, 75.

75. Iribarne, J.V.; Dziedzic, P.J.; Thomson, B.A. *Int. J. Mass Spectrom. Ion Phys.*, **1983**, *50*, 331.
76. Smith, R.D.; Loo, J.A.; Ogorzalek Loo, R.R.; Busman, M.; Udseth, H.R. *Mass Spectrom. Rev.*, **1991**, *10*, 359.
77. Guevremont, R.; Siu, K. W. M.; Le Blanc, J. C. Y.; Berman, S. S. *J. Am. Soc. Mass Spectrom.*, **1992**, *3*, 216.
78. Shinoda, K. (Ed.) *Solvent Properties of Surfactant Solutions*; Marcel Dekker, Inc.: New York, 1967.
79. Wagner, J. Master's Thesis, Georgia Institute of Technology, 1987.
80. Vestal, M. *Ion Formation from Organic Solids*; Springer Series in Chemical Physics, Vol. 25, Springer: New York, 1983.
81. (a) Röllgen, F.W.; Bramer-Weger, E.; Bütering, L. *J. Phys. (Paris)*, **1987**, *48*, C6-253.
(b) Schmeizeisen-Redeker, G.; Bütering, L.; Röllgen, F.W. *Int. J. Mass Spectrom. Ion Proc.*, **1989**, *90*, 139.
82. Berry, R.S.; Rice, S.A.; Ross, J.A. *Physical Chemistry*; John Wiley & Sons Ltd: New York, 1980, p.994.
83. Tanford, C. *The Hydrophobic Effect*; John Wiley & Sons, Inc.: New York, 1973.
84. Shelton, R.S.; Van Campen, M.G.; Tilford, C.H.; Lang, H.C.; Nisonger, L.; Bandelin, F.J.; Rubenkoenig, H.L. *J. Am. Chem. Soc.*, **1946**, *68*, 753.
85. Callahan, J. H.; Hool, K.; Reynolds, J. D.; Cook, K. D. *Int. J. Mass Spectrom. Ion Proc.*, **1987**, *75*, 291.
86. Ligon, W. V.; Dorn, S. B. *Int. J. Mass Spectrom. Ion Proc.*, **1985**, *63*, 315.
87. Ligon, W. V.; Dorn, S. B. *Int. J. Mass Spectrom. Ion Proc.*, **1986**, *68*, 337.
88. Caprioli, R. M.; Moore, W. T.; Petrie, G.; Wilson, K. *Int. J. Mass Spectrom. Ion Proc.*, **1988**, *86*, 187.
89. Blades, A.T.; Ikonomou, M.G.; Kebarle, P. *Anal. Chem.*, **1991**, *63*, 2109.
90. Rosen, M. J. *Surfactants and Interfacial Phenomena*; Wiley-Interscience: New York, 1978; p. 98.
91. Hutchinson, E.; Manchester, K.E.; Winslow, L. *J. Phys. Chem.*, **1954**, *58*, 1124.

92. Muller, N. *Reaction Kinetics in Micelles*; Plenum Press: New York, 1973; p. 1.
93. Lindman, B.; Wennerström, H. In *Topics in Current Chemistry*; Springer-Verlag: Heidelberg, 1980; Vol. 87, p. 1.
94. Chowdhury, S.K.; Chait, B.T. *Anal. Chem.*, **1991**, 63, 1660.
95. Zeman, I. *J. Chromatography*, **1988**, 426, 452.
96. Shaw, D. J. (Ed.) *Introduction to Colloid and Surface Chemistry*; Butterworth Inc.: Boston, 1980.
97. De Pauw, E.; Pelzer, G.; Dao Viet, D.; Marien, J. *Biochem. Biophys. Res. Commun.*, **1984**, 123(1), 27.
98. Ligon, W.V. *Anal. Chem.*, **1986**, 58, 485.
99. Mirza, U.A.; Cohen, S.L.; Chait, B.T. *Anal. Chem.*, **1991**, 65, 1.
100. Born, M. *Phys. Z.*, **1920**, 1, 45.
101. Aue, D.H.; Webb, H.M.; Bowers, M.T. *J. Am. Chem. Soc.*, **1976**, 98:2, 318.
102. de Visser, C; Somsen, G.J. *J. Chem. Thermodyn.*, **1972**, 4, 313.
103. Tamaki, K. *Bull. Chem. Soc. Jpn.*, **1974**, 47, 2764.
104. Marcus, Y. *Ion Solvation*; John Wiley & sons Ltd.: Chichester, 1985.
105. Tamaki, K.; Ohara, Y.; Kurachi, H.; Ariyama, M.; Odaki, H. *Bull. Chem. Soc. Jpn.*, **1974**, 47, 384.
106. Desnoyers, J.E.; Perron, G. *J. Solution Chem.*, **1972**, 1, 199.
107. Reynolds, J.D.; Cook, K.D. *J. Am. Soc. Mass Spectrom.*, **1990**, 1, 233.
108. Raffaelli, A.; Bruins, P. *Rapid Commun. Mass Spectrom.*, **1991**, 5, 269.
109. Horvath, A.L. *Handbook of Aqueous Electrolyte Solutions*; John Wiley & Sons Ltd.: Chichester, 1985.
110. (a) Bjerrum, N. *Kgl. Danske Videnskab. Selskab, Mat. Fys. Medd.*, **1926**, 7, 1.
(b) Kraus, C.A. *J. Phys. Chem.*, **1956**, 60, 129.
111. Nancollas, G.H. *Interactions in Electrolyte Solutions*; Elsevier Publishing Company: Amsterdam, 1966.

112. (a) Fuoss, R.M. *J. Am. Chem. Soc.*, **1958**, *80*, 5059.
 (b) Fuoss, R.M. *Proc. Natl. Acad. Sci. USA*, **1978**, *75*, 16.
 (c) Fuoss, R.M. *J. Phys. Chem.*, **1978**, *82*, 2427.
113. Harned, H.S.; Owen, B.B. in *"The Physical Chemistry of Electrolytic Solutions"*, Reinhold Publishing Corporation: New York (1958) Third Edition p. 161
114. Ikonomou, M.G.; Blades, A.T.; Kebarle, P. *Anal. Chem.*, **1988**, *60*, 1300.
115. Arnett, E.M. *Acc. Chem. Res.*, **1973**, *6*, 404.
116. Lumry, R.; Rajender, S. *Biopolymers*, **1970**, *9*, 1125.
117. Wauchope, R.D.; Haque, R. *Can. J. Chem.*, **1972**, *50*, 133.
118. Arnett, E.M.; McKelvey, D.R. *Solute-Solvent Interactions*; Marcel Dekker: New York, 1969.
119. Lias, S.G. *J. Phys. Chem. Ref. Data*, **1988**, *17(1)*, 5.
120. Böttcher, C.J.F. *Theory of Electric Polarization*; Elsevier Scientific Publishing Co.; Amsterdam, Netherlands, 1973.
121. Su, T.; Bowers, M. *Int. J. Mass Spectrom. Ion Phys.*, **1973**, *12*, 347.
122. Jones, III, F.M.; Arnett, E.M. *Prog. Phys. Org. Chem.*, **1974**, *11*, 263
123. Compilation of thermodynamic parameters (1993) provided by Professor J.E. Bartmess.
124. Smith, R. D., Loo. A. J., Edmonds, C. G., Barinaga, C. J., Udseth, H. R. (1990) *Anal. Chem.*, **62**, 882.
125. Cheng. Z. L.; Siu, K. W. M.; Guevremont, R.; Berman, S. S. *J. Am. Soc. Mass Spectrom.*, **1992**, *3*, 281.
126. Siu, K.W.M.; Gardner, G.J.; Berman, S.S. *Org. Mass Spectrom.*, **1989**, *24*, 931.
127. Mackay, G.I.; Tanner, S.D.; Hopkinson, A.C.; Bohme, D.K. *Can. J. Chem.*, **1979**, *57*, 1518.
128. Stryer, L. *Biochemistry*; W.H. Freeman and Company: New York; 1988.
129. O'Hair, R.O.J.; Bowie, J.H.; Gronert, S. *Int. J. Mass Spectrom. Ion Proc.*, **1992**, *117*, 23.

130. Bull, H.B.; Breese, K. *Arch. Biochem. Biophys.*, **1971**, *161*, 665.
131. Chowdhury, S.K.; Katta, V.; Chait, B.T. *J. Am. Chem. Soc.*, **1990**, *112*, 9012.

VITA

Mona Shahgholi was born in Tehran, Iran to a most loving family. She completed her elementary education in an American school and her high school education in a French school. She then obtained her Bachelor's of Science degree in Physiology from Oklahoma State University. Her next stop was Colorado where she spent a few years working in a geochemical laboratory while taking a variety of chemistry courses at University of Colorado at Denver. She decided to abandon the latter for a career in art which took her to California. There, she obtained an Associate degree in Graphic Communications from Pasadena City College and worked as a graphic artist and photographer for some time. Encouragement from her former professor at University of Colorado, Dr. John Lanning, brought her back to Colorado where she obtained her Master's of Science degree in Chemistry, in December of 1989, under the guidance of Dr. Larry Anderson and Dr. John Lanning. While working on her Master's degree she obtained a "student worker" position at the NIH Clinical Mass Spectrometry Research Resource. Her experiences at this job led to her decision to pursue a PhD in mass spectrometry which finally brought her to the University of Tennessee at Knoxville. The next stop for her is Washington, D.C., for a post-doctoral position at the Naval Research Laboratory.

The road has been long and rough at times, with soft spots far apart; however, the opportunity to make new friends and associates has made it all worthwhile.