with the electrospray source installed.

The protein samples used to produce the spectra in Figures 2-4 were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The sample used for the data shown in Figure 5 was provided by Dr. Christian Schwabe of the Medical University of South Carolina.

RESULTS AND DISCUSSION

The combination ion source gave comparable performance in liquid SIMS mode to the standard Nermag FAB ion source (data not shown), indicating that the hemispherical repeller was an adequate substitute for the curved metal strip repeller on the standard FAB ion source. The results of sample analysis in electrospray mode are shown in Figures 2-5. Figure 2 shows data obtained from analysis of bovine insulin. The measured molecular weight (5731.7 ± 1.5) was within 0.034% of the calculated value (5733.6). Figure 3 shows results obtained for equine myoglobin; the measured molecular weight (16944 ± 5) was within 0.037% of the calculated molecular weight (16950.5). The accuracy of these measurements is limited by the acquisition of nominal mass data and could likely be improved by acquisition of "profile" data and measurement of mass to charge ratios to tenths of a unit. Figure 4 shows data obtained on bovine serum albumin, the largest protein examined with this ion source. The measured molecular weight (66541 ± 39) was within 0.4% of the calculated weight (66267). The difference between the measured and calculated masses for this protein are comparable to those reported by other investigators (*4*, *7*) and could be due to bound ions (e.g. Ca^{2+}) other than protons and/or modifications of the protein. Figure 5 shows data obtained on a sample of porpoise relaxin (a protein structurally similar to insulin) for which only a partial sequence was known from Edman degradation. The sample spectrum contained one major and several minor series of peaks. Manual calculations on the mixture data (assuming molecular weight for relaxin in the 5000-6000 range) permitted identification of a major peak series (with charge states of +4 to +7) of molecular weight 6057.9. Components of masses 5986.4 (+3 to +7), 5883 (+4 to +6), 5726 (+3 to +6), 5629 (+5, +6), 5478 (+6; charge assignment based upon assumption that this peak was from a relaxin related molecule), and 5395 (+5, +6) were also observed. If these multiple components were the result of proteolytic trimming occurring in the isolation process, the mass differences of the components could be used to gain information on terminal amino acids (relaxin consists of two chains connected by disulfide bonds). For example, the difference of 71 mass units between components I and II would suggest a terminal alanine.

CONCLUSIONS

A combination electrospray-liquid SIMS ion source has been constructed that allows use of both modes of ionization without the need for physical reconfiguration of the instrument. The electrospray data obtained on samples of bovine insulin (mol wt 5733.6; error 0.034%), equine myoglobin (mol wt 16950.5; error 0.037%), and bovine serum albumin (mol wt 66267; error 0.4%) are comparable to previously published data (*4*, *7*). The electrospray source also performed satisfactorily on a second tandem quadrupole instrument which required a longer desolvation capillary, indicating that the design should be adaptable to a variety of instruments.

ACKNOWLEDGMENT

We thank S. K. Chowdhury and B. T. Chait for helpful discussion of their electrospray design. Special thanks go to Clifton Harvey for his expert machine work in construction of the ion source. We thank Vicki Wysocki, Virginia Commonwealth University, Richmond, VA, for the opportunity to test the ion source on her instrument.

LITERATURE CITED

(1) Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. *Anal. Chem.* **1985**, *57*, 675-679.
(2) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64-71.
(3) Aberth, W.; Straub, K. M.; Burlingame, A. L. *Anal. Chem.* **1982**, *54*, 2029-2034.
(4) Chowdhury, S. K.; Katta, V.; Chait, B. T. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 81-87.
(5) Callahan, J. H.; King, F. L.; Ross, M. M.; Wysocki, V. H. *Proc. Annu. Conf. Am. Soc. Mass Spectrom.* **1990**, *38*, 898-899.
(6) Chait, B. T. Presented at the ASMS Fall Workshop on Electrospray Ionization, Nov. 5-6, 1990, Chicago, IL.
(7) Baczynski, L.; Bronson, G. E. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 533-535.

RECEIVED for review February 6, 1991. Accepted April 15, 1991. This work was supported in part by NIH Grant EY-08239. This work was presented at the ASMS Fall Workshop on Electrospray Ionization, Nov 5-6, 1990, Chicago, IL.

Method for the Electrospray Ionization of Highly Conductive Aqueous Solutions

Swapan K. Chowdhury and Brian T. Chait*

Laboratory of Mass Spectrometry, The Rockefeller University, 1230 York Avenue, New York, New York 10021

INTRODUCTION

The electrospray phenomenon (also known as electrohydrodynamic atomization) is a process of disintegration of a liquid surface in the presence of a strong electric field into a spray of fine, highly charged droplets. A number of studies have been carried out, going back more than 70 years, aimed at gaining an understanding of the fascinating physical processes governing the spray (*1-3*). Considerable interest in the electrospray process has also arisen because it has found wide applications for such diverse purposes as electrostatic emulsification (*2e*, *4*), electrostatic painting (*5*), paint spraying (*2e*, *6*), fuel atomization in combustion systems (*7*), crop spraying (*2e*, *8*), and a method for sample preparation for β-counting experiments (*9*) and ^{252}Cf plasma desorption mass spectrometry (*10*). The most recent resurgence of interest in electrospray has arisen in connection with the technique of electrospray ionization mass spectrometry (*11-17*). In this technique, solutions of involatile organic molecules and biopolymers (such as proteins and DNA) are electrosprayed at atmospheric pressure to produce a large number of small highly charged droplets containing the component(s) of interest. The solvents are rapidly evaporated from the droplets, and the residual biopolymer ions are transported through differentially pumped orifices or capillaries into a mass spectrometer where the mass-to-charge ratio (m/z) values of the ions are accurately determined. By using electrospray

0003-2700/91/0363-1660$02.50/0

ionization (ESI) mass spectrometry, molecular masses of many peptides and proteins can be determined with an accuracy of better than 0.02% (*12, 13, 15–17*).

Despite its wide application and long history, a detailed understanding of the mechanism of the electrospray phenomenon continues to provide challenges to investigators. Recently, Smith (*18*) and Hayati et al. (*19*) investigated the effects of a large number of experimental parameters on the onset and stability of electrospray. The parameters included solution properties such as surface tension, dielectric constant, viscosity, and conductivity, as well as applied voltage, flow rate, and capillary diameter. Smith observed that the onset potential for electrospray of a liquid increases with the square root of the surface tension of the liquid and found an upper limit of surface tension (~0.05 N/m) above which stable electrospray in air could not be obtained (*18*). The failure to spray liquids with surface tensions > 0.05 N/m was explained by the requirement that the field necessary for the onset of the eletrospray exceeds that required for the ionization of air. Therefore, the process becomes corona limited for liquids having high surface tensions. Smith also investigated the effects of liquid conductivity on electrospray and observed no upper limit of the conductivity required to produce stable electrospray. In contrast, Hayati et al. (*19*) observed that conductivity was an important parameter in the electrostatic disintegration of a liquid surface and found that a stable spray could only be obtained when the conductivity of the liquid was between 10^{-6} and 10^{-8} Ω^{-1} m^{-1} (*19a*). The conductivity of the distilled water used in the present experiment is 6×10^{-4} Ω^{-1} m^{-1}, and the surface tension is 0.073 N/m. These values are higher than the upper limits for stable electrospray provided by Smith (*18*) and Hayati et al. (*19*).

Several reports concerning the electrospray of pure water exist in the literature. Sample and Bollini (*20*) reported the electrostatic atomization of distilled water, which they called "harmonic electrical spraying". The flow rates used in this latter study ranged between 135 and 400 μL/min, resulting in the formation of large droplets (d = 140–429 μm). These flow rates are much higher than is commonly used in conjunction with mass spectrometry (*12–17*). Kozhendov and Fuks (*3*) have suggested that the spray obtained by Sample and Bollini may not be true electrohydrodynamic atomization. Two other reports have been made (*21, 22*) on the electrospray of water without the addition of alcohol or surfactants or without assisting the spray by some other means (*13, 23, 24*). These reports (*21, 22*), however, do not provide detailed descriptions of the conditions under which electrospray was obtained and the relationship between the nature of the spray and the stability and sensitivities of ion signals detected mass spectrometrically.

In the electrospray ionization mass spectrometry of peptides and proteins, the analyte of interest is usually dissolved in an aqueous acidic solution containing a substantial proportion (typically 50%) of an organic solvent such as methanol (*12, 13, 15–17*). The presence of methanol in aqueous solutions lowers both the surface tension and the conductivity of the solution. Such solutions produce stable electrospray and generate stable and reproducible ion signals in the mass spectrometer. It is desirable, however, to have the ability to electrospray purely aqueous solutions because many proteins are not soluble in solutions containing a large proportion of organic solvent. Indeed, organic solvents are commonly used to precipitate proteins from aqueous solutions (*25*). We have also found it necessary to spray protein solutions with compositions ranging from 100% water to water containing varying amounts of organic solvents in order to gain an understanding of the role of solvent composition on the mass spectrometric sensitivity and charge–state distribution of protein ions in the ESI mass spectra (*26*). These investigations were motivated by the observation that different proteins yield widely different mass spectrometric sensitivities when electrosprayed from solutions containing ~50% methanol (*15, 27*). We report here the results of an investigation of the electrospray of 100% water, acidified water, and aqueous protein solutions containing small amounts of acid.

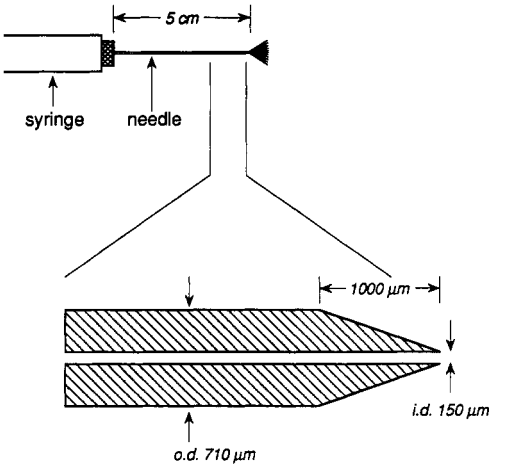

Figure 1. Shape and dimensions of a specially modified spray needle used for the electrospray of water, acidified water, and aqueous protein solutions.

EXPERIMENTAL SECTION

The electrospray ionization mass spectrometer and the procedure adopted to acquire mass spectra have been described previously (*15*). Briefly, the sample solution was electrosprayed from a syringe needle in ambient air. The resulting highly charged droplets and solvated ions were transported into the vacuum of the quadrupole mass spectrometer through a 20-cm-long, 0.5-mm-i.d. heated metal capillary tube for mass-to-charge ratio (m/z) analysis. The spectra were acquired by using a commercial data system, Vector-1 (Teknivent, St. Louis, MO), on an IBM AT compatible computer. The flow rate of the analyte solution was dictated by the amounts of acetic acid present and varied from 0.2 μL/min for aqueous solutions containing 3–4% acetic acid to 1.5 μL/min for pure water. The spray solutions were forced through the needle by a Harvard Apparatus syringe pump (Model 2400-001). The electrospray was performed by applying a dc voltage of 3–5 kV to the syringe needle relative to the metal capillary tube through which the solvated ions and droplets enter the vacuum of the mass spectrometer (*15*). The distance between the tip of the syringe needle and the capillary tube ranged between 4 and 10 mm. Water and methanol were obtained from Burdick & Jackson (Muskegon, MI), ultrapure acetic acid from J. T. Baker and Co. (Phillipsburg, NJ), and horse heart heart cytochrome C (Catalog No. C3256) from Sigma Chemical Co. (St. Louis, MO). The conductivities of various solutions were determined using a Model CDM 2d conductivity meter from Radiometer (Copenhagen, Denmark).

RESULTS AND DISCUSSION

Our initial attempts to spray aqueous solutions of proteins without added organic solvents from standard syringe needles (26s gauge and 22s gauge) were unsuccessful. These needles were used routinely and successfully for the electrospray of aqueous solutions containing a large proportion of organic solvent (*15*). In order to determine conditions under which pure water or aqueous protein solutions can be electrosprayed, we have investigated the effect of the variation of size (i.d. and o.d.) and shape of the syringe needle on the electrospray. The flat tip of a replaceable needle (22s gauge, 710-μm o.d., 150-μm i.d.) of a standard 100-μL syringe was sharpened by electropolishing to a final shape in several steps. The electropolishing was carried out in a solution containing water, glycerol, and phosphoric acid (1:1:1, v/v). The final shape

Table I. Spray Conditions[a] as a Function of Spray Voltage and Flow Rate for the Electrospray of Water

flow rate, μL/min	voltage on the spray needle, kV	spray current, nA	comments
2.0			no steady spray at any voltage
1.5	3.20	90	fluctuating
	3.50	350	steady
	3.70	1250	fluctuating
1.0	3.10		no spray
	3.17	20	steady
	3.35	65	fluctuating

[a] The distance between the spray tip and the transport capillary tube was 6.7 mm.

of the syringe needle tip is shown in Figure 1. As the tip of the syringe needle was made increasingly sharp, the water exiting the needle tended to form finer and finer droplets when a high voltage was applied. For the final conical shape (Figure 1), stable electrospray from pure water in ambient air was obtained. The optimum voltage at which stable electrospray of pure water was obtained decreased as the tip was made sharper. For a distance between the syringe needle and the capillary tube of 6.7 mm and a flow rate of 1.5 μL/min, a voltage of 3.5 kV was required to obtain a stable electrospray from pure water. The spray condition and the measured spray current as a function of the applied voltage are given in Table I. As the conductivity of the solution was increased by the addition of HCOOH or CH_3COOH to water or to aqueous protein solutions, it was necessary to reduce the flow rate to obtain stable electrospray. Thus, the optimum flow rate decreased from 1.5 μL/min for pure water (conductivity = $6.0 \times 10^{-4}\ \Omega^{-1}\ m^{-1}$) to 0.20 μL/min for water containing 3.0% acetic acid (conductivity = $0.13\ \Omega^{-1}\ m^{-1}$). The decrease in the optimum flow rate for electrospray with the increase in solution conductivity has previously been reported by Smith (*18*).

As pointed out above, the onset potential required to achieve steady electrospray decreased as the needle tip was made sharper. This observation is in agreement with the prediction of Smith that the onset voltage for electrohydrodynamic disintegration is proportional to $T^{1/2}r^{1/2} \ln (4h/r)$, where T is the liquid surface tension, r is the radius of the needle tip, and h is the distance between the needle tip and a planar counter electrode (*18*). Thus, for $h = 5$ mm and for a constant value of T, a change of capillary diameter from 700 to 200 μm decreases the required onset potential by a factor of 1.4. If we assume that the shape of the Taylor cone (*2a*) does not change as a function of the capillary diameter, the reduction in the required applied potential yields a reduction in the field at the tip. The onset of the spray, therefore, can occur from a sharp needle tip, as demonstrated in the present investigation, before the start of a corona discharge (*3*, *19*). It is noteworthy that Smith (*18*) was able to electrospray distilled water from an unmodified tip when the surrounding atmosphere was SF_6, a highly efficient electron scavenger (*28*) that prevented corona discharge.

Although a stable spray with fine droplets of pure water was obtained at a needle voltage of 3.5 kV, no ions from pure water were detected in the mass spectrometer until the needle voltage was increased to ~4.0 kV whereupon the spray became unsteady. When a small amount of acid (0.01–4%) was added, a stable ion signal in the mass spectrometer was observed at a needle voltage of ~3.5 kV. The ESI mass spectrum of water containing 1.0% v/v acetic acid is shown in Figure 2. The spectrum, acquired with the transport capillary tube heated

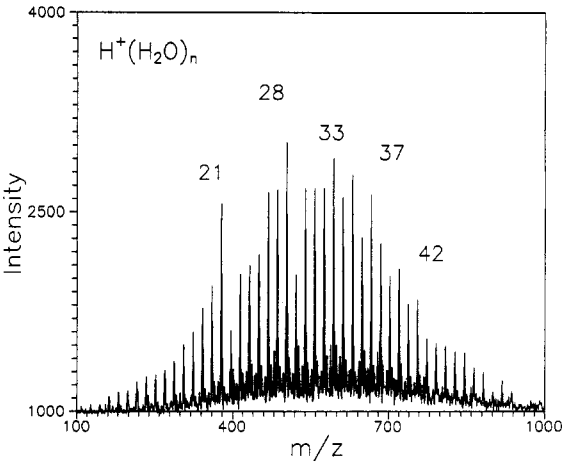

Figure 2. Electrospray ionization mass spectrum of water containing 1.0% acetic acid. The transport capillary tube was heated to 80 °C. The number (n) on an ion peak denotes the number of water molecules attached to H^+. Flow rate = 0.2 μL/min. The spectrum is an average of 26 scans each acquired in 28 s.

to a temperature of 80 °C, exhibits a series of ions, $H^+(H_2O)_n$, with n ranging from 6 to 52. A feature of the spectrum is the relatively high intensities of ions containing 21 and 28 water molecules relative to the ions containing 22 and 29 water molecules, respectively. Several investigators have previously observed enhancement of the intensities of the $H^+(H_2O)_{21}$ (*29–32*) and $H^+(H_2O)_{28}$ (*32*) ions in supersonic free jet expansion experiments, where the ions were generated by corona discharge before the expansion (*30*, *31*) or by electron impact after the expansion (*29*, *32*). It is known that, in neutral clusters, 21 water molecules can be arranged in a clathrate cage structure: a pentagonal dodecahedron with a oxygen atom at each corner and a water molecule trapped inside (*33*, *34*). As pointed out by Searcy and Fenn (*30*), a similar cage-like structure may be also possible for the protonated cluster. Holland and Castleman (*35*) have proposed a model based on the high mobility and bonding effects of the "excess" proton in water to explain the higher stability of $H^+(H_2O)_{21}$. These authors suggested that protonated water clusters containing 26, 28, and 30 water molecules may also possess special geometries. Kassner and Hagen (*34*) have predicted magic numbers of 21, 37, and 50. In the present investigation, water cluster ions, $H^+(H_2O)_n$, with $n = 21$, 28, 33, 35, 37, and 42 were found to have enhanced stability (Figure 2).

In the measurement shown in Figure 2, no potential difference was applied between the exit of the transport capillary tube and the skimmer (*15*). The potential difference, when applied, causes the exiting ions from the capillary tube to undergo energetic collisions with the neutral gases present in this region (*13*, *15*). The resulting collisional activation produces a shift in the maximum of the solvation-state distribution toward lower values of n. An increase in the temperature of the transport capillary tube also shifts the solvation-state distribution toward lower values of n. Water clusters formed with ions such as Na^+ and NH_4^+ can also be generated. Thus, the present investigation demonstrates that electrospray of pure water or water containing small amounts of acid or other electrolytes provides an intense source of large ionic clusters of water molecules that offers opportunities for further detailed investigation of structures and stabilities of such species. It should be noted that the present measurements do not provide information concerning the relative contributions to cluster formation of the electrospray ionization process itself and that due to condensation of ambient moisture during the jet expansion. To our knowledge, *unassisted* electrospray ionization mass spectra of aqueous solutions without the addition of any organic solvent have not

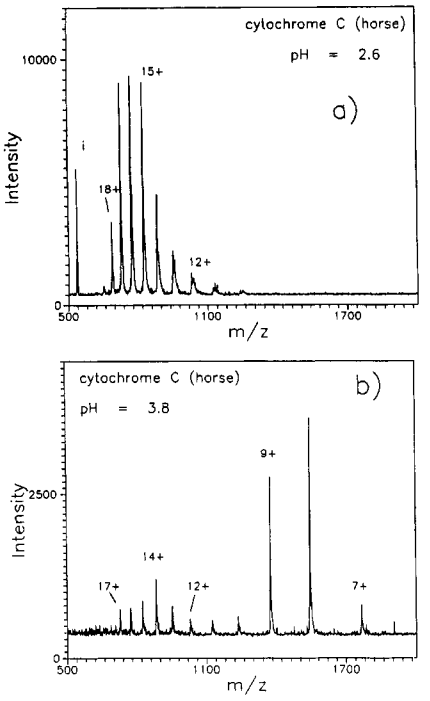

Figure 3. Electrospray ionization mass spectra of horse heart cytochrome *c* obtained from 10 μM aqueous solutions containing different amounts of acetic acid. *n*+ represents an ion of cytochrome *c* containing *n* protons. (a) 3% aqueous acetic acid, pH = 2.6, and flow rate = 0.2 μL/min. The ion denoted as i arises from an unidentified impurity. (b) 0.01% aqueous acetic acid, pH = 3.8, flow rate = 0.8 μL/min.

been previously reported in the literature.

There are a number of situations in which it is advantageous to electrospray aqueous protein solutions not containing organic solvents. One important situation arises during studies of proteins that are not soluble or tend to aggregate in aqueous solutions containing a large proportion of organic solvents (for example, chicken egg lysozyme precipitates when the ethanol content of the solution exceeds 25% and hen egg albumin irreversibly precipitates at a composition of 40% ethanol). Another important situation arose during our studies of the effects of protein conformation on the electrospray ionization mass spectra, studies that included proteins that are rapidly denatured in the presence of organic solvents. In a recent communication, we have reported the electrospray ionization mass spectra of bovine heart cytochrome *c* obtained from aqueous solutions without any organic solvent (*26*). These investigations provided information regarding the charge-state distribution of protein ions observed in the mass spectra as a function of their conformation in solution. The investigation could not be performed with protein solutions containing organic solvent because the presence of even small amounts of organic solvent causes cytochrome *c* to unfold. Results from an investigation with horse heart cytochrome *c* are shown in Figure 3 obtained from aqueous solutions containing 3% (Figure 3a) and 0.01% (Figure 3b) acetic acid with pH values of 2.6 and 3.8, respectively. No other organic solvent or buffer was added to the spray solutions. The two mass spectra are strikingly different. In the top panel, ions are produced with a single charge-state distribution with charges ranging from 18+ to 11+. The charges 18+ and 11+ denote $(M + 18H)^{18+}$ and $(M + 11H)^{11+}$, respectively, where M represents horse heart cytochrome *c*. In contrast, the bottom spectrum (Figure 3b) exhibits ions from the two discrete distributions of charge states. One distribution, also observed in the top figure, centers around 14+ and ranges from 17+ to 12+, while the other ranges from 10+ to 7+, with 8+ being the most intense ion. The formation of two discrete charge-state distributions (Figure 3b) results from two different conformational states of horse heart cytochrome *c*. At the lower pH, the majority of the protein is in a highly charge unfolded state (Figure 3a). When the pH is increased from 2.6 to 3.8, a large fraction of cytochrome *c* molecules converts to a tighter conformation, with fewer basic groups available for protonation, thus producing a second distribution of ions with lower charge states (*26*).

CONCLUSIONS

The present investigation demonstrates that the electrospray of 100% water or water containing small amounts (0.01–5%) of organic acid, such as formic acid and acetic acid, can be performed without nebulization despite the high conductivity and surface tension of the solutions. Electrospray of aqueous solutions was obtained by the use of a specially modified syringe needle tip (Figure 1). The onset potential required to obtain stable electrospray decreases with a reduction in the spray tip outside diameter. Thus, with the sharp capillary tip, the spray of aqueous solutions can be produced before the onset of a corona discharge.

ACKNOWLEDGMENT

We thank Gladys McMilleon for typing the manuscript.

LITERATURE CITED

(1) Zeleny, J. *Phys. Rev.* **1920**, *16*, 102; **1917**, *10*, 1; **1914**, *3*, 69.
(2) (a) Taylor, J. *Proc. R. Soc. London 1964*, *280*, 383. (b) Vonnegut, B.; Neubauer, R. L. *J. Colloid Sci.* **1952**, *7*, 616. (c) Macky, W. A. *Proc. R. Soc. London A* **1931**, *133*, 565. (d) Burayev, T. K.; Vereshchagin, I. P. *Fluid Mech.-Soviet Res.* **1972**, *2*, 56. (e) Bailey, A. G. *Atomisation Spray Technol.* **1986**, *2*, 95.
(3) Kozhenkov, V. I.; Fuks, N. A. *Russ. Chem. Rev.* **1976**, *45*, 1179 and references therein.
(4) Hughes, J. F.; Roberts, J. M. C. *Int. J. Cosmet. Sci.* **1984**, *6*, 103.
(5) Suzuki, Y. Eur. Pat. EP 363103, 11 April, 1990. Mirchandani, S. M.; Prabhu, P. K. *Paintindia* **1989**, *39*, 36. Elmoursi, A. A. *IEEE Trans. Ind. Appl.* **1989**, *25*, 234. Van den Abbeele, A. *Gatuano* **1968**, *37*, 743.
(6) Lundqvist, S.; Fredholm, O.; Loustrand, K. G. *Strif., Conf. Ser.-Inst. Phys.* **1971**, 260. Kleber, W. *Tech. Dig.* **1967**, *9*, 550.
(7) Childs, R. E.; Mansout, N. N. *J. Propul. Power* **1989**, *5*, 641. Yule, A. J.; Aval, S. M. *Fuel* **1989**, *68*, 1558. Mao, C. P.; Oechsle, V.; Chigier, N. *J. Fluids Eng.* **1987**, *109*, 64.
(8) Watanabe, M.; Hara, M.; Arai, S.; Makino, T. *Jpn. Kokai Tokkyo Koho* **1988**, *7*. Steiner, A. M.; Fuchs, H. *Seed Sci. Technol.* **1987**, *15* (3), 707.
(9) Bruninx, E.; Rudstam, G. *Nucl. Instrum. Methods* **1961**, *13*, 131.
(10) McNeal, C. J.; Macfarlane, R. D.; Thurston, E. L. *Anal. Chem.* **1979**, *51*, 2036. Sundqvist, B.; Macfarlane, R. D. *Mass Spectrom. Rev.* **1985**, *4*, 421. Chait, B. T.; Field, F. H. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 420. Roepstorff, P. *Acc. Chem. Res.* **1989**, *22*, 421.
(11) (a) Dole, M.; Mach, R. L.; Hines, R. L.; Mobley, R. C.; Ferguson, L. P.; Alice, M. B. *J. Chem. Phys.* **1968**, *49*, 2240. (b) Mach, L. L.; Kralik, P.; Rhende, A.; Dole, M. *J. Chem. Phys.* **1970**, *52*, 4977.
(12) (a) Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. *Anal. Chem.* **1985**, *57*, 675. (b) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F. *Mass Spectrom. Rev.* **1990**, *9*, 37.
(13) Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882.
(14) Ikonomou, M. G.; Blades, A. T.; Kebarle, P. *Anal. Chem.* **1990**, *62*, 957.
(15) (a) Chowdhury, S. K.; Katta, V.; Chait, B. T. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 81–87. (b) Chowdhury, S. K.; Katta, V.; Chait, B. T. *NATO ASI Ser*; Standing, K. G., Ed.; Plenum Press: New York, 1991, in press.
(16) Henry, K. D.; Williams, E. R.; Wang, B. H.; McLafferty, F. W.; Shabanowitz, J.; Hunt, D. F. *Proc. Natl. Sci. U.S.A.* **1989**, *86*, 9075.
(17) Covey, T. R.; Bonner, R. F.; Shushan, B. I.; Henion, J. D. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 249.
(18) Smith, D. P. H. *IEEE Trans. Ind. Appl.* **1986**, *IA-22*, 527.
(19) (a) Hayati, I.; Bailey, A. I.; Tadros, Th. F. *Nature* **1986**, *319*, 41. (b) Hayati, I.; Bailey, A. I.; Tadros, Th. F. *J. Colloid Interface Sci.* **1987**, *117*, 205. (c) Hayati, I.; Bailey, A. I.; Tadros, Th. F. *J. Colloid Interface Sci.* **1987**, *117*, 222.
(20) Sample, S. B.; Bollini, R. *J. Colloid Interface Sci.* **1972**, *41*, 185.
(21) Drozin, V. G. *J. Colloid Sci.* **1955**, *10*, 158.
(22) Luttgens, U.; Rollgen, F. W.; Cook, K. D. The 38th ASMS Conference Proceedings, 1990, 132.
(23) Thomson, B. A.; Iribarne, J. V. *J. Chem. Phys.* **1970**, *71*, 4451.
(24) Bruins, A. P.; Covey, T. R.; Henion, J. D. *Anal. Chem.* **1987**, *59*, 2642; **1988**, *60*, 1948.
(25) England, S.; Seifter, S. In *Methods Enzymol.* **1990**, *182*, 285.
(26) Chowdhury, S. K.; Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 9012.

(27) Jardine, I. *Nature* **1990**, *345*, 747.
(28) (a) Christophorou, L. G. *Adv. Electron. Electron Phys.* **1978**, *46*, 55. (b) Chen, C. L.; Chantry, P. J. *J. Chem. Phys.* **1979**, *71*, 3897. (c) Grimsrud, E. P.; Chowdhury, S.; Kebarle, P. *J. Chem. Phys.* **1985**, *83*, 1059.
(29) Lin, S.-S. *Rev. Sci. Instrum.* **1973**, *44*, 516.
(30) Searcy, J. Q.; Fenn, J. B. *J. Chem. Phys.* **1974**, *61*, 5282.
(31) Beuhler, R. I.; Friedman, L. *J. Chem. Phys.* **1982**, *77*, 2549.
(32) Dreyfuss, D.; Wachman, H. Y. *J. Chem. Phys.* **1982**, *76*, 2031.
(33) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Itacha, NY, 1960; p 469.
(34) Kassner, J. L., Jr.; Hagen, D. E. *J. Chem. Phys.* **1976**, *64*, 1860.
(35) Holland, P. M.; Castleman, A. W., Jr. *J. Chem. Phys.* **1980**, *72*, 5984. Yang, X.; Castleman, A. W., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 6845.

RECEIVED for review January 18, 1991. Accepted April 26, 1991. This work was supported in part by Grants RR00862 and RR07065, Division of Research Resources, NIH, and Grant GM38274, National Institutes of General Medical Sciences, NIH.