ELECTROSPRAY WINGS FOR MOLECULAR ELEPHANTS

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by

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The grant from NSF in 1960 started us on the road that led to Stockholm in 2002. Co-Principal Investigator, Michel Boudart was then hosting Jacques Deckers, a young Ph.D. who had done his doctoral research on ions in flames with Van Tiggelen at the University of Louvain. Jacques had had experience with vacuum systems and was very excited about our beam project so Michel turned him over to my tender love and care, (or me to his, I'm not sure which!). With Bob Drake and Michel looking over our shoulders we began designing an apparatus. One of the lessons from the experiments of the Kistiakowsky and Becker groups was the need for lots of pumping speed. When the salesman from NRC showed us the performance characteristics of its 32 inch (in diameter) oil diffusion pumps, Jacques' enthusiasm overcame my reticence (or timidity) so the main chamber of our system became a long horizontal tank resting on and evacuated by two of those big pumps. In retrospect I think that in choosing such big pumps as the heart of our system we were either much wiser or more fortunate than we knew. Those who work with vacuum systems learn early that there is no such thing as too much pumping speed. Sooner or later they always wish they had more. It's nice not to have to worry about the vapor pressure of finger prints, even nicer to be able to sustain disasters. We arrived one Monday morning to find the apparatus completely filled with some 700 gallons of water from a failed cooling coil! We drained the system and easily decanted the water from the silicone pump oil. After cleaning, the interior surfaces of the pumps, pipes and chamber, all of which were made of mild steel, sported a beautifully uniform beige coat of rust! We then closed up the chamber and ran the forepumps for a couple of days, passing a small flow of air through the system to help dry things out. With fingers crossed we then started the diffusion pumps and got the best vacuum we had ever obtained! Figure 1 shows a picture of that apparatus after it was moved to Yale.

Meanwhile Dame Fortune had smiled at me in another way. Not long after the beam project started, Professor Soo, who had been teaching thermodynamics to the mechanical engineers, sudden1y announced he would leave at the end of the first semester. Bob Drake, not knowing I had barely passed the

Figure 1. The "big" molecular beam system, with two "32" diffusions pumps and one "16" Jet Booster Pump, after it was moved from Princeton to Yale. The people, from left to right: S.P. Venkateshan and S. B. Ryali, both postdocs from India.

only course I had taken in the subject, asked me if I would accept a temporary appointment as a lecturer and teach the sophomore course on thermodynamics in the second semester. After a deep breath and a small prayer I said yes. Things must have gone pretty well because at the end of the term he asked if I would consider a permanent position on the faculty. All of a sudden I had the chance to realize what I had long hoped for, the opportunity to become a first class citizen in an academic community. Moreover, because my appointment as SQUID's director was "with rank of professor" I automatically became an instant full professor of Mechanical Engineering, a transmutation that would be much more difficult in today's world. I also continued to run SQUID until 1962 when, by mutual agreement of all parties, its headquarters moved to the University of Virginia where John Scott became director.

After an extensive effort in design and construction we finally assembled the molecular beam apparatus and got it working well. The first meaningful experiment was by Bob Koros, one of Michel Boudart's graduate students. He measured the sticking coefficient of water molecules on ice at liquid nitrogen temperatures. The simplicity and success of the experimental design were strong evidence of both the achievability and utility of high beam intensities from free jet sources. Bob simply suspended a small target from a calibrated helical spring of glass and measured the time-dependent extension of the spring with a cathetometer viewing the target through a window in the vacuum system. Nearby baffles, filled with liquid nitrogen, maintained the target at low temperature. The beam flux of water molecules, from a source containing at water vapor at pressure of a few torr, was sufficiently intense to de-
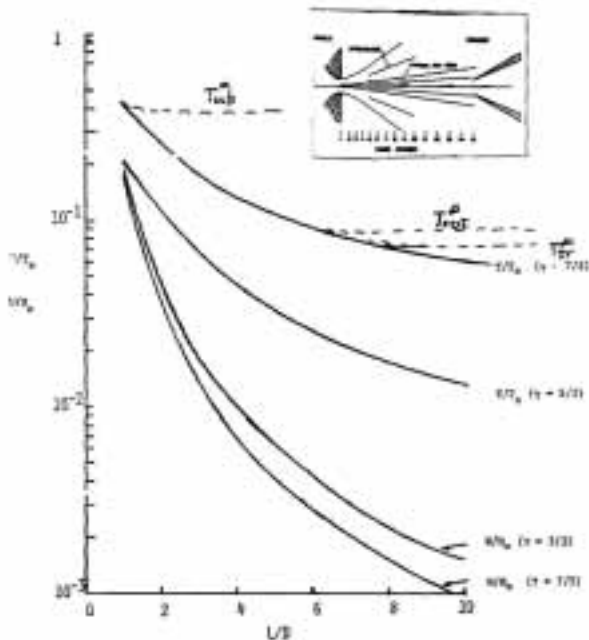
Figure 2. The axial distribution of gas properties in a supersonic free jet, normalized by their source values. The abscissa distance is in terms of nozzle diameters. The top two solid curves show the temperature distribution for a monatomic and a diatomic gas diatomic gas with rotational and vibrational degrees of freedom in equilibrim. Note that because the exchange of energy between translation and vibration is very slow, i.e. requires many collisions per quantum, the "vibrational temperature" (for a diatomic gas like nitrogen). "freezes" much earlier in the expansion than does the rotatational temperature. The bottom pair of curves show the axial distribution of gas density, also for a monatomic and a diatomic gas.

posit in a few hours enough mass of ice to extend the calibrated spring by measurable amounts. In a subsequent analogous experiment Jim Anderson, another Boudart student, found that oxygen molecules incident on a heated germanium wafer reacted to form a volatile GeO molecule. Above a threshold value that I've forgotten, the reaction probability of 0.04, was independent of the target temperature and the intensity of the incident beam. In that case we used the spring balance to measured the loss of mass in the germanium wafer. These preliminary experiments clearly demonstrated the utility of the apparatus whose design and performance were described and characterized in a 1963 paper [12].

Over the next four years at Princeton and the following twenty at Yale this equipment gave rise to some 65 papers. We confirmed the high intensities of beam molecules predicted by Kantrowitz and Grey and first realized by Becker and Bier. Moreover, our hope that the seeding technique could produce beams of molecules with supra thermal translational energies was abundantly realized by ourselves and others [13a]. Figure 2 shows some examples of the acceleration and the energies we obtained. Compargue and his group in France have since achieved translational energies as high as 45 eV.[13b]

The net result has been that studies of molecule-molecule and molecule-surface interactions, by ourselves and others, reached new levels of energy, utility and sophistication.

An originally unanticipated virtue of free jet expansions was their ability to produce and maintain populations of molecules under non-equilibrium but steady-state conditions which some investigators have characterized as "new states of matter!"[14] For present purposes it will be useful to show the distribution of conditions encountered by molecules of a gas undergoing a free jet expansion from a simple orifice into vacuum. Such expansions are "self-similar" in that they scale in terms of the orifice diameter. That is to say, at a distance of any particular number of diameters along the jet axis, the *equilibrium* state of a particular gas will be the same, no matter what the diameter of the jet may be. Thus, at 5 mm downstream from an orifice one mm in diameter the equilibrium state of the jet gas is the same as in a jet of the same gas at 5 m downstream from an orifice l m in diameter. As it happens, however, the expansion rate from small diameter orifices is so fast that the collision-induced processes and reactions involved in maintaining equilibrium in the jet gas may not be rapid enough for the gas to reach equilibrium before the gas density gets so low that the collision induced processes are arrested. The net result is that the terminal state of the jet gas may be far from equilibrium. This ability of free jet expansions from small nozzles to produce steady state conditions in gases that are far removed from equilibrium is one of the reasons that these jets have proved to be such interesting research tools.

Figure 2 shows the axial distribution of density (N/N_0) and temperature (T/T_0) in supersonic free jets from a monatomic and a diatomic gas where subscript "o" refers to the value in the source when the gas is at rest. The curves show the values first obtained by Owen and Thornhill from solutions of Euler's equation for monatomic species (Cp/Cv = 5/3) and for diatomic gases with rotational (but no vibrational) degrees of freedom (Cp/Cv = 7/5) [15]. We had no means of measuring the gas density "N" but we could and did obtain values of the centerline temperature at any point by locating the skimmer at that point, and "chopping" the extracted beam into pulses with a rotating shutter. The signal-vs-time output from a fast response detector located at a carefully measured distance downstream from the shutter was readily transformed into a velocity distribution and thus the translational temperature of the jet molecules at the skimmer entrance. From such velocity analysis at varying values of nozzle-skimmer distance we determined the distribution of translational temperatures of jet molecules along the jet axis. For monatomic and diatomic gases, e.g. argon and nitrogen, the experimental values fell right on the theoretical curves out to distances at which the jet gas densities became so low that the collision frequency was not high enough to maintain equilibrium. Because the jet expansion is adiabatic, and because vibrational relaxation times for many species are much longer than the sojourn time of the molecules in the jet, the vibrational energies for such molecules remain "frozen" at near the source values. Thus, as shown by a dashed line in Figure 2, the vibrational temperature for nitrogen "freezes" at an L/D value
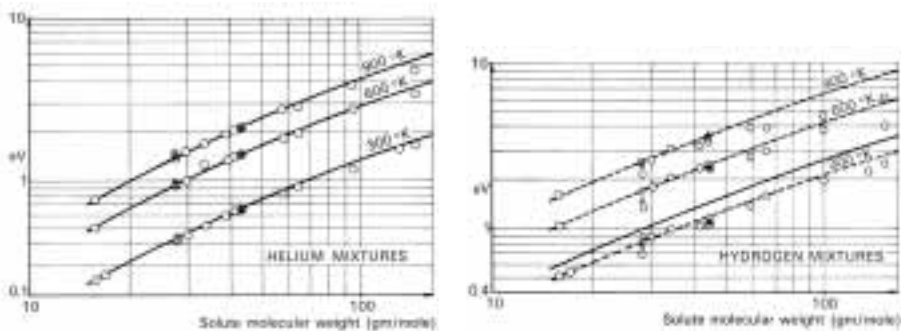
Figure 3. Some results on the acceleration of heavy molecules by a carrier gas comprising helium in 3a and hydrogen in 3b. Note that, as is clear in 3b for hydrogen, with increasing difference in molecular weight between seed and carrier species there can be appreciable slip resulting in lower velocities for the seed species than would be the case if the velocities of the two species remained in velocity equilibrium. The dotted lines in 3b assume no relaxation of hydrogen's rotational energy. The solid line at 300 K shows what would have happened if there were no slip and the rotational and translational "temperature" had remained in equililbrium. In Dole's experiments the differences in molecular weight between seed and carrier species were much greater than for any mixture in Figure 3. We estimate that the actual velocities of some of his large polymer molecules were as much as 40 per cent less than he thought they were.

of only 1.7 because in a single collision between two nitrogen molecules the probability of transferring a single quantum of vibrational energy into translational energy is only 1/5000. For rotational energy that probability doesn't "freeze" until an L/D value of about 6 is reached. Finally, even translational temperature becomes frozen at an L/D value of about 7.5, after with the flow is essentially collisionless. By means of an energy balance on the jet molecules it is thus possible to determine the relaxation rates for rotational degrees of freedom in polyatomic molecules.[16] It turns out that this approach is extremely effective for measurement of such rates at much higher gas temperatures than can be accommodated in the widely used instruments based measurements of the velocity of sound. In the present context the important result of our extensive studies of free jet expansion by velocity analysis is that the translational temperature distributions obtained analytically by Owen and Thornhill and shown in Figure 2 have been experimentally confirmed by ourselves and others. It will emerge in what follows that these findings played a crucial role in the development of Electrospray Ionization Mass Spectrometry.

Having developed the ability to measure velocity distributions at any point in the jet we, and subsequently others, were able to verify our hope that heavy molecules seeded at low levels into a light carrier gas could be accelerated to high velocities and thus high translational energy. [13a]. Figure 3 shows some early examples of the acceleration and the energies we obtained. Compargue and his group in France have since achieved translational energies as high as 8 eV [13b]. An important result of this seeding technique has been that studies of molecule-molecule and molecule-surface interactions, by ourselves and others, reached new levels of energy, utility and sophistication.

Free jet expansions have also revolutionized the optical spectroscopy of polyatomic molecules. Our studies showed that such expansions cool rotational degrees of freedom without affecting the vibrational modes. Thus, by performing absorption or emission spectroscopy on polyatomic molecules in a free jet, one can eliminate the rotational broadening of vibrational lines. Unfortunately, we were not knowledgeable enough to realize the spectroscopic implications of rotational cooling so it was Wharton, Smalley and Levy who provided the earliest demonstration of this effect, obtaining the first ever spectrum of NO_2 in which the vibrational lines could be clearly distinguished and identified without rotational broadening.[17] Now the free jets used in optical spectroscopy far outnumber those used as molecular beam sources in scattering experiments!

Because they can produce extremely low temperatures in gases at fairly high densities, free jet expansions can bring about supersaturation of almost any gaseous mixture for very short periods of time. Thus they have become sources of a wide variety of uncommon species such as HeBr and ArI and all kinds of aggregates, pure and mixed, even including helium. Consequently, by 1984 free jet expansions had began to play what has turned out to be a major role in the rapid development of so-called "cluster science and technology".[18] Indeed, the Scoles group at Princeton {19} and later the Toennies group at Goettingen [20] have found remarkable effects in the emission spectra of species contained in clusters of superfluid liquid helium where the absence of viscosity means that atoms can rotate freely!

In 1967, for a variety of reasons, I decided to accept an invitation to join the Chemical Engineering group in a newly organized "Department of Engineering and Applied Science" at Yale. The "beam machine" was dismantled in Princeton and reassembled at Yale in a somewhat different configuration. The picture in Figure 1 was in fact taken at Yale. The very large size of the apparatus had turned out to provide an unexpected dividend. Because our published results had many interesting implications in chemistry, many chemists visited our laboratory in both Princeton and New Haven. Frequently intimidated by the very size of our apparatus, they would listen to our story and say "Very nice, but not for us." The net result was that for a long time we enjoyed a relative absence of competition! In time, of course, we and others learned how to achieve similar results with much smaller and more compact gear so that free jet expansions have now became very commonplace tools in departments of chemistry and physics as well as engineering. It is perhaps appropriate to note that these supersonic free jets have played an important role in research that has helped at least five other investigators win Nobel prizes in Chemistry! [21]

In 1968, the year after I arrived at Yale, Malcolm Dole published his now famous paper describing his attempt to measure the molecular weights of oligomers of synthetic polymers based on electrospray ionization.[22] He chose to look at polystyrene molecules because relatively monodisperse samples were readily available over a wide range of molecular weights. That paper caught the eye of Professor Seymour (Sandy) Lipsky in Yale's Medical School.

With characteristic enthusiasm Sandy showed it to Csaba Horvath, one of my new Yale colleagues in Chemical Engineering who was working with Sandy on HPLC, then known as "High Pressure Liquid Chromatography." Sandy, who was also a mass spectrometrist, was quite excited because he thought that Dole's approach might make possible the mass spectrometric analysis of proteins and other complex molecules of biochemical importance. This cherished but elusive goal had long seemed a nearly impossible dream because all the then common methods for ionizing any atom or molecule depended upon an appropriately energetic gas phase encounter between a neutral molecule and and electron, photon or ion of some other atom or molecule. But large polyatomic molecules, especially the complex and fragile species of biological interest, could not be vaporized without extensive fragmentation and decomposition. With characteristic enthusiasm Sandy showed Dole's paper to Csaba. Noting the references to our Princeton papers Csaba told him that the John Fenn of those references had just moved to Yale so Sandy showed the paper to me. Always looking for new ways to apply our free jet technology I was readily seduced by Dole's results and Sandy's enthusiasm. Thus began a friendship with Sandy that lasted until his death and a romance with mass spectrometry that will doubtless last until mine. That romance led to the now popular *Electrospray Ionization (ESI)* which burst into prominence at about the same time as the other now most widely used method for ionizing large non-volatile molecules, *Matrix Assisted Laser Desorption Ionization (MALDI),* began to show its potential. A brief history of each of these techniques will be summarized in the next two sections of this report. MALDI's story will be told first even though ESI's was the first to begin.

IONIZATION BASED ON "ENERGY-SUDDEN" METHODS

One class of techniques for producing intact ions from complex and non-volatile molecules was embodied in what might be called the "energy sudden" approach based on an idea originally proposed in 1974 by Beuhler *et al.* [23]. On the grounds of rate theory for unimolecular decomposition they argued that sufficiently rapid heating could vaporize complex molecules before they had time to decompose. In some proof-of-principle experiments on the vaporization of small peptides they showed that decomposition indeed decreased with increasing heating rates but they never were able to heat rapidly enough to eliminate substantial fragmentation. The several techniques based on this idea include so-called "Pyrolysis Mass Spectrometry" (PMS) [24], pre-MALDI versions of Laser Desorption (LD) [25], Fast Atom Bombardment (FAB) [26], Fast Ion Bombardment, (usually called SIMS for "Secondary Ionization Mass Spectrometry) [27], in which rapid energy addition is achieved by incidence of high energy atoms, ions, or photons and Plasma Desorption (PD) [28] in which the sudden energy comes from the decay of a radioactive isotope, usually Californium (^{252}Cf). Introduced by MacFarlane and Torgerson PD was the first of these techniques to gain appreciable use.[28] Its name stems from the idea that the energy released by the nuclear

disintegration formed a small blob of plasma on the sample-bearing surface. As many as 30 or more analyte ions are ejected from that plasma when the analyte is dispersed in an appropriate matrix, typically nitrocellulose. The ions are then accelerated through a drift region so that their masses can be determined by time of flight (TOF) analysis for which a convenient zero-time marker is provided by back-scattered decay products of the Cf disintegration.

All these "Energy Sudden" methods depend on the nearly instantaneous achievement of high-energy density in a sample dispersed in or on a solid or liquid surface. Thus they can be regarded as extensions of the rapid heating idea of Beuhler *et al*. They all could produce intact ions from many complex molecules including peptides and small proteins. However, their procedures were complex and awkward, and because their processes were highly irreversible the yields of intact ions were very low. Even so, during the 70's and 80's they provided substantial accumulations of mass spectral data on a variety of complex, non-volatile molecules, giving rise to a literature much too large for review or comment here.

By far the most important legacy of these energy-sudden methods is so-called Matrix Assisted Laser Desorption Ionization or MALDI introduced in 1987 by Tanaka (29) and expanded in 1988 by Karas and Hillenkamp [30]. In MALDI the analyte molecules are dispersed on a surface in a thin layer of matrix, usually an organic acid. The energy of an incident pulse of laser photons is absorbed mostly by the matrix to form a jet of matrix vapor that lifts analyte molecules from the surface and by mechanisms still not well understood, transforms some of them into ions that are mostly singly charged. Because those ions are all produced at a well defined location in an exceedingly short time their mass analysis is most effectively achieved by Time-Of-Flight methods, as in the case of PD. MALDI is one of the two ionization methods for biomolecules that promise to dominate the MS scene for the foreseeable future. Recently MALDI-TOF in vacuo has been supplemented by MALDI at atmospheric pressure with subsequent introduction of the resulting ions into any of several types of mass analyzers.

IONIZATION BASED ON INTENSE ELECTRIC FIELDS

In 1966, when Dole first had the idea that led to his 1968 paper, there was little evidence of any useful solution to the problem of vaporizing large polar molecules. However, in 1951 the first step had been taken along one of two paths that ultimately led to successful "soft" ionization methods for complex molecules, though it was not fully appreciated at the time. In that year Mueller discovered that when a sharp metal point was at a sufficiently high voltage in vacuo, the electric field at the tip of that point could be intense enough to extract an electron from a nearby gaseous atom or molecule to form a positive ion [31], a discovery that led to his development of Field Ion Microscopy (32). The first use of such points as sources of ions for mass analysis was reported three years later by Inghram and Gomer [33], but only with gaseous molecules. The ion currents were too small to stir up much in-

terest but they were larger than could be accounted for by the flux of molecules from the ambient gas into the region occupied by the point. Beckey then suggested that this apparent excess in ion formation rate could be accounted for by molecules that struck the surface of the wire at some distance from its tip and then arrived at the tip region by surface diffusion. In 1963 he showed that arrays of "whiskers" on thin wires could produce much higher currents than single needles and that non-volatile species present on the surfaces of such whiskers could migrate to the tip by surface diffusion to be desorbed as intact ions. Beckey went on to develop this discovery into "Field Desorption Ionization Mass Spectrometry" (FDIMS) and in 1977 published a book on the subject [34]. Emitter electrodes comprising wires with dense arrays of whiskers are now available off the shelf at relatively low cost but dosing them with sample and positioning them in the vacuum system are time-consuming and tedious tasks. Moreover, ion currents depend so strongly upon both the temperature of the emitter and the applied voltage that substantial manual skill is required to find and maintain the right combination for a particular sample. That control function might now be made much less tedious by computers but solving the control problem does not deal with an even more important difficulty. The ions from field desorption are released from high voltage into high vacuum. Consequently, their kinetic energies are so high that their mass analysis required relatively large and expensive magnetic sector instruments. For these and other reasons FDIMS a la Beckey has never been widely practiced. However, from the perspective of history's hindsight one can argue that the ability of intense fields at the surface of a tiny charged droplet, which play a vital role in Dole's ESI, is simply an obvious extension of the discoveries by Mueller and Beckey on the nature of Field Ionization at a sharp tip, i.e. a surface with a very small radius of curvature. Maybe so, but it is also quite clear that nobody recognized these possibilities at the time, and that Dole arrived at the ESI approach by a logic that depended in no way on the field ionization ideas of Mueller and Beckey. In other words Dole was a true pioneer in that his ESI ideas were breaking brand new ground.

Fields intense enough to desorb solute ions at useful rates from a condensed phase can also be achieved without the array of very sharp needles or "whiskers" required by Beckey's approach. In 1972 Evans and Hendricks found that a high voltage applied to the surface of a non-volatile conducting liquid in vacuo would produce a sharply pointed cone [35]. The field at the tip of such a cone was intense enough to desorb charge-bearing molecules and clusters of the liquid. If the liquid was a solution, the desorbed ions included solute species. These observations led to so-called "Electrohydrodynamic Ionization" (EHI} that was introduced to the mass spectrometry community in 1974 by Simons *et al.* [36]. In EHI a sample solution is electrosprayed from a small bore tube maintained at high potential relative to the surroundings, just as had been taught by Zeleny and adopted by Dole. The difference is that in Dole's ESI the droplets are dispersed into gas at near atmospheric pressure. In EHI the sample solution is dispersed into vacuum. In that case the solvent must have a very low vapor pressure in order to avoid "freeze dry-

ing". But as Dole had recognized, evaporation of solvent is the *sine qua non* for the formation of solute ions from charged droplets and such evaporation requires a source of enthalpy. This dilemma has never been completely resolved in EHI because no satisfactory method has yet been found by which the enthalpy needed to vaporize solvent from a droplet can be supplied to such a droplet in vacuo. Moreover, as in the case of FDI, any ions produced by EHI in vacuo have such high kinetic energies that their mass analysis generally requires magnetic sector instruments which are large, heavy and expensive. For these reasons EHIMS has never become widely used despite fairly extensive research that has been reviewed in detail by Cook [37].

In sum, Malcolm's Dole's paper in 1968 was the seed which, after an extended period of germination, ultimately blossomed into Electrospray Ionization (ESI), one of the two "soft" ionization methods that have made the precision, sensitivity and elegance of mass spectrometry readily available for the study of biomolecules and their reactions. The germination and development of Dole's ESI will be briefly described in what follows.

ELECTROSPRAY IONIZATION

The other of the two dominant methods for ionizing large molecules is Electrospray Ionization (ESI), the subject of the 1968 paper by Malcolm Dole and his colleagues [22] that Sandy Lipsky showed me in 1969. In fact Dole had first proposed his electrospray idea two years earlier (1966) at the International Symposium on Macromolecular Chemistry in Tokyo. Those two communications comprise a testament to Dole's remarkable acumen in realizing that the production and behaviour of highly charged droplets from solutions of non-volatile solute species in volatile solvents might produce intact gaseous ions of those species. Perhaps even more remarkable was the extent of his insight on the roles of ESI's several component processes. For example, he clearly recognized the need to disperse the charged droplets in a gas at relatively high (atmospheric) pressure. Such gas would provide the enthalpy required to vaporize solvent from those highly charged droplets. Moreover, collisions of the ions with molecules of that gas would reduce their initially high kinetic energies to those of the ambient neutral gas molecules, thereby avoiding the need for mass analyzers capable of accommodating high energy ions, e.g. magnetic sector instruments.

As most mass spectrometrists now know, the essence of Dole's idea was to take advantage of what happens during the evaporation of solvent from a droplet that has a net electric charge. In 1882 Rayleigh had analyzed the behaviour to be expected of such a droplet and found that as the solvent evaporated the density of charges on the droplet surface would increase to a critical value, now known as the "Rayleigh Limit", at which Coulomb repulsion would overcome surface tension. The resulting instability would cause the droplet to break up into a plurality of offspring droplets [38]. Thirty years later John Zeleny made the first reported observation of this "Rayleigh Instability" in pioneering experimental studies on the production and behaviour of
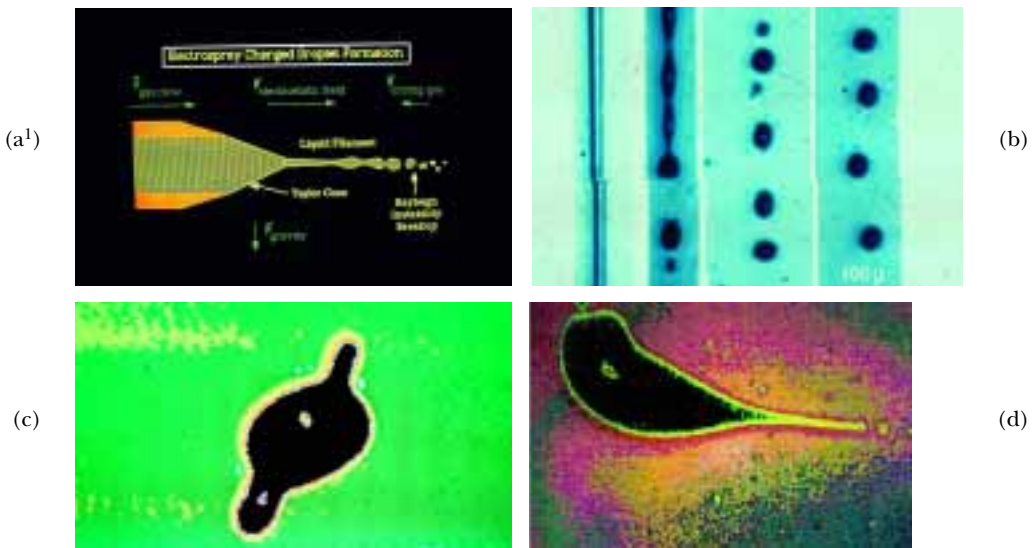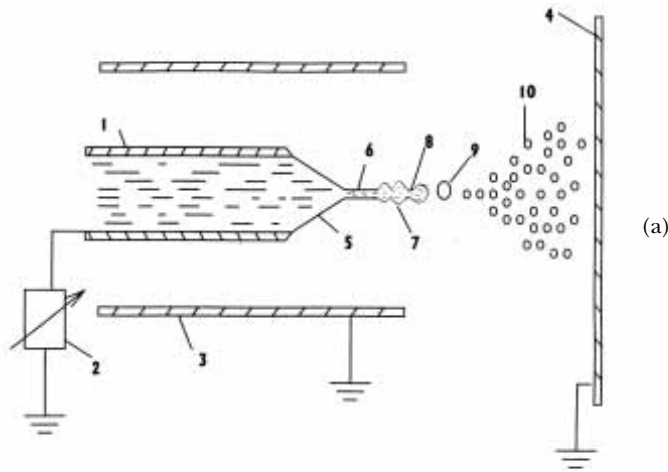
(a)

(a¹)

(b)

(c)

(d)

Figure 4. How a flow of conducting polar liquid is dispersed into highly charged droplets by an intense electric field at the exit tip of a small tube at high potential relative to an opposing electrode. Upper left hand panel *a* show schematically the conical meniscus formed by the emerging liquid as the result of competition between the surface tension of the liquid and forces due to the interaction of dipoles in the liquid with the applied field. This meniscus is known as a "Taylor Cone" because it was first characterized theoretically by G.I.Taylor. [41] When the liquid is electrically conducting, a small flow of the liquid emerges from the tip of the cone as a very narrow column or jet. Interaction between surface tension and viscosity produces so-called varicose waves which grow in amplitude until they truncate the jet into a sequence of segments of equal length that are molded by surface tension into droplets nearly uniform in size.[42] Because the droplets contain excess cations or anions on their surface, depending on the polarity of the field, they repel each other with the result that their trajectories diverge to form a conical "electrospray" of charged droplets. This sequence was captured in the photographs by Gomez and Tang shown in adjacent upper right hand panel *b.* If the liquid is volatile, evaporation shrinks the droplet thereby bringing the surface charges closer together until the resulting Coulomb repulsion overcomes surface tension and the droplets disrupt into a plurality of smaller droplets, a phenomenon characterized and predicted theoretically by Lord Raleigh in 1882. Two years more than a century later, Gomez and Tang captured on film, for the first time, the images in the lower two panels *c* and *d* showing a droplet in the throes of what is now known as a Rayleigh Instability, and sometimes referred to as a Coulomb explosion.
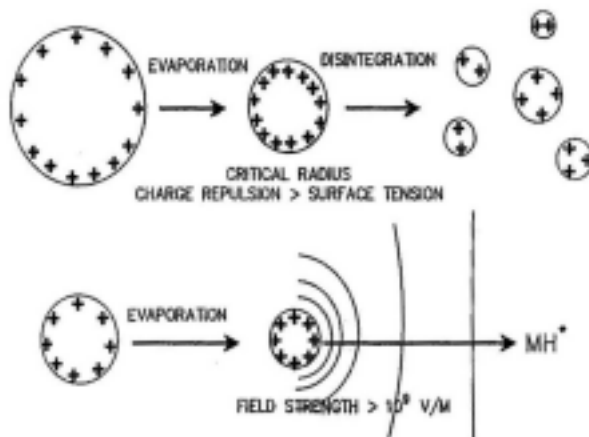
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Figure 5. The upper sequence of sketches attempts to portray Dole's idea of how solute ions are formed. He assumed that the offspring droplets resulting from a first Rayleigh Instability would continue to evaporate solvent so that they too would undergo a Rayleigh Instability and disrupt. If the original solution were sufficiently dilute, a sequence of such evaporation-disruption episodes would ultimately produce ultimate droplets so small that each would contain only one solute molecule. As the last of the solvent evaporated from the ultimate droplet the remaining solute molecules would retain some of the droplet charge and thus become a free gas phase ion. This scenario embodies what is sometimes referred to as the "Charged Residue Model" (CRM) for ES ion formation. The lower sequence of sketches portrays the so-called "Ion Evaporation Model" scenario later proposed by Iribarne and Thomson (46). It argues that before ultimate droplets containing only one solute molecules are formed, the field at a droplet's surface becomes sufficiently intense to lift a solute ion from the droplet surface into the ambient gas.

charged droplets carried out, first at the University of Minnesota and then at Yale [39]. He passed a stream of conducting volatile liquid through a small bore thin walled tube or "needle" (because it often comprised a short length of hypodermic needle tubing) maintained at a high potential relative to an opposing counter electrode. The resulting intense electric field at the tube tip dispersed the emerging liquid into a fine spray of charged droplets. Zeleny was able to see the break-up of charged droplets as solvent evaporated, just as Rayleigh had predicted. Figure 4a is a schematic representation of how the droplets are formed. The emerging liquid at the tube exit forms a conical meniscus now known as a "Taylor Cone" because its formation was predicted theoretically in 1964 by G. I. Taylor [41]. In truth, Taylor's treatment applied to a non-conducting liquid of neutral molecules with dipole moments for which there is no electric current and no liquid flow. Even so, the Taylor name is still applied even when the liquid is conductor because it contains anions and cations. In that case a thin jet emerges from the tip of the cone. As the result of another kind of instability, also first characterized by Rayleigh for uncharged liquids [42], the interaction between viscosity and surface tension produces so-called "varicose waves" on the surface of such a liquid jet. Those waves grow in amplitude until they truncate the jet into a series of uniform droplets as illustrated in Figure 4b. (That same kind of instability causes the break-up into droplets of a solid jet of water issuing from a

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garden hose or, on a smaller scale, the tiny stream of water from a tap that is incompletely turned off.) In the electrospray case the droplets all have excess charges of the same sign, (anions or cations, depending on the polarity of the applied field). Coulomb repulsion thus results in a divergence of their trajectories to form a conical "electrospray" of charged droplets. Figure 4b is a series of four photographs made by Tang and Gomez [40] illustrating, from left to right, the undisturbed jet, the appearance and growth of the varicose waves, the resulting droplets, and the beginning of the divergence of the droplet trajectories (due to Coulomb repulsion} to form a conical spray. We believe Figures 4c and 4d by Tang and Gomez are of historic significance as the first ever photographs showing droplets undergoing a Rayleigh instability when the Coulomb repulsion of the surface charges overcomes the surface tension that tries to contain the droplet liquid in a spherical shape.

Dole's scenario further assumed that the "offspring" droplets resulting from a Rayleigh instability would continue to evaporate until they too would reach the Rayleigh limit and break up into still smaller droplets. If the original solution were sufficiently dilute, a succession of such Rayleigh instabilities would ultimately produce droplets so small that each one would contain only one solute molecule. That lone molecule would retain some of its droplet's charge to become a free gas phase ion as the last of the solvent evaporated. The upper sequence in Figure 5 attempts to illustrate this process. (The lower sequence illustrates an alternative proposal by Iribarne and Thomson which will be described later.)

In the 1968 paper that described this scenario Dole also presented preliminary experimental results obtained in an attempt to reduce his idea to practice. As taught by Zeleny, a solution of polystyrene molecules was infused through a small bore tube maintained at high potential relative to a counter electrode. Dole was always generous and conscientious in acknowledging the work of others so it is curious that though he refers to Rayleigh's analysis of droplet instability he never mentioned Zeleny in any of his papers, even though his method of producing charged droplets seems to derive directly from Zeleny's experiments. His account of the electrospray experiments in his autobiography simply says: "I got this idea from learning about the electrospraying of paint on to automobile bodies while working as a consultant for a paint company in Chicago" [43]. Such "electrospraying" of paint consisted in maintaining a potential difference between the sprayer and the object being painted. The result was enough charge on the paint droplets to attract them toward the object being painted, thereby decreasing substantially the loss of paint to the surroundings by convection currents. Similar charging of droplets was practiced by "crop dusting" airplanes to decrease aggregation and increase adhesion of insecticides to the plants.

But Dole had another problem to solve. The mass spectrometers then available, even the most expensive magnetic sector instruments, were unable to "weigh" singly charged ions with masses larger than about ten thousand Daltons. The oligomer ions of interest to Dole could have molecular weights up to a million or more and he had apparently not taken into account the
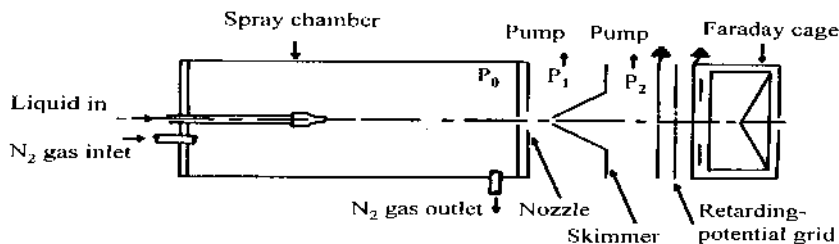
Figure 6. Schematic representation of Dole's original apparatus. The electrospray forms at the tip of the tube through which liquid enters the spray chamber concurrent with the nitrogen bath gas. Most of that gas leaves the chamber near the end plate containing the nozzle through which a small portion of the mixture of bath gas and ions enters the vacuum system in the form of a supersonic free jet. A small fraction of the ion-bearing jet gas passes though a skimmer into a second vacuum stage, thence through a retarding-potential grid into a Faraday cage connected to a galvanometer.

possibility of extensive multiple charging which then had not yet been discovered. The problem he thus faced was how to "weigh" such large ions after they had been produced. Moreover, as he also mentioned in his autobiography, he had found that the Magnetic Electron Multiplier (MEM) on his Bendix mass spectrometer did not respond to his ES ions because "Although these ions had the same kinetic energy as light ions their velocity decreased in the ratio of the square root of their mass. We proved the mass limitation of the MEM in a separate experiment." [44] That comment strongly suggests that the ions Dole had produced from polystyrene oligomers did not have extensive multiple charging. Therefore, their mass/charge ratios were too high for available analyzers so he had to find another method for "weighing" them. That is how our free jet studies entered the picture.

As noted earlier, we had shown that during such free jet expansions of mixtures comprising a very dilute solution of heavy "seed" molecules in a light carrier gas. e.g. argon in helium, the heavy seed species were accelerated like dust particles in a windstorm to almost the velocity that would be reached by the light carrier gas alone [13]. Consequently, the translational kinetic energy of the argon molecules after expansion was higher than would be achieved in a jet of pure argon by a factor roughly equal to the ratio of the molecular weights of the two species, i.e. 10 in the case of argon accelerated by helium. This acceleration effect was important to Dole because it seemed to provide a way to weigh the large polymer ions he hoped to produce. As shown in the sketch of his apparatus in Figure 6 (from his 1968 paper) a variable potential grid was placed between the skimmer (that passed a collimated, ion-containing beam from the free jet source into a vacuum chamber) and a sensitive galvanometer in a Faraday cage contained in that chamber. The only ions that could pass through the grid and reach the galvanometer were those with a translational kinetic energy $(mv^2/2)$ exceeding the product of the charge on those ions and the potential on the grid. The translational velocity v of the stopped ions was equal to the readily calculated velocity reached by the carrier gas in the free jet. Thus, scanning the voltage applied to the grid should
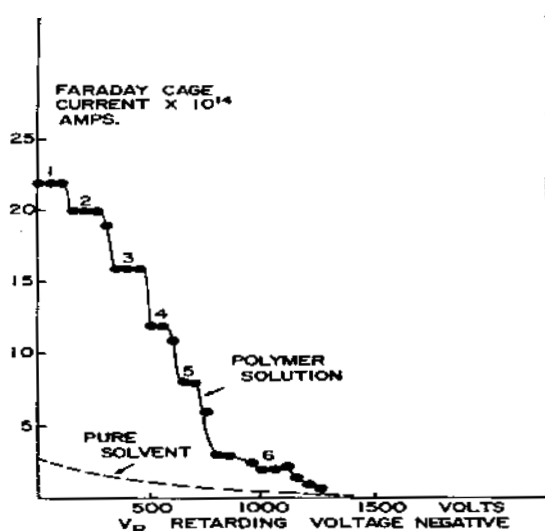
Figure 7. A current-voltage curve obtained by Dole when the electrosprayed solution comprised 0.005 wt per cent each of polystyrene samples having a nominal molecular weight of 51,000. The solvent comprised 3 parts of benzene to 2 parts of acetone by volume. The distances from the needle to the first aperture, the first aperture to the second aperture, and the second aperture to the nozzle were respectively 2, 3, and 3 inches.

produce a current-voltage curve with a decrease in current to the galvanometer at every value of the voltage corresponding to the energy of an ion species in the beam. Figure 7 shows the curve Dole obtained with a fractionated sample of polystyrene having a nominal Mr of 51,000 at a concentration of 0.01 % by weight in 60:40 (v:v) benzene:acetone. Also reported were results with polystyrene having a nominal Mr of 411,000 and with a mixture of the two. These results showed a certain consistency with expectations but, possibly for reasons to be described, were not convincing enough to persuade other investigators to confirm or extend them.

ELECTROSPRAY IONIZATION AT YALE

Early Efforts
A year or so after Sandy showed me Dole's paper I persuaded a new graduate student, Mike Labowsky, to repeat Dole's experiments in our much bigger vacuum system with much faster pumps than Dole had. We also had a better understanding of free jet expansions and realized that Dole had overlooked two of their features which were crucial in his experiments: (1) For the very large differences in molecular weight between the carrier gas and the seed species that he was using, there would have been a substantial amount of "slip" as suggested by the results in Figure 2. Thus, the actual velocities of his heaviest ions could have been as much as 50 per cent lower than he had reckoned. (2) During the adiabatic expansion that occurs in a free jet the tem-

perature of the gas nose-dives. In the case of nitrogen, for example, at an axial distance downstream from the orifice of only ten nozzle diameters (ca 1 mm in Dole's experiments) the absolute temperature of the gas drops to about five per cent or less of its source value! Thus, the expanding gas rapidly becomes supersaturated with solvent vapor resulting from the evaporation of the ES droplets before the expansion. Ions are famously effective as condensation nuclei so that any ES ions in Dole's experiments must have been substantially resolvated to an unknown extent. Consequently, the actual masses of the unsolvated ions must have been much lower than Dole's measurements had indicated. In order to avoid such resolution problems Mike modified the Dole arrangement so that (with reference to Figure 2) the bath gas of dry nitrogen was introduced at the exit-nozzle end of the spray chamber. Consequently, drying gas flowed "upstream", counter-current to the flux of charged droplets and ions from the spray needle, leaving the spray chamber through an exit port at its "entrance end", i.e. opposite to the end containing the nozzle leading into the vacuum system. Thus the solvent vapor from the evaporating droplets was carried out of the system so that the gas expanding into vacuum through the nozzle was free of solvent and any other "junk" that was not ionized. This counter-current flow of bath gas solved the problem of ion resolution during the free jet expansion, greatly reduced system fouling, and has become a feature of the most widely used ESIMS systems. It will be described later in more detail. One can also avoid resolution of ions during expansion by raising the temperature of the ion gas mixture to a value high enough so that after free jet expansion the gas temperature remains above "the dew point" for the ions. This "fix" was demonstrated by Chowdhury *et al.* [45] and has been incorporated in some commercial instruments. It has had limited use because, unlike a countercurrent gas flow, it does not prevent uncharged "junk" from entering the vacuum system. Much more frequent cleaning is therefore required.

As already mentioned, Dole's paper apparently failed to persuade other investigators to confirm his experiments which though simple in principle were really very difficult and demanding in practice. For example, large macroions incident on the first dynode of a multiplier do not produce secondary electrons unless their incident velocities are very high, either because of multiple charging or high accelerating voltages. As implied by reference 21b it seems highly likely that Dole's ions did not have many charges. Consequently, in his later experiments he used mobility measurements to characterize his macroions.
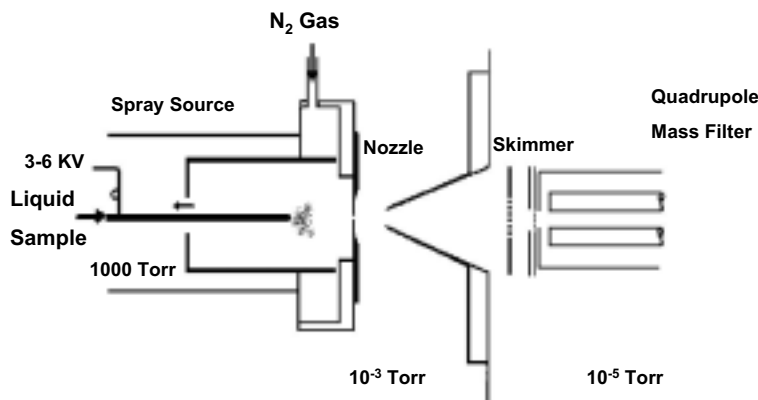
In 1969 at the age of 66, just two years before mandatory retirement at Northwestern, Dole had accepted an offer to become Welch Professor of Chemistry at Baylor University in Waco, Texas where he was assured there would be no mandatory retirement as there was at Northwestern. At Baylor he continued his electrospray experiments using mobility measurements rather than mass analysis to characterize the ions. He presented a short paper on that work at the 33rd Annual Meeting of American Society of Mass Spectrometery (ASMS) in San Diego in 1985 where I also gave a paper that

included a discussion of the cooling and resultant resolvation that occurs in free jets. Dole came up to me afterward to introduce himself and to express his gratitude for finally understanding why there didn't seem to be any reasonable relation in his experiments between the size of the molecules and the mobilities of their ES ions. He said "Now I realize that we have been measuring the mobilities of highly solvated ions!" That was our only face-to-face encounter but we subsequently exchanged several letters and had a cordial relationship. I was delighted when he was later able to attend the first ASMS Workshop on ESIMS which was in Chicago. I didn't see him there because I had not been invited but by that time the "electrospray revolution" was well on its way so Dole had the satisfaction of realizing how fruitful his ideas had become before he passed on to his ultimate reward some months later. He would have been amazed if he had known that by 2001 the number of papers *per year* on ESI in the archival journals would reach 1500 and still be growing!

There was a hiatus in ESI studies at Yale after 1975 when, for understandable reasons, Mike Labowsky got discouraged. The retarding potential method of determining ion mass was most exasperating. Our only available means for measuring the very small ion currents was an old vibrating-reed electrometer that on its best days was crankier than a model T Ford! Mike became interested in the evaporation behaviour of the cloud of ES droplets and thus in the evaporation and combustion of a cloud of interacting fuel droplets, an important and challenging practical problem of interest to several of my colleagues in Chemical Engineering. He developed some unique computational techniques which together several important papers on this classic problem comprised his PhD thesis.

In 1982, Masamichi Yamashita, a young scientist I had met in Japan, came to my lab as a postdoc. When he arrived, "Gado" as he preferred to be called, asked for some suggestions about what he might work on. I mentioned that it might be interesting to take another look at ESI, but instead of trying to make ions out of big molecules, to explore whether Dole's idea would work with molecules of low enough mass for their ions to be analyzed with a small quadrupole mass spectrometer that had been a detector in some of our molecular beam experiments. It had an upper limit for mass/charge ratio of only 500 or so. Gado liked the idea and off he went. Not only was he an extraordinary experimentalist, with sound instincts and magic hands, he knew everything from the biology of sea urchins to the practice of digital electronics (already in 1982!). In a relatively short time he assembled what was really the world's first ESI Mass Spectrometer from the bits and pieces of molecular beam apparatus in our precious "junk pile". (My Scottish heritage of "Waste not, want not" makes it very difficult for me to throw anything away that might have some conceivable use!)

That first apparatus is shown schematically in Figure 8 and illustrates the arrangement for providing a counter-current flow of drying gas. We first tried electrospraying methanol-water mixtures and obtained spectra showing peaks corresponding, for example, to protons solvated by various combinations and numbers of methanol and water molecules that could be varied widely simply

N₂ Gas

Spray Source

Nozzle

Skimmer

Quadrupole
Mass Filter

3-6 KV

Liquid

Sample

1000 Torr

10^{-3} Torr

10^{-5} Torr

Figure 8. The first electrospray mass spectrometer built at Yale. Sample solution was sprayed from the hypodermic needle into a counter-current flow of dry nitrogen. The needle was at high potential relative to the cylindrical electrode and the end plate containing the orifice into the vacuum system. A center portion of the resulting free jet passed through the skimmer into a second vacuum chamber containing a quadrupole analyzer.

by adjusting the flow rate and/or temperature of the counter current drying gas. We then added some solutes to the solvent and were rewarded with the spectrum shown Figure 9a. It was obtained with a solution of several tetra-alkyl ammonium (and one phosphonium) halides at concentrations of a few parts per million. These results were especially gratifying because those solutes cannot be vaporized without extensive, even catastrophic decomposition. Beginning to get excited, Gado dissolved a vitamin B tablet in methanol-water and obtained the spectrum in Figure 9b showing a clearly defined peak for intact ions of every species in that tablet (i.e. with an Mr value under 500.)

The spectra in Figures 9a and 9b were exciting to us because they clearly demonstrated that ESI could produce intact ions of each of several species in a plurality of fragile molecules in a dilute solution while avoiding both fragmentation and interference between them. The spectra also raised second thoughts about the mechanism of ionization. We had been tacitly assuming that Dole's "Charged Residue Model" (CRM) accounted for the formation of the large ions he had found as well as the small ions that we had found in the results just described. In our early experiments, as well as in Dole's, there were always many more analyte (solute) molecules than charges in the ES flux. Consequently, as our results accumulated we had become increasingly uneasy about the adequacy of the CRM. The spectra in Figure 9 heightened that concern because we could not understand how the sequence of Rayleigh instabilities could produce, for each of several species in the same solution, singly charged ultimate droplets containing only one molecule of that species. Moreover, if the CRM model is to explain the results, the relative number of those droplets for each species would have had to be in direct proportion to the relative concentration of that species! How could the droplet know how to program its sequence of Rayleigh instabilities so as to produce a distribution of ultimate droplets such that the number of those ultimate
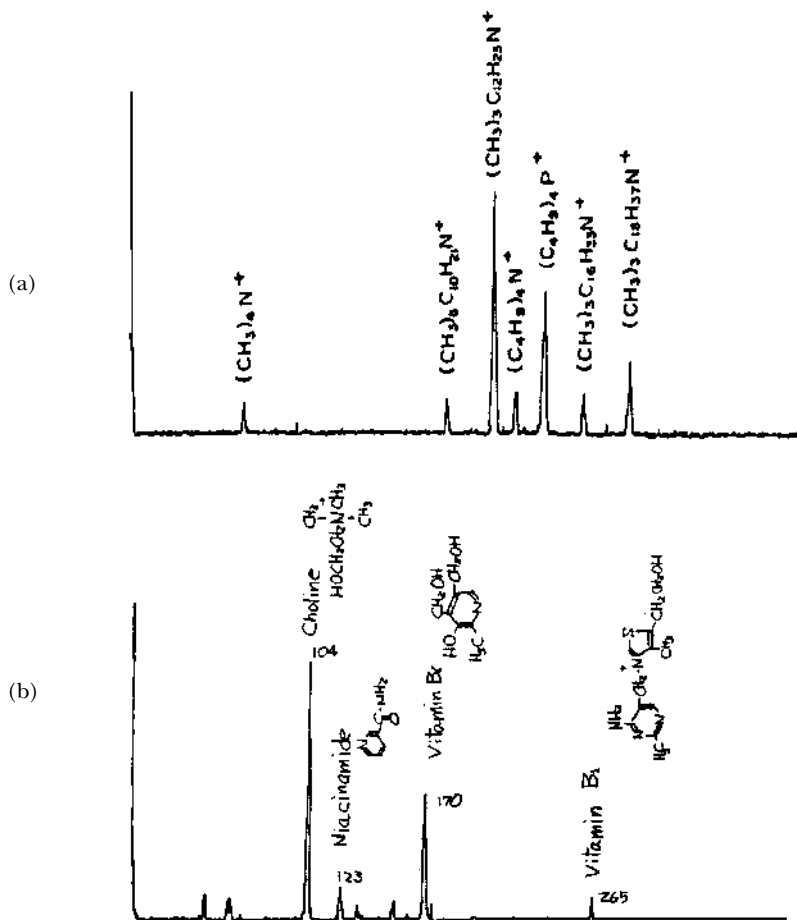
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Figure 9. (a) The mass spectrum obtained with the apparatus of Figure 8 with a dilute solution (ppm) of quarternary ammonium halides and one quarternary phosphonium halide in 50-50 methanol water. (b) The mass spectrum obtained with a solution obtained by dissolving a vitamin B tablet in 50-50 methanol water.

droplets containing one molecule of any species x is always directly proportional to the concentration of species x in the original solution? Of course, that would happen if the droplet subdivision continued until all the droplets contained only one solute molecule. But that scenario somehow seems unlikely, especially when rapid evaporation from all droplets is occurring from the start. Moreover, if the subdivision process were to produce ultimate droplets for each ionizable species in a solution, one should then always find a spectral peak for every species in such a solution and that just doesn't happen.

These conceptual problems seemed to be resolved when we learned about some papers by Iribarne and Thomson, meteorologists at the University of Toronto [46]. Interested in the possibility that charged droplets of sea water might be a source of some of the ions found in the atmosphere, they had car-

ried out experiments showing that evaporation of charged droplets could indeed produce gas phase ions of solute species in those droplets. The charged droplets in those experiments were produced by pneumatically nebulizing a conducting liquid. In such nebulization the droplets become charged as a result of statistical fluctuations in the distribution of anions and cations among those droplets so that roughly equal numbers of positively and negatively charged droplets are formed. In their first experiments only aerodynamic forces were used to nebulize the liquid, as in a perfume atomizer. Then they found that 3500 volts applied to an "induction electrode" (a cm away from the nebulizing zone) would result in all the droplets, and the ions from their evaporation, having the same sign, positive or negative, depending on polarity of that electrode. Iribarne and Thomson called their technique "Atmospheric Pressure Ion Evaporation" (APIE) a name which literally refers more to the mechanism of ion formation from charged droplets rather than to the method of producing the droplets. Moreover, both the earlier Electrospray Ionization (ESI) of Dole and the later Thermospray Ionization (TSI) of Marvin Vestal [47], also depend upon vaporization of charged droplets but their names do not identify any ion-forming mechanism. The essential differences between these three "spray" techniques consist mostly in the way the charged droplets are produced. Therefore, it seems more appropriate to give them names that relate to those characteristic differences, i.e. "ESI" for Electrospray Ionization, "ASI" for Aerospray Ionization, and "TSI" for Thermospray Ionization. These names relate specifically to the way droplets are produced in terms of the kind of energy used to produce them, (i.e. electrical, aerodynamic, and thermal) and will be used hereinafter.

The ASI technique of Iribarne and Thomson has never become has as widely used as ESI. However, their Ion Evaporation Mechanism (IEM) for ion formation from charged droplets, set forth in their papers, was a milestone contribution and has become a formidable competitor for Dole's Charged Residue Model (CRM) with each one having its champions. Both models assume that a sequence of evaporation steps followed by Rayleigh instabilities leads to smaller and smaller droplets. In the CRM this sequence continues until the droplets become so small that each one contains a single solute molecule that becomes an ion by retaining some of its droplet's charge as the last of the solvent evaporates. The upper sequence of steps in Figure 5 attempts to illustrate this process. The IEM holds that before the droplets become small enough to contain only one solute molecule, the field strength at the droplet surface becomes intense enough to "lift" a surface ion from the droplet into the ambient gas. In other words the surface field can evaporate an ion from the droplet surface. The lower sequence of steps in Figure 5 attempts to represent this mechanism. There is no unanimity in the ESI community as to which model is correct. The feeling in this corner is that under most circumstances the IEM is more consistent with more experimental observations than is the CRM. However, sometimes the CRM seems more likely to apply, especially in the case of very large analyte molecules.

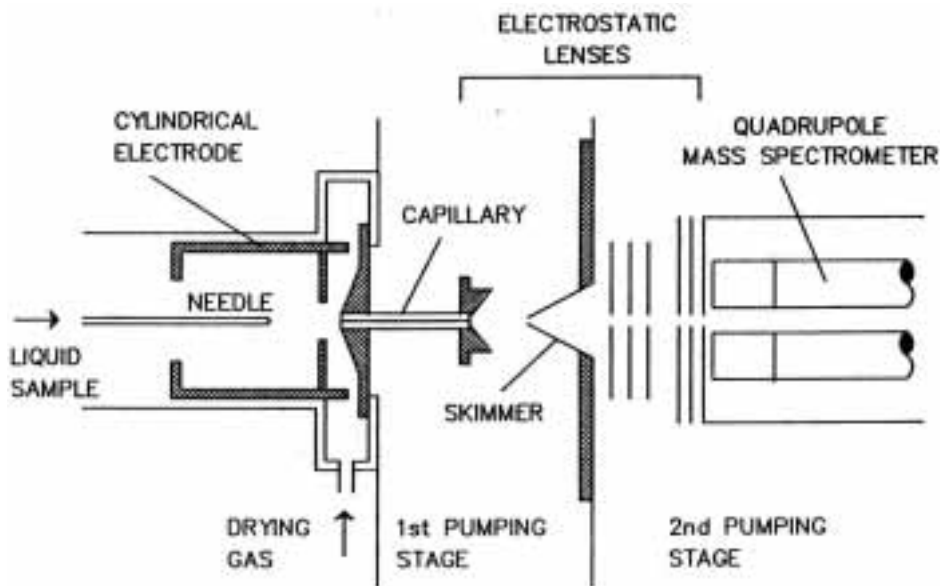A bit more needs to be said about TSI, the Thermospray Ionization

Figure 10a. Schematic diagram of the second ESIMS apparatus. It features the same counter current flow of drying gas shown in Figure 8 which excludes solvent vapor from the ion-gas mixture that enters the vacuum system by way of a free jet expansion. A novel feature of this apparatus is that the ion-gas mixture enters the free jet expansion at the exit of glass capillary 4 rather than through the simple flat-plate orifice of Figure 4. Each end of that capillary is metallized so that it can be maintained at any desired potential. Thus the field required to "electrospray" the sample solution can be achieved with the spray needle at while the metallized entrance of the capillary is at the required potential "below" ground. The ions entering the capillary are then in a potential well. The rapid flow of gas through the capillary then drags the ions out of that potential well to any desired potential at which the metallized exit end of the capillary is maintained. Thus, all external parts of the apparatus can be at ground, posing no hazard to an operator.

Technique introduced by Marvin Vestal and his colleagues beginning in 1978. The last of the charged droplet sources to appear, TSI became the first to be widely used, in part because of its effectiveness and in part because it was the first to become available commercially as an add-on to existing mass spectrometers. This sequence of events would seem to fulfill that passage in the scripture which says "The last shall be first." (That passage continues by saying "And the first shall be last", which apparently is being fulfilled by ESI because it was the first to be introduced and now would seem likely to be the last of the spray techniques to be abandoned, if it ever is!)

TSI consists in passing analyte solution through a capillary tube whose walls are hot enough to vaporize 90 per cent or more of the solvent. The resulting expansion produces the same kind of shear and acceleration forces on the liquid that occur in aerodynamic nebulization, e.g. as in ASI or a perfume atomizer. The result is a dispersion of droplets in vapor that emerges from the exit of the capillary as a supersonic jet into ambient solvent vapor at a pressure of 10 to 15 torr in a chamber whose walls are hot enough to maintain the vapor in a superheated state. The already mentioned statistical fluctuations in
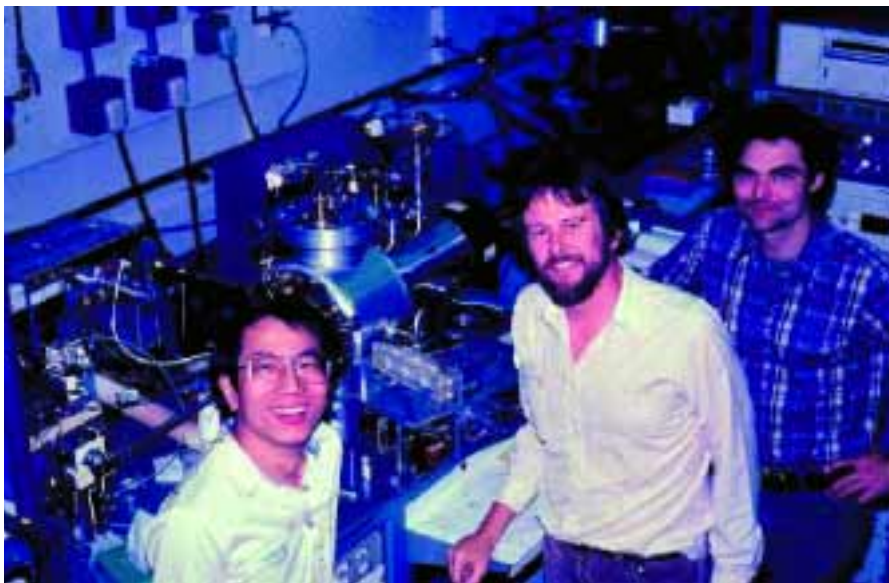
Figure 10b. Photograph of the apparatus of Figure 10a showing (left to right) Chin Kai Meng (graduate student), Robert Dreyer (chromatographer from the Medical School) and Craig Whitehouse, (graduate student who did much of the design and assembly of the instrument).

the distribution of cations and anions give rise to equal numbers of positively and negatively charged droplets as in ASI. Evaporation of the droplets in the superheated vapor brings about the sequence of Rayleigh instabilities common to all the techniques based on charged droplets. That sequence leads to the formation of gaseous ions by either the CRM of Dole or the IEM of Iribarne and Thomson. The mixture of ions in vapor flows past an aperture through which ions of the desired polarity are driven by an applied field into the vacuum chamber housing a mass analyzer. For several years in the 1980's TSI sources became the preferred hyphen in LC-MS, but they have now been almost completely replaced by ESI sources.

Meanwhile we were achieving ever better results with ESI as we climbed the learning curve and Sandy Lipsky was keeping in close touch with our progress. He was a good friend of Brian Green of VG Analytics, a British manufacturer of mass spectrometers that has since evolved into what is now Micromass, Ltd. Brian would stop by New Haven and visit Sandy every few months and on most of those visits Sandy brought him by our laboratory. As our results with small molecules began to get better and better, Brian became more and more intrigued and asked us if the technique would work with larger molecules. We said we thought it might but couldn't be sure because our little quadrupole analyzer had an upper limit of about 500 for mass/charge ratio. Brian then arranged for VG to lend us a used quadrupole analyzer that could weigh ions with mass/charge ratios up to 1500 or so. That instrument was incorporated in a new system for which much of the design and
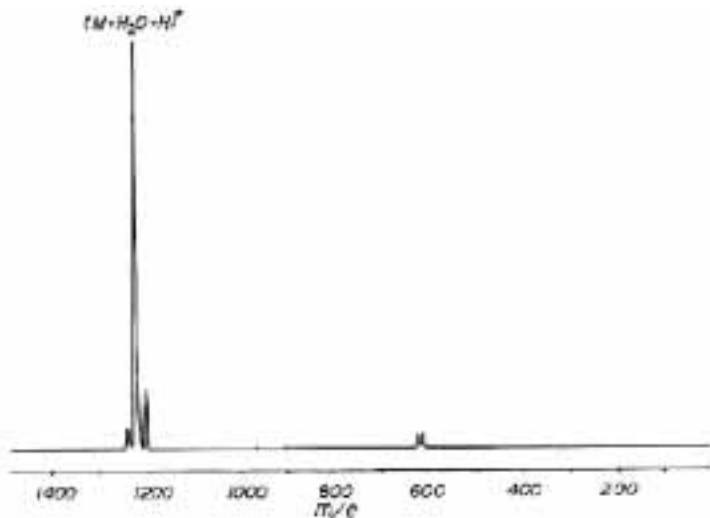
[M+H₂O+H]⁺

1400   1200   1000   800   600   400   200

m/e

Figure 11a shows a mass spectrum obtained with a solution of the cyclic peptide, cyclosporin A, in 50–50 methanol-water. The dominant peak is for a singly charged molecule with one adduct water molecule. The small peak on the right is due to the bare ion. The tiny one on the left is due to the parent molecule with two adduct water molecules. (Because of the "polarity" of the chart recorder in these experiments, m/z values increase from right to left, the reverse of normal practice.

assembly was done by Craig Whitehouse. Craig had worked in Sandy's lab after finishing college and Sandy urged me to take him on for graduate study in Chemical Engineering. The design of this new system is shown schematically in Figure 10a and is pictured in Figure 10b. It has some novel features that deserve comment.

In light of the results obtained with Gado's machine, and with Csaba and Sandy hovering in the background, we increasingly viewed ESI as a possibly more potent hyphen in LC-MS than the already widely used TSI. An electric field sufficiently intense to disperse analyte solution emerging from the spray needle into a fine spray required a potential difference of up to several kilovolts between that tip and the orifice leading into the vacuum system. Thus, one was faced with a trilemma in LC-MS operation. Either (1) the LC system would have to be maintained at several kV above ground, (2) the MS at several kV "below" ground, or (3) the power supply would require enough capacity to maintain the required potential difference in spite of the leak to ground of current through the conducting path comprising the flow of liquid from the LC. Options (1) and (2) were not feasible but option (3) was being used in some systems. We then had a wild idea that turned out to be a very effective solution to this problem. We replaced the simple orifice into the vacuum system by a glass capillary tube as shown in Figure 10a. That glass tube was metallized at each end to provide an electrical contact. The inlet end of the capillary would be maintained at the required potential below ground so the ions entering the tube would be in a potential well. We guessed, correctly as it turned out, that the fairly high velocity flow of bath gas through the glass (di-
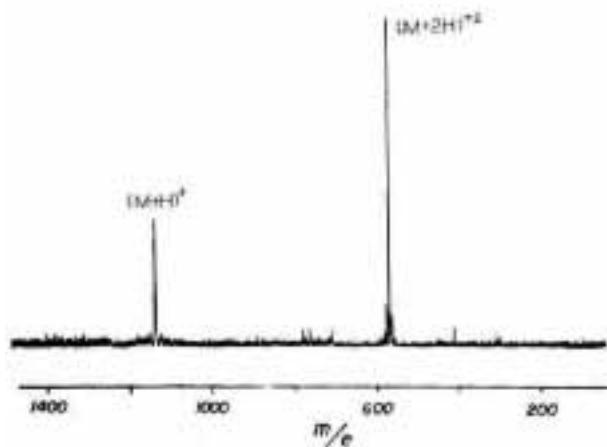
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$[M+2H]^{+2}$

$[M+H]^+$

m/e

Figure 11b shows the spectrum obtained with a solution of gramicidin S, another cyclic peptide with a molecular weight close to that of cyclosporin A.

electric) tube into the vacuum system could drag the ions out of that potential well up to whatever potential might be desired at the metallized exit end of that tube. It worked like a charm. Indeed, the gas flow could raise the ions to an exit potential of several tens of kV, sufficient to inject the ions into a magnetic sector analyzer. At the same time all exposed external parts and surfaces of the instrument could be maintained at ground potential, thereby posing no hazard to an operator.

Eager to flex our muscles with the new apparatus we tried it out with a couple of cyclic peptide samples from friends in the Medical School, Cyclosporin A and Gramicidin S with molecular weights of 1184 and 1141 respectively. Figure 11a shows the spectrum obtained with a solution of l.0 g/L of Cyclosporin A in 85:15 (v:v) acetonitrile:water. The dominant peak at 1203 corresponds to singly protonated parent molecules with one water of hydration. The small satellite peaks on either side are due to ions with one more and one less water molecule. The small triplet peak near mass 600 is from doubly protonated ions of the same species. (The right to left increase in mass/charge ratio is the opposite of custom due a quirk of the available chart recorder.) Figure 11b shows the spectrum for 1.0 g/L of Gramicidin S in 50:50 (v:v) methanol water. Here the peak is much larger for the ions that are doubly rather than singly protonated. These differences can be understood in terms of the IEM mechanism and the greater hydrophobicity of cyclosporin. A molecule of the latter can therefore be more easily removed from an aqueous environment can one of the former. Consequently, one charge can provide enough "lift" to remove the ion from the droplet surface. This observation seems to support the Ion Evaporation Model of Iribarne and Thomson. As the droplet shrinks, the charges get close enough together so that two of them can attach to a single gramicidin molecule thus providing more lift, in part because of the two charges and in part because the surface field is stronger, owing to the higher surface charge density.
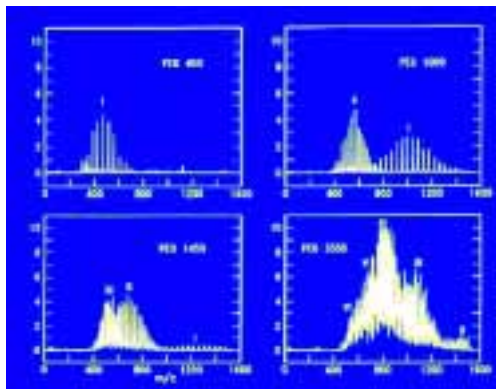
Figure 12a. Spectra obtained with the apparatus of Figure 10a for Poly(ethylene glycol)-oligomers with nominal molecular weights of 400, 1000, 1450 and 3350.

Peaks for doubly charged ions also dominated in similar spectra for the peptides bleomycin and "substance P". In the spectra for renin substrate and insulin chain B we found evidence of triple protonation. This propensity for multiple charging was quite provocative because the effective mass range of any analyzer increases by a factor equal to the number of charges per ion. In order to explore the extent to which such multiple charging can occur, and to identify and evaluate the controlling factors, we carried out an extensive study of ES ion formation with poly (ethylene glycol) (PEG) oligomers. Samples with a range of nominal molecular weights (Mr values) were kindly provided by Union Carbide and Chemical Corp. Each one comprised a mixture of oligomers with a Gaussian-like distribution of Mr's having a FWHM roughly 15 per cent of the nominal Mr of the most abundant oligomer. These species retain their chemical and structural similarity over a wide range of Mr values. It is known that Na^+ ions bind to the oxygen atoms in the oligomer chain with an energy of 2.05 eV and there is always enough sodium around so that spectra of samples from the manufacturer (Union Carbide and Chemical Corp) comprised oligomers with varying numbers of Na^+ adducts that increased with increasing molecular weight. (However, one can change these adducts by appropriate additions to the sample, e.g. to K^+ by adding KOH or KCl). Figure 12a shows mass spectra for PEG samples having nominal Mr values from 400 to 3350 for which the individual peaks are clearly resolvable in the original spectra and correspond to oligomers with varying numbers of Na+ adducts. Figure 12b shows spectra for samples with nominal Mr values of 8000 and 17,500 wherein the peaks are much too close together to be resolved (with our analyzer) because of the superposition of a wide distribution of oligomer sizes on a wide distribution of charge states for each oligomer.[48] A reviewer of that first paper on these results dismissed them outright, saying that the spectra in Figure 12b were "not mass spectra but were due to dirt in the system!" However, we have found over the years that
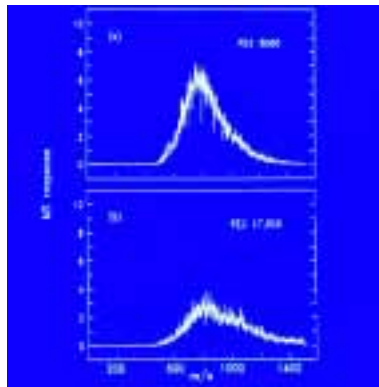
Figure 12b. Spectra obtained with the apparatus of Figure 10a for Poly(ethylene glycol)-oligomers with nominal molecular weights of 8000 and 17,500.

for polymers like these one can correctly and usefully assume that the m/z value at the maximum or peak value of the envelope of the overlapping peaks is a reasonably reliable estimate of the most probable m/z value for the most abundant oligomer in the sample. We used this assumption to great advantage in a later study which showed that intact ions could be obtained from PEG oligomers with Mr values of at least 5,000,000! [49] Others have since shown that ESI can produce intact ions of species with molecular weights of over 100 million! [50].

The message of these PEG experiments was clearly that to study the ESIMS behaviour of large molecules we needed samples of pure compounds in which all the molecules had the same molecular weight. Obvious examples of such compounds are natural peptides and proteins which for many other reasons, of course, are far more interesting and rewarding subjects of study than synthetic polymers. We began to get promising results with such compounds in late 1987 and presented the results shown in Figure 13 in June 1988 at the 36th ASMS Conference on Mass Spectrometry and Allied Topics in San Francisco. Inspection of those spectra shows that each peak in the spectrum for any one species differs from an adjacent peak only by one adduct charge, usually a proton in the case of these peptides and proteins. The first reviewer of a preliminary paper on these results asserted that they showed the method to be worthless for two reasons: (1) The total ion current was divided among so many peaks that the signal/noise ratio for any one peak would inevitably be low and (2) the multiplicity of peaks for each species in a mixture would inevitably make the spectra too congested to be interpreted. As I have often done, and still do, that reviewer underestimated the power of a computer. One day I commented to my then student, Matthias Mann, that each peak in one of these multiple peak spectra was really an independent measure of the parent ion mass. Therefore, there should be some way of averaging those independent values to get a more reliable and accurate measure of the parent
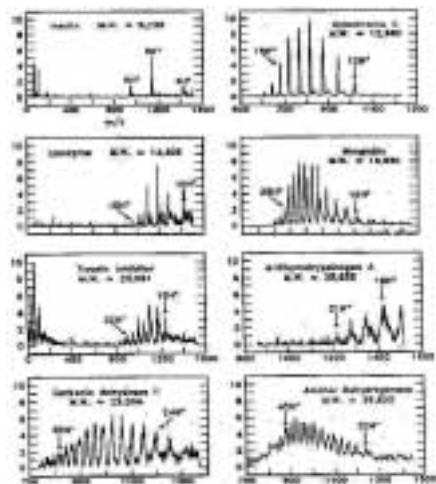
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Figure 13. Spectra obtained with the apparatus of Figure 10a for a variety of protein molecules.

molecule mass than any single peak spectrum could provide. Two days later he had worked out a computer algorithm that transformed the multiple peaks into a single peak that would be obtained if all the ions had a single massless charge. The m/z value for that peak is thus the Mr value for that species. Figure 14 shows an enlargement of the spectrum for cytochrome C in the upper right hand corner of Figure 13. The insert shows the result of the deconvolution by the algorithm Matthias had worked out, i.e. the peak that would be obtained if all the ions had a single massless charge. The m/z value on the abscissa for the apex of that peak is thus the true molecular weight of the parent neutral molecule. It is noteworthy that the scale for the ordinate value, which represents the relative abundance of the ions in any peak, is the same on the insert as in the multi-peak spectrum. Clearly, values for both total signal and signal/noise are much higher in the deconvoluted spectrum than in the parent spectrum as measured. It is now taken for granted that computers can deconvolute what seem to be hopelessly complex mass spectra to obtain precise and accurate Mr values for very large molecules in an extremely complex mixture of species.

However, not everyone was impressed. A reviewer of a reputable journal to which we submitted a manuscript on the results with poly(ethylene glycols), refused to approve publication of the protein results on the grounds that the protein spectra clearly showed that the method was worthless! In the first place the ion currents would be distributed over so many peaks that signal/noise ratios would inevitably be too small. Moreover, the multiplicity of peaks for each species would make the spectrum of any mixture hopelessly complex and uninterpretable! But as I myself have done all too often, that critic underestimated the power of the computer. (In fairness I should admit that the manuscript he saw didn't include any discussion of the algorithm
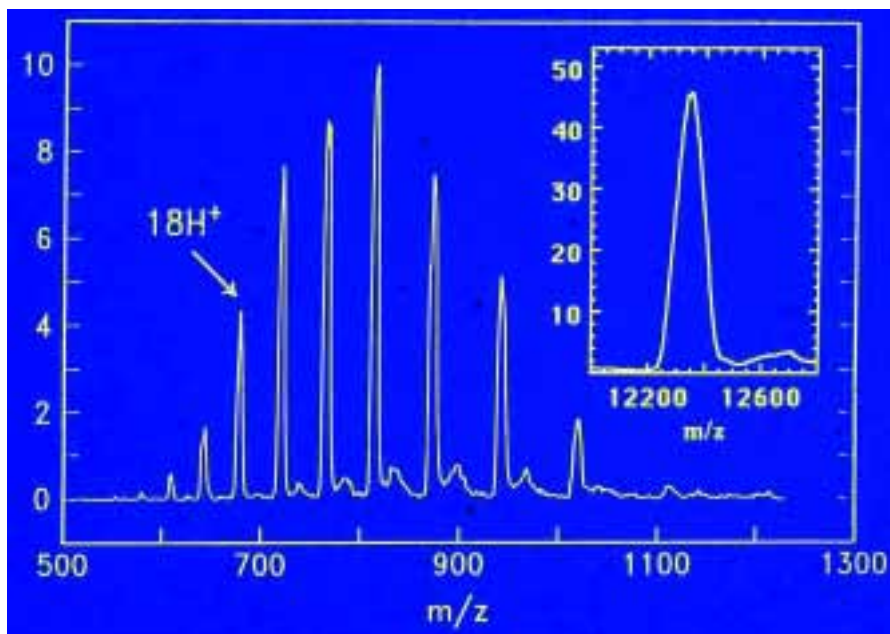
Figure 14. Spectrum for Cytochrome C , i.e. the right hand panel of Figure13. The insert shows the result of applying Matthias Mann's deconvolution algorithm which transforms the multiple peaks of the measured spectrum to what would be obtained if all the ions comprised the parent molecule with a single massless chargwe. Thus the abscissa value of the single peak is the molecular weight of the parent molecule. Note that the ordinate scale of relative abundance is the same for both the measured and deconvoluted spectra. Clearly both the effective signal and the signal/noise ratio are both much higher in the deconvoluted spectrum.

that had not yet then been worked out but the fact that such spectra could be obtained at all was surely worth publication at that time.

Funding agencies were also generally somewhat less than enthusiastic in response to our proposals. For example, when we submitted one for research to extend the results shown in Figures 9a and 9b, the reviewer simply said: "It is impossible for this man to obtain these results with his equipment!" I was reminded of the slogan once promoted by a company which said: "Difficult tasks we can finish quickly. The impossible ones take a little longer!"

There were only a handful of people at the session of the ASMS meeting at which we presented these results. Nor were there many questions or comments from the floor. However, when those results were published in 1989 [51], they began what has become a "flood" of papers and ignited what has been referred to as the "Electrospray Revolution!" Some idea of the dimensions of that flood can be gained from Figure 15 which was prepared by my good friend and former colleague, Prof. Juan de la Mora at Yale. It shows how the publications on ESIMS have been increasing with time. The sudden rise began in 1990, a year or so after we first presented the protein results and reaching over 1500 per year in 2001 and still climbing! (The first complete
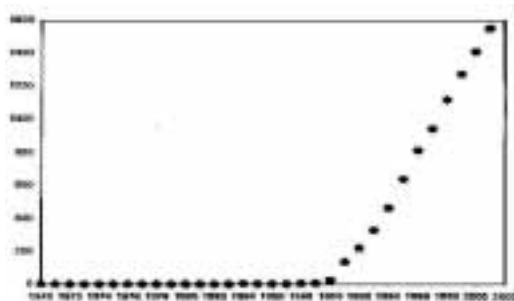
Figure 15. Number of publications on ESIMS from 1970 to 2002.

paper on the method and results appeared in the 10/6/89 issue of Science and is by far the most cited publication that ever came out of our lab (51). Moreover those numbers reflect only a small fraction of the total ESIMS activity, most of which is being carried out by the pharmaceutical companies and is not published.

In view of the flood of papers on ESIMS revealed in Figure 15, even a rudimentary summaray of what they have taught would clearly take much more than a little longer. Consequently, I will stop here with a final word of appreciation to the many who have made possible the preparation of this paper. I would especially emphasize the extent of my gratitude and thanks to my eager and able colleagues without whose efforts I would not be here today. Nor could I be writing this had I not had the support of numerous sponsors who over the years have provided the funds, (albeit sometimes unwittingly!) without which we could have done nothing. They include the Department of Energy, the National Science Foundation, the National Institutes of Health, the Army Research Office, the American Cancer Society, the du Pont Company, and the Jeffress Foundation. Finally, of course, I want to express my eternal gratitude to the Nobel Foundation and its marvelously helpful and gracious staff. They, along with what must have been generous and abundant approval from my peers and colleagues in the fellowship of scientists, have transformed my wildest dreams into a reality even more unbelievable than "flying elephants!"

REFERENCES

1. J. W. Mullen, M. R. Irby, J. B. Fenn, Third Internat'l Symp. on Combustion, Flames, & Explosive Phenomena, Williams and Wilkins, Baltimore (1949).
2. S. Datz and E. H. Taylor, J. Chem. Phys. 25, 395 (1956).
3. E. W. Becker and K. Bier, Z. Naturforsch, A9, 975 (1954).
4. A. Kantrowitz and J. Grey, Rev. Sci. Instrum. 22, 328 (1951).
5. G. B. Kistiakowsky and P. Schlichter, Rev. Sci. Inst. 22, 333 (1951).
6. T. H. Johnson, Nature 113, 745 (1927); Phys. Rev. 31, 103 (19280).

7.   I. Estermann, O. Frisch and O. Stern, Zeit. f. Physik 73, 348,(1931).

8.   J. E. Scott, Jr. and J.E. Drewry, Proc. 3rd. Intern. Symp. Rarefied Gas Dynamics, Paris 1963 Vol.I. p. 516, Academic Press, New York.

9.   Fraser, R.G.J, "Molecular Rays", Macmillan, New York (1931).

10. E. W. Becker, K. Bier, and H. Berghoff, Z. Naturforsch, A10, 565 (1955).

11. H. R. Murphy and D.R. Miller, J. Phys. Chem. 88, 4474 (1984).

12. J. Deckers and J.B. Fenn, Rev. Sci. Inst. 34, 96 (1963.)

13a. N. Abuaf, J. B. Anderson, R.P. Andres, J. B. Fenn, Science 35, 997 (1967); cf. "Molecular Beam Engineering at Intermediate Energies", Entropie 18, 314 (1967).

13b. R. Campargue, J.B. Anderson, J.B. Fenn, B.B. Hamel, E.F. Muntz, J.R. White, in "Nuclear Energy Maturity" (P Zaleski, ed.p. 5, Pergamon Oxford (1975).

14   E. Kolb and D.R. Herschbach, J. Phys. Chem. 88, 4488 (1984).

15. P. L. Owen and C. K. Thornhill, Aero Res. Council (U.K.) R & M No. 2616.

16. R. J. Gallagher, J. Chem. Phys. 60, 3487 (1974).

17. R.E. Smalley, L. Wharton, and D.H. Levy, J. Chem. Phys. 63, 4977 (1975).

18. S. B. Ryali, J.B. Fenn, Ber. Bunsenges. Phys. Chem. 88, 4451 (1984).

19. S. Goyal, D.L. Schutt, G. Scoles, Phys. Rev. Lett. 69, 933 (1992).

20. M. Hartmann, R.E. Miller, J.P. Toennies, A. Vilesov, Phys. Rev. Lett. 75, 1566 (1995).

21. Dudley Herschbach, Yuan Lee and John Polanyi (1986); R.F. Curl, Jr., H.W. Kroto and Richard Smalley (1996); Ahmed Zewail (1999).

22. M. Dole, L.L. Mach, R. L. Hines, R. C. Mobley, R.C. Ferguson, M.B. Alice; J. Chem. Phys. 49, 2240 (1968).

23. R.J. Beuhler, E. Flanigan, L.J. Green, L. Friedman, J. Am. Chem. Soc. 96, 3990 (1974).

24. H.L.C. Meuzelaar, M.A. Posthumus, P.G. Kistemaker, J. Kistemaker, Anal. Chem. 45, 1546 (1973).

25. E. Unsoeld, F. Hillenkamp, R. Nitsche, Analysis, 4, 115 (1976); M.A. Posthumus, . P. G. Kistemaker, Ten Noever de Brauw Meuse, Anal. Chem. 45, 1546 (1973).

26. M. Barber, R.S. Baordolli, G.J. Elliot, R. D. Sedgwick, A.N. Tyler, J. Chem. Soc. Chem. Commun. 325 (1981).

27. A. Benninghoven, D. Jaspers, W. Sichtermann, Appl. Phys. 11, 35 (1976).

28. D.F. Torgerson, AR. P. Skowronski, R. D. Macfarlane, Biochem. Biophys. Res. Commun. 60, 616; B.U.R. Sundquist, R.D. Macfarlane, Mass Specctrom Reviews, 42, 421 (1988).

29. K. H. Tanaka, H. Wake, Y. Ido, S. Akita, Y. Yoshida and I. Yoshida, Rapid Commun. Mass Spectrom. 8, 2, (1988).

30. M. Karas, F. Hillenkamp, Anal. Chem. 60, 2299 (1988).

31. E.W. Mueller, Z. Physik 131, 136 (1951).

32. E.W. Mueller, Electronics and Electron Physics Vol XIII, 83ff Academic Press, N.Y. (1960).

33. M. Ingram and R. Gomer, J. Chem. Phys. 22, 1279 (1954).

34. H.D. Beckey, "Principles of Field Ionization and Field Desorption Mass Spectrometry," Pergamon Press, Oxford, 1977.

35. C.A. Evans, Jr., C.D. Hendricks, Rev. Sci. Instr. 43, 1527 (1972).

36. D.W. Simons, B.N. Colby, C.A. Evans, Jr., Int'l J. Mass Spectrom. Ion Phys. 15, 291 (1974).

37. K.D. Cook, Mass Spectrom. Revs. 5, 467 (1986).

38. Rayleigh, Lord, Phil. Mag. 14, 184 (1882).

39. J. Zeleny, Phys. Rev. 10, 1 (1917).

40. A. Gomez, K. Tang, Phys. Fluids 6, 404 (1994).

4l. G.I. Taylor, Proc. Roy. Soc., A 280, 383 (1964).

42. Rayleigh, Lord, "The Theory of Sound", Chap. XX, Dover, N.Y. (1945).

43. M. Dole, "My Life in the Golden Age of America," P. 169 Vantage Press, NY (1989) 44. ibid. p. 167.

45. S. K. Chowdhury, V. Katta, B. T. Chait, Rapid. Commun. Mass. Spectrom. 1, 53 (1990).

46. J.V. Iribarne, B.A. Thomson, J. Chem. Phys. 64, 2287 (1976).

47. C. R. Blakley, M. J. McAdams, M.L. Vestal, J. Chromatog. 158, 264 (1978); Anal. Chem. 52, 1636 (1980). J. Am. Chem. Soc. 102, 5931 (1980).

48. S.F. Wong, C.K. Meng, J.B. Fenn, J. Phys. Chem. 92, 546 (1988).

49. T. Nohmi, J. B. Fenn, J. Am. Chem, Soc.114, 3241 (1992).

50. S. F. Fuerstenau, J. Mass Spectrom.Soc. Japan 51, 50 (2003).

51. J. B. Fenn, M. Mann, C. K. Meng, S.F. Wong, C.M. Whitehouse, Science 246, 6, 64 (1989).