Electrospray and MALDI Mass Spectrometry

Fundamentals, Instrumentation, Practicalities, and Biological Applications

Second Edition

Edited by
Richard B. Cole

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Foreword: Desorption Ionization and Spray Ionization: Connections and Progress

Mass spectrometry has the advantage of a broad definition. Only in a tangential way is it a form of spectroscopy. Rather, it is the field dealing with the study of a particular state of matter, the gaseous ionic state. This means that much chemistry and even physics and biology falls within its province. Practical applications come with this, driven by advances in instrumentation and techniques; but less obviously, so much fundamental science—kinetics and thermochemistry and reaction mechanisms—also emerges. Nowhere is this range more evident than in the methods and phenomena involved in the conversion of samples into gas-phase ions.

This Foreword starts with a consideration of the driving forces that have led to the elaboration of improved methods of ionization. A high point was reached with the introduction of the electrospray ionization (ESI)[1,2] and matrix-assisted laser desorption ionization (MALDI)[3,4] methods of the mid-1980s for the ionization of biological molecules. These methods are the prototypes of the two broad classes of ionization methods: spray ionization and desorption ionization. The first edition of this book[5] was concerned chiefly with ESI and its applications as developed in the decade after its introduction. The present volume deals with subsequent developments in ESI and also with MALDI. The forces driving improvements in ionization continue to operate, and emerging applications and variations on ESI and MALDI will be evident throughout this volume. In this Foreword, I cover the topics listed below. These are topics that I find fascinating and which appear to have potential to grow in importance in the future. Alternative examples could have been chosen and the astute reader will find many such in this volume.

1. Forces driving the development of ionization methods
2. Spray and desorption ionization
3. Ambient ionization
4. Molecular imaging
5. Enhanced analysis
6. Fundamental science
7. Opportunities and unsolved problems

FORCES DRIVING THE DEVELOPMENT OF IONIZATION METHODS

Over the past 50 years, mass spectrometry (MS) has been repeatedly transformed and its applications extended by new methods of ionization. These methods have had a cumulative effect as each has added to the capabilities available from previous work. The clear importance of the objectives involved, together with the use of striking names and

acronyms, has given ionization methods great visibility, both amongst mass spectrometrists and among the wider scientific public. Earlier ionization methods that received significant attention include field desorption, plasma desorption, molecular ("static") SIMS, laser desorption, fast atom bombardment, and thermospray. Some of these methods are now little known, but all were significant in laying the groundwork for new capabilities.

It is useful to consider the several *desiderata* that drove these developments. The most prominent was the mandate to ionize and so record mass spectra of thermally unstable, nonvolatile compounds of biological origin and of increasing molecular weight. This task has served for several decades as a singularly powerful driving force in mass spectrometry, one that was effectively satisfied by the nearly simultaneous development of MALDI and ESI in the mid-1980s. However, developing mass spectrometric methods for high-molecular-weight compounds has not been the only driving force in the development of ionization methods. An early consideration was that ionization be accompanied by limited and simplified fragmentation. One of the great successes of chemical ionization[6] is the fact that it allows, through choice of the reagent ion, control over the thermochemistry of the ionization reaction and hence over the degree of dissociation seen in the mass spectrum. A third driving force has been the wish to examine aqueous solutions directly, especially after the introduction of aqueous-phase chromatography for biomolecules. The thermospray method[7] satisfied this requirement before being supplanted by the related but far more convenient electrospray method. There are additional sample characteristics and analytical questions that have driven the development of ionization methods. One was the desire to achieve atomic, then molecular, and then biochemical analysis of the surfaces of materials. This problem was first addressed in the 1960s by laser desorption and by secondary ion mass spectrometry (SIMS). Successes were hard won but came in the special cases of sub-monolayers of organics on metals using SIMS[8,9] and with the introduction of early non-UV-active matrices for both SIMS and LD.[10] These early matrices (typically inorganic or organic salts) had dramatic effects on the intensities and quality of mass spectra and they led to the fast atom bombardment, liquid matrix method.[11,12] All this work contributed to the energy-absorbing matrix work embodied in the MALDI method.[3,4] A different type of analytical task that contributed to ionization method development was the desire to achieve depth profiling in solids. The use of polyatomic projectile ions, most notably C_{60}^+, is the most recent contribution to resolving this problem.[13] Elemental and isotopic analyses present other analytical requirements that have driven ionization method development. There are special issues here too which place exceptional demands on ionization methods, including the avoidance of molecular ion formation in elemental analysis and issues of source stability and high ionization efficiency in isotopic analysis. Plasma ionization and glow discharge ionization are the common solutions to the problems of elemental analysis, while high-precision isotopic analysis often employs electron ionization (lower-mass elements) or thermal ionization (higher-mass elements).

The previous paragraph represents a brief survey of the forces that drove the development of ionization methods from around 1965 through the introduction of the commercial ESI and MALDI sources in the late 1980s. Subsequent efforts have been motivated by attempts to (i) increase the throughput of mass spectrometry by decreasing the time and effort taken in sample preparation and ionization, (ii) ionize biological compounds while preserving features of the mass spectra which are associated with higher-order structure and biological activity, not merely with overall mass and charge, (iii) reduce the complexity of ESI spectra by simplifying the charge-state distribution, either during ionization or by subsequent modification of the set of molecular ions that are observed, and (iv) ionize intact clusters, especially protein complexes while retaining the

solution-phase cluster composition and structure. Dealing with each in turn: (i) Successful high-throughput methods in ESI involve miniaturization and automation of standard ESI nanospray ion sources[14,15]; in MALDI they involve automation of the matrix deposition,[16] that is, automated control of the laser position and of the sampling process to find "sweet" spots. (ii) Preservation of features characteristic of the native structures of biomolecules calls for gentle methods of ionization. This can be achieved using versions of ESI in which one starts with very small droplets and uses mild desolvation conditions. The use of an air amplifier[17] or of the high-velocity method of electrosonic spray[18–20] yields small initial droplets and proteins are ionized to give charge-state distributions dominated by a single charge state. These same methods achieve objective (iii) , simplification of the ESI mass spectra. Remarkable progress in the ionization of intact complexes has also been achieved.[21,22]

SPRAY IONIZATION AND DESORPTION IONIZATION

It is convenient to organize the ionization methods used in organic and biological MS into the broad groups shown in Table 1.

These methods are distinguished in various important ways. For example, only one type, electron ionization, completely avoids bimolecular chemical processes, and this is the reason for its intrinsically high reproducibility. EI normally gives radical cations (less commonly, radical anions), and these often fragment too readily and by too-complex routes, even in the small molecules to which EI is applicable. Chemical ionization solves this problem but still imposes the onerous requirement that the sample be vaporized before it can be ionized. By far the largest fraction of all studies on biological compounds is performed using ESI and MALDI. As noted, these are the exemplars of the modern desorption ionization (DI) and the spray ionization (SI) classes of methods. In spray ionization, the sample is introduced as a solution that is nebulized, with the resulting charged droplets being made to undergo desolvation and the resulting gas-phase ions being mass analyzed. In desorption ionization, by contrast, condensed phase samples are examined by the impact of energetic particles. A wide range of such energetic projectiles is available, leading to a rich variety of ionization methods of the DI type.

The desorption ionization event involves the input of sufficient energy to dislodge the analyte from the surface and also to ionize it, if it is not already charged. These requirements

Table 1 Classification of Ionization Methods for Organic Compounds

Method	Abbreviation	Example(s)	Analyte State	Characteristics
Electron ionization	EI	Electron impact; electron capture	Vapor phase	Highly reproducible spectra
Chemical ionization	CI	Chemical ionization	Vapor phase	Control of internal energy and fragmentation
Desorption ionization	DI	SIMS; MALDI	Solid phase	Matrix to protect analyte; singly charged ions
Spray ionization	SI	Thermospray ionization; electrospray ionization	Solution	Multiply charged ions; little fragmentation

call for significant energy input, and this in turn demands that the sample be protected from the direct effects of the energy. This is done using a matrix that is present in great excess (typically 1000-fold) and which transforms the incoming energy into a form suited to gentle desorption of analyte molecules from the surface. Coupling of the incoming energy (whether provided by a translationally excited atom, a UV-photon, or another source) into the internal modes of the locally and temporarily excited surface is a key step in the ablation process that results in desorption. Returning to the two steps involved in desorption ionization, namely desorption or phase change and ionization or charge-state change, one notes that it has long been known[23] that precharged species (e.g., organic cations when the organic compound is in the form of an organic salt) give exceptionally high yields in such DI experiments as LD and SIMS. If the analyte is neutral when it leaves the surface, subsequent gas-phase chemical reactions are needed to yield ions and yields drop correspondingly.

The spray ionization experiments do not require matrix because this is essentially provided by the solvent in which the sample is carried. However, the solvent must be removed, a demanding task if the analyte itself is to be preserved intact. Water, the solvent for biological molecules, is difficult to remove although the evaporation processes in ESI are remarkably successful.

The most striking difference between the desorption and spray ionization methods is the charge state in which the analyte is formed. In the DI experiments, the coulomb energy cost of removing multiply charged ions from surrounding counterions means that spectra are dominated by singly charged ions. In SI experiments, on the other hand, the evaporation of water (typically) from a microdroplet that has ionic constituents (buffer, a non-neutral pH) means for the same reason that the analyte tends to take a number of charges that is roughly in proportion to the size of the molecule. This dramatic difference in charge states has had a profound effect on the most appropriate type of mass analyzer for each type of ionization method. The DI methods demand an instrument with a large mass/charge range, whereas the SI methods yield ions for molecules of the same mass using mass analyzers with much smaller mass/charge ranges. It will be seen below (next section) that some newer DI methods yield highly charged ions.

In both the DI and the SI methods, the amount of internal energy acquired by a molecule during ionization affects the degree of fragmentation, as well as the level of molecular disruption that is not seen as fragmentation but which leads to loss of biological activity through changes in structure. The loss of these structural features is undesirable in the gas-phase ions because it precludes experiments such as H/D exchange to determine the number of active sites in the biomolecule that might have been responsible for biological activity. If biological ions can be generated in the gas phase with their original solution-phase structures, then molecular cross sections can be obtained by ion mobility measurements,[24] thereby providing valuable information on 3D structures and folding.

If one is successful in minimizing the input of internal energy during ionization, then spectra will be dominated by the ionized molecule, e.g. the singly protonated molecule in positive ion MALDI, or the multiply deprotonated molecule in negative ion ESI. The successful generation of a form of ionized molecule which retains the mass of the original condensed-phase molecule allows determination of its molecular weight. Structural characterization normally requires the formation of fragment ions and in both the DI and SI methods this is achieved by activation followed by dissociation. Since both the precursor and product masses are of interest, two stages of mass analysis (i.e., tandem mass spectrometry) are needed. This can be done using the same analyzer after a temporal delay (as in ion traps) or by using two separate mass analyzers. The activation methods

available[25] include the common collisional activation in the electron-volt collision energy range (multiple collisions) or in the kilo-electron-volt range (single collisions) as well as single- or multiphoton activation, surface collisions (surface-induced dissociation), or electron capture or electron transfer activation and dissociation. Note that the electron capture[26] and electron transfer[27,28] processes change the charge state as well as cause internal energy uptake and are suited to structural studies on multiply charged ions. The type of MS/MS experiment[29] selected depends on the type of mass analyzer, and this choice is affected by the ionization method itself, as already noted.

AMBIENT IONIZATION

Recently, it was been shown that an electrospray emitter can be used to provide translationally excited projectiles (charged microdroplets) which serve as projectiles for desorption and ionization of condensed-phase analytes present on surfaces. This hybrid technique, DESI (desorption electrospray ionization),[30] is applicable to analysis of samples in the ambient environment. The practical advantage is that the sample can be examined directly, without any preparation; hence the experiment is extremely fast (typically <5 s) and can be conducted in a high-throughput fashion and tandem mass spectrometry can be used for identification of components of complex mixtures. These features provide the speed of analysis and high chemical specificity that are needed in applications such as public safety monitoring.

The DESI method is placed in the context of conventional and newer vacuum-based desorption ionization methods in Table 2. Consideration of the data on kinetic energy per nucleon makes clear just how gentle the method is. DESI was introduced simultaneously with another ambient ionization method, DART (direct analysis in real time),[31] in which the initial projectile is a metastable atom that arrives at the surface as a water ion. The relationship between these methods and the older method of atmospheric-pressure MALDI (AP-MALDI)[32] is summarized schematically in Figure 1. These new types of experiments illustrate the rapid developments still taking place in ionization methods in mass spectrometry. This reinforces the need for a thorough understanding of the essentials of ionization methodology, such as that provided in this book. Second, it illustrates a merging of the DI and SI methods into new forms that carry the advantages of both. The separate consideration of

Table 2 Velocities, Radii, and Kinetic Energies of Projectiles in Some Desorption Ionization Methods

Method	Projectile Velocity	Projectile Size	Kinetic Energy per Nucleon
DESI	0.1 km/s	~3 μm	0.6 meV/u
Massive cluster ionization (MCI)[a]	5 km/s	~80 μm	100 meV/u
Electrospray droplet impact (EDI)[b]	11 km/s	~20 μm	1 eV/u
Molecular SIMS with C_{60}[c]	10–100 km/s	~1 nm	80–300 eV/u

Source: Adapted from Venter, A.; Sojka, P. E.; Cooks, R. G. Droplet dynamics and ionization mechanisms in desorption electrospray ionization mass spectrometry. *Anal. Chem.* **2006**, *78*, 8549–8555.

[a]Mahoney, J. F.; Perel, J.; Lee, T.D.; Martino, P. A.; Williams, P. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 311–317

[b]Hiraoka, K.; Asakawa, D.; Fujimaki, S.; Takamizawa, A.; Mori, K. *Eur. Phys J. D*, **2006**, *38*, 225–229

[c]Winograd, N. *Anal. Chem.* **2005**, *77*, 143a–149a.

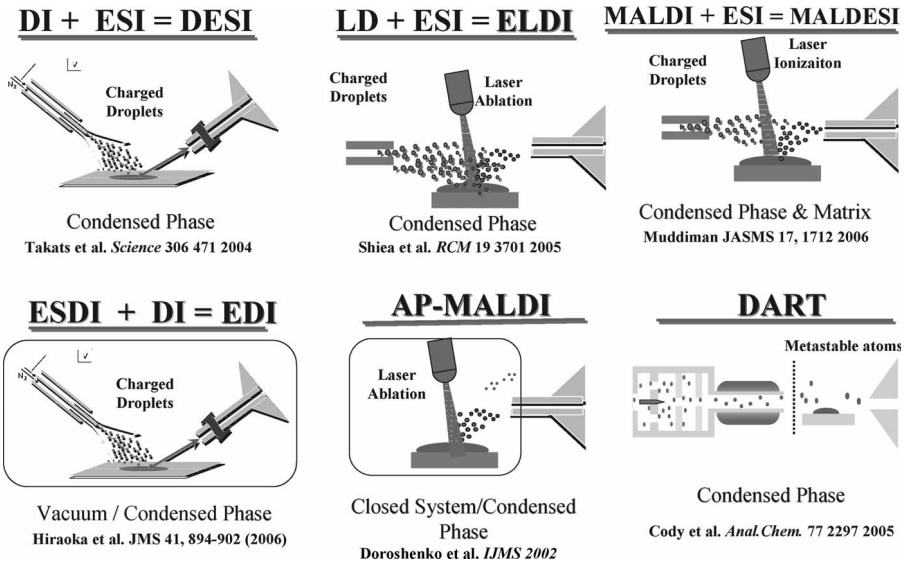


Figure 1. Ambient ionization methods for ionizing condensed phase samples on surfaces using droplets, atom beams, electrical discharges and laser beams. For comparison, some vacuum methods are also shown (See color insert).

these two main classes of ionization method is no longer appropriate, and this edition of Richard Cole's book provides a strong basis for understanding the current literature in both DI and ESI and for contributing to the development of the newer methods that will inevitably follow.

It is noteworthy that chemical reagents can be included in the spray solvent (or gas) in DESI (and related methods) to cause ionization of specific classes of compounds.

MOLECULAR IMAGING

Imaging of materials by mass spectrometry has been an important topic since at least the 1960s, when ion microscopes and ion microprobes were used for spatial elemental analysis. These experiments were based on sputtering in vacuum using energetic (kilo-electron volt) primary ions; that is, they were SIMS experiments. Using primary ion beams of different fluxes, it is possible to sputter without revisiting damage sites in order to perform 2D analysis. By alternating between dynamic and static SIMS, one can "mill" through a surface to perform 3D elemental or molecular analysis. With the introduction of TOF mass analyzers in place of the elegant sectors of the earlier ion microscopes, the spatial resolution of these devices has improved to a few tens of nanometers. At the same time, the use of SIMS to image molecules has improved with the introduction of cluster ion sources. As a result of these developments, TOF SIMS imaging[33] has acquired excellent capabilities in terms of sensitivity and resolution (sub-micron allowing subcellular structural studies), although the quality of the molecular information obtained is limited, in part because there is often significant fragmentation and in part because some instruments do not have MS/MS capabilities. More recently, imaging using laser beams has become a significant topic, especially the MALDI-based experiments.[34] These latter experiments have a spatial resolution that is in the 100 μm range, but they make up for this by providing information

more rapidly and they give mass spectra that provide molecular-structure information (through fragmentation) on high-molecular-weight compounds.

The MS imaging methods are often contrasted favorably with optical methods because they are "label-free." However, in the SIMS method the samples are "fixed," and in MALDI they are treated with matrix; in both experiments the sample is introduced into the vacuum environment for imaging. These requirements are disadvantages that to some extent offset the label-free characteristics of the method. With the development of the ESI-based DESI method has come a new imaging procedure performed on unmodified biological materials in the ambient environment.[35] These advantages offset the still limited spatial resolution of the method (~200 μm). Applications to human tissue have been reported, and the location of cancer margins by the patterns of phospholipids is especially significant. The lack of sample preparation, surface specificity, and MS/MS capabilities of DESI imaging provide advantages. DESI imaging and MALDI imaging appear to be highly complementary methods, with the former ionizing phospholipids particularly effectively and the latter giving higher-quality protein data. The considerable promise of the methods in due course in clinical practice is illustrated by the human liver cancer data shown in Figure 2.

Looking at the current state of imaging MS, MALDI, and DESI, it can be argued that mass resolution is a lower priority than increased chemical information that can be derived through MS/MS experiments and improved molecular interpretation (structure–spectrum correlations). This latter undertaking requires further development of knowledge of ion chemistry, especially for classes of molecules like lipids that have not been thoroughly investigated by mass spectrometry. Complementing this analytical–organic chemistry task are the interlocked bioinformatics tasks of data collection, reduction, and manipulation that are needed to maximize the power of imaging MS. Another requirement in MS imaging is to develop more specific analytical methods—for example, methods in which

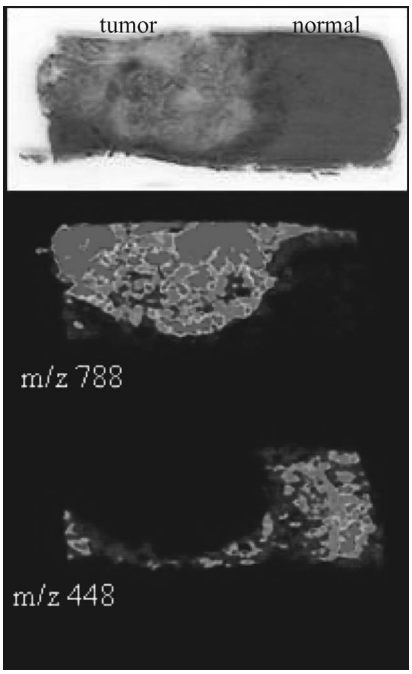


Figure 2. Human liver sample. (**a**) H&E (hematoxylin and eosin) stain. (**b**) Negative ion images of phosphatidylserine (ion of m/z 788). (**c**) Chenodeoxycholic acid (ion of m/z 448). (Unpublished DESI imaging data of Wiseman, Caprioli, Puolitaival, and Cooks; false color intensity; tissue shown is 17 mm by 8 mm by 12 μm.) (See color insert).

images are recorded for particular compounds on the basis of MS/MS signals (product ion or multiple reaction monitoring experiments). Such a front-end capability will reduce the quantity of raw data processed and increase the informing power of the questions asked during an imaging experiment as well as increasing the quality of the quantitative data obtained.

ENHANCED ANALYSIS

The range of applications of ESI and MALDI has been extended to a remarkable degree over the past several years. Extensions are to larger and more complex molecular systems, especially to biological problems of increasing complexity. This progress has been based on technological factors (as is so frequently the case in mass spectrometry), especially improvements in mass analyzers and in methods of ion activation. In regard to mass analyzers, notable developments are FT-ICR instruments of higher field strength and the introduction of the Orbitrap mass spectrometer with its unique nonmagnetic field high-performance mass analyzer. However, most ultra-high-mass work has used simpler systems, including hybrid time-of-flight instruments.

The development of activation methods for MS/MS has seen the extension of the electron capture dissociation (ECD) method from ICR to Paul ion trap instruments, providing users of modest-sized mass spectrometers the advantages of a fragmentation method that is complementary to collision-induced dissociation and, hence, access to high protein/peptide sequence coverage. Electron transfer dissociation (ETD), the more versatile chemical analog of ECD, has rapidly impacted biological analysis since its introduction in 2004.[27,28] This experiment is complementary to the more traditional collision-induced dissociation experiment and is rapidly making a large contribution to increasing the information on post-translational modifications in proteins. Also worthy of note are McLuckey's experiments on charge-changing collisions,[36] experiments that allow the charge state of a collection of ions to be altered to suit the measurement at hand—whether it is a high-accuracy mass measurement or collision-induced dissociation or an ion–molecule reaction. His remarkable "ion parking" experiment,[37,38] based on the strong velocity dependences of charge-changing reaction cross sections, means that trapped ions can be resonantly excited to give them high translational energies, thereby protecting them from reaction. This allows the reversal of the characteristic dispersion of molecules over a range of charge states in ESI so that quantitatively all molecules are "parked" in a chosen charge state. The ability to move at will between charge states—like scale transposition in a musical piece—is sure to have a large impact on mass spectrometry and the development has important implications for top-down proteomics.

Other noteworthy developments in the realm of analytical applications are:

(a) *High-mass Complexes*. Some of the most impressive applications of mass spectrometry over the past decade have been to protein complexes.[21,22] Recently, experiments in which micelles incorporate proteins have yielded to the ESI methodology.[21]

(b) *Inorganic Compounds*. Application of ESI to inorganic complexes and organometallic compounds is generally straightforward and a valuable way of characterizing these classes of compounds. Limitations are met in terms of the reactivity of many of these compounds with air and water, but nonaqueous solutions can be sprayed and MALDI is also useful.

(c) *Chiral Analysis*. The growing importance of chirally pure drug substances has led to great emphasis on enantiomeric control in synthesis and to increased demands for chiral analytical methods. This latter need has been satisfied in large part by chiral stationary phases for liquid chromatography. However, mass spectrometry and especially ESI can be used for chiral analysis.[39] One experiment involves generating a cluster ion involving a selected chiral reference (R), the analyte (A), and a metal salt (M). The resulting cluster ion (e.g., $[MR_2A - H]^+$ is mass selected and dissociated by competitive loss of the chiral reference or the analyte. The ratio of the two product channels depends on the chirality of the reference, and a plot of the abundance ratio versus % enantiomeric excess is linear. Unknown enantiomeric excess values can be quickly read from a calibration curve.

FUNDAMENTAL SCIENCE

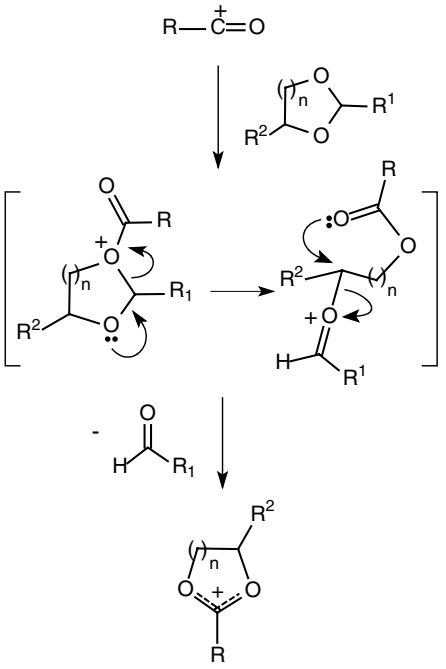
Fundamental aspects of ESI and MALDI and the fundamental chemistry that they facilitate are large subjects. A recent book[25] has an extensive coverage of these topics, and there is also much on these subjects in the present volume. Some additional comments on particular topics are as follows:

(a) *Fundamentals of Field Ionization*. By levitating a charged droplet and applying a strong electric field, the Taylor cone phenomenon and associated ion emission processes have been studied with unprecedented precision.[40]

(b) *Binding Affinities*. ESI experiments are a powerful source of information on fundamental thermochemical quantities. Amongst these, none is more important in biochemistry than the binding affinity. The ESI experiment offers different methods to characterize this quantity: examination of ligand exchange ion/molecule reactions provides intrinsic (solvent free) values; alternatively, examination of solutions containing competitive ligands allows relative binding affinities to be established under appropriate conditions.[41]

(c) *Reaction Mechanisms of Organic Ions*. Somewhat lost sight of in the bioanalytical applications of ESI is its role in providing mechanistic connections between the vacuum (isolated phase) environment and the ordinary solution environment. Some reactions, like the Eberlin transacylation shown in Scheme 1, have been studied in the vacuum environment, the ESI interface, under ambient pressure conditions, and in solution.[42] The polar transacetalization (Scheme 1) parallels the condensed-phase transacetalization. The reactant acylium ion and the cyclic ionic acetal product mimic the free reactant carbonyl compound and the cyclic acetal product of the condensed-phase reaction, respectively.

(d) *Retention of Biological Activity: Native Protein Configuration*. The already remarkable reach of ESI mass spectrometry into the biological sciences would be enhanced if the ionization method were even more gentle. For some years there has been evidence of retention of native protein structures in the gas phase, starting with McLafferty's data on H/D exchange in proteins.[43] The most direct evidence of retention of biological activity comes from electrosonic spray ionization (ESSI) followed by mass selection and ion soft landing into an aqueous medium.[44] Demonstrations of enzymatic activity—for example, phosphorylation of typical substrates using protein kinase A—were successful.



Scheme 1. Ionic transacetalization (Eberlin reaction) in the gas phase under high vacuum and at atmospheric pressure. (Adapted from Cooks, R. G.; Chen, H.; Eberlin, M. N.; Zheng, X.; Tao, W. A. Polar acetalization and transacetalization in the gas phase: The Eberlin reaction. *Chem. Rev*. **2006**, *106*, 188–211.)

(e) *Cluster Science*. The use of electrospray and to a more limited extent, MALDI, to generate noncovalently bound clusters illustrates the role of mass spectrometry in cluster science. The evaporation step that the electrosprayed droplets undergo imposes a Le Chatelier driving force in favor of clustering. The clustering of simple coding amino acids is a case in point with the clustering of proline to form a magic number dodecamer[45] being a feature of the ESI mass spectrum. The proposed structure of the proline 12-mer in its protonated form is shown in Figure 3.

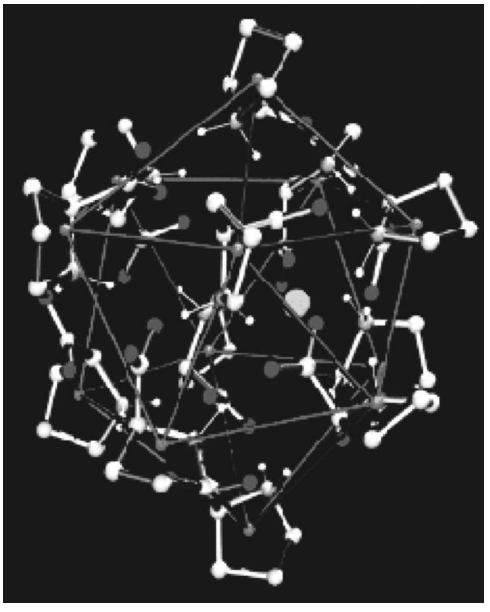


Figure 3. Calculated structure of the protonated form of the homochiral proline-12 icosahedral cluster. (From Clemmer, D. E., et al. *J. Am. Chem. Soc.*, **2006**, *128*, 15988–15989.)

OPPORTUNITIES AND UNSOLVED PROBLEMS

In spite of the spectacular progress in ESI and MALDI addressed throughout this volume, there remain major challenges and hence opportunities. Notable amongst them are:

(a) *Low Efficiency of Ionization*. Virtually all mass spectrometry ionization methods except for thermal ionization of low ionization energy elements have very low ionization efficiency (conversion of atoms/molecules of sample to gas-phase ions entering the vacuum system of the mass spectrometer). The best reported numbers are for nanospray where values are on the order of 0.1%. Obviously, there is much room for improvement. Optimization of the transfer of droplets and ions from the atmospheric-pressure region into the vacuum region is needed. Ion funnels[46] have had considerable success in the collection and transfer of free ions. The collection and transfer of droplets remains to be improved.

(b) *Higher Ion Currents*. Efficiency of ionization is important when sample sizes are limited. When this is not the case, higher-quality mass spectral data can be obtained by increasing ion currents. This cannot be done by simply increasing solution flow rates, although it might be achieved using multiple sprays.[47]

(c) *Hydrated Ions*. It is remarkably difficult to create micro-solvated ions by electrospraying solutions.[48] This is because solvents like water bind strongly to other water molecules and conditions which lead to desolvation tend to produce completely "dry" ions. This is just as well; otherwise the value of ESI in chemical analysis would be much reduced. Because of the enormous importance of solvation phenomena, along with attempts to understand solvated ion structures both spectroscopically[49,50] and through *ab initio* calculations,[51] new routes to the preparation of solvated ions would be of great value. One approach is based on the observation that electrospraying pure solvent in the presence of the vapors of an organic compound allows the uptake of that molecule into the charged microdroplets of solvent.[52]

(d) *Simulations at Higher Pressures*. The remarkable successes of ion simulation programs in assisting in the development of mass spectrometers has been based on the ability to calculate ion trajectories in vacuum, allowing for the effects of space charge and collisions. Both the widely used program SIMION and other more specialized programs have been used. However, at higher pressures than commonly encountered in mass analyzers, pneumatic effects on ion motion are not insignificant and appropriate computational procedures are still being developed. These pressures include those met in ESI and related ion sources, so clearly ion source development has proceeded without the aid of simulations. As such simulations become available, one can expect rapid progress in addressing issues like those mentioned in a) and b) above.

(e) *Multiplexed High-Throughput Analysis*. In spite of the value of ESI-based mass spectrometry, it is often coupled with slow chromatographic methods making the mass spectrometry experiment fast enough to be done in a serial fashion. Exceptions to this are the use of an array of micromachined electrospray sources[15] and the four-LC column MUX systems.[53] Multichannel mass spectrometers, in which multiple ESI ion sources are connected to multiple analyzers and multiple detectors, have been reported[54] but not yet commercialized.

(f) *Vapor Phase Analysis*. In an experiment patented by Fenn and Ffurstenau,[55] it was shown that ESI is applicable to these analytes by an extractive process in which the droplets of the electrosprayed solvent pick up vapor-phase compounds present in air. It is interesting to note that one of the variants on the DESI experiment, extractive electrospray ionization (EESI), uses a related principle to examine solutions for particular analytes.[56] In this experiment, a pure solvent spray, subjected to a high potential to give charged droplets, intersects the spray of a liquid. The advantages of this experiment, which appears to work by a solvent transfer process, is that the analyte is transferred from a solution that might be very complex, and hence difficult to handle by conventional ESI, into a pure solvent. The same concept has recently been applied by Zenobi and Chen to the analysis of nonvolatiles in breath[57] and to a high-throughput analysis of practical materials like foodstuffs for contamination and spoilage.[58] These compounds are presumably present in the form of an aerosol.

(g) *Detection of High-Mass Ions*. With the problem of creating high-mass ions well in hand, the limitations in high-mass MS lie increasingly in the detection step. Several innovative solutions are being developed: A cryo-detector in which the enthalpy of ion impact is measured has been developed and commercialized.[59,60] Another approach is to combine the detection and mass measurement step using laser elastic scattering from ions trapped in electromagnetic traps. The mass/charge ratio is measured from the ion frequency.[61] The limitations to this type of detector are that the size of the ion must not be too small. In addition, the experiment is effectively limited to the examination of small numbers of ions.[62]

(h) *Stand-Off (Remote) Detection*. The ability to move ions some meters, from the source of an ambient mass spectrometer to the mass spectrometer, has provided a modest stand-off capability that could be valuable in several areas of MS application. This is a convenience in many potential applications, not the least of which are those clinical applications in which the mass spectrometer would be intrusive but the DESI probe could be used to generate and then sample ions.

(i) *Miniature (Fieldable) Mass Spectrometers*. Given the importance of ESI for the detection and identification of biological compounds, there would be great advantages in several areas of application (environmental, clinical, food safety, public safety, etc.) if ESI or related methods like DESI could be adapted to small mass spectrometers. There has been progress in producing portable instruments based on laser desorption[63] and progress with DESI on a transportable instrument.[64]

The richness of the subject of ionization in mass spectrometry is the result of the not-always-easy coexistence of fundamental chemistry and physics with applied science; most certainly the role of instrumentation is an important factor in the continuous invention and re-invention that has characterized mass spectrometry in the past three decades. In searching for reasons for the vigor of mass spectrometry, one might argue that the scale of the instrumentation (in terms of physical size but even more in terms of complexity) has been appropriate: Mass spectrometers and their components are sufficiently complex that the instrumentation development is a difficult and rewarding scientific and technological undertaking, but it is not so complex that it can only be done with large budgets in a few places. (Parenthetically, this situation might change, and in the area of miniature instrumentation is already changing, to favor those with access to semiconductor and MEMS fabrication facilities.) Especially in the area of development of ion sources, the cottage

industry aspect of instrumental mass spectrometry has been an engine for progress, as seen throughout the development of the desorption ionization and spray ionization methods. This book appears at the right time to help spur this progress; it is a work of importance, and the community owes Richard Cole its thanks as well as its congratulations.

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Foreword to the First Edition

When Richard Cole asked me to write a foreword for this volume, I was highly flattered, but hardly eager. That reticence was due in part to a pile of deferred chores that grows as relentlessly as the universal entropy. A more forbidding inhibition was that in a world now teeming with electrospray (ES) users, I really didn't know what to say that hadn't already been said many times in many ways. Even so, the deadline was then far away and distant commitments always loom small, so I said yes. Tomorrow is now here and none of the nine Muses have come to my rescue. In desperation I have succumbed to banality by presenting "The Inside Story" of what has been called the electrospray revolution or "Things About Electrospray You Won't Find in the Papers!" What follows, therefore, is a highly personal account of how I became involved in the developments that led to the basis for this book.

The prologue to the story was written in 1937 at Berea College in Kentucky, where I was a senior majoring in chemistry. Jobs of any kind were scarce, so I applied for graduate study at several universities and was lucky enough to be accepted, with financial support, at both Northwestern and Yale. At first, I leaned toward Northwestern because its chemistry department was supposed to be better and its pay definitely was. But for various reasons, including a door-to-door free ride to Connecticut with my gear, I decided that fate wanted me in New Haven rather than Evanston. As a result, my chance to meet Malcolm Dole, then an Assistant Professor at Northwestern, was delayed half a century. At the time, I knew only that he had written a textbook on electrochemistry, not that he was at Northwestern. When I arrived at Yale, oddly enough as it turned out, John Zeleny was still on the Physics Faculty. Thirty years earlier, he had led the definitive pioneering studies on electrostatic dispersion of liquids into charged droplets. I was oblivious to that work for another 35 years, when I learned about Dole's electrospray technique and found that its roots were in Zeleny's investigations.

Meanwhile, I finished graduate study in 1940 and spent the next dozen years in applied research and development at Monsanto Chemical Company in Anniston, Alabama, Sharples Chemicals in Wyandotte, Michigan, and, beginning in 1945, at Experiment, Inc., a contract research and development company in Richmond, Virginia. There I became involved in combustion research for Project Bumblebee, a Navy program to develop a ramjet-powered antiaircraft missile. The experience led, in 1952, to the directorship of Project SQUID, another Navy program, administered by Princeton, on pure and applied research in "those fields of science relating to jet propulsion": combustion, fluid flow, and heat transfer. In 1955, the Navy arranged for me to spend a year at the London Branch of the Office of Naval Research. While there I came across a 1954 paper by E. W. Becker and K. Bier describing the production of intense beams of hydrogen molecules from rarefied supersonic flows as proposed in a 1951 paper by A. Kantrowitz and J. Grey. I had been musing about using molecular beam scattering experiments to study combustion reactions, but was discouraged because the expected reaction cross sections were too small for measurement with beam intensities available from the effusive sources of Otto Stern, Nobel Laureate and father of molecular-beam research. Moreover, the activation energies for the most interesting reactions were above 1 eV and would require source temperatures above

5000 K, much too high to be feasible. The Becker–Bier results indicated that the intensity barrier could be scaled with source gas flows that were convective as opposed to effusive. It occurred to me that if heavier reactant molecules like oxygen or chlorine were seeded into flows of hydrogen or helium, they should be accelerated to the high flow velocities achievable with those light gases. Calculations showed that, depending on their mass, such seed molecules could reach translational energies of several electron volts or more with modest source temperatures.

Back from London, I found Princeton's Department of Mechanical Engineering willing to provide a home for a research project on this idea. A proposal to NSF was fortunate enough to win support for a program that started in the fall of 1959. To make a long story short, we found that supersonic free jets from very small orifices could indeed produce intense beams of molecules with energies as high as 10 eV or more. Alas, we also found that these high translational energies were not effective in promoting chemical reaction. Meanwhile, we had learned that supersonic free jets had many other valuable features that have, ever since, played a key role in my research. Another major byproduct of the beam project was my transition from Director of Project SQUID to Professor of Mechanical Engineering, an achievement undreamt of in the philosophy of a young chemistry major at Berea College, 23 years earlier.

In 1967 I decided to accept an invitation to join Yale's newly organized Department of Engineering and Applied Science. About that time, the Bendix Corporation, producers of time-of-flight (TOF) mass spectrometers, was looking for a way to make intact ions from large polymer molecules so they could sell their instruments to the burgeoning plastics industry. They interested Malcolm Dole in the problem and he came up with his now-famous idea of using Zeleny's technique to disperse dilute solutions of macromolecules as a fine spray of charged droplets into bath gas at atmospheric pressure. He reasoned that evaporation of solvent from such a droplet would increase its surface-charge density up to the Rayleigh limit, at which Coulomb repulsion overcomes surface tension and the droplet breaks up into offspring droplets. Each offspring droplet would repeat that sequence, ultimately producing droplets containing only one solute molecule, which would become a free ion by retaining some droplet charge after all solvent had evaporated. This charged-residue model (CRM) for ion formation is one of the two models most often invoked to explain the formation of ES ions. The other is the ion-evaporation model (IEM) proposed some years later by Iribarne and Thomson. As discussed in the chapter by Kebarle and Ho, neither model has achieved unanimous acceptance.

Under Dole's leadership, a group at Bendix assembled an apparatus to test his idea. Sample solution was injected through a small tube or needle into a flow of dry-bath gas (nitrogen) through a cylindrical glass chamber. The needle was maintained at several kilovolts, relative to a plate constituting the end wall of the chamber. The resulting high field at the needle tip dispersed the emerging liquid into small charged droplets that drifted down the potential gradient concurrently with the flow of bath gas toward the end plate. A small orifice admitted some of the resulting mixture of ions and gas into the vacuum system as a supersonic free jet. I find it interesting that even though the Bendix people were expert in TOF techniques, a retarding potential method was chosen for mass analysis. The underlying idea was that during free-jet expansion, the ions would be accelerated to the easily calculated velocity of the nitrogen bath gas. Well downstream of the orifice, where the jet-gas density is too low to have any further effect on ion velocity, the ions passed through a set of grids on their way to a Faraday-cup electrode monitored by a sensitive electrometer. A scan of the grids' potential produced a current–voltage curve in which a

dip occurred when that potential became equal to the kinetic energy of some of the arriving ions, thus preventing them from contributing to the current at the Faraday cup. The mass of the excluded ions was readily obtained from this measured value of their energy along with a value of the velocity taken as equal to that calculated for the nitrogen in the jet. Promising results for polystyrene oligomers with molecular weights (M_rs) up to 500,000 were presented in the *Journal of Chemical Physics* in 1968, the year after I arrived at Yale.

Not an avid reader of the literature, I was unaware of Dole's paper, but it stirred the interest of Professor Seymour "Sandy" Lipsky in the Medical School. A long-time mass spectrometrist, he was excited by the possibility that Dole's technique might work with large biomolecules. Sandy showed the paper to Csaba Horvath, a colleague of mine in Chemical Engineering, who had been working closely with him on the development of HPLC, a now-invaluable methodology to which they made major contributions. Dole had kindly referenced some of our Princeton papers on the acceleration of heavy molecules by light carrier gases in free-jet expansions. When Csaba saw those references, he told Sandy that I was now at Yale, so Sandy tracked me down to show me the paper. Always on the lookout for new applications of free jets, I was very much intrigued and managed to interest a new graduate student, Mike Labowsky, in repeating Dole's experiments, which by then had been confirmed in a new apparatus at Northwestern and reported in a 1970 *Journal of Chemical Physics* paper. Our vacuum system was much bigger than Dole's and had much higher pumping speeds. Moreover, we had had more experience with free jet expansions. I realized that, in his experiment, the concurrent flow of bath gas and droplets meant that solvent vapor was present in the unheated mixture of gas and ions that entered the vacuum system. It was, therefore, highly likely that adiabatic cooling during free jet-expansion resolvated the ions to an appreciable extent, thus adding to their masses by an unknown amount. As a good chemical engineer, Mike knew that desolvation would be much more effective with bath gas flowing countercurrent to the drift of droplets and ions toward the end plate. Moreover, resolvation would be avoided, because only dry bath gas would then enter the free jet. With these changes, he obtained results somewhat different from and more reproducible than Dole's.

Both Dole and we had been persuaded by the work of R. Beuhler and L. Friedman that ions as large as we hoped we were producing would not generate secondary electrons at a dynode unless they were accelerated to about half a million volts. Therefore, we did not attempt to use ion-multiplier detectors, thus depriving ourselves of the mega-fold gain in sensitivity that mass spectrometrists take for granted. The currents of ions we could get into the vacuum system were very small and the vibrating-reed electrometer we used to measure them was very balky. Moreover, we knew from our earlier studies that there could be substantial slip effects during acceleration of very heavy molecules by a lighter carrier gas. Indeed, we estimated that the actual velocities of Dole's macroions were probably as much as 40% less than he had assumed. For these and other reasons, we abandoned further experiments. By that time, Dole had retired from Northwestern and moved to Baylor University, where he continued his experiments. However, instead of retarding potential measurements of mass, he was using mobility measurements to characterize the ions.

In 1981, our group was joined by Masamichi "Gado" Yamashita, a young scientist I had met during a stay at the University of Tokyo. During discussions about a possible project, I suggested that it might be interesting to take another look at Dole's ES ionization. Instead of macromolecules as analytes, we would use species with molecular weights less than 400 so they could be "weighed" with a small quadrupole mass analyzer we had in the laboratory.

Gado was a marvelous experimentalist and soon had converted a small "minibeam" apparatus into our first ES mass spectrometer. In an extensive set of exploratory experiments, with species small enough for our analyzer, he found that almost any solute organic molecule containing polar atoms such as O, S, N, and P would produce ions comprising the parent molecule with an anion or cation adduct. He also found that inorganic cations and anions could be produced, but generally in much lower abundances than he routinely obtained with organic solutes. As we later learned, the Aleksandrov group at the University of Leningrad (now St. Petersburg) had also begun to investigate ESI at about the same time as we did. From the fragmentary reports that we later obtained, it appeared that their emphasis was on ions from inorganic solutes.

One day in 1982 Sandy Lipsky, who had kept abreast of our work, brought VG's Brian Green to visit our lab. Brian was very interested and asked whether the technique would work with larger species. We pointed out that the upper mass limit for our analyzer was only 400 u so he arranged for VG to lend us a quadrupole with a mass range up to 1500 u. By that time, Gado had gone back to Japan and Craig Whitehouse, who had been working in Sandy Lipsky's lab, became a graduate student in Chemical Engineering. After joining my group, he designed and built a new system incorporating the VG analyzer and a modified version of Gado's electrospray source. That new system gave beautifully clean spectra for gramicidin S and cyclosporin, two cyclic decapeptides with almost the same M_r value. A provocative difference was that most cyclosporin ions had one charge, whereas most of those from gramicidin S had two. Moreover, for some slightly larger peptides, we obtained ions with three charges. Such multiple charging was most intriguing, so we decided to explore the phenomenon further with poly(ethylene glycol)s. They were attractive as test species because their oligomers were linear polymers whose composition and structure were essentially the same no matter what their size. (Moreover, samples over an M_r range from 200 to 20,000 were available at no charge from Union Carbide!) We found that the number of charges (Na^+) per oligomer increased with size, reaching at least 23 for an M_r of 20,000. Oddly enough, when we presented the data at the 1987 ASMS meeting in Denver, the only interest shown was by Charles McEwen, coauthor (with Barbara Larsen) of Chapter 5 and one of the first to recognize the virtues of ESI. He said right away that those PEG results were by far the most significant at the meeting. In contrast, a reviewer of our later paper dismissed the bands of overlapping peaks for large oligomers as spectra of dirt in the system, not of PEG oligomers!

To avoid the spectral complexity due to the wide distribution of molecular weights in PEG samples, we turned to nature to obtain samples comprising large molecules all of the same size, that is, pure proteins. We soon obtained spectra with the coherent sequences of peaks that have become the cachet of ES spectra for large molecules. The initial reaction of most mass spectrometrists to such peak multiplicity was one of horror. They were convinced that the resulting spectral complexity would make interpretation difficult, if not impossible. Moreover, the distribution of total charge among so many peaks would inevitably decrease signal/noise! They were wrong on both counts because they had reckoned without the powers of modern computers (as I still do!). One afternoon, I remarked to Matthias Mann, then a graduate student in my lab, that each of these multiple peaks really constituted an independent measure of parent ion mass. One should therefore be able to use signal-averaging methods to obtain a more accurate and reliable value of M_r. Two days later he came back with a deconvolution algorithm that allowed our little quadrupole, with a nominal mass limit of 1500 u, to determine M_r values up to 30,000 or more with an accuracy of 0.01%! At that time, M_r values from most other methods seldom had accuracies better than 5% to 10%. We presented these findings in San Francisco at the 1988 ASMS meeting;

the rest, as they say, is history. There were seven ES papers at that meeting: three from Henion's lab, two from Dick Smith's, and two from ours. Six years later, in 1994, at Chicago that number had climbed to over 300 where it remained in 1995 at Atlanta. I'm told that in the archival journals covered by the Citation Index, the term "electrospray" appeared in the title and/or abstract of over 300 papers in both 1994 and 1995. I'm sure Dole never dreamed that the seed he planted would bear so much fruit. Unfortunately, germination took so long that he didn't live to see the full magnitude of the stampede that began after our results with proteins became known.

My only face-to-face encounter with Malcolm Dole was in 1985 at the San Diego ASMS Meeting. At a session in which I gave a paper, I saw him in the audience and asked him to stand up and be recognized. Even though electrospray was not yet famous, the audience was generous in its applause, for which he (and I!) were grateful. My paper included some discussion of the importance of countercurrent drying gas to keep solvent vapor out of the free-jet expansion to avoid resolvation of the ions. At the end of the session, Dole came up to say he was delighted to learn about resolvation in the free jet, a possibility of which he had been unaware. He thought such resolvation might well account for an anomaly in his drift-tube experiments that had been puzzling him for a long time—the small differences between mobilities of ES ions from large and small molecules. (I now suspect that multiple charging of the larger ions, the extent of which didn't become clear until several years later, was as much or more to blame.) After our 1989 paper on ESMS of large biomolecules appeared in *Science*, Dole wrote me a nice note thanking me for the references to his papers and asking for a reprint. I sent it right away, along with one of our papers in *Mass Spectrometry Reviews* that included a more complete and complimentary account of his work. Three months later, I received a very apologetic note saying that he had taken the reprints along on a cruise and had somehow lost them. He would be most grateful if I could find another copy of each. He enclosed a twenty dollar bill to pay for whatever costs I might incur! I returned his money with the reprints and received a very gracious note of thanks, along with a copy of his privately printed autobiography. In it he made specific mention of my asking him to stand up and take a bow at the San Diego meeting, a gesture that was obviously very meaningful to him. He also quoted, clearly with great appreciation and satisfaction, my sometime remark to the effect that his electrospray idea was "extremely ingenious." Unfortunately, I never saw or heard from Dole again. Having not been invited, I did not attend the Electrospray Workshop in November 1991 at which he was present. I was delighted to learn that he had received much well-deserved attention, so that before he passed away some months later, he could begin to realize the abundance of the harvest from the seeds he had planted.

There is a presumption in many cultures that the longer one has lived in the past, the further can he or she see into the future. That may be one reason why Editor Cole suggested that my views on electrospray's future would be welcome. However, there have been so many surprises in its past that my crystal ball sees only great risks in any attempt to predict its future. Who would have dreamed 20 years ago that by now investigators would be able to examine the behavior of biopolymer ions in the gas phase, to determine their masses with accuracies approaching parts per billion, to study the kinetics and dynamics of their inter- and intramolecular reactions and processes, and to obtain detailed information on their composition, structure, and conformation that would provide insight on these properties in solution, *in vivo* as well as *in vitro*? Who could have anticipated that living organisms could be ionized, transferred into vacuum, recovered, and found to retain their viability? Yet this *tour de force* was recently accomplished with viruses by Siuzdak and his colleagues at Scripps Institute in La Jolla, California. With these so recently unbelievable achievements

of the electrospray methodology already in hand, who would dare to imply any limit on its possibilities by undertaking to guess what the future might reveal? Instead, I would simply urge investigators to exorcise their inhibitions and exercise their imaginations. May the platform of past accomplishments, so ably described in this volume, serve as a launching pad for their flights of fancy into the future. Let them dare to take off for any star whose twinkle beckons. Let them hearken to the words of Robert Browning in "Andrea del Sarto": "Ah, but a man's reach should exceed his grasp, or what's a heaven for?"

Richmond, Virginia JOHN. B. FENN

Preface

One dozen years have passed since the book *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation, and Applications* was first published. During that time, the expansion of mass spectrometry has taken on global proportions. In addition to its habitual place in chemistry and physics laboratories, advanced mass spectrometry instrumentation has truly become a staple in biological and biochemical research facilities. In many ways, the biochemical revolution, sparked by past efforts such as the human genome project, has driven the development of mass spectrometry over the past two decades. But the benefits of this progress are indeed far-reaching, and they are being reaped in the traditional areas of chemistry and physics from which mass spectrometry arose, in addition to the more recently emerged frontier domains such as proteomics, metabolomics and materials science. The purpose of this new book is to update experts and inform novices about the most recent trends in modern mass spectrometry. The chapters are written in a style intended to serve as both critical reviews and tutorials. The content is designed to provide a thorough coverage of the major topics in current day mass spectrometry. The intent was to make this volume highly suited both as a wide-ranging reference tome and as a classroom tool to support a graduate-level course in Advanced Mass Spectrometry.

The development of electrospray ionization and matrix-assisted laser desorption/ ionization as "soft" mass spectrometric ionization methods occurred virtually in parallel. The sudden growth of each began at almost the same time and was spurred primarily by the new possibilities that their advent provided for analyses of biopolymers, especially proteins. The impact of the two approaches on analytical chemistry—and in particular, biomolecule analysis—was both major and complementary. The overlapping histories of development of electrospray and MALDI over the past quarter century are like two sides of the same coin; for this reason, both have been included in this new volume.

Part I of the book is dedicated to explaining fundamental aspects of the electrospray process. First, the detailed fundamentals of electrospray are considered from a mechanistic viewpoint (Chapter 1). Special attention is then given to the root causes of the observed selectivity of ionization in electrospray (Chapter 2). Inherent to electrospray ionization sources are electrochemical processes that are explained in detail in Chapter 3. The ES fundamentals section is completed with a comparative inventory of source hardware (Chapter 4).

Part II turns to the MALDI side, and opens with an overview of ionization mechanisms in MALDI (Chapter 5). This is followed by a presentation of the progressive development of MALDI hardware up to the present (Chapter 6). As practitioners know, the art of MALDI lies in sample preparation, and an overview of matrices is given in Chapter 7. The special application of MALDI to obtaining two-dimensional images of the spatial distribution of compounds on surfaces is presented in Chapter 8.

Each of the remaining chapters considers both electrospray and MALDI in addressing the respective topic of interest. Part III examines the coupling of these ionization techniques to various mass analyzers. First up, quadrupoles, quadrupole ion traps, linear quadrupole ion traps, and the Orbitrap are discussed in Chapter 9. The development of MALDI

and electrospray has also led to a renaissance in time-of-flight technology as detailed in Chapter 10. Ultra-high-resolution Fourier transform ion cyclotron resonance mass spectrometers as well as magnetic sector mass analyzers are examined in Chapter 11. Last comes Chapter 12's presentation on ion mobility spectrometry that is particularly oriented toward biological issues.

In Part IV of the book, analytical and practical issues of electrospray and MALDI are confronted. Chapter 13 presents an analytical comparison between electrospray and MALDI, with atmospheric-pressure chemical ionization thrown in for good measure. An investigation into the factors that influence charge state distributions in electrospray and MALDI appears in Chapter 14. Next comes a detailed overview of the utility of electrospray and MALDI for investigating noncovalent interactions that are preserved as gas-phase ions (Chapter 15). Attention then turns to discussion of the various ion activation techniques that are used to provoke dissociation of the typically stable intact precursors generated by electrospray and MALDI in preparation for tandem mass spectrometry experiments (Chapter 16). Chapter 17 presents a primer on interpretation of mass spectra specifically targeting decompositions of the even-electron ions produced by soft ionization techniques.

Part V targets applications that are organized according to specific compound classes of analytes. The role of mass spectrometry in peptide and protein characterization and in proteomics is the subject of Chapter 18. Next, the topic of carbohydrate analysis by ESI and MALDI is tackled in Chapter 19. This is followed by an examination of ESI and MALDI applications to lipid analysis (Chapter 20). Finally, the important subject of drug discovery is addressed in Chapter 21, including *in vitro* ADME (absorption, distribution, metabolism and excretion) profiling and pharmacokinetic screening.

I must thank all of the contributing authors who, in my humble opinion, rank among the finest mass spectrometrists in the world. Their dedication and perseverance throughout this project was truly remarkable. Out of necessity, the lives of scientists in the 21st century have become more multifaceted than ever, and the time and effort that is dedicated to a project like this book must be carved out of schedules that are fuller than the day has hours. Nevertheless, this group of consummate professionals delivered their contributions in extraordinary fashion. In this same vein, I extend my utmost gratitude to Professor R. Graham Cooks for agreeing to write the Foreword for this new edition. It is important to point out that each of the chapters was reviewed by independent experts in the field, whom I thank enormously for their time and effort. I must also thank my super-assistants Ms. Ashley A. Smith, Mr. Kevin M. McAvey and Ms. Priya Bariya for their vital help with the assembling and formatting of the final product. Finally, I thank Dr. Dominique M. Custos for *terra firma* support throughout the entire project.

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