REVIEW

Ambient Mass Spectrometry

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A recent innovation in mass spectrometry is the ability to record mass spectra on ordinary samples, in their native environment, without sample preparation or preseparation by creating ions outside the instrument. In desorption electrospray ionization (DESI), the principal method described here, electrically charged droplets are directed at the ambient object of interest; they release ions from the surface, which are then vacuumed through the air into a conventional mass spectrometer. Extremely rapid analysis is coupled with high sensitivity and high chemical specificity. These characteristics are advantageously applied to high-throughput metabolomics, explosives detection, natural products discovery, and biological tissue imaging, among other applications. Future possible uses of DESI for in vivo clinical analysis and its adaptation to portable mass spectrometers are described.

The application of mass spectrometry (MS) to the identification of chemical compounds in a mixture, including determining the structural composition of large biomolecules, depends on much more than the resolving power of the analyzer used for discriminating mass/charge (m/z) ratios. Often, the main limitation is getting the sample of interest into the vacuum environment of the spectrometer in the form of ions suitable for mass analysis. This problem was solved, for the case of samples in the solution phase, with the introduction of electrospray ionization (ESI) (*1*). ESI is a method where the solution is nebulized to create a fine spray of droplets under conditions in which solvent evaporation occurs as the droplets traverse the atmospheric interface, hence introducing molecular ions into the analyzer.

A critical development for the analysis of condensed-phase samples was that of the desorption/ionization (DI) methods, where molecules embedded in a substrate and introduced into the vacuum system are rapidly desorbed and ionized using energetic charged particles or laser beams. High-energy sputtering methods such as SIMS (secondary ion MS) (*2*) can be used to produce intact molecular ions. Larger molecules such as proteins are also amenable to DI methods if they are embedded in a frozen solvent (typically ice) or in an ultraviolet (UV)-absorbing matrix that can be rapidly volatilized with a laser pulse, as in MALDI (matrix-assisted laser desorption/ionization) (*3*). Although vacuum conditions are a simple choice for creating and maintaining ions, this environment is not absolutely necessary. Ions can in fact be generated in air; an atmospheric pressure version of the MALDI experiment (*4*) was an important progenitor of ambient MS experiments, even though it did not have unimpeded access to the sample nor the lack of sample preparation that characterize more recent methods.

Recently, a new family of techniques has emerged that allows ions to be created under ambient conditions and then collected and analyzed by MS. In the desorption electrospray ionization (DESI) method (*5*), a fine spray of charged droplets hits the surface of interest, from which it picks up small organic molecules and large biomolecules, ionizes them, and delivers them—as desolvated ions—into the mass spectrometer. DESI can be considered an atmospheric pressure version of SIMS, being especially close to versions that use C_{60} projectiles (*6*) or massive water clusters as primary impacting particles (*7*). In the DART (direct analysis in real time) method, an electrical potential is applied to a gas with a high ionization potential (typically nitrogen or helium) to form a plasma of excited-state atoms and ions, and these desorb low–molecular weight molecules from the surface of a sample (*8*). Other closely related methods have also been introduced. Desorption atmospheric pressure chemical ionization (DAPCI) (*9*), a variant of DESI that uses gas-phase projectile ions generated by an atmospheric pressure corona discharge in the vapor of toluene or another compound, produces ions by a heterogeneous (gaseous ion/adsorbed analyte) charge-transfer mechanism. Electrospray-assisted laser desorption/ionization (ELDI) (*10*) uses a laser for the desorption of neutral molecules from an ambient surface and uses charged droplets produced by electrospray for post-desorption ionization of the ablated neutral molecules. In atmospheric solids analysis probe (ASAP) (*11*), another variant on atmospheric-pressure DI methods for solids analysis, a heated gas jet is directed onto the sample surface, and desorbed species are ionized by corona discharge in the gas phase.

Here we focus on the DESI method, on which there is the most literature, while noting cases in which the applications of the other methods yield comparable results. The ambient ionization methods retain the signature advantages of MS—speed, chemical specificity, low detection limits, and, via the MS/MS experiment, applicability to complex mixtures. However, these characteristics are now implemented in a direct experiment that requires no sample preparation. Applications to high-importance samples—such as traces of explosives on luggage, drug metabolites in urine, lipids in intact tissue, and active ingredients in

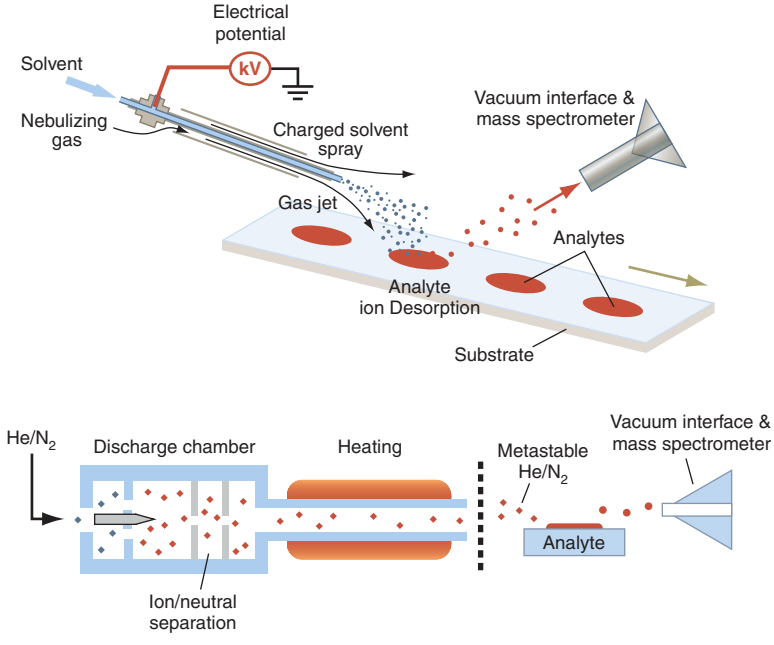

Fig. 1. DESI (upper) and DART (lower) analyses for ambient high-throughput mass spectrometric analysis of unprepared samples (skin, bricks, urine spots, clothing, tissue, etc.).

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pharmaceutical tablets—have quickly followed the introduction of the methodology.

Implementation

The essential aspects and the simple instrumentation of the DESI experiment are illustrated in Fig. 1. The condensed phase–to–gaseous ion transfer that is a feature of DI methods is achieved by using charged droplets (for larger molecules) or primary ions (for smaller molecules), either of which is produced by ESI (*1*). The sample of interest is in the solid phase—not in the solution phase as it is in ESI—and, in contrast to the established DI methods like SIMS and MALDI, the sample is not under vacuum. Figure 1 also summarizes the DART experiment, which differs because it uses a gas rather than a solvent to generate the energetic agents that desorb and ionize the analyte molecules.

DESI applies to both large and small molecules. The charged microdroplets used as projectiles in DESI pick up proteins and other large biomolecules from the surface, ionize them, and transport them to the mass spectrometer. This process gives the mass spectra of proteins in the solid phase, which typically closely resemble the ESI spectra of protein solutions. In addition, gas-phase solvent ions in the spray protonate or otherwise react with analyte molecules on the surface, generating ions from compounds that have low desorption energies, including volatile and semivolatile compounds (e.g., aromatic hydrocarbons and pesticides), low-polarity molecules of smaller size (e.g., terpenes and lipids), low–molecular weight polar compounds (e.g., amino acids and drug molecules), and certain inorganic ions (e.g., perchlorate). In MS, free gas-phase analyte ions are characterized by their *m/z* ratio and sometimes, in more detail, by recording their dissociation products (MS/MS spectra) and their ion-molecule reactivity. Similar to other atmospheric ionization methods (*1*, *4*, *10*, *11*), DESI causes minimum fragmentation; that is, it is a soft ionization technique that produces low-energy, intact molecular ions. This feature is associated with fast collisional cooling of nascent ions at atmospheric pressure and ambient temperature.

Analytical Performance and Characteristics

The type of ions observed in DESI, DART, and other ambient MS methods depends on the nature of the sample, substrate, and reagent. For example, the explosive RDX (hexahydrotrinitro-1,3,5-triazine) is observed as the chloride adduct $[RDX + Cl]^-$ when electrosprayed with a dilute HCl solution, but is observed as the protonated molecule $[RDX + H]^+$ when sprayed with pure water. The ionized molecules observed in the mass spectrum are conveniently mass-selected and individually examined by recording their dissociation products in the form of MS/MS spectra. Figure 2 illustrates the DESI mass spectrum of a dry urine spot on paper (2 μL of urine), showing the complex nature of this mixture. Even minor components can be identified by recording their MS/MS spectra; for example, the isolation of the ion with *m/z* = 214 and the measurement of its product spectrum allows its identification as aspartyl-4-phosphate. Experiments of the type illustrated in Fig. 2 can be performed at a rate of one per second. There is no preparation of the biological fluid other than its deposition on the surface.

Not only is DESI a very rapid method, but it is well suited to trace analysis. Luggage screening in airports is a task where very high sensitivity must be combined with high chemical specificity (low false positives, low false negatives), while maintaining immunity to matrix effects and achieving very high throughput rates. Success has been achieved in laboratory experiments with several classes of explosives and their compositions (Fig. 3) (*12*). Under artificial conditions, detection limits into the femtomole (fmol) range have been observed for some of these compounds (Fig. 3). Analogous data have been reported for DART, including cocaine detection on banknotes (*8*). Limit of detection (LOD) values in the low fmol range have been reported for proteins by using DESI (*13*) and ELDI (*10*).

DESI operating characteristics can be chosen to favor the ionization of small or large molecules. Small molecules are often seen well in the positive ion mode by using a spray voltage of 5 kV, a tip-to-sample distance of 5 mm, and an incident angle of 40° to the surface normal. Oligosaccharides and proteins require smaller tip-to-sample distances and steeper impact angles. These conditions are associated with the need to have droplets hit this type of sample to cause ionization ("droplet pick-up" mechanism). Both small and macromolecule analytes were examined from a variety of surfaces including paper, plastics, and glass surfaces, with no significant differences in the spectra. Polymer analyses included determinations of molecular weights of industrial polymers such as polyethylene glycol (*14*), as well as those of proteins and oligosaccharides (*13*).

Complex processes are involved in producing gas-phase ions from condensed-phase samples through impact of charged aqueous droplets, gas-phase ions, or metastable atoms. It is well known that molecules at surfaces under vacuum can be simultaneously desorbed and ionized by charge transfer (electron, proton, or other ion) using primary ions with low translational energies. This low-energy heterogeneous process known as chemical sputtering (*15*) occurs in the ambient DI methods also, even though projectile ion energies are so low that reaction exothermicity must be the source of the desorption endothermicity. The involvement of liquid droplets in DESI introduces an additional and fundamentally different mechanism of ion formation. The charged droplets pick up molecules as they splash off the surface, and the secondary droplets produce gaseous ions by well-known ESI mechanisms of direct ion emission (ion evaporation model) or complete evaporation of the neutral solvent molecules (charge residue model) (*16*). Because the secondary droplets contain the analyte and move through the normal ESI atmospheric interface,

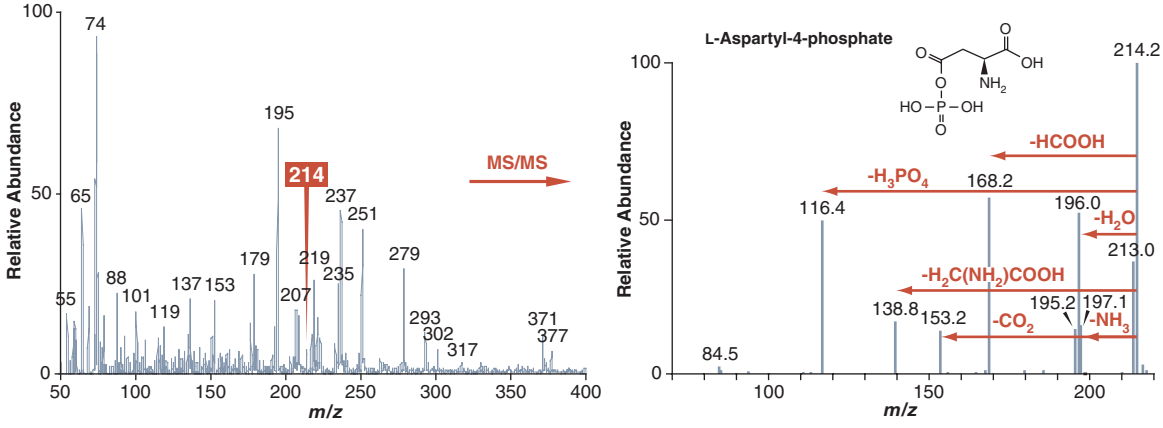

Fig. 2. DESI mass spectrum of dried, 2-μL, raw urine spots on paper, sprayed with 1:1 methanol:water containing 1% acetic acid. The product ion MS/MS spectrum identifies one of the minor components, with *m/z* = 214, as aspartyl-4-phosphate.

Limit of detection
(5s sampling)

Explosives	Ions observed	LDD (pg)	Substrate
TNT	M^- $(M - NO)^-$	10	Plastic
RDX	$(M + H)^+$	1	Paper
RDX	$(M + A)^-$	10	Paper Metal Plastic
HMX	$(M + A)^-$	10	Metal
PETN	$(M - H)^-$ $(M + A)^-$	100	Paper
TATP	$(M + Na)^+$	1000	Paper

A, anion: Cl^-, acetate, trifluoro-acetate, nitrate

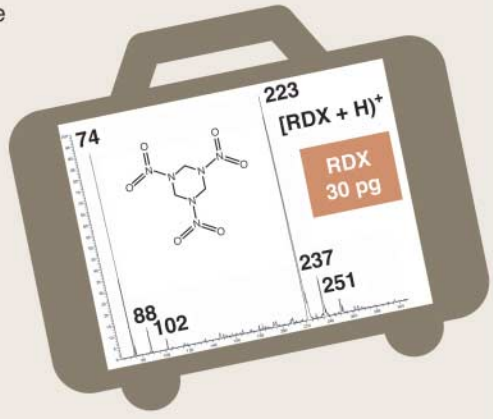

Fig. 3. Explosives detected at low levels on various surfaces, in the positive- and negative-ion modes, showing the mass spectrum of 30-pg RDX (TNT, trinitrotoluene; RDX, hexahydrotrinitro-1,3,5-triazine; HMX, octahydro-MX H1,3,5,7-tetranitro-1,3,5,7-tetrazocine; PETN, pentaerythritol tetranitrate; TATP, triacetonetriperoxide).

it is expected and observed that the DESI and ESI spectra of proteins are very similar, even though the protein being analyzed is in a different physical phase. Momentum transfer from impacting droplets may contribute to the desorption of larger molecules such as proteins, together with electrostatic repulsion caused by charge accumulation on the surface, whereas the force responsible for transfer to the mass spectrometer is suction from the vacuum system.

One consequence of these mechanistic considerations is that the range of applications and the ion formation mechanism of DESI (and presumably of ELDI, although this has not been investigated fully) is wider than but includes those of DAPCI and DART, because the conditions of the electrospray used in DESI can be selected to favor either dry ion or droplet impact by adjusting the distance to the sample or the nebulizing gas flow rate. It is the presence of charged aqueous liquid droplets that allows the ionization of highly polar species independent of molecular weight, therefore readily allowing DESI ionization of proteins, peptides, carbohydrates, polar lipids, and nucleic acids (*13*). As a consequence, the overwhelming majority of known molecular or ionic species can be ionized and detected by DESI-MS, from single metal ions to large proteins and from unsaturated hydrocarbons to complex polysaccharides.

Quantitative accuracy of the ambient MS methods is limited by matrix effects, which vary with the analyte. Internal standards increase accuracy and precision for solutions but cannot be used for solid samples. The surface-derived ion current is somewhat transitory in nature and is perhaps associated with surface-charging effects and the turbulent nature of high-velocity gas streams. Although detection limits are remarkably low, peak stability is not high, so some averaging of data is necessary for quantitation, the precision of which can be as low as a few percent relative standard deviation (RSD) but commonly is as high as 20%.

Applications

DESI experiments can be performed on a very wide range of samples to give information on many types of compounds (polar and nonpolar, low and high molecular weight) present in a wide range of matrices and in various physical states. As a consequence, the range of applications of DESI is extraordinarily wide and covers many scientific fields (Fig. 4). Applications include the following: (i) the examination of native surfaces for forensics, security, and environmental studies; (ii) the examination of biological surfaces, especially imaging of particular compounds in intact tissue; and (iii) high-throughput examination of solutions (after transfer onto a surface like paper), for example, in disease-state recognition using metabolomics.

Several groups have reported encouraging results with ambient MS of intact pharmaceutical products and in drug discovery (*17*–*23*). Pharmaceutical applications include high-throughput DESI analysis (rates of up to 10 tablets per second for simple MS scans and about 1 per second for more complex experiments, in which MS/MS spectra are recorded to confirm the identity of particular ions in the original mass spectra) (*17*). These experiments suggest the potential value of ambient MS in a wider range of industrial and laboratory process-monitoring applications, including its use as a chromatographic detector (*24*). Related applications are to natural products, including active components present in plant tissue. This type of study, e.g., on alkaloids in *Conium maculatum* (*25*), illustrates a number of important features of this ambient ionization method: (i) Mass and tandem mass spectral data take only a few seconds to acquire. (ii) The samples are examined as collected; there is no need for extraction or other work-up of the plant material. (iii) Isomers and congeners are readily distinguished. (iv) Trace constituents can be observed. (v) Quantitative reproducibility is adequate (20% RSD).

Recent advances in ambient ionization methods are providing sensitive, high-throughput means of analyzing biological samples at atmospheric pressure. Biological and biochemical applications of DESI include pharmacokinetics (*5*) and high-throughput medical screening. The best established high-throughput DESI experiments are in

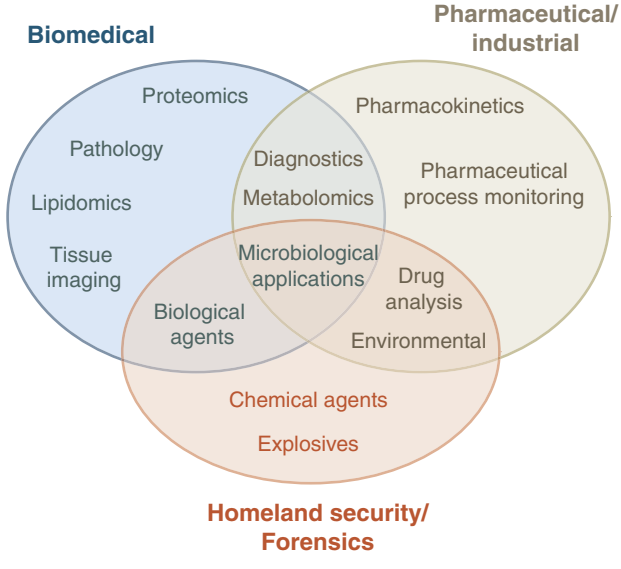

Fig. 4. Areas of application of the ambient MS method of DESI, grouped into three broad overlapping classes.

metabolomics, where quantitative pattern recognition techniques have been applied. By using principal component analysis, for example, it has been found that mice in different disease states can be distinguished by DESI urine analysis. This method identifies many more metabolites, and it is much faster than are corresponding nuclear magnetic resonance measurements made on the same samples (*26*). Although it is not necessary to identify particular up- or down-regulated metabolites to distinguish specific subpopulations, tandem mass spectrometry can provide this information (Fig. 2), and there is the potential to contribute to a more fundamental understanding of metabolism by focusing attention on particular compounds that participate in known metabolic pathways. Surveys for inborn errors of metabolism require high sensitivity, minimal sample clean up, chemical specificity, and high-throughput capabilities, all demonstrated characteristics of the DESI method.

The application of DESI to microbiological sample screening (for toxicity, clinical microorganism identification, etc.) has seen limited application, but information-rich spectra are recorded for bacteria and bacterial spores even in the presence of growth media. Distinctions between species are readily made, and it remains to be seen whether the more interesting subspecies distinctions can be made, and if so, in what times and at what levels.

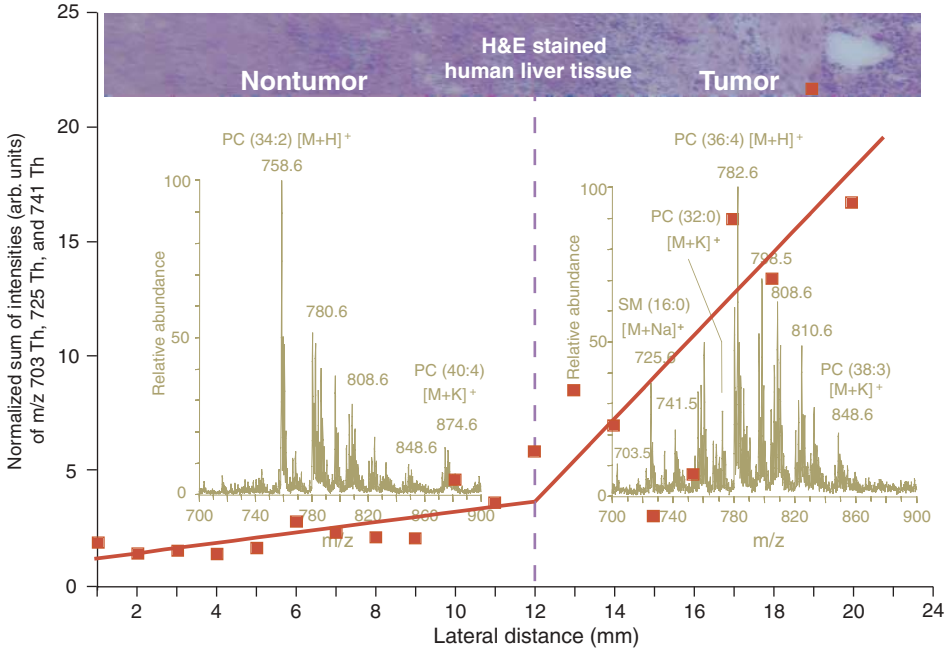

Fig. 5. Direct tissue profiling of human liver adenocarcinoma using DESI in the positive-ion mode. The tissue was sectioned and untreated, and it was subjected to a spray of 1:1 methanol:water containing 0.1% ammonium hydroxide. Adapted from (*32*).

Biological Tissue Analysis

Among the many biomedical problems to which DESI could be applied, in vivo real-time tissue imaging by MS could prove to be the most challenging and the most useful. Like other DI methods, DESI gives information on the spatial distribution of analytes on a surface, not only on their molecular identity but on the amounts present. Biological imaging by MS is achieved currently either through the irradiation of a thin, matrix-coated tissue section using a laser (*27*, *28*) [UV- or infrared (IR)-MALDI] or by particle bombardment as in SIMS (*6*, *29*, *30*) and now DESI (*31*). Each of these methods provides spatially defined chemical information on a tissue sample that can be correlated to cell morphology or the biological state of the tissue. Chemical imaging of this type is not only complementary to traditional histopathological protocols but has the potential to provide specific chemical information that reveals disease progression and prognosis.

The simplest way to perform DESI imaging is to use a microprobe beam of solvent microdroplets and to raster this across the surface. Tissue imaging by DESI shows only modest spatial resolution (spot sizes 0.5 to 1.0 mm), but it removes the constraints of the high vacuum met in SIMS imaging (*29*) and that of sample preparation, which is a requirement for MALDI imaging (*30*). This allows for analysis of the sample surface in the free ambient environment at atmospheric pressure.

DESI-MS studies of various biological tissues and whole organs, including cancerous human liver tissue, revealed differences in the distributions of compounds, including elevated levels of certain phospholipids in the tumor region as compared with the nontumor region (Fig. 5) (*31*). These initial results suggest, for the longer term, the development of ambient MS techniques for routine use in surgery or histology.

Reactive DESI

It is straightforward to add reagents to the DESI spray solvent to mediate the interfacial reactions of solution-phase reactants with condensed-phase analytes to yield gas-phase ions. These experiments (so-called reactive DESI) add chemical selectivity to the DESI experiment, just as the ability to choose a chemical ionization gas adds chemical selectivity to conventional chemical ionization MS. A wide range of reaction types can be used, including redox reactions of metal complexes (*32*), covalent modification of particular functional groups, and the formation of noncovalent complexes (*5*). Specific examples include the formation of specific enzyme substrate complex ions by doping the spray solvent with substrate (*5*) and the formation of cyclic boronates for identification of the cis-diol functional group. For example, when solid-phase glucose, glycosides, steroids, and other compounds with the cis-diol functionality are subjected to a spray containing phenylboronic acid, cyclic boronates are generated and easily recognized by the mass shift seen (*33*). Future applications are likely to focus on drug target screening, using reactive DESI in a high-throughput fashion to screen for drug candidate activity through the formation of noncovalent complexes.

Future

The emergence of MS as a tool for the biological sciences is the result of a series of remarkable developments, occurring over several decades. They include the following: (i) sensitive mass analyzers capable of appropriate resolution and mass range, (ii) MS/chromatography combinations, (iii) tandem mass spectrometry, and (iv) new ionization methods. MS is now an indispensable tool in the fields of proteomics, lipidomics, and metabolomics; on the basis of the detection, identification, quantification, and structural characterization of peptides, lipids, and metabolites derived from biological sources. In addition to these small-molecule applications, intact biomolecules such as proteins and protein complexes (enzyme-substrate, protein-protein, and protein-DNA) (*34*, *35*) are increasingly falling within the scope of MS, which is providing information such as molecular weight, stoichiometry, and binding affinity. All of these developments seem likely to be accelerated by the advent of ambient MS techniques, which allow compounds ranging from biopolymers to small drugs to endogenous

biochemicals to be analyzed in unprepared samples, very rapidly and with high specificity.

The rapid development of ambient MS likely will accelerate interest in miniature mass spectrometers. We can foresee handheld mass spectrometers equipped with DESI ion sources. The current trend in MS miniaturization is driven by the desire to perform in situ chemical analysis and facilitated in large measure by the development of small ion-trap analyzers, which have led to tandem mass spectrometers in the 10-kg (total system) weight range (*36*). These developments have been extended to instruments fitted with ambient ion sources, and a portable DESI ion-trap system based on a cylindrical ion-trap analyzer has been built. The drive toward miniature instruments, in parallel with the drive toward ambient mass spectrometers, has created technology that now allows ambient ionization methods to be used with instruments small enough to serve as personal mass spectrometers for individuals. It is likely that chemical measurements could be made soon by individuals with the ease with which, a generation ago, mathematical computational power became widely available with the development of the electronic calculator and the personal computer. Suitable ambient mass spectrometers would allow many of the environmental, pharmaceutical, and physiological measurements described in this article, and these could be of intense personal interest to individuals. The confluence of ambient and miniature MS has the potential to change not just MS but the whole subject of analytical chemistry.

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REVIEW

Probing Cellular Chemistry in Biological Systems with Microelectrodes

R. Mark Wightman

Over the past 20 years, the technological impediments to fabricating electrodes of micrometer dimensions have been largely overcome. These small electrodes can be readily applied to probe chemical events at the surface of tissues or individual biological cells; they can even be used to monitor concentration changes within intact animals. These measurements can be made on rapid time scales and with minimal perturbation of the system under study. Several recent applications have provided important insights into chemical processes at cells and in tissues. Examples include molecular flux measurements at the surface of single cells and through skin—which can offer insights into oxidative stress, exocytosis, and drug delivery—and real-time brain neurotransmitter monitoring in living rats, which reveals correlations between behavior and molecular events in the brain. Such findings can promote interdisciplinary collaborations and may lead to a broader understanding of the chemical aspects of biology.

Microelectrodes, sometimes termed ultramicroelectrodes, are chemical sensors with micrometer or smaller dimensions. Very small microelectrodes that report concentrations on the basis of potential changes across chemically selective membranes at their tip, termed potentiometric electrodes, have been in use for decades. However, this review concerns voltammetric microelectrodes, microscopic devices that sense substances on the basis of their oxidation or reduction and that were introduced in the early 1980s (*1*). More recently, voltammetric microelectrodes—dynamic devices that allow control of chemical environments as well as chemical sensing—have been used in a variety of unusual applications, including fabrication of microstructures and investigation of chemical fluctuations at the surface of single biological cells and in the living brain. They offer considerable advantages relative to voltammetric electrodes of conventional (millimeter) size. The small double-layer capacitance of microelectrodes enables chemical events occurring on the submicrosecond time scale to be monitored (*2*). Their small currents facilitate measurements in highly resistive media such as solvents without electrolyte and supercritical fluids (*3*). Unlike electrodes of conventional size, microelectrodes can be used in measurements on longer time scales, when the distance across which reactive molecules diffuse to the electrode is much greater than the electrode dimensions. Thus, very small electrodes can have markedly enhanced fluxes, which can enhance signal-to-noise ratios in trace metal ion determinations (*1*). In general, microelectrodes allow chemical measurements

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