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SPECIAL FEATURE: PERSPECTIVE

Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms and applications in forensics, chemistry, and biology

Zoltán Takáts,* Justin M. Wiseman and R. Graham Cooks*

Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

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Analytical characteristics of DESI are summarized. Examples of applications to small and large molecules, to *in situ* analysis, and to high-throughput analyses are presented. Evidence is provided for both a heterogeneous charge-transfer mechanism and a droplet pick-up mechanism of ionization. The speed, lack of the need for sample preparation, selectivity, and sensitivity of DESI are all demonstrated and discussed. Instrumentation is also discussed. Forensic applications as well as emerging areas of application including tissue imaging are given emphasis. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: electrospray ionization; desorption ionization; metabolomics; natural products; high throughput; ionization mechanisms; *in vivo* analysis; *in situ* analysis; chemical profiling

INTRODUCTION

The development of mass spectrometry (MS) can be seen, from one perspective, to be based on the invention and utilization of ion sources of ever greater power and more general applicability. Such a simplified picture is useful, even though it ignores important work in the development of mass analyzers, in the field of gas-phase ion chemistry, and in the important topics of ion kinetics and thermochemistry. It is most strikingly shown by the almost simultaneous introduction, some two decades ago, of two new ionization methods. One of these methods, applicable to the analysis of solids, is the particularly useful desorption ionization (DI) method known as *matrix assisted laser desorption ionization* (*MALDI*),[1] and the second, applicable to the analysis of solutions, *electrospray ionization* (*ESI*).[2,3] Both methods have had enormous consequences for the utilization of MS in biology and the life sciences. An argument can be made that the next major advances in MS will be based on the availability of instruments that operate in the ambient environment. The ability to record mass spectra of ordinary samples in the lab or field, while they are simultaneously subject to chosen chemical and physical operations and environments, should make MS qualitatively more valuable and open many new areas of application for the technique.

The aims of this Perspective article are to describe the first ambient MS method, desorption electrospray ionization (DESI),[4] to evaluate its performance at this early stage of its development and to describe the instrumentation and some of its applications. In addition, we provide some information relevant to the ionization mechanism and preview emerging areas of application. The distinguishing feature of DESI is that it allows ambient MS. This term is used to describe capabilities that are broader than those encompassed by the term atmospheric pressure MS. Atmospheric pressure MALDI, first described 5 years ago,[5,6] was the first ionization technique in which a condensed-phase sample was examined at atmospheric pressure. Prior to the first report on DESI and the more recently described direct analysis in real-time (DART)[7] method, samples examined by MS were not in the free ambient environment nor could they be subjected to arbitrarily chosen processing actions or conditions while mass spectra were being recorded.

DESI is a novel method that allows MS to be used to record spectra of condensed-phase samples under ambient conditions; it is applicable to solid samples, including complex biological materials, but it can also be applied to liquids, to frozen solutions, and to adsorbed gases. The

*Correspondence to: Zoltán Takáts or Graham Cooks, Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA.
E-mail: takats@purdue.edu; cooks@purdue.edu
Contract/grant sponsor: InProteo LLC (Indianapolis, IN).
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method has high sensitivity, is virtually instantaneous in response time, and is applicable to small-molecule organic compounds as well as to proteins and other biological molecules. Self-evidently, no ionization method can give ions for *all* the molecular or ionic constituents of a biological sample (e.g. tissue sample) under a single set of conditions. Nonetheless, DESI is still able to give massive amounts of chemical information that can be correlated with the biological status of the sample. For instance, as discussed later for the case of liver tissue sections, most of the detected species are phospholipids, and their distribution gives sufficient information to distinguish between non-tumor and adenocarcinoma cells.

The DESI experiment combines features of ESI with those of the family of DI methods.[1] The connection with the spray ionization family of methods is both methodological and instrumental. An electrospray emitter is used to create gas-phase solvent ions, ionic clusters, and charged microdroplets, which are directed at the sample. An electrical potential of several kilovolts is applied to the spray solution and pneumatic nebulization is used to assist in desolvation. With the important exception of the physical state of the sample, the DESI experiment closely resembles the ESI experiment. The relationship of DESI with the DI methods is also close, at least at the phenomenological level. All the DI methods (plasma desorption, PD; laser desorption ionization, LDI; matrix assisted laser desorption ionization, MALDI; secondary ion mass spectrometry, SIMS; fast atom bombardment, FAB) involve the impact on condensed-phase samples of projectiles, which include photons (laser desorption, including MALDI), translationally excited atoms (FAB), and energetic ions (SIMS). The projectiles used in SIMS include polyatomic ions,[8–12] and it is well established that their use significantly increases the efficiency of sputtering. DI experiments are conducted in a high-vacuum environment, while DESI is performed in air, which is the principal feature that distinguishes it from other DI methods. This also implies significant differences in the fundamental processes involved; the momentum-transfer collisions upon which traditional SIMS and other DI sputtering mechanisms are based[13] are not necessarily applicable in the high-pressure environment of DESI, since the projectiles can have only low kinetic energies. One mechanism that might be involved in DESI has emerged from studies of ion/surface scattering processes in vacuum;[14,15] it is the efficient, low-translational-energy process known as *chemical sputtering*.[14–16] In this process, ions are generated by heterogeneous electron, proton, or other ion transfer from the arriving projectile to the analyte on the surface. The surface group is charged and, if also given enough momentum, is released from the surface. This process occurs at very low impact energy for exothermic reactions and at higher energies for endothermic reactions.

In contrast to ion/surface interactions, charged droplet/surface interactions have not been studied extensively. Under high vacuum, massive cluster impact ionization (MCI) was observed[11,12,17] when multiply charged glycerol clusters impact solid surfaces bearing analyte. Unlike any other DI technique, this experiment produces multiply charged ions from macromolecular analytes.[18] The formation of multiply charged ions has been tentatively associated with the shock-wave-generated secondary droplets and solution-phase, ESI-like ionization of macromolecules.[18] As discussed later on, the similarities between ESI and DESI spectra of proteins indicate an ESI-like droplet pick-up mechanism for ion formation in DESI.

DESI allows organic molecules present at sample surfaces to be analyzed by MS without requiring, in most cases, any sample pretreatment. Ionization is effected by spraying the sample with an electrically charged aqueous mist, achieved by directing a pneumatically assisted electrospray at the surface to be analyzed. The ions released from the surface are transported through air at atmospheric pressure for some distance before they reach the atmospheric interface of the mass spectrometer. DESI ion sources are readily connected to any mass spectrometer equipped with an atmospheric pressure interface. Depending on the capabilities of the mass spectrometer, tandem MS, selected ion monitoring, exact mass, and other types of measurement are possible. In an important variant on the DESI method, selected chemicals can be added to the spray solution to provide specificity for the ionization of particular types of analytes. In all cases, the sample remains fully accessible to observation as well as additional physical and chemical processing during analysis.

Initial applications, discussed or cited here, are the identification of natural products in plant material, high-throughput analysis of pharmaceutical preparations, drugs and drug metabolite identification, and quantitation in blood and other biological fluids, as well as the direct monitoring of biological tissue for biomarkers and *in vivo* analysis. The DESI method is applicable to the analysis of proteins and protein complexes, carbohydrates, and oligonucleotides, as well as industrial polymers and small organic molecules. Protein DESI spectra are almost identical in appearance to ESI spectra, but the samples are quite different. **I**n ESI, the protein is dissolved in an appropriate solvent, while in DESI, the protein being analyzed is either deposited on a selected surface as the solid or is found within a tissue or other complex material. Another focal point of DESI applications is forensics and public safety. Explosives,[19] toxic industrial compounds, and chemical warfare agents are detected with high sensitivity and specificity on surfaces of a variety of common types of materials including paper, plastics, luggage, etc. as the pure compounds, in formulated mixtures (e.g. in the form of plastic explosives), and in complex solid and liquid matrixes. In these types of applications, the sensitivity, specificity, speed of response, and lack of sample preparation are all commanding advantages.

INSTRUMENTATION, METHODS, AND EXPERIMENTAL

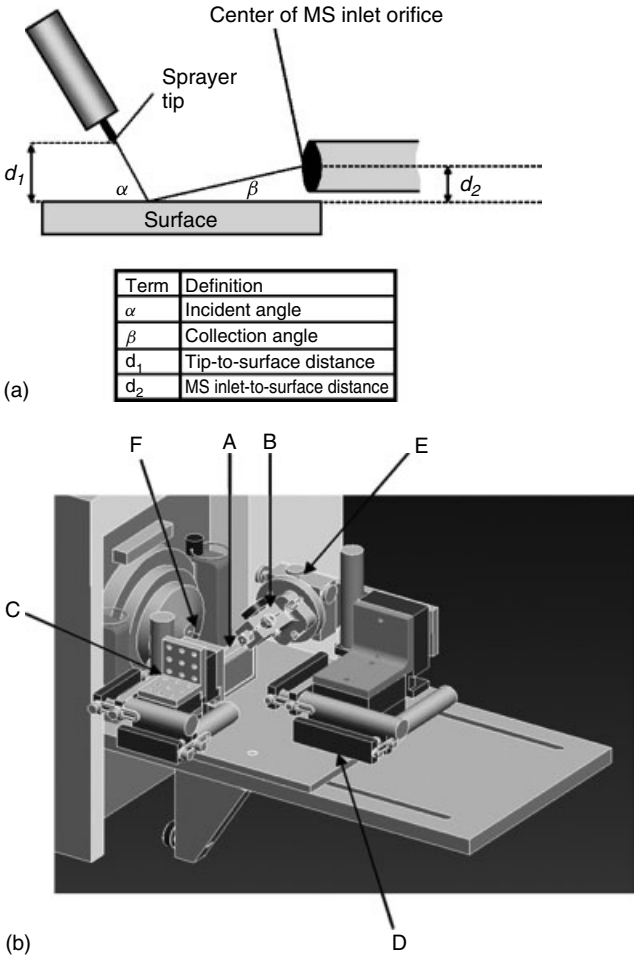
The nature of the instrumental tasks involved in DESI reflects the dual character of the method. On the one hand, DESI can be considered an atmospheric pressure DI method that involves the construction of an appropriate ion source that can be fitted to existing mass spectrometers. On the other

hand, DESI is likely to broaden the general application area of MS to such an extent that it will demand the development of new mass spectrometer systems suitable for the emerging applications. This presentation focuses on the former aspect.

DESI ion source

In many applications, samples are spotted onto a surface, which is mounted on a target planchette, by thin sample sections or even three-dimensional objects, are examined. A suitable ion source is basically a pneumatically assisted micro-electrospray source equipped with a surface holder and a positioning device. The source comprises two main parts, a sprayer assembly and a surface assembly, both mounted on a source base (Fig. 1). The sprayer itself is mounted onto a vertical rotating stage, which in turn is mounted onto a 3D linear moving stage. The linear movement is used not only to change the sprayer-to-MS or sprayer-to-surface distance but also to compensate for the different angles at which the sprayer is used. The rotating stage allows selection of impact angles of 0° to 90°. The surface holder is mounted on a separate 3D moving stage. In one version, the surface holder can carry 1 × 5 cm large disposable surface slides, which lie in a thin piece of stainless steel (SS) embedded into the surface holder PTFE block. The SS piece is connected to an external high-voltage power supply (Analytica of Branford) to provide an appropriate surface potential. The range of surface potentials available is identical to the range for the ion source. In a different surface holder design, the surface holder is a PEEK-coated aluminum block, which has a built-in heater cartridge and thermometer. This surface holder provides controlled surface temperature using an external temperature controller (Omega), allowing access to temperatures in the range from ambient to 300 °C. The newest DESI ion sources (Prosolia, Indianapolis, commercial prototype) features two CCD cameras and a light source.

For high-throughput applications, the surface assembly is replaced by a moving belt system. The position of the belt is optimized so as to expose the samples (e.g. pharmaceutical tablets) to the spray in an optimal and reproducible fashion.



Term	Definition
α	Incident angle
β	Collection angle
d_1	Tip-to-surface distance
d_2	MS inlet-to-surface distance

Figure 1. (a) Definitions of terms used in conjunction with DESI. (b) DESI ion source (prototype omnispray (Prosolia)) mounted on a Thermo Finnigan LTQ instrument. A: surface holder block; B: sprayer; C: 3D moving stage for surface alignment; D: 3D moving stage for sprayer alignment; E: rotating stage for sprayer; F: mass spectrometer inlet.

DESI wand

The laboratory source design described above does not take full advantage of the most advantageous feature of DESI, the capability for the investigation of virtually any sample in the ambient environment. In order to fully utilize this capability and to provide free access to the sample surface, a so-called DESI-wand system can be used. The wand system comprises two main elements – one is a miniaturized, rugged DESI source, while the other is a long ion-transfer tube that transfers analyte ions from the sample surface to the mass analyzer. The ion-transfer capillary can be combined with the gas and liquid supply tubing of the source to form a compact probe, which ends in the DESI source. One can imagine this type of device being used for *in situ* chemical analysis during surgery, e.g. a situation in which a conventional ion source would not be useful.

Fieldable mass spectrometers

While there has been rapid progress in the development of small, low-power mass spectrometers for in-field applications,[20] these instruments are not fitted with atmospheric interfaces and, hence, are not suited for DESI. Development of new, fieldable mass spectrometers on which DESI experiments can be done is a task currently being undertaken in this laboratory.

The main challenge in designing such an instrument is to provide efficient ion transfer from the ion source to the fore vacuum region of the mass spectrometer without increasing the pumping capacity of the vacuum system. Ions can be transferred through capillaries at atmospheric pressure,[21] using both insulator and conductor materials. However, in the case of insulators, charge buildup occurs on the inner surface of capillary, can result in poor transmission. Although metal capillaries do not suffer from this phenomenon, extensive neutralization can cause poor transmission efficiency.

DAPCI (desorption atmospheric pressure chemical ionization)

Desorption atmospheric pressure chemical ionization (DAPCI) is a variant of DESI, which employs gaseous ions of volatile compounds as the primary probe beam in a DI experiment. In the first reported experiment,[19] the primary ions were produced by corona-discharge ionization of vapors carried by a high-velocity nitrogen jet. DAPCI experiments were carried out using the same surface and source assemblies as those used in DESI experiments except that the sprayer was replaced by a corona-discharge ion source. The corona-discharge ion source used is similar to that used in a previously reported electrosonic spray ionization (ESSI) source;[22] however, the sprayer capillary was replaced by a pointed SS wire (10 cm long, 0.1-mm diameter) and the nitrogen gas included a variable concentration of a reagent vapor such as toluene. Volatile reactants were directly mixed into the high-velocity nitrogen flow using a T-junction and fused silica capillary introduced into the gas line. High voltage was applied directly to the SS needle.

Sample preparation

In the case of biological tissues, no sample treatment was necessary. Freshly cut surfaces of plant tissues or microtome sections of animal tissues were directly exposed to the spray. In a few experiments, whole organs were examined. In the case of biological fluids (e.g. blood, urine, plasma) or other solutions, 1–5 μl of sample was deposited on the desired surface and allowed to dry. CAUTION: In the case of biological fluids and intact bacterial cells, the surface has to be chosen carefully, because aerosol formation from these samples may present a biohazard. A good surface for these samples is etched glass; plain PTFE surfaces should be avoided.

RESULTS AND DISCUSSION

Analytical performance and parameters

While it is impossible to use a single set of numbers to represent the performance of a complex mass spectrometer system, some quantitative data for a few specific experiments provide a summary of the capabilities of DESI when used with an ion trap mass spectrometer, such as the Thermo LTQ instrument. Limits of detection (LODs) are 10–100 pg for RDX, HMX, TNT, and pentaerythritol-tetranitrate (PETN), where these explosives are present on paper, leather, plastic, and a variety of other ordinary surfaces. Note that the sample is largely unused after the experiment and also that these are not extrapolated detection limits; they refer to total amounts of sample placed on these surfaces. In these as well as in most other experiments, the total time for analysis is typically 5 s; in many cases, this includes the time needed to record MS/MS spectra as well as the primary mass spectra. The sensitivity of DESI in protein and peptide analysis is a little lower than that of MALDI. Absolute detection limits for 3-s experiments with the enzyme lysozyme fall in the range 10–50 pg and with bradykinin, in the 1-pg region. The upper end of the mass/charge range of DESI has not been determined, since it was limited by the mass spectrometers that have been employed. However, proteins like lysozyme or cytochrome *c* with molecular masses in the range of 10–20 kDa give spectra from which molecular weights can be determined. An additional performance characteristic of interest is the sampling area which is typically *ca* 4 mm^2, although much smaller areas have been sampled in some tissue-imaging studies.[23]

There are three analytical performance characteristics on which adequate information is not yet available. These are suppression effects, namely, the degree to which analytes suppress the ionization of other analytes present in the same material; overall yields (i.e. the fraction of the original material that is converted into gas-phase ions at the detector of the mass spectrometer); and the surface sensitivity, i.e. the sampling depth. Additional comments on each of the above analytical characteristics are made at appropriate points in the text.

Spectral characteristics

Spectral characteristics of DESI are very similar to those of ESI in the cases of typical electrospray analytes like peptides, proteins, and drug molecules (Fig. 2). DESI spectra feature multiply charged ions, alkali metal adducts, and noncovalent complexes originating in the condensed phase, similar to

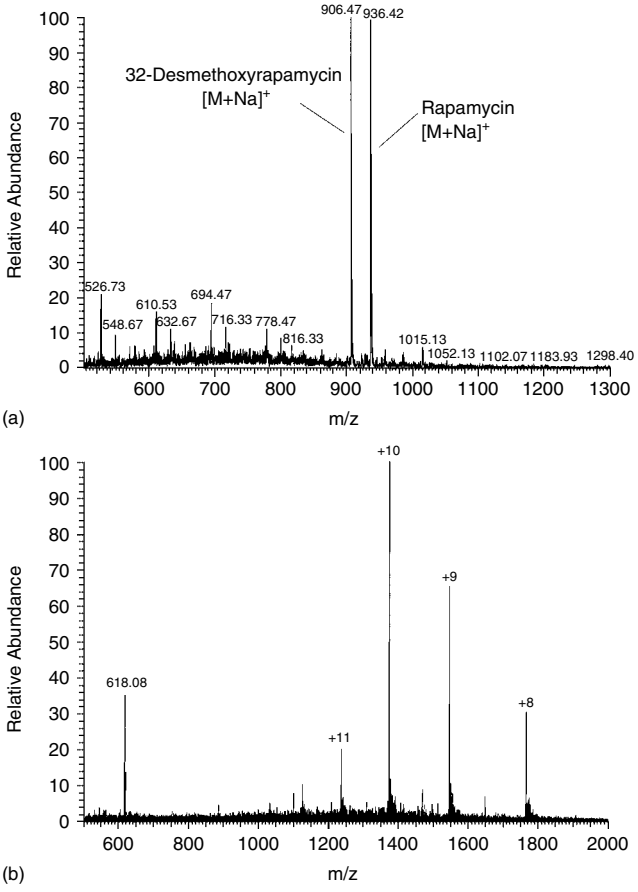


Figure 2. (a) Positive ion DESI spectrum of human serum spiked with 60 μg/l rapamycin and an equal amount of 32-desmethoxy rapamycin. Ions at *m/z* 906 and 936 correspond to the sodiated molecules of each compound. (b) Positive-ion DESI spectrum of 10-ng equine cytochrome *c* deposited on a PMMA surface.

observations made for ESI. There are marked similarities between the spectra recorded by the two techniques in the case of proteins, and this is ascribed to the similarity in the latter stages of the mechanisms of ion formation, which in both cases proceed via analyte-containing charged droplets. Solvent adduct formation is a general feature of the DESI ionization of proteins; however, it can be minimized by appropriate choice of instrumental settings (heated capillary temperature, tube lens voltage). The available data suggest that the charge-state distributions of protein ions are shifted toward lower charge states in the case of DESI compared to ESI.[24] This phenomenon, together with a more pronounced tendency for metal adduct formation, can be associated with the lower specific charging of offspring droplets in DESI. Comparisons of DESI and ESI in ion mobility experiments reveal similar charge states but somewhat more gentle ionization conditions in DESI.[24]

DESI can also be used to ionize molecules that are generally not ionized by electrospray, e.g. nonpolar compounds such as cholesterol, carotene, and TNT. Spectral characteristics of DESI in such cases are similar to those of corona discharge or atmospheric pressure chemical ionization (APCI). Besides the generally observed protonation/deprotonation processes, electron abstraction and electron capture are also observed for these types of samples. A general finding is the absence of multiply charged species in these types of analytes.

Dynamic range and quantitation

Tests of dynamic range were made both for individual analytes and for mixtures. A dynamic range of 5 orders of magnitude was observed in the case of peptides melittin and bradykinin (Fig. 3). In the case of mixtures (such as tryptic digests), the dynamic range is considerably smaller, and suppression effects can be observed (Fig. 3(b)). Similar effects were observed in the case of intact proteins. Suppression can be minimized by decreasing solvent flow rates and increasing the nebulizing gas flow rate. Since both actions decrease droplet size[25,26] in the spray, this favorable effect is associated with the smaller primary droplets produced under these conditions.

The quantitative accuracy of DESI appears to be typical of that of many other mass spectrometric experiments, with relative standard deviations (RSDs) of about 5%. Lower values are attainable by use of appropriate internal standards (Fig. 4). In the case of samples deposited on solids from solution, internal standards can be added to the solution prior to deposition. In the case of natural surfaces, however, use of internal standards is not straightforward. The surface of the sample can be doped with an internal standard by means of, e.g. electrospray deposition, or the internal standard can be directly added to the sprayed solvents, but these methods do not give strictly quantitative results. Fortunately, the investigation of natural surfaces often requires only relative quantitative determinations of related chemical species, rather than absolute quantitative distributions of entirely different species. Since the peak ratios in DESI spectra show high reproducibility in the case of natural surfaces (*vide infra*), DESI spectra are still highly informative.

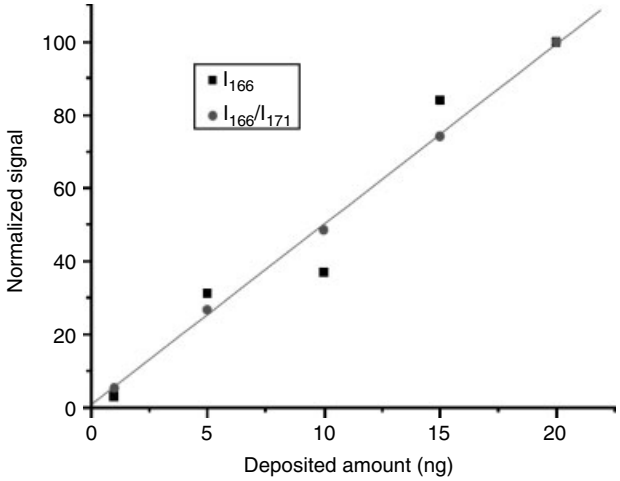


Figure 4. Feasibility of quantitative determinations by DESI with or without using internal standards. Various amounts of phenylalanine (Phe) were deposited onto a glass surface together with a standard amount (5 ng) of Phe-d_5. One series of data shows intensity of $[Phe + H]^+$ ions (squares), while the other set shows the intensity ratio of analyte and internal standard.

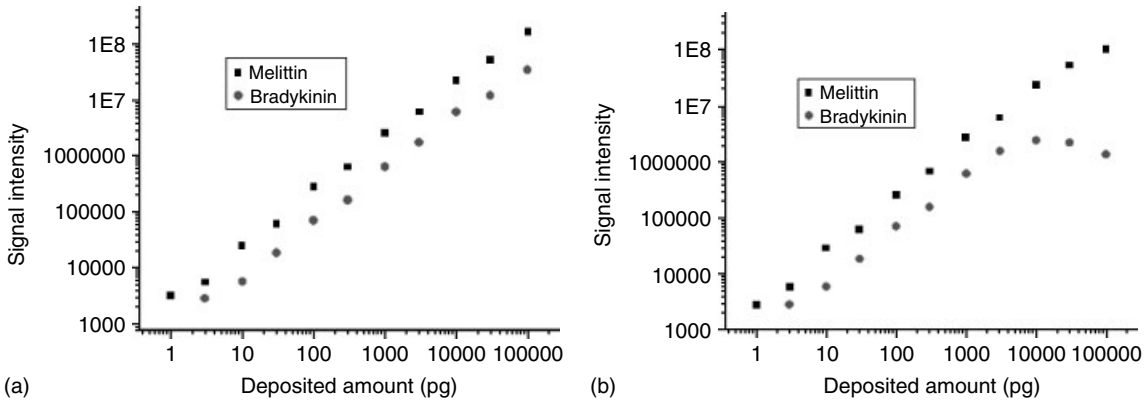


Figure 3. Signal intensity *versus* amount of peptide deposited. Data sets shown in (a) were recorded separately, while in (b), the peptide solutions were mixed prior to deposition. (a) Illustration of the 5-orders-of-magnitude dynamic range of DESI for a pure compound. (b) Strong suppression effect at higher surface concentrations (peptides were deposited in 1-μl solvent onto PMMA surface, methanol/water 1 : 1 was used as solvent, at 0.5 μl/min flow rate).

Spatial resolution

DESI allows chemical imaging of surfaces, an experiment in which spatial resolution is an important performance parameter. Parameters influencing spatial resolution include the incident angle (α), the spray tip-to-surface distance (d_1), the spray tip diameter, the solvent flow rate, the nebulizing gas flow rate, and the orifice diameter of the entrance capillary into the mass spectrometer. The effects of tip size and d_1 on resolution are shown in Fig. 5. As shown in the figure, a spot size of 50 μm can be achieved by using a 1-μm nanospray tip. Note that the actual resolution achieved is limited not only by the available emitter tip sizes but also by signal intensity, which was observed to be proportional to the size of sampled area. A further significant problem in imaging applications is contamination of the surface in the close vicinity of the sampled area. One surface-scanning strategy developed to overcome these problems and to achieve optimal resolution and minimum contamination used $\alpha = 60^\circ$, with each data point being interrogated until the complete disappearance of signal (exhaustive ionization). The spray was rastered across the surface, as shown in Fig. 6.

Some applications (e.g. explosives detection from surfaces) require sampling from as large an area as possible, as opposed to a very small area. Increasing the sampled area gives rise to problems too. Firstly, emitter tip sizes cannot be freely increased, since stable electrosprays cannot be achieved for tip sizes larger than 1 mm. The other problem is the collection of ions from larger areas into the mass spectrometer. The experimental setup shown in Fig. 7 offers a solution to these problems. The depicted device is expected to be capable of interrogating an area of ~10 cm^2.

Surface effects

Both the chemical composition and the texture of a surface dramatically affect the ionization process. Particularly important with regard to DESI ionization is the electrical conductivity of the surface. Since the DESI mechanism involves the landing and release of charged particles at a surface, neutralization at the surface must be avoided. Neutralization is most likely in the case for conductive materials, such as metals or graphite, which must either be carefully isolated or floated at a potential that is equal to or lower than the spray voltage. (For the effect of surface potential on the ionization process, see discussion of mechanisms in the following below.) In the case of insulators, the electrostatic properties of the surface are also very important. Signal stability depends strongly on whether the surface prefers the polarity of the spray or not. For instance, PTFE is a highly electronegative polymer and gives excellent signal stability in the negative-ion mode, while PMMA gives better performance in the positive-ion mode.

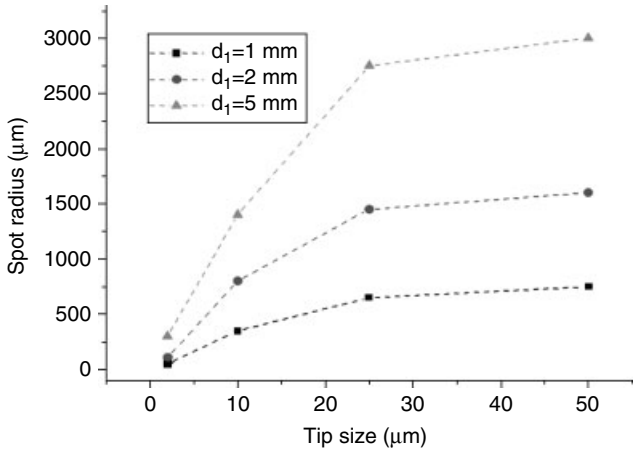


Figure 5. Diameter of sampled spot as a function of spray tip diameter in the case of different tip-to-surface distances (d_1). Impact angle (α) was 90° in all cases (Methanol/water 1 : 1 solvent containing 0.01 mM Fe–dipyridyl complex was sprayed onto paper surface at 0.1 μl/min. Traces were evaluated by densitometry).

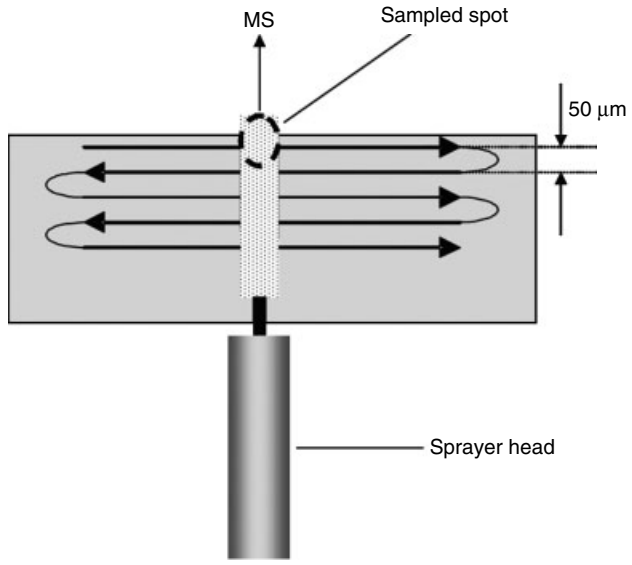


Figure 6. Surface-scanning strategy for maximum spatial resolution. The orthogonal diameter of the sampled spot can be decreased to 50–70 μm. This spot can be scanned along the surface in a controlled fashion. The surface is then moved by 50 μm and rescanned. The surface area scanned in the first line is not sampled again and the sprayer–MS distance is kept constant.

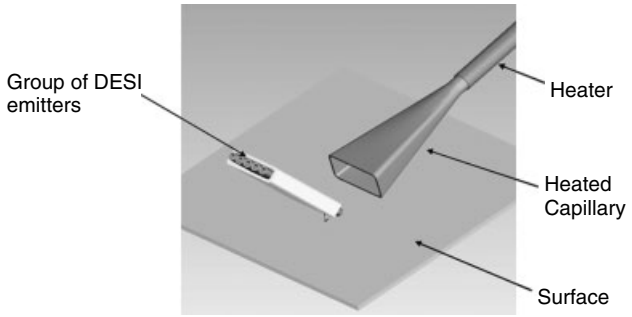


Figure 7. Concept of a DESI ion source for the interrogation of a larger surface area. All applications aimed at the trace-level detection of species on different objects (e.g. detection of explosive residues on suitcases or traces of drug in a car trunk) require this kind of feature. The multiple sprayers used for sampling are as large as 10–20 cm^2. A funnel-shaped mass spectrometer inlet is utilized to collect the spatially distributed ions.

The chemical nature of the surface is also an important parameter. When the sample is deposited from solution, the nature of the surface may affect crystallization of the analyte, causing uneven distribution of the analyte on the surface. High affinity of analyte molecules to the surface results in a loss of sensitivity. For instance, nucleic acids cannot be ionized from the positively charged Amersham Hybond-N+ (Amersham Biosciences) membrane. PTFE is a generally applicable surface, in part because of its low affinity toward most analytes.

Surface roughness was also observed to have an effect on ionization efficiency. Microscope glass slides were used as DESI substrate before and after HF etching. Etching was found to dramatically increase signal stability and to eliminate 'sweet spot' effects. In agreement with this consideration, rough surfaces like paper give high sensitivity and are often the substrates of choice for DESI.

Table 1 summarizes the optimized instrumental parameters for a wide variety of different compounds and gives the corresponding LOD values.

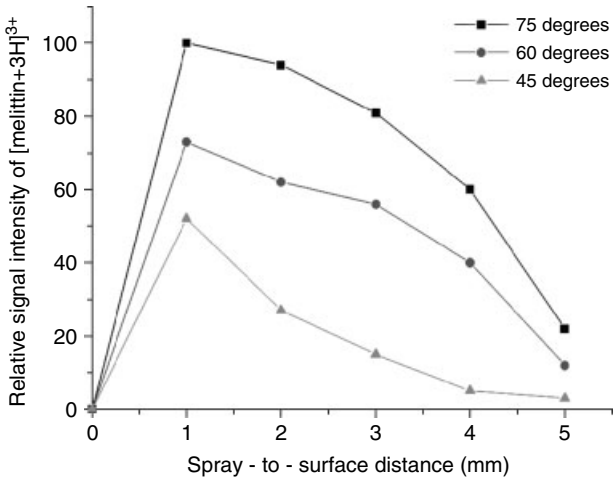


Figure 8. Dependence of signal intensity of triply charged melittin ions on the spray position at various spray impact angles (10-ng melittin was deposited on a PMMA surface, 1 μl/min methanol/water was sprayed).

Dependence of DESI on instrumental parameters

A remarkably large number of parameters affect DESI performance. One result is that operators usually go through a learning process during which they produce data of increasing quality. The parameters of most importance include geometric parameters (α, β, d_1, d_2; see Fig. 1(a)), spray parameters (gas and liquid flow rates, high voltage), chemical parameters (sprayed solvent, solvent used for deposition of sample), and surface parameters (composition, temperature, potential).

With regard to geometric parameters, α and d_1 have direct effects on the ionization process, while the other two parameters have important effects on the collection efficiency and, hence, on the sensitivity of the method. The optimal setting is generally 5–10° for β and 0–2 mm for d_2. The dependence of signal intensity on the parameters α and d_1 is shown in Fig. 8. In the case of melittin and other peptides and proteins, low d_1 values are preferred, clearly indicating the essential role of charged droplets in the ionization process. (As d_1 is increased, evaporation becomes

Table 1. Analytical performance in desorption electrospray ionization mass spectrometry

Compound	Detected species (most abundant)	LOD absolute amount/ concentration pg; pg/mm^2	Surface	Solvent	α (°)	d_1 (mm)
Melittin	$[M+3H]^{3+}$	0.1; 0.05	PMMA	MeOH/Water	72	1
Bradykinin	$[M+2H]^{2+}$	0.7; 0.04	Glass	MeOH/Water	80	1
Angiotensin	$[M+2H]^{2+}$	1.2; 0.05	PMMA	MeOH/Water	75	2
Insulin	$[M+4H]^{4+}$	150; 30	PMMA	MeOH/Water	70	1
Cytochrome c	$[M+9H]^{9+}$	500; 40	Glass	MeOH/Water 0.1% AcOH	65	1
Lysozyme	$[M+8H]^{8+}$	400; 40	PMMA	MeOH/Water	70	1
Apomyoglobin	$[M+10H]^{10+}$	1000; 80	PMMA	MeOH/Water	75	1
NADH	$[M-H]^-$	250; 20	PTFE	1 mM NH_3 in water	67	2
Phenylalanine	$[M+H]^+$	10; 1	Glass	Water	65	2
Raffinose	$[M-H]^-$	1200; 100	PTFE	1 mM NH_3 in water	70	2
Glucose	$[M+Na]^+$	800; 90	Glass	0.1 mM Nacl in water	64	2
Chitohexaose	$[M+H]^+$	150; 20	Glass	Water	60	1
Loratadine	$[M+Na]^+$	50; 5	PMMA	MeOH/Water	65	2
Rapamycin	$[M+Na]^+$	100; 10	PMMA	0.1 mM Nacl in water	60	3
TNT	$M^{-\bullet}$	10; 1	Paper	Water	35	6
RDX	$[M+H]^+$	20; 1	PTFE	MeOH/Water 0.1% AcOH	40	7
HMX	$[M+Cl]^-$	50; 5	Paper	1 mM HCl in water	35	6
PETN	$M^{-\bullet}$	100; 10	SS	MeOH/Water	40	4
Cholesterol	$[M+H]^-$	150; 10	PMMA	MeOH/Water	30	8
β-Carotene	$M^{-\bullet}$	88; 50	Glass	Water	25	8
Coronene	$[M+H]^+$	2000; 100	Glass	Methanol	30	10

more complete.) Also clearly shown in the data is the fact that high α values are preferred, although a considerable increase of chemical noise is observed above 80°. The mechanistic implications of the data of Fig. 8 are described in the section on mechanism.

Spray parameters also influence the spectral characteristics of DESI. These parameters determine the size distribution, average charging, and velocity of impacting droplets and ions. The effect of these parameters on signal intensity is shown in Fig. 9. Interestingly, the signal intensity does not drop to zero in the absence of an electrospray high voltage. It is known[27] that the sprayer used here also works in the sonic spray mode, generating charged droplets without application of a high voltage. The ion currents are considerably lower in this case; however, this mode of operation may find application in areas where the use of high voltage is inappropriate, e.g. for *in vivo* applications. The observed saturation of the response curve at high voltage is similar to that of simple ESI itself. Turning to another parameter, the gas flow rate has a dual role in DESI. High gas flow rates decrease the initial droplet size and increase the velocity of impacting droplets. These phenomena are advantageous up to a point; because smaller droplet sizes favor enhanced desolvation efficiency and droplets having higher velocity produce more offspring droplets upon impact. Above a certain limit, however, the small size and high velocity will cause droplet evaporation before impact with the surface, a result that appears to correlate with the lack of ion formation in the case of peptides and proteins. For those analytes for which gaseous projectiles are responsible for ionization, this consideration is not valid, although the high gas velocity decreases the sampling efficiency of the mass spectrometer.

Solvent flow rate is also expected to have an effect on droplet size distribution and on the average charge carried by the droplets. At low solvent flow rates, the droplet size may be too small for survival of the droplets from the spray tip to the surface, a phenomenon similar to that described in the case of high gas flows. High solvent flow rates result in the formation of larger droplets, which might cause inefficient desolvation and, in extreme cases, accumulation of liquid on the surface. These constraints define a clear optimal working range for solvent flow rates which is summarized, together with other optimum values, in Table 2.

The effects of the nature of the surface have already been discussed. Adjustable surface parameters include temperature and the surface float potential. Elevated surface temperatures increase the ion yield, as shown in Fig. 10. The ion current, however, shows a strong decrease with temperature, in the case of melittin at temperatures above ~120°. This phenomenon can be associated with the complete evaporation of droplets on impact with the hot surface. This breakpoint temperature strongly depends on the solvent system, spray parameters, and geometrical parameters. For β-carotene, the ionization efficiency increases with surface temperatures above the boiling point of the solvent, indicating that ionization occurs by gas-phase proton transfer or chemical sputtering (Fig. 10(a)). In the case of particular samples, thermal degradation might also need to be considered. Surface potential is also an important parameter. In the case of metals (or other conductive

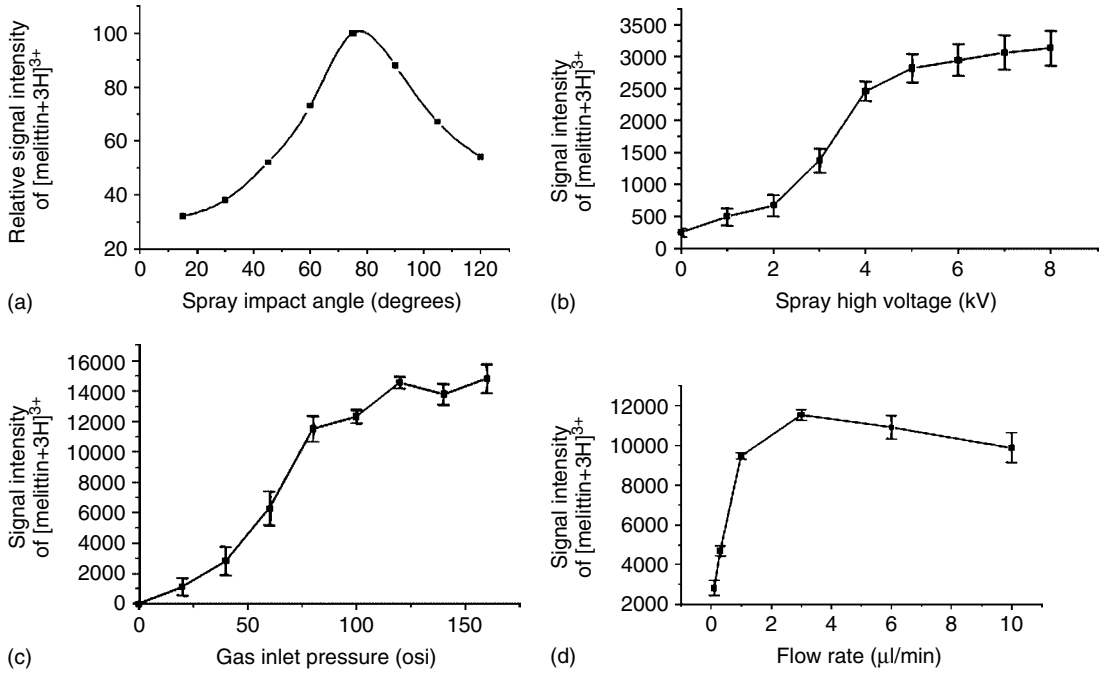


Figure 9. Dependence of signal intensity of triply charged melittin ions on (a) spray impact angle, (b) spray high voltage (the data show a strong similarity with the voltage dependence of ESI), (c) nebulizing gas inlet pressure (measured at the regulator), and (d) solvent flow rate. In each case, 10-ng melittin was deposited on a PMMA surface; 1 µl/min methanol/water was sprayed in cases (a)–(c); nebulizing gas was used at 10 bar inlet pressure in cases (a), (b) and (d); 3-kV spray voltage was used in cases (a), (c) and (d); and 80° spray impact angle was used in cases (b)–(d). Spray tip-to-surface distance was 1 mm in all cases. The sprayer used is described in detail in Ref. 22.

Table 2. Optimum parameters in desorption electrospray mass spectrometry

Parameter	Optimal setting	
Analyte type	Peptides, proteins, carbohydrates, nucleic acids	Explosives, lipids, aromatic hydrocarbons
Electrospray voltage	1–4 kV	3–8 kV
Electrospray flow rate	0.1–3 μl/min	–
Nebulizing gas linear velocity	>350 m/s	–
Heated capillary temperature	200–350 °C	200 °C
Tube lens potential	200–250 V for proteins	30–150 V for small molecules
Capillary inlet sample distance	1–2 mm	2–8 mm
Tip sample distance	1–2 mm	5–8 mm
Incident angle (α in Fig. 1)	60–90 degree	20–50 degree
Collection angle (β)	<10 degree	10–15 degree

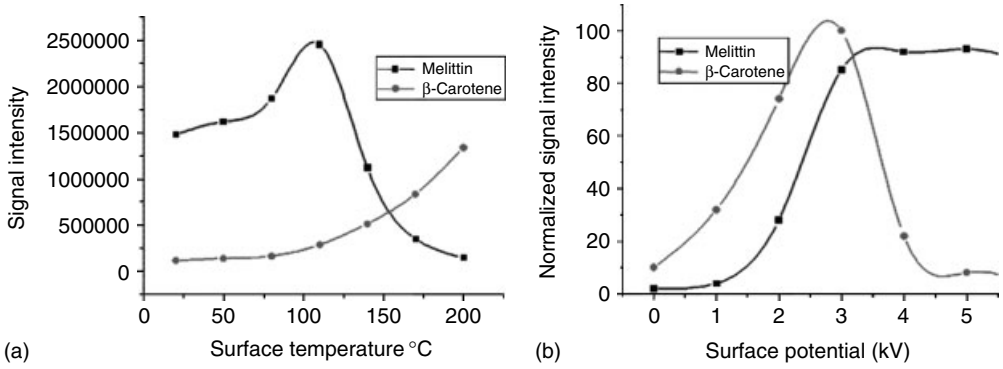


Figure 10. Dependence of signal intensity on (a) surface temperature and (b) surface potential in the cases of melittin and carotene. 1-ng melittin and 100-ng carotene were deposited on (a) PEEK and (b) SS surface (methanol/water 1 : 1 was sprayed at 1 μl/min).

surfaces), the application of a voltage onto the surface is necessary to obtain acceptable signal intensity. The signal intensity does not actually drop to zero in the case of grounded metal surfaces. This residual signal is associated with fusion of charged droplets not undergoing surface impact and offspring-neutralized droplets from the surface. When the difference between the surface potential and the high voltage on the spray drops below ~3 kV, the signal intensity for carotene (and other low-polarity compounds like cholesterol or TNT) decreases dramatically, while the intensity of melittin ions remains practically unchanged. The mechanistic implications of these and other observations are taken up further in the next section.

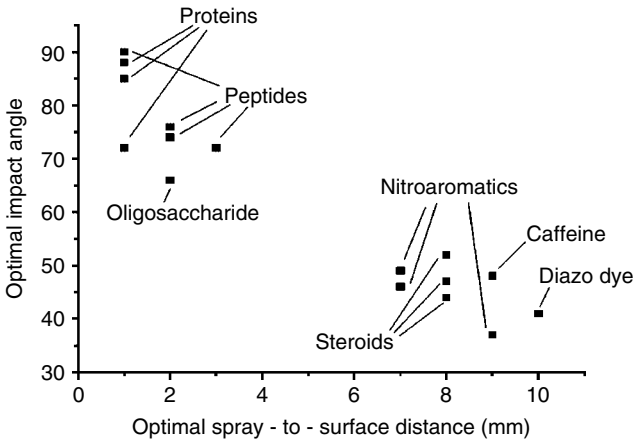


Figure 11. Optimal combinations of spray impact angle and spray position for different compounds (glass surface, 10 ng of each compound, 1 μl/min methanol/water; optimization was performed to obtain the best S/N).

Mechanistic considerations

The data just cited, the optimized values shown in Table 2, and even more clearly, the data of Fig. 11, show that analytes can be divided into two subgroups on the basis of experimental conditions which optimize DESI. The analytes in each group are similar to those traditionally grouped as typical electrospray analytes and APCI analytes in the case of LC–MS applications. These two groups appear to correspond to analytes that are ionized by different mechanisms of ionization in DESI.

Analytes in the electrospray group, such as peptides or proteins, show electrospray-like spectra in DESI, indicating an ion formation mechanism that is proposed to involve the formation of charged droplets containing analyte species. The observed formation of multiply charged ions virtually excludes gas-phase ionization mechanisms. Formation of specific, solution-phase complexes between sprayed species and molecules on the surface[4] also supports a mechanism involving charged droplets containing the analyte. Optimum parameters for the ionization of peptides include a short d_1 distance and moderately high gas and liquid flow rates. These parameters again suggest that impact of charged droplets on the surface is essential for the ionization of these analyte species. The first event when a droplet impacts

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on a surface is the spreading of the liquid to cover an area having a diameter 3–10 times larger than the original droplet diameter. During this process, offspring droplet formation ('jetting') occurs around the edge of expanding liquid film.[28] This phenomenon is similar to the shock-wave-induced secondary charged droplet formation, which was described in the case of the MCI technique.[12] The process is enhanced by the increasing velocity of impacting droplets. At the velocities used in DESI (estimated to be on the order of a few hundred m/s), the jetting may consume most of the liquid. Some of the net charging of the droplets is presumably lost to the surface; thus, the specific charge of offspring droplets will be lower than that of the original droplet. The offspring droplets are assumed to be transferred into the mass spectrometer by the gas jet of the spray, by coulombic effects, and by dynamic drag from the vacuum interface. The importance of the pneumatic contribution to this mechanism is supported by the fact that peptides still undergo ionization, even when the surface is kept at the spray potential using the same high-voltage power supply (Fig. 12). In such a case, charging of secondary droplets occurs in the region between the analyte surface and the mass spectrometer inlet.

Analytes in the 'APCI group', such as cholesterol, carotene, or TNT, are not ionized under the experimental conditions just described. However, this set of compounds is ionized when the potential difference between sprayer and surface exceeds 2 kV (Fig. 10(b)). Ionization of these analytes apparently does not require or involve charged droplets. The spectral characteristics and dependence of signal intensity on d_1 and on the surface temperature indicate that the main ion formation process is charge transfer between solvent ions and analyte molecules. These heterolytic charge-transfer reactions include the exchange of a proton or electron between a gas-phase ion and a surface molecule. The occurrence of purely gas-phase ion–molecule reactions cannot be completely excluded in these cases, and it is expected that their contribution to the overall signal intensity will be greater for compounds of greater vapor pressure under given conditions.

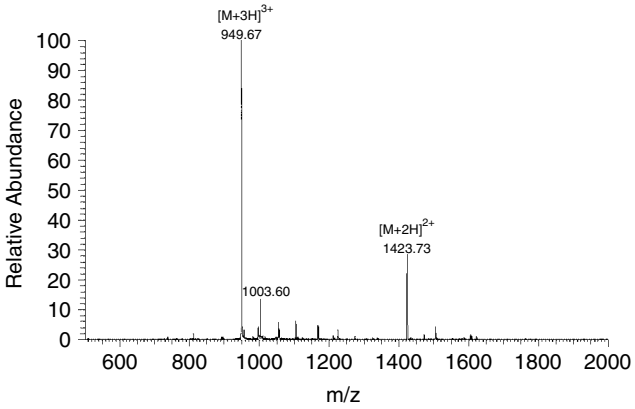

Figure 12. DESI spectrum of 10-pg melittin on a SS surface held at the same voltage (5 kV) as the sprayer. Methanol/water 1 : 1 was sprayed at 1 µl/min, $\alpha = 85°$, $d_1 = 2$ mm. Note that the lack of a potential difference between sprayer and surface results in more intense cluster formation.

Ionization by heterogeneous charge transfer (chemical sputtering, see Ref. 14) usually involves proton-transfer reactions and, as expected, it is controlled by the relative proton affinity (PA) of the species involved. As an example, coronene ($PA = 861$ kJ/mol) is readily ionized by methanol ($PA = 754$ kJ/mol); however, when spraying with acetone ($PA = 816$ kJ/mol), no coronene species was observed in the positive-ion DESI spectrum. In order to provide further evidence for this heterogeneous charge exchange mechanism, a DAPCI ion source[19] was constructed to produce gaseous ions of volatile solvents by means of corona-discharge ionization of the vapor. The resulting ions are carried by a supersonic nitrogen gas jet to the surface, similar to the case in DESI. DAPCI ionization of cholesterol, carotene, coronene, and other compounds using protonated methanol reagent ions leads to results identical to those recorded for these analytes by DESI. In the negative-ion mode, when using toluene anions as reagent, TNT readily undergoes ionization as shown in Fig. 13. The TNT signal intensity was highly dependent on the high voltage of the electrospray source, strongly implicating the corona discharge as the primary source of electrons for the electron capture ionization. The spectrum shows that the species responsible for carrying the electrons was identified in this case. As expected, TNT was not observed to form positive ions in DESI ionization, since its proton affinity is considerably lower than that of methanol. In the case of peptides, carbohydrates, nucleotides, and other typical ESI analytes, however, no signal was observed using DAPCI. The two experiments (i.e. applied surface potential and DAPCI) provide clear evidence for the existence of the two ionization mechanisms described. There is additional data to suggest that an additional mechanism plays a role in some instances. One such case is that of the ionization of piperidine alkaloids where the signal intensity is considerably higher in the case of 1 M aqueous ammonia solution than in the neutral solution. Since all the detected piperidine alkaloids have a considerable vapor pressure at ambient temperature, and all of them are highly basic, the

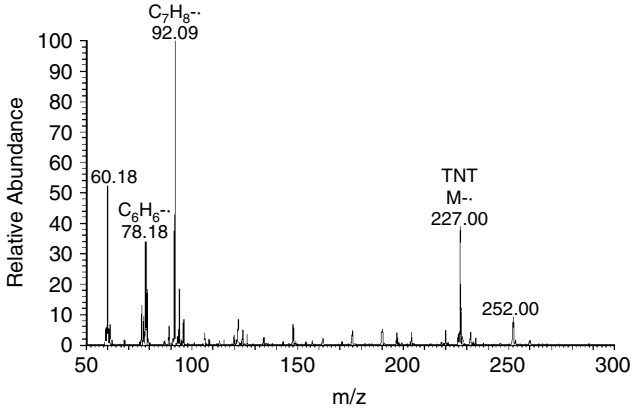

Figure 13. Negative-ion DAPCI mass spectrum of 10-pg TNT on paper, using ionized toluene as the electron carrier. Nitrogen gas containing 100-ppm toluene vapor was used at 10 bar inlet pressure, resulting in ~400 m/s linear gas velocity. A 0.1-mm diameter, tapered SS wire was used as corona-discharge needle, replacing the spray tip in the ESSI sprayer described in Ref. 22 The spectrum features $M^{-\bullet}$ ions of TNT and toluene.

function of ammonia in this case seems to be to deprotonate the analyte, thus increasing its vapor pressure and enhancing a gas-phase ionization process.

Analytical applications

Application areas of DESI analysis include environmental applications, high-throughput analysis, clinical diagnostics, food analysis, forensics, and many others. A potentially important and broad area of application of DESI is forensics, in which on-location analysis, speed, specificity, and wide applicability are all important. Applications include monitoring for public safety (explosives, chemical agents, biological pathogens, etc.) as well as monitoring for drugs of abuse, poisons, and in overdose situations. The ability to monitor selected compounds in complex matrices, including biological matrices, is a significant advantage as is the applicability to both small and large molecules and the ability to perform spatial imaging for particular molecules.

High-throughput analysis

DI methods are intrinsically very efficient tools for high-throughput analysis, since the analysis time is usually short and sample cross-contamination is minimal. The spatial resolution of currently used DI techniques, especially MALDI, allows the rapid, sequential analysis of hundreds of samples located on a single sample holder. DESI retains the advantages of DI methods with regard to high-throughput applications. The additional advantage of applicability to natural surfaces enormously broadens the potential range of applications of DESI. High-throughput applications of DESI have been demonstrated in detail[29] by recording full mass spectra on pharmaceutical tablets at a rate of 3 samples/s using a moving-belt sample carrier. The spectra show RSD values between 2 and 5%, depending on the analyte and the sampling speed.

Explosives detection

Detection of explosives has become a significant problem in analytical chemistry owing to threats to civil society and to increasing environmental problems associated with residues of explosives. Explosives detection requires techniques that are fast, sensitive, selective, and capable of analyzing a large number of samples in a high-throughput fashion. The main advantage of MS compared to other methods is its very high specificity. The main advantage of DESI when compared to other MS ionization methods is its speed, a consequence of the fact that it does not require any surface pretreatment and can be performed under ambient conditions. DESI spectra of the various explosives listed in Table 1 were recorded in both the positive- and negative-ion modes. Note that protonation, deprotonation, and electron attachment and detachment can all be used to detect various explosives. Formation of other adducts is also possible using reactive DESI experiments in which a particular ion is generated in the spray solution by addition of an appropriate reagent. A simple example is the addition of NaCl to generate the chloride adduct of RDX by Cl^- attachment; similar procedures could be used to attach acetate, trifluoroacetate, and other anions. It is a simple matter to confirm suspected explosives by MS/MS experiments. Detection limits as low as 10–100 fg were achieved, depending on the surface chosen.

PETN, a representative of the important class of polyol-nitrate explosives, gives $(M - H)^-$ and $(M - NO_3)^-$ ions in negative-ion DESI experiments from paper. The practical value of DESI in this area of forensics is further shown by the fact that DESI is capable of detecting traces of explosives present on a wide variety of surfaces including paper, plastic, glass, leather, and skin. The method is fast (it takes a few seconds to obtain spectra, including confirmatory MS/MS spectra), highly selective, and its detection limits are in the sub-nanogram range in all of the studied cases. DESI coupled to portable mass spectrometers appears capable of revolutionizing transportation security in the near future.

Chiral analysis

Mass spectrometry has only recently become of interest as a method of quantitative chiral analysis.[30,31] In a typical procedure, a mixture of the analyte, chiral reference, and a metal salt are electrosprayed and the resulting metal complexes, which include the analyte and a chiral reference compound as ligands, are mass selected and dissociated in an MS/MS experiment. The fragmentation data is treated using the kinetic method formalism,[32] and calibration plots are used to measure enantiomeric excess (ee). The common advantages of speed and sensitivity of MS are combined in this application with the ability to make quantitative measurements. The DESI technique allows this type of measurement to be made for analytes present on solid surfaces. These are precisely the types of chiral analytes that are most likely to be encountered in the pharmaceutical industry.

Plant tissue analysis

In vivo analysis of plant tissues was used in early demonstrations of the capabilities of DESI. Plant tissues can be interrogated by simply directing the spray onto native or cut tissue surfaces. Several plants (poison hemlock, *Conium maculatum*; deadly nightshade, *Atropa belladonna*; jimsonweed, *Datura stramonium*) known to contain a wide variety of alkaloids which show different distributions in the different types of plant tissues were investigated.[33] DESI spectra of various tissues of several plants showed protonated alkaloids as abundant ions. Besides alkaloids, ordinary metabolic products such as amino acids, polar lipids, or carbohydrates were also detected. In all cases, virtually all previously described alkaloids were detected, and the corresponding peaks were identified by MS/MS experiments. Although the quantitative determination of constituents in natural samples is troublesome, the reproducibility of spectra obtained from the same type of tissue provides semiquantitative information on the alkaloid content of plant parts. Possible application areas of plant analysis by DESI include toxicology and pharmacognosy.

Proteomics

Identification of proteins by mass spectrometric analysis of tryptic digests (the so-called *bottom-up strategy*) has become one of the most common applications of MS. Currently,

different mass spectrometric methodologies are used for this purpose, including various methods that combine chromatography with ESI and MALDI. The electrospray-based methods give good sequence coverage and feature multiply charged peptide ions, which are better subjects for MS/MS identification than the corresponding singly charged ions seen in MALDI. The main disadvantage of these methods is their high time demand, especially for the chromatographic steps, although these are sometimes done in parallel on multiple samples. In contrast, MALDI is fast and can be fully automated; however, the sequence coverage is usually worse than that for ESI.

Protein sequencing by DESI combines the advantages of the two techniques, since it is fast and easy to automate, but the spectral features are similar to those of electrospray. Equine cytochrome *c* was chosen as a model protein for the demonstration of DESI analysis of tryptic digests. A positive-ion DESI spectrum of a cytochrome *c* tryptic digest is shown in Fig. 14. Twenty-six ions were identified as tryptic fragments of the protein and these identifications were confirmed by MS/MS for each ion, the inset of the figure showing the MS/MS spectrum of an arbitrarily chosen peptide. The time required to record the data for protein identification using DESI was 10 s in the case of 50-ng cytochrome *c* digest deposited on PTFE. Recording all MS/MS spectra of the peptide peaks took 85 s. Theoretically, DESI can be applied to the interrogation of sample spots deposited in array format as well as in line, as in a tape format.

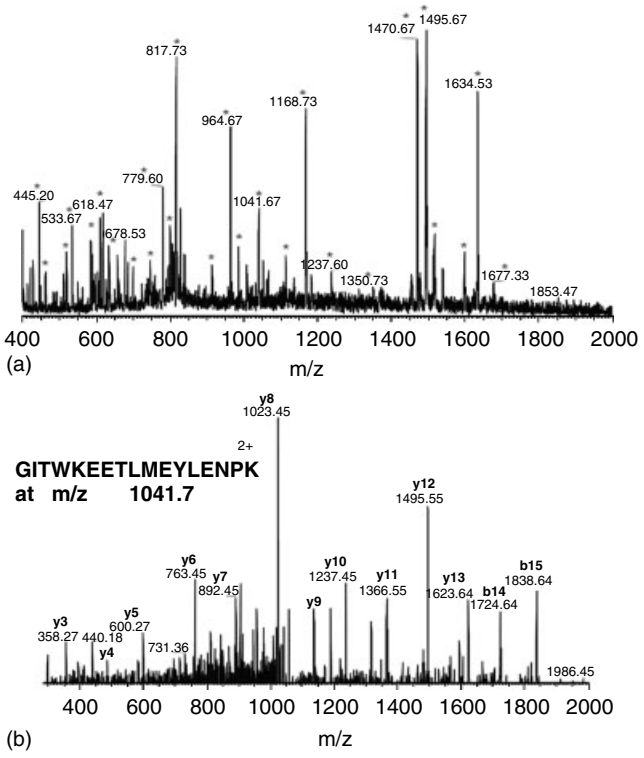

Figure 14. (a) DESI spectrum of cytochrome *c* tryptic digest and (b) MS/MS spectrum of one of the tryptic peptides. Equine cytochrome *c* was digested with TPCK-treated trypsin in NH_4HCO_3 buffer using 100 : 1 protein trypsin ratio. Digest solution containing 50-ng cytochrome *c* digest was deposited onto PTFE surface. The dried spot was analyzed by DESI using methanol/water.

Metabolites and diagnostics

During the past decade, MS has become an important tool in the field of medical diagnostics. It is now widely used for the diagnosis of inborn errors of metabolism and is an essential tool for pharmacokinetic analysis and drug metabolism studies. In contrast to other areas of application, MS-based diagnostic methods still suffer from the requirement of complex sample preparations.

Biological fluids can be directly analyzed by DESI in the form of dried spots on paper or other appropriate surface. DESI spectra of samples like dried blood, urine, or plasma spots typically show hundreds of peaks corresponding to low-molecular-weight constituents of these samples. Individual assignments are confirmed readily by MS/MS experiments. The time needed for such a DESI analysis is on the order of 10–30 s. Quantitative determinations, e.g. of blood constituents, are feasible using isotope-labeled internal standards which are added to blood samples prior to deposition on the substrate. Diagnosis of metabolic disorders, especially neonatal screening for inborn errors of metabolism, can be performed by recording DESI spectra of dried blood spots. Many diagnostically relevant metabolic constituents of blood, including amino acids, acylcarnitines, bile acids, glucose, creatinine, bilirubin, etc. are readily seen.

DESI analysis of body fluids for both drug metabolism and pharmacokinetic studies is being driven by its simplicity and ease of automation. A few experiments have shown that DESI of whole blood (dried onto etched glass) allows recognition of drugs and metabolites when these are taken orally. One example is the antihistamine loratadine (and its main metabolite).[4] In another application, DESI analysis of dried whole blood showed components that are not present in plasma but are present in the cytoplasm of erythrocytes. For example, rapamycin (sirolimus) is detected in dried whole blood spots at clinically relevant levels. Rapamycin and its derivatives usually give alkali-metal ion adducts in positive-ion mode, similar to other macrolids. Quantification of rapamycin in blood was also performed by adding 1-ppm 32-desmethoxy rapamycin to blood as internal standard (Fig. 2).

Blood proteins have also been detected from dried blood or serum samples. The positive-ion DESI spectrum of dried serum spots showed multiply charged serum albumin ions in the high mass range, while the DESI spectrum of whole blood features ions corresponding to heme and to the hemoglobin α and β chains (Fig. 15). The resolution achieved in these spectra makes the detection of hemoglobin mutations feasible. Besides the diagnostics of hemoglobinopathies, the method appears to have potential value for the detection of traces of blood in stool or urine for the diagnosis of colorectal cancer or kidney diseases, respectively.

DESI analysis of urine samples is also straightforward. In metabolomics and pharmacological studies on urine, an important advantage of DESI over other ionization methods is that salts do not need to be removed from the sample. Samples can be examined over extended periods of time

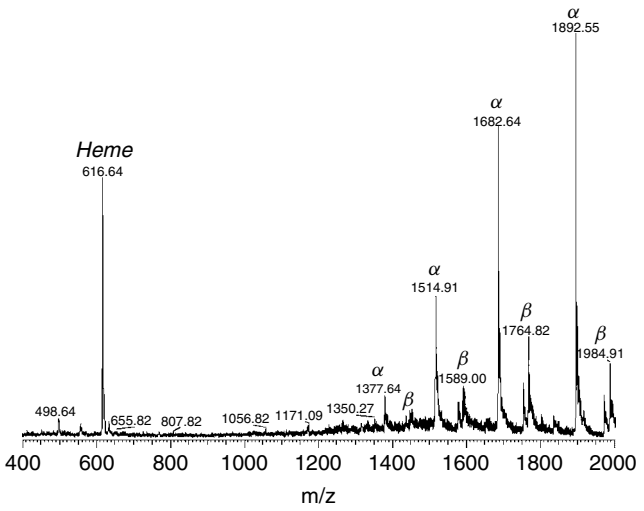

Figure 15. Spectrum of a whole blood sample diluted 10× with distilled water. A 1-μl sample of the diluted solution was dispensed onto a PTFE surface and allowed to air dry. Methanol/water 1 : 1 was sprayed at 1 μl/min. The spectrum shows ions corresponding to heme and hemoglobin α and β chains.

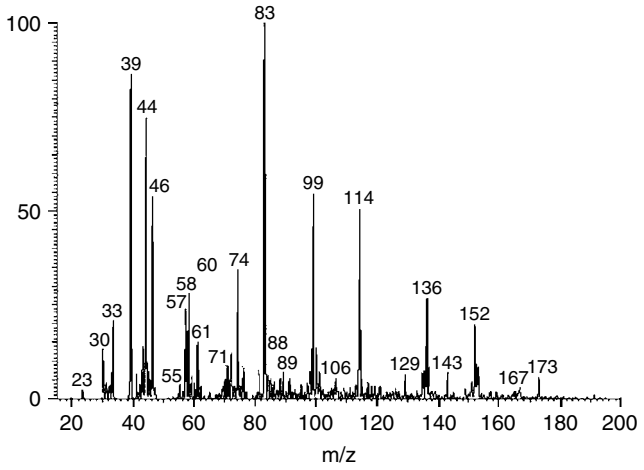

Figure 16. Direct analysis of human urine using DESI without sample pretreatment. Ions *m/z* 61, 83, and 99 correspond to the various adducts of urea, ions at *m/z* 114, 136, and 152 are similar adducts of creatinine, while *m/z* 106 is protonated serine.

without the loss of performance characteristic of other methods. Note that this advantage appears to be greatest when paper is used as the DESI substrate. The DESI spectrum of a human urine sample on paper analyzed without sample pretreatment shows many metabolites (Fig. 16). Most of the abundant peaks have been identified and confirmed by tandem MS.

Healthy and diseased rats raised under controlled conditions gave distinctive DESI spectra in direct urine analysis. Principal component analysis (PCA) based on DESI and NMR data allow ready distinction of rats with different health conditions.[34]

Analysis of intact skin is related to body fluid analysis, since in these cases, it is not the constituents of the dermal tissue that are analyzed, but excretion of glands present in the skin. The negative-ion DESI spectrum of skin shows various fatty acids and other lipids. Drugs and drug metabolites excreted with perspiration can be easily detected from the skin surface using DESI.

ADVANCED TOPICS AND CONCLUSIONS

Although MS is likely to remain the main method used to study ions generated by DESI, the fact that it generates ions in the gas phase at atmospheric pressure makes DESI compatible with ion-mobility measurements. The simplicity of the DESI source also matches well with the simple IMS instrument. Both operate in air at atmospheric pressure, have high sensitivity, and are rapid experiments. For these reasons, combinations of DESI with ion mobility are beginning to be realized, starting with a rather more complex experiment in which DESI is followed by reduced pressure ion mobility spectrometry and then by quadrupole and time-of-flight MS.[24] This experiment allowed a comparison of DESI and ESI as ionization methods for the same proteins; the ion mobility measurements gave information on unfolding of the multiply charged protein ions in particular charge states. Striking similarities were observed between the two ionization methods, although DESI appeared to be somewhat gentler in this particular comparison.

Applications of MS to microorganism characterization have developed in several stages.[35] A generation ago, very fine work was done on the identification of bacterial cultures using pyrolysis MS, a method that provided highly reproducible mass spectra that proved to be characteristic of species and even particular strains of microorganism.[36] In spite of their highly reproducible nature, most molecular information is lost in these experiments. Subsequently, MALDI and ESI have been used to characterize microorganisms by recognizing characteristic fragment ions in the MALDI mass spectra and by characterizing their coat proteins.[37] The importance of microorganism identification in connection with biosafety has increased recently, with the growing need for very rapid identification of harmful microorganisms, including those in air, water, and foodstuffs. The characteristics of DESI make it likely that it will be valuable in providing sensitive and useful information of this type; what is most valuable is the fact that the DESI experiment can be done in parallel with other experiments; e.g. DESI could be performed directly on growing cultures in petri dishes. An early indication of the potential success of this type of application is found in the fact that characteristic spectra are recorded for different microorganisms. Figure 17 shows a mass spectrum of a dried sample of *Escherichia coli* cells. It is expected that bacteria on skin, in foodstuffs, and in biological fluids will also be amenable to analysis by these methods.

Of all the emerging and possible areas of application of DESI, the one in which biological samples are imaged for the spatial distribution of particular compounds is perhaps the most exciting. The potential of MS for chemical imaging is now well recognized,[38] and it is now increasingly being used to image specific biological compounds in biological

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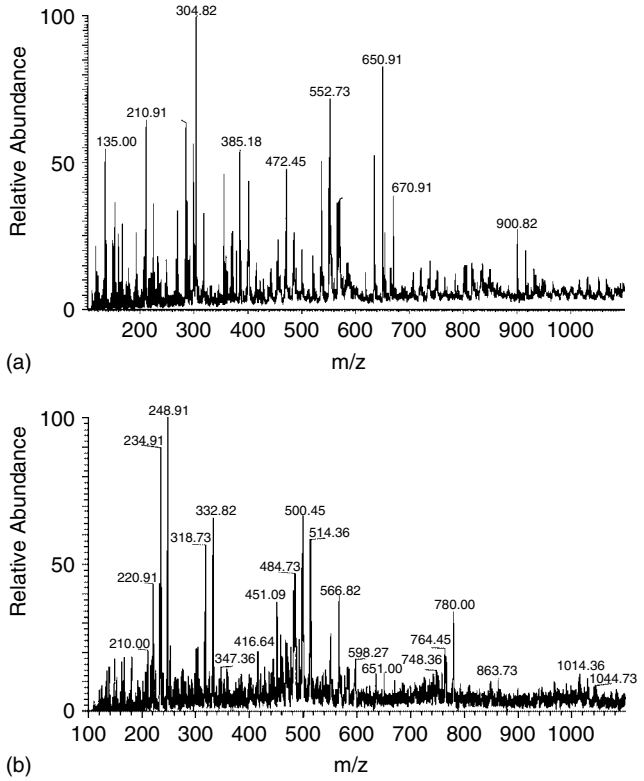

Figure 17. Positive-ion DESI spectrum of dried (a) *E. coli* and (b) *Pseudomonas aeruginosa* cells. Bacteria were cultured on LB-agar; freshly harvested cells were dried onto PTFE target, and DESI spectra were taken by using 1 μl/min methanol/water spray.

tissue. These experiments are being done using two main methods; SIMS in which focused high-energy (keV) ion beams are the primary projectiles and MALDI/TOF in which lasers are used to impact the sample of interest.[38–45] Both of these methods have given exciting data with high chemical specificity and with a spatial resolution on the order of 50 μm or less. However, both methods are demanding with regard to sample preparation. The promise of no sample preparation is one advantage that DESI offers, however it is acknowledged that some sample preparation may be required under certain circumstances. Another advantage is the fact that the sample is fully accessible to manipulation during the imaging experiment and that imaging of living systems is at least conceivable. Certainly, *in vivo* analysis is possible and a few simple examples have already been reported[4] including analysis of skin and wound surfaces. The main challenge in developing a DESI-imaging capability is to achieve spot sizes that are appropriate to the problem in hand. Using fine capillaries, spot sizes of *ca* 50 μm can be reached (*vide supra*); to improve resolution further, it will be necessary to use new approaches, the most interesting of which involves hybrid methods in which DESI is used to define a larger area of sample and to provide a mechanism for ionization and ion transport from this region, but in which a fine laser spot or needlepoint is used to define a region by its unique thermal or electrical potential and to distinguish ions arising from a subarea within the larger area. For some types of problems, the current spot sizes of a few millimeters will likely prove adequate; a case in point would be dermatological examination of skin lesions for pathological features. This could be done at or below the surface and would be a minor procedure compared to surgical removal and conventional microscopic pathological examination. Very recent results in which biological tissues have been examined for their lipidic content in carcinogenicity testing[23] are highly promising, showing that high-quality mass spectra can be recorded on untreated tissue almost instantaneously with spot sizes of a few hundred micrometers.

The ambient MS method described here should help drive the next revolution in MS, making the uniquely sensitive and specific general-purpose technique of MS accessible to a wider range of scientists studying additional types of problems. The development of ambient MS, especially if successfully implemented on miniature mass spectrometers, could be an almost perfect tool for explosives screening at airports or in the field. It would also open potential applications by lay individuals in chemical sampling of their environment, including medical, environmental, and product screening by and for themselves. The development of personal mass spectrometers would increase general scientific literacy and could touch off changes in physiological monitoring that might be significant.

Acknowledgements

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