Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization

the H_2 peak in the MAS NMR spectrum gives an occupancy of about 0.5 H_2 molecules per small cage on average. In contrast, estimates of occupancy from volumetric measurements performed during hydrate dissociation give on average 1.0 molecules of H_2 per small cage. One explanation for this discrepancy between NMR and volumetric measurements is that there was a loss of H_2 during NMR measurements, because the MAS sample rotors were open to the atmosphere. This explanation is supported by repeat NMR experiments that show that the size of the H_2 peak can vary among samples. Based on the NMR line width of H_2, some small cages may be doubly occupied, whereas other small cages are singly occupied.

Our results show that promoter guest molecules can be used to store hydrogen in a binary clathrate hydrate at low pressures. Storage capacities might be increased by optimization of the promoter system. In the case of sII binary hydrogen hydrate, with double occupancy of the small cavities by H_2 and the large cavities partially occupied by THF, the mass of hydrogen could be up to 4%.

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Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization

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A new method of desorption ionization is described and applied to the ionization of various compounds, including peptides and proteins present on metal, polymer, and mineral surfaces. Desorption electrospray ionization (DESI) is carried out by directing electrosprayed charged droplets and ions of solvent onto the surface to be analyzed. The impact of the charged particles on the surface produces gaseous ions of material originally present on the surface. The resulting mass spectra are similar to normal ESI mass spectra in that they show mainly singly or multiply charged molecular ions of the analytes. The DESI phenomenon was observed both in the case of conductive and insulator surfaces and for compounds ranging from nonpolar small molecules such as lycopene, the alkaloid coniceine, and small drugs, through polar compounds such as peptides and proteins. Changes in the solution that is sprayed can be used to selectively ionize particular compounds, including those in biological matrices. In vivo analysis is demonstrated.

An awkward feature of mass spectrometry (MS) is that the sample must be introduced into vacuum or into an inaccessible region closely coupled to the vacuum system. Here we describe a simple approach that allows ambient sampling for MS analysis. Electrosprayed (ES) aqueous droplets are directed at a surface of interest in air. The sample can be moved continuously or reoriented in space while MS analysis proceeds. The microdroplets act as projectiles and desorb ions from the surface as a result of electrostatic and pneumatic forces (*1*). The desorbed gas-phase ions are transferred to the distant mass spectrometer via an atmospheric pressure ion-transfer line.

This new method, termed desorption electrospray ionization (DESI), is related to other spray ionization methods, including electrospray ionization (ESI) (*2*, *3*), and to the desorption ionization methods, such as secondary ion mass spectrometry (SIMS) and laser desorption (*4*, *5*). No matrix is needed to perform the experiment—an advantage shared with laser desorption from porous silicon surfaces (*6*)—and the production of multiply charged biological ions is advantageous in extending the mass range, as is the case with ESI.

In its simplest form, the desorption electrospray experiment (Fig. 1) uses an aqueous spray directed at an insulating sample or an analyte deposited on an insulating surface such as polytetrafluoroethylene (PTFE). The desorbed ions are sampled with a commercial ion trap mass spectrometer equipped with an atmospheric interface connected via an extended and preferably flexible ion-transfer line made either of metal or an insulator.

Examination of insulator surfaces is highly unusual in MS; however, the fast nebulizing gas jet in the DESI experiment transports the charged microdroplets and allows them to impact the surface and to carry away analyte molecules. The underlying processes might be related to those occurring in versions of SIMS that use clusters as projectiles (*7–11*)

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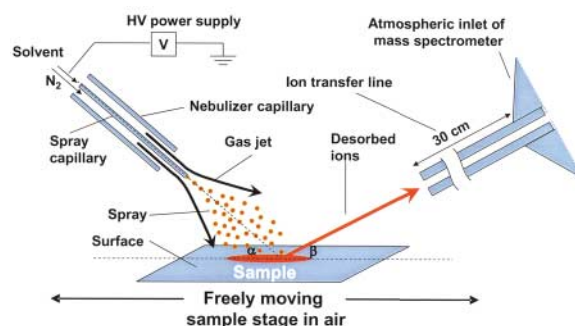
Fig. 1. Schematic of typical DESI experiment. The sample solution was deposited from solution and dried onto a PTFE surface, and methanol-water (1:1 containing 1% acetic acid or 0.1% aqueous acetic acid solution) was sprayed at a flow rate of 3 to 15 μl/min under the influence of a high (4 kV) voltage. The nominal linear velocity of the nebulizing gas was set to 350 m/s.

or impact desolation of droplets on surfaces (*12*) in vacuum environments.

A broad range of analytes have been examined, from simple amino acids through drug molecules, alkaloids, terpenoids, and steroids, to peptides and proteins. The methodology seems to be particularly promising for forensic and public-safety applications, including analysis of dried blood, detection of explosives, and monitoring of chemical warfare agents (*13*), as illustrated by two experiments. In one experiment, the explosive RDX was desorbed from an insulating tanned leather surface, to give the mass spectrum shown in Fig. 2A. In the other experiment, nitrile gloves that were exposed for less than 1 s to dimethyl methylphosphonate vapors (DMMP, a chemical warfare agent simulant), and then washed and dried, gave a mass spectrum (Fig. 2B) that unequivocally indicates the presence of trace levels of DMMP.

New applications of MS might emerge from such simple sampling procedures. In particular, process analysis and other high-throughput experiments are much simplified over standard MS methods. Initial experiments with pharmaceuticals show analysis rates of 20 samples per second (*1*). Optimum experimental conditions are summarized in table S1 (*1*). The ultimate sensitivity of the DESI method has not been determined, but lysozyme present in amounts ranging from 10 to 50 pg could be detected.

A feature of DESI relative to traditional desorption ionization methods, and indeed other MS methods, is the ease with which chemical reagents can be supplied to the site of analysis. This allows the generation of specific reaction products that can be used to confirm analyte identification. Biochemical reactions, including the formation of noncovalent complexes between enzymes and substrates, also serve this purpose. For example, when the lysozyme substrate hexa-(*N*-acetyl) chitohexaose was included in the solution sprayed onto a lysozyme sample, the enzyme-substrate complex was seen at mass-to-charge (*m/z*) ratios of 1944 and 2220 (fig. S3) (*1, 14–16*). Another example of a specific chemical reaction used to confirm MS identification is the formation of metal complexes between an analyte on the surface and a metal ion introduced into the spray solution. Uses for this capability include experiments (*1*) in which the chirality of amino acids is measured via diastereomeric complex ion formation and fragmentation (*17, 18*).

Both MALDI (matrix-assisted laser desorption/ionization) (*19–21*) and SIMS (*5, 22, 23*) can be used to image biological materials in experiments usually done in vacuum. The exceptions are atmospheric pressure (AP)–MALDI (*21, 24*) and AP-laser ablation (*25*), but in both of these methods the sample is strictly positioned relative to the rest of the ion source and is inaccessible and not manipulated during the experiment. Working under ambient conditions, DESI can be used for the spatial analysis of native surfaces, such as plant or animal tissues. The potential for this type of application is illustrated by the DESI spectrum of a seed section of poison hemlock (*Conium maculatum*) (Fig. 3A). The peak at *m/z* 126 is due to coniceine, which is known to be present in this particular plant species. The
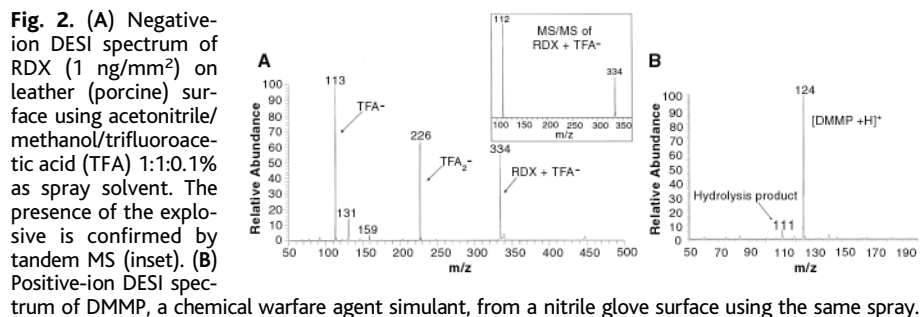
Fig. 2. (**A**) Negative-ion DESI spectrum of RDX (1 ng/mm²) on leather (porcine) surface using acetonitrile/methanol/trifluoroacetic acid (TFA) 1:1:0.1% as spray solvent. The presence of the explosive is confirmed by tandem MS (inset). (**B**) Positive-ion DESI spectrum of DMMP, a chemical warfare agent simulant, from a nitrile glove surface using the same spray.
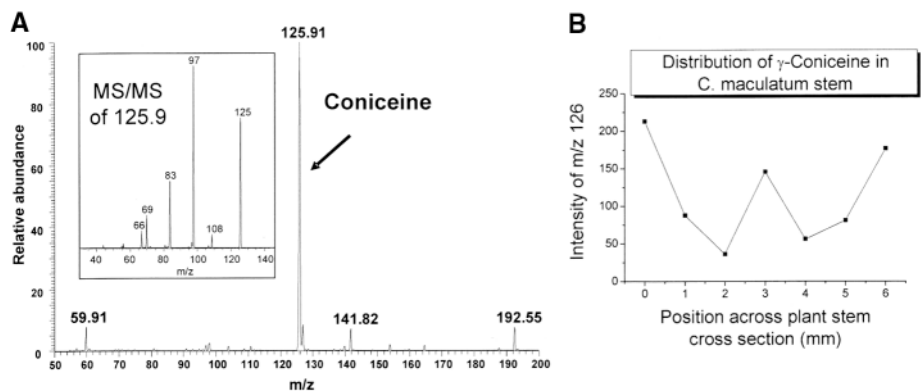
Fig. 3. (**A**) Positive-ion DESI spectrum of *Conium maculatum* seed section with the sample held under ambient conditions. The signal at *m/z* 126 corresponds to protonated γ-coniceine (molecular weight 125), an alkaloid present in the plant. The inset shows the MS/MS spectrum of *m/z* 126. (**B**) The intensity distribution of *m/z* 126 across a stem cross section. (**C**) DESI mass spectrum of the tomato shown in photograph S2. (*1*) The peak at *m/z* 536 is due to lycopene and/or other carotenoids. Methanol-water was sprayed onto the tomato surface and desorbed ions were transferred to the ion trap mass spectrometer.

Fig. 4. DESI spectrum recorded by spraying methanol-water onto the finger of a person 50 min after taking 10 mg of the over-the-counter antihistamine Loratadine (*m/z* 383/385). Other peaks, e.g., at *m/z* 245, are either due to the lab air background or are associated with the detergent that was used for hand washing before the experiment. Note that mass assignments are from the instrument data system and are accurate to just 0.5 *m/z* units.
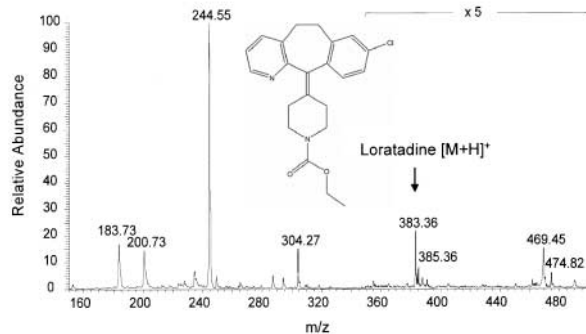
possibility of in situ imaging was demonstrated by scanning the spray spot across a cross section of the plant stem (Fig. 3B). Similarly, the DESI spectrum collected from tomato (*Lycopersicon esculentum*) skin also indicates the localization of characteristic compounds, including lycopene at m/z 536 (Fig. 3C).

Quantitative results can be obtained by using appropriate internal standards in experiments where the sample is deposited on a target surface; however, quantification by any method is intrinsically difficult in the analysis of natural surfaces. Sprayed compounds used as internal standards yielded semiquantitative results (relative standard deviation values of ~30%) for spiked plant tissue surfaces.

We have also used DESI for in vivo sampling of living tissue surfaces. An aqueous-alcohol DESI spray was directed onto the finger of a person who had taken 10 mg of the over-the-counter antihistamine Loratadine. About 40 min after taking the tablet, the molecule became detectable directly on the skin or in saliva and its surface concentration remained above the detection limit for another 50 min. A typical spectrum taken directly from skin (photograph S3) is shown in Fig. 4. This example is representative of the novel applications of MS once its vacuum constraints are lifted.

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Vibrational Energy Transfer Across a Reverse Micelle Surfactant Layer

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In a suspension of reverse micelles, in which the surfactant sodium dioctyl sulfosuccinate (AOT) separates a water nanodroplet from a bulk nonpolar CCl_4 phase, ultrafast vibrational spectroscopy was used to study vibrational energy transfer from the nanodroplet through the AOT interfacial monolayer to the surrounding CCl_4. Most of the vibrational energy from the nanodroplet was transferred to the polar AOT head group within 1.8 picoseconds and then out to the CCl_4 within 10 picoseconds. Vibrational energy pumped directly into the AOT tail resulted in a slower 20- to 40-picosecond transfer of energy to the CCl_4.

The flow of heat between two bulk phases separated by an interfacial monolayer is usually a simple function of the thermal conductivities of the two phases. However, when the heat source is within a few molecular diameters of the interfacial layer, the interfacial thermal conductivity becomes important. The flow of vibrational energy across the interfacial monolayer can depend on how and where the energy is deposited in the system, and different excitations may travel across the interface along different pathways and with different rates.

We present an example of such a situation in a reverse micelle (*1*) system. We used nonlinear vibrational spectroscopy with picosecond time resolution to monitor the flow of energy across surfactant molecules that separate nanodroplets of confined water from a nonpolar liquid phase. We found that vibrational energy deposited in the water could be transferred to the polar surfactant head groups and then to the nonpolar phase in 10 ps; conversely, energy deposited directly in the alkyl surfactant tails was transferred to the nonpolar phase on a longer 20- to 40-ps time scale.

A number of laboratories have studied the ultrafast dynamics of micelle-confined water (*2*). Ultrafast infrared (IR) (*3*, *4*) or THz (*5*) spectroscopies are notable because they do not require the use of extrinsic dopants. Seifert and co-workers (*3*, *4*) looked at sodium dioctyl sulfosuccinate (AOT) surfactant reverse micelles with a water:AOT ratio in the 10 to 55 range. They excited the OH stretch (ν_{OH}) of the confined water (a broad band peaked near 3500 cm^{-1}) with IR pulses, somewhat longer than the ν_{OH} lifetime, that create thermalized confined water. The subsequent cooling of the confined water over hundreds of picoseconds, the nonexponential cooling process, and its dependence on micelle diameter could be explained with ordinary heat conduction theory for a hot water droplet suspended in a colder nonpolar bath (*4*). The success of that explanation indicated that the AOT interface thermal conductivity had little effect on the flow of heat from the water to the nonpolar phase. As a consequence of the rather large water:AOT ratios, the average distance between the heat source and the interfacial layer was large enough that heat diffusion from the interior of the water droplet limited the micelle cooling rate (*4*).

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