

**SPECIAL FEATURE:
TUTORIAL**

Established and emerging atmospheric pressure surface sampling/ionization techniques for mass spectrometry

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The number and type of atmospheric pressure techniques suitable for sampling analytes from surfaces, forming ions from these analytes, and subsequently transporting these ions into vacuum for interrogation by MS have rapidly expanded over the last several years. Moreover, the literature in this area is complicated by an explosion in acronyms for these techniques, many of which provide no information relating to the chemical or physical processes involved. In this tutorial article, we sort this vast array of techniques into relatively few categories on the basis of the approaches used for surface sampling and ionization. For each technique, we explain, as best known, many of the underlying principles of operation, describe representative applications, and in some cases, discuss needed research or advancements and attempt to forecast their future analytical utility. Copyright © 2008 John Wiley & Sons, Ltd.

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INTRODUCTION

The introduction of the technique known as *desorption electrospray ionization (DESI)* in the fall of 2004¹ appears to have been the catalyst for interest in and the rapid emergence of a considerable variety of ambient surface sampling and ionization combinations for use with MS.² In MS, the desorption or ablation and ionization of atoms and molecules from surfaces under vacuum is a well-established field of study and an application that continues to expand and mature. Techniques like secondary ion mass spectrometry (SIMS) and matrix-assisted laser desorption ionization (MALDI) have become mainstays for surface chemical interrogation in a wide range of fields, including geochemistry, material sciences, and biology.^{3–5} The interest or, maybe better stated as, the intrigue in the use of ambient surface sampling (not strictly limited to ‘desorption’ methods) and associated ionization techniques might be broadly summarized by two general statements. First, these ambient sampling/ionization combinations liberate the analysis from the constraints of getting the surface to be sampled into the vacuum system. And second, performing the sampling and ionization under ambient conditions presents an opportunity to study materials under many

real-world conditions. Thus, gone are the many constraints on the sizes of surfaces and the volatility of materials to be studied. Now, it is possible to analyze living tissue and insulating materials. Also, avenues for exploitation of many traditional chemistries for enhancement of the analysis at hand have opened up. These techniques also appear to allow the analysis of many materials directly from surfaces without extensive preparation or certain adverse matrix effects. If these early reports are true, these methods hold promise in greatly simplifying and speeding up many types of mass spectrometric measurements.

The number and type of ambient, or atmospheric pressure (AP), techniques suitable for sampling analytes from surfaces, forming ions from those analytes, and subsequently transporting these ions into vacuum for interrogation by MS are rapidly expanding. Cooks and coworkers⁶ recently overviewed this area, which they termed *ambient desorption ionization mass spectrometry*, focusing on providing a basic classification of the many related techniques on the basis of the desorption and ionization processes involved. ‘Desorption’ with respect to these ambient processes is a term that might imply the inclusion of only methods in which the rapid addition of energy to a condensed-phase sample (e.g. heat, photons, droplet or gas impact) results in the liberation of species on a surface into the gas phase. However, both they as well as we include in this area surface-sampling probes that use a direct liquid extraction method to remove material from a surface. As such, we prefer the more general phrase

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AP-surface sampling. No matter what the surface-sampling process is, it is either intertwined with an ionization process, or ionization occurs as a discrete process subsequent to the sampling. This latter case we will refer to here as a secondary ionization process. A ready reference compilation explaining existing AP-ionization techniques⁷ and the basics of ion/surface collisions⁸ are invaluable references as one explores this area of AP-surface sampling/ionization.

In this tutorial article, we sort this vast array of techniques into relatively few categories on the basis of the surface-sampling and ionization approaches as summarized in Table 1. These categories are not set in stone, and might be different depending on one's perspective (e.g. organization based on the ionization *vs* desorption process), and the categories and placement of particular techniques will certainly evolve as techniques mature and become better understood. For each technique we explain, as best known, many of the underlying principles of operation, describe representative applications and, in some cases, discuss needed research or advancements and attempt to forecast their future analytical utility. Limitations were set so that only those methods in which both the surface-sampling and ionization process take place at atmospheric pressure are discussed. While many of these techniques first appeared in the last several years, some were first demonstrated much further back, and a few have been proven methods for almost two decades.

THERMAL DESORPTION/IONIZATION

Thermal desorption atmospheric pressure chemical ionization (TD/APCI) is probably the oldest and most established of the AP-surface sampling/ionization techniques in use with MS (Fig. 1). Introduced in the mid-1970s, commercially available in the 1980s, and largely forgotten in the 1990s, this approach has had multiple reincarnations in recent years.

In this approach to surface sampling, heat is used to liberate the sample intact from the condensed phase to the vapor phase. Typically, this heating is accomplished through the use of a heated gas passing over the sample and/or a resistively heated sample surface. Given the nature of the desorption process, this approach is limited to relatively low mass species (*ca* 2000 Da or less) that can be liberated intact into the gas phase by heat; thermally labile, highly polar, and high-molecular-mass species are typically not amenable to vaporization by heating. Once in the gas phase, the sample can be ionized by any number of ion/molecule chemistries. Typically, this has been atmospheric pressure chemical ionization (APCI).⁹ Unexpectedly, some ionic species can be liberated into the gas phase directly as ions through the use of heat. This has been shown conclusively to be a thermal evaporation of ions under vacuum for quaternary ammonium and phosphonium salts.¹⁰ Chen *et al.*¹¹ recently showed that some organic salts could be thermally desorbed as ions into the gas phase under ambient conditions, a process they termed *atmospheric pressure thermal desorption ionization (APTDI)*.

With a secondary ionization by APCI, a reagent-ion population is generated by initiating an ion/molecule

reaction cascade though the use of a β -emitter like ⁶³Ni or, more typically today, with a corona discharge. A high voltage (*ca* 5 kV or less) applied to a sharp metal electrode, the corona discharge electrode, ionizes the nitrogen in air in positive-ion mode. This then undergoes a series of reactions with nitrogen and water vapor that produces hydronium ion-water clusters as the major reagent-ion species. Hydronium ion-water clusters can protonate molecules with a gas-phase basicity (or proton affinity) higher than that of water. In negative ion mode, where a negative high voltage is applied to the corona electrode, electrons are emitted from the electrode, typically producing a large population of OH⁻ as the reagent ion. The OH⁻ will ionize by proton transfer all species in the gas phase that have higher gas-phase acidity than water. In some situations, the gas-phase ion chemistry is not that simple. In positive-ion mode, ionization can also occur by charge transfer and cation attachment, and by electron capture and anion attachment in negative-ion mode. In either ionization mode, the introduction of 'doping' agents to the area of discharge can be used to alter the gas-phase ion chemistries to influence the ions ultimately formed.⁹

The basic TD/APCI approach can be traced back at least to the work of Horning and coworkers in the mid-1970s.¹² In that early work, samples on a platinum wire were immersed in a heated gas stream, desorbing the material into the gas phase, which was subsequently ionized by APCI. Commercial triple quadrupoles such as the Sciex Aromic 9100 Cargo Evaluation System and the British Aerospace/Sciex CONDOR Contraband Detection System became available in the mid to late 1980s with two methods of acquiring samples: direct vapor detection and collection of trace particle residue or vapors followed by TD and corona discharge APCI (Fig. 2).^{13,14} In some cases, these units were equipped with a heated surface sampler and heated transfer line to sample materials at distances up to 10 m remote from the mass spectrometer.¹⁵ Such remote sampling capability in relation to newer AP-surface sampling/ionization techniques is referred to as 'non-proximate' detection.¹⁶ These early systems were touted for their ability to rapidly analyze (near real time) for targeted analytes in complicated matrices without the need for chromatography or a sample cleanup stage. Selectivity in detection was gained through the use of tandem mass spectrometry (MS/MS) or (MSⁿ where n=2) utilizing two or more selected reaction monitoring (SRM) transitions along with their respective abundance ratios as additional components in confident identification. The application area was typically the detection of drugs and explosive materials.

Some of these dedicated TD/APCI-MS instruments survived in the 1990s and were demonstrated for use in applications such as the detection of controlled substances on banknotes associated with illicit drug sales.¹⁷ At least one plug-and-play TD/APCI unit, though not widely advertized, has been commercially available since at least the early 2000s,¹⁸ and demonstrated for detection of drugs on money.¹⁹ Recently, researchers have begun to show that commercial plug-and-play corona discharge APCI sources designed for liquid introduction can be used to sample materials

Table 1. Compilation of AP-surface sampling/ionization techniques

Dominant surface-sampling process							
Surface-sampling/ ionization approach	Mechanism	Driving force	Dominant ionization process	Technique name	Acronym	Notes	Selected references
Thermal desorption/ ionization	Thermal desorption	Heated gas flow, surface or combination	Liberation of organic salts from surface APCI – corona discharge	Atmospheric pressure thermal desorption ionization Thermal desorption/atmospheric pressure chemical ionization Atmospheric pressure solids analysis probe Laser diode thermal desorption	APTDI TD/APCI ASAP LDTD	Analysis of some organic salts is possible for thermal desorption with a secondary ionization Commercially available; analyte is deposited on a glass capillary and inserted into the source Commercially available; analyte is deposited in stainless-steel sample wells heated using IR laser Generally used for detection of low mass and volatile analytes Commercially available; analysis from a wide range of surface types is possible Choice of solvent vapor can dramatically affect ionization AC plasma AC plasma DC plasma	11 18,19 21 23 33,35 24 41 43 44 48
Laser desorption (ablation)/ ionization	Laser desorption (ablation)	Laser beam surface impact	Secondary ionization by ICP Secondary ionization by ESI	Desorption atmospheric pressure chemical ionization Direct analysis in real time Desorption atmospheric pressure photoionization Plasma-assisted desorption/ionization Dielectric barrier discharge ionization Atmospheric pressure glow discharge desorption ionization Laser ablation/inductively coupled plasma Laser desorption/atmospheric pressure chemical Ionization Laser desorption/electrospray ionization Electrospray-assisted laser desorption/ionization	DAPCI DART DAPPI PADI DBDI APGDDI LA/ICP LD/APCI LD/ESI ELDI	Commercially available; useful for elemental analysis of the sample Used for analysis of proteins and peptides in gels Can form multiply charged ions from proteins	54–56 62 69

Table 1. (Continued)

Surface-sampling/ ionization approach	Dominant surface-sampling process			Technique name	Acronym	Notes	Selected references
	Mechanism	Driving force	Dominant ionization process				
				Laser ablation with electrospray ionization	LAESI	Use of IR laser allows analysis of 'wet' biological samples	76
				Infrared laser assisted desorption electrospray ionization	IR LADESI		77
				Matrix-assisted laser desorption electrospray ionization	MALDESI		75
				Atmospheric pressure matrix-assisted laser desorption ionization	AP-MALDI	Commercially available; easily coupled with a variety of mass spectrometers	79–81
Liquid and gas jet desorption/ ionization	Droplet/ liquid jet/gas impact	Charged droplet/gas jet surface impact	ESI-like	Desorption electrospray ionization	DESI	Commercially available; analysis from a wide range of surface types is possible	1,2
		Neutral droplet/gas jet surface impact	SSI-like	Desorption sonic spray ionization	DeSSI	No voltage DESI; high gas flow velocity needed	109
		Charged liquid stream surface impact	ESI-like	Easy ambient sonic spray ionization	EASI		113,114
		Gas jet surface impact		Jet desorption electrospray ionization	JeDI	High-velocity solvent stream used for sampling	116
		Extraction using a confined liquid stream with liquid microjunction surface contact	Secondary ionization by ESI	Neutral desorption extractive electrospray ionization	NDEESI	Sampling geometry similar to DESI; no spray solvent or high voltage needed	118
Liquid extraction surface- sampling probe/ ionization	Confined liquid extraction	Extraction using a confined liquid stream with liquid microjunction surface contact	ESI APCI – corona discharge	Liquid microjunction surface-sampling probe	LMJ-SSP	Use of other liquid introduction ionization sources possible	120,131,133
		Extraction by confined liquid stream with a sealed surface contact	ESI	Sealing surface-sampling probe	SSSP	Commercially available; use of other liquid introduction ionization sources possible	119,123

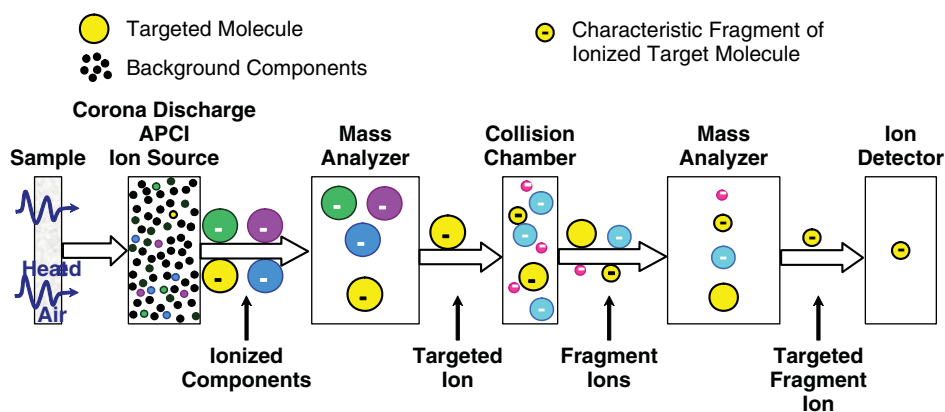


Figure 1. Schematic illustration of a generic surface-sampling TD/corona discharge APCI system using SRM for targeted compound detection.

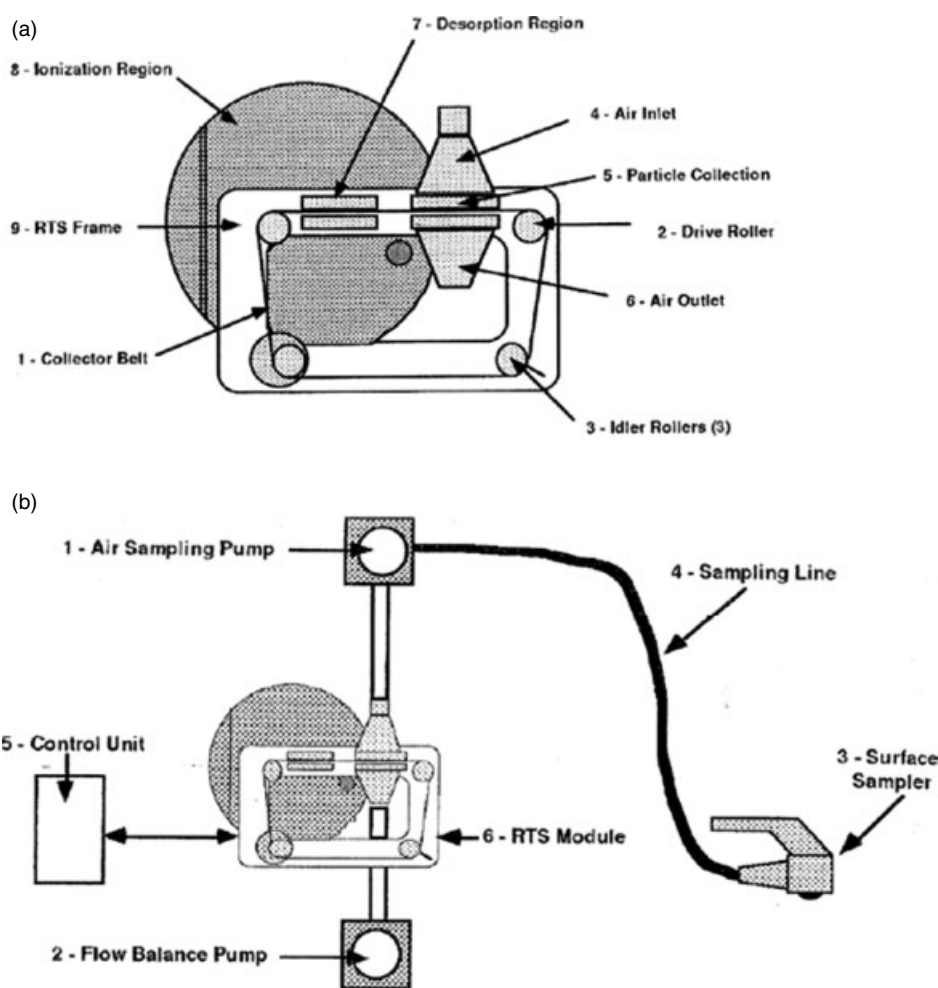


Figure 2. (a) Real-time sampler (RTS) and (b) remote sampling components of the British Aerospace/Sciex CONDOR Contraband Detection System. Used with permission from Ref. 14.

from surfaces. For example, Popov *et al.*²⁰ simply placed a cotton swab containing an explosive (e.g. trinitrotoluene, TNT; cyclotrimethylenetrinitramine, RDX; or pentaerythritol tetranitrate, PETN) into a heated nebulizer probe and thermally desorbed the material, which was then ionized by corona discharge APCI. McEwen and coworkers^{21,22} took a more 'refined' approach, modifying a flange on a commercial corona discharge APCI source to allow insertion of a glass

melting point capillary into a heated gas stream (100–500 °C) emerging from the heated nebulizer for the rapid analysis of liquids and volatile and semivolatile solid materials (Fig. 3). They termed this approach an *atmospheric pressure solids analysis probe (ASAP)*. Analyte deposited on the capillary is thermally desorbed by the gas, ionized by APCI, and analyzed by MS. The technique has proved suitable for the types of compounds typically amenable to ionization

by APCI, including lipids, capsaicins, and carotenoids from fresh biological samples, polymer additives, fatty acids, and drugs such as cocaine and acetylsalicylic acid. Rather than targeted compound detection by MS/MS with quadrupole instruments, they have used to date high-resolution accurate mass spectrometers that are more suitable for a discovery mode of operation where the species to be detected may not be known.

A new commercial form of TD/APCI has emerged that has been named *laser diode thermal desorption (LDTD)*.²³ This plug-and-play source uses an IR laser to thermally desorb samples that have been deposited onto stainless-steel sample wells in a specially designed 96-well plate (Fig. 4). The plate is held in line with the laser beam that fires sequentially at the back of each well, thermally desorbing the sample. Before the laser fires at a particular well, a transfer tube is driven by a piston into that well. Thermally desorbed species are carried by heated air through the transfer tube and are ionized by a corona discharge source at the inlet of the mass spectrometer. The LDTD-APCI source requires optimization of carrier gas flow as well as laser power and duration for optimal performance. Wu *et al.*²³ demonstrated this technique as a high-throughput method for the analysis of cytochrome P450 inhibition assays.

The technique known as *direct analysis in real time (DART)* may also be categorized as a variation of TD/APCI.²⁴ In this case, He gas flowing through a probe is subjected to a discharge at a needle electrode, producing ions, electrons, and metastable species (Fig. 5). Perforated electrodes downstream act to remove ions from the gas stream, while neutral metastable species are carried by the gas through a heated chamber, passing through a grid electrode before entering the ambient atmosphere. The grid electrode prevents ion-ion and ion-electron recombination and also acts as a source of electrons, either through Penning ionization of a neutral species or through surface Penning ionization.²⁵ The exiting gas flow is directed at the entrance of the mass spectrometer

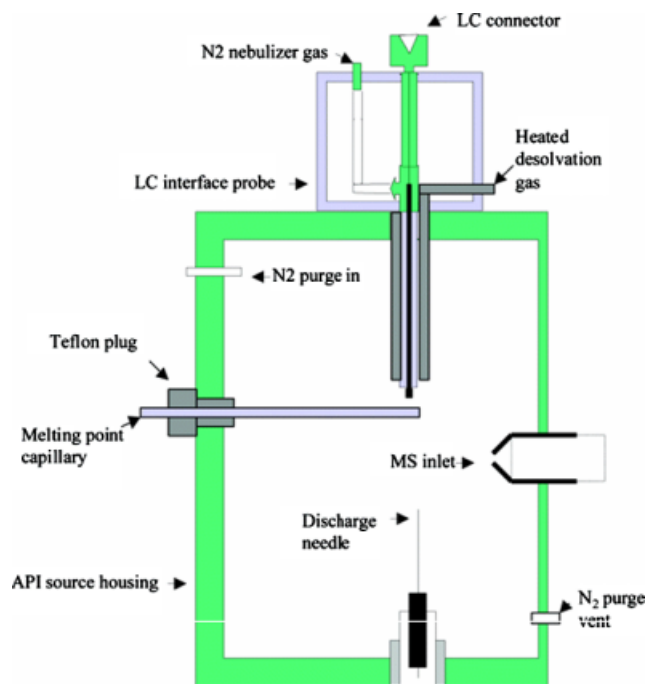


Figure 3. Cross-sectional drawing of an atmospheric pressure LC/MS ion source modified for ASAP analysis. Used with permission from Ref. 21.

and the sample surface to be analyzed is placed between the two.

The ionization process in DART is a variation of APCI in which the reagent-ion population originates from the gas-phase reactions of the metastable He atoms ($\text{He}^* (2^3S_1)$) produced in the discharge. Once in the ambient atmosphere, He^* reacts rapidly and efficiently with ambient species to produce a reagent-ion population that can ionize the gas-phase analytes. When even traces of water vapor are present, as is the case in ambient air, protonated water clusters become one of the dominant reagents, serving as a source of

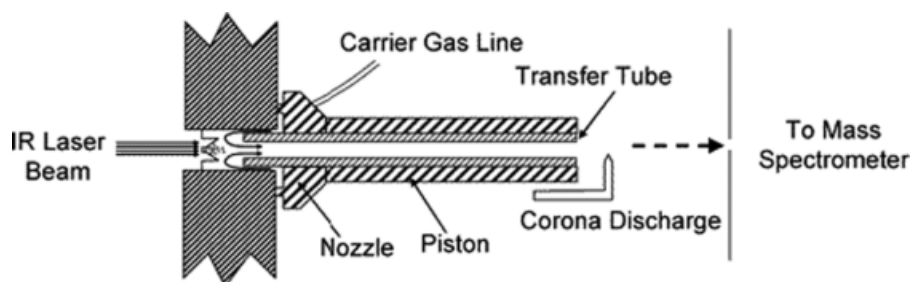


Figure 4. LDTD-APCI laser diode thermal desorption. Used with permission from Ref. 23.

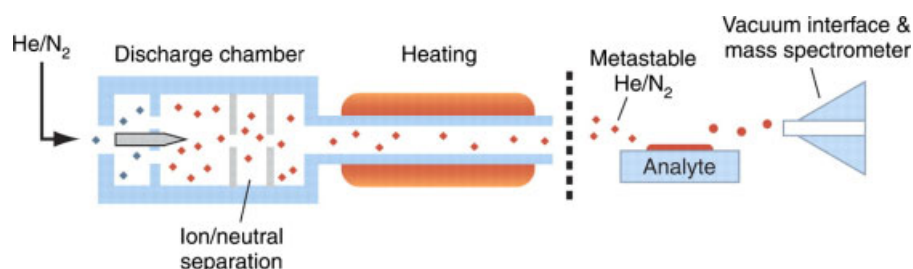


Figure 5. Schematic illustration of a DART ion source. Used with permission from Ref. 2.

protons for protonation of gas-phase species. Electrons may undergo electron capture by O_2 , producing O_2^- that reacts with desorbed analyte, forming negative ions. The exact distribution of reagent ions formed was shown to depend on the gas used (e.g. N_2 rather than He), the ion polarity, and the presence or absence of dopants in the gas stream.²⁴

The surface-sampling or desorption process involved in DART has not been explicitly stated in the literature, but the reported data is consistent with TD as a dominant process. Many species can be observed without substantial heating of the gas stream, but, in general, signal levels can be significantly improved by heating the gas up to 200 °C or more. Desorption resulting from sputtering or bombardment by ionized water clusters and metastable species has been proposed to contribute.²⁴ However, the very limited lifetime of He^* at AP, and the fact that when a low kinetic energy metastable atom interacts with a surface most of the excitation energy is used to eject electrons from the surface,²⁶ brings that proposition into question.

The main argument used against a pure TD process in DART has been the ability to analyze materials that have 'no significant vapor pressure', such as the organic salts. However, as discussed above,^{10,11} some of these types of species have already been shown conclusively to desorb by thermal means, intact into the gas phase. Furthermore, compounds that do have low vapor pressures, like the explosive RDX, can be calculated to be present at part-per-trillion (ppt) to low part-per-billion (ppb) levels by volume in room air just a few tens of degrees above ambient.²⁷ Given the exceptional detection levels of today's mass spectrometers, and the 'in-beam' nature of the technique, the ability to detect compounds that can be effectively ionized at ppb concentrations or lower is not unexpected.

Regardless of mechanistic aspects of the desorption process, DART has been successfully used in the analysis of both polar and nonpolar compounds from a wide range of surface types. These applications include the analysis of compounds separated on thin layer chromatography (TLC) plates,²⁸ characterization of counterfeit drug samples,²⁹ reaction monitoring in drug discovery applications,³⁰ characterization of fatty acid methyl ester ions from whole bacterial cells,³¹ and the analysis of self-assembled monolayers on gold.³² As a tool for the rapid analysis of individual samples, DART has merit similar to ASAP. Variations in the reagent-ion plasmas and source geometries may prove one technique more suited than another for particular sample types. For quantitative work and higher throughput, the sample introduction in DART will almost certainly become automated and the entire source closed or vented. This will allow the safe analysis of all sample types and make the current open-air system suitable for use in more regulated work environments. A safety enclosure might be expected for all 'open source' AP-surface sampling and ionization sources in the future.

The technique termed *desorption atmospheric pressure chemical ionization* (DAPCI), is also, in some incarnations, a simple TD/APCI system.^{33,34} In DAPCI, as defined and used by Cooks' group,³⁵ the DAPCI emitter is aimed at the surface to be analyzed as in DESI. In this case, the emitter is composed of a capillary with a taper-tip stainless-steel

electrode aligned coaxially within it and projecting from it. An inert sheath gas, into which a solvent vapor is in some cases introduced, is supplied to the capillary and flows through it at a high velocity. A high-voltage power supply is used to apply a voltage (typically ± 3 to 6 kV) to the electrode; this induces a corona discharge at the tip of the electrode, ionizing the introduced solvent vapor. The sheath gas may be heated in some cases.

Ionization mechanisms in DAPCI are similar to other APCI sources. As in all APCI systems, the reagent-ion species formed can be influenced by the means of initiating the reagent-ion plasma, and by the particular solvent vapor and sheath gas used.^{1,36} Desorption mechanisms in DAPCI are in some cases unclear. When the sheath gas is heated, TD is certainly a dominant process.^{33,34} In other cases, the high-velocity gas might actually liberate minute particles from the surface that can then be ionized in the gas phase. However, desorption/ionization has been reported even without heat or high-velocity gas.³⁷ In such cases, it has been proposed that static charge buildup on the dielectric sample surface facilitates ion desorption. If true, this same mechanism might contribute to desorption in ambient temperature DART and all other related techniques in which the reagent-ion population (charged particles) impinges onto a dielectric surface being analyzed. Further inquiry regarding this possible mode of desorption is warranted.

Among the applications to date, what has been called *DAPCI-MS* has been used for desorption/ionization and detection of low-mass ions, including drug molecules,^{33,34,37} explosives,^{1,36-39} molecular markers for spoilage in meats,³⁷ adulterants in food products,³⁷ and agricultural chemicals.³⁷ DAPCI-MS has also been used to differentiate between samples of tea obtained from various manufacturers.⁴⁰

The technique that has been termed *desorption atmospheric pressure photoionization* (DAPPI) is similar to DAPCI except that the reagent-ion population is initiated by a photoionization process rather than by a corona discharge (Fig. 6).⁴¹ Haapala and coworkers⁴¹ introduced this source using a microchip nebulizer to deliver a heated solvent vapor jet at the sample surface, followed by ionization in the gas phase from photoinitiated ion-molecule reactions using a UV lamp. In the Haapala and coworkers⁴¹ DAPPI configuration, TD is the dominant surface-sampling process. The ionization process is the same as that in APPI used with liquid introduction ion sources.⁴² As such, the choice of solvent (dopant) vapor dramatically affected the ionization of test compounds. Analytes such as anthracene, testosterone, methylenedioxymethamphetamine (MDMA), and verapamil were most effectively ionized when using toluene, acetone, or a 50/50 acetone/toluene mixture as dopants.

So-called plasma-assisted desorption/ionization techniques have also emerged, which appear to have, at least in part, a TD sampling component. Two similar techniques are those referred to as *plasma-assisted desorption/ionization* (PADI)⁴³ and *dielectric barrier discharge ionization* (DBDI)^{44,45} (Fig. 7). The desorption/ionization plasma in both experiments is typically created in a flowing stream of He by applying an alternating voltage between two electrodes,

one of which is covered by a dielectric layer. The resulting nonequilibrium plasma consists of species like He^* , ions, radicals, and electrons. The sample surface to be analyzed is placed in direct contact with the plasma jet created, exposing the sample to all these plasma species, which results in analyte desorption and ionization. Mass spectra obtained contain mainly the molecular ionic species like protonated molecules and ion adducts of the analyte. In some cases fragment ions are observed.

The initial publications on these techniques acknowledge a lack of a detailed understanding of both the desorption and the ionization processes involved. Ratcliffe *et al.*,⁴³ speculate that 'a combination of energy transfer from metastable helium, ion impact, and radical-surface interactions contribute to the [desorption] mechanisms. . .'. These processes may contribute to desorption, but one might anticipate that

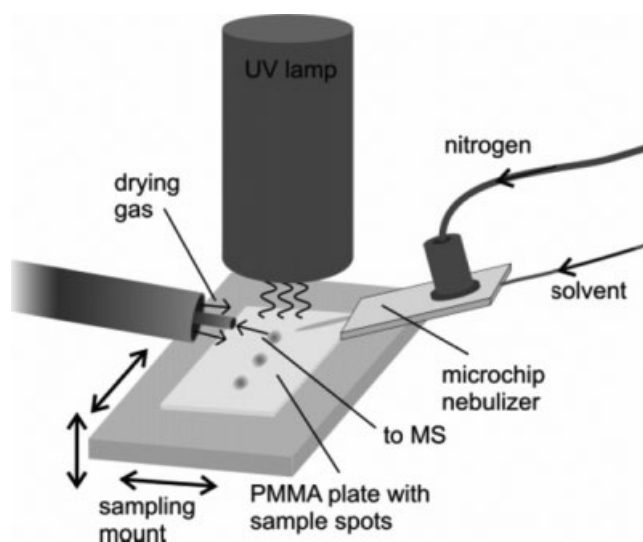


Figure 6. Schematic view of the DAPPI setup. Used with permission from Ref. 41.

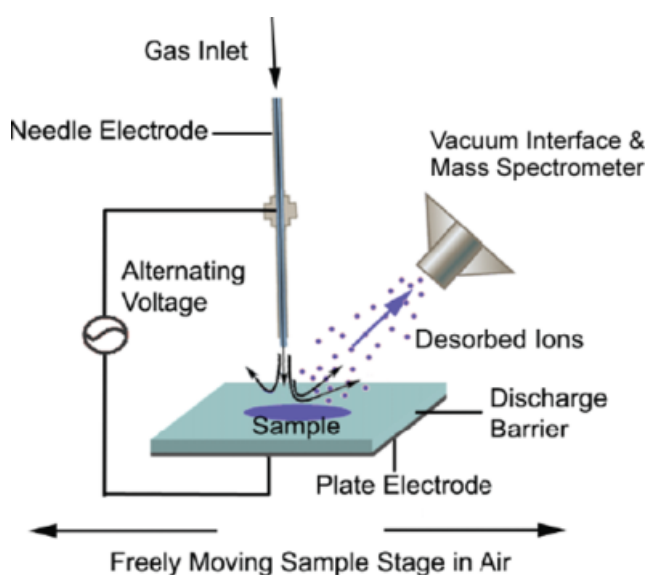


Figure 7. Schematic diagram of DBDI ion source. Used with permission from Ref. 44.

these same processes could result in modification of the analytes (such as radical addition reactions) on the surface or gas phase.

Because these are so-called cold plasma techniques, TD is perhaps erroneously overlooked as a contributor to the desorption process. Ratcliffe *et al.*⁴³ acknowledged, however, that operation of their source at powers above 7 W actually charred the sample surface, proving that these sources can produce a 'hot' plasma. To minimize the visible thermal damage to the surface, they typically operated their source at <5 W, which produced an elongated plasma jet about 4–5 mm in length. At <3 mm they could not visually detect any surface damage. Stoffels *et al.*⁴⁶ actually derived an equation to calculate the temperature of these types of plasmas on the basis of plasma jet length during work to develop an ambient-temperature plasma technique to modify biomaterials. They also determined the temperature from optical spectral measurements and computation at different plasma powers. They found, in fact, that at 3 W the plasma temperature was about 100 °C, at 5 W about 150 °C, and at 7 W about 200 °C. In fact, the detection of the explosives TNT, RDX, and PETN by Na *et al.*⁴⁵ using one of these plasma desorption/ionization sources was consistent with, but not conclusive for, TD as the sampling mechanism. They found that detection levels improved as the vapor pressure of the explosive (PETN < RDX < TNT)⁴⁷ increased, with TNT showing the lowest level of detection. Also of note, Hieftje's group recently described an AP glow discharge (constant current) source for surface sampling, which we will term *atmospheric pressure glow discharge desorption ionization (APGDDI)*, that is acknowledged to have a TD component from plasma heating of the sample surface.⁴⁸ In 1990, Lubman and coworkers⁴⁹ described an AP glow discharge that used a heated bath gas and ionization region for TD of solid material from a probe, which was subsequently ionized in the plasma.

Ratcliffe *et al.*⁴³ speculate that ionization in positive-ion mode is a combination of 'direct electron ionization, metastable Penning ionization and ion-molecule reactions.' In the negative-ion mode they believe ionization may '... proceed via direct and dissociative electron attachment to oxygen species, e.g., O_2^- , which then react with analyte molecules. . .'. to produce molecular ionic species like $(\text{M} - \text{H})^-$. In general, the mass spectra observed and the application space of these plasma-assisted desorption/ionization techniques are similar to TD with other APCI ionization methods. However, the ability of plasma of a relatively small diameter (*ca* 1 mm) to interact with the surface may provide desorption/ionization spatial resolution useful in particular types of spot-sampling scenarios or possibility chemical imaging. More detailed study of the desorption processes involved is needed.

LASER DESORPTION (ABLATION)/IONIZATION

The basic laser desorption (ablation)/ionization source for surface sampling, whether at vacuum or at AP, is relatively simple in concept.⁵⁰ A pulsed laser beam is focused at a small spot on the surface to be analyzed; material is desorbed (ablated)/ionized by the laser pulse; and the ions

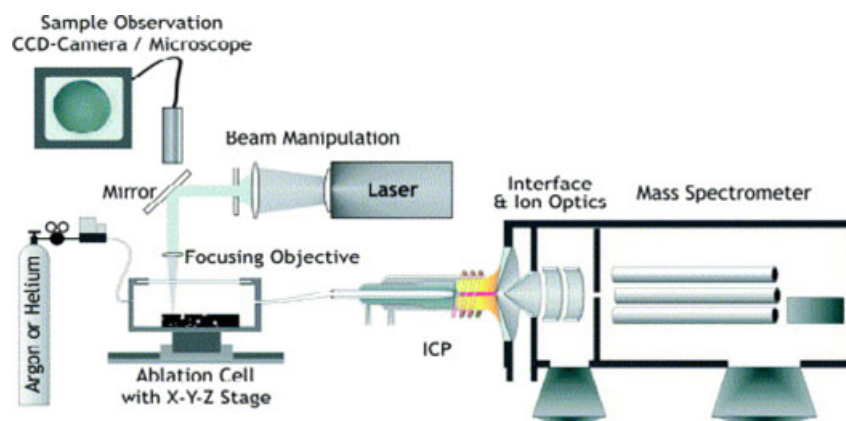


Figure 8. Schematic of LA-ICP/MS setup. Used with permission from Ref. 54.

generated are analyzed by a suitable mass spectrometer. The exact mechanisms of the desorption and ionization processes are often far from simple and not always fully understood.^{50,51} In general, as the laser pulse is absorbed, the solid surface under analysis is excited and the analyte is desorbed along with other species in the immediate area. The desorbed material from the surface, including the analyte, forms a reactive region just above the surface, sometimes termed a *selvedge*, that can participate in the proton and electron transfer, cationization, and radical reactions which ultimately produce the final ionic products measured by the mass spectrometer. The exact desorption/ionization pathways followed depend on the material properties of the compounds and on the laser irradiation parameters, and are beyond the scope of this discussion. What is important to note is that even in the best cases, many more neutral species than ions are generated from a typical laser desorption (ablation). Therefore, using a distinct secondary ionization process following the desorption (ablation) allows the desorption (ablation) and ionization conditions to be independently optimized. This leads to increased overall ionization efficiency, provides flexibility in the types of ions created (e.g. atomic or molecular), can reduce sample preparation requirements (e.g. no added chemical matrix), and opens up a variety of new application opportunities.

To aid our current classification scheme and the associated discussion, we have divided the atmospheric pressure laser desorption ionization (AP-LD/I) topic into two subareas, viz. atmospheric pressure laser desorption (ablation) with secondary ionization (AP-LD/SI) and atmospheric pressure matrix-assisted laser desorption ionization (AP-MALDI). Thus, the AP-MALDI section includes, for the purposes of this overview, the AP-LD/I techniques that do not involve a distinct secondary ionization process. The 'matrix' that absorbs the laser energy includes the analyte itself (sometimes called direct LD/I), a distinct chemical matrix species admixed with the analyte (the classic MALDI experiment), or the support surface for the analyte (e.g. water in tissue, see below).

Atmospheric pressure laser desorption (ablation) with secondary ionization (AP-LD/SI)

The oldest and most established of the AP-LD/SI type systems is *laser ablation inductively coupled plasma (LA-ICP)*,

first introduced in 1985,⁵² and which has continued to mature and expand in use ever since.^{53,54,55} These systems have been commercially available for a number of years⁵⁶ and are often configured for automated analysis that includes simple spot sampling as well as surface imaging (Fig. 8).⁵⁴ In a typical laser ablation inductively coupled plasma mass spectrometry (LA-ICP/MS) experiment, the sample is placed in a closed ablation chamber that is flushed with argon or helium. The laser beam is focused on the sample surface through a window in the ablation chamber. With the appropriate laser conditions, material is ablated from the sample surface and carried in the argon gas stream to the ICP. The ICP is a separate excitation source in which the laser-generated particles are desolvated, vaporized, atomized, and ionized.⁵⁷ The atomic ions created are then extracted by an atmosphere-to-vacuum interface and guided into the mass analyzer.

Because the ICP is an atomic ion source, LA-ICP/MS is confined to elemental analysis of the sample under study. However, major, minor, and trace element compositions can be measured and isotopic ratios determined. Sub-nanogram per gram detection limits for laser spot sizes above 100 μm are obtained for many elements.⁵³ The spot size examined can be reduced to just a few micrometers, but because much less material is sampled, detection levels suffer. These overall attributes have made LA-ICP/MS an important tool in geology for applications such as geochronological studies, elemental analysis of fluid inclusions in ores, and spatially resolved isotope ratio measurements, among many others.⁵⁴ Other scientific fields of study also make use of the technique, including material science (e.g. trace element distributions in ceramics), environmental science (e.g. trace elements in tree rings and soil), forensics and archeology (e.g. elemental 'fingerprinting' items like glass and pottery), and biology (e.g. trace element distribution in human tissue like hair and nails).^{53,54,55}

The use of LA-ICP/MS in biological studies is in fact rapidly growing.^{58,59} Becker's group⁵⁹ has been a champion of this area, applying LA-ICP/MS to the analysis of phosphoproteins and trace elements in proteins separated in gel electrophoresis and to map trace element distributions in thin tissue sections. An excellent example is the use of LA-ICP/MS to image the distribution of copper in a 20- μm -thick thin tissue section of a whole human brain hemisphere (110 \times 65 mm) (Fig. 9).⁵⁹ The concentration of copper present

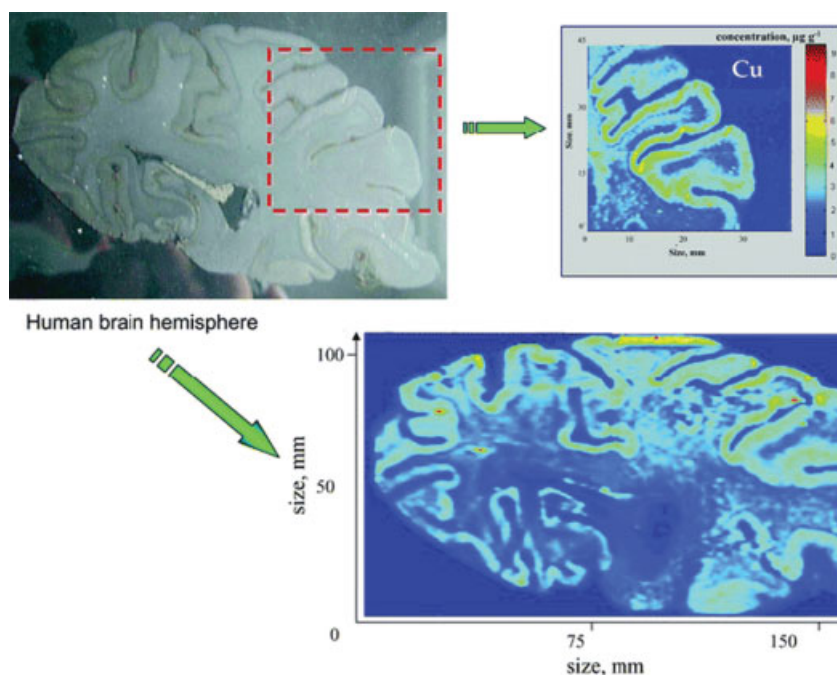


Figure 9. Cu images in part of (top, right) and the whole (bottom, right) human hemisphere measured by LA-ICP/MS compared with the light photograph of the thin tissue section. Use with permission from Ref. 59.

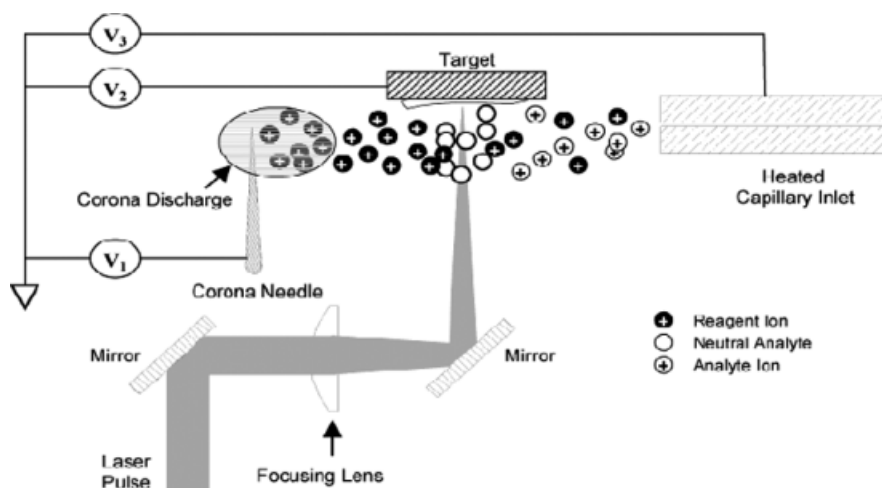


Figure 10. Schematic representation of LD-APCI source. Used with permission from Ref. 64.

was calibrated using synthetic matrix-matched standards and found to be on average in the range of 3–7 $\mu\text{g/g}$.

Another exciting possibility in LA-ICP/MS was illustrated by Becker's group,⁶⁰ namely, the use of the 'near-field effect'⁶¹ to beat the wavelength limitation of the laser light to achieve laser desorption (ablation) spot sizes on the order of hundreds of nanometers. This spatial sampling resolution is sufficient for spot sampling within, and imaging, of individual cells or organelles. Such sampling resolution would certainly find application in other fields like geology and material science as well. Note that it should be possible to take advantage of this same near-field effect in the other AP-LD/I methods discussed below to achieve nanometer scale spatial sampling resolution.

Laser Desorption Atmospheric Pressure Chemical Ionization (LD/APCI) is an AP-LD/SI technique that uses laser desorption to sample a surface and secondary ionization by

an APCI process to produce molecular ionic species of the species sampled (Fig. 10). Harrison and coworkers^{62–65} have done most of the recent work in this area with an emphasis on directly analyzing proteins and peptides in polyacrylamide gels. However, Kolaitis and Lubman⁶⁶ had already demonstrated in 1986 the use of LD/APCI for the analysis of small biological molecules like amino acids, vitamins, and catecholamines. Others have more recently used AP-LD/APCI for the analysis of analytes separated on TLC plates.⁶⁷

With the approach used by Harrison's group, an IR laser (10.6 μm) is used to desorb neutral molecules from a sample at AP, followed by ionization in the gas phase with corona discharge APCI. Secondary ionization by corona discharge APCI proved efficient, at least in part, because of the number of high density reagent ions in the region of the desorption plume. The analytical utility for this approach was demonstrated by the analysis of horse heart cytochrome

c that had been loaded onto a gel and electrophoresed. The gel spots were excised, washed, dried, and rehydrated in a trypsin-containing solution. After a 20-h incubation at 37 °C, the gel pieces were analyzed. The LD/APCI-MS mass spectra obtained clearly showed a number of peptides that matched with most of those expected for the digestion of this protein.

While the possibility of direct analysis from the gels is exciting, it is somewhat troubling that high gel-loadings were needed to detect a good signal (no signals were observed for below 500 pmol of protein loaded) and that extensive workup of the gel was needed before analysis. However, it is very early in this research area. Gel analysis has lost favor with the MS community to multidimensional liquid chromatography. If a straightforward and rapid analysis method for gels (or even another high resolution planar separation medium), which did not require an extensive, even if automated, preparation scheme, was available, it would almost certainly be welcomed. Gel technology is firmly established in the biological community and this LD/APCI-MS development would take advantage of that established infrastructure.

An interesting preliminary report in the LD/APCI area is from Hieftje's group.⁶⁸ They showed that a commercially available LA system used for coupling with ICP/MS (see above)⁵⁶ could also be coupled with their constant current AP glow discharge source (a type of APCI) to form molecular ionic species from the ablated species on the surface. This report is significant because this LA system coupled to an APCI source (or other secondary ionization method like ESI that produces molecular ionic species), simply by feeding the carrier gas outlet into the AP ion source, readily presents users with an automated unit ready to be applied to any number of sample types for spot sampling and imaging.

The first use of *laser desorption electrospray ionization* (LD/ESI) was reported by Shiea *et al.*⁶⁹ under the name *electrospray-assisted laser desorption/ionization* (ELDI). The LD/ESI technique works in much the same way as LD/APCI, except that the desorbed (ablated) materials (gaseous analytes or particles) are ionized through reaction with the charged solvent droplets, protonated solvent species, or gas-phase ions created in the ESI process (Fig. 11). The Shiea group ELDI setup consisted of a 337-nm nitrogen laser operating at 20 μ J per pulse and a focused laser spot size of 100 μ m \times 150 μ m with the incident angle of the laser beam

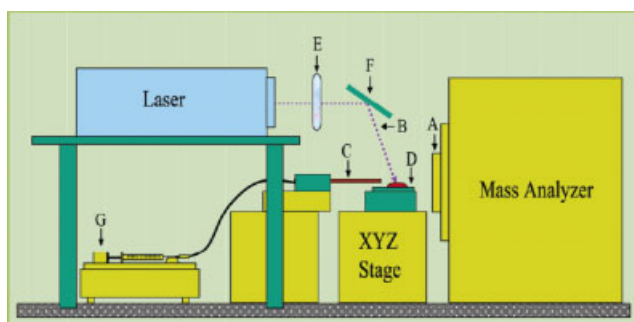


Figure 11. Detailed schematic of ELDI setup, (a) sampling skimmer, (b) laser beam, (c) electro-spray capillary, (d) sample plate, (e) focusing lens, (f) reflecting lens, (g) syringe pump. Used with permission from Ref. 72.

set at 45°. ⁷³ The laser-ablated material was ionized by interaction with the charged species in the electrospray plume positioned overhead the point of ablation. This secondary ionization process is similar to the 'fused droplet' ESI process described by Shiea's group⁷⁰ and sometimes called *extractive ESI* by others.⁷¹ Electrospray charging of airborne gases and particles is known to be a highly efficient process in other fields of use.⁷² Besides the apparent high secondary ionization efficiency, the other advantage of using ESI is the ability to form multiply charged species from macromolecular species like proteins. The other secondary ionization processes are limited to forming singly charged ions. The observation of the typical multiply charged envelope of ions from LD/ESI of proteins indicates that at least some of the laser-desorbed protein is dissolved in the charged electrospray droplets and proceeds to form ions by the typical ESI route.⁷³ Using this technique, it was possible to perform direct characterization of chemical compounds like amines on TLC plates⁷⁴ and detect intact proteins in dried biological fluids (e.g. blood, tears, saliva, and serum), bacterial cultures, and tissue⁷³ without a chemical matrix added to the samples to enhance the desorption process. Using a very similar experimental setup, Muddiman's group added a chemical matrix to the samples and named the resulting process *matrix-assisted laser desorption electrospray ionization* (MALDESI).⁷⁵ They reported the need to add a matrix, because they could not reproduce the 'matrix-less' ELDI results from Shiea's group. The reasons for this discrepancy are unclear.

While the ELDI and MALDESI techniques use a UV laser source, Nemes and Vertes⁷⁶ introduced what they called *laser ablation with electrospray ionization* (LAESI) technique, which uses an IR laser. Murray's group⁷⁷ also reported LD/ESI using an IR laser and they referred to the technique as *infrared laser assisted desorption electrospray ionization* (IR LADESI). This acronym (*vs* LAD/ESI or simply LD/ESI) could be confusing to some as 'DESI' is not involved in the desorption process. Using a mid-IR laser, Nemes and Vertes⁷⁶ were able to examine biological samples that have resonant frequencies in the IR because of the inherent water in the samples. Thus, the IR-LD/ESI approach allows for direct analysis of 'wet' biological samples. While the technique seems to work with samples containing high water content (e.g. fruit), it is limited with dried samples or less water-rich tissues like bone, nail, or dry skin.

Atmospheric pressure matrix-assisted laser desorption ionization (AP-MALDI)

AP-MALDI was first reported by Burlingame and coworkers in 2000.⁷⁸ In the last couple of years, the number of publications that describe the use or study of AP-MALDI has increased significantly as the commercial plug-and-play technology⁷⁹ has become more widely available. A more detailed discussion of the technique and applications is beyond the limited space of this tutorial article and the interested reader is referred to the reviews^{80,81} and some of the broad overview articles^{82,83} that appeared on the subject. A schematic of a commercial AP-MALDI source coupled to an LCQ ion trap is shown in Fig. 12. The coupling with ion trap mass spectrometers, orthogonal acceleration

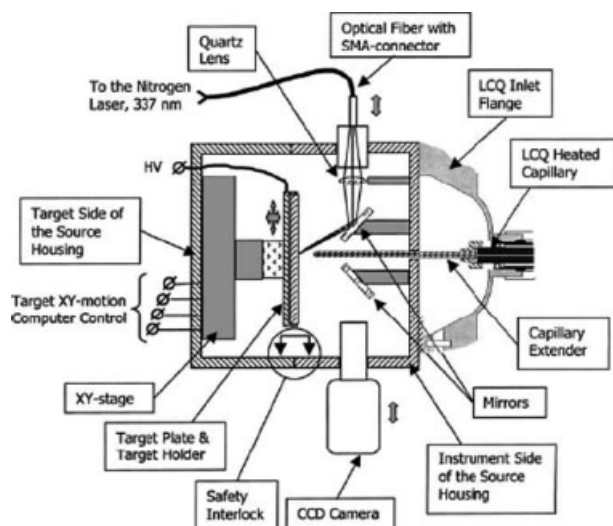


Figure 12. Schematic of AP-MALDI setup. Used with permission from Ref. 82.

time-of-flight mass spectrometers, and hybrid instruments takes advantage of their high duty cycle and the capability to perform MS/MS and MSⁿ (in the case of the ion trap) experiments. Thus, many of the AP-MALDI application papers are taking advantage of MSⁿ to provide quality structural information from analytes. The newest commercial AP-MALDI sources are configured for fully automated sample analysis and chemical imaging of a surface.⁷⁹

The mechanism of ion generation in AP-MALDI is believed to be the same as in vacuum MALDI (although that issue is not completely settled)⁸⁴ and is believed to be as efficient.⁸⁰ One difference between vacuum MALDI and AP-MALDI mass spectra is that the latter generally contain less fragmentation, because of the rapid collisional cooling that occurs at AP. This same process benefits all the LD/I techniques. The poorer detection levels compared to vacuum MALDI that were apparent early in the development of the technique have largely been overcome.

Different lasers have been used in AP-MALDI, but the most common is the 337-nm UV nitrogen laser. As in AP-LD/SI discussed above, there has also been a move toward the use of IR lasers in AP-MALDI.^{85–89} For example, Vertes' group⁸⁷ has used a 2940-nm IR laser for sampling tissue *in situ*, which utilizes the inherent water in samples like fruit or tissue as a matrix. They have demonstrated the ability to image the distribution of sugars in cross section of fruits and plants. A potential issue with IR-MALDI is lower ion yields compared with UV-MALDI. However, the secondary ionization processes discussed above should open a way to circumvent this limitation.

LIQUID AND GAS JET DESORPTION/IONIZATION

As mentioned above, *DESI*, introduced in late 2004,^{1,90} seems to be the technique that triggered a rapid increase in AP-surface sampling/ionization research and applications. A schematic rendering of a typical *DESI* setup is shown in Fig. 13.⁹¹ A pneumatically assisted ESI source is mounted above the surface to be analyzed. In many cases, an external

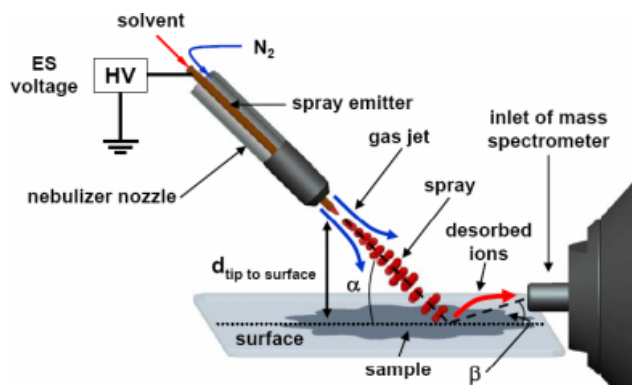


Figure 13. Schematic rendering of a typical *DESI*-MS setup. Used with permission from Ref. 91.

atmospheric sampling capillary is added to the atmospheric sampling inlet of the instrument to extend over and rest very close to or upon the region to be sampled. This arrangement allows a larger surface area and a variety of sample geometries to be easily analyzed. Often the surface of interest is mounted upon a moveable stage for aid in proper sample positioning, to improve the reproducibility of analysis, and to provide the ability to image the surface. As in ESI, a voltage of ~4 kV is typically applied to the emitter. This high voltage, solvent flow and coaxial nebulizing gas combine to create a high-velocity spray of charged solvent microdroplets. This spray is directed at the surface to be interrogated, creating an elliptical impact region where dissolution and desorption/ionization of the analyte takes place.

The size of the *DESI* spray impact region and its point of impact relative to the sampling capillary are largely controlled by the distance of the *DESI* spray tip from the surface, the angle α , and solvent and gas flow rates.⁹⁰ Adjustment of these parameters is carried out to optimize detection, but the optimum setting can vary to some degree among analyte and surface types. Typically, the emitter is mounted 1–3 mm above the surface to be analyzed, and at an angle of 45–60 degrees relative to it. Spray-to-capillary distance typically ranges from 2–3 mm. A geometry-fixed *DESI* system that eliminates sample-to-sample parameter adjustment has been developed for spot sampling, but it may not be suitable for applications like surface imaging.⁹² Any solvent appropriate for use in ESI can be used. Solvent additives typical for ESI (e.g. volatile acids and bases), as well as additives not usually used in ESI (e.g. nonvolatile salts like NaCl), or other chemical reagents can be used to enhance detection, a process often referred to as *reactive DESI*.³⁸ Typical solvent flow rates range from 1.5 to 10 $\mu\text{l}/\text{min}$, and gas flow velocities from ~150 to 500 m/s. The spray/surface and analyte/surface interactions are also important parameters relating to the efficient desorption/ionization and sampling of analyte ions into the mass spectrometer.^{90,96,93}

In *DESI*, the charged solvent droplets impacting the surface dissolve some of the analyte, and charged secondary droplets containing the analyte are subsequently liberated from the surface by mechanical force of the impacting droplets or nebulizing gas and/or by electrostatic forces.

Once an analyte is present in the charged droplets leaving the surface, the normal mechanisms giving rise to gas-phase ions from analytes in droplets during ESI are presumably in operation.⁹⁴ Analyte ions and secondary charged droplets resulting from impact (which subsequently liberate analyte ions) are carried into the atmospheric sampling capillary of the mass spectrometer by the drag forces created by the vacuum system. Note, however, that depending on the analyte and the precise operating conditions, it appears that other ionization mechanisms can also be at work leading to formation of species like radical cations that are more typically observed with an APCI source.^{1,90} Thus, DESI may operate as a mixed ionization mode source under certain conditions. The dominant desorption/ionization process in most DESI experiments has been referred to as “droplet pick-up”,⁹⁵ or what might even more descriptively be explained as a liquid–solid microextraction/liquid film-droplet sputtering process. Under typical operation conditions, the DESI spray impacts and wets a small elliptically shaped area on the surface. This impact area has three major regions (Fig. 14).⁹⁶ The first small inner region of the impact area, centered on-axis from the sprayer tip to the surface, is the most effective desorption/ionization region. The second zone is composed of solvent jets that originate from the inner region, and the third region consists of large, slow-moving droplets on the periphery of the larger impact region. These outer two regions are much less effective in generating gas-phase ions from the species on the surface. This wetted area continues to be impacted by droplets from the spray, a situation in which the physical processes typical for droplets impacting thin wetted films apply, including splashing, jetting, and secondary droplet formation.^{97,98} A modeling study investigating droplet collisions with a liquid thin film in DESI has confirmed the droplet pickup mechanism, showing that the secondary droplets leaving the surface of the film after primary droplet impact contain both liquid from the thin film and liquid from the impacting droplet.⁹⁵

The numerous literature reports on DESI-MS demonstrate its potential to be a powerful tool for a wide range of surface-sampling applications. A perspective article covers

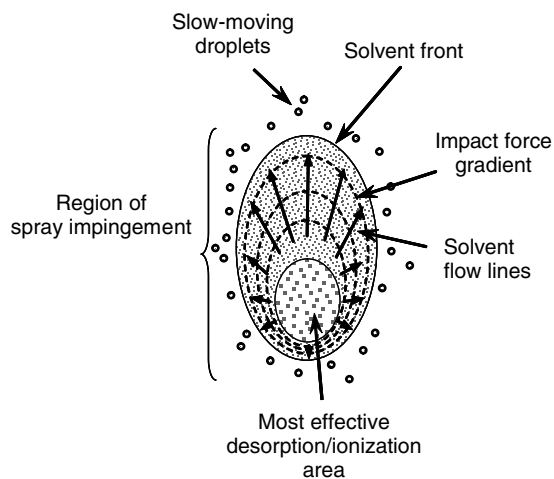


Figure 14. Idealized representation of the DESI impact plume region. Used with permission from Ref. 96.

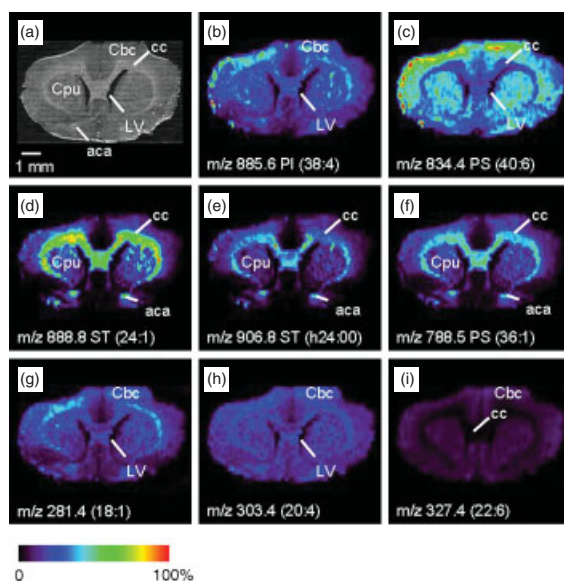


Figure 15. Selected molecular ion ($M - H$)⁻ images of specific lipids from analysis of a 13 × 10 mm² area of rat brain tissue section. (a) Optical image of the coronal section of the rat brain prior to analysis. Cc, corpus callosum; Cpu, striatum; Cbc, cerebral cortex; LV, lateral ventricle; aca, anterior part of anterior commissure. (b)–(i) Images of phosphatidylinositol (PI) (38 : 4; b), phosphatidylserine (PS) (40 : 6; c), sulfatide (ST) (24 : 1; d), ST (h24 : 1; e), PS (36 : 1; f), oleate (18 : 1; g), arachidonate (20 : 4; h), and docosahexenoate (22 : 6; i). Used with permission from Ref. 101.

many of these potential applications.⁹⁰ Some uses that may be exceptionally valuable are the analysis of analytes on planar separation media,⁹⁹ high-throughput sample analysis,¹⁰⁰ and chemical imaging.

Chemical imaging with DESI is a particularly interesting application that has been so far used to profile phospholipid distributions in rat brain thin tissue sections,^{101,102} analyte bands on TLC plates,¹⁰³ and inked lettering and images on paper.^{102,104,105} To obtain the rat brain images shown in

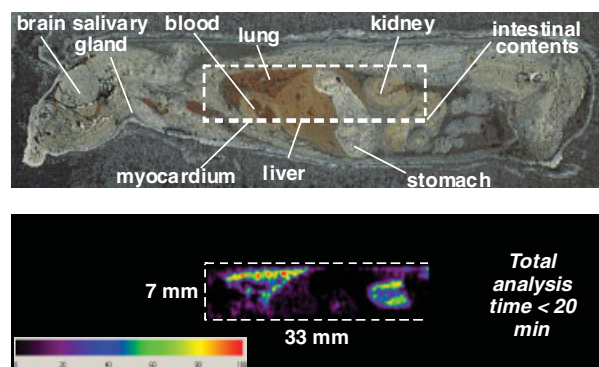


Figure 16. Optical image (upper) and DESI-MS/MS image (lower) of propranolol in 40-µm sagittal tissue section of mouse dosed IV with propranolol (7.5 mg/kg) and sacrificed 20 min post dose. SRM detection of drug using 30 ms dwell time. Surface scan rate: 0.5 mm/s. DESI solvent: 80/20 (v/v) ACN/H₂O at 5 µl/min, 500 µm spaced lanes. Used with permission from Ref. 108.

Fig. 15, the tissue sections were mounted on a motorized translational sample stage, and the image acquired by conducting a series of lane scans of the sample while acquiring mass spectra. Following data acquisition, the spatial intensity distributions of selected ions were mapped using the BioMap image analysis software.¹⁰⁶ Lateral spatial resolution was estimated as $<500\ \mu\text{m}$, but there are indications that given the appropriate operating parameters, surface type, and analyte characteristics, one can achieve resolutions better than $100\ \mu\text{m}$ with DESI-MS.¹⁰⁷ Even with this modest spatial resolution, DESI has the potential to become a valuable analytical method for identifying the distribution of endogenous and even exogenous compounds in tissues. In the latter case, for example, Kertesz and Van Berkel¹⁰⁸ have shown the ability to image, at relatively rapid speeds, the spatial location of propranolol in regions of whole body thin tissue sections from mice dosed at pharmacologically relevant levels (Fig. 16). However, for this application aimed at determining the drug and metabolite distribution in small animals for drug discovery purposes, the detection levels and sensitivity of the technique will need significant improvement before it can be a practical tool.

By simply turning off the variable high voltage in the DESI experiment and providing sufficient nebulizing gas velocity, one creates what some have called a *desorption sonic spray ionization (DeSSI)* source.^{90,109,110} Sonic spray ionization (SSI) is a liquid solution introduction ionization source that uses only the forces of a high-velocity nebulizing gas to generate gas-phase ions from species in solution.^{111,112} Because no voltages are used, the spray plume is nearly neutral, consisting of a distribution of positively and negatively charged droplets. This has the advantage of allowing the analysis of both positive and negative ions without the need to rapidly switch high voltages in the source. Confusingly, this same DeSSI technique is now also being called *easy ambient sonic spray ionization (EASI)* by at least one research group.^{113,114}

One can view the high voltage and nebulizing gas velocities in DESI and DeSSI as variable parameters that at one extreme (high voltage and low gas velocity) is the DESI source and at the other extreme (no voltage and high gas velocity) is DeSSI. However, one source creates unipolar spray droplets with significant ion current and the other a bipolar, near neutral plume. These differences may influence the desorption process and certainly do relate to the ionization process. Understanding desorption and ionization processes ongoing in these two techniques may lead to better

understanding of issues like surface charging and analyte alteration in DESI.^{110,115} Most species that are observed with DeSSI are observed with DESI, but the opposite does not appear to be true. Empirically, we have seen that preformed ions and very basic or acidic compounds in particular are amenable to DeSSI; these also work well in DESI (and ESI). Less polar analytes may not be as amenable to DeSSI. Often the chemical noise can be lower in DeSSI, leading to better signal/noise levels. More experiments are required to determine which technique might be best for specific types of analytes, and under what analysis conditions.

At the extreme of high liquid flow in these 'spray' desorption/ionization sources is the technique termed *jet desorption ionization (JeDI)*.^{116,117} In JeDI, a fused silica capillary with an inner diameter of $1\text{--}20\ \mu\text{m}$ is used to generate a highly collimated continuous liquid jet oriented in a position relative to the surface, similar to the liquid droplet/gas jet emitter in DESI. A voltage of 4 kV is applied at the emitter to the solution that is pumped through the emitter at flow rates ranging from 0.05 to 0.15 ml/min. The high-velocity jet stream produced in JeDI continuously erodes the sample surface and generates gas-phase ions. The ability to depth profile, and spatial resolution as good as a few micrometers are some of the intriguing features of this technique. Scans across lines of a peptide deposited on a glass slide showed a spatial sampling resolution from 1 to 5 μm .

In the technique termed *neutral desorption extractive electrospray ionization (NDEESI)*, the general desorption and sampling geometry of the DESI-type experiment are again retained, but both the liquid and high voltage are eliminated from the experiment. Only a moderate velocity (*ca* 10 m/s), room temperature gas jet is available to accomplish the desorption step (Fig. 17).¹¹⁸ Ionization of material desorbed from the surface is accomplished by a secondary ESI process, although there seems no reason, other than analyte type and the type of ions desired, that prevents another ionization process, such as any of the APCI techniques, from being used. For the most part, small, volatile compounds like amines derived from spoiled meat have been analyzed, although some much less volatile explosives have been analyzed directly from the skin. Ultimately, a successful analysis is limited by what can be aerosolized and transported to the ion source by the gas stream aimed at the sample. Thus, volatile or semivolatile species would seem most amenable to analysis. Species that are liberated from the surface as particles by the gas stream and transported to the ionization source might also be observed in the mass spectra.

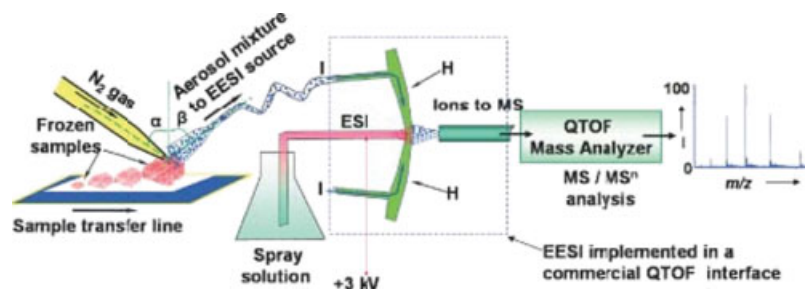


Figure 17. Schematic diagram of the atmospheric pressure neutral desorption extractive electrospray ionization MS. Used with permission from Ref. 118.

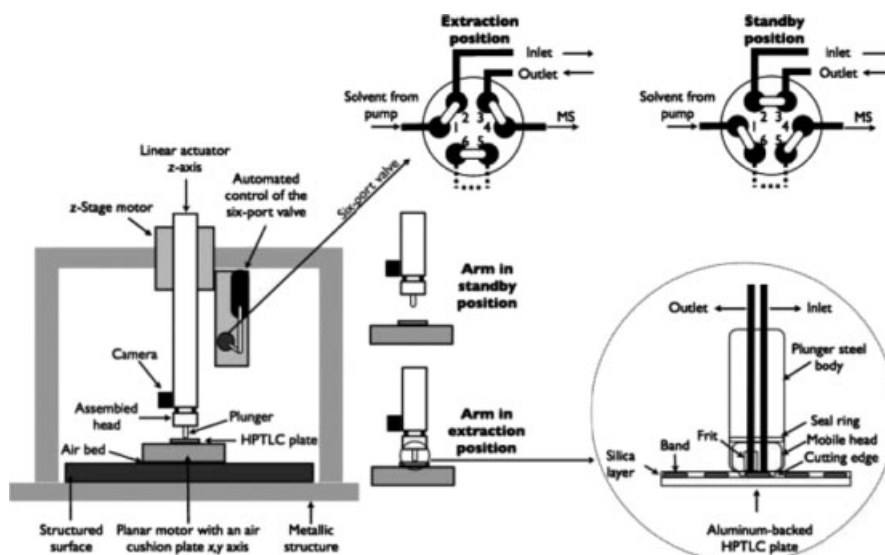


Figure 18. Schematic of the automated Luftmann sealing surface-sampling probe. Used with permission from Ref. 126.

LIQUID EXTRACTION SURFACE-SAMPLING PROBE/IONIZATION

We categorize here liquid extraction surface-sampling probes as those systems that directly reconstitute or extract an analyte from a surface by contacting the surface with a confined liquid stream. The stream is both brought to the surface and is then carried on to the ionization source through a probe acting as a liquid conduit. This contrasts with the normal liquid introduction ion source system where a liquid solution of the analyte enters the ion source through a tubing connection either via continuous infusion of the sample solution, from a solution injection into the flow stream, or as the eluant from a liquid-phase separation. There are two basic types of surface-sampling probes currently being used, viz. a *sealing surface sampling-probe (SSSP)*¹¹⁹ and a *liquid microjunction surface-sampling probe (LMJ-SSP)*.¹²⁰ While the emphasis in each case has been on coupling with ESI, the use of APCI has been demonstrated,^{121,122} and there is no fundamental limitation to using other liquid introduction ionization sources such as APPI, ICP, or any other. Once in solution and carried into the source, the analyte ionization is governed by the processes fundamental to the particular ionization source. Thus, these surface-sampling probes can be applied to all species that can be dissolved and conducted into the probe and subsequently ionized by the respective ionization method being used. Though not yet demonstrated, it should also be possible to integrate sample concentration and cleanup and separations with these sampling probe systems. This added analysis flexibility might prove valuable, for example, when analyzing complex mixtures or sample matrices that cause signal suppression.

Sealing surface-sampling probe

At least two elution approaches with the same basic type of sealing surface-sampling probe concept have appeared in the literature.^{119,121} However, the probe and elution concept by Luftmann¹¹⁹ has been most often used because it is available commercially.¹²³ These sealing surface-sampling probes have been used exclusively to date for the analysis of analytes

separated on TLC plates. As such, the applications have been limited to relatively low molecular-mass compounds like caffeine or other pharmaceuticals, or components of plant extracts.^{124–126} One would expect, however, that the same type of probe or possibly a probe with a different sealing mechanism (e.g. an o-ring *vs* knife edge seal) could be used to sample from many other types of surfaces. This might include any number of hard, nonporous materials such as glass, teflon, or various plastics, or soft, sometimes porous, biological materials such as skin or thin tissue sections.

A schematic of the Luftmann type sealing sampling probe and eluting scheme is shown in Fig. 18 for a recently described automated version of the device.¹²⁶ The inlet capillary of a stainless-steel plunger which seals to the surface to be sampled is connected to an HPLC pump. The outlet capillary is connected to the ion source of the mass spectrometer. The zone of interest on the TLC plate is positioned under the probe, and the surface sealed by compression with the knife edges on the probe. By switching the valve from standby to extraction mode, the solvent that was previously flowing directly to the ion source travels to the surface, extracts the analyte from the surface, and then carries it onto the ion source. Between elution steps, the probe is moved to a docking station, where the sampling face of the probe is automatically cleaned of accumulated particulate material from the surface sampled by pressured air. The probe typically has a diameter of 2 or 4 mm. A 2 × 4-mm elliptical sampling head has also been used to better match TLC band size and shape for more effective sampling. The reported limits of detection are lower picogram levels for the amount of material applied to the plate. Assuming that the complete analyte band on the plate is sampled with a 4-mm sampling probe, this equates to detection levels around 5–50 fg/mm². Quantification with isotopically labeled internal standards provided better than 5% relative standard deviation (RSD) and relaxed the requirement for exact positioning of the sampling probe relative to the band on the plate to achieve quality quantitative results.

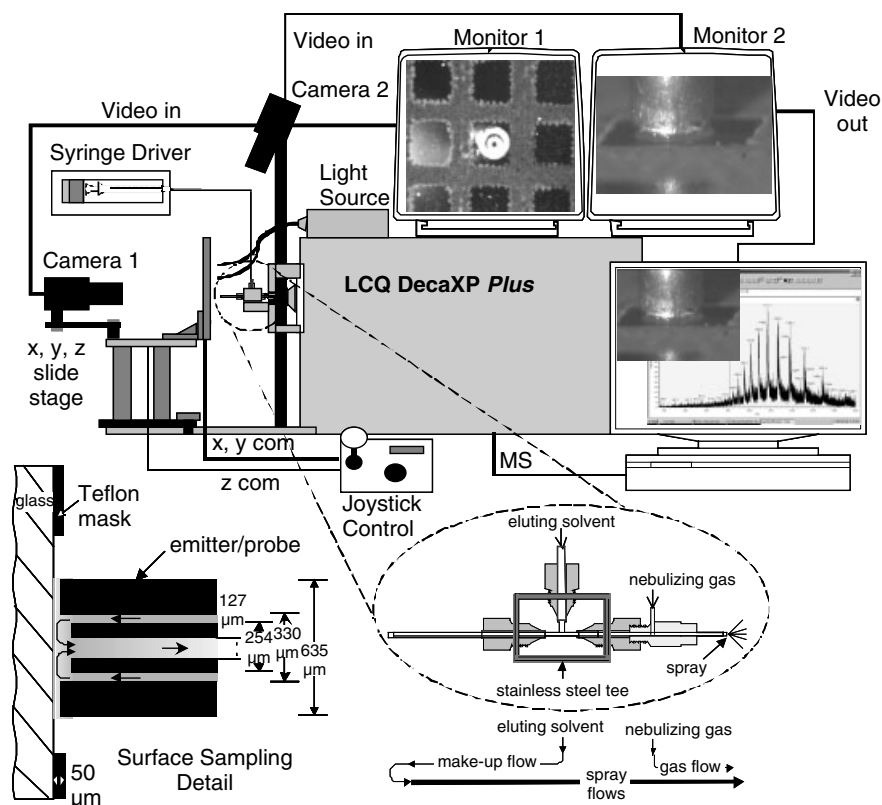


Figure 19. Schematic illustration of the complete ES emitter/surface-sampling probe ion trap mass spectrometer system. Blowup inserts show scale drawing of surface-sampling detail and the details of the emitter/sampling probe solvent and gas flow paths. Monitors show images of sampling probe at the array surface forming a liquid microjunction. Used with permission from Ref. 131.

Liquid microjunction surface-sampling probe (LMJ-SSP)

The genesis of the LMJ-SSP can be traced back to Wachs and Henion¹²⁷ and Lev and coworkers¹²⁸ who first reported on a coaxial tube sampling end for a probe used to sample analytes from surfaces in conjunction with ESI. In these probes, annular space between the inner and outer capillary tubes facing the sample was used to deliver solvent to the surface. The inner tube was used to draw liquid from the surface to the ES source. This design is essentially a 'sipper' source that could be configured to sample material at, in, or just above a surface. Figure 19 shows a schematic illustration of a complete LMJ-SSP/ESI-MS system, including details of the surface-sampling process. To date, analytical applications of the LMJ-SSP for surface analysis have involved the sampling and analysis of dried drugs or proteins or solutions thereof from wells on microtiter plates,¹²⁷ drugs captured in solid-phase extraction cards,¹²⁹ a variety of dyes, inks, or pharmaceuticals on paper or separated on hydrophobic reversed-phase (RP) (C8 and C18) TLC plates,^{120,130} and surface-deposited and affinity-captured proteins.¹³¹ These applications include our work showing automated lane scanning with TLC plates and automated imaging of inked lettering on paper.¹³² Detection levels reported on the more sensitive mass spectrometers have typically been in the range of 10–100 fmol/mm². Quantification with isotopically labeled internal standards has also been demonstrated with typically better than 5% RSD.

One successful application of the LMJ-SSP has been the read out of developed hydrophobic RP TLC plates.^{120,130} Important in this work was the fact that commercial RP C8 or RP C18 TLC plates were used and no postdevelopment processing other than drying of the plates was necessary prior to analysis. However, this application also points to a current limitation to the technique. Only hydrophobic RP plates could be successfully analyzed; normal-phase plates and wettable RP plates were not successfully analyzed. With these phases, a fraction of the eluting solvent flowed radially out of the probe into the thin-layer separation phase by capillary action. This moved the analyte at the sampling spots out from the vicinity of the probe. This same limitation exists with the current probe design when attempting to sample from any surface type in which the liquid is conducted out from the probe into the surface at a rate faster than it is aspirated back into the probe.

Just recently, a LMJ-SSP combined with ESI-MS has been demonstrated as a viable means to sample and detect exogenous compounds from thin tissue sections.¹³³ A stable sampling LMJ, a prerequisite for successful surface sampling, could be formed with liver, brain, and other organs using various solvents (viz. methanol, acetonitrile, water, and additives). Imaging of individual organs such as a liver or a brain was possible with a readout resolution roughly the size of the sampling end of the probe (*ca* 500 μm). Imaging whole body sections was postulated to be more difficult, because different tissue types can require a change in conditions required to maintain the optimum

LMJ. This is a particular problem in regions where the tissue is absorbent. Experimenting with different tissue thicknesses and tissue mounting methods may find tissue surface characteristics more amenable to imaging by this approach. Nonetheless, it already appears that the LMJ-SSP might be used as a relatively rapid discovery tool in applications like drug discovery. When used in a spot-sampling mode, signal levels are maintained for >30 s, providing ample time for multiple 'discovery' type scans such as full, precursor ion and neutral loss scans, in addition to targeted detection and confirmation modes like SRM and product ion scans. Controlled experiments will also need to be devised and carried out to determine the level, if any, of matrix effects or signal suppression/enhancement associated with sampling/ionizing material associated with a particular tissue. The results from such studies will help to determine whether the LMJ-SPP might be a useful quantitative tool in this same application.

CONCLUSIONS

Seemingly new AP-surface sampling/ionization techniques for MS have been emerging in the literature at a rapid rate over the last several years. This explosion in the number of techniques, and the associated acronyms for these techniques, has presented a challenge to those interested in appreciating the field as a whole. In this tutorial article, as summarized in Table 1, we have attempted to simplify this field by sorting the vast array of techniques into four main subcategories based primarily on the approach used for surface sampling (i.e. heat, photons, droplet or gas impact, and direct liquid extraction) that are further differentiated on the basis of the ionization process (e.g. APCI, ICP, and ESI). When this is done, one sees that many of the techniques are simple variations on an existing theme and that some AP-surface sampling/ionization techniques like TD/APCI and LA/ICP have been established for as long as two decades. As other techniques come on the scene it should be possible to place them within this classification scheme. However, these categories are not hard and fast and might look somewhat different, if, for example, they were based primarily on the ionization process *versus* the desorption process. As was apparent from the discussion above, an understanding of the desorption mechanisms, and, in a few cases, the ionization processes are required to more accurately categorize some of the techniques. With this better understanding, the placement of some of these techniques might also change and it will be possible to better appreciate their analytical potential, and suggest modifications or alternatives that continue to push forward the area of AP-surface sampling/ionization. In any case, AP-surface sampling/ionization has established roots in MS and, by all indications, will continue to be an area of rapid evolution and growth for the foreseeable future.

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