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REVIEW

Ion sources for mass spectrometric identification and imaging of molecular species

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The ability to transfer molecular species to the gas phase and ionize them is central to the study of natural products and other molecular species by mass spectrometry (MS). MS-based strategies in natural products have focused on a few established ion sources, such as electron impact and electrospray ionization. However, a variety of other ion sources are either currently in use to evaluate natural products or show significant future promise. This review discusses these various ion sources in the context of other articles in this special issue, but is also applicable to other fields of analysis, including materials science. Ion sources are grouped based on the current understanding of their predominant ion formation mechanisms. This broad overview groups ion sources into the following categories: electron ionization and single photon ionization; chemical ionization-like and plasma-based; electrospray ionization; and, laser desorption-based. Laser desorption-based methods are emphasized with specific examples given for laser desorption postionization sources and their use in the analysis of intact microbial biofilms. Brief consideration is given to the choice of ion source for various sample types and analyses, including MS imaging.

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1 General characteristics of ion sources

Many analytical techniques have been applied to characterize the natural products found in living organisms, such as plants and microbial communities. Mass spectrometry (MS) is one of the more extensively used techniques for the characterization of small molecule natural products due to its potential for rapid analysis, high throughput, and accuracy in identification of a wide variety of sample types.[1–5] The ability to transfer molecular species from the condensed phase to the gas phase and in the process, ionize them is central to their analysis and a few methods have traditionally dominated natural products analysis by MS, as described elsewhere in this issue. The choice of technique to transfer to the gas phase and ionize is ideally governed by the chemical and physical properties of the target molecule and the biological substrate in which it is found. However, more traditional methods often utilize extraction and purification strategies that are not always ideally suited for specific analyses. MS imaging strategies are rapidly developing to probe intact, native biological samples, thereby preserving spatial distributions of molecular species.[6–12]

This review attempts a brief overview of some of the more popular and/or promising ion sources that are increasingly applied to natural products MS, but which are also applicable to other fields of analysis, including materials science. Ion sources are grouped into common modes of ionization of neutral species, ranging from electron ionization to electrospray ionization. Ion sources are categorized by how they transfer neutrals into the gas phase, using strategies ranging from evaporation to nebulization to laser desorption. The most common types of precursor ions that are formed are given for each class of ion source. Precursor, molecular, or pseudomolecular ions include: the less stable, odd electron radical cations $M^{\bullet+}$ of an analyte M; the more stable, even electron protonated species MH^+,

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including multiply protonated or deprotonated species $(MH_n)^{n+}$ or $(M–H_n)^{n-}$; those associated with alkali metal ions or other metals such as $(M + Na)^+$, $(M + K)^+$, or $(M + Ag)^+$; adduct ions such as $(M + NH_4)^+$ or cluster ions such as $M(H_2O)_nH^+$; and negative ions formed by electron attachment M^-, deprotonation $(M–H)^-$, or clustering. The extent to which an ion source imparts internal energy to the precursor ions, where "soft" and "hard" ionization correspond to low and high internal energy, respectively, is also considered as it qualitatively predicts the extent of precursor ion fragmentation.[13]

The pressure range at which the ions are formed is highly relevant: ion sources under high vacuum of $<10^{-5}$ mBar do not permit ion-neutral gas phase collisions. By contrast, sources operating at atmospheric pressure (AP) allow many gas phase collisions, facilitating ion cooling, ion–molecule reactions, and/or removal of adducts *via* collision induced dissociation. AP sources also permit intact samples to be analyzed under ambient conditions,[12] while vacuum sources induce sample dehydration prior to analysis.

The various ion sources can be further categorized based on their compatibility with sample pretreatment and separation strategies, such as clean up, extraction, and chemical derivatization. An ion source's potential for coupling to gas or liquid chromatography (GC and LC, respectively) are only briefly addressed as this issue is dealt with in other contributions to this issue. Ambient pressure or AP sources, particularly those based on electrospray ionization, are discussed in detail elsewhere in this issue and are only briefly described here. The reader is also referred to a table comparing the general characteristics and applications of many of these ion sources.[8] For example, some ion sources are particularly well suited to MS imaging analyses of intact biological samples, such as intact leaves or microbial communities.[6,7,9–12,14,15] However, the issue of what source to choose for a particular problem cannot be easily summarized. The reader is referred to the many excellent articles in this issue which discuss in great and expert detail the application of specific ion sources to specific analytical problems in natural products discovery.

The review ends with some brief comments on the diversity of ions source, multimode ion sources, and comments on choosing an ion source for specific analyses. More specialized reviews of each class of ion source are cited throughout, with emphases on the most recently published reviews. Finally, this review is meant as a brief overview and is neither a comprehensive review of all available ion sources nor of the relevant literature. The authors apologize in advance for any omissions.

2 Ionization of gaseous neutrals: Electron Ionization (EI *vs.* Single Photon Ionization (SPI)

Electron ionization (EI) and single photon ionization both result in ionization of desorbed neutrals to form radical cations. Fig. 1 shows a schematic comparing theses two ion sources. EI is historically the ion source most widely used in organic mass spectrometry.[16] EI involves evaporation or mild heating of a sample to form gaseous neutrals, which are then ionized by collisions with 70 eV kinetic energy electrons to produce radical cations, $M^{\bullet+}$. EI data is highly reproducible between different instruments, which has allowed the collection of what are probably the most extensive, highest quality mass spectral libraries[17] whose use facilitates identification of known compounds and helps determination of unknowns. Those analytes most suited for EI analysis are relatively volatile and thermally stable. EI is usually coupled to GC for chromatographic separation, necessitating sample pretreatment and extraction of natural products from their native source.

EI is a hard ionization technique whose mass spectra are often so dominated by fragment ion peaks that the precursor ion is often either low intensity or not present. EI analysis suffers the further disadvantage of not being well suited to the study of thermally labile molecules, such as alkaloids or those

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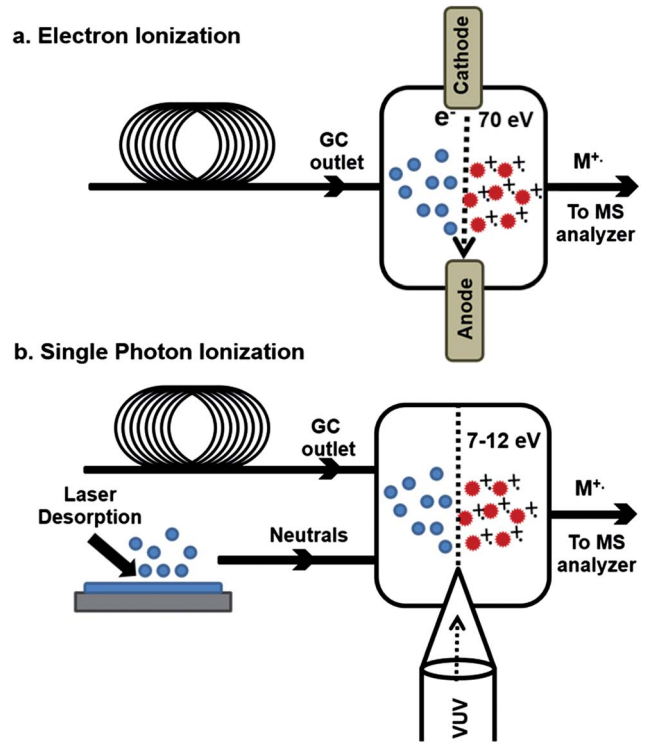

Fig. 1 A schematic of (a) electron ionization (EI) and (b) single photon ionization (SPI) sources. The figure also shows the dominant radical cation precursor produced by the ionization process.

with molecular weights above ~500 Da. While fragmentation can be reduced slightly by lowering the kinetic energy of the ionizing electrons, this comes with a severe loss in signal due to the reduced ionization efficiency.[16] Supersonic cooling of the neutrals prior to ionization[18] or more commonly, chemical derivatization with trimethyl silane or other compounds that facilitate analyte volatilization, are both used to reduce fragmentation in EI-MS and enhance precursor ion signal.

Single photon ionization (SPI) also generates radical cations from gaseous neutrals, but it uses vacuum ultraviolet (VUV) radiation rather than electrons for ionization, as shown in Fig. 1.[19,20] SPI is a much softer ionization technique than EI and usually produces an intense molecular ion signal. SPI generally occurs when gas phase analyte neutrals have ionization energies below the energy of irradiating photons. VUV photons at various energies can be generated by a laser, a rare gas discharge lamp, or a synchrotron light source, but ~10 eV is the most widely used photon energy for SPI. Higher photon energies show signal enhancement, but fragment and other low mass ion signals also increase. Photon energies below 10 eV are insufficient to ionize many species, since natural products typically have ionization energies in the range of 7–10 eV.[7] SPI induces less fragmentation than EI because ~10 eV photons impart only a few eV of excess internal energy into the precursor, reducing fragmentation compared to the ~60 eV of excess energy imparted by 70 eV EI.[7,13,19,20] The ~10 eV photon energy is therefore an optimum balance between sensitivity and fragmentation while excluding ionization of water, carbon dioxide and other uninteresting, but abundant species with high ionization energies. The reduced ion signal from fragmentation and background species decreases chemical noise in SPI for improved sensitivity, especially compared to EI. Furthermore, clusters of pure analyte, analyte/solvent, or analyte/matrix have lower ionization energies than do isolated molecules, permitting SPI of such clustered species at slightly lower VUV photon energies.[21] SPI in vacuum can additionally dissociate clusters to both protonated and non-protonated ions,[21] although ~10 eV VUV photons are probably not sufficiently energetic to desolvate large, multiply charged droplets commonly formed in some ion sources (see below).

SPI has the ability to selectively ionize target analytes using different VUV photon energies,[19,20] as reported for the analysis of aerosols,[25] combustion products,[26] and chemical stimulants.[27] Although a wide range of target analytes and samples can be studied using SPI, selectivity can be introduced by the use of low photon energy VUV sources, such as the fluorine excimer laser. Chemical derivatization of high ionization energy analytes with a low ionization energy chromophore will create a complex whose ionization energy approaches that of the chromophore, thereby allowing selective detection of the complex from a mixture by SPI using a low photon energy source.[7]

The extent of fragmentation for SPI depends on how much internal energy is imparted to species when they are transferred into the gas phase[13] (*i.e.*, transfer line temperature when coupled to GC), since internal hot neutrals will dissociate more than cold neutrals upon SPI.[24] The energy of the VUV photons used for SPI also affects the fragmentation of the analyte, but only to a lesser degree than the initial internal energy of the neutrals when the VUV photon energy is within a few eV of the ionization energy threshold.

A wide variety of analyses have been performed by SPI,[19,20] such detection of the aromatic vapors from individual coffee beans.[22] SPI yields depend on the photoionization cross section of the analyte, which only vary by an order of magnitude for different organic species compared to a variation of several orders of magnitude for EI.[19] This makes SPI potentially more quantifiable compared to EI and many other ionization methods.[23]

The most significant disadvantage of SPI is that commercial instruments are only beginning to be embraced by analysts, leading to a relative low utilization of the method compared to other more popular ion sources. Furthermore, SPI is performed under vacuum and adaptation to intermediate or atmospheric pressure fundamentally alters the ionization event (see below). Finally, the use of mass spectral libraries to assist in compound identification with SPI is far less well developed than it is for EI.[20]

3 Chemical, plasma, and related ionization methods

Chemical ionization was developed as the first soft ionization alternate to EI,[28] but is now available on relatively few commercial instruments. Chemical ionization proceeds most

commonly *via* proton transfer to or from reagent ions formed by EI *via* thermodynamically favorable gas phase ion-neutral collisions that occur at elevated gas pressures. Proton affinities for reagent and analyte predict proton transfer as well the amount of excess energy available for subsequent molecular dissociation. Analytes are introduced in chemical ionization by evaporation or rapid heating of a solid probe, again allowing facile coupling to GC.

The significance of chemical ionization lay not so much in its relatively infrequent current utilization, but rather because it serves as a mechanistic template for some of the more popular chemical, plasma and related ionization techniques discussed in this section and shown in Fig. 2.[30] Nevertheless, this analogy is limited in the case of AP ionization, where higher pressures introduce critical differences from traditional chemical ionization in terms of the types of ion–molecule reactions, collision frequency, ion source residence time, and hydrodynamic flow.[36]

Proton transfer reactions play a key role in ionization of analytes under elevated pressure conditions, as described below. For example, proton transfer reaction MS is a subset of chemical ionization in which the reagent ions are protonated water clusters (which themselves play an important role in many chemical ionization-like sources).[29] Proton transfer reaction MS has potential for application to the analysis of volatile natural products given its expanding usage in gas and aerosol sampling.

Atmospheric pressure chemical ionization (APCI, see Fig. 2) is the most popular of the chemical ionization-type sources and is available on the majority of mass spectrometers coupled to LC. Like chemical ionization, APCI generally forms singly charged precursor ions *via* proton transfer which in the positive and negative modes are protonated MH^+ and deprotonated $(M–H)^-$, respectively, formed by gas phase ion-molecule reactions with the reagent ions.[30] Analyte ions can also form in APCI *via* adduction of reactant gas, solvent, and/or clusters thereof as well as by other mechanisms (see below). Reagent ions in APCI are formed by a direct current corona discharge emanating from a needle located adjacent to the solvent/analyte aerosol formed in a nebulizer. APCI is best suited for the analysis of polar to relatively non-polar compounds with mass up to 1500 Da.

It has been argued that many of the other AP-based ion sources can be considered variants of APCI.[30] Atmospheric pressure photoionization (APPI) is mechanistically similar to chemical ionization and APCI, but is initiated by SPI induced by continuous VUV discharge lamps.[31–34] Direct VUV SPI of the analyte (similar to what occurs in vacuum) is possible in APPI, it typically occurs with low efficiency.[36] Efficient absorption of VUV by air and/or solvent molecules indicates that the initial photoionization event in APPI occurs either *via* more abundant solvent or dopant species, which initiate an eventual proton transfer to the analyte. Dopants are VUV photoionizable compounds, such as toluene, that are introduced in large relative concentrations to facilitate the ionization of analyte molecules *via* ion–molecule interactions. Furthermore, it has been argued that water and/or other solvent clusters can play an important role in proton transfer in APPI and related methods.[35] APPI in the negative mode produces radical anions as well as deprotonated species. Radical anions can be produced by direct electron capture or by charge transfer from excited gas anions of O_2^- or other atmospheric gases. It has been observed that APPI is generally more suited to study nonpolar compounds compared to APCI.[31]

Both APPI and APCI are highly sensitive to the experimental conditions and properties of solvents, additives, dopants, and buffer components present in the sample that can strongly influence the selectivity and sensitivity to specific analytes. APCI, APPI, and the other chemical ionization-like methods all suffer from a high background of chemical noise due to efficient ionization of gases, solvents and impurities. Thus, these chemical ionization-like methods also require sample cleanup or extraction of the target analyte from complex matrices prior to injecting on an LC column in order to avoid unwanted matrix interferences.

Several variants of APPI have been described. Micro-APPI is one of the earlier variants of APPI which renders the method compatible with microfluidic separation systems.[33] Desorption APPI (DAPPI) relies on a nebulizer microchip to deliver a heated jet of vaporized solvent for desorption, permitting efficient ionization of nonpolar and neutral compounds on surfaces, such as pharmaceuticals in tablet form.[37] Capillary APPI (cAPPI) and capillary photoionization (CPI) are variants of APPI specifically designed for coupling to chromatographic interfaces in which APPI occurs within the confined volume of a capillary, but differ in the types of VUV source and whether or not they employ a VUV window.[38,39] Both capillary APPI and CPI display improved sensitivity compared to APPI by improved ion transmission in the MS and reduction in unfavorable ion–molecule reactions. Capillary APPI is well suited to the analysis of volatile compounds while CPI can detect both volatile and nonvolatile compounds regardless of polarity since it provides additional sample heating. Atmospheric pressure laser ionization (APLI) replaces the VUV discharge lamp in APPI with a nanosecond pulsed laser operating at 248 or 266 nm, making the initiating ionization event a resonant enhanced multiphoton (REMPI) process.[36]

The original APCI source is driven by a corona discharge and the strategy of using plasmas to initiate ionization at AP has been widely expanded into new ion sources. Direct analysis in real time (DART) utilizes a He or N_2 plasma glow discharge at AP to induce positive or negative ion formation *via* interaction of neutrals with electronically excited (metastable) atoms or molecules in a process known as Penning ionization, as shown in Fig. 2.[40,41] Negative ions are produced either by electron capture due to electrons produced by Penning ionization or by ion–molecule reactions with negative ions formed from atmospheric water and oxygen, leading to deprotonation. Many of the disadvantages of other chemical ionization-like methods remain since ionization in DART can also proceed *via* many (or all) of the aforementioned secondary ion–molecule reactions.[40] Often, the metastable species efficiently ionize atmospheric moisture to form protonated water clusters, which then facilitate proton transfer to the analyte. Abundant non-protonated molecular ions have also been observed in DART analyses,

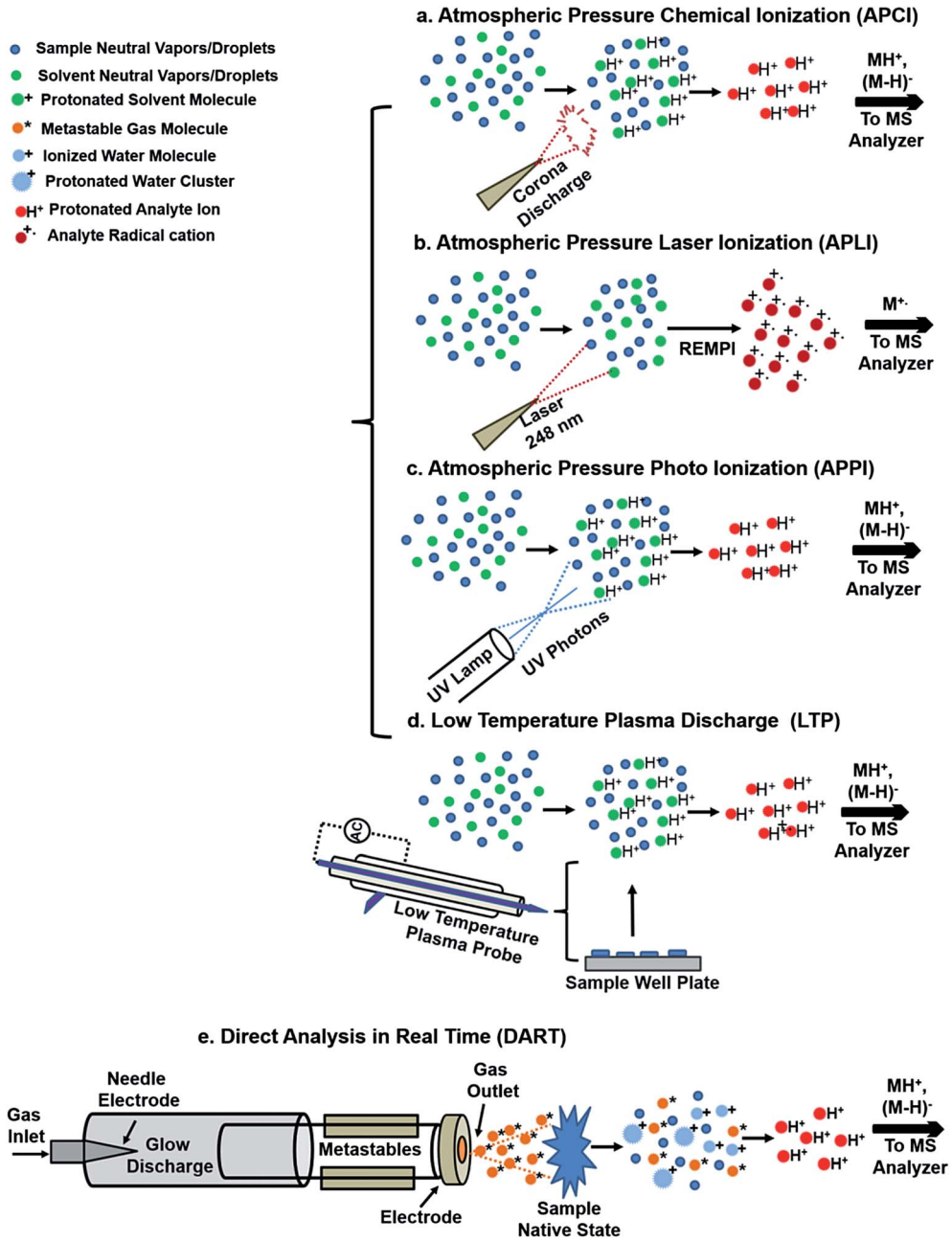

Fig. 2 A schematic of various chemical ionization-like sources: (a) atmospheric pressure chemical ionization (APCI), (b) atmospheric pressure laser ionization (APLI), (c) atmospheric pressure photoionization (APPI), (d) low temperature plasma (LTP), (e) direct analysis in real time (DART). The dominant precursor ions produced by each source are also shown.

often for nonpolar species, such as alkanes. Intact molecular ions are favored under certain experimental conditions in the DART source, which can also be tuned to enhance fragmentation. DART can analyze a wide variety of solid, liquid and gaseous samples with little pretreatment, including the analysis of natural products directly within leaves, microbial communities, or other complex biological matrices in which they are produced.[42,43]

Another AP plasma based ion source that is well suited for the analysis of nonpolar analytes is low temperature plasma (LTP) discharge, which utilizes a non-equilibrium plasma for desorption/ionization.[44] LTP employs a high frequency alternating current between specially designed electrodes to produce a dielectric-barrier discharge in helium, as shown in Fig. 2. Penning ionization is also evoked in LTP, specifically to form N_2^+ that undergoes subsequent ion-molecule reactions to form analyte ions.[44] Potential desorption mechanisms in LTP (and DART) of solid samples include thermal desorption, sputtering, and surface reactions. LTP is useful for studying low mass compounds over a relatively wide polarity range compared to APCI.

It should already be clear from the above discussion that many of the ion sources in this section have common mechanistic features. Furthermore, the atmospheric pressure plasmas used in APCI, DART, LTP, and related methods can generate electrons, radicals, clusters, nanoparticles, UV photons, and VUV photons in addition to atomic and molecular ions of both polarities.[45,46] While the mechanistic implications radicals have been considered in at least some chemical ionization-like methods,[36,40] VUV photons and the other energetic species also generated in plasmas have been largely ignored. Understanding how APCI and other AP sources function requires sophisticated computer simulations that takes into account the density and interaction of these energetic particles as well as their hydrodynamic and electrostatic flow from the source into the mass spectrometer.[30,47] Furthermore, radicals, ions, and/or VUV photons with surfaces can modify sample surfaces during MS analysis.[45,46,48] Thus, caution must be taken when claims are made that the aforementioned ion sources are non-destructive of solid samples.

4 Electrospray Ionization-based methods (ESI)

Fig. 3 shows a schematic comparing electrospray ionization (ESI), perhaps the most common ion source currently in use.[30]

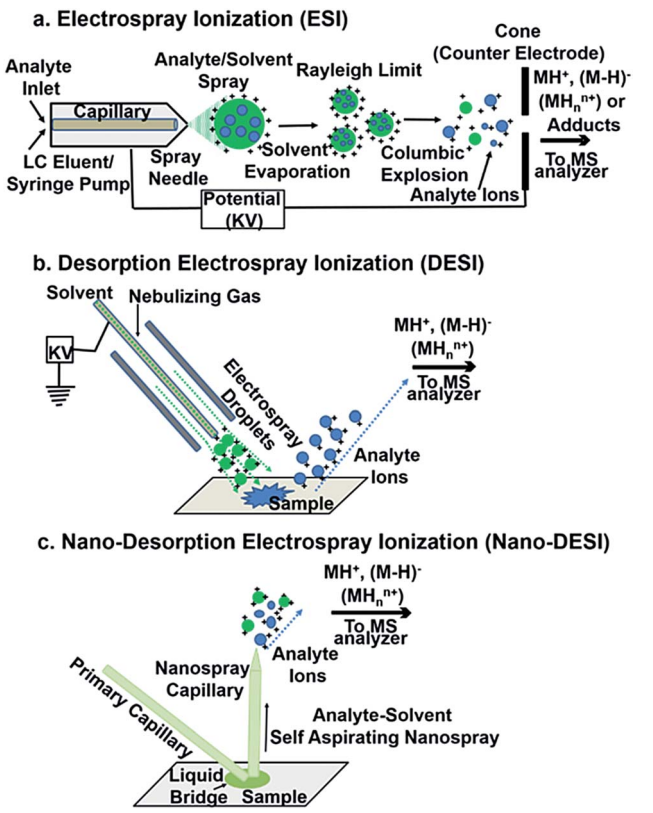

Fig. 3 A schematic of (a) electrospray ionization (ESI), (b) desorption electrospray ionization (DESI), and (c) nanoDESI. The dominant protonated and deprotonated ions produced by each source are also shown.

ESI and related sources are only described briefly in this section with relatively few examples because they are covered in a much more thorough fashion elsewhere in this special issue. ESI involves nebulization of a liquid feed, but differs from APCI in that ionization occurs from liquid droplets rather than in the gas phase. ESI applies a strong electric field to the sample solution passing through a capillary and the field induces charge accumulation on the liquid surface at the end of the capillary. This charging produces a Taylor cone, which breaks down to release highly charged droplets that are dispersed in space as micron and sub-micron sized droplets by flowing gas.[30,49,50] These droplets are then desolvated by passing through a curtain of heated inert gas which, *via* collisions and Coulombic explosion, leads to the formation of ions. ESI produces a wide range of ions varying from protonated and deprotonated molecules in positive and negative mode, respectively, to multiply charged ions for large molecules, such as $(MH_n)^{n+}$ or $(M–H_n)^{n-}$, and pseudomolecular ions such as $(M + Na)^+$, $(M + K)^+$, $(M + NH_4)^+$ and/or $(M + Cl)^-$. ESI is a soft ionization technique with very little fragmentation observed in the mass spectra. Ions are produced at AP and samples are often required to be pretreated and extracted into the liquid phase for inclusion in the liquid eluent of an LC feed. ESI can analyze a wide range of analytes, but is most effective for polar samples. ESI has the distinct advantage of forming multiply charged ions, enabling the study of large molecules at lower *m*/*z* values, which facilities their mass analysis. However, this leads to the observation that a given compound can display ions in multiple charge states, requiring mathematical deconvolution to convert the raw data back to mass spectra of singly charged masses *vs.* intensities.

Desorption electrospray ionization (DESI) reconfigures an ESI source so that the capillary is directed at a solid sample and the AP-interface of the mass spectrometer is positioned to collect the species desorbed and/or entrained from the sample's surface by the impinging charged droplets.[3,12,41,51] Studies of the desorption/ionization mechanism for DESI continues to evolve, with recent investigations suggesting that DESI occurs through wetting of the sample surface by the spray plume, extraction of the analyte into the wet surface film, momentum transfer from impacting particles and gas to form progeny charged droplets, and transport of said droplets towards the AP interface of the MS analyzer. DESI produces mass spectra similar to ESI with generation of singly or multiply charged precursor ions and like ESI, DESI is also used to study a wide variety of analytes ranging from small to very large molecules (~100 kDa). Amino acids, alkaloids, steroids, other drugs, peptides, proteins, and a wide range of other analytes have been analyzed by DESI, which also permits imaging analysis of intact biological samples.[12,14]

One variant of DESI is nanoDESI, in which analyte is desorbed into a solvent bridge formed between two capillaries and the analysis surface (Fig. 3).[52] One capillary supplies solvent to the sample and the second capillary transports the dissolved analyte to the mass spectrometer, allowing variation of the size of the sampled area. Another ESI variant is liquid DESI,[41] in which the sample solution is sheeted onto a surface and then the normal DESI spray is performed. Finally, a proximal heated

probe has been demonstrated to induce spatially confined thermal desorption from a sample surface for introduction in an ESI source.[53]

Paper spray transports a solvated sample by wicking in paper or another porous material that is cut to a sharp point to facilitate a process categorized as mechanistically similar to ESI.[12] Whole blood, dried blood, and tissue samples have been analyzed for hormones, drugs, and lipids by paper spray with minimal sample preparation.

5 Laser Desorption (LD)-based methods

LD-based ion sources are widely used for MS analyses of natural products, both for pretreated samples as well as for MS imaging of samples in native form.[6–8,10,54] These methods mostly employ nanosecond pulse length lasers in the ultraviolet (260–380 nm) and less commonly, the mid-infrared (~3000 nm). The laser wavelength must be strongly absorbed by the solid sample, analyte, and/or an added strong light absorbing matrix species to induce efficient desorption.[54] The mid-IR wavelength has the advantage that it is resonant with the water naturally present in native biological samples, allowing efficient desorption without the addition of matrix.[63,64] Ultrashort pulse lasers also avoid the need for a strong light absorber (see below). Ions can form directly in laser desorption ionization from a solid target. Alternatively, most of the ion sources discussed above can be used for postionization of neutrals that have been laser desorbed into the gas phase.

5.1 Matrix assisted and other methods of direct laser desorption ionization (LDI)

Matrix-assisted laser desorption ionization (MALDI) is by far the most popular of the LDI methods and another article in this issue details its use for natural products analysis. Samples are mixed with an organic matrix and are then irradiated with laser pulses, leading predominantly to singly charged protonated (or deprotonated) species. The mechanism of desorption ionization in MALDI is an explosive event that is a combination of molecular desorption, particle ejection, proton transfer, gas phase collisions, photoionization, charged droplet/cluster decay, and/or other phenomena.[55–59] MALDI is a soft ionization method where singly charged molecular ions are readily observed and multiply charged ions similar to those formed by ESI can be detected under certain conditions.[59] A matrix that absorbs most of the incident laser energy and a high matrix to analyte ratio are both typically required. MALDI was originally performed only on samples under high vacuum conditions, but is now also routinely performed at intermediate[60] and atmospheric pressures.[61,62] MALDI performed with UV lasers dominates due to lower laser costs and generally higher ion yields. However, mid-IR lasers allow MALDI without the addition of matrix compounds *via* excitation of water.[63,64] Both wavelength regions are widely used for MS imaging of samples in their native form.[6,8,10,15] The wide range of commercial instruments and sample preparation strategies have facilitated many natural products analyses with MALDI.

LDI can also be enhanced by the close association of metal or semiconductor coatings, nanoparticles or nanostructures with a sample.[65–73] The mechanism of desorption, ionization, and fragmentation is associated with the unique optical, electronic, and thermal properties that arise in such nanostructures. Phenomena such as enhancement of the excitation field in the proximity of the nanostructured surface, various confinement effects based on the dimensions of the nanostructures, plasmon resonances, in-plume reactions, and adsorbate–solvent and adsorbate–surface interactions all likely affect the ion formation.[73] Altering the surface chemistry of the nanostructures can promote selective capture of certain molecules and improve sensitivity.[67,68,73] The extent of ion decomposition and the level of fragmentation can be controlled by adjusting the laser fluence, incidence angle, or polarization. Pharmaceutical, metabolite, tissue imaging, biofilms, single cell and other potential sources of natural products have been analyzed by nanostructure-based LDI. However, the utility and efficacy of the technique can be limited by the fashion in which the nanostructures are prepared and introduced to the sample.

Laser spray ionization (LSI), and related methods[59,74] also utilize laser desorption of a matrix–analyte mixture in a fashion similar to MALDI except that unique matrix compounds and ion source conditions are used to detect multiply charged ions similar to those produced by ESI. LSI and related ion sources operate in both positive and negative ion modes, with ions produced under vacuum or at intermediate pressures.

Moving beyond the nanosecond pulse length lasers employed above, ultrashort pulse lasers display additional advantages for LDI. Laser pulses at 800 nm wavelength and sub-100 fs pulse lengths have been used for fs-LDI of purified samples and intact biological material *via* a nonresonant desorption event that avoids the need to add a matrix compound.[75–78] Femtosecond laser ablation can remove sample from a solid while doing minimal damage to the remaining material, a remarkable effect that has motivated fs laser applications in laser surgery and micromachining of intact biological samples.[79–82] For example, ablation with 800 nm, ~75 fs laser pulses can remove material from bacterial biofilms and bovine eye tissue with minimum chemical modification to the underlying sample, indicating that depth profiling should be feasible with this method.[83,84] An LDI instrument with 800 nm, <100 fs laser pulses has been recently described (see below) and its ion source region is shown in Fig. 4.[78,85] Combined with the increasing availability of reliable and lower cost fs pulse length lasers, these capabilities are opening up new possibilities for laser desorption that have yet to be explored for natural products analyses. However, fs lasers are apparently being considered by at least one instrument manufacturer for AP ionization.[86]

5.2 LD combined with postionization of neutrals

Laser desorption of neutrals has been coupled with most of the ionization sources described above. For example, LD has been

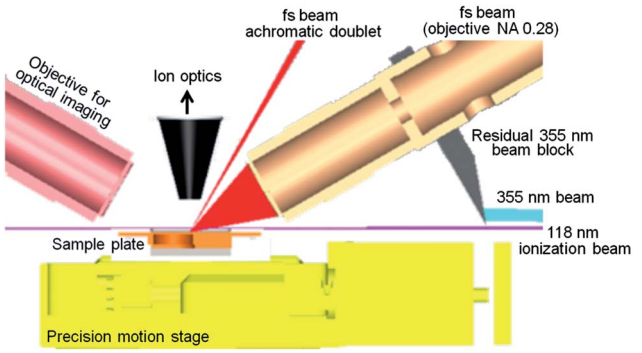

Fig. 4 A schematic showing the femtosecond laser desorption postionization (fs-LDPI) source with 10.5 eV VUV for single photon ionization. The configuration is similar for nanosecond LDPI. Adapted from ref. 78.

coupled with EI,[19] but the strategy is much more effective when supersonic cooling is incorporated into the instrument.[18,87] Laser desorption postionization (LDPI) utilizes a nanosecond UV (ns) or femtosecond (fs) laser for desorption followed by SPI with a VUV light source[7,19,54,88] or resonant multiphoton ionization with a ns UV laser.[54,89,90] Pulsed LD of neutrals can also be achieved with 1.064 or 10.6 μm wavelength IR lasers.[19,54,88–91]

A fs-LDPI imaging source is shown in Fig. 4, but the configuration is similar to that used for ns-LDPI.[7,92,93] An 800 nm, ~75 fs pulse length ultrafast beam from a Ti:sapphire laser is focused onto a sample under vacuum by an achromatic doublet lens with a focused beam diameter of ~25 μm and long depth of focus ideally suited for rough biological surfaces. An alternative objective (NA 0.28) is available to focus the fs laser beam to a 7 μm diameter, permitting high spatial resolution *via* sample movement by the high accuracy, 3D motion stage. 10.5 eV VUV radiation is generated by third harmonic generation of the 355 nm radiation from a pulsed Nd:YAG laser.[19,93]

Samples need not be subjected to extraction or extensive treatment prior to analysis by LDPI-MS, although the vacuum environment will induce dehydration. Even rough, thick, and/or electrically insulating surfaces can be analyzed by ns- or fs-LDPI, including intact eye lens tissue[84] and antibiotic treated bacterial biofilms.[92,94] For example, ns-LDPI-MS was used to probe a single bacterial biofilm consisting of two genetically engineered strains of *Escherichia coli* grown on the same substrate under identical conditions, then analyzed in intact form.[95] 10.5 eV photons ionized the (ns, UV) laser desorbed neutrals to obtain the mass spectrum shown in Fig. 5(a). The two *E. coli* strains (labeled citrine and tomato) showed similar mass spectral peaks, but with varying signal intensity. Processing using principal component analysis, as shown in Fig. 5(b), clearly distinguished the two strains and indicated which peaks contributed to the separation. The peaks were tentatively assigned to low mass metabolites preferably produced in one strain over the other, thereby demonstrating the ability of the technique to map metabolites in intact biological systems.

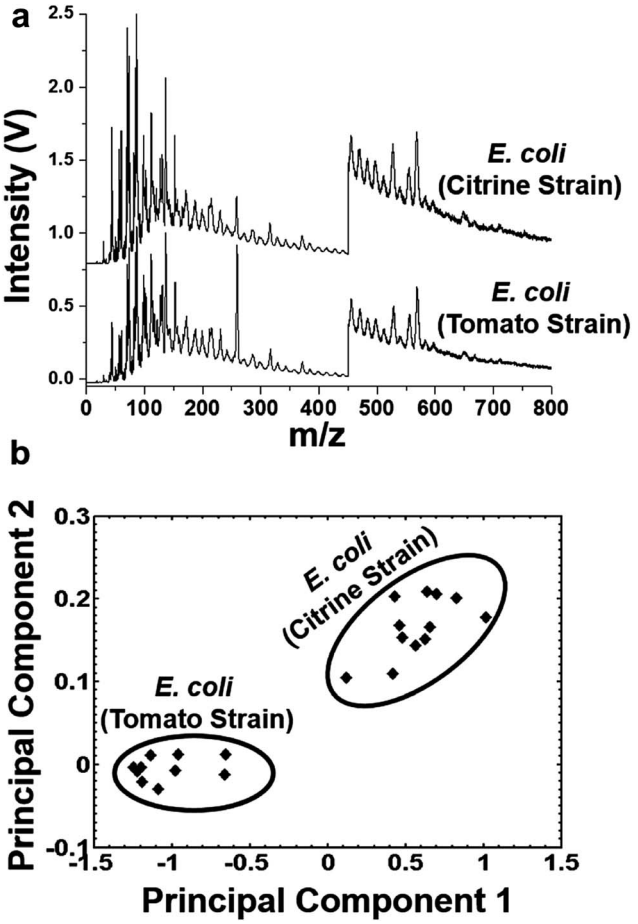

Fig. 5 A nanosecond LDPI study of genetically similar *Escherichia coli* strains in intact coculture biofilms. (a) MS data wherein the two strains show little visually discernable difference. b) Principal component analysis performed on the data resulted in clear separation of the two strains. Adapted from ref. 95.

Photon energy tunable VUV radiation available at synchrotron light sources has also been used for ns-LDPI analysis.[19,21,91,92,95–97] Tunable VUV provides the distinct advantage of selectively ionizing different classes of compounds; a recent study of organic–mineral soil samples showed that tuning the VUV photon energy and the UV laser parameters allowed control of fragmentation of the organic analyte and enabled compounds identification with less ambiguity.[98]

Overall, ns-LDPI does not display the interfering intense matrix peaks that appear in MALDI, permitting more useful information to be obtained from the low mass region ($m/z < 300$) of the spectra. However, LDPI suffers a lack of a commercially available ion source.

An example of imaging of an intact sample by fs-LDPI using 800 nm, ~75 fs laser desorption and 10.5 eV SPI is shown in Fig. 6 for a yeast - *E. coli* coculture biofilm grown on membranes.[78] The top panel (a) of the total ion counts (TIC) displays three distinct regions attributed to "pure" yeast culture (left), "pure" *E. coli* (right), and a mixed region (center). The three panels display the MS images for (b) m/z 93 which appeared mainly in the mixed region, (c) m/z 258 which

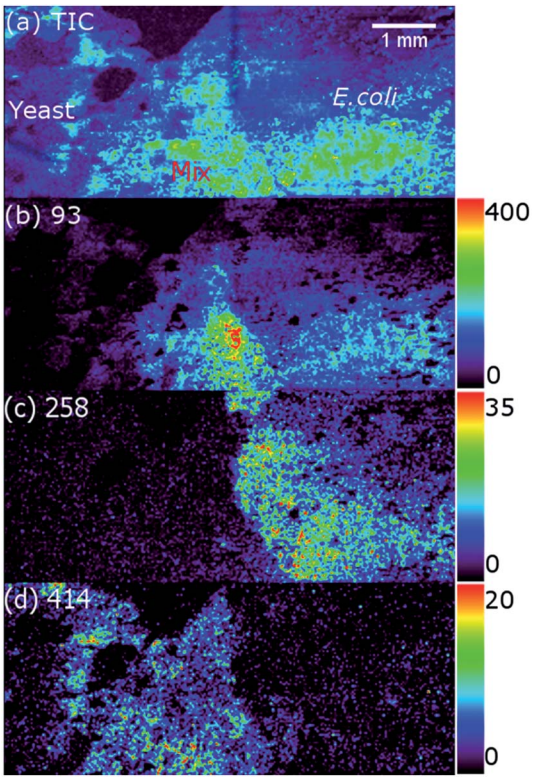

Fig. 6 Ion images from fs-LDPI of blotted co-cultured yeast - *E. coli* biofilm: (a) total ion current (TIC), (b) *m/z* 283, (c) *m/z* 258, and (d) *m/z* 414. The color bar on the right indicates signal levels. Adapted from ref. 78.

appeared mainly in the *E. coli* region, and (d) *m/z* 414, which appeared mainly in the mixed region and also in the yeast region with lower abundance. While this instrument does not possess the tandem MS capabilities needed for full chemical identification, LDPI can be readily coupled to such capabilities just as is possible with vacuum MALDI.[10,15]

Several imaging ion sources are based on mid-IR laser desorption at AP, in which the water in a biological sample replaces added matrix, leading to recoil-induced material expulsion to deliver intact sample particulates into a gaseous plume for postionization.[63] Mid-IR laser ablation is well suited for the analysis of intact biological samples, but desorption efficiency depends on the homogeneity of the water content in the sample. Laser ablation electrospray ionization (LAESI) uses a mid-IR laser beam to produce the gaseous plume that then interacts with highly charged electrospray droplets emitted from an ESI source to produce ESI-like ions (see above).[10,99,100] LAESI is commercially available and is well suited to the analysis of natural product distributions within intact plant samples[99,100] and microbial biofilms.[10] Laser ablation atmospheric pressure photoionization (LAAPPI) is similar to LAESI except that the ablation plume is first desolvated by an orthogonal hot solvent jet, then ionized by an APPI source.[101] LAAPPI efficiently ionizes neutral and nonpolar compounds from the analysis of intact biological samples, making it complimentary to LAESI, which itself is better suited to the analysis of polar compounds.

Mid-IR laser ablation metastable-induced chemical ionization (IR-LAMICI) produces sample neutrals that interact with a reactive, ambient temperature metastable plume to induce chemical ionization in the gas phase.[102] The precursor ions generated by IR-LAMICI are protonated or deprotonated species in the positive and negative ion modes, respectively. This ion source also provides an AP-plasma-based platform for small molecule imaging, with analysis of algal tissues for natural products and imaging of pharmaceutical tablets demonstrated using IR-LAMICI.

Ultrashort pulse laser ablation of neutrals has been used instead of mid-IR laser ablation for coupling to ESI in a method referred to as laser electrospray ionization (LESI).[103,104] LESI has at least one advantage over LAESI in that LESI's non-resonant ablation efficiency will not depend upon water content (see above).

The laser desorption methods described above generally expose a sample to pulsed laser radiation on the same side from which desorption occurs. Alternatively, the laser light can shine through a transparent substrate for optical absorption and desorption from the opposite surface.[105] Back laser irradiation of an opaque, thin foil can create a shock wave resulting in laser-induced acoustic desorption (LIAD) of neutral molecules from the opposite surface.[106] Postionization is required in LIAD and has been demonstrated using both SPI[106] and chemical ionization.[107,108]

6 Secondary Ion Mass Spectrometry (SIMS)

SIMS is perhaps the original MS imaging method: a sample surface is bombarded with a focused beam of high energy primary ions which causes sputtering of the sample surface resulting in the ejection of positive and negative "secondary" ions.[11,109] The desorption/ionization event in SIMS is a highly energetic process that often leads to extensive fragmentation. However, the extent of fragmentation tends to be lower for static *versus* dynamic SIMS, the distinction between the two defined by $\sim 10^{13}$ ions cm^{-2} total flux of primary ions impacting a given sample area. SIMS analyzes samples under vacuum and the method is not readily coupled to chromatography. However, SIMS is capable of the highest spatial resolution possible with any MS technique: ion beams can be focused to <20 nm enabling subcellular resolution, albeit at the cost of enhanced fragmentation.[9] SIMS is also the only MS imaging method that has been fully developed for depth profiling.[11,109]

SIMS was originally performed with atomic primary ion beams, but C_{60}, Bi_3, and other cluster ions beams have been shown to produce more secondary ion signal that is representative of intact molecular species while imparting less damage to the remaining sample.[6,11,54,109] SIMS imaging of individual biological cells has been reported using these cluster ion sources.[9,110,111] Ionization efficiency can be aided in some cases by evaporation of a metal overlayer[112] or addition of an ionic liquid

matrix to the sample surface.[113] Recent work is moving to yet more massive $Au_{\sim 400}^{n+}$,[114] Ar_n^{n+},[115,116] and water cluster ion beams that permit increasing soft ionization and/or improved depth profiling (often in dual beam configurations). Finally, postionization methods, such as EI, SPI and resonant multiphoton ionization, have been combined with SIMS to enhance ion yields in a method generally known as secondary neutral mass spectrometry.[54,117–119]

7 Comparing and choosing ion sources

The above narrative attempts to herd the vast menagerie of ion sources into corrals constituting a few general classes. Non-scientific concerns have lead in some cases to the renaming of what are arguably minor source modifications, thereby generating new acronyms that are daunting to the newcomer to MS. This situation is exacerbated by the combinations of different ionization modalities into single sources to increase capabilities.[30,120] One recent example is the combination of EI and SPI in a single instrument for quasi-simultaneous acquisition of complementary fragmentation information[121] and sampling of liquid feeds.[122] SPI and chemical ionization have also been combined in a single ion source.[123]

To paraphrase a recent comparison of MS ion sources for crude oil analysis, "*there is no single method or ionization technique that allows for the accurate characterization of all the components present*"[36] in a biological sample, either in its intact form or following extensive sample pretreatment. Furthermore, the overlapping capabilities of sources indicate that many ion sources can often be used to detect the same species, albeit with significant operational differences. Thus, there is often no single "best" choice of ion source to address a given natural products analysis. Rather, the choice of ion source is often dictated by the local availability of instrumentation and expertise.

Finally, desorption/ionization is an early step in identification and quantification of natural products and other molecular species by MS. Sample extraction and pretreatment, chromatographic separation, exact mass measurements for elemental formula determination, tandem MS for fragmentation and structural identification, ion mobility measurements to distinguish isomeric compounds, comparison with databases of MS or other associated data (*i.e.*, metabolomic, proteomic, or genomic), multivariant analyses, imaging processing, and other strategies are all critical steps in natural products analyses that are discussed elsewhere is this issue.

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