Emerging Capabilities in Mass Spectrometry of Natural Products

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ARTICLE TYPE

Emerging Capabilities of Mass Spectrometry for Natural Products

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A brief history of mass spectrometry in natural products research serves to identify themes which have driven progress in this area of application and in mass spectrometry itself. This account covers six decades of ionization methods, starting with traditional electron ionization and progressing through today's ambient ionization methods. Corresponding developments in mass analyzers are indicated, ranging from sector magnetic fields, through hybrid quadrupole mass filters to miniature ion traps. Current capabilities of mass spectrometry in natural products studies include direct *in situ* analysis, mass spectrometry imaging, and the study of biosynthetic pathways using metabolomic information. The survey concludes with a discussion of new experiments and capabilities including ion soft landing, preparative mass spectrometry, and accelerated ionic reactions in confined volumes.

1 Introduction

The connections between mass spectrometry (MS) and the study of natural products were cemented during the years 1960 - 1980. These developments were important to both subjects. The chemical properties of natural products provide a useful set of compounds needed to test and extend mass spectrometric techniques. Alkaloids, a large and structurally diverse class of nitrogen-containing compounds, were a proving ground given their chemical diversity and relatively high concentrations in natural sources. Further, their ready ionization to give radical cations ($[M^{\bullet+}]$) which undergo charge/radical-site driven fragmentation provided structurally characteristic mass spectra in low mass resolution, electron ionization experiments. The advent of high resolution MS (HRMS), first using sector mass spectrometers and later fourier transform ion cyclotron resonance (FTICR) and time-of-flight (TOF) mass analyzers, allowed molecular formulae to be proposed from accurate mass measurements. Molecular formulae, used in combination with fragmentation patterns, helped to reveal the molecular structures of many natural products. When softer ionization methods such as field desorption (FD) and chemical ionization (CI)[1] were developed, the high proton affinities of alkaloids meant that they yielded abundant molecular ions ($[M+H]^+$) even from complex mixtures, while other components were more poorly ionized. Selection of these ions in a first stage of mass analysis followed by collision-induced dissociation (CID) and identification of the product ions in a second stage of mass analysis, *viz.* in an MS/MS experiment, represented the beginning of direct analysis of complex mixtures.[2] This application soon spread from natural products and has been a major feature of MS ever since. The essential concept, conversion of a set of neutral molecules in a structurally analogous set of ions which can act as surrogates for the neutral compounds, is the foundation of complex mixture analysis by MS.

The controlled generation of fragments from mass-selected ions that allowed MS/MS was first implemented on sector instruments. These instruments had a magnetic and an electric sector which mutually cancelled dispersions due to velocity and gave high resolution mass spectra. However, when operated separately, the electric sector served to mass analyze product ions generated from precursor ions mass-selected by the magnetic sector. Electric sectors are poor mass analyzers and tandem quadrupole mass filters ('triple quadrupoles') more appropriate to MS/MS were soon introduced.[3] The high energy collisions of sector instruments (keV) and the low energy collisions of triple quadrupoles (ca. 100 eV) have similar efficiency and give similar structural information even though the mechanisms of ion activation and the resulting ion internal energy distributions are quite different.[4]

Terpenoids are among the classes of natural products amenable to separation by gas chromatography (GC). This form of chromatography was the first to be combined with on-line MS analysis and it allowed powerful new experiments to be performed. Most notable were experiments in which the mass analyzer was set to monitor only ions of a particular m/z ratio. This experiment, single (or selected) ion monitoring (SIM), greatly increases the sensitivity of detection of particular compounds eluting from the GC with a loss in the specificity of identification which is often acceptable. When additional specificity is required, the mass analyzer can be used to monitor several specific ions, an experiment known as multiple ion monitoring. An analogous MS/MS experiment, single (or selected) reaction monitoring (SRM) and the plural version, multiple reaction monitoring (MRM) have become mainstays of quantitative proteomics.[3, 5] The extension of chromatography/mass spectrometry combinations to LC/MS has greatly impacted natural products research although it was driven

by pharmaceutical and protein science not particularly by natural products chemistry.

The desire to ionize refractory compounds has been a continuous driving force in MS. Many natural products, like most biological molecules of any type, are not sufficiently volatile or thermally stable to allow MS analysis by the traditional EI and CI methods. The desorption ionization methods solved this problem by generating gas-phase ions directly from thermally labile and non-volatile solids. These methods started with field desorption (FD)[6] and proceeded through secondary ion mass spectrometry (SIMS)[7] and laser desorption. They included variants that employ a matrix to facilitate ionization and avoid direct interaction of the analyte molecules with the energy source. The most important of these is matrix assisted laser desorption ionization (MALDI).[8] MALDI allowed for desorption and ionization of large molecules (>2000 Da) and aptly coupled to TOF instrumentation, yielded a mass-to-charge limit exceeding 10,000 Th (Thomson, unit of *m/z*). A parallel development over the same period of 1968 – 1988 was that of the spray ionization methods, which began with interfacing of LCs to thermospray[9] ion sources and culminated with the highly successful electrospray ionization (ESI) method.[10] ESI provided the ability to easily produce ions from solutions, including natural product extracts, successfully ionizing polar, non-volatile compounds. Both MALDI and ESI have been widely applied in natural products in recent decades with great success and are the ubiquitous ionization techniques of today.‡

2 MS of Natural Products

The interface between mass spectrometry and natural products research was founded by the work of Djerassi,[11] Biemann,[12, 13] and others[14] primarily in studies of alkaloids and terpenoids. EI coupled to magnetic sectors provided low resolution mass spectra from which molecular weights could be ascertained. Furthermore, fragmentations resulting from EI allowed for correlation of spectrum and structure. The structural information conveyed via spectrum-structure correlations was systematically studied using model compounds, including alkaloids. The early 1960's saw EI-MS application in such typical studies as the determination of indole derived alkaloids via retro Diels-Alder fragmentation,[15] the exploration of unsaturation and substitution patterns of triterpenes,[16] and the discovery of aspidosperma alkaloids in *Rhyazya strictia* and *Aspidosperma quebrancho blanco*.[17]

2.1 High Mass Resolution

High resolution mass spectrometry (HRMS), the accurate measurement of *m/z*, allowed for the differentiation of molecular ions at the same nominal *m/z* in low resolution spectra[18] and the ability to generate molecular formulae. HRMS measurements were performed using double-focusing sector instruments, *e.g.* those of Nier-Johnson geometry which were used to record full high resolution mass spectra by scanning the field strength or in the case of Mattauch-Herzog geometry focal plane instruments, by using a non-scanning mode with a photographic plate placed at the focal plane of the instrument. The generation of molecular formulae was accomplished using peak matching[19] which allowed a number of possible formulae, not usually only a single possibility, which then could be winnowed using additional data (*e.g.* fragmentation, isotopic patterns or any other relevant information). Henningsamine was identified in this way from the bark of *Strychnos henningsii* at a concentration that would have been insufficient for traditional means of characterization. Its presence was detected using a few milligrams of adequately purified material that provided the correct molecular formula, $C_{23}H_{26}N_2O_4$, with an error of less than 1 ppm, a measurement on a par with even today's experimental results.[20] The structures and molecular formulae of vinblastine and vincristine found in *Vinca rosea* were similarly derived, the latter being of medical significance as an early chemotherapeutic agent.[21] The 1960s - 1980s saw the proposed structures of hundreds of natural products, a good number derived at least in part by MS from the combination of fragmentation and accurate mass measurements. Acquiring accurate mass measurements was and continues to be a critical application of mass spectrometry in natural products research.

2.2 MS/MS

An important step forward from examining essentially pure compounds was made when complex alkaloid mixtures were subjected to soft ionization and the CID products of mass-selected ions were measured. Such MS/MS experiments allowed natural products in complex mixtures to be identified based on characteristic fragmentations in low resolution mass spectra. One profound advantage of MS/MS was the increase in the signal-to-noise ratio (Fig. 1a) compared to the normal mass spectrum, an advantage that increases with the number of stages of MS analysis in MS^n experiments. The increase in signal-to-noise allowed the detection and identification of compounds that are not observable because of chemical noise in the full mass spectrum. The phenomena was demonstrated in the analysis of coniine (*m/z* 128), found in *C. maculatum*, which was indistinguishable from noise in the full mass spectrum but readily detected in the MS/MS scan (Fig. 1b).[22]

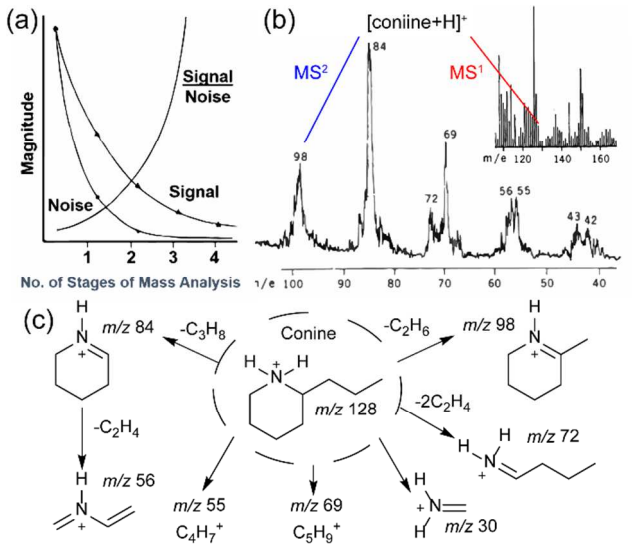


Fig. 1 (a) Signal-to-noise advantage of MS/MS with increasing number of stages of mass analysis (b) MS^1 of *C. maculatum* (left) and coniine fragments detected in an MS/MS experiment (CI-MIKES (right) [adapted from ref. [22]] (c) Structural information provided via fragmentation of coniine

The identification of natural products and generation of chemical structure via interpretable fragmentation is represented for coniine by the pathways shown in Fig. 1c. Furthermore, the ability to detect natural products directly from the material from which they originate clearly has advantages over traditional means of characterization.

The advantages of MS/MS paved the way for the direct analysis of plant materials, introduced into the vacuum system and performed for the first time in the mid 1970's. An early example of direct analysis was the detection of cocaine and cinnamoylcocaine from *Erythroxylum coca*, performed by directly inserting plant tissue into the ionization source of the mass spectrometer.[23] However, most early MS/MS was performed using crude plant extracts that allowed previously unknown plant constituents to be quickly identified, such as mescaline as a constituent of *Opuntia spinosior* and *Trichocereus peruvianus*.[22]

2.3 MS Instrumentation and Ionization

MS instrumentation, particularly mass analyzers, developed from sectors to quadruples and time-of-flight spectrometers to ion traps of various types. The development from sectors to double-focusing sectors had a profound influence during the 1960s - 1980s. Reverse-geometry instruments such as the MIKES and its precursor/product ion scan gave way to triple quadruples (QqQ). Time-of-flight (TOF) MS allowed for the detection of large ions (>1000 Th) providing new fields of research in natural products and in protein science. Multianalyzers (hybrids) of all combinations were also built including but not limited to multiple sector instruments, sector-quadruples, and quadruple-TOF hybrids. The development and refinement of ion traps (*i.e.* Paul, Kingdon and Penning) started in the 1980's and are the ancestors to the high performance traps of today the FT-ICR, orbitrap, and quadruple ion traps.

3 Current Capabilities of Mass Spectrometry

3.1 MS/MS Scan Modes

The common structural motifs of natural products are well suited to recognition using the various MS/MS scan modes as they often fragment predictably via characteristic losses of neutrals from the ionized molecules. There are in principle five types of MS/MS scan modes.[24] The full 2D data domain, which has two dimensions of mass and one of intensity and is a collection of all precursor/product/intensity combinations. An experiment already mentioned, the MRM experiment (●→●), which has zero dimensions of information in mass but provides highly specific and often quantitative information on the amount of a particular compound. There are also three well-known one-dimensional experiments, the common product ion scan (●→○) which characterizes a particular ion (as a surrogate for a neutral molecule) through a set of fragment ions and their abundances. Surveys scans that can be used to assess an unknown sample for compounds of the same class can be done using either neutral loss (○→○) or precursor ion scans (○→●). Neutral loss scans recognize compounds of the same functional class by the characteristic neutral fragment losses and precursor ion scans do so by formation of characteristic fragment ions. Neutral loss scans allowed the detection of a set of compounds related to artemisinin in *Artemisia annua*, simplifying a complex full mass spectrum (obtained from plant extract) to a set of signals for structurally-related ions (Fig. 2). Then product ion MS/MS spectra were used to confirm these novel Artemisinin analogues and to assign tentative structures based on functional groups and molecular weights.[25] Similarly precursor ion scans recognize common product fragments as arising from particular precursor ions. Precursor ion scans of *Senecio sp.* plant extracts which possess a common fragment, *m/z* 120 ($C_8H_{10}N$), viz. ○→ 128, coupled with subsequent product ion scans (●→○) revealed the presence and structures of multiple pyrrolizidine alkaloids.[26] The various MS scans allow complex mixtures to be surveyed. Applications of the higher order MS^n experiments, where n>2, have not been widely utilized in natural products research. Nevertheless, they possess unique structural information. Sequential precursor scans (○→●→●) are more selective versions of precursor ion scans which define the product ion by a particular further fragmentation. Information of this nature can be used to distinguish isobaric and isomeric ions both of which commonly occur in natural products.[24]

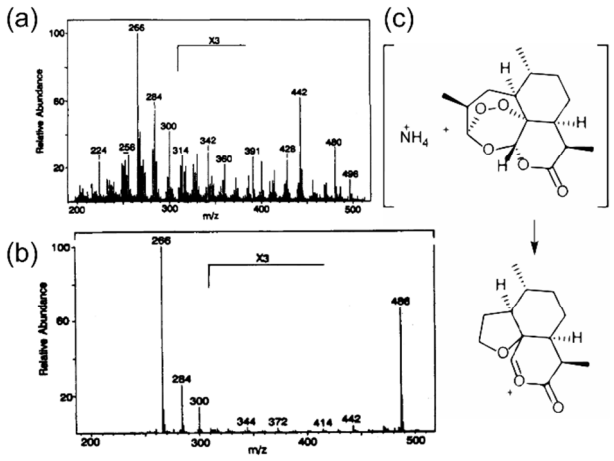


Fig. 2 (a) Desorption chemical ionization (ammonia reagent gas) of *A. annua* leave extract (b) Neutral loss spectrum for loss of 77 Da revealing Artemisinin-related compounds [modified from reference[25]] (c) Ammonium adduct of artemisinin, *m/z* 300 fragments by neutral losses (77 Da total) to the proposed ion structure, *m/z* 233 shown.

3.2 Ambient Ionization

Ambient ionization refers to the ability to produce ions outside of the mass spectrometer in the native environment (pressure, temperature, humidity) from samples requiring little to no sample pretreatment.[27] A large set of techniques possessing these characteristics has been developed since the first reported method, desorption electrospray ionization (DESI), in 2004.[28, 29] Currently, they number more than forty[30] while being divisible into classes based on the ionization/ desorption agent employed.[31] Natural products have been studied using a number of ambient ionization methods including DAPCI,[32, 33] DART,[34-36] ELDI,[37] LTP,[38] and nano-DESI[39]. Regardless of the particular method, a hallmark of ambient ionization is rapid analysis, requiring only seconds to record mass spectra. Rapid analysis is allowed by the relaxation of requirements for sample pretreatment. Ambient ionization methods display near universal applicability with high sensitivity allowing for the detection of virtually all compound classes in minute quantities, even when present in complex

mixtures. Importantly, the ions produced by most ambient methods allow for MS/MS (*e.g.* using CID) and hence compound identification.[27] Furthermore, certain ambient ionization methods allow mass spectrometry imaging which gives the spatial distribution of compounds in two or three dimensional space.

3.3 Direct Ambient Ionization of Intact Plant Material

Phytochemicals represent a diverse range of compounds and are of great interest to natural product chemists and mass spectrometrists alike. The analysis of plant material for phytoconstituents by ambient ionization minimizes or in extreme examples removes the need for sample preparation. The ability to investigate natural products in this way allows the identification of plant constituents within seconds by analyzing intact plant material, directly.

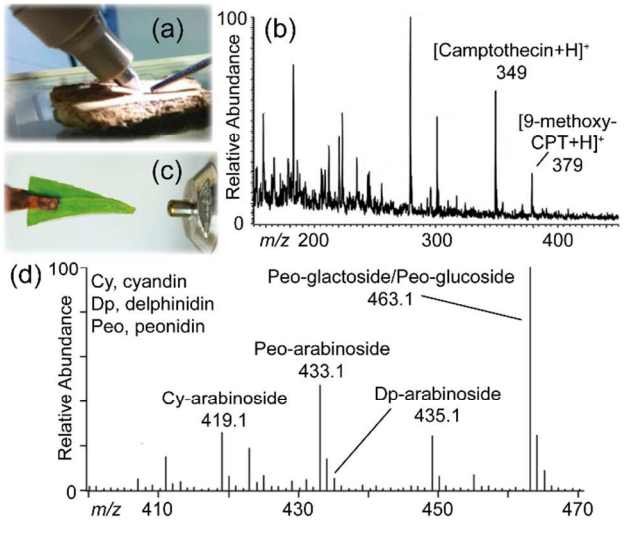


Fig. 3 (a) Photograph of DESI-MS analysis of *N. nimmoniana* bark showing sprayer on left and secondary droplet transfer tube on right (b) resulting mass spectrum showing ionized camptothecin and a 9-methoxy derivative. [Modified from reference[40]] (c) Photograph of leaf spray (LS) MS analysis of green onion showing tissue triangle held in front of MS entrance (d) LS-MS spectrum of cranberry fruit revealing anthocyanin constituents [Modified from reference[41]]

Direct detection of natural products from their biological source is facilitated by the ability to analyze surfaces without pretreatment, typically sampling constituents via thermal desorption or solvent extraction. For example, DESI uses a stream of charged droplets which contacts the sample surface, dissolving analytes which are subsequently carried to a MS in splashed secondary microdroplets.[30] The roots of *Datura stramonium* were analyzed by DESI-MS, without any prior treatment, allowing the detection of 15 tropane alkaloids simultaneously.[42] Similarly, the glycosides responsible for the sweet taste of *Stevia rebaudiana* leaves could be readily detected[43] and the poisonous alkaloid coniceine confirmed in the seeds of *Conium maculatum*.[28] Notably, these examples required only seconds to obtain a full mass spectrum containing the reported constituents. The application of ambient ionization MS is not limited to a particular type of plant material, being equally applicable to leaves, roots, stems, bark and flowers. The direct rapid analysis of leaves, stems, and bark from *Nothapodytes nimmoniana* (Fig. 3a) confirmed the presence of camptothecin, an anti-tumor drug, and allowed an estimate of its concentration in tree bark.[40]

The direct analysis of plant material by ambient ionization is exemplified in leaf spray (LS),[41] a recent ambient ionization method. Leaf spray, derived from paper spray (PS),[44] is a radical departure from previous ionization techniques as ionization occurs directly from the sample (Fig. 3b). Voltage and solvent, if necessary, are applied to either an innate or created edge in the plant material. Extracted constituents are sprayed from the high electric field at the point producing ions.[41] Leaf spray analysis showed anthocyanin constituents in cranberry fruit (Fig. 3c), glycosides in *S. rebaudiana* leaves,[45] and the allergens of *Toxicodendron radicans*.[37] Furthermore, LS experiments done on related plants can be used to rapidly determine chemical difference by subtraction of the spectra. The specific production of a novel polyhydoxylated, monoterpene-containing pyrrolidine alkaloid in *Hibiscus moscheutus* but not in *H. syriacus* was recently demonstrated in this way.[46]

3.4 MS imaging using ambient ionization

Mass spectrometry imaging by ambient ionization methods such as LAESI[47], nano-DESI[48] and DESI provides relative abundances of molecules in two[49] or three[50] dimensional space. The combination of chemical information obtained by MS and spatial information allow complex biological questions to be investigated. For example, the localization of active compounds can be determined easily: an early example is the distribution of coniceine within the stem of *C. maculatum*. The relative abundance of coniceine was found to vary 4-fold over ~3 mm simply by analyzing across the stem's surface using DESI-MS.[42] Furthermore, the location of constituents can be correlated with data from independent analytical techniques to reveal biological relationships. The analysis of an algal sample, shown in Fig. 4a, revealed the 2D spatial distribution of anti-fungal bromophycolides which correlated with coloration differences.[51]

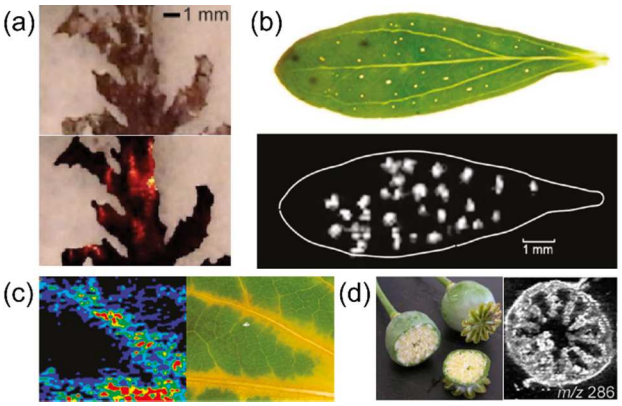


Fig. 4 (a) (Upper) Photograph of algae subjected to DESI-MS imaging and overlay of bromophylic A/B ion image, *m/z* 701 (lower). [Modified from reference[51]] (b) *H. perforatum* leaf (upper) and DESI-MS ion image, *m/z* 535, corresponding to hyperforin (lower). [Modified from reference[52]] (c) Photograph of *Katsura* leaf (left) and corresponding ion image of a chlorophyll catabolite (right) [modified from reference[53]]. (d) (Left) Photograph of *P. somniferum* capsule and imaged imprint (right) revealing the distribution of morphine. [modified from reference[52]]

Direct imaging of plant leaves has also been used in the detection of phytochemicals and their 2D spatial distribution.[54] Ambient

mass spectrometry imaging allowed the detection of endogenous[55] and exogenous compounds such as mapping the distribution of nicotine in rat brain tissue.[56] The simultaneous acquisition of chemical and spatial information by MS imaging allows complex questions to be addressed concerning the synthesis, storage, and biological relevance of natural products.

3.5 Imprint Imaging of Intact Plant Material using Ambient Ionization

The nature of biological materials can preclude direct analysis by ambient ionization. In order to acquire higher quality data in such cases, the constituents can be transferred onto a suitable surface by imprinting then ambient ionization imaging can be applied. Caution must be taken while transferring constituents so as to preserve the spatial relationships that exist in the original material. The catabolites of chlorophyll analyzed from senescent *Katsura* leaves were detected by DESI-MS, directly; however, signal intensity improved after transferring the constituents onto a porous PTFE surface (Fig. 4c).[53] Similar methodology has been applied in the analysis of *Hordeum vulgare*,[57] capsules of *Papaver somniferum* (Fig. 4d),[52] leaves of *Darura stramonium*,[52] and flower petals of *Catharanthus roseus*[58] and *Hypericum perforatum.*[52] The petals of *H. perforatum* were imprinted and imaged by DESI-MS; the detection of hypericin was found to correlate with the black glands which exist along the edges of the petals, whereas hyperforin (Fig. 4b) was found to occur in bands and/or spots throughout the petal.

3.6 Ambient MS Analysis of Microorganisms

Although previous sections of this report have focused on plant derived natural products, natural products derived from microorganisms are equally important. Ambient ionization provides the same advantages in the analysis of bacteria and fungi as with plant materials, *viz.* rapid analysis without the need for extensive sample pretreatment. Ambient ionization has been utilized for *in vitro* detection of endogenous lipid constituents, primary metabolites, and natural products.

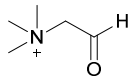
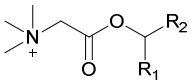
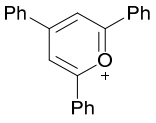
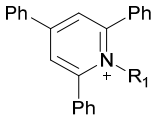
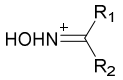
The *in vitro* detection of microorganisms and their secondary metabolites is facilitated by eliminating preparative steps. Bacteria directly analyzed from culture and/or biofilms represent the majority of work performed to date.[59] Nanospray desorption electrospray ionization (nano-DESI) has been used in detecting rhamnolipids of *P.* aeruginosa[39] directly from Petri dishes. Glycolipids and metabolites of *Synechococcus sp.*[60] and surfactin, a surfactant and antibiotic, produced by *B. subtilis* have been studied *in vitro*.[39, 61] Ambient ionization MS allows differentiation of bacterial genera[62] and species[63] using multivariate statistics. Fungi, relatively understudied using ambient ionization, have also been used to detect triterpenoids in *Ganoderma lucidum* and *Antrodia camphorate* using electrospray laser desorption ionization (ELDI).[37]

Ambient MS has proven useful in understanding biosynthetic pathway of natural products.[64] The combination of chemical information obtained from MS and genetics provides deeper insights into the biochemistry of natural products.[65] Furthermore, imaging of microorganisms, directly or via imprints, allows one to monitor inter-organism chemical processes occurring at their interface.[66] The combination of metabolite and spatial information creates a "dynamic" image of metabolism allowing the assessment of environmental impacts, culturing conditions, inter-organisms interactions over time.

3.7 Reactive Ambient Ionization

Reactive variants of ambient ionization are capable of combining ionization with functional group specific chemical derivatization. This simultaneous derivatization experiment takes no longer than direct ionization so has proven beneficial in the analysis of complex mixtures.[30] Reactive ambient ionization can be used to minimize the complexity of the mass spectra obtained on samples not subjected to prior separation or pretreatment. The use of appropriate reagents produce charged derivatives of particular analytes that are efficiently ionized from the sample mixture.[67] Similarly, analytes obscured by interfering species can be distinguished by a characteristic mass-to-charge shifts observed upon reaction with the appropriate reagent.[68] Reactive ambient ionization can be performed in a targeted manner were a known functional group is the explicit target,[69] as illustrated in Table 1. The combination of functional group specific derivatization with functional group specific MS/MS should be a powerful new discovery method.[70]

Table 1 Reactive ionization: some reagents, targets, and charged products

Reagent	Target	Product
 Betaine Aldehyde	 Alcohol	 Ester
 Pyrylium	 Amine	 Pyridinium
 Hydroxylamine	 Aldehyde/Ketone	 Oxime
$AgNO_3$	Alkenes	Silver Adduct

4 Emerging Capabilities of Mass Spectrometry: Miniature Mass Spectrometers

Miniaturization of mass spectrometers has been pursued for two decades with the first description of a miniature mass analyzer in 1991.[71] Subsequent improvements in vacuum pumps, detection systems, and control electronics allowed reduced in the dimensions, weight, and power requirements for the whole mass spectrometer system. Self-sustainable MS systems have been developed for sector, TOF, and ion traps mass analyzers.[72, 73] The most successful miniaturized mass spectrometer designs employ simplified ion trap geometries that have evolved from traditional 3D and linear traps. The advantages of miniaturized ion traps are numerous including operation at higher pressures, an intrinsic capability for MS/MS, large *m/z* range, and sensitivity similar to that of commercial instruments.[71, 73, 74] The recent development of miniature mass spectrometers utilizing miniaturized ion traps coupled with ambient ionization sources is producing the first generation of self-sustainable mass spectrometers capable of *in situ* analysis.

4.1 Miniature MS Applications

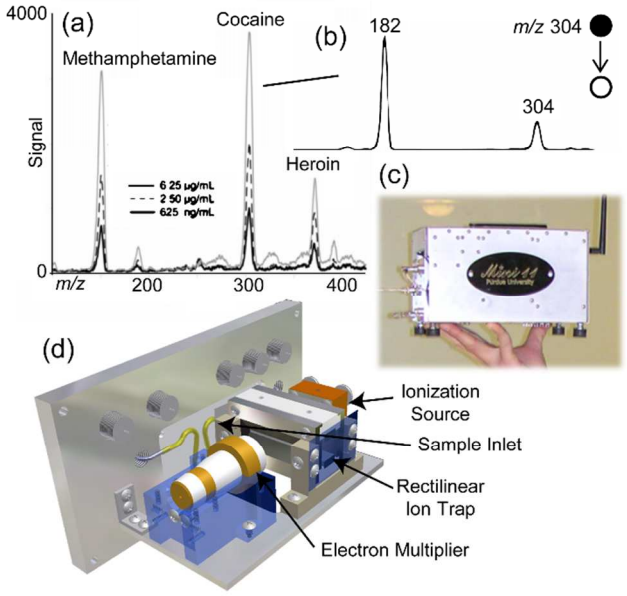


Fig. 5 (a) Detection of cocaine, heroin, and methamphetamine in full scan MS using the Mini 10 illustrated. (b) MS/MS product ion spectrum of cocaine using Mini 10 [modified from reference[75]] (c) Photograph of Mini 11 (d) Schematic of Mini 10, rectilinear ion trap MS.

Current use of MS in natural products relies on in-field sampling and transportation, and then laboratory analysis. Although this process certainly yields important results, it is a time-consuming procedure during which the rediscovery of already known compounds is certain. The ability to bring the mass spectrometer to the sample allows experiments to be conducted more efficiently and allowing precious biological resources in remain in their native environment.[76] The variety of compounds that can be analyzed using miniature mass spectrometers is nearly universal. These instruments have been demonstrated to be able to detect, identify, and in some cases quantify pharmaceuticals,[77] agrochemicals,[78] and proteins[77] among other compound classes. The detection of cocaine (*m/z* 304) in full scan and product ion MS/MS modes using the Mini 10 is illustrative (Fig. 5).[75] Detection of natural products using a portable mass spectrometer is illustrated by the detection of DDT, a pesticide, and alachlor from a cornstalk leaf.[78]

4.2 Coupling of Ambient Ionization with Miniature MS

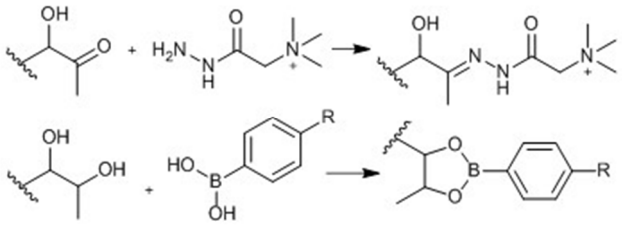
The coupling of ambient ionization sources with miniature MS results in the ideal MS system for *in situ* detection of natural products. Significant obstacles in regards to performance, especially pumping requirements, were overcome with the introduction of the discontinuous atmospheric pressure interface (DAPI).[74] This provided sensitivity of the same order of magnitude as lab-scale instruments while requiring much lower pumping speed.[79] The integration of DAPI and other improvements in mini MS technology have allowed DESI, PS, and low temperature plasma (LTP)[38] ionization sources to be successfully coupled to miniature ion traps, such as Purdue's Mini 10[80] and Mini 11.[77] The time required to sample, ionize, and detect analytes using ambient ionization coupled to with a miniature MS is typically a few seconds.[81] Future iterations in miniature mass spectrometer designs are likely to fully integrate ambient ionization methods, providing a truly self-sustainable instrument for *in situ* analysis.

4.3 Ion Soft Landing and Preparative Mass Spectrometry

This presentation has discussed mass spectrometry's primary function in chemical analysis based on the detection of ions and measurement of *m/z* ratios. However, mass spectrometry deals with chemical materials and includes the capability for ion collection. The collection of ions can be performed by "landing" ions onto a surface at relatively low energy, hence the term ion soft landing. Ion soft landing was first demonstrated in 1977[82] in the modification of surfaces in vacuum. Organic cations can be soft landed the neutral or charged products collected.[83, 84] Furthermore, soft landed proteins can maintain their original structure and biological activity.[85, 86] Preparative mass spectrometry has also been demonstrated in the ambient environment using ambient ion deposition.[87]

4.4 Accelerated Ionic Reactions in Confined Volumes

Future application in MS of natural products is likely to take advantage of the accelerated rates of derivatization reactions carried out in confined volumes, such as droplets. The recent observation of very rapid, small-scale chemical reactions in the secondary droplets generated in DESI indicates that reaction rates are elevated by several orders of magnitude. Reagent concentrations increase and changes in pH heighten the already favoured acid/base chemistry within charged droplets, the former increasing the frequency of intermolecular collisions.[30] The phenomenon of increased rate reaction has also been found in other spray-based ionization methods such as electrospray ionization (ESI).[88] Chemical synthesis by electrospray[89] allows millisecond time scale derivatization of natural products, such as in the modifications of cis-diols and ketones shown in Scheme 1. This should improve the analytical capabilities of mass spectrometry as well as pointing the way to small scale synthesis.



Scheme 1 (Upper) Reaction of a hydroxyketone with Girard's T reagent to form a charged product. (Lower) Selective reaction of cis-diol with phenylboronic acid. Both reactions occur on the millisecond time scale.

5 Conclusions

Many of the current capabilities of MS have arisen from developments in ionization methods and in instrumentation. The early work on complex mixture analysis by MIKES/CID and the ambient ionization experiments of today both arose from a desire to minimize sample workup. They differ most significantly in their vacuum requirements: ambient ionization allows complex materials, including whole plant components to be ionized in the open environment. The use of miniature mass spectrometers, especially when coupled with ambient ionization, has the

potential for significant impact in natural products research.

The intertwined relationship that exists between mass spectrometry and natural products, both in the past and present, is likely to continue into the future. Future growth in ambient ionization in natural products research is likely to focus on the analysis of biological materials. A dramatic new capability is the ability to perform essentially instantaneous mass spectrometric analysis on complex materials. *In situ* detection, identification, and dereplication of natural products at their sources seems increasingly likely. The preparative and synthetic capabilities of mass spectrometry will undoubtedly be useful in future natural products research. Preparative MS may allow for the creation of analytical standards currently unobtainable at sufficient quantity and purity for subsequent experimentation. Synthetic MS could be used to carry out rapid, small scale chemical reactions so as to instantaneously derivatize compounds or survey natural products for bioactivity through rapid screens using condensed phase chemical reactions, carried out by MS in the ambient environment. If current capabilities are widely applied, natural products and mass spectrometry should continue to enjoy a strong synergistic relationship, potentially contributing to significant discoveries.

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Notes and references

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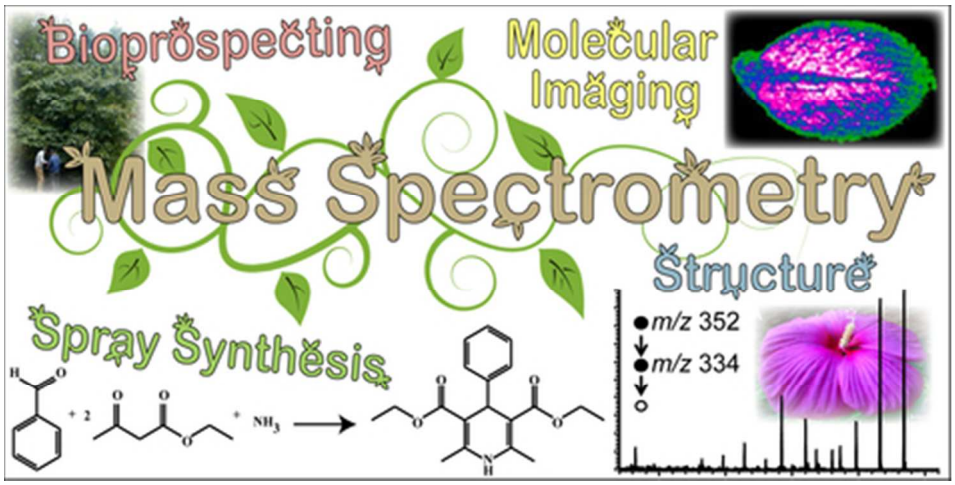

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Mass spectrometry has a rich history in natural products research. This is likely to grow as new *in situ* methods of bioprospecting, structure analysis, molecular imaging, and rapid small scale MS synthesis take hold.