UC Berkeley College of Chemistry

Chemistry 105

Instrumental Methods in Analytical Chemistry

Matrix-Assisted Laser Desorption/Ionization (MALDI)

Final Paper

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1 Introduction

MALDI, or Matrix-Assisted Laser Desorption/Ionization, is an analytical technique used for soft ionization (ionization without fragmentation) of particles for use in mass spectrometry, especially biopolymers or other macromolecules that are difficult to ionize without fragmentation. By irradiating a sample with a high-energy (usually UV) laser pulse, a plume of ions can be produced that can be analyzed (this is known as Laser Desorption Ionization). MALDI improves upon this process by greatly increasing the cutoff on analyte mass, as well as providing a consistent crystal matrix that greatly increases the consistency of results between laser pulses[1].

The technique was first described in a seminal paper by Karas et. al.[2], in which the authors describe what they call "'Matrix-Assisted' Laser Desorption" for the first time:

> The mass spectrum of a mixture of alanine (Ala) and Trp taken at Trp threshold irradiance is shown in Figure 1. A strong signal of the Ala quasi-molecular ion was observed in addition to that of Trp. It is important to note that its desorption took place at an irradiance of about a tenth of that necessary for obtaining spectra of alanine alone.
>
> Tryptophan thus must be regarded as an absorbing matrix resulting in molecular ion formation of the nonabsorbing alanine. This kind of "matrix-assisted LD" has also successfully been applied to reproducibly desorb other nonvolatiles, e.g., stachyose. (Karas, 1985)

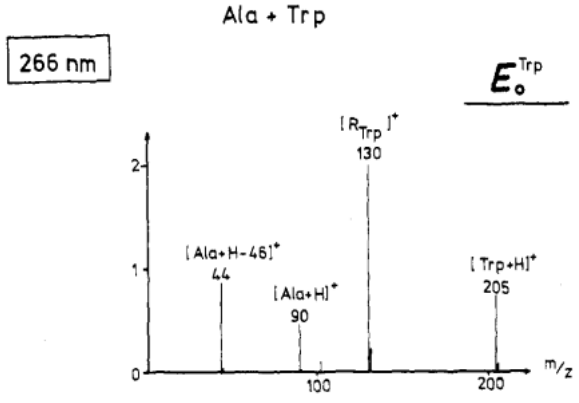


Figure 1: "LD mass spectrum of a 1:1 mixture of alanine and tryptophan at 266 nm and threshold irradiance for tryptophan." (Karas, 1985)

Through the use of a 266 nm Nd-YAG pulsed laser, Karas et. al. discovered that a mixture of alanine and tryptophan was more effective at producing alanine molecular ions than a pure solution of alanine, and proposed that the tryptophan was absorbing the laser more effectively and transferring the energy to the alanine.

In a followup paper by the same authors, they determined that the use of a matrix for laser desorption mass spectrometry was extensible to much larger biomolecules, and were characterized by "high quasimolecular ion yield with little or no fragmentation and only a few signals in the low mass range"[3]. Most notably, matrix-assisted LD of mellitin (a polypeptide consisting of 26 amino acids, with a molecular mass of 2843 Da) produced the mass spectrum seen in Figure 2, demonstrating the amazing capability of MALDI to produce molecular ions of biomacromolecules with minimal amounts of fragmentation.

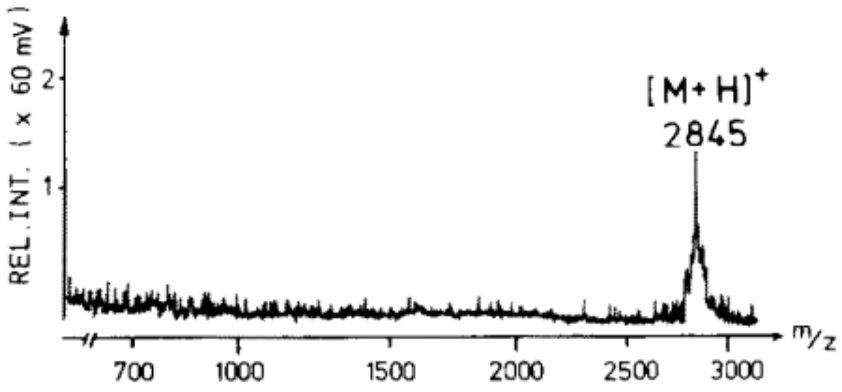


Figure 2: "Matrix-LD spectra of mellitin/NicAc accumulated from 12 single laser shots." (Karas, 1987)

The first breakthrough in this method came in 1988, when Tanaka et. al.[4] detailed what they described as the "ultra fine metal plus liquid matrix method", using 300 Å cobalt nanoparticles in glycerol as a matrix for the sample, and using a 337 nm nitrogen laser for ionization. A solution of the analyte would be mixed with this matrix solution and vacuum dried, a process that increased molecular weight precision by about an order of magnitude yet was one or two orders of magnitude faster to perform than competing methods of the time (including sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) and gel permeation chromatography (GPC)). The authors supposed that this phenomenon was due to the unique qualities of the cobalt nanocrystals (low specific

heat, high photoabsorptivity, and large surface area per volume) that enhanced the speed of sample heating by the laser, and thereby increased the rate of molecular ion formation, while the glycerol allowed for constant replenishment of unionized analyte molecules to the location of laser incidence. Through this method, Tanaka et. al. were consistently able to ionize lysozymes with molecular masses as high as 34,000 Da, and observed the production of ions of $m/z \geq$100,000 – the highest known at that time. In 2002, Koichi Tanaka received the Nobel Prize in Chemistry, "for [his] development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules".

The technique began to come into its own with the discovery by Beavis and Chait[5] that cinnamic acid derivatives couple nicely with Nd-YAG or nitrogen UV lasers to greatly increase mass resolution, and to enable the determination of polypeptide molecular weights with as little as one pmol of analyte. The cinnamic acid derivatives, including nicotinic, ferulic, and caffeic acid, absorb the UV radiation strongly and subsequently enter the gas phase, producing an abundance of molecular ions with minimal fragmenting. Cinnamic acid derivatives, such as α-cyano-4-hydroxycinnamic acid, are still used as choice matrix compounds today. Using modern techniques and instrumentation, the upper mass limit for MALDI is around 350,000 Da, and in some cases requires as little as 250 fmols of sample[6].

2 Principles

The mechanism behind MALDI relies on three principle steps. To begin with, a matrix-analyte solution must be prepared, and a cocrystal must be formed. This cocrystal is then excited using a laser to produce a plume of cocrystal particles that subsequently interact to form molecular ions. Finally, the molecular ions are analyzed using a mass spectrometer.

2.1 Cocrystal Formation

The first step of MALDI requires the formation of a matrix-analyte cocrystal. A sample of analyte is dissolved into a solution of the matrix compound and spotted onto a plate. The solvents then evaporate, producing a recrystallized matrix with interstitial analyte particles; this substance is known as a cocrystal. Choice of matrix compound is key to obtaining a quality spectrum: matrix compounds must fit a very specific set of criteria. Matrix compounds are generally low in mass, to ensure that they will easily vaporize upon laser incidence, though they must not be volatile enough to evaporate spontaneously. They must have high UV absorbance, which generally indicates the presence of a conjugated π system, because their sole purpose is to absorb radiation more effectively than the analyte molecules in order to transfer its energy to them. They are generally polar, to aid in the creation of an aqueous matrix-analyte solution, and are often acidic, to act as a proton source for ionization of the analyte.

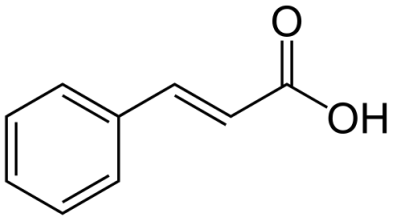
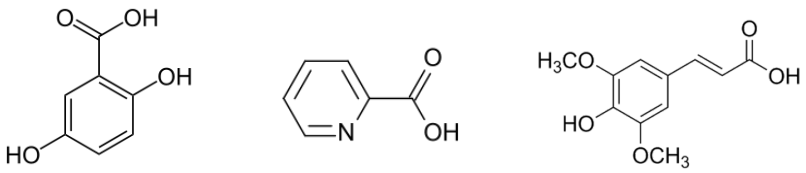


Figure 3: Cinnamic acid, of which many common matrix compounds are derivatives

It is immediately apparent from examining the structure of cinnamic acid (figure 3) why it is a common foundation for many matrix compounds. It meets every necessary characteristic for an effective MALDI matrix: relatively small size, conjugated π system, polar functional group, and acidic proton.



(a) Gentisic acid *(b) Picolinic acid* *(c) Sinapic acid*

Figure 4: Some common matrix compounds for UV MALDI-MS

The choice of matrix compound can greatly influence the capabilities of MALDI, and

specific compounds can be chosen to increase the sensitivity of the method to particular analytes. For example, gentisic acid (figure 4a) is particularly effective at detecting peptides containing boronic acid groups[7], while picolinic acid matrixes (figure 4b) have been experimentally determined to effectively detect oligonucleotides[8], and sinapic acid (figure 4c) is considered a good matrix choice for peptides with masses in excess of 10,000 Da[5].

2.2 Laser Excitation, Ablation, and Analyte Ionization

The next step involves the firing of a pulsed laser at the matrix cocrystal. The laser is generally a UV laser; common choices include a nitrogen laser (λ=337 nm) or a frequency-tripled Nd-YAG laser (λ=355 nm). The laser vibrationally couples with the chromophore of the matrix molecule, inducing a localized desorption of the matrix cocrystal and creating a gaseous plume of matrix and analyte ions. The exact mechanism of this ablation is not precisely known, but it is suspected to occur either because of sublimation after photochemical excitation of matrix molecules or due to a sharp increase in pressure caused by rapid expansion of units in the crystal lattice.

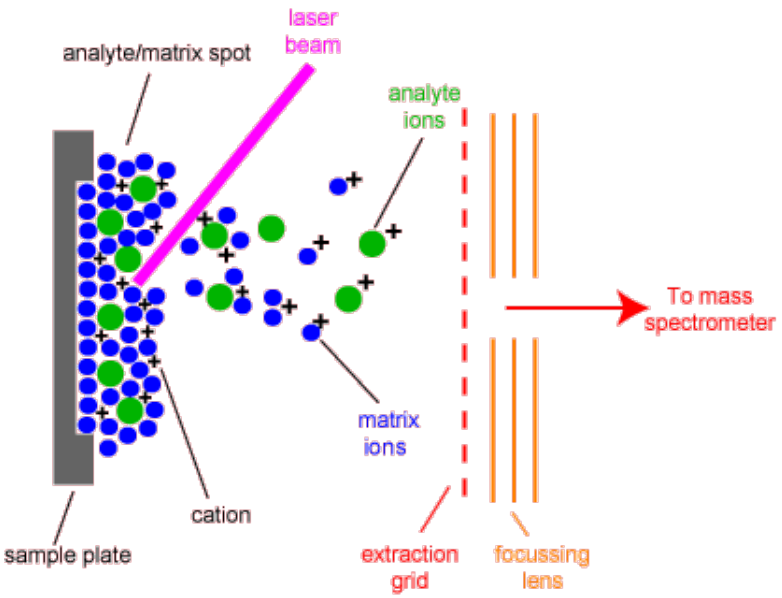


Figure 5: The mechanism of MALDI laser incidence

In the hot plume formed by the laser, quasimolecular ions of the analyte molecule are formed. Though the precise mechanism of this ionization is still debated, it is likely that

ablated species in the gaseous plume serve to aid in this process via proton transfer from the acidic matrix compound to the analyte ion. This process forms the quasimolecular ion $[M+H]^+$, generally the most common ion produced by MALDI, though multiply-charged ions, such as $[M+nH]^{n+}$ can be formed, especially with specific matrix-laser combinations.

Variations on this procedure, while uncommon, do exist. Infrared lasers occasionally see use due to their softer ionization mode[9]. Additionally, variations in matrix compound choice can produce alternate quasimolecular ions, such as the $[M-H]^-$ anion via deprotonation of the analyte by a basic matrix compound.

2.3 Mass Spectrometry

The most common mass analyzer paired with MALDI is the time-of-flight (TOF) mass spectrometer. MALDI produces ions in short bursts due to the use of a pulsed laser, and produces a wide variety of ion masses that require a detector with a broad mass range. Because it is a soft ionization technique that does not induce significant fragmentation in the analyte, MALDI-TOF is often paired with another variety of mass spec with a hard ionization method in a process known as tandem mass spectrometry (MS-MS) that induces fragmentation in the analyte after it has already been analyzed by a soft ionization method, in an effort to glean as much information as possible from both methods of ion production. While other methods of analyzing ions produced by MALDI exist, such as Fourier transform mass spectrometry (FTMS), they are much less commonly used.

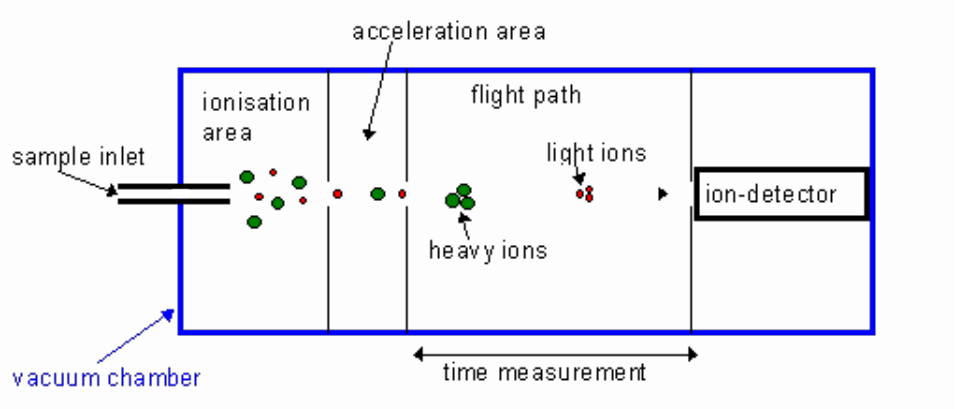


Figure 6: Schematic of a time-of-flight (TOF) mass spectometer

In time-of-flight mass spectrometry, a constant electric field is applied to a group of ions with varying masses and charges in order to categorize them by their mass-to-charge ratio. For a particle with charge q in an electric field with a potential difference (voltage) V, the particle has an electric potential energy:

$$PE = qV$$

When the charged particle is accelerated through the potential difference, this potential energy is turned into kinetic energy such that

$$PE = KE = \frac{1}{2}mv^2$$

where m is the mass of the particle and v is the final velocity after the particle exits the electric field. The particle, now at constant velocity v, travels the length ell of a time-of-flight tube, at the end of which it is detected and the time t it took to travel the tube is measured (a longer ℓ here serves to increase the resolution of the detector; for this purpose, many TOF detectors utilize an "ion mirror"[1] to reflect the ions and double the path length). The relationship between the path length and the particle's velocity and time of transit is

$$v = \frac{\ell}{t}$$

By combining the previous equations, we derive the relation

$$t = \frac{\ell}{\sqrt{2V}}\sqrt{\frac{m}{q}}$$

or

$$\frac{m}{q} = \frac{2t^2V}{\ell^2}$$

by which we can derive the mass-to-charge ratio of an ion in terms of its flight time.

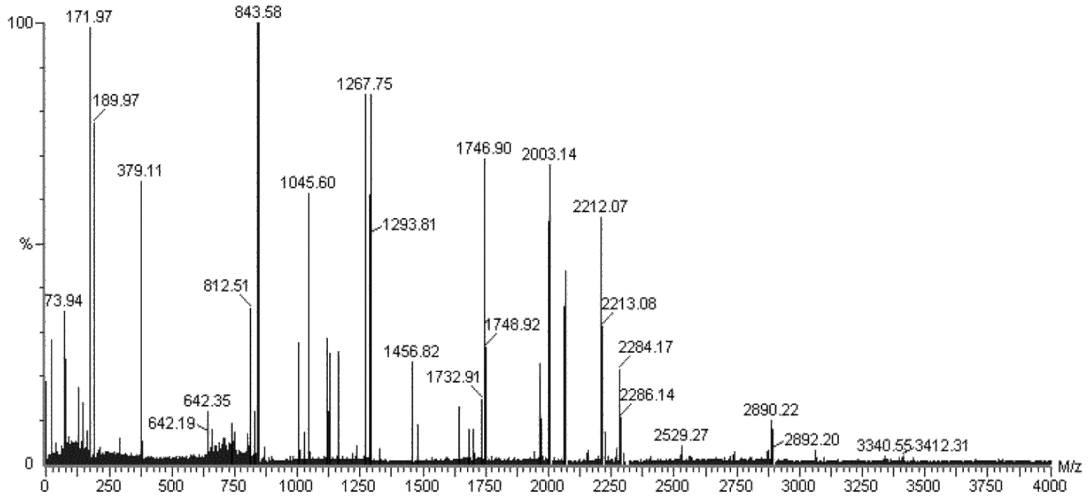
[1] A TOF MS that utilizes an ion mirror is known as a "reflectron"; TOF mass spectrometers that do not utilize an ion mirror are referred to as "linear".

3 Proven Capabilities

MALDI excels at soft ionization of macromolecules, especially biopolymers like peptides, proteins, or DNA, though it is often used in polymer chemistry to characterize the degree of polymerization of a compound. Particular combinations of lasers and matrix compounds have been experimentally determined to be particularly efficacious for analysis of specific compounds, though the mechanism of the matrix-analyte interaction is not yet fully understood. However, its effectiveness is limited by the minimal amount of multiple ionization is produces, requiring an associated mass spectrometer to have a large mass range, which inevitably leads to lower mass sensitivity.

3.1 Peptide Mass Fingerprinting

Peptide mass fingerprinting is an analytical technique that allows for the identification of proteins (i.e., from a genome) without time-consuming analysis of the peptides from scratch. By cleaving a protein into its substituent peptides, analyzing these fragments with MALDI-TOF to determine their masses, and referencing the peptide masses against a database of known peptide masses and sequences of proteins in a genome, it is possible to identify protein sequences to a high degree of accuracy without ever determining more than the masses of the peptides in question. However, this method requires that the protein sequence in question must exist in the database, and becomes exponentially more complicated the more proteins are mixed into the sample. While a mixture containing only one or two proteins can be feasibly calculated using only MALDI-TOF analysis, any more than that generally requires the use of tandem mass spectroscopy (MS-MS), the additional data of analyte fragmentation patterns from a hard ionization method serving to give additional specificity to the database search[10].



(a) A MALDI-TOF mass spectrum of enkephalinase

Observed	Expected	Calculated	Error	Start - End	Miss	Peptide
812.51	811.50	811.55	-0.05	355 - 361	0	LKPILTK
828.46	827.45	827.44	0.01	120 - 126	0	DVLQEPK
1002.54	1001.53	1001.54	-0.01	127 - 135	0	TEDIVAVQK
1027.59	1026.59	1026.51	0.07	104 - 111	0	YSNFDILR
1119.66	1118.66	1118.64	0.01	674 - 683	0	LLPGLDLNHK
1129.59	1128.59	1128.53	0.05	709 - 718	0	TDVHSPGNFR
1162.63	1161.62	1161.56	0.06	366 - 374	0	DLQNLMSWR
1267.75	1266.74	1266.66	0.07	375 - 385	0	FIMDLVSSLSR
1284.66	1283.66	1283.64	0.02	426 - 437	0	LYVEAAFAGESK
1292.81	1291.80	1291.72	0.08	438 - 448	0	HVVEDLIAQIR
1456.82	1455.81	1455.70	0.11	397 - 409	0	ALYGTTSETATWR
1477.74	1476.74	1476.71	0.03	480 - 492	0	IGYPDDIISNENK
1643.85	1642.84	1642.82	0.02	262 - 275	0	LPIDENQLSLEMNK
1683.78	1682.77	1682.84	-0.06	294 - 307	1	NDPMLLYNKMTLAK
1732.91	1731.90	1731.81	0.09	240 - 254	0	EACTAYVDFMISVAR
1967.12	1966.12	1966.04	0.07	104 - 119	1	YSNFDILRDELEVILK
2002.14	2001.13	2001.08	0.05	551 - 568	0	NQIVFPAGILQPPFFSAR
2888.21	2887.21	2887.33	-0.12	569 - 595	0	QSNSLNYGGIGMVIGHEI THGFDDNGR

No match to: 741.35, 1325.62, 1698.92, 1746.90, 2064.07, 2889.22

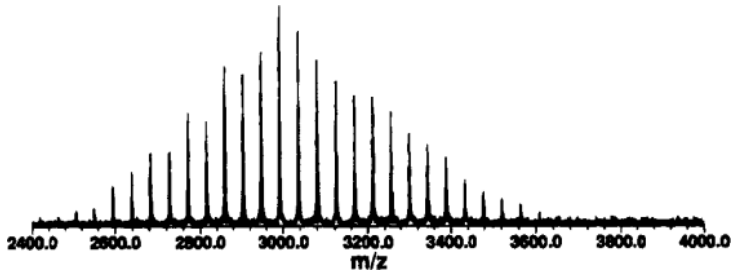
(b) A mass fingerprint of enkephalinase from figure 7a

Figure 7: Peptide mass fingerprinting of enkephalinase against the National Center for Biotechnology Information protein database identifies it positively, $p < 0.05$[11]

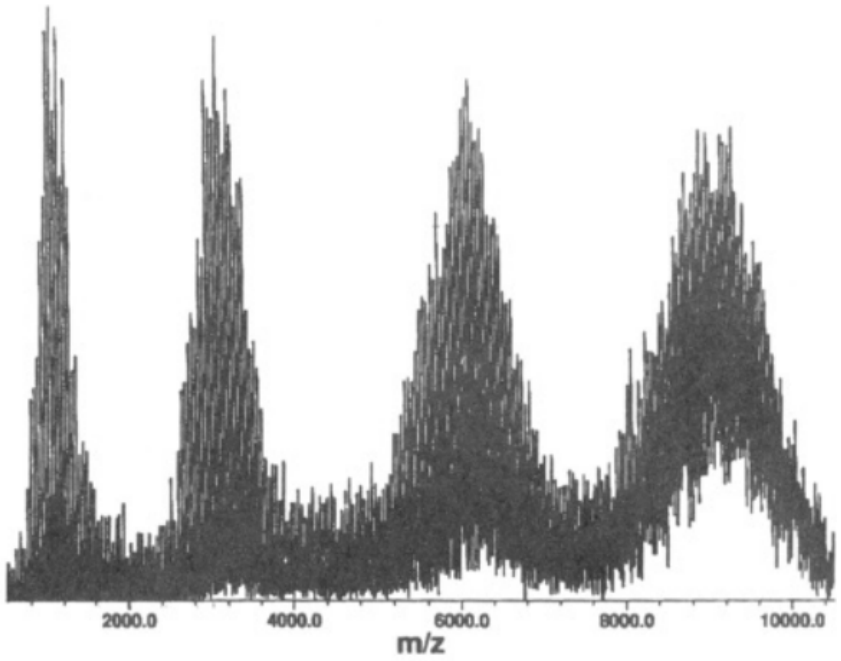
3.2 Polymer Molar Mass Distribution

Though a majority of its applications lie in the sphere of biochemistry, MALDI is also a useful tool in polymer chemistry. MALDI can be used to determine the molar mass distribution or degree of polymerization of extended polymers that would fracture if ionized by conventional means. Because polymers with the same composition but differing degrees of polymerization often have different properties (for instance, higher tensile strength and higher melting point), identifying the molar mass distribution of a polymer

is of great interest to polymer chemists.



(a) A MALDI-FT mass spectrum of PEG-3000, showing a roughly Gaussian fit around 3000 m/z



(b) A MALDI-FT mass spectrum of an equimolar mixture of PEG-1000, PEG-3000, PEG-6000, and PEG-9000

Figure 8: MALDI-FTMS analysis of poly(ethylene glycol) (PEG) samples with standardized weights reveals their molar mass distributions[12]

4 Competition

While there exist a plethora of ionization methods used for mass spectrometry, such as Chemical Ionization (CI), Electron Ionization (EI), Inductively-Coupled Plasma (ICP), and Desorption Electrospray Ionization (DESI), and many more, few of them are capable of soft ionization of macromolecules like MALDI is, giving it a distinct niche. Because soft- and hard-ionization techniques can be used as complements to each other in MS-MS, many of these ionization techniques are distinctly orthogonal to MALDI in use. Combin-

ing MALDI with these techniques allows for the accurate determination of molecular mass that soft ionization provides, while still providing structural information via fragmentation from hard ionization. In fact, of the commonly-used and commercially-available MS-coupled ionization techniques, only ESI (Electrospray Ionization) is capable of producing quasimolecular ions of large molecules without fragmentation, and thus competes with MALDI.

ESI chiefly utilizes an electrospray apparatus that employs a strong electric field to nebulize a solution containing the analyte. The solution typically contains an electrolyte to increase the dispersive power the electrospray, and is usually an acid (e.g. acetic acid) to double as a proton source for analyte ionization. It is important that the solvent be a volatile compound that will rapidly evaporate; individual droplets will continue to shrink due to evaporation (increasing the droplet charge density) until the Coulombic forces are strong enough that they tear the droplet apart. This cycle of evaporation and fission occurs until the charged analyte ions are desorbed, either because the droplets are small to allow them to desorb into the gas phase or because of the evaporation of nanodroplets containing only one analyte ion each[13].

While MALDI and ESI overlap greatly in function, however, there are advantages and disadvantages to each. Most notably, ESI is significantly more capable than MALDI of producing multiply-charged ions, which greatly increases the effective range of the mass analyzer by dividing the m/z ratio by an integral factor. Because many mass analyzers have "sweet spots" in which they are much more sensitive, increasing the range of mass-charge ratios produced in this manner greatly enhances the accuracy of the instrument, as well as allowing it to analyze compounds that are multiplicatively larger than compounds analyzed using MALDI. While this process does significantly complicate the spectra relative to MALDI's singly-charged ion spectra, the increase in sensitivity and detector range as a result is generally considered more important. Because of the differing sample preparation methods required for ESI and MALDI, either of them can be used as soft ionization sample preparation methods based on the availability of instruments and preparation materials; however, this is largely a matter of convenience and is not an

indication of the quality of either method. Both MALDI and ESI instrumentation are widely available today, and as such both see a significant amount of usage when performing soft ionization mass spectrometry; however, ESI's inherent advantage at analyzing more massive compounds is clearly a mark against MALDI.

5 Outlook

Soft ionization of macromolecules is an incredibly useful technique with a variety of applications in a range of fields; it is unlikely that uses for MALDI will wane in the coming years, as there will always be call for methods to analyze polymers or sequence polypeptides. However, in the future it is unlikely that we will find many applications for MALDI that cannot be accomplished at least as effectively with ESI. Right now, there is significant usage of both techniques, but this can be chalked up to varying availability of instruments and preparation materials for both methods. MALDI's principle advantage is that the spectra it creates are simpler and easier to analyze than the multiple charges created by ESI; however, interpreted properly (such as by a computer), ESI can provide more information over a wider mass range. Over time, it is likely that we will see a gradual shift away from MALDI and toward ESI, due to the latter's greater capabilities within the niche they both occupy. While we can still expect to see usage of MALDI in the near future, there are currently few fundamental advantages of the technique over ESI.

6 Conclusion

MALDI (Matrix-Assisted Laser Desorption/Ionization) is an incredibly powerful analytical technique that allows for the "soft ionization" (i.e. ionization without fragmentation) of compounds, a procedure that is especially useful for analyzing macromolecules that would be fragmented by other methods. This method is especially useful for analysis of biological macromolecules (such as polypeptides) and other polymers, where it allows for feats such as "peptide fingerprinting" by mass spectra or calculation of the molar mass

distribution of a specific sample. Specific combinations of matrix compounds, lasers, and mass spectrometers allow for the technique to be tuned for analysis of specific compounds. However, while soft ionization of macromolecules provides powerful solutions to many problems in biochemistry and polymer chemistry, MALDI is not the only technique for performing this ionization. ESI (Electrospray Ionization) also performs many of the same functions as MALDI, and is often more effective at creating mass spectra of heavier molecules due to its greater tendency to produce multiply-charged ions, something that multiplicatively reduces the m/z ratio of an ion and thus significantly increases the effective mass range of any detector it is paired with. While MALDI is still useful, and often provides spectra that are easier to analyze than ESI, the latter's greater range is likely more important within the niche they both occupy.

Despite this fact, however, MALDI is still fundamentally a powerful analytical technique, and one of only a few that solves a very specific problem with a wealth of applications in a variety of fields.

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