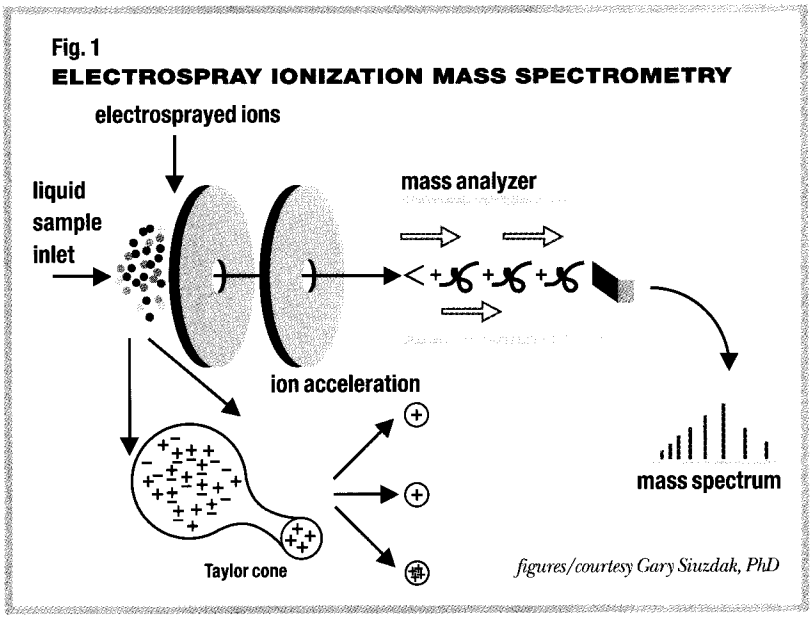
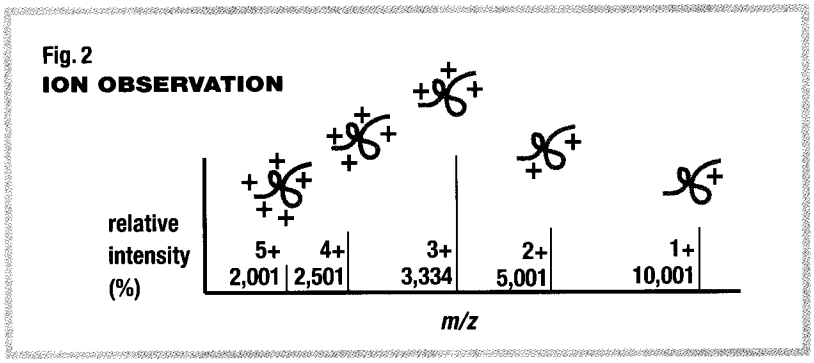
# Innovations Continue in Mass Spectrometry

Commercial availability of mass spectrometry instruments has made the analysis of biomolecules routine.




By Gary Siuzdak, PhD

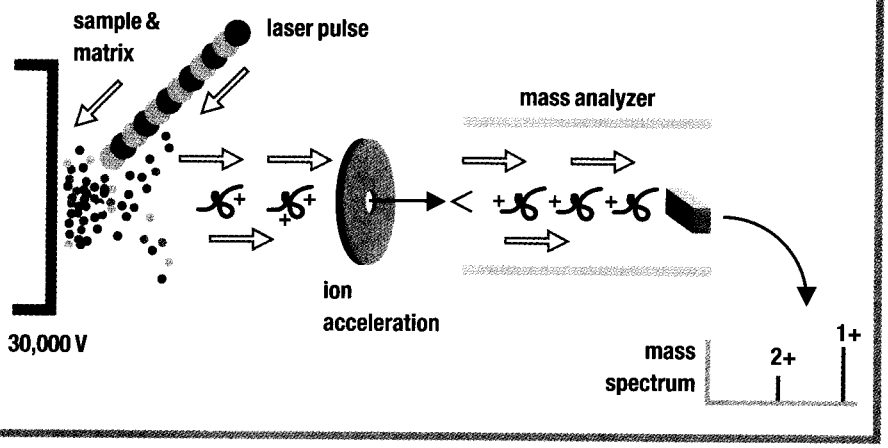
Mass spectrometry (MS) has emerged as an important tool in biochemical research.[1] Scientists have continued to develop and establish this technique into what it is today—a highly sensitive tool capable of analyzing samples ranging in size from small molecules to whole viruses. Moreover, since electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) MS have been added to the repetoire of research methods, the demand for this instrumentation has exploded.




Now, the commercial availability of MS instruments that offer picomole to femtomole sensitivity and enable the analysis of biological fluids with a minimum amount of sample preparation has made the analysis of a large variety of compounds—proteins, peptides, carbohydrates, oligonucleotides, natural products, drugs and drug metabolites—routine.

The developmental stage of MS has not stopped. Innovations such as nanospray, curved reflectrons and electrospray with orthogonal spraying continue to expand its capability. Extending beyond simple molecular weight characterization, these "mild" ionization methods can be applied to many new applications, such as: ▷

**Fig. 3**
**MATRIX-ASSISTED LASER DESORPTION/IONIZATION (MALDI) MASS SPECTROMETRY**




- protein-protein interactions,
- dynamic viral analysis,
- high sensitivity protein sequencing,
- routine DNA sequencing,
- protein folding,
- high throughput analysis in combinatorial chemistry and
- drug discovery.

**Mass Spectrometry Defined**

MS is the art of measuring atoms and molecules to determine their molecular weight. Such mass or weight information is sometimes sufficient, frequently necessary and always useful in determining the identity of a species.[2]

For descriptive purposes, an analogy can be drawn between a mass spectrometer and a prism. In the prism, light is separated into its various wavelength components and is then detected with an optical receptor, such as an eye. Similarly, in a mass spectrometer, generated ions are separated in the mass analyzer and detected by an ion detector, such as an electron multiplier. The key to MS's recent success is how electrospray and MALDI have allowed ions to be generated in the gas phase with great efficiency and without decomposition.

**More on Electrospray**

ESI is a method used to produce gaseous ionized molecules from a liquid solution. This is done by creating a fine spray of highly charged droplets in the presence of a strong electric field (Fig. 1). The sample solution is sprayed from a region of a strong electric field at the tip of a metal nozzle maintained at approximately 4,000 V, and the highly charged droplets are then electrostatically attracted to the mass spectrometer inlet. Either dry gas, heat or both are applied to the droplets before they enter the vacuum of the mass spectrometer, thus causing the solvent to evaporate from each droplet. As the droplet decreases in size, the electric field density on its surface increases. The mutual repulsion between like charges on this surface becomes so great that it exceeds the forces of surface tension, and ions begin to leave the droplet through what is known as a "Taylor cone."[3] The ions are then directed into an orifice through electrostatic lenses leading to the mass analyzer. Because it is a continuous ionization method, it is suitable for use as an interface with high performance liquid chromatography or capillary electrophoresis.

*Both ESI and MALDI-MS are sensitive tools for mass measurements and can provide surprisingly large amounts of other information as well.*

Electrospray ionization, routinely used for peptides, proteins, carbohydrates, small oligonucleotides, synthetic polymers and lipids, is conducive to the formation of singly charged small molecules, but is also well known for producing multiply charged species of larger molecules. This is an important feature since the mass spectrometer measures the mass-to-charge ratio (m/z), making it possible to observe large molecules with an instrument having a relatively small mass range.

Fig. 2 illustrates how the ions from a protein of mass 10,000 Da can be observed, where each of the peaks can be associated with different charge states of the protein's molecular ions. Fortunately, computer programs available with electrospray mass spectrometers facilitate molecular weight calculations.

**What is MALDI?**

MALDI-MS permits the analysis of both low and high molecular weight compounds, including carbohydrates, lipids, peptides, proteins and oligonucleotides, as well as synthetic molecules and polymers with high sensitivity. MALDI allows for the ionization and transfer of a sample from a condensed phase to the gas phase through laser vaporization of a solid matrix (Fig. 3).

More specifically, ion formation is accomplished by directing a pulsed laser beam to a sample suspended or dissolved in a matrix. The matrix plays a key role in this technique by absorbing the laser light energy and causing the matrix material to vaporize (the vaporized matrix will carry some of the sample with it).

Once in the gas phase, the matrix may play a role in the ionization of the analyte molecules. Electrostatic lenses will direct the charged molecules from the ionization sources into the mass analyzer. Uncharged molecules will often react with the matrix or other molecules to produce charged species, transferred electrostatically into the mass analyzer. Once the molecules in the sample are vaporized, time-of-flight mass analysis is often used to separate the ions according to their m/z.

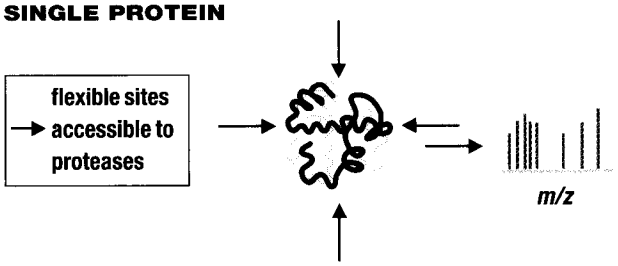
In short, the efficient and directed energy transfer during a matrix-assisted, laser-induced desorption event allows for relatively small quantities of sample to be analyzed. In addition, the utility of MALDI for the analysis of heterogeneous samples makes it very attractive for the mass analysis of biological samples.

**Applications**

Both ESI and MALDI-MS are sensitive tools for mass measurements and can provide surprisingly large amounts of other information as well. Initially, both methods were used primarily to obtain accurate molecular weight information on molecules that were traditionally difficult or impossible to analyze (i.e., proteins, oligonucleotides and carbohydrates). However, as a result of their improved sensitivity and accuracy, MALDI and ESI were quickly applied to even more interesting problems.

For example, the ability to analyze complex mixtures has made MALDI and ESI useful for the examination of proteolytic digests, an application otherwise known as protein mass mapping. Through the appli- ▷

**Fig. 4**
**THEORETICAL PROTEOLYSIS OF A SINGLE PROTEIN**




providing a viable alternative to existing analytical techniques that typically require extensive sample preparation and optimization time, the disposal of biohazardous waste or a significant amount of sample.

Another application that has generated excitement involves electrospray's ability to produce and mass analyze biological noncovalent complexes in the gas phase. The possibility of correlating condensed phase intermolecular interactions with mass spectrometry has captured the attention of many researchers.

cation of sequence-specific proteases, protein mass mapping allows for the identification of protein primary structure.[4] Performing mass analysis on the resulting proteolytic fragments yields information on fragment masses with accuracy approaching +/- 5ppm, or +/- 0.005 Da for a 1,000 Da peptide. The protease fragmentation pattern is then compared with the patterns predicted for all proteins within a database and matches are statistically evaluated. Since the occurrence of Arg and Lys residues in proteins is statistically high, trypsin cleavage (specific for Arg and Lys) generally produces a large number of fragments that, in turn, offer a reasonable probability for unambiguously identifying the target protein.

Protein mass mapping has also been used for studying higher order protein structure by combining limited proteolytic digestion, mass analysis and computer-facilitated data analysis.[5] In the analysis of protein structure, enzymes are used to initially cleave surface accessible regions of the protein or protein complex (Fig. 4). These initial cleavage sites are then identified using accurate mass measurements combined with the protein's known structure and the known specificity of the enzyme. Computer-based sequence searching programs allow for the identification of each proteolytic fragment that can be used to map the protein's structure. This approach has also been used to examine viral protein capsid structure.[6]

MS is also playing an increasingly important role in the molecular characterization of combinatorial libraries.[7] Crucial to distinguishing the most active component or obtaining structure-activity relationships of compounds in a library is an efficient qualitative and quantitative assay. Toward this end, ESI and MALDI have been useful for the qualitative, and more recently, the quantitative screening of combinatorial libraries.

In addition, mass spectrometry does not involve chromophores or radiolabelling, being useful for large molecules, ESI is an important tool for qualitative and quantitative analysis of small biomolecules. In our lab, we recently developed an ESI-based method to quantitatively examine small molecules (steroids) at the attomole level ($100 \times 10^{-18}$ moles) (Fig. 5).

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For the first time, MS can be used as a tool to observe complexes in the gas phase taken from an aqueous environment, thereby providing insights into specific noncovalent associations in solution. The selectivity of MS may eventually help avoid impurity problems associated with immunoaffinity procedures and also facilitate drug screening. Examples include the observation of the heme globin complex, an oligonucleotide duplex, cell surface carbohydrate association, catalytic antibody-inhibitor interactions and the analysis of whole viruses.

Until now, small molecule quantitative analysis has been left to traditional techniques such as electron ionization MS however, any compounds are too thermally fragile to survive its ionization process. Fortunately, in addition to

## Conclusion

The development of ESI and MALDI-MS has been the platform for routine biomass analysis, resulting in their application toward a wide range of biochemical problems. The complementary nature of ESI and MALDI-MS has made it increasingly vital that researchers and laboratorians have access to both types of instrumentation. Independently, ESI and MALDI-MS can help answer many questions, yet together they represent a formidable tool with new levels of sensitivity, accuracy and mass range. ■

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## References

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**Fig.5**
**MASS SPECTRUM OF THREE DIFFERENT STEROID SULFATES USING HIGH SENSITIVITY ESI (NANOESI)**