The Physics behind Mass Spectrometry

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Introduction

Mass spectrometry (MS) is an analytical technique that measures the mass/charge \((m/z)\) ratio of gas phase ionized molecules to calculate molecular weight (Carey and Giuliano 2011). A classic mass spectrometer has three parts: an ion source, mass analyzer, and detector (Ekman et al 2009). As with most modern scientific instrumentation, mass spectrometers operate by the application of physical phenomena, notably electricity and magnetism (Wilkins and Trimpin 2011). Mass spectrometry has far ranging applications involving forensics, clinical chemistry, imaging, and nanotechnology. Advances in the last thirty years have enabled larger molecules in more complex solutions to be analyzed, opening the door to extensive work in protein and other biochemical research.

Figure 1. Schematic of a mass spectrometer including an ion source, mass analyzer, and detector (Carey and Giuliano 2011).
Ion Sources

Mass spectrometry measures the mass/charge ratio of ions, thus ionization of the sample must occur before analysis can begin. The creation of gas phase ions occurs in the aptly named ion source. Multiple ionization methods have been developed over the years, but the most common will be discussed here.

Electron Ionization (EI), one of the first methods developed in 1921 by Dempster, directs an electron beam into a vapor held at low pressure to create ions (Ekman et al 2009). The electron beam is produced by heating a metal filament aimed across the ion source chamber. Low magnetic fields are used to make the electrons spiral along their path and increase the chance for an interaction with the analyte. The interaction causes one, or possibly more, electrons to be removed and the formation of a positive radical molecular ion (M+). Each molecule has its own unique ionization energy (IE), the amount of energy that must be applied for a molecule to lose an electron. Ionization will not take place if the IE is not reached, but the standard value of 70eV suffices for most compounds. During the process of ionization, excess energy may spread over the chemical bonds of the ion and can break bonds if the excess energy is greater than the bond energy. The appearance energy (AE) of an ion is the energy necessary to create fragment ions. Multiple fragmentations can occur to dissipate the excess internal energy of an ion. Fragmentation, as will be discussed later, can be used as a “fingerprint” to identify the sample (Wilkins and Trimpin 2011).

Electrospray Ionization (ESI), developed by Dole and coworkers in 1968 and coupled with MS by Yamashita and Fenn in 1984, remains a popular method to this day. To begin, the sample is dissolved in a polar, volatile solvent (e.g. methanol or acetonitrile) and injected into the source with a needle (Scripps 2011). A high electric potential exists between the injection needle
and the nozzle leading to the mass analyzer and causes the liquid to form a Taylor cone. In the case of positive ionization, positive ions gather at the tip and a spray of charged droplets is removed from the Taylor cone by the electric field. Evaporation assisted by nitrogen gas breaks up the droplets that can then enter the vacuum of the mass analyzer. As the molecules evaporate and become smaller, the charge density of the droplets increases; eventually, the repulsion between like charges overcomes the surface tension, a.k.a. the Rayleigh limit, and a Coulombic explosion ensues releasing the charged ions (University of Bristol 2005).

![Figure 2. Schematic of ESI (University of Bristol 2005).](image)

Despite the previous use of the word “explosion,” this is a gentle ionization method and results in little fragmentation. Analysis of ESI is more complicated than other methods since it produces a multiple-charged ion spectrum that must be transformed into a single peak.

First used by Karas, Hillenkamp, and coworkers in the 1980s, Matrix-Assisted Laser Desoprtion/Ionization (MALDI) in one of the most widely used methods. In MALDI-MS, the analyte is dilutely embedded in a matrix of low mass, highly light absorbing molecules (Ekman
et al 2009). A UV laser pulse is then directed at the matrix under high vacuum conditions and the absorbed energy causes breakup and ionization of the sample into the gas phase. MALDI-MS is very sensitive and is capable of detecting large biomolecules in impure samples.

**Mass Analyzers**

Once the sample has been ionized, the ions need to be separated. Magnetic and/or electric fields can be used to separate ions based on their $m/z$ ratio.

Time-of-Flight (TOF) mass analyzers separate ions based on the time it takes an ion to reach the detector. Published in 1946 by Stephens, TOF analyzers uses the equation

$$t_{\text{TOF}} = \frac{L}{v} = L\sqrt{\frac{m}{2qV_a}} \propto \sqrt{\frac{m}{z}}$$  \hspace{1cm} (1)

where $L$ is the length of the field-free zone, $v$ is the velocity of the ion, $m$ is the mass of the ion, $q$ is the charge of the ion, $V_a$ is the electric potential energy, and $z$ is the charge state, to calculate the time difference from the start signal to detection (Ekman et al 2009). Pulsed ion sources, like MALDI, make timing easier than continuous ion sources, such as ESI. One problem with TOF analyzers is that the initial velocities of the ions are not uniform. Time-lag focusing can be used to correct this. For the first few hundred nanoseconds of ionization the extraction region is kept field free, but the sample plate potential is then raised producing an electric field. The slower ions closer to the field with experience a higher electric potential than the faster farther ions and increase kinetic energy. This can be modeled by equation 2 with $V$ approximating the sample plate as a finite line charge

$$E_i = \frac{1}{2}mv^2 = qV + \frac{1}{2}mv_0^2$$  \hspace{1cm} (2)

$$V = \sum_i \frac{kq_i}{d_i}$$  \hspace{1cm} (3).
The ions with the same $m/z$ with get closer together in the field free region and will reach the detector at the same time if tuned properly.

Magnetic/electric sector analyzers use electric and magnetic fields to focus ions of a specific $m/z$ onto the detector. Upon exiting the ion source, a magnetic field is applied that does not change the ion velocity, but moves the ions into a circular path with radius $r$ given by

$$\frac{m}{q} = \frac{B^2 r^2}{2V_u}$$  \hspace{1cm} (4)$$

where $m$ is the mass of the ion, $q$ is the charge of the ion, $B$ is the magnetic field strength, $r$ is the radius, and $V_u$ is the potential difference (Ekman et al 2009). Next, an electric sector applies a perpendicular electric field that focuses ions with the same kinetic energy to the same position at the exit slit. Depending on the instrument, either the electric sector or the magnetic sector may be adjusted, by increasing the voltage or magnetic field strength respectively, to focus the desired $m/z$ through the detector slit.

**Figure 3.** Magnetic/Electric sector mass analyzer (University of Bristol 2005).
Fourier Transform Mass Spectrometry (FTMS) also uses magnetic fields to move ions in circular paths (Ekman et al 2009). Ions with charge $q$ and mass $m$ moving in a uniform electric field of strength $B$ circulate within the analyzer with a cyclotron frequency of

$$f_c = \frac{qB}{2\pi \cdot m} \propto \frac{1}{m/z} \quad (5)$$

All ions with the same frequency also have the same $m/z$ regardless of velocity. The ions must initially have a high enough velocity to surpass the high magnetic field difference between the ion source and analyzer. Once in the analyzer, a pulsed radio frequency (RF) excites the ions into larger orbits (Scripps 2011). A small current is induced on the detector every time the ion spins by. The detector can pick up a range of frequencies and produces a time spectrum. A Fourier transformation of the time spectrum produces a frequency spectrum that can be related to a mass spectrum. FTMS are known for their high accuracy and resolution, both of which improve as the magnetic field is increased.

**Detectors**

Detectors convert the energy of ions into a current signal that can be interpreted by a computer. The impact of ions is usually associated with the discharge of electrons or photons that can be detected by electronic devices. An electron multiplier (EM) amplifies weak current by using a series of secondary emission electrodes to produce a higher current at the anode. When the ions hit the electrodes, energy is transferred to the electrons in the plate and secondary electrons are emitted, this can result in up to $10^6$ amplification (Scripps 2011). Solid-state detectors (SSD), made of silicon or germanium, emit electrons in the presence of ionizing radiation (Ekman et al 2009). The benefit of using an SSD is that the mass, charge, and energy can be independently measured instead of the ratio of mass to charge or energy to charge.
**Interpretation**

The interpretation of mass spectra is relatively simple compared to other analytical techniques such as NMR. The mass spectrometer plots $m/z$ versus relative intensity, figure 3, and if the change $z$ is 1, then the mass of the molecule can be taken directly from the spectrum.

![Mass Spectrum](image)

**Figure 4.** N, N-diethylmethylamine mass spectrum (Virtual Text of Organic Chemistry 2007).

Figure 3 also illustrates fragmentation of the molecule. The molecular weight (MW) of N, N, diethylmethylamine is 87 daltons; yet, multiple peaks of lesser MW were detected. This is because the compound broke down during ionization. Individual compounds have a unique fragmentation relatively independent of the instrument, and thus an unknown sample can be compared to databases of known spectra and identified.

**Applications**

Mass spectrometers provide more than just the molecular weight of a sample: time dependent studies give kinetic information; assays detect the presence of certain compounds; hydrogen/deuterium exchange can provide information on protein structure and stability. Researchers are continuing to adapt MS in new and exciting ways.
One of the frontiers of research in which MS is being applied is protein biophysics. Proteins are complex molecules involved in many biochemical reactions that sustain life. They aid in molecular transport, cell signaling, provide structural integrity, and catalyze reactions. The function of a protein is dependent upon its folded structure; correct folding corresponds to healthy cell function, while misfolding can lead to disease (Dobson 1999). Despite the importance of proteins and their functions, science has an incomplete picture of how proteins achieve their tertiary structure from the primary amino acid sequence. If scientists were able to accurately predict the folded structure, then there would be a surge of protein technologies with applications in medicine, agriculture, and biotechnology. Mass spectrometry has become an increasingly popular technique for characterizing protein stability and energetics. Researchers at the University of Arkansas are currently developing HDX-PEPS (Hydrogen Deuterium Exchange-Protein Equilibrium Population Snapshot) method coupled with electrospray ionization mass spectrometry (ESI-MS) to probe the global structure of a denatured protein by measuring the free energy change between the native and denatured state (Liyanage et al 2009).

The PEPS method is an equilibrium-based method where the population of the open and closed state is measured by observing the mass and intensity changes during HDX as the globally protected region of the protein is subjected to systematic denaturation. Denaturation is initiated by adding guanidine hydrochloride (GdHCl). HDX can probe the entire protein backbone by allowing the deuterium to exchange with the backbone amide protons. When the protein is folded, part of the peptide backbone is buried in the hydrophobic core along with the amide protons associated with this region. This prevents deuterium from exchanging with these protons, thus allowing a differentiation between the closed and open state. The standard equation for the exchange rate of globally protected amide hydrogens defined as
\[
k_{\text{ex}} = \left( \frac{k_{\text{op}} k_{\text{int}}}{k_{\text{op}} + k_{\text{cl}} + k_{\text{int}}} \right)
\]

(5)
can be simplified to \(k_{\text{ex}} = k_{\text{op}}\) when \(k_{\text{op}}\) and \(k_{\text{cl}} << k_{\text{int}}\), where \(k_{\text{op}}, k_{\text{cl}},\) and \(k_{\text{int}}\) refer to the rate of opening, rate of closing, and the intrinsic exchange rate of an unprotected amide proton respectively. Under these conditions, every time the protein unfolds from the closed to open state all the globally protected amide protons get exchanged because the unfolded state lives much longer than the time needed to exchange a fully exposed amide proton. Hence, a short HDX time provides accurate population information as long as this time is shorter or comparable to the lifetime of both the open and closed states.

Since the amount of deuterium exchange is different for the open and closed states, mass spectrometry can be used to detect the populations of these states. The state with the higher mass is the open state since more hydrogens are exposed because the hydrophobic center becomes solvent accessible as the protein unfolds. As the protein is denatured, the unfolding protein (higher mass) increases in the open state intensity while the intensity of the closed state declines.

**Figure 5.** Mass spectra as mutant V66W denatured.
Hence the apparent folding constant (the folding constant at a given GdHCl) should be given by equation 6 if the intensities of the closed and open states are truly representative of the population information.

\[ K_{f}^{\text{app}} = k_{cl}/k_{op} = [\text{Closed}]/[\text{Open}] = I_{\text{closed}}/I_{\text{open}} \]  

(6)

Correction has to be made for the amount of closed state moving to the open state during the HDX time \( t \). Hence the experimental equilibrium constant is given by

\[ K_{f}^{\text{app} (\text{experimental})} = I_{\text{closed}}/I_{\text{open}} = (k_{cl}e^{-k_{ext}t}) / (k_{op} + k_{cl}(1-e^{-k_{ext}t})) \]  

(7)

with the denominator representing how many molecules get exchanged moving to the open state and the numerator representing what remains in the closed state after exchange time \( t \). Once the equilibrium constant was calculated for each concentration of GdHCl, the linear extrapolation method (LEM) was used to calculate the physiologically relevant folding energy by the equation

\[ \Delta G_{\text{app}} = \Delta G_{\text{H2O}} + mC \]  

(8)

where \( \Delta G_{\text{app}} = -RT\ln K_{f}^{\text{app}} \), \( \Delta G_{\text{H2O}} \) is the folding energy at 0M denaturant, \( C \) is the molar concentration of the denaturant, and \( mC \) is the \( \delta\Delta G_{\text{H2O}} /\delta C \).

**Figure 6.** V66W Folding Energy. \( \Delta G = 1.95 \text{ kcal/mol} \), calculated by HDX PEPS.
One ΔG value in itself is not meaningful; however, the comparison of folding energy values for mutants of a protein can be used to understand the role of specific amino acids in the overall structure. For instance, will adding a polar amino acid into the hydrophobic core destabilize the interior, causing loss of function? Can disulfide bridges be formed by introducing cystines in the desired location? Renaturation of proteins from the denatured to native state proves that the primary structure has all the information to make the correct tertiary structure. The electrostatic interactions between the amino acids must then be responsible for the protein’s shape. A thorough understanding of the intramolecular forces will allow scientists to create models to predict how designed amino acid sequences fold, paving the way for custom-made drugs and industrial enzymes.

Conclusion

Mass spectrometry has become an integral tool in modern science. In 2002, John B. Fenn and Koichi Tanaka shared the Nobel Prize in Chemistry for their separate work in mass spectrometry, Fenn for ESI and Tanaka for MALDI (Nobel Prize 2011). The manipulation of electromagnetic fields and an understanding of ion physics allowed these scientists to create one of the most widely used analytical instruments in use today. Its popularity results from several distinct advantages over past techniques. One of the strongest assets of mass spectrometry is the amount of sample needed; nano-ESI and MALDI-MS work on as small as the picogram/liter scale (Scripps 2011). Mass spectrometers also can be coupled with most chromatography systems to separate the desired analyte out of a complex, high performance liquid chromatography (HPLC) being the most common. New advances in instrumentation and methods continue to increase the applications and sensitivity of mass spectrometers and will do so for many years to come.
Bibliography


