MS Goals and Applications

• Several variations on a theme, three common steps
  – Form gas-phase ions
    • choice of ionization method depends on sample identity and information required
  – Separate ions on basis of m/z
    • “Mass Analyzer”
    • analogous to monochromator, changing conditions of analyzer results in different ions being transmitted
  – Detect ions
    • want (need) high sensitivity
      – “Resolution”

MS Goals and Applications

• All MS experiments are conducted under vacuum, why?
  – Mean free path (\(\lambda\)):
    \[
    \lambda = \frac{RT}{\sqrt{2} \pi d^2 N_A P} \approx \frac{5 \text{ cm}}{\text{mtoorr}}
    \]

• Ion Optics: Electric and magnetic fields induce ion motion
  – Electric fields most common: Apply voltage, ions move
  – Magnetic fields are common in mass analyzers. “Bend” ions paths (Remember the right hand rule?)
**MS Figures of Merit:**

**Resolving Power and Resolution**

- Relate to ability to distinguish between m/z
  - Defined at a particular m/z

- **Resolving Power, Resolution...**
  - Variety of definitions
  - $\Delta m$ at a given m

  \[
  \text{Resolving Power} = \frac{m}{\Delta m}
  \]

  \[
  \text{Resolution} = \frac{m}{\Delta m}
  \]

**MS Components: Mass Analyzers**

- **Magnetic Sector Mass Analyzers**
  - Accelerate ions by applying voltage (V)
  - velocity depends on mass and charge (m/z)

  \[
  KE = \frac{1}{2} mv^2
  \]

  - Electromagnet introduces a magnetic field (variable)
  - The path on an ion through the sector is driven by magnetic force and centripetal force

  - For an ion to pass through, These must be equal

  \[
  F_m = BzeV = \frac{mv^2}{r} = F_c \quad \Rightarrow \quad \frac{m}{z} = \frac{B^2 r^2 e}{2V}
  \]

  - For a given geometry (r), variation in B or V will allow different ions to pass
  - “Scanning” B or V generates a mass spectrum
MS Components: Mass Analyzers

• In practice, ions leaving the source have a small spread of kinetic energies (bandwidth?)
  \[ R = \frac{m}{\Delta m} \leq 2000 \text{ for mag. sector alone} \]

• Result is a spread in paths through magnetic field
  – leads to broadened bands and decreased resolution

• Problem is minimized using *Double Focusing MS*
  – Two sectors:
    • Electrostatic sector focuses on the basis of translation energy: “Energy Analyzer”
    • Magnetic sector focuses on the basis of momentum: “Momentum Analyzer”
  – Results in better M/Z discrimination and higher resolution (up to 100,000!).
  – Often more $$$
MS Components: Mass Analyzers

- **Quadrupole Mass Filter**
  - Opposing AC voltage applied between pairs of rods
  - $U_{dc} + V \cos \omega t$ and $-(U_{dc} + V \cos \omega t)$
  - Because of positive potential superimposed on AC, quad acts as high-pass mass filter in plane with positive DC offset
  - Because of negative potential, quad acts as a low-pass mass filter in plane with negative DC offset.

- By changing AC and DC potentials, different $m/z$ will have "stable" trajectories
  - acts like a "notch" filter!
  - Tunable up to $m/z \sim 4000$ with unit mass resolution

- Many benefits over Double Focusing
  - Smaller, Less Expensive
  - More Rugged
  - Possible to "scan" spectra in $<0.1$ sec

- Can’t get the high resolution like double focusing!
MS Components: Mass Analyzers

- **Ion Traps**
  - Ions are "stored" and selectively cycled out

- **Quadropole Ion Trap (QIT)**
  - Similar concept to quadropole
  - RF and DC electric fields
  - Only certain $m/z$ are "stable"

- **FT-Ion Cyclotron Resonance (FT-ICR)**
  - Magnetic field traps ions
  - RF pulse is added to augment motion
  - Current at receiver relates to $m/z$
    - [http://www.magnet.fsu.edu/education/tutorials/magnetacademy/fticr/](http://www.magnet.fsu.edu/education/tutorials/magnetacademy/fticr/)

---

MS Components: Mass Analyzers

- **Time of Flight Mass Analyzer:**
  - "Pulse" of ions are accelerated into analyzer
    - Very small range of kinetic energies (ideally all have same KE)
    - Since masses vary, velocity must also vary
  - Ions enter a field-free region, the *drift tube*, where they are separated on the basis of their velocities
    - Lighter ions (smaller $m/z$) arrive at the detector first, heavier ions (larger $m/z$) arrive later

MS Components: Mass Analyzers

- Potential for very fast analysis (sub millisecond)
- Simple instrumentation
- Resolution depends on applied voltage (kinetic energy) and flight time
  - use internal standards to calibrate
  - Resolution is enhanced by use of **reflectron**
    - Like a concave "ion mirror"

MS Components: Detectors

- Two common types of detectors:
  - Faraday Cup
  - Electron Multiplier

- **Faraday Cup**
  - Ions are accelerated toward a grounded "collector electrode"
  - As ions strike the surface, electrons flow to neutralize charge, producing a small current that can be externally amplified.
  - Size of this current is related to # of ions in
  - No internal gain → less sensitive
**MS Components: Detectors**

- **Electron Multiplier**
  - Analogous to PMT
  - Durable, applicable to most analyzers
  - Ions strike surface of dynode
    - Generate electrons
    - $>1 \text{ e}^-/\text{ion}$
  - Ejected electrons are accelerated to other dynodes
    - $>1 \text{ e}^- \text{out/e}^-\text{in}$
  - Current is related to number of ions in times large gain (10$^7$ or so)

- **Single channel vs. array detectors**
  - “Single” m/z vs “whole spectrum at a time”
  - Often a tradeoff between sensitivity and speed

- **Microchannel Plate**
  - Converts ions to electrons
    - Gains approaching electron multipliers
    - $\sim 10^4$ for single, more if “stacked”
  - Electrons can be detected in two dimensions.
    - One approach: convert electrons to photons and use optical detection (i.e. camera!)
MS Components: Sources

- Ion sources are the component with the greatest number of variations
- Choice of source depends on identity of analyte
  - solid/liquid
  - organic/inorganic
  - reactive/nonreactive
- Common requirements of sources
  - produce ions!
    - Ideally small spread in kinetic energies
    - Produce ions uniformly, without mass discrimination
  - Accelerate ions into analyzer
    - Series of ion optics

MS Components: Atomic Sources

- **Inductively Coupled Plasma**: Atmospheric pressure discharge
- Relatively high argon flow rate (Liters per minute)
- After ignition, coupling of ionic charge with RF magnetic field “forces” ions to move
  - Heating results, plasma is sustained
MS Components: Atomic Sources

• **The ICP as an ionization source:**
  - High temperature in the source results in the formation of ions
    • best for atomic mass spec.
  - Challenges:
    • How do we get from atmospheric pressure in the ICP to vacuum in the MS without filling the MS with argon?
    • How do we keep the high temperature of the ICP from melting/ionizing components of the MS instrument?

MS Components: Atomic Sources

• Pressure is reduced by inserting a cooled cone (sampler) into the plasma. This allows only a small fraction of the plasma material to pass.
  - mechanical pump maintains lower pressure of ~1 torr
• A small fraction of this material passes through a second cone (the skimmer) into the high vacuum chamber
  - ion optics accelerate the ions into the mass analyzer
• Typically used with quadrupoles.
  - Unit mass resolution up to ~1000-2000
  - Large LDR
• Isobaric Interference
• Polyatomic ions
• Matrix effects (refractory oxides…)

http://iramis.cea.fr/Images/astImg/886_1.jpg
MS Components: Hard vs Soft Sources

- Parent or Molecular Ion Formation
  - needed to establish molecular weight

- Hard (energetic) sources leads to excited-state ions and fragmentation
  - Good for structural information

- Soft sources cause little fragmentation
  - Good for molecular weight determination

Molecular Ionization Sources

- More exist than are on this list!
- Need to transfer energy to analyte and ultimately produce ions.
  - Mechanism determines the extent of fragmentation

<table>
<thead>
<tr>
<th>Basic Type</th>
<th>Name and Acronym</th>
<th>Ionizing Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas phase</td>
<td>Electron impact (EI)</td>
<td>Energetic electrons</td>
</tr>
<tr>
<td>Desorption</td>
<td>Chemical ionization (CI)</td>
<td>Reagent gaseous ions</td>
</tr>
<tr>
<td></td>
<td>Field ionization (FI)</td>
<td>High-potential electrode</td>
</tr>
<tr>
<td></td>
<td>Field desorption (FD)</td>
<td>High-potential electrode</td>
</tr>
<tr>
<td></td>
<td>Electrospray ionization (ESI)</td>
<td>High electrical field</td>
</tr>
<tr>
<td></td>
<td>Matrix-assisted desorption-ionization (MALDI)</td>
<td>Laser beam</td>
</tr>
<tr>
<td></td>
<td>Plasma desorption (PD)</td>
<td>Fission fragments from $^{252}$Cf</td>
</tr>
<tr>
<td></td>
<td>Fast atom bombardment (FAB)</td>
<td>Energetic atomic beam</td>
</tr>
<tr>
<td></td>
<td>Secondary-ion mass spectrometry (SIMS)</td>
<td>Energetic beam of ions</td>
</tr>
<tr>
<td></td>
<td>Thermospray ionization (TS)</td>
<td>High temperature</td>
</tr>
</tbody>
</table>

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**MS Components: Molecular Sources**

- **Electron Ionization (EI or Electron Impact):**
  - Sample is vaporized by heating and “leaked” into source
  - Electrons are formed at a hot filament and accelerated across the path of the sample gas
  - As electrons “impact” gas molecules, ionization may occur (electrostatic repulsion). Forms “molecular ion”
    \[
    M + e^- \rightarrow M^{+} + 2e^-
    \]

- **EI cont’d**
  - High energy of electrons results in excited state ions
    - energy may be lost through collisions or reactions
    - Results in fragmentation of molecular ion to form daughter ions
      - Reactions may be unimolecular (fragmentation, rearrangement) or bimolecular
    - “Hard” ionization source
    - Fragmentation pattern is characteristic of molecule → Structure Identification

- **Chemical Ionization (CI):**
  - Excess of small, gaseous molecule is added to ionization chamber
  - Odds of collision of e⁻ produced by filament with the additive >> than with analyte
  - Result is production of ionized additive species
  - These less-energetic ions serve to ionize analyte
MS Components: Molecular Sources

- CI Example: methane
  - Forms $\text{CH}_4^+$, $\text{CH}_3^+$, $\text{CH}_2^+$ by ionization
  - These ions react to form primarily $\text{CH}_5^+$ and $\text{C}_2\text{H}_5^+$
  - Analyte (MH) is ionized by proton transfer or hydride transfer

\[
\begin{align*}
\text{CH}_5^+ + \text{MH} \rightarrow & \text{MH}_2^+ + \text{CH}_4 \\
\text{C}_2\text{H}_5^+ + \text{MH} \rightarrow & \text{MH}_2^+ + \text{C}_2\text{H}_4 \\
\text{C}_2\text{H}_5^+ + \text{MH} \rightarrow & \text{M}^+ + \text{C}_2\text{H}_6
\end{align*}
\]

- Result is a spectrum dominated by (M+1)$^+$ or (M-1)$^+$ peaks and little fragmentation
- Soft Ionization Source!

• **Field Ionization**
  - Gas flows past “emitter” subject to large electric field
  - Electron tunneling causes ionization
  - Little fragmentation

MS Components: Molecular Sources for Nongaseous Samples

• Applicable to large molecules, nonvolatile species

• **Electrospray Ionization (ESI):**
  - Atmospheric pressure method
  - Sample is pumped through a needle that is held at high voltage compared to cylindrical electrode
  - Produces fine spray of charged droplets
  - As solvent evaporates, charge density increases → ionization
  - Often produces multiply charged ions: good for large molecules!
    - Making elephants fly!
MS Components: Molecular Sources for Nongaseous Samples

• **Matrix-Assisted Laser Desorption/Ionization (MALDI)**
  - Sample is placed in a matrix containing a good optical absorber (chromophore), solvent is removed
  - Sample is irradiated with a pulsed laser. Absorption by matrix aids in sublimation/ionization of analyte (HOW?)
  - Essentially no fragmentation! Good for big molecules (biopolymers, etc.)

*Figure 5.11* The MALDI mass spectrum of a mouse monoclonal antibody. The matrix and van der Waals and the laser radiation used was 266 nm. (Reprinted from Kauer and Fenselau, with permission from Elsevier.)

• **Fast Atom Bombardment (FAB):**
  - Molecule dispersed in a glycerol matrix, bombarded by a beam of atoms from an *atom gun* (energetic)
  - Energy transfer results in production of positive and negative ions, matrix helps to aid ejection

*Figure 5.12* Schematic FAB ionization source. The sample, dissolved in solvent, is spread in a thin film on the end of a metal probe and bombarded by fast-moving argon atoms. Both positive and negative ions are produced.
“New” Ionization Methods

- **DESI** – Desorption Electrospray Ionization
  - Minimal sample prep
  - Imaging capabilities

- **DART** – Direct Analysis in Real Time
  - Interaction between metastables and analyte
    \[ M^* + A \rightarrow A^{*'} + M + e^- \]
  - No sample prep!

- **APCI** - Atmospheric Pressure Chemical Ionization
  - Typically coupled with HPLC
Hyphenated MS Techniques

- **Tandem MS (MS-MS):**
  - Multiple MS (often quads) coupled together.
  - Each serves a different purpose
  - Soft ionization source produces parent ions that are filtered by the first MS
  - Field-free region is filled with inert gas to allow collisions and fragmentation, producing “daughter ions”
  - Daughter ions are analyzed
  - Since each MS can be scanned, several applications are possible: separations,

**Ion Mobility Spectrometry – Mass Spectrometry**

- Separation in two dimensions
  1. Size to charge
  2. Mass to charge

- Application particularly for large molecules
Hyphenated MS Techniques

- **GC-MS**
  - Need to deal with the presence of carrier gas and the pressure difference b/w GC and MS
    - Capillary GC is usually no problem
    - Packed Column GC can be a problem
      - Use “jet separator” to remove carrier gas
  - Typically combined with quads, but also ion-trap detectors: fast scans for rapid separations
  - Detection modes: Total ion chromatogram, Selected ion chromatogram or Mass spectra
    - Possible 3-D data containing separation and identification!

Hyphenated MS Techniques

- **LC-MS**
  - HUGE difference b/w LC and MS conditions
  - Interface is critical
    - Many variations (thermospray, electrospray), nothing is ideal (yet)
    - Most common are ESI and Atmospheric Pressure Chemical Ionization (APCI)
Hyphenated MS Techniques

- **CE-MS**
  - CE is probably best suited for coupling to MS
    - low volume flow rates
  - ESI is most common
    - “End” of the capillary is metalized
    - Allows application of potential for both separation and ionization
      - $E_{\text{injection}} > E_{\text{ionization}} > \text{ground}$

![Diagram of CE-MS setup](image)

Strategies for Quantitation (not exclusive to MS)

- Key challenges involve two considerations
  - Instrument limitations
  - Sample limitations
- Ideally, choose the simplest method that provides required level of accuracy and precision
  - Basic calibration curve
- Internal Standards
  - Deal with precision issues by measuring a relative signal of Int. Standard and Analyte
    - Internal Standard and Analyte are different species!
Strategies for Quantitation (not exclusive to MS)

- Standard Additions
  - Often components present in an analyte sample (other than the analyte itself) also contribute to an analytical signal, causing **matrix effects**.
    - It is difficult to know exactly what is present in a sample **matrix**, so it is difficult to prepare standards.
  - Possible to minimize these effects by employing **standard additions**
    - Add a known amount of standard to the sample solution itself.
    - Perform the analysis.
    - The resulting signal is the sum of the signal for the sample and the standard.
    - By varying the concentration of the standard in the solution, it is possible to extract a value for the response of the unknown itself.

---

Strategies for Quantitation (not exclusive to MS)

- Graphical Approach to Standard Add's:

<table>
<thead>
<tr>
<th>Hg added (ppm)</th>
<th>Current (μA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.66</td>
</tr>
<tr>
<td>9.36</td>
<td>6.76</td>
</tr>
<tr>
<td>18.72</td>
<td>8.83</td>
</tr>
<tr>
<td>28.08</td>
<td>10.86</td>
</tr>
<tr>
<td>37.44</td>
<td>12.8</td>
</tr>
</tbody>
</table>

- Unknown concentration is derived by extrapolating line to x-intercept.
Strategies for Quantitation
(not exclusive to MS)

• Isotope dilution - more MS exclusive
  – Artificially change isotope ratios of a sample by spiking with isotope-enriched standard
    • Standard has same identity as analyte, but different, and known, isotopic abundance.
    • Analyte has natural abundance (typically)
  – Measured isotope ratio from MS reflects combination of analyte and spike signal
    • Signal at m/z for isotope A = f(C_{unk}F_X + C_{spike}F_X,spike)
    • Signal at m/z for isotope B = f(C_{unk}F_X + C_{spike}F_X,spike)
      C = total concentration of all isotopes of element
      F_X = Fractional abundance of isotope X
  – Since we know F_X, F_{X,spike}, and C_{spike}, a little algebra gets us to C_{unk}