20.1 Mass Spectrometry Fundamentals

- general idea behind the instrument
- electron impact ionization with an example mass spectrum
- block diagram and timing of events within an instrument
- isotope effects on a spectrum
- determination of a molecular formula
- spatial and temporal schemes for tandem mass spectrometry
- collision-induced ionization, dissociation, and charge exchange
A neutral molecule is introduced into a vacuum at a low number density. It is excited with sufficient energy to eject an electron and form an ion.

If the ion is formed with excess energy, bonds can be broken. In each unimolecular fragmentation reaction, one of the fragments will pick up the charge. If these secondary ions still have sufficient excess energy, they too can fragment.

All of the ions are extracted from the ionization region by an applied electric potential and separated according to their mass-to-charge ratio. After separation, the ions are detected and a plot of abundance versus m/z is constructed. This is called a mass spectrum, in analogy to an optical spectrum.

The mass spectrum can be used to determine molecular structure, to quantify single compounds or mixtures of compounds, or to study ion-molecule reactions.
Electron Impact Ionization

• creation of the molecular ion
  \[ \text{ABCD} + e^- \rightarrow \text{ABCD}^+ + 2e^- \]

• fragmentation of the molecular ion
  \[ \text{ABCD}^+ \rightarrow \text{AB}^+ + \text{CD}^+ \text{ (even electron ion - favored)} \]
  \[ \text{ABCD}^+ \rightarrow \text{AB} + \text{CD}^+ \text{ (odd electron ion - less likely)} \]

• rearrangement followed by fragmentation
  \[ \begin{array}{c}
  \text{A} \overset{\text{---}}{\text{D}}^+ \\
  \text{B} \quad \text{C}
  \end{array} \rightarrow \text{BC} + \text{AD}^+ \]

• further fragmentation
  \[ \text{CD}^+ \rightarrow \text{C}^+ + \text{D} \]
  \[ \text{AD}^{+\cdot} \rightarrow \text{A}^{+\cdot} + \text{D} \]

Fragmentation reactions are endothermic and require excited molecular ions. The fragments do not recombine because they are at a low number density in a vacuum. Rearrangements are slower than direct fragmentation, thus ion abundance is lower.
The molecular ion, CH$_3$OH$^{+\bullet}$, has an odd number of electrons.

Even fragments: 31 is loss of H$^{\bullet}$ and 15 is loss of OH$^{\bullet}$

Odd fragment: 30 is loss of H$_2$

Mass 29 is loss of H$_2$ from CH$_2$OH$^{+}$.

Masses at 33 and 34 are due to isotopes of carbon and oxygen.
All mass spectrometers operate under a vacuum. This ensures that the ions can travel from the source to the detector without collision.

The required spectrometer pressure can be computed using the collision cross-section of the ion. The mean free path of an ion is given by,

\[ L = \frac{kT}{\sqrt{2P\sigma}} \]

where \( k \) is Boltzmann's constant, \( T \) the temperature in Kelvin, \( P \) the pressure in Pascals, and \( \sigma \) the cross-section.

A spectrometer with a 1-meter length, requires a pressure of \( 6.5 \times 10^{-3} \) Pa, at room temperature and a typical cross-section of \( 4.5 \times 10^{-19} \) m\(^2\). This is \( \sim 5 \times 10^{-5} \) torr, which requires a diffusion pump.

For a source potential of 1,000 V, a singly-charged ion gains $1.6 \times 10^{-16} \text{ J}$ of kinetic energy.

$$\frac{mv^2}{2} = \left(10^3 \text{ V}\right)\left(1.6 \times 10^{-19} \text{ C}\right) = 1.6 \times 10^{-16} \text{ J}$$

A 100 amu ion has a mass of $1.66 \times 10^{-25} \text{ kg}$. Its velocity is computed from the above equation to be $4.4 \times 10^4 \text{ m s}^{-1}$.

At this velocity the ion leaves a 1-cm source in 0.23 $\mu$s and travels the 1-meter length of the spectrometer in 23 $\mu$s.

As a result ions fragment before leaving the source region.
Most elements have stable isotopes. Since isotopes have different masses, their presence will add peaks to the spectrum.

Units: exact mass, atomic mass units (amu); average mass, Daltons (Da); and, mass to charge ratio, Thomsons (Th).

<table>
<thead>
<tr>
<th>isotope</th>
<th>mass (amu)</th>
<th>abundance</th>
<th>average mass (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1\text{H}$</td>
<td>$1.007825$</td>
<td>$0.99985$</td>
<td>$1.00794$</td>
</tr>
<tr>
<td>$^2\text{H}$</td>
<td>$2.014$</td>
<td>$0.00015$</td>
<td></td>
</tr>
<tr>
<td>$^{12}\text{C}$</td>
<td>$12.000000$</td>
<td>$0.9890$</td>
<td>$12.011$</td>
</tr>
<tr>
<td>$^{13}\text{C}$</td>
<td>$13.003355$</td>
<td>$0.0110$</td>
<td></td>
</tr>
<tr>
<td>$^{16}\text{O}$</td>
<td>$15.994915$</td>
<td>$0.9976$</td>
<td>$15.9994$</td>
</tr>
<tr>
<td>$^{17}\text{O}$</td>
<td>$16.999133$</td>
<td>$0.0004$</td>
<td></td>
</tr>
<tr>
<td>$^{18}\text{O}$</td>
<td>$17.999160$</td>
<td>$0.0020$</td>
<td></td>
</tr>
<tr>
<td>$^{35}\text{Cl}$</td>
<td>$34.968852$</td>
<td>$0.7577$</td>
<td>$35.453$</td>
</tr>
<tr>
<td>$^{37}\text{Cl}$</td>
<td>$36.965903$</td>
<td>$0.2423$</td>
<td></td>
</tr>
</tbody>
</table>
Isotope Peaks in CH$_3$OH

$M^+ = 32$ Da
This nominal mass is given by $^{12}$CH$_4$$^{16}$O (CH$_4$O)
The abundance of this isotopic composition is
0.9890$\times$(0.99985)$^4$$\times$0.9976 = 0.986035

$M^{+}+1 = 33$ Da
This nominal mass can be obtained with three different isotopic substitutions (you need to include the four permutations of HHHD):
$^{13}$CH$_4$O, (CHHHDO, CHHDHO, CHDHHO, CDHHHO), CH$_4$$^{17}$O
The ion abundance involves all six ways to get the mass
0.0110$\times$(0.99985)$^4$$\times$0.9976
+ 4$\times$[0.9890$\times$0.00015$\times$(0.99985)$^3$$\times$0.9976]
+ 0.9890$\times$(0.99985)$^4$$\times$0.0004 = 0.011954 or 1.21% of $M^+$

$M^{+}+2 = 34$ Da
This nominal mass can be obtained with two different isotopic substitutions (you need to include the six permutations of HHDD):
CH$_2$D$_2$O, CH$_4$$^{18}$O, $^{13}$CH$_3$DO, CH$_3$D$^{17}$O
The ion abundance is 0.001977 or 0.20% of $M^+$
Multiple High-Abundance Isotopes

Of elements commonly found in organic compounds, sulfur and the halogens have several stable isotopes.

\[ ^{32}\text{S} \ (0.9503), \ ^{33}\text{S} \ (<1\%), \ ^{34}\text{S} \ (0.0422), \ ^{36}\text{S} \ (<1\%) \]
\[ ^{35}\text{Cl} \ (0.7577), \ ^{37}\text{Cl} \ (0.2423) \]
\[ ^{79}\text{Br} \ (0.5069), \ ^{81}\text{Br} \ (0.4931) \]

With halogens it is possible to have the most intense peak occur at a higher mass than M⁺. As an example consider CCl₄ and ignore \(^{13}\text{C}\).

<table>
<thead>
<tr>
<th>mass</th>
<th># of (^{35}\text{Cl})</th>
<th># of (^{37}\text{Cl})</th>
<th>abundance</th>
<th>normalized</th>
</tr>
</thead>
<tbody>
<tr>
<td>152</td>
<td>4</td>
<td>0</td>
<td>0.326</td>
<td>0.782</td>
</tr>
<tr>
<td>154</td>
<td>3</td>
<td>1</td>
<td>0.417</td>
<td>1</td>
</tr>
<tr>
<td>156</td>
<td>2</td>
<td>2</td>
<td>0.200</td>
<td>0.480</td>
</tr>
<tr>
<td>158</td>
<td>1</td>
<td>3</td>
<td>0.043</td>
<td>0.102</td>
</tr>
<tr>
<td>160</td>
<td>0</td>
<td>4</td>
<td>0.003</td>
<td>0.008</td>
</tr>
</tbody>
</table>

The strongest odd mass peak is 155 with an abundance of 0.005.
Double focusing scanning and Fourier transform ion cyclotron resonance instruments are capable of generating mass resolutions greater than 100,000. This allows the direct determination of the molecular formula from \( M^+ \).

If a high resolution spectrometer is not available, the intensity of the \( M^+ \), \( M^++1 \) and \( M^++2 \) peaks can often be used to distinguish among possibilities. As an example, the following table lists all combinations of C, H, N and O that yield 28 amu.

<table>
<thead>
<tr>
<th>fragment</th>
<th>( M^++1 )</th>
<th>( M^++2 )</th>
<th>avg. mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{N}_2^+ )</td>
<td>0.74</td>
<td>0.00</td>
<td>28.0062</td>
</tr>
<tr>
<td>( \text{CO}^+ )</td>
<td>1.15</td>
<td>0.20</td>
<td>28.0049</td>
</tr>
<tr>
<td>( \text{CH}_2\text{N}^+ )</td>
<td>1.53</td>
<td>0.00</td>
<td>28.0187</td>
</tr>
<tr>
<td>( \text{C}_2\text{H}_5^+ )</td>
<td>2.28</td>
<td>0.01</td>
<td>28.0313</td>
</tr>
</tbody>
</table>

The required amplitude precision is \( \sim 0.5\% \) (1:200).
Tandem Mass Spectrometry (1)

A first mass analyzer is used to isolate a "precursor ion." This ion is then sent into a collision cell where it fragments further to create a set of product ions. This new set of ions is examined by a second step of mass analysis.

With scanning instruments, the ions are transferred through space between the various steps. With trapping instruments the steps occur at different times using one sample cell. The first step includes ejection of all ions except the one of interest. This is followed by adding a collision gas to the cell. The ions produced by collisions are examined in the third step.

• product ion scan - the mass spectrum of the product ion is determined. This is used for structural determination.
• precursor scan - a product ion is selected and all precursor ions are examined to see which will fragment into the product ion. This is useful for mixture analysis, e.g. R-A⁺ → R + A⁺, where R varies.
• neutral loss scan - by linking the two analyzers it is possible to identify all precursor ions losing the same neutral fragment, e.g. CO₂.
• selected ion monitoring - once appropriate precursor and product ions have been identified this scan can be used for quantification, e.g. chromatographic detector.

Collision-Induced Dissociation

In the following, A is the precursor ion, N is the neutral collision gas, and B and C are product ions derived from A.

- **unreactive gas:**
  \[ \text{A}^+ + \text{N} \rightarrow \text{B}^+ + \text{C} + \text{N} \]

- **reactive gas:**
  \[ \text{A}^+ + \text{N} \rightarrow \text{AN}^+ \]

- **charge exchange:**
  \[ \text{A}^{+}\cdot + \text{N} \rightarrow \text{A} + \text{N}^{+}\cdot \]

- **charge transfer:**
  \[ \text{A}^{2+} + \text{N} \rightarrow \text{A}^{+}\cdot + \text{N}^{+}\cdot \]

- **ionization:**
  \[ \text{A} + \text{N} \rightarrow \text{A}^{+}\cdot + \text{N} + e^- \]

- **charge stripping:**
  \[ \text{A}^{+}\cdot + \text{N} \rightarrow \text{A}^{2+} + \text{N} + e^- \]

- **coulomb explosion:**
  \[ \text{A}^{2+} + \text{N} \rightarrow \text{C}^+ + \text{B}^+ + \text{N} \]