In a mass spectrometer
- The chemical substance undergo ionization to produce charged particles (ions – in the gas phase)
- Then the mass of the charged ions are measured using electrical and/or magnetic fields

Mass spectrometers are used for
- characterization of unknown compounds,
- quantitative analysis of known compounds (detection of very small quantities of material - $10^{-12}$ g)

Example:

$$H_2O$$
Total mass of a water molecule is mass of two hydrogens (approx. 1 Da per hydrogen) and one oxygen (approx. 16 Da per oxygen). Total = 18 Da

If we put some water vapour into the high vacuum chamber of a mass spectrometer and pass a beam of electrons through the water vapour, some of the electrons will hit the water molecules and remove an electron → $[H_2O]^+$
Ions are produced......compound must be ionised

Some of the collisions will produce other ions and fragments. Generally, for water, the only fragments are $[OH]^+$, $O^+$ and $H^+$.

The mass spectrum of water will show peaks that can be assigned to masses of $1$ – $H^+$, $16$ – $O^+$, $17$ – $[OH]^+$, $18$ – $[H_2O]^+$

The "mass-to-charge" ratio ($m/z$) of an ion is the mass of the ion divided by the charge on the ion.

**MS Instrumentation**

- **Mass Spectrometer**
  - **INLET SYSTEM**
  - **ION SOURCE**: form gas-phase ions from sample,
  - **ANALYSER**: separate ions based on their mass-to-charge ratio ($m/z$),
  - **DETECTOR**: measure the abundance of the ions according to $m/z$.
  - **DATA OUTPUT**: mass spectrum

**Ionisation**

- Molecules gain or lose electrons such that they acquire a positive or negative charge
- ionisation process can produce
  - molecular ion: same molecular weight and elemental composition of the starting analyte
  - fragment ion: corresponds to smaller piece of the analyte molecule
- Common molecular ion products of ionisation
  - Molecular ions ("parent ions") $M^+$ or $M^-$
  - Protonated molecules $[M + H]^+$
  - Simple adduct ions $[M + Na]^+$
- Different ionisation techniques depending on chemical & physical properties of molecule of interest
Ionisation

- Range of methods are used:
  - Electron Impact Ionisation (EI) (also known as Electron Bombardment and Electron Ionisation)
  - Electrospray Ionisation (ESI)
  - Atmospheric Pressure Chemical Ionisation (APCI)
  - Fast Atom Bombardment (FAB)
  - Matrix Assisted Laser Desorption Ionisation (MALDI)
  - Inductively Coupled Plasma (ICP)

Look at four in more detail

Electron Impact Ionisation

- High vacuum ionisation chamber
- Sample in the gas phase is bombarded with electrons
- Electron beam (70eV) will knock out electrons in the sample molecule, leaving the molecule with a positive charge and usually an unpaired electron, a radical cation.

\[
M + e^- \rightarrow M^+ + 2e^-
\]

- EI ionisation is high energy. Additional energy of the molecular ion may result in decomposition or “fragmentation”.

Chemical ionisation

- High vacuum ionisation chamber
- CI process initiated with a reagent gas such as methane (CH\(_4\)), isobutane [CH(CH\(_3\))\(_3\)] or ammonia (NH\(_3\)) (initially ionised by electron ionisation)

\[
CH_4 + e^- \rightarrow CH_4^+ + 2e^- \quad \text{- ionisation}
\]

\[
CH_4^+ \rightarrow CH_3^+ + H^- \quad \text{- fragmentation}
\]

- These ions collide with neutral molecules
  \[
  CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3
  \]
  \[
  CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2
  \]

Chemical ionisation

- Get similar reactions for C\(_4\)H\(_{10}\) and NH\(_3\)
- Produce CH\(_5^+\), C\(_4\)H\(_9^+\) and NH\(_4^+\) respectively
- These act as acids to protonate the sample

\[
M \rightarrow MH^+
\]

- CI is a softer ionisation method than EI – useful when EI doesn’t give a parent (molecular) ion

Electrospray ionisation

- (most common mode)
  - Sample passed through the stainless steel capillary into small-diameter tip held at high potential
  - Emerging solution dispersed into fine spray of charged droplets all at same polarity
  - Solvent evaporates away, shrinking droplet size, increasing charge concentration at droplet’s surface

- At Rayleigh limit, coulombic repulsion overcomes surface tension, droplet explodes – forms smaller lower charged droplets
- Shrinking and explosion continues until individually charged ‘naked’ analyte ions formed.

Courtesy www.ionsource.com

At Rayleigh limit, coulombic repulsion overcomes surface tension, droplet explodes – forms smaller lower charged droplets
- Shrinking and explosion continues until individually charged ‘naked’ analyte ions formed.

Courtesy www.ionsource.com
Electrospray ionisation

- This is the softest ionisation method, normally no fragmentation occurs
- Multiply charged ions are often formed for large molecules – hence useful for large proteins and DNA molecules

\[ m/z = \frac{[M + H]^+}{[M + 2H]^{2+}} = \text{mass} + 1 / \text{mass} + 2 \times 2 \]

MALDI

- Matrix Assisted Laser Desorption/Ionisation
- sample mixed with a matrix material which absorbs laser light strongly
- mixture spotted onto metal (or polymer) plate \(\rightarrow\) allowed to dry
- sample molecules become incorporated into the crystallized matrix
- plate inserted into the sample target region of the mass spectrometer under high vacuum
- matrix/sample spot bombarded with pulsed laser beam
- laser excitation results in localized sublimation of portions of the matrix/sample spot,
- Matrix absorbs laser pulse \(\rightarrow\) energy transferred to sample
- Desorption of gaseous plume of matrix and sample ions.

Mass Analysers

- Following ionisation, ions in the gas phase enter the mass analyser.
- Separates ions on the basis of their mass/charge ratio.
- Ions are separated by magnetic fields, electric fields or by the time taken by the ion to travel a fixed distance

Analysers

- Measure mass-to-charge ratio rather than mass alone
- Different types:
  - Time-of-flight
  - Ion trap
  - quadrupole mass filters
  - magnetic sectors
  - Fourier transform ion cyclotron resonance spectrometers

Time-of-flight analyser

- Electric field in source extracts and accelerates the ions
- Accelerated ions (with same kinetic energy) pass into field-free drift tube
- Kinetic energy = \( \frac{1}{2}mv^2 \)
- The lower the mass, the greater the velocity and the shorter the flight time
- Ions separated in time and collected by the detector. Travel time converted into \( m/z \) value

Quadrupole Analyser

- Uses two electric fields applied at 90° to separate ions
- One field is DC, the other is AC oscillating at radiofrequency
- Effect of the two fields is to produce a resonance frequency for each \( m/z \) value.
- Ions which resonate can pass through and be detected.

From http://elchem.kaist.ac.kr/vt/chem-ed/ms/quadrupo.htm
Ion trap analyser

- Consist of 3 electrodes
  - Ring electrode
  - Entrance endcap electrode
  - Exit endcap electrode
- Form cavity to trap ions
- Ions enter trap through inlet focusing system
- Various voltages applied to trap and eject ions according to m/z values

Magnetic Sector Analyser

- Ions are accelerated (electric field) and pass into a magnetic field.
- Charged particle travelling through a magnetic field will experience a force and tend to travel in a circular motion depending on the m/z and speed of the ion.
- Only ions of a particular m/z will reach the detector at a particular magnetic field

Mass Spectrometer

- Allows mass spectrometer to generate a signal current from ions by generating secondary electrons, which are further amplified
- Electron multiplier
- Photomultiplier

Summary

- Sample must be volatilised before analysis
- Ions are formed using several techniques (EI, CI, Electrospray) – may produce fragments (also ionised)
- Ions are accelerated in high vacuum using electric fields
- Ions are deflected (analysed) using either magnetic or electrical fields
Combinations of technologies:
- MALDI-TOF-MS
- ESI-ion trap-MS (QTrap)
- ESI-triple Q-MS (Tandem MS) – most common
- ESI-Q-TOF-MS

Analysis:
- select single ions/fragments (Q) (SI monitoring, SIM)
- MRM (multiple reaction monitoring):
  Q1 filters the analyte mass (= precursor ion)
  Q2 fragments this mass
  Q3 filters the mass of one of its fragments (= product ion)

Tandem MS (MS/MS)
- Powerful way to obtain structural information and quantitation
- Used for smaller molecules (eg, drugs) up to MW = 3000
- Very high sensitivity (7 orders of magn) c.f. TOF (4 orders)
- Common example: triple quadrupole
  - First quadrupole used to select precursor ion
  - Second quadrupole (Rf only) used as a collision cell for fragmentation of precursor ion (collision induced dissociation, collision with inert gas)
  - Third quadrupole generates spectrum of resulting product ions
- May use TOF analyser in place of third quadrupole

Sample introduction
- Capillary infusion – gas chromatography, liquid chromatography

Vacuum
- All mass spectrometers require a vacuum
- Necessary to permit ions to reach the detector without colliding with other gaseous molecules → reduction in resolution and sensitivity

Mass Spectrum
- The “mass spectrum” usually contains a number of peaks of differing intensities.
- Bar graph with mass to charge ratio on x-axis and relative intensity / relative abundance on the y-axis
- Most abundant ion is the base peak – assigned a value of 100%
- Intensity of other ions expressed as relative abundance

Molecular ion (MI) should be the ion of highest mass in the spectrum
- Need to try to ensure that this is not a fragment (may be the case if molecule undergoes total fragmentation) → decrease fragmentation energy, if possible
- MI can be used to determine the molecular formula of a compound.
Mass Spectrum
Masses are graphed or tabulated according to their relative abundance.

Isotopes
- Many elements with heavier isotopes of appreciable abundance
- MI and fragments are accompanied by a cluster of peaks – low abundance and higher mass
- Useful for providing information
- Classification
  A – atoms with only one natural isotope e.g. F, P, I
  A+1 – atoms with isotopes 1 mass unit higher e.g. C, H, N
  A+2 – atoms with isotopes 2 mass units higher e.g. Cl, Br, O, S

Isotopes – A+1 elements
- $^{13}$C is most useful
- Abundance is small but the greater the number of C atoms the greater is the probability of a $^{13}$C
- e.g. For CH₄ (m/z 16) the $^{13}$C isotope at m/z 17 is about 1.1% of the $^{12}$C intensity
- For a larger hydrocarbon (e.g. C₃₀H₆₂) the contribution is much greater.
- Ratio of M+1/M gives an estimate of number of carbons – M+1 peak will be 1.1% of the parent for every carbon atom

Isotopes – A+2 elements
- Easier to recognise due to higher abundance of ions for Br, Cl and S – characteristic patterns
- Prediction should be easier
  e.g. HCl is a 3:1 mixture of $^{35}$Cl at m/z 36 and $^{37}$Cl at m/z 38
- A+2 isotope clusters can provide information about presence of halogens

Isotopes
- A chemically pure sample will produce a mass spectrum with a mixture of ions
- The elements are often not isotopically pure
- Exact masses are not integers due to the slight mass difference between a proton (1.67262 x 10⁻²⁷ kg) and a neutron (1.67493 x 10⁻²⁷ kg) and the fact that electrons have mass (9.10956 x 10⁻³¹ kg)

Isotopes – A+2 elements
- e.g. CH₃Cl Molecular ion peaks
  

Isotopes – A+2 elements
- e.g. CH₃Cl Molecular ion peaks

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Atomic weight</th>
<th>Predicted abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{35}$Cl</td>
<td>35.007</td>
<td>100</td>
</tr>
<tr>
<td>$^{36}$Cl</td>
<td>36.007</td>
<td>0.7</td>
</tr>
<tr>
<td>$^{37}$Cl</td>
<td>37.008</td>
<td>0.3</td>
</tr>
<tr>
<td>$^{38}$Cl</td>
<td>38.009</td>
<td>0.1</td>
</tr>
<tr>
<td>$^{39}$Cl</td>
<td>39.010</td>
<td>0.1</td>
</tr>
<tr>
<td>$^{40}$Cl</td>
<td>40.009</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Relative Intensity

<table>
<thead>
<tr>
<th>Mass</th>
<th>Relative Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>51</td>
<td>100</td>
</tr>
<tr>
<td>52</td>
<td>100</td>
</tr>
<tr>
<td>53</td>
<td>100</td>
</tr>
</tbody>
</table>
The presence of two peaks, two mass units apart, the second a third as high as the first, probably indicates the presence of both isotopes of chlorine, $^{35}\text{Cl}$ and $^{37}\text{Cl}$. This should be seen for any chlorine containing fragment.

The presence of a bromine containing fragment in the sample is indicated by two peaks of equal height, 2 mass units apart, corresponding to $^{79}\text{Br}$ and $^{81}\text{Br}$.

The $^{12}\text{C}$ and $^{13}\text{C}$ isotopes of carbon occur in the ratio of 99:1. This means that ions containing carbon produce two peaks, one 99 times higher than the other.
Isotopes – unknown compounds

- Mass spectra and isotope distribution gives information on elemental composition
- Molecular ion:
  - M (monoisotopic ion) – where all elements are of "lowest isotope"
  - M+1 from the +1 isotope of one A+1 element
  - M+2 from the +2 isotope of one A+2 element or from two +1 isotopes of A+1 elements
  - M+3
- Can get overlap of different isotope combinations and also overlap from other ions e.g. loss of hydrogen radical → M-1; fragment may then overlap with the M and M+1

Fragmentation

- Electron impact (EI)
  - Molecule is bombarded with highly energetic electrons – knock weakly bound electrons out of molecule
  - → radical cation: an unpaired electron and a positive charge
  - Usually not a bonding electron – therefore likely from a lone pair
    - e.g. NH$_3$ → NH$_3^+$
  - Ketone → R$_2$C=O$^+$
  - Unknown molecule → Radical cation → Radical + cation
    - M → M$^+$ → X + Y$^+$

Examples – EI fragmentation

Largest peak – base peak is at 43
Peak at 58 (50%)
Highest mass at 114 – molecular ion (C$_7$H$_{14}$O)
Pheromone is heptan-2-one

Examples – EI fragmentation

C$_7$H$_{14}$O
Main fragmentation is to a C$_5$H$_{11}$ radical (not observed as it isn’t charged) and a cation C$_2$H$_3$O$,^+$ which forms the base peak.

Examples – EI fragmentation

Molecular ion of the bromo-amide has two peaks:
- at 213 and 215 respectively. Might be loss of H$_2$ from molecular ion.
But, the base peak also has the same pattern – suggests presence of bromine. Smaller fragment at 155, 92 etc lack the pattern – therefore lack the bromine.
**Chloroquine:**

A: EI mass spectrum – double molecular ion peak at 319 and 321 – arises from the presence of chlorine.

B: CI mass spectrum – little or no fragmentation – double molecular ion peak at 319 and 321.

From: Pharmaceutical Analysis (D.G. Watson)

---

**El Mass Spectra of Alkanes**

More stable carbocations will be more abundant.

---

Simple alkanes tend to undergo fragmentation by the initial loss of a methyl group to form a (m-15) species. This carbocation can then undergo stepwise cleavage down the alkyl chain, expelling neutral two-carbon units (ethene). Branched hydrocarbons form more stable secondary and tertiary carbocations, and these peaks will tend to dominate the mass spectrum.

---

**Mass Spectra of Alcohols**

- Alcohols usually lose a water molecule.
- \( M^+ \) may not be visible.

---

The molecular ion represents loss of an electron and the peaks above the molecular ion are due to isotopic abundance. The base peak in toluene is due to loss of a hydrogen atom to form the relatively stable benzyl cation.

---

The benzyl cation is thought to undergo rearrangement to form the very stable tropylium cation, and this strong peak at \( m/z = 91 \) is a hallmark of compounds containing a benzyl unit. The minor peak at \( m/z = 65 \) represents loss of neutral acetylene from the tropylium ion and the minor peaks below this arise from more complex fragmentation.
Summary

- The fragmentation pattern of a compound is dependent on the energy required to break its bonds. Essentially, more stable bonds are more difficult to break. Thus the fragmentation process tends to produce smaller, more stable species.

How large a molecule?

- Multiple charging means that proteins can be readily observed, e.g., dehydrogenase enzyme.

Hyphenated Techniques

- Mass Spectrometry is a very powerful detector for separation techniques.
  - e.g., gas chromatography
  - liquid chromatography
- GC-MS
- LC-MS or HPLC-MS
- LC-MS-MS

GC-MS

- Problem: vacuum of mass spectrometer
- Gas chromatography – use capillary GC columns – low gas flow
- Very successful technique for analysing drugs in biological fluids, environmental contaminants etc.

A mixture of compounds is separated by gas chromatography, then identified by mass spectrometry.
**LC-MS**

Liquid chromatography – produces large amounts of solvent vapour when converted to gas phase. Must be removed.

In electrospray ionisation heat and drying gas are needed to increase the rate of droplet evaporation as the sample solution is sprayed from a needle held at high voltage → ions.

**LC-MS Application**

- Most common ionization is the electro-spray
- When other ions co-elute the analyte molecules don’t ionise as much due to competition (ion-suppression)
- This is a major problem in quantitative analysis using LC-MS
- Solution: use an internal standard with the same retention time to estimate this reduction and correct
- Use isotopically labelled compound as an internal standard (common approach in both GC-MS and LC-MS unlike GC and LC with other detectors. Why?)

Eg. Analyte: D-Glucose (C₆H₁₂O₆) (M.Wt – 180)
Internal standard: D-Glucose-¹³C₆ (M.Wt-186) or D-Glucose- d₁₂ (M.Wt-192)