Introduction & Definitions

GroEL: 14-mer (802.5 kDa) and 13-mer

14-mer

13-mer

12000 14000 16000 18000 20000 22000 m/z
Types of compounds allowed

Almost all compounds can be analyzed by MS: peptides, proteins, lipids, oligonucleotides, sugars, polymers, organometallics, nanoparticles, ....

but the results strongly depend on the mode of ionization and the type of instrument used
A mass spectrometer is an accurate balance for charged molecules.
Why the charge?

In the presence of an electric field $\vec{E}$ or magnetic field $\vec{B}$

- Neutrals are not affected
- Ions can be disturbed and their velocity changed:
  - Focused (Ion Guide)
  - Deflected and accelerated (Mass Analyseur)
Fondamentals en MS

• Mass: comes from protons, neutrons and electrons

• Charge: comes either from an excess of protons (+) or excess of electrons (-) or lack (+) of electrons

• Proton $H^+$ on an analyte: $[M+H]^+$

• Analyte with a lack of proton: $[M-H]^{-}$

• Multiple charged possible (larges molecules, organometallics…): ex: $[M+30H]^{30+}$ for a protein with 30 charges

Only charged species are detected in MS
MS: key features

- A mass spectrometer measures the masses of individual molecules that have been converted to ions, i.e. electrically charged.

- MS allows ionization and analysis of very diverse chemical structures in a wide MW range (1-1'000'000 Da).

- Separation of compounds with very close MW ($\Delta M < 0.005$ Da).

- Ionization produces also fragments useful for structural characterization.

- Mass analysers can be placed in tandem.

- Sensitivity and resolution are in constant increase (Attomole $10^{-18}$).

- Destructive technique.
Sir J.J. Thomson, 1897 demonstrates that an electron has a mass \( m_e \) and a charge, and measures for the first time the ratio \( -e/ m_e \) of an electron:

\[
-e/ m_e = -1.758820150 \times 10^{11} \text{ C/kg}
\]

- **Electron mass:** \( 9.109 \times 10^{-31} \text{ kg} \) or \( 5.489 \times 10^{-4} \text{ ua} \)
  
  \( \text{(1 ua} = 1.660538 \times 10^{-27} \text{ kg}) \)

- **Electron charge:** \( e = -1,602 \times 10^{-19} \text{ coulomb} \)

- **Charge of the ion** \( Z = q \ e \quad q: \text{number of charges (+ or –)} \)
Electron

• Mass of electron = 0.0005489 Da
  – That’s 9.11 x 10^{-28} grams
  – Small but measurable by HRMS

• Gain/lose proton H^+
  – H atom weight: 1.007824
  – H^+ weight: 1.007276

• 1-4 ppm $m/z$ error for small molecules if the electron mass is not considered!
Mass Terminology: Units of Mass

Atomic mass unit, U

- $1/12^{th}$ of the mass of $^{12}\text{C} = 1.660538 \times 10^{-27}$ kg
- Units known as a dalton (Da)
- $m/z$ unit is in kg/Coulomb = $u/e = Da/e = \text{Thomson (Th)}$
- **amu** terminology no longer accepted, but incredibly common!
Mass Terminology: Reality

Use approved terminology!

- IUPAC Gold Book, MS terms Wiki etc…

- Mass spec Desk Reference (O. David Sparman)

for extended discussion on terms in MS (including conflicting uses)
What is a mass?

- **monoisotopic mass**: Sum of the exact masses of the most abundant isotope of each element present.
- **nominal mass**: Sum of nominal masses of the elements in the empirical formula calculated from integer atomic weights.
- **most abundant mass**: Mass of the most abundant isotope of the envelope.
- **average mass**: Centroid of the complete isotopic envelope.

**Example: Glucagon**
- Formula: $C_{153}H_{224}N_{42}O_{50}S$
- Nominal Mass: 3480
- Monoisotopic Mass: 3481.5997
- Most Abundant Mass: 3483.6048
- Average Mass: 3483.7806
Mass Terminology: Monoisotopic mass

- Calculated mass using precisely known mass value for the most abundant isotope of each element present

Ex: Dextromethorphan

\[ ^{12}\text{C}_{18}^{1}\text{H}_{26}^{14}\text{N}_{16}^{16}\text{O}, \ [\text{M+H}]^+ \]

\[ = [18*12.0000 + 26*1.0078 + 1*14.0031 + 1*15.9949] + 1.0073 = 272.20009 \]
Mass Terminology: Monoisotopic mass

Ex: C1000, 12000.000
Mass Terminology: Exact mass

- Calculated mass using precisely known mass value for a specific isotope for each element present

Ex: Dextromethorphan

$^{12}\text{C}_{18}^{1}\text{H}_{26}^{15}\text{N}^{16}\text{O}$, $[\text{M+H}]^+$

$= [18 \times 12.0000 + 26 \times 1.0078 + 1 \times 15.0001 + 1 \times 15.9949] + 1.0073 = 273.1979$
Isotopique Distribution

Group of isotopologues with their mass and each of their relative intensity

\[ A = \frac{n!}{(a)! \cdot (b)! \cdot (c)!} \cdot (r_1)^a \cdot (r_2)^b \cdot (r_3)^c \cdots \]

Where:

- \( n \) number of atoms of the element considered
- \( a, b, c \) are numbers of each type of isotope \((a + b + c = n, C_aH_bO_c \ldots)\)
- \( r_1, r_2, r_3 \) are relative abundances of each isotope
Isotopic distribution, example with C100

\[
M: \quad A = \frac{100!}{100! \cdot 0!} (0.989)^{100} (0.011)^0 = 0.3308
\]

\[
M + 1: \quad A = \frac{100!}{99! \cdot 1!} (0.989)^{99} (0.011)^1 = 0.3679
\]

\[
M + 2: \quad A = \frac{100!}{98! \cdot 2!} (0.989)^{98} (0.011)^2 = 0.2026
\]

\[
M + 3: \quad A = \frac{100!}{97! \cdot 3!} (0.989)^{97} (0.011)^3 = 0.0708
\]

\[
= \frac{0.3308}{0.3679} \times 100 = 89.9\
\]

\[
= \frac{0.3679}{0.3679} \times 100 = 100\
\]

\[
= \frac{0.2026}{0.3679} \times 100 = 55.0\
\]

\[
= \frac{0.0708}{0.3679} \times 100 = 19.2\
\]

complex calculation for molecules having many elements and with many isotopes!
How does isotopic distribution change with mass?

- Monoisotopic mass dominates up to MW ~1100
- For MW >7000, the monoisotopic peak disappear
## Isotopes

<table>
<thead>
<tr>
<th>Element</th>
<th>Isotopes</th>
<th>A (masse)</th>
<th>Abundance (%)</th>
<th>Δm (A+1/A)</th>
<th>Δm (A+2/A)</th>
<th>Δm (A+3/A)</th>
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<tbody>
<tr>
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<td>1.0078250</td>
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<td>97.3</td>
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</tr>
</tbody>
</table>

S, Si, Cl and Br have a characteristic abundance of A+2
Isotopes

• The isotope pattern is a signature of a given elemental composition

• Isotope pattern filtering is a major tool in data reduction:
  – Are measured exact masses of isotope peaks consistent with the proposed elemental composition?
  – Are intensities of +1 and +2 isotope peaks consistent with the proposed elemental composition?
Example for CS

$\Delta m C^{12-13}: 1.0033548$

$\Delta m S^{32-33}: 1.0000007$

$C^{13} S^{32}$

44.9754

$C^{12} S^{33}$

44.9715

$C^{12} S^{34}$

45.9678
Example for C=S=O

\[ \Delta m \text{S}^{32-33}: 1.0000007 \]
\[ \Delta m \text{C}^{12-13}: 1.0033548 \]

\[ \text{\(^{13}\text{C}^{32}\text{S}^{16}\text{O}\)} \]

\[ \text{\(^{12}\text{C}^{32}\text{S}^{16}\text{O}\)} \quad 59.96699 \]

\[ \text{\(^{12}\text{C}^{33}\text{S}^{16}\text{O}\)} \]

\[ \text{\(^{12}\text{C}^{34}\text{S}^{16}\text{O}\)} \]

\[ \text{\(^{12}\text{C}^{32}\text{S}^{18}\text{O}\)} \]

\[ \text{\(^{12}\text{C}^{34}\text{S}^{16}\text{O}\)} \quad 61.962 \]

\[ \text{\(^{12}\text{C}^{32}\text{S}^{18}\text{O}\)} \]

\[ \text{\(^{14}\text{C}^{32}\text{S}^{16}\text{O}\)} \]

\[ \Delta m \text{S}^{32-34}: 1.99641 \]
\[ \Delta m \text{C}^{12-14}: 2.00324 \]
\[ \Delta m \text{O}^{16-18}: 2.00425 \]
Isotope Spacing: multiple charges

- As the charge state increases, spacing between isotopes decreases as $1/z$

- Spacing for fine structure also decreases

Resolution needs to be increased to make fine structure visible for multiply-charged ions
Mass accuracy

- Achieving a good mass accuracy depends on:
  - Quality of calibration
  - Resolution
  - Signal intensity (S/N ratio)
  - Possible chemical interferences

- Accurate mass can be used to determine **elemental composition**

- Higher mass accuracy allows fewer possible compositions

- A 1-3 ppm mass accuracy is usually sufficient for elemental formula assignment of molecules < 300 Da

- The isotopic pattern provides additional informations to discriminate between structures with similar masses
HRMS role for small molecules

• Identify compound
  Use measured mass as accurately as possible
  As measurement becomes less exact, more combinations are possible

• Eliminate interferences in quantification
  Interferences distort LRMS quantification
  HRMS is more discriminating

• Avoids further MS/MS fragmentation
  Useful for ions difficult to fragment
Resolution
Mass Terminology: Resolution

- $\Delta m$: must be defined (half peak? 10% valley?)
- FWHM: Full Width at Half Maximum
- Resolution=Resolving Power (RP): $M/\Delta m_{50\%}$
## Resolution

<table>
<thead>
<tr>
<th>m/z</th>
<th>1.5</th>
<th>0.7</th>
<th>0.1</th>
<th>0.02</th>
<th>0.005</th>
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<tr>
<td>200</td>
<td>133</td>
<td>286</td>
<td>2000</td>
<td>10,000</td>
<td>40,000</td>
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<td>500</td>
<td>333</td>
<td>714</td>
<td>5000</td>
<td>25,000</td>
<td>100,000</td>
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<tr>
<td>1000</td>
<td>667</td>
<td>1429</td>
<td>10,000</td>
<td>50,000</td>
<td>200,000</td>
</tr>
</tbody>
</table>

- **RP< 2,000** is low resolution
- **RP> 10,000** is generally agreed to be high resolution
Resolution

• Resolution alone is not enough: mass accuracy also needs to be high

• High resolution mass spectrometry: many acronyms but HRMS most commonly accepted

• HRMS generally not enough to provide unambiguous formulas
  – MS/MS may be needed
  – NMR
Resolution: $[\text{M+H}]^+$ Bosentan $\text{C}_{27}\text{H}_{29}\text{N}_5\text{O}_6\text{S}$

Theor. $[\text{M+H}]^+$ 552.1916

Separation of compounds with close Mass

\[ m/z = 1000 \text{ (ex: 1000 and 1000.4)} \]

1000 Da compounds different of 0.4 Da can only be fully resolved with \( R = 6000 \)
Mass Terminology: Mass Defect

- $^{12}$C is the only isotope with an integer exact mass (12.0000)
  Used to set mass scale for all other elements & isotopes

- All others have a mass defect: difference between exact mass and integer mass

- As total mass increases, the mass defect increases
  (~0.05 per 100 Da)
  - Nominal mass 300 is actually likely near 300.15
  - Nominal mass 800 is actually likely near 800.45
Mass Defect: Examples

• Positive mass defect: Hydrogen 1.0078
  - Gain ~0.008 Da per hydrogen
  - Typically, many H present in a molecule: 40 H yield positive mass defect of about +0.3 Da

• Negative mass defect: Cl (34.96885), Br (78.91834), S (31.97207), O (15.9949)
  - 0.031 Da lost per Cl
  - 0.082 Da lost per Br
Peptide Mass Defect

Can be used to discard non-peptidic compounds

Matrix-alkali cluster masses

Mass Defect, summary

• Low mass defect may indicate halogens or rings/double bonds (insaturated, few hydrogens)

• High mass defect may indicate many hydrogens

• Look for exact mass change in \( m/z \) from metabolite transformation:
  Ex: Oxidation is +15.9949 (MD slightly more negative than parent compound)
MS instrumentation
MS instrumentation | 3 steps

1. Ionization
   - Ionization source
     - e.g. EI, CI, ESI, APCI, APPI, MALDI

2. Separation (m/z)
   - Analyzer
     - e.g. quadrupole, ion trap, time-of-flight

3. Detection
   - Detector
     - e.g. photomultiplier, microchannel plate, electron multiplier

4. Pumping
   - (10^{-6}-10^{-7} Torr)
Ion Sources
**2 main Ion sources Types**

- **Molecular analysis**
  - Electron impact ionization (EI) and Chemical ionization (CI), *gas phase*
  - Atmospheric pressure chemical ionization (APCI), *liquid*
  - Atmospheric pressure photoionization (APPI), *liquid*
  - Electrospray ionization (ESI), *liquid*
  - Matrix assisted laser desorption/ionization (MALDI), *solid*
  - Novel ion sources: DESI, DART…

- **Elemental analysis**
  - Thermal desorption
  - Inductively coupled plasma (ICP)

→ All sample states are compatible with MS
→ Positive and negative ions (except for EI)
Fast Atom Bombardment (FAB)

- Developed 1980s

- Sample is mixed with a liquid matrix (glycerol) on a Cu electrode

- A beam of atoms (Ar, Xe ou He) is focused on the sample

- The gaz is ionized by a potential difference (8-35 kV)

- Ions (Ar\(^+\)...\) are accelerated, the analyte is desorbed and ionized, then transmitted to the mass analyzeur

- The matrix absorbed part of the energy of the incident ions: FAB is quite soft

- FAB is no longer used (replaced by MALDI)
Electron impact ionization

Interaction of a molecule with electron

\[ \text{A} + e^- \rightarrow \text{A}^{\text{+\#}} + 2e^- \]

Then fragmentation process

- Even e ion (cation)
- Odd e ion (cation radical)
- Radical
- Molecule

ISIC-EDCH MS course - 2017
EI Fragmentation: energy of electrons

(a) 70 eV

(b) 12 eV

H₃C-[CH₂]₁₁-0-[CH₂]₁₁-CH₃

High energy (70 eV)

Low energy (12 eV)

Vékey et al. 1996
El Fragmentation: molecular structure

Aromatic compound
resists dissociation and
gives MW ion

Cyclic compound
fragments easily and only little
molecular ion is observed

Fragmentation depends on the chemical structure of the analyte
EI Fragmentation, internal Energy ($E_{int}$)

- Fragmentation depends on the $E_{int}$ of the precursor ion and its mode of formation (ionization type)

- Compound with high $E_{int}$ have a EI spectra showing a lots of fragment ions

- Fragments are produced by 2 types of competitive and successive reactions: direct clivages (fast) et rearrangements (slow)

- In order to fragment ions in MS/MS their $E_{int}$ often needs to be increased. This is done with a secondary excitation and by producing ion-neutral molecules (gas) collisions (CID)
Advantages and disadvantages of the EI

Advantages

- Reproducible: libraries of EI spectra for identification
- Sensitive: High ionization efficiency
- Nonselective: all vaporized molecules can be ionized
- Extensive in-source fragmentation: molecular information deduced

Disadvantages

- Only cations are formed: no negative polarity
- Radical cation: rearrangement processes complicate mass spectra
- Sample must be volatile: limited to low MW compounds (< 600 Da)
- Nonselective: complex mass spectra
- Strong ionization: extensive fragmentation induces loss of the molecular ion
Chemical ionization: CI

Interaction of a molecule with a reagent ion $R^+$ in large excess

- Similar to EI but higher pressure and simultaneous introduction of $R$ and $A$
- Maintaining a large excess of $R$ compared to $M$ ($10^3$-$10^4$ fold) ensures its preferential ionization

Reagent gas can be $\text{NH}_3$, $\text{CH}_4$, $\text{H}_2$
Chemical ionization (CI)

• Reagent species is ionized by high-pressure electron ionization; maintaining a large excess of R compared to M ensured preferential ionized R

\[ R + e^- \rightarrow R^{++} + 2e^- \]  
(Primary ion formation, ex CH4^+, NH3^+)

• Collisions between reagent ions (ionization plasma)

\[ R + R^{++} \rightarrow RH^+ + R-H^- \]  
(Secondary ion formation, ex NH4^+ or CH5^+ ions)

• Collision of reagent ions with gas-phase analyte yields analyte ions

Product ion formation

\[ M+ RH^+ \rightarrow [M+H]^+ + R \]  
proton transfer → protonation

\[ M+ RH^+ \rightarrow [M+RH]^+ \]  
adduct formation (electrophile addition)

\[ M + R^{++} \rightarrow R + M^{++} \]  
charge exchange

\[ M + RH^+ \rightarrow M^+ + RH \]  
charge exchange

\[ M + [R-H]^+ \rightarrow [M-H]^+ + R \]  
H abstraction
Chemical ionization (CI)

• Observation of \([M+H]^+\) depends on proton affinity (PA) and implies that \(PA(M) > PA(R)\)

• Negative CI (NCI): the analyse must capture an electron (so contain acidic groups or electronegative elements such as halogens)

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

• CI causes less fragmentation than EI. The degree of fragmentation of \([M+H]^+\) will depend on the internal energy of the products ions
Atmospheric pressure CI (APCI)

- The sample is introduced in liquid state (Infusion or LC); the analyte must be volatile and thermally stable.
- The droplets are dried using heat (up to 450 °C) + nebulizer / auxiliary gas flow, yielding vapors of both solvent and analyte.
- A high voltage is applied to the corona needle* and the electric discharge constitutes a constant source of electrons.
- Electrons ionize solvent and analyte (air serves as reagent gas). A plasma is created around the corona end. Radical ions generated participate in analyte ionization.

* The ionisation chamber is the counter electrode, ΔV 3-6 kV
Atm. Pres. Photoionization (APPI)

- Gas phase ionization occurs by high energy photons from a Kr lamp causing either direct or indirect (dopant-assisted) photoionization. (experimentally, you can view APPI as an APCI source where the corona discharge has been replaced with a Kr lamp)

- Vapourization occurs via nebulization ($N_2$) and heating to 350-550°C. (Analyte must be thermally stable!)

- The analyte can be directly photoionized by photons from the Kr lamp (10-10.6 eV) if its Ionization Energy (IE) is below 10.6 eV.

- A dopant is generally added to aid the photoionization process; abundant photoions formed serve as primary reagents for gas-phase ion-molecule reactions.

- Very useful ionization technique for non-polar analytes that are difficult to ionize with ESI.

- Singly charged ions only.
Energetics in APPI mechanisms

**PhotoMate™ lamp**
Krypton 10.0 eV, 10.6 eV

<table>
<thead>
<tr>
<th>Dopant</th>
<th>Ionization Energies (IE, eV)</th>
<th>Proton Affinity (PA, kJ mol(^{-1}))</th>
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<tbody>
<tr>
<td>Toluene</td>
<td>8.83 eV</td>
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<tr>
<td>THF</td>
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<tr>
<td>Acetone</td>
<td>9.70 eV</td>
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<td>Chlorobenzene</td>
<td>9.06 eV</td>
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**Solvent**

<table>
<thead>
<tr>
<th>Solvent</th>
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<td>Acetonitrile</td>
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<td>Water</td>
<td>12.61 eV</td>
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</table>

- The photons from the Kr lamp can only photoionize compounds of IE<10.6 eV
- Dopants should have IE < 10.6 eV to be ionized
- Common HPLC solvents like H\(_2\)O, CH\(_3\)OH and CH\(_3\)CN are NOT ionized and cannot aid ion formation
- Sensitivity is higher with MeOH than ACN, due do its lower PA
APPI in positive mode

• **Direct APPI:**

\[ M + h\nu \rightarrow M^+\cdot + e^- \]  
(M is ionized to \( M^+\cdot \) if its IE is <10–10.6 eV)

and subsequently

\[ M^+\cdot + SH \rightarrow [M+H]^+ + \cdot S \]  
\( M^+\cdot \) may abstract a hydrogen to form \([M+H]^+\)

This last mechanism involves Proton Affinity (PA) properties and occurs from a reagent ion to a neutral molecule having higher PA

• **Dopant APPI:**

\[ D + h\nu \rightarrow D^+\cdot + e^- \]  
(The photoionizable dopant is in excess and yields many \( D^+\cdot \) ions)

and subsequently

\[ D^+\cdot + M \rightarrow M^+\cdot + D \]  
M is ionized via charge (electron) transfer

\[ D^+\cdot + M \rightarrow [M+H]^+ + [D-H]\cdot \]  
M is ionized via proton transfer from dopant or solvent
APPI in negative mode

**Electron capture**

\[ \text{D} + \text{hv} \rightarrow \text{D}^+ + e^- \]
\[ \text{M} + e^- \rightarrow \text{M}^- \]
\[ \text{S} + e^- \rightarrow \text{S}^- \]

Direct capture of a photoelectron occurs for compounds having high EA

**Charge exchange**

\[ \text{M} + \text{S}^- \rightarrow \text{M}^- + \text{S} \]

**Proton transfer**

\[ \text{M} + [\text{X-H}]^- \rightarrow [\text{M-H}]^- + \text{X} \]  (to form a deprotonated molecule)

Because Oxygen is ubiquitous in API sources and it has a high EA, it captures electrons to yield superoxide ions \( \text{O}_2^- \); those can serve as deprotonation reagents due to their basic properties

- Negative APPI may be applied to ionization of electron deficient analytes, including those containing heteroatoms (Boron)
Ranges of applicability of APPI

- **Still quite unexplored**
- ESI
- Syagen Photoionization
- APCI

MW

Nonpolar

Ionic

Polarity

ISIC-EDCH MS course - 2017
APPI Sources

Now proposed by most vendors

Thermo

PhotoMate light source

Waters

Agilent
APPI: negative mode

- NO₂ withdrawing groups create a region of reduced electron density: the electron affinity of the molecule is increased and electron capture favoured in Neg. mode

- In positive mode, as NO₂ groups increase analyte’s IE, the cation is more difficult to be generated

> X50 gain in sensitivity in negative mode

Theoretical

APPI+, M⁺

APPI-, M⁺
APPI: Fragmentation

- Fragmentation is observed depending on the compound
APPI: range of MW

- Successfull ionization of analytes above 3 kDa
- Large range of mass range covered: 70 to 4000 Da
- Successfull ionization of a large diversity of chemical structures

Increasing MW

MW ~3535 Da
APPI: summary

- Large applicability of APPI in terms of MW range and types of compounds: more than 1500 compounds could be ionized by APPI in our Facility

- APPI is a smooth ionization technique compared to IE. Limited neutral losses (H₂O, COOH, NO₂, CH₃, t-Bu, N₂, N₃)

- Can be interfaced with UPLC with post-column addition of the dopant

- APPI/APCI Dual-Mode ionization sources (Waters) to acquire both APCI and APPI data in the same analysis

Electrospray ionization (ESI)

Very soft ionisation method

- Sample is introduced in liquid state and ions are liberated directly from solution
- The solution is sprayed using an electrical field towards a negatively charged plate
- Droplet are formed, evaporate with gas & heat and become smaller and smaller
- Ions emerge from charged droplets and are accelerated by the electrical field
- ESI is smoother compared to EI and CI and produces multiply-charged ions
Adducts and MW identification

**Positive ESI:**
- \([\text{M+H}]^+\)
- \([\text{M+Na}]^+\)
- \([\text{M+NH}_4]^+\)
- \([\text{M+K}]^+\)
- \([\text{M+H+CH}_3\text{OH}]^+\)
- \([\text{M+H+CH}_3\text{CN}]^+\)

**Negative ESI:**
- \([\text{M-H}]^-\)
- \([\text{M+Cl}]^-\)
- \([\text{M-H+HCO}_2\text{H}]^-\) (Formic acid)
- \([\text{M-H+CH}_3\text{CO}_2\text{H}]^-\) (Acetic acid)
- \([\text{M-H+CF}_3\text{CO}_2\text{H}]^-\) (TFA)

MW = 192

MW = 192

![Molecule](image)
Nano-Electrospray

- Conventional (sub-micro) ESI at flow rate 100 – 400 nL/min for nano-UPLC column (down to 75 um I.D.)

- Nano ESI at flow rate ~20-300 nL/min (Nanomate, Advion): Chip-based infusion, 400 nozzles/ chip
Protein analysis by ESI-FT-MS:
10.3 kDa (GroES monomer)

\[ z = \frac{1}{\Delta m} = 10 \]

\([M+10H]^{10+}\)
Matrix Assisted Laser Desorption Ionization (MALDI)

- Analytes are embedded in a UV absorbing matrix crystals
MALDI mechanism

- Upon irradiation matrix-analyte clusters are transferred in the gas phase. The plasma generated is called MALDI plume.
MALDI mechanism

- The laser pulse is strongly absorbed by the matrix
- Rapid heating of the matrix causes sublimation and expansion into gas phase
- The ionization mechanism is gas-phase proton transfer
- Soft ionization: matrix absorbs the laser energy and prevents fragmentation
- Efficient ionization: matrix isolates molecules and prevents clusters
- Allows analysis of large intact biopolymers
- Allows analysis of complex samples
- LDI (no matrix) possible
Pulsed Laser

- Laser are pulsed (duration 1 to 10 ns), either in IR or in UV regions.

### UV
- Laser N$_2$: 3 ns pulse, $\lambda = 337$ nm
- Laser Nd-YAG: 15 ns pulse, $\lambda = 355$ nm

### IR
- Er-YAG: 200 ns pulse, $\lambda = 2.94$ um
- CO2 laser: 70 ns pulse, $\lambda = 10.6$ um

- Softer desorption method
- For large and labile compounds (high MW proteins (>500 kDa) and DNA (>1000 nucleotides))

- Most commonly found in MALDI sources
MALDI targets

- Sample is mixed with matrix and 1 µL is deposited on the target
- Many different protocols: Dried-droplets (DD), Sandwich...
Properties of the matrices

- Strong absorption of the laser irradiation (\(\lambda\, 337\, \text{nm} / 355\, \text{nm}\))
- Should have a good solubility in the solvent compatible with the sample
- Homogeneous solid-state mixing with the analyte for a good co-crysallisation
- Matrices are often acidic, therefore act as a proton source to favor proton transfer to the analyte
- Matrices are functionalized with polar groups, allowing their use in aqueous solutions

Structure of the most versatile matrices

- **HCCA** (\(\alpha\)-Cyano-4-HydroxyCinnamic Acid)
- Sinapinic Acid (SA)
- 2,5-DiHydroxy Benzoic Acid (DHB)
Ambient Ionization

- Developed since 2005

- Open-air noninvasive real-time ESI/APCI ionization without sample preparation
  - ASAP
  - DART
  - Thermal Desorption APPI
  - DESI, laser ablation ESI (LAESI), Surface Acoustic Wave Nebulization, paper spray….

- Many applications: illegal drugs, explosives, forensic, food, natural products, environmental contaminations…
Atmospheric Solids Analysis Probe (ASAP)

- Sample deposited onto glass tube
- Desorption using a hot N2 flux
- Ionization from plasma generated by a corona discharge

**Ion formation**

**Charge transfer**
\[
N_2 + e^- \rightarrow N_2^{+\cdot} + 2e^- \quad N_2^{+\cdot} + 2N_2 \rightarrow N_4^{+\cdot} + N_2
\]
\[
M + N_2^{+\cdot} \rightarrow M^{+\cdot} + N_2
\]

**Proton transfer (MH\(^{+}\))**
\[
N_4^{+\cdot} + H_2O \rightarrow H_2O^{+\cdot} + 2N_2
\]
\[
H_2O^{+\cdot} + H_2O \rightarrow H_3O^+ + HO^-
\]
\[
H_3O^{+\cdot} + M \rightarrow [M+H]^+ + H_2O
\]
Front end Optics
Front-end Optics

- Transmission of ions from Atmospheric pressure to the high vacuum region of the mass analyzer
  - Heated capillaries, orifices, lenses, skimmers
  - Ion Funnels
  - RF-only multipoles
- Considerations
  - $m/z$ range focused efficiently
  - $m/z$ range transmitted (cutoffs?)
  - Effect of focalisation/transmission on $m/z$ resolution?
Ion Funnels

- Series of closely-spaced flat ring electrodes of fixed i.d. with alternating RF voltage
  - Stacked Ring Ion guide (SRIG), ....
  - RF potentials of opposite polarity are applied on adjacent electrodes
  - The resulting potential $V^*$, proportionnal to the square of the amplitude of the local Electrical field, radially confines ions inside the ion guide

$$V^*(r, z) = \frac{ze |E_{rf}(r, z)|^2}{4m\omega^2}$$

$r, z$ radial and axial positions
$E_{rf}$ Electrical field
$z$ ion charge state
$e$ elementary charge
$m$ ion mass
$\omega = 2\pi f$ (f is the Rf frequency)
• Electrodes gradually narrowed towards exit

• The spacially dispersed ion cloud is efficiently focused to a much smaller radial size

• RF waveforms provide radial focusing

• DC voltage added to drive ions along the z axis toward exit

• High transmission efficiency from atmospheric pressure ion source to high vacuum but it needs enhanced pumping

RF-only Multipoles

- Focusing effectiveness: Quad > Hex > Octa

- $m/z$ range passes: Octa > Hex > Quad

- Many varieties of Multipoles exist: Round, square, or hyperbolic rods

- RF-only Quad use:
  - Ion guide: links between lens and quadrupole mass analyzers (prefilters)
  - Collision cells: Quad are the most common format for performing CID fragmentation
Vacuum System
Vacuum

• At $P_{\text{atm}}$, many collisions occur: background molecules cause ion deviation

• Long way from source to detector for analyte:
  
  $< 1\text{m for Q and IT}$
  $> 1\text{m for TOF}$
  $> 1\text{km for FT-MS}$

<table>
<thead>
<tr>
<th>Analyser</th>
<th>Mean free path (L)</th>
<th>Pressure (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadrupole</td>
<td>50 cm</td>
<td>$&lt; 10^{-4}$</td>
</tr>
<tr>
<td>Ion Trap</td>
<td>50 cm</td>
<td>$&lt; 10^{-4}$</td>
</tr>
<tr>
<td>TOF</td>
<td>50 m</td>
<td>$&lt; 10^{-6}$</td>
</tr>
<tr>
<td>Magnetic Sector</td>
<td>50 m</td>
<td>$&lt; 10^{-6}$</td>
</tr>
<tr>
<td>FT-MS (Orbitrap, ICR)</td>
<td>5 km</td>
<td>$&lt; 10^{-8}$</td>
</tr>
</tbody>
</table>

• Vacuum is a critical factor in high resolution to achieve high transmission of ions

• Ion trapping systems (Orbitraps, ICR) are especially demanding due to increased collision probabilities while held in the trap
Vacuum

• **Foreline/rotary pumps (roughing pumps)**
  - A piston rotates in a compression chamber, moving gas from the inlet to the exhaust port
  - Reduce the pressure within a particular region to 0.1-1 Pa (10^{-3} - 10^{-2} Torr)
  - Monitored using a Pirani gauge (filament)

• **Turbomolecular pumps**
  - Spinning of blades at ~60,000 rpm (200-500 L/s typically)
  - Monitored using a Penning gauge (high tension)
Differentially pumping regions

- The transmission depends on the size of the orifice
- The transfer capillary aids in transmissions of ions (keep ions focused and stop them from diffusing from the expansion cone)
Mass analyzers:
Separate ions according to their $m/z$
MS analyzers

- Ion trap (IT)
- Quadrupole (Q)
- Time-of-flight (TOF)
- Orbitrap
- ICR
- Magnetic sector
Classification of mass analyzers

\[ M \frac{d\vec{V}(\vec{r}, t)}{dt} = q\vec{E}(\vec{r}, t) + q[\vec{V}(\vec{r}, t) \times \vec{B}(\vec{r})] \]

Field Free Based Analyzers

Electric Field Based Analyzers

Magnetic Field Based Analyzers

- Linear/reflectron TOF
- Linear/reflectron TOF
- Linear/reflectron TOF

- Quadrupole
- Quadrupole
- Quadrupole

- Ion trap
- Ion trap
- Ion trap

- Orbitrap
- Orbitrap
- Orbitrap

- Magnetic sector
- Magnetic sector
- Magnetic sector

- Ion cyclotron resonance (ICR)
- Ion cyclotron resonance (ICR)
- Ion cyclotron resonance (ICR)
## Comparison of mass analyzers

<table>
<thead>
<tr>
<th>Type</th>
<th>Resolving Power (RP)</th>
<th>Mass Accuracy (ppm)</th>
<th>Ion sampling</th>
<th>Scan Rate</th>
<th>Scan efficiency*</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadrupole</td>
<td>2-5000</td>
<td>100</td>
<td>Continuous</td>
<td>&lt;1 sec</td>
<td>Low (Full scan), High (SIM)</td>
<td>$</td>
</tr>
<tr>
<td>Ion Trap</td>
<td>4000</td>
<td>100</td>
<td>Pulsed</td>
<td></td>
<td></td>
<td>$</td>
</tr>
<tr>
<td>TOF</td>
<td>20-50,000</td>
<td>5</td>
<td>Pulsed</td>
<td>&lt;0.1 sec</td>
<td>Mid-High</td>
<td>$$</td>
</tr>
<tr>
<td>Orbitrap</td>
<td>240,000</td>
<td>&lt;2</td>
<td>Pulsed</td>
<td>Depends on RP</td>
<td>Low-Mid</td>
<td>$$$</td>
</tr>
<tr>
<td>ICR</td>
<td>500,000</td>
<td>&lt;2</td>
<td>Pulsed</td>
<td>Depends on RP</td>
<td>Low-Mid</td>
<td>$$$$</td>
</tr>
<tr>
<td>Magnetic Sector (Double-focusing)</td>
<td>100,000</td>
<td>&lt;5</td>
<td>Continuous</td>
<td>~1 sec</td>
<td>Low (Full scan), High (SIM)</td>
<td>$$$$</td>
</tr>
</tbody>
</table>

* Ions made vs Ions detected
Characteristics of mass analyzers

- **m/z range**
  - Each mass analyzer has its m/z range
  - 100-2000 Da common range for Q but can be increased for biopolymers analysis (up to 8000 for some Q)

- **Resolving power**: how it varies across m/z
  - Quadrupole: peak width is constant so RP increases with increasing m/z
  - TOF: RP is constant across m/z (peaks get wider)
  - Orbitrap: RP decreases as √ of increase in m/z
  - ICR: RP decreases linearly with increase in m/z

- **Time for achieving high resolution**
  - Should be compatible with LC peaks (else infusion)

- **Duty cycle**: number of different m/z monitored at the same time
  - Quads, sectors: only one m/z reaches the detector at a time, wasting all other m/z at that time
  - TOF: package of ions pushed towards the detector

- **Costs**: to buy, to own (maintenance, spare parts…)

\[
RP = \frac{m}{\Delta m}
\]
Quadrupole (Q)
Quadrupole, Historical

• 1953: W. Paul and H.S. Steinwedel describe the quadrupole mass analyzer (Bonn University). Got the 1989 Nobel Prize in Physics for the work on ion trapping

• 1968: Finnigan introduces the first instrument with Q technology

• 1978: Building of the first triple quadrupole

• 1982: The first commercial triple quadrupole is launched by Finnigan and Sciex
Quadrupole (Q) Mass Analyzer

- 4 parallel metal rods of opposite polarity
- A RF voltage is applied between one pair of rods and the other
- A Direct Current (DC) voltage is superimposed on the RF voltage
- Ions travel between the rods: only ions of a certain $m/z$ will reach the detector for a given ratio of RF and DC voltages applied
Quadrupole (Q) Mass Analyzer

- By continuously varying the applied voltage, the operator selects an ion with a particular \( m/z \) or can scan for a range of \( m/z \) values
- Other ions with unstable trajectories collide with the rods
- Ion trajectories can be modeled by Mathieu differential equations derived from Paul equations

Rods can be cylindric or hyperbolic

Ions transmitted: stable trajectory
Ions ejected: unstable trajectory
Ion Motion: effect of DC

Velocity V and Acceleration A

\[
\mathbf{v} = (v_x, v_y, v_z) = \frac{dx}{dt}, \frac{dy}{dt}, \frac{dz}{dt}
\]

\[
\mathbf{a} = (a_x, a_y, a_z) = \frac{d^2 x}{dt^2}, \frac{d^2 y}{dt^2}, \frac{d^2 z}{dt^2}
\]

\[
F = m \mathbf{a}
\]

\[
E_p = e \ U
\]

\[
E_c = \frac{1}{2} \ m V^2
\]
Ion Motion: effect of AC

- **AC, no DC**
  - Charge + more stable
  - Charge – less stable

- **AC + DC**
  - Charge + more stable
  - Charge – less stable

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Quadrupole Mass Analyzer

Potentials applied on the rods

U: Direct potential (DC)
V: RF voltage amplitude

Quadrupoles are operated at fixed frequency but variable V

Typically
U: 500-2000 V
V: 0-3000 V

\[ \Phi_0 = + (U - V \cos \omega t) \]

\[ -\Phi_0 = -(U - V \cos \omega t) \]

\( \omega = 2\pi v \) where \( v \) is the frequency of the RF field

(180° out of phase)
The Equation of Ion Motion

\[ F_x = ma = m \frac{d^2x}{dt^2} = -ze \frac{d\Phi}{dx} \]

\[ F_y = ma = m \frac{d^2y}{dt^2} = -ze \frac{d\Phi}{dy} \]

\[ F_z = 0 \]

\[ \frac{d^2x}{dt^2} + \frac{2ze}{mr_0^2} (U - V \cos \omega t) x = 0 \]

\[ \frac{d^2y}{dt^2} + \frac{2ze}{mr_0^2} (U - V \cos \omega t) y = 0 \]
Matthieu Equations

The trajectory of an ion will be stable if the values of $x$ and $y$ never reach $r_0$ (thus never hits the rods)

\[ x < r_0 \]
\[ y < r_0 \]

\[
\frac{d^2 u}{d\xi^2} + \left(a_u - 2q_u \cos 2\xi\right)u = 0 \\
\xi = \frac{\omega t}{2} \quad u = x \\
\xi = \frac{\omega t}{2} \quad u = y
\]

\[
\begin{aligned}
\left\{
\begin{array}{l}
a_u = a_x = -a_y = \frac{8zeU}{m\omega^2 r_0^2} \\
q_u = q_x = -q_y = \frac{4zeV}{m\omega^2 r_0^2}
\end{array}
\right.
\end{aligned}
\]

Define stable and unstable regions
Stability Diagram: \( a_u \) and \( q_u \) diagram

\[
\begin{align*}
a_u &= a_x = -a_y = \frac{8zeU}{m\omega^2 r_0^2} \\
q_u &= q_x = -q_y = \frac{4zeV}{m\omega^2 r_0^2}
\end{align*}
\]

Overlay of the 2 diagrams

Values of U and V such that \( x \) and \( y \) are \( < r_0 \)

This overlay gives regions where the ions have stable trajectories according to both \( x \) and \( y \) axes simultaneously

Only this Area is used in MS; this area depends on the mass
Stability Diagram

- In practice, quadrupoles are operated at fixed frequency but variable Vc
Stability Diagram

\[ U = a \frac{m \omega^2 r_0^2}{z^2 e} \]

DC component

\[ V = q_u \frac{m \omega^2 r_0^2}{z^2 e} \]

AC component

- Frequency is fixed; U/V ratio is kept constant
- Scanning a U/V ratio line allows the successive detection of masses m1, m2, m3 when reaching stability area
- The higher the slope of U/V is, the better is the resolution
- If U=0, all the masses above m1 will have a stable trajectory
- Highest detectable \( m/z \) ~4000 Th; resolution ~ 2000-3000

\( \text{a/q} = \frac{U}{V} \)
Mode Scan

Quadrupole mass analyzer is scanned sequentially passing all m/z

Mode SIM

Quadrupole Mass Analyzer is allowing only ions of a single m/z to pass
In SRM both Q1 and Q3 are parked at a single m/z
In MS/MS, precursor ions are selectively isolated and fragmented in Q2 to generate a mass spectrum of product ions.

⇒ Structural informations
Ion Trap (IT)
Ion Trap Mass Analyzers

Classic Ion Trap

Linear Ion Trap

R 2’000-10’000
3D Ion Trap

- Consist of a Ring electrode and 2 end caps electrodes
- Principles is very similar to quadrupole
- Ions are stored by RF and DC fields
- Scanning of RF amplitude can eject ions of specific m/z
- High sensitivity in full scan
- Mass range 0-4000 m/z
- Mass accuracy typically 0.1 u
3D ion Trap

• The quadripolar field is created between the ring and end-cap electrodes

• RF is applied on the Ring electrode: trapping voltage

• A resonance voltage is applied to end-cap electrodes to eject ions

• Mass separation in time (in space for quadrupoles)

• Resolution depends on the scan speed
Ions maintain stable trajectories inside the device as a result of the application of a RF voltage to the ring electrode. Ions are attracted and repelled on the X/Y plane, depending on the polarity.

V (RF amplitude) can be varied but $\omega$ is fixed (ex 760 kHz, 760’000/ sec).

The RF applied on end-cap electrodes is of opposite polarity to ring electrode. Ions entering in the trap oscillates in Z-axis between the end-cap electrodes but being at the same time attracted and repelled by the ring electrode.

A potential well is created to trap the ions.
Ion Trajectories
3D Ion Trap, Helium

- Ions are trapped and repelled each other: their trajectory expand with time.

- Helium in the trap works as a dumping gas: following collision of ions with He atoms, ions lose their momentum and part of their Ec is converted in energy of vibration and heat, and rapidly dissipated.

- The net loss of Ec occurs without fragmentation, therefore ions stay centered in the trap.

- In theory, ions can stay indefinitely in the trap. To be detected ions should be ejected.

   A RESONANCE frequency is applied to end-cap electrodes to destabilize and eject ions of a given m/z.
Matthieu Equations

- The trajectory is stable if ion coordinates never reach or exceed $r_0$ and $z_0$

\[
\alpha_z = -2\alpha_r = \frac{16zeU}{m\omega^2(r_0^2 + 2z_0^2)}
\]

\[
q_z = -2q_r = \frac{8zeV}{m\omega^2(r_0^2 + 2z_0^2)}
\]

- In most 3D Trap, $U=0$ ($a_r=a_z=0$)
Stability Diagrams in 3D Ion Trap

$$q_z = -2q_r = \frac{8zeV}{m\omega^2\left(r_0^2 + 2z_0^2\right)}$$

- For a given charge $z$ and a given trap geometry ($r_0$ & $z_0$), $q$ increases with $V$ and decreases with $m$

- Area of stability in the x,y and z axis

- Generally $U=0$, so $a=0$: the diagram is reduced to 1D
Stability Diagrams in 3D Ion Trap

- At $U = 0$, increasing $V$ will destabilize low $m/z$ ions.

Ions trapped with stable trajectory up to $q_z$ of 0.908

Stability diagrams for $m/z = 10$, 50, and 100 in $V(\text{RF}) - U(\text{DC})$ space.
Mass analysis in the Trap

Can be done by:

• Ejection at the stability limit

• Resonant ejection
3D versus 2D Ion Trap

- 50X ion storage capacity
- 20X higher ion injection efficiency
- Efficient coupling with other mass analyzers (hybrides MS)

Advantages of a 2-D Ion trap
Time of Flight (TOF)

(see HRMS course later for more details)
TOF: the Basics

Mass analysis is achieved because ions of different \( m/z \) have different velocities and therefore reach the detector at different times.

If 2 ions of different \( m/z \) start at the same location, with same kinetic energy and travelling in the same direction:

the ion with the lower \( m/z \) will travel faster and reach the detector first.

We can measure unknown \( m/z \) by calibrating the travel time vs. \( m/z \) for a given Ec.
TOF: starting with a Pulse

- TOFs need a $t_0$ starting gate to give a start to the pack of ions

- Pulsed sources (such as MALDI) therefore have an inherent compatibility advantages: laser pulse initiates ion formation in very short time
**TOF: Time**

Time = Distance (Flight length) / velocity

\[ M \frac{d\bar{V}(\bar{r}, t)}{dt} = 0 \]

The time \( t \) needed to cover the distance \( L_d \) before reaching the detector is \( t = L_d/V \)

**Velocity = from accelerating voltage**
• Ions are accelerated by a **difference of potential** $V_s$ applied between a first electrode and the **extraction grid**

• Their potential energy is converted into kinetic energy

\[
E_p = E_c \quad \Rightarrow \quad qV_s = \frac{1}{2} mV^2
\]
TOF: time & resolution

- Replacing $V$ by $L/ \tau$ gives

$$t^2 = \sqrt{\frac{2qVs}{m}}$$

- Relation between $m/z$ and flight time is

$$\frac{m}{z} = \left(\frac{2eVs}{L^2}\right)t^2$$

- Resolution of TOFs depends on the flight length $L$ and the thickness of an ion packet approaching the detector

- To increase resolution, use longer flight tubes and high acceleration voltages (at least 20 kV to keep sensitivity high)

- Factors affecting resolution:
  - length of ion formation pulses (time distribution)
  - size of the volume where ions are formed (space distribution)
  - variations of the kinetic energy of the ions
TOF: resolution

To reduce the kinetic energy spread among ions with the same $m/z$ ratio leaving the source:

- Time lag (100 ns-1us) between ion formation and extraction is introduced: the **Pulsed Extraction Delay**

- Ion Mirror or **Reflectron**
• Ions with more Ec (hence more velocity) will penetrate more deeply than ions with lower Ec: they will then reach the detector at the same time

• Reflectron increases the mass resolution at the expense of sensitivity and introduces a mass range limitation (≈ 8000 m/z)
Orbitrap (OT)
Orbitrap: Background

- Newest mass analyzer
  - Announced ASMS 1999, published 2000*
  - Commercial launch ASMS 2005

- Thermo is the sole supplier
  - All informations come via Thermo
  - Patent expires soon!

- Completely changed the LC-HRMS landscape
  - Better performance than TOF
  - Easier to use than FTICR

Orbitrap: Fundamentals

- Ions are trapped in ultra-high vacum
- To trap ions, OT uses electrostatic fields + electrodes:
  - 1 central spindle
  - 2 halves of outer shell
  - End electrodes
- Radial field between outer and spindle electrodes drives ions towards spindle

 Ion energy, centrifugal force & tangential injection put ions into orbit around spindle
(electrostatic attraction compensates the centrifugal force)
Orbitrap: Fundamentals

- The axial field (spindle axis) drives axial oscillation between 2 halves of outer shell
  - The frequency of this detected oscillation is the key signal
  - This Frequency is proportional to $m/z$
- Ions confined axially by end electrodes
- Fourier Transform used to convert signal to $m/z$ data

Fourier Transformation has been a part of the evolution and performance improvement of OT across generations.
Orbitrap: ion motion

\[ \omega = \sqrt{\frac{k}{m / z}} \]

- Very long mean free path (many km!)
Orbitrap: Capabilities

- High performance instrument competitive with FT-ICR mass analyzers
- Mass resolving power up to 240’000 at 400 m/z
- Resolution depends on the transient length: 512 ms for 240K on a Qexactive HF
- High mass accuracy (1-2 ppm)
Ion Cyclotron Resonance
ICR

(More detailed in the FT-ICR course given by Y.O. Tsybin, september)
Ion Cyclotron Resonance (ICR) Mass Analyzers

Superconducting Magnet (3 to 21T)

2 excitation electrodes

2 detection electrodes

2 trapping plates
ICR: equations and ion motion

\[ \vec{F} = q \cdot (\vec{v} \times \vec{B}) = \frac{m \cdot v^2}{r} ; \quad \frac{m \cdot v}{r} = m \cdot \omega_c = q \cdot B ; \]

Angular velocity = \( \omega_c \)

Only the angular velocity \( \omega_c \) is used in MS

Determining the mass consist in determining the frequency
ICR MS : ion excitation

• Ions introduced with different Ec and at different time are not in phase and turn in an orbit on a small radius trajectory.

• Ions of same m/z need to acquire a coherent motion on the same orbit: this is done by a resonante excitation.

• A sinusoidal pulse (AC) at the same frequency $\omega_c$ of the ion m/z is applied on excitation electrodes.
  - Resonance absorption of the wave
  - Energy transferred = Increase in the Ec of the ion: the motion becomes coherent with an increase in the radius of the trajectory.

• Other m/z ions are not in resonance with this AC and cannot absorb the energy: they stay in the ICR trap.
ICR: ion detection by induced current

• The packets of ions of same \( m/z \) passing close to the detection plate create an induced current \( \Delta Q \)

\[ \Delta Q = \frac{2zeV_y}{d} \]

\( \Delta Q \) increases with charge: a multiply-charged ion gives a more intense ICR signal

\[ V_y = \frac{zeB \cdot r}{m} \]

\( r \) = cyclotronic radius

The bigger is the radius of orbit, the more intense will be the signal
ICR Cell versus Orbitrap

\[ \omega \approx \frac{B}{m/z} \]

\[ \omega = \sqrt{\frac{k}{m/z}} \]

FT-ICR

M1 < M2 < M3

B 3T

m/z 28 Th: \( \Omega_c \) 1.65 MHz

m/z 4000 : \( \Omega_c \) 11.5 kHz
ICR: Transient signal

• Complex signal depending on the sample complexity

• Resolution depends on the observation time, linked to the relaxation time

• Very high cell vacuum is needed to limit ion-molecule collisions
ICR: Fourier transform

transient

Fourier Transform

cyclotron equation

mass/charge
ICR: Capabilities

- High performance mass analyser
- Mass resolving power >200’000, typically 500K
- High mass accuracy (≈ 1 ppm)
- Sensitive enough to detect 10 ions in the cell
- Limited number of ions in the cell (10^6): limited dynamic range
- Expensive (> 1M $)
Magnetic and Electrostatic sectors
Magnetic Sector Analyzers

The particle moves in a circular orbit (perpendicular to the field)

\[ F = z e v B \] (Lorentz Force)
\[ F = \frac{m v^2}{r} \] (Centrifuge Force)

\( v \): initial velocity

- The charged particle in a magnetic field is equilibrated when both forces equilibrates each others

\[
(1) \quad \frac{m v^2}{r} = z e v B
\]

\[
(2) \quad r = \frac{m v}{q B}
\]

- Introducing the kinetic energy: \( E_c = \frac{1}{2} m v^2 = q V_s \) and \( m v^2 = 2 q V_s \)

Rearranging equations gives

\[
\left( \frac{m}{z} \right) = \frac{(r B)^2}{2 V_s}
\]

\( m/z \) of ions that reach the detector can be varied by changing either \( B \) or the applied \( V_s \) of the ion optics

\( r \): fixed radius of the flight tube
\( V_s \): potential difference for ion acceleration in the source (typically 10 kV)
Magnetic Sector Analyzers

\[
\left( \frac{m}{z} \right) = \frac{(rB)^2}{2V_s}
\]

higher-mass ions are less deflected than lower-mass ions

\[ m/z_1 > m/z_2 > m/z_3 \]

• Ions entering from the source are then deflected in the magnetic sector at different positions depending on their m/z.

• B needs to be increased to detect \( m/z_1 \) and decreased for \( m/z_3 \) : scanning the magnet B enables ions of different m/z to be focused to the detector.

• A mass spectrum is obtained by fixing r and varying B or Vs.

• Technically it’s easier to keep B constant and «play» with the initial velocity or Ec of ions (through Vs).

\[
r = \frac{\sqrt{2mE_c}}{qB}
\]
Magnetic and electrostatic simple-focalisation Sector Analyzers

- **Ions of similar \( m/z \) and Ec**
  
  Ions of same \( m/z \) and Ec enter in the B sector with a different angle \( (a) \): ions staying longer time in B have a more bended trajectory:

  Magnetic sector analyzers with simple focusing have detectors in F0

- **Ions of similar \( m/z \) and different Ec**

  - All ions leaving the source do not have the same Ec and as the magnet has no energy focusing, ions of different Ec are brought to different foci. This dispersion alters the mass resolution

  Ec dispersion is further controlled with an electrostatic analyser
Electrostatic Sector Analyzers

- Electric sectors separate ions **according to their Ec and not their mass**

\[
z E = \frac{mv^2}{r}
\]

(E: intensity of the electric field)

So

\[
r = \frac{2 E_c}{z E}
\]

The trajectory is independent of the mass, depends only on E and on Ec (acceleration voltage / charge)

- As in magnetic sector instruments, E is fixed and r is controlled by Ec, by varying the acceleration potential from the ionisation source

- Coupling magnetic sector and electrostatic sector allows kinetic energy distribution of ions of same m/z generated in a magnetic sector to be corrected in the electrostatic sector
Double focalisation Sector Analyzers

- Ions are separated according to their \( m/z \), then focalized in the electrostatic sector

- The sectors can be reversed: first E then magnetic sector

- The detection is made on 1 point by scanning B or Vs (easier)

- Magnetic instruments function at constant \( R \) (\( m/\Delta m \approx 18000 \)): as a result, \( \Delta m \) varies in proportion to \( m \)

- When designed and aligned properly, \( R \) can reach 100000
Ion detection
Ion detection: 3 main types

Detectors allow a mass spectrometer to generate a signal (a current) from incident ions, by generating secondary electrons, which are further amplified by:

• Channeltrons

• Discrete or continuous Dynodes

• MCP
Continuous dynode electron multiplier

Vacuum-tube structure that multiplies incident charges by secondary emission of electrons

Electrons produced in cascade are attracted towards the anode (positive gradient) and collected

Amplification $10^5 - 10^6$

The final result is a measurable current proportional to the number of particles that hit the cathode
Discrete dynode electron multiplier

Series of 1 conversion dynode + 12-20 dynodes with good secondary emission properties

A cascade of electrons is created and the final flow of electrons provides an electrical current at the end of the EM, further increased by an electronic amplification.

Conversion dynode: Ions +: -5kV Ions -: + 5KV

Dynodes are held at decreasing negative potentials by a chain of resistors.
Microchannel plate detectors (MCP)

- Each channel is covered by a semiconductor substance
- Electron path inside very short: very fast response detector
- Plate input side kept negative 1 kV compared to outside side
- Well suited for TOF analyzers which need precise arrival times and narrow pulse widths
Microchannel Plate Detector
Microchannel Plate Detector (MCP)

High electron gain!

Disadvantages:
- Fragile (should never apply HV without conditioning)
- Sensitive to air
- Large microchannel plates are expensive
Sample introduction

- Direct infusion
- HPCL / UPLC
- GC
Sample introduction methods

- Flow injection analysis
- Infusion of a sample in the mass spectrometer
- For pure compounds or low complexity samples
Possible hyphenation with MS

chromatographic methods

- GC
- SFC
- HPLC/UPLC
- CE
- TLC

Ions in gas phase

Gas phase

Liquid phase

On solid phase
Importance of LC/GC

- Reduces complexity of mixtures
- Removes chemical interferences/impurities
- Increases sensitivity
- Separate isobaric compounds prior to MS
- Fully automated – direct coupling to the MS or to a MALDI plate spotter
- High throughput analysis
Sample preparation

- LLE
- SPE
- Ultrafiltration
- Precipitation
- Chemical modification

Metabolites extraction

Compound derivatization
LC-MS: dimensionality

High throughput Chromatographic dimension Spectroscopic dimension

Multidimensional Analysis

Intensity Chromatographic dimension

2D

3D

m/z Time

ISIC-EDCH MS course - 2017
High Performance LC (HPLC)

- **Pump**
- **Réservoir de phase mobile**
- **Mobile Phase**
- **Injector**
- **Column**
- **Stationary Phase**
- **Oven**
- **Detector**
- **Data acquisition**
- **Data treatment**
Reversed-phase Chromatography

- Hydrophobic Retention
- Polar compounds not retained

Schematic representation of Reverse Phase HPLC

**Mechanism Partitioning**

<table>
<thead>
<tr>
<th>Stationary Phase</th>
<th>Mobile Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octadecylsilyl</td>
<td>Water</td>
</tr>
<tr>
<td>Octyl silyl</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Cyano</td>
<td>Methanol</td>
</tr>
<tr>
<td>Amino</td>
<td>THF</td>
</tr>
<tr>
<td>C4</td>
<td>Buffers</td>
</tr>
<tr>
<td></td>
<td>Additives</td>
</tr>
</tbody>
</table>

Supports RPLC
Ultra Performance LC (UPLC)

HPLC

UPLC

5 \mu m
Analytical Particles

60 \mu m Human Hair
(very fine hair)

1.7 \mu m
ACQUITY UPLC Particles
Optimal Particle Size distribution
for Maximum Efficiency at a given Pressure

High pressure: ca 1000 bar
Advantages of UPLC

HPLC

Column Xbridge BEH C18
150 x 4.6 mm I.D., 5 µm
5-40% ACN in 60 min
at 1 mL/min

40 min run

UPLC

Column Acquity BEH C18
150 x 2.1 mm I.D., 1.7 µm
5-40% ACN in 60 min
at 0.35 mL/min.

5 min run

Time reduced to 5 min!
same resolution & same selectivity
Detection: acquisition frequency

HPLC

UPLC

MS 1Hz (1 spectra/s)

MS 10Hz

ISIC-EDCH MS course - 2017
Gas Chromatography (GC)

- The sample is vaporized (250°C) and injected into the head of the column, transported by a flow of inert gas.
- The column contains a stationary phase adsorbed onto the surface of inert solid (glass).
GC: general

- Method of choice for the separation of volatile & semi-volatile components
- High resolution of the columns due to the length (30m, N≈ 10^6)
- Drawbacks: T °C limited to 350°C and boiling points of analytes < 500°C
- Sampling sample: the smallest (< 3 μl) for best peak shape and max resolution.
- No acid for the silica column
- Capillary column: fused silica tubing with ID 100 um coated with a thin film of stationary phase
  Ex: 5% phenyl-methyl 95% dimethylpolysiloxane column) suitable for a wide range of applications but other stationary phases can be used depending on the application
GC-MS, applications

- Food chemistry: flavors, stabilizers, colorants...
- Essential oils, plant extracts
- Biology: pheromones, hydrocarbons...
- Forensic Sciences: poisons, toxines, drug (amphetamines...)
- Environnement: pesticides, oils, fuels, water contaminants...
- Pharmacology
- Petroleochemistry
Merci pour votre attention.

Y a-t-il des questions ?"