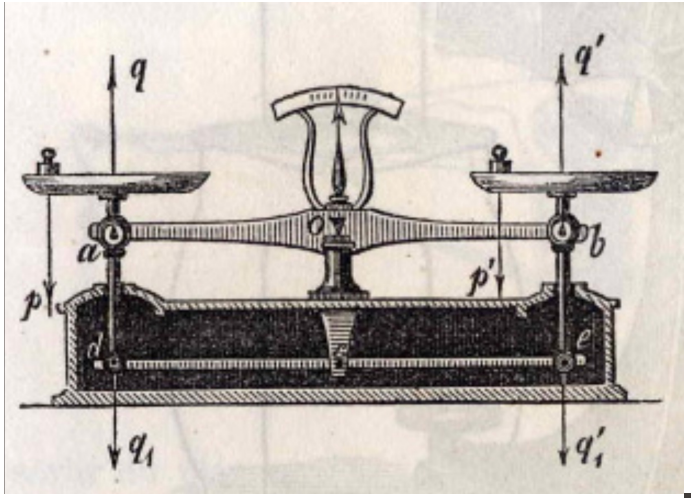
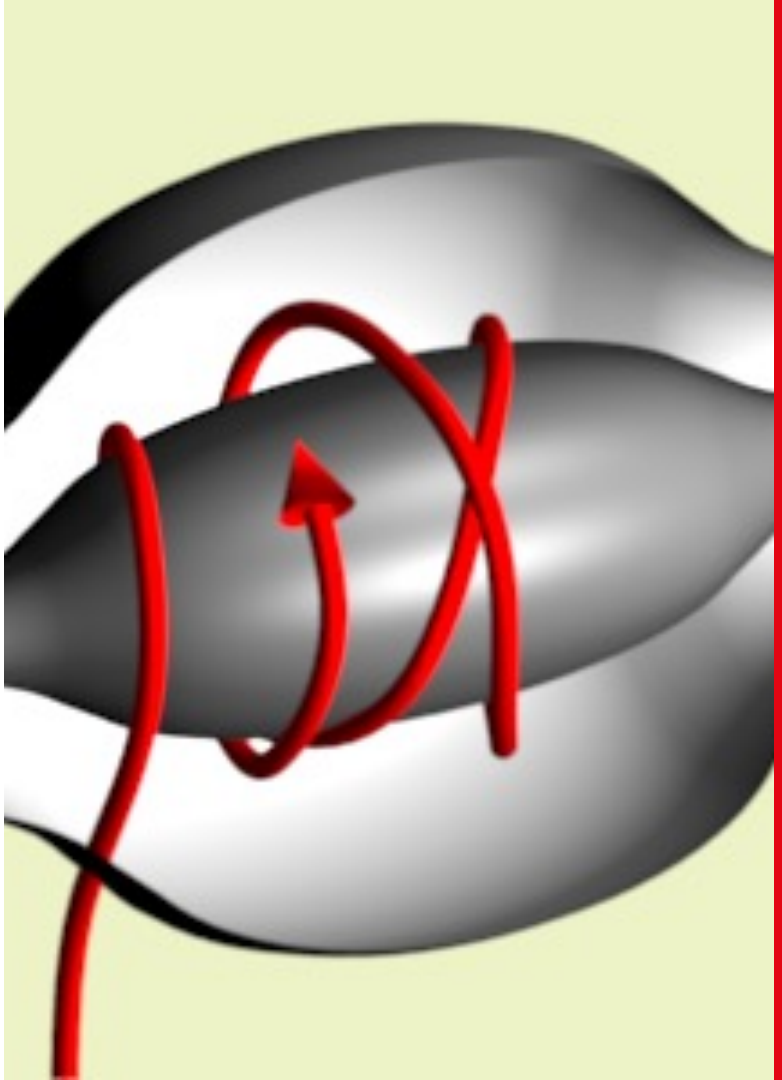


Part 1 : Mass spectrometry

Chapter 1 : Mass spectrometry Introduction

CH - 314 Structure elucidation

Dr MER Pascal Miéville
Assistant : Leander Choudhury



What is mass spectrometry?

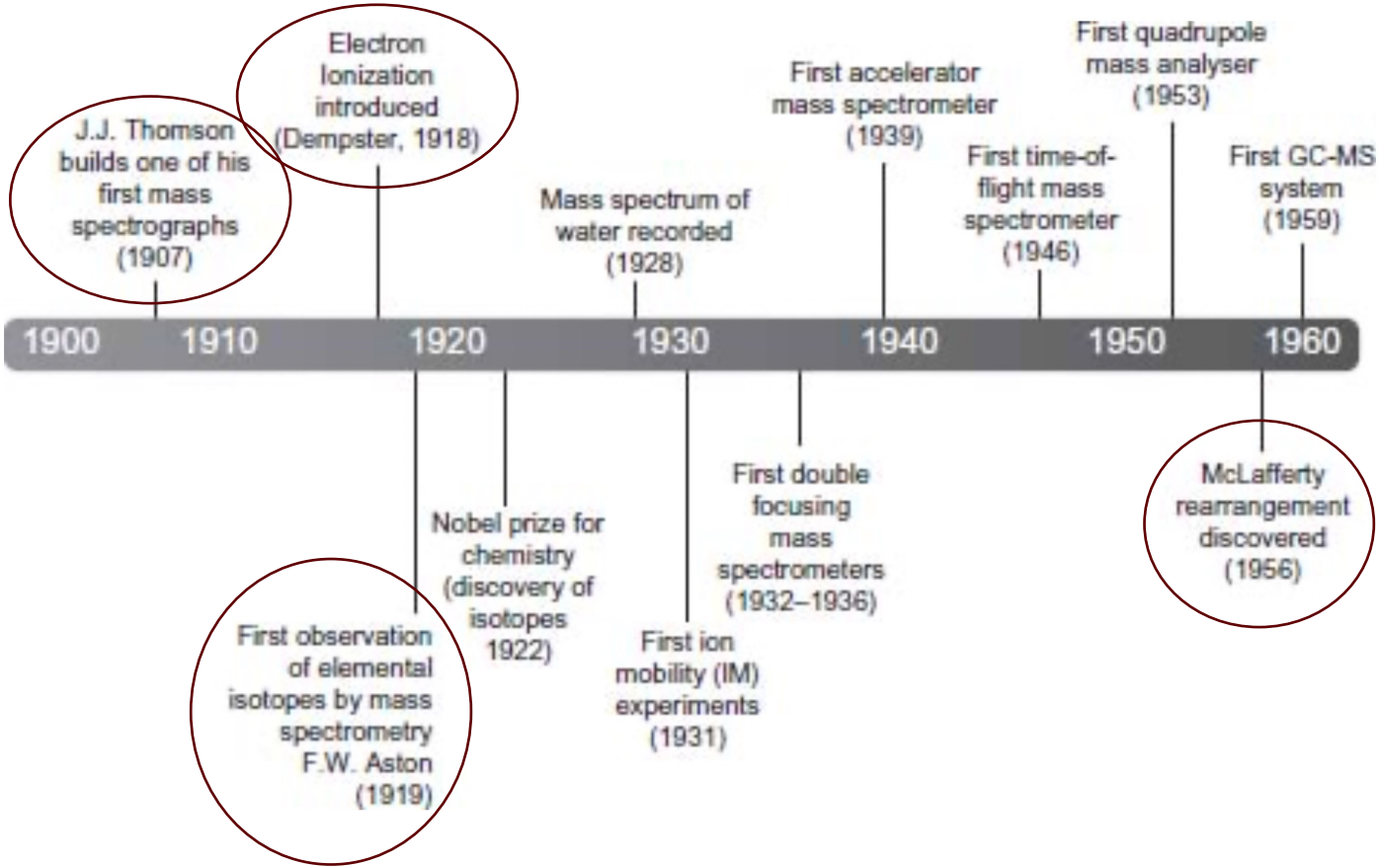
What is MS ?

- **Mass spectrometry (MS)** is an analytical technique that forms ions from atoms or molecules and measures their mass-to-charge ratios (m/z).
- It can provide information about molecular and elemental composition and also quantify the abundance of individual chemical components.
- It can be used to analyze solids, liquids, and gases with very high sensitivity.
- A mass spectrometer measures the **mass-to-charge ratio (m/z)** of **ions** in the **gas phase**.
- In mass spectrometry, *m* refers to the mass of the ion in atomic mass units, which are given the symbol *u* or *Da* (daltons, after John Dalton).
- By definition, 1 *u* (or *Da*) = 1/12 mass of the carbon-12 atom ($m(^{12}C)$). z is the integer charge state of an ion (+1, +2, −1, −2, etc.) of total absolute charge *ze*, where *e* is the elementary charge in coulombs (1.60×10^{-19} C).

1.2 A brief history of mass spectrometry

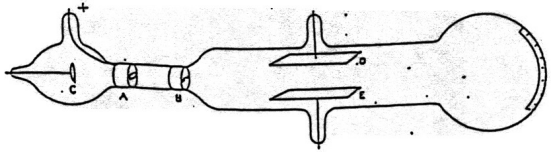
A brief history of MS

1900 to 1960

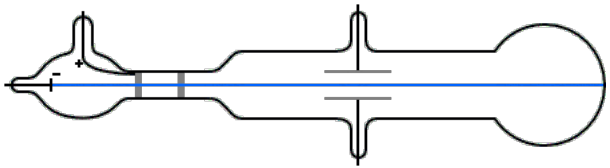


A brief history of MS

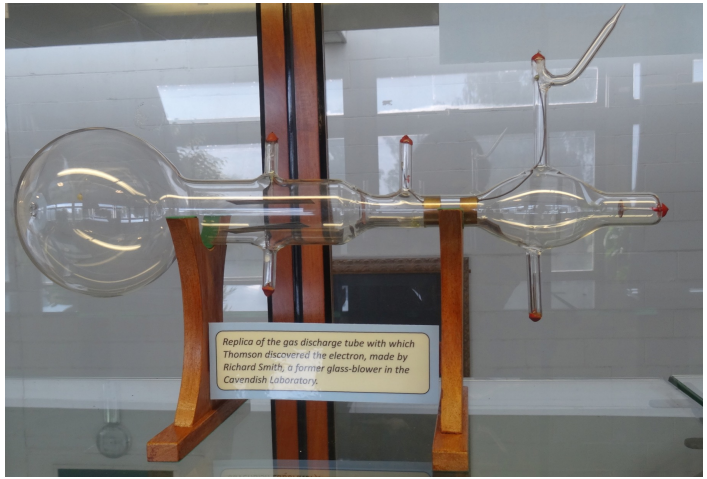
J. J. Thompson mass spectrometer



J.J. Thomson - Philosophical Magazine, 44, 293 (1897)



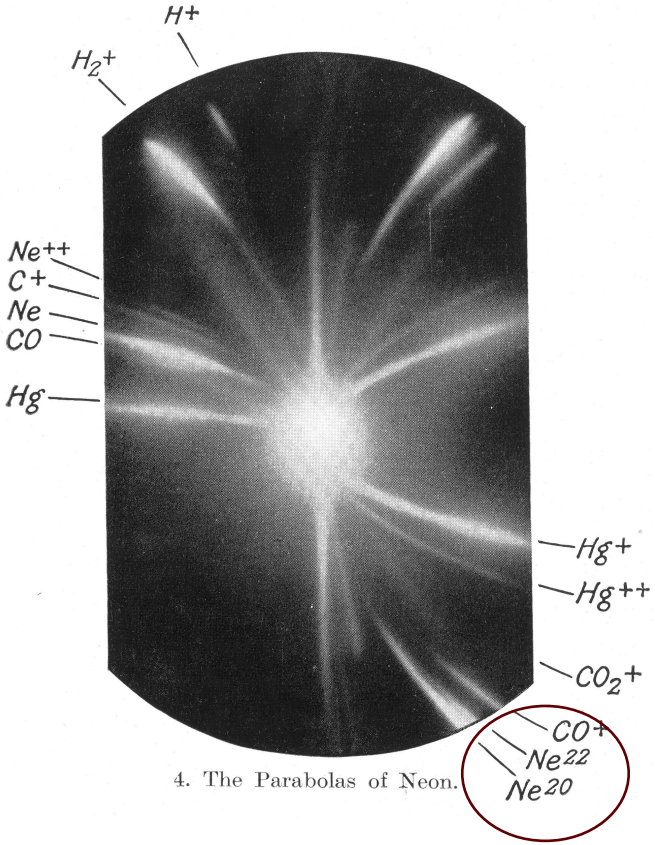
https://en.wikipedia.org/wiki/J._J._Thomson



A brief history of MS

J. J. Thompson mass spectrometer

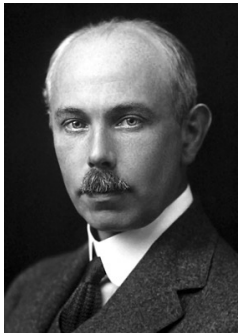
Thomson's separation of neon isotopes by their mass was the first example of mass spectrometry, which was subsequently improved and developed into a general method by F. W. Aston and by A. J. Dempster

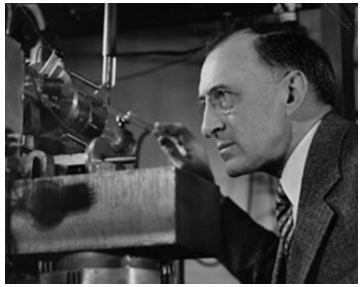


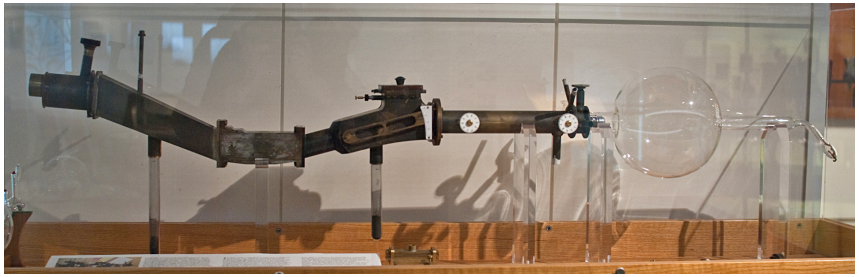
4. The Parabolas of Neon.

A brief history of MS

W. Aston and by A. J. Dempster







Replica of Francis William Aston's third mass spectrometer.
D.Sc, F. W. Aston M. A. (1 December 1919). "LXXIV. A positive ray spectrograph". The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science. ***38*** *(228): 707–714. doi:10.1080/14786441208636004.*

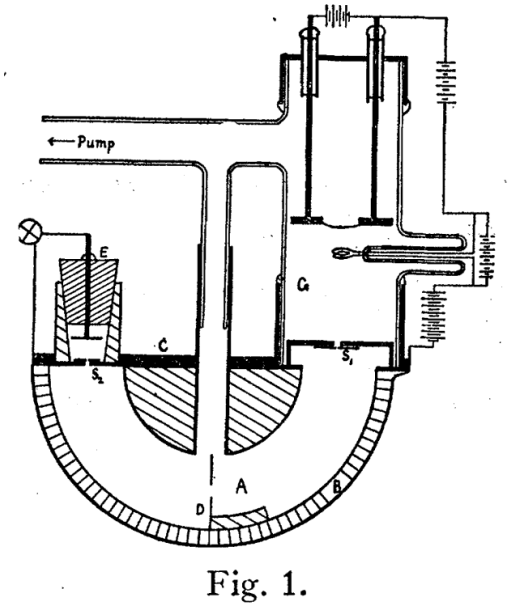
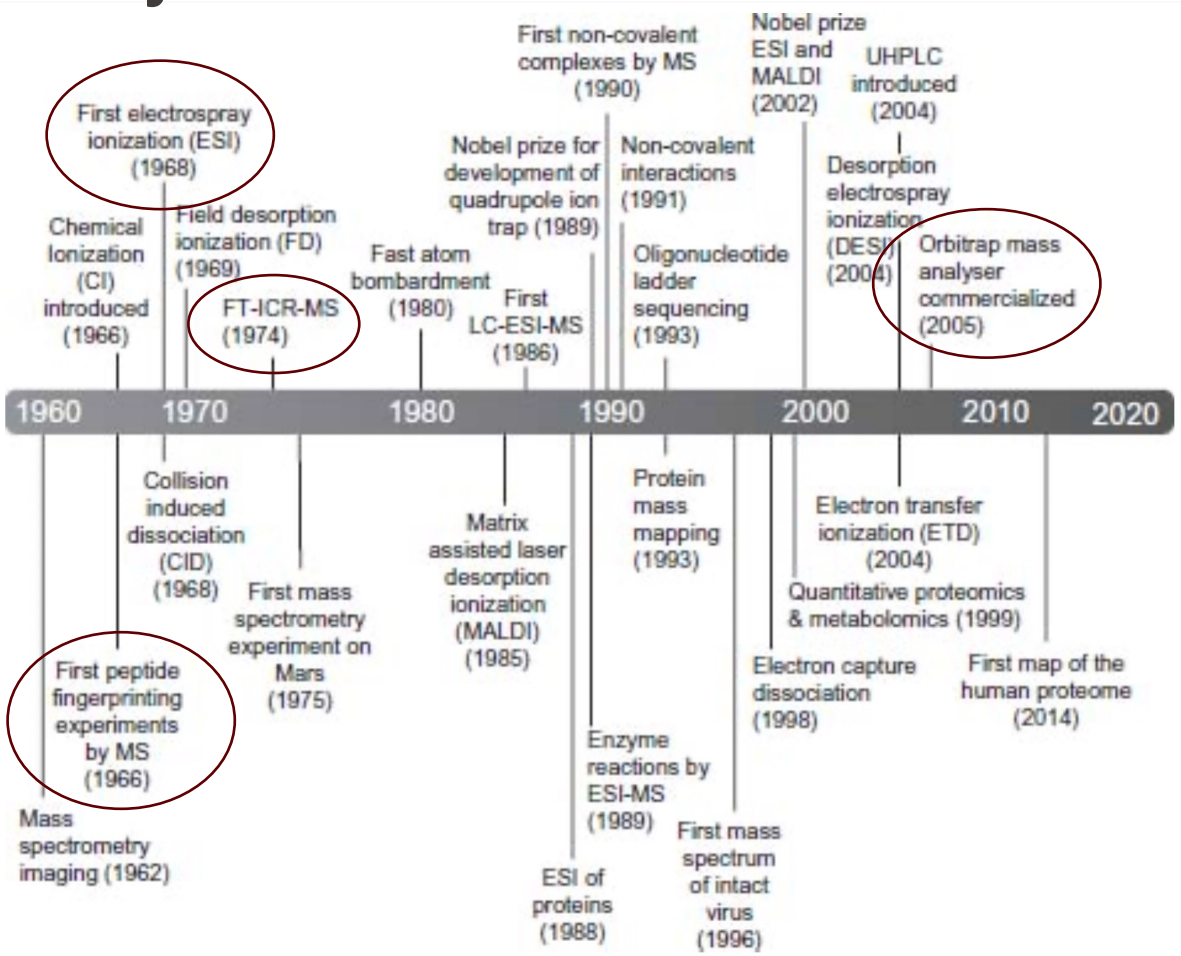


Fig. 1.

Dempster's 180 degree magnetic sector mass analyzer.
A new Method of Positive Ray Analysis
A. J. Dempster Phys. Rev. **11**, 316 –
Published 1 April, 1918
https://doi.org/10.1103/PhysRev.11.316

A brief history of MS

1960 to 2020



A brief history of MS

Some trends in MS (not exhaustive)

Trend	Why it matters	References
▪ **1. Single-cell & ultrasensitive proteomics**	New ion sources, microfluidic sample preps and sub-zeptomole Orbitrap/TOF hybrids now push proteome coverage below 1 pg, enabling true single-cell and spatially resolved assays.	https://www.nature.com/articles/s41586-025-08584-0
▪ **2. Trapped-ion mobility & hybrid analyzers**	Adding an orthogonal ion-mobility dimension boosts peak capacity and sequencing speed while preserving duty cycle, crucial for top-down and “proteoformics”.	

A brief history of MS

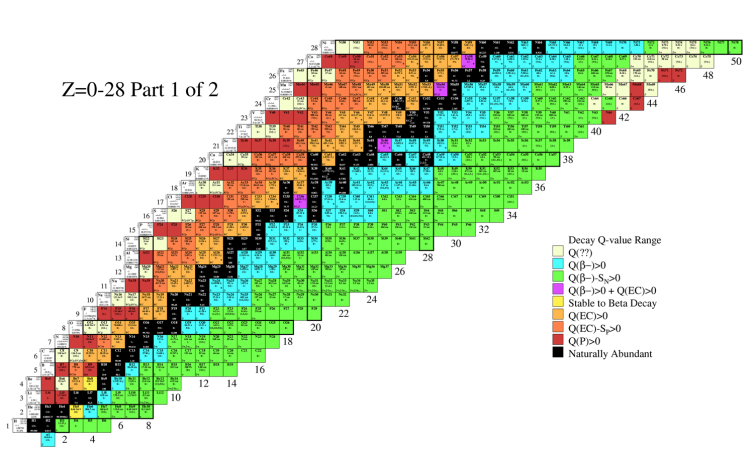
Some trends in MS

Trend	Why it matters	References
▪ **3. Ambient & imaging MS**	Vacuum-free ionization (DESI, LAESI, nanoDESI) and high-resolution MS imaging are moving analyses out of the lab, mapping metabolites in tissues, plants and food with <10 µm resolution.	https://pubs.rsc.org/en/content/articlehtml/2024/an/d4an00644e https://chemistry-europe.onlinelibrary.wiley.com/doi/epdf/10.1002/ansa.70007
▪ **4. AI-driven data treatment & instrument control**	Deep-learning models accelerate spectral deconvolution, peak identification and predictive maintenance; first demonstrations couple miniature MS with on-board DL for structural calls.	https://www.sciencedirect.com/science/article/pii/S2772577425000771

A brief history of MS

Some trends in MS

Trend	Why it matters	References
▪ **5. Miniaturisation & field-deployable MS**	MEMS quadrupoles, low-power pumps and ambient sources deliver suitcase-size instruments for on-site forensics, planetary probes and point-of-care diagnostics.	https://www.sciencedirect.com/science/article/pii/S2772577425000540
▪ **6. Charge-Detection MS (CDMS) & single-particle weighing**	CDMS extends mass range to gigadalton viruses, lipid nanoparticles and intact mRNA (>3 MDa), providing mass/particle distributions inaccessible to conventional MS.	https://www.sciencedirect.com/science/article/pii/S232905012500049X



Atomic and molecular mass

- Isotopically averaged mass
- nominal mass
- monoisotopic mass
- Mass is an intrinsic and differentiating component of matter

Atomic and molecular mass

Isotopically averaged mass

When referring to the **atomic mass** of a particular **element** it is common to use either **the isotopically averaged mass of all of the naturally occurring isotopes**, or the **mass of a single isotope**.

The former, **equivalent to the dimensionless relative atomic mass A_r** , is the one shown on most periodic tables, and is widely employed to derive **relative molecular mass M_r** in many chemical calculations.

Using ethanol (C_2H_6O) as an example, the relative atomic masses (to three decimal places) of the constituent elements are $A_r(C)$ = 12.011, $A_r(H)$ = 1.008, $A_r(O)$ = 15.999, and thus the relative molecular mass (M_r) of ethanol is 46.069.

Table 2. List of Elements in Atomic Number Order.

At No	Symbol	Name	Atomic Wt	Notes
1	H	Hydrogen	1.0080(2)	3, 5
2	He	Helium	4.002 602(2)	1, 2
3	Li	Lithium	6.94(6)	3, 5
4	Be	Beryllium	9.012 1831(5)	
5	B	Boron	10.81(2)	3, 5
6	C	Carbon	12.011(2)	5
7	N	Nitrogen	14.007(1)	5
8	O	Oxygen	15.999(1)	5
9	F	Fluorine	18.998 403 162(5)	
10	Ne	Neon	20.1797(6)	1, 3
11	Na	Sodium	22.989 769 28(2)	
12	Mg	Magnesium	24.305(2)	5
13	Al	Aluminium	26.981 5384(3)	
14	Si	Silicon	28.085(1)	5
15	P	Phosphorus	(30.973 761 998(5)	
16	S	Sulfur	32.06(2)	5
17	Cl	Chlorine	35.45(1)	3, 5
18	Ar	Argon	39.95(16)	1, 2, 5
19	K	Potassium	39.0983(1)	
20	Ca	Calcium	40.078(4)	
21	Sc	Scandium	44.955 907(4)	
22	Ti	Titanium	47.867(1)	
23	V	Vanadium	50.9415(1)	
24	Cr	Chromium	51.9961(6)	
25	Mn	Manganese	54.938 043(2)	
26	Fe	Iron	55.845(2)	
27	Co	Cobalt	58.933 194(3)	
28	Ni	Nickel	58.6934(4)	2
29	Cu	Copper	63.546(3)	2

https://iupac.qmul.ac.uk/AtWt/

Atomic and molecular mass

nominal mass / monoisotopic mass

Let's (re-)define first :

- **Atomic mass :** $\boldsymbol{m_a}$ mass can be given in kg or often in dalton (Da) or unified atomic mass unit (u).
- **One dalton** is equal to +1/12 the mass of a carbon-12 atom in its natural state, given by the atomic mass constant $m_u = m(^{12}C)/12 = 1$ Da, where $m(^{12}C)$ is the atomic mass of carbon-12.
- **The atomic mass** $\boldsymbol{m_a}$ mostly comes from the combined mass of the protons and neutrons in the nucleus, with minor contributions from the electrons and nuclear binding energy.
- The **relative isotopic mass** $\boldsymbol{rm_a}$ can be obtained by dividing the atomic mass m_a of an isotope by the atomic mass constant m_u, yielding a dimensionless value.

Atomic and molecular mass

nominal mass / monoisotopic mass

For small molecules (< 900 Da) most modern mass spectrometers can easily resolve the 1 Da mass difference caused by the presence of the different isotopes of their constituent elements (for example ^{13}C versus ^{12}C).

For this reason, single **isotope masses** are normally used in mass spectrometry of small molecules rather than the isotopically averaged mass.

The two commonly encountered types of **isotopic mass are** :

- **Nominal mass (m_N)** mass of an ion or molecule calculated using the **relative isotopic mass** (rm_a) of the most abundant constituent elemental isotope rounded to the **nearest integer value** and **multiplied by the number of atoms of each element** *(n)*.

$$m_N = \sum_{elements}(round(rm_a, 0) * n)$$

- **Monoisotopic mass:** exact mass (m_e) of an ion or molecule calculated using the **relative isotopic mass** (rm_a) of the **most abundant isotope** of each element **multiplied by the number of atoms of each element** *(n)*.

$$m_e = \sum_{elements}(rm_a * n)$$

Atomic and molecular mass

nominal mass / monoisotopic mass

- For ethanol (C_2H_6O) the **nominal mass** of the molecule is 46 Da ($m_a(C)$ = 12 Da, $m_a(H)$ = 1 Da, $m_a(O)$ = 16 Da)
- The **monoisotopic molecular mass** is 46.043 Da ($m_a(C)$ = 12.000 Da, $m_a(H)$ = 1.008 Da, $m_a(O)$ = 15.995 Da, to three decimal places).
- In mass spectrometry, the difference between the nominal mass and monoisotopic mass is known as the **mass defect**.

https://physics.nist.gov/cgi-bin/Compositions/stand_alone.pl

Atomic Weights and Isotopic Compositions for All Elements

Isotope		Relative Atomic Mass	Isotopic Composition	Standard Atomic Weight	Notes
1 H	1	1.007 825 032 23(9)	0.999 885(70)	[1.007 84, 1.008 11]	m
D	2	2.014 101 778 12(12)	0.000 115(70)		
T	3	3.016 049 2779(24)			
2 He	3	3.016 029 3201(25)	0.000 001 34(3)	4.002 602(2)	g,r
	4	4.002 603 254 13(6)	0.999 998 66(3)		
3 Li	6	6.015 122 8874(16)	0.0759(4)	[6.938, 6.997]	m
	7	7.016 003 4366(45)	0.9241(4)		
4 Be	9	9.012 183 065(82)	1	9.012 1831(5)	
5 B	10	10.012 936 95(41)	0.199(7)	[10.806, 10.821]	m
	11	11.009 305 36(45)	0.801(7)		
6 C	12	12.0000000(00)	0.9893(8)	[12.0096, 12.0116]	
	13	13.003 354 835 07(23)	0.0107(8)		
	14	14.003 241 9884(40)			
7 N	14	14.003 074 004 43(20)	0.996 36(20)	[14.006 43, 14.007 28]	
	15	15.000 108 898 88(64)	0.003 64(20)		
8 O	16	15.994 914 619 57(17)	0.997 57(16)	[15.999 03, 15.999 77]	
	17	16.999 131 756 50(69)	0.000 38(1)		
	18	17.999 159 612 86(76)	0.002 05(14)		
9 F	19	18.998 403 162 73(92)	1	18.998 403 163(6)	
10 Ne	20	19.992 440 1762(17)	0.9048(3)	20.1797(6)	g,m
	21	20.993 846 685(41)	0.0027(1)		

Atomic and molecular mass

Isotopically averaged mass / nominal mass / monoisotopic mass
How to choose ?

- For small molecules, where isotope separation is achieved but the mass measurement is accurate to 1 Da only, **nominal mass** is normally used. => EtOH = 46 Da
- Where accurate mass measurements (> 3 decimal places) are made with isotopic resolution, then **monoisotopic mass** is used. => EtOH = 46.043 Da
- In cases where isotopic resolution is not achieved by the spectrometer, **isotopically averaged mass** is employed in order to ensure proper comparison between measured and calculated values. => EtOH = 46.069 Da
- This put in evidence two essential and different features of mass spectrometers :
  - **Precision (number of digits)**
  - **Resolution (capacity to separate signals)**

Atomic and molecular mass

Mass is an intrinsic and differentiating component of matter

- The elements that comprise all chemical species have, **by definition, a unique proton, neutron, and electron composition**, and each **sub-atomic particle** has a **unique, non-integer mass in the unbound state**

Sub-atomic particle	Mass (Da) (unbound state)
Proton	1.0073
Neutron	1.0087
Electron	0.0005

- **If nuclear mass, and thus atomic and molecular mass**, were simply derived from the sum of the unbound masses of the appropriate number of protons and neutrons for the given elements in a compound, **mass spectrometry would be limited in its ability to determine elemental composition**

Atomic and molecular mass

Mass is an intrinsic and differentiating component of matter

- Sub-atomic particles **bound in the nucleus** of an element **do not have exactly the same mass as in the unbound state.**

Mass (Da)	di-oxygen ($^{16}O_2$)	Sulfur (^{32}S)
Free state	*32.2640*	*32.2640*
Bound state	31.9898	31.9720

Mass (Da)	di-nitrogen ($^{14}N_2$)	Silicon (^{28}Si)
Free state	*28.2317*	*28.2317*
Bound state	28.0061	27.9769

- **A small amount of the mass of elemental nuclei is lost due to energy released during its formation** (nuclear binding energy).
- **The amount released differs according to the size of the nucleus.**

The mass spectrometer

- What characterizes the mass spectrometer
- The use of electric and magnetic fields to manipulate ions
- Sample inlets
- Ionization sources
- Mass analysers
- Detectors
- HRMS

The mass spectrometer

The use of electric and magnetic fields to manipulate ions

- All mass spectrometers use electric or magnetic fields to accelerate and/or manipulate ions to measure their m/z.
- The relationship between the mass, charge, and force applied by an electromagnetic field is described principally by two basic equations:

- $\vec{F} = m\vec{a}$ (Newton's second law)
- $\vec{F} = \overrightarrow{F_E} + \overrightarrow{F_M} = Q\vec{E} + Q(\vec{v} \times \vec{B}) = Q(\vec{E} + \vec{v} \times \vec{B})$ (Lorentz force law)
- $Q = ze$ (Total charge of the ion)

Where:

$\vec{F}$ is the force applied to the ion by the electromagnetic field

m is the mass of the ion

$\vec{a}$ is the acceleration

z is the number of elementary charges in the ion

e is the unitary charge, e = 1.602176634×10^{-19} C (https://physics.nist.gov/cgi-bin/cuu/Value?e)

$\vec{E}$ is the electric field

$\vec{v}\times\vec{B}$ is the vector cross product of the ion velocity and applied magnetic field

The mass spectrometer

The use of electric and magnetic fields to manipulate ions

In the context of ions:

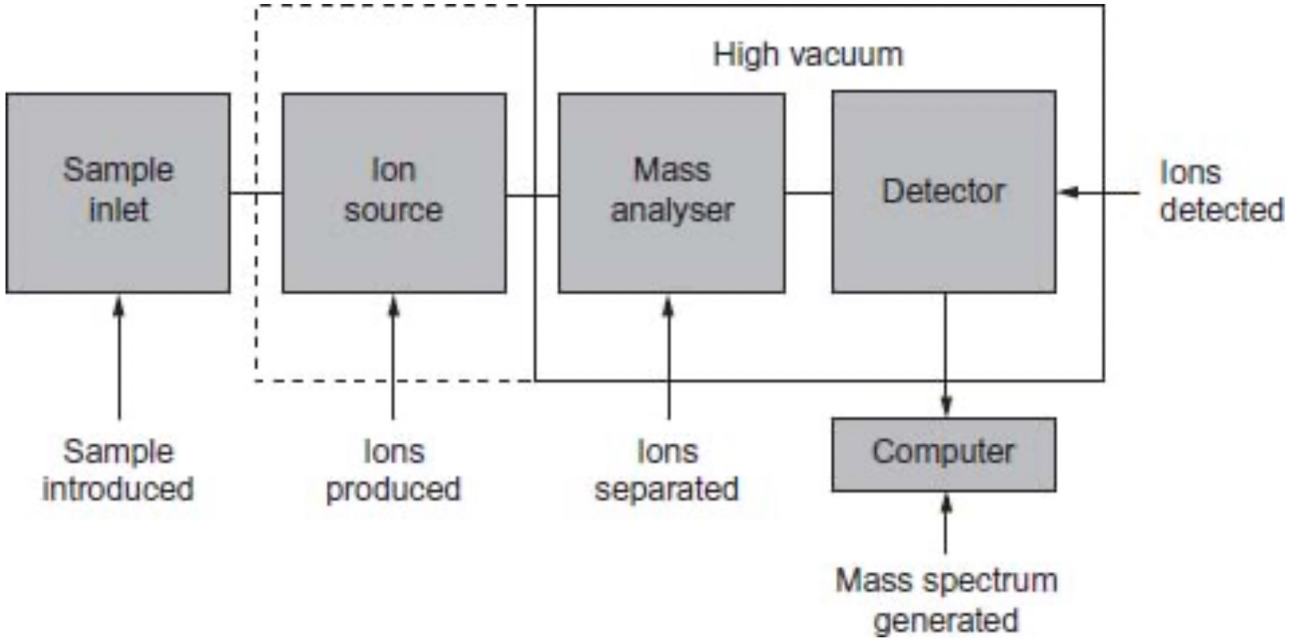
- **Newton's second law** demonstrates that an applied electromagnetic force $\vec{F}$ causes an ion to accelerate in a way that is dependent on mass, m.
- **The Lorentz force** equation shows that this acceleration is also dependent on the total charge of the ion, Q.
- By equating these two equations and replacing Q by ze :

$$ze\left(\vec{E} + \vec{v} \times \vec{B}\right) = m\vec{a}$$
$$\boldsymbol{(m/ze)\vec{a} = \vec{E} + \vec{v} \times \vec{B}}$$

- We obtain the **classical differential equation of motion for a particle.**
- Together with the particle's initial conditions, it completely determines the particle's motion in space and time in terms of m/ze.
- Since e is a constant, it is sufficient and relevant in terms of molecular structure information **to express the result in the dimensionless $\boldsymbol{m/z}$.**

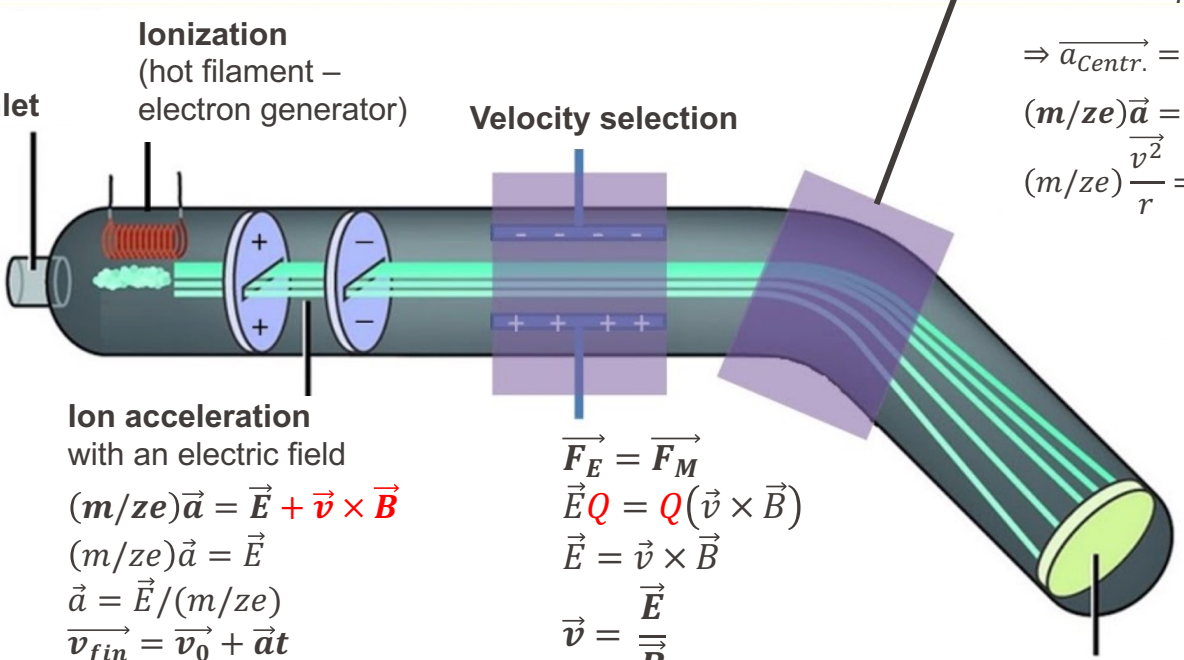
The mass spectrometer

The main components of a mass spectrometer



The mass spectrometer

The Bainbridge spectrometer



Ion's deflection

Centripetal force in a circular motion

$$\overrightarrow{F_{Centr.}} = \frac{m\overrightarrow{v^2}}{r} \text{ and } \vec{F} = ma$$

$$\Rightarrow \overrightarrow{a_{Centr.}} = \frac{\overrightarrow{v^2}}{r}$$

$$(\boldsymbol{m/ze})\vec{a} = \vec{E} + \vec{v} \times \vec{B}$$

$$(m/ze)\frac{\overrightarrow{v^2}}{r} = \vec{E} + \vec{v} \times \vec{B}$$

Electric deflection

$$(m/ze)\frac{\overrightarrow{v^2}}{r} = \vec{E}$$

$$\boldsymbol{r = \frac{(m/ze)\overrightarrow{v^2}}{\vec{E}}}$$

Magnetic deflection

$$(m/ze)\frac{\overrightarrow{v^2}}{r} = \vec{v} \times \vec{B}$$

$$\boldsymbol{r = \frac{(m/ze)\vec{v}}{\vec{B}}}$$

The mass spectrometer

The ionization sources (will be discussed more in details)

- Many ionization sources are available for mass spectrometers, although six are routinely used for the majority of applications.

  - Electron ionization (EI)
  - Chemical ionization (CI)
  - Electrospray ionization (ESI)
  - Atmospheric chemical ionization (APCI)
  - Inductively coupled plasma ionization (ICP)
  - Matrix assisted laser desorption ionization (MALDI)

- They differ in the physical mechanism of ion formation and in their suitability for different types of sample (based on polarity, volatility, and other chemical and physical properties).

The mass spectrometer

The ionization sources (will be discussed more in details)

- The ionic species formed are not always of the same type; for example, EI leads to the production of radical cations ($M^{+\cdot}$) from vaporized samples, whereas ESI forms protonated ($[M+H]^+$) or deprotonated molecules ($[M-H]^-$) and is used for the analysis of compounds from solution.

$$M + e^-_{(fast)} \rightarrow M^{+\cdot} \quad \text{(EI)}$$
$$M + H^+ \rightarrow [M+H]^+ \quad \text{(ESI positive)}$$
$$M - H^+ \rightarrow [M-H]^- \quad \text{(ESI negative)}$$

- In general, protonated/deprotonated molecules or radical cations/anions are the most common charged species formed in mass spectrometers, but the mechanisms by which they are produced can differ significantly.

The mass spectrometer

The mass analyzer (will be discussed more in details)

- The mass analyzer is at the heart of the mass spectrometer, and is designed to separate ions of different m/z.
- Different mass analyzer designs exist, see table below.

Mass analyser	Abbrev.	Date
Double focusing magnetic sector	Sector	1932
Time-of-flight	TOF	1946
Quadrupole	Q	1953
Ion trap	IT	1953
Ion-cyclotron resonance	FT-ICR	1974
Orbitrap	Orbi	2005

The mass spectrometer

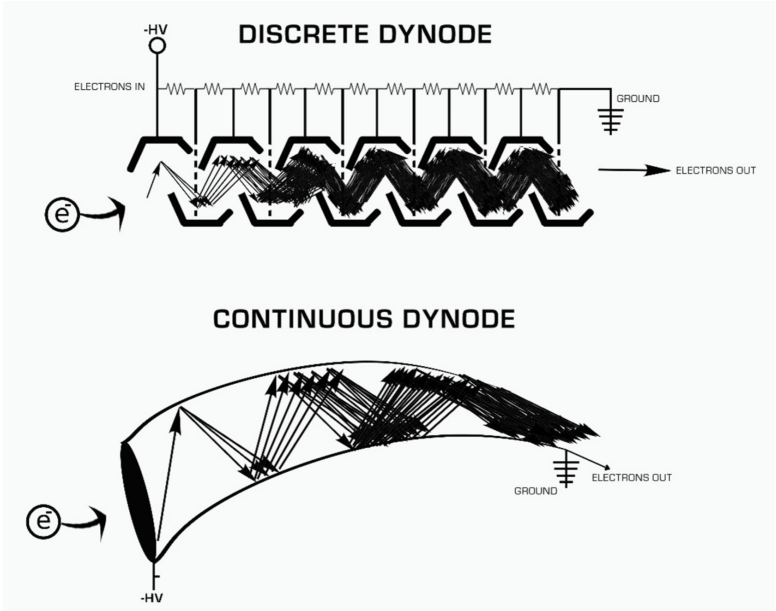
The detector (will not be discussed more in details later in the course)

- Electron multipliers (EM)
- Faraday cup (FC)
- Photomultiplier conversion dynode
- Array detectors

The mass spectrometer

The detector – Electron multiplier (EM)

- A **dynode electron multiplier (EM)** is a charged-particle detector that amplifies the signal generated by a single incoming ion through a cascade of **secondary electron emissions**.
- When an energetic ion strikes the **first dynode** (typically a metal surface biased at high voltage), it releases several low-energy secondary electrons due to kinetic energy transfer.
- These electrons are then accelerated toward the **next dynode**, which is held at a progressively higher potential. At each dynode stage, the number of electrons multiplies exponentially.



Rev. Sci. Instrum. 18, 739–749 (1947) https://doi.org/10.1063/1.1740838

The mass spectrometer

The detector – Electron multiplier (EM)

Pulse Height Distribution & Noise Discrimination

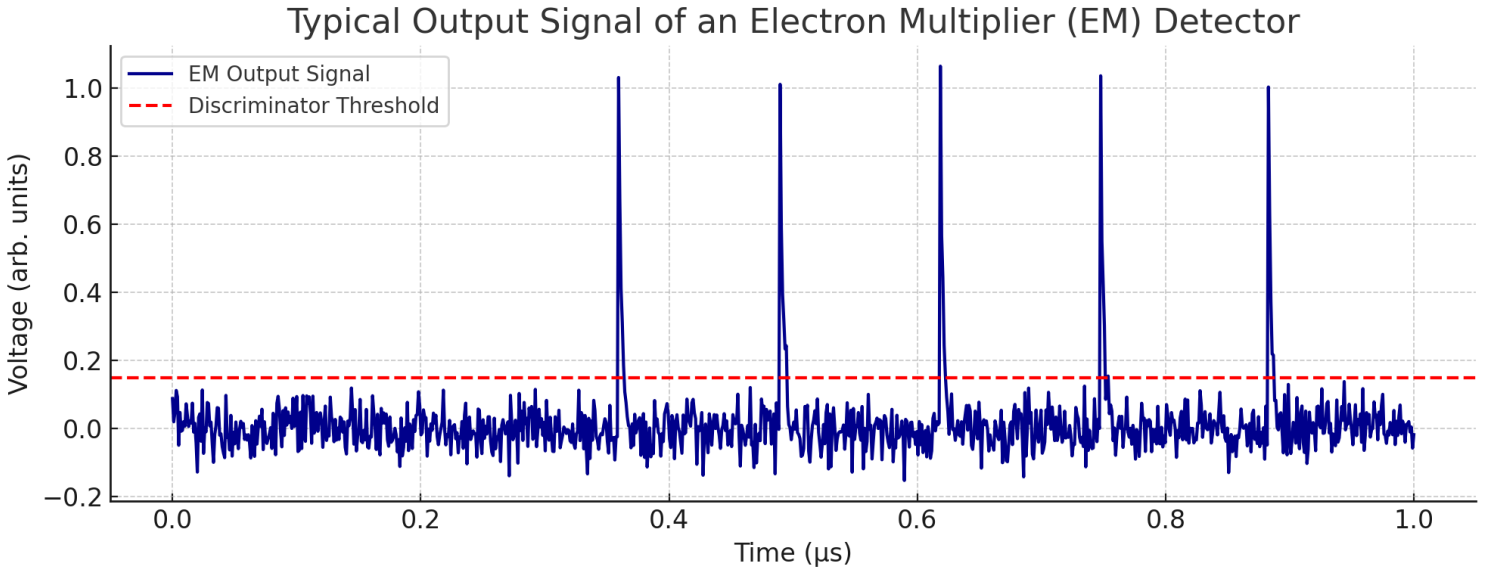
- The total electron gain G of an EM with n dynodes and a secondary emission coefficient δ per stage n is:

$$G = \delta^n$$

- **Typical gains range from 10^5 to 10^8**, allowing **single-ion detection** as discrete voltage pulses.
- The **output signal** of an electron multiplier consists of a sequence of voltage pulses.
- The **pulse height distribution (PHD)** is stochastic due to variations in the secondary electron yield and cascade amplification. The PHD typically shows two distinct regions:
  - **Low-amplitude region:** Dominated by **electronic noise**, showing an exponentially decreasing signal.
  - **High-amplitude region:** Represents **true ion events**, typically broader due to variability in gain per impact.
- To distinguish real ion impacts from background noise, a **discriminator threshold** is set just above the noise floor. Pulses exceeding this threshold are counted as ion events.

The mass spectrometer

The detector – Electron multiplier (EM)



The mass spectrometer

The detector – Electron multiplier (EM)

Detection Limitations

Electron multipliers have an effective **dynamic range** up to approximately 10^6 counts per second (Hz).

Beyond this, performance degrades due to two main phenomena:

Dead Time (τ):

After an ion impact, the detector requires a short recovery period (typically 10–100 ns) during which it cannot register new events. This leads to count loss at high flux rates.

The corrected count rate R for non-paralyzable systems is:

$$R = \frac{r}{1 - r\tau}$$

where: r is the observed count rate and τ is the dead time.

Pile-up or Coincidence Effects:

When multiple ions arrive within a time window shorter than the pulse width, they may produce a single indistinguishable signal — leading to **undercounting**. This “quasi-simultaneous arrival” effect becomes prominent at high ion fluxes.

The mass spectrometer

The detector – The Faraday cup (FC)

- A Faraday Cup (FC) is a **direct-current ion detector** that operates on the principle of **charge collection**.

- When a beam of ions **strikes the conductive metal cup** (typically stainless steel, graphite, or platinum), the ions are neutralized upon contact with the inner surface, transferring their charge to the conductor. This accumulated charge results in a small current, which is measured by a sensitive electrometer connected to the cup.

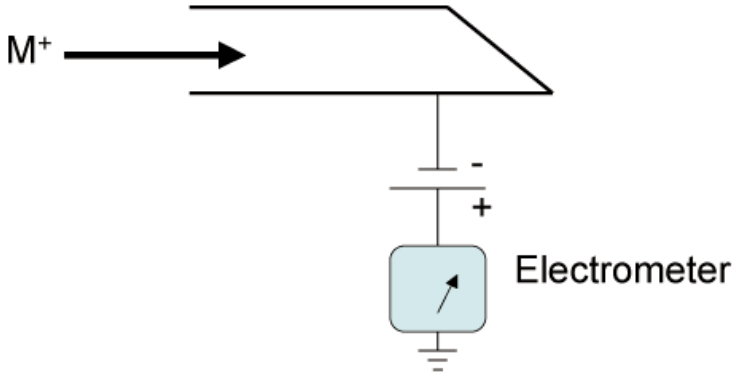
- The measured current I is directly proportional to the **ion flux** φ :

$$I = q \cdot \varphi$$

where:
q is the charge of the ion (C),
φ is the ion arrival rate or ion flux (ions/s).

The mass spectrometer

The detector – The Faraday cup (FC)



Faraday cup with an electron-suppressor plate in front

- To suppress secondary electrons emitted upon impact (which would otherwise cause signal loss), the FC includes a **suppressor electrode** — a negatively biased grid placed in front of the cup, typically at –100 V to –300 V. This bias ensures that ejected electrons are repelled back into the cup, preserving charge accuracy.

Rev. Sci. Instrum. 27, 696–702 (1956), https://doi.org/10.1063/1.1715674

The mass spectrometer

The detector – The Faraday cup (FC)

- Faraday Cups are **absolute detectors**, requiring no prior calibration for ion identity or gain.
- Their linear response and robustness make them ideal for **high-flux, quantitative applications**, such as isotope ratio mass spectrometry (IRMS) or ion beam profiling.
- However, they are inherently **less sensitive** than electron multipliers: their detection limit is in the **picoampere to femtoampere range**, corresponding to ~10^5–10^7 ions/s, depending on setup and noise floor.
- Because FCs lack gain mechanisms, they are not suitable for single-ion detection. Nonetheless, they offer superior **linearity, stability**, and **dynamic range** (up to 10^9 Hz), making them the preferred choice for accurate, long-duration measurements in systems where ion current is sufficiently high.

The mass spectrometer

The detector – The photomultiplier tube (PMT) with conversion dynode

- A **photomultiplier tube (PMT) with conversion dynode** is a hybrid ion detection system that combines the ion sensitivity of an electron multiplier with the ultra-low-light amplification of a photomultiplier. It operates by **converting incoming ions into photons**, which are then detected by the PMT.

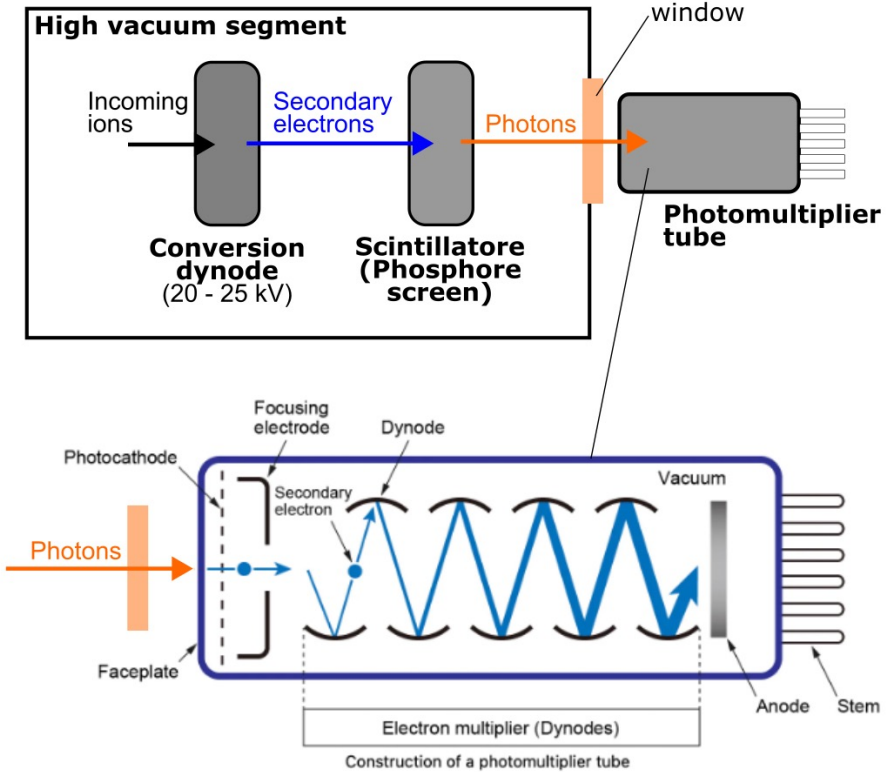
- The process begins when a high-energy ion strikes a **conversion dynode**—a surface held at a large positive potential (typically a polished aluminum foil at +20–55 kV relative to ground). The ion impact liberates several high-energy secondary electrons, which are accelerated toward a **scintillator** (e.g. phosphor screen). Upon striking the phosphor, these electrons generate a brief burst of visible photons.

- These photons are collected by a **photomultiplier tube**, typically positioned behind a vacuum viewport (window) or light guide. The PMT contains a photocathode and a cascade of dynodes that amplify the signal by secondary emission, with total gains reaching 10^6–10^8. Each ion event produces a sharp, discrete voltage pulse suitable for **single-ion counting**.

The mass spectrometer

The detector – The photomultiplier tube (PMT) with conversion dynode

- **Incoming ions** (black arrow) strike the conversion dynode held at high positive potential.
- The impact releases **secondary electrons** (blue arrow), which are accelerated onto a phosphor screen.
- The **phosphor scintillator emits visible photons** (orange arrow), which are directed into a photomultiplier tube (PMT).
- The **PMT converts the photon** burst into an **amplified electrical pulse**, enabling fast, sensitive, single-ion detection.



Construction of a photomultiplier tube

The mass spectrometer

The detector – The photomultiplier tube (PMT) with conversion dynode

- PMT conversion dynode detectors offer **exceptional time resolution (sub-nanosecond)** and are capable of operating at **very low background noise levels**, owing to the photon-based detection mechanism. Their sensitivity rivals that of dynode electron multipliers, but with higher immunity to electronic interference and greater tolerance for high kinetic energy ions (e.g., >10 keV).

- They are ideal for **time-of-flight mass spectrometry (TOF-MS)**, particularly in high-resolution or pulsed-laser desorption setups, where fast response and low false-positive rates are critical.

- However, like all single-ion detectors, they are limited by **dead time** (typically 10 – 30 ns) and **pile-up** effects at high count rates. Their complex optical assembly and need for vacuum-isolated photon transmission introduce additional design considerations.

- In summary, conversion-dynode PMTs provide **fast, high-sensitivity, and low-noise** ion detection with robust gain and temporal fidelity, making them a benchmark choice in precision TOF and high-speed mass spectrometric analysis.

The mass spectrometer

The detector – The array detector

- An **array detector** in mass spectrometry is a position or time-sensitive detection system composed of multiple discrete sensing elements (pixels), enabling the **simultaneous detection of a spatially or temporally dispersed ion beam** most commonly in **time-of-flight (TOF)** or **imaging MS** setups.

- Array detectors are **multi-pixel ion sensors** used to record the **arrival position, intensity, or timing** of ions across a dispersed plane or axis. Unlike single-point detectors (e.g., Faraday cups or electron multipliers), they capture **entire mass spectra or ion images in parallel**.

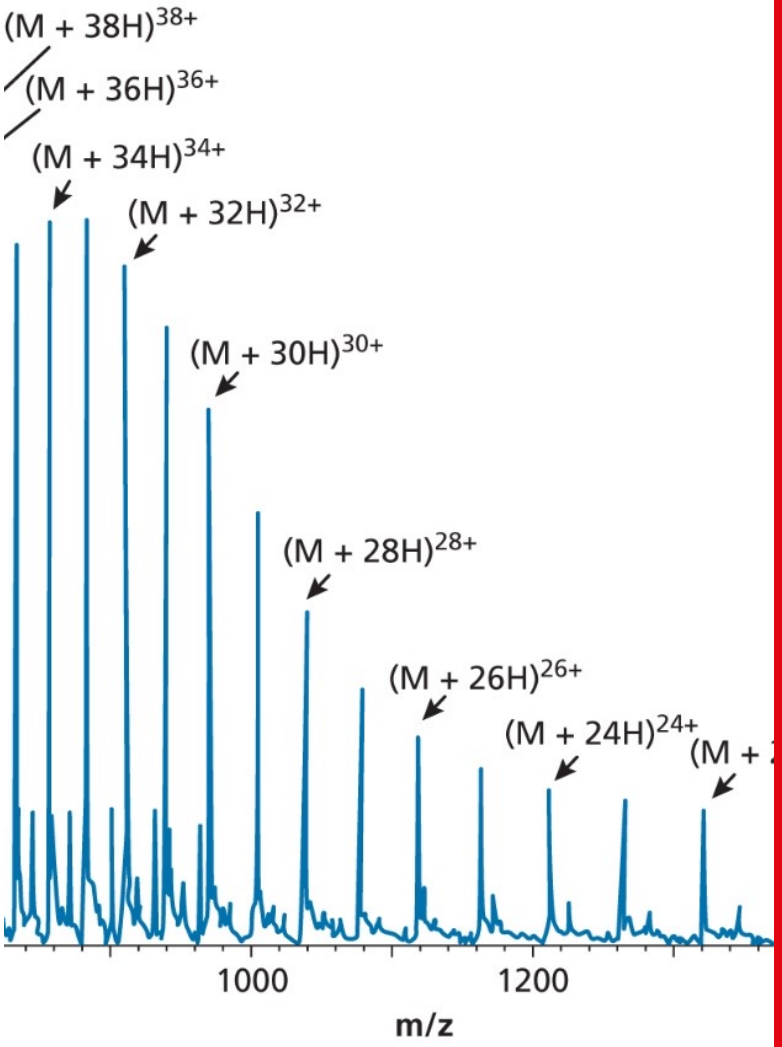
- In TOF-MS or MALDI imaging, ions are dispersed based on flight time or position and strike a **planar detection surface**. Array detectors register:

  - **Time-of-flight:** Each pixel captures the arrival time of ions (via TDC or delay-line systems).
  - **Intensity:** The charge or light output per pixel correlates to ion abundance.
  - **Spatial location:** Position-sensitive systems resolve ion beam profiles or create molecular images.

The mass spectrometer

What characterizes a MS spectrometer ? (almost right for any analytical instrument)

- Sensitivity
- Resolution
- Selectivity
- Domain of application
- Throughput
- Cost of acquisition
- Cost of operation



The mass spectrum

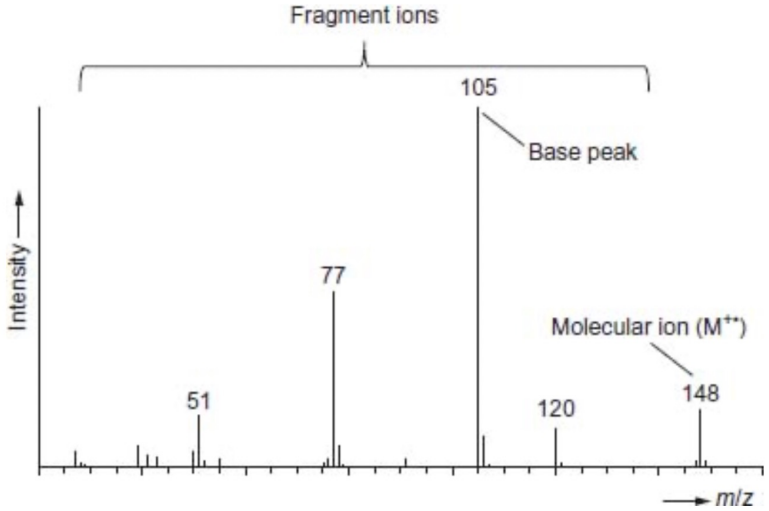
- Isotopic distributions
- Continuum and centroid spectra
- Charge state

The mass spectrum

- The output of a mass spectrometer is known as a mass spectrum (plural spectra).
- This is a plot of relative ion abundance on the vertical axis versus m/z on the horizontal axis.

Electron ionization (EI) generated **mass spectrum**, therefore contains a **radical cation** arising from removal of an electron from the molecule. This is known as the **molecular ion (M+•)**.

The spectrum also displays **fragment ions** caused by dissociation of M+• in the EI source. The most abundant ion (m/z 105 in this example) is termed the **base peak**.



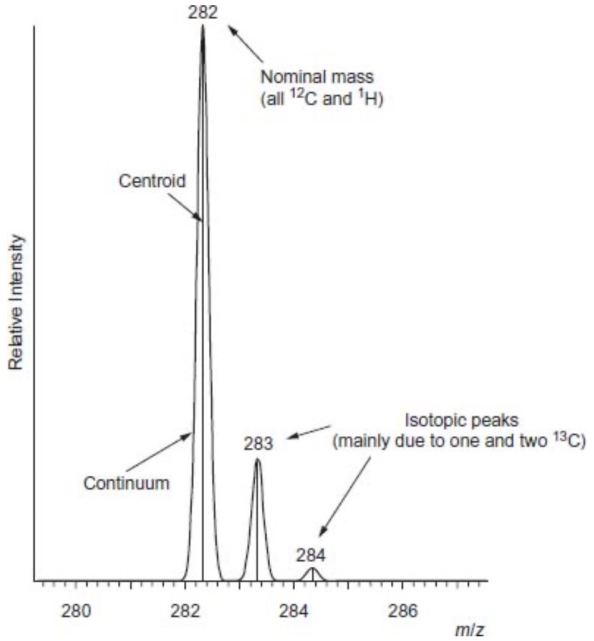
The mass spectrum

Isotopic distributions

- For small molecules, most mass analyzers are easily able to separate the 1 Da mass difference caused by the presence of heavier isotopes of the same element.
- This allows the measurement of isotopic ratios within the sample molecule.

- Close-up of the molecular ion of the hydrocarbon eicosane, $C_{20}H_{42}$.

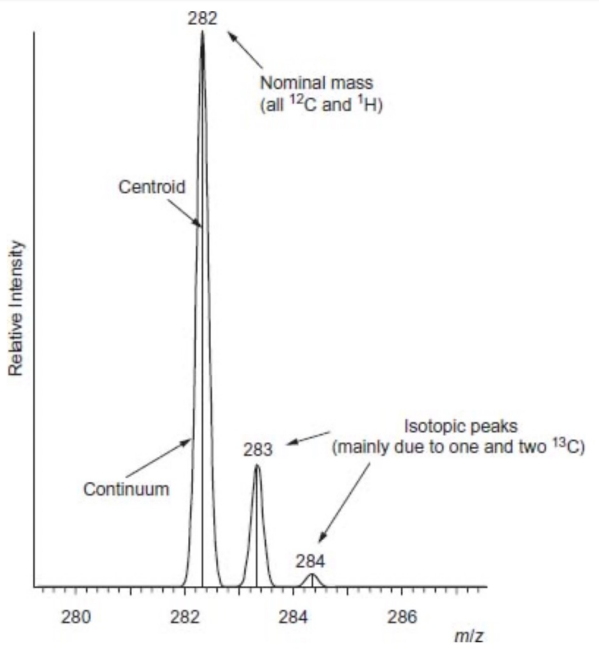
- The nominal mass, 282, is indicated, as are the isotopic peaks at 283 and 284, which arise due to the natural presence of heavier isotopes of carbon and hydrogen.



The mass spectrum

Isotopic distributions

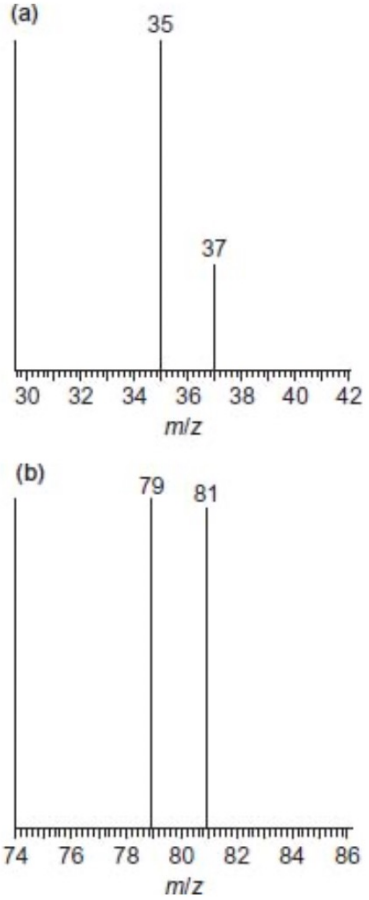
- The principal contribution to the isotopic peaks is from ^{13}C, which has a natural abundance of 1.07%. Deuterium (^{2}H) constitutes only 0.01% of hydrogen, and thus is only a very minor contributor to the signals at 283 and 284.
- The natural abundances of ^{14}C and ^{3}H are far too low to detect, and so the ion at 284 arises principally due to the presence of two ^{13}C atoms in the molecule..



The mass spectrum

Isotopic distributions

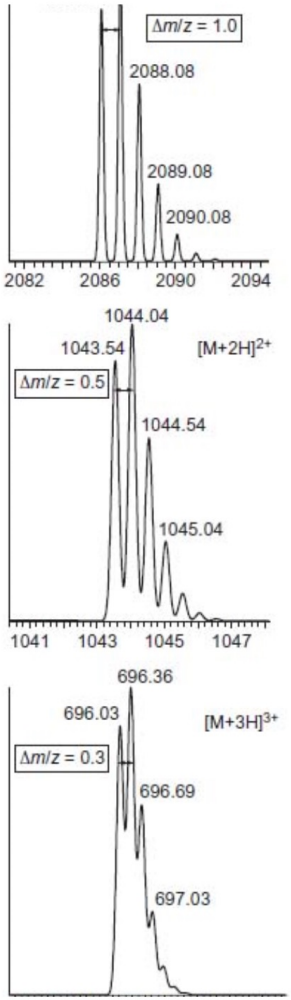
- The **halogens** Cl and Br produce **very distinctive isotopic patterns** due to $^{35}Cl/^{37}Cl$ and $^{79}Br/^{81}Br$.
- Which makes identifying the presence of these elements in molecules relatively straightforward.
- Likewise, some metals, such as chromium, iron, nickel, copper, zinc, and tin, have characteristic and often complex isotopic distributions. Tin, for example, has ten naturally occurring stable isotopes, all at appreciable concentrations.



The mass spectrum

Continuum & centroid spectra

- A mass spectrum is acquired by the instrument's detector as a continuous analogue response across the *m/z* scale.
- This signal is digitized for handling by a computer, which is able to reconstruct a continuum display by joining the discrete data points across the peak with a smoothed line.
- The result, known as a **continuum spectrum**
- The **advantage of this display is that it reveals the resolution achieved** in the measurement: in other words, how well neighboring *m/z* peaks are separated.
- The disadvantage is that data files are often large (megabytes of data) due to the high number of data points collected.

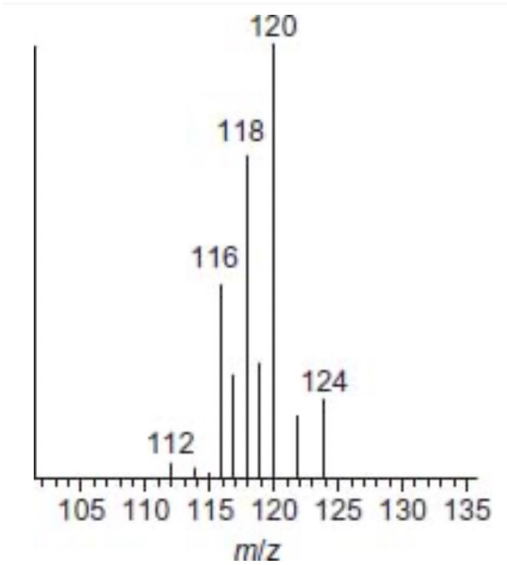


Examples of continuum spectra at different charges showing the difference in resolution

The mass spectrum

Continuum & centroid spectra

- An alternative way to display (and save) the data is as **a centroid spectrum**.
- This shows only the centre of each peak as a ‘stick’ plot, with one *m/z* and one intensity value.
- The centroid spectrum is often used for small molecules where unit mass resolution is achieved and nominal mass accuracy reported.
- In cases where it is necessary to show how well m/z peaks are resolved, a continuum spectrum is required.



Example of centroid spectrum showing the isotopic distribution of Tin (Sn). Resolution is not visible.

The mass spectrum

Charge state

- So far, we have only considered mass spectra where the **ions are singly charged, *z* = 1.**
- **This is almost always the case for small molecules ionized by EI.**
- **Larger molecules, such as peptides and proteins**, usually exhibit **higher charge states** when ionized by **electrospray ionization (ESI)**.
- This raises the question of how the charge state of an ion can be determined.
- If the mass of the molecule is known then the problem is a trivial one, as the charge will be that fraction of the mass at which the ion appears on the m/z scale.

  - For example, a peptide of mass 2085.07 Da will give rise to a singly-protonated ion ($[M+H]^+$) at *m/z* 2086.07, whereas the doubly-protonated ion ($[M+2H]^{2+}$) will be at *m/z* 1043.54, and the triply-protonated ion ($[M+3H]^{3+}$) at *m/z* 696.03

  - Note the need to take into account the mass of each added proton.

The mass spectrum

Charge state

- If the **molecular mass of the analyte is unknown**, then we **cannot** use this information to determine the charge state.
- **Providing that some degree of isotopic resolution is seen in the spectrum**, then the **separation of peaks in the isotopic cluster can be utilized to identify the charge stat**e without any prior knowledge of molecular mass.
- This is because we know that the mass separation between neighboring isotopic peaks (*Δm/z*) is 1 *Da* (the mass of the neutron).

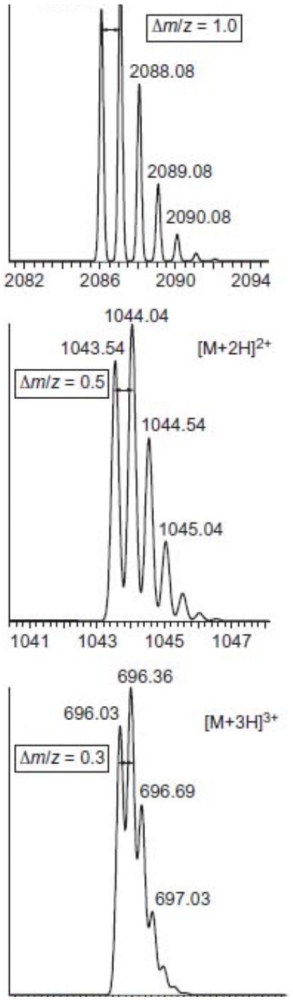
  - When the charge state z = 1 on the m/z scale, isotopic peaks will be separated by 1 (1/1).
  - When the charge state z = 2, isotopic peaks will instead be separated by Δm/z = 0.5 (1/2),
  - For z = 3 the m/z separation between isotopic peaks will be Δm/z = 0.33 (1/3).
  - …

The mass spectrum

Charge state

- The spectra on the right show the $[M+H]^{+}$, $[M+2H]^{2+}$, and $[M+3H]^{3+}$ for the hypothetical peptide of mass 2085.07 discussed previously.

- Since we know the m/z values from the measurement and can identify the charge state from isotopic separation in the spectrum, we can determine the mass of multiply-charged ions.



Questions ?