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Course handout

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Physicochemical Analysis Techniques II

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Preface

This course in Spectroscopy has been designed specifically for second-year undergraduate students in Chemistry (L2 Chemistry). It aims to provide a clear and structured introduction to the fundamental principles that govern interaction between light and matter. Spectroscopy is an essential part of modern chemical education, serving as both a theoretical foundation and a practical analytical tool for understanding molecular and behavior structure.

The objective of this course is to familiarize students with the major spectroscopic techniques used in chemical analysis, such as ultraviolet-visible (UV-Vis), infrared (IR), Raman, and nuclear magnetic resonance (NMR) spectroscopy. Emphasis is placed on both the conceptual understanding of physical principles and the practical interpretation of experimental data.

Since 2018, I have had the privilege of teaching this course to undergraduate chemistry students. Over the years, I have continuously refined its content to make it more accessible and relevant to learners at this level. The present handout (polycopied course material) summarizes the key concepts, definitions, and examples discussed in lectures and work directed sessions. It is intended as a learning companion to help students grasp the essential aspects of spectroscopy and to encourage further exploration of its numerous scientific and industrial applications.

I sincerely hope that this material will assist students in developing not only a solid understanding of spectroscopic methods but also an appreciation for the power of spectroscopy as a window into the microscopic world of atoms and molecules.

SUMMARY

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CHAPTER 1: INTRODUCTION TO SPECTRAL METHODS

CHAPTER 2: ABSORPTION LAWS AND APPLICATION OF BEER-LAMBERT'S LAW TO UV-VISIBLE SPECTROPHOTOMETRY

CHAPTER 3: ATOMIC ABSORPTION SPECTROPHOTOMETRY

CHAPTER 4: INFRARED SPECTROSCOPY

CHAPTER 5 : NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

CHAPTER 6 : MASS SPECTROSCOPY

General Introduction

Spectroscopy is a broad field of science that investigates how matter interacts with electromagnetic radiation (light). By analyzing these interactions, spectroscopy allows us to probe the physical, chemical, and electronic structure of materials, from simple atoms to complex molecules and condensed matter. It is a central tool in physics, chemistry, astronomy, and many applied sciences.

What is Spectroscopy?

At its core, spectroscopy examines the absorption, emission, or scattering of electromagnetic radiation by atoms, molecules, or solids. When light interacts with matter, energy can be transferred between the electromagnetic field and the internal energy states of the matter. The nature of these energy exchanges reveals valuable information about the material's properties.

- Absorption spectroscopy measures how much light of specific wavelengths is absorbed by a sample. Peaks in the absorption spectrum correspond to transitions between energy levels.

- Emission spectroscopy examines light emitted by a sample, often after excitation, revealing which transitions release energy.

- Scattering spectroscopy investigates how the light is deflected by the sample, offering complementary structural and dynamic information.

Why Spectroscopy is Important

Spectroscopic techniques are essential for several reasons:

1. Structural information: Spectroscopy can identify functional groups in molecules, bond lengths and angles, and conformational states.

2. Quantitative analysis: By measuring how much light is absorbed or emitted, one can determine concentrations of substances.

3. Dynamic processes: Time-resolved spectroscopy tracks how molecular systems evolve over time after excitation (e.g., chemical reactions, energy transfer).

4. Material characterisation: In solid-state physics and materials science, spectroscopy helps to understand band structure, defects, and electronic properties.

5. Astrophysics and remote sensing: Spectroscopy is critical for identifying chemical composition in planetary atmospheres, stars, and galaxies, based on their emission or absorption lines.

Fundamental Concepts

To understand spectroscopy, one must be familiar with key fundamentals:
- Electromagnetic spectrum: Ranges from gamma rays and X-rays (high energy, short wavelength) to radio waves (low energy, long wavelength). Different parts of the spectrum probe different types of transitions (e.g. UV–visible for electronic transitions, IR for vibrational, microwave for rotational).

Energy levels and transitions: In atoms and molecules, electrons, vibrations, and rotations occupy discrete energy levels. Transitions between these levels correspond to absorption or emission of photons with energies matching the difference.
- Selection rules: Not all transitions are allowed — quantum mechanical rules determine which energy transitions are observable.

- A Spectral line shapes and broadening: Real spectra include effects like Doppler broadening, pressure broadening, and instrumental broadening, which influence resolution and information content.

I. Definition

Spectroscopy is the scientific discipline that concerne the study of electromagnetic radiation emitted, absorbed, or scattered by matter. Such interactions correspond to transitions of matter from one quantum state to another. The analysis of radiation into its different frequencies is performed with instruments called spectrographs or spectrometers, which make it possible to obtain the corresponding electromagnetic spectra.

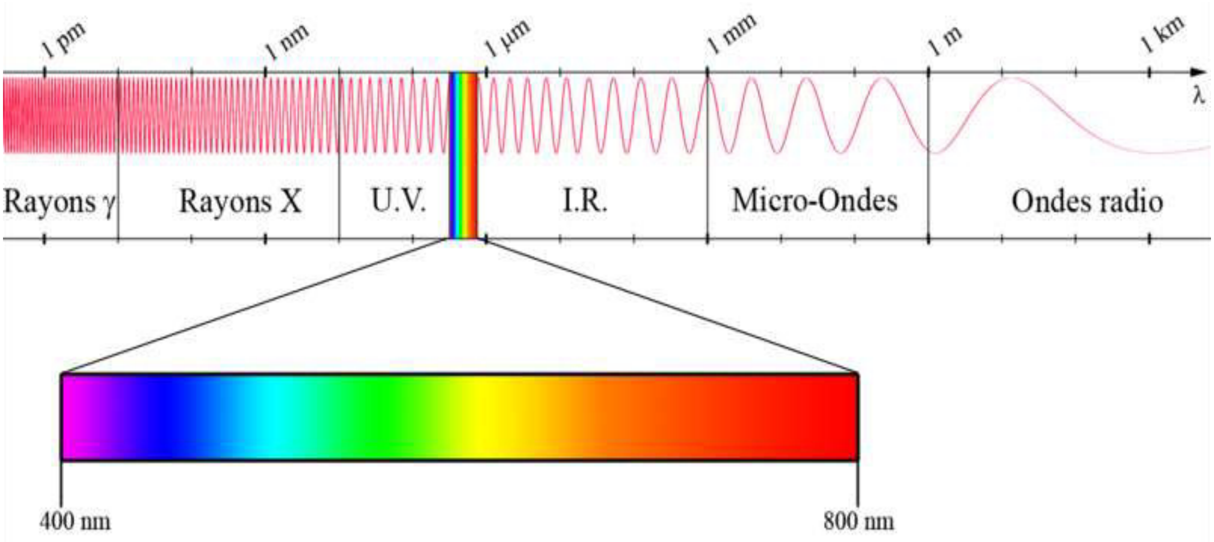


Figure 1: Electromagnetic spectrum

I.2. Interaction of Electromagnetic Waves with Matter

According to Max Planck's hypothesis, energy exchanges between matter and radiation occur in discrete packets called quanta. The relationship is given by the well-known equation: $E = h\nu$, where h is Planck's constant, equal to 6.624×10^{-34} joule·seconds.

In simpler terms, the energy radiated by matter is proportional to the frequency of the radiation with which it interacts.

I.3. Different forms of energy

Molecules can store and exchange energy in four distinct modes of motion:

Translational motion

Rotational motion

Vibrational motion

Electronic motion (linked to electron cloud deformation).

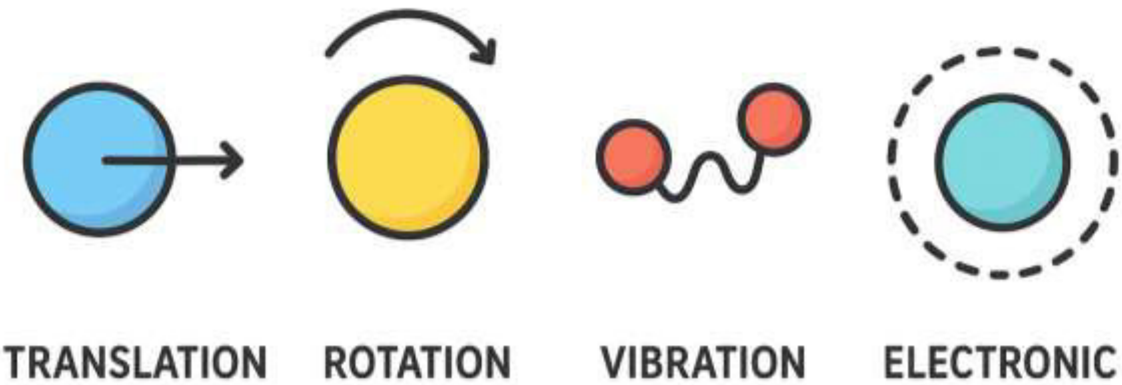


Figure 2: Different forms of energy

As a first approximation, the uniform translational movement of the entire molecule can be separated, since it is not quantized.

Because nuclei are thousands of times heavier than electrons, their motion is much slower. This distinction allows us to apply the Born-Oppenheimer approximation, where nuclei are treated as fixed when analyzing electronic motion.

Thus, the total molecular energy can be written as: $E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$, with magnitudes generally ordered as $E_{electronic} >> E_{vibrational} >> E_{rotational}$.

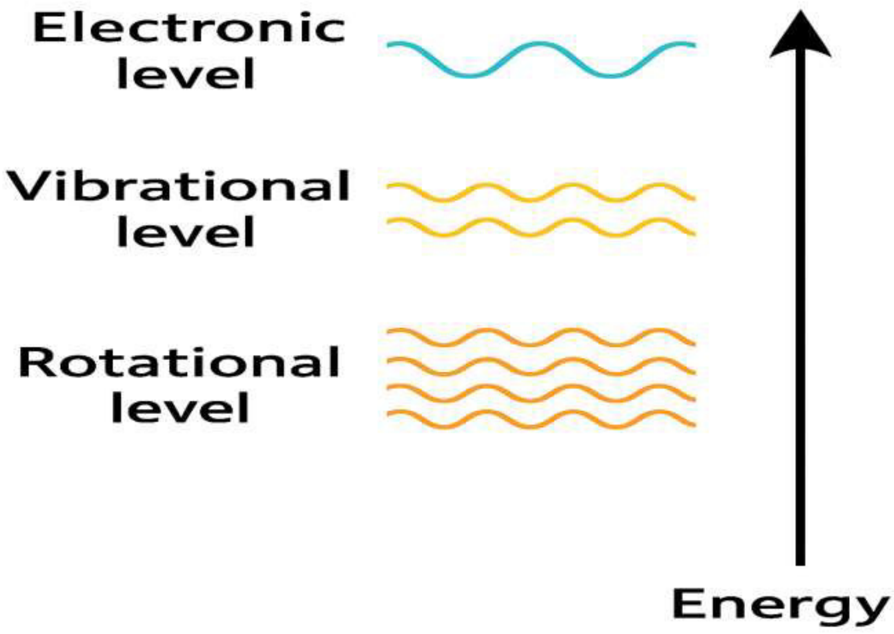


Figure 3: Energy level diagram

IV.1. Types of Spectra

When matter interacts with electromagnetic radiation, transitions occur that manifest as line or band spectra.

IV.2.1. Line spectra:

In atoms, variations in electronic energy give rise to discrete spectral lines, each corresponding to a monochromatic radiation.

IV.2. Band Spectra:

In molecules, transitions involve not only electronic levels but also vibrational and rotational sublevels. This results in groups of closely spaced transitions that appear experimentally as bands.

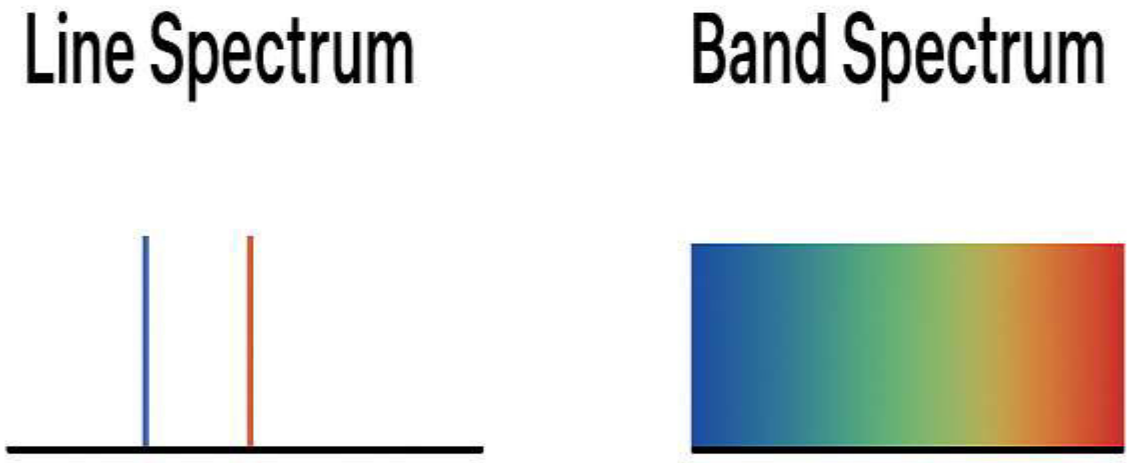


Figure 4: Types of Spectra

V. Electromagnetic Spectrum

The visible region of solar light represents only a very small part of the total electromagnetic spectrum. Beyond it lie invisible regions, characterized by different wavelengths, frequencies, and energies, ranging from gamma rays to radio waves.

The spectrum therefor, provides a continuous classification of all known electromagnetic waves.

VI. Different Types of Spectroscopies

Spectroscopy investigates the various possible energy transitions predicted by quantum theory. These transitions span a very wide energy range across the electromagnetic spectrum, giving rise to highly diverse techniques, such as:

VI.1. Gamma-ray spectroscopy

Involves very high-energy photons capable of affecting atomic nuclei. These rays are hazardous to biological tissues but provide characteristic nuclear signatures.

VI.2. X-ray spectroscopy

Related to the excitation of inner-shell electrons, largely independent of chemical bonding. Extensively used for qualitative and quantitative elemental analysis.

VI.3. UV-visible spectroscopy

Involves transitions in outer electronic shells, widely applied in both atomic and molecular studies. Applications include astronomy, chemical analysis, and materials characterization.

VI.4. Infrared (IR) spectroscopy

Sensitive to vibrational and rotational transitions of molecules, making it fundamental for molecular structure determination.

VI.5. Microwave spectroscopy

Focused mainly on molecular rotational transitions, providing precise information about bond lengths and molecular geometry.

VI.6. Radio-wave spectroscopy

Includes nuclear magnetic resonance (NMR), which is based on the transitions between spin states of nuclei under a strong magnetic field, offering critical insights into molecular and biological systems.

Note: Optical spectroscopies involve X-rays, UV, visible, and IR radiation, while microwave and radio-frequency spectroscopies are often referred to as "Hertzian spectroscopies." In the optical domain, transitions usually occur via electric dipole interactions, while in the radio-frequency domain, magnetic dipole interactions often dominate.

I. Introduction

Absorption spectroscopy in the ultraviolet-visible (UV-Visible) region is an essential analytical method, widely used in chemistry, biology, material sciences, and environmental studies. It is based on the interaction of matter with light, providing insight into the electronic structure of molecules.

When a light beam passes through a sample, certain wavelengths, usually between 200 and 800 nm, are absorbed by the electrons in molecular orbitals. These electrons are then excited to vacant orbitals of higher energy. The intensity and position of the observed absorption bands provide valuable information on the presence of chromophores and the electronic structure of the molecule.

This technique is highly appreciated because it is rapid, non-destructive, and versatile. It allows both qualitative analyses (identification of functional groups) and quantitative analyses (determination of concentrations using the Beer-Lambert law).

II. Principle and Selection Rules

A UV-Visible transition (typically between 180 and 750 nm) corresponds to the excitation of an electron from an occupied molecular orbital (ground state) to a vacant orbital of higher energy (excited state). The material absorbs a photon whose energy exactly matches the energy difference between these two levels: $\Delta E = h\nu = hc/\lambda$.

However, not all energetically possible transitions are allowed. Only those that induce a change in the electric dipole moment of the system are observed. These conditions are expressed through selection rules, derived from quantum mechanics.

II.1. Spin Selection Rule

A transition is allowed only if the total spin remains unchanged ($\Delta S = 0$). Thus, singlet → singlet transitions are allowed, while singlet → triplet transitions are forbidden (though they may occur weakly due to spin–orbit coupling).

II.2. Laporte Rule (Symmetry Rule)

In systems with a center of inversion, transitions between orbitals of the same parity (g → g or u → u) are forbidden. Only g → u or u → g transitions are allowed. For example, pure d–d transitions in octahedral complexes are forbidden, but may become partially allowed through vibronic coupling.

II.3. Orbital Overlap

The intensity of an absorption band also depends on the overlap between the involved orbitals. $\pi \rightarrow \pi^*$ transitions are generally strong and intense, whereas $n \rightarrow \pi^*$ transitions are weaker because orbital overlap is less effective.

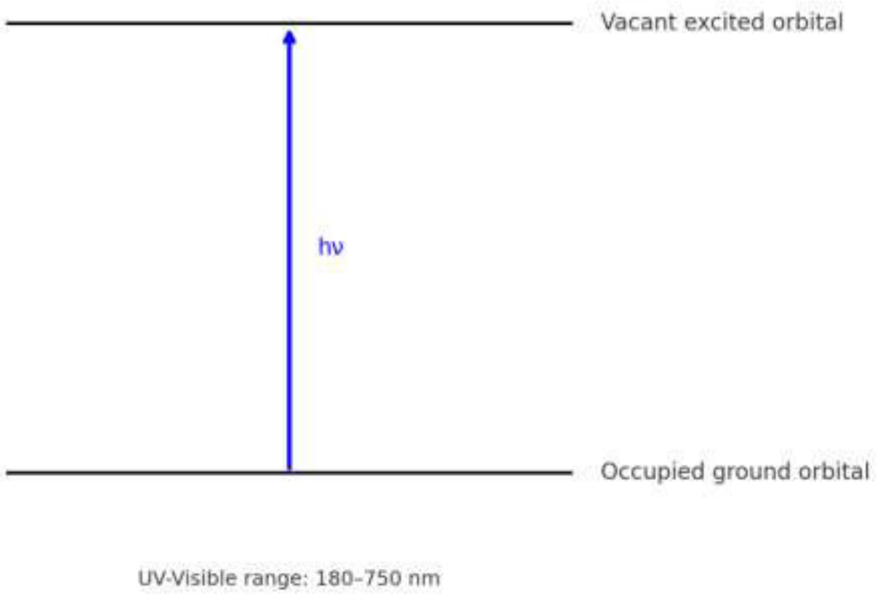


Figure 1: Electronic transition during UV-Visible absorption

III.1. Electronic Transitions

III.1.1. σ → σ* Transition

The strong stability of σ bonds in organic compounds makes the excitation of an electron from a bonding molecular orbital (σ) to an antibonding orbital (σ*) require a large amount of energy. As a result, the corresponding absorption band is intense and located in the far-UV region, around 130 nm. Because of its high energy, this transition is not commonly observed in typical UV-Vis spectroscopy applications, but it is important for saturated hydrocarbons and systems with only σ bonds.

Scheme

Orbitale liante σ ————————

↑ hν

Orbitale antiliante σ* ————————

III.1.2. n → π* Transition

This transition involves the excitation of a nonbonding electron (n), usually found as alone pair on a heteroatom (O, N, S, Cl, etc.), to an antibonding π* orbital. It typically occurs in molecules containing heteroatoms within unsaturated systems (such as C=O groups). The absorption band corresponding to this transition is weak because it is formally forbidden by selection rules. Nevertheless, it is often observed in the near-UV region and provides crucial structural information.

Scheme

Orbitale non liante n ————————

↑ hν

Orbitale antiliante π* ————————

III.1.3. n → σ* Transition

This transition results from the transfer of a nonbonding electron (n) on a heteroatom (O, N, S, Cl, etc.) to an antibonding σ* orbital. It is commonly observed in alcohols, ethers, amines, and halogen derivatives. The corresponding absorption band has a medium intensity and is typically found at the extreme limit of the near-UV region. Although less intense than π → π* transitions, these absorptions are useful for identifying the presence of specific functional groups.

Scheme

Orbitale non liante n ————————

↑ hν

Orbitale antiliante σ* ————————

III.1.4. π → π* Transition

One of the most important and common transitions in organic spectroscopy is the π → π* transition. It occurs in molecules containing isolated double bonds or conjugated systems. This transition produces strong absorption bands located in the range of 165–200 nm. In conjugated systems, the energy gap decreases, shifting the absorption to longer wavelengths. As a result, π → π* transitions play a central role in understanding color and chromophores in organic chemistry.

Scheme

Orbitale liante π ————————

↑ hν

Orbitale antiliante π* ————————

IV. Chromophores

The absorption of a photon in the UV-Visible domain can often be attributed to electrons belonging to small groups of atoms known as chromophores. Chromophores are functional groups responsible for characteristic absorption bands in a molecule. Examples include C=C, C=O, C=N, C≡C, and C≡N.

The exact absorption wavelength depends on the nature of the orbitals involved in the transition.

For instance, a C=O group typically exhibits an $n \rightarrow \pi^*$ transition, while conjugated C=C bonds favor $\pi \rightarrow \pi^*$ transitions. Understanding chromophores is essential in correlating molecular structure with absorption spectra, and it explains why certain compounds absorb light in the UV or visible regions, sometimes imparting color to the molecule.

V. Influence of Substituents and Spectral Shifts

V.1. Auxochromes

An auxochrome is a functional group that, when bonded to a chromophore, alters both the wavelength and the intensity of absorption.

Common auxochromes: –OH, $–NH_2$, $–OCH_3$, –Cl.

By resonance or inductive effects, they extend electron delocalization in the chromophore.

As a result, auxochromes often increase the absorption wavelength (λmax) and sometimes the intensity of the absorption band.

V.2. Bathochromic Effect (Red Shift)

A bathochromic shift corresponds to the displacement of the absorption maximum to longer wavelengths (lower energy).

It is generally caused by enhanced conjugation, the introduction of an auxochrome, or solvent interactions.

Example: Conjugation of a carbonyl group with a C=C double bond can shift absorption from ~170 nm to ~220 nm.

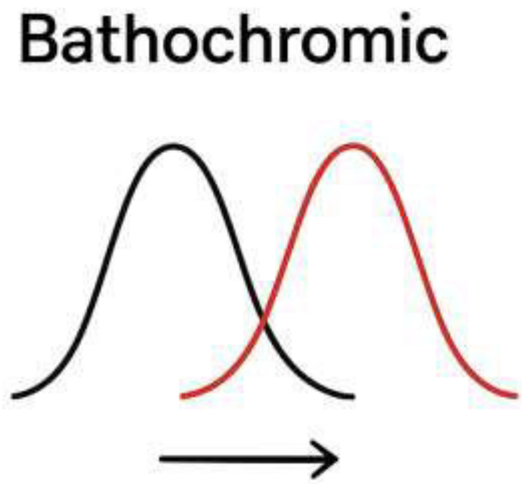


Figure 2: Bathochromic effect

V.3. Hypsochromic Effect (Blue Shift)

A hypsochromic shift is the movement of the absorption maximum to shorter wavelengths (higher energy).

It may occur when conjugation is reduced or when solvents stabilize the ground state more strongly than the excited state.

Example: Transitioning from a polar solvent to a nonpolar one may produce a hypsochromic shift.

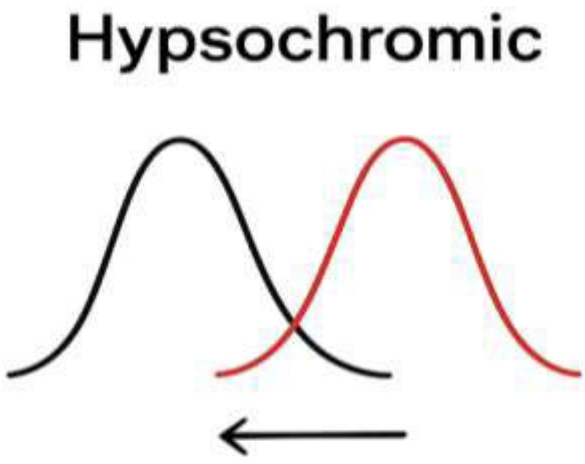


Figure 3: Hypsochromic effect

V.4. Hyperchromic Effect

A hyperchromic effect refers to an increase in absorption intensity.

It occurs when substituents or structural modifications raise the probability of an electronic transition.

Example: The –OH group on benzene increases absorption intensity due to enhanced resonance.

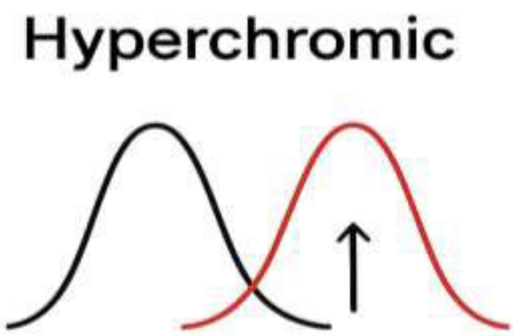


Figure 4: Hyperchromic effect

V.5. Hypochromic Effect

A hypochromic effect represents a decrease in absorption intensity.

It is often due to steric hindrance, molecular aggregation, or restricted conjugation.

Example: π–π stacking between aromatic rings reduces the absorption intensity in solution.

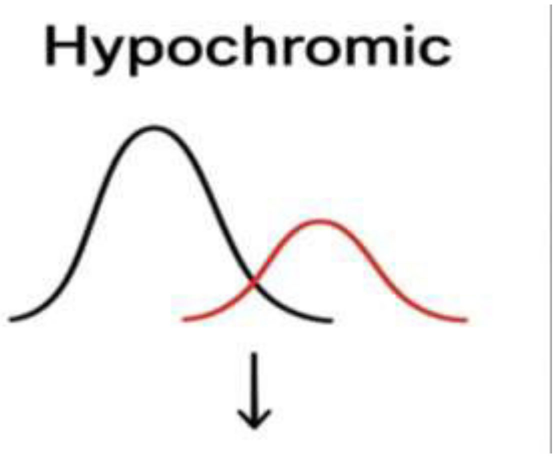


Figure 5: Hypochromic effect

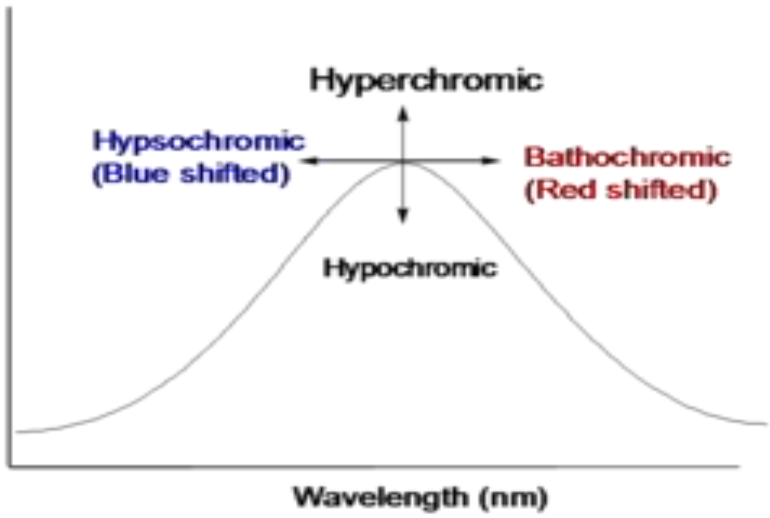


Figure .6: Global effect

Table .1: Main Chromophores and their Absorption Characteristics

Chromophore	Typical Transitions	λmax Range (nm)	Comments
C=C (double bond)	$\pi \rightarrow \pi^*$	160 – 190	Strong absorption in the far UV
C≡C (triple bond)	$\pi \rightarrow \pi^*$	170 – 200	Weak intensity, symmetry-forbidden transition
C=O (carbonyl group)	$n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$	270 – 300 ($n\rightarrow\pi^*$), 180 – 200 ($\pi\rightarrow\pi^*$)	Highly sensitive to conjugation and solvent
C=N (imine)	$n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$	250 – 290	Similar to carbonyl, often weaker
C≡N (nitrile)	$\pi \rightarrow \pi^*$	180 – 190	Sharp and low intensity band
Aromatic ring (benzene)	$\pi \rightarrow \pi^*$	180 – 260	Multiple bands, λmax increases with conjugation
Nitro group ($-NO_2$)	$n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$	270 – 320	Strong auxochromic influence
Azo group (–N=N–)	$\pi \rightarrow \pi^*$	350 – 500	Responsible for coloration in azo dyes

Conjugated dienes (C=C–C=C)	π → π*	220 – 270	λmax increases with conjugation
Conjugated polyenes	π → π*	250 – 500+	Large bathochromic shifts, possible visible coloration

Summary of the Chromophores Table:

1. **Simple Multiple Bonds (C=C, C≡C)**

   Double and triple bonds contain π electrons that can undergo excitation (π → π*).

   They absorb in the far UV region (160–200 nm).

   The intensity depends on symmetry and conjugation: the more conjugated the system, the more the absorption shifts toward the visible region (**bathochromic effect**).

2. **Carbonyl Groups (C=O) and Imines (C=N)**

   These groups contain non-bonding (n) electrons.

   Two transitions are possible:

   n → π* (weak intensity, ~270–300 nm).

   π → π* (intense, ~180–200 nm).

   Strongly affected by conjugation and solvent polarity.

3. **Nitrile Groups (C≡N)**

   Exhibit π → π* transitions, very localized around 180–190 nm.

   Bands are sharp and of low intensity.

4. **Aromatic Rings (Benzene and Derivatives)**

Show several π → π* transitions.

Absorb between 180–260 nm. Extended conjugation (naphthalene, anthracene, etc.) shifts absorption into the visible region.

This explains why many dyes are based on aromatic systems.

5. **Nitro (–NO_2) and Azo (–N=N–) Groups**

Strongly influence electronic transitions.

NO_2: both n → π* and π → π* transitions, ~270–320 nm.

N=N: π → π* transitions, ~350–500 nm → responsible for coloration in azo dyes.

6. **Conjugated Systems (Dienes, Polyenes)**

The longer the conjugated chain, the higher the λmax.

Long polyenes absorb in the visible region (e.g., β-carotene → orange).

General rule: conjugation shifts absorption to longer wavelengths (bathochromic shift).

VI. How to interpret a UV-Visible spectrum?

A UV-Visible spectrum represents the absorbance of a molecule as a function of the incident wavelength. Interpretation is based on two aspects:

Position of absorption maxima (λmax): Provides information about the nature of the electronic transitions (π→π*, n→π*, σ→σ*) and the structure of the chromophore.

Intensity of absorption bands (A or ε): Indicates the probability of the electronic transition. A stronger band reflects a more allowed transition.

VI.1. Practical uses

Identification of functional groups (comparison with reference λmax tables).

Monitoring chemical kinetics (variation of absorbance over time).

Purity control and quantitative assays in analytical chemistry.

VI.2. Chromatic circle (Color perception):

When a molecule absorbs light at a given wavelength in the UV-Vis domain, the human eye perceives the complementary color of the absorbed light.

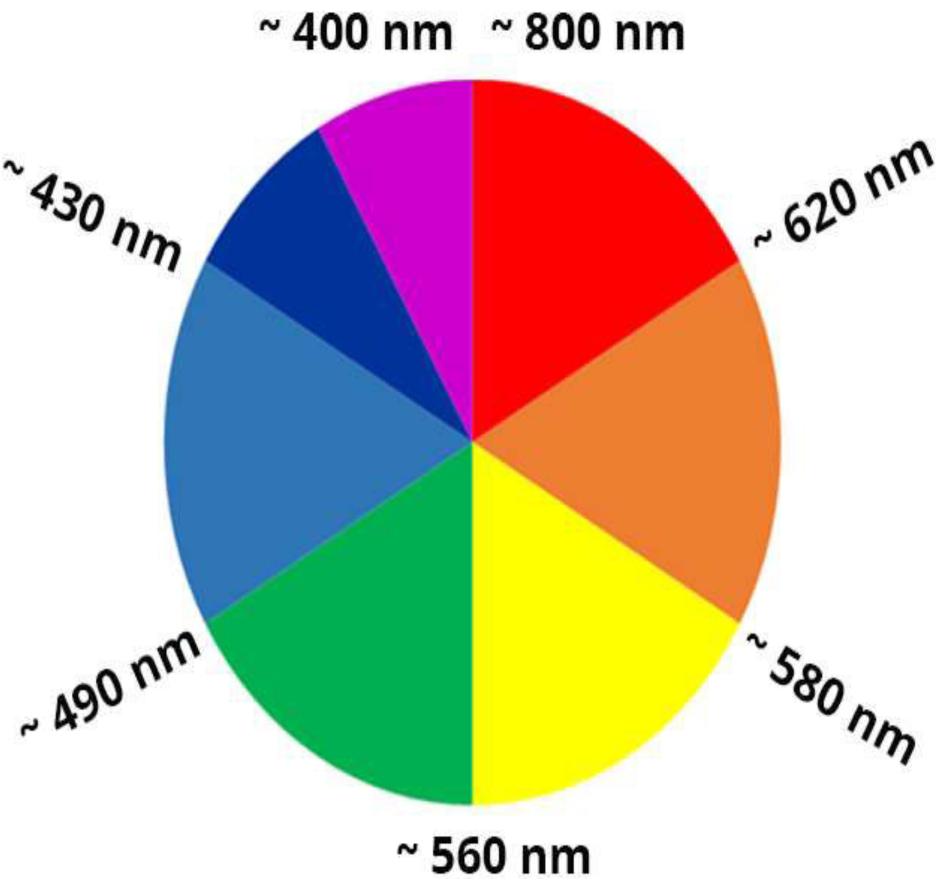


Figure.7: Chromatic circle

Example relations:

Table .2: Color perception

Absorption wavelength (nm)	Absorbed color	Observed (complementary) color
~450 nm (blue)	Blue	Orange
~500–520 nm (green)	Green	Red
~650 nm (red)	Red	Green
~580 nm (yellow)	Yellow	Violet

VI.3. Quantitative analysis (Beer-Lambert law)

The Beer-Lambert law describes the linear relation between absorbance and concentration:

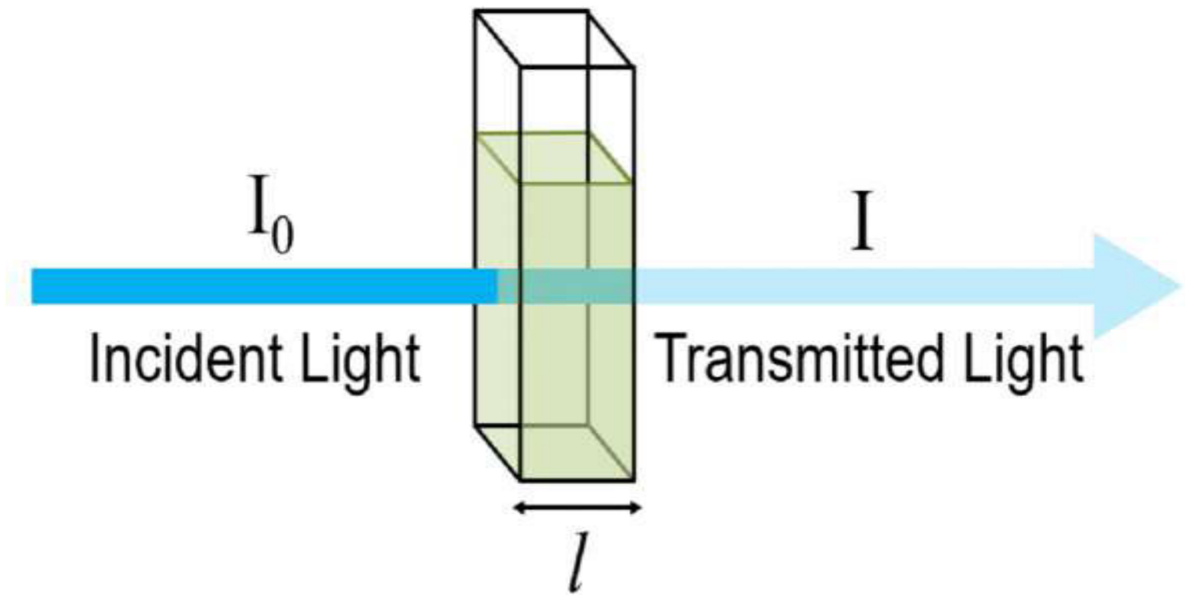


Figure.8: Beer-Lambert Law | Transmittance and Absorbance

$$\boldsymbol{A = \varepsilon l C}$$

Where:

A = absorbance (no unit)

ε = molar extinction coefficient ($L \cdot mol^{-1} \cdot cm^{-1}$)

l = path length of the cell (cm)

C = concentration of the solution ($mol \cdot L^{-1}$)

Conditions for validity:

Solution must be homogeneous and not too concentrated.

Absorbance must remain within the linear range ($A < 2$).

Applications:

Determining unknown concentrations.

Quantitative monitoring of chemical or biological reactions

VI.5. Additivity law of absorbances

In a mixture of absorbing species, the total absorbance at a given wavelength is the sum of the individual absorbances:

$$\boldsymbol{A_\lambda = \sum_{i=1}^{n} A_{i,\lambda}\ ,\quad A_\lambda = \sum_{i=1}^{n} \varepsilon_{i,\lambda}\, C_i\, l}$$

Applications:

Analysis of mixtures.

Simultaneous quantification of multiple compounds (by measuring at different λmax)

I. Introduction

Atomic Absorption Spectrophotometry (AAS) is a reference analytical method widely used to measure the concentration of metallic elements in various samples. Its importance lies in its high specificity and sensitivity. For example, AAS is used to monitor toxic heavy metals such as lead or cadmium in drinking water, or to ensure the correct nutritional composition of food products. The technique was first formalized by Alan Walsh in the 1950s, and since then, improvements in optics, electronics, and computing have made it one of the most robust and reliable tools in modern analytical chemistry.

In this lecture, we review the theoretical basis of AAS, the design of the instrument, the nature of the flame and the graphite furnace as atomizers, the sources of interferences, and a wide range of applications.

II. Principle and Theory

The fundamental principle of AAS is the absorption of electromagnetic radiation by free atoms. When atoms are in the gaseous state, they can absorb photons of very specific wavelengths that correspond to electronic transitions unique to each element. As shown in Figure 1, the instrument is designed to produce free atoms and measure how much of the light emitted by a source lamp is absorbed.

This behavior is quantitatively described by the Beer–Lambert law:

$$\boldsymbol{A = log\frac{I_0}{I} = k\,c\,l}$$

where A is the absorbance, I_0 is the incident light intensity, I the transmitted intensity, c the concentration of analyte, l the path length of the cell, and k a proportionality constant.

Because the atomic absorption lines are extremely narrow (picometers), the technique offers excellent selectivity and minimizes spectral overlap.

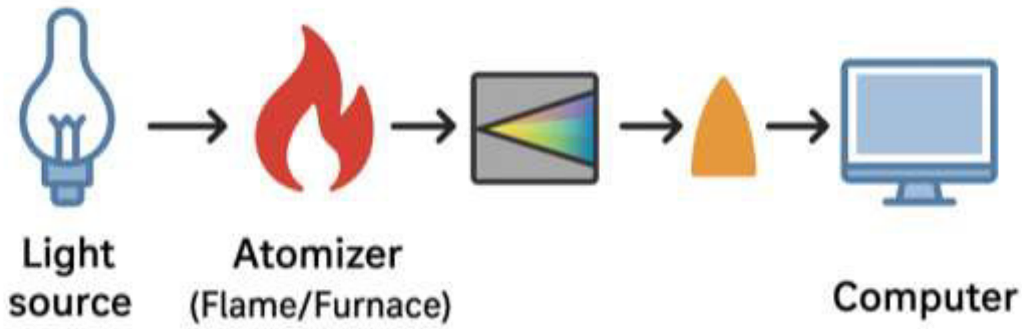


Figure.1: Diagram of an Atomic Absorption Spectrophotometer

III. Instrumentation

The instrumentation of AAS includes several modules working together (Figure 1). The light source, usually a hollow cathode lamp, emits the characteristic wavelength of the element being analyzed. The sample solution is introduced into the nebulizer, which generates an aerosol that passes into the atomizer (flame or furnace). The monochromator isolates the absorption line of interest, and the detector converts the transmitted light into an electrical signal that is processed by a computer.

This modular design ensures specificity, reproducibility, and precision in quantitative analysis.

IV. Flame Characteristics

The flame atomizer (Figure 2) is the most traditional means of generating free atoms. A mixture of fuel gas (commonly acetylene) and oxidant (air or nitrous oxide) creates a high-temperature zone. The flame is divided into three regions:

- Primary combustion zone: where initial burning occurs with excess fuel.

Interzonal region: where atomization is most efficient; ideal for measurements.
Outer zone: hottest region, dominated by oxidation processes.

The choice of flame composition determines the maximum achievable temperature. For example, an air-acetylene flame reaches ~2300 °C, while a nitrous oxide–acetylene flame reaches ~3000 °C. As illustrated in Figure 2, knowing these regions is essential to position the optical path correctly.

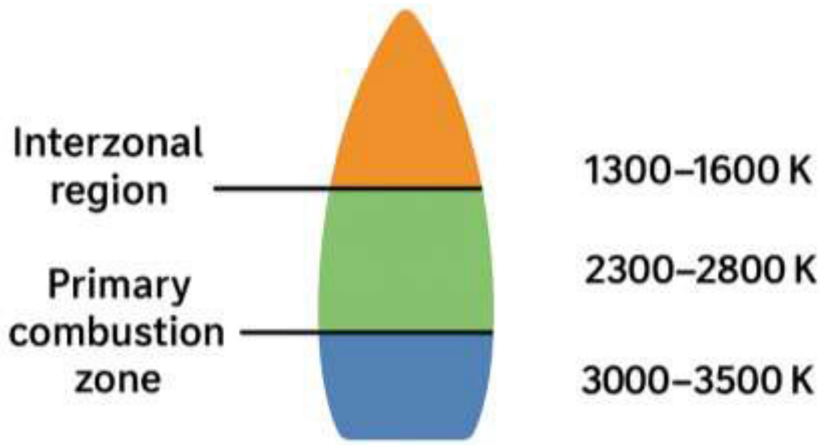


Figure .2: Structure of a Flame used in AAS

V. Graphite Furnace

The graphite furnace (Figure 3) provides electrothermal atomization with significantly higher sensitivity than flames. A small aliquot of sample (5–50 µL) is injected into a graphite tube, and the furnace is electrically heated in controlled stages:

Drying: removes solvent.
Ashing: decomposes organic matrix.
Atomization: generates free atoms at 2000–2800 °C.
Cleaning: eliminates residues and impureties.

The technique achieves detection limits in the ppb range. However, as shown in Figure 3, the process requires careful temperature programming to avoid losses or incomplete atomization.

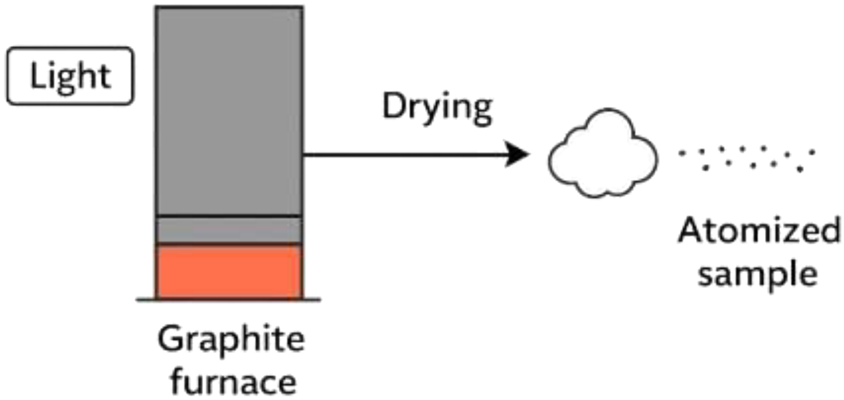


Figure.3: Graphite Furnace and Atomization Steps

VI. Interferences

AAS measurements can be affected by several interferences (Figure 4): Spectral interferences: overlapping emission/absorption lines, or broadband molecular absorption.

Chemical interferences: formation of thermally stable compounds (e.g., metal oxides).

Physical interferences: changes in viscosity, density, or surface tension affecting nebulization.

Electrical interferences: lamp instability or furnace current noise. Correction techniques include background subtraction using a deuterium lamp, Zeeman effect correction, and matrix modifiers that prevent analyte loss. As illustrated in Figure 4, identifying the interference type is crucial for applying the proper correction strategy.

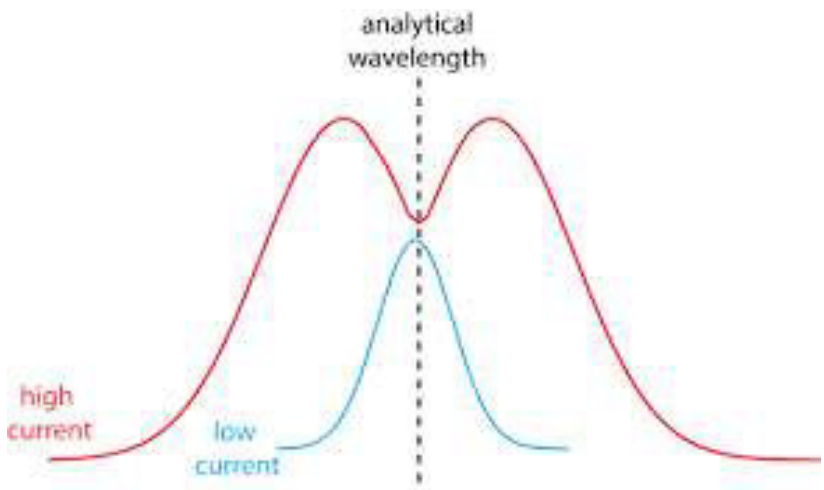


Figure .4: Diagram of interferences

VII. Applications

AAS is widely applied across scientific and industrial fields. Environmental chemistry uses AAS for monitoring heavy metals in air, water, and soil. In food science, the technique measures micronutrients (Fe, Zn, Cu) critical for nutrition. Clinical laboratories employ AAS to detect trace metals in blood and tissues. In metallurgy, alloy composition is routinely checked, while in pharmaceuticals, AAS is a tool for raw material quality control.

Table.1: Compares the flame and graphite furnace

System	Benefits	Drawbacks
Flame AA	• Ease of use • Fast analysis	• Requires gases (can be expensive) • Open-flame source (limits throughput → analyst must be present) • Combustible gases • Requires large amount of sample (5–7 mL/min) • Ionization interferences (requires use of matrix modifier → adds to sample preparation) • Lower sensitivity

		• One element at a time • Requires ventilation
Furnace AA	• Better detection limits • Uses small amounts of sample (µL)	• Analysis time is longer (several minutes → heating stages) • Furnace program can be complex • Droplet must be optimized for viscosity • Consumables required (tubes, caps and holders, tips) • One element at a time • Requires ventilation

Atomic Absorption Spectrophotometry has retained its relevance for decades due to its simplicity, robustness, and cost-effectiveness. Although modern methods like ICP-OES and ICP-MS provide multi-element capabilities, AAS continues to dominate routine trace metal analysis in laboratories worldwide. The choice of atomizer (flame vs furnace), the correction of interferences, and rigorous calibration ensure reliable results.

Table 2: ICP-OES method

System	Benefits	Drawbacks
ICP-OES	• Sample throughput (can run over night, simultaneous measurement of all elements at all wavelengths) • Good detection limits (especially when considering speed compared to Furnace AA) • Excellent precision • Excellent LDR	• Sample introduction techniques can add to analysis time (and cost) • Requires large amounts of sample • Prone to interferences (Physical and Spectral) • Correction can get complex • Requires ventilation • Plasma must be optimized for

	• Can handle complex matrices (e.g., high TDS) • Uses inert gas (Ar) • Easy routine operation	organics/petrochemical products • Can be difficult to maintain

Table 3: ICP-OES method

System	Benefits	Drawbacks
ICP-MS	• Excellent detection limits • Sample throughput (can run overnight, simultaneous measurement of all elements) • Excellent LDR • Capable of isotopic analysis • Uses small amounts of sample • Uses inert gas	• Cost • Environmental considerations (clean lab)

Detection Limits and Measurement Range:

The relative measurement range and detection limits of atomic spectroscopy technologies are summarized below.

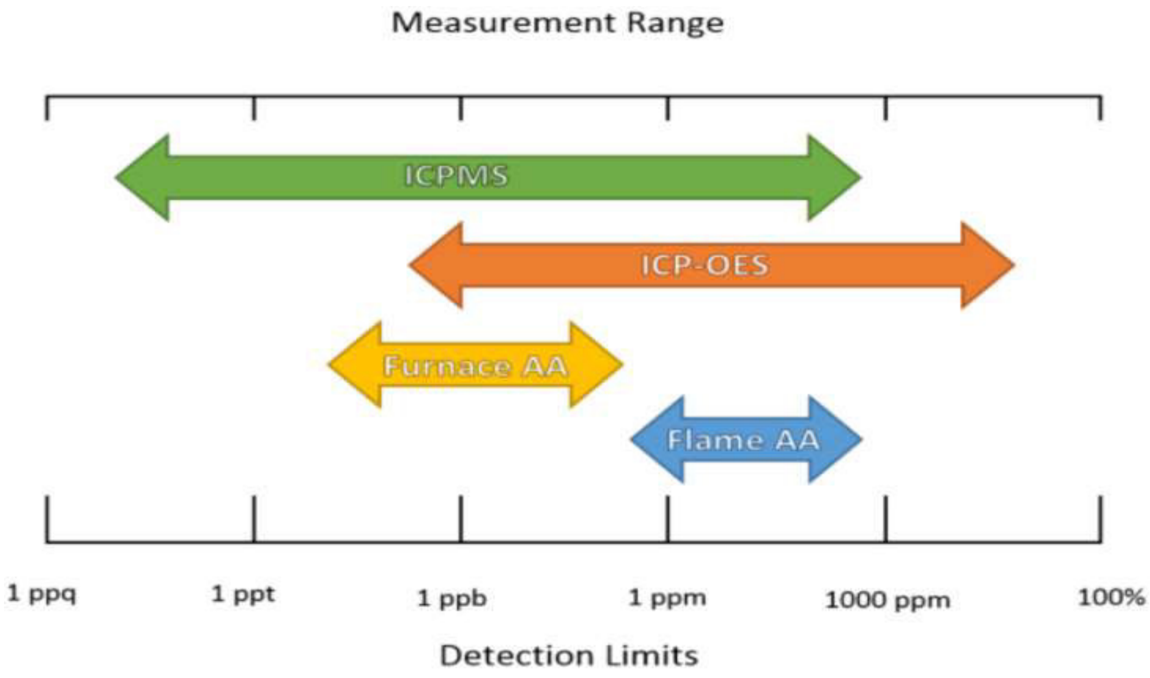


Figure 5: Limits and Measurement Range

VIII. Appendix: Detailed Description of the Instrument

Figure 5 provides a global overview of the AAS instrument. The hollow cathode lamp produces element-specific light, which passes through the sample aerosol generated by the nebulizer and atomized in the flame or furnace. The monochromator selects the characteristic wavelength, and the detector measures the transmitted intensity. The computer processes the signal to provide quantitative results.

Each component is described in detail:

Hollow cathode lamp: element-specific emission.

Nebulizer: aerosol generation.

Atomizer: flame or furnace to create free atoms.

Monochromator: wavelength isolation.

Detector: light-to-electricity conversion.

Data system: signal processing and concentration output.

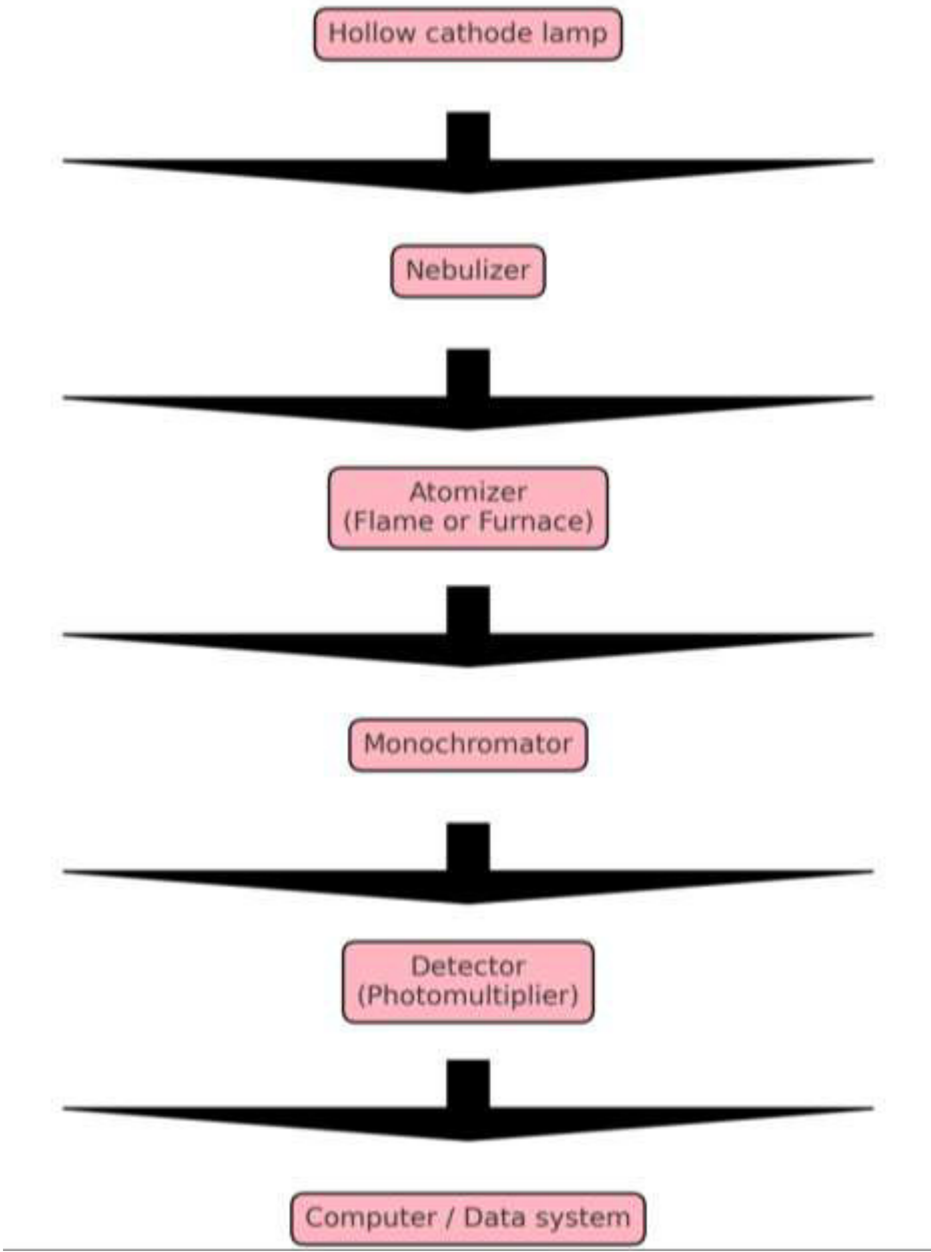


Figure 6: Detailed diagram of the Atomic Absorption Spectrophotometer

I- Introduction

Infrared (IR) spectroscopy is one of the most widely used analytical techniques in organic and inorganic chemistry. It provides essential information about the molecular structure by analyzing how molecules absorb infrared radiation and undergo vibrational transitions. Unlike other spectroscopic methods that focus mainly on electronic or nuclear properties, IR spectroscopy highlights the dynamic behavior of chemical bonds, making it a powerful tool for structural elucidation.

The mid-infrared region, in particular, is of major interest because it contains absorption bands that correspond directly to fundamental vibrational modes of most functional groups. As a result, IR spectra act as molecular fingerprints, allowing the identification of compounds with a high degree of specificity.

The objective of this chapter is to familiarize students with the principles of IR spectroscopy, the nature of molecular vibrations, the interpretation of characteristic absorption bands, and the basic instrumentation used to record IR spectra. Through a combination of theoretical explanations and practical examples, this course aims to develop the ability to analyze and compare infrared spectra in the context of organic chemistry.

II. Presentation of the Mid-Infrared Spectrum

II.1. Position of Infrared within the Electromagnetic Spectrum

The electromagnetic spectrum covers all types of radiation, from gamma rays to radio waves.

Infrared radiation lies between visible light (400–800 nm) and microwaves.

Its wavelength range extends approximately from 0.78 µm to 1000 µm, which corresponds to wavenumbers between 12,800 cm^{-1} and 10 cm^{-1}.

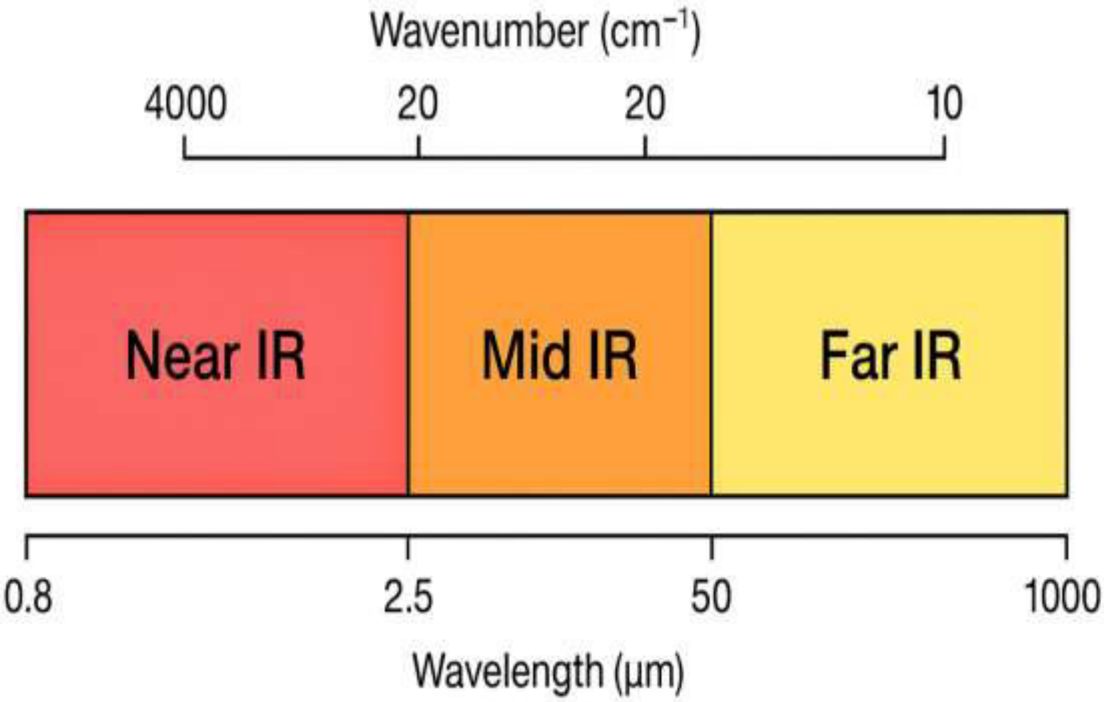


Figure 1: Infrared spectrum regions and wavelength ranges

To facilitate its study, infrared radiation is generally divided into three sub-regions:

Near-infrared (NIR): 14,000–4000 cm^{-1} (0.7–2.5 µm). Used in rapid analytical chemistry (food industry, polymers).

Mid-infrared (MIR): 4000–400 cm^{-1} (2.5–25 µm).

The richest region in structural information for organic molecules.

Far-infrared (FIR): 400–10 cm^{-1} (25–1000 µm). Mainly used to study lattice vibrations and metal–ligand bonds.

Among these, the mid-infrared region is by far the most widely exploited in organic spectroscopy.

II.2. Representation of an Infrared Spectrum

An infrared spectrum is a curve relating:

- The x-axis: the wavenumber (cm^{-1}), a quantity proportional to the energy of the radiation. This unit is preferred over wavelength because it allows a direct reading of vibrational frequencies.
- The y-axis: the absorption intensity, expressed as transmittance (%) or absorbance (A).

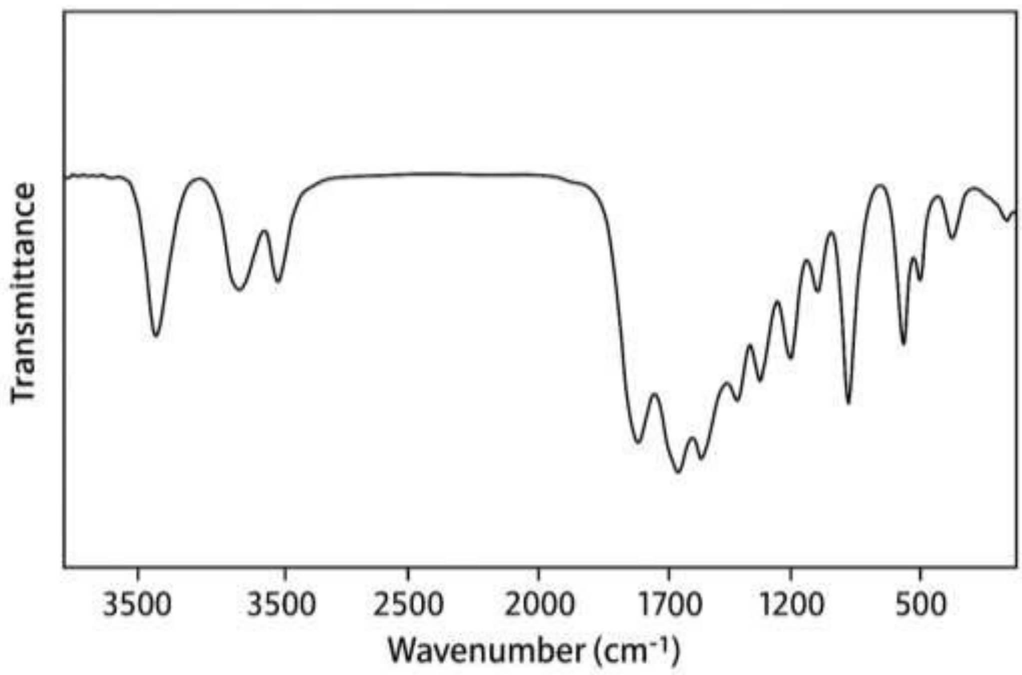


Figure 2: Infrared spectrum

The spectrum thus displays absorption bands at various positions, each corresponding to a specific molecular vibration.

Example:

A C=O bond absorbs strongly around 1700 cm^{-1}.

An O–H bond (alcohol or carboxylic acid) absorbs in the region 3200–3600 cm^{-1}.

II.3. Infrared Selection Rule

Not all vibrations are visible in IR spectroscopy. For a vibration to be IR-active, it must:

Induce a change in the dipole moment of the molecule.

Example:

The asymmetric stretching vibration of CO_2 → IR-active.

The stretching vibration of N_2 or O_2 is symmetric (no dipole change) → IR-inactive. This rule explains why some bonds absorb strongly while others produce no signal.

II.4. Division of the IR Spectrum into Interpretation Zones

The mid-infrared spectrum is divided into two main regions:

Functional group region (4000–1500 cm^{-1}):

This region shows the characteristic bands of functional groups (O–H, N–H, C=O, C=C, etc.). It allows the determination of the chemical functions present in a molecule.

Fingerprint region (1500–400 cm^{-1}):

This region contains numerous narrow and complex bands, unique to each molecule. Even if two compounds have the same functional groups, their fingerprint regions will differ.

This uniqueness makes it possible to identify a molecule unambiguously by comparison with spectral databases.

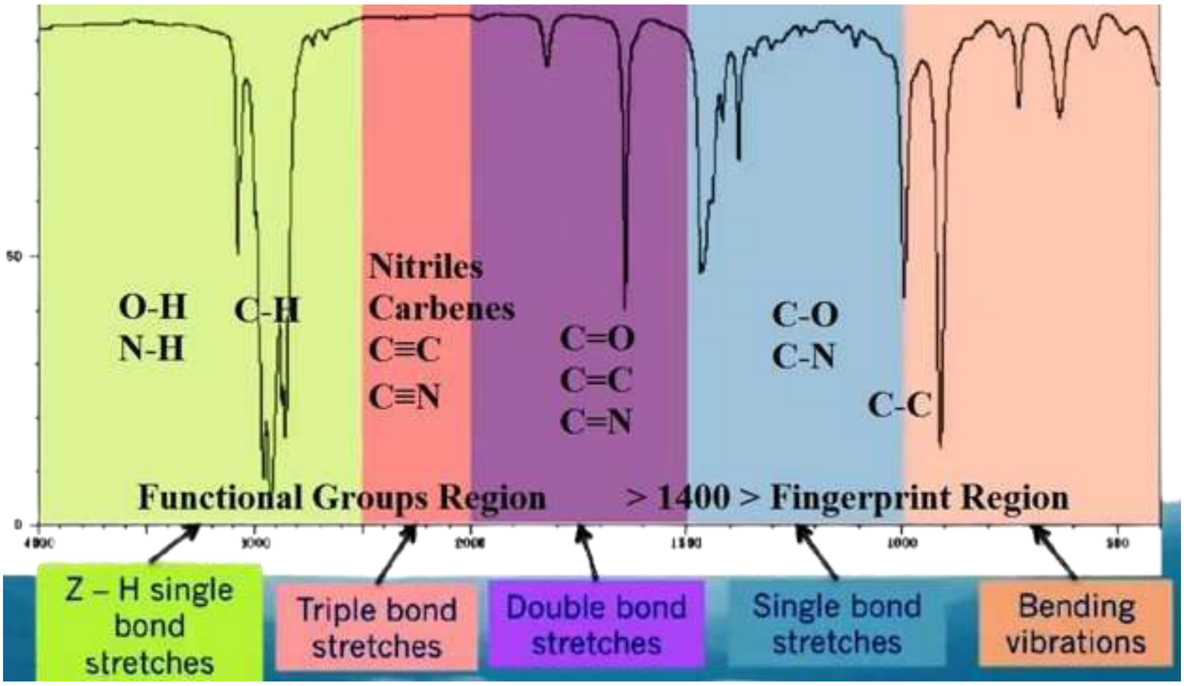


Figure 3: IR Spectrum Zones

II.5. Importance of the IR Spectrum in Chemical Analysis

The mid-infrared spectrum serves as a true molecular identity card. Its applications are multiple:

Identification of functional groups: for example, distinguishing an alcohol from an aldehyde.

Checking the purity of a product: a pure spectrum shows sharp bands, whereas a mixture shows additional bands.

Monitoring chemical reactions: the appearance or disappearance of bands reflects the transformation of reactants into products.

Quality control in pharmaceutical, food, and petrochemical industries.

Summary

The mid-infrared spectrum is at the core of IR spectroscopy. It provides general information on functional groups (the functional group region) and specific information on molecular identity (the fingerprint region).

Its interpretation is the essential first step in any analysis by infrared spectrometry.

III. Origin of Absorptions in the Mid-Infrared

III.1. Nature of the Interaction between Radiation and Matter

When infrared radiation passes through a sample, part of its energy may be absorbed.

This absorption occurs only if the frequency of the radiation matches the natural vibrational frequency of the molecule.

In such cases, resonance takes place between the incident radiation and the atomic motion within the molecule.

The absorbed energy is then used to promote the molecule from its ground vibrational state to an excited vibrational state.

As a result, the IR spectrum does not appear as a continuous curve but as a set of absorption bands, each corresponding to a specific molecular vibration.

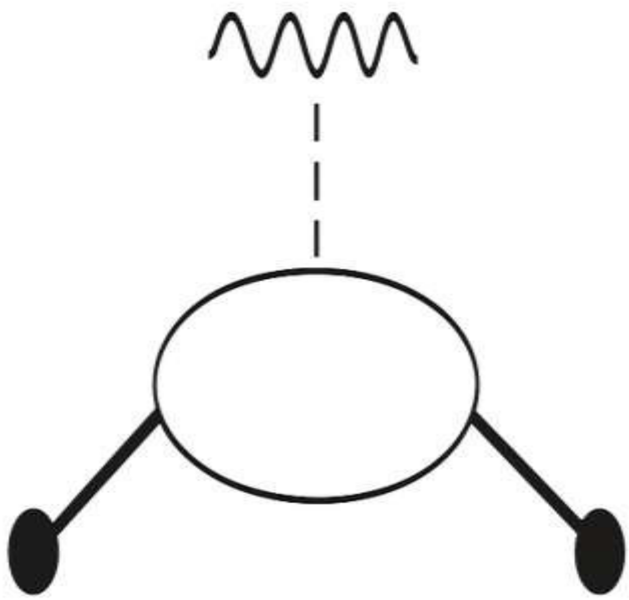


Figure 4: Simplified model of vibrational interaction

III.2. Quantum View of Vibrational Transitions

From a quantum mechanical perspective, vibrational energy levels are discrete and quantized.

Each chemical bond can be approximated as a harmonic oscillator, with energy levels separated by defined intervals.

Absorption occurs only if the photon energy exactly matches the difference between two vibrational levels.

The most common transition is the fundamental transition ($v = 0 \rightarrow v = 1$).

Transitions to higher levels ($v = 2$, $v = 3$ …) exist but are less probable and typically weaker in intensity.

III.3. Types of Molecular Vibrations

Atoms in a molecule are not static; they oscillate around their equilibrium positions. Two main families of vibrations are observed:

Stretching vibrations: periodic variation in bond length.

Symmetric stretching: atoms move simultaneously in opposite directions relative to the center.

Asymmetric stl &retching: one atom approaches while the other moves away.

Bending vibrations: periodic variation of the bond angle.

These include scissoring, rocking, wagging, and twisting motions.

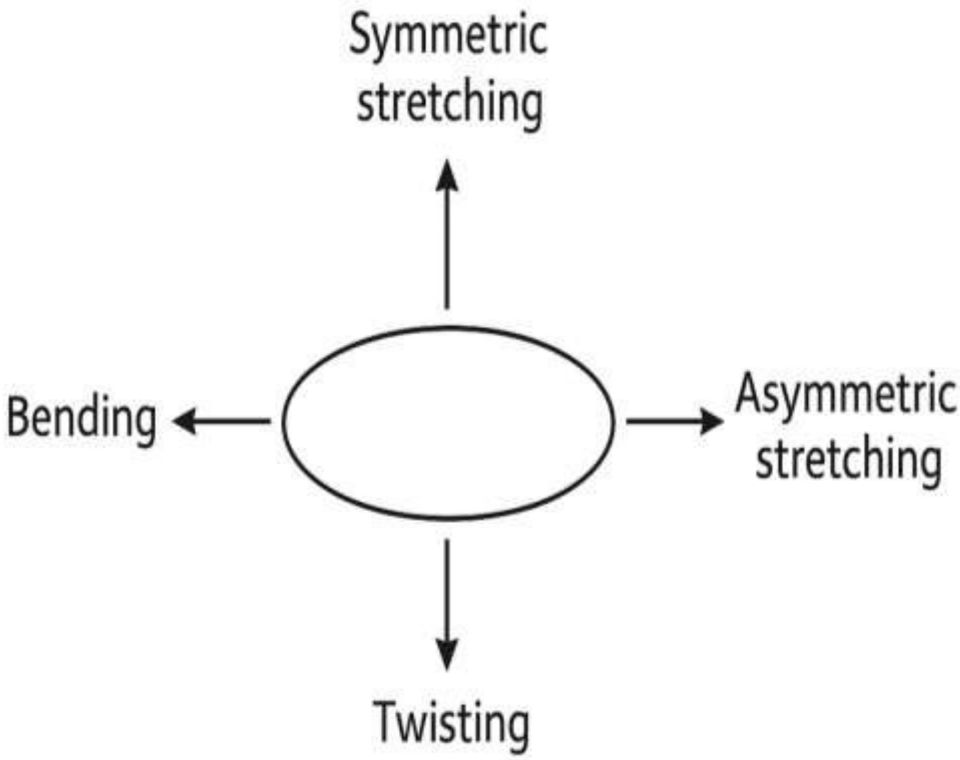


Figure 5: Origin of mid-infrared absorptions

Each type of vibration has a characteristic frequency, determined by the nature of the bond and the masses of the atoms involved.

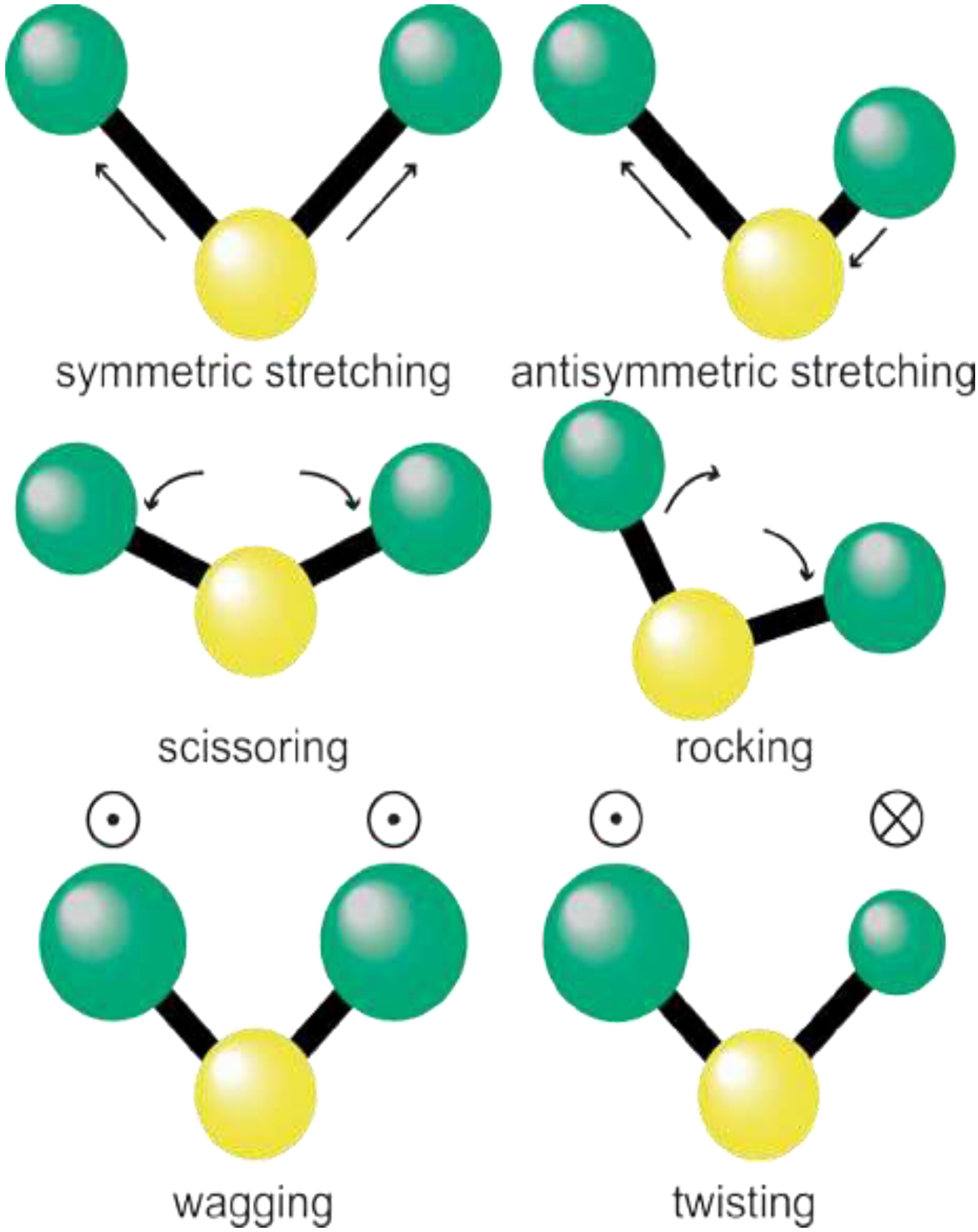


Figure 6: Vibration types

III.4. Factors Influencing Absorption Frequency

The position of absorption bands in an IR spectrum depends on several factors:

Bond strength (force constant k): the stronger the bond, the higher the absorption frequency.

Example: a C≡C bond absorbs at a higher frequency than a C=C bond.

- Atomic masses: according to Hooke's law, bonds involving light atoms (H, C) vibrate at higher frequencies than bonds involving heavier atoms (Cl, Br).
- Chemical environment: an isolated C=O bond absorbs differently than a C=O bond engaged in hydrogen bonding (e.g., in carboxylic acids).

III.5. Selection Rules and Band Intensity

For a vibration to be detected in IR spectroscopy, it must induce a change in the dipole moment of the molecule.

This is the condition for IR activity. Thus:

Homonuclear diatomic molecules (N_2, O_2, Cl_2) are IR-inactive because their vibrations do not produce a dipole change.

Polar molecules (CO, HCl, H_2O, asymmetric CO_2) exhibit strong IR bands.

The intensity of a band is directly proportional to the magnitude of the dipole moment variation during the vibration.

III.6. Vibration–Rotation Coupling

In practice, polyatomic molecules rarely exhibit isolated vibrational lines.

Each vibrational transition is accompanied by rotational transitions, leading to complex patterns known as vibration–rotation bands. These bands are especially observed in gases and provide fine spectroscopic resolution.

III.7. Band Broadening and Shapes

Infrared bands are not infinitely sharp. Their width and shape depend on several factors:

Temperature: thermal motion broadens spectral features.

Intermolecular interactions: hydrogen bonding (e.g., O–H in water) causes pronounced broadening, producing wide absorption bands.

Physical state: in the gas phase, bands are sharper, while in liquids and solids intermolecular forces generate broader signals.

III.8. Characteristic Examples

Free O–H stretching (alcohol vapor) appears as a sharp band near 3650 cm^{-1},

While hydrogen-bonded O–H (in water or carboxylic acids) produces a very broad band in the range 3200–3500 cm^{-1}.

C–H stretching frequencies vary depending on carbon hybridization:

Alkanes: 2850–2960 cm^{-1}

Alkenes: 3020–3100 cm^{-1}

Aromatics: ≈3030 cm^{-1}

C=O stretching is intense and sharp around 1700 cm^{-1}, but its exact position varies depending on the functional group:

Aldehydes: 1720–1740 cm^{-1}

Ketones: 1705–1720 cm^{-1}

Esters: 1735–1750 cm^{-1}

Amides: 1650–1700 cm^{-1}

Summary

Absorptions in the mid-infrared region originate from vibrational transitions of molecules, governed by quantum principles and influenced by atomic masses, bond strengths, and chemical environments.

The detailed analysis of absorption bands allows not only the identification of chemical bonds but also insights into their structural surroundings. This makes infrared spectroscopy a powerful tool in analytical and structural chemistry.

IV. Vibration-Rotation Bands in the Mid-Infrared

IV.1. Introduction to Vibration-Rotation Bands

When a molecule absorbs infrared radiation, the transition involves not only the vibrational levels but also the rotational levels. Thus, a vibrational transition is usually accompanied by rotational transitions, leading to more complex spectral structures known as vibration-rotation bands.

These bands are particularly visible in the spectra of gaseous molecules, where rotations are relatively free and well defined. In condensed phases (liquid or solid), intermolecular interactions attenuate these fine structures, resulting in broader absorption bands.

IV.2. Quantum Origin and Selection Rules

The energy levels of a polyatomic molecule combine three main contributions:

Electronic energy (Eelec), generally much higher.

Vibrational energy (Evib), associated with bond stretching and bending motions.

Rotational energy (Erot), corresponding to the rotation of the molecule about its axes.

When a molecule undergoes a vibrational transition (induced by IR absorption), it simultaneously changes its rotational state due to the coupling between these motions. This generates a series of so-called vibration-rotation transitions, which appear as multiplets in the spectrum.

These transitions follow quantum selection rules:

$\Delta v = \pm 1$: fundamental vibrational transition (from ground v=0 to excited v=1).

$\Delta J = \pm 1$: associated rotational transitions (P and R branches).

$\Delta J = 0$: Q branch transitions, absent in linear molecules but present in some polyatomic species.

"Forbidden" transitions (e.g., $\Delta v = 2$ overtones) may also appear with weaker intensity due to anharmonicity.

IV.3. Structure of Vibration-Rotation Bands

A vibration-rotation band is typically divided into three branches:

P branch: transitions with $\Delta J = -1$, observed at lower frequencies than the band center.

Q branch: transitions with $\Delta J = 0$, absent for linear molecules but present in nonlinear ones.

R branch: transitions with $\Delta J = +1$, observed at higher frequencies than the band center.

Together, they form a characteristic "comb-like" pattern, especially evident in diatomic molecules.

IV.4. Rotational Constant and Line Spacing

The spacing between adjacent lines in a branch is directly related to the rotational constant B of the molecule, which depends on its geometry and mass distribution. Therefore, vibration- rotation spectroscopy provides valuable structural information such as internuclear distances in diatomic species.

IV.5. Factors Influencing Vibration-Rotation Bands

The shape and resolution of vibration-rotation bands depend on several parameters:

Temperature: at higher temperatures, more rotational levels are populated, broadening the band and enriching its structure.

Molecular symmetry: linear molecules (e.g., CO, HCl) show simple structures, while nonlinear polyatomic molecules (e.g., H_2O, NH_3) display more complex patterns.

Physical state: in the gas phase, the P, Q, and R branches are clearly resolved, while in liquids or solids these details are lost and only broad bands remain.

IV.6. Case Studies and Examples

Diatomic heteronuclear molecules such as HCl and CO exhibit highly regular spectra with symmetrical P and R branches.

Linear polyatomic molecules such as CO_2 (asymmetric stretch at 2350 cm^{-1}) show well- defined fine structures including P, Q, and R branches.

Nonlinear molecules like H_2O and NH_3 produce more complex spectra with overlapping vibrational bands.

IV.7. Practical Applications

Atmospheric gas analysis: detection of CO_2, CH_4, N_2O via their fine vibration-rotation features.

Astrophysics: studying planetary atmospheres and interstellar environments through their infrared rotational-vibrational signatures.

Fundamental chemistry: determining rotational constants to obtain precise information on bond lengths and molecular dynamics.

Summary

Vibration-rotation bands in the mid-infrared provide a direct manifestation of the coupling between rotational and vibrational motions in molecules. They carry rich information about molecular structure, symmetry, and interactions. Although less visible in condensed phases, they are crucial in gas-phase studies and represent a bridge between infrared spectroscopy and pure rotational spectroscopy.

V. Simplified Model of Vibrational Interactions

V.1. Introduction to the Simplified Model

The interpretation of infrared spectra relies on an understanding of molecular vibrations. Real molecules, however, are complex systems with many degrees of freedom.

To make the analysis manageable, simplified models are used that approximate molecular vibrations while still explaining the key spectroscopic features.

The basic model is the harmonic oscillator, in which a chemical bond is treated as a spring that can stretch and compress. Although this model is not entirely accurate, it provides simple relationships between vibrational frequencies, atomic masses, and bond stiffness.

V.2. Harmonic Oscillator and Hooke's Law Applied to Molecules

In the harmonic oscillator model, a chemical bond is treated as a spring obeying Hooke's law.

The potential energy of a vibration is expressed as:

$$\boldsymbol{E = \frac{1}{2} k\, x^2}$$

Where k is the force constant of the bond and x is the displacement from equilibrium.

The vibrational frequency ν is related to the force constant and the reduced mass μ of the two atoms:

$$\nu = (1 / 2\pi c) \cdot \sqrt{(k / \mu)}$$

With μ = (m1·m2)/(m1 + m2). This relation shows that:

The stronger the bond (high k), the higher the vibrational frequency;
The heavier the atoms, the lower the vibrational frequency.

For example, an O–H bond vibrates at a higher frequency than a C–Cl bond.

V.3. Limitations of the Harmonic Model and Anharmonicity

The harmonic model assumes that a bond can stretch indefinitely, which is unrealistic. In reality, a bond breaks when stretched too far. To represent this behavior more accurately, the Morse potential is used:

$$V(x) = De\,[1 - e^{(-ax)}]^2$$

Where De is the dissociation energy and (a) is a parameter linked to bond stiffness. Consequences of anharmonicity include:

Vibrational levels are no longer equally spaced.
“Forbidden” transitions (Δv = ±2, ±3, …) become allowed, explaining the presence of overtone bands.
The real spectrum is therefore richer than the one predicted by the harmonic model.

V.4. Normal Modes and Vibrational Coupling

In polyatomic molecules, vibrations do not involve a single bond but rather coordinated motions of several atoms.

These are referred to as **normal modes of vibration**.

Examples:

In H_2O, the symmetric stretch, asymmetric stretch, and bending mode.

In CO_2, the symmetric stretch, asymmetric stretch, and bending vibrations.

Normal modes may interact with one another, producing **vibrational coupling** phenomena:

Fermi resonance: when a fundamental vibration is close in energy to an overtone or combination band, the bands mix and shift.

Anharmonic couplings: interactions between vibrations of different bonds that alter band positions and intensities.

V.5. Spectroscopic Consequences

Vibrational interactions result in:

Frequency shifts relative to expected values (coupling effects).

The appearance of additional bands corresponding to combination transitions.

Intensity changes, where some bands are strengthened or weakened depending on the degree of coupling.

These phenomena explain why real IR spectra are often more complex than idealized predictions.

V.6. Applications and Significance of the Simplified Model

Despite its limitations, the simplified model plays a central role:

It allows approximate prediction of band positions based on bond types.

It provides a framework to interpret discrepancies between theory and experimental spectra.

It serves as both a pedagogical and practical tool for rapid IR spectrum analysis.

Furthermore, incorporating anharmonicity and coupling effects bridges the gap between theoretical models and experimental observations, making structural interpretation more accurate.

Summary

The simplified model of vibrational interactions is a fundamental tool for understanding the origin of infrared absorption bands. Although based on approximations (harmonic oscillator, limited anharmonicity), it establishes general rules relating vibrational frequencies to atomic masses, bond stiffness, and normal mode interactions. This model is therefore essential for interpreting IR spectra and extracting structural information about molecules.

VI. Instrumentation

VI.1. Introduction to IR Instrumentation

The quality and reliability of infrared spectra depend heavily on the instrumentation used. From the first dispersive spectrometers to modern Fourier-transform infrared (FTIR) instruments, the technology has evolved significantly. FTIR systems now provide faster, more sensitive, and more precise measurements, making IR spectroscopy a routine and versatile analytical tool.

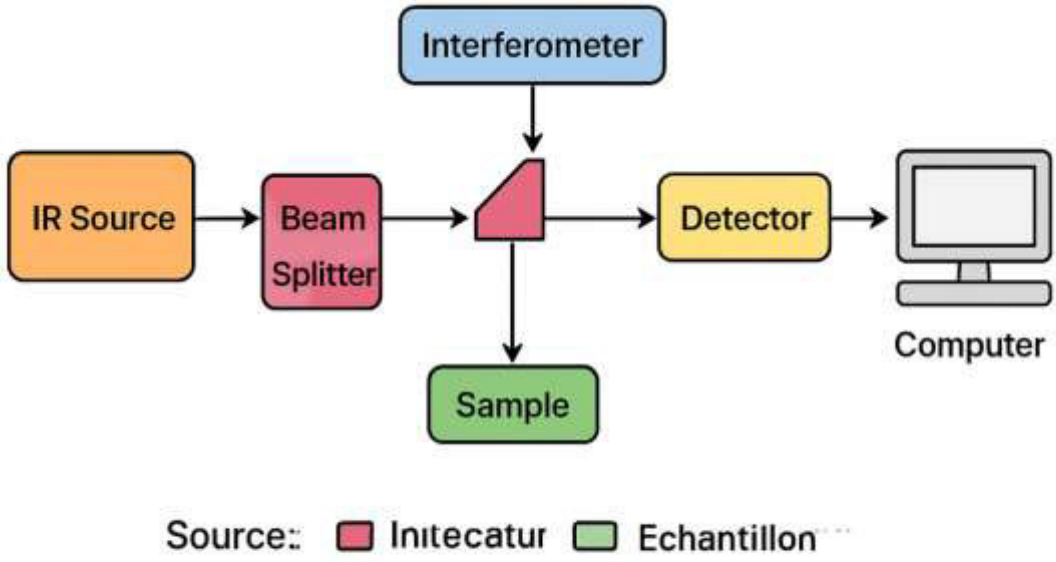


Figure 7: Schematic representation of a Fourier Transform Infrared (FTIR) spectrometer

VI.2. Fundamental Components of an IR Spectrometer

An IR spectrometer is composed of several essential parts:

Radiation sources: generate infrared radiation over the desired spectral region. Common sources include:

* Nernst glower (heated rare-earth oxides).
* Globar (silicon carbide rod heated electrically).
* Modern, stable, long-lasting IR lamps.

Dispersion and interference systems:

* Traditional spectrometers used prisms and gratings to disperse radiation.

FTIR instruments use a Michelson interferometer, which splits and recombines radiation by interference, enabling simultaneous analysis of all frequencies.

Sample holders and cells: adapted to the sample's physical state.

* Transmission cells (KBr pellets for solids, NaCl cuvettes for liquids).
* Reflection accessories.
* ATR (Attenuated Total Reflectance), enabling direct analysis of solids and liquids with minimal preparation.

Detectors: convert infrared radiation into electrical signals.

* Thermal detectors: thermocouples, bolometers.
* Pyroelectric detectors (DTGS).
* Quantum detectors (MCT, HgCdTe): highly sensitive and fast, used in advanced research.

VI.3. Types of Spectrometers

Dispersive spectrometers: separate frequencies using prisms or gratings. They provide accurate results but require slow mechanical scanning.

Fourier-transform infrared spectrometers (FTIR): use a Michelson interferometer. The recorded signal (interferogram) is mathematically transformed by Fourier transform to produce the spectrum.

Advantages: speed, high resolution, superior signal-to-noise ratio, and ability to co-add spectra.

Sample Preparation Techniques

Solids:

* KBr pellets (powdered sample mixed with KBr, then pressed).
* Thin films deposited on transparent supports.

Liquids:

* NaCl, KBr, or CaF_2 cuvettes transparent to IR.
* Direct ATR analysis without complex preparation.

Gases:

* Long-path gas cells (10–20 cm) with IR-transparent windows.

- Advanced IR Techniques

ATR (Attenuated Total Reflectance): enables direct analysis of solids and liquids with minimal preparation. Widely used in quality control.

Diffuse Reflectance (DRIFTS): suitable for powdered samples and heterogeneous surfaces.

Micro-IR: combines IR spectroscopy with optical microscopy, allowing localized analysis.

In-line and in situ IR: monitors chemical reactions and industrial processes in real time.

- Practical Applications of IR Instrumentation

Pharmaceutical industry: identification of active ingredients and quality control.

Food industry: detection of additives and monitoring of composition.

Polymers and materials: identification of plastics and verification of manufacturing processes.

Environmental monitoring: detection of atmospheric pollutants (CO_2, CH_4, VOCs).

Scientific research: investigation of surfaces, thin films, and complex materials.

Summary

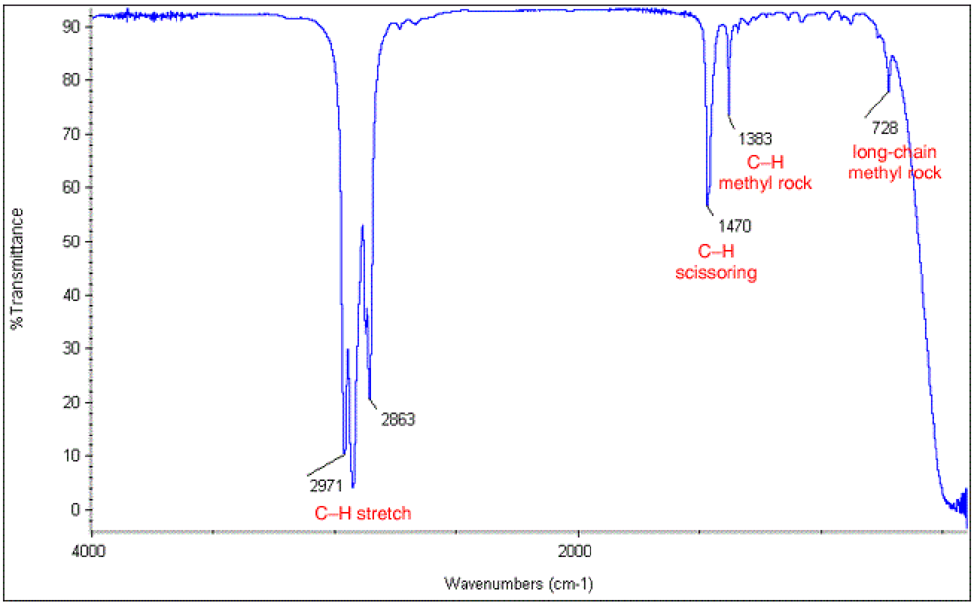
Instrumentation is at the core of infrared spectroscopy.

Technological advances, especially FTIR and ATR, have greatly expanded the scope of applications.

Modern IR spectrometers now provide reliable qualitative and quantitative analyses, making them indispensable tools in industry, research, and quality control.

VII. Spectrum comparison

VII.1. Alkanes



The spectra of simple alkanes are characterized by absorptions due to C–H stretching and bending (the C–C stretching and bending bands are either too weak or of too low a frequency to be detected in IR spectroscopy). In simple alkanes, which have very few bands, each band in the spectrum can be assigned.

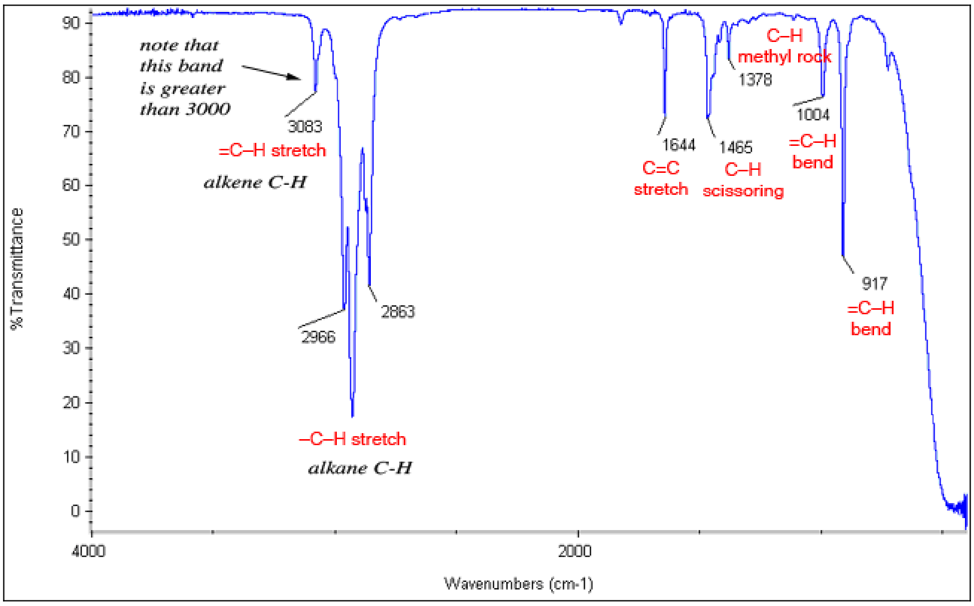
C–H stretch from 3000–2850 cm^{-1}

C–H bend or scissoring from 1470-1450 cm^{-1}

C–H rock, methyl from 1370-1350 cm^{-1}

C–H rock, methyl, seen only in long chain alkanes, from 725-720 cm^{-1}.

VII.2. Alkenes



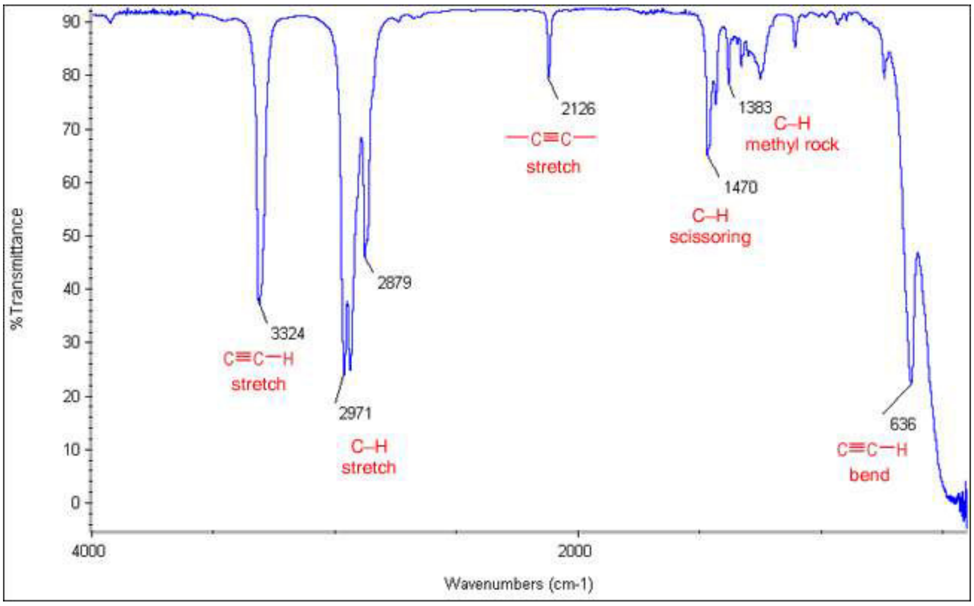
Alkenes are compounds that have a carbon-carbon double bond, –C=C–. The stretching vibration of the C=C bond usually gives rise to a moderate band in the region 1680-1640 cm^{-1}.

Stretching vibrations of the –C=C–H bond are of higher frequency (higher wavenumber) than those of the –C–C–H bond in alkanes.

This is a very useful tool for interpreting IR spectra: Only alkenes and aromatics show a C-H stretch slightly higher than 3000 cm^{-1}. Compounds that do not have a C=C bond show C-H stretches only below 3000 cm^{-1}.

The strongest bands in the spectra of alkenes are those attributed to the carbon-hydrogen bending vibrations of the =C–H group. These bands are in the region 1000-650 cm^{-1} (Note: this overlaps the fingerprint region).

VII.3. Alkynes

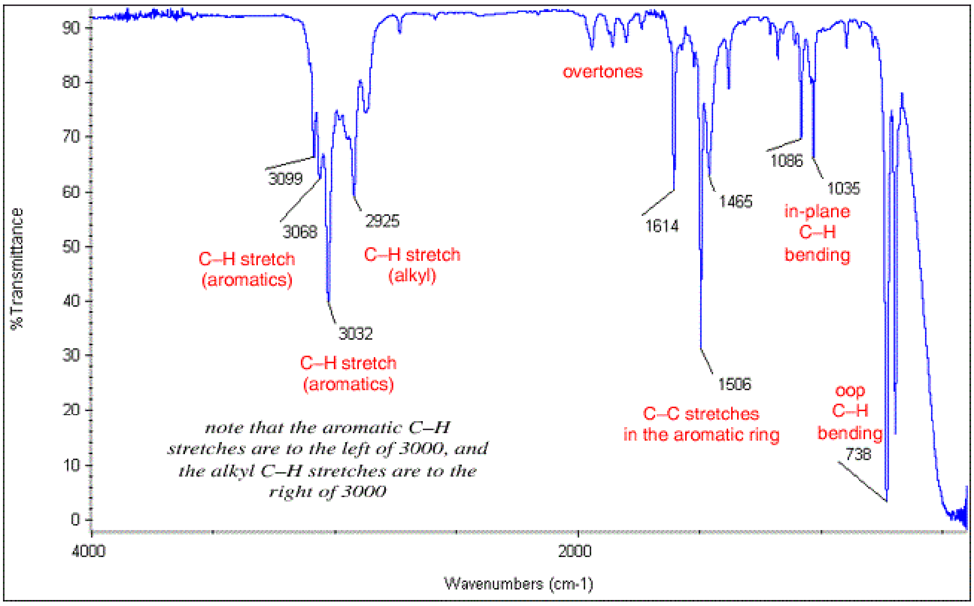


Alkynes are compounds that have a carbon-carbon triple bond (–C≡C–). The –C≡C– stretch appears as a weak band from 2260-2100 cm^{-1}. This can be an important diagnostic tool because very few organic compounds show an absorption in this region.

A terminal alkyne (but not an internal alkyne) will show a C–H stretch as a strong, narrow band in the range 3330-3270 cm^{-1}. (Often this band is indistinguishable from bands resulting from other functional groups on the same molecule which absorb in this region, such as the O-H stretch.)

A terminal alkyne will show a C–H bending vibration in the region 700-610 cm^{-1}.

VII.4. Aromatic molecule



The =C–H stretch in aromatics is observed at 3100-3000 cm^{-1}. Note that this is at slightly higher frequency than is the –C–H stretch in alkanes.

This is a very useful tool for interpreting IR spectra: Only alkenes and aromatics show a C–H stretch slightly higher than 3000 cm^{-1}. Compounds that do not have a C=C bond show C–H stretches only below 3000 cm^{-1}.

Aromatic hydrocarbons show absorptions in the regions 1600-1585 cm^{-1} and 1500-1400 cm^{-1} due to carbon-carbon stretching vibrations in the aromatic ring.

Bands in the region 1250-1000 cm^{-1} are due to C–H in-plane bending, although these bands are too weak to be observed in most aromatic compounds.

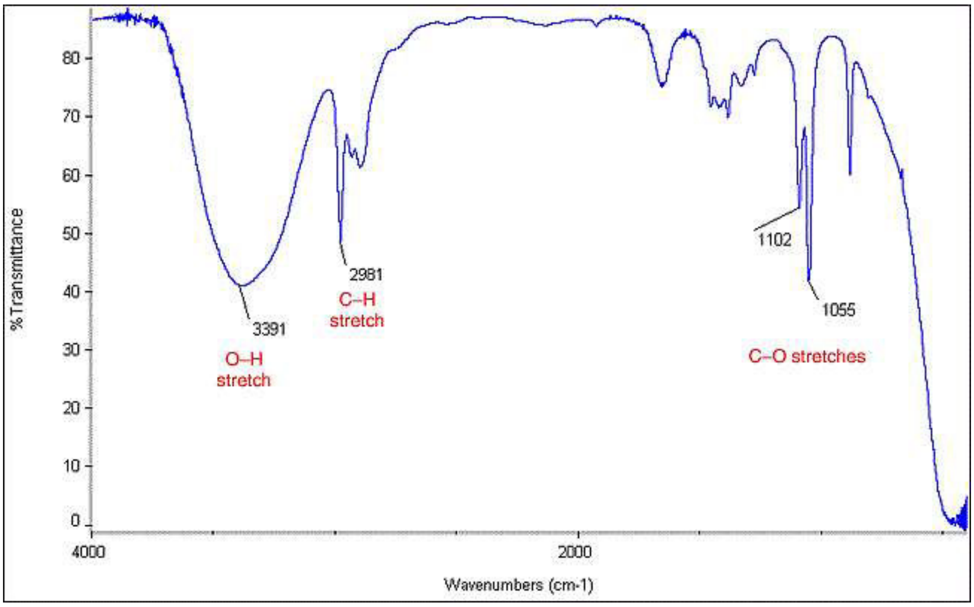
Besides the C–H stretch above 3000 cm^{-1}, two other regions of the infrared spectra of aromatics distinguish aromatics from organic compounds that do not have an aromatic ring:

2000-1665 cm^{-1} (weak bands known as "overtones")

900-675 cm^{-1} (out-of-plane or "oop" bands)

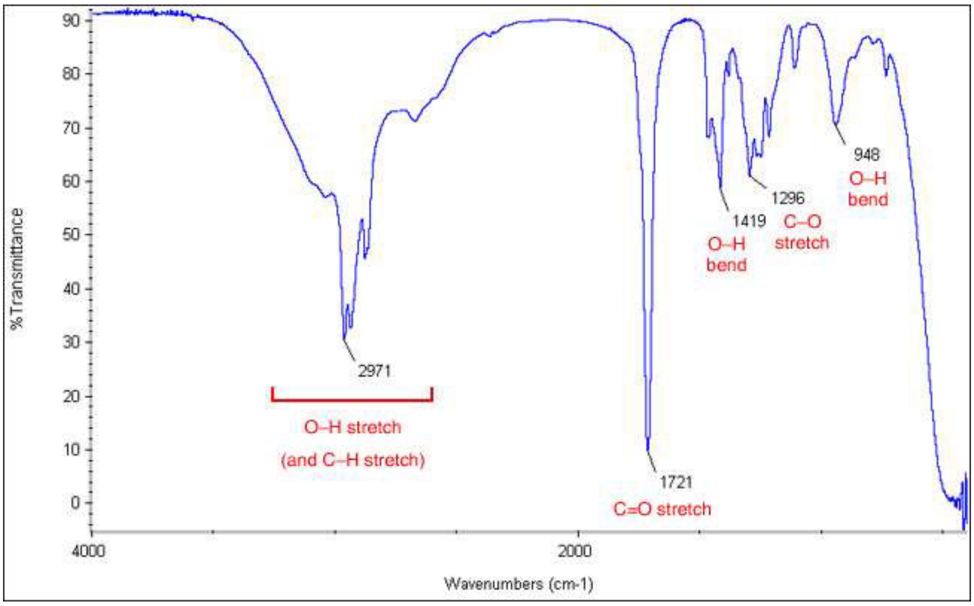
Not only do these bands distinguish aromatics, but they can be useful if you want to determine the number and positions of substituents on the aromatic ring. The pattern of overtone bands in the region 2000-1665 cm^{-1} reflect the substitution pattern on the ring. The pattern of the oop C–H bending bands in the region 900-675 cm^{-1} are also characteristic of the aromatic substitution pattern.

VII.5. Alcohol



Alcohols have characteristic IR absorptions associated with both the O-H and the C-O stretching vibrations. When run as a thin liquid film, or "neat", the O–H stretch of alcohols appears in the region 3500-3200 cm^{-1} and is a very intense, broad band. The C–O stretch shows up in the region 1260-1050 cm^{-1}.

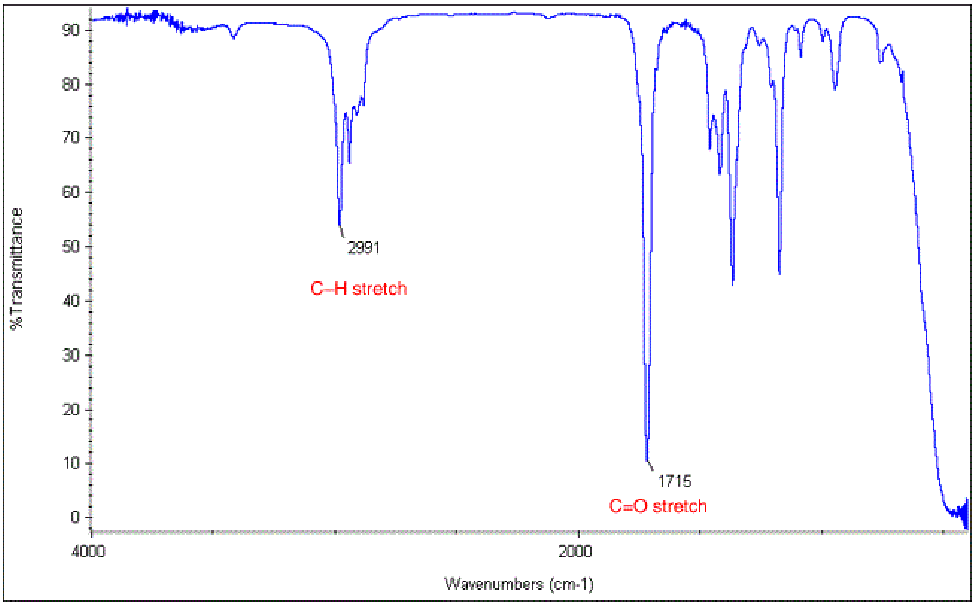
VII.6. Carboxylic acid



Carboxylic acids show a strong, wide band for the O–H stretch. Unlike the O–H stretch band observed in alcohols, the carboxylic acid O–H stretch appears as a very broad band in the region 3300-2500 cm^{-1}, centered at about 3000 cm^{-1}. This is in the same region as the C–H stretching bands of both alkyl and aromatic groups. Thus a carboxylic acid shows a somewhat "messy" absorption pattern in the region 3300-2500 cm^{-1}, with the broad O–H band superimposed on the sharp C–H stretching bands. The reason that the O–H stretch band of carboxylic acids is so broad is becase carboxylic acids usually exist as hydrogen-bonded dimers.

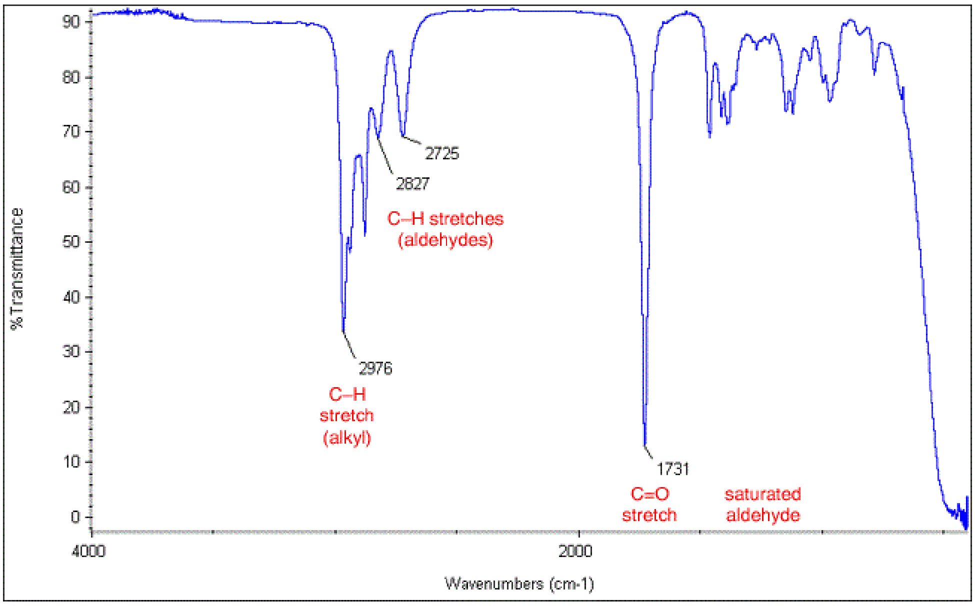
The carbonyl stretch C=O of a carboxylic acid appears as an intense band from 1760-1690 cm^{-1}. The exact position of this broad band depends on whether the carboxylic acid is saturated or unsaturated, dimerized, or has internal hydrogen bonding. The C–O stretch appears in the region 1320-1210 cm^{-1}, and the O–H bend is in the region 1440-1395 cm^{-1} and 950-910 cm^{-1}, although the 1440-1395 band may not be distinguishable from C–H bending bands in the same region.

VII.7. Ketone



The carbonyl stretching vibration band C=O of saturated aliphatic ketones appears at 1715 cm^{-1}. Conjugation of the carbonyl group with carbon-carbon double bonds or phenyl groups, as in alpha, beta-unsaturated aldehydes and benzaldehyde, shifts this band to lower wavenumbers, 1685-1666 cm^{-1}.

VII.8. Aldehyde



The carbonyl stretch C=O of saturated aliphatic aldehydes appears from 1740-1720 cm^{-1}. As in ketones, if the carbons adjacent to the aldehyde group are unsaturated, this vibration is shifted to lower wavenumbers, 1710-1685 cm^{-1}.

Another useful diagnostic band for aldehydes is the O=C–H stretch. This band generally appears as one or two bands of moderate intensity in the region 2830-2695 cm^{-1}. Since the band near 2830 cm^{-1} is usually indistinguishable from other C–H stretching vibration bands (recall that the C–H stretches of alkanes appear from 3000-2850 cm^{-1}), the presence of a moderate band near 2720 cm^{-1} is more likely to be helpful in determining whether or not a compound is an aldehyde.

VII.9. Amine

The N–H stretches of amines are in the region 3300-3000 cm^{-1}. These bands are weaker and sharper than those of the alcohol O–H stretches which appear in the same region. In primary amines (RNH_2), there are two bands in this region, the asymmetrical N–H stretch and the symmetrical N–H stretch.

Secondary amines (R_2NH) show only a single weak band in the 3300-3000 cm^{-1} region, since they have only one N–H bond. Tertiary amines (R_3N) do not show any band in this region since they do not have an N–H bond.

(A shoulder band usually appears on the lower wavenumber side in primary and secondary liquid amines arising from the overtone of the N–H bending band: this can confuse interpretation. Note the spectrum of aniline, below.)

The N–H bending vibration of primary amines is observed in the region 1650-1580 cm^{-1}. Usually, secondary amines do not show a band in this region and tertiary amines never show a band in this region. (This band can be very sharp and close enough to the carbonyl region to cause students to interpret it as a carbonyl band.)

Another band attributed to amines is observed in the region 910-665 cm^{-1}. This strong, broad band is due to N–H wag and observed only for primary and secondary amines.

The C–N stretching vibration of aliphatic amines is observed as medium or weak bands in the region 1250-1020 cm^{-1}. In aromatic amines, the band is usually strong and in the region 1335-1250 cm^{-1}.

Summary:

N–H stretch 3400-3250 cm^{-1}

1° amine: two bands from 3400-3300 and 3330-3250 cm^{-1}

2° amine: one band from 3350-3310 cm^{-1}

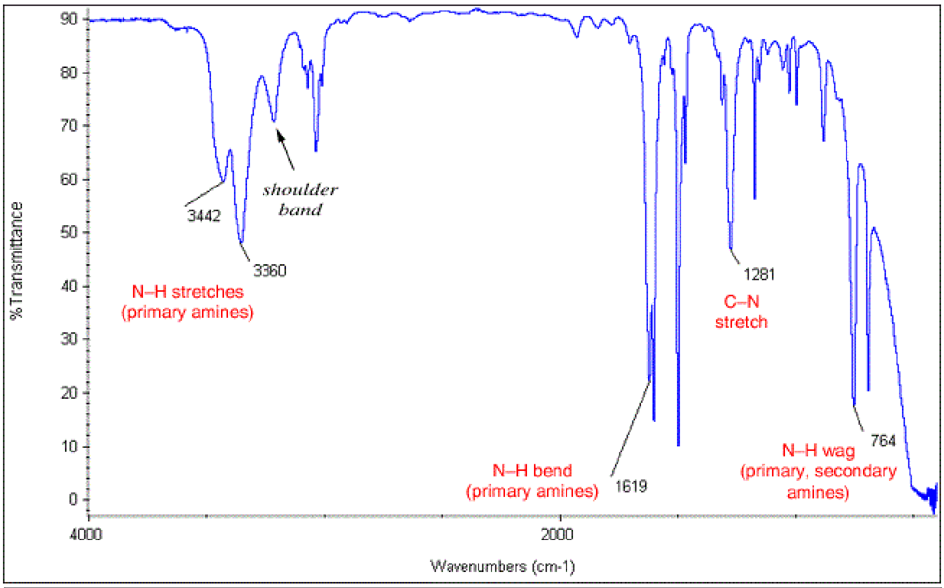
3° amine: no bands in this region

N–H bend (primary amines only) from 1650-1580 cm^{-1}

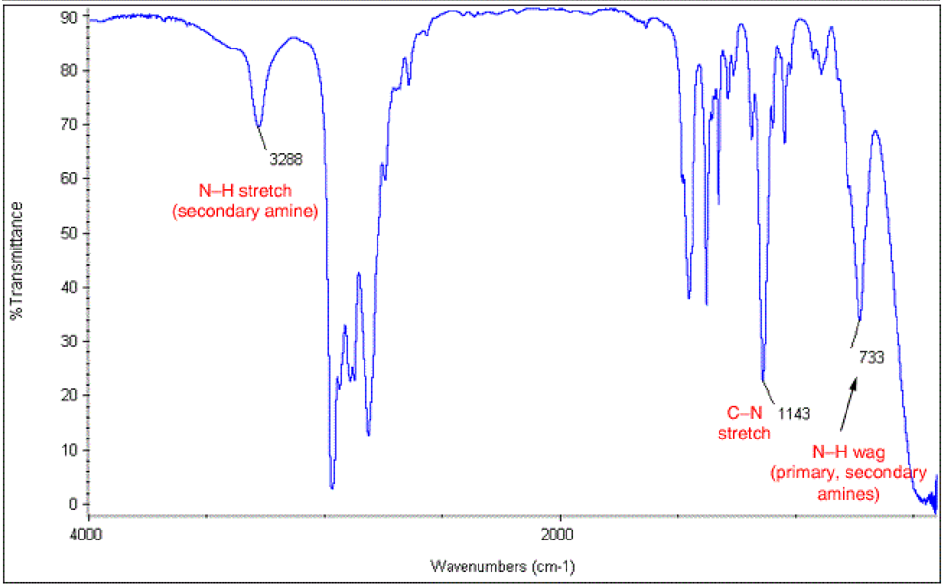
C–N stretch (aromatic amines) from 1335-1250 cm^{-1}

C–N stretch (aliphatic amines) from 1250–1020 cm^{-1}

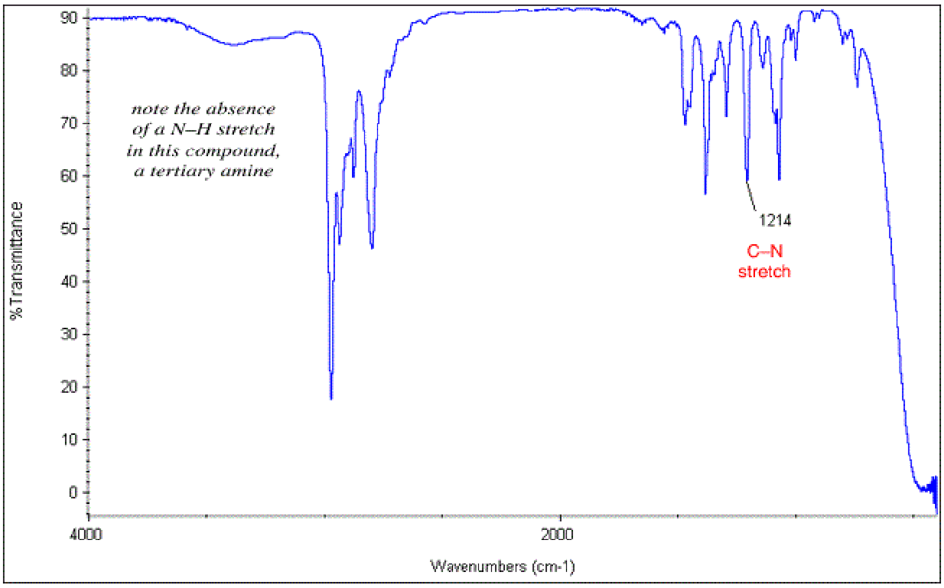
N–H wag (primary and secondary amines only) from 910-665 cm^{-1}.



90
80
70
60
50
40
30
20
10
0
% Transmittance
4000
2000
Wavenumbers (cm-1)
3442
3360
N–H stretches
(primary amines)
shoulder
band
N–H bend
(primary amines)
1619
1281
C–N
stretch
764
N–H wag
(primary, secondary
amines)



90
80
70
60
50
40
30
20
10
0
% Transmittance
4000
2000
Wavenumbers (cm-1)
3288
N–H stretch
(secondary amine)
C–N
stretch
1143
733
N–H wag
(primary, secondary
amines)



90
80
70
60
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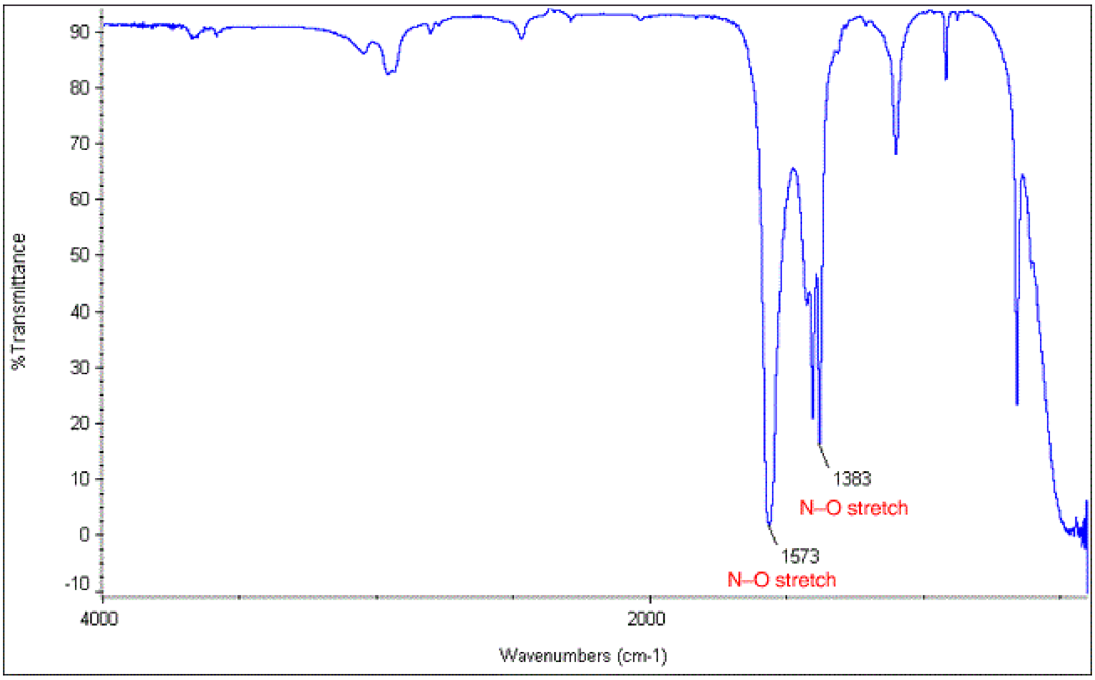
30
20
10
0
% Transmittance
4000
2000
Wavenumbers (cm-1)
note the absence
of a N–H stretch
in this compound,
a tertiary amine
1214
C–N
stretch

VII.10. Nitro groups

The N–O stretching vibrations in nitroalkanes occur near 1550 cm^{-1} (asymmetrical) and 1365 cm^{-1} (symmetrical), the band at 1550 cm^{-1} being the stronger of the two.

If the nitro group is attached to an aromatic ring, the N–O stretching bands shift to down to slightly lower wavenumbers: 1550-1475 cm^{-1} and 1360-1290 cm^{-1}.



VIII. Conclusion

Infrared spectroscopy is one of the most powerful and versatile analytical methods for the identification and characterization of chemical compounds. Throughout this chapter, we explored the theoretical and practical foundations of the technique, ranging from the origin of mid-infrared absorptions to modern instrumentation and advanced applications.

Each section highlighted a key aspect:

The presentation of the mid-infrared spectrum and its essential features.

The explanation of molecular absorptions related to vibrational and rotational motions.

Simplified models that provide insight into vibrational interactions.

The role of characteristic absorption bands in identifying organic functional groups.

Significant advances in instrumentation, particularly FTIR and ATR techniques.

The importance of spectral comparison for authentication, quality control, and scientific research.

Technological progress has broadened the scope of IR spectroscopy applications: from pharmaceuticals and materials chemistry to environmental sciences, food quality control, and even in situ industrial monitoring.

In conclusion, infrared spectroscopy has become an indispensable tool in analytical chemistry, providing speed, precision, and reliability for both research and industrial purposes.

I.1. Introduction

Nuclear Magnetic Resonance (NMR) is a modern spectroscopic technique that plays a central role in chemical analysis. It is considered one of the most powerful tools for determining the structure of organic and inorganic molecules without destroying them. Unlike other analytical methods, NMR provides precise information about the atomic environment, spatial arrangement, and internal interactions within a molecule.

Proton NMR (1H NMR) is the most widely used, as hydrogen is the most abundant atom in organic molecules. Each proton in a molecule has a specific electronic environment, resulting in distinct signals in the NMR spectrum. These signals constitute a true “fingerprint” of the molecule.

The main information provided by proton NMR includes:

The position of the signals (chemical shift δ), which reveals the nature of the chemical environment of the protons;

The area of the signals (integration), which indicates the relative number of equivalent protons;

The multiplicity of the signals (spin-spin coupling), which provides information on neighboring protons;

The coupling constant (j), which gives insights into the proximity and interactions between protons.

Beyond the simple identification of small molecules, NMR plays a fundamental role in medicinal chemistry, biology, and materials science. For example, it is used to elucidate the structure of proteins, monitor reactions in real time, and control the purity of pharmaceutical products.

Thus, NMR is not only an essential analytical tool for chemistry students, but also a gateway to advanced fields of scientific research.

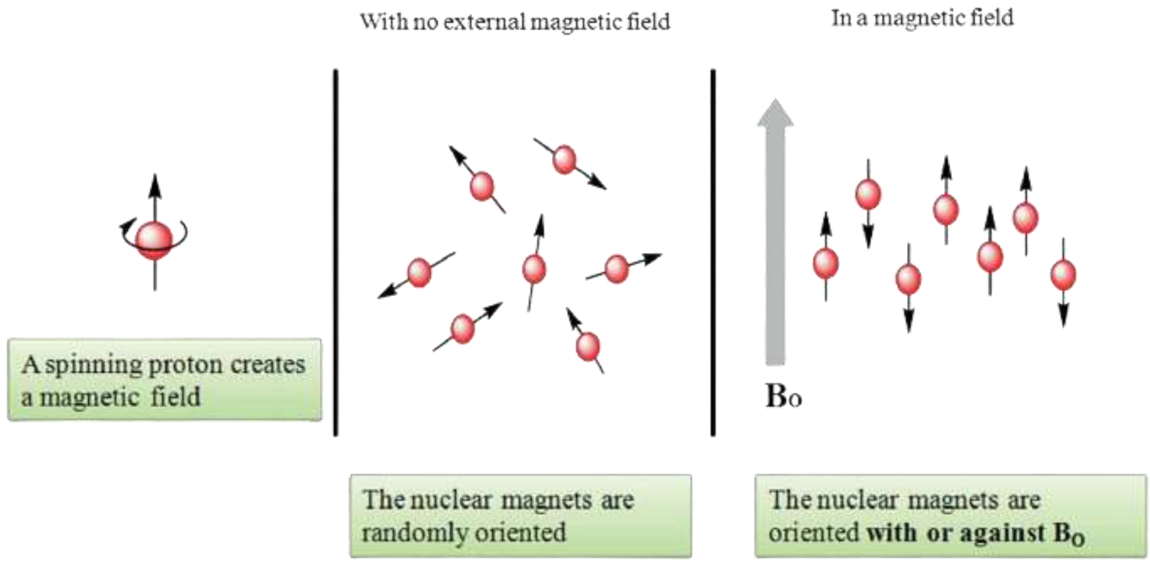


Figure 1: Simplified diagram of the principle of NMR: interaction between proton spin and the external magnetic field (B_0)

II. Theoretical Principle of NMR

Nuclear magnetic resonance is based on a fundamental property of atomic nuclei: spin. Certain nuclei, such as hydrogen (1H), possess a magnetic moment associated with this spin. In the presence of a strong external magnetic field (denoted B0), these nuclei behave like tiny magnets that can align either with the field (lower energy state) or against it (higher energy state).

This distribution leads to two distinct energy states, separated by an energy difference proportional to the strength of the magnetic field B_0. When a radiofrequency wave corresponding exactly to this energy difference is applied, the nuclei absorb the energy and switch from the ground state to the excited state. This phenomenon is called resonance. After excitation, the nuclei gradually return to their initial state by releasing the absorbed energy. This relaxation results in the emission of a signal measurable by the spectrometer detector. Mathematical analysis of these signals makes it possible to construct the NMR spectrum.

Two important phenomena characterize nuclear relaxation:

-spin-lattice relaxation (T1), which corresponds to the transfer of energy from the excited spins to their environment;

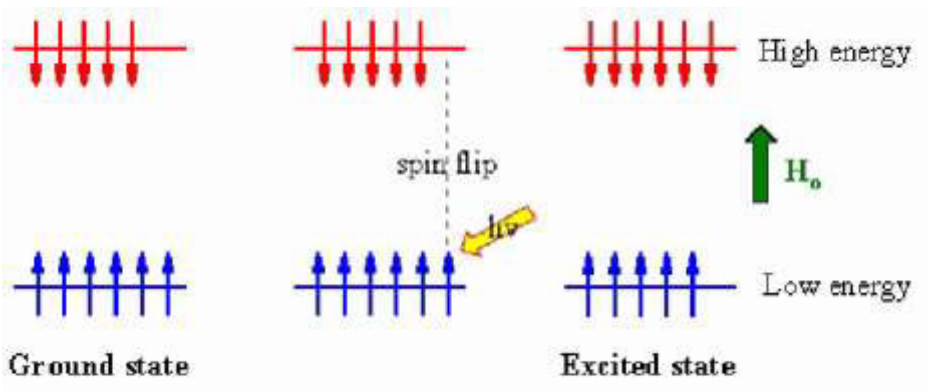
spin-spin relaxation (T2), which corresponds to the loss of coherence between the spins.

In practice, the resonance frequency (called the Larmor frequency) is given by the relation:

$$\mathbf{N = (\gamma \times B0) / (2\pi)}$$

Where γ is the gyromagnetic ratio, characteristic of each type of nucleus. Thus, the stronger the magnetic field, the higher the resonance frequency. This is why modern NMR spectrometers use very powerful superconducting magnets.

Understanding this principle is essential, as it explains the origin of chemical shifts and couplings observed in proton NMR spectra.



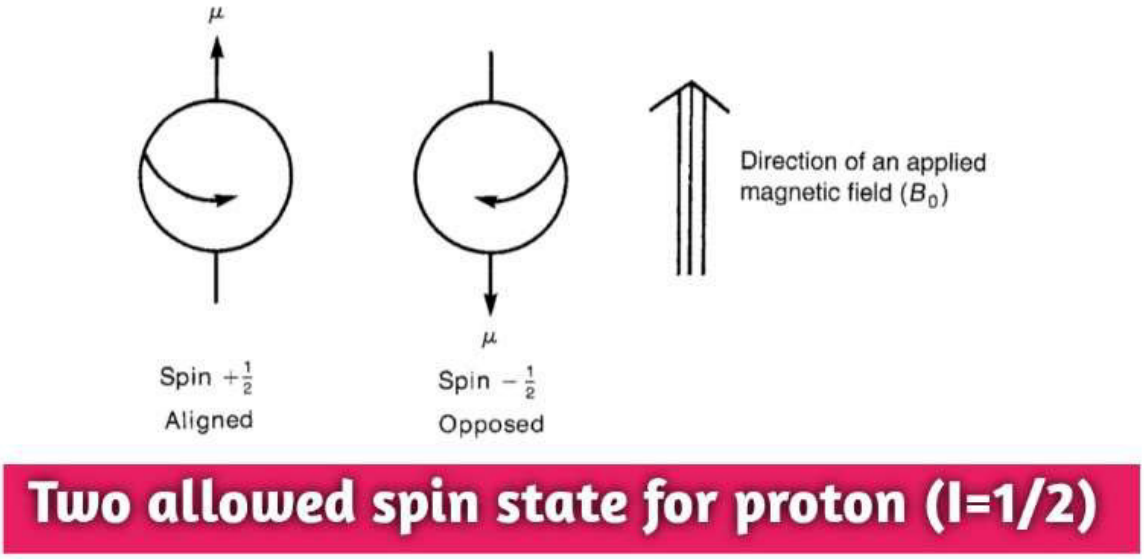


Figure 2: Representation of the two energy states of a proton in the presence of an external magnetic field (B_0)

III. Chemical Shift (δ)

The chemical shift is one of the fundamental parameters in NMR spectroscopy. It corresponds to the position of a signal in the NMR spectrum, expressed in parts per million (ppm), and reflects the electronic environment of the observed proton.

Electrons surrounding a nucleus generate small local magnetic fields that oppose the external field B_0. This phenomenon is called the shielding effect. Thus, two protons located in different chemical environments do not resonate at the same frequency:

- a proton strongly surrounded by electrons is more "shielded," and its signal appears at high field (low δ);
- a proton bonded to electronegative atoms or involved in π bonds is "deshielded," and its signal appears at low field (high δ).

The value of the chemical shift depends not only on the nature of neighboring atoms but also on conjugation effects, anisotropic interactions of aromatic rings, and even intermolecular interactions such as hydrogen bonding.

To standardize measurements, NMR spectra use an internal reference. Most often, tetramethylsilane (TMS) is employed, whose signal is arbitrarily set at $\delta = 0$ ppm. All other signal positions are then measured relative to this standard.

In practice, the chemical shift scale in proton NMR generally extends from 0 to 12 ppm:

- $\delta \approx$ 0–2 ppm: protons of saturated alkanes (strongly shielded);

- $\delta \approx$ 2–4 ppm: protons near electronegative atoms (O, N, halogens);

- $\delta \approx$ 4–6 ppm: vinylic protons (C=C bonds);

- $\delta \approx$ 6–9 ppm: aromatic protons;

- $\delta \approx$ 9–12 ppm: aldehydic and carboxylic protons (strongly deshielded).

Thus, the analysis of chemical shifts provides valuable first indications about the nature of the chemical groups present in a molecule.

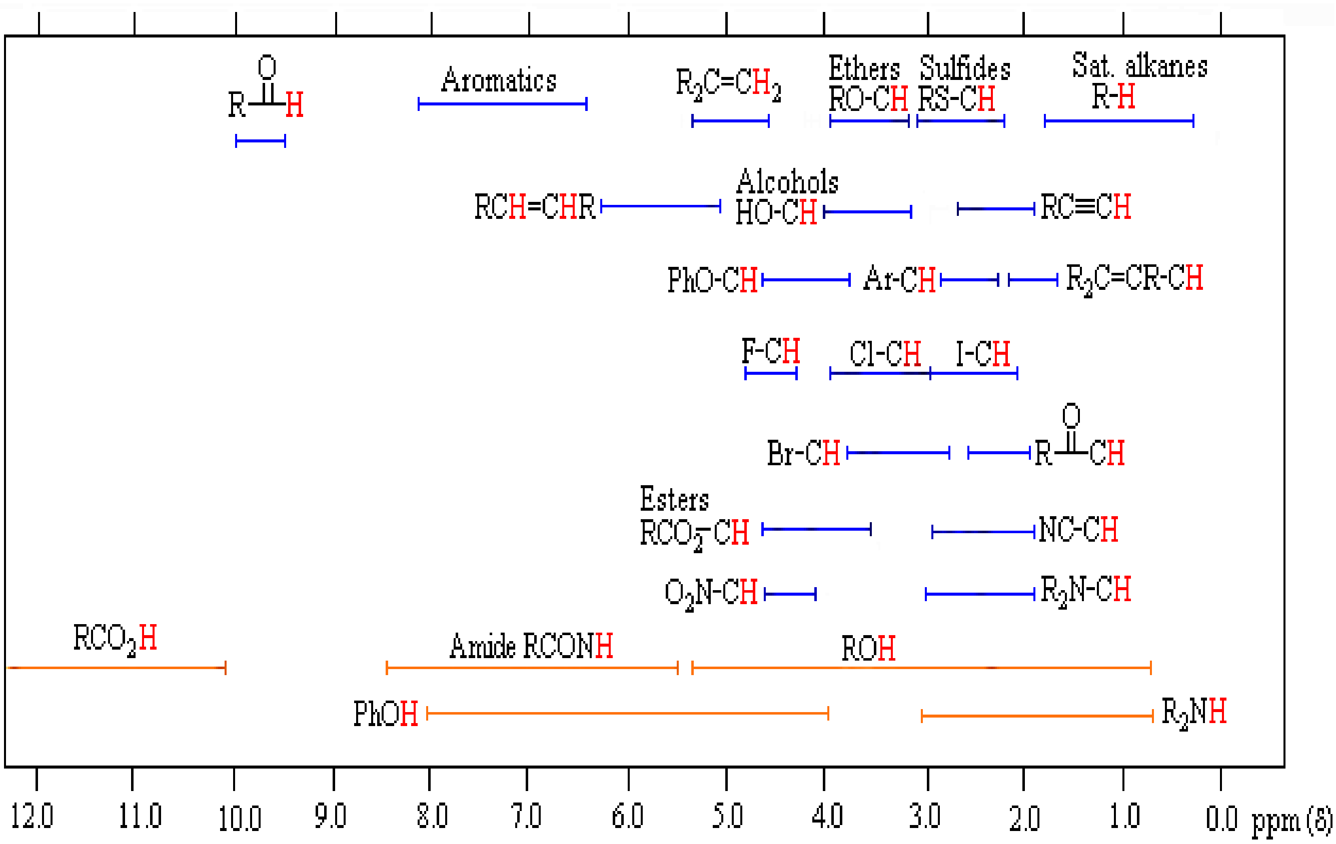


Figure 3: Typical scale of chemical shifts (δ) in proton NMR with characteristic regions

IV. Integration of NMR Signals

In proton NMR spectroscopy, integration of the signals corresponds to measuring the area under each peak. This information is directly proportional to the number of protons responsible for that signal. Thus, integration is an essential tool for establishing the formula and structure of a molecule.

In practice, NMR spectrometers generate an integration curve superimposed on the spectrum. This curve usually takes the form of a step trace, where each plateau corresponds to a group of equivalent protons. The relative height of each plateau makes it possible to determine the ratio between the different types of protons present.

For example, if the integration of a spectrum shows a ratio of 3:2:1 for three distinct signals, this means that the protons responsible for these signals are present in a relative ratio of 3, 2, and 1. This result may correspond to a molecule with a CH3 group, a CH2 group, and an isolated proton.

It is important to note that integration gives only relative ratios, not absolute numbers. To obtain the exact correspondence with the molecular formula, the integration data must be compared with the chemical composition determined by other methods (elemental analysis, mass spectrometry, etc.).
Integration is therefore an essential step in spectral analysis, as it links the observed signals to the actual proportion of protons present in the studied molecule.

Integration tells us the ratio of protons by measuring the area under each peak of the signal.

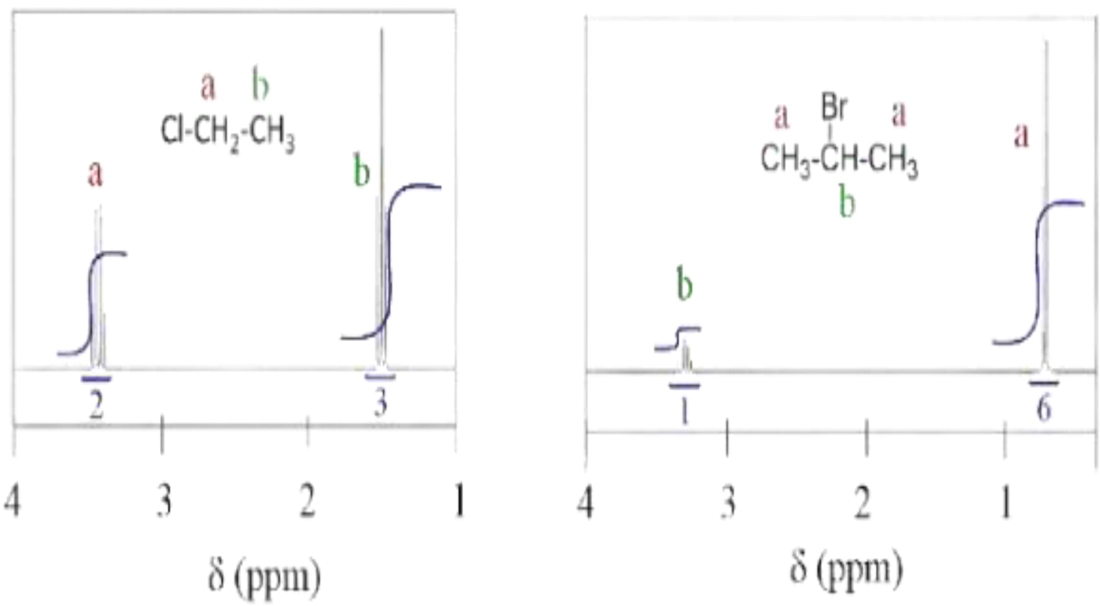


Figure 4: Example of an NMR spectrum with a step-shaped integration curve, illustrating the ratios between equivalent protons

V. Multiplicity and Spin-Spin Coupling

In addition to their position and integration, NMR signals possess a fine structure called multiplicity. This multiplicity results from spin-spin coupling, that is, magnetic interactions between neighboring protons. It provides valuable information on the connectivity and proximity of hydrogen atoms in a molecule.

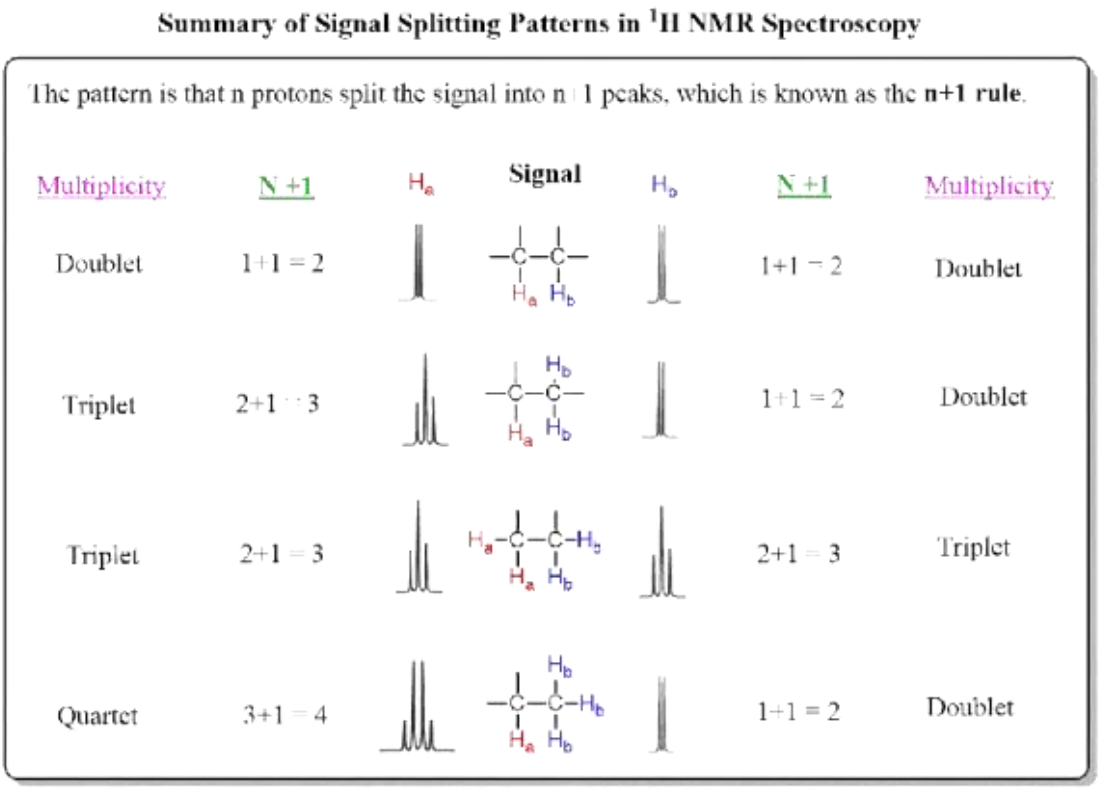
When a proton is located near other nonequivalent protons, its signal splits into several peaks. The number of peaks obtained follows the "n + 1" rule: a proton coupled to n neighboring protons will appear as a multiplet consisting of (n + 1) lines. For example:

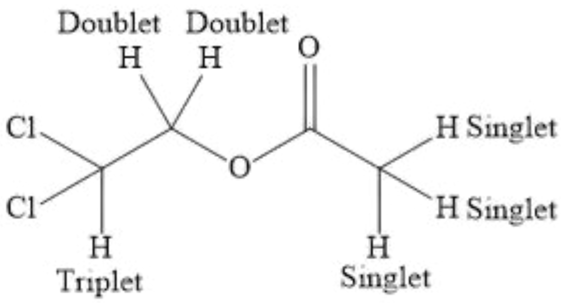
a proton with no neighbors appears as a singlet;
a proton coupled to one neighbor appears as a doublet;
a proton coupled to two equivalent neighbors appears as a triplet;
a proton coupled to three equivalent neighbors appears as a quartet.

The distance between the lines of a multiplet corresponds to the coupling constant J, expressed in hertz (Hz). This constant reflects the intensity of the interaction between spins and depends on the nature of the bonds and the molecular geometry. For example, vinylic protons display different coupling constants depending on whether they are in cis, trans, or geminal configuration.

The study of multiplicity thus makes it possible to distinguish different types of environments and confirm atomic connectivities. Combined with chemical shifts and integration, it constitutes a powerful tool for elucidating the structure of organic molecules.

However, in some complex cases (mixtures of couplings or overlapping signals), interpretation may become difficult and require advanced techniques such as two-dimensional NMR.





Number of equivalent hydrogen atoms causing splitting	Multiplicity	Relative peak intensities
1	doublet	1:1
2	triplet	1:2:1
3	quartet	1:3:3:1
4	quintet	1:4:6:4:1
5	sextet	1:5:10:10:5:1
6	septet	1:6:15:20:15:6:1

Figure 5: Examples of typical multiplets: singlet, doublet, triplet, and quartet, illustrating the (n + 1) rule

VI. Coupling Constants (J)

The coupling constant J is an essential parameter in NMR spectroscopy, as it provides information on the magnetic interaction between neighboring protons. It corresponds to the distance, expressed in hertz (Hz), that separates the lines of a multiplet observed in an NMR spectrum.

The value of J depends directly on the nature of the chemical bonds connecting the protons concerned and on their spatial orientation. Consequently, it provides valuable structural information that complements that provided by chemical shift and integration.

The main situations encountered are:

- Germinal coupling (2J): interaction between two protons bound to the same carbon (–CH2–). The value of J generally varies between –10 and +18 Hz, depending on local geometry and substituent effects.
- Vicinal coupling (3J): interaction between protons separated by three bonds (–CH–CH–). The value of J strongly depends on the dihedral angle between the C–H bonds (Karplus relation). Typically, it is about 0 to 12 Hz for aliphatic systems.

- Long-range coupling (4J, 5J, etc.): interactions between protons separated by four or more bonds. These couplings are often weaker (less than 3 Hz), but can be observed in aromatic or heteroaromatic rings.

One of the most characteristic examples concerns vinylic protons:
- in cis configuration, 3J is generally between 6 and 12 Hz;

- in trans configuration, 3J is between 12 and 18 Hz.

Thus, measuring coupling constants makes it possible not only to confirm atomic connectivity but also to deduce conformational information about the geometry of molecules.

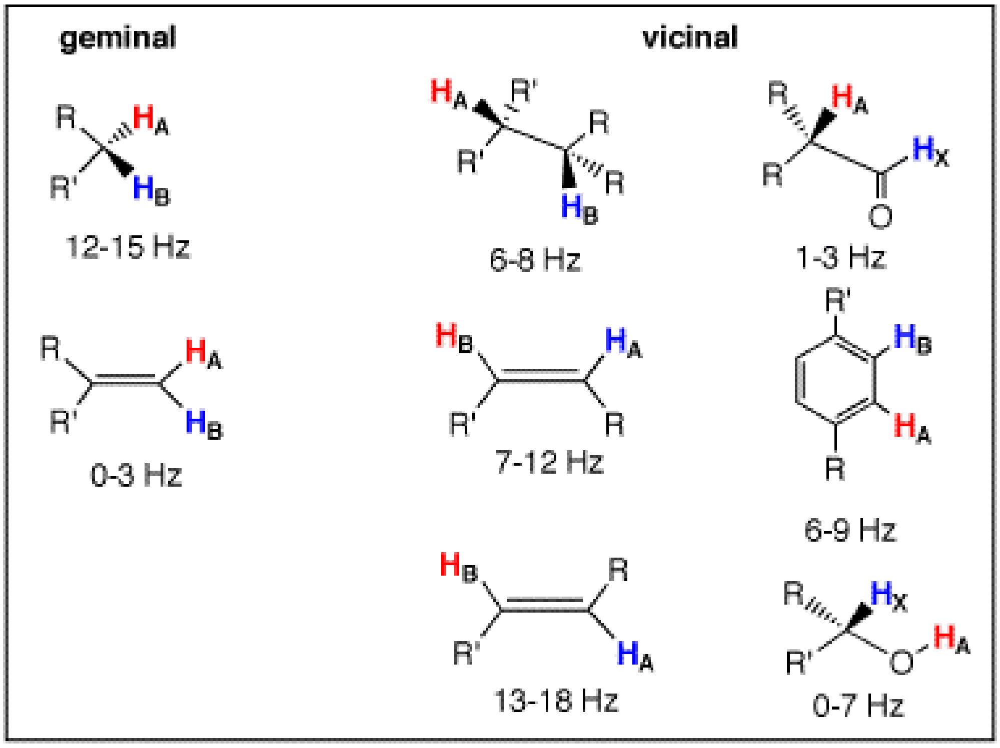


Figure 6: Illustration of the main coupling constants: germinal (2J), vicinal (3J, cis/trans), and long-range

VII.. Solvents and References in NMR

The choice of solvent is a crucial step in NMR spectroscopy, as it must dissolve the studied molecule while not interfering with spectral analysis. Indeed, protons from the solvent could generate undesirable signals in the spectrum.

To avoid this problem, deuterated solvents are used, in which hydrogen atoms are replaced by deuterium (2H). Since deuterium has a different resonance frequency, it does not disturb the proton NMR spectrum. Moreover, deuterated solvents play an important role in stabilizing the magnetic field through the deuterium signal used for spectrometer locking.

Among the most commonly employed deuterated solvents are:

- deuterated chloroform (cdcl3), suitable for many organic compounds;
- deuterated dimethyl sulfoxide (DMSO-d6), used for polar molecules and

biological compounds;

- deuterated methanol (CD_3OD), often used for polar and water-soluble compounds;
- deuterated water (D_2O), essential for the study of biomolecules in aqueous solution.

As for the internal reference, tetramethylsilane (TMS) is the most commonly used compound. Its unique signal, corresponding to 12 equivalent strongly shielded protons, is arbitrarily set at $\delta = 0$ ppm. This choice is motivated by several reasons:

- TMS is chemically inert and does not react with most compounds studied;
- it is soluble in most organic solvents;
- it provides an intense, sharp signal well separated from most organic signals.

In some cases where TMS cannot be used, other secondary references may be employed, such as the residual proton signal of the deuterated solvent itself (for example, the chcl3 signal in cdcl3, fixed at $\delta = 7.26$ ppm).

The judicious choice of solvent and reference is therefore indispensable for obtaining clear, interpretable, and reproducible proton NMR spectra.

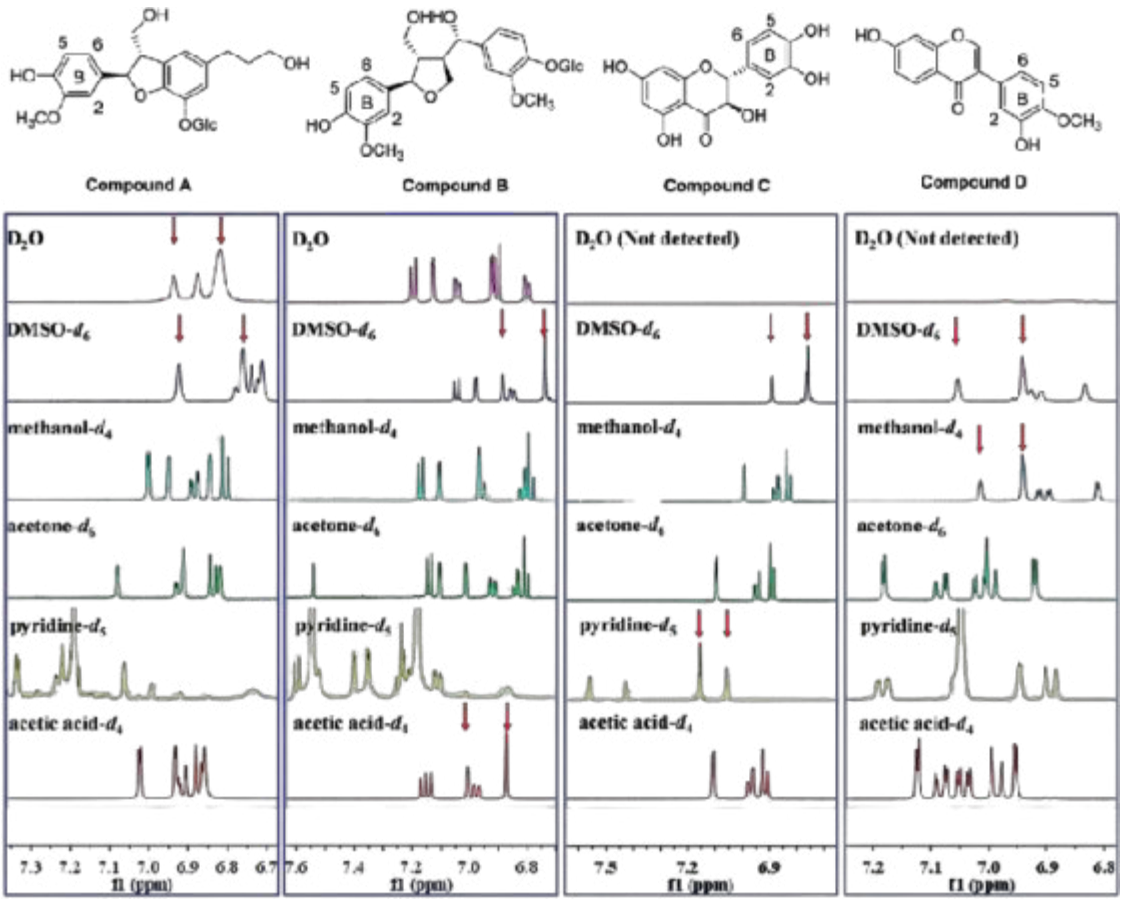


Figure 7: Examples of commonly used deuterated solvents in NMR and position of the TMS reference signal

VIII. NMR Spectrometers and Their Components

An NMR spectrometer is a sophisticated instrument that generates, detects, and analyzes nuclear magnetic resonance signals. Its design relies on several essential elements, each playing a defined role in spectrum acquisition.

The main components of an NMR spectrometer are:

1. The superconducting magnet: the heart of the instrument. By using superconducting alloys cooled with liquid helium, it can produce a strong, stable, and homogeneous magnetic field. Fields used generally range between 200 and 1000 mhz for proton NMR. The stronger the field, the higher the spectral resolution.

2. The probe: it contains the detection coil in which the sample dissolved in a deuterated solvent is placed. The probe allows both excitation of nuclei by the radiofrequency pulse and detection of the signal emitted during relaxation.

3. The radiofrequency (RF) generator: it sends very precise radiofrequency pulses capable of inducing resonance of nuclei according to the applied magnetic field.

4. The detection system: it captures signals emitted by relaxing nuclei. These signals are then converted into digital data and analyzed by computer after Fourier transformation.

5. The control unit and computer: they ensure programming of pulse sequences, data collection, and spectrum processing.

The proper functioning of an NMR spectrometer depends on precise calibration, continuous temperature control, and perfect magnetic field homogeneity. These conditions guarantee the acquisition of reliable and reproducible spectra.

Thanks to technological advances, modern spectrometers allow not only one-dimensional (1D) measurements but also multidimensional (2D, 3D) experiments very useful in biomolecular chemistry and materials science.

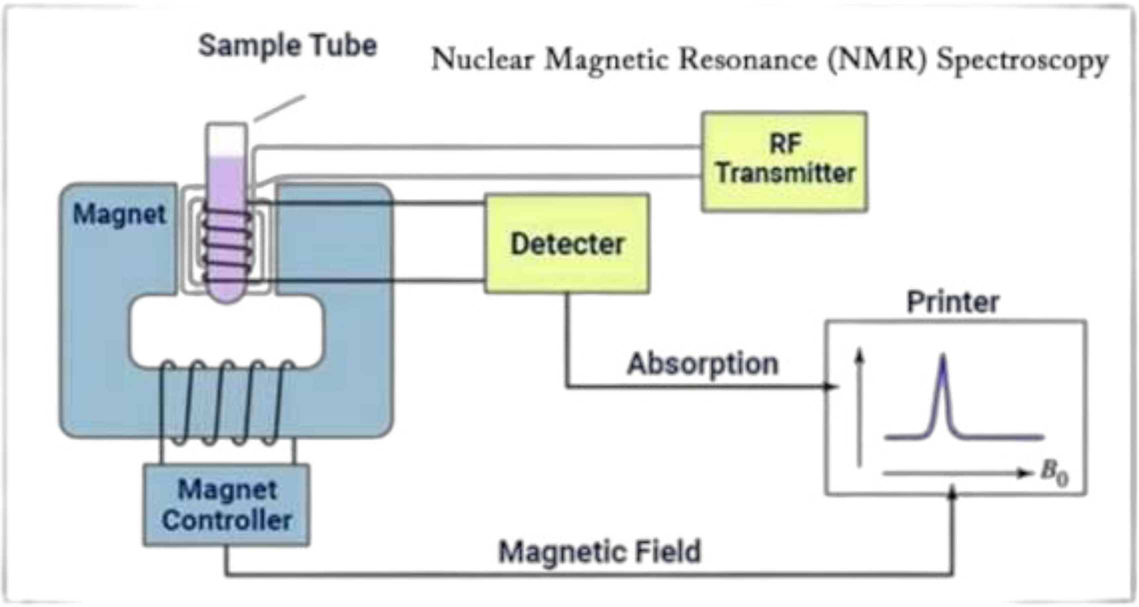


Figure 8: Simplified diagram of an NMR spectrometer with its main components: magnet, probe, RF generator, and detection system.

IX. Applications of Proton NMR in Organic Chemistry

Proton NMR is a fundamental tool for the analysis and characterization of organic compounds. It provides valuable information on the chemical environment of hydrogen atoms, thus making it possible to determine the structure and dynamics of molecules.

Among the main applications, we distinguish:

1. Identification of functional groups: characteristic chemical shifts (δ) make it possible to recognize the presence of groups such as alcohols, aldehydes, carboxylic acids, amines, aromatics, etc. For example, a signal around δ = 9–10 ppm is typical of an aldehydic proton.

2. Determination of molecular structure: through the combined analysis of chemical shift, integration, and spin-spin coupling, it is possible to reconstruct the

architecture of a molecule and confirm a proposed structure. Proton NMR is thus a complementary tool to IR spectroscopy and mass spectrometry.

3. Analysis of sample purity: NMR spectra make it easy to detect the presence of impurities, residual solvents, or by-products. This application is particularly useful in synthetic organic chemistry.

4. Study of stereochemistry and conformation: coupling constants (J) and correlation experiments (COSY, NOESY) provide information on molecular geometry, relative configuration of stereogenic centers, and preferred conformations adopted in solution.

5. Monitoring chemical reactions: NMR makes it possible to monitor the progress of a reaction in real time by identifying the disappearance of reactants and the appearance of products. It is a preferred tool for optimizing reaction conditions.

6. Applications in biomolecular chemistry: proton NMR is widely used for studying peptides, proteins, and nucleic acids. It provides information on secondary and tertiary structure as well as on noncovalent interactions with ligands.

In summary, proton NMR is a versatile and indispensable technique that finds applications in all areas of organic chemistry, from academic research to the pharmaceutical industry.

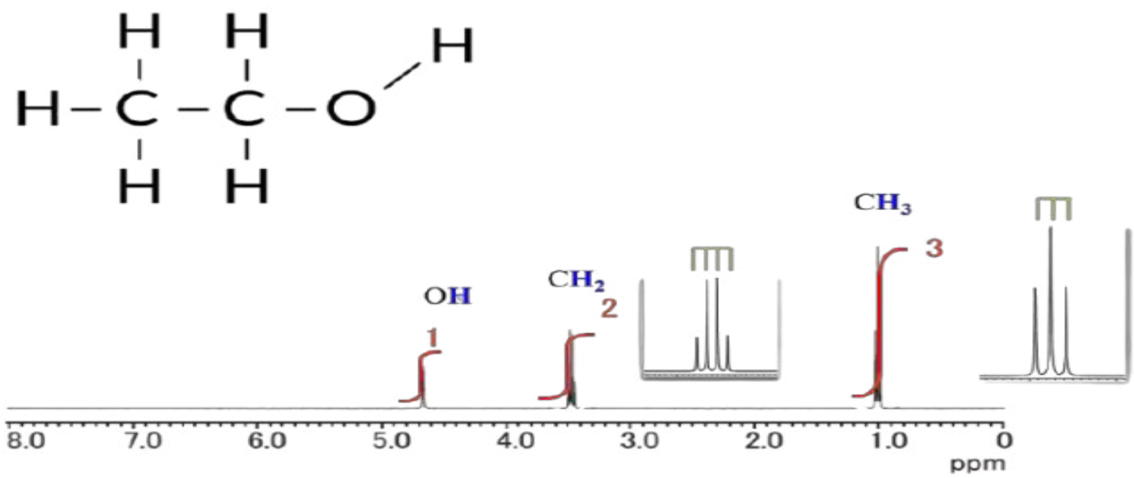
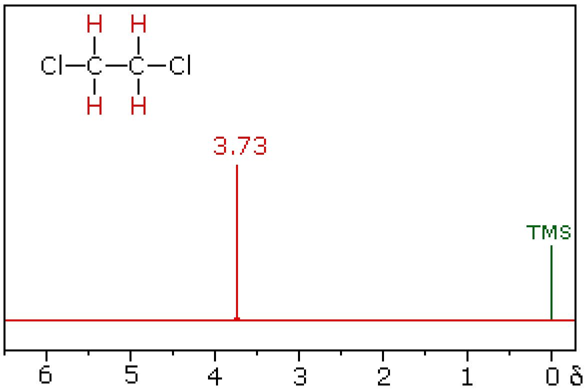


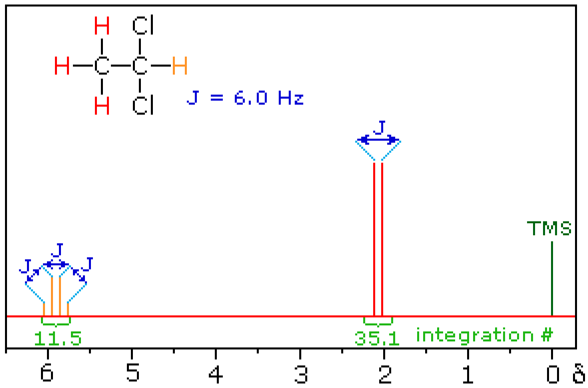
Figure 9: Example of an application of proton NMR in the identification of functional groups and molecular structure determination

X. NMR spectra comparison

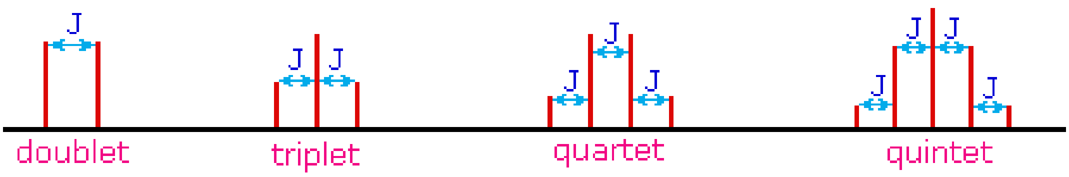
X.1. Spin-Spin Interactions

The NMR spectrum of 1,1-dichloroethane (below right) is more complicated than we might have expected from the previous examples. Unlike its 1,2-dichloro-isomer (below left), which displays a single resonance signal from the four structurally equivalent hydrogens, the two signals from the different hydrogens are split into close groupings of two or more resonances. This is a common feature in the spectra of compounds having different sets of hydrogen atoms bonded to adjacent carbon atoms. The signal splitting in proton spectra is usually small, ranging from fractions of a Hz to as much as 18 Hz, and is designated as **J** (referred to as the coupling constant). In the 1,1-dichloroethane example all the coupling constants are 6.0 Hz, as illustrated by clicking on the spectrum.



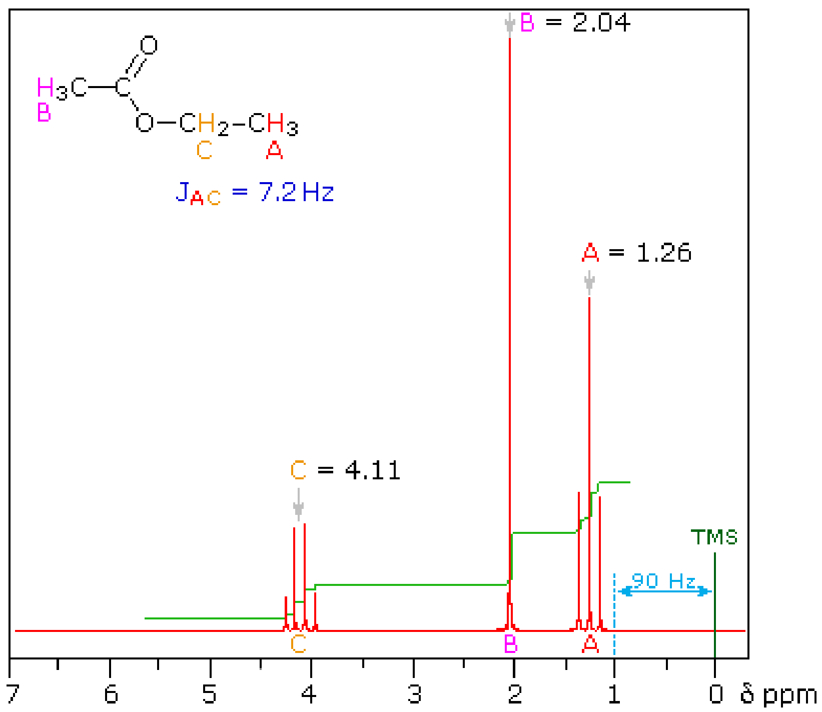


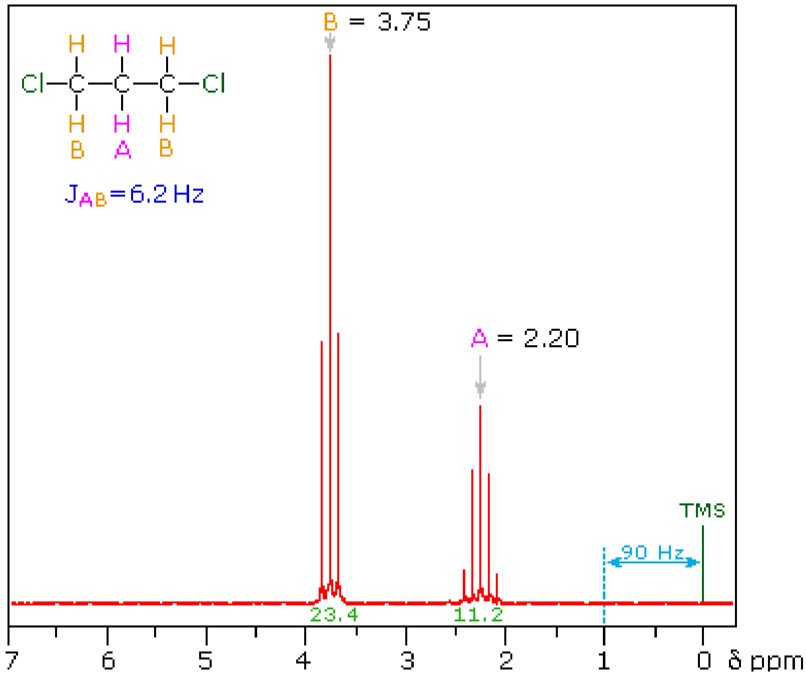
The splitting patterns found in various spectra are easily recognized, provided the chemical shifts of the different sets of hydrogen that generate the signals differ by two or more ppm. The patterns are symmetrically distributed on both sides of the proton chemical shift, and the central lines are always stronger than the outer lines. The most commonly observed patterns have been given descriptive names, such as **doublet** (two equal intensity signals), **triplet** (three signals with an intensity ratio of 1:2:1) and **quartet** (a set of four signals with intensities of 1:3:3:1). Four such patterns are displayed in the following illustration. The line separation is always constant within a given multiplet, and is called the **coupling constant (J)**. The magnitude of J, usually given in units of Hz, is magnetic field independent.



The splitting patterns shown above display the ideal or "**First-Order**" arrangement of lines. This is usually observed if the spin-coupled nuclei have very different chemical shifts (i.e. Δν is large compared to J). If the coupled nuclei have similar chemical shifts, the splitting patterns are distorted (second order behavior). In fact, signal splitting disappears if the chemical shifts are the same. Two examples that exhibit minor 2nd order distortion are shown below (both are taken at a frequency of 90 MHz). The ethyl acetate spectrum on the left displays the typical quartet and triplet of a substituted ethyl group. The spectrum of 1,3-dichloropropane on the right demonstrates that equivalent sets of hydrogens may combine their influence on a second, symmetrically located set.

Even though the chemical shift difference between the A and B protons in the 1,3-dichloroethane spectrum is fairly large (140 Hz) compared with the coupling constant (6.2 Hz), some distortion of the splitting patterns is evident. The line intensities closest to the chemical shift of the coupled partner are enhanced. Thus the B set triplet lines closest to A are increased, and the A quintet lines nearest B are likewise stronger. A smaller distortion of this kind is visible for the A and C couplings in the ethyl acetate spectrum.



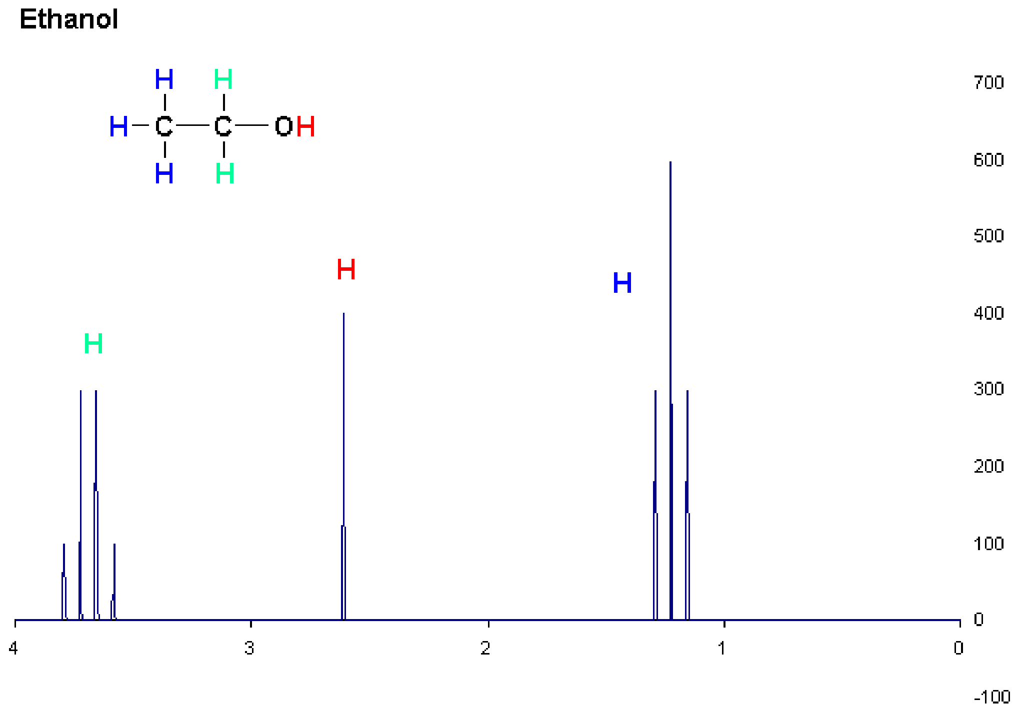


What causes this signal splitting, and what useful information can be obtained from it ?

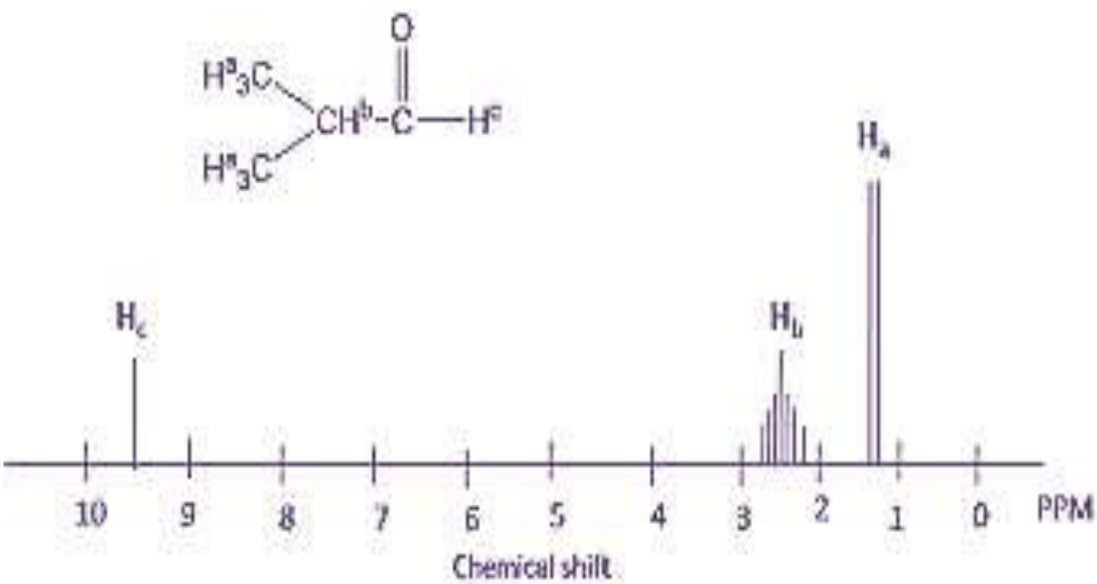
If an atom under examination is perturbed or influenced by a nearby nuclear spin (or set of spins), the observed nucleus responds to such influences, and its response is manifested in its resonance signal. This spin-coupling is transmitted through the connecting bonds, and it functions in both directions. Thus, when the perturbing nucleus becomes the observed nucleus, it also exhibits signal splitting with the same J. For spin-coupling to be observed, the sets of interacting nuclei must be bonded in relatively close proximity (e.g. vicinal and geminal locations), or be oriented in certain optimal and rigid configurations. Some spectroscopists place a number before the symbol J to designate the number of bonds linking the coupled nuclei (colored orange below). Using this terminology, a vicinal coupling constant is 3J and a geminal constant is 2J.

For students: observe and interpret

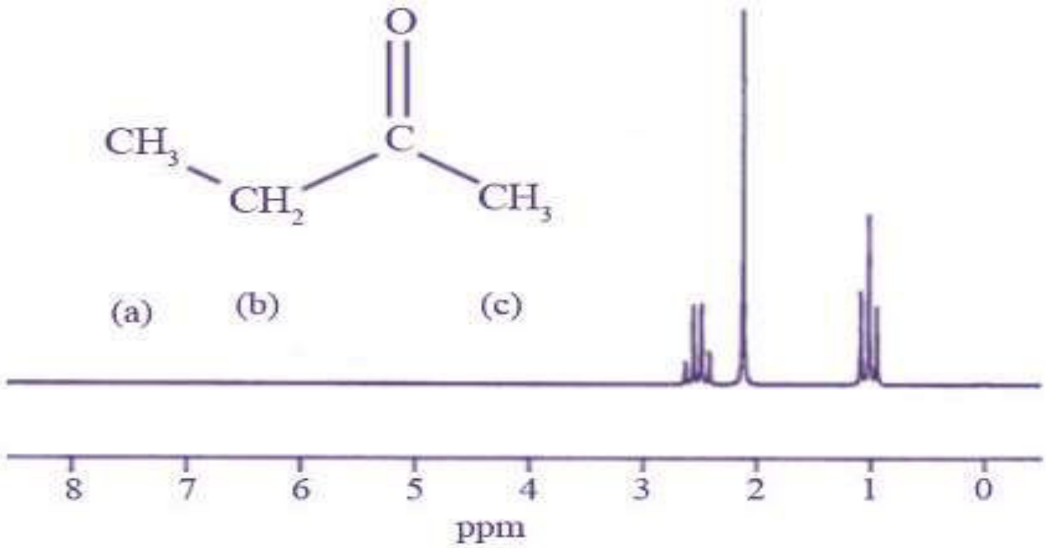
Alcohol



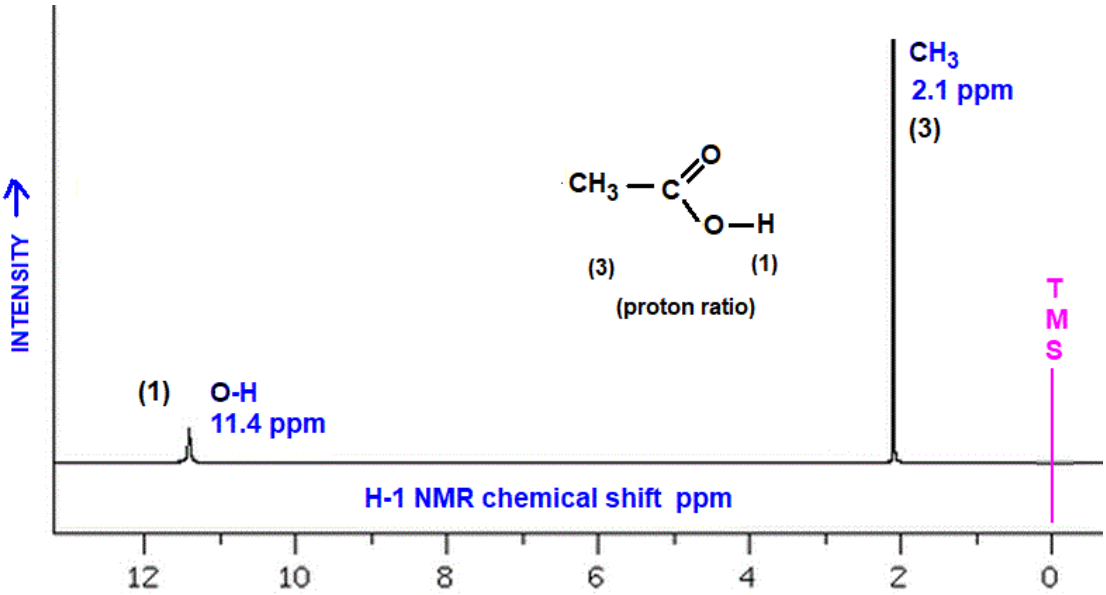
Aldehyde



Ketone



Carboxylic acid



XI. Conclusion and Perspectives

Proton NMR spectroscopy (1H) occupies a central place in the arsenal of modern analytical techniques. It combines high structural precision with versatile applicability, ranging from the simple identification of small organic molecules to the detailed study of complex biological macromolecules.

Throughout this course, we have seen that the information contained in an NMR spectrum results from several key parameters: chemical shift, integration, multiplicity, and coupling constants. The combined interpretation of these data makes it possible to construct an accurate picture of molecular structure.

NMR is also a constantly evolving technique. Advances in superconducting magnets, cryogenic probes, and multidimensional methods continually expand its possibilities. These developments open the way to new applications, particularly in:

- Medicinal chemistry and drug discovery;

- Analysis of materials and polymers;

- Structural biology and the study of biomolecular interactions;

- Real-time monitoring of dynamic processes and chemical reactions.

Looking ahead, the combination of NMR with other analytical techniques, such as IR spectroscopy, mass spectrometry, or X-ray diffraction, will provide an even more comprehensive understanding of matter at the molecular level.

Thus, proton NMR remains an essential method, not only as a pedagogical tool for chemistry students but also as a cutting-edge technique in scientific research and industry.

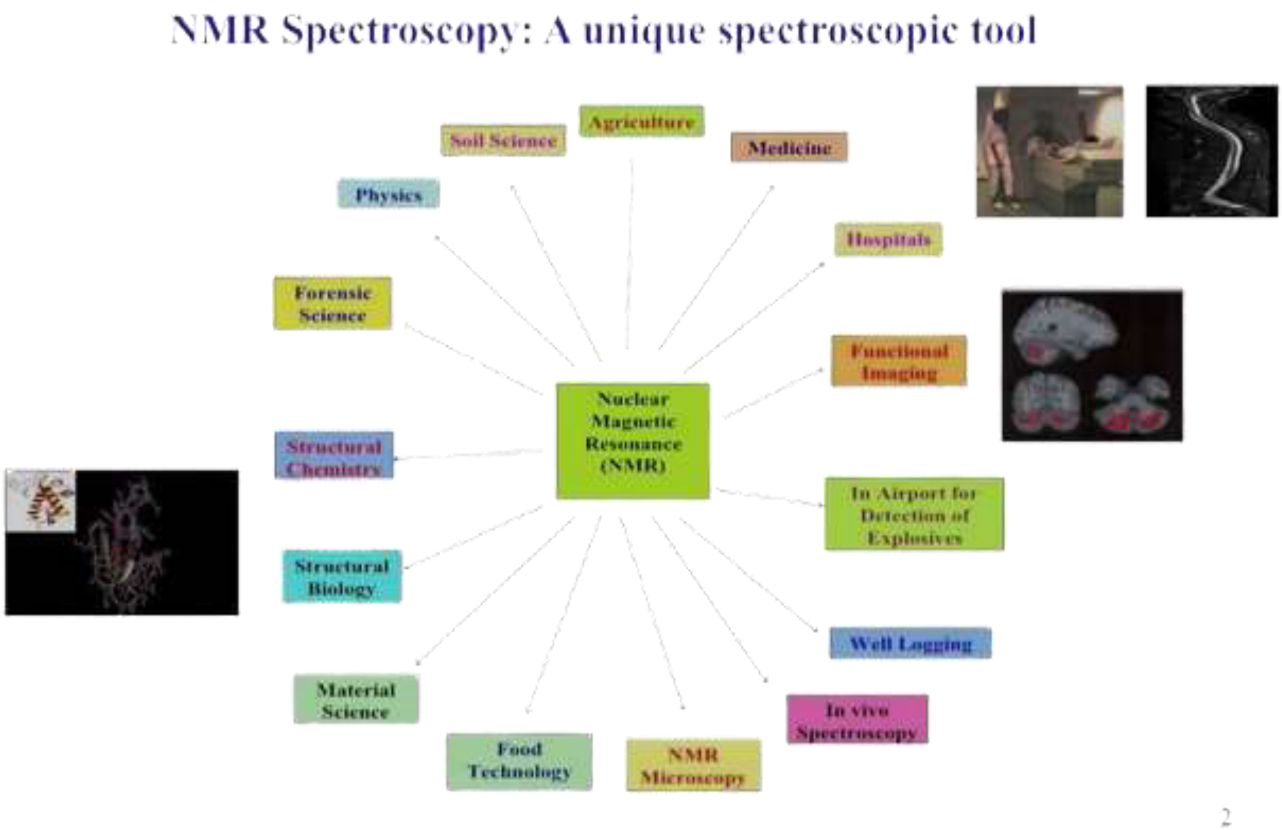


Figure 10. Summary diagram of the main applications of proton NMR and future perspectives

I. Introduction

Mass spectrometry (MS) is one of the most powerful and versatile analytical techniques available in modern science. Its ability to provide information about the mass, structure, and abundance of molecules has made it indispensable in chemistry, biology, medicine, environmental sciences, and material research. Unlike other spectroscopic methods that rely on interactions between molecules and electromagnetic radiation, mass spectrometry measures the mass-to-charge ratio (m/z) of ions with high accuracy, allowing both qualitative and quantitative insights into a wide variety of samples.

The basic operation of a mass spectrometer involves four major steps: ionization of the sample, separation of ions, detection, and spectrum generation. Although instrument designs may differ significantly, every mass spectrometer shares a common architecture built around these essential functions. To fully understand how MS works and how to interpret its results, it is crucial to examine each component and its role in detail.

Section 1: Structure of a Mass Spectrometer.

Section 2: The Ion Source focuses on the different ionization techniques, which determine how neutral molecules are converted into ions. From classical electron ionization (EI) to modern methods such as electrospray ionization (ESI) and MALDI, this section emphasizes the importance of selecting the right ionization strategy depending on the analyte.

Section 3: The Mass Analyzer explores the various technologies used to separate ions according to their m/z ratio. Quadrupoles, TOF, ion traps, Orbitrap, and FT-ICR analyzers are presented, each with its advantages, limitations, and applications, from routine analysis to ultra-high-resolution studies.

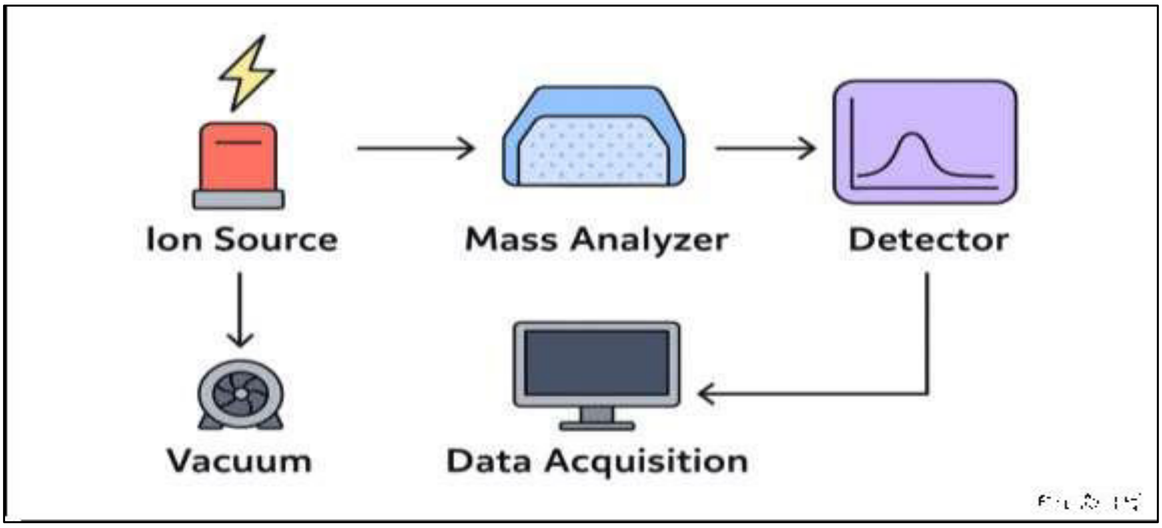
Section 4: The Detector explains how the separated ions are finally converted into measurable signals. Different detection systems, including electron multipliers, microchannel plates, and Faraday cups, are discussed, with attention to their sensitivity, robustness, and analytical roles.

Section 5: The Mass Spectrum presents the final output of mass spectrometry. It explains how to interpret the molecular ion peak, fragment ions, isotopic patterns, and tandem spectra. The section also highlights applications in structural elucidation, quantitative analysis, and advanced fields such as proteomics, metabolomics, and forensic science.

Together, these five sections provide a comprehensive overview of mass spectrometry. From the physical principles underlying ionization and mass analysis to the practical interpretation of spectra, this course aims to equip students with both theoretical knowledge and practical skills. Mastering these concepts will allow researchers to confidently apply MS across scientific disciplines and to appreciate its central role in modern analytical science.

I. Mass Spectrometer Structure

Structure of a Mass Spectrometer introduces the general architecture of the instrument, describing the three essential building blocks: the ion source, the mass analyzer, and the detector. It also highlights the supporting systems, such as vacuum technologies and data acquisition software that ensure reliable operation.



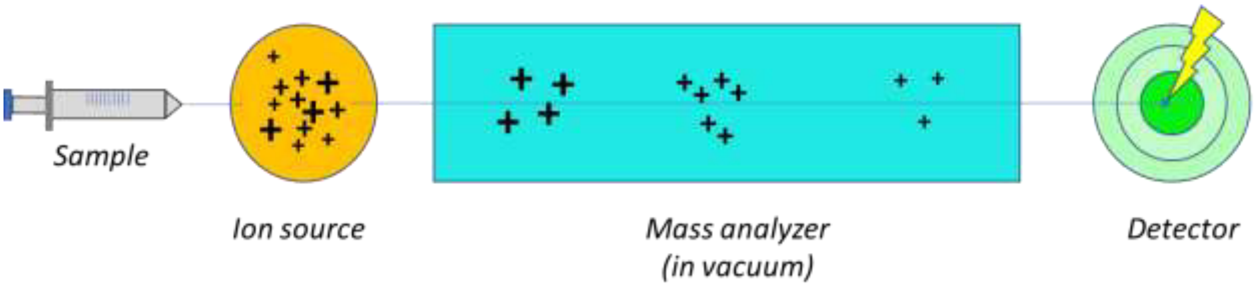


Figure 1: Structure and Principe of a mass spectrometer

II. The Ion Source

The ion source constitutes the gateway of the mass spectrometer. Its primary role is to transform neutral molecules into charged species that can be accelerated, focused, and analyzed by subsequent components. The choice of ionization method determines the type of ions generated, their stability, and the richness of structural information that can be extracted. Without efficient ionization, even the most advanced mass analyzer cannot yield reliable spectra.

II.1. Electron Ionization (EI)

Electron Ionization is a hard ionization technique where a beam of electrons, typically accelerated at 70 eV, collides with neutral molecules in the gas phase. The collision results in ejection of an electron, forming positively charged molecular ions (M^+). Because the imparted energy is high, EI frequently induces extensive fragmentation, producing a characteristic pattern of ions that serves as a molecular fingerprint. Although fragmentation can complicate interpretation, it is extremely useful for structure elucidation, which explains why EI spectra have been systematically compiled in large databases for compound identification. EI is best suited for small to medium volatile organic molecules, such as hydrocarbons, esters, and aromatic compounds. However, its use is limited for biomolecules or thermally labile species.

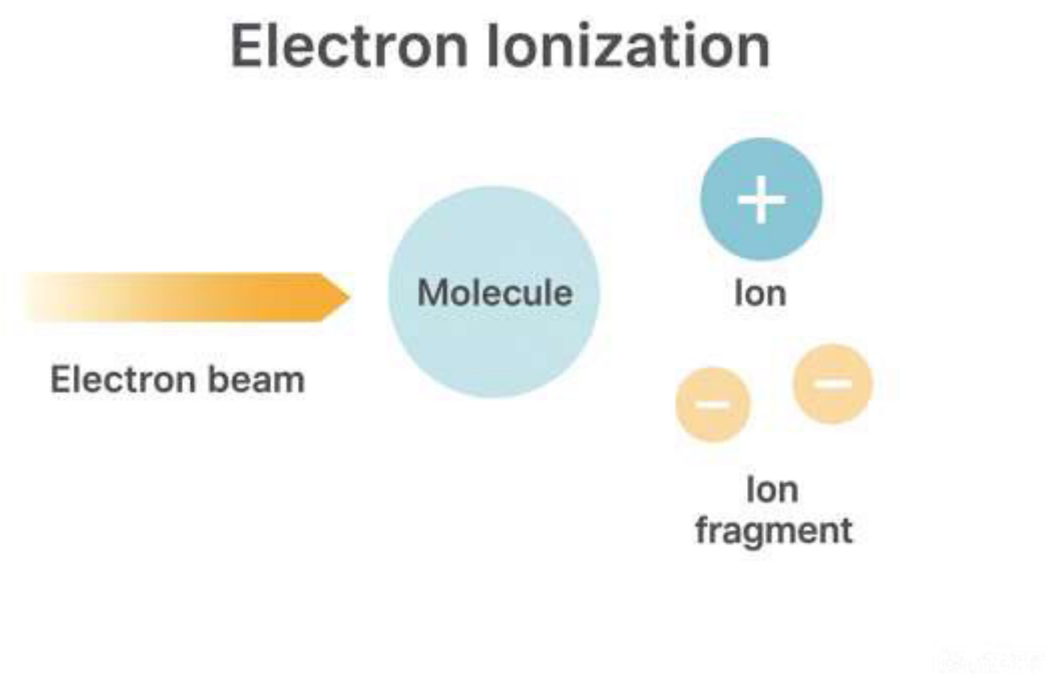


Figure 2: Schematic representation of an Electron Ionization (EI) source

II.4. Chemical Ionization (CI)

Chemical Ionization was developed as a softer alternative to EI. Instead of direct impact, a reagent gas (commonly methane, isobutane, or ammonia) is first ionized by electron bombardment. The ionized gas molecules then react with analyte molecules through ion-molecule reactions, producing ions with reduced fragmentation. This makes CI especially valuable for preserving the molecular ion peak, thus improving molecular weight determination. Softness, however, comes at the expense of reduced fragmentation information. Applications of CI are often found in environmental chemistry and pharmaceutical analysis, where unambiguous determination of molecular weights is crucial.

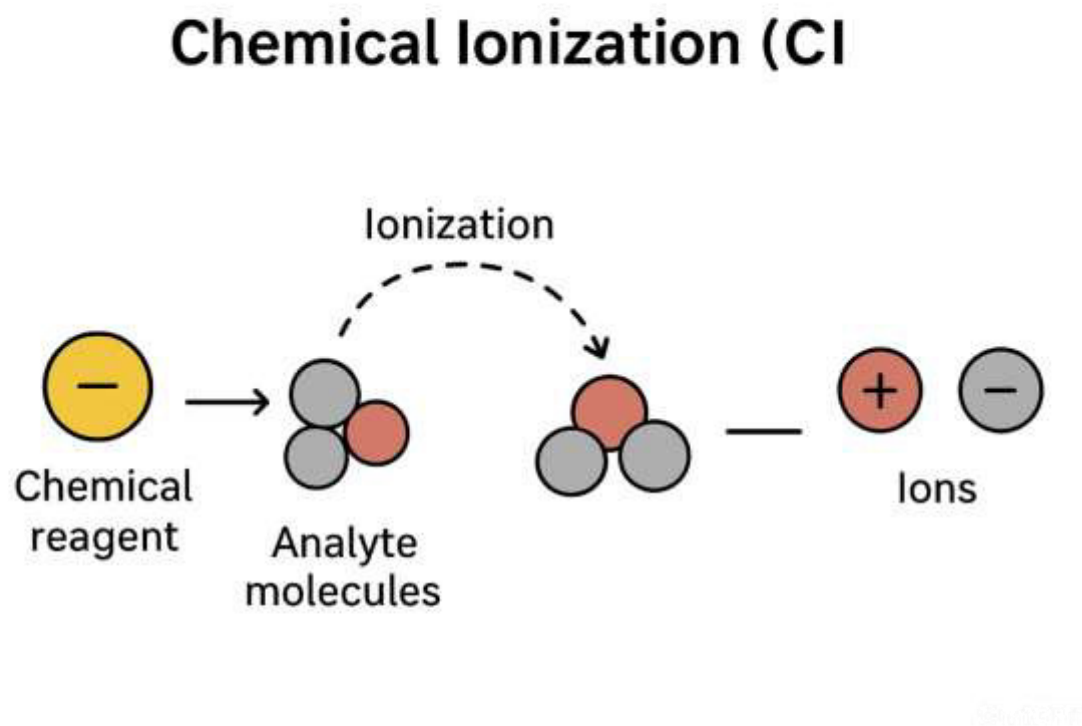


Figure 3: Simplified diagram of a Chemical Ionization (CI) source

II.5. Electrospray Ionization (ESI)

Electrospray Ionization revolutionized the field by enabling direct ionization of large biomolecules under ambient conditions. In ESI, the analyte solution passes through a fine capillary under high voltage (1–5 kV), producing a mist of charged droplets. Solvent evaporation and Coulombic repulsion eventually release ions into the gas phase, often carrying multiple charges. This multiple charging drastically reduces the mass-to-charge ratio, allowing even very large proteins or nucleic acids to be analyzed with instruments of limited m/z range. ESI is highly compatible with liquid chromatography (LC-MS), making it a cornerstone in proteomics, metabolomics, and pharmaceutical research. Its limitations include sensitivity to salts and matrix effects, which can suppress ion formation.

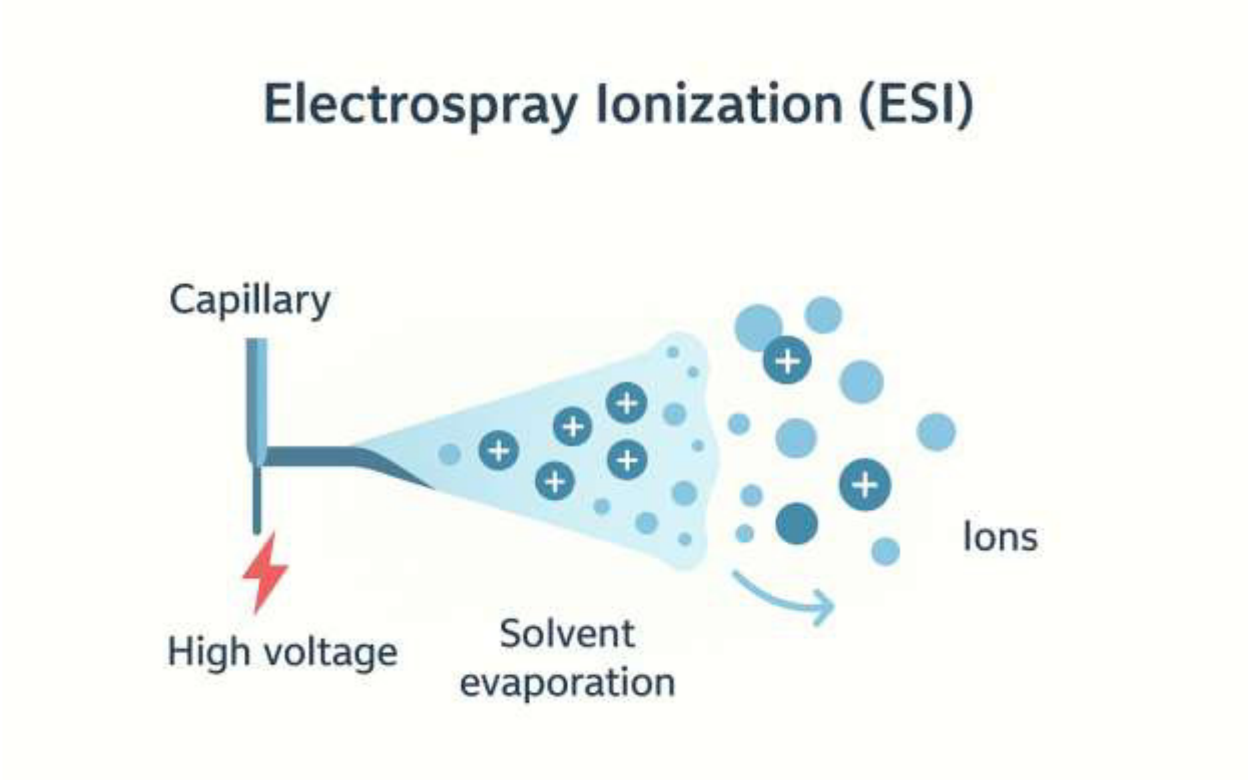


Figure 4: Diagram showing the principle of Electrospray Ionization (ESI)

II.6. Matrix-Assisted Laser Desorption/Ionization (MALDI)

Matrix-Assisted Laser Desorption/Ionization (MALDI) is another transformative soft ionization method, particularly suited for high-mass biomolecules and polymers. The analyte is mixed with an organic matrix that strongly absorbs laser radiation. Upon laser irradiation, the matrix vaporizes and transfers energy to the analyte, facilitating its ionization with minimal fragmentation. MALDI typically produces singly charged ions, which simplifies spectral interpretation. Applications of MALDI range from polymer characterization to biological tissue imaging, where MALDI imaging mass spectrometry (MALDI-MS) enables spatial distribution mapping of biomolecules directly from tissue slices.

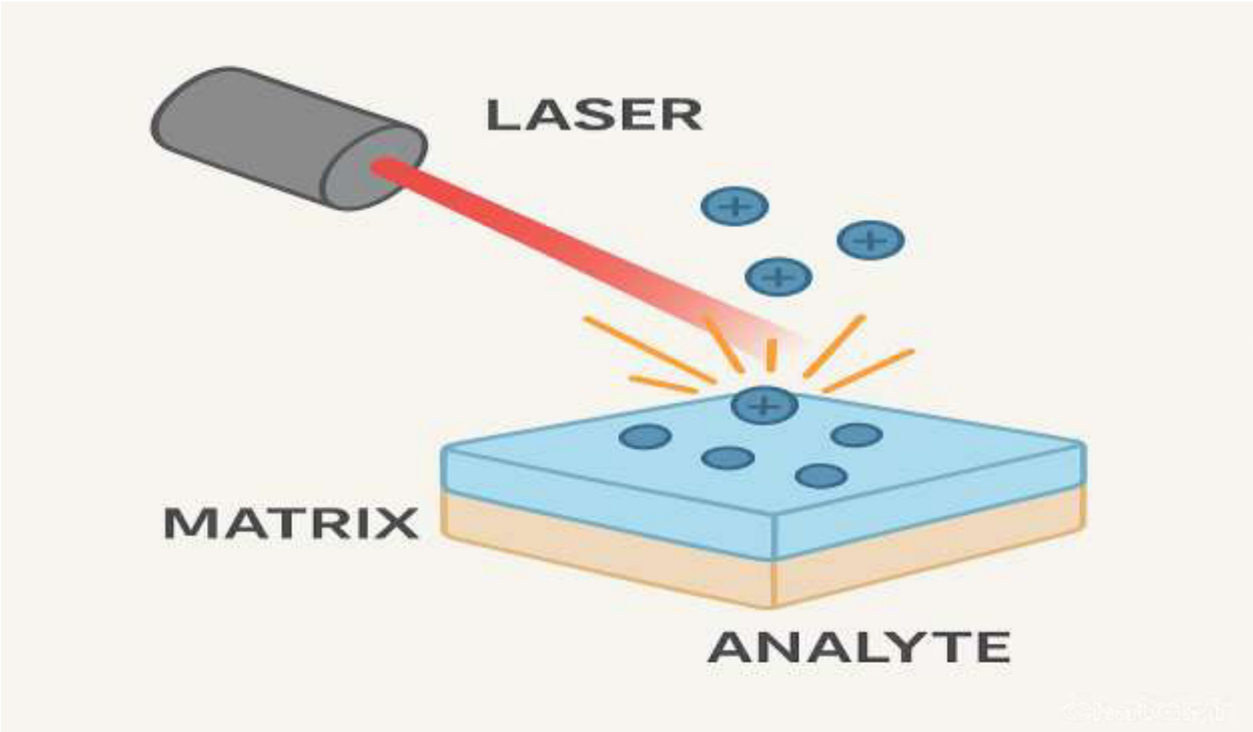


Figure 5: Representation of a MALDI ionization source

II.7. Summary and Comparison

The ion source is the heart of the mass spectrometer, dictating the nature of the ions available for analysis. Hard ionization methods such as EI are unparalleled for structural elucidation of small molecules due to their rich fragmentation patterns, whereas soft ionization techniques like CI, ESI, and MALDI preserve molecular integrity, enabling studies of complex biomolecules. In practice, the selection of the ionization technique depends on analyte properties, analytical goals, and instrument compatibility. The diversity of ion sources ensures that mass spectrometry remains one of the most versatile and powerful analytical tools in modern science.

III. The Mass Analyzer

The mass analyzer is the centerpiece of a mass spectrometer, as it performs the critical task of separating ions according to their mass-to-charge ratio (m/z). The performance of the entire instrument depends heavily on this component, since it dictates resolution, accuracy, acquisition speed, and sensitivity. Depending on the type of analyzer used, researchers can achieve anything from simple qualitative identification to extremely precise quantitative and structural studies. A wide variety of mass analyzers exist, each based on different physical principles, and their choice is guided by the nature of the analyte, the analytical objectives, and the available resources.

III.3. Quadrupole Analyzer

The quadrupole analyzer is one of the most widely used designs due to its simplicity, robustness, and cost-effectiveness. It consists of four parallel rods arranged in a square configuration. By applying a combination of direct current (DC) and radiofrequency (RF) voltages, a stable oscillating electric field is created. Only ions with a particular m/z ratio will maintain a stable trajectory and reach the detector, while all others collide with the rods and are filtered out. This selectivity makes the quadrupole an excellent tool for targeted analysis and routine monitoring, such as in environmental testing, clinical diagnostics, and quality control in the pharmaceutical industry. Its main limitation lies in its relatively low resolution compared to high-end analyzers.

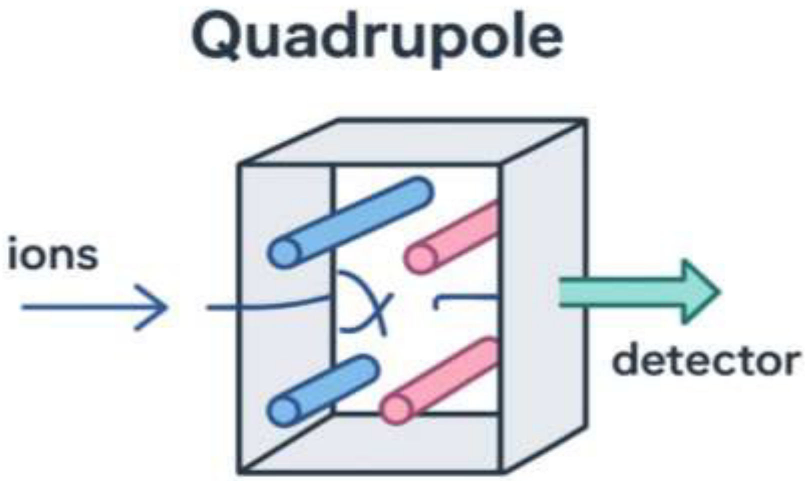


Figure 6: Schematic diagram of a quadrupole mass analyzer

III.2. Time-of-Flight (TOF) Analyzer

The Time-of-Flight analyzer operates on a straightforward yet powerful principle. Ions are accelerated by an electric field to the same kinetic energy. Because lighter ions move faster than heavier ones, they reach the detector earlier. By precisely measuring the flight time of ions over a fixed distance, the instrument calculates their m/z values. TOF analyzers are particularly valued for their unlimited mass range, rapid acquisition rates, and compatibility with pulsed ionization techniques such as MALDI. They are essential in high-throughput applications, proteomics, and polymer research. However, their resolution may be affected by initial energy dispersion, which is usually corrected using reflectron technology.

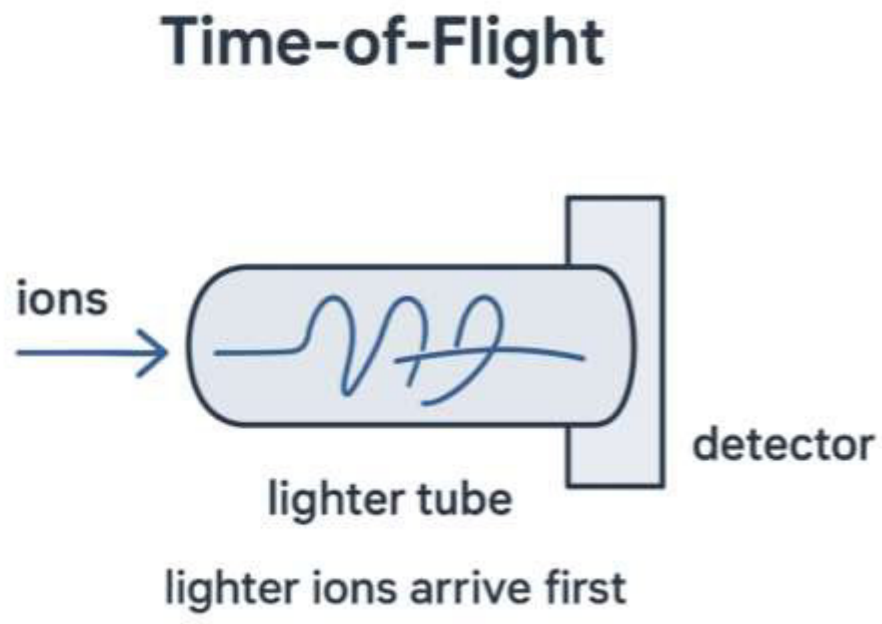


Figure 7: Principle of a Time-of-Flight (TOF) analyzer

III.3. Ion Trap Analyzer

Ion traps represent a versatile class of analyzers that capture ions in a confined space using an oscillating electric field. Once trapped, ions can be manipulated in various ways: they can be isolated, fragmented, and sequentially ejected to the detector. This enables multiple stages of mass spectrometry (MS^n), where detailed structural information can be obtained by fragmenting selected precursor ions. Ion traps are widely used in structural elucidation, pharmaceutical research, and studies requiring tandem MS. Their relatively compact size and cost make them attractive, but they suffer from limited mass range and space-charge effects when too many ions accumulate.

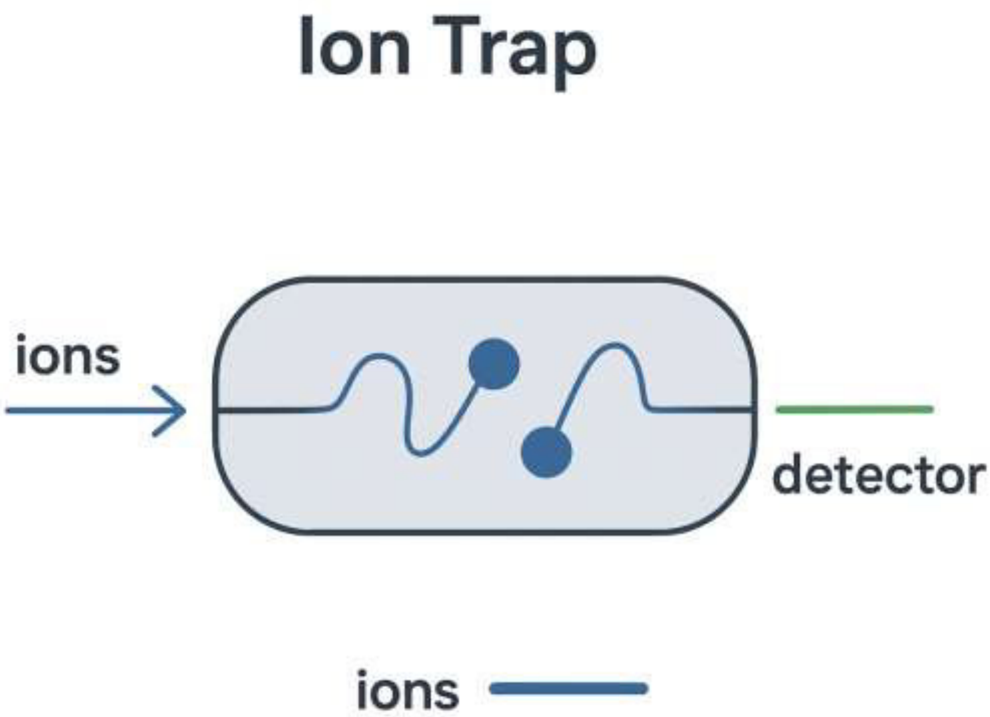


Figure 8: Representation of an Ion Trap analyzer

III.4 Orbitrap Analyzer

The Orbitrap analyzer is a high-resolution instrument that has transformed modern mass spectrometry. It consists of an electrostatic field in which ions are trapped and made to oscillate around a central spindle-shaped electrode. The oscillation frequency of the ions is directly related to their m/z ratio. By detecting these frequencies, Orbitrap instruments achieve extremely high resolution and mass accuracy, often within a few parts per million (ppm). Their stability and reproducibility make them indispensable in proteomics, metabolomics, and advanced clinical studies. They are, however, more expensive than quadrupoles and require careful calibration.

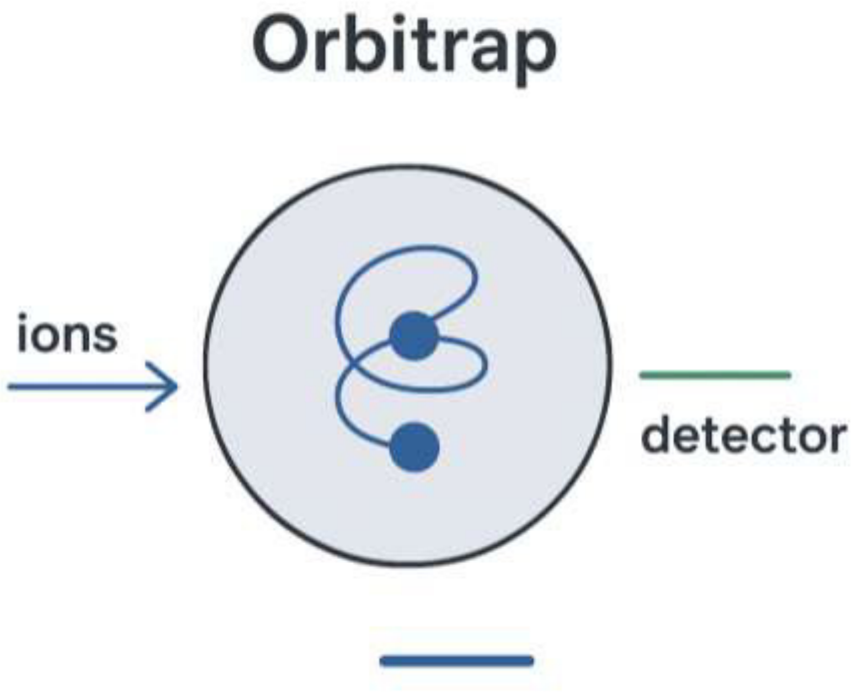


Figure 9: Simplified diagram of an Orbitrap analyzer

III.5 Fourier Transform Ion Cyclotron Resonance (FT-ICR)

The FT-ICR analyzer represents the pinnacle of resolution and mass accuracy in mass spectrometry. It uses a powerful magnetic field to trap ions, which move in circular orbits according to their m/z-dependent cyclotron frequency.

The frequencies are detected and transformed into mass spectra through Fourier Transform mathematics. FT-ICR provides unmatched resolution and can distinguish ions with extremely close masses, making it the gold standard for fundamental research, structural biology, and complex mixture analysis. However, its high operational cost and large instrument footprint restrict its use to specialized laboratories.

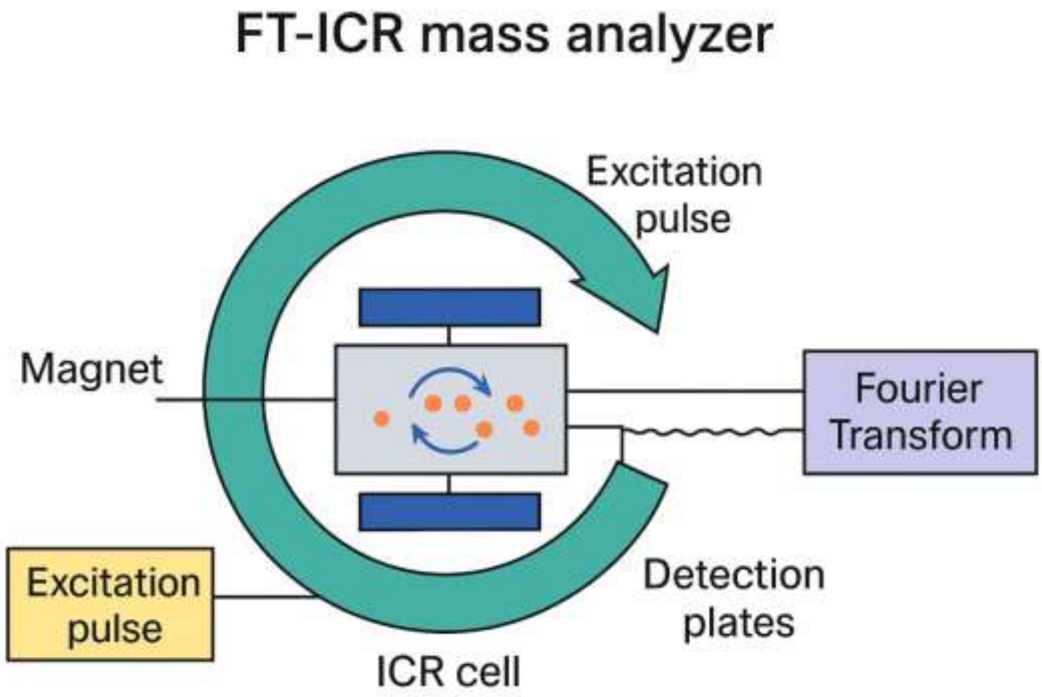


Figure 10: Diagram of an FT-ICR mass analyzer

Summary

The mass analyzer defines the analytical performance of a spectrometer. Quadrupoles are reliable for routine applications, TOF analyzers offer unmatched speed, ion traps enable advanced fragmentation studies, and Orbitraps provide high-resolution data for biomolecular research. FT-ICR stands at the top, delivering unparalleled mass accuracy for the most demanding applications. The diversity of analyzers ensures that mass spectrometry can be adapted to a wide range of scientific and industrial challenges, making it one of the most powerful analytical techniques of the 21st century.

IV. The Detector

The detector is the final yet crucial component of a mass spectrometer. Its role is to convert the flux of ions that have been separated by the analyzer into a measurable electrical signal. The sensitivity, dynamic range, stability, and response time of the detector directly influence the quality of the mass spectrum. A variety of detectors exist, each with specific advantages depending on the type of experiment and instrument design.

IV.1. Electron Multiplier Detectors (EMD)

Electron multiplier detectors are among the most commonly used in mass spectrometry. Their principle relies on the impact of an ion on a surface, which releases secondary electrons. These electrons are then accelerated towards another surface, producing a cascade of additional electrons. This process results in signal amplification, allowing detection of even single ions. EMDs offer excellent sensitivity and fast response times, making them ideal for routine analysis and high-resolution instruments. Their main limitation is the limited lifespan due to gradual degradation of the multiplier surface.

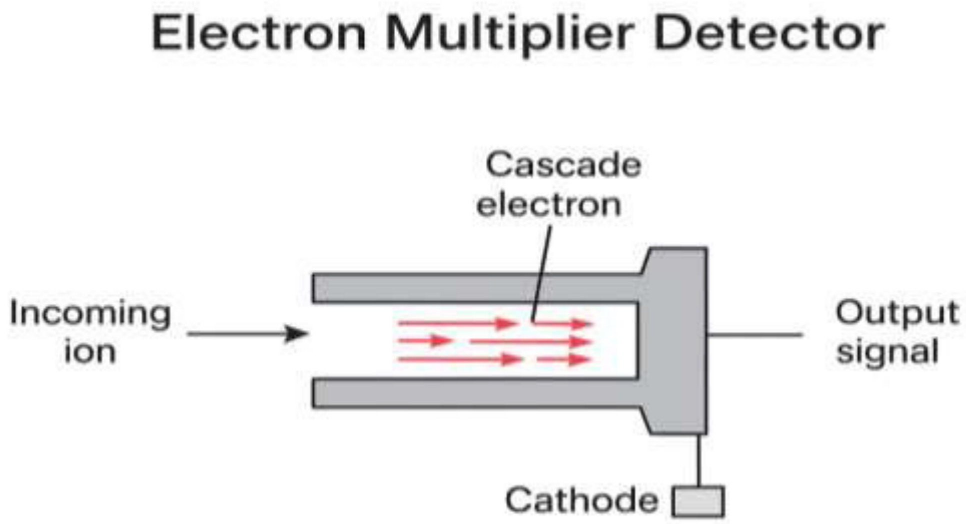


Figure 11: Schematic of an electron multiplier detector

IV.2. Channel Electron Multipliers (CEM)

Channel electron multipliers are a specific type of electron multiplier built as a single narrow tube or channel. When ions enter the channel, they trigger a cascade of electrons that travel down the channel, amplifying the signal. CEMs are characterized by high sensitivity, low noise, and compact design. They are widely employed in quadrupole and TOF instruments. However, their sensitivity decreases with aging, and their performance depends on the geometry of the channel.

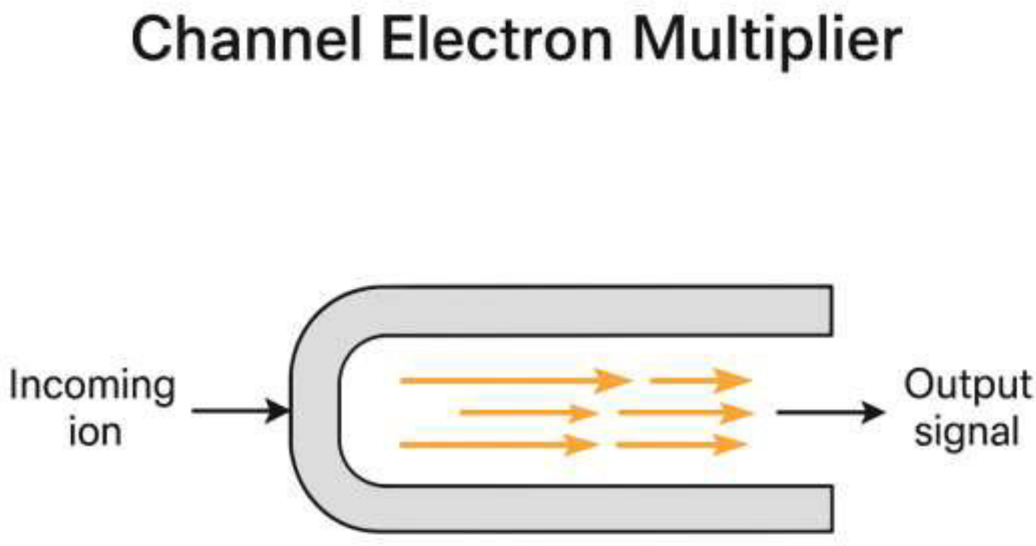


Figure 12: Diagram of a channel electron multiplier (CEM)

IV.3. Dynode Detectors

Dynode detectors use a series of metal plates (dynodes) at increasing potentials to deflect ions and generate secondary electrons at each impact. The signal is amplified step by step, and finally collected at the anode. Dynode-based detectors provide stable signals suitable for quantitative analysis, but they are generally less sensitive than electron multipliers. They are still valuable in instruments where robustness and reproducibility are prioritized.

IV.4. Microchannel Plates (MCP)

Microchannel plates consist of a thin disk with millions of microscopic glass channels, each acting as an independent electron multiplier. When ions strike the plate, each channel produces an amplified signal. MCPs are extremely fast and allow spatial detection of ions, making them suitable for imaging mass spectrometry, such as MALDI-TOF imaging. Their advantages include large surface area and high temporal resolution, but their cost and susceptibility to wear limit their lifetime.

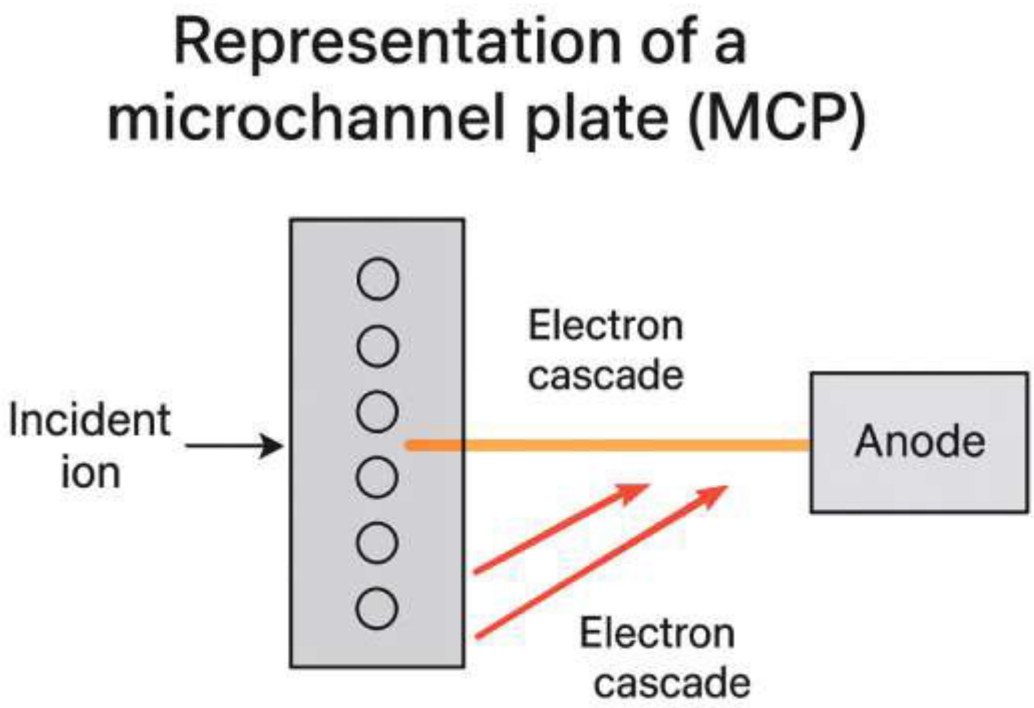


Figure 13: Representation of a microchannel plate (MCP)

IV.5 Faraday Cup Detectors

The Faraday cup is one of the simplest and most robust detectors in mass spectrometry. It consists of a conductive cup that collects incoming ions. The resulting current is measured and directly proportional to the number of ions. Although the Faraday cup is less sensitive compared to electron multipliers, it offers unmatched stability and quantitative accuracy. It is particularly important in isotope ratio mass spectrometry and applications requiring absolute quantification.

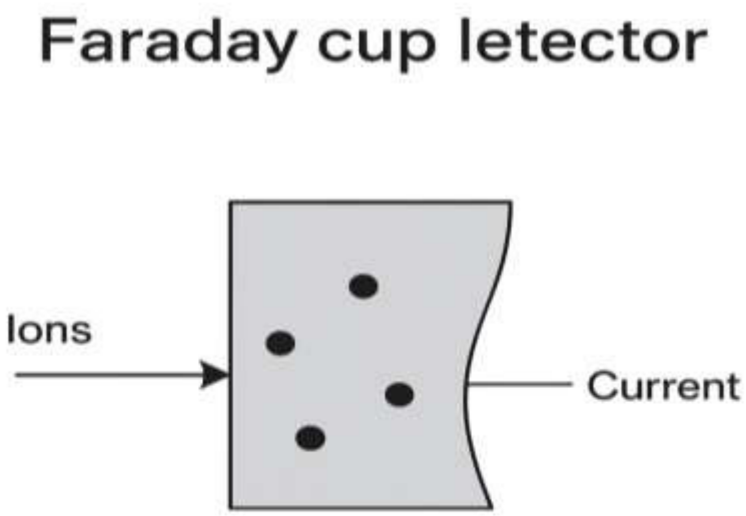


Figure 14: Schematic of a Faraday cup detector

Summery

Detectors represent the final stage of the mass spectrometer, yet their performance determines the accuracy and quality of the data obtained. Electron multipliers and channeltrons are preferred for high-sensitivity applications, MCPs enable ion imaging, dynodes provide stability for quantitative studies, and Faraday cups ensure robust measurements for isotope analysis. The choice of detector must therefore be aligned with the analytical objective, making this component as critical as the ion source or the mass analyzer in the overall architecture of a mass spectrometer.

V. Mass Spectrum

V.1 General Characteristics of a Mass Spectrum

A typical mass spectrum contains a variety of peaks, each corresponding to ions with specific m/z values. The most important peak is often the molecular ion peak (M^+ or $[M+H]^+$), which represents the intact molecule. Depending on the ionization method, adducts such as $[M+Na]^+$ or $[M+K]^+$ may also appear. In addition, fragment ions provide structural information, as their appearance reflects specific bond cleavages within the molecule.

Another key aspect of spectra is isotopic distribution. Elements such as chlorine, bromine, and sulfur produce distinctive isotope patterns due to their natural isotopic abundances. For instance, chlorine produces a characteristic 3:1 ratio between the 35Cl and 37Cl isotopes, while bromine shows a 1:1 ratio between 79Br and 81Br. These patterns can be used as diagnostic markers for the presence of specific elements.

Baseline noise and instrument sensitivity also play an important role in interpreting spectra. A clear baseline ensures that minor peaks can be distinguished, which is critical when analyzing trace compounds or complex mixtures.

V.2 Quantitative Aspects of Mass Spectrometry

While the mass spectrum is most often associated with qualitative analysis, it can also be used quantitatively. The height or area of a peak is proportional to the number of ions detected, which in turn reflects the abundance of the analyte in the sample. However, absolute quantification requires careful calibration. Internal standards are commonly used to account for variations in ionization efficiency and instrument response. By generating calibration curves, analysts can relate peak intensities to known concentrations, allowing precise quantification of analytes.

Quantitative mass spectrometry plays a central role in pharmaceutical research, where drug concentration in biological fluids must be measured accurately. It is also widely applied in environmental science, where pollutants and toxins must be detected at trace levels.

V.3 Structural Information from Fragmentation Patterns

One of the most powerful aspects of mass spectrometry is its ability to reveal structural information. Fragment ions are generated when molecular ions break apart along specific bonds. These fragmentation pathways are not random but follow predictable rules based on chemical stability.

For example, α-cleavage near heteroatoms such as oxygen or nitrogen produces characteristic fragment ions. The McLafferty rearrangement, another common process, involves the transfer of a hydrogen atom and the cleavage of a bond, generating a distinctive fragment. Neutral losses of small molecules such as H_2O, CO_2, or NH_3 are also diagnostic of functional groups like alcohols, carboxylic acids, and amines.

By carefully analyzing fragmentation patterns, chemists can deduce structural features such as the presence of functional groups, ring systems, and side chains. For small organic molecules, this approach can often lead to complete structural elucidation.

V.4 High-Resolution and Accurate Mass Spectra

High-resolution mass spectrometry (HRMS) provides exact mass measurements with accuracy often within a few parts per million (ppm). This capability allows unambiguous determination of molecular formulas. For example, two compounds with the same nominal mass (e.g., 46 Da) could correspond to C_2H_6O (ethanol) or CH_4O_2 (methanol oxide). A high-resolution spectrum distinguishes between these based on their exact masses.

HRMS is essential in fields such as metabolomics, where thousands of small molecules must be identified within complex biological samples. It also plays a key role in forensic science, enabling the detection of designer drugs or other unknown compounds. The ability to resolve isobaric species (compounds with the same nominal mass but different exact masses) makes HRMS one of the most powerful analytical tools available.

V.5 Tandem Mass Spectrometry (MS/MS and beyond)

Tandem mass spectrometry (MS/MS) adds another dimension to spectral analysis by introducing a fragmentation stage after mass selection. In this approach, a precursor ion is selected, fragmented in a collision cell, and the resulting product ions are analyzed. This generates a product ion spectrum that provides detailed structural information.

In proteomics, peptide sequencing relies on MS/MS, where fragmentation produces series of b- and y-ions that reveal the amino acid sequence. In lipidomics, characteristic neutral losses can identify specific headgroups. Multi-stage experiments (MS^3, MS^4, etc.) further extend this principle, enabling structural elucidation of complex natural products.

MS/MS has become indispensable in biomedical research, drug discovery, and environmental science, offering unprecedented insight into molecular structures and interactions.

V.6 Practical Applications of Mass Spectra

Mass spectra have broad applications across nearly every field of science. In pharmaceuticals, they are used to monitor drug metabolism, detect impurities, and ensure quality control. In clinical diagnostics, mass spectra can reveal biomarkers for diseases such as cancer, diabetes, and cardiovascular disorders.

In environmental monitoring, they are essential for detecting pesticides, heavy metals, and organic pollutants.

Food safety applications include the identification of contaminants, adulterants, and allergens. Forensic scientists rely on mass spectra to identify drugs, explosives, and toxic substances in criminal investigations. The versatility of the mass spectrum makes it an indispensable tool in modern analytical science.

V.7. What does a mass spectrum look like?

The mass spectrum is a graph that displays the mass-to-charge ratio (m/z) on the x-axis and the relative intensity (number of ions detected) on the y-axis. An electron ionization (EI) mass spectrum of water is shown below. We'll explain what this tells us in a minute.

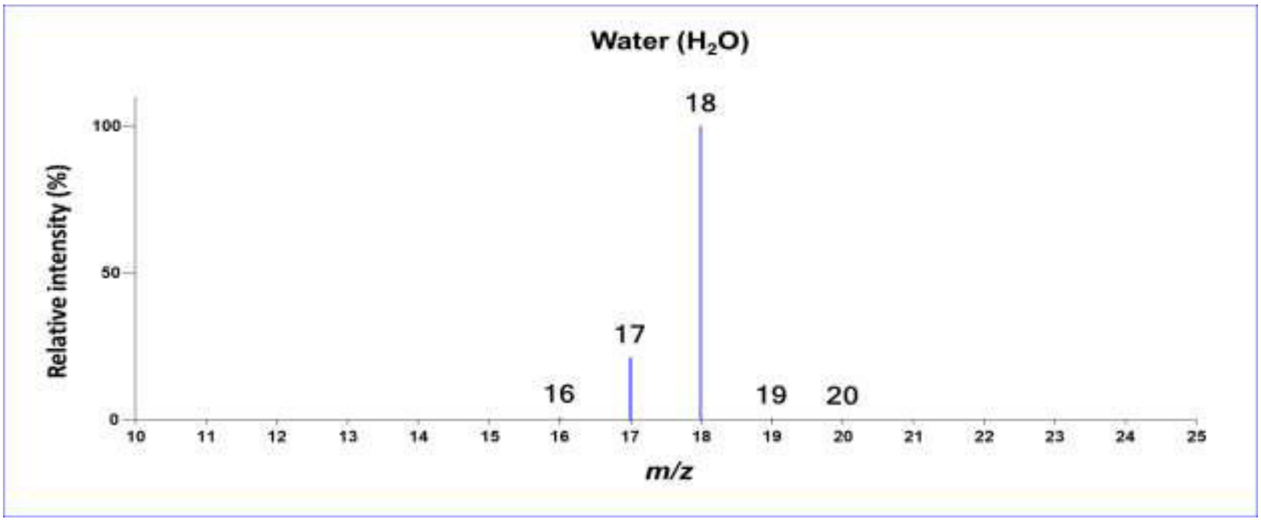


Figure 15: An electron ionization mass spectrum of water

Atoms have characteristic masses that represent the amount of matter in an atom of each element. For example, the most common forms of carbon, hydrogen, and oxygen on earth have integer masses of 12, 1, and 16, respectively, but the masses aren't exactly integers[3].

Carbon (^{12}C) has a mass of 12 (12.0000)

Hydrogen (^{1}H) has a mass of 1 (1.0078)

Oxygen (^{16}O) has a mass of 16 (15.9949)

Nitrogen (^{14}N) has a mass of 14 (14.0031)

Water (H_2O) has two hydrogen atoms and one oxygen atom, so the integer mass of water is Mass of water = (2 x 1) + (16 x 1) = 18

We can see that the largest peak in the water mass spectrum is at *m/z* 18. What are the other peaks in the mass spectrum?

Isotopes

Atoms are made up of protons (positive charge), neutrons (uncharged) and electrons (negative charge). Here's a schematic diagram of a helium (He) atom:

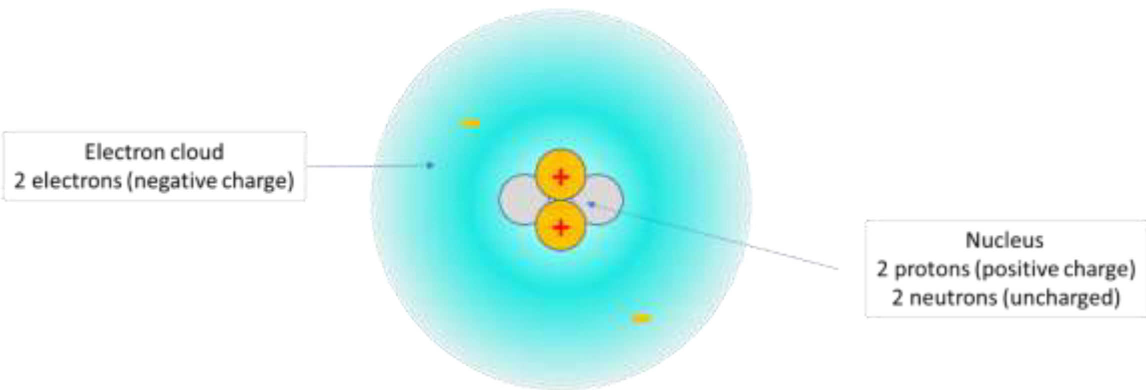


Figure 16: A schematic diagram of a helium (He) atom

Helium Atom Diagram

2 protons (+): mass = 1.007825

2 electrons (-): mass = 0.0005

2 neutrons (0): mass = 1.00866

Exact mass = 4.002600

Charge: 2 protons (+) + 2 electrons (-) = 0 charge

The mass of the helium atom is approximately 4, but it isn't exactly an integer. The *exact mass* of the helium atom is 4.002600. The exact mass of the atom is smaller than the sum of the masses of the protons, neutrons and electrons because of the nuclear binding energy[4]. This is called the "mass defect ".

Not all atoms of a given element have the same number of neutrons, so some atoms of a particular element can have different masses. Atoms of an element that have different numbers of neutrons are called *isotopes*. Helium has another stable isotope, helium-3 (^{3}He), that has one proton and 2 neutrons and an exact mass of 3.016029. Helium-3 is very rare on earth, with a relative abundance of only about 0.000137%.

Most carbon on earth has 6 protons and 6 neutrons, so it has a mass of 12 (exactly). About 1.1% of the carbon on earth has 7 neutrons, and an integer mass of 6+7 = 13. Carbon-13 (^{13}C) atoms have an exact mass of 13.003355.

The relative amounts of the different isotopes are additional information we can get from a mass spectrum. Some elements, such as chlorine and bromine have very distinctive isotope patterns that are easy to recognize in a mass spectrum:

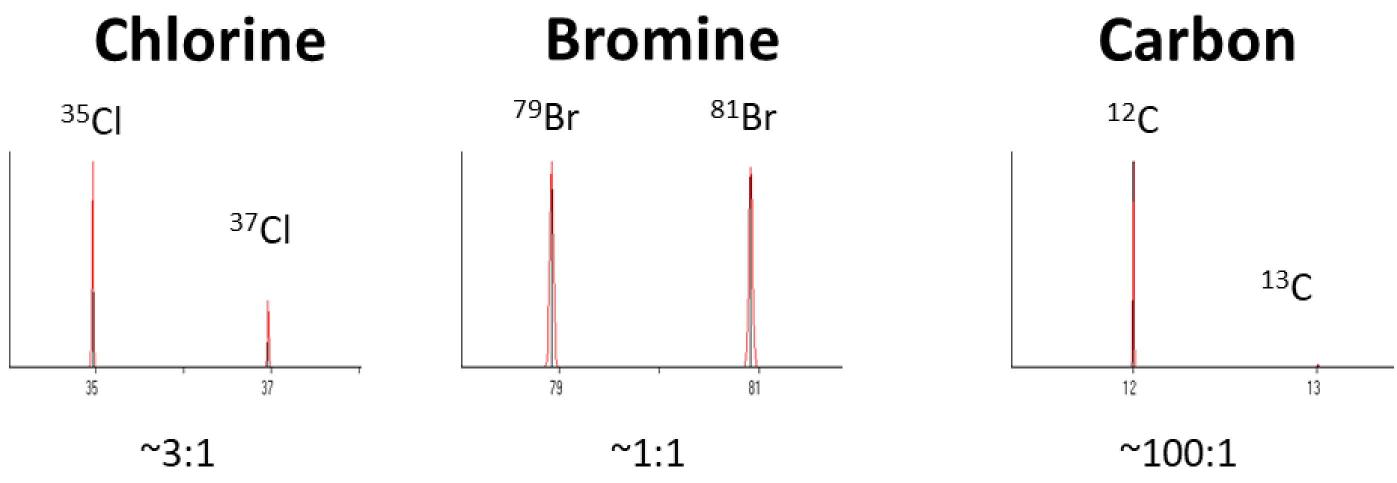


Figure 17: Isotopes for chlorine, bromine, and carbon

A tiny fraction of the hydrogen on earth has an extra neutron, giving it an integer mass of 2 (let's ignore the exact mass for now), and a fraction of the oxygen on earth has two extra neutrons, giving it a mass of 18. That's why we see small peaks at *m/z* 19 and 20 in the mass spectrum of water. What are the other peaks at *m/z* 16 and 17?

V.8. Fragment ions

As we explained above, an electron ionization source shoots a high-energy beam of electrons into a cloud of gas in a vacuum chamber. This knocks an electron off of the atom, creating an ion. If we knock an electron off of the helium atom in the diagram above, we have removed a negative charge. That means that we have two protons (+) and one electron (-), so there is a net positive charge. We show this with a plus sign, for example He^+. The electron beam doesn't have enough energy to break apart the nucleus of the atom, but it does have enough energy to break apart atoms in a molecule. Some of the water molecules were shattered when the electron beam hit them, so some of the ions are $H2O^+$ (*m/z* 18), but some are OH^+ (*m/z* 17) and some are O^+ (*m/z* 16). Some are also H^+ (*m/z* 1), but they aren't shown in the water mass spectrum in the figure above.

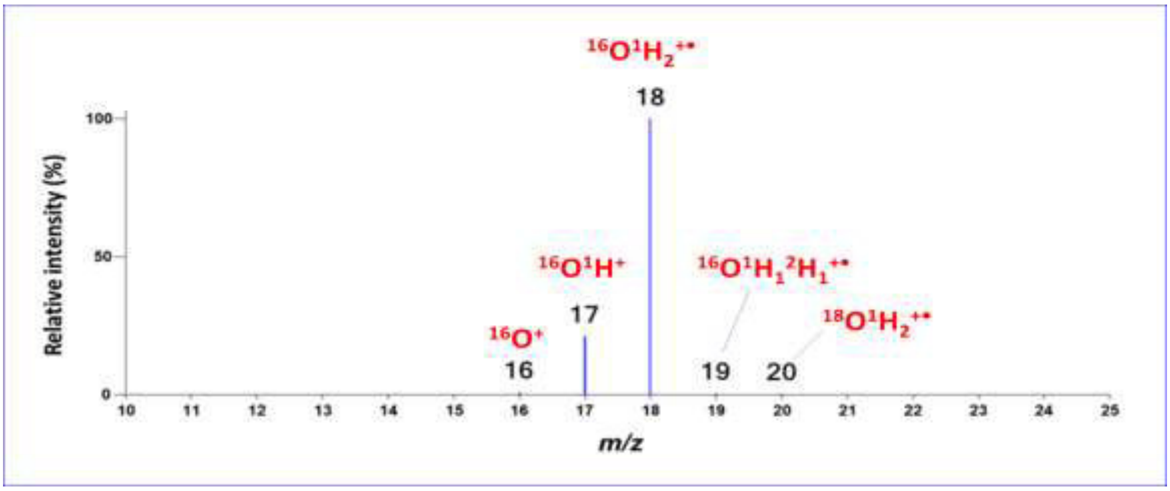


Figure 18 Electron ionization mass spectrum of water with isotopes and fragments labeled

Here's an EI mass spectrum for a more complicated molecule: a drug of abuse.

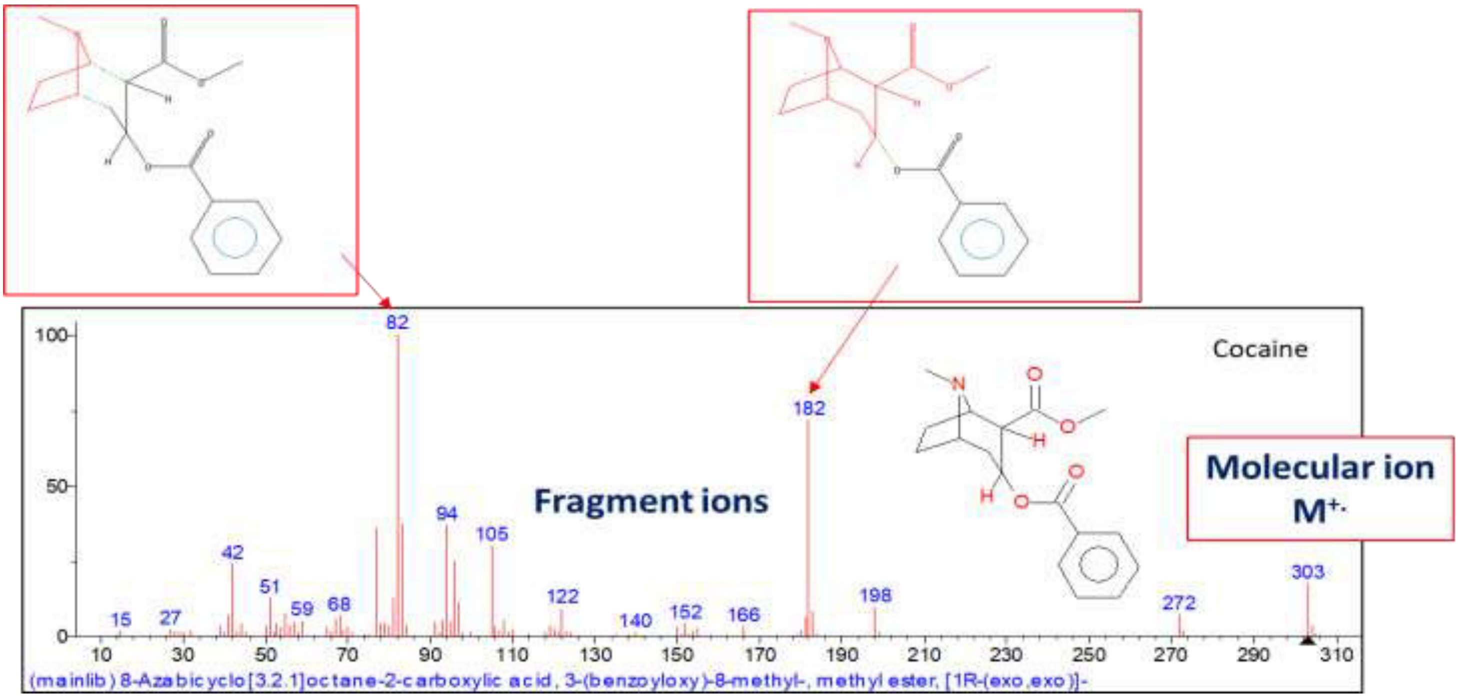


Figure 19: An electron ionization mass spectrum of a drug of abuse

The unfragmented molecule ($C_{17}H_{21}NO_4$) is at *m/z* 303. This is called the **molecular ion**. The fragment ions represent different parts of the molecule. You can try to piece these "puzzle pieces" together to figure out the chemical structure. The most common way to identify molecules from EI mass spectra is to search databases of mass spectra to look for compounds with matching fragment patterns[5]. Databases are available with mass spectra for hundreds of thousands of chemical compounds.

Summary

The mass spectrum is the ultimate output of mass spectrometry, transforming ionized molecules into interpretable data. By analyzing peak positions, intensities, isotopic distributions, and fragmentation patterns, scientists gain insight into molecular structures, compositions, and abundances. High-resolution methods and tandem MS have further expanded the interpretative power of mass spectra, enabling breakthroughs in chemistry, biology, medicine, and environmental science. As data processing and computational methods advance, the interpretation of mass spectra will continue to become more powerful, cementing mass spectrometry's role as one of the most essential techniques in modern science.

General Conclusion

Spectroscopy remains one of the most fundamental and versatile analytical tools in modern science. By studying the interaction between electromagnetic radiation and matter, it provides a direct connection between theory and experiment, allowing scientists to probe the structure, composition, and dynamics of atoms, molecules, and materials.

Each region of the electromagnetic spectrum reveals different aspects of matter: ultraviolet and visible spectroscopy uncovers electronic transitions, infrared spectroscopy explores vibrational modes, microwave spectroscopy studies rotational motion, and X-ray spectroscopy examines core electrons and crystal structures. Together, these methods build a comprehensive understanding of how matter behaves at the microscopic level.

Beyond its scientific significance, spectroscopy has countless applications in everyday life — from identifying chemical compounds and monitoring environmental pollution to developing new materials and exploring the composition of distant stars and galaxies.

For students, learning spectroscopy provides not only practical laboratory skills but also a deeper appreciation of the quantum mechanical principles governing the natural world. As technology advances, new spectroscopic methods continue to emerge, offering higher sensitivity, resolution, and speed.

In conclusion, spectroscopy is far more than a measurement technique; it is a universal language of science that enables us to “see the unseen.” Mastering its principles allows future scientists to explore and understand the fundamental nature of matter, bridging the gap between observation and understanding.

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