Molecular Spectroscopy:
Principles, Techniques and Applications

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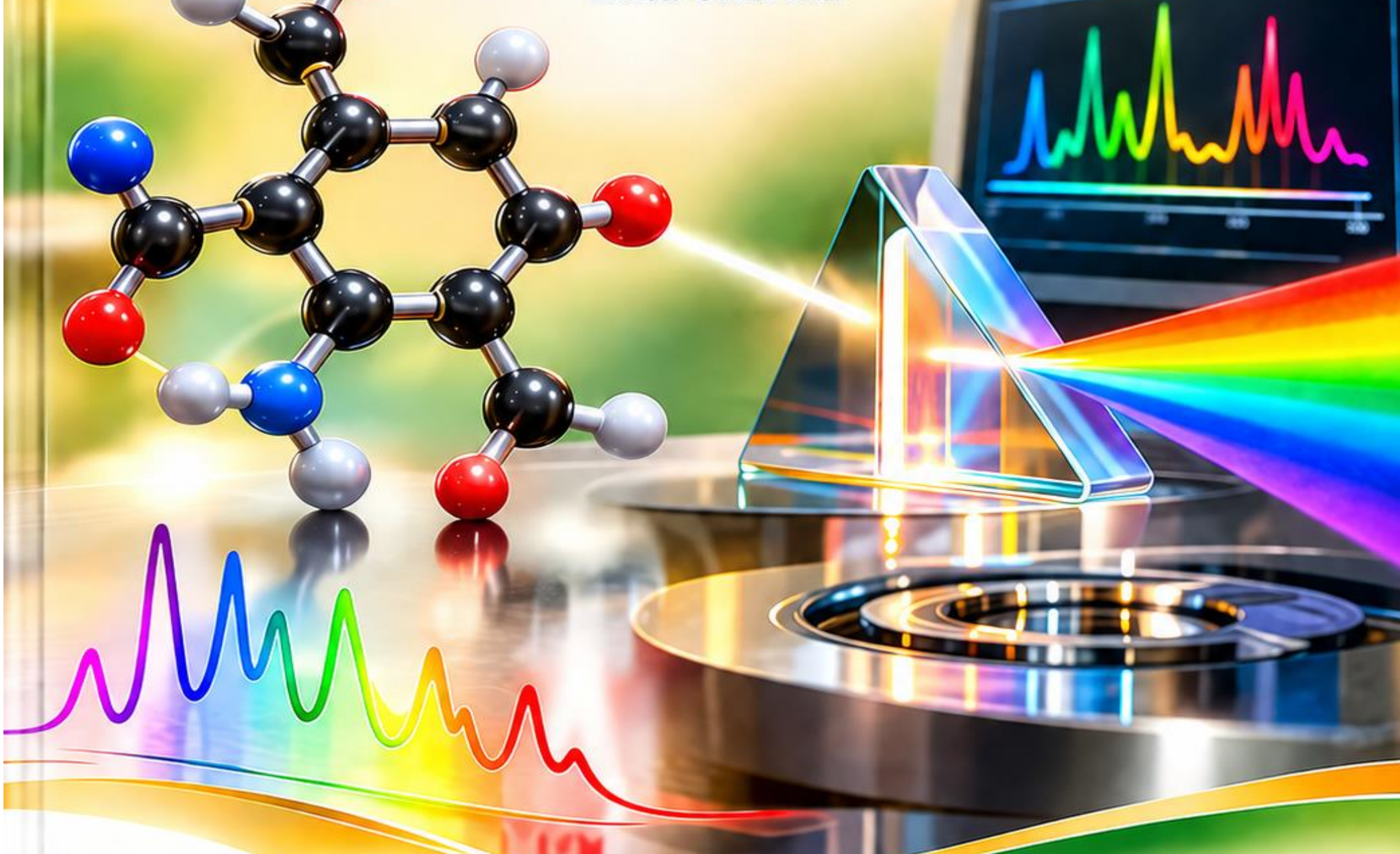

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Molecular Spectroscopy: Principles, Techniques and Applications

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Acknowledgment

It gives me immense pleasure to present *Molecular Spectroscopy: Principles, Techniques and Applications*, a scholarly volume intended to provide a comprehensive and accessible understanding of the fundamental principles, experimental techniques, and diverse applications of molecular spectroscopy. The preparation of this book has been a rewarding academic endeavour, and I would like to express my sincere gratitude to all those who contributed to its successful completion.

I extend my heartfelt appreciation to my mentors, colleagues, collaborators, and academic peers for their insightful discussions, valuable suggestions, and constant encouragement. Their expertise and constructive feedback have enriched the content and helped present the concepts of molecular spectroscopy in a clear, systematic, and scientifically meaningful manner.

I also acknowledge the invaluable contributions of researchers and scientists worldwide whose pioneering work has shaped the development of molecular spectroscopy. The remarkable advances in techniques of spectroscopy have greatly expanded our ability to understand molecules and their interactions. Their research and published literature have served as important sources of knowledge and inspiration for this book.

I am sincerely grateful to my institution for providing a supportive academic environment, research facilities, and encouragement that made this contribution possible. I also extend my appreciation to the authors, reviewers, contributors, and publishing team for their dedication, professionalism, and meticulous efforts in bringing this volume to completion.

Special appreciation is extended to the broader scientific community for its continued pursuit of knowledge, innovation, and technological advancement. The interdisciplinary nature of molecular spectroscopy has made it an essential component of modern chemistry, pharmaceutical sciences, materials science, biochemistry, environmental science, and related fields.

Finally, I express my deepest gratitude to my family and friends for their unconditional love, patience, understanding, and constant encouragement. Their unwavering support and belief in me have been a continual source of strength and motivation throughout this academic journey.

This book is a reflection of the collective contributions of researchers, educators, scientists, and academicians dedicated to advancing the understanding and application of molecular spectroscopy. It is my sincere hope that *Molecular Spectroscopy: Principles, Techniques and Applications* will serve as a valuable reference for students, researchers, academicians, teachers, and professionals, while encouraging further exploration and innovation in the field of molecular sciences.

Editors

- **Tripta Kumari**
- **Shilpa Sharma**
- **Ekta Sharma**

Preface

Molecular Spectroscopy: Principles, Techniques and Applications is intended to provide a clear and comprehensive understanding of the fundamental principles, instrumental techniques, and practical applications of molecular spectroscopy. Spectroscopy is an essential area of modern chemistry that provides valuable information about molecular structure, composition, bonding, and interactions.

This book presents the basic concepts of molecular spectroscopy in a systematic and accessible manner, covering important spectroscopic techniques such as ultraviolet-visible (UV-Visible), infrared (IR), Raman, and nuclear magnetic resonance (NMR) spectroscopy. The fundamental principles, instrumentation, spectral interpretation, and applications of these techniques are discussed to help readers develop both theoretical understanding and practical insight.

The book has been designed to bridge the gap between fundamental concepts and their applications in chemistry, pharmaceutical sciences, biochemistry, materials science, and related disciplines. Emphasis has been placed on clear explanations, relevant examples, and important applications to make the subject easier to understand for students and useful for researchers and academicians.

With the rapid advancement of analytical and spectroscopic techniques, molecular spectroscopy continues to play a vital role in research, pharmaceutical analysis, structural elucidation, quality control, environmental studies, and the characterization of chemical and biological systems. A sound understanding of these techniques is therefore essential for students and professionals working in the molecular sciences.

It is hoped that this book will serve as a useful academic and reference resource for undergraduate and postgraduate students, researchers, teachers, academicians, and professionals. The authors/editors sincerely hope that the contents of this volume will encourage readers to explore the fascinating world of molecular spectroscopy and contribute to further learning, research, and scientific advancement.

Editors

- **Tripta Kumari**
- **Shilpa Sharma**
- **Ekta Sharma**

Chapter 1: Introduction to Spectroscopy

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Abstract

Spectroscopy is a fundamental analytical science concerned with the interaction of electromagnetic radiation with matter. It provides valuable information about the structure, composition, concentration, and properties of substances by studying the absorption, emission, transmission, reflection, and scattering of radiation. Atoms and molecules possess discrete energy levels, and interaction with electromagnetic radiation can produce transitions between these levels. The resulting spectra are characteristic of individual substances and can therefore be used for identification and structural investigation. This chapter introduces the fundamental concepts of spectroscopy, including the interaction of electromagnetic radiation with matter, different types of molecular and atomic transitions, and the electromagnetic spectrum. Major spectroscopic techniques, including ultraviolet-visible (UV-Visible), infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry, are introduced in relation to their analytical applications. The chapter also discusses the advantages of spectroscopic methods and their applications in structure elucidation, quantitative analysis, pharmaceutical and industrial quality control, medicine, forensic science, agriculture, environmental monitoring, biochemistry, material science, space science, and research and development. The chapter provides a general foundation for science students to understand the importance and wide-ranging applications of spectroscopy in modern science.

Keywords

Spectroscopy; electromagnetic radiation; electromagnetic spectrum; absorption; emission; scattering; transmission; reflection; electronic transitions; vibrational transitions; rotational transitions; NMR; infrared spectroscopy; UV-Visible spectroscopy; mass spectrometry; structure elucidation; quantitative analysis.

1.1 Introduction to Spectroscopy

Spectroscopy is the study of the interaction between electromagnetic radiation and matter. It is one of the most powerful analytical tools used in chemistry, physics, biology, pharmaceutical sciences, environmental science, and material science. Spectroscopic techniques help scientists understand the structure, composition, concentration, and properties of substances by examining how they interact with different forms of electromagnetic radiation. Depending on the spectroscopic technique, the interaction may involve the absorption, emission, transmission, reflection, or scattering of radiation by atoms, ions, molecules, or materials [1,3].

At the atomic and molecular level, matter possesses quantized energy levels. This means that atoms and molecules can exist only in specific energy states. When electromagnetic radiation interacts with matter, the energy of the radiation may be absorbed by the substance if it corresponds to an allowed transition between two energy levels. The resulting transition may involve electrons, molecular vibrations, molecular rotations, or nuclear spins, depending on the energy of the radiation involved [1,3].

The spectrum obtained from a substance contains characteristic information about its structure and composition. Because different atoms and molecules possess different energy levels, their spectra are generally characteristic of the substances from which they originate. For this reason, a spectrum is often described as a fingerprint of a substance and can be used for identification and structural investigation [1,2].

Spectroscopic analysis may involve several important techniques, including Ultraviolet-Visible (UV-Visible) spectroscopy, Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, and Mass Spectrometry (MS) [2,3]. Each method is based on a different type of interaction and therefore provides different types of information. UV-Visible spectroscopy is particularly useful for studying electronic transitions, IR spectroscopy provides information about molecular vibrations and functional groups, NMR spectroscopy provides information about the chemical environment of nuclei, and mass spectrometry provides information related to molecular mass and fragmentation patterns [1–3].

The importance of spectroscopy extends far beyond the identification of organic compounds. Modern spectroscopic methods are routinely used in pharmaceutical research and quality control, environmental monitoring, forensic investigations, food analysis, medical diagnostics, agricultural studies, industrial process monitoring, material characterization, and fundamental scientific research [3,5].

1.2 Advantages of Spectroscopic Methods

The major advantages of employing spectroscopic methods for **structure determination, identification, and analysis of substances** include the following [2,3]:

i) Rapid Analysis and Permanent Record

Spectroscopic methods generally require relatively little time to obtain analytical information. The spectrum obtained from a sample can be recorded as a permanent analytical record in the form of a chart or digital spectrum. This record can be stored, compared with reference spectra, and used for subsequent interpretation and identification of substances [2].

ii) Requirement of Very Small Quantities of Sample

Only a very small quantity of substance is often required for spectroscopic analysis [3]. This is particularly advantageous when the sample is expensive, difficult to obtain, available only in limited quantities, or isolated in small amounts from natural sources.

iii) High Sensitivity and Reliability

Spectroscopic methods are highly sensitive and can provide reliable information for establishing the structure of compounds and analyzing mixtures of closely related substances [1]. Their ability to detect characteristic spectral features makes them valuable for both qualitative and quantitative analysis.

iv) Detection of Short-Lived Reaction Intermediates

Spectroscopic techniques can be used for the detection and identification of short-lived reaction intermediates [5]. Such intermediates may exist only for a very short period and may not be sufficiently stable to isolate by conventional chemical methods. Rapid spectroscopic measurements can provide information about their formation and disappearance during a reaction.

v) Non-Destructive Nature of Many Spectroscopic Methods

During many spectroscopic measurements, the substance is not chemically altered and can therefore be recovered after analysis [2]. This is an important advantage when working with valuable or limited samples. However, this characteristic is not applicable to every technique. In particular, mass spectrometry involves ionization of the sample and may result in fragmentation; therefore, the original sample is generally not recovered unchanged.

vi) Continuous Analysis and Reaction Monitoring

Continuous or repeated spectroscopic analysis is often possible, making spectroscopy particularly useful for studying the rates of chemical reactions [3]. Changes in spectral intensity with time can be monitored to follow the disappearance of reactants, formation of products, or changes in reaction intermediates.

1.3 Electromagnetic Radiation

Electromagnetic radiation is a form of energy that travels through space and can interact with matter. It is characterized by properties such as wavelength, frequency, and energy. Electromagnetic radiation is associated with discrete energy units called **quanta or photons**.

The energy of a photon is directly proportional to its frequency and inversely proportional to its wavelength. The relationship is expressed as:

$$E = h\nu = hc/\lambda$$

where, E: Energy of Photon

h: Planck's Constant (6.625×10^{-34}Js)

ν: frequency of electromagnetic radiation

c: speed at which light travels in vacuum (3×10^8 m/s)

λ: Wavelength of electromagnetic radiation [1,4]

Thus, electromagnetic radiation having a higher frequency has greater energy, whereas radiation having a longer wavelength has lower energy. Different regions of the electromagnetic spectrum therefore possess different energy ranges and can produce different types of transitions in atoms and molecules [1,4].

The relationship between wavelength and frequency is:

$$c=\nu\lambda$$

Therefore, wavelength and frequency are inversely related. This relationship is fundamental to understanding why different regions of the electromagnetic spectrum interact with matter in different ways.

1.4 Interaction of Electromagnetic Radiation with Matter

When electromagnetic radiation interacts with matter, several processes can occur depending on the energy of the radiation and the nature of the substance. The major forms of interaction relevant to spectroscopy include absorption, emission, scattering, transmission, and reflection [1,3].

1.4.1 Absorption

Absorption occurs when a molecule, atom, or other substance absorbs energy from electromagnetic radiation. The absorbed energy causes the system to move from a lower-energy state to a higher-energy state.

The energy absorbed must correspond to the energy difference between the two allowed states. Thus:

$$\Delta E = E2 - E1 = h\nu$$

where E1 represents the lower-energy state and E2 represents the higher-energy state.

Absorption is the fundamental process involved in techniques such as UV-Visible and IR spectroscopy [3]. In UV-Visible spectroscopy, absorption generally produces electronic transitions, whereas IR absorption is primarily associated with molecular vibrational transitions.

1.4.2 Emission

Emission occurs when an atom or molecule in an excited state returns to a lower-energy state and releases energy in the form of electromagnetic radiation [4].

The emitted radiation may possess a characteristic wavelength or set of wavelengths. These wavelengths can be used to identify the species responsible for the emission. Emission spectroscopy is therefore useful in the analysis of atoms and molecules.

The general process can be represented as:

Excitation → Higher-energy state → Relaxation → Emission of radiation

The wavelength of emitted radiation depends on the energy difference between the relevant energy levels.

1.4.3 Scattering

Scattering occurs when electromagnetic radiation interacts with particles or molecules and is redirected from its original path. Scattering can occur with or without a change in the energy of the radiation [4].

Two important types of scattering are:

a) Rayleigh Scattering

In Rayleigh scattering, the scattered radiation has essentially the same energy and frequency as the incident radiation. Therefore, there is no significant change in the wavelength of the radiation.

b) Raman Scattering

In Raman scattering, interaction between radiation and molecular vibrations results in a change in the energy of the scattered radiation. The resulting Raman spectrum provides information about molecular vibrational characteristics [4].

1.4.4 Transmission

Transmission occurs when electromagnetic radiation passes through a substance without being significantly absorbed [1]. The amount of radiation transmitted through a sample can be measured and compared with the incident radiation.

Transmission measurements are particularly important in spectrophotometric techniques, where the relationship between incident and transmitted radiation can be used to determine absorbance.

1.4.5 Reflection

Reflection occurs when electromagnetic radiation encounters a surface and is redirected back from that surface [1]. Reflection spectroscopy can provide information about the surface characteristics and composition of materials and is particularly important in surface and material analysis.

1.5 Types of Transitions

Different regions of electromagnetic radiation possess different energy ranges. Consequently, different types of transitions occur when radiation interacts with matter [1,3].

a) Electronic Transitions

Electronic transitions involve changes in the electronic energy states of atoms or molecules. They generally require relatively high energy and are associated particularly with the **UV-Visible region** of the electromagnetic spectrum.

b) Vibrational Transitions

Vibrational transitions involve changes in the vibrational energy of molecules. They are primarily associated with the **infrared region** and provide important information about chemical bonds and functional groups.

c) Rotational Transitions

Rotational transitions involve changes in the rotational energy of molecules. They are primarily associated with the **microwave region** of the electromagnetic spectrum.

d) Nuclear Spin Transitions

Nuclear spin transitions involve changes in the spin states of atomic nuclei in an external magnetic field. These transitions form the basis of **Nuclear Magnetic Resonance (NMR) spectroscopy** and occur in the radiofrequency region [1,3].

Thus, spectroscopy covers a wide range of electromagnetic radiation, with each region providing a different type of structural or analytical information.

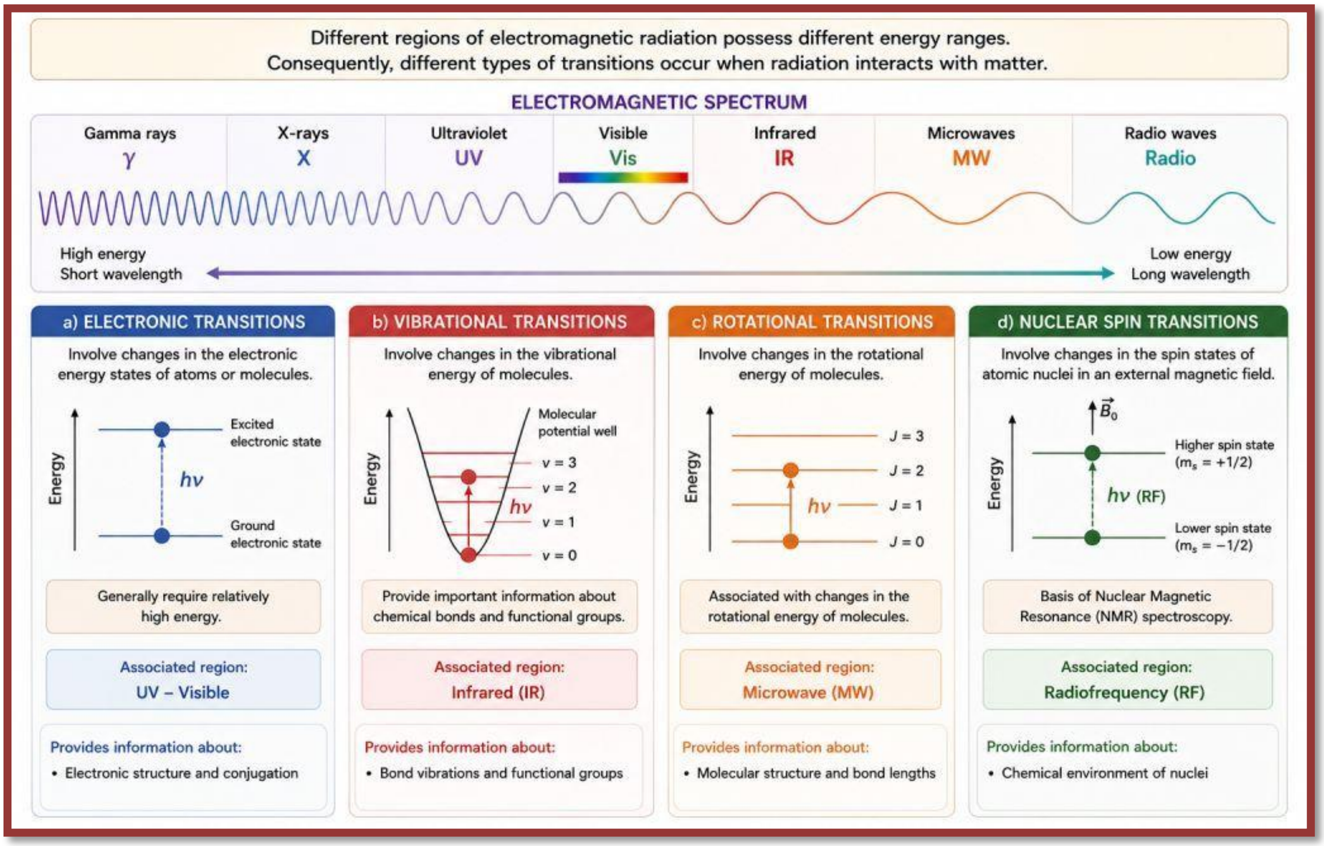

Figure 1.1: Diferent Types of Transitions

1.6 Electromagnetic Spectrum

The **electromagnetic spectrum** is the complete range of electromagnetic radiation arranged according to wavelength, frequency, or energy. It can be described in increasing order of wavelength or, equivalently, decreasing order of frequency and energy [4].

The major regions of the electromagnetic spectrum include gamma rays, X-rays, ultraviolet radiation, visible radiation, infrared radiation, microwaves, and radio waves.

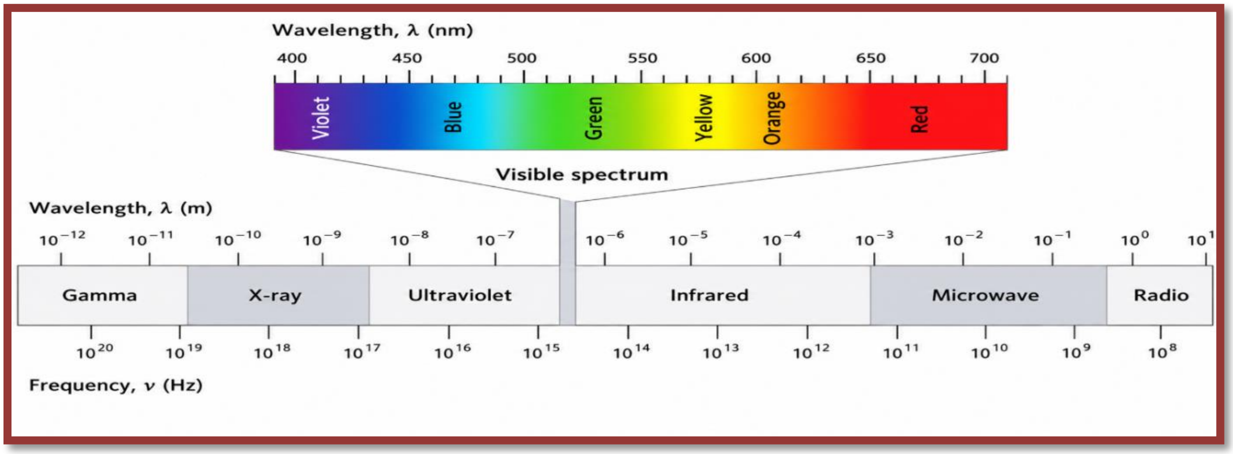

Figure1.2: Approximate ranges of wavelengths and frequencies of electromagnetic radiations

Region	Wavelength	Application
Gamma rays	<0.01 nm	Nuclear studies
X-rays	0.01–10 nm	Medical imaging
UV	10–400 nm	Electronic transitions
Visible	400–700 nm	Color analysis
IR	700 nm–1 mm	Vibrational analysis
Microwave	1 mm–1 m	Rotational studies
Radio waves	>1 m	NMR

Table 1.1: Wavelengths of different regions of Electromagnetic Spectrum and their applications [1,4]

The boundaries between the different regions are approximate and may vary slightly depending on the scientific or instrumental convention used [1,4]. Nevertheless, the classification is useful for understanding the relationship between radiation energy and the type of transition produced.

1.7. Applications of Spectroscopy

Spectroscopy has a wide range of applications because different spectroscopic techniques provide complementary information about substances. It is used for structural determination, qualitative identification, quantitative analysis, quality control, reaction monitoring, and characterization of biological and material systems [1–5].

1.7.1 Structure Elucidation

Spectroscopy plays a crucial role in determining the structure of molecules [1,3]. Different spectroscopic techniques provide different structural information, and their combined use can provide a comprehensive understanding of an unknown compound.

a) NMR Spectroscopy

NMR spectroscopy helps identify the arrangement of atoms within molecules and provides information about the number and chemical environment of hydrogen and carbon atoms. Chemical shifts, signal splitting, and signal intensities can be interpreted to obtain information about molecular structure.

b) IR Spectroscopy

IR spectroscopy is particularly useful for identifying functional groups such as –OH, –NH, C=O**,** and other characteristic chemical bonds. The positions and intensities of absorption bands provide information about the molecular vibrations associated with these groups.

c) Mass Spectrometry

Mass spectrometry provides information about molecular mass and fragmentation patterns. The molecular ion or related mass information can help determine molecular weight, while characteristic fragmentation patterns provide additional structural information.

d) Identification of Unknown Compounds

The combined use of UV, IR, NMR, and mass spectrometry can be particularly valuable for identifying unknown organic compounds encountered in research and analytical laboratories [1–3].

1.7.2 Quantitative Analysis

Spectroscopy is widely used to determine the concentration of substances present in a sample [3,4]. Quantitative spectroscopic analysis is based on measuring a property of the sample that varies predictably with concentration.

In UV-Visible spectroscopy, quantitative analysis commonly uses the Beer–Lambert law**,** which states that under appropriate conditions, absorbance is proportional to concentration and path length.

$$A = \varepsilon c l$$

where:

A = Absorbance

ε = Molar absorptivity

c = Concentration

l = Path length

Applications include determination of drug concentration in pharmaceutical preparations, estimation of biological substances such as glucose, and analysis of metal ions and other chemical species in solution [3,4].

1.7.3 Identification of Compounds

Each compound may produce characteristic spectral features that can be used for identification [1,2]. Spectral comparison with reference materials is an important approach in qualitative analysis.

a) IR Spectroscopy

IR spectra contain a characteristic fingerprint region, generally considered approximately 1500–500 cm^{-1}, which can be particularly useful for comparison and identification of compounds.

b) NMR Spectroscopy

NMR provides characteristic chemical shifts and splitting patterns that reflect the chemical environments of nuclei within molecules.

c) Mass Spectrometry

Mass spectra provide characteristic molecular-mass information and fragmentation patterns that can help distinguish and identify compounds.

The complementary information obtained from these techniques makes spectroscopy highly valuable for the identification of unknown substances [1–3].

1.7.4 Industrial Applications

Spectroscopy is extensively used in different industries for research, development, quality control, and process monitoring [3].

a) Pharmaceutical Industry

In the pharmaceutical industry, spectroscopy is used for:

- Drug formulation and development
- Identification of pharmaceutical substances

- Purity testing
- Detection of impurities
- Stability studies
- Quality control of raw materials and finished products

Spectroscopic methods can provide rapid analytical information and are therefore important throughout the development and manufacturing of pharmaceutical products.

b) Quality Control

Spectroscopic methods are widely used to ensure the consistency, identity, purity, and quality of products. Raw materials can be examined before production, while intermediate and final products can be analyzed to detect variations or impurities [3].

c) Polymer Industry

In the polymer industry, spectroscopy is used for:

- Analysis of polymer composition and structure
- Identification of additives
- Monitoring polymerization reactions
- Investigation of chemical changes during processing
- Characterization of polymer materials

1.7.5 Food Industry

Spectroscopy has numerous applications in food science, including:

- Detection of adulterants
- Nutritional analysis
- Identification of food components
- Quality assessment
- Analysis of flavour and aroma compounds [3]

1.7.6 Medical Applications

Spectroscopic methods have important applications in medical science and clinical analysis.

Imaging Techniques

Magnetic Resonance Imaging (MRI) is based on the principles of nuclear magnetic resonance. It is widely used for imaging soft tissues such as the brain, muscles, and internal organs [5].

Clinical Diagnostics

Spectroscopic methods can be used in the analysis of blood, urine, and other biological samples. Spectral measurements can provide information about biochemical constituents and can assist in the investigation of disease-related changes and biomarkers [5].

Drug Monitoring

Spectroscopic approaches can be used to measure drug concentrations and investigate drug metabolism. Such information is useful in pharmaceutical and biomedical research and can contribute to understanding the behaviour of therapeutic substances within biological systems [5].

1.7.7 Forensic Applications

Spectroscopy is an important analytical tool in forensic science. It can assist in the identification and characterization of unknown materials encountered during criminal investigations [3].

Applications include:

- Identification of drugs and controlled substances
- Analysis of poisons
- Examination of suspicious materials
- Analysis of fibres
- Examination of blood and biological materials
- Identification of unknown substances
- Investigation of explosive-related materials

Spectral information can be compared with reference data to help establish the identity or composition of evidence.

1.7.8 Agricultural Applications

Spectroscopy is increasingly useful in agricultural science and analysis. Applications include:

- Soil nutrient analysis
- Detection of pesticide residues
- Analysis of agricultural products
- Monitoring plant health
- Characterization of plant components [3]

Spectroscopic measurements can provide rapid analytical information and can support monitoring of soil and plant systems.

1.7.9 Space and Astronomical Applications

Spectroscopy is one of the most important analytical methods used in astronomy and space science. Light emitted or absorbed by stars, planets, and other astronomical objects contains information about their chemical composition and physical properties [3].

By studying emission and absorption spectra, scientists can obtain information about:

- Composition of stars
- Composition of planets and other celestial bodies
- Physical characteristics of astronomical objects
- Distant galaxies
- Presence of particular atoms and molecules

Because the spectral characteristics of atoms and molecules are distinctive, spectroscopy allows scientists to investigate objects that cannot be directly sampled in conventional laboratories.

1.7.10 Environmental Applications

Spectroscopy plays a major role in environmental monitoring and pollution analysis [5].

Detection of Pollutants

Spectroscopic methods can be used to identify and monitor pollutants in the environment. Important applications include the detection of harmful gases such as: Carbon monoxide (CO), Nitrogen dioxide (NO_2), Sulfur dioxide (SO_2)

Spectroscopic monitoring can help assess air quality and pollution levels.

Water Quality Testing

Spectroscopy can be applied to water-quality analysis for the detection and estimation of chemical contaminants. Applications include analysis of heavy metals such as:Lead (Pb), Mercury (Hg), Cadmium (Cd).

Spectroscopic methods can also be used for the analysis of organic contaminants and other substances present in water.

Soil Analysis

Spectroscopic methods are useful in the analysis of soil composition. Applications include:

- Determination of nutrient content
- Detection of pesticide residues
- Analysis of soil contaminants
- Assessment of soil characteristics [5]

1.7.11 Biochemical and Biological Applications

Spectroscopy is extensively used in biochemistry and molecular biology for studying biological molecules and biochemical processes [5].

Important applications include:

- Protein structure analysis
- DNA and RNA studies
- Determination of biomolecule concentration
- Enzyme activity studies
- Enzyme kinetics
- Investigation of metabolic pathways
- Study of molecular interactions

Different spectroscopic techniques provide complementary information about biomolecules. For example, UV-Visible spectroscopy can be used for quantitative measurements, while other techniques can provide information about molecular structure and chemical environments.

1.7.12 Material Science Applications

Spectroscopy is an important analytical tool in material science and nanotechnology [3]. The properties of materials are strongly influenced by their composition, structure, surface characteristics, and electronic properties.

Applications include:

- Characterization of nanomaterials
- Surface analysis
- Study of semiconductors
- Characterization of metals
- Investigation of material composition
- Analysis of structural and chemical changes

Spectroscopic methods can therefore assist in the development and characterization of new materials with specialized properties.

1.7.13 Research and Development

Spectroscopy has a central role in scientific research and technological development [3]. It is used in fundamental research as well as in the development of new products and technologies.

Important applications include:

- Fundamental research in chemistry and physics
- Development of new drugs
- Development of advanced materials
- Investigation of reaction mechanisms
- Characterization of newly synthesized compounds
- Monitoring chemical reactions
- Investigation of molecular interactions

The ability to obtain structural and compositional information rapidly makes spectroscopy particularly valuable during the development and optimization of new scientific and industrial processes.

1.8 Importance of Combining Spectroscopic Techniques

No single spectroscopic technique provides all possible information about a substance. Different methods investigate different properties and transitions. Therefore, combining several techniques can provide a more complete understanding of an unknown compound [1–3].

For example, UV-Visible spectroscopy may provide information about electronic transitions and conjugated systems, while IR spectroscopy can identify important functional groups. NMR spectroscopy can provide detailed information about the chemical environment and arrangement of atoms, while mass spectrometry can provide molecular-mass and fragmentation information.

The combined interpretation of these techniques is therefore a powerful approach to structure elucidation and compound identification. In modern analytical laboratories, spectroscopic methods are frequently used together with chromatographic separation techniques to analyze complex mixtures.

1.9 Conclusion

Spectroscopy is a powerful analytical technique based on the interaction of electromagnetic radiation with matter. Different spectroscopic methods provide complementary information about the structure, composition, concentration, and properties of substances. Its advantages, including rapid analysis, high sensitivity, small sample requirements, and reliable results, make spectroscopy an essential tool in modern scientific analysis.

The applications of spectroscopy extend across pharmaceuticals, medicine, environmental science, forensic science, agriculture, food science, material science, biochemistry, and research. The combined use of techniques such as UV-Visible, IR, NMR, and mass spectrometry provides comprehensive information for compound identification, structure elucidation, quantitative analysis, and quality control, making spectroscopy an indispensable part of modern science and technology.

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Chapter 2: Ultraviolet (UV) Spectroscopy

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Abstract

Ultraviolet–visible (UV–Vis) spectroscopy is a fundamental analytical technique based on the absorption of ultraviolet and visible electromagnetic radiation by atoms, ions and molecules, producing electronic transitions between molecular energy levels. This chapter introduces the fundamental principles of UV spectroscopy, including photon energy, electronic transitions, chromophores, auxochromes, spectral shifts and the Beer–Lambert law. It also describes the construction and operation of UV–Vis spectrophotometers, including light sources, monochromators, sample cells, detectors, signal-processing systems and readout devices, together with single-beam and double-beam configurations. Calibration requirements, common sources of analytical error and practical precautions are discussed to help students understand reliable spectrophotometric measurements. The chapter further surveys applications of UV spectroscopy in pharmaceutical, chemical, biochemical, environmental, food, clinical, agricultural, forensic, industrial and research settings. Emphasis is placed on the relationship between molecular structure, wavelength of maximum absorption (λmax), absorbance and concentration, enabling students to appreciate both the qualitative and quantitative uses of UV–Vis spectroscopy. Overall, the chapter provides a student-oriented foundation for understanding how light can be used to investigate and quantify chemical substances.

Keywords: Ultraviolet spectroscopy; UV–visible spectroscopy; electronic transitions; chromophore; auxochrome; Beer–Lambert law; spectrophotometer; monochromator; detector; absorbance; transmittance; quantitative analysis

2.1 Introduction

Ultraviolet (UV) spectroscopy is one of the most widely used analytical techniques in chemistry, pharmaceutical sciences, biochemistry, environmental science, food science, and material science. It is based on the interaction of ultraviolet radiation with matter, resulting in the absorption of electromagnetic energy by molecules. This absorbed energy promotes electrons from lower-energy molecular orbitals to higher-energy orbitals, producing characteristic absorption spectra that provide valuable qualitative and quantitative information about the substance under investigation.

Ultraviolet spectroscopy generally covers the wavelength range of 190–400 nm**,** while visible spectroscopy extends from 400–800 nm**.** Because most modern instruments measure both UV and visible regions, the technique is commonly referred to as UV–Visible (UV–Vis) spectroscopy**.**

UV spectroscopy is particularly useful for compounds containing chromophores**,** such as aromatic rings, carbonyl groups, conjugated double bonds, nitro groups, and azo groups. These groups absorb UV radiation at characteristic wavelengths, allowing identification and estimation of chemical substances. The technique is rapid, accurate, economical, and non-destructive, making it indispensable in quality control laboratories, pharmaceutical industries, academic research, environmental monitoring, and clinical laboratories [1].

Only UV and visible radiation are commonly employed in UV–Visible spectroscopy.

2.2 Principle of UV Spectroscopy

UV spectroscopy is an analytical technique that measures the absorption of ultraviolet radiation by atoms, ions, or molecules as a function of wavelength.The absorbed energy excites electrons from lower-energy molecular orbitals to higher-energy orbitals. Each compound exhibits a characteristic absorption spectrum that can be used for identification and quantitative analysis.[1]

The fundamental principle of UV spectroscopy is based on the absorption of ultraviolet radiation by molecules containing electrons capable of electronic excitation.When ultraviolet radiation passes through a sample photons possessing the correct energy are absorbed;electrons are promoted from the ground state to an excited state;the amount of absorbed radiation depends on the molecular structure and concentration of the analyte.

Mathematically,

$$E=h\nu=\lambda hc$$

where:

E = Energy of a photon (Joules, J)

h = Planck's constant = 6.626×10−34 J

ν (nu) = Frequency of radiation (Hertz, Hz or s−1

c = Speed of light = 3.00×108 m

λ (lambda) = Wavelength of radiation (meters, m)

Shorter wavelengths possess higher energy and can induce higher-energy electronic transitions.[3]

2.3 Electronic Transitions

The absorption of UV radiation results in the excitation of electrons from occupied molecular orbitals to unoccupied anti-bonding orbitals.[1,3]

The important electronic transitions are:

a) **σ → σ* Transition:** The **σ → σ*** transition is the highest-energy electronic transition in UV spectroscopy. It involves the excitation of an electron from a bonding sigma (σ) orbital to an anti-bonding sigma (σ*) orbital. This transition requires very high energy and therefore occurs at wavelengths below **200 nm** in the vacuum ultraviolet region. It is commonly observed in saturated hydrocarbons containing only single covalent bonds, such asmethane and ethane.

b) **n → σ*Transition:** The **n → σ*** transition occurs in compounds containing atoms with lone pair (non-bonding) electrons. In this transition, a non-bonding (n) electron is excited to an anti-bonding sigma (σ*) orbital. It typically requires radiation in the wavelength range of **180–260 nm** and is commonly observed in compounds such as alcohols, amines, andhalides.

c) ***π* → *π** Transition:** The ***π* → *π**** transition occurs in compounds containing doublebonds or other π-electron systems. In this transition, an electron is promoted from a bonding pi (π) orbital to an anti-bonding pi (π*) orbital. It is commonly observed in alkenes, aromatic compounds, and conjugated dienes. Compared with σ → σ* transitions, π → π* transitions require less energy and generally occur in the ultraviolet region. The presence of conjugation shifts the absorption to longer wavelengths, making these transitions especially important in UV-visible spectroscopy for the identification and analysis of unsaturated and aromatic compounds

d) **2n → *π** Transition:** The n → π* transition occurs in molecules containing both non-bonding (lone pair) electrons and π-anti-bonding (π*) orbitals. In this transition, a non-bonding (n) electron is excited to an anti-bonding π* orbital. It is commonly observed in compounds containing carbonyl groups (aldehydes, ketones, carboxylic acids, and esters), nitro compounds, and azo compounds. Among the four types of electronic transitions, the n → π* transition requires comparatively less energy and therefore occurs at longer wavelengths in the ultraviolet region, making it an important transition in UV-visible spectroscopy for the identification of functional groups.

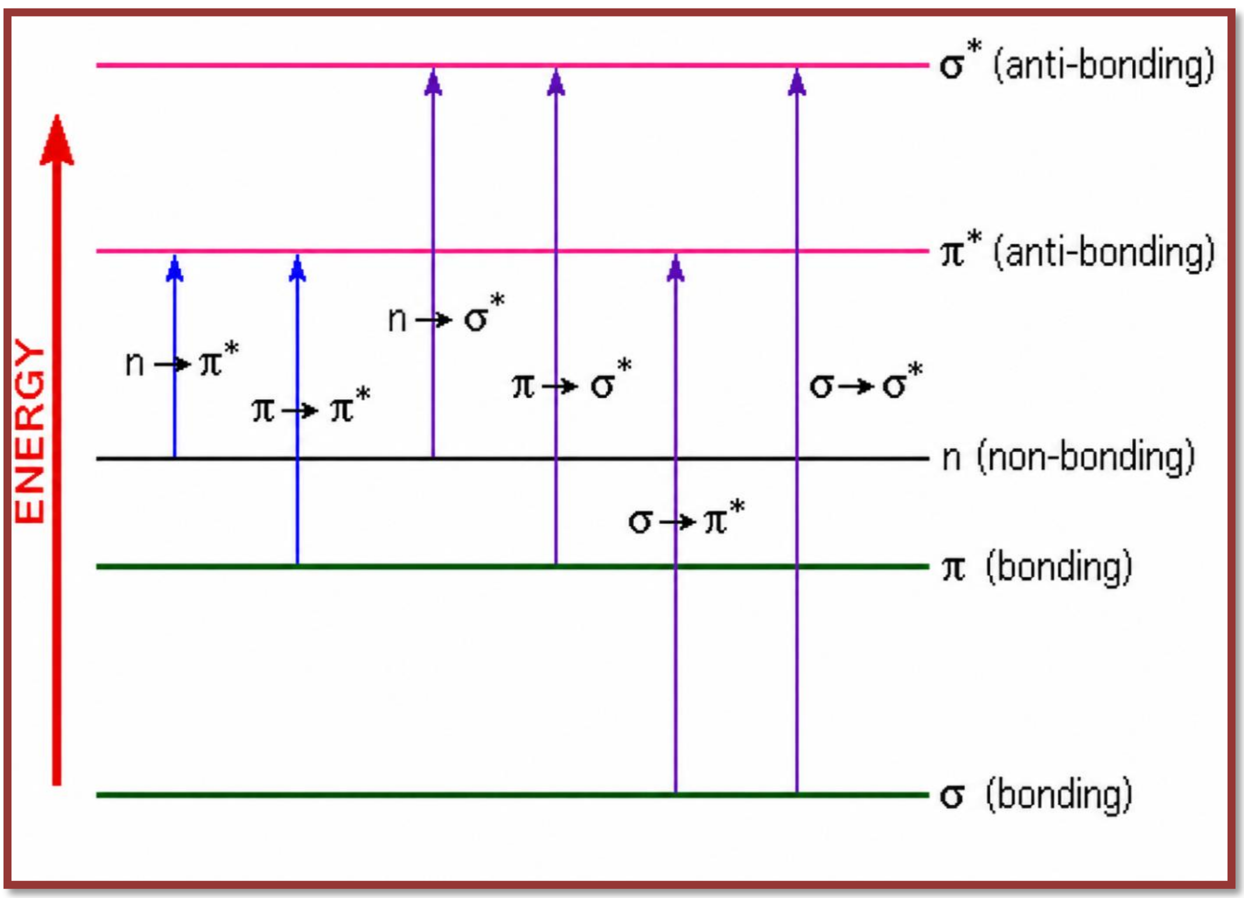


Figure 2.1: Different types of Electronic Transitions

2.4 Concept of Chromophore and Auxochrome:

Chromophores: A chromophore is the part of a molecule responsible for the absorption of ultraviolet or visible radiation. Chromophores determine the wavelength at which maximum absorption occurs.Common chromophores include:Alkene(C=C), Alkyne (C≡C), Carbonyl (C=O), Azo compounds (N=N), Nitro compounds (NO_2), Aromatic ring (Benzene)

Auxochromes: Auxochromes are groups that do not themselves absorb strongly in the UV region but modify the absorption characteristics of a chromophore when attached to it.

Common auxochromesinclude:–OH, $–NH_2$, –OR, –SH. Auxochromes generally increase absorption intensity;shift the absorption maximum (λmax) to longer wavelengths (bathochromic or red shift).[3]

2.4.1 Types of Absorption Shifts

a) **Bathochromic Shift (Red Shift):** A bathochromic shift, also known as a red shift, is the movement of the absorption maximum **(**λmax**)** towards a longer wavelength in the UV-visible spectrum. This shift occurs when the energy required for an electronic transition decreases. It is commonly caused by increased conjugation**,** solvent effects, or the presence of auxochromes (such as –OH and $–NH_2$), which enhance electron delocalization. A typical example is the conversion of benzene to aniline, where the amino group ($–NH_2$) acts as an auxochrome, increasing conjugation and shifting the λmax to a longer wavelength.

b) **Hypsochromic Shift (Blue Shift):** A hypsochromic shift**,** also known as a blue shift**,** is the movement of the absorption maximum **(**λmax**)** towards a shorter wavelength in the UV-visible spectrum. This shift occurs when the energy required for an electronic transition increases. It is commonly caused by decreased conjugation**,** protonation of functional groups**,** or steric hindrance, which reduces the extent of electron delocalization within the molecule. As a result, the compound absorbs light of higher energy and shorter wavelength.

c) **Hyperchromic Effect:** The hyperchromic effect refers to an increase in the intensity of absorption (absorbance) in the UV-visible spectrum without necessarily changing the wavelength of maximum absorption (λmax). This effect occurs when the probability of electronic transitions increases, resulting in stronger absorption of ultraviolet or visible light. It is commonly caused by the presence of auxochromes, which enhance electron delocalization, and increased conjugation, which facilitates electronic transitions.

d) **Hypochromic Effect:** The hypochromic effect refers to a decrease in the intensity of absorption (absorbance) in the UV-visible spectrum. This effect occurs when the probability of electronic transitions is reduced, leading to weaker absorption of light. It is commonly caused by steric effects**,** which hinder molecular planarity and reduce conjugation, and hydrogen bonding**,** which can alter the electronic environment of chromophores and decrease absorption intensity.

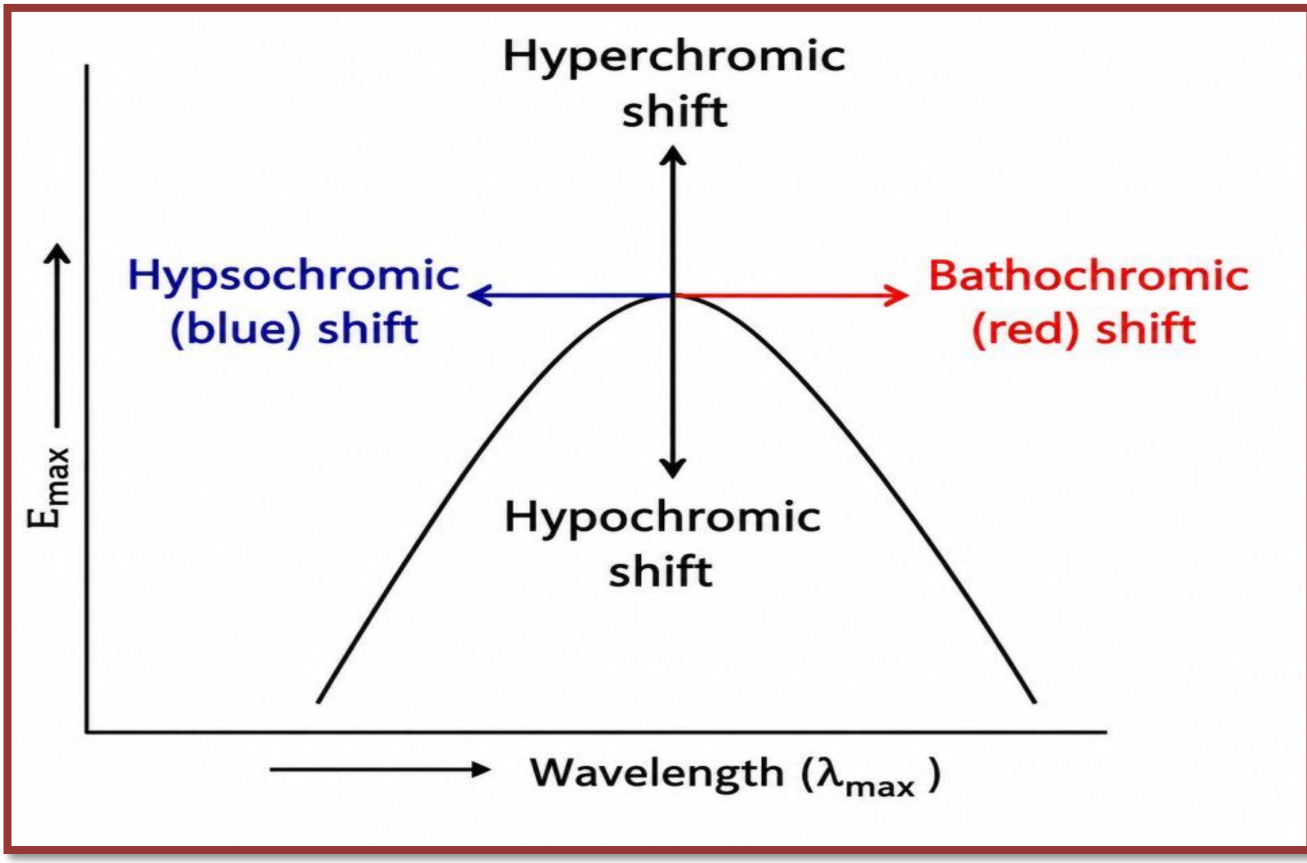


Figure 2.2: Different types of Absorption Shifts

2.5 Beer–Lambert's Law

The Beer–Lambert law forms the basis of quantitative UV analysis under conditions in which the relationship between absorbance and concentration remains linear. [2,6]

It statesthat:The absorbance of a solution is directly proportional to the concentration of the absorbing species and the path length of the sample through which the radiation passes.

Mathematically,

$$A=\varepsilon cl$$

where:

A = Absorbance

ε = Molar absorptivity (L mol^{-1} cm^{-1})

c = Concentration (mol L^{-1})

l = Path length (cm)

2.5.1 Relationship between Absorbance and Transmittance

Absorbance is related to the intensity of incident and transmitted light by:

$A=\log(I_0)/I=-\log T$

where:

I_0 = Intensity of incident light

I = Intensity of transmitted light

T = Transmittance (T=I/I0)

As absorbance increases, transmittance decreases, meaning less light passes through the sample.

2.6 Instrumentation of UV Spectroscopy

A UV spectrophotometer is an analytical instrument designed to measure the absorption of ultraviolet and visible radiation by a sample. The instrument compares the intensity of incident light with the intensity of transmitted light after passing through the sample. From these measurements, the absorbance and concentration of the analyte can be determined.[1,3]

The basic components of a UV spectrophotometer are:

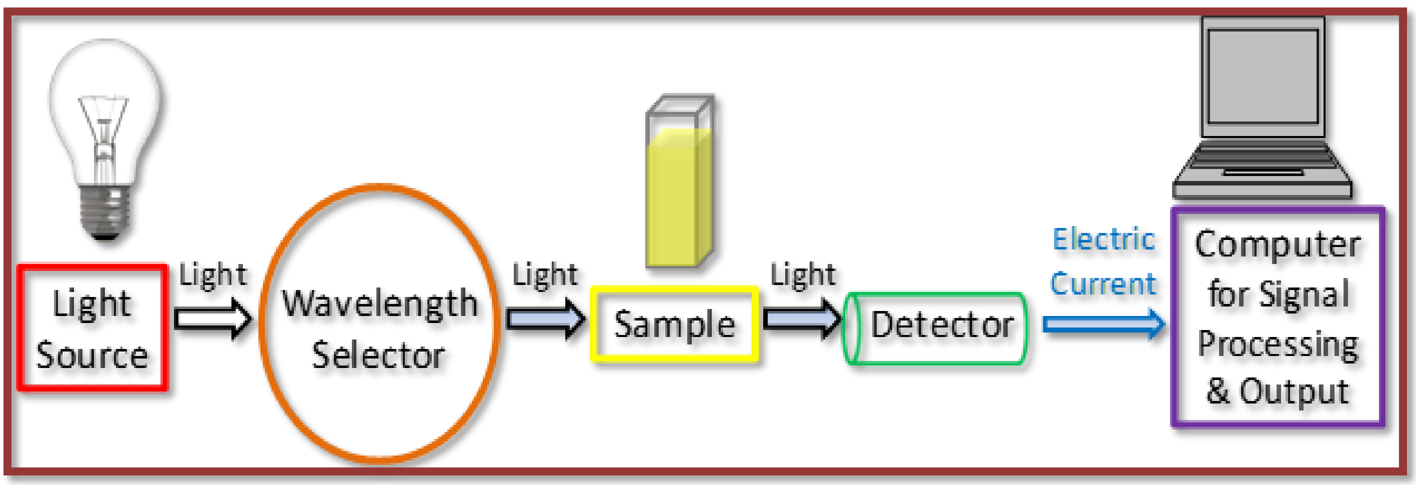


Figure 2.3: Block Diagram of UV Spectrophotometer

2.6.1 Components of UV Spectrophotometer

a) Light Source: The light source provides continuous radiation covering the ultraviolet and visible regions.

An ideal light source should:

- Produce stable radiation
- Cover a wide wavelength range
- Have high intensity
- Exhibit long operational life

Types of Light Sources

Light Source	Wavelength Range	Application
Deuterium lamp	190–400 nm	UV region
Hydrogen discharge lamp	190–350 nm	UV spectroscopy
Tungsten lamp	320–1100 nm	Visible region
Xenon arc lamp	190–900 nm	UV–Visible spectroscopy

Table 2.1: Different types of light sources in UV-Visible Spectroscopy

Deuterium Lamp: The deuterium lamp is the most commonly used UV source because it provides a stable continuous spectrum in the ultraviolet region.

Advantages:

- High stability
- Continuous UV spectrum
- Long service life

b) Monochromator

The monochromator isolates a narrow band of wavelengths from the broad spectrum emitted by the light source thereby permits controlled wavelength selection [4].

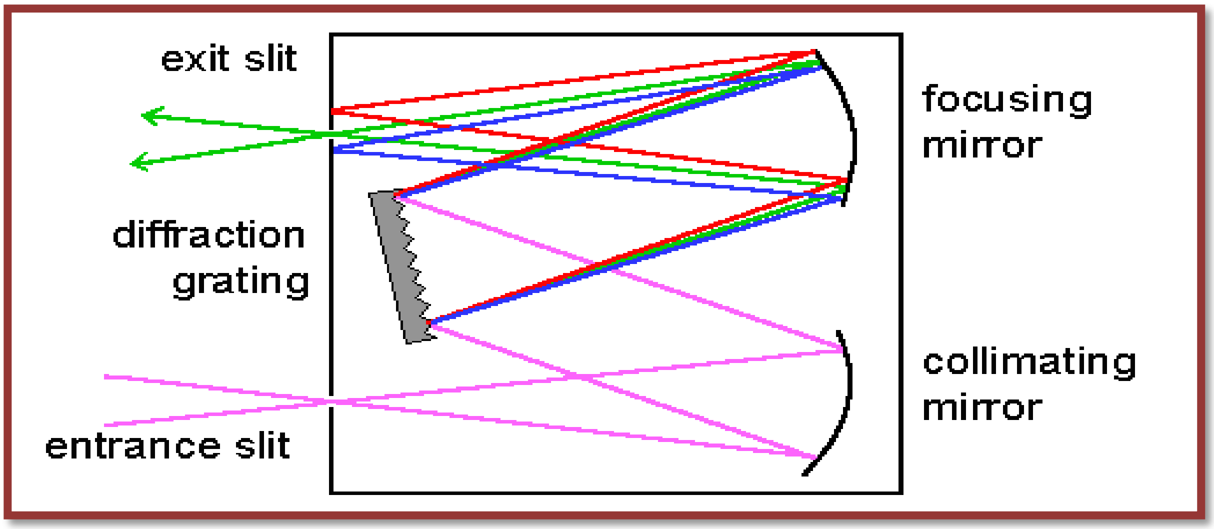
Its functions are:

- Separate different wavelengths

- Select the desired wavelength
- Improve spectral resolution

Components of Monochromator

- Entrance slit
- Collimating mirror
- Prism or diffraction grating
- Focusing mirror



- Exit slit

Figure 2.4: Monochromator

Prism Monochromator

A prism monochromator separates polychromatic light into its component wavelengths based on the principle that different wavelengths are refracted by different amounts when passing through a prism. It has a simple design and provides good wavelength separation, making it suitable for many spectroscopic applications. However, prism monochromators exhibit non-lineardispersion, meaning that the separation between wavelengths is not uniform across the spectrum. They also offer comparatively lower resolution, making them less suitable for applications requiring precise wavelength selection.

Diffraction Grating Monochromator

A diffraction grating monochromator is the most commonly used dispersing element in modern UV-Visible spectrophotometers. It separates light by diffraction and interference through a large number of closely spaced parallel grooves, producing a spectrum with linear dispersion. Compared to prism monochromators, diffraction gratings provide higher resolution, betterwavelength accuracy, and more uniform wavelength separation across the UV-Visible region. These advantages make diffraction grating monochromators the preferred choice for modern analytical instruments.

c) Sample Holder (Cuvette)

The sample holder, commonly known as a cuvette, is used to hold the sample solution during UV-Visible spectroscopic analysis. It allows the monochromatic light beam to pass through the sample so that the amount of absorbed light can be measured. An ideal cuvette should be transparent to UV radiation to ensure accurate transmission of light, chemically inert so that it does not react with the sample, and should have a uniform optical path length (typically 1 cm) to provide precise and reproducible absorbance measurements. Quartz or fused-silica cuvettes are generally preferred for UV measurements because they transmit ultraviolet radiation effectively; ordinary glass and many plastics have substantially greater UV absorption and are therefore unsuitable at shorter wavelengths[4]

d) Detector

The detector is a vital component of a UV-Visible spectrophotometer that converts the transmittedlight passing through the sample into an electrical signal proportional to the intensity of the transmitted light. This electrical signal is then processed to determine the absorbance or transmittance of the sample. An ideal detector should have high sensitivity to detect even low levels of light, low noise to ensure accurate and reliable measurements, fast response for rapid signal detection, and high stability to provide consistent and reproducible results during analysis. Commonly used detectors in UV-Visible spectroscopy include photomultiplier tubes (PMTs),photodiodes, and photodiode array (PDA) detectors [5].

e) Signal Processor (Amplifier)

The signal processor (amplifier) receives the weak electrical signal generated by the detector and amplifies it so that it can be accurately measured and analyzed. In addition to signal amplification, it performs noise reduction to improve signal quality, analog-to-digital conversion to convert the electrical signal into digital form, and data processing to calculate parameters such as absorbance and transmittance. These functions ensure accurate, reliable, and reproducible spectroscopic measurements.

f) Readout Device

The readout device displays the processed signal in a user-friendly format. It provides important analytical information such as absorbance, transmittance, selected wavelength, and the absorption spectrum of the sample. Modern UV-Visible spectrophotometers are equipped with computerized display systems that automatically generate absorption spectra, store experimental data, and perform quantitative calculations, making the analysis faster, more accurate, and easier to interpret.

2.6.2 Working of a UV Spectrophotometer

The operation of a UV spectrophotometer involves the following steps [1]:

1) The light source emits UV radiation.

2) The monochromator selects a desired wavelength.
3) The monochromatic beam passes through the sample.
4) Molecules absorb part of the radiation.
5) The transmitted light reaches the detector.
6) The detector converts light into an electrical signal.
7) The signal is amplified and processed.
8) The instrument displays absorbance versus wavelength.

2.6.3 Types of UV Spectrophotometers

Single-Beam UV Spectrophotometer

A single-beam instrument uses only one light path. The blank and the sample are measured separately.

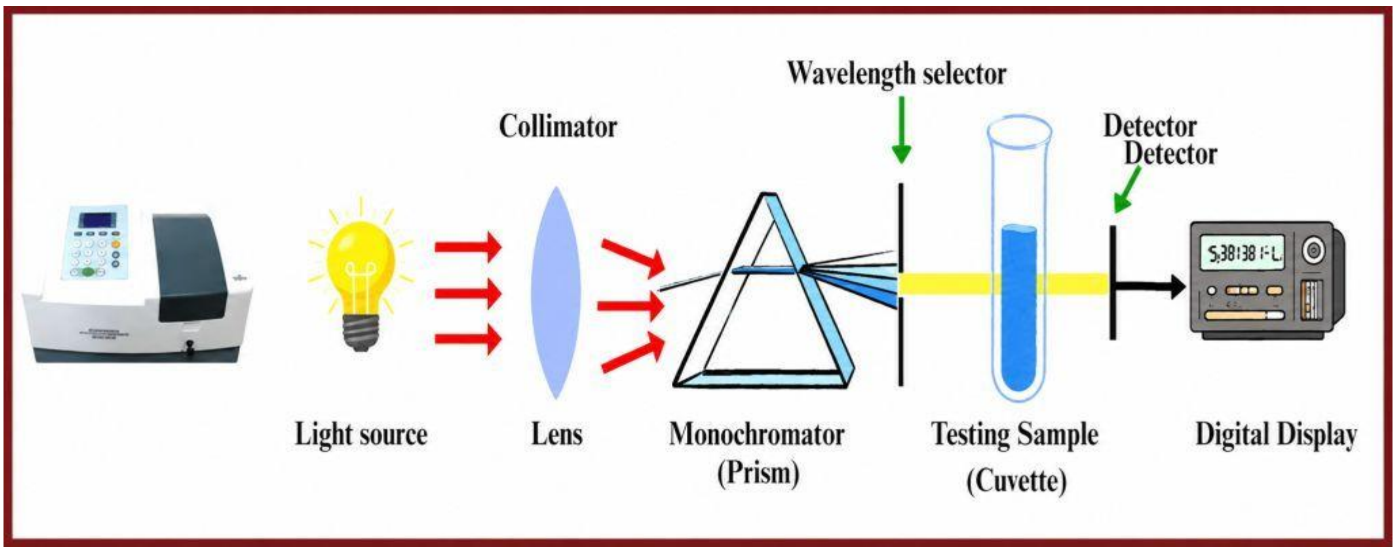


Figure2.5: Single-Beam UV Spectrophotometer

Double-Beam UV Spectrophotometer

A double-beam instrument splits the light into two beams: Sample beam and Reference (blank) beam. Both are measured simultaneously or alternately.

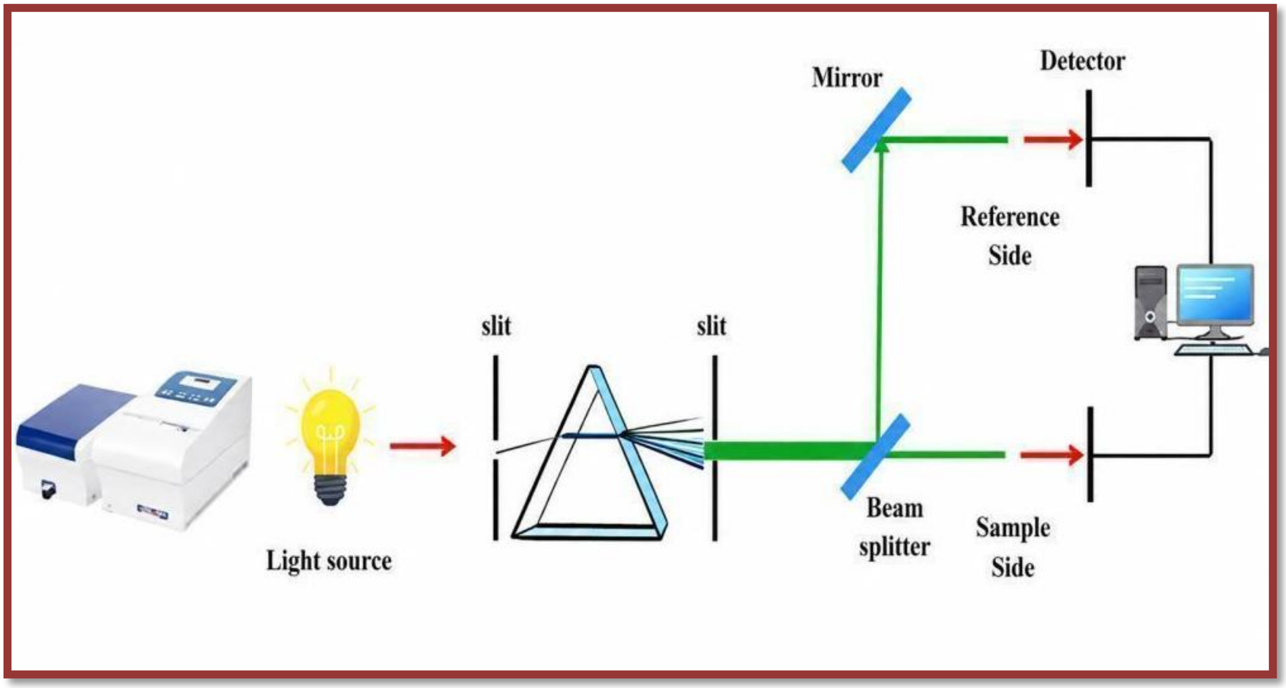


Figure2.6: Double-Beam UV Spectrophotometer

Type of Spectrometer	Advantages	Disadvantages
Single-Beam Instrument	Simple construction Low cost Easy maintenance High light intensity	Blank and sample measured separately More susceptible to source fluctuations Lower accuracy than double-beam instruments
Double-Beam Instrument	High accuracy Automatic correction for lamp fluctuations Better baseline stability Suitable for research applications Continuous comparison with reference	More expensive Complex optical system Higher maintenance requirements

Table 2.2: Advantages and Disadvantages of Single and Double beam Spectrometer

2.6.4 Comparison of Single-Beam and Double-Beam Instruments

Feature	Single Beam	Double Beam
Light path	One	Two
Cost	Low	High
Accuracy	Moderate	High
Stability	Lower	Excellent
Speed	Slower	Faster
Blank correction	Manual	Automatic
Research suitability	Limited	Excellent

Table 2.3: Comparison of Single-Beam and Double-Beam Instruments

2.6.5 Calibration of UV Spectrophotometer

Proper calibration and performance verification are essential for accurate and reproducible measurements [6].

Common calibration parameters include:

- Wavelength accuracy
- Photometric accuracy
- Baseline stability
- Stray light
- Resolution
- Detector response

Standard reference materials and certified reference solutions can be used to verify wavelength and photometric performance; holmium-based wavelength references and suitable absorbance standards are commonly employed in spectrophotometric performance checks [6].

2.7Sources of Error in UV Spectroscopy

Common sources of error include:

- Dirty or scratched cuvettes
- Fingerprints on cuvettes
- Air bubbles in the sample
- Incorrect blank solution
- Instrument drift
- Improper wavelength selection
- Stray light
- Highly concentrated samples
- Turbid or particulate samples
- Temperature variations

Proper instrument maintenance and careful sample preparation minimize these errors.

2.8 Applications of UV Spectroscopy

Ultraviolet (UV) spectroscopy is one of the most versatile analytical techniques used in scientific laboratories. It provides rapid, accurate, sensitive, and non-destructive analysis of a wide range of compounds. Applications extend across pharmaceutical sciences, chemistry, biochemistry, environmental science, food science, forensic science, agriculture, and clinical diagnostics.

2.8.1 Pharmaceutical Applications

The pharmaceutical industry is one of the largest users of UV spectroscopy because of its simplicity, accuracy, and cost-effectiveness.

(a) Quantitative Estimation of Drugs

UV spectroscopy is routinely used to determine the concentration of drugs in bulk materials and pharmaceutical formulations according to the Beer–Lambert law [1].

Examples include:Paracetamol, Aspirin, Ibuprofen, Diclofenac sodium, Ciprofloxacin, Metformin, Atorvastatin

(b) Quality Control

UV spectroscopy is extensively employed for:

- Assay of active pharmaceutical ingredients (APIs)
- Uniformity testing
- Dissolution studies
- Stability studies
- Identification of impurities
- Content uniformity testing

(c) Dissolution Testing

During dissolution testing, the amount of drug released from tablets or capsules is measured at predetermined time intervals using UV spectroscopy.

(d) Stability Studies

UV spectroscopy helps determine degradation caused by light, heat, humidity, oxidation, hydrolysis. This information is essential for establishing shelf life.

2.8.2 Chemical Applications

In analytical chemistry, UV spectroscopy is widely used for:

- Identification of organic compounds
- Detection of conjugated systems
- Determination of reaction completion
- Monitoring chemical kinetics
- Estimation of equilibrium constants
- Analysis of transition metal complexes

2.8.3 Biochemical Applications

Many biomolecules absorb ultraviolet (UV) radiation because they contain aromatic amino acids and nucleic acid bases [1, 7, 8]. Therefore, UV spectroscopy is widely employed in biochemical research and analysis for various applications, including protein estimation, DNA quantification, RNA quantification, enzyme activity studies, and determination of protein purity.

Protein Analysis: Proteins containing tyrosine, tryptophan, and phenylalanine absorb strongly near 280 nm**.**

DNA Analysis: DNA exhibits maximum absorption at approximately 260 nm**.**

The A_{260}/A_{280} ratio is commonly used to assess nucleic acid purity [8].

2.8.4 Environmental Applications

UV spectroscopy is used for monitoring environmental pollutants.

Examples include: Nitrate determination, Nitrite estimation, Phosphate analysis, Detection of pesticides, Heavy metal complex analysis, Industrial wastewater monitoring.

2.8.5 Food Industry

Applications include: Vitamin estimation, Food additive analysis, Color measurement, Sugar analysis, Beverage quality control, Detection of food adulterants.

Examples: Vitamin A, Vitamin B_2, Vitamin C.

2.8.6 Clinical Applications

Hospitals and diagnostic laboratories use UV spectroscopy for:

- Blood glucose estimation
- Cholesterol analysis
- Bilirubin determination
- Urea estimation
- Creatinine analysis
- Enzyme assays

2.8.7 Agricultural Applications

UV spectroscopy assists in:

- Fertilizer analysis
- Soil nutrient determination

- Pesticide residue analysis
- Plant pigment estimation

2.8.8 Forensic Applications

UV spectroscopy plays an important role in forensic investigations.

Applications include:

- Drug identification
- Poison detection
- Ink analysis
- Document examination
- Fibre analysis
- Explosive residue studies

2.8.9 Industrial Applications

Industries use UV spectroscopy for:

- Dye analysis
- Polymer characterization
- Paint quality control
- Petrochemical analysis
- Cosmetic product testing

2.8.9 Research Applications

Researchers employ UV spectroscopy for: Structure elucidation, Reaction kinetics, Complex formation studies, Nanomaterial characterization, Protein folding studies, Ligand binding investigations

2.9Advantages of UV Spectroscopy

UV spectroscopy offers numerous advantages that make it one of the most commonly used analytical techniques.

- Rapid analysis.

- Simple operation.
- High sensitivity.
- Good accuracy and precision.
- Non-destructive technique.
- Requires only a small sample volume.
- Minimal sample preparation.
- Suitable for both qualitative and quantitative analysis.
- Relatively inexpensive instrumentation.
- Wide range of pharmaceutical applications.
- High reproducibility.
- Can be easily automated.
- Compatible with flow-injection and chromatographic systems.
- Suitable for routine quality control.
- Environmentally friendly due to low reagent consumption.

2.10 Limitations of UV Spectroscopy

Despite its advantages, UV spectroscopy has several limitations.

- Applicable only to compounds that absorb UV or visible radiation.
- Poor specificity for complex mixtures without prior separation.
- Interference from impurities with overlapping absorption spectra.
- Turbid samples cause scattering and inaccurate results.
- Solvents must not absorb at the analytical wavelength.
- High concentrations may deviate from Beer–Lambert law.
- Limited structural information compared with IR, NMR, or mass spectrometry.
- Quartz cuvettes are required for UV measurements, increasing cost.

- Instrument performance may be affected by stray light and lamp aging.
- Highly coloured samples may produce inaccurate absorbance values.

2.11Precautions during UV Analysis

To obtain accurate and reproducible results, the following precautions should be observed:

- Use clean and scratch-free quartz cuvettes for UV measurements.
- Handle cuvettes by the frosted sides to avoid fingerprints.
- Remove air bubbles before analysis.
- Use high-purity solvents and reagents.
- Prepare fresh standard and sample solutions.
- Always run a blank before sample analysis.
- Select an appropriate analytical wavelength (λmax).
- Keep the instrument properly calibrated.
- Avoid highly concentrated samples; dilute if necessary.
- Close the sample compartment during measurement to prevent stray light.
- Maintain a constant laboratory temperature where possible.
- Ensure the sample is free from suspended particles.

2.13 Conclusion

Ultraviolet–visible spectroscopy is a fundamental analytical method that connects the interaction of electromagnetic radiation with molecular structure to practical chemical measurement. The technique is based on electronic excitation following absorption of UV or visible radiation, and its analytical usefulness depends on the nature of the absorbing species, the selected wavelength, sample concentration and optical path length. Concepts such as chromophores, auxochromes, electronic transitions and spectral shifts help explain why different substances absorb at different wavelengths, while the Beer–Lambert law provides the foundation for quantitative measurements under suitable conditions.

A sound understanding of the instrument is equally important. The light source, wavelength-selection system, sample cell, detector and signal-processing system work together to convert optical absorption into a measurable analytical signal. Proper calibration, appropriate cuvette selection, correct blanking, clean samples and control of instrumental and environmental variables are essential for reliable results. Although UV spectroscopy has limitations in specificity and structural information, its speed, simplicity, sensitivity and relatively low cost make it valuable across pharmaceutical, chemical, biochemical, environmental, food, clinical, agricultural, forensic, industrial and research laboratories. For general science students, UV–Vis spectroscopy therefore provides an important introduction to the wider field of instrumental analysis and demonstrates how fundamental principles of light and matter can be applied to real analytical problems.

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Chapter 3: Nuclear Magnetic Resonance (NMR) Spectroscopy

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Abstract:

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful and versatile analytical technique widely employed for the identification, characterization, and structural elucidation of organic, inorganic, and biologically important compounds. This chapter provides a comprehensive overview of the fundamental concepts and practical aspects of NMR spectroscopy, with particular emphasis on ^{1}H and ^{13}C NMR. The theoretical basis of NMR is discussed in terms of nuclear spin, interaction of nuclei with an external magnetic field, energy-level transitions, radiofrequency irradiation, resonance, and relaxation. The chapter further describes the principle and instrumentation of conventional continuous-wave (CW) and modern Fourier Transform (FT) NMR spectrometers, including their major components and modes of operation. Particular attention is given to sample preparation, selection of deuterated solvents, and factors affecting spectral quality. The interpretation of ^{1}H NMR spectra is discussed through the concepts of chemical and magnetic equivalence, number of signals, chemical shift, shielding and deshielding, inductive and anisotropic effects, hydrogen bonding, and hybridization. Spin–spin coupling, signal splitting, the (n + 1) rule, common multiplicities, and coupling constants are also explained with representative examples. Overall, the chapter highlights the importance of NMR spectroscopy as an indispensable tool for establishing molecular connectivity, identifying chemical environments, and supporting the structural characterization of organic compounds.

Keywords

Nuclear Magnetic Resonance (NMR); ^{1}H NMR; ^{13}C NMR; Fourier Transform NMR; Continuous-Wave NMR; nuclear spin; chemical shift; shielding; deshielding; spin–spin coupling; coupling constant; structural elucidation; spectroscopy.

3.1 Introduction to Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most important and versatile analytical techniques used for the identification, characterization, and structural elucidation of organic, inorganic, and biological molecules. It provides detailed information about the number, type, and chemical environment of specific nuclei present in a molecule, making it an indispensable tool in chemistry, pharmaceutical sciences, biochemistry, medicinal chemistry, polymer science, and materials research. Unlike many other spectroscopic techniques, NMR not only identifies functional groups but also establishes the complete molecular framework, stereochemistry, molecular conformation, and intermolecular interactions.

The NMR Spectroscopy was introduced by Felix Bloch & Edward Purcell in 1946and is the most powerful tool for structure elucidation & identification of organic substances. [1]

It is based on the interaction of **nuclear spins** with an external magnetic field and radiofrequency radiation.[1,3]

It is of two types:

Proton Magnetic Resonance Spectroscopy (PMR or ^{1}H-NMR)

Proton NMR spectroscopy involves the study of hydrogen nuclei (^{1}H), which are present in the majority of organic compounds. Since hydrogen has a natural abundance of approximately 99.98%, ^{1}H-NMR is highly sensitive and is the most commonly employed form of NMR spectroscopy. It provides information regarding:

- Number of chemically distinct hydrogen atoms.
- Chemical environment of each proton.
- Relative number of protons through signal integration.
- Neighbouring hydrogen atoms through spin-spin splitting.
- Molecular structure and functional groups.

Carbon (^{13}C) Nuclear Magnetic Resonance Spectroscopy (^{13}C-NMR)

Carbon-13 NMR spectroscopy is based on the resonance of the ^{13}C isotope, which has a nuclear spin of I = 1/2 and a natural abundance of approximately 1.1%. Although less sensitive than proton NMR, ^{13}C-NMR provides detailed information regarding the carbon skeleton of organic molecules. It is extensively used to identify different types of carbon atoms such as methyl, methylene, methine, quaternary, carbonyl, aromatic, and aliphatic carbons. Together with ^{1}H-NMR, it provides complete structural characterization of organic compounds.

3.2 Theory of NMR:

The theory of Nuclear Magnetic Resonance is based on the magnetic properties of atomic nuclei. Not all atomic nuclei exhibit NMR behaviour. Only those nuclei possessing a non-zero nuclear spin quantum number ($I \neq 0$) can interact with an external magnetic field and produce an NMR spectrum.

Nuclear spin arises due to the combined angular momentum of protons and neutrons present within the atomic nucleus. Since both protons and neutrons possess intrinsic spin, the overall nuclear spin depends upon the number and arrangement of these particles. Depending upon the mass number (A) and atomic number (Z), nuclei may possess different values of spin quantum number.

The NMR activity of nuclei follows the general rules shown below:

Mass No.	Atomic No.	Examples	Resultant Nuclear Spin	NMR Activity
Odd	Odd or Even	$^{1}_{1}H$, $^{13}_{6}C$, $^{15}_{7}N$, $^{19}_{9}F$, $^{31}_{15}P$	½	NMR Active
Even	Odd	$^{2}_{1}H$, $^{10}_{5}B$, $^{14}_{7}N$	1	NMR Active
Even	Even	$^{12}_{6}C$, $^{16}_{8}O$, $^{32}_{16}S$	0	NMR Inactive

Table 3.1: Nuclear spin and NMR activity [1,4]

Thus, nuclei having odd mass number or odd atomic number possess a resultant nuclear spin and are therefore NMR active, whereas nuclei having both even mass number and even atomic number possess zero spin and are NMR inactive.

The magnitude of nuclear spin is represented by the spin quantum number (I). The possible values include: I = 0, ½, 1, 3/2, 5/2,7/2. Among these, nuclei with I = 1/2 are the most commonly encountered in organic chemistry because they produce simple, easily interpretable NMR spectra.

If an external magnetic field is applied, the number of possible orientations calculated by: **(2I+1)** [1]

For Example: Hydrogen has Spin Quantam Number, I=1/2

So Possible Orientations is (2xI/2+I)= 2 i.e. (+1/2) and (-1/2)

Hence, a proton can adopt two possible orientations:

α-spin (+1/2): Parallel to the applied magnetic field (lower energy state).

β-spin (−1/2): Antiparallel to the applied magnetic field (higher energy state).

The energy difference between these two orientations is very small under normal conditions but increases proportionally with the strength of the external magnetic field. When radiofrequency radiation having energy exactly equal to this energy difference is applied, nuclei absorb the energy and undergo a transition from the lower energy state to the higher energy state. This process is called resonance, and the resulting absorption forms the basis of Nuclear Magnetic Resonance spectroscopy.

3.3 Principle of NMR Spectroscopy:

The principle of Nuclear Magnetic Resonance (NMR) spectroscopy is based on the magnetic properties of certain atomic nuclei, such as ^{1}H, ^{13}C, ^{19}F and ^{31}P, which possess a non-zero nuclear spin. A spinning charged nucleus generates a magnetic moment and behaves like a tiny bar magnet.

When a sample containing NMR-active nuclei is placed in a strong external magnetic field (H_0), the nuclei orient themselves in specific energy states. For a nucleus with spin **I = ½**, such as ^{1}H and ^{13}C, two orientations are possible:

Parallel to the applied magnetic field → lower-energy state (α-state)

Antiparallel to the applied magnetic field → higher-energy state (β-state)

The difference in energy between these two states is called **energy separation (ΔE)**. Under normal conditions, slightly more nuclei occupy the lower-energy state than the higher-energy state.

When radiofrequency (RF) radiation of the appropriate frequency is applied, the nuclei absorb this energy and undergo a transition from the lower-energy state to the higher-energy state. This process is called resonance. The frequency of RF radiation required for resonance depends on the type of nucleus and the strength of the applied magnetic field. [1,3]

The energy difference between the two spin states is given by:

$$\Delta E = h\nu = \gamma h H_0 / 2\pi$$

where

ΔE: energy difference between two magnetic spins

h: Planck's Constant

ν:frequency of radiation absorbed

H_0:Strength of applied field

γ: Nuclear constant/ Gyromagnetic ratio (Ratio of angular momentum of an atomic system to its magnetic moment) For proton its value is 26750. It has different value for different nuclei.

After absorption of RF energy, the excited nuclei return toward their lower-energy state through a process called relaxation. During this process, the nuclei produce a weak RF signal. This signal is detected by the receiver coil of the NMR spectrometer and converted into an **NMR spectrum**.

The exact position and intensity of signals in the spectrum provide valuable information about the chemical environment of the nuclei. In ^{1}H NMR, for example, differences in electronic shielding cause protons in different chemical environments to resonate at different positions, expressed as their ***chemical shifts (δ)*** in ppm.

Thus, NMR spectroscopy allows the determination of the number, chemical environment, connectivity and molecular structure of nuclei present in a compound. The resonance frequency is directly related to the strength of the applied magnetic field:

$$\nu \propto H_0$$

Therefore, increasing the magnetic field strength increases the resonance frequency. This fundamental relationship between nuclear spin, magnetic field, RF radiation and resonance forms the basis of NMR spectroscopy.

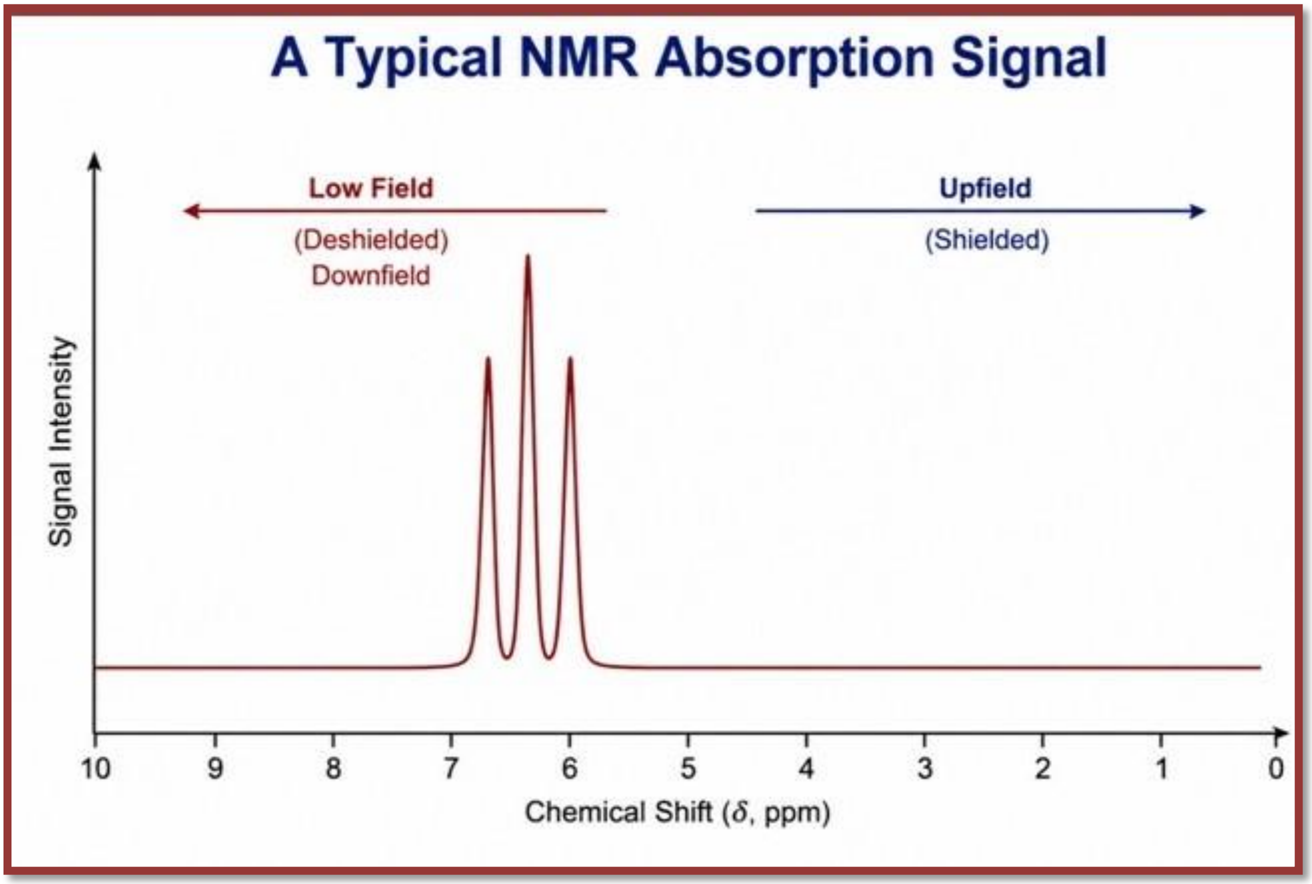


Figure 3.1: A Typical NMR Absorption Signal. [2]

3.4 Instrumentation of NMR:

Nuclear Magnetic Resonance (NMR) spectrometers are sophisticated analytical instruments used to study the magnetic properties of atomic nuclei and to obtain structural information about molecules. NMR spectroscopy is based on the absorption of radiofrequency (RF) radiation by nuclei possessing nuclear spin when they are placed in a strong magnetic field. The absorbed energy produces a resonance signal, which is detected, processed, and converted into an NMR spectrum.

An NMR spectrometer mainly consists of a strong magnetic field, a sample holder, radiofrequency transmitter and receiver systems, and an electronic data-processing system. Depending on the method used to excite the nuclei and record the signal, NMR spectrometers are broadly classified into two types:

3.4.1 Sweep or Continuous Wave (CW) NMR Spectrometer: In a Sweep or Continuous Wave (CW) NMR spectrometer, the sample is continuously exposed to radiofrequency radiation while the magnetic field is slowly varied or swept over a selected range. Alternatively, the magnetic field may be kept constant while the radiofrequency is varied.

At a particular combination of magnetic field strength and radiofrequency, the resonance condition is satisfied. At this point, the nuclei absorb RF energy and undergo a transition from the lower-energy spin state to the higher-energy spin state. This absorption is detected by the receiver and appears as an absorption signal in the NMR spectrum.

The CW spectrometer scans the resonance conditions one at a time. Therefore, individual signals are recorded sequentially, and the complete spectrum is built up peak by peak. The magnetic field sweep must be controlled carefully to obtain accurate chemical-shift values and well-resolved signals.

3.4.2 Fourier Transform (FT) NMR Spectrometer:

Fourier Transform (FT) NMR spectroscopy is the modern and widely used method of recording NMR spectra. In this technique, the sample is exposed to a short and intense pulse of radiofrequency radiation containing a broad range of frequencies. This pulse excites all nuclei that satisfy the required resonance conditions simultaneously.

After the RF pulse is removed, the excited nuclei return toward their equilibrium state through relaxation processes. During this process, they produce a weak RF signal known as the **Free Induction Decay (FID)**. The FID is recorded as a function of time. The signal obtained from the sample is initially in the time domain, where signal intensity is measured as a function of time. A mathematical procedure called **Fourier Transform** converts the time-domain FID into a frequency-domain spectrum**,** which is the conventional NMR spectrum showing resonance signals at different chemical shifts.[2,3]

The basic sequence of operation in FT-NMR is:

RF pulse → Simultaneous excitation of nuclei → Relaxation → FID generation → Signal detection → Fourier Transform → NMR spectrum

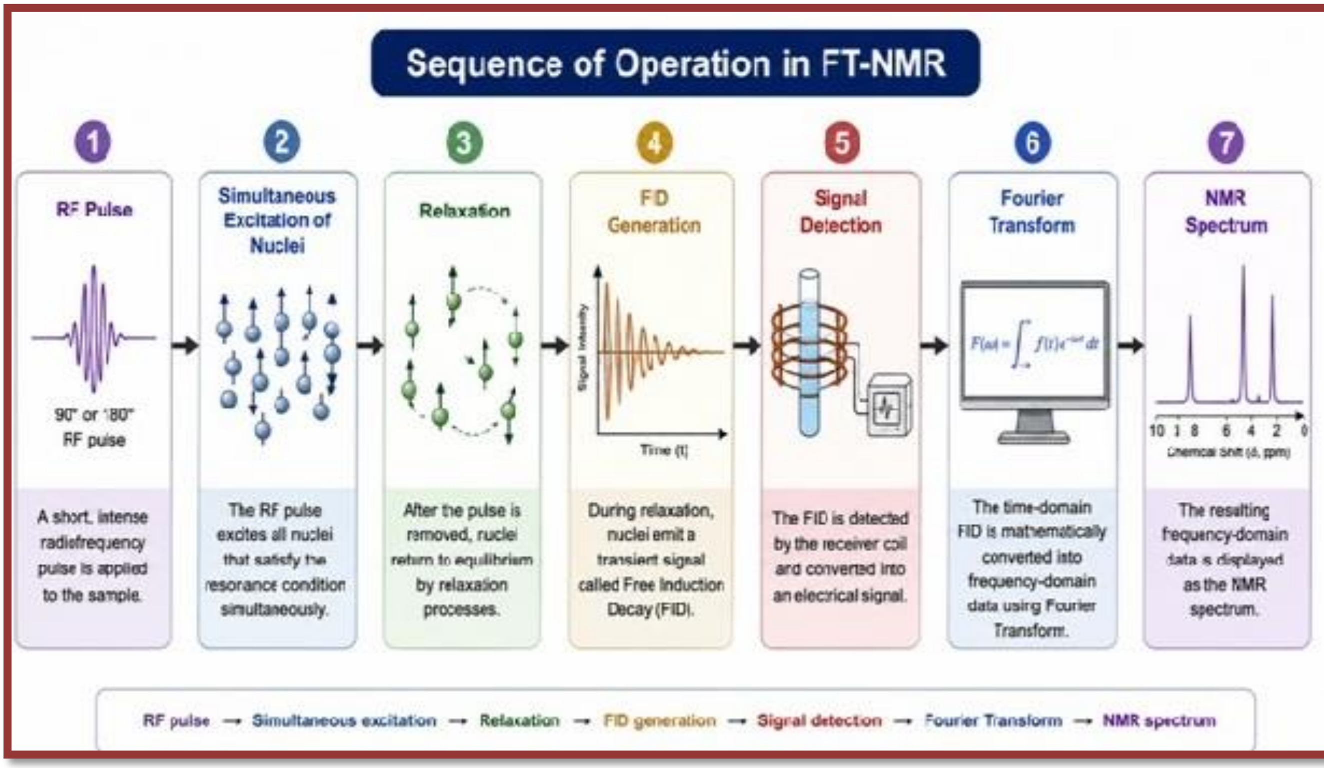


Figure 3.2: Basic sequence of operation in FT-NMR

FT-NMR spectrometers are widely used in modern spectroscopy because of several important advantages:

a) **Excellent signal-to-noise ratio:** Multiple scans can be accumulated and averaged, which improves the signal-to-noise ratio and produces clearer spectra.

b) **Detection of low-concentration samples:** The signal-averaging capability allows weak signals from samples present at low concentrations to be detected.

c) **Rapid data acquisition:** Since many frequencies are excited simultaneously, the complete spectrum can be obtained much faster than with the conventional CW method.

d) **Suitable for complex molecules:** FT-NMR is particularly useful for studying large and structurally complex organic molecules, biomolecules, polymers, and natural products.

e) **Multidimensional NMR capability:** FT-NMR provides the basis for advanced techniques such as COSY, HSQC, HMBC, NOESY, and other multidimensional NMR experiments.

3.4.3 Instrumentation for Sweep or Continuous Wave (CW) NMR Spectrometer

The major components of a Sweep or Continuous Wave NMR spectrometer are the sample holder, permanent or superconducting magnet, magnetic coils, sweep generator, radiofrequency transmitter, radiofrequency receiver, and readout/data-processing system.

The function of each component is discussed below.

a) Sample Holder

The sample holder is usually a thin-walled glass tube designed to contain the sample solution. In conventional NMR spectroscopy, a cylindrical glass tube of approximately 5 mm diameter is commonly used, with a sufficient length to place the sample properly within the magnetic field region.

The substance under investigation is generally dissolved in a suitable solvent. Deuterated solvents, such as $CDCl_3$, D_2O, or DMSO-d_6, are commonly used because they minimize interference from ordinary hydrogen-containing solvent molecules and can also provide a signal for maintaining field-frequency stability.

The sample tube is positioned in the central region of the magnet, where the magnetic field is highly homogeneous. During measurement, the sample may be rotated slowly to average out small variations in the magnetic field and improve spectral resolution.

b) Permanent Magnet / Superconducting Magnet

The magnet is one of the most important components of an NMR spectrometer because it provides the strong, stable, and highly homogeneous magnetic field required for nuclear resonance.

Modern high-resolution NMR instruments generally use superconducting magnets, which can produce very strong and stable magnetic fields. The strength of an NMR instrument is often expressed in terms of the corresponding proton resonance frequency, such as 60 MHz, 100 MHz, 300 MHz, 400 MHz, 500 MHz, 600 MHz, etc.

A stronger magnetic field generally provides better spectral dispersion and can improve the ability to distinguish between closely spaced resonance signals [2,5].

c) Magnetic Coils

Magnetic coils are used to generate, adjust, and maintain magnetic fields in the NMR instrument. They assist in producing a uniform magnetic environment around the sample.

In CW NMR instruments, the coils can be used in conjunction with the sweep system to vary the magnetic field gradually over the desired range. Additional field-correction coils, commonly called shim coils, are used to improve the homogeneity of the magnetic field. Good magnetic-field homogeneity is essential because even small variations in the field can broaden the resonance signals and reduce the resolution of the spectrum.

d) Sweep Generator

The sweep generator is an important component of a CW NMR spectrometer. It produces a controlled and gradually varying magnetic field that is superimposed on the main magnetic field.

The sweep generator changes the magnetic field systematically over a selected range while the radiofrequency is kept constant. As the magnetic field is swept, different nuclei reach their resonance conditions at different field strengths.

When the resonance condition is reached, the nuclei absorb RF energy, producing an absorption signal. The sweep generator therefore allows the spectrometer to scan different resonance positions and construct the complete NMR spectrum.

e) Radiofrequency Transmitter

The radiofrequency transmitter generates the RF radiation required to excite the nuclei. In a CW NMR spectrometer, the transmitter produces continuous radiofrequency radiation at a selected frequency.

The RF radiation is applied to the sample through an RF coil. When the energy of the applied RF radiation matches the energy difference between the nuclear spin states, resonance occurs and the nuclei absorb energy.

The frequency of the RF radiation is carefully controlled because the resonance frequency depends on the magnetic field strength and the type of nucleus being studied.

f) Radiofrequency Receiver

The radiofrequency receiver detects the weak electromagnetic signal produced by the nuclei after they absorb RF energy and subsequently return toward their equilibrium state.

The detected signal is very weak and therefore requires amplification and processing. The receiver converts the nuclear signal into an electrical signal that can be further processed by the electronic system.

The sensitivity and stability of the receiver are important for obtaining high-quality NMR spectra, particularly when analyzing samples present at low concentrations.

g) Readout and Data-Processing System

The readout system receives the electrical signal from the RF receiver and processes it to produce the final NMR spectrum.

In a conventional CW instrument, the detected signal is amplified and recorded as a function of the applied magnetic field or frequency. The resulting absorption signals are displayed as peaks in the spectrum.

In modern instruments, a computer-based data system is used for signal processing, storage, display, and analysis. The software can determine chemical shifts, peak intensities, integrations, coupling patterns, and other spectral parameters.

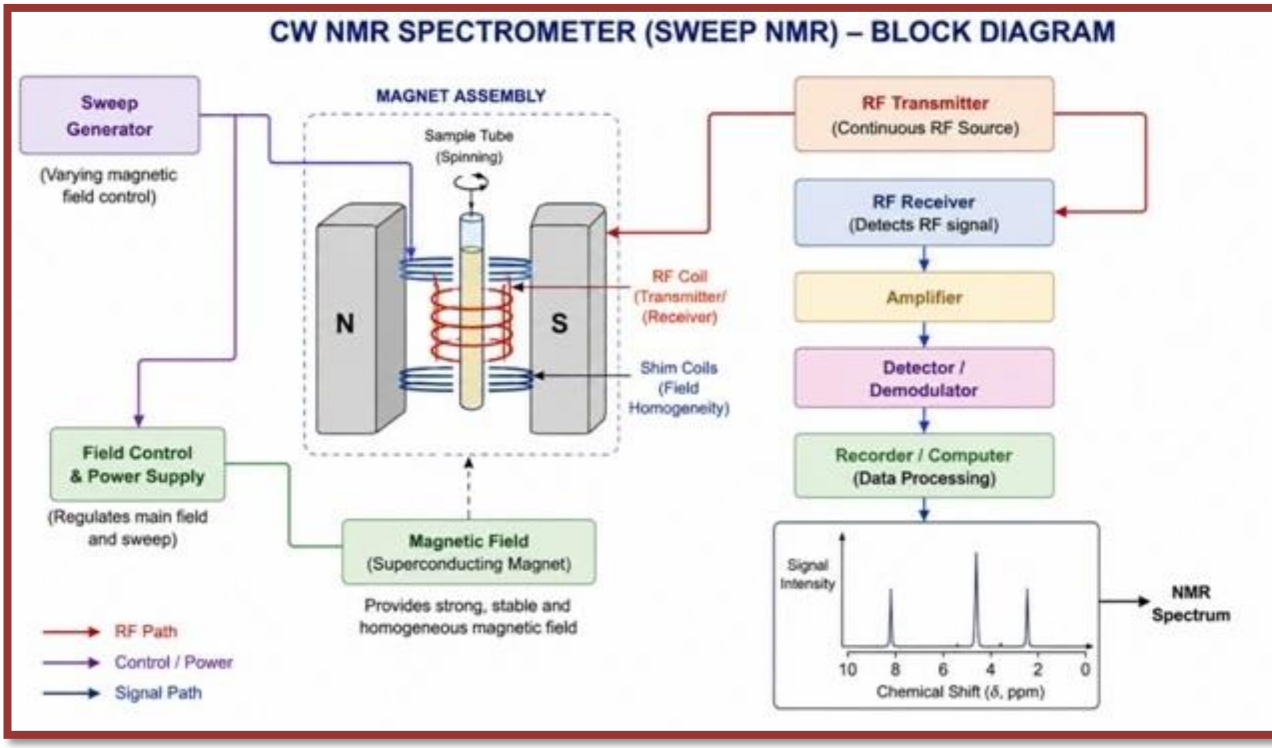


Figure 3.3: Block Diagram of a Sweep/Continuous Wave (CW) NMR Spectrometer [2,5]

3.4.4 Working of a CW NMR Spectrometer

In a CW NMR spectrometer, the sample is placed in a strong and homogeneous magnetic field. The nuclei possessing spin become oriented with respect to the magnetic field and occupy different energy states.

Continuous RF radiation is then applied to the sample. At the beginning of the experiment, the magnetic field is gradually varied by the sweep generator. When the energy of the applied RF radiation becomes equal to the energy difference between the nuclear spin states, the nuclei absorb RF energy and resonance occurs.

The absorbed energy causes transitions between the nuclear spin energy levels. As the magnetic field continues to sweep, nuclei in different chemical environments reach resonance at different positions. These resonance signals are detected by the RF receiver.

The detected signals are amplified and processed by the readout system. Finally, the signals are displayed as peaks in the NMR spectrum. The position of each signal is expressed as its chemical shift (δ), which provides information about the chemical environment of the nucleus.

Thus, the basic operation of a CW NMR spectrometer can be represented as:

Sample → Strong magnetic field → Continuous RF radiation → Magnetic-field sweep → Nuclear resonance → Signal detection → Signal processing → NMR spectrum

3.4.5 Working of an FT-NMR Spectrometer

In an FT-NMR spectrometer, the sample is placed in a strong magnetic field and is subjected to a short RF pulse. Unlike the CW method, the RF pulse contains a broad range of frequencies and excites many nuclei simultaneously.

After the RF pulse, the excited nuclei begin to return to their equilibrium state. During this relaxation process, the nuclei generate a time-dependent signal called the Free Induction Decay (FID).

The FID is detected by the receiver and sent to the computer. Since the FID contains information from many resonance frequencies simultaneously, it cannot directly be interpreted as a conventional spectrum.

The computer applies a mathematical operation known as Fourier Transform to convert the FID from the time domain into the frequency domain. The resulting frequency-domain spectrum contains the resonance signals at their respective chemical-shift positions.

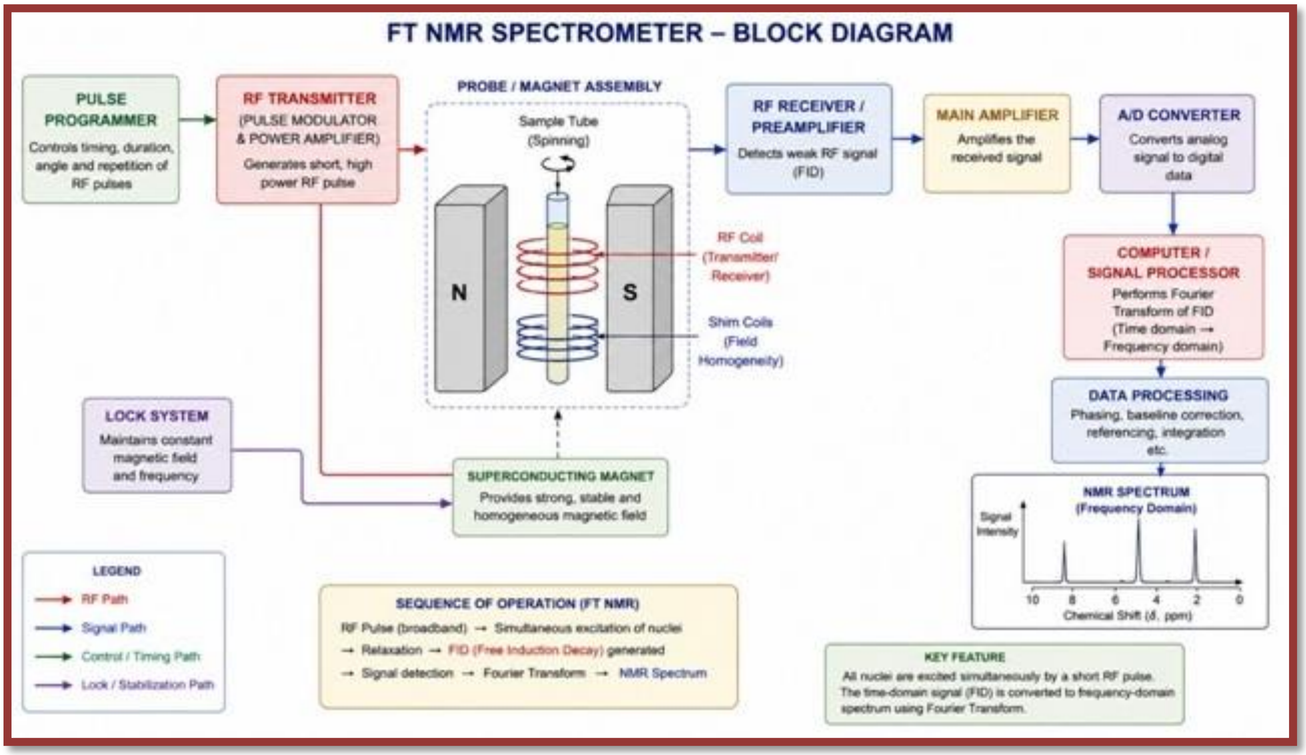


Figure 3.4: Block Diagram of a Fourier Transform (FT) NMR Spectrometer [2,5]

3.4.6 Advantages of FT-NMR over CW-NMR

a) **Faster data acquisition:** Many nuclei are excited simultaneously, so the spectrum is obtained rapidly.

b) **Improved sensitivity:** Repeated scans can be accumulated and averaged to improve the signal-to-noise ratio.

c) **Detection of weak signals:** Low-concentration samples and weak resonances can be detected more effectively.

d) **Suitable for complex molecules:** It is highly useful for large organic molecules, biomolecules, polymers, and natural products.

e) **Advanced NMR experiments:** FT-NMR forms the basis of modern one-dimensional and multidimensional NMR techniques.

f) **Digital data processing:** Spectra can be stored, processed, expanded, integrated, and analyzed using computer software.

3.5Sample Preparation in NMR: NMR sample preparation is important for obtaining a good-quality spectrum. The sample should be properly dissolved in a suitable solvent, sufficiently concentrated, pure, and free from suspended particles.

3.5.1 Solvents Used in NMR: Commonly used solvents in NMR spectroscopy include carbon tetrachloride (CCl_4), carbon disulfide (CS_2), deuteriochloroform ($CDCl_3$), hexadeuteriobenzene (C_6D_6), and deuterium oxide (D_2O)**.**

Deuterated solvents are preferred in NMR because hydrogen atoms are replaced by deuterium (^2H)**.** This minimizes interference from solvent proton signals in the ^1H NMR spectrum. The deuterium signal can also be used by the spectrometer for field-frequency stabilization.

Solvent	Formula	Important feature
Carbon tetrachloride	CCl_4	Non-polar and relatively inert solvent
Carbon disulfide	CS_2	Non-polar solvent for suitable organic compounds
Deuteriochloroform	$CDCl_3$	Most commonly used solvent for many organic compounds
Hexadeuteriobenzene	C_6D_6	Useful non-polar aromatic solvent
Deuterium oxide	D_2O	Polar solvent used for water-soluble compounds

Table 3.2: Features of Different types of Solvents Used in NMR Spectroscopy

3.5.2 Requirements of NMR Solvent: The solvent selected for NMR analysis should have the following properties:

- It should dissolve the sample completely and provide adequate concentration.

- It should be chemically inert and should not react with the sample.
- It should produce minimum interfering signals in the region of interest.
- It should be sufficiently pure and dry.
- It should have suitable viscosity and physical properties for NMR analysis.

For routine NMR analysis, the concentration required depends on the type of nucleus, sample, and sensitivity of the instrument. Higher concentrations are generally required for less sensitive nuclei such as ^{13}C.

3.5.3 Sample Purity and Concentration: The sample should beas pure as possible**,** because impurities can produce additional signals and make interpretation of the spectrum difficult.

The sample should have an appropriate concentration to obtain sufficient signal intensity. If the concentration is too low, the signals may be weak and may be difficult to distinguish from noise. Excessively high concentrations may also cause changes in viscosity, aggregation, or chemical shifts.

The sample should be:

- Pure and free from unwanted impurities.
- Completely dissolved in the selected solvent.
- Homogeneous and free from suspended particles.
- Prepared at an appropriate concentration.
- Placed in a clean, dry NMR tube, commonly a 5 mm tube for routine liquid-state NMR.
- Free from air bubbles as far as possible.

Thus, proper selection of solvent, sample purity, adequate concentration, and correct sample preparation are essential for obtaining a high-quality NMR spectrum. [2]

3.6 Proton NMR (PMR)

Proton Nuclear Magnetic Resonance (^{1}H NMR), also known as Proton Magnetic Resonance (PMR), is the most commonly used form of NMR spectroscopy. It is based on the magnetic properties of the ^{1}H nucleus (proton). Since hydrogen is present in a large number of organic compounds, ^{1}H NMR spectroscopy is one of the most important techniques for determining molecular structure. [1]

When a sample containing hydrogen nuclei is placed in a strong magnetic field, the proton nuclei occupy different spin energy states. On irradiation with radiofrequency radiation, the nuclei absorb energy when the resonance condition is satisfied. The resulting signals provide information about the chemical environment of the hydrogen atoms.

The ^1H NMR spectrum provides several important types of structural information, including:

a) **Number of signals:** Indicates the number of different types of hydrogen environments present in the molecule.

b) **Chemical shift (δ):** Gives information about the electronic and chemical environment surrounding each proton.

c) **Integration:** Indicates the relative number of protons responsible for each signal.

d) **Spin-spin splitting:** Provides information about neighboring, coupled protons.

e) **Coupling constant (J):**Gives information about the interaction between coupled nuclei.

In general, chemically different sets of protons produce signals at different chemical-shift positions. Therefore, the number of signals in a ^1H NMR spectrum can provide an initial idea about the symmetry and structural environment of a molecule.

For example, if all hydrogen atoms in a molecule are chemically equivalent, they will normally give a single resonance signal. If hydrogen atoms occupy different chemical environments, they generally produce separate signals.

3.7 Number of Signals: Equivalent and Non-equivalent protons

The number of signals observed in a ^1H NMR spectrum depends primarily on the number of chemically distinct proton environments present in the molecule.

Protons can be broadly classified as:

1) **Equivalent protons**

2) **Non-equivalent protons**

The identification of equivalent and non-equivalent protons is important because it helps in predicting the number of signals expected in the NMR spectrum.

3.7.1 Equivalent Protons: Protons are said to be equivalentwhen they occupy the same chemical environment and therefore have the same chemical shift. Equivalent protons normally give one NMR signal**.**

For example, when several hydrogen atoms are related by symmetry and experience identical electronic environments, they are chemically equivalent and contribute to the same resonance signal.

Characteristics of Equivalent Protons

Equivalent protons generally:

- Experience the same chemical environment.

- Have the same chemical shift (δ).
- Produce one signal in the NMR spectrum.
- Have identical magnetic properties with respect to the relevant coupled nuclei.
- Do not normally split one another because they have the same resonance frequency.

The equivalence of protons can be considered in two important ways: chemicalequivalenceandmagnetic equivalence. [1,2]

a) Chemically Equivalent Protons

Protons are chemically equivalent when they are in identical chemical environments and have the same chemical shift.

They are often related by a symmetry operation within the molecule. Such protons are indistinguishable by ^1H NMR spectroscopy and therefore appear at the same chemical-shift position.

Example 1: Methane (CH_4) : In methane, all four hydrogen atoms are chemically equivalent because of the high symmetry of the molecule. They experience the same chemical environment and therefore give one ^1H NMR signal.

CH_4 → One type of proton → One signal

Example 2: Methyl group ($–CH_3$): The three hydrogen atoms of a simple methyl group are generally chemically equivalent because rapid rotation about the C–H/C–C bonds and molecular symmetry make them experience essentially the same environment. Therefore, they normally contribute to **one signal**.

$–CH_3$ → Three equivalent protons → One signal

b) Magnetically Equivalent Protons

Magnetic equivalence is a more restrictive condition than chemical equivalence. Two nuclei are magnetically equivalent when:

- They have the same chemical shift, and
- They have identical coupling constants (J) with every other relevant nucleus in the spin system.

Thus, magnetically equivalent protons not only experience the same chemical environment but also have identical magnetic interactions with other coupled nuclei.

Magnetic equivalence is important when interpreting complex splitting patterns because chemically equivalent protons are not necessarily magnetically equivalent in every molecule.

All magnetically equivalent protons are chemically equivalent, but chemically equivalent protons are not necessarily magnetically equivalent.

Therefore, magnetic equivalence must be considered when predicting the detailed splitting pattern of an NMR spectrum.

Examples of Equivalent Protons

Methane:CH_4 → all 4 H are equivalent → **1 signal**

Methyl group:CH_3–R → all 3 H are generally equivalent → **1 signal**

Symmetrical molecules: When a molecule contains a plane or other symmetry element that makes two or more protons indistinguishable, those protons may produce the same NMR signal.

3.7.2 Non-equivalent Protons

Protons are called non-equivalent when they experience different chemical environments and therefore have different chemical shifts. Non-equivalent protons generally produce separate signals in the ^{1}H NMR spectrum.

For example, in a molecule containing a methyl group, a methylene group, and a hydroxyl group, these protons experience different chemical environments. Consequently, they generally appear at different chemical-shift positions[1,2].

Non-equivalent protons can also interact with one another through spin-spin coupling, causing splitting of their NMR signals.

Characteristics of Non-equivalent Protons

Non-equivalent protons generally:

- Experience different chemical environments.
- Have different or distinguishable chemical shifts.
- Produce separate resonance signals.
- May undergo spin-spin coupling with neighboring non-equivalent protons.
- Can provide information about the connectivity and neighboring groups in a molecule.

There are two important forms of non-equivalence:

a) **Chemical Non-equivalence:** Protons are chemically non-equivalent when they experience different chemical environments and have different chemical shifts.

For example, in ethanol: **CH_3–CH_2–OH**, the three different proton groups are:

CH_3 protons: three equivalent protons within the methyl group.

CH_2 protons: two protons belonging to the methylene group.

OH proton: one proton attached to oxygen.

These groups experience different chemical environments and therefore generally produce different signals.Thus, ethanol normally gives three major proton environments: **CH_3 → CH_2 → OH**. The corresponding signals occur at different chemical-shift values.

b) **Magnetic Non-equivalence:** Magnetic non-equivalence occurs when nuclei may have thesame or very similar chemical shifts but do not have identical coupling relationships with other nucleiin the spin system.In other words, chemical equivalence is not sufficient for magnetic equivalence. For nuclei to be magnetically equivalent, they must have the same chemical shift and identical coupling constants with all relevant neighboring nuclei.Magnetic non-equivalence is particularly important in molecules with complex structures, especially when symmetry is incomplete or when protons are coupled differently to other nuclei. It can result in complex multiplets and may make the spectrum more difficult to interpret.

Example: Proton Signals in Ethanol: Ethanol has the molecular structure:

CH_3–CH_2–OH

It contains three different types of protons:

A. Methyl protons (CH_3): The three protons attached to the methyl carbon are generally equivalent to one another. Therefore, they give one signal**.**

Number of protons = 3

B. Methylene protons (CH_2): The two protons attached to the methylene carbon form another proton environment and generally give one signal in the ordinary ^1H NMR spectrum of ethanol.

Number of protons = 2

C. Hydroxyl proton (OH): The hydroxyl proton forms a third proton environment and generally gives one signal**.**

Number of protons = 1

Therefore, ethanol generally shows three proton signals corresponding to:

CH_3 (3H) → CH_2 (2H) → OH (1H)

The exact position and appearance of the OH signal can vary because the proton can undergo exchange, particularly in the presence of water, acids, bases, or other exchangeable protons. Consequently, OH signals may sometimes appear broad and may not show the expected coupling with neighboring protons.

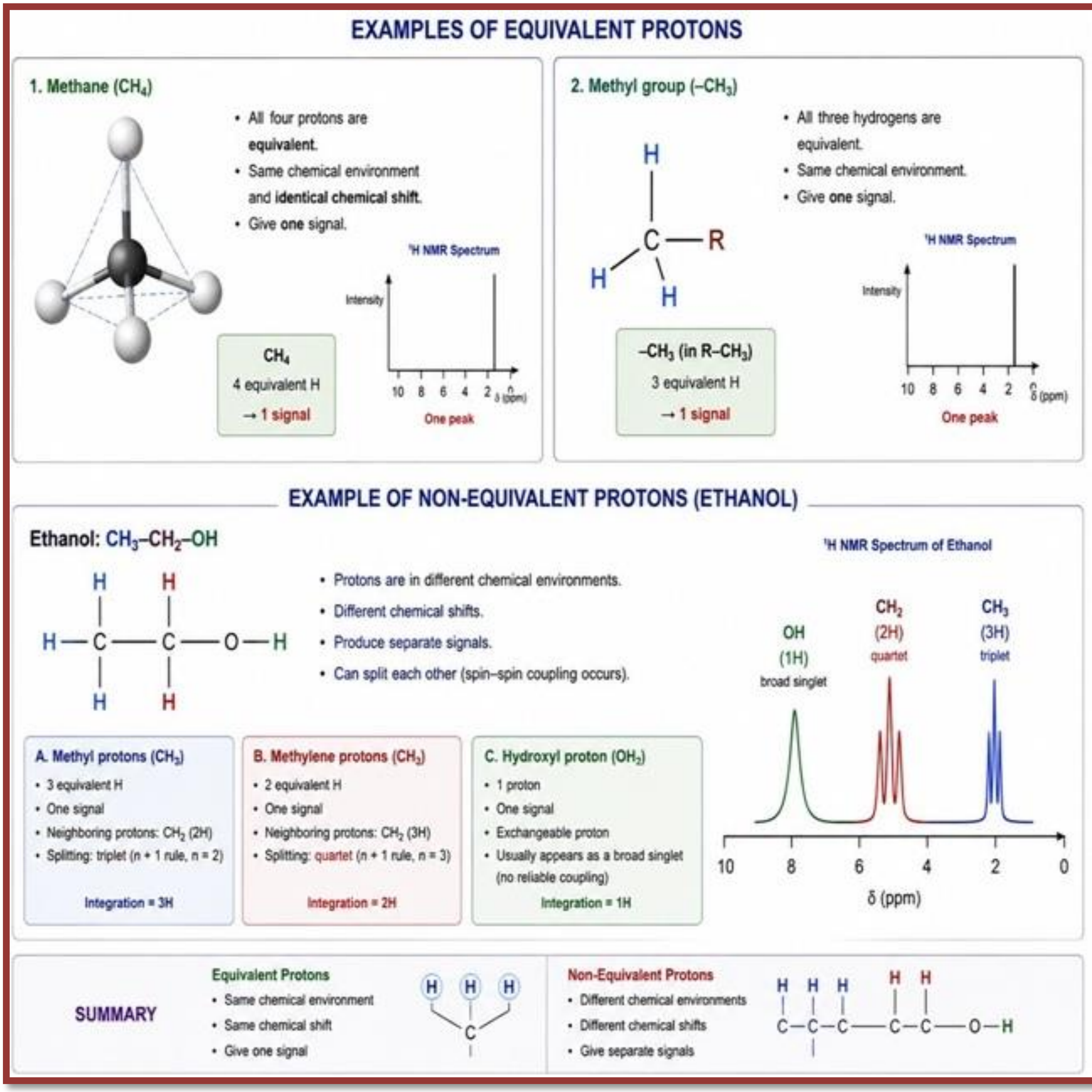


Figure 3.4: Equivalent and Non-Equivalent Protons

3.7.3 Relationship Between Proton Equivalence and Number of Signals

The number of ^1H NMR signals is determined by the number of chemically distinct proton environments rather than simply by the total number of hydrogen atoms.

For example:

Compound	Different proton environments	Expected number of ^{1}H NMR signals
CH_4	1	1
$CH_3–CH_3$	1	1
$CH_3–CH_2–OH$	3	3
$CH_3–CH_2–CH_3$	2	2
Benzene (C_6H_6)	1	1

Table 3.3: Examples showing Relationship Between Proton Equivalence and Number of Signals

Thus, symmetry reduces the number of signals, while the presence of different chemical environments increases the number of signals.

3.7.4 Importance of Determining the Number of Signals

Determining the number of proton signals is one of the first steps in interpreting a ^{1}H NMR spectrum. It helps in:

- Identifying different proton environments in the molecule.
- Recognizing molecular symmetry.
- Estimating the number of different structural groups present.
- Distinguishing between possible structural isomers.
- Providing a starting point for interpreting chemical shifts, integration, and splitting patterns.

Therefore, the number of signals, together with chemical shift, integration, and spin-spin splitting, provides valuable information for determining the structure of an unknown organic compound.

3.8 Position of Signals and Chemical Shift

In Proton Magnetic Resonance (PMR) or ^{1}H NMR spectroscopy, the position of an absorption signal depends on the chemical environment of the proton. When a molecule is placed in a strong external magnetic field, the electrons surrounding the nuclei circulate in response to the applied magnetic field. This circulation produces a secondary or induced magnetic field.

The induced magnetic field may either oppose or reinforce the applied magnetic field at the position of a proton. As a result, protons in different chemical environments experience different effective magnetic fields and therefore absorb radiofrequency radiation at different positions in the NMR spectrum.

The two important effects are shieldingand deshielding**.**

3.8.1 Shielding: When the induced magnetic field opposes the applied magnetic field, the effective magnetic field experienced by the proton decreases. The proton is said to be shielded. A shielded proton requires a higher applied magnetic field to reach resonance at a fixed radiofrequency. Therefore, it appears toward the upfield region of the NMR spectrum, corresponding to **a** lower chemical-shift value**.**

3.8.2 Deshielding: When the induced magnetic field reinforces the applied magnetic field, the effective magnetic field experienced by the proton increases. The proton is said to be deshielded. A deshielded proton requires a lower applied magnetic field to reach resonance at a fixed radiofrequency. Therefore, it appears toward the downfield region of the spectrum, corresponding to a higher chemical-shift value.

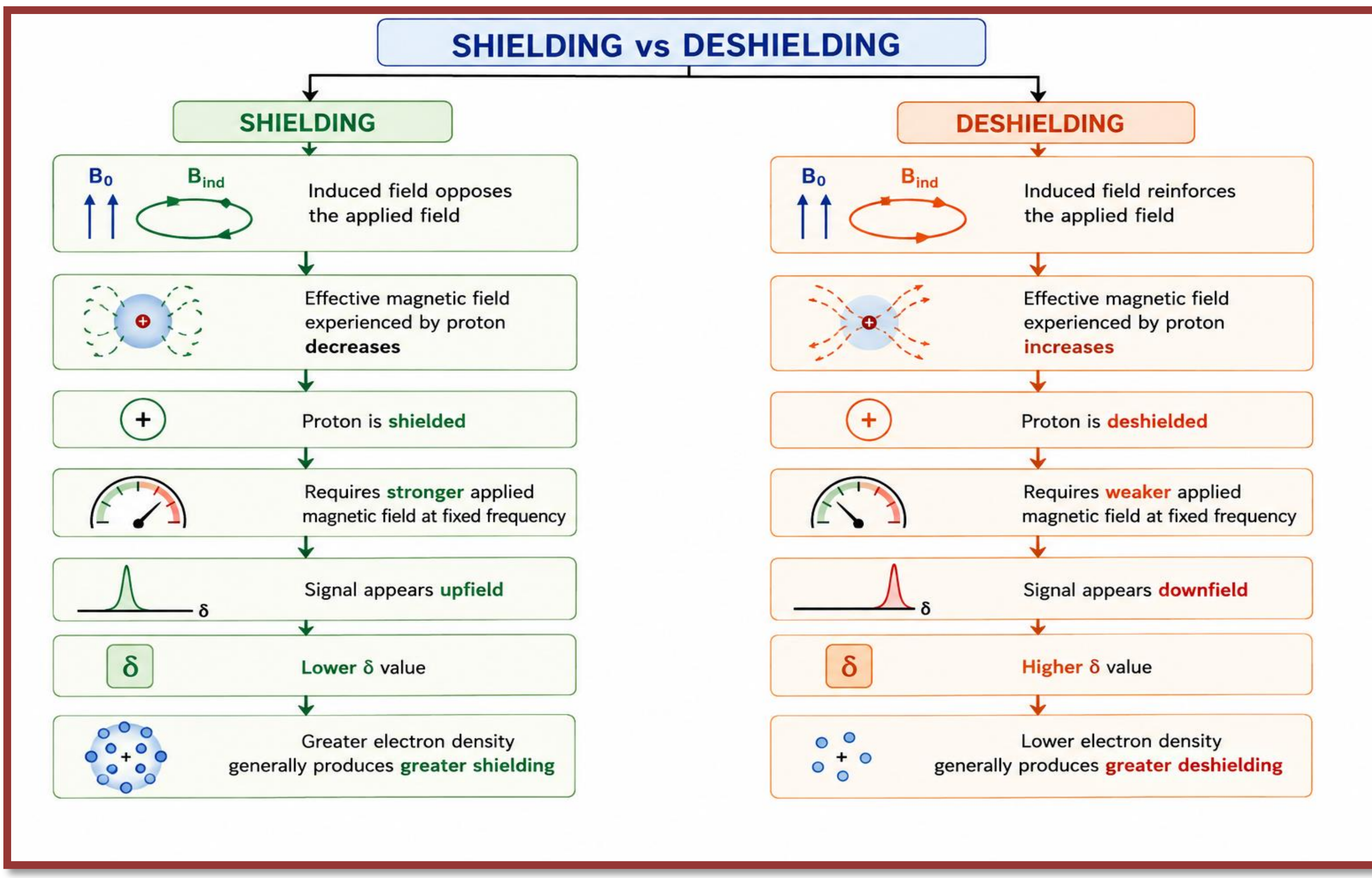


Figure 3.5: Shielding and Deshielding of Protons. [2,3]

3.8.3 Reference Standard: Tetramethylsilane (TMS)

The field strengths or resonance frequencies at which different types of protons absorb are measured relative to a standard reference signal. The most commonly used reference compound in ¹H NMR spectroscopy is tetramethylsilane (TMS), $(CH_3)_4Si$.The position of the absorption signal of TMS is assigned as 0.00 ppm on the δ (delta) scale. The difference between the resonance position of a sample proton and that of TMS is called the chemical shift**.** Thus:

Chemical shift = Difference in resonance position of sample proton relative to TMS

Chemical shift is one of the most important parameters in ¹H NMR spectroscopy because it provides information about the electronic environment surrounding a proton. [1–3]

3.8.4 Factors Influencing the Choice of TMS as Reference Standard

Tetramethylsilane (TMS) is widely used as the reference standard in ¹H NMR spectroscopy because it possesses several desirable properties.

a) Twelve Equivalent Protons: TMS contains four identical methyl groups. All twelve hydrogen atoms are chemically equivalent. Therefore, they produce asingle, sharp and intense NMR signal**.**

b) Highly Shielded Protons: The electronegativity of silicon is lower than that of carbon. Consequently, the methyl protons of TMS are highly shielded relative to most protons in ordinary organic compounds. Therefore, the TMS signal appears at an extreme upfield position and can conveniently be assigned the value of0.00 ppm**.**Most organic proton signals consequently occur downfield from TMS.

c) Chemical Inertness: TMS is relatively chemically inert and does not readily react with most organic compounds used for routine NMR analysis. It therefore generally does not interfere with the sample.

d) Volatility: TMS has a relativelylow boiling point (approximately 27 °C; about 300 K)**.** Therefore, it can readily be removed from the sample by evaporation after the spectrum has been recorded.

e) Zero Reference on the δ-Scale: The resonance signal of TMS is assigned the value:**δ = 0.00 ppm.** It therefore provides a convenient reference point for reporting the chemical shifts of other protons.

f) Strong and Sharp Signal: Because TMS contains twelve equivalent protons, it produces a strong signal even when used in a relatively small amount. Only a small concentration of TMS is generally required for routine NMR measurements.

When D_2O is used as the solvent, an alternative water-soluble reference compound such as sodium 3-(trimethylsilyl)propionate (TSP)may be used. [1,2]

3.9 Expressing the Chemical Shift

Chemical shift can initially be expressed as a difference in resonance frequency between the sample signal and the reference signal. The resonance frequency of a nucleus is related to the applied magnetic field according to:

$$\nu = \gamma B_0 / 2\pi$$[1]

where:

ν = resonance frequency,

γ = gyromagnetic ratio of the nucleus,

B_0 = applied magnetic field strength.

The difference between the resonance frequency of a sample proton and the reference compound is represented as $\Delta\nu$, usually expressed in hertz (Hz).

Therefore:

Chemical shift ∝ frequency difference between sample and reference

However, reporting chemical shifts simply in Hz is inconvenient because the numerical value depends on the magnetic-field strength and operating frequency of the NMR spectrometer.

Chemical Shift in Relation to Applied Field and Radiofrequency: The resonance frequency of a proton increases with increasing magnetic-field strength. Therefore, the frequency difference between two proton signals also depends on the operating frequency of the NMR spectrometer.For example, the same proton may show a frequency difference of several hundred hertz on one instrument and a different number of hertz on another instrument operating at a different frequency.

Thus:

Chemical shift in Hz ∝ strength of applied magnetic field ∝ operating radiofrequency

To make chemical-shift values independent of the spectrometer frequency, the shift is expressed in parts per million (ppm).

The conversion is:

$$\delta \text{ (ppm)} = [\text{Observed shift (Hz)} \times 10^6] / [\text{Operating frequency (Hz)}]$$

The use of ppm allows chemical shifts obtained from different NMR instruments to be directly compared.

3.9.1 Scales Used to Express Chemical Shift:

Two scales have historically been used for expressing chemical shifts:

1) **δ (delta) scale**
2) **τ (tau) scale**

δ (delta) scale: δ=Observed shift (in Hz)×10^6/Radiofrequency used in spectrometer (in Hz)

Important features of the δ scale are:

a) The TMS signal is assigned 0.00 ppm.

b) Most ^1H NMR chemical shifts occur approximately between 0 and 10 ppm.

c) A small δ value indicates a more upfield or more shielded proton.

d) A large δ value indicates a more downfield or more deshielded proton.

e) The δ scale increases in the downfield direction.

Thus: **Upfield ← 0 ppm → increasing δ → Downfield**

τ (tau) scale: The τ scale was used in earlier NMR literature and is related to the δ scale by: $\tau = 10 - \delta$

On the τ scale:

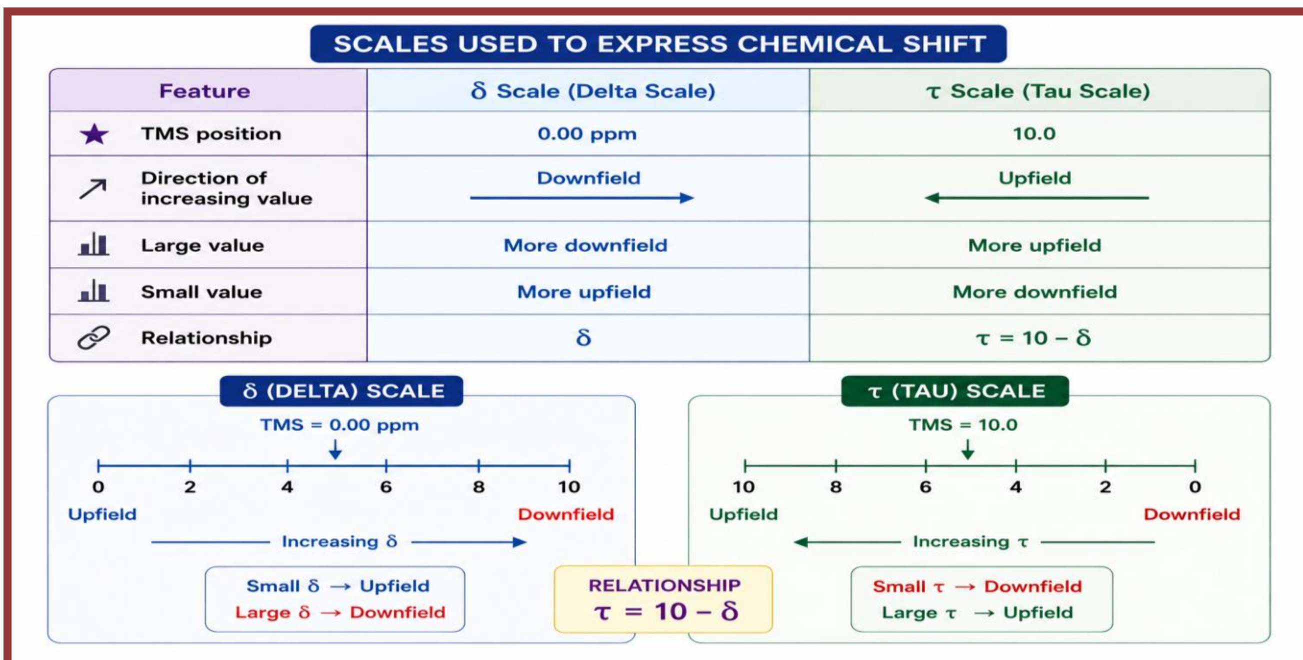
a) TMS is assigned a value of 10.0.

b) Most proton chemical shifts occur approximately between 10 and 0.

c) A small τ value corresponds to a downfield/low-field signal.

d) A large τ value corresponds to an upfield/high-field signal.

Therefore, the direction of the τ scale is opposite to that of the δ scale.


SCALES USED TO EXPRESS CHEMICAL SHIFT

Feature	δ Scale (Delta Scale)	τ Scale (Tau Scale)
TMS position	0.00 ppm	10.0
Direction of increasing value	Downfield →	← Upfield
Large value	More downfield	More upfield
Small value	More upfield	More downfield
Relationship	δ	τ = 10 − δ

Figure 3.6: Scales used to express Chemical Shift

The δ scale is now universally preferred for reporting chemical shifts.

3.9.2Factors Affecting Chemical Shift of Protons:

1) Inductive Effect: The inductive effect is one of the important factors affecting the chemical shift of a proton. Electronegative atoms or groups attached near a proton can withdraw electron density through sigma (σ) bonds.Atoms such as fluorine, chlorine, oxygen, and nitrogen are more electronegative than carbon and hydrogen. When such atoms are present near a proton, they withdraw electron density and reduce the electron density around the proton.

Reduced electron density results in:

Less electron density → less shielding → greater deshielding → higher δ value → downfield shift

The magnitude of the effect generally depends on:

- Electronegativity of the attached atom.
- Distance between the electronegative atom and the proton.
- Number of electronegative atoms present.
- Nature of the surrounding molecular structure.

2)Effect of Halogen Substitution: The effect can be illustrated by the following approximate chemical shifts

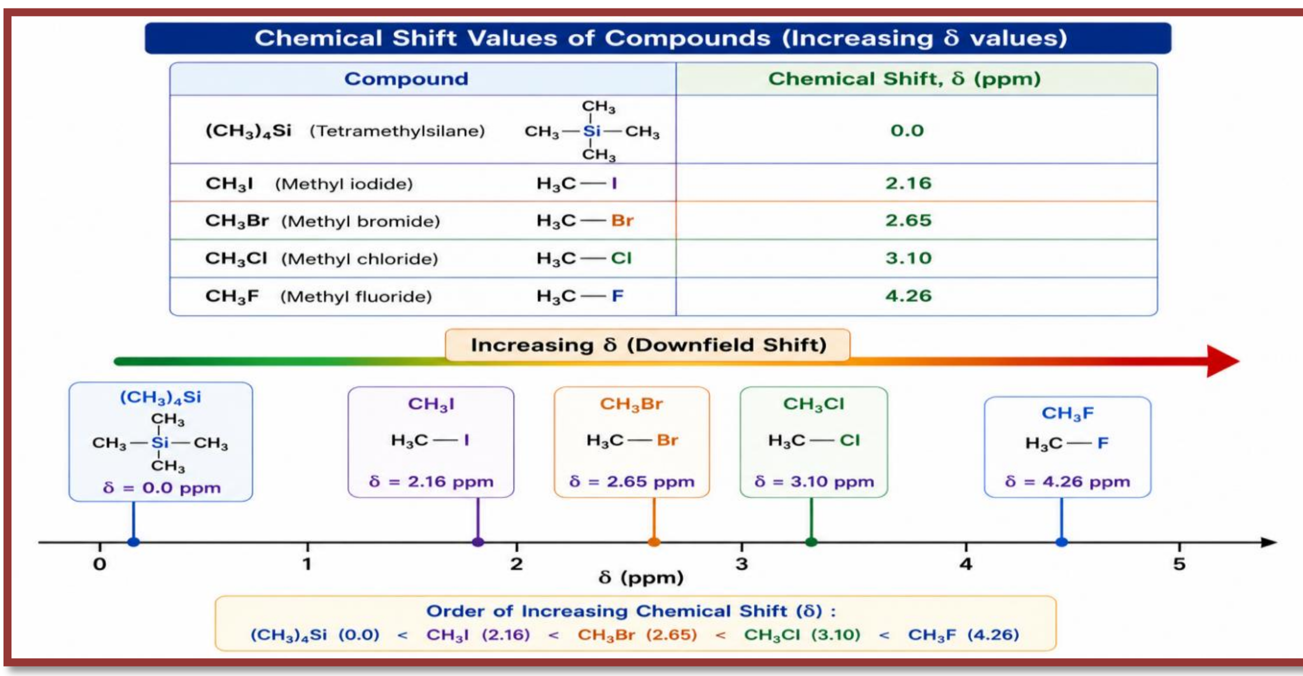


Figure 3.7: Chemical shift values of compounds (increasing δ values) [1,5]

The progressive increase in chemical shift reflects the increasing electron-withdrawing influence of the halogen, with fluorine producing the strongest inductive effect among these examples.

Greater electron withdrawal → lower electron density → greater deshielding → higher δ

Conversely, greater electron density around a proton generally results in greater shielding and a lower chemical-shift value. [1,5]

3) **Anisotropic effect:** The **anisotropic effect** is an important factor responsible for characteristic chemical shifts in compounds containing π-electron systems, such as alkenes, aromatic compounds, and alkynes.

When a molecule containing π electrons is placed in an external magnetic field, the π electrons circulate and generate a **local induced magnetic field**. This induced field is not uniform in all directions. Therefore, depending on the position of the proton relative to the π system, the proton may experience either additional shielding or additional deshielding.

This directional dependence of the induced magnetic field is called **magnetic anisotropy**. [2]

Examples

Aromatic rings: Aromatic protons are strongly deshielded and generally appear around **6–8.5 ppm**.

Alkenes: Vinylic protons generally appear around **4.5–6.5 ppm**.

Alkynes: Terminal alkyne protons generally appear around **2–3 ppm**.

The anisotropic effect explains why the chemical shift cannot be predicted solely from the electronegativity of the atoms attached to a proton.

4) **Hydrogen Bonding**: Hydrogen bonding can significantly influence the chemical shifts of **exchangeable protons**, particularly **–OH and –NH protons**. Hydrogen bonding generally decreases electron density around the proton and causes **deshielding**, resulting in a shift toward the downfield region.

The position of an –OH or –NH signal is therefore not always fixed and can depend on factors such as concentration, temperature, solvent, and the presence of other hydrogen-bonding groups.

a) Effect of Concentration

At **low concentration**, intermolecular hydrogen bonding is generally reduced. Consequently, the proton experiences less deshielding and may appear relatively upfield.

At **higher concentration**, intermolecular hydrogen bonding may become more significant, resulting in greater deshielding and a downfield shift.

b) Effect of Temperature

Increasing temperature generally weakens or reduces intermolecular hydrogen bonding. As a result, the extent of deshielding may decrease and the exchangeable proton signal may shift upfield.

c) Intramolecular Hydrogen Bonding

In intramolecular hydrogen bonding, the hydrogen bond is formed within the same molecule. Such hydrogen bonding is less dependent on concentration because the donor and acceptor groups are held within the same molecule.

Therefore, dilution generally has a much smaller effect on an intramolecularly hydrogen-bonded proton than on an intermolecularly hydrogen-bonded proton.

Examples of Downfield Hydrogen-Bonded Protons

Some characteristic examples include:

Salicylate/phenolic OH: approximately **δ 10–12 ppm**

Enol of β-diketone: approximately **δ 11–16 ppm**

Carboxylic acid: approximately **δ 10–13 ppm**, often appearing as a broad signal due to hydrogen bonding and proton exchange.

Therefore:

Hydrogen bonding → decreased electron density → deshielding → downfield shift [5]

5) Hybridization:

The hybridization of the carbon atom attached to hydrogen has a significant influence on the chemical environment and chemical shift of the proton.The three common types of carbon hybridization encountered in organic compounds are: sp^3, sp^2, sp

The percentage of s-character is approximately:

sp^3 = 25% s-character

sp^2 = 33% s-character

sp = 50% s-character

Greater s-character makes the carbon atom relatively more electronegative and can influence the distribution of electron density around the attached proton. However, the observed chemical shift is also strongly affected by **magnetic anisotropy**, especially in π-bonded systems.

a) Protons Attached to sp^3-Hybridized Carbon:

In saturated compounds such as alkanes, the carbon atoms are sp^3 hybridized.

Example: $CH_3–CH_3$

The C–H bonds are relatively rich in electron density, and the protons are generally more shielded than protons attached to sp^2-hybridized carbons.Typical chemical shifts for simple alkyl protons are approximately:**δ = 0.5–2.0 ppm**. Therefore, alkyl protons generally appear in the upfield region.

b) Protons Attached to sp-Hybridized Carbon

In alkynes, the carbon atoms are sp hybridized and have approximately 50% s-character.

Example: HC≡CH

Because of the high s-character, the carbon atom has greater effective electronegativity. On the basis of electronegativity alone, one might expect the proton to be strongly deshielded.However, the observed chemical shift of an alkyne proton is relatively moderate because of the anisotropic effect of the C≡C triple bond. Circulation of π electrons around the triple bond produces a local magnetic field that provides shielding in the region where the terminal alkyne proton is located.Consequently, terminal alkyne protons generally appear at approximately:δ = 2–3 ppm.

This illustrates that chemical shift is determined by a combination of electronic effects and magnetic anisotropy, rather than hybridization alone.

c) Protons Attached to sp^2-Hybridized Carbon

Protons attached to sp^2-hybridized carbon are commonly found in alkenes and aromatic compounds**.**

Alkenes

In alkenes, such as ethene:$CH_2=CH_2$**.** The carbon atoms are sp^2 hybridized. The protons are affected by both the increased s-character of the carbon and the anisotropic magnetic field generated by the C=C π system.Vinylic protons generally appear at approximately:δ = 4.5–6.5 ppm

Aromatic Compounds

In benzene, the six carbon atoms are sp^2 hybridized and form a delocalized π-electron system.Example: C_6H_6. The circulation of aromatic π electrons produces a strong anisotropic magnetic field that significantly deshields the aromatic protons. Consequently, aromatic protons generally appear at approximately:δ = 6–8.5 ppm

Type of proton	Example	Approximate δ (ppm)	General position
sp^3 C–H	CH_3–CH_3	0.5–2.0	Upfield
sp C–H	HC≡CH	2–3	Moderate
sp^2 C–H, alkene	CH_2=CH_2	4.5–6.5	Downfield
sp^2 C–H, aromatic	C_6H_6	6–8.5	Strongly downfield

Table 3.4: Approximate Chemical-Shift Ranges Based on Hybridization

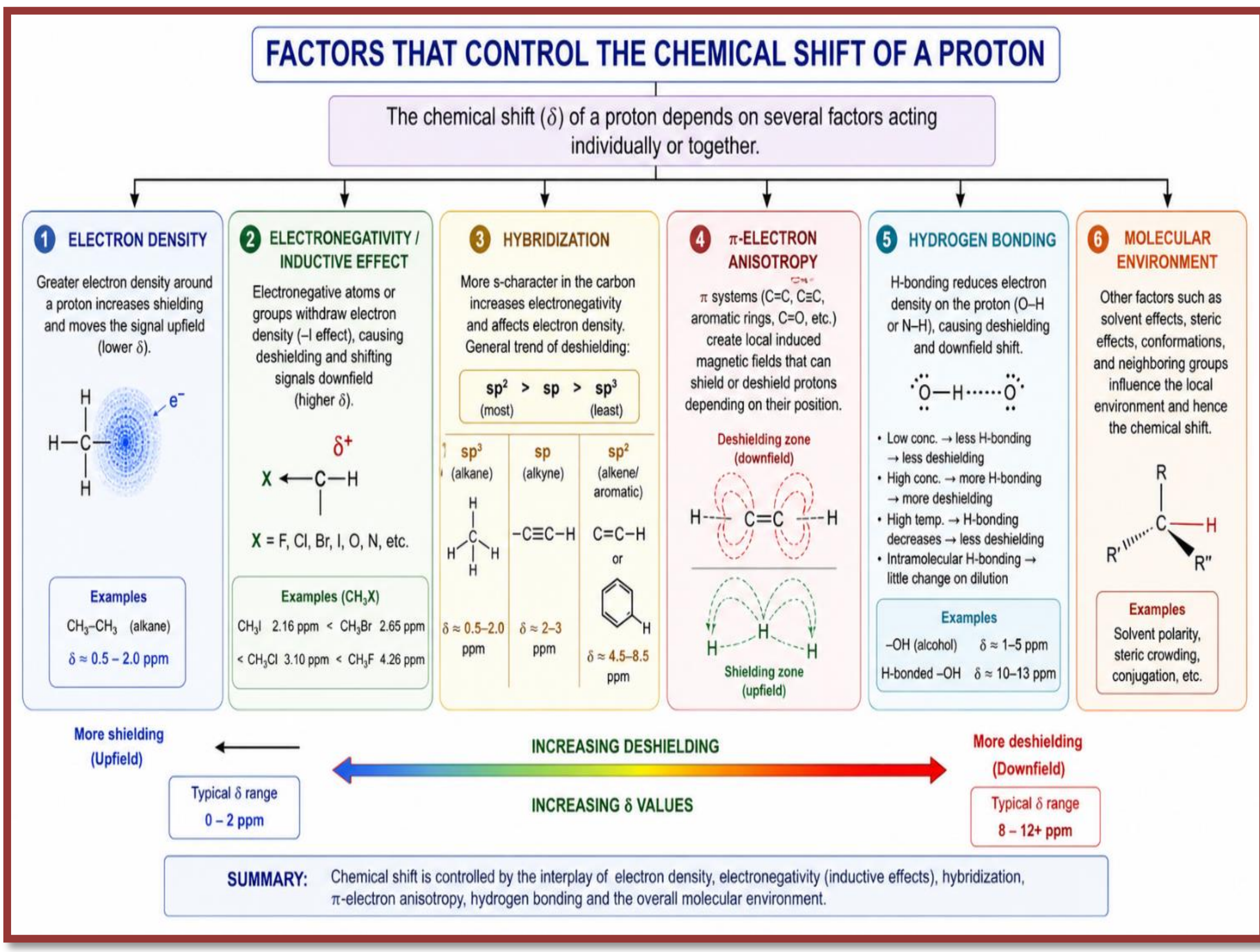


Figure 3.8: Factors Controlling the Chemical Shift of Protons

3.10 Spin–Spin Coupling / Spin–Spin Splitting

In a ^1H NMR spectrum, chemically equivalent protons generally give the same resonance signal. However, a signal produced by a group of equivalent protons may not always appear as a single line. Instead, it may be divided into two or more closely spaced lines. This phenomenon is known as splitting of the NMR signal or spin–spin splitting.

Consider the molecule 1,1,2-tribromoethane ($CHBr_2$–CH_2Br). It contains two different types of protons, represented as H^a and H^b. Therefore, two different proton environments are present and two absorption signals are expected. However, each of these signals is further divided into a group of lines. The H^a signal appears as a triplet, while the H^b signal appears as a doublet. Thus, a total of five lines are observed in the spectrum, as shown in Figure 3.9.

This observation demonstrates that a proton signal may appear as a multiplet rather than a single peak because of interaction with neighboring, non-equivalent protons. [1,2]

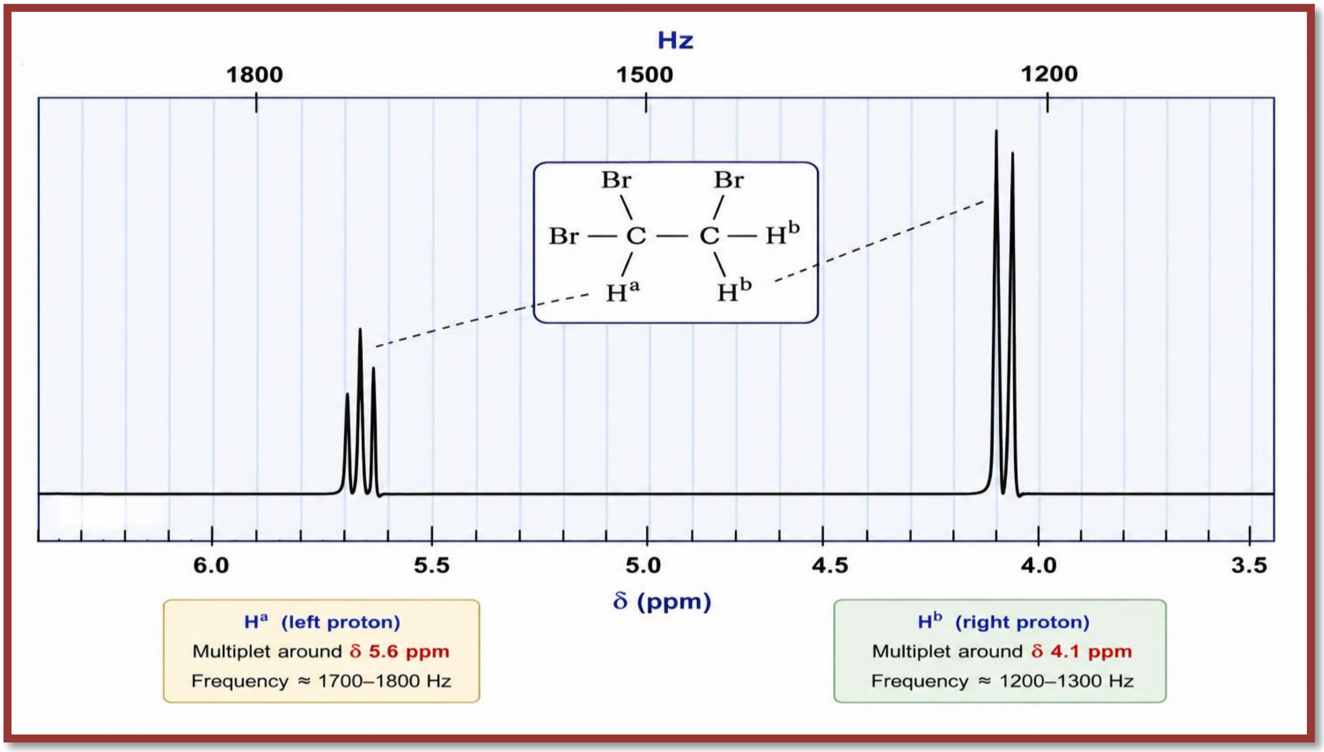


Figure 3.9: PMR spectra of 1,1,2-tribromoethane [1]

Explanation of Splitting

The splitting of a proton signal occurs because of the magnetic interaction between a proton and neighboring non-equivalent protons**.** This interaction is calledspin–spin coupling.A proton can exist in two possible spin orientations with respect to the external magnetic field. The neighboring protons can therefore produce different local magnetic environments at the proton being observed. As a result, the resonance signal is split into several closely spaced lines.For a simple first-order spectrum, the number of lines produced by a group of equivalent protons is commonly predicted by the **(n + 1) rule**:

Number of lines = n + 1

where:n = number of equivalent neighboring protons

Example: 1,1,2-Tribromoethane$CHBr_2$–CH_2Br

Let:H^a = proton attached to $CHBr_2$andH^b = two protons attached to CH_2Br

a) Splitting of H^b Protons

The H^b protons are adjacent to the $CHBr_2$ proton (H^a). Therefore, they are coupled to one neighboring proton**.**

Here:n = 1, According to the (n + 1) rule:

Number of lines = 1 + 1 = 2

Therefore, the H^b signal appears as a doublet**.**

H^b → 1 neighboring proton → (1 + 1) = 2 → Doublet

The relative intensities of a simple doublet are:1 : 1

b) Splitting of H^a Proton

The H^a proton is adjacent to two equivalent H^b protons. Therefore, it is coupled to two neighboring protons**.**

Here:n = 2, According to the (n + 1) rule:

Number of lines = 2 + 1 = 3

Therefore, the H^a signal appears as a triplet.

H^a → 2 neighboring protons → (2 + 1) = 3 → Triplet

The relative intensities of a simple triplet are:1 : 2 : 1

Thus, the spectrum of 1,1,2-tribromoethane contains:

H^a → Triplet → 3 linesH^b → Doublet → 2 lines

Total = 3 + 2 = 5 lines

3.10.1Spin–Spin Coupling

The splitting of NMR signals arises from the interaction between the magnetic moments of neighboring nuclei. This interaction is called spin–spin couplingorJ-coupling.When two non-equivalent protons are close enough to interact through chemical bonds, the spin state of one proton influences the magnetic environment experienced by the other proton. Consequently, the resonance signal of one proton is divided into multiple lines. Spin–spin coupling is transmitted through the bonding electrons and is therefore also referred to as through-bond coupling.

For example, in: $CHBr_2$–CH_2Br

the H^a proton interacts with the two H^b protons, and the H^b protons interact with H^a. This mutual interaction produces a triplet and a doublet, respectively.

Common Splitting Patterns:

The number and relative intensities of lines in a simple first-order multiplet can be predicted using the **Pascal's triangle** relationship.

Number of neighboring protons (n)	Number of lines (n + 1)	Multiplicity	Relative intensities
0	1	Singlet	1
1	2	Doublet	1 : 1
2	3	Triplet	1 : 2 : 1
3	4	Quartet	1 : 3 : 3 : 1
4	5	Quintet	1 : 4 : 6 : 4 : 1
5	6	Sextet	1 : 5 : 10 : 10 : 5 : 1
6	7	Septet	1 : 6 : 15 : 20 : 15 : 6 : 1

Table 3.5: Pascal’s Triangle to determine relative intensities of splitted peaks [1,2]

Singlet: When a proton has no coupling to neighboring non-equivalent protons, its resonance signal appears as a single line called a singlet.

For: **n = 0**, According to the (n + 1) rule:0 + 1 = 1 line

Therefore: Singlet → 1 line**,** a singlet has no splitting caused by neighboring equivalent protons.

Doublet: When a proton or group of equivalent protons is coupled to one equivalent neighboring proton, the signal is divided into two lines.

For: n = 1, n + 1 = 1 + 1 = 2

Therefore, the signal is a doublet. The two lines generally have equal intensity:1 : 1

Triplet: When a proton or group of equivalent protons is coupled to two equivalent neighboring protons, the signal is divided into three lines.

For: n = 2, n + 1 = 2 + 1 = 3, Therefore, the signal is a triplet.

The relative intensity of the three lines is:1 : 2 : 1, the middle line is therefore approximately twice as intense as either outer line.

Quartet: When a proton or group of equivalent protons is coupled to three equivalent neighboring protons**,** four lines are observed.

For: n = 3, n + 1 = 3 + 1 = 4, Therefore, the signal is a quartet**.**

The relative intensities are:1 : 3 : 3 : 1

A common example is the CH_2 group of an ethyl group: CH_3–CH_2–

The CH_2 protons are coupled to three neighboring CH_3 protons and therefore commonly appear as a quartet, while the CH_3 protons are coupled to two CH_2 protons and commonly appear as a triplet.

3.10.2 Rules for Splitting of Proton Signals

The following general rules are useful for understanding proton signal splitting:

a) Splitting is caused by spin–spin coupling

Splitting occurs because of magnetic interaction between coupled, non-equivalent nuclei.

b) Non-equivalent protons generally cause splitting

Protons in different chemical environments can interact with one another and cause splitting when they are sufficiently coupled.

c) Equivalent protons do not normally split one another

Protons that are chemically and magnetically equivalent have the same resonance frequency and do not produce mutual splitting in a simple first-order spectrum.

For example, the three protons of a simple CH_3 group do not split one another.

d) Protons on the same carbon

Protons attached to the same carbon may be equivalent and therefore do not split each other. However, if they are chemically non-equivalent, such as in certain CH_2 groups of unsymmetrical molecules, they can exhibit geminal coupling and may produce more complex splitting patterns.Therefore, the statement that protons on the same carbon can never split each other is not generally correct.

e) Vicinal coupling

The most commonly observed proton–proton coupling occurs between protons attached to adjacent carbon atoms**.** This is called vicinal coupling**.**

For example:CH_3–CH_2–

The CH_3 and CH_2 protons can couple with one another and produce the characteristic triplet–quartet pattern.

f) Long-range coupling

Coupling is not restricted exclusively to adjacent carbon atoms. Long-range coupling can occur through three or more bonds, particularly in unsaturated and aromatic systems. However, such coupling is usually smaller than ordinary vicinal coupling and may not always be resolved in a spectrum.

3.11 Coupling Constant (J)

The coupling constant (J) is the frequency difference between adjacent lines of a multiplet resulting from spin–spin coupling. It is a quantitative measure of the strength of the coupling interaction between two nuclei.

The coupling constant is expressed in Hertz (Hz)**.**

For example, if the individual lines of a doublet are separated by 7 Hz, then:J = 7 Hz

Similarly, the spacing between adjacent lines of a simple triplet or quartet represents the corresponding coupling constant.

Characteristics of Coupling Constant

1) **Unit:** Coupling constants are expressed in **Hz**.

2) **Independent of magnetic-field strength:** The value of J remains essentially constant when the same coupling is observed on NMR spectrometers operating at different magnetic-field strengths.

3) **Represents interaction between nuclei:** J provides a quantitative measure of spin–spin coupling.

4) **Related to molecular structure:** The magnitude of J depends on factors such as the number of bonds between the nuclei, molecular geometry, and the types of atoms involved.

5) **Useful for structural determination:** Coupling constants can provide information about the arrangement and stereochemical relationship of nuclei in a molecule.

Coupling Constant and Chemical Shift: Difference

It is important to distinguish between **chemical shift (δ)** and **coupling constant (J)**.

Chemical Shift (δ)	Coupling Constant (J)
Indicates the chemical environment of a nucleus	Indicates interaction between coupled nuclei
Expressed in ppm	Expressed in Hz
Measured relative to a reference such as TMS	Measured as spacing between adjacent lines of a multiplet
Influenced strongly by shielding/deshielding	Influenced by bonding and molecular geometry
Helps identify the type of proton	Helps identify neighboring nuclei and their relationship

Table 3.6: Differences between Chemical Shift and Coupling Constant

Importance of Splitting in ^{1}H NMR

Spin–spin splitting is highly useful in determining molecular structure. It provides information about the number of neighboring protons and their relationship within the molecule.

- The splitting pattern can help to:
- Determine the number of neighboring protons.
- Identify adjacent proton-containing groups.
- Establish connectivity between carbon atoms.
- Distinguish between different structural possibilities.
- Determine coupling relationships and, in suitable cases, stereochemical information.

Factors Influencing Coupling Constant (J)

Coupling constant **J** can have **+ve & –ve values.**

The number of bonds intervening between coupling nuclei because the coupling is transmitted via the electrons of these bonds. So, it is convenient notation to indicate this number as a superscript to the symbol for coupling constant.

a) **Direct Coupling:Direct coupling** would be a one-bond coupling (**^1J**).

b) **Geminal Coupling:**Coupling between protons on a -CH_2 group → **^2J**

c) **Vicinal Coupling:**Between protons on adjacent carbons → **^3J**

d) **Long Range Coupling:**Coupling over more than three bonds is called as long range coupling(^4J, ^5J)

Conclusion

Nuclear Magnetic Resonance spectroscopy represents one of the most informative and reliable analytical techniques for the structural characterization of chemical compounds. The fundamental concepts of nuclear spin, magnetic-field interaction, radiofrequency absorption, resonance, and relaxation provide the basis for understanding NMR spectra. Both ^{1}H and ^{13}C NMR offer complementary information regarding the chemical environments and molecular framework of organic compounds.

The development of Fourier Transform NMR has significantly improved the speed, sensitivity, and efficiency of spectral acquisition compared with conventional continuous-wave NMR. Proper sample preparation, including appropriate solvent selection, concentration, purity, and use of suitable NMR tubes, is essential for obtaining high-quality spectra. Interpretation of ^{1}H NMR spectra through chemical shift, integration, signal multiplicity, shielding and deshielding effects, and spin–spin coupling provides valuable information about proton environments and molecular connectivity. The coupling constant further contributes to understanding the interaction and spatial relationships between coupled nuclei.

Thus, NMR spectroscopy integrates theoretical principles with practical spectral interpretation to provide detailed structural information that cannot be obtained readily from a single analytical technique. Its ability to establish molecular connectivity, distinguish chemically different environments, and provide information about molecular structure makes it an indispensable technique in modern chemical research, particularly in organic, medicinal, pharmaceutical, and materials chemistry.

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Chapter 4: Infrared (IR) Spectroscopy

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Abstract

Infrared (IR) spectroscopy is a powerful and widely used analytical technique for the identification, characterization, and structural investigation of chemical compounds. It is based on the interaction of infrared radiation with molecules, resulting primarily in vibrational transitions when the radiation frequency matches the natural vibrational frequency of a chemical bond. This chapter provides a comprehensive overview of the fundamental principles and applications of IR spectroscopy, beginning with the position and subdivisions of the infrared region in the electromagnetic spectrum. The Near-IR, Mid-IR, and Far-IR regions are discussed along with their characteristic applications. Particular emphasis is placed on molecular vibrations, including stretching and bending modes such as symmetric stretching, asymmetric stretching, scissoring, rocking, wagging, and twisting. The concepts of molecular energy levels, vibrational frequency, Planck's equation, Hooke's law, reduced mass, and bond strength are explained to establish the relationship between molecular structure and vibrational frequency. The chapter further discusses vibrational modes of diatomic, linear polyatomic, and non-linear polyatomic molecules, together with IR selection rules and the importance of changes in molecular dipole moment for IR activity. Fundamental vibrations, overtones, combination bands, difference bands, vibrational coupling, degeneracy, and Fermi resonance are also described. The principles and components of dispersive and Fourier Transform Infrared (FTIR) spectrometers, including radiation sources, wavelength selectors, sample cells, detectors, and signal-processing systems, are presented. Finally, the interpretation of IR spectra, functional-group identification, fingerprint-region analysis, and applications in pharmaceutical analysis, quality control, structural elucidation, purity testing, research, and industry are discussed. Overall, the chapter highlights the significance of IR spectroscopy as a rapid, sensitive, non-destructive, and reliable technique for molecular identification and chemical analysis.

Keywords

Infrared spectroscopy; IR spectroscopy; FTIR; molecular vibrations; vibrational modes; stretching vibrations; bending vibrations; functional group region; fingerprint region; Hooke's law; selection rules; dipole moment; vibrational frequency; fundamental vibrations; overtone bands; combination bands; Fermi resonance; IR spectrometer; pharmaceutical analysis; structural identification; quality control.

4.1 Introduction to Infrared (IR) Spectroscopy

Infrared (IR) spectroscopy is one of the most widely used analytical techniques in chemistry, pharmacy, biochemistry, and material sciences for the identification and characterization of organic and inorganic compounds. It is based on the interaction of infrared radiation with matter, particularly the absorption of infrared radiation by molecules. The absorbed energy causes molecular vibrations, producing a unique spectrum that serves as a molecular fingerprint for the identification of compounds.

IR spectroscopy has become an indispensable tool in pharmaceutical industries because it enables rapid, accurate, and non-destructive analysis of drugs and pharmaceutical formulations. It is extensively employed for the identification of raw materials, detection of impurities, quality control, structural elucidation, compatibility studies, and stability testing. Modern Fourier Transform Infrared (FTIR) spectroscopy has further enhanced the sensitivity, speed, and accuracy of infrared analysis, making it an essential analytical technique in research laboratories and industrial quality assurance. The term **infrared** literally means **"below the red"** region of the visible spectrum. The infrared region lies immediately beyond the red end of visible light and before the microwave region in the electromagnetic spectrum. Although infrared radiation is invisible to the human eye, it can be detected as heat because its energy causes molecular vibrations rather than electronic excitation. Infrared radiation covers the wavelength range from **0.8 µm to 200 µm**, corresponding to a **wavenumber range of 12500–5 cm^{-1}**. Since the energy of infrared radiation is relatively low compared with ultraviolet or visible radiation, it is insufficient to promote electrons to higher electronic energy levels. Instead, it causes atoms within molecules to vibrate by stretching or bending their chemical bonds. Every chemical bond vibrates at a characteristic frequency; therefore, every molecule exhibits a unique infrared spectrum that can be used for its identification [2,4].

Position of Infrared Region in the Electromagnetic Spectrum

The electromagnetic spectrum consists of different types of electromagnetic radiations arranged according to their wavelength or frequency. Starting from the highest-energy radiations, the sequence is:

Gamma rays → X-rays → Ultraviolet → Visible → Infrared → Microwaves → Radio waves

Infrared radiation occupies the region between visible light and microwaves. Compared with ultraviolet radiation, IR possesses lower energy and longer wavelengths. Consequently, IR radiation induces molecular vibrations rather than electronic transitions. Because molecular vibrations are highly characteristic of specific functional groups, IR spectroscopy is considered one of the most reliable techniques for functional group identification and structural analysis.

4.2 Subdivisions of Infrared Region

The infrared region is conventionally divided into three major regions according to wavelength and wavenumber.

Region	Wavenumber (cm^{-1})	Wavelength (µm)	Description
Near IR	>4000	0.8–2.5	Overtone and combination bands
Mid IR	4000–650	2.5–15.4	Fundamental molecular vibrations
Far IR	<650	15–200	Rotational and lattice vibrations

Table 4.1: Subdivisions of IR Region [2]

4.2.1 Near Infrared Region (NIR)

The near infrared region extends from **12500 to 4000 cm^{-1} (0.8–2.5 µm).** Absorptions observed in this region are primarily due to overtone and combination vibrations rather than fundamental vibrations. Although these absorption bands are relatively weak, near infrared spectroscopy has become increasingly important in pharmaceutical quality control, food analysis, agriculture, and biotechnology because it permits rapid, non-destructive analysis without extensive sample preparation.

Common applications include:

- Moisture determination
- Pharmaceutical tablet analysis
- Protein estimation
- Process analytical technology (PAT)
- Online monitoring of manufacturing processes

4.2.2 Mid Infrared Region

The mid infrared region extends from **4000 to 650 cm^{-1} (2.5–15.4 µm)** and is the most important region for analytical purposes. Most organic molecules absorb infrared radiation in this region because the energy corresponds to their fundamental vibrational frequencies. Nearly all pharmaceutical compounds are characterized using this region since it contains characteristic absorptions for functional groups such as: O–H, N–H, C–H, C=C, C≡N, C=O, C–O, C–N. Consequently, mid-IR spectroscopy is routinely used for qualitative identification of compounds.

4.2.3 Far Infrared Region

The far infrared region lies below **650 cm^{-1} (15–200 µm).**

This region mainly provides information regarding:

- Rotational transitions
- Heavy atom vibrations
- Metal-ligand bonding
- Crystal lattice vibrations

Far IR spectroscopy is particularly useful in inorganic chemistry, coordination chemistry, polymer science, and solid-state pharmaceutical research.

4.2.3 Functional Group Region

The **functional group region** extends approximately from **4000 to 1400 cm⁻¹.**This region contains highly characteristic absorption bands produced by stretching vibrations of functional groups.

Since each functional group absorbs within a definite frequency range, this portion of the spectrum is primarily used for determining the functional groups present in an unknown compound. For example, the presence of a strong absorption near **1700 cm^{-1}** immediately suggests the presence of a carbonyl group [2,3].

Functional Group	Characteristic Absorption (cm^{-1})
O–H	3200–3600
N–H	3300–3500
C–H	2850–3100
C≡C	2100–2260
C≡N	2210–2260
C=O	1650–1750
C≡C	2100–2260
C–O	1000–1300

Table 4.2 Different functional groups and their characteristic absorption Bands

4.2.3 Fingerprint Region

The fingerprint region extends approximately from 1500 to 400 cm^{-1} Unlike the functional group region, this portion consists of numerous closely spaced absorption bands arising from complex skeletal vibrations. The fingerprint region is unique for every compound because even molecules possessing identical functional groups exhibit different absorption patterns.

Consequently, the fingerprint region is extensively used for:

- Identification of unknown compounds
- Comparison with authentic reference standards
- Purity determination
- Detection of structural isomers

Modern spectral libraries contain thousands of fingerprint spectra, allowing computerized identification within seconds [1,3].

4.3 Interaction of Infrared Radiation with Molecules

4.3.1 Frequency: Frequency is defined as thenumber of complete wave cycles passing through a fixed point in one second**.** It is represented by the Greek letter ν (nu)and is expressed inHertz (Hz)ors^{-1}**.** Mathematically,

$$\nu = \lambda c$$

where:

- ν = Frequency (Hz)
- c = Speed of light (3×10^8 m s^{-1})
- λ = Wavelength (m)

Since frequency is inversely proportional to wavelength, radiation having shorter wavelengths possesses higher frequencies and consequently higher energy [2].

When infrared radiation passes through a sample, not all frequencies are absorbed. Only those frequencies that exactly match the natural vibrational frequencies of chemical bonds are absorbed. The remaining radiation passes through the sample without absorption.

The absorbed radiation causes molecular vibrations, including:

- Stretching vibrations
- Bending vibrations

A detector records the transmitted radiation, and the instrument plots absorbance (or transmittance) against wavenumber (cm^{-1}) to produce the characteristic infrared spectrum of the compound [1, 2].

4.3.2 Molecular Energy Levels

Every molecule possesses three principal types of energy:

1. **Electronic Energy** – Highest energy
2. **Vibrational Energy** – Intermediate energy
3. **Rotational Energy** – Lowest energy [3,4]

The relationship is represented as:

Electronic (Highest energy)
↑
Vibrational
↑
Rotational (Lowest energy)

Electronic transitions require ultraviolet or visible radiation, whereas infrared radiation possesses sufficient energy only to induce vibrational and rotational transitions. Therefore, IR spectroscopy is often described as vibrational spectroscopy or vibration–rotation spectroscopy.

4.3.3 Energy of Infrared Radiation

According to Planck's quantum theory, the energy of electromagnetic radiation is directly proportional to its frequency. The relationship is expressed as:

$$E = h\nu = hc/\lambda$$ **[1,2]**

where:

- **E** = Energy of radiation
- **h** = Planck's constant
- **ν** = Frequency
- **c** = Speed of light
- **λ** = Wavelength

Thus, higher-frequency radiation possesses greater energy.

4.3.4 Vibrational Transitions in IR Spectroscopy

The energy required for molecular transitions follows the order:

Electronic > Vibrational > Rotational [4]

Since infrared radiation possesses intermediate energy, it promotes molecules from one vibrational energy level to another, often accompanied by simultaneous rotational transitions. When the frequency of the incident IR radiation matches the natural vibrational frequency of a chemical bond, resonance occurs, and the molecule absorbs energy. This absorption increases the amplitude of bond vibration without breaking the bond. Consequently, every molecule exhibits characteristic absorption bands that form its unique infrared spectrum. This spectrum provides valuable information regarding molecular structure, functional groups, and chemical identity, making infrared spectroscopy one of the most powerful analytical techniques in modern pharmaceutical and chemical analysis [2,5].

4.4 Applications of Infrared (IR) Spectroscopy

Infrared (IR) spectroscopy is one of the most versatile analytical techniques used in chemistry, pharmacy, biochemistry, forensic science, environmental science, and material sciences. Since every molecule exhibits a unique infrared absorption spectrum, IR spectroscopy provides valuable qualitative and quantitative information regarding molecular structure and composition. The technique is rapid, non-destructive, highly sensitive, and requires only a small amount of sample. Consequently, it has become an indispensable tool in pharmaceutical research, quality control laboratories, and industrial manufacturing.

The major applications of IR spectroscopy are discussed below.

a) **Identification of Organic and Inorganic Compounds**

One of the most important applications of IR spectroscopy is the identification of unknown compounds**.** Every molecule possesses a characteristic arrangement of atoms and chemical bonds, which vibrate at specific frequencies. As a result, each compound produces a unique infrared spectrum that acts as its molecular fingerprint.

The IR spectrum of an unknown sample can be compared with reference spectra available in spectral libraries or pharmacopoeias. If the absorption bands match, the identity of the compound can be confirmed accurately. This property makes IR spectroscopy one of the fastest and most reliable methods for qualitative analysis.

For example:

- Ethanol exhibits a broad O–H stretching band around **3300 cm^{-1}.**
- Acetone shows a strong carbonyl (C=O) absorption near **1715 cm^{-1}.**
- Benzene exhibits characteristic aromatic C=C stretching vibrations between **1450 and 1600 cm^{-1}.**

Thus, IR spectroscopy is highly useful in assigning unknown compounds to their respective chemical classes such as alcohols, aldehydes, ketones, esters, carboxylic acids, amines, amides, nitriles, and aromatic compounds [1,3].

b) **Determination of Functional Groups**

IR spectroscopy is extensively used for the identification of functional groups present in organic molecules. Every functional group absorbs infrared radiation within a characteristic frequency range due to its specific bond vibrations. The presence or absence of these characteristic peaks allows chemists to determine the functional groups present in a molecule without carrying out complicated chemical tests. For example, the appearance of a sharp absorption around **2250 cm⁻¹** indicates the presence of a nitrile (C≡N) group, while a broad band between **3200 and 3600 cm⁻¹** suggests the presence of hydroxyl (O–H) groups [2,5].

c) **Determination of Molecular Structure**

Besides identifying functional groups, IR spectroscopy provides valuable information regarding the overall molecular structure of organic compounds. It helps determine the presence of various chemical bonds such as: C–H , C–C, C=C, C≡C, C=O, C–O, C–N, N–H, O–H, C≡N, C≡O. By analyzing all absorption bands collectively, chemists can propose the probable molecular structure of unknown compounds. IR spectroscopy is therefore frequently used together with Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) for complete structural elucidation [2, 5].

d) **Structural Confirmation of Synthesized Compounds**

IR spectroscopy plays a crucial role in synthetic organic chemistry and medicinal chemistry by confirming the successful synthesis of new compounds. After completion of a chemical reaction, the disappearance of reactant peaks and the appearance of new absorption bands confirm product formation. For example:

- During esterification, disappearance of the broad O–H absorption of carboxylic acid and appearance of ester carbonyl absorption near **1740 cm⁻¹** confirms ester formation.
- Formation of Schiff bases is confirmed by the appearance of the characteristic **C=N stretching band**around **1610–1660 cm⁻¹** and disappearance of aldehyde carbonyl peaks.

Thus, IR spectroscopy is widely used to verify reaction completion during pharmaceutical synthesis [4,5].

e) **Purity Testing**

Pure compounds exhibit well-defined characteristic absorption bands. The presence of impurities often introduces additional peaks or causes distortion of existing peaks. IR spectroscopy is therefore routinely employed for:

- Detection of impurities
- Assessment of product purity
- Comparison with standard reference spectra
- Quality assurance during pharmaceutical manufacturing

Pharmaceutical industries commonly compare the IR spectrum of a manufactured drug with that of an official pharmacopoeial standard before product release [4, 5].

f) **Identification of Isomerism**

IR spectroscopy helps distinguish between various types of isomerism because different isomers possess different vibrational frequencies. It is useful in identifying:

- Structural isomers
- Positional isomers
- Geometrical (cis-trans) isomers
- Conformational isomers

Although isomers may have identical molecular formulas, differences in bonding arrangement alter the vibrational frequencies, producing distinguishable IR spectra. For example, **cis** and **trans** alkenes exhibit characteristic out-of-plane C–H bending vibrations at different frequencies [4].

g) **Study of Hydrogen Bonding**

Hydrogen bonding significantly influences the vibrational frequencies of O–H and N–H bonds. IR spectroscopy can distinguish between: Free hydroxyl groups, Intramolecular hydrogen bonding, Intermolecular hydrogen bonding

Hydrogen bonding generally causes:

- Broadening of absorption bands
- Shift towards lower frequencies
- Decrease in stretching frequency

For example, alcohols containing hydrogen bonding exhibit broad O–H absorption bands between **3200–3500 cm^{-1},** whereas free hydroxyl groups show sharp peaks near **3600 cm^{-1}.** This application is extremely useful in studying proteins, polymers, pharmaceuticals, and biological macromolecules [5].

h) **Determination of Molecular Symmetry**

The intensity and position of infrared absorption bands depend upon changes in molecular dipole moment during vibration. Highly symmetrical molecules often exhibit fewer IR-active vibrations because some vibrational modes do not produce dipole moment changes. For example:

- Carbon dioxide (CO_2) shows IR-inactive symmetrical stretching.
- Asymmetrical stretching produces strong IR absorption.

Thus, IR spectroscopy provides valuable information regarding molecular symmetry and bonding characteristics [4].

i) **Pharmaceutical Applications**

Infrared spectroscopy has extensive applications throughout pharmaceutical industries. It is routinely employed for:

- Identification of Active Pharmaceutical Ingredients (APIs)
- Drug-excipient compatibility studies
- Detection of counterfeit medicines
- Quality control testing
- Stability studies
- Identification of polymorphic forms
- Characterization of drug degradation products
- Authentication of herbal medicines
- Analysis of pharmaceutical formulations

Modern FTIR spectrometers are incorporated into pharmaceutical quality control laboratories because they provide rapid, reproducible, and highly accurate results [2,5].

j) **Applications in Research and Industry**

Apart from pharmaceutical analysis, IR spectroscopy finds widespread applications in numerous scientific disciplines [2].

These include:

- Polymer characterization
- Food quality analysis
- Environmental pollution monitoring
- Forensic investigations
- Petrochemical analysis
- Cosmetic quality control
- Textile industry
- Agricultural research
- Biomedical diagnostics
- Nanotechnology and biomaterials research

4.5 Molecular Vibrations & Modes of Vibration

Infrared (IR) spectroscopy is based on the principle that molecules absorb infrared radiation when the frequency of the incident radiation matches the natural vibrational frequency of their chemical bonds. Atoms in a molecule are not stationary; instead, they are in continuous motion due to thermal energy. These atoms vibrate about their equilibrium positions, and the nature of these vibrations determines the absorption pattern observed in an IR spectrum.

A chemical bond between two atoms may be compared to a **spring** connecting two masses. Just as a spring stretches and compresses when force is applied, chemical bonds also undergo periodic stretching and bending vibrations. These vibrations occur at specific frequencies depending on the bond strength, bond length, and masses of the bonded atoms. When infrared radiation of the appropriate energy interacts with a molecule, it excites these vibrational motions, resulting in the absorption of radiation and the formation of an infrared spectrum.

Different molecules possess different vibrational frequencies because they differ in atomic masses, bond strengths, molecular geometry, and chemical environment. Consequently, each molecule exhibits a unique IR spectrum, which forms the basis for molecular identification and structural characterization [2].

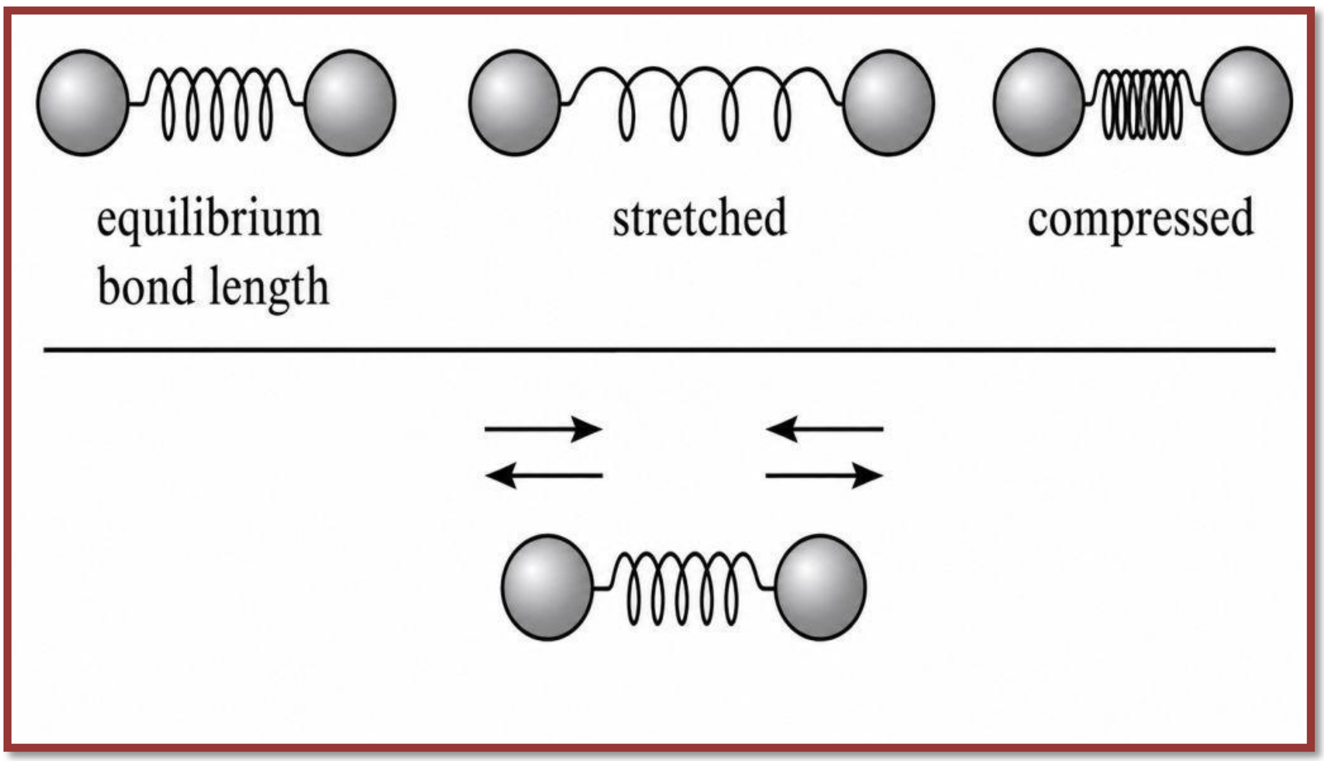

Figure 4.1: Molecular vibrations in a molecule. [2]

Types of Molecular Vibrations

Molecular vibrations are broadly classified into two major categories:

1. **Stretching Vibrations**
2. **Bending (Deformation) Vibrations**

Stretching vibrations involve changes in **bond length**, whereas bending vibrations involve changes in **bond angle**. Generally, stretching vibrations require more energy than bending vibrations and therefore appear at higher frequencies in the infrared spectrum.

4.5.1 Stretching Vibrations (Bond Stretching)

Stretching vibration refers to the periodic movement of atoms along the axis of the chemical bond, causing the bond length to increase and decrease alternately without changing the bond angle. In other words, the atoms move back and forth along the line joining their nuclei while remaining on the same bond axis.

For example, consider the vibration of a hydrogen molecule (H–H):

Compressed bond

H———H

↓

Equilibrium bond length

H—————————H

↓

Extended bond

H—————————————H

The vibration consists of continuous compression and extension of the bond, similar to the oscillation of a spring. Because stretching vibrations involve changes in bond length, they require relatively large amounts of energy. Consequently, they absorb infrared radiation at higher frequencies than bending vibrations [2].

Characteristics of Stretching Vibrations

- Change occurs in bond length.
- Bond angle remains unchanged.
- Atoms move along the bond axis.
- Higher energy is required.
- Absorption bands appear at higher wavenumbers.

- More intense than bending vibrations.

Types of Stretching Vibrations

Stretching vibrations are of two types:

1. Symmetric Stretching
2. Asymmetric Stretching

Symmetric Stretching

In symmetric stretching, the bonded atoms move simultaneously either towards or away from the central atom. Thus, both bonds stretch or compress together while maintaining molecular symmetry. All atoms move in the same direction relative to the central atom. Symmetric stretching generally requires slightly less energy than asymmetric stretching.

Characteristics

- Simultaneous movement of atoms.
- Bond lengths change equally.
- Molecular symmetry is maintained.
- Lower frequency than asymmetric stretching.
- May be IR inactive if no dipole moment change occurs.

Asymmetric Stretching

In asymmetric stretching, one bond is compressed while the other is simultaneously stretched. Thus, atoms move in opposite directions relative to the central atom. One bond becomes shorter while the other becomes longer. Asymmetric stretching produces greater changes in dipole moment and therefore generally gives stronger infrared absorption bands.

Characteristics

- Opposite movement of atoms.
- One bond stretches while another compresses.
- Higher energy than symmetric stretching.
- Appears at higher frequency.
- Usually IR active.

Property	Symmetric Stretching	Asymmetric Stretching
Direction of movement	Same	Opposite
Bond change	Simultaneous	Alternate
Energy	Lower	Higher
Frequency	Lower	Higher
Dipole moment change	Small	Large
IR activity	Sometimes inactive	Usually active

Table 4.3: Comparision between Symmetric and Asymmetric Stretching

4.5.2 Bending Vibrations (Deformation Vibrations)

Unlike stretching vibrations, bending vibrations involve changes in bond angles rather than bond lengths. During bending vibrations:

- Bond length remains almost constant.
- Bond angle changes periodically.
- Atoms move either within or outside the molecular plane.

Since less energy is required to alter bond angles than bond lengths, bending vibrations occur at lower frequencies [4].

Characteristics of Bending Vibrations

- Bond length remains unchanged.
- Bond angle changes continuously.
- Lower energy required.
- Lower frequency absorption.
- More common in complex molecules.

Types of Bending Vibrations

Bending vibrations are divided into two categories:

1. **In-plane bending**
2. **Out-of-plane bending**

In-Plane Bending

In in-plane bending, atoms move within the same plane as the molecule. These vibrations are commonly observed in: Alkenes, Aromatic compounds, Carbonyl compounds

In-plane bending consists of two types.

(a) Scissoring: During scissoring, two atoms move toward and away from each other within the molecular plane, resembling the blades of a pair of scissors.

Characteristics

- Atoms move towards each other.
- Occurs within molecular plane.
- Common in CH_2 groups.

(b) Rocking: During rocking, both atoms move in the same direction while remaining within the molecular plane. Both atoms rock together in one direction.

Out-of-Plane Bending

In out-of-plane bending, atoms move above and below the molecular plane. These vibrations are particularly useful in identifying substitution patterns in aromatic compounds.

Out-of-plane bending consists of two types.

(a) Twisting: During twisting, one atom moves upward while the other moves downward relative to the molecular plane. The two atoms twist in opposite directions.

(b) Wagging: During wagging, both atoms move simultaneously upward and downward together. Both atoms swing together like the tail of a wagging animal.

Type	Direction of Motion	Plane of Motion
Scissoring	Towards each other	In-plane
Rocking	Same direction	In-plane
Twisting	Opposite directions	Out-of-plane
Wagging	Same direction	Out-of-plane

Table 4.4 Comparison of Bending Vibrations

All types of mode of vibrations described above are shown in figure 4.2.

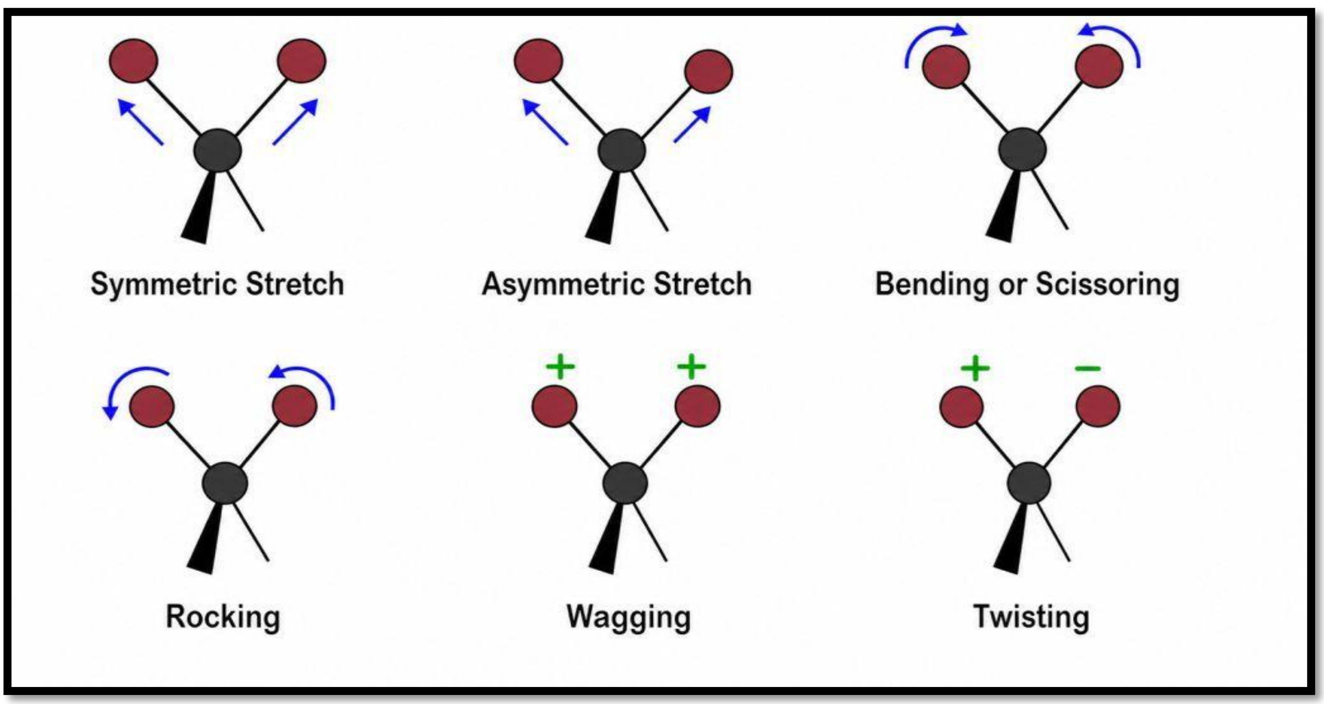

Figure 4.2: Different modes of vibrations in molecule [2,5]

4.5.3 Energy Requirement of Molecular Vibrations

The energy required for different molecular vibrations follows the order:

Asymmetric Stretching > Symmetric Stretching > Bending Vibrations

Similarly, the approximate frequency order is:

Stretching Vibrations > Bending Vibrations

Therefore,

- Stretching vibrations generally appear in the higher wavenumber region (4000–1500 cm^{-1}).
- Bending vibrations mainly appear in the fingerprint region (1500–400 cm^{-1}) [2,5].

4.6 Calculation of Vibrational Frequency (Hooke's Law)

One of the fundamental principles of Infrared (IR) spectroscopy is that molecules absorb infrared radiation when the frequency of the incident radiation matches their natural vibrational frequency. The frequency at which a chemical bond vibrates depends primarily on two factors: the strength of the bond and the masses of the bonded atoms. These relationships can be explained mathematically using Hooke's Law, which models a chemical bond as a spring connecting two atoms.

Hooke's Law was originally developed to describe the behavior of mechanical springs. Since a chemical bond behaves similarly to a spring that stretches and compresses during molecular vibrations, the same principle can be applied to estimate the vibrational frequencies of molecules. Although real molecules are more complex than ideal springs, Hooke's Law provides an excellent approximation for understanding the factors that influence vibrational frequencies in IR spectroscopy.

The Hooke's Law model explains why strong bonds such as C≡C and C≡N absorb at higher frequencies than single bonds such as C–C, and why bonds involving lighter atoms vibrate faster than those involving heavier atoms [1].

Hooke's Law Equation

The vibrational frequency of a chemical bond is given by the Hooke's Law equation:

$$\nu = 1/2\pi c \sqrt{k/\mu} \quad [1]$$

Where: ν = Vibrational frequency (cm^{-1})

k = Force constant (bond strength)

μ = reduced mass $\mu = m1+m2/m1m2$ where m_1 and m_2 are masses of the two atoms

c = Speed of light (3×10^{10} cm s^{-1})

The equation indicates that the vibrational frequency depends directly on the square root of the bond strength and inversely on the square root of the reduced mass.The vibrational frequency of a bond is expected to increase when the bond strength increases & also when the reduced mass of the system decreases [5]. As electronegativity increases, bond strength also increases, frequency also increases [2].

4.7 Selection Rules

4.7.1 Fundamental Vibrations: Infrared (IR) spectroscopy is based on the absorption of infrared radiation by molecules. A molecule absorbs IR radiation when the energy of the radiation corresponds to the energy difference between two allowed vibrational energy levels of the molecule. When a molecule in its **ground vibrational state** absorbs IR radiation and moves to the **first excited vibrational state**, the transition is generally:

$$v=0 \rightarrow v=1$$

The absorption band produced by this transition is called a **fundamental absorption band**. Fundamental bands are generally the strongest and most intense bands in an IR spectrum [2].

$$\text{Fundamental vibrational transition: } \Delta v=+1$$

However, for real molecules, weak overtone and combination transitions may also occur because molecular vibrations are not perfectly harmonic. The number of possible fundamental vibrational modes depends upon the number of atoms in the molecule and its geometry.

So, **No. of fundamental vibrational modes = No. of normal modes of vibration**

A **normal mode of vibration** is an independent and characteristic way in which the atoms of a molecule vibrate collectively.

4.7.2 Vibrational Modes of Diatomic Molecules

A diatomic molecule contains two atoms. Therefore: $3N=3(2)=6$. For a linear diatomic molecule: Translational modes are 3 and Rotational modes are 2 So number of Vibrational modes will be $6-3-2=1$.

$$\text{Number of Vibrational modes of a diatomic molecules}=1$$

This vibration is a **stretching vibration**, in which the distance between the two atoms changes periodically. Examples include: HCl , CO, N_2, O_2. However, the presence of a vibrational mode does not necessarily mean that it will produce an IR absorption band. The vibration must satisfy the **IR selection rule**, namely that it must produce a change in the molecular dipole moment.

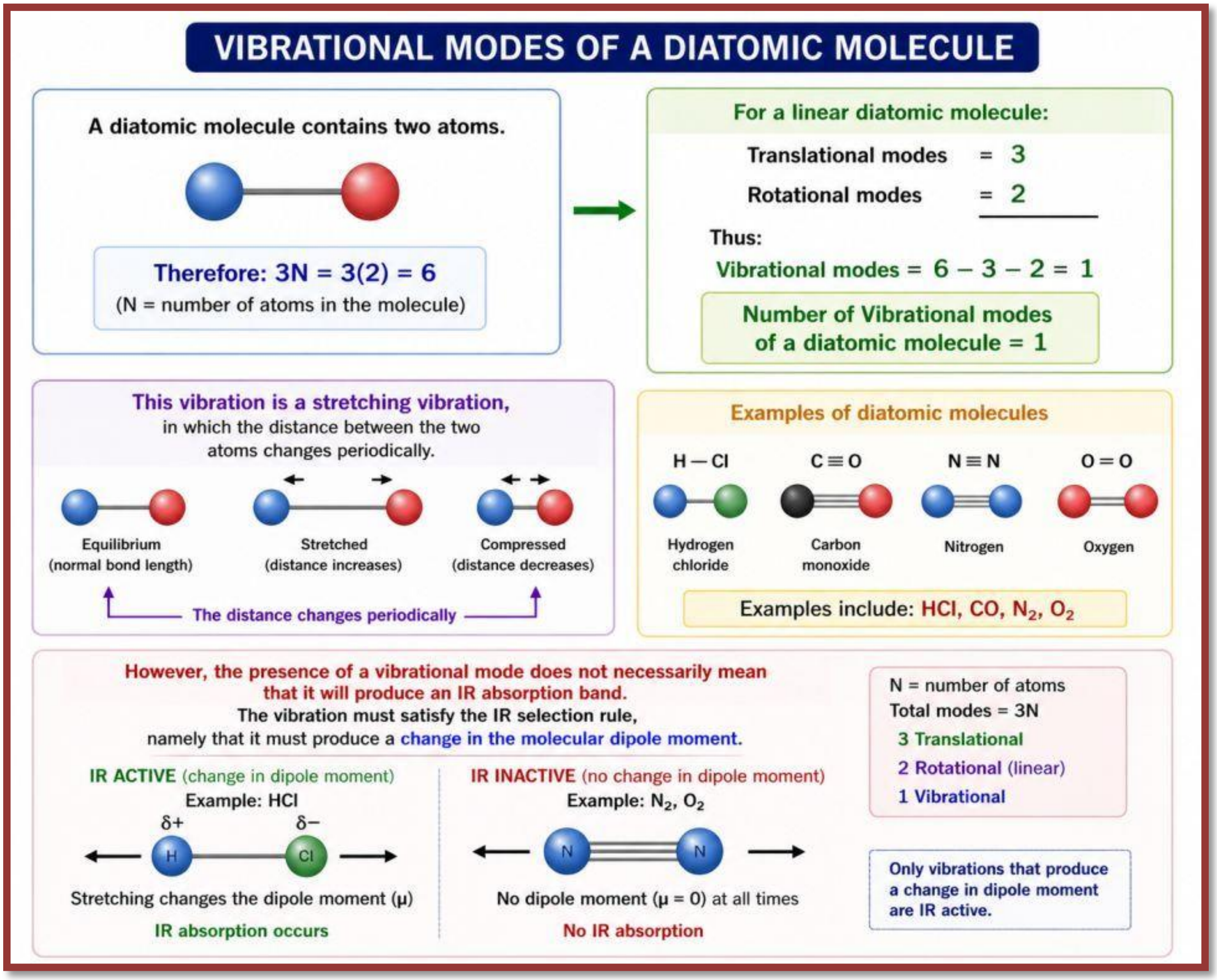

Figure 4.3: Vibrational Modes of a Diatomic Molecule

4.7.3 Vibrational Modes of Polyatomic Molecules

Polyatomic molecules may be either:

1. **Non-linear**
2. **Linear**

The number of fundamental vibrational modes differs for these two types.

A. Non-linear Polyatomic Molecules: For a non-linear molecule containingN atoms total degrees of freedom are 3N. Out of these 3 correspond to translation and 3 correspond to rotation. Therefore:

Number of vibrational modes=3N−6

Example 1: H_2O, CH_4

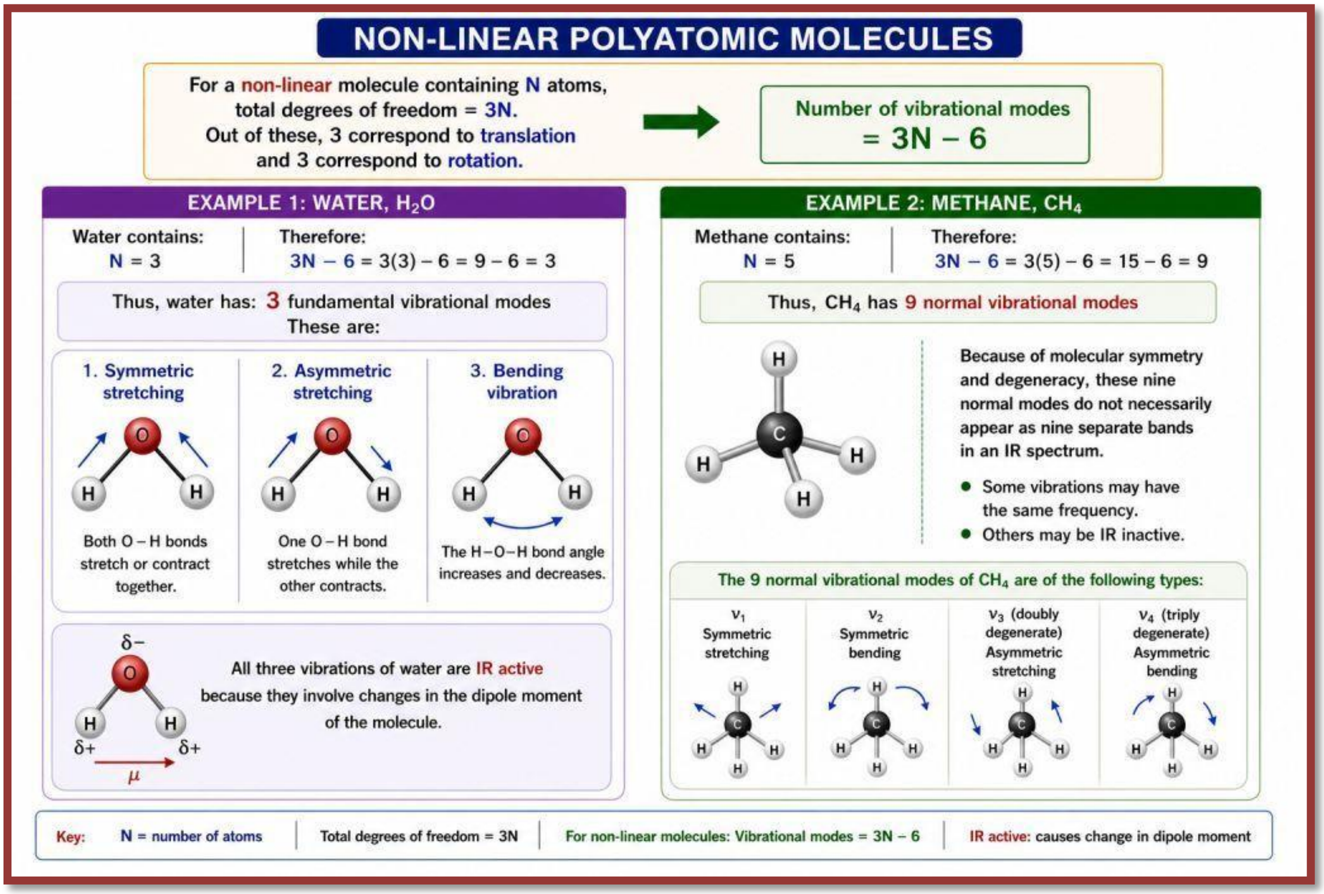

Figure 4.4: Vibrational Modes of Non-Linear Polyatomic Molecule

B. Linear Polyatomic Molecules

For a linear molecule containing **N atoms**, total degrees of freedom = 3N, Translational degrees of freedom = 3, Rotational degrees of freedom = 2. Therefore:

$$\textbf{Number of vibrational modes} = 3N - 5$$

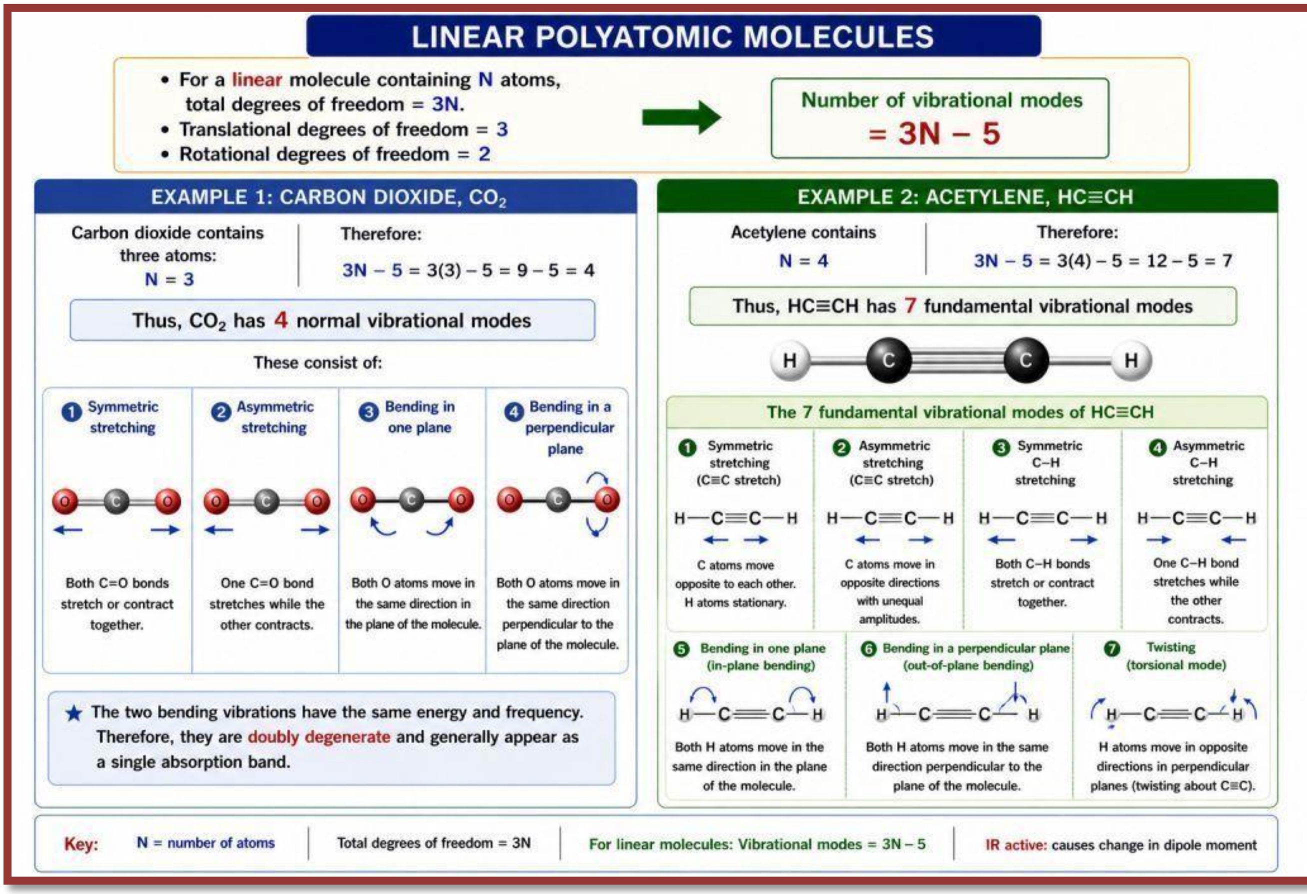

Figure 4.5: Vibrational Modes of Non-Linear Polyatomic Molecule

4.7.4 Actual Observations: The theoretical number of normal vibrational modes does not always correspond to the number of absorption bands actually observed in an IR spectrum [2,3].

This difference can arise for several reasons.

(a) Some Vibrations are IR Inactive: A normal mode may exist theoretically but may not produce a change in dipole moment. Such a vibration will not absorb IR radiation. For example, the symmetric stretching vibration of CO_2 is IR inactive.

(b) Some Absorption Bands are Very Weak: Certain vibrations may produce only a very small change in dipole moment. As a result, the corresponding absorption band may be extremely weak and may not be detected clearly. The intensity of an IR absorption band depends largely on the magnitude of the dipole moment change during vibration.

(c) Overlapping of Bands: Two or more vibrations may occur at very similar frequencies. Their absorption bands may overlap, resulting in one broad or unresolved absorption band instead of separate bands.

Therefore, number of observed bands may be less than the number of normal modes

(d) Degeneracy: Two or more different vibrational motions may possess exactly the same energy and frequency. Such vibrations are called degenerate vibrations. For example, CO_2 has two degenerate bending modes, but they correspond to the same vibrational frequency and appear as one fundamental absorption band.

(e) Absorption outside the Measured Spectral Range: A vibration may have a frequency outside the IR region being examined by the instrument. The commonly used mid-infrared region is approximately: 4000−400 cm−1 or approximately:2.5−25 μm. Therefore, vibrations outside the selected measurement range may not be observed.

(f) Instrumental Limitations: Weak bands may not be resolved because of:

- low detector sensitivity
- poor spectral resolution
- noise
- sample concentration
- interference from solvents or atmospheric water and carbon dioxide

Molecular symmetry plays an important role in determining whether a vibration is IR active.

Type of molecule	Nature of vibration	IR activity
Centrosymmetric molecule	No change in dipole moment	IR inactive
Centrosymmetric molecule	Produces a change in dipole moment	IR active
Non-centrosymmetric molecule	Generally more vibrations can produce dipole changes	May be IR active

Table 4.5: Selection rules for IR active vibrations[3]

4.8 Intensity & Position of IR Bands

The intensity of an IR absorption band depends primarily on the change in dipole moment during vibration.A vibration is IR active when it produces a change in the dipole moment of the molecule.The greater the change in dipole moment during the vibration, the stronger the interaction with IR radiation and, consequently, the more intense the absorption band.

Mathematically, the IR intensity is related to:

$(\partial Q/\partial \mu)2$

where:

- μ = dipole moment of the molecule
- Q = normal coordinate of vibration

Thus:

$$\text{IR intensity} \propto (\partial Q/\partial \mu)2$$

Therefore:Greater the change in dipole moment during vibration, greater will be the intensity of the IR absorption band.It is important to distinguish between the difference in dipole moment between two stationary vibrational states and the change in dipole moment as the molecule vibrates. For IR absorption, the relevant selection rule is the variation of dipole moment with the vibrational coordinate.

4.8.1 Classification of IR Band Intensity

The intensity of an absorption band is often described qualitatively as:

Description	Approximate relative intensity
Most intense / very strong	Close to 100%
Very strong	Approximately 80–100%
Strong	Approximately 60–80%
Medium	Approximately 40–60%
Weak	Approximately 20–40%
Very weak	Less than approximately 20–30%

Table 4.6: Relative Intensity of IR Absorption bands

These values are approximate and may vary depending on the method used to record and present the spectrum.In an IR spectrum plotted as % transmittance versus wavenumber**,** a strong absorption appears as a deep downward band because less radiation is transmitted through the sample. In an absorbance spectrum, a stronger absorption appears as a higher peak.

4.8.2 Fundamental Vibrational Bands

A fundamental vibrational transition generally corresponds to excitation from the ground vibrational state to the first excited vibrational state:

$$v=0 \rightarrow v=1$$

For an ideal harmonic oscillator, the vibrational selection rule is:

$$\Delta v=\pm 1$$

Therefore, the fundamental absorption is normally the strongest vibrational absorption observed in an IR spectrum [2,4].However, real molecular vibrations are not perfectly harmonic. Because of anharmonicity, additional transitions may occur.

These additional features include:

- Overtones
- Combination bands
- Difference bands
- Vibrational coupling or interaction effects
- Fermi resonance

These bands are generally weaker than the fundamental absorption bands, although their intensities can change significantly when interactions occur [3,5].

4.8.3 Overtone Bands or Harmonics

These are high frequency bands which may occur at or near integral multiple of the fundamental band frequency. The overtone which appears at double the frequency of the fundamental band is called first overtone (v = 0 to v = 2). The overtone which appears at 3 times i.e v = 0 to v = 3 is called as 2nd overtone. Probability of overtones rapidly decreases as vibrational level increases. Intensity of overtones will be lower than the fundamental vibrations. e.g.:Strong absorption at 800 cm^{-1} & at 1750 cm^{-1} will also give rise to weaker absorption at 1600 cm^{-1} & 3500 cm^{-1}. These are called overtones. [3]

Characteristics of Overtone Bands

- They are usually weaker than fundamental bands.
- Their intensity decreases rapidly for higher overtones.

- Their positions are approximately integral multiples of the fundamental frequency.
- They arise because of vibrational anharmonicity.
- The first overtone is generally stronger than the second or higher overtones.

4.8.4 Vibrational Coupling and Coupling Peaks

Vibrational coupling occurs when two vibrational modes interact with each other. This interaction is most significant when:

1. The vibrations have similar frequencies.
2. The vibrating groups are close to each other.
3. The vibrations have compatible symmetry.
4. The molecular motions are able to influence one another.

As a result of coupling, the original vibrational frequencies may shift, split, or redistribute in intensity [3].

The coupled vibrations can produce new spectral features and may result in:

- Combination bands
- Splitting of absorption bands
- Shifts in vibrational frequencies
- Changes in band intensity

The term coupling peaks is often used informally for spectral features arising from vibrational interaction. More precisely, coupling refers to the interaction between normal vibrational modes rather than simply the production of "beats."

A common example is the interaction between vibrations of two similar functional groups located close together in a molecule.

4.8.5 Combination Bands: A **combination band** occurs when two or more vibrational modes are excited simultaneously. A no. of weak absorption occur in IR spectra due to sum of 2 or more vibrational frequencies. e.g.: $(x + y)$ cm^{-1} [5]. Combination bands are usually weak because they involve simultaneous excitation of more than one vibrational mode.

Their occurrence is mainly due to:

- anharmonicity
- vibrational interactions

- coupling between normal modes

Combination bands can occasionally become relatively intense if they interact with a nearby fundamental vibration.

4.8.6 Difference Bands: Some of the absorptions recorded in IR spectra corresponds to the difference between 2 vibrational frequencies i.e $(x - y)$ cm^{-1}. In this case, the molecule already existing in one excited vibrational state absorb enough additional energy. Difference bands are usually weak because relatively few molecules occupy excited vibrational states at ordinary temperatures.Their intensity may increase at higher temperatures because a larger number of molecules occupy excited vibrational levels [3]

3. Fermi Resonance/Fundamental Overtone interaction: Fermi resonance is a special type of interaction that occurs when:

1. A fundamental vibration and an overtone or combination band have nearly the same energy or frequency.
2. The interacting vibrations possess compatible symmetry.

Under these conditions, the two vibrational states interact strongly.

As a result:

- The positions of both bands may shift.
- The bands may move apart in frequency.
- The intensity of the fundamental band may decrease.
- The normally weak overtone or combination band may become much stronger.
- The intensity is redistributed between the interacting bands.

This phenomenon is commonly described as intensity borrowing.

Thus, Fermi resonance does not simply involve a literal transfer of energy from one peak to another. Instead, the vibrational states interact and mix, producing new observed states with redistributed energies and intensities [5].

4.9 IR Spectrometers: Infrared (IR) spectrometers are analytical instruments used to measure the interaction of infrared radiation with a sample. When infrared radiation passes through a substance, certain frequencies are absorbed by the molecules because they correspond to the vibrational and rotational motions of chemical bonds. The resulting spectrum provides valuable information about the molecular structure and functional groups present in the sample.

IR spectrometers can broadly be classified into two main types based on how infrared frequencies are handled:

1. **Dispersive IR Spectrometers**
2. **Fourier Transform Infrared (FTIR) Spectrometers**

Dispersive IR Spectrometers

Dispersive IR instruments separate polychromatic infrared radiation into individual wavelengths or frequencies before measuring the absorption of the sample. A **prism or diffraction grating** is commonly used as the wavelength-dispersing element.

Dispersive instruments are further classified as:

- **Single-beam IR spectrometers**
- **Double-beam IR spectrometers**

Fourier Transform Infrared (FTIR) Spectrometers

FTIR instruments operate on a different principle from dispersive instruments. Instead of separating IR radiation into individual frequencies, an FTIR instrument allows many frequencies to interact with the sample simultaneously.An interferometer**,** usually a Michelson interferometer, produces an interference pattern called an interferogram. This interferogram contains information about all the frequencies present in the radiation. A mathematical operation called the Fourier transform converts the interferogram into the conventional IR spectrum.

FTIR instruments have several advantages, including:

- High speed of analysis
- High sensitivity
- Good wavelength accuracy
- Better signal-to-noise ratio
- Ability to collect the complete spectrum simultaneously
- Small sample requirements [2,3]

4.9.1Dispersive Spectrophotometers

4.9.1.1 Single-Beam IR Spectrometer:A single-beam IR spectrometer is one of the simplest types of dispersive IR instruments. It measures the intensity of infrared radiation transmitted through the sample and compares it with a previously measured reference or background spectrum.

The basic components of a single-beam instrument include:

1. IR radiation source

2. Chopper
3. Sample holder
4. Monochromator
5. Detector
6. Amplifier and recorder/display

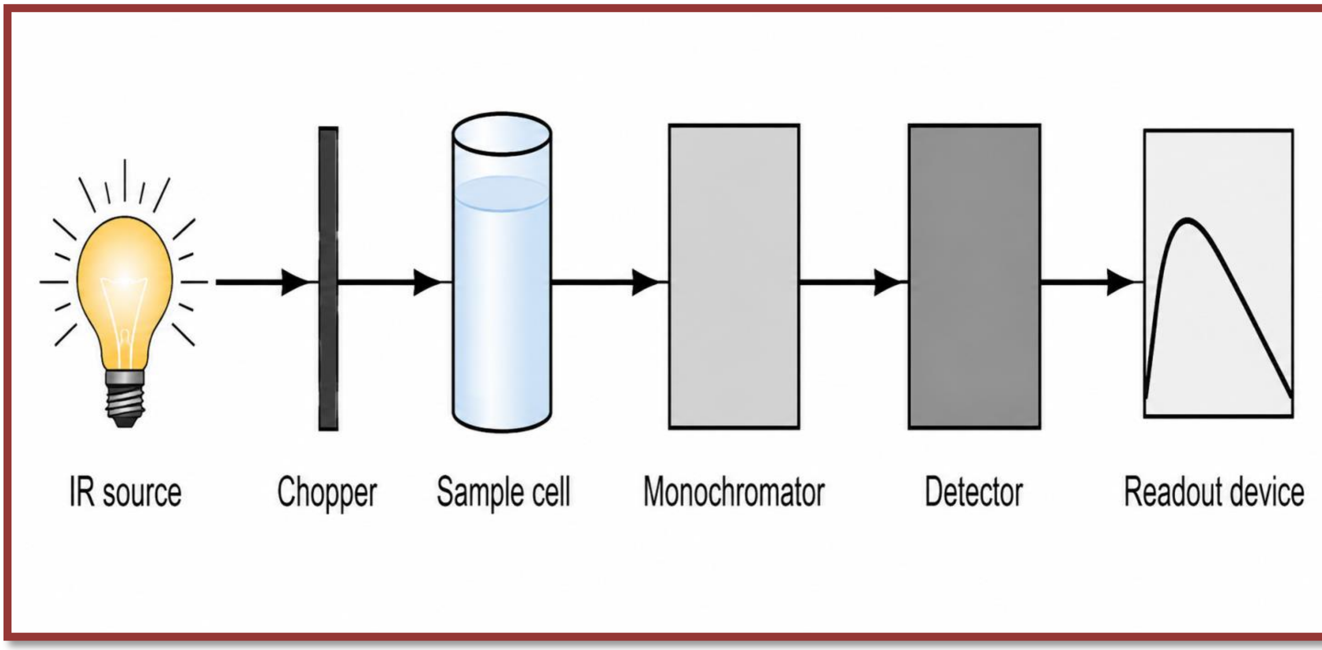

Figure 4.6: Single Beam IR Spectrometer.[2]

Working of a Single-Beam Instrument

The IR radiation produced by the source passes through the optical system and is directed toward the monochromator. The monochromator selects a particular wavelength or frequency from the polychromatic radiation.The selected radiation then passes through the sample. Molecules present in the sample absorb specific IR frequencies corresponding to their molecular vibrations. The radiation that is not absorbed reaches the detector.The detector converts the transmitted IR radiation into an electrical signal. The signal is amplified and processed to produce an IR spectrum.In a single-beam instrument, a background measurement is normally recorded first. The sample measurement is then compared with this background to determine the percentage transmittance or absorbance [2].

4.9.1.2 Double Beam IR Spectrometer: A double-beam IR spectrometer is designed to compensate for fluctuations in the intensity of the radiation source and other instrumental variations.

In this arrangement, the IR radiation is divided into **two beams**:

- One beam passes through the **sample**
- The other passes through the **reference**

The intensities of the two beams are compared by the instrument. This arrangement improves the stability and reliability of measurements.

The major components of a double-beam IR spectrophotometer include:

1. Radiation source
2. Chopper
3. Monochromator
4. Beam splitter/optical system
5. Sample cell
6. Reference cell
7. Detector
8. Amplifier
9. Recorder or computer-based data system

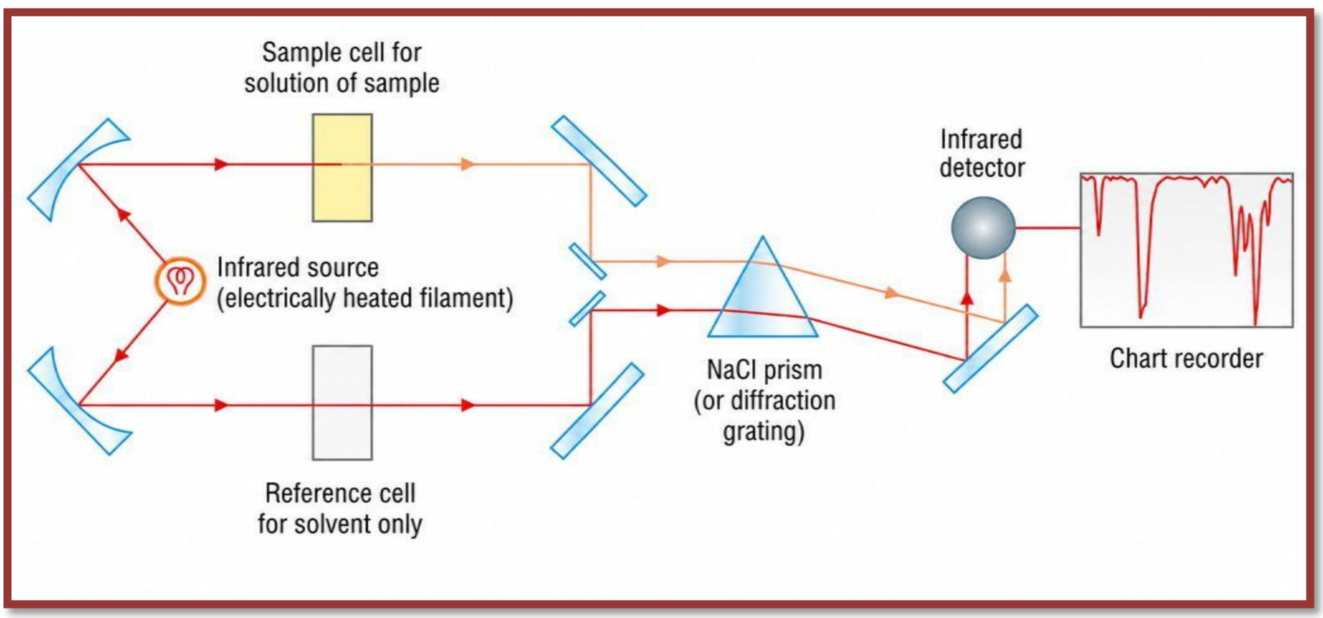

Figure 4.7: Double Beam IR Spectrophotometer.[2,5]

These can be explained as below:

a) **Sources of Radiation:** IR instruments require a source of radiant energy which emits IR radiation which must be steady, intense enough for detection and extend over the desired wavelength. An inert solid is electrically heated to a temperature in the range of 1500–2000 K. This heated material will then emit IR radiation. [4] **Types: Nernst Glower:** Wide range, stable ,**Globar:** Silicon carbide rod, high intensity, **Mercury Arc:** For far IR, **Incandescent Wire:** Low intensity, long life, **CO_2 Laser:** Narrow, powerful source [4,5]

b) **Wavelength Selector (Monochromator):** The monochromator is an optical component that separates polychromatic infrared radiation into its individual wavelengths or frequency components.

The basic function can be represented as:

Polychromatic IR radiation → Monochromator → Selected wavelength

A monochromator generally contains:

- Entrance slit
- Dispersing element
- Exit slit
- Optical components such as mirrors

Types: **Prism**:NaCl, KBr (moisture sensitive)**, Grating:**Gratings are rulings made on materials like glass, quartz or alkyl halides They are more stable, wide range

c) **Sample Cell/ Cuvette:** Made of NaCl/KBr, narrow with thickness: 0.01–1 mm**.** Aqueous solvents not used (absorbs IR & dissolves salts) only organic solvents are used. The choice of solvent depends on the spectral region of interest, sample solubility, safety considerations, and compatibility with the cell material.

d) Detectors: Detectors convert the radiation into electrical signal. It is of various types:

Thermal Detectors: Work by heating effect; For Example: **Thermocouple:** Measures temperature change. **Bolometer:** Resistance changes with heat; **Golay Cell:** Fast optoacoustic detector.

Photon Detectors: Based on photon interaction (faster, more sensitive), used in near IR region. When radiations fall on these they go to higher level and produce signal. [2,3]

4.9.2 Measurement of IR Spectrum:

The measurement of an IR spectrum involves passing infrared radiation through a sample and determining which wavelengths or frequencies are absorbed.

The resulting spectrum is usually expressed as:

- **Percentage transmittance (%T)** versus wavenumber, or
- **Absorbance (A)** versus wavenumber.

The infrared region is commonly represented in terms of wavenumber (cm^{-1}) rather than wavelength.For mid-IR spectroscopy, the commonly studied region is approximately:4000–400 cm^{-1}. The spectrum provides information about the chemical bonds and functional groups present in the sample.

4.9.3 Steps Involved in the Working of IR Spectroscopy

a) Selection of an appropriate IR source: An appropriate source of infrared radiation is selected according to the wavelength range required for the analysis. The source should produce stable and sufficiently intense radiation.

b) Generation of infrared radiation: The radiation is generated by a suitable IR source, such as a **heated filament, Nernst glower, or Globar**.The source produces radiation over a broad range of infrared wavelengths.

c) Division or optical handling of the radiation: In a double-beam instrument, the radiation is divided into two paths so that one beam passes through the sample and the other through the reference.In some instruments, a rotating chopper alternately directs radiation through the sample and reference paths. This permits the detector to compare the two signals.

d) Passage through sample and reference: One beam passes through the sample cell while the reference beam passes through the reference cell or background path.The molecules in the sample absorb specific frequencies of IR radiation.

The amount of radiation absorbed depends on:

- Nature of the chemical bonds
- Concentration of the sample
- Path length
- Molecular environment
- Frequency of the incident radiation

e) Function of the chopper: The chopper is used in double-beam systems to alternately direct radiation through the sample and reference paths. This allows the detector and electronic system to compare the signals and compensate for fluctuations in source intensity.The exact chopping frequency depends on the design of the instrument; therefore, a value such as 10 rotations per second should be regarded as instrument-specific rather than universal**.**

f) Detection of radiation: After passing through the sample, the transmitted IR radiation reaches the detector.The detector converts the radiation into an electrical signal. The magnitude of the signal corresponds to the intensity of radiation reaching the detector.

g) Amplification and recording: The electrical signal produced by the detector is usually very small. Therefore, it is amplified and processed electronically.Older instruments may use a pen recorder, whereas modern instruments generally use a computer-based data acquisition system.The final output is an IR spectrum showing absorption or transmittance as a function of wavenumber.

h) Interpretation of the spectrum: The resulting spectrum is interpreted to identify functional groups and structural features of the compound.

Different bonds absorb infrared radiation at characteristic frequencies. For example:

- **O–H stretching:** commonly appears in the broad region around 3200–3600 cm^{-1}
- **C–H stretching:** commonly occurs around 2850–3100 cm^{-1}
- **C=O stretching:** commonly occurs around 1650–1750 cm^{-1}
- **C–O stretching:** commonly occurs around 1000–1300 cm^{-1}

The exact position depends on the molecular environment.

4.10 Fingerprint Region: The fingerprint region is the portion of the infrared spectrum that generally extends from approximately 1500 to 400 cm^{-1}**.** It is called the fingerprint region because the pattern of absorption bands in this region is highly characteristic of an individual molecule, much like a fingerprint is characteristic of a person.

The absorption bands in this region are usually complex and closely spaced because they arise from several types of molecular vibrations, including C–C, C–O, C–N and other skeletal stretching and bending vibrations. Small differences in molecular structure can produce significant changes in the position and intensity of these absorption bands [1,3].

Importance of the Fingerprint Region

a) **Identification of compounds:**The fingerprint region is mainly used to identify unknown compounds by comparing their IR spectrum with a standard or reference spectrum. If the absorption pattern, including the positions and relative intensities of the major bands, closely matches the reference spectrum, it provides strong evidence that the compounds are the same.

b) Confirmation of compound identity: The fingerprint region is particularly useful for confirming whether two compounds are identical or different. Even when two compounds contain the same functional groups and show similar absorptions in the functional-group region, their fingerprint regions are usually different because of differences in their overall molecular structures.

c) Useful when functional groups are already known: The functional-group region, approximately **4000–1500 cm^{-1}**, is generally used first to determine the major functional groups present in a compound. Once these groups have been identified, the fingerprint region can be examined in greater detail to confirm the identity of the compound.

d) Differentiation of closely related compounds: Compounds having similar functional groups may show very similar absorption bands above 1500 cm^{-1}. However, their fingerprint patterns below 1500 cm^{-1} can be significantly different. Therefore, this region is useful for distinguishing isomers and structurally related compounds.

e) Reference-spectrum matching: Modern IR instruments and spectral databases allow an unknown spectrum to be compared with large collections of reference spectra. A close match in the fingerprint region provides strong supporting evidence for compound identification.

4.11 Interpretation of IR Spectra of Simple Organic Compounds

IR spectroscopy helps identify functional groups by characteristic absorption bands as shown in table 3. Functional group region (4000–1500 cm^{-1}) shows specific peaks like O–H (3200–3600 cm^{-1}), C=O (~1700 cm^{-1}), and C≡N (~2200 cm^{-1}). The fingerprint region (1500–400 cm^{-1}) is unique for each compound. Interpretation involves identifying key peaks and matching them with known functional group frequencies.

Functional Group	Bond/Type	Frequency (cm^{-1})	Nature of Peak
Alcohol	O–H	3200–3600	Broad, strong
Carboxylic acid	O–H	2500–3300	Very broad
Amine (1°)	N–H	3300–3500	Two sharp peaks
Amine (2°)	N–H	3300–3500	One sharp peak
Alkane	C–H (sp^3)	2850–2960	Strong
Alkene	C–H (sp^2)	3000–3100	Medium
Alkyne	C–H (sp)	~3300	Sharp
Alkyne	C≡C	2100–2260	Weak–medium

Nitrile	C≡N	2210–2260	Strong
Alkene	C=C	1620–1680	Medium
Aldehyde	C=O	1720–1740	Strong
Ketone	C=O	~1715	Strong
Carboxylic acid	C=O	~1700	Strong
Ester	C=O	1735–1750	Strong
Amide	C=O	1630–1690	Medium
Alcohol/Ether	C–O	1000–1300	Strong
Nitro compound	NO_2	1500 & 1350	Strong (two peaks)
Aromatic ring	C=C	1450–1600	Medium

Table 4.7: Functional groups and their characteristic IR absorption Frequencies. [1,2,5]

Let us take some examples

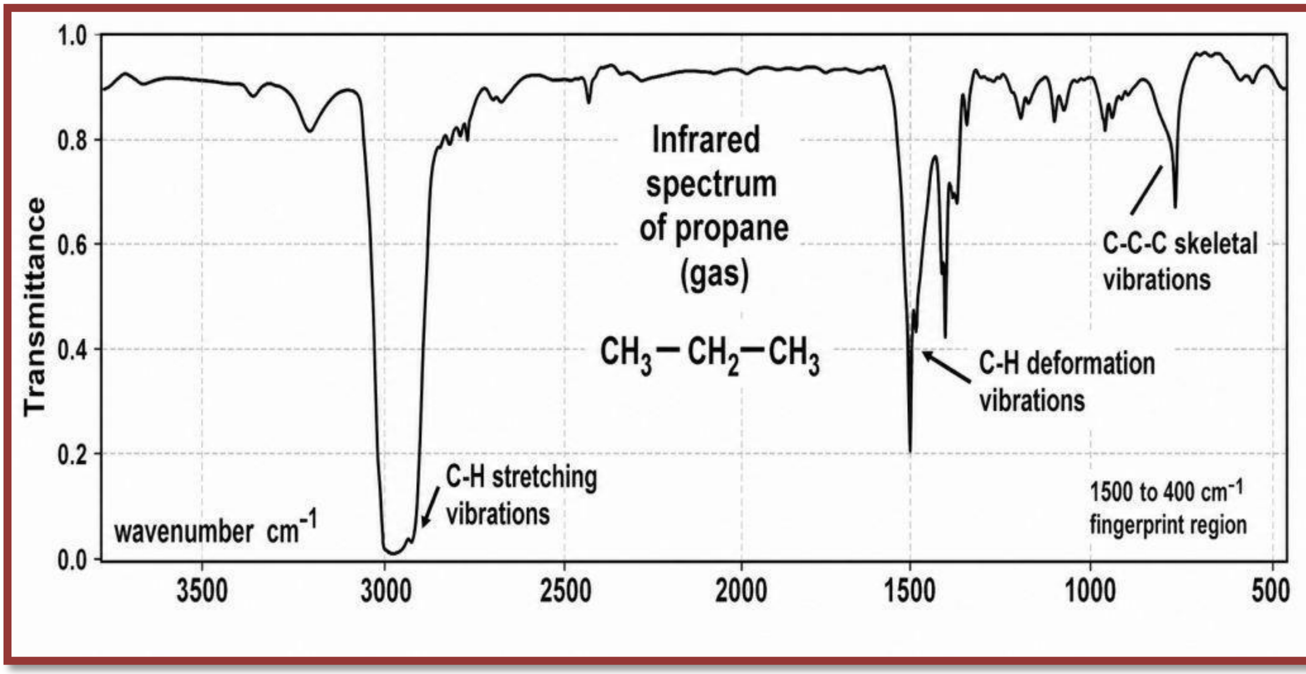

Figure 4.8: IR Spectrum of Propane (CH3-CH2-CH3).[1]

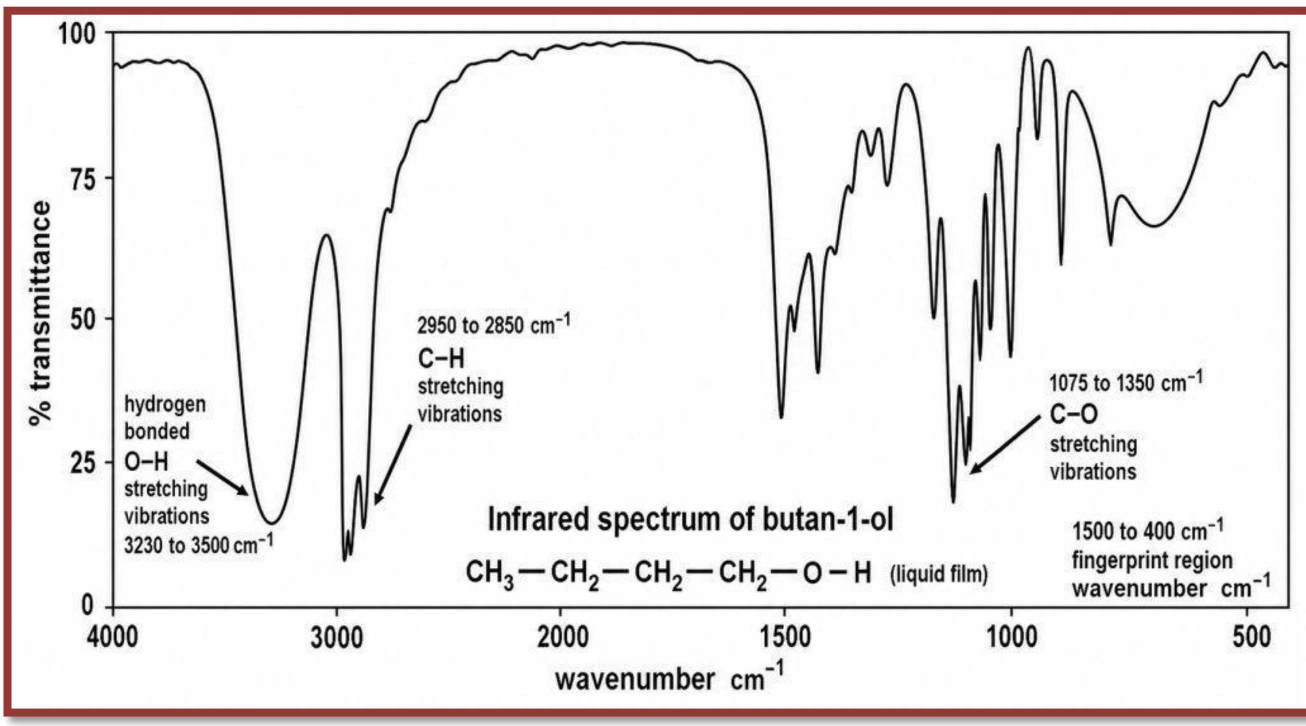

Figure 4.9: IR Spectrum of 1-Butanol (CH3-CH2-CH2-CH2-OH). [1]

Conclusion

Infrared spectroscopy is an essential analytical technique for understanding molecular structure and identifying chemical compounds through their characteristic vibrational behavior. The absorption of infrared radiation occurs when the energy of the radiation corresponds to an allowed molecular vibrational transition, making the resulting spectrum a characteristic molecular fingerprint. The study of stretching and bending vibrations provides important information about bond strength, bond length, molecular geometry, and chemical environment.

The number and nature of vibrational modes depend on the number of atoms and the geometry of a molecule. Diatomic, linear polyatomic, and non-linear polyatomic molecules therefore exhibit different numbers of normal modes. However, the theoretical number of vibrational modes does not necessarily correspond to the number of experimentally observed absorption bands because of IR inactivity, weak absorptions, overlapping bands, degeneracy, instrumental limitations, and spectral-range restrictions. The IR selection rule, particularly the requirement for a change in molecular dipole moment during vibration, is fundamental to understanding IR activity and band intensity. The functional-group region provides valuable information for recognizing characteristic chemical groups such as O–H, N–H, C–H, C=O, C≡N, C=C, and C–O, whereas the fingerprint region is particularly useful for confirming compound identity and distinguishing closely related structures. Phenomena such as overtones, combination bands, difference bands, vibrational coupling, and Fermi resonance further demonstrate the complexity of real molecular vibrational spectra.

Modern FTIR spectroscopy has significantly improved the speed, sensitivity, accuracy, and practicality of IR analysis. Its applications extend from fundamental chemical research to pharmaceutical quality control, drug identification, purity testing, structural confirmation, drug–excipient compatibility studies, stability assessment, forensic analysis, polymer characterization, food analysis, environmental monitoring, and biomedical research.

Thus, IR spectroscopy combines fundamental concepts of molecular vibration with powerful instrumental techniques to provide rapid and reliable chemical information. Its versatility, minimal sample requirements, non-destructive nature, and ability to generate highly characteristic spectra make it an indispensable technique in modern chemistry, pharmaceutical sciences, material science, and related fields.

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Chapter 5: Mass Spectroscopy

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5.1 Indroduction to Mass Spectroscopy:

Mass spectrometry (MS) is one of the most powerful and versatile instrumental analytical techniques used for the identification of chemical compounds, determination of molecular mass, structural elucidation, and qualitative and quantitative analysis. It has become an essential tool in modern analytical chemistry because of its high sensitivity, selectivity, speed, and ability to provide molecular-level information from very small quantities of a substance. Mass spectrometry is particularly important in organic chemistry, pharmaceutical chemistry, medicinal chemistry, biochemistry, natural-product chemistry, environmental chemistry, forensic science, food analysis, clinical chemistry, proteomics, metabolomics, and polymer science.

The fundamental difference between mass spectrometry and many conventional spectroscopic techniques is that MS does not primarily depend upon the interaction of electromagnetic radiation with molecules. Instead, it involves the conversion of molecules into gas-phase ions, separation of these ions according to their mass-to-charge ratio (m/z), and detection of the separated ions. The resulting information is presented as a mass spectrum, which can provide information about the molecular mass, molecular formula, isotopic composition, and structural characteristics of the analyte.

In a typical mass spectrometric experiment, the sample is introduced into the instrument and subjected to an appropriate ionization process. The neutral molecules are converted into positively or negatively charged species. These ions are then accelerated and introduced into a mass analyser. The mass analyser separates the ions according to their m/z values. Finally, the separated ions are detected and their signals are processed by a computer to produce the mass spectrum.

The general sequence can be represented as:

Sample introduction → Ionization → Ion acceleration → Mass separation → Ion detection → Data processing → Mass spectrum

The basic operation and sequence of a mass spectrometer are described in the source material as sample introduction followed by ionization, acceleration, mass separation, detection, and generation of the spectrum.

A mass spectrum is generally represented as a plot of m/z on the horizontal axis and relative abundance or intensity on the vertical axis. The most intense peak is called the base peak and is assigned a relative intensity of 100%. Other peaks are expressed relative to this peak. The molecular ion or molecular-related ion may provide information about molecular mass, whereas fragment ions provide information about the structure of the molecule.

Mass spectrometry is applicable to a very wide range of substances. Small organic molecules, pharmaceuticals, pesticides, natural products, metabolites, peptides, proteins, lipids, polymers and other chemically important substances can be studied by suitable MS techniques. Modern instruments can also be coupled with separation methods such as gas chromatography and liquid chromatography, producing powerful hyphenated techniques such as GC–MS and LC–MS.

The importance of MS has increased considerably with the development of modern ionization methods and high-performance mass analysers. Soft ionization techniques such as electrospray ionization allow relatively large and thermally unstable molecules to be analysed, while high-resolution instruments can provide accurate mass measurements useful for molecular-formula determination. Thus, mass spectrometry should not be regarded simply as a method for determining molecular weight. It is a comprehensive analytical technique capable of providing molecular, structural, qualitative, quantitative and compositional information [5].

5.2 Principle of Mass Spectrometry

The fundamental principle of mass spectrometry involves three major operations:

1. Formation of ions
2. Separation of ions according to m/z
3. Detection of the separated ions

The basic principle described in the source is that a sample is converted into ions, the ions are separated according to their mass-to-charge ratios, and the separated ions are detected to generate a mass spectrum [1].

The simplified sequence is:

Sample → Ionization → Ion acceleration → Mass separation → Detection → Mass spectrum

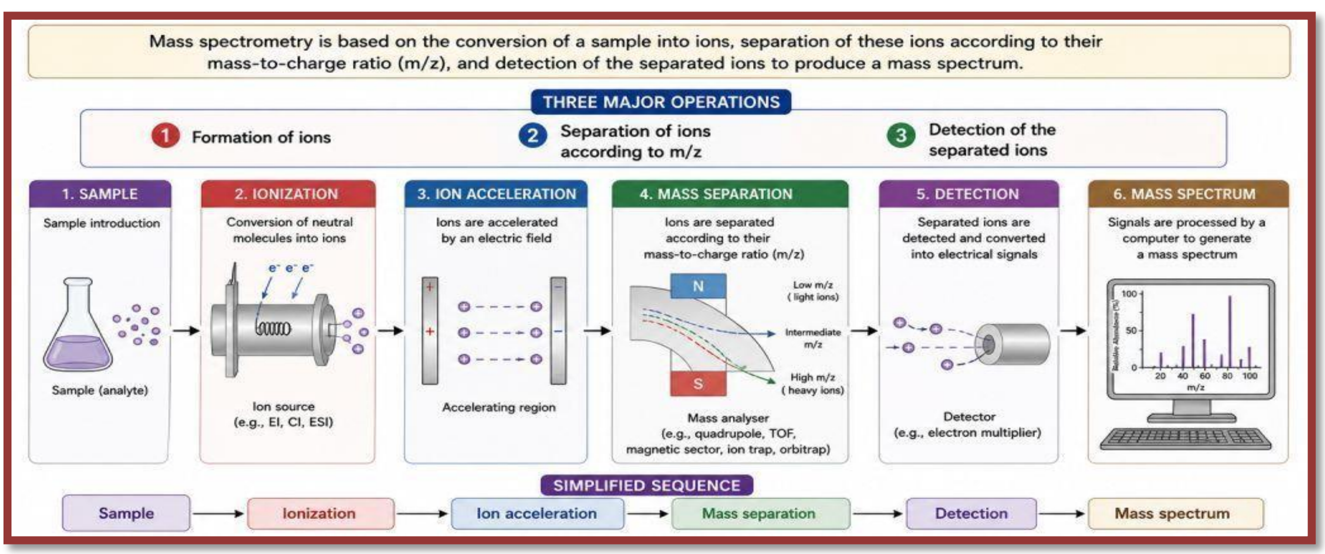

Figure 5.1: Principle of Mass Spectrometry

5.2.1 Ion Formation

The first requirement of MS analysis is the formation of charged particles. Most chemical compounds in their normal state are electrically neutral. A mass spectrometer, however, operates on charged particles. Therefore, the analyte must be converted into ions.

Depending upon the ionization technique, an analyte may:

- Lose an electron
- Gain an electron
- Accept a proton
- Lose a proton
- Form an adduct with another species
- Become multiply charged

For example, electron ionization produces a molecular radical cation:

$$M+e^{-}\rightarrow M^{+\cdot}+2e^{-}$$

In electrospray ionization, protonation may occur:

$$M+H^{+}\rightarrow[M+H]^{+}$$

or deprotonation may occur:

$$M-H^{+}\rightarrow[M-H]^{-}$$

The type of ion produced depends strongly on the ionization method and chemical nature of the analyte [5].

5.2.2 Acceleration of Ions

Once ions are generated, they must be transferred into the mass analyser. In many mass spectrometers, electric fields are used to accelerate the ions. The ions acquire kinetic energy and move through the instrument under controlled conditions.

The motion of an ion depends on several factors, including:

- Its mass
- Its charge
- Applied electric field

- Applied magnetic field, where applicable
- Instrument geometry

The controlled movement of ions is essential for their subsequent separation according to m/z [1].

5.2.3 Separation According to m/z

The key analytical function of the mass analyser is to distinguish ions having different mass-to-charge ratios.

The quantity measured by a mass spectrometer is:

$$m/z$$

where:

- m = mass of the ion
- z = number of elementary charges carried by the ion

For a singly charged ion:

z=1and therefore the measured m/z approximately corresponds to the mass of that ion.

For multiply charged ions, the observed m/z is lower than the actual molecular mass. Multiply charged ions are especially important in the analysis of large molecules such as proteins and other biomolecules.

5.2.4 Detection

After separation, the ions reach the detector. The detector converts the arrival of ions into electrical signals. The magnitude of the signal is related to the abundance of the ions reaching the detector. The computer processes these signals and generates the mass spectrum [1].

5.3 Theory of Mass Spectrometry

The theoretical basis of mass spectrometry is the conversion of neutral molecules into charged species followed by their controlled movement and separation under electric and/or magnetic fields. Depending on the ionization technique, a molecule may lose an electron, accept a proton, lose a proton, or form another type of charged species.

The theory can be understood through four important concepts:

1. Ion formation
2. Mass-to-charge ratio
3. Ion acceleration and separation

4. Fragmentation

5.3.1 Formation of Ions: Ion formation is the central event in mass spectrometry. Different ionization techniques produce different types of ions.

Electron Ionization: In electron ionization (EI), gaseous molecules are exposed to high-energy electrons. An electron is removed from the molecule to produce a molecular radical cation:

$$M + e^- \rightarrow M^{+\cdot} + 2e^-$$

The molecular ion may contain excess internal energy. Consequently, it may undergo cleavage of chemical bonds and produce fragment ions.

For example: $M^{+\cdot} \rightarrow A^+ + B^\cdot$

The fragment ions produced during EI are often characteristic of the structure of the original compound. EI is therefore highly useful for structural identification and library-based identification of volatile organic compounds. The source specifically describes EI as producing molecular radical cations and extensive fragmentation and notes its common application in GC–MS [5].

5.3.2 Soft Ionization: Some molecules are easily fragmented by high-energy ionization methods. For such compounds, softer ionization methods are preferred. In soft ionization, the energy transferred to the molecule is relatively low, so the molecular ion or molecular-related ion can survive with less fragmentation.

Important soft ionization techniques include:

- Chemical ionization
- Electrospray ionization
- MALDI
- Atmospheric-pressure chemical ionization

These techniques are particularly important for pharmaceutical compounds, peptides, proteins and other thermally labile molecules [1].

5.3.3 Molecular Ion: The molecular ion is the ion corresponding to the intact molecule after ionization, particularly in electron ionization. It is commonly represented as: $M^{+\cdot}$

The molecular-ion peak can provide information about the molecular mass of the compound. However, the molecular ion may be absent or weak in some mass spectra because the molecule may undergo extensive fragmentation. Therefore, molecular-mass determination must take into account the ionization method used [1].

5.3.4 Molecular-Related Ions: In softer ionization techniques, the intact molecule may appear in the spectrum in forms other than the radical cation. Common examples include:

$$[M+H]^+ \quad [M-H]^-$$

Other adduct ions may also occur, depending on the sample, solvent, mobile phase and ionization conditions. Such molecular-related ions are particularly common in electro-spray ionization [1].

5.3.5 Mass-to-Charge Ratio: The mass spectrometer separates ions according to their mass-to-charge ratio.

$$m/z$$

For a singly charged ion: z=1, so:

$$m/z \approx m$$

For a doubly charged ion: z=2 and:

$$m/z = 2m$$

For example, if a molecule of mass 1000 Da produces a doubly charged ion, the observed m/z would be approximately 500, ignoring the mass contribution of the ionizing species [2].This principle is particularly important for high-molecular-weight biomolecules because they can produce multiple charge states.

5.3.6 Fragmentation: Fragmentation is one of the most useful aspects of mass spectrometry for structural elucidation. After ion formation, an energized ion may undergo cleavage of one or more chemical bonds. The resulting fragment ions provide information about the molecular structure.

Fragmentation depends on:

- Molecular structure
- Bond strength
- Stability of the resulting ions
- Functional groups
- Position of substituents
- Ionization energy
- Instrumental conditions

The source emphasizes that fragmentation can provide information about molecular mass, molecular formula when accurate mass is available, functional groups and structural features, characteristic fragmentation pathways, and compound identity.

Importance of Fragmentation

The molecular ion alone may only provide information about molecular mass. Fragment ions can provide additional structural information.Thus:

Molecular ion → Molecular mass

Fragment ions → Structural information

Complete fragmentation pattern → Structural fingerprint

5.3.7 Base Peak: The base peak is the most intense peak in a mass spectrum. It is assigned a relative abundance of 100%.The base peak does not necessarily represent the molecular ion. It may correspond to a particularly stable fragment ion.

Therefore, the following distinction is important:

- **Molecular ion:** indicates the intact molecular species under the relevant ionization conditions.
- **Base peak:** the most abundant detected ion.

These two peaks may be the same or different [3].

5.3.8 Isotopic Peaks: Naturally occurring isotopes can produce additional peaks in a mass spectrum. For example, carbon occurs predominantly as ^{12}C but also contains a smaller proportion of ^{13}C. Consequently, compounds containing carbon may show an M+1 isotope peak. Elements such as chlorine and bromine produce particularly characteristic isotope patterns. Such patterns can assist in recognizing the presence of these elements and can support molecular-formula determination [1].

5.4 Instrumentation of Mass Spectrometer

A typical mass spectrometer consists of several interconnected components:

Sample inlet → Ion source → Mass analyser → Detector → Data system

The source material identifies these five major components and describes their individual functions. Each component performs a specific function in the overall analytical process.

5.4.1 Sample Introduction System: The sample introduction system transfers the analyte into the mass spectrometer under suitable conditions. The method depends on the nature of the sample.

For volatile compounds: Volatile and thermally stable compounds can often be introduced directly into the ion source or separated by gas chromatography before entering the mass spectrometer.

For non-volatile compounds: Less volatile and thermally sensitive compounds are often introduced through liquid chromatography and ionized using techniques such as electrospray ionization. The source notes that volatile compounds may be introduced directly or through GC, whereas less volatile and thermally sensitive compounds can be introduced using LC coupled to ESI.

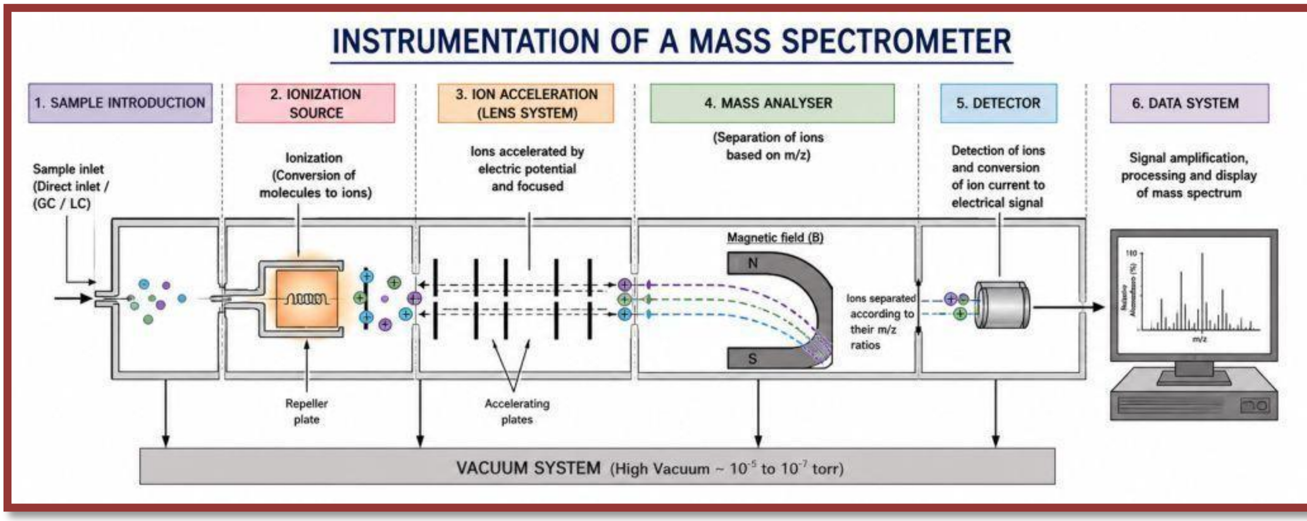

Figure 5.2: Instrumentation of Mass Spectrometer

5.4.2 Ion Source: The ion source is responsible for converting neutral analyte molecules into ions.

The selection of an ion source depends on:

- Volatility
- Molecular mass
- Polarity
- Thermal stability
- Chemical structure
- Desired fragmentation
- Nature of the sample

Important ionization techniques are discussed below.

A. Electron Ionization (EI)

Electron ionization is a high-energy ionization technique widely used for volatile organic compounds. The sample must generally be present in the gas phase. A beam of energetic electrons interacts with the analyte molecule and removes an electron:

$$M + e^- \longrightarrow M^{+\cdot} + 2e^-$$

The resulting molecular radical cation can undergo extensive fragmentation.

Characteristics of EI

- High-energy ionization
- Reproducible spectra
- Extensive fragmentation
- Useful structural information
- Commonly used with GC–MS
- Suitable for volatile and thermally stable compounds

The source identifies EI, commonly at 70 eV, as a technique producing molecular radical cations and extensive fragmentation [5].

B. Chemical Ionization (CI): Chemical ionization is a relatively softer technique. In CI, a reagent gas is first ionized. The reagent-gas ions then interact with the analyte and produce ions through processes such as proton transfer. A commonly encountered product is: $[M+H]^+$

Because less energy is transferred directly to the analyte, fragmentation is generally reduced compared with EI.

Advantages of CI

- Reduced fragmentation
- Strong molecular-related ion
- Useful molecular-mass information
- Complementary to EI

The source describes CI as a softer technique that generally produces less fragmentation than EI and can provide useful molecular-mass information [4].

C. Electrospray Ionization (ESI): Electrospray ionization is a soft ionization technique widely used in LC–MS.

It is particularly suitable for:

- Polar compounds
- Ionic compounds
- Thermally labile substances

- High-molecular-weight compounds
- Peptides
- Proteins
- Pharmaceutical compounds

In ESI, a solution containing the analyte is converted into highly charged droplets. Solvent evaporation and subsequent processes ultimately produce gas-phase ions.

For positive-ion mode:

$$M+H+ \rightarrow [M+H]^{+}$$

For negative-ion mode:

$$M-H^{+} \rightarrow [M-H]^{-}$$

ESI can also produce multiply charged ions, making it possible to analyse very large molecules within the mass range of the instrument.The source identifies ESI as particularly suitable for polar, ionic, thermally labile and relatively high-molecular-weight compounds and notes its extensive use in LC–MS and biological analysis [1].

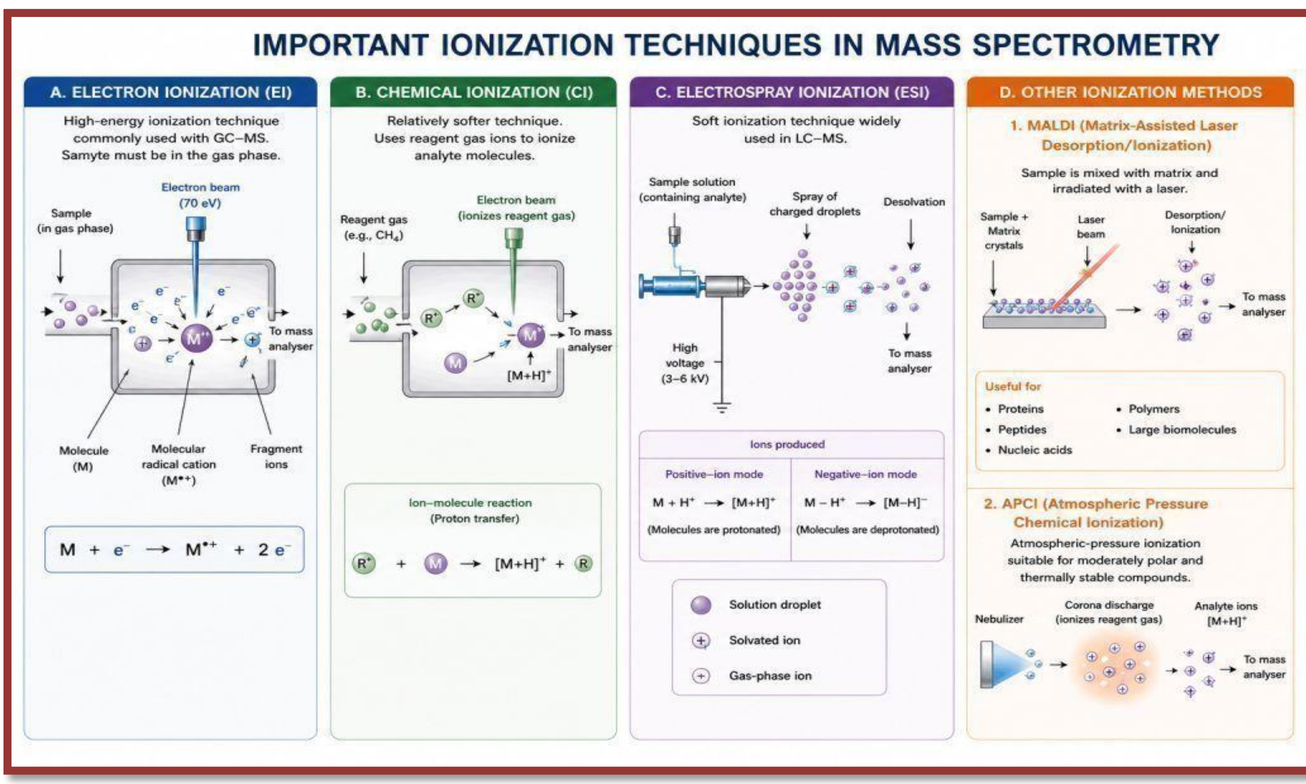

Figure 5.3: Different Ionization Techniques in Mass Spectrometry

D. Other Ionization Methods: Modern MS instruments may also employ other ionization methods depending upon the application.

Matrix-Assisted Laser Desorption/Ionization (MALDI): MALDI is widely used for large biomolecules and polymers. The analyte is mixed with an appropriate matrix and irradiated with a laser. The matrix assists in the desorption and ionization process.

MALDI is particularly useful for:

- Proteins
- Peptides
- Nucleic acids
- Polymers
- Large biomolecules

Atmospheric Pressure Chemical Ionization (APCI)

APCI is another atmospheric-pressure ionization method commonly used for compounds that are less suitable for ESI, particularly moderately polar and relatively thermally stable compounds [4].

Comparison of Important Ionization Techniques

Ionization technique	Nature	Typical application	Fragmentation
EI	Hard ionization	GC–MS, volatile organic compounds	Extensive
CI	Softer than EI	Molecular-mass information	Relatively low
ESI	Soft ionization	LC–MS, pharmaceuticals, biomolecules	Generally low
MALDI	Soft ionization	Proteins, peptides, polymers	Generally low
APCI	Soft/atmospheric-pressure ionization	Moderately polar compounds	Relatively limited

Table 5.1: Comparison of Important Ionization Techniques

5.4.3 Mass Analyser: The mass analyser is one of the most important components of a mass spectrometer. Its primary function is to separate ions according to their m/z values [1].

The source identifies the following major types:

1. Magnetic-sector analyser
2. Quadrupole analyser
3. Time-of-flight analyser
4. Ion-trap analyser
5. Orbitrap analyser

A. Magnetic-Sector Analyser: A magnetic-sector analyser uses a magnetic field to influence the trajectories of charged ions.Ions with different m/z values are deflected by different amounts under the applied field. Appropriate adjustment of the field therefore permits ions of selected m/z values to reach the detector.Magnetic-sector instruments can provide high mass accuracy and resolution and have historically played an important role in high-performance mass spectrometry.

B. Quadrupole Mass Analyser: A quadrupole consists of four parallel rods to which radio-frequency and direct-current electrical potentials are applied.The oscillating electric field causes ions of different m/z values to follow different trajectories.Under suitable operating conditions, ions with a particular m/z range have stable trajectories and pass through the quadrupole to the detector, whereas other ions are destabilized and do not pass through.The source describes the quadrupole as using oscillating electric fields to selectively transmit ions of particular m/z values.

Advantages

- Compact design
- Rapid scanning
- Good quantitative performance
- Widely used in GC–MS and LC–MS
- Particularly useful in routine analytical laboratories

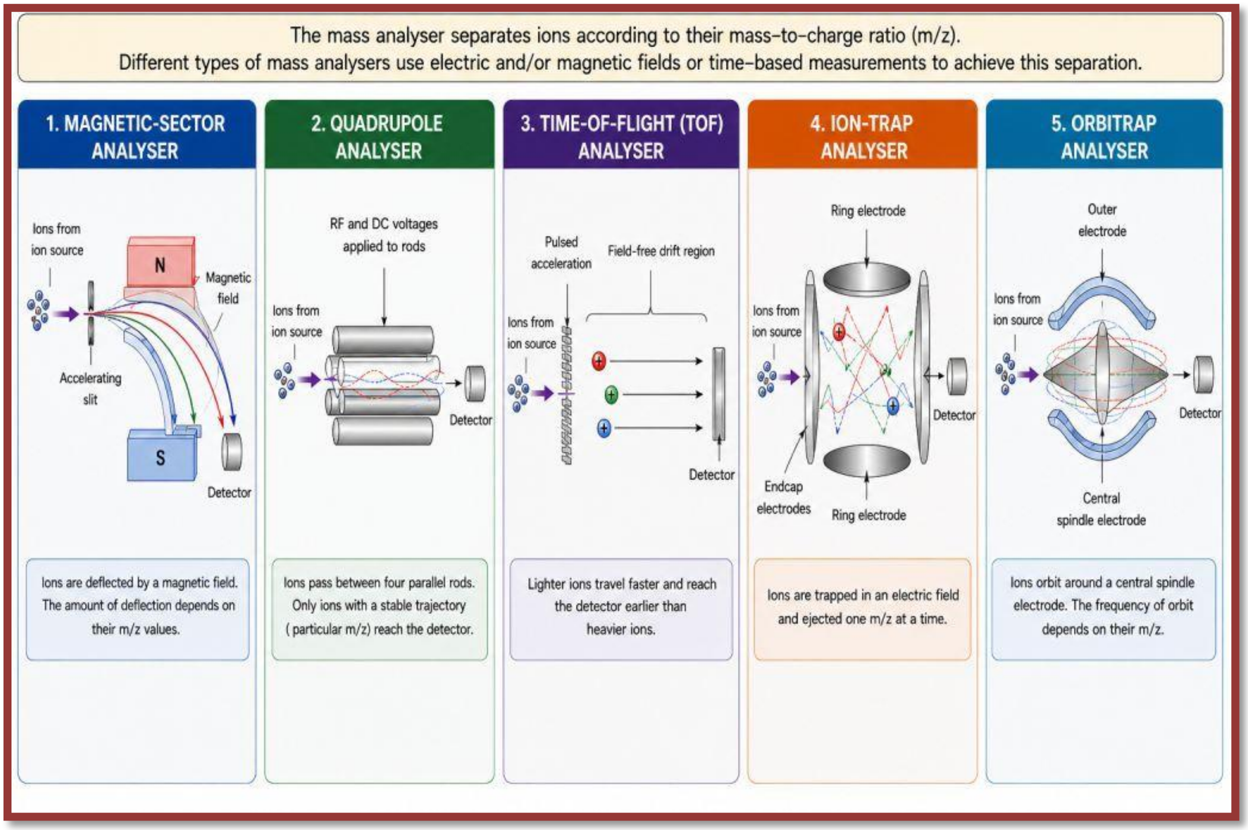

Figure 5.4: Types of Mass Analysers in Mass Spectroscopy

C. Time-of-Flight (TOF) Analyser: In a TOF analyser, ions are accelerated to approximately the same kinetic energy.Their velocities then depend on their mass-to-charge ratios.Lighter ions travel faster, while heavier ions travel more slowly.Therefore:

Lower m/z → shorter flight time

Higher m/z → longer flight time

The flight time is measured and converted into m/z.The source specifically notes that lighter ions generally reach the detector faster than heavier ions in TOF analysis.

TOF instruments are particularly useful when:

- Rapid analysis is required
- A broad mass range is needed
- High-mass ions are being studied
- MALDI is used as the ionization technique

D. Ion-Trap Analyser: An ion trap confines ions using electric fields. Ions can be accumulated and subsequently ejected according to their m/z values. Ion traps are especially useful for tandem mass spectrometry because selected ions can be isolated and fragmented repeatedly.

E. Orbitrap Analyser: The Orbitrap is a high-resolution mass analyser in which ions move around a central electrode under the influence of an electrostatic field. The frequency of ion oscillation is related to its m/z value.

Orbitrap instruments can provide:

- High mass resolution
- High mass accuracy
- Accurate-mass measurements
- Detailed analysis of complex mixtures

These characteristics make them particularly valuable in proteomics, metabolomics, pharmaceutical analysis and natural-product research [1,2].

5.4.4 Detector: The detector receives the ions after separation by the mass analyser and converts the ion signal into an electrical signal.Common detectors include:

- Electron multipliers
- Microchannel plates
- Other ion-detection devices

The source notes that the detector converts ion arrival into an electrical signal and that the signal intensity is related to the abundance of the corresponding ions.

Electron Multiplier: An electron multiplier amplifies the signal generated by an incoming ion. The initial impact produces secondary electrons, which generate additional electrons through successive collisions. This produces an amplified electrical signal [5].

Comparison of Important Mass Analysers

Mass analyser	Basic principle	Important characteristic
Magnetic sector	Deflection in magnetic field	High resolution/accuracy in suitable configurations

Quadrupole	Stability in oscillating electric fields	Fast, compact and widely used
TOF	Measurement of ion flight time	Broad mass range and rapid acquisition
Ion trap	Storage and controlled ejection of ions	Useful for MS/MS
Orbitrap	Measurement of ion oscillation frequencies	High resolution and accurate mass

Table 5.2: Comparison of Important Mass Analyzers

5.4.5 Data Processing System: The detector produces electrical signals that are transferred to a computer.

Modern MS software can:

- Generate mass spectra
- Determine peak positions
- Measure peak intensities
- Identify molecular-related ions
- Compare spectra with databases
- Determine accurate masses
- Assist in molecular-formula determination
- Process MS/MS data
- Perform quantitative calculations

The source specifically identifies computer processing as the stage in which detector signals are converted into mass spectra and used for peak identification, molecular-mass determination and spectral comparison [4,5].

5.5 Mass Spectrum

A mass spectrum is the graphical representation of the ions detected during a mass spectrometric analysis.Generally:

X-axis → m/z

Y-axis → Relative abundance/intensity

The highest-intensity peak is assigned an abundance of 100% and is called the **base peak**. Other peaks are expressed relative to the base peak.

A mass spectrum may contain:

- Molecular ion
- Molecular-related ions
- Fragment ions
- Isotope peaks
- Adduct ions
- Multiply charged ions

The interpretation of these peaks provides information about the chemical identity and structure of the analyte [4].

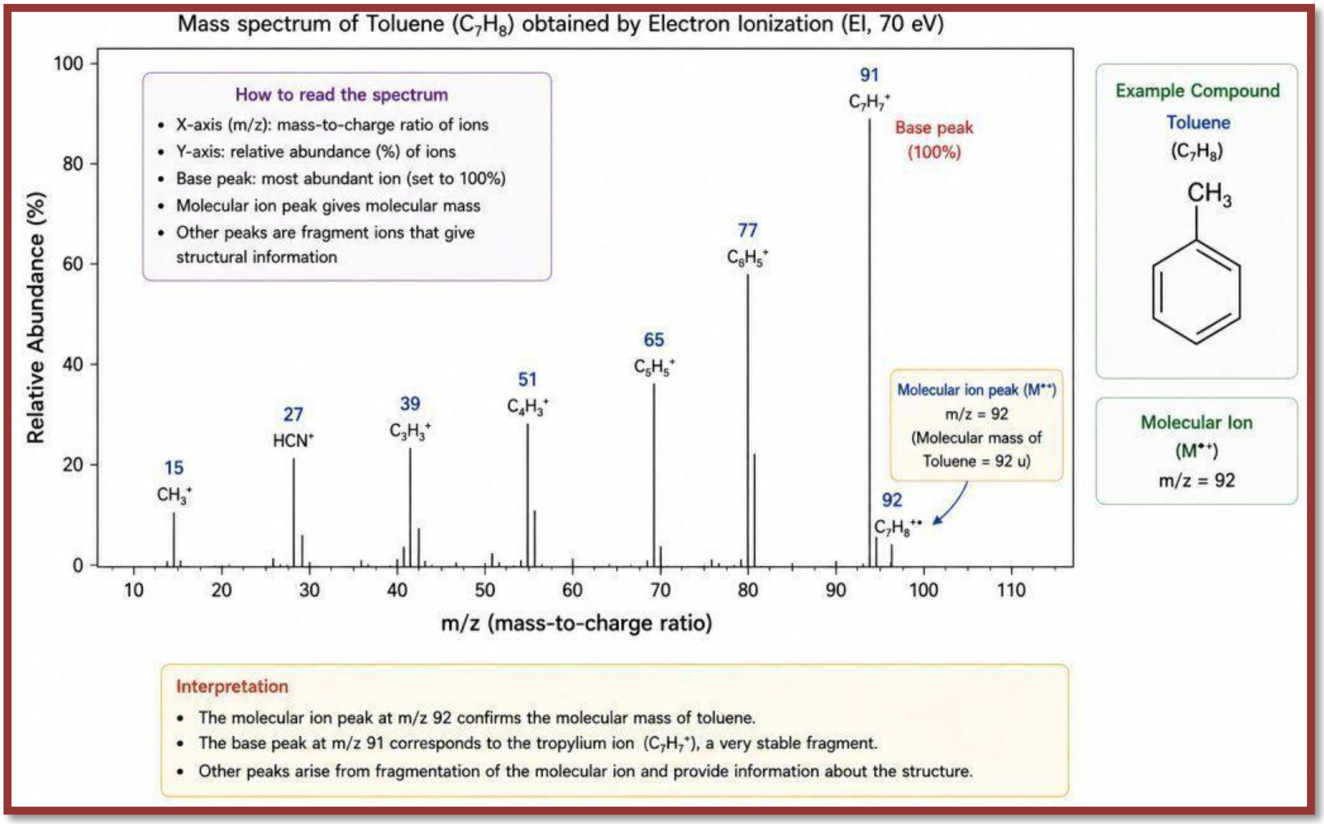

Figure 5.5: Mass Spectrum of Toluene (C_7H_8)

5.5.1 Molecular Ion Peak: The molecular ion peak represents the intact molecule after ionization under appropriate conditions.

For electron ionization: $M^{+\cdot}$

The molecular ion can provide molecular-mass information.However, some compounds undergo extensive fragmentation and may produce a very weak molecular ion or no observable molecular-ion peak [5].

5.5.2 Base Peak: The base peak is the most intense peak in the mass spectrum.Its intensity is arbitrarily assigned a value of:100% .All other peaks are expressed relative to this intensity.The base peak may correspond to the molecular ion or to a fragment ion.

5.5.3 Fragment Peaks: Fragment peaks arise when the molecular ion or precursor ion breaks into smaller ions.These peaks are particularly important for structural identification because different compounds can produce characteristic fragmentation patterns.

5.6 Applications of Mass Spectrometry

Mass spectrometry has extensive applications in chemical, pharmaceutical, biological, environmental and forensic sciences. The source identifies molecular-mass determination, structural elucidation, identification of unknown compounds, molecular-formula determination, pharmaceutical analysis, natural products, chromatography–MS, and quantitative analysis as major applications [1,4,5].

5.6.1 Determination of Molecular Mass: One of the fundamental applications of MS is the determination of molecular mass.The molecular ion or molecular-related ion can provide information about the molecular weight of an unknown compound. This information can be combined with elemental analysis and other spectroscopic techniques to establish the identity of a compound [5].

5.6.2 Structural Elucidation: Mass spectrometry provides structural information through fragmentation.It can help determine:

- Molecular mass
- Molecular formula
- Structural fragments
- Functional-group-related fragmentation
- Characteristic cleavage pathways
- Identity of unknown substances

MS is frequently used along with IR, NMR and UV–Visible spectroscopy for complete structural characterization [4].

5.6.3 Identification of Unknown Compounds: An unknown compound can be identified by comparing its experimental mass spectrum with spectra of known compounds in spectral libraries.This approach is especially valuable in:

- Organic analysis
- Environmental analysis
- Forensic science
- Pharmaceutical analysis
- Food chemistry
- Natural-product research

Library matching is particularly powerful when combined with chromatographic separation [4].

5.6.4 Molecular Formula Determination: High-resolution mass spectrometry can measure mass with high accuracy.Accurate mass measurements can help distinguish between possible molecular formulas having similar nominal masses.For example, two compounds may have the same nominal molecular mass but different exact masses. High-resolution MS can therefore provide valuable information for determining possible elemental compositions.The source identifies HRMS as particularly useful for molecular-formula determination in natural-product and pharmaceutical research [5].

5.6.5 Pharmaceutical and Medicinal Chemistry: Mass spectrometry has become an important technique in pharmaceutical research and development.It is used for:

- Drug identification
- Drug purity assessment
- Drug-metabolite identification
- Pharmacokinetic studies
- Drug degradation studies
- Pharmaceutical quality control
- Bioanalysis
- Therapeutic-drug monitoring

LC–MS and LC–MS/MS are particularly useful because pharmaceutical compounds are often present in complex biological matrices such as plasma, serum and urine.The source specifically identifies drug identification, purity assessment, metabolite identification, pharmacokinetic studies and pharmaceutical-compound analysis as important applications [1,4].

5.6.6 Natural Products: Natural products frequently contain complex mixtures of chemically related compounds. MS is highly useful for their characterization.Applications include:

- Identification of plant metabolites
- Characterization of alkaloids
- Analysis of flavonoids
- Identification of terpenoids
- Characterization of phenolic compounds
- Analysis of microbial metabolites
- Detection of bioactive compounds

LC–MS can be particularly useful because many natural products are polar and thermally unstable [1,4].

5.6.7 Biochemistry and Biomolecular Analysis: Mass spectrometry is extensively used in the study of:

- Peptides
- Proteins
- Amino acids
- Lipids
- Carbohydrates
- Metabolites
- Biomarkers

Its high sensitivity allows analysis of very small quantities of biological material.The source specifically identifies peptides, proteins, lipids and metabolites among important biological applications [1].

5.6.8 Proteomics: Proteomics involves the large-scale study of proteins.Mass spectrometry can be used to:

1. Identify proteins.
2. Determine peptide sequences.
3. Detect post-translational modifications.

4. Compare protein expression.
5. Characterize protein interactions.
6. Study disease-associated changes.

Tandem MS is particularly valuable because peptide precursor ions can be selected and fragmented to obtain sequence-related information [1].

5.6.9 Metabolomics: Metabolomics involves the study of small molecules present in biological systems.MS-based metabolomics can detect and characterize a large number of metabolites in biological samples [1].Applications include:

- Disease biomarker discovery
- Drug-response studies
- Metabolic-pathway studies
- Nutritional studies
- Toxicological investigations

5.6.10 Chromatography–Mass Spectrometry: One of the most important developments in analytical chemistry is the coupling of chromatography with mass spectrometry.The source identifies GC–MS and LC–MS as major examples.

GC–MS: Gas chromatography separates volatile components before they enter the mass spectrometer [1].It is particularly useful for:

- Volatile organic compounds
- Solvents
- Essential oils
- Flavour compounds
- Fragrance compounds
- Pesticides
- Forensic substances
- Environmental pollutants

LC–MS: Liquid chromatography separates compounds before they enter the MS [1].It is particularly suitable for:

- Polar compounds
- Non-volatile compounds
- Thermally unstable compounds
- Pharmaceuticals
- Biomolecules
- Metabolites
- Natural products

Thus, chromatography provides separation, while MS provides mass-based detection and identification.

5.6.11 Tandem Mass Spectrometry (MS/MS): Tandem mass spectrometry involves at least two stages of mass analysis.

A simplified process is:

Ion formation → Selection of precursor ion → Fragmentation → Analysis of product ions

The first stage selects a precursor ion. The selected ion is then fragmented, and the resulting product ions are analysed.MS/MS provides substantially greater selectivity and structural information than a single-stage mass analysis.It is widely used for:

- Drug analysis
- Metabolite identification
- Peptide sequencing
- Protein analysis
- Biomarker analysis
- Trace-level quantitative analysis

The source specifically notes the high sensitivity and selectivity of tandem MS for quantitative analysis [1].

5.6.12 Environmental Analysis: Mass spectrometry is widely used for monitoring environmental contaminants.Examples include:

- Pesticide residues
- Industrial pollutants

- Organic contaminants
- Hydrocarbons
- Pharmaceutical residues
- Persistent organic compounds

GC–MS and LC–MS can provide highly sensitive analysis of contaminants present at low concentrations [4].

5.6.13 Food Analysis: MS is used in food science for:

- Detection of pesticide residues
- Identification of food contaminants
- Analysis of flavours and fragrances
- Detection of adulterants
- Nutritional analysis
- Authentication of food products
- Identification of metabolites

Chromatography coupled with MS is especially useful when the sample contains many chemically related components [5].

5.6.14 Forensic Science: Mass spectrometry has important forensic applications.It can assist in identifying:

- Drugs of abuse
- Toxic substances
- Poisons
- Unknown chemicals
- Explosives-related residues
- Biological compounds

GC–MS is particularly important for the identification of volatile and semi-volatile forensic compounds [4].

5.7 Advantages of Mass Spectrometry

The major advantages of mass spectrometry include:

1. High sensitivity: MS can detect very small quantities of analytes.

2. High selectivity: Measurement of m/z provides highly selective detection.

3. Molecular-mass information: MS can provide direct information about molecular or molecular-related ions.

4. Structural information: Fragmentation patterns provide information about molecular structure.

5. Accurate-mass determination: High-resolution instruments can provide highly accurate mass measurements.

6. Wide applicability: MS can be applied to small organic molecules as well as large biomolecules.

7. Coupling with separation techniques: GC–MS and LC–MS combine separation with mass-based detection.

8. Quantitative capability: Modern MS, especially MS/MS, permits sensitive quantitative analysis.

9. Small sample requirement: Only small quantities may be required for many analyses.

10. Rapid analysis: Modern instruments can acquire large amounts of analytical information rapidly.

5.8 Limitations of Mass Spectrometry

Although MS is extremely powerful, it also has certain limitations.

1. High cost: Mass spectrometers, particularly high-resolution instruments, can be expensive to purchase and maintain.

2. Skilled operation: Proper operation and interpretation require trained personnel.

3. Sample preparation: Complex samples may require extensive extraction, purification or chromatographic separation.

4. Matrix effects: Components of the sample matrix can suppress or enhance ionization, particularly in ESI.

5. Ionization dependence: Not all compounds ionize equally efficiently using a particular ionization technique.

6. Fragmentation complexity: Complex molecules may produce complicated spectra that require considerable expertise to interpret.

7. Isomer discrimination: Compounds having identical molecular masses may require complementary techniques or advanced MS/MS approaches for complete differentiation.

8. Complementary techniques may be necessary: MS does not always provide complete structural information by itself. IR, NMR, UV–Visible spectroscopy and chromatographic information may therefore be required.

5.9 Conclusion

Mass spectrometry is a highly sensitive and versatile analytical technique based on the formation, separation and detection of ions according to their mass-to-charge ratio (m/z). It provides information about molecular mass, molecular formula, isotopic composition and molecular structure. The source material establishes ion formation, m/z-based separation, fragmentation, instrumentation and major applications as the central concepts of MS.

The development of different ionization methods, including EI, CI and ESI, has enabled the analysis of compounds having widely different physical and chemical properties. Similarly, the development of quadrupole, TOF, ion-trap, magnetic-sector and Orbitrap analysers has expanded the analytical capabilities of MS.

The coupling of mass spectrometry with chromatography, particularly GC–MS and LC–MS, has further increased its usefulness for the analysis of complex mixtures. Modern MS and MS/MS systems are now indispensable in pharmaceutical analysis, natural-product research, proteomics, metabolomics, environmental analysis, forensic science and many other areas.

Therefore, mass spectrometry is not merely a technique for measuring molecular mass; it is a comprehensive analytical platform capable of providing qualitative identification, quantitative determination, molecular-formula information and structural elucidation, often at very low analyte concentrations. When combined with complementary techniques such as IR, NMR, UV–Visible spectroscopy and chromatography, MS provides a powerful approach for the complete characterization of chemical and biological substances.

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