# ORGANIC SPECTROSCOPY TECHNOLOGY AND APPLICATIONS

Editor:
**Manisha C. Kotadiya**

**Bentham Books**

# Organic Spectroscopy Technology and Applications

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## Organic Spectroscopy Technology and Applications

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ISBN (Online): 978-981-5313-23-9

ISBN (Print): 978-981-5313-24-6

ISBN (Paperback): 978-981-5313-25-3



First published in 2024.

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# PREFACE

Today, spectroscopy is the most widely used tool for determining and analyzing molecular structures. As a result, understanding spectroscopy has become essential for all organic chemistry students. The book covers an introduction to electromagnetic radiation, UV-visible, infrared, Raman, 1H NMR, and mass spectroscopy, as well as a discussion on the field of spectroscopy, structural analysis, and problem-solving techniques.

The material of this book is structured in such a way that students may progressively get a comprehensive understanding of the said approaches. The book contains several illustrated diagrams, including spectra and diagrams of the instruments used in the studied approaches. A few chapters also discuss the solved problems.

The material has been given in a thorough, lucid, and methodical manner that is simple to grasp. I feel that learning *via* problem-solving techniques instills skills and gives a good grasp of the topic.

We drew material for this book from a variety of sources, including review articles, spectroscopic reference works, spectrum catalogues, and other publications. Although individual acknowledgement is not possible, I take great delight in expressing my gratitude to all contributors to the aforementioned source.

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# CHAPTER 1

# An Electromagnetic Spectrum

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**Abstract:** Spectroscopy has historically played an important role in the development of atomic theory, and it is currently widely utilised in basic research. In forensic laboratories, spectroscopic methods are commonly employed for quantitative and qualitative analysis. This chapter gives an overview of the fundamental concepts of spectroscopy as they apply to analytical measurements.

**Keywords:** Absorption maximum, Absorption spectra, Electromagnetic radiation, Emission spectra, Energy levels, Frequency, Rays, Rotational energy, Transitional energy, Vibrational energy, Wavenumber.

## INTRODUCTION

In the field of physical science known as spectroscopy, molecules, and electromagnetic radiation are examined in relation to one another for calculating energy.

The term "spectroscopy" refers to the study of differences seen between molecular energy state and energy absorbed or released by molecules or atoms. The "spectrophotometer" is the name of an instrument used for this kind of particular research.

The graph of absorbance/transmittance *vs*. $\tilde{\nu}/\lambda$ is obtained as a result [1].

### Electro-magnetic Radiation

In space, energy is conveyed in the form of electromagnetic radiation [1, 7]. As shown in Fig. (**1**), electromagnetic radiation is a combination of electrical and magnetic components.

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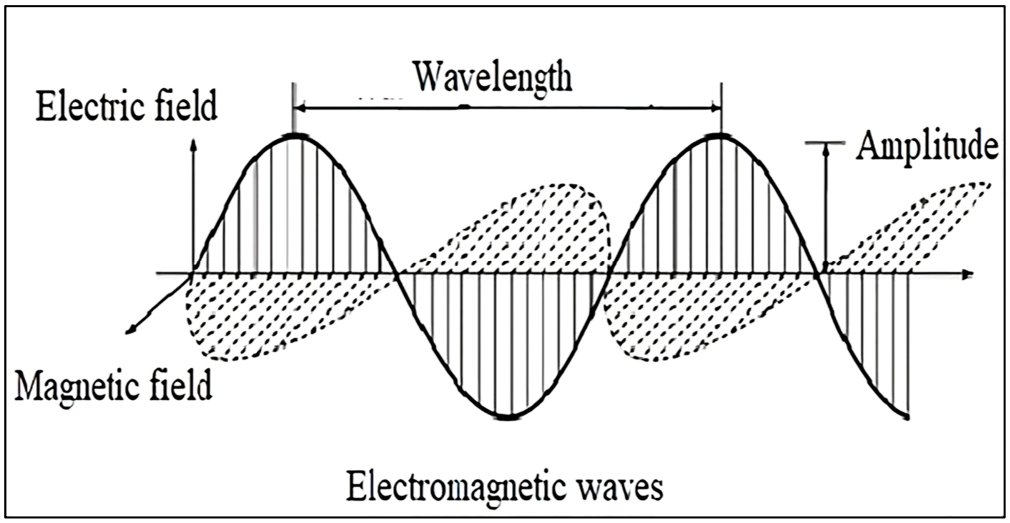


**Fig. (1).** Electromagnetic waves.

It is crucial to understand which element of "EMR" interacts with the molecule when it is exposed to it during irradiation in spectroscopy [1].

***Units of EMR [2]***

1) Wavelength:

The wavelength is depicted by the paint "xy" on the wave. Wavelength refers to the distance of two crests or valleys." It was represented by ". It is calibrated in mm, cm, *etc*. [1].

$$1\mu = 10^{-4} \text{ cm} = 10^{-6} \text{ m}$$

$$1\text{Å} = 10^{-8} \text{ cm} = 10^{-10} \text{ m}$$

2) Wave number:

The term "wave number" refers to the total number of full waves present in each individual box. It is expressed as & and has a unit of measurement of cm-1.

$\vartheta = 1/\lambda$ $\text{cm}^{-1}$ or “K” (Kaysers)

3) Frequency: Frequency is defined as the quantity of waves flowing through at a specific location and specific moment. Frequency is represented by the letter "v," and its units include nm, cm, m, *etc*.

$$\upsilon \propto 1/\lambda$$

$$\upsilon = c/\lambda$$

$$\upsilon = c\vartheta$$

c = velocity of light

### *Theory of EMR*

The quantum theory states that light has both a wave and a particle character [3, 10]. It is also crucial to consider the energy stream's composition. This idea, known as a "light quanta packet," was supported by the scientist Max Plank.

According to the Einstein-Plank connection, particles are seen as energy bundles called "photons."

$$E = h\nu \quad \textbf{(1)}$$

where h=6.63 × 10-27 erg.sec & h= plank constant

Therefore, energy is given as per the below equation.

$$E = hc/\lambda \quad \textbf{(2)}$$

Energy has a direct relation with frequency and wave number but a reciprocal relation with ‘wavelength’.

Numericals:

1. Conversion- λ into wavenumbers in $cm^{-1}$:

(i) 6.0 μ and (ii) $10^3$ nm

Calculation:

Wavenumber, $\vartheta = 1/\lambda$, $\lambda = 6\ \mu = 6*10^{-4}$cm

CHAPTER 2

# Ultraviolet and Visible Spectroscopy

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**Abstract: Ultraviolet and Visible** spectroscopy was one of the first instrumental methods for analysis. It may be used to characterise a wide range of materials. The UV-Vis provides information based on the degree of absorption or transmittance of a variable wavelength of beam light and sample responses. The general law known as Beer's law may be used to quantitatively quantify the absorption of radiant energy by materials. The UV-VIS spectrometer is easy to operate and maintain. It may be used in both qualitative and quantitative assessments. Metal and its nanoparticles are commonly measured at wavelengths ranging from 200 to 700 nm. The UV/Vis spectrum can also help us understand the delicate mechanism of complexation between templates, monomers, and cross-linkers during polymerization. It is a rapid, easy, and low-cost characterisation approach. The spectrum may be used to investigate the composition and structure of materials. These findings have applications in academics, business, medical laboratories, and chemical analysis of environmental samples.

**Keywords:** Absorption bands, Bathochromic shift, Beer’s law, Chromophore, UV-Vis, Electronic store, Filter, Hypsochromic shift, Monochromator, Spectrometer, Spectrum.

## INTRODUCTION

The other term, **electronic spectroscopy**, is frequently chosen since it more accurately describes how transitions between a molecule's electronic energy levels occur when ultraviolet/visible radiation interacts with it.

Although often with extremely short wavelengths, all organic substances absorb UV light. Even with this constraint, researchers observe that almost all organic compounds exhibit some UV absorption above 200 nanometres.

The ability to measure the degree of aromatic conjugation or multiple bonds present in molecules is the power of electronic spectroscopy. It is difficult to manage ultraviolet light that has wavelengths below 200 nm and is rarely employed as a standard instrument for structural analysis [1].

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## Colour and Light Absorption-the Chromophore Concept

To the human eye, compounds that absorb visible light at a wavelength from 400 to 800 nm appear coloured. The precise colour is a complicated effect in which wavelengths of the components deduct from white light. Yellow and orange are the most prevalent colour among organic compounds because when violet is subtracted from white light, the complementary colour is left behind. Yellow and orange appear to the human eye as yellow or orange. Progressive darkening *via* yellow, orange, red, green, blue, violet, and finally black results from the absorption of light in the range of 400 nm onward.

### *Chromophores*

A chromophore is a covalently bonded unsaturated group that absorbs in the visible or ultraviolet spectrum. As an illustration, consider C=C, C=N, C=0 and N=N, *etc*. Only when a molecule absorbs light in the visible range (400–800 nm), it shows colour. A chromophore absorbs photons in the visible or ultraviolet range, it may or may not give colour. Chromophores with n electrons go through n to n* transitions, whereas those with n and non-bonding electrons go through n- a* and n-n* transitions. There are several elements that affect the absorption's wavelength and intensity, hence there are no set rules for the identification of a chromophore.

### *Auxochromes*

An auxochrome is a group that can improve a chromophore's ability to impart colour without really being a chromophore; examples are -OR, -NH2, -NR2, *etc*. The concept of auxochromes has been altered in light of contemporary thinking, much like it was for chromophores. Auxochromes have a synergistic effect and can extend the conjugation of a chromophore by sharing nonbonding electrons. In a real sense, this causes the auxochrome to become a part of a new, extended chromophore; the effect is only notably different from that of extending the conjugation by introducing an additional chromophore to a chromophore [2].

In Table **1**, the absorption band ( $\varepsilon_{max}$) is given.

### ***Theory Involved In Electronic Spectroscopy***

Electronic transitions occurring within a molecule are the source of UV-visible absorption spectra. These transitions from the ground state to the higher energy state (excited state), which include the promotion of valence electrons, are known as electronic excitations to the absorption of incident radiation in the UV-visible

zone. Due to the quantization of molecules' different energy levels, a specific electronic excitation can only occur when a wavelength of light matching the necessary quantum of energy is absorbed.

**Table 1. Maximum absorption.**

| Chromophore | Wavelength (nm) | Intensity ($\varepsilon_{max}$) |
|---|---|---|
| $C=C$ | 175 | 14000 |
| $C\equiv C$ | 175 | 10000 |
| $C=O$ | 160 | 18000 |
| $C\equiv N$ | 165 | 5 |
| $R-NO_2$ | 200 | 5000 |
| $C=C-C=C$ | 217 | 20000 |
| $C=C-C\equiv C$ | 220 | 7500 |
| $C=C-C=O$ | 220 | 10000 |

## ***Electronic Transition***

The electronic transitions are [3]:

(1) σ to σ∗ (alkenes)

(2) π to π∗ (alkenes, carbonyl compounds, alkynes, azo compounds)

(3) n to σ∗ (oxygen, nitrogen, sulphur, and halogen compounds)

(4) n to π∗ (carbonyl compounds)

The order of energy required for various electronic transitions is σ to σ∗ > n to σ∗ > π to π∗ > n to π∗ (Fig. 1).

**CHAPTER 3**

# IR Spectroscopy

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**Abstract:** Infrared spectroscopy is a method that analyzes the vibrations of atoms in a molecule. An infrared spectrum is generally acquired by sending infrared light through a sample and calculating how much of the incident radiation is absorbed at each energy level. The frequency of vibration of a section of a sample molecule resembles the energy at absorption. The fundamental concepts and terminology of infrared spectroscopy are presented in this chapter. The vibrations of molecules will be examined in this section since they are critical to the understanding of infrared spectra. Various applications of IR spectroscopy are also discussed in brief.

**Keywords:** Bending vibrations, FTIR, Fermi resonance, IR, Monochromator, Mid-IR, Modes of vibration, Spectrum, Spectrometer, Stretching vibrations, Vibrational frequency.

## INTRODUCTION

### Important Terms [1]

**(i) Infrared Spectroscopy:** The interaction between infrared light and materials is investigated in infrared spectroscopy.

Mainly three regions in infrared spectroscopy are covered as follows:

**(ii) The mid**- (fundamental) infrared region (IR or MIR) extends from 4000 $cm^{-1}$ ($\lambda$ = 2.5 μm) to 400 $cm^{-1}$ (25 μm). It is surrounded by the **far-IR region** (FIR) from 400 $cm^{-1}$ (25 μm) to 10 $cm^{-1}$ (1 mm) and **near-IR region** (NIR) from 12500 $cm^{-1}$ (800 nm) to 4000 $cm^{-1}$ (2.5 μm).

**(iii) Spectrum** – A graph that is plotted between the intensity of light and Wavelength/Wavenumber.

**(iv) Spectrometer** – Measurement of the spectrum is done by an instrument known as a spectrometer.

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**(v) Infrared Spectrometer** – An instrument that is used to measure an infrared spectrum.

**(vi) FTIR** – A particular kind of infrared spectrometer is the Fourier Transform Infrared.

## Infrared Absorptions

A certain property, namely a change in the molecule's electric dipole moment during vibration, is required for a molecule to exhibit infrared absorption [2]. This is the infrared spectroscopy selection rule. A homonuclear diatomic molecule is an illustration of a "infrared-inactive" molecule. In contrast, a heteronuclear diatomic molecule is an illustration of an "infrared-active" molecule and is shown in Fig. (**1**) Such a molecule experiences a dipole moment change when the bond stretches and contracts.

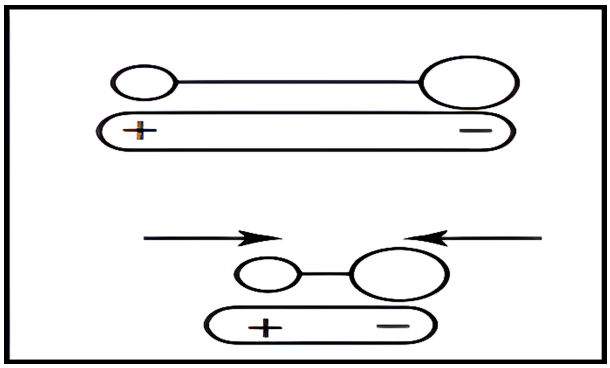


**Fig. (1).** Dipole moment of a heteronuclear diatomic molecule.

There are a number of factors that contribute to the stretching of infrared absorptions since they are not indefinitely thin. Bands can also get broader as a result of molecular collisions. The finite duration of the energy levels that lead to the transition is another cause of line broadening.

### *Modes of Vibration* [3]

The change in molecular dipoles brought on by vibrations and rotations can be used to explain how infrared radiation interacts with matter. A molecule can be considered a collection of masses connected by spring-like bond qualities to start with a simple model. As an example, diatomic molecules have 3D translational and 2D rotational motion, respectively. The molecular atoms can also move in relation

to one another; for example, bond lengths can change one atom that can leave its current plane. This is an explanation of the bending and stretching motions that make up vibrations**.**

Two categories of triatomic molecules—linear and non-linear—can be identified when considering molecules with three atoms as a starting point. $CO_2$ and $H_2O$ are two illustrations of linear and non-linear triatomic, respectively. There are three translational degrees of freedom for both $CO_2$ and $H_2O$. Since there is no discernible energy involved in spinning around the O=C=O axis, carbon dioxide has just two degrees of rotational freedom compared to water's three. When these are subtracted from 3N, $CO_2$ (or any other linear molecule) has 3N-5 degrees of freedom, and water has 3N-6 (or any non-linear molecule).

Various degrees of freedom are given in Table **1**.

**Table 1. Degree of freedom [3].**

| Types of Degree of Freedom | Linear | Non-linear |
|---|---|---|
| Translational | 3 | 3 |
| Rotational | 2 | 3 |
| Vibrational | 3N-5 | 3N-6 |
| Total | 3N | 3N |

## ***IR Absorption Band* [4]**

Hooke’s law (Frequency)

$$\varpi = 1/2\pi c \times \sqrt{K/\mu} \quad \textbf{(1)}$$

$\varpi$ = Frequency

$K$ = force constant of the bond

$\mu$ = reduced mass ($m_1 \times m_2 / m_1 + m_2$)

$m_1$ and $m_2$ are the masses of the two atoms

**CHAPTER 4**

# Mass Spectrometry

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**Abstract:** Mass spectrometry is the most effective method for determining molecular structure. It typically offers information on a substance's molecular weight, but it may also show atomic mass units and up to ten-hundredths of atomic mass units depending on the mass analyzer's accuracy. Furthermore, it offers information on the ions (positive charged) created in between the ionization process, related to the molecule's chemical structure and the type of bonds. During these years, the technique paired with the use of software and databases, has been spectacular, enhancing ionization processes and ion analysis. This chapter will demonstrate how mass spectrometry has aided in quality control analysis.

**Keywords:** Bioanalytical applications, Instrumentation, Ionization techniques, Molecular ions.

## INTRODUCTION

It is an analytical technique that produces free gaseous ions that are then exposed to electric and magnetic fields in a high vacuum for mass/charge ratio analysis. Although it was initially developed for physicochemical exploration, mass spectrometry has observed widespread application in the interpretation of small biomolecules and more complex organic compounds since the 1950s. This was the first implementation of mass spectrometry in research. Combined gas chromatography-mass spectrometry could analyze large molecules of compounds that could be derivatized to increase volatility (GC-MS).

Mass spectrometry has become central to getting rid of many unwanted hurdles in biopolymer analysis because of substantial analytical advancements and advances in equipment in recent years. These advancements allow for the determination of molecular masses of large biomacromolecules allowing for the identification, for example. supramolecular biopolymer interactions *etc*.

The adoption of novel ionization techniques including fast atom bombardment (FAB), plasma desorption (PD), and thermo-spray (TSP) that allowed the

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production of gas phase ions from polar and charged biopolymers was a major reason for this advancement [1].

## Instrumentation and Various Techniques of Mass Spectrometry [2-8]

### (i) Sampling and Ionization Methods

### Prior- Requirement

In this, ions are exposed to electric fields and magnetic fields in a vacuum. A compound must be charged just before spectrometry analysis for this purpose. Furthermore, these ions must be transmitted to the gas phase in a mass spectrometer's vacuum system. In general, mass spectrometry can be employed to examine ions that are in free form under a high vacuum.

The method of ionization chosen is determined by the analyte properties. Hard ionization is carried out to obtain structural information. The "soft ionization" techniques are used to obtain mass spectra of molecules.

### (a) Gas- Phase Ionization Method

Ionization by electron impact (EI) is the traditional "hard" ionization technique that includes an electron beam travelling to the sample. When neutral molecules of analyte clash, an additional electron can get pushed off, leading to either a positive-charged analyte ion or, more typically, fragment ions belonging to a specific chemical substructure. EI is typically performed with energetic electrons of 70 eV. (Fig. **1**). Reduced fragmentation may result from lowering this energy, but it also causes decreased sensitivity.

EI is the most ancient and well-studied ionization method, and it can be used on any volatile/thermostable substance. EI mass spectra are highly reproducible ("fingerprinting") and are frequently used in conjunction with mass spectral library resources. The fragmentation pattern can also be used to obtain structural information.

### (b) Chemical Ionization

A number of these ions can form analyte ions by reacting with analyte molecules. The reagent is an energy mediator, which controls the energy transfer to the sample substance. The fragmentation is reduced when compared to EI, and $[M+H]^+$ is obtained.

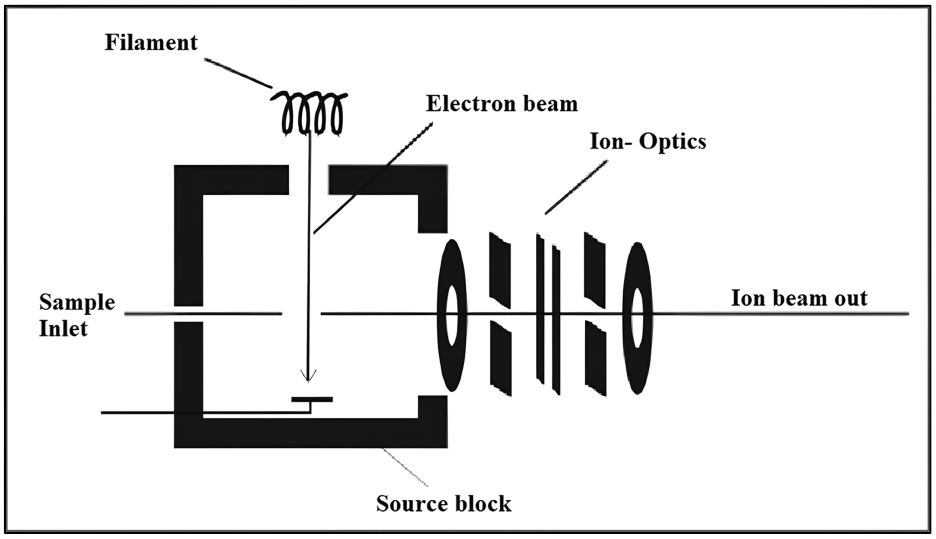


**Fig. (1).** Electron Ionization: Set up for Ionization.

## (c) Soft Ionization Methods

"Soft" ionization strategies had to be developed in order to identify intact molecular ions (Field desorption). Electron tunneling induced by a high-voltage emitter is at the heart of FD. As the emitter's filament gets heated up, the sample being studied evaporates into a gaseous phase. The majority of the time, intact molecule ions are discovered. This technique is restricted to low molecular weight compounds that must also be thermostable to a certain extent. Plasma desorption is another soft ionization method that has been developed.

## (d) Fast Atom Bombardment

It is especially useful for studying ionic compounds with molecular weights (>or= 10kDa). The unknown substance is dissolved in a low-volatility liquid like glycerol/ meta-nitrobenzyl alcohol, and then deposited on a target. The fast, heavy atoms (for example, Xe) or ions (for example, $^{131}Cs$) are fired at the target continuously, also known as secondary ion mass spectrometry in the latter case (SIMS). Analyte molecules and fragments, and cluster ions are desorbed.

This quick and easy ionization technique is quite tolerant of sampling variations and is suitable for different substances to be analyzed. It is limited by a high chemical background, making differentiation in-between lower molecular weight substances and the background difficult.

**CHAPTER 5**

# A Brief Account on Recent Advancements and Applications of NMR Spectroscopy

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**Abstract:** The present book chapter has highlighted the various types of NMR spectroscopy, including solid-state NMR, solution-state NMR, and their applications in hybridization with other spectroscopic techniques in pharmaceutical analysis, drug discovery, and impurity profiling. NMR has been a powerful tool for both drug discovery and the study of the structure and dynamics of biomolecules in conjunction with X-ray crystallography. Along with other chemometric techniques like principal component analysis, discriminant analysis, and partial least square regression are used. In the pharmaceutical sector, NMR has been found to be much important in determining the purity of medications with their various variants, the validity of herbal products/heparin, fingerprinting of isotopes, *etc*.

**Keywords:** Applications of NMR, NMR spectroscopy, Nuclear magnetic resonance, Organic compounds, Solid-state NMR.

## INTRODUCTION

### Background

Organic compounds have been generally made from carbon and hydrogen and have a huge range of applications in the field of medicine and many others. Chemists are generally involved in the synthesis followed by the characterization of organic compounds, which include their determination or structural elucidation. The knowledge of their structure has been the key parameter for understanding the reactivity of the compounds along with their structure, physical properties, and chemical properties [1]. Various methods to characterize compounds are elemental analysis, spectroscopical, non-spectroscopical, electrochemical, microscopical methods, *etc*. [2] The characterization of transition metal complexes has been accomplished on solid materials (X-ray methods) and in solutions (electronic

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spectroscopy and nuclear magnetic resonance - NMR spectroscopy) and neutral complexes are mostly characterized by mass spectrometry [3, 4]. The coordination chemistry of oxazolines and the transition metal complexes of boron have been well characterized using solid-state NMR and X-ray diffraction techniques [5, 6]. For the structural insights of organolithium compounds, NMR has emerged as an important technique along with other techniques of characterization [7].

Due to its accuracy and precision, NMR has been regarded as the much important analytical tool for quality control and standardization of pharmaceutical, chemical, and medicinal substances [8]. The sample of NMR should be completely soluble and it must have the detectable nuclei such as hydrogen, fluorine, boron, *etc*. whilst other techniques are challenged by specific response factors and chemical and/or mechanical consideration. In the field of metabolomics, NMR has been found as an important detection technique for multiple metabolites without their separation. Further, cell metabolic alterations due to the administration of the metal-based antitumor drug have been well studied using NMR tools [9]. NMR is mainly used to study structures and dynamics of biomolecules and is also utilized efficiently in drug discovery as it can locate drug-ligand binding sites on target proteins [10, 11]. With the help of NMR spectroscopy, we can characterize chemical constituents either as the individual or as a mixture. This has helped a lot in the physical analysis of different components of wastewater, leading to environmental damage and human health problems [12]. NMR can also be used for detecting impurities, especially microimaging is used to study the dissolution of tablets and whole-body imaging is widely used in clinical diagnostics [13]. The differential techniques of solid-state NMR spectroscopy have maximized the anisotropic interactions in the solid state, which are sometimes unavailable in the liquid state [14].

$^{1}$H NMR spectroscopy has been one of the most powerful tools for elucidating the types and number of protons, whereas $^{13}$C NMR has been used to determine the type and number of carbon atoms in the compound of interest. It has been used to study a wide variety of nuclei such as $^{1}$H, $^{15}$N, $^{19}$F, $^{13}$C, $^{31}$P, and $^{27}$Al. $^{1}$H NMR spectroscopy has been used to investigate the hydrogen bond, ionization states of small compounds in organic or inorganic solids, and acidity of polymers and rubbers. Nitrogen nucleus is the third most important probe (after $^{1}$H & $^{13}$C) for investigation of the structure by NMR and it is used as a probe in molecular structure and reactivity of organic, bioorganic, and inorganic molecules, viz. specific application of $^{15}$N NMR for the determination of site of protonation in vitamin $B_{12}$, along with other organic transformations such as tautomerism, complexation, protonation, *N*-alkylation, regio-isomerism, and changes in configuration or conformation [15]. Recent advancements in the NMR

spectroscopy have also utilized deep learning to improve the analysis of complex magnetic resonance data, particularly in the study of large biomolecules [16].

## APPLICATIONS OF NMR SPECTROSCOPY

NMR spectroscopy has been a highly useful analytical technique with abundant works of literature published on its applications in the field of pharmaceutical analysis. The fragment-based drug discovery (FBDD) has been the *in silico* innovative strategy for hit identification against undruggable drug targets with large molecular weights [17]. Herein, the NMR spectroscopy along with the X-ray diffraction has been applied to reveal the 3-dimensional insights of the complex of ligands bound with the protein. Drug discovery processes heavily rely on structure-based drug design techniques that are based on X-ray crystallographic data or computationally generated pharmacophore models. NMR has been the best method for providing many types of data that impact medicinal chemistry decisions in FBDD [18, 19]. The NMR spectroscopy along with other analytical techniques has been used to screen the adulterants (especially, phosphodiesterase type 5 (PDE-5) enzyme inhibitors and their illegal analogues) among the natural products or marketed supplements used to treat erectile dysfunction [20]. The advantage of using the NMR technique has been higher accuracy, precision, and reproducibility with the limitation of low sensitivity in comparison with mass spectrometry techniques (LC-MS and GC-MS).

NMR has been growing increasingly for the identification and quality check of pharmaceutical products or drugs, at present playing an important and demanding role in impurity profiling, structural elucidations, and determination of the amount of impurities present in pharmaceutical products. Not only this but also, the quantification of many ambiguous compounds and substances without any need for previous separation can be attained [21]. NMR can be coupled with other separation techniques to increase its analytical efficiency to identify the unknown substance. For example, NMR has been used in combination with liquid-chromatography (LC-NMR) for the identification of metabolites of drugs using LN-NMR [22].

CHAPTER 6

# Raman Spectroscopy

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**Abstract:** Raman spectroscopy is a type of spectroscopy that is used to detect vibrational, rotational, and other low-frequency modes in a system. It is based on the Raman scattering (inelasticity) of monochromatic light. When laser light interacts with molecular vibrations, the energy of the laser photons is moved up or down. The energy shift reveals information about the system's vibrational modes. This chapter defines Raman spectroscopy, and its various varieties, and explains some of its most important and advanced uses.

**Keywords:** Raman spectroscopy, Rayleigh scattering, Stokes scattering, Resonance raman spectroscopy, Stimulated raman scattering.

## INTRODUCTION

Raman spectroscopy is a powerful tool for studying pharmacological and therapeutically important compounds. It is employed in a variety of applications that need non-destructive microscopic chemical analysis and imaging. It may be used to quickly determine the chemical content and sample chemical structure, for any form of substances like solid, liquid, gas, gel, slurry and even for powder. It lends very well to *in vivo* measurements. The most extensively utilized techniques for giving information about the structure and properties of molecules by providing specific features of their vibrational transitions are Raman spectroscopy and infrared absorption spectroscopy. Raman analysis can quickly offer critical information [1].

Raman spectroscopy is a widespread method for analyzing molecular structure, and it is now thought to be useful in conjunction with infrared spectroscopy. The difference in energy between the scattered photon and the incident photon is known as the Raman Shift 1.

The Raman Shift refers to the energy differential between the incident and dispersed light. The x-axis is the wavenumber of the Raman shift ($cm^{-1}$), and the vertical axis is the intensity of the scattered light in a spectrum.

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Various materials have distinct vibrational modes and hence varied Raman spectra. Raman spectroscopy is thus a helpful method for material identification. There is a significant distinction to be made between Raman spectra obtained from gases and liquids and those obtained from solids (like crystals). Crystals, on the other hand, are made up of molecules with different vibrational energy levels. As a result, the entire crystal lattice vibrates at the macroscopic level. These macroscopic vibrational modes are referred to as phonons [2-3].

If the incoming photon's energy is insufficient to excite the molecule from its ground state to its lowest electronic state, the molecule will be stimulated to some virtual state between the two states. Yet, the electron of that excited molecule cannot remain in the virtual state for long and must return to the ground state promptly. If the electron returns to its original state (ground state in this case), the wavelength of the released photon becomes the same as the wavelength of the incident light photon. Rayleigh scattering is a sort of scattering phenomenon.

On the other hand, the electron can move to a virtual state that is different from where it is excited, resulting in an energy difference between the emitted photon and the incident photon, and thus a shift in wavelength (and thus an energy shift) for the emitted photon as compared to the incident photon. This is referred to as Raman scattering. Stokes scattering, the energy of the released photon is less than the incident photon. Anti-Stokes scattering occurs when the incident photon has a higher amount of energy than the output photon [4].

**Rayleigh Scattering**

The stimulated molecule's electron returns to its original state. Raman Spectroscopy examines changes in a molecular bond's polarizability, which is a measure of a bond's deformability in an electric field. Photon interaction with a molecule can cause deformation of the molecule's electron cloud. This deformation is referred to as a shift in polarizability. This factor is mostly determined by how easily electrons in the bond may be moved, resulting in a transient dipole. When there is a high concentration of loosely held electrons in a bond, the polarizability increases, and the group or molecule produces a strong Raman signal.

As a result, Raman is often more sensitive to a molecule's molecular framework than a single functional group, as in IR Spectroscopy. For example, when photons interact with molecules containing bonds between homonuclear atoms such as C-C, S-S, and N≡N bonds, the polarizability changes, giving only the raman shift, not

FTIR. This is not to be confused with a molecule's polarity, which is a measure of the separation of electric charge within a molecule.

Fig. (**1**) shows the diagrammatic view of Rayleigh scattering.

### *Stokes Scattering*

The stimulated molecule's electron relaxes back in higher vibrational state than it was in the previous state. The photon has hv-ΔE energy and that is inelastic in nature.

### *Anti-Stokes Scattering*

The excited molecule's electron begins in a vibrationally excited state. The excited molecule's electron relaxes back to a lower vibrational state than it had before. The photon escapes with energy hv + ΔE and that is super elastic [5].

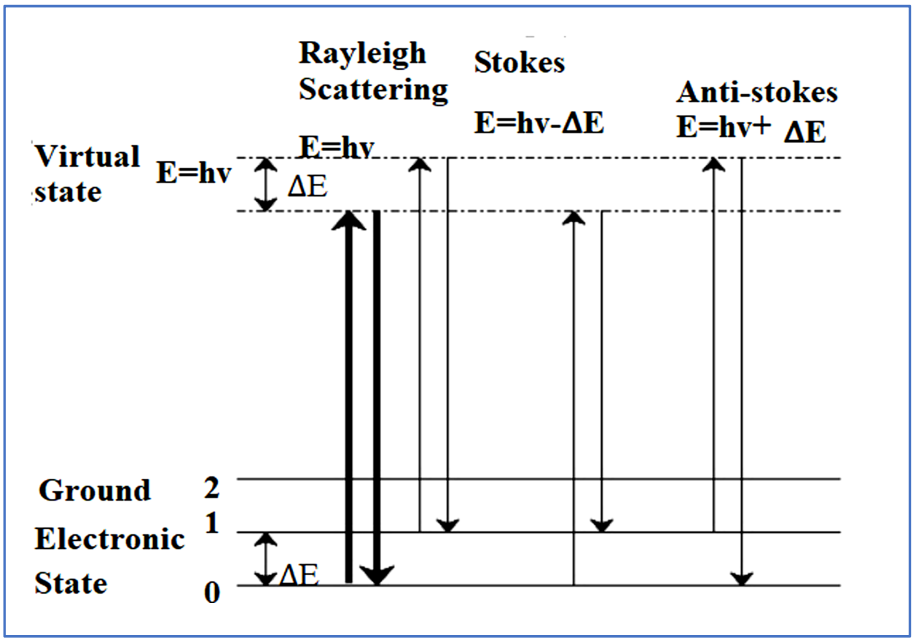


**Fig. (1).** Rayleigh scattering, Stokes scattering and anti-Stokes scattering.

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