

Spectroscopy

**Presented By:-
Samarpan Mishra**

Spectroscopy

Spectroscopy means studying a substance by seeing how it interacts with energy or radiation, such as light.

Spectroscopy Techniques

- 1. UV-Visible Spectroscopy**
- 2. IR Spectroscopy (Infrared Spectroscopy)**
- 3. NMR Spectroscopy**
- 4. Mass Spectrometry (MS)**
- 5. Raman Spectroscopy**

1. UV-Visible Spectroscopy

Introduction:-

UV-Visible (UV-Vis) Spectroscopy is an analytical technique that measures how much ultraviolet or visible light a chemical substance absorbs. This interaction excites valence electrons to higher energy levels, allowing scientists to identify molecules and determine their exact concentrations in a solution.

Principle:-

- ❑ **UV-Visible spectroscopy** is based on the **absorption of ultraviolet (UV) or visible light by molecules.**
- ❑ When UV or visible light passes through a sample, the molecules **absorb certain wavelengths of light.** This absorption causes electrons in the molecule to move from a **lower energy level to a higher energy level.**
- ❑ Different compounds absorb different wavelengths. Therefore, the absorption pattern can help in the **identification and estimation of compounds.**



Working of UV-Visible Spectroscopy

The basic working can be understood in **6 simple steps**:

Light source → Monochromator → Sample → Detector → Signal processing → Spectrum

Step 1: Light source - A light source produces UV or visible radiation.

- ☐ Deuterium lamp → commonly used for UV region
- ☐ Tungsten-halogen lamp → commonly used for visible region

Step 2: Monochromator - The monochromator selects a **particular wavelength** of light from a light source.

Step 3: Sample - The selected light passes through the sample solution kept in a **cuvette**.

Step 4: Absorption - The compound present in the sample absorbs some of the light.

Step 5: Detector - The detector measures the amount of light that **passes through the sample**.

Step 6: Result - The instrument calculates **absorbance** and produces a spectrum showing **absorbance versus wavelength**.



Applications:

- 1) Identification of phytoconstituents
- 2) Quantitative estimation of plant constituents
- 3) Quality control of crude drugs
- 4) Detection of adulteration
- 5) Standardization of herbal drugs
- 6) Study of isolated natural products
- 7) Monitoring extraction and purification

2. IR Spectroscopy (Infrared Spectroscopy)

Introduction:-

IR spectroscopy helps us identify a compound by studying the vibrations of its chemical bonds.

Principle:-

IR spectroscopy is based on the **absorption of infrared radiation by molecules**, When IR radiation falls on a molecule, its chemical bonds **vibrate (stretch or bend)** at specific frequencies.



Step-by-step

1. IR source produces infrared radiation.
2. The radiation is directed toward the sample.
3. The sample is prepared and placed in the sample holder.
4. Molecules absorb specific frequencies of IR radiation.
5. The absorbed energy causes **vibrations** of chemical bonds.
6. Radiation that is not absorbed reaches the detector.
7. The detector records the amount of IR radiation absorbed at different frequencies.
8. The instrument produces an **IR spectrum**.
9. The characteristic peaks are studied to identify **functional groups and the compound**.



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3. NMR Spectroscopy

Introduction

NMR spectroscopy is an analytical technique, Used to determine the structure of a compound by studying the behaviour of certain atomic nuclei in a strong magnetic field.

In Pharmacognosy and Phytochemistry, NMR is especially useful for identification and structural characterization of isolated natural products.

Principle

NMR spectroscopy is based on the absorption of radiofrequency radiation by certain atomic nuclei placed in a strong magnetic field.



Working of NMR Spectroscopy

The working can be understood in simple steps:

- 1) **Sample preparation:-** The compound is dissolved in a suitable **deuterated solvent** and placed in an NMR tube.
- 2) **Magnetic field:-** The sample is placed inside a **strong magnetic field**.
- 3) **Nuclei align:-** Nuclei such as ^1H and ^{13}C align with or against the magnetic field.
- 4) **Radiofrequency applied:-** Radiofrequency radiation is applied to the sample.
- 5) **Energy absorption:-** The nuclei absorb the required amount of energy and undergo **resonance**.
- 6) **Return to original state:-** When the radiofrequency is removed, the nuclei return toward their original energy state.
- 7) **Signal produced:-** During this process, the nuclei produce signals that are detected by the instrument.
- 8) **Spectrum obtained:-** The computer converts these signals into an **NMR spectrum**.
- 9) **Structure determination:-** The position and pattern of the signals provide information about the chemical environment of atoms, helping determine the structure of the compound.



Applications in Pharmacognosy

- 1) Identification of natural products
- 2) Structure determination
- 3) Structural confirmation
- 4) Detection of adulteration

4. Mass Spectrometry (MS)

Introduction

Mass spectrometry tells us the molecular mass and helps determine the structure of a compound by converting it into ions and measuring them according to their mass.

It is especially useful in Pharmacognosy and Phytochemistry for identification and characterization of natural products.

Principle

Mass spectrometry works on the principle of converting molecules into charged ions and separating these ions according to their mass-to-charge ratio (m/z).



Working of Mass Spectrometry

The working can be understood in **6 simple steps**:

- 1. Sample introduction-** The sample containing the compound is introduced into the mass spectrometer.
- 2. Ionization-** The molecules are converted into **charged particles called ions**.
- 3. Acceleration-** The ions are accelerated using an **electric field** so that they move through the instrument.
- 4. Separation-** The ions are separated according to their **mass-to-charge ratio (m/z)**.
- 5. Detection-** A detector detects the separated ions and measures their abundance.
- 6. Mass spectrum-** The computer produces a **mass spectrum**, which is a graph of:
 - **X-axis** → m/z
 - **Y-axis** → **Relative abundance/intensity**

The peaks provide information about the **molecular mass and structure** of the compound.



Applications of Mass Spectrometry

1. Identification of natural products
2. Determination of molecular mass
3. Identification of unknown compounds
4. Analysis of plant extracts
5. Detection of adulteration

5. Raman Spectroscopy

Introduction

Raman spectroscopy helps us identify a substance by studying the change in wavelength of scattered light.

Principle

Raman spectroscopy is based on the Raman effect, in which a small amount of incident light is scattered by molecules at a different wavelength due to molecular vibrations.

Example

Think of shining a torch on a ball.

- ❑ Most light comes back in the same form.
- ❑ A very small part interacts differently with the ball.

In Raman spectroscopy, this small change in scattered light gives information about the molecule.



Working of Raman Spectroscopy

- 1. Laser produces light** :- A strong laser beam is used as the light source.
- 2. Laser falls on the sample** :-The laser light is directed onto the sample.
- 3. Molecules interact with light** :-The molecules in the sample interact with the laser light.
- 4. Scattering occurs** :-Most light undergoes Rayleigh scattering, while a very small portion undergoes Raman scattering.
- 5. Raman light is collected** :-The Raman-scattered light is collected by the instrument.
- 6. Filter removes Rayleigh light** :-A filter removes the much stronger Rayleigh-scattered light.
- 7. Detector detects Raman light** :-The detector measures the Raman-scattered light.
- 8. Spectrum is produced** :-The computer produces a Raman spectrum.
- 9. Identification** :-The characteristic peaks are compared with reference data to help identify and characterize the substance.



Applications of Raman Spectroscopy

1. Identification of natural compounds
2. Identification of functional groups
3. Authentication of crude drugs
4. Detection of adulteration
5. Study of molecular structure



Thank you