

# HIGHLIGHTS OF SPECTROSCOPIC TECHNIQUE AND THEIR APPLICATION

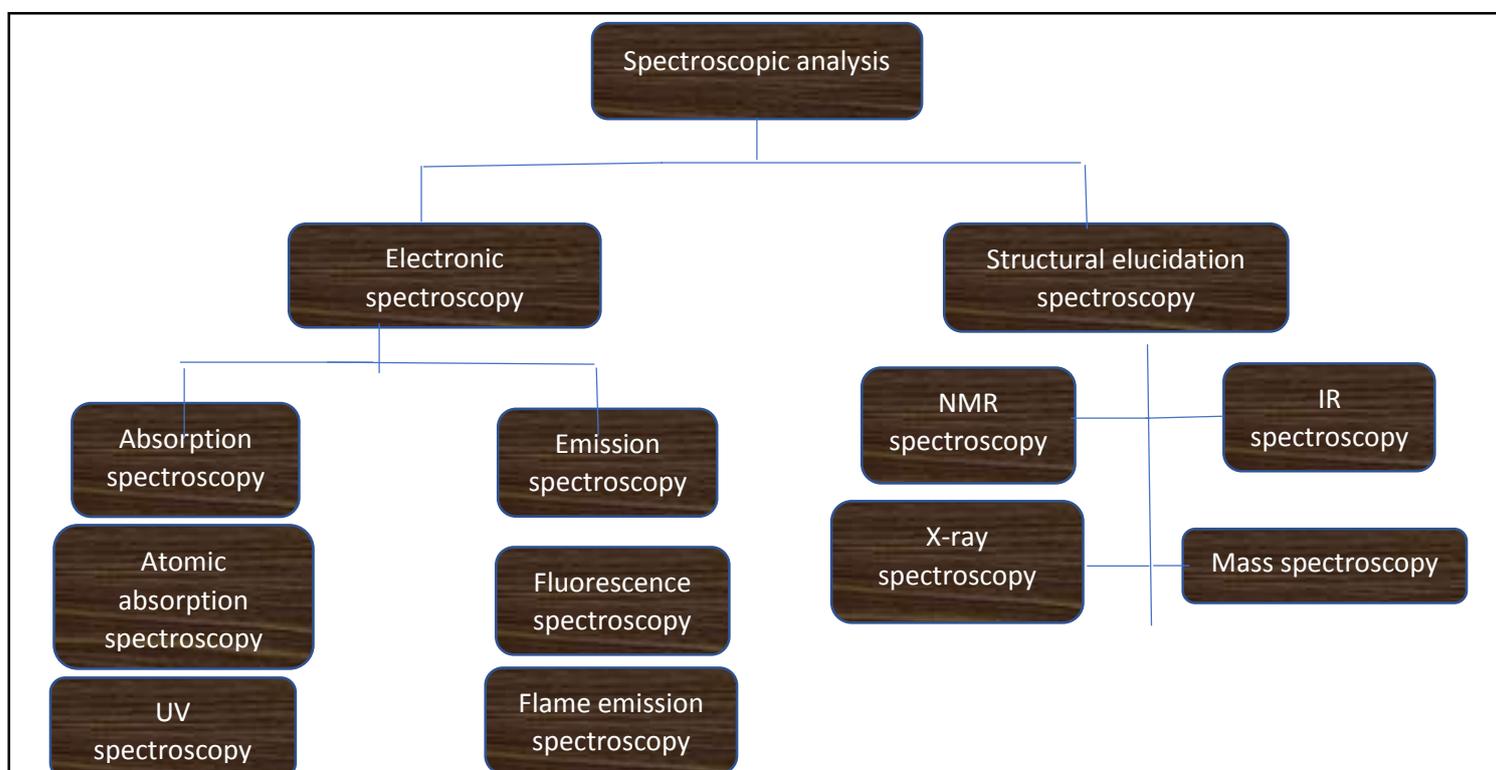
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**Abstract:** spectroscopic method is broadly employed for the evaluation of chemical structure of a compound (analyte) accurately. In all methods molecule identified because of interaction with electromagnetic radiation. The electric and magnetic property of radiation is reacted with similar properties of chemical compound, therefore the analyte is identified and characterized for the presence of atom, bond, functional group, basic nucleus, molecular formula and molecular weight. This article gives information of importance of analytical technique and their application. The investigation implies content of basics for interpretation of spectrum, laws, sampling techniques, sample cell, mechanism involved in solvent utilization, appearance of spectra and application of technique. this review also includes significant aspect of various spectroscopic method and represent quantities and qualitative parameters of compound, entire spectroscopic principal, instrumentation and application.

**Keywords:** spectrum (spectra), interpretation, application, compound(analyte), atom, molecule.

**INTRODUCTION:** electromagnetic radiation in which matter collaborate with high electric radiation. It has to do with atoms or molecules absorbing, emitting, or dispersing electromagnetic energy. The outcome of such an engagement provides us with analytical knowledge.(1)

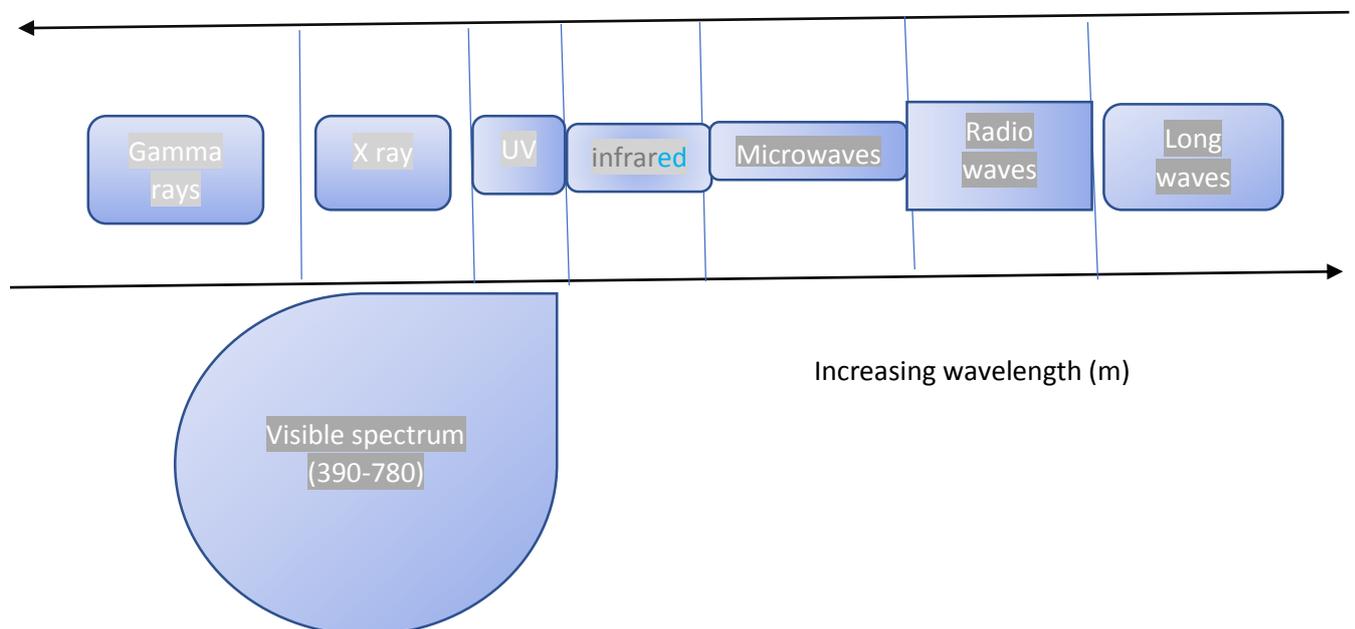


## ✓ *UV-Visible Spectroscopy:*

This spectroscopy determines analyte concentration by measuring light absorption throughout a range via a liquid sample (1).

One of the most dynamic used techniques in pharmaceutical analysis is UV-Visible spectrophotometry. It provides information related to how much UV or visible radiation a material in solution absorbs. Ultraviolet-Visible spectrophotometers are instruments that measure the ratio, function of ratio, and intensity of two beams of light in the ultraviolet range (2) UV. spectroscopy is one of the earliest analytical techniques, and it serves as the foundation for a variety of perfect approaches for determining micro and semi micro amounts of analyte in a sample (2). A system will get aroused once energies is applied before reverting to its original state. (3) Optical spectroscopy studies the interaction of light by matter . It is a non-destructive test, which provides rapid result analysis, and it can be used for qualitative and quantitative analysis of sample. (4). UV-VIS spectrometry is applied for the absorbing different wavelength. (5) Light, in literal terms, is a unit of power that flows across space at a very high rate. Light is radiant energy, which is an electromagnetic radiation that flows across space. The intensity of light vibrates between a trough and a fro as a recorded of time, just same as a pulse. An electromagnetic radiation among the wavelength is the distance between two peaks or troughs. Nanometre (nm) is the unit of measurement.(6)

Increasing frequency(HZ)

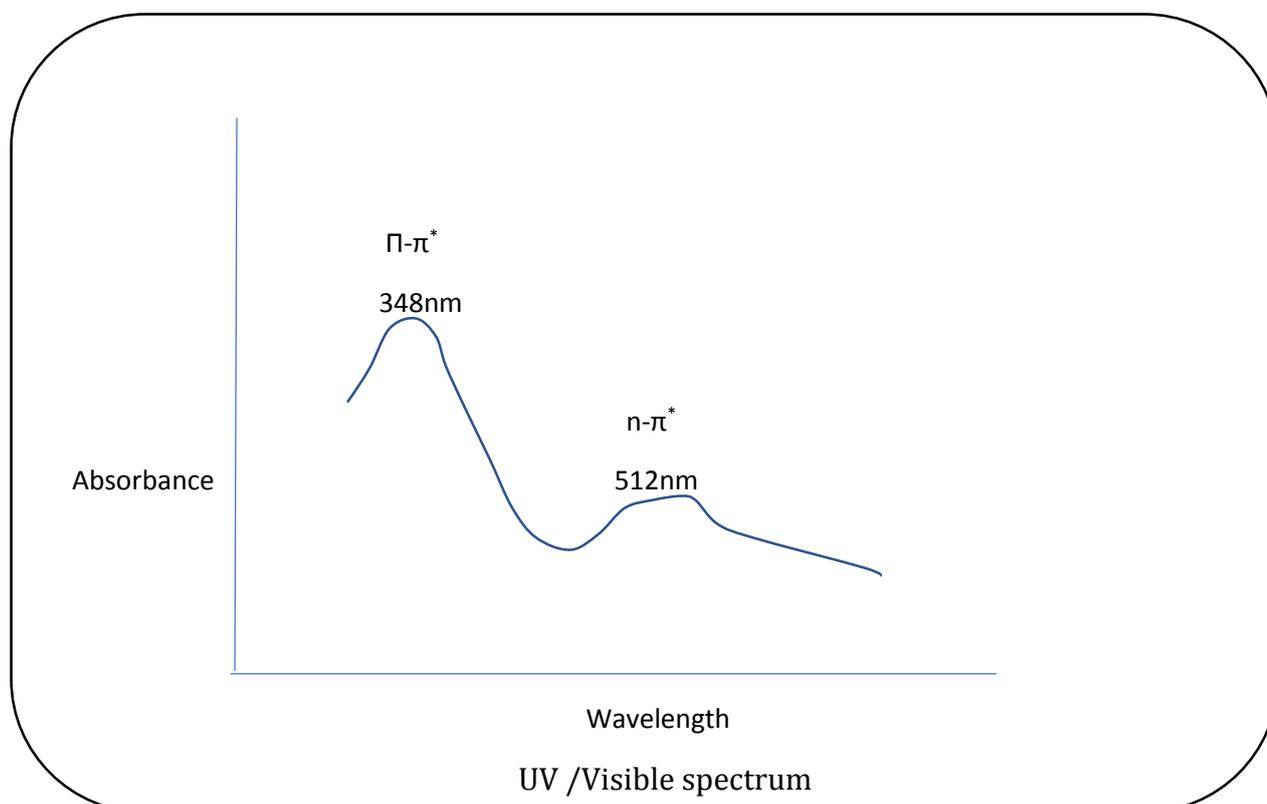


**Visible spectrum (390-780nm) represent only small portion of the whole electromagnetic spectrum.**

Each colour has its own wavelength, such as 660 nm for red and 520 nm for green. As a result, each light component has its own wavelength. A spectrum is defined as the total of all

components, or wavelengths. A spectrum, in further detail, is a representation of radiant energy dispersion.(6)

Sr.no	Parameter	UV/visible spectroscopy
1	Electromagnetic radiation	Ultraviolet and visible radiation
2	Light source	D2/w lamp
3	Monochromators	Filter/prism/grating.
4	Sample cell /cuvette	Cylindrical/rectangle.
5	Solvent use	Polar solvent like water ,methanol
6	Sample analysed	Absorption
7	Chemical properties of sample analysed	Chromophore
8	Principle	Change occurs at electronic level of molecule.
9	Concentration range	$\mu\text{g}-\text{mg}$
10	Detector	Phototube ,photovoltaic cell, photo multiplier tube
11	Appearance of spectra	Absorption vs wavelength
12	Qualitative respect	$\lambda$ max
13	Quantitative respect	Concentration vs absorption
14	Interpretation law	Beer-lambert law
15	Radiation range	200-800nm



- **PRINCIPLE:**

Beer's law: asserts that as the number of absorbing molecules increases, the intensity of a parallel monochromatic beam drops exponentially. To put it another way, the concentration is proportional to the absorbance. (2)

Lambert's law: It says that when a beam of parallel monochromatic light goes through a material of uniform thickness, the intensity of the beam diminishes exponentially. The Beer-Lambert law is obtained by combining these two laws. When a beam of light passes through a transparent cell containing an absorbing material, the intensity of the light is reduced, which may be stated mathematically in Eqn.1.

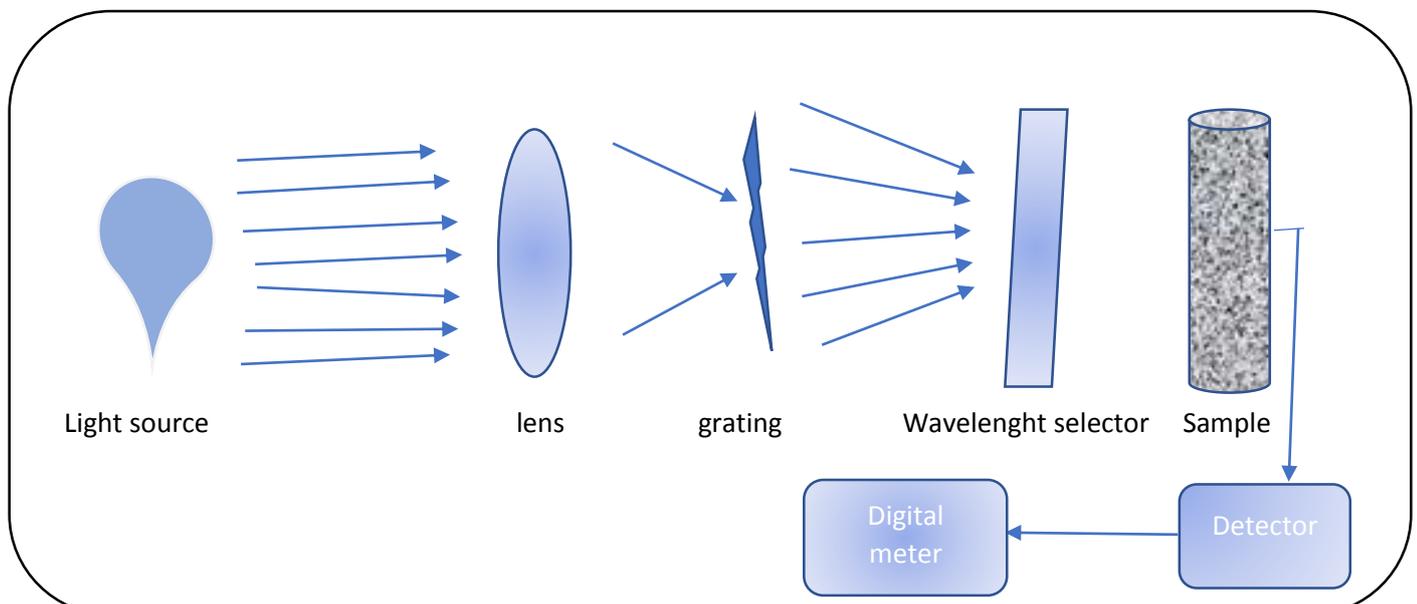
$$A = a \cdot b \cdot c \quad \text{Eqn. 1}$$

Where A is the absorbance (optical density), a is the absorptivity or extinction coefficient, b is the path length of radiation over a sample (cm), and c is a concentration of solute (substance) in solution (7)

- **INSTRUMENTATION:**

A spectrophotometer is made up of four pieces in the majority of cases:

1. A UV spectroscopy enlighten light source. A gas-filled bulb, such as xenon, or compilation of the two basic lamps, such as tungsten and deuterium, is occasionally used for most of time.
2. The sample must be maintained in a proper sampling container. - Liquid samples are kept in cuvettes manufactured of quartz, borosilicate glass, or a combination of materials. Acrylic plastic In contrast, UV rays do not pass via glass or acrylic material, thus they should only be used for aesthetics.
3. measurements of visible region - Samples can be mounted in a compatible holder and inserted into the microscope's beam axis.
- 4)Using a spectrophotometer, the transmitted light is measured .To be successful,



Region	Wavelength
Far( or vacuum)ultraviolet	10-200nm
Near ultraviolet	200-400nm
Visible	400-750nm
Near infrared	0.75-2.2µm
Mid infrared	2.5-50 µm
Far infrared	50-1000 µm

- **APPLICATION:**

- 1) Formaldehyde is the most common aldehyde found in the environment (HCHO). Because of its cytotoxicity, it is one of the deadliest air pollutants. Spectroscopy is often used in formaldehyde analysis since formaldehyde or its vapours can induce a wide variety of illnesses and disease.(8)
- 2) Synthesized a series of noble metal nanoparticles UV/vis spectroscopy is only one method for investigating the swelling and dowelling dynamics of polymer micro gels and composite related to the title with very small particle sizes. (9)
- 3) UV spectroscopic method is useful for multicomponent analysis. UV spectrophotometric methods are mostly utilised for multicomponent analysis, lowering the time and cost of analysis by reducing the time and effort required to separate interferes and allowing the determination of a greater number of analyte. (10)
- 4) one of the Common applications for difference UV spectroscopy include the determination Number of aromatic amino acids exposed to solvent, detection of conformational changes Found in proteins, amino acid which has aromatic amino acids in active sites of enzymes.
- 5) carbon nanotubes are detected using the UV-Vis spectra , which utilize the Beer Lambert equation to show the strength of absorption at a certain wavelength to the concentration of carbon nanotubes floating in the solution .(12)
- 6) Aside from pharmacological assays, difference spectroscopy is also utilised in biopharmaceutical formulation development to assess protein structure and analyse the structure's response to formulation composition. (13)
- 7) application of UV–Vis spectra gives information about optical properties of the chromophore and idea regarding inspect difference in absorbance spectra of amino acid .(14)
- 8) main application of UV-VIS spectrum analysis is a key development trend for water quality monitoring, since it assesses the degree of water pollution by building a correlation model of absorbance, organic matter, and inorganic matter concentrations. UV-Vis absorption gives clear idea about quantitative detection of water quality also gives quality detection.(15)
- 9) UV/VIS spectrophotometry is used in the food sector, dyes and beverage sector to evaluate and enhance product quality and consistency. This approach may also be used to study

the effects of packing material and stabilisers, as well as chemical deterioration and degradation processes.(6)

- 10) UV visible spectra is applicable to find the purity of olive oil, which declare the form compound to be categories as Extra Virgin oil. the Standards are in occur for the evaluation of olive oil as well as virgin oil based on the absorbance mechanism of specific molecules in the UV spectra. oil containing fatty acid with unsaturation leads to catalysis and oxidation. due to oxidation of the free acid leads to formation of peroxides. Therefore peroxide ion reason for distraitment and rancidity of compound .(6)

### ✓ *INFRA-RED SPECTROSCOPY:*

In the year 1800, Mr. Herschel found infrared radiation during preparation. Herschel was looking at the energy levels linked with visible spectrum light wavelengths. The visible spectrum of the rainbow hues, i.e. the visible spectrum from blue to red with similar wavelengths or frequencies, was shown using a prism.(16)

When IR light – 780-2500 nm – passes through a sample, infrared (IR) spectroscopy assesses the wavelength and intensity of the energy absorbed. It is traversed by This method is widely utilised in a variety of industries. both in the pharmaceutical, chemical, and polymer industries, as well as Academia.(17)

Infrared radiation is a part of the electromagnetic spectrum with wavenumbers ranging from about 13,000 to 10 cm<sup>-1</sup>, or wavelengths ranging from 0.78 to 1000 m. At high frequencies, it is bound by the visible red end, while at low frequencies, it is confined by the microwave area.

Wavenumbers or wavelengths are commonly used to describe IR absorption sites

The number of waves per unit length is known as wavenumber. (18) The visible range (380–780 nm) and infrared range (800–2500 nm) of electromagnetic waves are separated by 800–2500 nm.

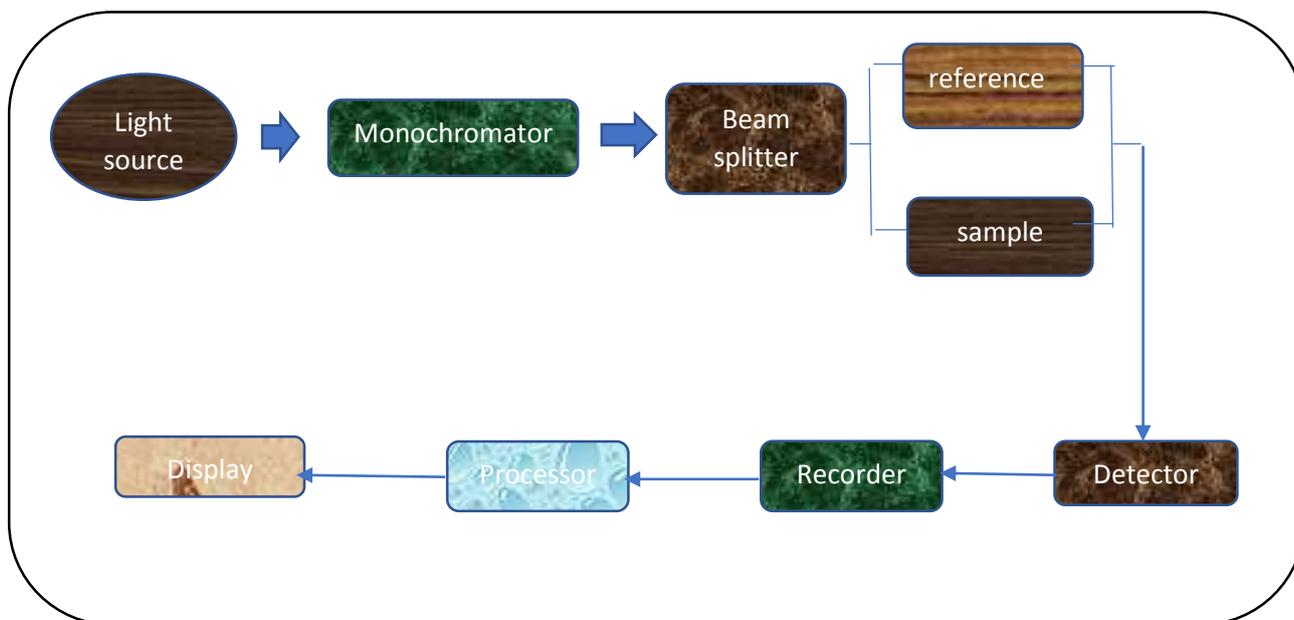
Near-infrared (IR) range (2500–25,000 nm) NIR (near-infrared) spectrum Absorption spectroscopy or absorption spectroscopy are two types of spectroscopy. The term "NIRS" refers to emission in the near-infrared spectrum. Compounds made of organic matter consists of a number of functional groupings, each of which has its own set of core values. IR detects vibration (molecular vibration).

Sr.No	Functional group	IR frequency (wavenumber cm <sup>-1</sup> )
1	C-H aliphatic (stretching)	29950-2840
2	C-H aromatic (stretching)	3100-3000
3	O-H	3700-3100
4	C=O	1750-1720
5	C-O	1150-1050

spectrophotometers are used to capture the IR spectrum. A graph of absorbance or transmittance (percent) vs. frequency is known as an infrared spectrum (cm<sup>-1</sup>). In many disciplines of research laboratories, the Fourier Transform infrared (FTIR) equipment

is often used to record IR spectrum for various objectives such as identification of newly created textile materials, chemical substances, and pharmaceuticals. Near-IR, mid-IR, and far-IR are the three portions of the infrared spectrum of electromagnetic radiation.(20)

Mid-Infrared (MIR) spectroscopy is the most direct method for studying a molecule's vibrational states. Because each frequency corresponds to a certain quantity of energy and a specific molecular motion (e.g. stretching, bending, etc.), the MIR can detect the molecular movements and bonds or functional groups present in the sample.(21)



***Diagram of IR spectrometer with two –beam absorption***

- ***PRINCIPLE:***

The absorption of photons by molecules is the basis for infrared (IR) and near-infrared (NIR) spectroscopy. When the energy of the photon to be absorbed equals the energy difference between two molecule vibrational energy levels, absorption occurs. (The basic vibrational modes are found in the mid-infrared spectral region, which is generally described as 2.5–50  $\mu$ m (200–4000  $\text{cm}^{-1}$ ). The near-infrared, on the other hand, encompasses the wavelength range of 0.75–2.5  $\mu$ m (4000 to 13333  $\text{cm}^{-1}$ )

and includes overtone and combination bands. The production of an oscillating dipole moment during the vibrational motion of the molecule is a general condition for permitting photon absorption. (The following equation can be used to explain this requirement mathematically:  $z\mu$

$zq$

$\neq 0$

where  $q$  is the normal coordinate and  $\mu$  is the dipole moment. When this molecule get fulfilled then it become active in IR (22) An interferometer is a device that sits inside the infrared light source. It serves as a guide The interferometer is made up of a moving mirror and an infrared sample .When it goes through a mirror, it affects the distribution of IR light. The result of radiation that gives the sample is also called as to as, "Interferogram, ". As a function of mirror position output the "Fourier transform" is a data-processing method .turns unprocessed data into predicted outcomes

the spectrum of a sample The amount of light produced is determined by The spectrum of the sample is predominantly in the infrared wavelength.in reference to delicate to a related spectra of IR with label -pattern loudly removed "A two beam" refers to both the reference and sample spectra." absorption spectrometer" is a term used to describe a device that measures(23)

- *APPLICATION:*

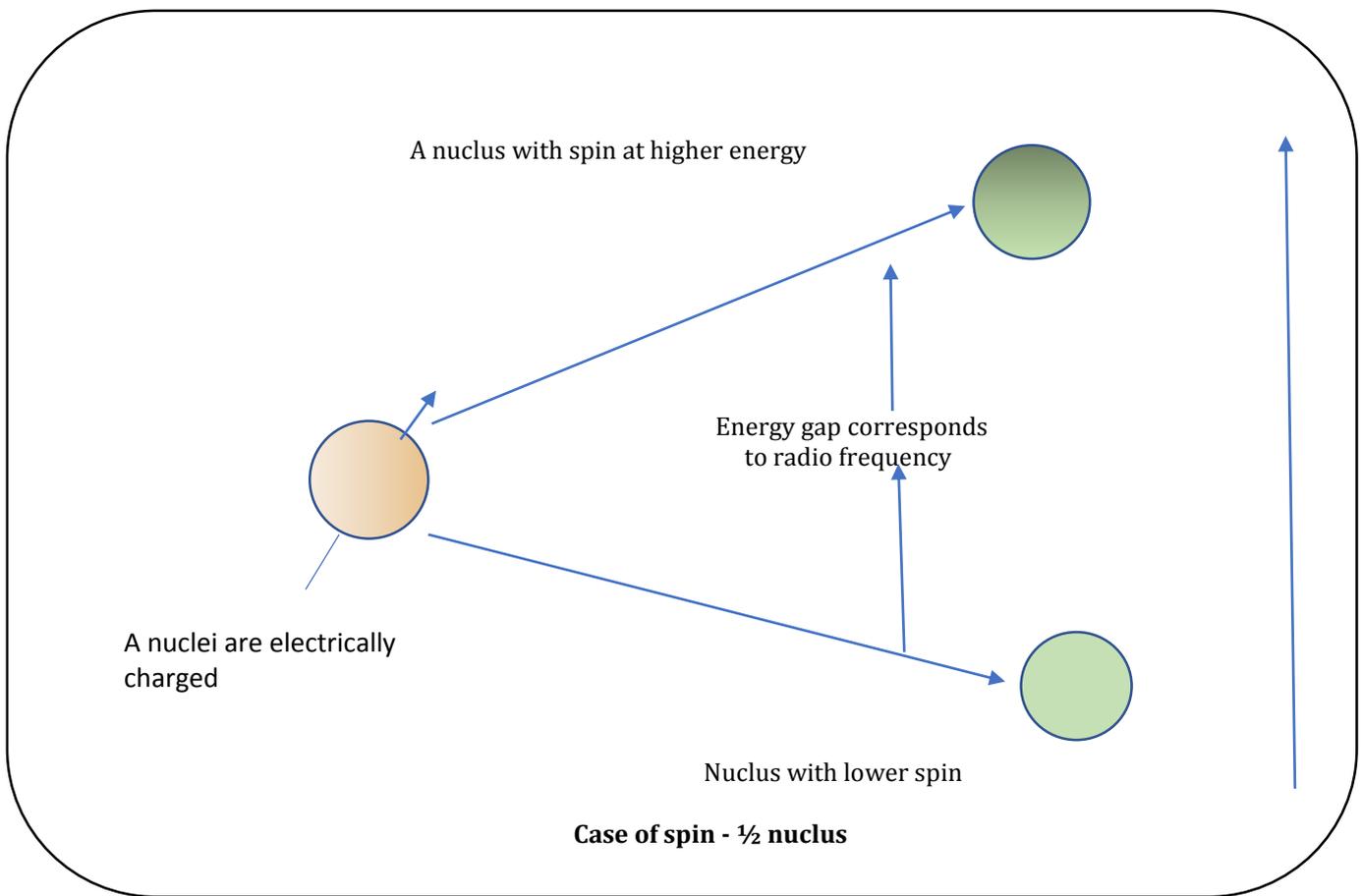
- 1) One of two options for developing an analytical application for identification and classification is to use supervised or unsupervised algorithms.
- 2) Each spectrum in the spectral collection used to train the identification/classification algorithm is assigned to a class in the supervised approach. There is no a priori assumption regarding the number of classes present in the sample set in the unsupervised technique. The algorithm must determine (or assist the user in determining) how the number of groups within the samples can be distributed (cluster analysis), classify the samples of the training set, and offer the model for further classification of unknown data all at the same time.(24)
- 3) They also observed that a harmonic vibrational approach has a low computational performance, which can be compounded by unfavourable growth with particle shape. Since scientists noted out, this severely restricts the feasibility of using similar methodologies to study complex molecules.. (25)
- 4) In comparison to other physicochemical methods of investigation, IR spectroscopy has a high chemical specificity . It's ideal for analysing chemically complicated biological substances qualitatively.(26)
- 5) the capacity to predict protein, fat, and moisture content in meat samples using NIR spectroscopy.(27)
- 6) The goal of using this characterisation approach is to figure out quality control, dynamic measurement, and monitoring applications including long-term unattended CO2 concentration measurements in greenhouses, growth chambers using infrared gas analyser's, and forensic analysis.(23)

- 7) As previously stated, bioactive chemical assessment should now be incorporated into a multidisciplinary study strategy for food research. In this sector, near-infrared (NIR) and mid-infrared (MIR) approaches are fast gaining traction. This spectroscopic approach can predict bioactive chemicals and antioxidant characteristics.(22)
- 8) Infrared spectroscopy is used to detect adulteration and discrimination in olive oil, as well as to monitor contaminations, processes, and recovery. The examination of functional components is the emphasis here.(22)
- 9) Because each frequency corresponds to a given amount of energy and a specific molecular motion (e.g. stretching, bending, etc.), the MIR can identify the types of molecular motions and bonds or functional groups present in the sample, allowing complex systems such as soil components to be characterised.(21)
- 10) Infrared spectroscopy is used in chemistry and industry for identification and characterization of molecule.(16)
- 11) In polymer manufacturing, IR spectroscopy use for to find degree of polymerization(16)
- 12) Cotton fibre contains certain contaminants. It is necessary to identify these contaminants. The method of IR spectroscopy is useful for identifying these contaminants.(20)
- 13) Recently a group of scientists investigated the use of closer spectroscopy (NIRS) to assess various biofuel features.(19)

#### ✓ *NMR SPECTROSCOPY:*

The structure of organic substances may be determined using NMR spectroscopy, which is a rather excellent method of elucidation. NMR spectroscopy makes use of the interaction between a tiny magnet in the nucleus and an external magnetic field to evaluate chemical bonds and the nuclear environment.(28) The signal acquired by NMR spectroscopy contains information on nuclei-electron interactions as well as nuclei-nuclei interactions, which can aid in determining the structure of a chemical complex.(28) The most powerful is NMR, which can provide a wide range of extremely informative two-dimensional (2D) spectra using just sub-milligram amounts of amorphous materials.

It gives comprehensive information on the local chemical environment, as well as the connectivity and stereochemistry of individual hydrogen and carbon atoms.(29) Proton nuclear magnetic resonance (NMR) spectroscopy provides a powerful tool for chemical profiling.(30)



- *PRINCIPLE:*

Many nuclei have spin, and all nuclei are electrically charged, according to the NMR principle. An energy transfer between the base energy and a higher energy level is achievable if an external magnetic field is provided (generally a single energy gap). When the ball back to its ground level, energy is reflect at the same wavelength as when the energy is convert at a frequency that corresponds to radio lable frequencies (Rf). The signal corresponding to this transfer is detected and processed in a variety of ways to produce an NMR spectrum for the nucleus in question. (31)

We used <sup>1</sup>H NMR spectroscopy in combination with chemometric approaches to do a biological compound investigation on a male crowd with normal , hyperuricaemia, and gout.(32)

- *NMR CHEMICAL –SHIFT PREDICTION:*

NMR chemical-shift prediction, as previously indicated, is quite useful in estimating recommended structures.

Empirical and quantum-mechanical (QM) approaches can be used to compute chemical shifts of <sup>13</sup>C, <sup>1</sup>H, <sup>15</sup>N, <sup>19</sup>F, and <sup>31</sup>P nuclei. The most widely used NMR spectrum predictors are HOSE codes neural networks and an incremental technique, which were examined.

The predictors can be included in expert systems (as outlined for ACD/Structure Elucidator and LSD), or they can be used independently as a commercial or free tool. The algorithms estimate <sup>13</sup>C

chemical shifts with an accuracy of 1.5–1.8 ppm, allowing virtually always for the selection of the most likely structure(s) among possibilities. The forecast is made in a short amount of time. (33)

- *APPLICATION:*

- 1) NMR can provide information on the three-dimensional structures of small molecules in solution, also molecule having high molar mass , and related to enzyme mechanism that can be used to help in drug development.(34)
- 2) NMR can be used to directly investigate biological samples and cell cultures without requiring significant sample preparation.(35)
- 3) The use of chiral alignment medium to distinguish enantiomers has a wide range of applications.(36)
- 4) DEPT [8] and SEMUT [9] both employ <sup>13</sup>C NMR spectroscopy. Methods for <sup>1</sup>H NMR spectroscopy editing and selective detection are discussed elsewhere.(37)
- 5) The most effective method for identifying the chemical characteristics of functional groups in biochemistry of macromolecules, as such catalytic groups at enzyme active

sites. Intermolecular interactions that aren't as strong as they should be. Allowing the investigation of weak functional interactions between macro biomolecules (e.g., those with dissociation constants in the micro molar to Mill molar range), which is impossible with current methods.(38)

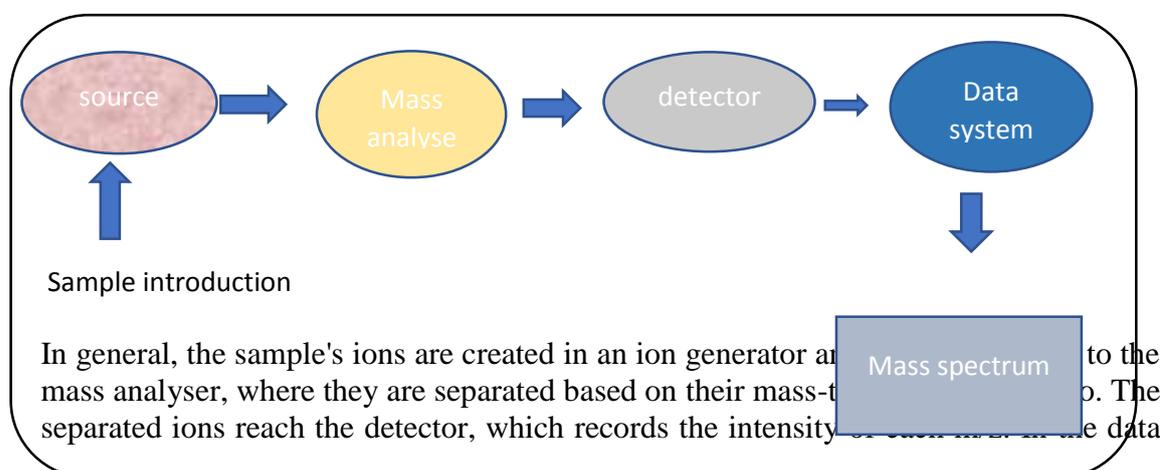
- 6) Verification and identification of organic molecules from a structure database using both <sup>1</sup>H and <sup>13</sup>C-NMR spectra were reported(33)
- 7) A comprehensive characterization of <sup>13</sup>C-labeled poplar plants was reported in an advanced NMR application for the identification of the composition of woody angiosperm plants, when these were preliminarily cultivated in a CO<sub>2</sub>-enriched environment and fed with glucose to roots.(39)
- 8) SSNMR (solid-state NMR spectroscopy) is a highly effective technology for analysing pharmacological dosage formulations. In the pharmaceutical business, the most extensively utilised use of solid state NMR is in the field of polymorphism or pseudo polymorphism. (31)

✓ *MASS SPECTROSCOPY:*

Absorption spectroscopy (MS) technology, particularly simultaneous mass spectrometry (MS/MS), has now been employed as a data defence collection engine in proteome (DDA).(40). Mass spectroscopy is a abbreviated method for studying the geographic dissemination of molecular species in a range of samples in an approximate manner. It can visualise hundreds of molecules in a single experiment without cataloguing them, including metabolites, lipids, fats, peptides, proteins, and glycan's.(41) Due to its great sensitivity, selectivity, accuracy, and throughput capabilities, mass spectrometry (MS) is one of the most powerful analytical instruments, and its application is rising in a variety of scientific domains.(42) The method was originally used to measure the masses of atoms in the early twentieth century, and one of its earliest contributions to science was demonstrating the existence of isotopes.(43)

• *INSTRUMENTATION:*

A vacuum system, an ion source, a mass analyser, an ion detector, and a data recording system are the five major components of a mass spectrometer.



recording system, the ion signal is amplified and processed, yielding the mass spectrum, which is a plot of intensity versus  $m/z$  normalised to the most abundant ion.

The widespread analysis tool, ion sensor, as well as other elements of a spectrograph all are managed to keep in a hollow tube that can reach increasing pressure as low as 10<sup>-9</sup> torr. The free energy of the fuel ions generated increases in air, which is critical for avoiding unwanted interactions.(44)

- *APPLICATION:*

- 1) MSI allows for the identification and mapping of a wide range of molecules on complicated surfaces without the need of labels, and it has become a popular molecular histology technique in pharmaceutical and medical research.(45)
- 2) Mass spectrometry is an extremely strong technique for determining the number of proteins in a sample quantitatively. In comparison to procedures like ELISA or PCR, however, its uptake into biology and medicine is more slower.(40)
- 3) To flexible correct sampling of the molecules of interest, minimum area is necessary for MALDI ionisation. Analyte are isolated from tissue sections and co-crystallized while the matrix crystallises.

- ✓ *X-RAY:*

X-ray absorption probes the unoccupied section of the electronic structure of the system because a core electron is stimulated to an empty state throughout the process. EXAFS analysis offers an accurate assessment of the local geometry for many systems because the excited electron has high kinetic energy in the EXAFS area. Using the multiple scattering concept, the XANES structure may also be modelled.(46)

Single-particle approaches within density functional theory can be used to correctly characterise X-ray absorption spectra. Both metal and ligand K-edge and band structure approaches, as well as multiple scattering computations, can be applied in many cases. The distribution of empty states in the final state of the absorption process is calculated by applying the final state rule. Calculating the final state density of states is complicated since band structure computations are performed in reciprocal space.(46)

In scientific community, X-ray spectroscopic is also known as basic or core-spectroscopy, it's become an invaluable tool for investigating a wide variety of channels.(47)

The use of X-ray spectroscopic to probe extreme properties of matter is fraught with difficulty. The first is due to the high amount of ambient radiation that occurs during interaction testing with high-intensity beams in the pita watts (PW) and mega joule classes (e.g., from bremsstrahlung owing to the presence of MeV electrons, material activation, etc.). The unfriendly environment has a direct influence on the selection of diagnostic techniques, but also appropriate tools and trial designs. As a result, the major question is whether high-resolution X-ray spectroscopy can be used well enough to be

considered a unique and potent biomarker for examining dense materials in challenging environments.(48)

- *PRINCIPLE:*

X-ray spectra methods are particle techniques that employ X-ray stimulus to get knowledge on the electrical or geometric structure of the system being studied. Matter absorbs X rays with energies ranging from a few tens of eV to a few tens of keV mostly due to the photoelectric effect. An electron at a closely confined quantum core level (such as the 1s, 2s, or 2p) of an atom absorbs a photon. During this procedure, an X-ray photon with appropriate energy is produced. A main electron moves to the higher level, null state, or the prograde. When an isotope is charged, one of its core ion levels is left unoccupied after a successful absorption (a core hole). The core hole is filled by an inner or valence shell electron during the decay of this intermediate excited state. When the excitation decays to the final position, an X ray particle, whose energy varies depending on the electricity levels involved with the process, or an Auger electron or an electron, is emitted. This chapter will not, however, address the latter occurrence. The investigation of the mentioned processes is done spectroscopy of X-ray absorption on X-ray emission (XAS and XES, respectively). XAS represents the unfilled electric distribution of stages of an atom, while XES represents the occupied density of state. (35)

- *APPLICATION:*

- 1) It has been widely employed in a variety of scientific domains, beginning with solid-state physics and progressing to chemical and biological research .biology and health sciences, as well as environmental sciences, archaeology, and art research .(46)
- 2) Study contains both basic systems like single molecules and more complex and diverse entities like cellular level.
- 3) uses include stress analysis of chloro peroxidase compounds, assessment of zinc ligand impact in different regions of Zn hyper accumulating plants, and chemical characteristics of enzymes and proteins, among many others.

- ✓ *ATOMIC ABSORPTION SPECTROSCOPY:*

A material must be converted to an atomic vapour in order to use atomic-absorption spectrometry. In the vapour phase, each of the component elements will absorb radiation with a small range of signals spread out over a long period of time a wavelength area that is extremely narrow (about 0.01 nm). (49)

In atomic absorption spectrophotometer (AAS), advancements were made in both the traditional flames AAS and the furnace AAS.

However, remarkable attempts were made to employ all sorts of procedures that allow for the synthesis of gaseous species with metals. (50)

Atomic absorption spectroscopy is a useful tool for analysing metals and non-metals components in inorganic compounds, both quantitatively and qualitatively. (51)

The methodology utilised in all of these systems was to spray the solutions into a flame and measure the absorption by the atoms in the flame at the wavelength of a specific phrase separated by a coupler, using a hollow cathode lamp or a spectral discharge lamp

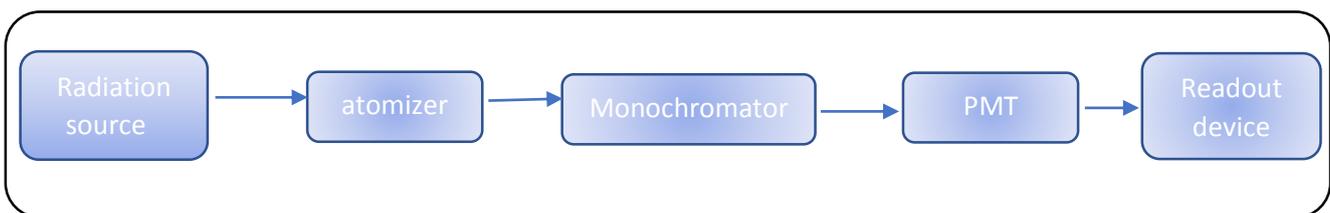
as a source. When steady bulbs were employed, the accuracy was generally good, with coefficients of variation ranging from 1 to 3%. The sensitivity has been found to be adequate—in some cases, more than adequate—for the aim of the approach, and atomic absorption methods have been found to be superior to most other methods in terms of simplicity and speed. Entanglements have been rare, yet they have occurred in several cases.(52)

- *ATOMIZATION:*

Both flame or electro thermal techniques can be used to successfully complete the atomization process. Thermal energy is employed in both procedures to evaporate the analyte material and disrupt the chemical connections between the component molecules. The atomization efficiency is the proportion of the molecule's atoms of concern that are transformed to the gas phase during the atomization process. In the analytical sensitivity of AAS, atomization efficiency is a critical major bottleneck. (51)

- ✓ *FLAME ATOMIZATION:*

A most common approach for creating free atoms in AAS is to fire the sample in a flame. The material to be evaluated in flame AAS must first be dissolved in a suitable solvent. A pneumatic nebulizer is used to turn the electrolyte solution into an aerosol, which is then fed into the flame. Alternative methods have been used, such as ultrasonic nebulization. The flame oxidant gas is used as an atomizing gas in mechanical nebulizer treatments. The oxidant gas differential pressure between the examined solution and the flame head pulls the solution via a sample funnel .(51)



Essential component of atomic absorption spectroscopy

Sr.no	Parameter	UV/visible spectroscopy	Atomic absorption spectroscopy	Fluorescence spectroscopy	Flame emission spectroscopy
1	Electromagnetic radiation	Ultraviolet and visible	Ultraviolet and visible	Ultraviolet and visible	No radiation source use
2	Light source	D2/W lamp	D2/W lamp	D2/W lamp	D2/W lamp
3	Monochromators	Filter/prism /grating	Filter/prism /grating	Filter/prism /grating	Filter/prism /grating
4	Solvent use	Polar solvent like water, methanol	Polar solvent like water, methanol	Polar solvent like water, methanol	Polar solvent like distilled water
5	Concentration range	$\mu$ -mg	$\mu$ -mg	$\mu$ -mg	$\mu$ -mg
6	Physical properties of sample analysed	Absorption	Absorption	Emission	Emission
7	Chemical properties through sample analysed	Chromophore	Valence electron	Fluorophore	Valence electron
8	Principle	Change occur at electronic level of molecule	Change occur at electronic level of atom	Change occur at electronic level of molecule	Change occur at electronic level of atom
9	Mechanism	Moving of electron from HOMO to LUMO	Moving of electron from HOMO to LUMO	Moving of electron from LUMO to HOMO	Moving of electron from LUMO to HOMO
10	Detectors	Photo tube, photovoltaic, photomultiplier	Photo tube, photovoltaic, photomultiplier	Photo tube, photovoltaic, photomultiplier	Photo tube, photovoltaic, photomultiplier
11	Appearance of spectra	Absorbance vs wavelength	Absorbance vs wavelength	%fluorescence vs wavelength	%flame intensity vs wavelength
12	Qualitative respect	Lambda max	Lambda max	Lambda max	Lambda max

*Comparison and difference of principle, instrumentation and application of electronic spectroscopy*

*CONCLUSION:* this study gives information of atomic and emission spectroscopy which include electromagnetic radiation, principle, instrumentation along with their application in research field. Without a doubt, this will assist you in gaining a succinct understanding of the foundations of each strategy. Researchers, scientists, chemists, and pharmacy students are among those who will benefit from this initiative. Also this review tells all compilation of application in various field such pharmaceutical industry, agriculture field.

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