Uttar Pradesh Rajarshi Tondon
open University

DCEBCH - 106

Spectroscopy

Spectroscopy

COURSE INTRODUCTION

The objective of this course is to enhance the knowledge and handling skills of **spectroscopy**. Because, spectroscopy is the main tools, that are very useful to determination of quantities and qualitative information for different physical, chemical, and biological parameters. In current scenario, due modern technological evolution, its make life easy. If we seen pharmaceutical and clinical industry, most to the thinks are based on the spectroscopic studies. This course contain brief introduction, principle, and instrumentation of varies spectroscopic methods to determination of biological and chemical samples. The course is organized into following blocks:

Block 1 covers the basics of Visible and IR spectroscopy

Block 2 deals the nutrition of NMR and Atomic spectroscopy

Block 3 describes in brief discussion of Luminescence and Electron spectroscopy

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Spectroscopy

Block

1

UV-Visible and IR spectroscopy

DCEBCH -106

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Introduction

This is the first block of Spectroscopy. This consists of three units.

Unit-1: In the first unit that have general introduction of spectroscopy. Before going to more detail the student's should learn more about basic concept of spectroscopy. The fundamental law of spectroscopy and electromagnetic radiation discuss in details. The role of spectra in molecular determination and interpretation has also disused. The origin of spectra and its role especially in biochemistry also discuss in this unit.

Unit-2: This unit covers the basic introduction of uv-visible spectroscopy. Spectroscopy governs by different laws such as Beer and Lambert law. The qualitative and quantitative analysis by UV-Visible spectroscopy is mentioned in this unit. The origin of spectra, electronic transition, and composition of color complex and application of UV-Visible described. The role of spectrometer in enzyme kinetics reaction is also discussed.

Unit-3: This unit covers the theory, components and principle of infrared spectroscopy the details study of its, application of FTIR in biochemistry is also mentioned here.

Unit-1: Spectroscopy

Contents

1.1. Introduction

Spectroscopy is the most important tools for the detection of organic and inorganic compounds. In spectroscopy, we study the interaction of radiation with matter to detect the nature of compound. The electromagnetic radiation change their frequency and wavelength when interact with matter resultant spectra is obtained. After studying this module, you shall be able to learn about the nature and properties of electromagnetic waves. You will be introduced to the classical picture of light and electromagnetic waves. The different ranges of electromagnetic radiation are found to be responsible for the different spectra of compounds. Spectroscopy generally contains all science such as physics, chemistry, astrophysics, life sciences, geology, or any other science. However, spectroscopy does not directly show us the molecules but its shows different peaks of radiation absorb by matter, thus it reveal a lots about it structure. The spectroscopy, which is the main tools in study of matter not only reveal structural properties of but also it useful in detection of unknown molecules and measurement of concentrations for analytical purposes. Generally the concept of spectroscopy is clearly appearing in the study of visible light dispersed according to its wavelength, for example by prism. In

prism, visible light pass through it reflect at different angle due to nature of prism particle. Spectroscopic data are often represented by an emission spectrum or absorption spectrum. Spectroscopy is a fundamental exploratory tools in the fields of physical, chemical and biological science at atomic and macro scale.

Objectives

- To understand the effects of electromagnetic radiation on the matter
- To understand the role of electromagnetic radiation in spectroscopy
- To discuss different types of spectroscopy and their characteristic feature
- To understand the applications of spectroscopy in biochemistry.

1.2. Electromagnetic radiation

Light is also described as electromagnetic wave because it changing electric field creates a changing magnetic field. A wave is defined as a disturbance which travels and spreads out through some medium. Wave only moves up and down, normal to the direction of propagation of the wave. Such waves are called transverse waves, and light waves are also transverse. The other kinds of waves are longitudinal, in which the motion is parallel to the direction of motion for example sound wave, which require a medium for their propagation that is known as mechanical waves. Transverse waves can travel in vacuum.

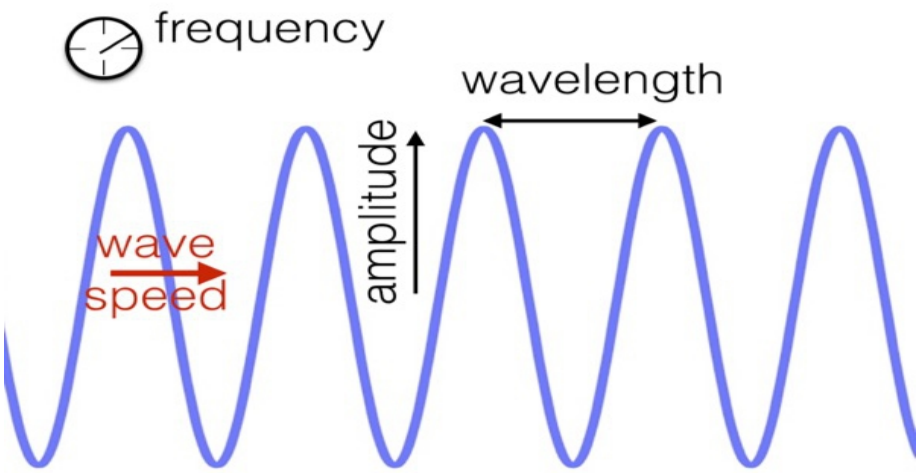


Fig.1.1: Spectra of light

However, the electromagnetic radiation is the source of radiated energy used in spectroscopic investigation of molecules. The electromagnetic spectrum encompasses a wide range of energies, of which visible radiation is

only a small part. Electromagnetic radiation consists of radio waves, microwaves, infrared waves, visible light, ultraviolet radiation, X-rays, and gamma rays.

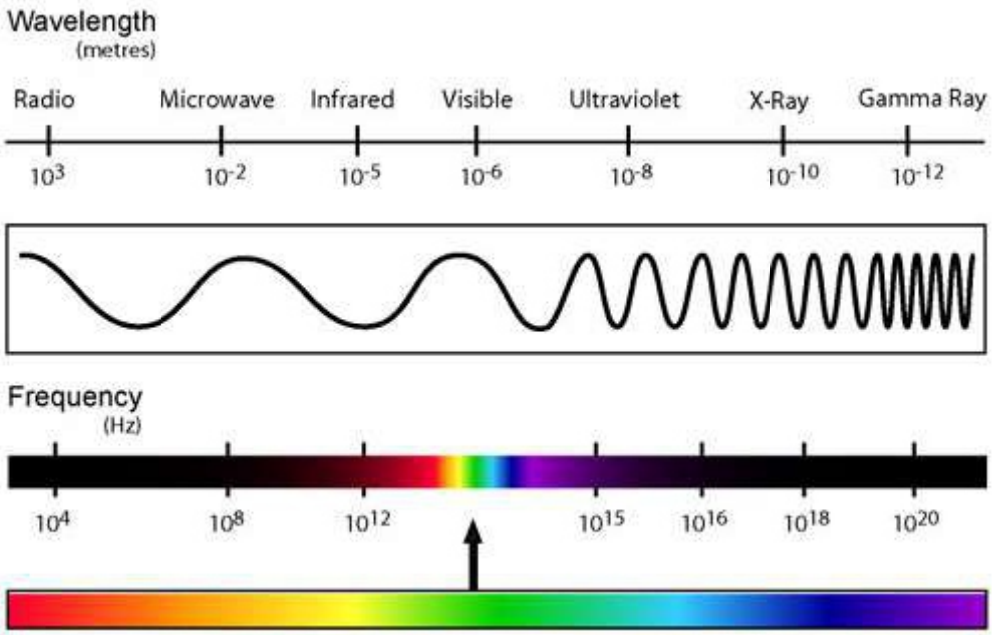


Fig.1.2: Diagram of the electromagnetic spectrum

Every radiation has different wavelength, frequency and energy. The wavelength refers for special distance between two consecutive peaks in sinusoidal waveform and is measured in nanometer (nm). The frequency (υ) of electromagnetic radiation defines the number of oscillator made by the wave within the timeframe of 1 second. If we consider the range of different wave length, the electromagnetic spectrum is useful to reveal in interpretation of frequencies, wavelengths and photon energies of radiation. The electromagnetic radiation cover frequencies form below 1 hertz to above 1025 Hz corresponding to wavelengths which are a few kilometers to a fraction of the size of an atomic nucleus in the spectrum of electromagnetic waves.

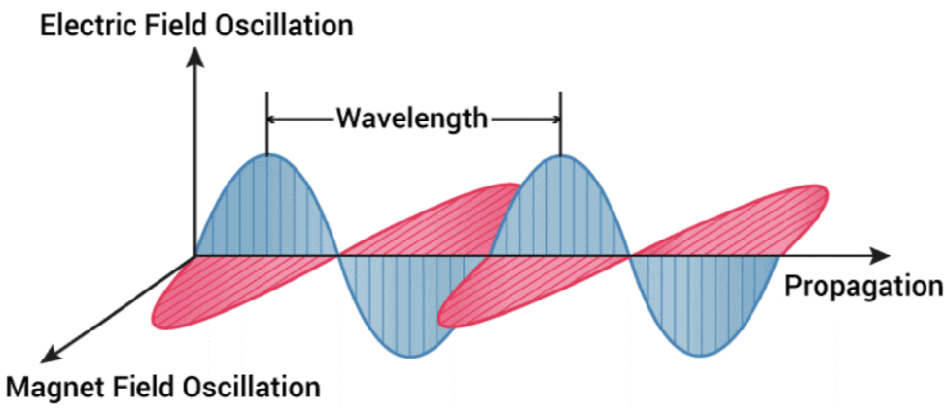


Fig.1.3: Nature of electromagnetic waves

The energy in electromagnetic radiation exists in the form of photons. The quantum phenomenon of electromagnetic radiation depends upon both; properties of the radiation and the appropriate structural part of the samples involves. The electromagnetic radiation is propagated through free space or through a medium and composed of both electric and magnetic waves. The electric and magnetic waves have oscillations that are perpendicular to each other and also to the direction of travel of the wave. There are many sources of electromagnetic radiation, both natural and man-made. Different electromagnetic radiations are given below in Table (1.1) along with their wavelength and frequency. As the wavelength of waves increases frequency get decreases.

Table1.1: Region of electromagnetic spectrum.

Electromagnetic Rays	Wavelength Range	Frequency range(Hz)
Gamma Rays	$< 10^{-12}$ m	$10^{20} – 10^{24}$
X-Rays	1 nm – 1 pm	$10^{17} – 10^{20}$
Ultraviolet	400 nm – 1 nm	$10^{15} – 10^{17}$
Visible	750 nm – 400 nm	$4 \times 10^{14} – 7.5 \times 10^{14}$
Near Infrared	2.5 μm – 750 nm	$1 \times 10^{14} – 4 \times 10^{14}$
Infrared	25 μm – 2.5 μm	$10^{13} – 10^{14}$
Microwaves	1 mm – 25 μm	$3 \times 10^{11} – 10^{13}$
Radiowaves	> 1 mm	$< 3 \times 10^{11}$

1.3. Types of electromagnetic radiation

The electromagnetic spectrum consists of all type of electromagnetic radiation. However, we know that radiation is energy that travel and spreads out in environment. It produce form different source that consist of their basic properties of electromagnetic. The visible light that comes from a lamp in our house, and the radio waves that come from a radio station are two types of example of electromagnetic radiation. Some other types of radiation produce from different sources and that make up the electromagnetic spectrum are microwaves, infrared light, ultraviolet light, X-rays and gamma-rays.

Radio waves: The radio wave is the lowest range of electromagnetic radiation having frequency about 30 billion hertz, or 30 gigahertz (GHz), and wavelengths greater than about 10 millimeters (0.4 inches). It is used primarily for communications including voice, data and entertainment media.

Microwaves: Microwaves fall in the range of the electromagnetic spectrum between radio and IR. It has frequency between 3 GHz up to about 30 trillion hertz. It use for high-bandwidth communications, radar and as a heat source for microwave ovens and industrial applications.

Infrared: Infrared is in the range of the electromagnetic (EM) spectrum between microwaves and visible light. Infrared EMR commonly interacts with dipoles present in single molecules, which change as atoms vibrate at the ends of a single chemical bond. Infrared light is invisible to human eyes, but we can feel it as heat if the intensity is sufficient. Infrared radiation is divided into several spectral range such as near-infrared (0.75–1.4 μm), short-wavelength infrared (1.4–3 μm), mid-wavelength infrared (3–8 μm), long-wavelength infrared (8–15 μm) and far infrared (15–1000 μm).

Visible light: Natural sources produce EM radiation across the spectrum. EM radiation with a wavelength between approximately 400 nm and 700 nm is directly detected by the human eye and perceived as visible light. It has frequencies of about 400 THz to 800 THz and wavelengths of about 740 nm (0.00003 inches) to 380 nm (0.000015 inches). More generally, visible light is defined as the wavelengths that are visible to most human eyes. Photosynthesis becomes possible in this range as well, for the same reason.

Ultraviolet: Ultraviolet light is in the range of the EM spectrum between visible light and X-rays. It has frequencies of about 8 × 1014 to 3 × 1016 Hz and wavelengths of about 380 nm. UV light is a component of sunlight; however, it is invisible to the human eye. It has numerous medical and industrial applications, but it can damage living tissue.

X-rays: X-rays are a type of radiation called electromagnetic waves. X-ray imaging creates pictures of the inside of your body. It is a penetrating form of high-energy electromagnetic radiation. Most X-rays have a wavelength ranging from 10 picometers to 10 nanometers, corresponding to frequencies in the range 30 petahertz to 30 exahertz X-rays are roughly classified into two types: soft X-rays and hard X-rays. Different types of X-rays are used for different purposes. For example, your doctor may order a mammogram to examine your breasts. Or they may order an X-ray with a barium enema to get a closer look at your gastrointestinal tract.

Gamma-rays: A gamma ray, also known as gamma radiation, is a penetrating form of electromagnetic radiation arising from the radioactive decay of atomic nuclei. Gamma rays have the smallest wavelengths and the most energy of any wave in the electromagnetic spectrum. Gamma-rays have frequencies greater than about 1018 Hz and wavelengths of less than 100 pm (4 × 10−9 inches). Gamma radiation causes damage to living tissue, which makes

it useful for killing cancer cells when applied in carefully measured doses to small regions.

Table1.2.: List of electromagnetic radiation of different types of spectroscopy techniques

Region of electromagnetic radiation (energy per photon)	**Process that occur and the corresponding spectroscopy techniques**
Gamma rays 10^5-10^6 eV/photon	Nuclear transitions, change of nuclear configuration **Mössbauer spectroscopy**
X-rays 10^2-10^4 eV/photon	Inner shell electronic transitions Electron **spectroscopy, XPS, Aüge**
Ultraviolet and visible rays 1-10^2 eV/photon	Valence shell electronic transitions in molecules **Electronic spectroscopy** also known **as UV-Vis spectroscopy**
Infrared rays 10^{-2}-1 eV/photon	Transition among vibrational levels of molecules Vibrational spectroscopy also known as **Infrared Spectroscopy**
Infrared rays 10^{-2} – 1 eV/photon	Transitions among rotational levels of molecules **Rotational spectroscopy**
Radio wave rays 10^{-9} – 10^{-6} eV/photon	Change of electron and nuclear spins in the presence of a magnetic field. **Nuclear Magnetic Resonance (NMR)** and **Electron Spin Resonance (ESR) spectroscopy**

1.4. Origin of spectra

In spectroscopy, we are interested in the absorption and emission of radiation by matter and its consequences. A spectrum is the distribution of photon energies coming from a light source:

• How many photons of each energy are emitted by the light source? Spectra are observed by passing light through a spectrograph.

• Breaks the light into its component wavelengths and spreads them apart (dispersion).

• Uses either prisms or diffraction gratings.

The absorption and emission of photons are governed by the Bohr condition $\Delta E = h\nu$. This relation and the prism (or diffraction grating) which casts white light into its components with known wavelengths bent by known angles, give us the tools we need to peer into the inner workings of atoms! Not merely check their size (atomic microscopy) but rather determine experimentally their internal energy states! How? By giving them a kick and seeing what light falls out.

Atomic Spectra Atomic spectra are line spectra. The peaks are sharp because only electronic transitions are possible in atoms, whereas in molecules, rotational and vibrational energy changes can also take place simultaneously. Molecular spectra are much more complex, even for the simplest molecule, hydrogen, as shown in Figure 1, where the emission spectrum of hydrogen molecule (top) is much more complex than that of atomic hydrogen (bottom).

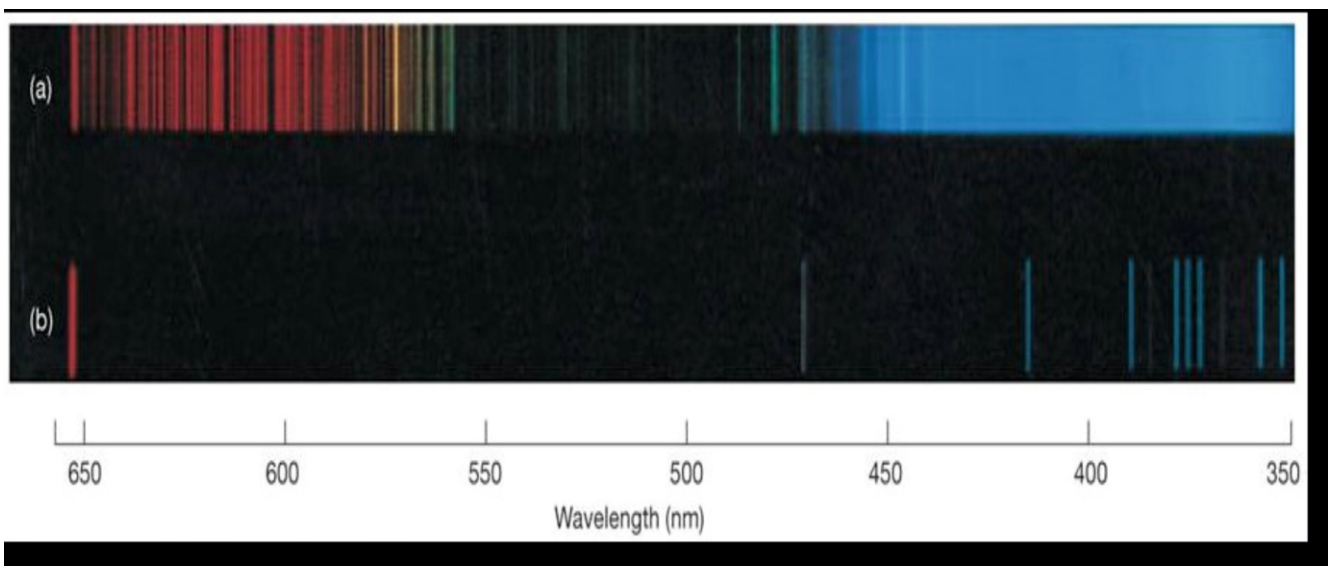


Fig.1.4: a) Molecular hydrogen b) atomic hydrogen

Hydrogen Spectrum

We know when the atom absorbs quantum of energy then it goes to exited state and after that when it return to their original or ground state then atom emit a light appeared as a series of discrete lines characteristic of the particular element series is called spectrum of that particular elements. For example, the familiar red light of neon signs is due to neon atoms which have been excited by an electrical discharge. Various colure in spectrum is occurs abortion of their corresponding color of light of definite wavelengths and the series of lines

is occurs that called a line spectrum. The line spectrum of each element is so characteristic of that element that its spectrum may be used to identify. This same procedure is followed by hydrogen atom thus the emission or absorption processes in hydrogen give rise to series particular wavelength of light, which are sequences of lines corresponding to its atomic transitions. The emission spectrum of atomic hydrogen has been divided into a number of spectral series, with wavelengths given by the Rydberg formula.

The hydrogen has single electron, the energy of electron in nth orbital of hydrogen atom, E_n is calculate by following formula

$$E_n = \frac{-Z^2 e^4 m}{8\varepsilon_0^2 h^2 n^2}$$

Where,

Z = atomic number

E = charge of electron

M = mass of electron

ε_0 = permittivity in vacuum and has the mass value 8.854 x 10^{-12} C^2N^{-1} m^{-2}

h = planck constant

n = the number of orbits

Thus the energy difference

$$\Delta E - E_2 - E_1 = \frac{-Z^2 e^4 m}{8\varepsilon_0^2 h^2 n_2^2} - \frac{-Z^2 e^4 m}{8\varepsilon_0^2 h^2 n_1^2}$$

$$\Delta E = \frac{-Z^2 e^4 m}{8\varepsilon_0^2 h^2} \left(\frac{1}{n_2^2} - \frac{1}{n_1^2}\right)$$

We can say that wave number of the radiation associated with the above energy difference can be express as

$$v = \frac{\Delta E}{hc} = \frac{-Z^2 e^4 m}{8\varepsilon_0^2 h^3 c} \left(\frac{1}{n_2^2} - \frac{1}{n_1^2}\right)$$

The term $\frac{-Z^2 e^4 m}{8\varepsilon_0^2 h^3 c}$ is called the Rydberg constants and is donated by symbol R_H for hydrogen

$$v = R_H \left(\frac{1}{n_2^2} - \frac{1}{n_1^2}\right)$$

The experimental value of Rydberg constant is 1.09677 x 10^7 m^{-1}, but the theoretical value of Rydberg constant is donated by 'R' .

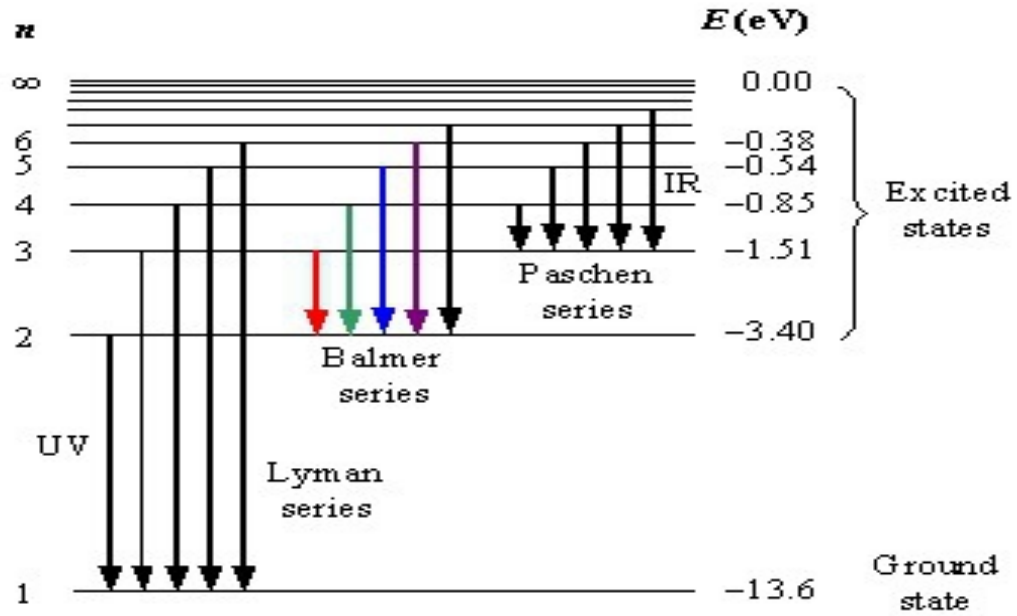


Fig.1.5: Energy level of the hydrogen atom with some of the transition between them that give rise to the spectral line

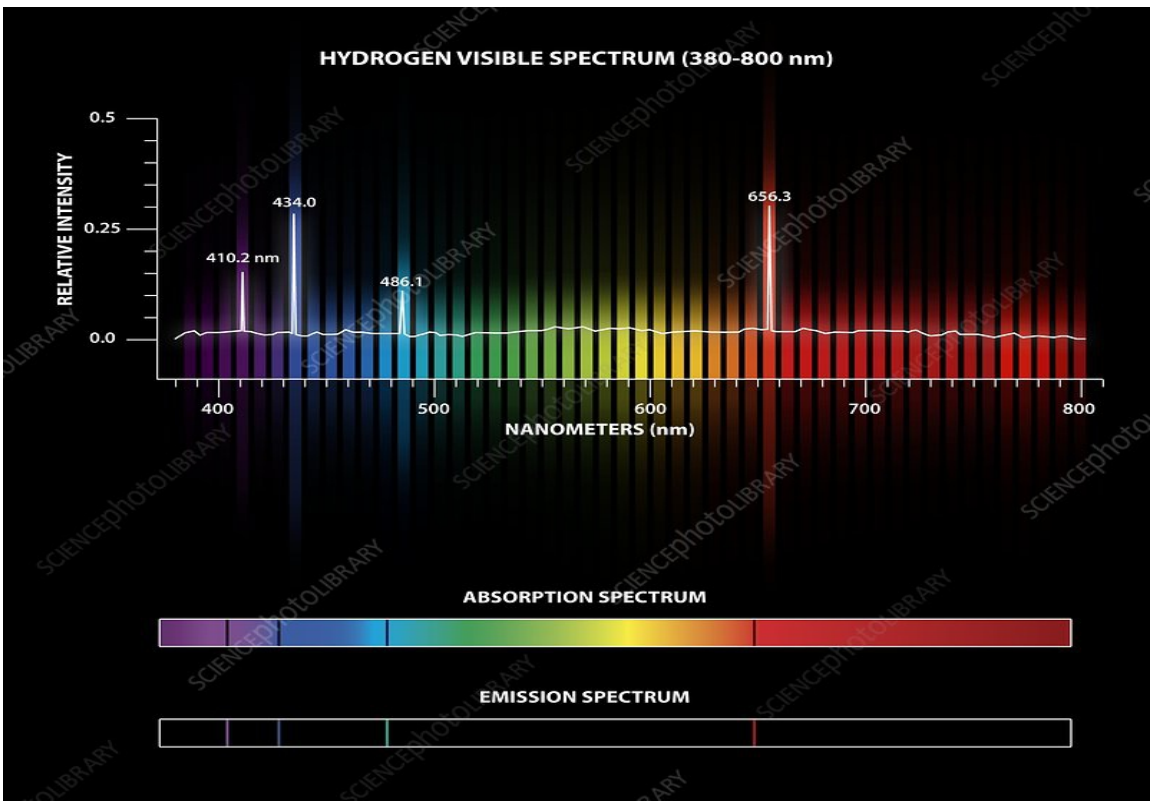
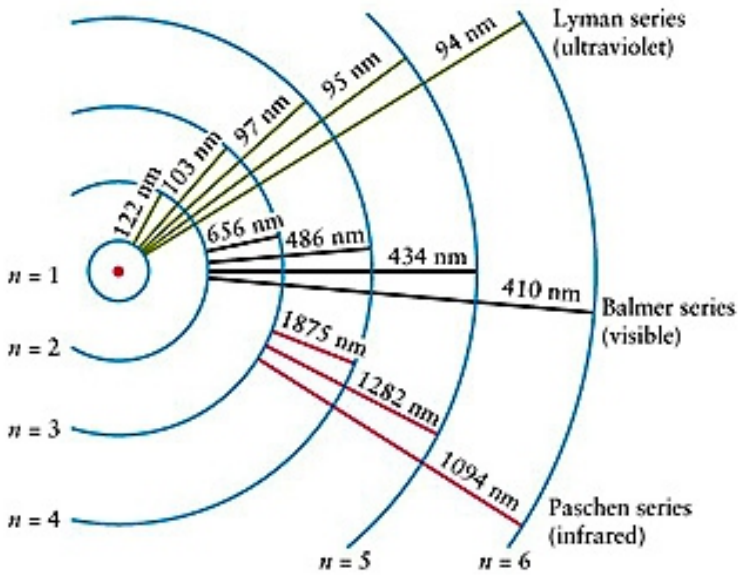


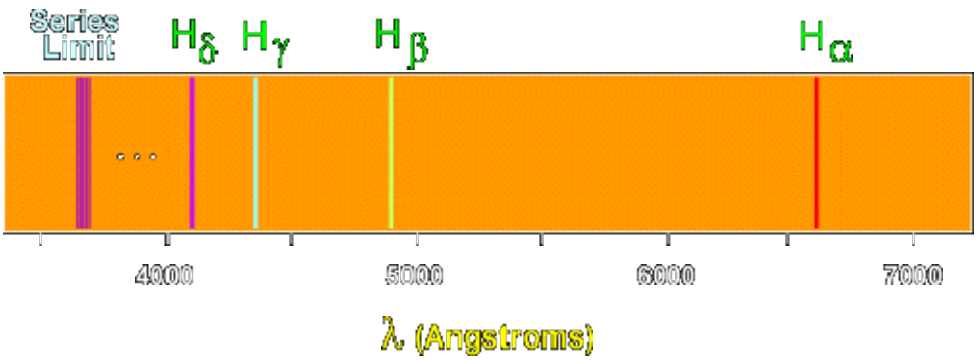
Fig.1.6: Hydrogen visible spectrum

These observed spectral lines are due to the electron making transitions between two energy levels in an atom. Thus, for example, the *Balmer Series* involves transitions starting (for absorption) or ending (for emission) with the first excited state of hydrogen, while the *Lyman Series* involves transitions that start or end with the ground state of hydrogen; the adjacent image illustrates the atomic transitions that produce these two series in emission. The various series of lines are named according to the lowest energy level involved in the transitions that give rise to the lines.

The Lyman series involve jumps to or from the ground state (n=1); the Balmer series (in which all the lines are in the visible region) corresponds to n=2; the Paschen series to n=3, the Brackett series to n=4, and the Pfund series to n=5.



The Lyman series is in the ultraviolet while the Balmer series is in the visible and the Paschen, Brackett, Pfund, and Humphreys series are in the infrared.



However, in 1914, Niels Bohr explains the theory of hydrogen spectra. According to this theory, the wavelengths of the hydrogen spectrum could be calculated by the following formula known as the Rydberg formula:

$$\frac{1}{\lambda_{vac}} = RH\left(\frac{1}{n_2^1} - \frac{1}{n_2^2}\right)$$

Where

λ_{vac} is the wavelength of the light emitted in vacuum in units of cm,

R_H is the Rydberg constant for hydrogen (109,677.581 cm^{-1}),

n_1 and n_2 are integers such that $n_1 < n_2$,

The series of spectral lines may be obtained using the values of n_1 and n_2 in the following table:

n1	n2	Name	Converge toward
1	$2 \rightarrow \infty$	Lyman series	91nm
2	$3 \rightarrow \infty$	Balmer series	365nm
3	$4 \rightarrow \infty$	Paschen series	821nm
4	$5 \rightarrow \infty$	Brackett series	1459nm
5	$6 \rightarrow \infty$	Pfund series	2280nm
6	$7 \rightarrow \infty$	Humphreys series	3283nm

1.5. Types of Spectroscopy

Spectroscopy is a fundamental tool of scientific study, with applications ranging from materials characterization to astronomy and medicine. Spectroscopy techniques are commonly categorized according to the wavelength region used, the nature of the interaction involved, or the type of material studied. The word spectroscopy comes from the Latin word *specere*, means "to look at" and the Greek word *skopia*, means "to see". So that in spectroscopy refers the techniques that employ light to produce properties of object. Spectroscopy is the technique that is widely useful in the detection of organic and inorganic compounds. This techniques based the phenomenon of the interaction of radiation with matter resulted spectra is obtained of particular compounds. The electromagnetic radiation is used and change their frequency and wavelength

when interact with matter resultant spectra is obtained. However, the light interacts with matter and probe certain features of sample to learn about it consistency or structure. Thus we can say that the Spectroscopy means the dispersion of light into component colours. In simple words, it is a method to measure how much light is absorbed by a chemical substance and at what intensity of light passes through it. In spectroscopy, the type of interaction between light and the material is usually. There are some common types of spectroscopy-

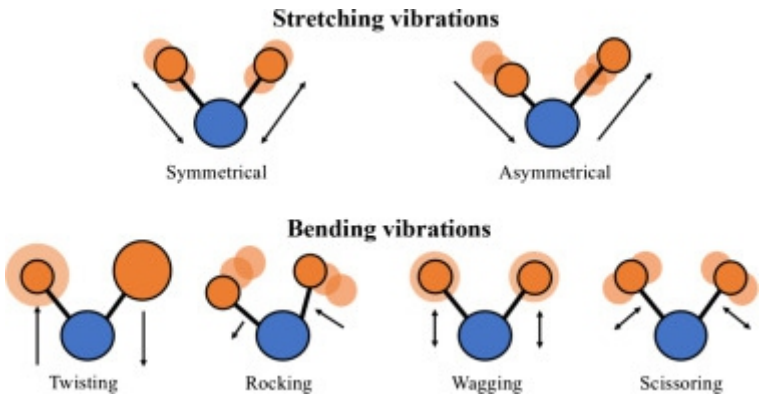
a. Infrared (IR) Spectroscopy

Infrared spectroscopy is the one of widely used techniques in the determination of information about molecules. The rays of the infrared region have longer wavelength whereas having a lower frequency than light. IR spectrometers typically measure the relative absorption of different frequencies in the IR region by a sample. Infrared spectroscopy is based on absorption spectroscopy. Infrared region most useful for analysis of organic compounds is wavelength range from 2,500 to 16,000 nm, with a corresponding frequency range from 1.9*1013 to 1.2*1014 Hz. Photon energies associated with this part of the infrared (from 1 to 15 kcal $mole^{-1}$) are not large enough to excite electrons, but may induce vibrational excitation of covalently bonded atoms and groups. The infrared spectrum rise from the fact that molecule absorbs incident radiation of a certain wavelength which will then be missing from the transmitted light. The recorded spectrum will show absorption. The photons in the infrared region of the electromagnetic spectrum have characteristic energies corresponding to those of molecular vibrations. The number of functional groups increases in more complex molecules, the absorption bonds becomes more difficult to assign. In IR spectroscopy, the fundamental frequencies observed are characteristics of the functional groups concerned, hence the term fingerprint. Once a spectrum is obtained, the main challenge is to extract the information it contains in abstract or hidden form. This requires the recognition of certain patterns, the association of these patterns with physical parameters, and the interpretation of these patterns in terms of meaningful and logical explanations. Identification of substances IR spectroscopy is used to establish whether a given sample of an organic substance is identical with another or not. This is because large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds will produce identical spectra is almost zero. So if two compounds have identical IR spectra then both of them must be samples of the same substances.

Vibration Mode

In order for a vibration mode in a sample to be "IR active", it must be associated with changes in the dipole moment. A permanent dipole is not necessary, as the rule requires only a change in dipole moment. A molecule can

vibrate in many ways, and each way is called a vibrational mode. IR radiation causes the excitation of the vibrations of covalent bonds within that molecule. These vibrations include the stretching and bending modes. Number of vibrational modes- Covalent bonds can vibrate in several modes, including stretching, rocking, and scissoring. The most useful bands in an infrared spectrum correspond to stretching frequencies, and those will be the ones we'll focus on



More complex molecules have many bonds, and their vibrational spectra are correspondingly more complex, i.e. big molecules have many peaks in their IR spectra. Atom, can vibrate in nine different ways. Asymmetrical diatomic molecules, e.g. CO, absorb in the IR spectrum. If the molecule is symmetrical, e.g. N_2, the band is not observed in the IR spectrum, but only in the Raman spectrum. Simple diatomic molecules have only one bond and only one vibrational band.

b. Ultraviolet-Visible (UV/Vis) Spectroscopy

The ultraviolet (UV) and visible regions of the electromagnetic spectrum correspond to electron energy level transitions in atoms and molecules. Instrument used to measure the absorbance in UV (200-400 nm) or Visible (400-800 nm) region is called UV- Visible spectrophotometer. A spectrophotometer records the degree of absorption by a sample at different wavelengths and the resulting plot of absorbance (A) versus wavelength (λ) is known as a Spectrum. The spectrophotometer instrumentation has following components such as

- Light Source
- Recorder
- Detector
- Sample compartment
- Wavelength selectors or Dispersing devices

UV-Visible used following radiations such as

UV Radiation – Wavelength range 220 – 380 nm

Visible Radiation – Wavelength range 380 – 780 nm

Coloured compounds absorb energy in both UV and visible region of the electromagnetic spectrum. Substances can be liquids or solids and measurements are made with instruments called SPECTROPHOTOMETERS or SPECTROMETERS. UV/Vis spectroscopy can therefore be used to probe the electronic structure of molecules in a sample, subsequently enabling identification of the compounds present. Modern instruments can be coupled to microscopes which allow solid samples and very small samples of solids and liquids to be analysed both qualitatively and quantitatively. UV/Vis spectroscopy is particularly useful for identifying peptide bonds, certain amino acid side chains, and certain prosthetic groups and coenzymes.

c. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear magnetic resonance spectroscopy is a technique used to measure the magnetic fields that exist around atomic nuclei. NMR spectroscopy uses radio waves to excite atomic nuclei in a sample. When nuclei start to resonate, this is detected by sensitive radio receivers. As the resonant frequency of an atomic nucleus depends on the electronic structure of the molecule of which it is a part, NMR spectroscopy provides detailed information about the structure and reaction state of molecules. It is hence a powerful tool for deducing the exact nature of monomolecular organic compounds.

d. Raman Spectroscopy

Raman spectroscopy is solely concerned with the inelastic scattering of photons, known as Raman scattering, where the apparent wavelength of a photon is changed when it interacts with the sample. Raman scattering uses a source of monochromatic light that is used to illuminate the sample. Raman scattering provides similar yet complementary data to IR spectroscopy. When light is scattered by molecule, the oscillating electromagnetic field of a photon induces a polarization of the molecular electron cloud which leaves the molecule in a higher energy state with the energy of the photon transferred to the molecule. This can be considered as the formation of a very short-lived complex between the photon and molecule which is commonly called the virtual state of the molecule. The virtual state is not stable and the photon is re-emitted almost immediately, as scattered light.

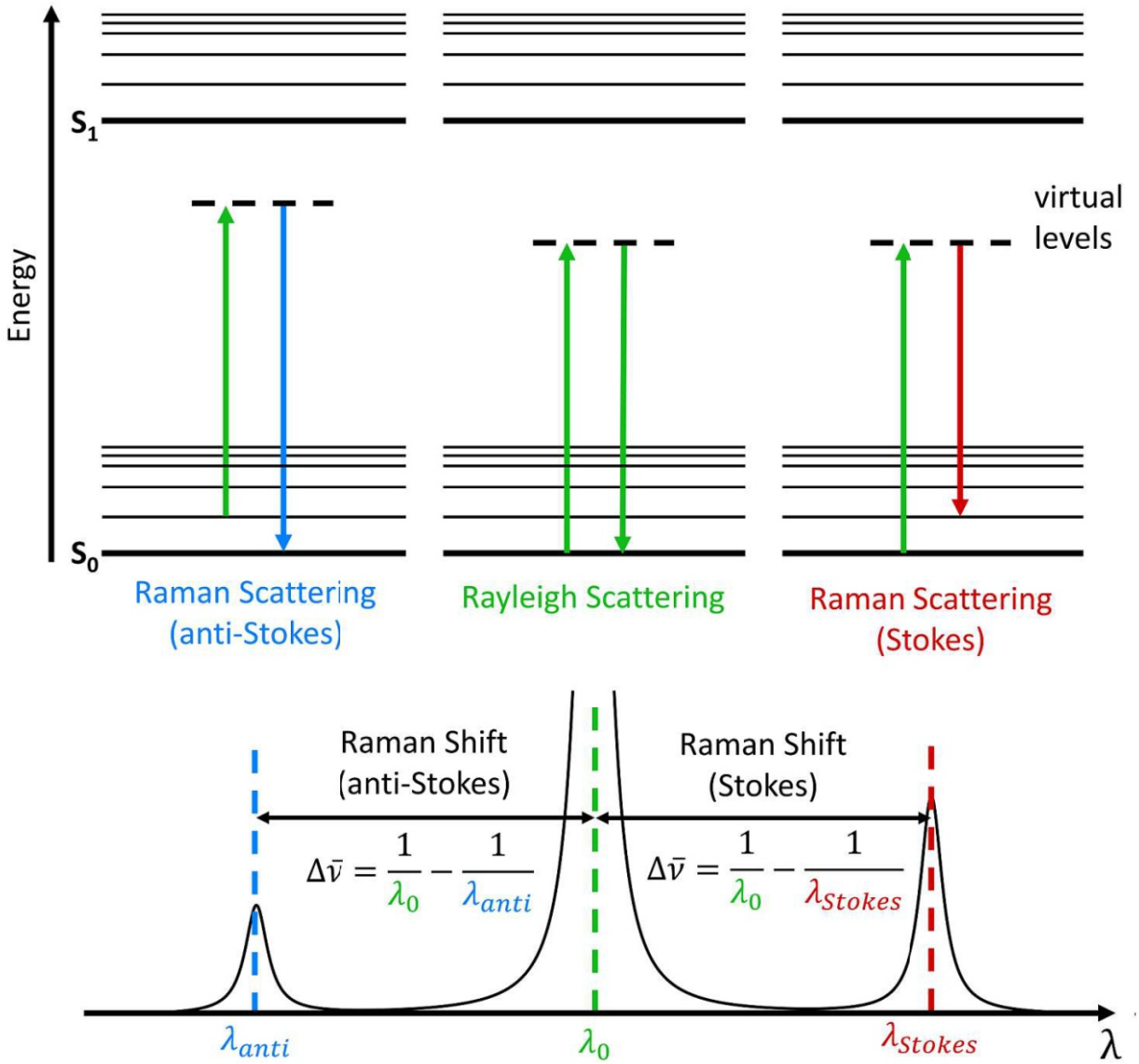


Fig.1.7: Stocks line in Raman scattering

e. X-Ray Spectroscopy

The use of X-ray spectroscopy began with the development of X-ray crystallography in 1912. Father-and-son team William Henry Bragg and William Lawrence Bragg showed that the diffraction patterns created by X-rays passing through crystalline materials could be used to deduce the nature of the crystal structure. X ray spectroscopy is non destructive techniques to analysis of crystalline material. X-ray absorption, X-ray diffraction and X-ray florescence are the three main field of X-ray spectroscopy. Amongst four techniques, the X ray diffraction (XRD) is most useful in elucidation of structure of compounds. The X-ray diffraction based on its scattering by crystal is extremely important as compared to other. By X- ray diffraction, one can be identify the crystal structure of determine the particle size. The XRD is

scarcely used for quantitative analysis but in some time it is also useful in quantitative analysis. The X- ray has significance aspect due to much shorter wavelength of about < 0.01 – 10 nm.

In XRD, the interaction of electromagnetic radiation with matter causes the electron in the exposed sample to oscillate. The secondary waves that are produced by accelerated electron have same frequency as incident radiation. This super-position of waves gives rise to phenomenon of interference. The interference gives rise to dark and white ring line or spot. Since the distance between atom and ions is of the order of 10-1 m (1 A˚), different methods are used to determine structure at atomic level. In this case X- ray region of electromagnetic radiation is required. In the same way, when an X- ray beam is diffracted off a crystal, the different parts of the diffracted beam will have seemingly stronger energy, while other parts will have seemed to lost energy.

$$n\lambda = 2dsin\theta$$

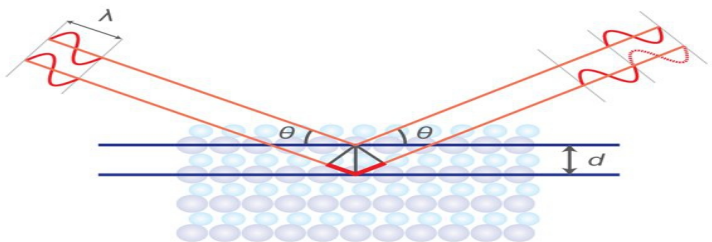


Fig.6.1: Schematic representation of Bragg's law interference

Where 'n' is an integer called the order of reflection, λ is the wavelength of x-rays, d is the characteristic spacing between the crystal planes of a given specimen and θ is the angle between the incident beams. By measuring the angles 'θ' under which the constructively interfering x-rays leave the crystal, the inter planar spacing 'd' of every single crystallographic phase can be determined.

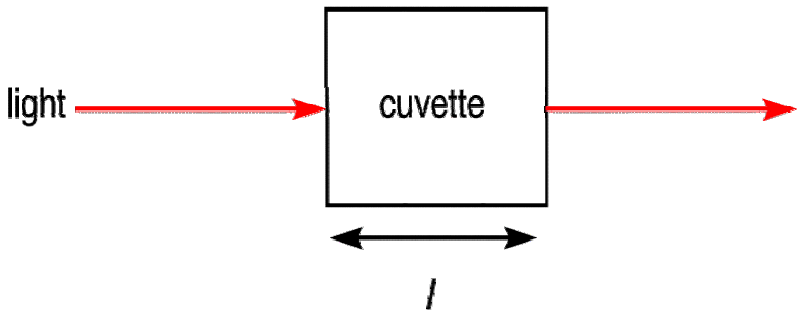
1.6. Fundamental law of spectroscopy

The Beer-Lambert law is a linear relationship between the absorbance and the concentration, molar absorption coefficient and optical coefficient of a solution. For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured. This is usually referred to as Io - that's I for Intensity.

The chance for a photon to be absorbed by matter is given by an extinction coefficient which itself depend on the wavelength (λ) of the photon. If the intensity Io passes through a sample with appropriate transparency and the path length (thickness) 'l', the intensity 'I' drops along the pathway in an exponential manner.

The absorbance of a transition depends on two external assumptions.

The characteristic absorption parameter for the sample is the extinction coefficient α. Which yield by the equation $I = I_0 e^{-\alpha d}$. The ratio of $T = I/I_0$ is called transmission.



$$A = \varepsilon cl$$

Where, A = Absorbance of sample, which display on the spectrophotometer.

ε = Molar absorption coefficient M^{-1} cm^{-1}

C = Molar concentration "M"

l = optical path length in cm

The Beer-Lambert law is valid for low concentrations only. The absorption and extinction coefficients are additives parameters, which complicates determination of concentrations in samples with more than one absorbing species.

The intensity of the light passing through the sample cell is also measured. For that wavelength, given the symbol 'λ'. If 'λ' is less than I_0, then the sample has absorbed some of the light (neglecting reflection of light off the cuvette surface). The absorbance of a transition depends on two external assumptions-

1. The absorbance is directly proportional to the concentration (C) of the solution of the sample used in the experiment.
2. The absorbance is directly proportional to the length of the light path, which is equal to the width of the cuvette.

1.7. Applications of spectroscopy in biochemistry

- The spectroscopy used for the study of molecular or atomic structure of a substance by observation of its interaction with electromagnetic radiation.
- Spectroscopy is the study of interaction of electromagnetic radiation with matter as a function of frequency Spectroscopy is the study of the exchange of energy between electromagnetic radiation and matter.

- Depending on the region of the electromagnetic spectrum used for the excitation, different processes occur in atoms and molecules leading to different spectroscopic technique

The main application of IR spectroscopy in biochemistry is to-

Qualitatively

- decreased the time to confirm compound identification 10- 1000 fold.
- examine what functional groups are present by looking at group frequency region - 3600 cm-1 to 1200 cm^{-1}.
- approximate frequency of many functional groups (C=O,C=C,C-H,O-H) can be calculated from atomic masses & force constants.
- fingerprint Region (1200-700 cm^{-1}) –
  - region of most single bond signals.
  - many have similar frequencies, so affect each other & give pattern characteristics of overall skeletal structure of a compound.
  - exact interpretation of this region of spectra seldom possible because of complexity uniqueness- complexity
- Computer Searches - many modern instruments have reference IR spectra on file (~100,000 compounds) - matches based on location of strongest band, then 2nd strongest band, etc

Quantitative Analysis

- not as good as UV/Vis in terms of accuracy and precision
- more complex spectra
- narrower bands (Beer's Law deviation)
- limitations of IR instruments (lower light throughput, weaker detectors)
- high background IR
- difficult to match reference and sample cells

1.8. Summary

In spectroscopy, we are interested in the absorption and emission of radiation by matter and its consequences. Atomic spectra are line spectra because only electronic transitions are possible in atoms, whereas in molecules, rotational and vibrational energy changes can take place simultaneously. Various colours in spectrum are occur due to absorption of their corresponding colour of light of definite wavelengths and the series of lines is occurs that called a line spectrum. The line spectrum of each element is so characteristic of that element that its spectrum may be used to identify. Infrared spectroscopy is based on absorption spectroscopy. Infrared region most useful for analysis of organic compounds is wavelength range from 2,500 to 16,000 nm, with a

corresponding frequency range from 1.9*1013 to 1.2*1014 Hz. The XRD and NMR would be leading to biochemistry regarding characterization and detection any biological compounds. The body of organism contains groups of chemicals, and every chemical have different characteristic character and gives their performances accordingly. The chemical phenomenon creates abnormality in organism due to presence of various functional groups or different chemical structure. Thus, the instrumentation techniques are found very useful in understanding of chemical nature and structure of compounds. The NMR with learning of XRD and NMR techniques student would create new idea to utilize these techniques for characterization of biological samples.

1.9. Terminal questions

Q.1. What do you understand for spectroscopy? Write the application of spectroscopy.

Answer:----------

Q.2. Discuss about Hydrogen Spectrum.

Answer:----------

Q.3. Write the phenomenon of X- ray spectroscopy.

Answer:----------

Q.4. Write the low of UV- visible spectroscopy.

Answer:----------

Q.5. Describe the electromagnetic radiation in spectroscopy.

Answer:----------

Q.6. XRD work on which law? Discuss briefly.

Answer:----------

1.10. Further readings

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Unit-2: UV-Visible spectroscopy

Contents

2.1. Introduction

In spectroscopy, we study the interaction of radiation with matter to detect the nature of compound. Spectroscopy is the most important tools for the detection of organic and inorganic compounds. Generally, the concept of spectroscopy is clearly appearing in the study of visible light dispersed according to its wavelength, for example by prism. In prism, visible light pass through it reflects at different angle due to nature of prism particle. Spectroscopic data are often represented by an emission spectrum or absorption spectrum. However, the light interacts with matter and probe certain features of sample to learn about it consistency or structure. To better understanding of the spectroscopy we should know about the electromagnetic radiation, the interaction of electromagnetic radiations with matter is a quantum phenomenon. The different ranges of electromagnetic radiation are found to be responsible for the different spectra of compounds. Electromagnetic radiation consists of radio waves, microwaves, infrared waves, visible light, ultraviolet radiation, X-rays, and gamma rays. Every

radiation has different wavelength, frequency and energy. The wavelength refers for special distance between two consecutive peaks in sinusoidal waveform and is measured in nanometer (nm). The frequency (υ) of electromagnetic radiation defines the number of oscillator made by the wave within the timeframe of 1 second. The quantum phenomenon of electromagnetic radiation depends upon both; properties of the radiation and the appropriate structural part of the samples involves. The electromagnetic radiation is propagated through free space or through a medium and composed of both electric and magnetic waves. The electric and magnetic waves have oscillations that are perpendicular to each other and also to the direction of travel of the wave. Spectroscopy is a fundamental exploratory tools in the fields of physical, chemical and biological science at atomic and macro scale.

Ultraviolet–visible spectroscopy is the one of the spectroscopic techniques. It refers to absorption spectroscopy or reflectance spectroscopy concern with ultraviolet and the visible spectral regions of electromagnetic radiation. Means this technique utilizes visible and adjacent region for analysis work and research into biological probe. The absorption or reflectance in the visible region affects the observed color of the chemical involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. There are four possible transition occurs but only two transition states excited with light form the UV/Vis spectrum for some biological molecules. The electromagnetic transition into molecules can be classified according to the participating molecular orbital.

Objectives

- To understand the effects of UV radiation on the matter
- To understand the principle and instrumentation of spectroscopy
- To understand the Origin of spectra of uv radiation
- To discuss about Electronic transition and color complexes of molecules
- To discuss the application of UV-Visible spectrometer in enzyme kinetics reaction

2.2. UV-Vis spectroscopy

UV-Visible is a vital tool for the characterization of the functional groups of molecules and complexes. It produces absorbance or transmittance spectra of molecules in the ultraviolet and visible region for quantitative and qualitative analysis of chemical species. The wavelength of absorption is

usually reported as λ_{max} which represents the wavelength at the highest point of the curve. Absorption in UV or visible region leads to excitation of bonding electrons. Thus the absorption peak can be correlated with the kind of bonds existing in species. The UV-Visible spectra of ligands and their synthesized complexes in solution were recorded by UV-Visible Instrument (Thermo Scientific) in laboratory. The stock solution of synthesized complexes (0.1 x 10^{-3}M) were prepared by dissolving appropriate and exactly weighted pure solid compound in 50 mL double distilled water. Working solutions were prepared by appropriate dilution of stock solutions. The spectra of working solutions were recorded at room temperature.

The absorbance at a particular wavelength is defined by the equation-

$$A = \log_{10}\frac{I_0}{I} = \varepsilon I_0 C$$

or

$$\varepsilon = \frac{A}{CI}$$

where,

A= absorption

I_0= the intensity of the incident radiation

I = the intensity of the transmitted radiation

ε = a constant for each absorbing material, known as the molar absorption coefficient (called the molar extinction coefficient in older texts) which having the units L $mol^{-1}cm^{-1}$, but by convention the units are not quoted.

❖ **The ultraviolet spectrum:**

Generally, an electronic transition occurs due to the absorption of EMR in the range of 10 nm to 780 nm. So this region is known as a ultra-violet visible region which is further divided into the following region.

- ***Far (vacuum) ultraviolet region(10-200 nm):*** The absorption below 200 nm (atomic absorption) appeared in this region. To study the specific system in this region, a vacuum is necessary, so this region is known as "vacuum- UV" region.
- ***Near UV region (200-380):*** The atmosphere is transparent in this region. The absorption due to p & d electronic transition appears in the range of the ultraviolet spectrum.
- *Visible region (380-780):* If enough no. of π bond in conjugation, absorption takes place in this region

❖ **Electronic transition**

Electronic transition in energy levels is possible by absorption of radiation due to occurrence following transaction. The electromagnetic transition into molecules can be classified according to the participating molecular orbital.

There are four possible transitions excited with light form the UV/Vis spectrum for some biological molecules such as-

$\sigma \rightarrow \sigma^*$, $\sigma \rightarrow$ □ * {high-energy, (λ_{max} <150 nm)},

$\eta \rightarrow \sigma^*$, □ $\rightarrow \sigma^*$ {non-bonding electrons (lone pairs), (λ_{max}=150-250 nm)}

$\eta \rightarrow$□*, □ $\rightarrow$□* {organic molecular UV-Vis, lone pairs and multiple, (λ_{max} = 200-600 nm)}

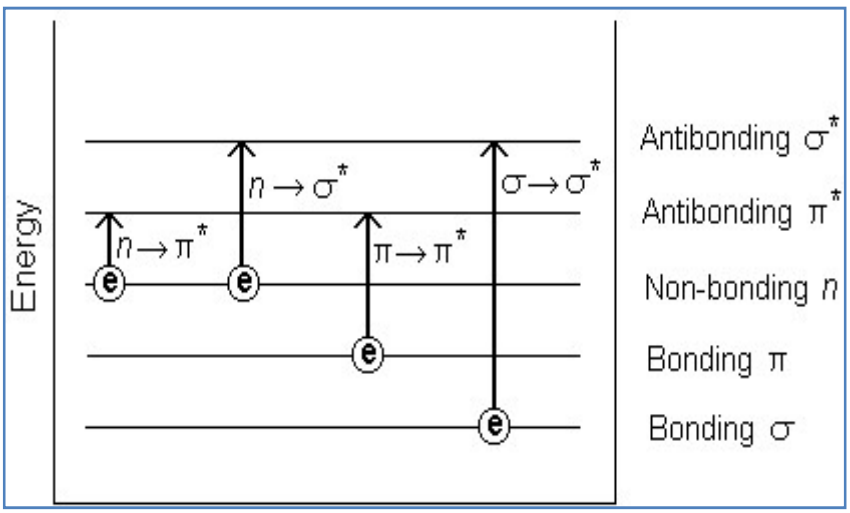


Fig.1.6: Electronic Transition in UV-Vis Spectroscopy

UV-Vis spectroscopy is used for both the quantitative and qualitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. In qualitative manner, UV-VIS spectroscopy is used to identify the functional group or confirm the identity of compound by matching the absorbance spectrum. The absorption or reflectance in the visible region affects the observed color of the chemical involved. The light source is a tungsten filament bulb for the visible part of the spectrum, and a deuterium bulb for the UV region. Since the emitted light consists of many different wavelengths, a monochromatic, consisting of either a prism or a rotating metal grid of high precision called grating, is placed between the light source and the sample. Wavelength selection can also be achieved by using colored filters as monochromators that absorb all but a certain limited range of wavelengths.

Spectroscopy analysis is commonly carried out in solutions but solids and gases may also be studied. The molecular structures which are responsible for interaction with electromagnetic radiation are called chromophores. In proteins, there are three types of chromophores relevant for UV/Vis spectroscopy:

- peptide bonds (amide bond);
- certain amino acid side chains (mainly tryptophan and tyrosine); and

- certain prosthetic groups and coenzymes (e.g. porphyrine groups such as in haem)

The UV extends from 100–400 nm and the visible spectrum from 400–800 nm. The 100–200 nm range is called the deep UV. Light source for deep UV range is more difficult so it is not commonly used for UV-Vis measurements.

The presence of several conjugated double bonds in organic molecules results in an extended p-system of electrons which lowers the energy of the π^* orbital through electron delocalisation. In many cases, such systems possess π^* -π^* transitions in the UV/Vis range of the electromagnetic spectrum. Such molecules are very useful tools in colorimetric applications.

The electronic transitions of the peptide bond occur in the far UV. The intense peak at 190 nm and the weaker one at 210–220 nm is due to the π^* -π^* and n -π^*transitions. A number of amino acids (Asp, Glu, Asn, Gln, Arg and His) have weak electronic transitions at around 210 nm. If protein has prosthetic groups and some metal–protein complexes, may have strong absorption bands in the UV/Vis range.

To know the UV-Visible spectroscopy phenomenon, student should know about some related to UV-Visible spectroscopies that are-

❖ Chromophore and auxochromophore

It is covalently unsaturated group responsible for absorption in the UV-Vis region. Some examples are C=C, C=O, N=N, C≡N, C≡C etc. Chromophores can be divided into two groups. (1) First type of chromophores contains sigma and pi electrons and undergo $\pi \rightarrow \pi^*$ transitions. Ethylenes and acetylenes are the example of such chromophores. (2) Second type of chromophores contains sigma, pi and nonbonding electrons. They undergo two types of transitions; $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$. Carbonyl, nitriles, azo compounds, nitro compounds etc. are the example of such chromophores.

Auxochromes are covalently saturated groups with one or more lone pair of electrons. These groups themselves do not show any characteristics absorption above 200 nm but when attached to a given chromophores usually cause a shift of the absorption band to longer wavelength with a simultaneous increase in the intensity of the absorption band. Auxochrome generally increase the value of wavelength as well as absorbance by extending the conjugation through resonance or hyper conjugation. Some common examples are halogens, –OH, -SH, -NH_2 and their derivatives such as –OR, -NHR, -NR_2 etc.

- **Bathochromic shift or Red shift**: Shift of absorption maxima to a longer wavelength is called bath chromic shift. n→π* transition of carbonyl compounds observes this type of shift.
- **Hypsochromic shift or Blue shift:** A shift of absorption maxima to shorter wavelength is called hypsochromic shift or blue shift. Generally it is caused due to the removal of conjugation or by changing the polarity of the solvents.
- **Hyperchromic shift:** Due to hyperchromic shift intensity of absorption maxima increases. For example, benzene shows B-band at 256 nm, ε_{max} at 200 whereas aniline shows B-band at 280 nm, ε_{max} 1430. The increase of 1230 in the value of aniline compared to that of benzene is due to the hyperchromic effect of the auxochrome NH_2.

❖ **Hypochromic shift:** It is defined as effect due to which the intensity of absorption maxima decreases. This is caused by reduction in conjugation. For example, aniline shows λ_{max} 280 nm and ε_{max} 1430 whereas the anilinium ion shows λ_{max} 254 nm and ε_{max} 160.

2.3. Principle of UV-Visible spectroscopy

When a photon hits a molecule, it is absorbed by molecules, given by an extinction with itself depend on the wavelength λ of photon. The photon is promoted into a more excited energetic state. UV-Visible light has enough energy to excite the electrons from ground state to a higher electronic state. The energy difference between the lower to higher energy level is called the *band gap*. The energy of the photon must exactly match the band gap for the photon to be absorbed. When the incident light intensity (I_0) passes through a sample with appropriate transparency through the path length (thickness) 'l', then the change in intensity occurs this is represented as observed intensity (I).

The characteristic absorption parameter for the sample is the extinction coefficient a, yielding the correlation $I= I_0$. The ratio $T= I/I_0$ is called transmission.

Thus, molecules with different chemical structures have different energy band gaps and different absorption spectra. The larger the band gap between the energy levels, the greater the energy required to promote the electron to the higher energy level, ultimately resulting in light of higher frequency, and therefore shorter wavelength, being absorbed.

2.4. Instrumentation of UV-Visible spectroscopy

Ultraviolet- visible spectroscopy involves the spectroscopy of photons in the UV-visible region. There is an interaction between UV-

visible light and sample to be analyzed. As a result of this interaction, some photons (photons of UV-Vis EMR) are absorbed and this absorption of UV visible is measured by an instrument named UV-visible spectrophotometer. It measures the intensity of light passing out through a sample (I), and compares it to the intensity of light before it passes through the sample (I_0). The ratio (I/I_0) is called the transmittance, and is usually expressed as a percentage (%T). From the transmittance (T), the absorbance can be calculated as, $A = -logT$.

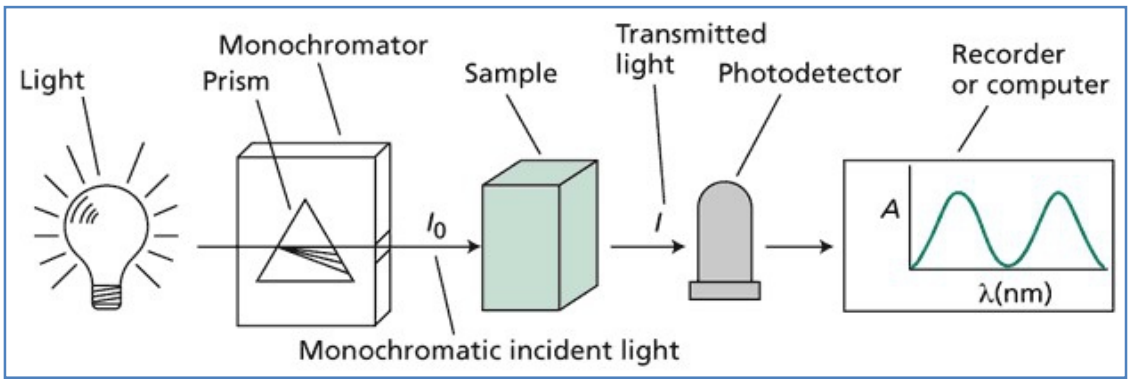


Fig.1.5. Scheme representation of working of spectrophotometer.

The components of spectrophotometer are as follows-

Light source: Light source is the basic part of spectrophotometer. It must be stable and provide continuous radiation. Light source must be of the sufficient intensity for the transmitted energy to be detected at the end of the optical path. Most commonly radiation source used in UV-Vis spectroscopy are as follows.

- **Deuterium lamp:** Deuterium arc lamp, which is continuous over the ultraviolet region (190–400 nm). The intensity of radiation of deuterium lamp is 3-5 times than the hydrogen lamp.
- **Hydrogen discharge lamp:** Hydrogen discharge lamp consists of two electrodes contain in deuterium filled silica envelope. This lamp covers a range from 160-375 nm. These lamps are stable, robust and widely used.
- **Tungsten lamps:** It is similar to house hold lamp in construction and provide a supply of radiation in wave length ranging from 320-2500 nm.
- **Xenon discharge lamp:** These are enclosed in a glass tube with quartz or fused silica and xenon gas is filled under pressure, contains two tungsten electrodes separated by a distance. An intense arc is formed between electrodes by applying high voltage.

- **Mercury arc lamps:** In mercury arc lamp, mercury vapor is stored under high pressure and excitation of mercury atom is done by electric discharge.

Monochromator: It is used to remove the radiation of desired wavelength from the wavelength of continuous spectra. Following types of monochromatic are used.

- Filters
- Prisms
- Gratings

Sample compartment: Cells or cuvettes are used for holding liquid sample. Sample holder should be transparent to the wavelength region to be recorded. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. Cells may be rectangular in shape or cylindrical with flat ends generally having 1 cm thickness.

Detector: Detectors convert light energy into electrical signals that are display on read out device. It measures the absorption of an analyzer via the intensity of transmitted light. Mainly three types of photosensitive devices are used-

Barrier layer cell / Photovoltaic cell

Phototubes / Photo emissive tube

Photomultiplier tube

Recorder: Signals from the detector are finally received by the recording device.

2.5. Beer-Lambert law

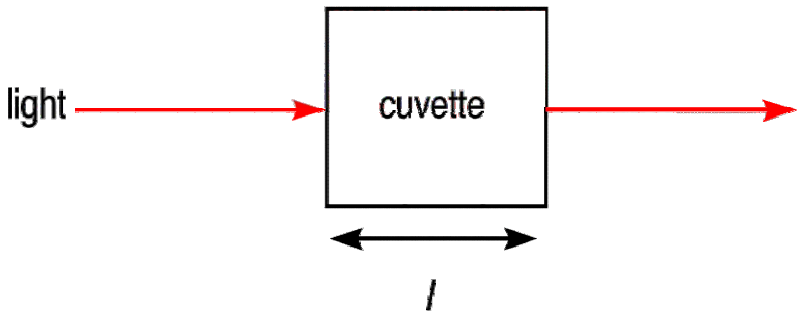
We know the biological samples mainly comprise aqueous solutions; the detection of substance present in sample is measured in molar concentration 'c'. The transition phenomenon in solution is governed by Lambert- Beer's law. The absorption of light by any absorbing material is governed by Beer-Lambert law. The Beer-Lambert law is a linear relationship between the absorbance and the concentration, molar absorption coefficient and optical coefficient of a solution. For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured. This is usually referred to as I_0 - that's 'I' for Intensity.

The chance for a photon to be absorbed by matter is given by an extinction coefficient which itself depend on the wavelength (λ) of the photon. If the intensity I_0 passes through a sample with appropriate transparency and

the path length (thickness) 'l', the intensity 'I' drops along the pathway in an exponential manner.

The absorbance of a transition depends on two external assumptions.

The characteristic absorption parameter for the sample is the extinction coefficient α. Which yield by the equation $I = I_0 e^{-\alpha d}$. The ratio of T = I/Io is called transmission.



$$A = \varepsilon cl$$

Where, **A** = Absorbance of sample, which display on the spectrophotometer.

ε = Molar absorption coefficient M^{-1} cm^{-1}

c = Molar concentration "M"

l = optical path length in cm

The Beer-Lambert law is valid for low concentrations only. The absorption and extinction coefficients are additives parameters, which complicates determination of concentrations in samples with more than one absorbing species.

The chance for a photon to be absorbed by matter is given by an extinction coefficient which itself depend on the wavelength (λ) of the photon. If the intensity I_0 passes through a sample with appropriate transparency and the path length (thickness) 'l', the intensity 'I' drops along the pathway in an exponential manner. The characteristic absorption parameter for the sample is the extinction coefficient α. Which yield by the equation $I = I_0 e^{-\alpha d}$. The ratio of $T = I/I_0$ is called transmission.

The intensity of the light passing through the sample cell is also measured. For that wavelength, given the symbol 'λ'. If 'λ' is less than I_0, then the sample has absorbed some of the light (neglecting reflection of light off the cuvette surface). The absorbance of a transition depends on two external assumptions-

3. The absorbance is directly proportional to the concentration (c) of the solution of the sample used in the experiment.

4. The absorbance is directly proportional to the length of the light path, which is equal to the width of the cuvette.

a. Qualitative and quantitative analysis

The qualitative analysis is carried out by the UV-Vis spectroscopy when the atom or molecules absorb UV radiation and identification is done by comparing the absorption spectra with the spectra of known compound. Qualitative analysis is done in UV/Vis regions to identify certain classes of compounds both in the pure state and in biological mixtures (e.g. protein-bound). The application of UV/Vis spectroscopy to further analytical purposes is rather limited, but possible for systems where appropriate features and parameters are known. Most commonly, this type of spectroscopy is used for quantification of biological samples either directly or via colorimetric assays. In many cases, proteins can be quantified directly using their intrinsic chromophores, tyrosine and tryptophan. The characteristic picks in protein spectrum are a band at 278/280 nm and another at 190 nm. The region from 500 to 300 nm provides valuable information about the presence of any prosthetic groups or coenzymes. Protein quantification by single wavelength measurements at 280 and 260 nm only should be avoided, as the presence of larger aggregates (contaminations or protein aggregates) gives rise to considerable Rayleigh scattering.

Difference spectra

Difference spectrum is obtained by subtracting one absorption spectrum from another. Difference spectra can be obtained in two ways: either by subtraction of one absolute absorption spectrum from another, or by placing one sample in the reference cuvette and another in the test cuvette. Difference spectra have three distinct features as compared to absolute spectra shown in Fig.

- difference spectra may contain negative absorbance values;
- absorption maxima and minima may be displaced and the extinction coefficients are different from those in peaks of absolute spectra;
- There are points of zero absorbance, usually accompanied by a change of sign of the absorbance values. These points are observed at wavelengths where both species of related molecules exhibit identical absorbance's (isosbestic points), and which may be used for checking for the presence of interfering substances.

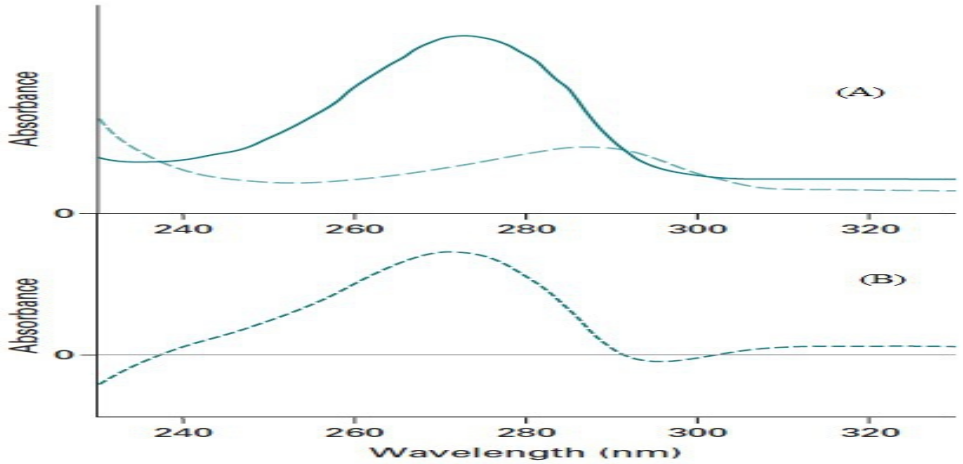


Fig. 1.6: (A) Absolute spectra of ubiquinone (solid curve) and ubiquinol (dotted curve), (B) Difference spectrum.

b. Origin of spectra

Ultra violet absorption spectra arise from transition of electron from lower energy level to higher both organic and inorganic species exhibit electronic translations in which outermost of bonding electrons are promotes to higher energy levels. The colour of compound is appeared due to absorption of visible region of light and it reflects the light of wavelength in the rest of the visible region. The main function of absorbed energy is to raise the molecule from the ground state energy E_0 to the higher excited state (energy E_1) that can be represented by following formula such as

$$\Delta E = E_1 - E_o$$

We know that

$$\Delta E = hv$$

$$hv = hc/\lambda$$

$$v = c/\lambda$$

where h = Planck's constant

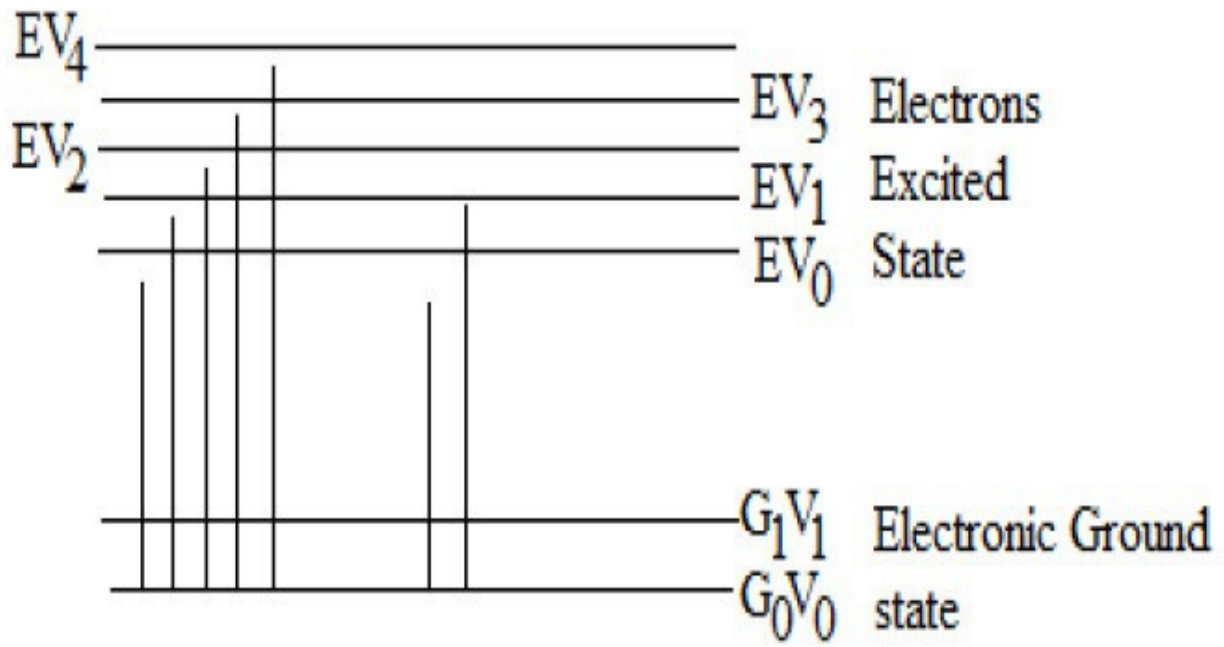
c = velocity of light

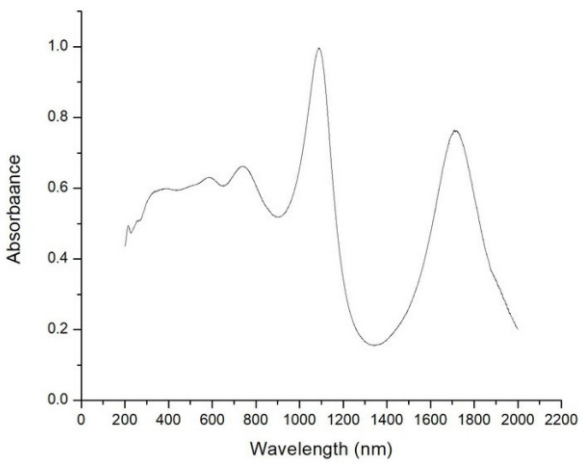
λ = wavelength of the absorbed ration.

ΔE depends upon how tightly the electrons are bound in the bonds and accordingly the absorption will occur in UV or particular region of visible range. However, the frequency of the absorbed light is associated with a particular line in the spectrum. The values of ΔE are very close to each other; the lines appear as a band. The existence of the bands in definite parts of the

spectrum produces the color. The total energy of the molecule is the sum of its electronic energy, its vibration energy and its notational energy. This is due to the fact that there may be three changes in the molecule as a result of absorption of radiation-

- There may be rotation of molecule
- There may be vibration of combining atoms, within the molecule
- There may be electronic transition from one orbit to another





2.8. Electronic transition

Ultraviolet–visible spectroscopy utilizes visible radiation and gives spectra when interact with biomolecule. The absorption or reflectance in the visible region affects the observed color of the chemical involved. In visible region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Ultraviolet and visible radiation interacts with matter

which causes electronic transitions that is promotion of electrons from the ground state to a high energy state. There are four possible transition ($n \rightarrow \pi^*, \pi \rightarrow \pi^*, n \rightarrow \sigma^* \; and \; \sigma \rightarrow \sigma^*$) are possible. But only two transition states ($n \rightarrow \pi^* \; and \; \pi \rightarrow \pi^*$) goes for excited with light form the UV/Vis spectrum for some biological molecules.

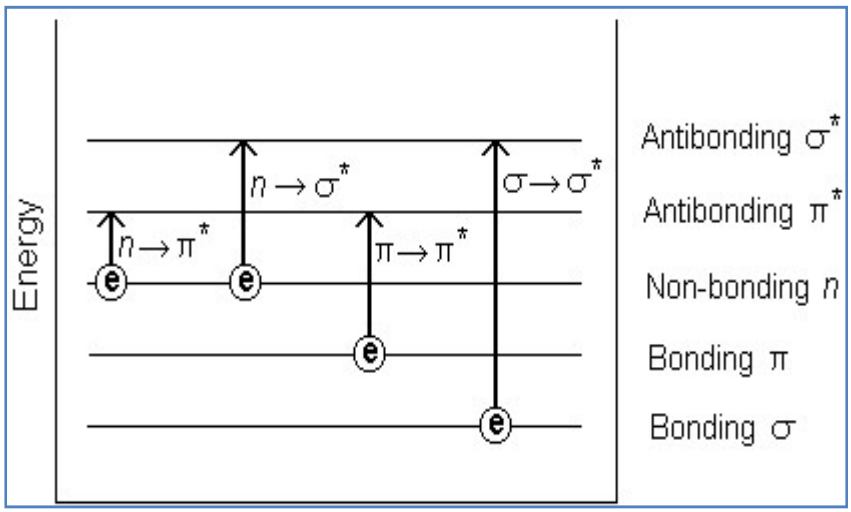


Fig.1.4: Electronic Transition in UV-Vis Spectroscopy

σ→σ* Transitions:

This type of transition takes place when an electron in a bonding sigma orbital is excited to the corresponding antibonding orbital i.e. $\sigma \rightarrow \sigma^*$ transitions, associated with all saturated hydrocarbons and high energy (150 nm) is required. As the energy of UV region is 200-400 nm, so for this type of transition we have to go in vacuum UV region. For example, methane shows an absorbance maximum at 125 nm which has only C-H bonds, and can only undergo $\sigma \rightarrow \sigma^*$ transitions. UV radiation around 150 nm; ethane shows A_{max} 135 nm. The region below 200 nm is called *vacuum UV region,* since oxygen present in air absorbs strongly at 200 nm and below. Similarly, nitrogen absorbs at 150 nm and below. Thus, an evacuated spectrophotometer is used for studying such high energy transitions (below 200 nm). Absorption maxima due to $\sigma \rightarrow \sigma$ transitions fall in deep UV, so they are less useful.

n→σ* Transitions:

Transition of electron takes place from non-bonding orbital to antibonding σ* orbital. For this transition energy required is 175 nm. This type of transition occurred when any hetero atom is present in saturated compounds such as ketone, amine, aldehyde, alcohol, etc. For example, methyl chloride shows absorption at 169 nm. Compounds containing non-bonding electrons on a hetero atom are capable of showing absorption due

to $n \rightarrow \sigma^*$ transitions. These transitions require lower energy than $\sigma \rightarrow \sigma^*$ transitions. Some organic compounds undergoing $n \rightarrow \sigma^*$ transitions are halides, alcohols, ethers, aldehydes, ketones etc. For example, methyl chloride shows absorption max A_{max} 173 nm, methyl iodide A_{max} 258 nm, methyl alcohol A_{max} 183 nm and water A_{max} 167 nm. In alkyl halides, the energy required for $n \rightarrow \sigma^*$ transition increases as the electronegativity of the halogen atom increases. This is due to comparatively difficult excitation of non-bonding *(n* or *p)* electrons on increase in the electronegativity. The difficult excitation means less probability of transition. The molar extinction coefficient 'ε*':* increases as the probability of the transition increases. Thus, methyl iodide shows A_{max} 258 nm, E_{max} 378 and methyl chloride A_{max} 173 nm, E_{max} -100.

π→π* Transitions:

This is associated with the transition of electron from non-bonding orbital to pi- antibonding orbital and energy required is more than 200 nm. It may be 800 nm depends upon conjugation present in that particular compound. Doubly and triply bonded hydrocarbons and aromatic compounds shows π→π* transition. The π→π* occurs in compounds containing one or more covalently unsaturated groups like C=C, C=O, NO_2 etc. $\pi \rightarrow \pi^*$ transitions require lower energy than n -σ* transitions. In unconjugated alkenes, this transition occurs in the range 170-190 nm; ethylene shows A_{max} 171 nm.

n→π* Transitions:

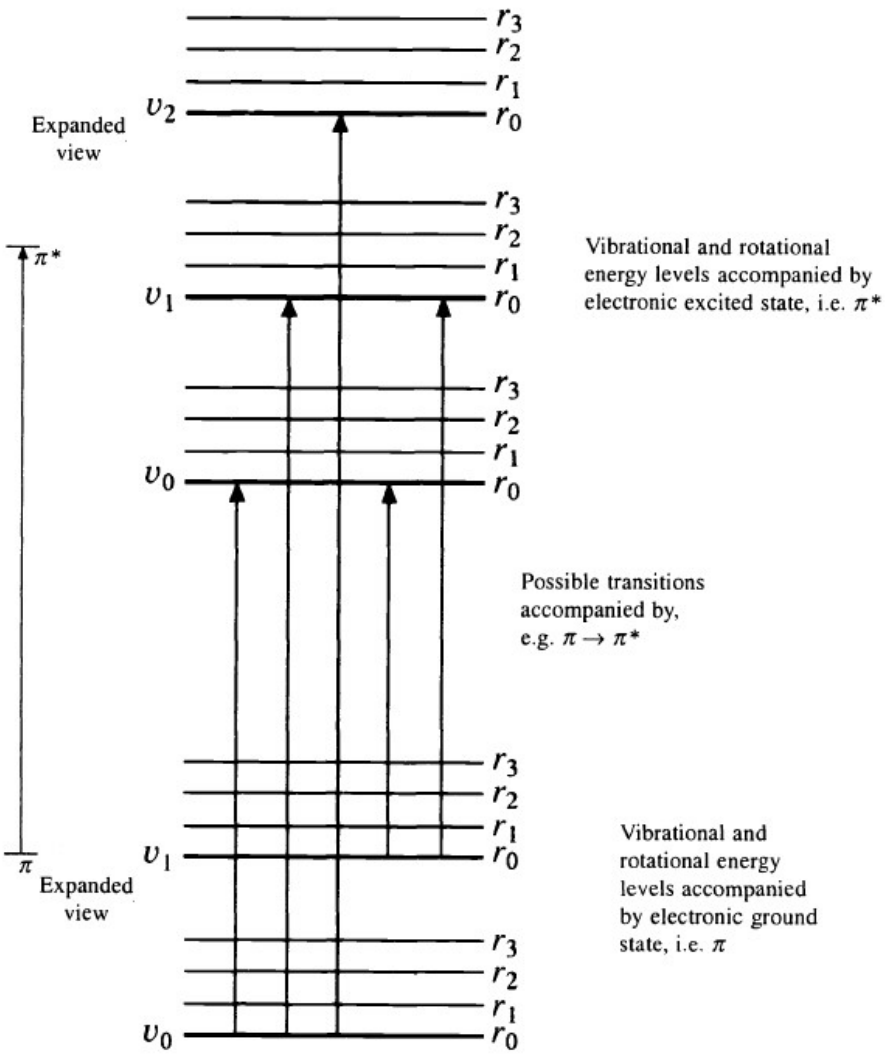
This electronic transition involve transition of electron from non-bonding orbital to pi- anti-bonding orbital and energy associated with it is more than 200 nm and extended up to 800 nm depending upon coupling conjugation present in that particular compound. Example of compound associated with this n→π* transition is carbonyl compound.

c. Formation of Absorption Band

Since the energy required for each electronic transition is quantized, the UV-visible spectrum is expected to exhibit a single, discrete line corresponding to each electronic transition. In practice, broad absorption bands are usually observed. In a molecule, each electronic energy level (either in ground state or in excited state) is accompanied by a large number of vibrational (v_0 , v_1 , v_2 etc.) and rotational (r_0 , r_1, r_2, etc.) energy levels which are also quantized . In complex molecules having many atoms there are still a large number of closer vibrational energy levels.

The radiation energy passed through a sample is sufficient to induce various electronic transitions as well as transitions in accompanying vibrational and rotational energy Ievels. However, these transitions have very small energy

differences, but the energy required to induce an electronic transition is larger than that required to cause transitions in the accompanying vibrational and rotational energy levels. Thus, the electronic absorption is superimposed upon the accompanying vibrational and rotational absorptions resulting in the formation of broad bands.



Schematic energy level diagram of a diatomic molecule

2.10. Calculation of Absorption Maximum

Woodward-Fieser rules for calculating λ_{max} in conjugated dienes and trienes: In 1941 Woodward introduced empirical rules for calculating and predicting λ_{max} for acyclic and six-membered dienes. Further, Fieser and Scott modified these rules with dienes and trienes. Summaries of both rules called Woodward-Fieser Rules.

Woodward-Fieser Rules for Calculating λ_{max} in Conjugated Dienes and Trienes

Base value for acyclic or heteroannular diene 214nm

Base value for homoannular diene 253nm

Increment for each:

Alkyl substituent or ring residue 5nm

Exocyclic conjugated double bond 5nm

Double bond extending conjugation 30nm

-OR (alkoxy) 6nm

-Cl, -Br 6nm

-OCOR (acyloxy) 0nm

-SR (alkylthio) 30nm

$-NR_2$ (dialkylamino) 60nm

In the same double bond is exocyclic to two rings

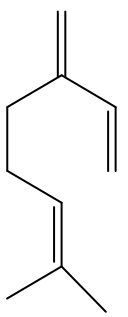
Simultaneously 10nm

Solvent correction 0nm

Calculated* λ_{max} of the compound Total = nm

*For π-π^* transition (K-band).

Example 1: Calculate the wavelength of the maximum UV absorption for



Myrcene

Since, it is an acyclic diene with one alkyl substituent, thus

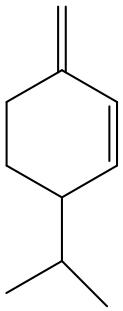
Base value 214nm

One alkyl substituent 5nm

Calculated λ_{max} 219nm

Observed λ_{max} 224nm

Example 2: Calculate the value of λ_{max} for β-phellandrene



This is a heteroannular diene with conjugated double bonds are not in the same ring with two ring residues and one exocyclic double bond, hence

Base value 214nm

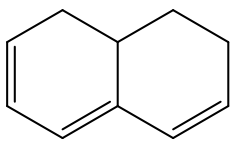
Two alkyl substituents (2 x 5nm) 10nm

One exocyclic double bond 5nm

Predicted λ_{max} 229nm

Observed λ_{max} 232nm

Example 3: Expected λ_{max} values of compound



This is a homoannular diene with conjugated double bonds are not in the same ring with two ring residues and one exocyclic double bond, hence-

Base value 253nm

Three alkyl substituents (3 x 5nm) 15nm

One exocyclic double bond 5nm

Predicted λ_{max} 273nm

2.11. Composition of colour complex

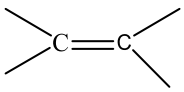
Chromatophores are pigment-containing cells or groups of cells, found in a wide range of animals including amphibians, fish, reptiles, crustaceans and cephalopods. Mammals and birds, in contrast, have a class of cells called melanocytes for colouration. Chromatophores are largely responsible for generating skin and eye colour in ectothermic animals and are generated in the

neural crest during embryonic development. Mature chromatophores are grouped into subclasses based on their colour (more properly "hue") under white light: xanthophores (yellow), erythrophores (red), iridophores (reflective/ iridescent), leucophores (white), melanophores (black/brown), and cyanophores (blue).

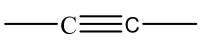
In terms of spectroscopy, chromophores are covalently unsaturated groups responsible for absorption in the UV or visible region.

Chromophores are covalently unsaturated group responsible for absorption in the UV-Vis region. Some examples are C=C, C=O, N=N, C≡N, C≡C etc. Chromophores can be divided into two groups. (1) This type of chromophores contain sigma and pi electrons and undergo π→π* transitions. Ethylenes and acetylenes are the example of such chromophores. (2) Second type of chromophores contain sigma, pi and nonbonding electrons. They undergo two types of transitions; π→π* and n→π*. Carbonyl, nitriles, azo compounds, nitro compounds etc. are the example of such chromophores. Characteristics of some common unconjugated chromophores are given in Table below.

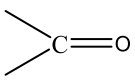
Chromophore Example λ_{max} (nm) Transition



Ethylene 171 π-π*



Acetylene 150 π-π*



Aectaldehyde 160 n-σ*

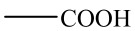
180 π-π*

290 n-π*

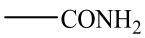
Acetone 166 n-σ*

188 π-π*

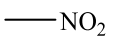
279 n-π*



Acetic acid 204 n-π*



Acetamide 178 π-π*

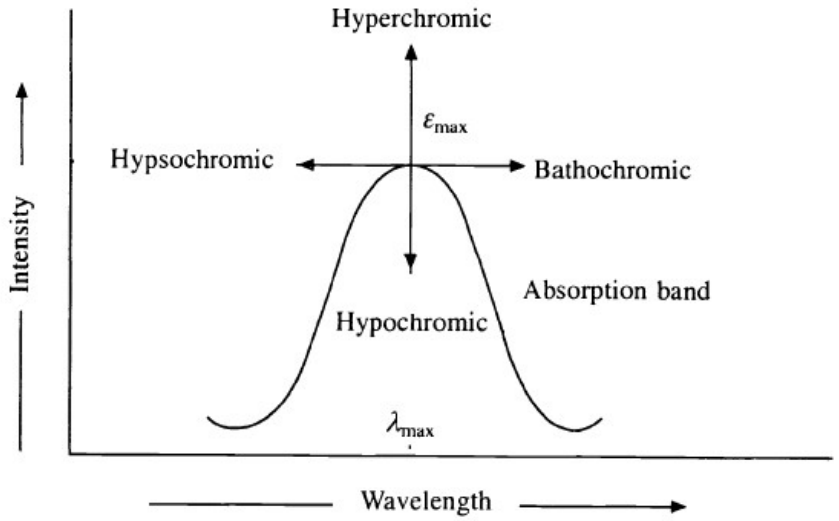


Niromethane 201 π-π*

274 n-π*

Auxochromes are covalently saturated groups with one or more lone pair of electrons. These groups themselves do not show any characteristics absorption above 200 nm but when attached to a given chromophores usually cause a shift of the absorption band to longer wavelength with a simultaneous increase in the intensity of the absorption band. Auxochrome generally increase the value of wavelength as well absorbance by extending the conjugation through resonance or hyper conjugation. Some common examples are halogens, –OH, -SH, $-NH_2$ and their derivatives such as –OR, -NHR, $-NR_2$ etc.

- **Bathochromic shift or Red shift**: Shift of absorption maxima to a longer wavelength due to the presence of an auxochrome or solvent effect is called a *bathochromic shift* or *red shift*. Example: Benzene shows λ_{max} 256 nm and Aniline shows λ_{max} 280 nm. Thus, there is a bathochromic shift of 24 nm in the λ_{max} of benzene due to the presence of the auxochrome NH_2. The n→π* transition of carbonyl compounds also observes this type of shift.



- **Hypsochromic shift or Blue shift:** A shift of absorption maxima to shorter wavelength is called hypsochromic shift or blue shift. Generally, it is caused due to the removal of conjugation or by changing the polarity of the solvents. Example: Aniline shows λ_{max} 280 nm, whereas anilinium ion (acidic solution of aniline) shows λ_{max} 254 nm. This hypsochromic shift is due to the removal of *n-π* conjugation of the lone pair of electrons of the nitrogen atom of aniline with the n-bonded benzene ring on protonation because the protonated aniline (anilinium ion) has no lone pair of electrons for conjugation.

- **Hyper chromic shift:** Due to hyperchromic shift intensity of absorption maxima increases. For example, benzene shows B-band at 256 nm, ε_{max} at 200 whereas aniline shows B-band at 280 nm, ε_{max} 1430. The increase of 1230 in the value of aniline compared to that of benzene is due to the hyperchromic effect of the auxochrome NH_2.

❖ **Hypochromic shift:** It is defined as effect due to which the intensity of absorption maxima decreases. This is caused by the introduction of a group which distorts the chromophore. For example, biphenyl shows λ_{max} 252 nm, ε_{max} 19,000, whereas 2,2'-dimethylbiphenyl shows λ_{max} 270 nm, ε_{max} 800. The decrease of 18,200 in the value of ε_{max} of 2,2'-dimethylbiphenyl is due to the hypochromic effect of the methyl groups which distort the chromophore by forcing the rings out of coplanarity resulting in the loss of conjugation.

2.12.Application of UV-Visible spectrometer in enzyme kinetics reaction

Enzyme activity is frequently investigated in the medicinal, biochemistry, and food science research fields to elucidate the rate of which reaction occurs and the affinity of the enzyme-substrate interactions. The rates of these reactions can be accurately measured using a UV-Visible spectrophotometer. The rates of these reactions can be accurately measured using a UV-Visible spectrophotometer. When an enzyme (E) binds with a substrate (S), an intermediate or enzyme/substrate complex (ES) is produced, which can further react and yield a by-product (P), shown in Scheme.

$$E + S \rightleftarrows ES \; E + P \longrightarrow$$

The Michaelis-Menten expression is commonly used to describe the rate (v) of the enzyme reaction.

According to this model when enzyme (E) combines with substrate (S) that form enzyme substrate (ES) complex. In reversible reaction this ES complex can be reverse and can proceed chemically to be give enzyme (E) and substrate (S) as.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

The rate constants k_1, k_2 and k_3 describe the rates association with each step of the catalytic process. This reaction shows the ES maintain their steady state because the ES remains approximately constant unit nearly all the substrate is used in backward reaction. It is know, that the initial velocity (V_o) of any enzyme at low substrate concentration is directly proportional to [S]. While at high substrate concentration the velocity tends to shift at maximum value, that is the rate becomes independent of [S].

The maximum velocity known as maximum velocity ($V_{max}/2$). The maximum velocity express in unite μmol min^{-1}. To describe this observation the Michaelis and Mound derived as equation that is known as Michaelis and Mound equation:

$$V_o = \frac{V_{max}\, X\, [S]}{K_m + [S]}$$

This equation describes the hyperbolic curve as shown the experimental data in Fig. 2.5. After solving the equation Michaelis and Mound defined a new constant, K_m called as Michaelis constant.

$$K_m = \frac{k_2 + k_3}{k_1}$$

K_m is refers to the stability of the ES complexes. It is equal to equal to the sum of the rates of breakdown of ES over its rate of formation. The k_2 value for many enzymes is found much greater than k_3 in this circumstances K_m becomes a measure of the affinity of an enzyme for its substrate since its value depends on relatives values of k_1 and k_2 for ES formation and dissociation, respectively. The k_m may be determined experimentally by the fact that its values are equivalent to the substrate concentration at which the velocity is equal to half of V_{max}. In addition, the K_m is equal to the sum of the rates of breakdown of the enzyme- substrate complex over its rate of formation, and is a measure of the affinity of an enzyme for its substrate. The K_m values for most of enzymes lies between 10^{-1} and 10^{-7} M.

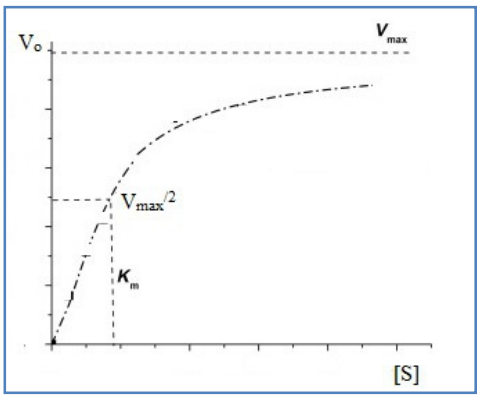


Fig.25: The relationship between [S] and V_o in enzyme catalytic reaction

2.13. Summary

There is an interaction between UV visible light and sample to be analyzed. By measuring the absorbance at specific wavelength impurities can be detected by UV visible spectroscopy. Most commonly, this type of spectroscopy is used for quantification of biological samples either directly or via colorimetric assays. In many cases, proteins can be quantified directly using their intrinsic chromophores, tyrosine and tryptophan. Protein spectra are acquired by scanning from 500 to 210 nm. The qualitative analysis is carried out by the UV-Vis spectroscopy when the atom or molecules absorb UV radiation and identification is done by comparing the absorption spectra with the spectra of known compound. Qualitative analysis is done in UV/Vis regions to identify certain classes of compounds both in the pure

state and in biological mixtures. There is an interaction between UV visible light and sample to be analyzed. By measuring the absorbance at specific wavelength impurities can be detected by UV visible spectroscopy.

2.14. Terminal questions

Q.1. What do you understand for UV- Visible spectroscopy?

Answer:----------

Q.2. Write the principle and application of UV-Visible spectroscopy.

Answer:----------

Q.3. UV-Visible work on which law? Discuss the Lambert-Beer's Law.

Answer:----------

Q.4. Briefly discuss chromophore and auxochrome in spectroscopy.

Answer:----------

Q.5. Discuss about electronic transition.

Answer:----------

Q.6. Discuss about bond in UV-Visible spectroscopy.

Answer:----------

2.15. Further readings

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Unit-3.IR spectroscopy

Contents

3.1. Introduction

Infrared spectroscopy deals with the recording of the absorption of radiations in the infrared region of the electromagnetic spectrum. The position of a given infra red absorption is expressed in terms of wavelength in micron (μ) or commonly in terms of wave number $\bar{v}$(cm^{-1}), since it is directly proportional to energy. The ordinary infrared region 2.5-15 μ (4000-667 cm^{-1}) is of greatest practical use to organic chemists. The region 0.8-2.5 μ (12,500-4000 cm^{-1}) is called the near infrared and the region 15-200 μ (667-50 cm^{-1}) the far infrared. Note that wave numbers are often called frequencies, although strictly it is incorrect. However, it is not a serious error as long as we keep in mind that $\bar{v} = \frac{1}{\lambda}$ and $v = \frac{c}{\lambda}$. The absorption of infrared radiation by a molecule occurs due to quantized vibrational and rotational energy changes when it is subjected to infrared irradiation. Thus, IR spectra are often called *vibrational-rotational spectra.* Unlike UV spectra, IR spectra have a large number of absorption bands and therefore provide plenty of structural information about a molecule. Bands observed in an IR spectrum correspond to various functional groups and bonds present in the molecule. Thus, IR spectroscopy is most widely used for the detection of functional groups and identification of organic compounds.

Objectives

- To discuss the infrared spectroscopy
- To know the theory and principle of IR
- Do discuss the different component of IR

3.2. Theory of Infrared spectroscopy

Infrared spectroscopy is an important tool for the organic chemist. It is one of the most widely used tools the detection of functional group in the pure compound as well as in mixture for the comparison purpose. Infrared refers to that part of the electromagnetic spectrum between the visible and microwave region. In a molecule; atoms join by covalent bonds are not fixed at one position but continual vibration with each other. The energy associated with this motion corresponds to frequencies in the region of infrared waves, which stretches from 4000-400 cm^{-1}. For the study of vibrational spectroscopy being with a diatomic molecule as the model vibrator as a harmonic oscillator. The diatomic molecules (two atoms join with each other by a covalent bond) obey Hooke's law. Vibration is 'to' and 'fro' motion of atoms connected by a bond in the molecule as well as its gravity centre. The elongation and compression of bond alternately resulting in the displacement of the atom from their equilibrium position Let the displacement be 'a', an external force 'F' is required for the displacement. A restoring force 'f' arises in the bond in opposite direction of the displacement, for the equilibrium position: i.e.

$F=-f$ ……. (i)

According to classical harmonic motion; the displacement of an atom is directly proportional to the applied force.

$F \alpha a$ …… (ii)

$F \alpha -a$ …….. (iii)

$F= -ka$ ……..…….(iv)

'k' is proportionality constant which measures the 'stiffness' at the bond, hence also known as " force constant" for the relevant bond.

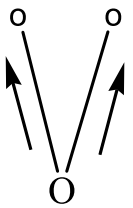
3.2.1. Origin of Infrared Spectra

IR absorption spectra originate from transitions in vibrational and rotational energy levels within a molecule. On absorption of IR radiation, vibrational and rotational energies of the molecule are increased. When a molecule absorbs IR radiation below 100 cm^{-1}, the absorbed radiation causes transitions in its rotational energy levels. Since these energy levels are quantized, a molecular rotational spectrum consists of discrete lines.

When a molecule absorbs IR radiation in the range 100-10,000 cm^{-1}, the absorbed radiation causes transitions in its vibrational energy levels. These energy levels are also quantized, but vibrational spectra appear as bands rather than discrete lines. Thus, a single transition in vibrational energy levels is accompanied by a large number of transitions in rotational energy levels and so

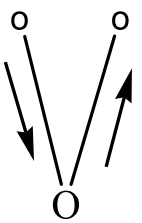
the vibrational spectra appear as vibrational-rotational bands instead of discrete lines. Organic chemists are mainly concerned with these vibrational-rotational bands, especially with those occurring in the region 4000-667 cm^{-1}.
Atoms in a molecule are not still but they vibrate. The two types (modes) of fundamental molecular vibrations known are: (a) stretching and (b) bending vibrations.

Stretching Vibrations
In stretching vibrations, the distance between two atoms increases or decreases, but the atoms remain in the same bond axis. Stretching vibrations are of two types:
(a) Symmetrical stretching. In this mode of vibration, the movement of atoms with respect to the common (or central) atom is simultaneously in the same direction along the same bond axis



Symmetric stretching (v_s) of CH_2

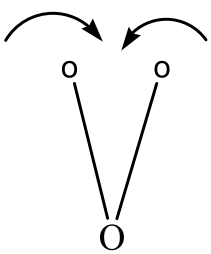
(b) Asymmetrical Stretching. In this vibration, one atom approaches the common atom while the other departs from it.



Asymmetric stretching (v_{vs}) of CH_2

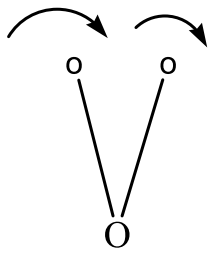
Bending Vibrations (Deformations)
In such vibrations, the positions of the atoms change with respect to their original bond axes. Bending vibrations are of four types:

(a) Scissoring. In this mode of vibration, the movement of atoms is in the opposite direction with change in their bond axes as well as in the bond angle they form with the central atom.



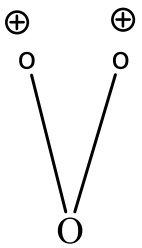
Scissoring of CH_2 in plane bending vibration

(b) Rocking: In this vibration, the movement of atoms takes place in the same direction with change in their bond axes. Scissoring and rocking vibrations are in-plane bendings.



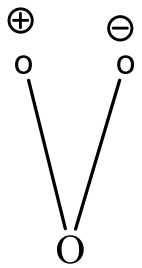
Rocking of CH_2 in plane bending vibration

Wagging: In this vibration, two atoms simultaneously move above and below the plane with respect to the common atom. Note: + and- signs indicate movements perpendicular to the plane of the paper.



Wagging; **Out-of-plane bending vibrations (CH_2)**

(d) ***Twisting:*** In this mode of vibration, one of the atom moves up and the other moves down the plane with respect to the common atom.



Twisting; **Out-of-plane bending vibrations (CH_2)**

3.2.2. Number of Fundamental Vibrations

The IR spectra of polyatomic molecules may exhibit more than one vibrational absorption bands. The number of these bands corresponds to the number of

fundamental vibrations in the molecule which can be calculated from the degrees of freedom of the molecule. The degrees of freedom of a molecule are equal to the total degrees of freedom of its individual atoms. Each atom has three degrees of freedom corresponding to the three Cartesian coordinates *(x, y* and z) necessary to describe its position relative to other atoms in the molecule. Therefore, a molecule having *n* atoms will have *3n* degrees of freedom.

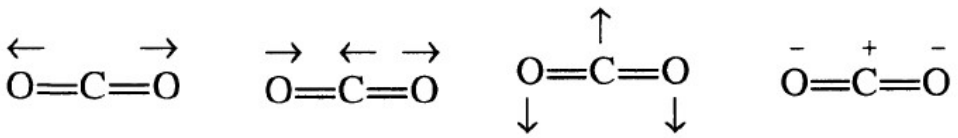
In case of a nonlinear molecule, three of the degrees of freedom describe rotation and three describe translation. Thus, the remaining ***(3n* - 3 - 3) = *3n* - 6** degrees of freedom are its vibrational degrees of freedom or fundamental vibrations, because

Total degrees of freedom (3n) = Translational + Rotational + Vibrational degrees of freedom

In case of a linear molecule, only two degrees of freedom describe rotation because rotation about its axis of linearity does not change the positions of the atom and three describe translation. Thus, the remaining ***(3n* - 2 - 3) = *3n* – 5** degrees of freedom are vibrational degrees of freedom or fundamental vibrations.

3.2.3. In carbon dioxide (CO_2) molecule

The number of vibrational degrees of freedom for the linear carbon dioxide (CO_2) molecule can be calculated as follows:
Number of atoms (n) = 3
Total degrees of freedom (3n) = 3 x 3 = 9
Rotational degrees of freedom = 2
Translational degrees of freedom = 3
Therefore, vibrational degrees of freedom = 9 - 2 - 3 = 4



Vibration ν_1 ν_2 ν_3 ν_4

Mode of vibration: Symmetrical stretching | Asymmetrical Stretching | In-plane bending | Out-of-plane bending

Infrared: Inactive | Active | Active | Active
Raman: Active | Inactive | Inactive | Inactive

The symmetrical stretching vibration ν_1 produces no change in the dipole moment of the molecule, hence it is IR inactive. On the other band, it produces change in polarizability, hence it is Raman active. The bending vibrations ν_2 occur at the same frequency and are equivalent (degenerate) and produce change in dipole moment, hence are IR active. Similarly, the asymmetrical

vibrations do not produce change in polarizability, hence are Raman inactive. Thus, the CO_2 molecule shows three fundamental bands, two in the IR spectrum and one in the Raman spectrum.

The carbon dioxide molecule is linear and has four fundamental vibrations (3 *x* 3)- 5 = 4. Thus, four theoretical fundamental bands are expected but actually it shows only two. The symmetrical Stretching vibration in carbon dioxide is IR inactive because it produces no change in the dipole moment of the molecule. The two bending vibrations are equivalent and absorb at the same wave number (667.3 cm^{-1}). Thus, the IR spectrum of carbon dioxide shows only two fundamental absorption bands, one at 2350 cm^{-1} due to asymmetrical stretching vibration, and the other at 667.3 cm^{-1} due to the two bending vibrations.

Ethane (C_2H_6) molecule

Non-linear molecule ethane (C_2H_6), the vibrational degrees of freedom can be calculated as:

Number of atoms (n) = 8

Total degrees of freedom (3n) = 3 x 8 = 24

Rotational degrees of freedom = 3

Translational degrees of freedom = 3

Hence, vibrational degrees of freedom = 24- 3- 3 = 18

Benzene (C_6H_6) molecule

Benzene (C_6H_6), the number of vibrational degrees of freedom can be calculated as follows:

Number of atoms (n) = 12

Total degrees of freedom (3n) = 3 x 12 = 36

Rotational degrees of freedom = 3

Translational degrees of freedom = 3

Therefore, vibrational degrees of freedom = 36 - 3 - 3 = 30

Thus, theoretically, there should be 30 fundamental vibrational bands in the IR spectrum of benzene.

3.2.4 Fingerprint Region

It is not possible for any two different compounds to have exactly the same IR spectrum (except enantiomers). Therefore, the IR spectrum of a compound is called its *fingerprint.* The region below 1500 cm^{-1} is called fingerprint region because every compound has unique absorption pattern in this region, just as every person has unique fingerprints. The fingerprint region contains many absorption bands caused by bending vibrations as well as

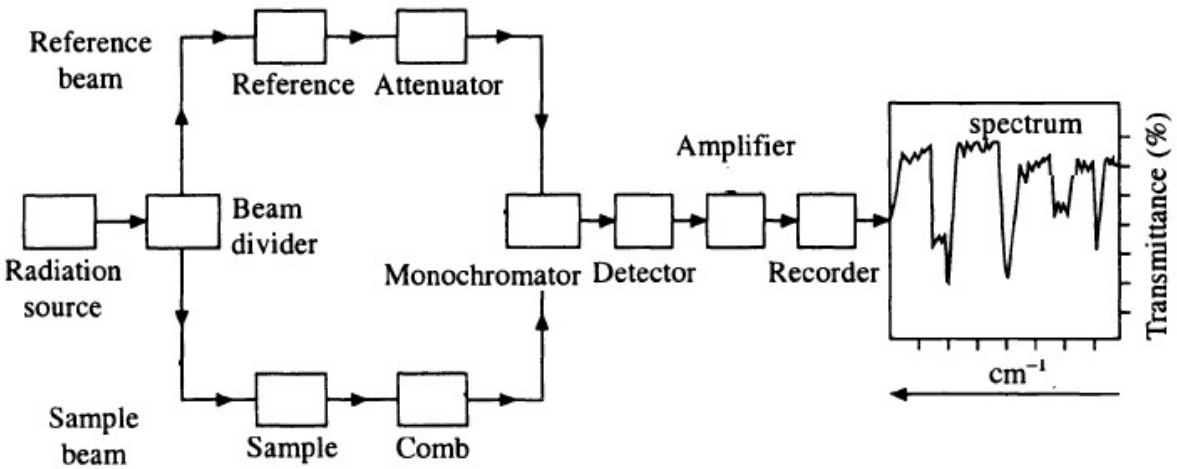
absorption bands caused by C-C, C-O like in alcohols, ethers, esters, etc. and C-N (e.g. in amines, amino acids, amides, etc.)

Stretching vibrations: Since the number of bending vibrations in a molecule is much greater than its stretching vibrations, the fingerprint region is rich in absorption bands and shoulders. Thus, the superimposability of IR bands of the spectra of any two different compounds becomes impossible in this region. However, similar compounds may show very similar spectra above 1500 cm^{-1}.

3.2.5 Instrumentation

Most IR spectrophotometers are double-beam instruments consisting of the following parts:

1. Radiation source
2. Sample and reference cells
3. Attenuator and comb (photometer)
4. Monochromator
5. Detector and amplifier
6. Recorder



Block diagram of IR-spectroscopy

1. Radiation Source

Infrared radiation is usually produced by electrically heating a Nernst filament (mainly composed of oxides of zirconium, thorium and cerium) or a globar (rod of silicon carbide) to 1000-1800°C. The infrared radiation of succesively increasing wavelength is used. The radiation from the source is divided into sample and reference beams of equal intensity by beam divider.

2. Sample and Reference Cells

Reference and sample beams pass through the reference cell and sample cell respectively. Glass and quartz cannot be used as windows of cells and optical

prisms, etc. because they absorb strongly in most of the IR region. Thus, alkali metal halides such as NaCl, NaBr, KCl and KBr are most commonly used as these are transparent to most of the IR region.

3. Attenuator and Comb (Photometer)

The reference beam passes through the attenuator and the sample beam through the comb. Then the two beams can be alternately reflected out of the optical system and to the entrance slit of the monochromator with the help of several mirrors. Thus, the photometer combines the reference and sample beams into a single beam of alternating segments. The comb allows balancing of the two beams.

4. Monochromator

The combined beam passes through the prism or grating of the monochromator which disperses the beam into various frequencies. Since the prism or grating rotates slowly, it sends individual frequency bands to the detector, thus allowing a scan of frequency bands. Gratings that give better resolutions than prisms consist of a series of parallel and straight thin lines on a smooth reflecting surface; the spacing between lines is of the order of few angstroms (Ä) depending on the desired wavelength range.

5. Detector and Amplifier

The detector is a thermocouple which measures radiant energy by means of its heating effect that produces current. Due to difference in the intensity of the two beams falling on the detector, an alternating current starts flowing from the detector to the amplifier where it is amplified and relayed to the recorder.

6. Recorder

It records IR spectra as a plot of wavelengths λ or wave numbers (*ν)* of absorbed radiations against the intensity of absorption in terms of transmittance '*T'* or absorbance 'A'. Presently we use the wave number unit as it is directly proportional to energy

$$T = \frac{I}{I_o}$$

$$T\%(\text{percentage transmittance}) = \frac{I}{I_o} \times 100$$

$$A = \log_{10} \frac{1}{T}$$

Where I_o is the intensity of the incident radiation and '*I'* the intensity of the radiation emerging from the sample.

At present, FT-IR (Fourier transform infrared) spectrophotometers have become common. The FT-IR instrument gives same information as a simple IR

spectrophotometer but the former is much efficient, as it is quick, has high sensitivity and requires very small quantity of the sample.

3.4. Sample Handling

Infrared spectra of compounds may be recorded in the vapour phase, as pure liquids in solution and in the solid state. The sample should be dry because water absorbs near -3710 cm^{-1} and about -1630 cm^{-1}.

In Solid State

As a mull or paste: About 2-5 mg of a solid is finely ground in an **agat mortar** with one or two drops of the mulling agent. The mull is examined as a thin film between two flat plates of NaCl. The most commonly used mulling agent is nujol (a high boiling petroleuro oil). When C-H bands interfere with the spectrum, another mulling agent, hexachlorobutadiene, may be used.

As a Liquid Film

A drop of neat liquid is placed between two flat plates of NaCl to give a thin film. Thick samples of neat liquids usually absorb too strongly to give satisfactory spectrum. This is the simplest of all sampling techniques.

In Vapour Phase

The vapour or gas is introduced into a special cell which is usually about 10 cm long and the walls of its both the ends are normally made of NaCl which is transparent to IR radiation. The vapour phase technique is limited because of the too low vapour pressure of most organic compounds to produce a useful absorption spectrum.

In solution

Usually, a 1-5% solution of the compound is introduced into a special cell of 0.1-1 mm thickness and made of NaCl.

3.4.Applications of IR Spectroscopy

Among all the properties of an organic compound, no single property gives as much information about the compound's structure as its infrared spectrum. Thus, IR spectroscopy is the most widely used method for structure determination of biomolecule in biochemistry. The basic reason why IR spectra are of such value to the biomolecule is that molecular vibrations depend on interatomic distances, bond angles and bond strengths, rather than on bulk properties of the compound.

Thus, these vibrational frequencies provide a molecular fingerprint which enables the identification of the compound either in the pure state or in mixtures. IR spectroscopy is especially used for detection of functional groups in organic compounds and for establishing the identity of organic compounds.

Use of IR spectroscopy to biochemistry as follows:

Detection of Functional groups

All functional groups absorb in a definite frequency region. Thus, the presence or absence of a band in a definite frequency region tells the presence or absence of a particular functional group in the compound.

Example: In the presence of a $\nu_{C=O}$ band in the region 1720-1740 cm^{-1} along with another band (usually two bands) in the region 2700-2900 cm^{-1} shows the presence of an aldehydic carbonyl group in the compound.

Purity of Samples

IR spectra of impure sample are usually blurred and have many bands which cannot be interpreted, whereas a pure compound gives a clear IR spectrum. For example, a sample of an alcohol containing a ketone as an impurity gives poor IR spectrum which shows additional absorption bands due to the carbonyl group.

Study of Hydrogen Bonding

IR spectroscopy is useful in detecting hydrogen bonding, in estimating the strength of hydrogen bonds and in distinguishing intermolecular and intramolecular hydrogen bondings.

1. **Orientations in Aromatic Compounds**

Absorptions in the region 675-900 cm^{-1} due to out-of-plane bending vibrations indicate the relative positions of substituents on the benzene ring. The position of absorption bands in this region depends on the number of adjacent hydrogen atoms on the ring.

2. **Progress of Reactions**

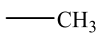
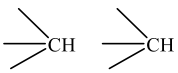
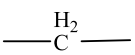
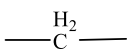
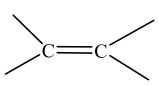
In most of the cases the progress of an organic reaction can be followed by IR spectroscopy. This is done by examining the IR spectra of portions of the reaction mixture withdrawn at certain time intervals. For example, in a reaction involving the oxidation of a secondary alcohol into a ketone, it is expected that the ν_{O-H} band near 3570 cm^{-1} will disappear and a new $\nu_{c=o}$ band will appear near 1715 cm^{-1} on completion of the reaction.

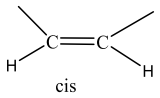
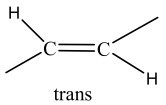
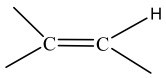
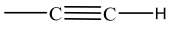
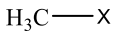
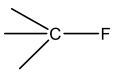
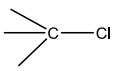
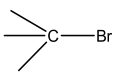
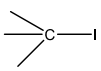
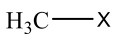
3.5. Interpretation of Infrared Spectra

The presence or absence of an absorption band indicates the presence or absence of a particular functional group in a compound. For example, appearance of an absorption band near 3330 cm^{-1} is indicative of an inter molecularly **hydrogen bonded** O-H group, similarly appearance of a band around 1700 cm^{-1} indicates the presence of a **carbonyl group (**C=O group). After tentative assignment of an absorption band to a particular group, it should be confirmed wherever possible by examination of other band(s) expected for that group. For example, the assignment of a **carbonyl band to an aldehyde**

should be confirmed by the appearance of a band or a pair of bands in the region 2700-2900 cm^{-1} due to **aldehydic** ν_{c-H}. Similarly, the assignment of a **carbonyl band to an ester** should be confirmed by the presence of a strong band due to $\nu_{c=o}$ in the region 1000-1300 cm^{-1}, etc., frequencies are given in list of table given below for respective frequencies. Usually, characteristic absorption bands of functional groups are used for their detection; it is rarely possible to deduce complete structure of a compound from its IR spectrum alone. In structure determination, IR spectroscopy is supplemented by chemical evidence and UV, NMR and mass spectral data for complete structure determination.

Some characteristic group frequencies along with effects of structural environments on them are discussed as follows:

Type	Group	Absorption frequency (cm^{-1})	Intensity*	Assignment and remarks
Alkanes		2840-3000	m→s	C-H stretch; two or three bands
		800-1200	w	C-C stretch; little value
Cycloalkanes				
		2840-3950	m	Asym. And Sym. C-H stretch, two bands
(cyclopropane)		3040-3060	m	Asym. C-H stretch
		2975-2985	m	Sym. C-H stretch
		1015-1045	m	Skeletal vibration
Alkenes		1620-1680	v	C=C stretch; diene, tiene.etc. 1650(s) and 1600(s)

 cis	1655-1660	m		
 trans	1670-1675	w		
	3000-3100	m	C-H stretch; almost the same position in the cis and trans isomer	
Alkynes		2100-2260	v	 stretch
		2100-2140	s	 stretch
Halogen Compounds X=F, Cl, Br, I		near 3000	s	Asym. And sum. C-H
		1000-1400	s	C-F stretch
		600-800	s	C-Cl stretch
		500-750	s	C-Br stretch
		-500	s	C-I stretch
		1441-3100	v	C-H Asym. bending
	X=F,Cl,Br,I	1255-1475	v	C-H Asym. bending
Aromatic	C-H	3000-3100	m	C-H stretch
	C-C	1600 ± 5	v	C=C skeletal stretch
		1580 ± 5	m	Sleletak stretch; present when ring is further conjugated
Alcohols and Phenols	O-H	3590-3650	v	Free O-H stretch
		3200-3600	v	Intermediate hydrogen bonded O-H stretch
		2500-3200	s	Intermediate hydrogen bonded O-H stretch
C-O		1000-1200	m→ s	C-O stretch

	-1050	s	C-O stretch pri. alcohol
	-1100	s	C-O stretch sec. alcohol
	- 1150	s	C-O stretch Ter. Alcohol
	-1200	s	C-O stretch phenols
O-H	1339-1420	s	In-plane O-H stretch
	650-769	s	Out-of-plane O-H bending

3.6. Summary

After study of this unit learner is able to define Electromagnetic Radiations, Electromagnetic spectrum and absorption of radiations, Absorption Laws, concepts of UV-Visible spectroscopy, Formation of Absorption Bands, Concepts of Chromatophore, Calculation of Absorption Maximum of organic compounds and theory and application of IR Spectroscopy for organic compounds to identification of compounds.

3.7. Terminal Questions

Q.1: What is IR spectroscopy? Discuss the different types of vibration in IR spectroscopy.

Answer: ----------

Q.2: Discuss the origin of IR-visible spectra.

Answer: ----------

Q.3: Write notes on:

(a) Stretching and bending vibrations

(b) Fingerprint region

Answer: ----------

Q.4: Discuss the factors which affect the IR absorption frequency of a functional group.

Answer: ----------

Q.5: Using IR spectroscopy, how will you distinguish the Intermolecular and intramolecular hydrogen bonding

Answer: ----------

3.8. Suggested readings

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DCEBCH - 106

Spectroscopy

Block

2

NMR and Atomic spectroscopy

DCEBCH -106

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Introduction

This is the second block of Spectroscopy. This consists of three units.

Unit-4: This unit covers the principle of NMR spectroscopy. The NMR types and measurement is briefly discussed here. The basics parameters such as chemical shift and spectral peaks are discussed. The application of NMR in biochemistry is also mentioned.

Unit-5: Atomic adsorption spectroscopy discussed in this unit. The Principle instrumentation and application of adsorption of atomic adsorption spectroscopy discussed in details.

Unit-6: Atomic adsorption spectroscopy discussed in this unit. The Principle instrumentation and application of adsorption of atomic emission of spectroscopy discussed in details. The role biochemistry also mentioned here.

Unit-4: NMR Spectroscopy

Contents

4.1. Introduction

This unit covers the nuclear magnetic resonance (NMR). NMR spectrum is obtained when the nuclei has odd number of proton in their nucleus. The splitting of signal determines the position and number of proton in any organic compounds. In this unit, all aspect of NMR has been described in detail. NMR spectra are unique, well-resolved, analytically tractable and often highly predictable for small molecules. Different functional groups are obviously distinguishable, and identical functional groups with differing neighboring substituents still give distinguishable signals. NMR has largely replaced traditional wet chemistry tests such as color reagents or typical chromatography for identification. A disadvantage is that a relatively large amount, 2–50 mg, of a purified substance is required, although it may be recovered through a workup. Preferably, the sample should be dissolved in a solvent, because NMR analysis of solids requires a dedicated magic angle spinning machine and may not give equally well-resolved spectra. The timescale of NMR is relatively long, and thus it is not suitable for observing fast phenomena, producing only an averaged spectrum. Although large amounts of impurities do show on an NMR spectrum, better methods exist for detecting impurities, as NMR is inherently not very sensitive - though at higher frequencies, sensitivity is higher.

Objectives:

- To learn phenomenon and working principle of NMR

- To develop the basic understanding of analytical tools that is very useful in detection or characterization of biological samples

4.2. NMR Spectroscopy overview

NMR stands for Nuclear Magnetic Resonance it is another form of absorption spectroscopy because under magnetic condition the sample absorbs certain wavelength of electromagnetic radiations. NMR is an analytical tool to determine the structure and purity of a sample as well as its molecular structure. Most studies in organic chemistry involve the use of ^{1}H, but NMR spectroscopy with ^{13}C, ^{15}N and ^{31}P isotopes is frequently used in biochemical studies. The resonance condition in NMR is satisfied in an external magnetic field of several hundred mT, with absorptions occurring in the region of radio waves (frequency 40 MHz) for resonance of the ^{1}H nucleus. However, in the NMR the magnet involved is not electron but nuclei of atom of element. Once the basic structure is known, NMR can be used to determine molecular conformation in solution in which the studying physical properties such as conformational exchange, phase changes, solubility, and diffusion are determined. The nuclei of molecules give rise to spectrum which absorbs electromagnetic radiation under magnetic condition. Most of the study shows in organic chemistry involve the use of ^{1}H NMR but NMR study with ^{13}C, ^{15}N and ^{31}P is frequently used in biochemical study. While the proton of nuclei is put under magnetic condition. The proton present in nuclei shows spin due to absorption of radio wave and act like small magnet. Resonance in this small magnate show spin and process is called NMR in other word we can say that, the nuclear magnetic resonance is the phenomenon of nucleolus in which proton and neutron spin about the axis due to electromagnetic radiation.

All nuclei carry a charge. In some nuclei charge “spin” on the nuclear axis and it circulation of nuclear charge generates a magnetic dipole along the axis. The angular moment of spinning charge can be describe in term of quantum spin numbers ***I;*** these have number values of 0, 1/2,1, 3/2 and so on**.** The intrinsic magnitude of generated dipole is expressed in term of magnetic moments ‘μ’. The H-nucleus is the most commonly studied by NMR spectroscopy because of its high natural abundance of 99.985% and its presence in the majority of organic compounds. NMR studying ^{1}H atom is called hydrogen or proton NMR spectrum. The proton NMR spectrum gives the information about the number of different types of protons and also the chemical environment around it.

NMR active nuclei

We know the nuclei are built up of protons and neutrons each possessing an angular momentum due to its motion about the centre of the nucleus. The total

angular momentum of a nucleus in its ground state is called as nuclear spin. This is characterized by a spin quantum number 'I', which may be integral, half-integral or 0. Only those nuclei which have non-zero spin number can absorb/emit radiofrequency (RF) radiation and hence are NMR active nuclei.

1. If the number of neutrons and the number of protons are both even, then the nucleus has NO spin (I = 0). For example ^{12}C, 16O, ^{32}S have no NMR signal because of even number of proton and neutrons.
2. If the number of neutrons and the number of protons are both odd, then the nucleus has an integer spin (I = 1, 2, 3 and so on). Example: ^{2}H, ^{10}B, ^{14}N are NMR active.
3. If the number of neutrons plus the number of protons is odd (odd mass number or we can say odd number of neutrons/protons and even number of protons/neutrons), then the nucleus has a half-integer spin (I = n/2, where n is an odd integer; I = 1/2, 3/2, 5/2 and so on) Example: ^{1}H, ^{13}C, ^{15}N, ^{31}P are NMR active.

Instrumentation

In NMR the samples in solution are contained in sealed tubes which are rotated rapidly in the cavity to eliminate irregularities and imperfections in sample distribution. In solid samples, the number of spin–spin interactions is greatly enhanced due to intermolecular interactions that are absent in dissolved samples due to translation and rotation movements. As a result, the resonance signals broaden significantly. However, high-resolution spectra can be obtained by spinning the solid sample at an angle of 54.7°. Advanced computer facilities are needed for operation of NMR instruments, as well as analysis of the acquired spectra.

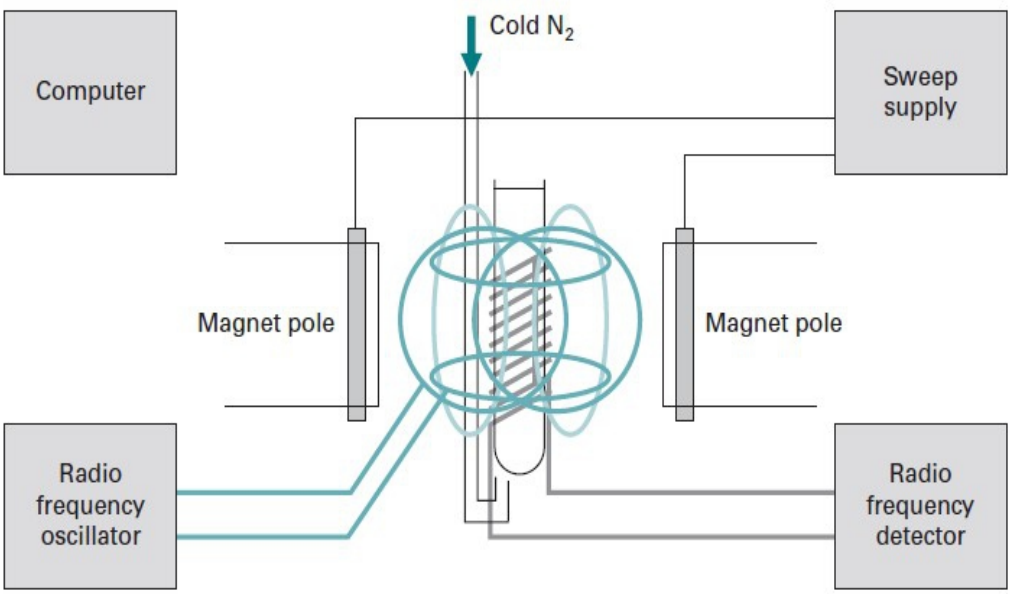


Fig. 6.4: Schematic diagram of an NMR spectrometer with cryoprobe.

4.3. Principle of NMR spectroscopy

The principle behind NMR is that many nuclei have charge "spin" when they have odd number of proton in their nuclei. When an external magnetic field applied and the nuclear spin occurs, the energy transfer is possible between the base energy to a higher energy level. The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in several ways and processed in order to yield an NMR spectrum. The molecular environment of proton governs the value to applied external field at which the nucleuses resonate. This gives recorded as chemical shift. The chemical field rises from the applied field including secondary field of about 0.15 – 0.2 mT at proton by interacting with adjacent bonding electron. .

Nuclear magnetic moment $\mu = g_N u_N [I(I+1)]^{1/2}$

Where I is angular momentum

Also nuclear spin angular momentum $= [I(I+1)]^{1/2} \frac{h}{2\pi}$

$$\Delta E = h\nu\, g u_N B_o \text{ (bore-Einstein)}$$

g_N = Nuclear g factor, characteristic nucleus

μ_N = nuclear magnetron = 5.05X 10^{-27} J T^{-1}

B_o = External magnetic field in tesla

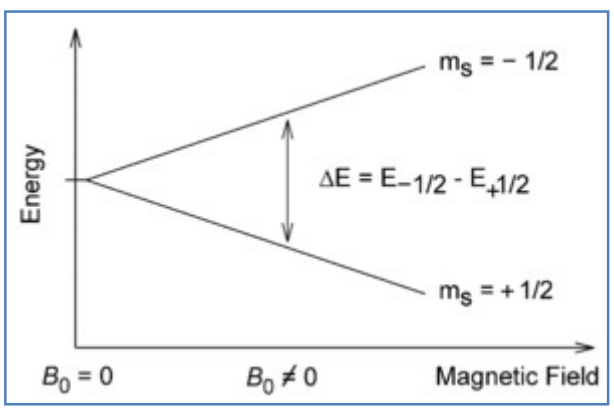


Fig.6.3: Representing the phenomenon of NMR in ^{1}H molecule.

4.4. Types of NMR

^{13}C NMR

Carbon forms the backbone of the organic molecules therefore C NMR is also important and gives valuable information about the molecule. The ^{13}C NMR is generated in the same fundamental principle such as proton ^{P}NMR

spectrum. Only 1.1 % of naturally occurring carbon is ^{13}C and actually an advantage because of less coupling. In ^{13}C NMR spectrum is occurs directly due to present of carbon skeleton in the molecule and the proton present in molecules does not play direct role. The ^{12}C do not absorb radio frequency energy but the other isotope i.e. ^{13}C absorbs the energy but the ^{13}C has a natural abundance of only about 1%. Therefore the sensitivity of ^{13}C is less than the 1H NMR and requires longer time to record

Key feature of ^{13}C NMR spectroscopy:

- The chemical shift value for ^{13}C NMR spectroscopy is □ = 0-200 ppm with respect to the internal reference standard.
- Due to low chance ratio ^{13}C - ^{13}C coupling is negligible; so proton decouple spectrum shows singlet for each non-equivalent carbon.
- The number of signals tell us how many different carbons or set of equivalent carbons
- The splitting of a signal tells us how much hydrogen is attached to each carbon. (N+1 rule)
- The chemical shift tells us the hybridization (sp^3, sp^2, sp) of each carbon.

^{1}HNMR

The H-nucleus is the most commonly studied by NMR spectroscopy because of its high natural abundance of 99.985% and its presence in the majority of organic compounds. NMR studying ^1H atom is called Hydrogen or proton NMR spectrum. The proton NMR spectrum gives the information about the number of different types of protons and also the chemical environment around it.

Key feature of ^{1}HNMR spectroscopy:

Calculate the No. of peaks (signals) observed in the spectrum.

It gives types of protons present in molecules.

- Intensity of the peaks(Signals)

It represents no. of protons present in individual peaks (area under curve).

- Multiplicity of the signals:

  It gives the information regarding the no. of proton present on the adjacent atom.

- Position of signals:

  It gives the information about the chemical atmosphere surrounding the protons.

4.5. NMR spectra measurement

4.5.1. NMR active nuclei

We know the nuclei are built up of protons and neutrons each possessing an angular momentum due to its motion about the centre of the nucleus. The total angular momentum of a nucleus in its ground state is called as nuclear spin. This is characterized by a spin quantum number I, which may be integral, half-integral or 0. Only those nuclei which have non-zero spin number can absorb/emit radiofrequency (RF) radiation and hence are NMR active nuclei.

4. If the number of neutrons and the number of protons are both even, then the nucleus has NO spin (I = 0). For example ^{12}C, 16O, ^{32}S have no NMR signal because of even number of proton and neutrons.

5. If the number of neutrons and the number of protons are both odd, then the nucleus has an integer spin (I = 1, 2, 3 and so on). Example: ^{2}H, ^{10}B, ^{14}N are NMR active.

6. If the number of neutrons plus the number of protons is odd (odd mass number or we can say odd number of neutrons/protons and even number of protons/neutrons), then the nucleus has a half-integer spin (I = n/2, where n is an odd integer; I = 1/2, 3/2, 5/2 and so on) Example: ^{1}H, ^{13}C, ^{15}N, ^{31}P are NMR active.

4.5.2 NMR phenomenon

Only those nuclei will show NMR phenomenon whose spin number I is greater than 0 (zero). In the nucleolus when the number of proton is equal to the number of proton; and neutron are paired the spin will be cancelled due to parallel and anti-parallel spin. Thus the net result of spin will be zero and such nuclei will not show NMR phenomenon. For example molecules of carbon and oxygen (C atomic number = 12, O atomic number = 16) have number of proton and neutron in their nucleus. Whereas as those molecules that have not paired proton and neutron or having unequal number of proton and neutron in their nucleus shows NMR phenomenon because such nuclei will have resultant spin (I) more than zero. Thus, resulting nuclei carry charge, then get spin and generate magnetic field. Thus we can say that nuclei act as a tiny bar magnet.

The magnitude and direction of the magnetic field generate by this nuclei is described by a vector called magnetic moment or magnetic dipole. The nuclei is placed in strong magnetic field it spin states shows two stages.

α-spin state: In this state nuclear spin aligned in the same direction as that of applied magnetic field. α-spin state is of lower energy.

β-spin state: In this state nuclear spins aligned themselves in the opposite direction of applied magnetic field. β-spin state is of higher energy.

When the electromagnetic radiation of proper frequency is passed, a nucleus with α-spin state absorbs this radiation and converted into higher energy state that is β-spin state. This process is called flipping of nucleus. When the proton is place at magnetic field then it starts processing a certain frequency in the radio wave region and thus it will capable of taking up one of the two orientations with respect to the axis of the external field.

- Alignment with the field
- Alignment against the field

If the proton is processing in the aligned orientation. It can pass into the opposed orientation by absorbing higher energy. It come backs to lower energy aligned orientation by losing energy. The transition between two energy states is called flipping of proton. The transition between two energy states can be brought about by the absorption of electromagnetic radiation in the radio wave region and resulting single peaks is obtained.

4.5.3. Solvent used in NMR:

The spectrum of NMR depends on the nature of solvent of samples. The solvent should have following character

- It should be chemically inert
- It should be magnetically isotropic in nature
- It should be free from any hydrogen (^1H) atom

Some solvent that are most commonly used in NMR study are

- Carbon tetrachloride (CCl_4)
- Carbon disulphide (CS_2)
- Deuterochloroform ($CDCl_3$)
- Deutero dimethyl sulfoxide (CH_3)SO
- Deuterobenzene (C_6D_6)

4.6. Chemical Shift and signals in NMR

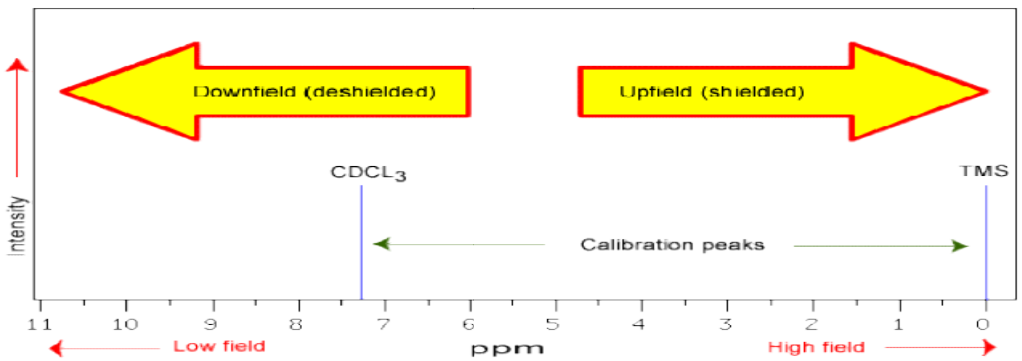
The chemical shift is the position of NMR signals which arises due to shielding and deshielding of electron in chemical bond is called chemical shift. Different types of proton in the compound have different electronic environment and proton gets absorb at different applied magnetic field. Chemical shift are measured in reference to a particular standard.

The shielding and deshielding proton by electron produced very small change in the strength of applied magnetic field. This small change in the field

strength can't be determined accurately. Therefore, chemical shift of proton are measured with reference to a solvent tetramethyl silane. Chemical shift δ is usually expressed in parts per million (ppm) by frequency, because it is calculated from:

$$\delta = \frac{v_{sample} - v_{ref}}{v_{ref}}$$

Where ν_{sample} is the absolute resonance frequency of the sample and ν_{ref} is the absolute resonance frequency of a standard reference compound, measured in the same applied magnetic field B_0. Since the numerator is usually expressed in hertz, and the denominator in megahertz, δ is expressed in ppm. When molecule is placed in external magnetic field electron will produce secondary magnetic field.



Down-field Up-field

←	Deshielding	Shielding	→
←	Don-field	Up-field	→
←	High frequency	Low frequency	→
←	High chemical shift values	Low chemical shift values	→

Secondary magnetic field → opposed → shielding → up-field

Secondary magnetic field → reinforced → shielding → up-field

Up-field/down-field shift is known as chemical shift.

TMS (tetramethyl silane) is considered has reinforce compound because it has following characteristic characters

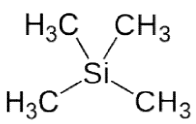


Fig 6.5 : Tetra methyl silane

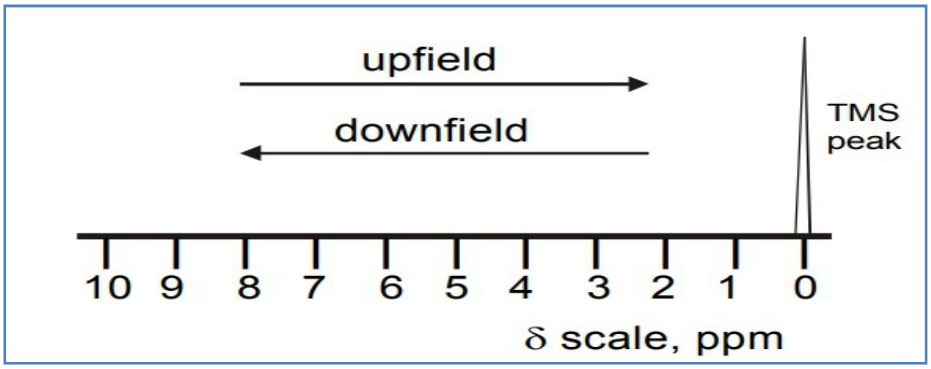


Fig.6.6 : Chemical shift in TMS

- It has zero delta values because it highly shielded.
- TMS has highest shielding than other organic compounds because it absorb at higher field. The reason behind this is that silicon is more electro positive (1.8) than carbon (2.5).
- It is easy miscible with many other organic compound.
- It highly volatile in nature (boiling point = 27 ˚C) so sample can be recorded easily.
- It does not take part in any chemical reaction.

The number of signals in NMR spectrum occurs by different set of chemically equivalent proton in molecules. The proton which has the same chemical environment is called equivalent proton and they show the chemical shift. The protons have equivalent chemical environment gives same signal whereas those protons have different chemical environment produces different signals. In other word we can say that the each signal in the NMR spectrum represents a set of equivalent proton. Thus, the NMR signal tells us the kind of proton present in the molecules and it position reveal the nature of proton. Chemically equivalent proton must be sterochemically equivalent and also have same chemical shift. Magnetically equivalent proton have same chemical shift and same coupling constant (J) to every other nucleolus in the molecules. Often, magnetically equivalent protons are chemically equivalent. For example CH_3CH_2OH gives three signals.

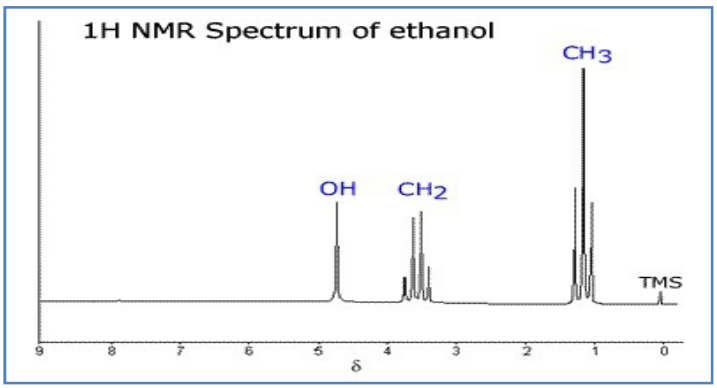


Fig. 6.7 : NMR spectrum of ethanol

4.7. Applications of NMR in biochemistry

NMR spectroscopy is the use of NMR phenomena to study the physical, chemical, and biological properties of matter. Chemists use it to determine molecular identity and structure. In each carbon the multiplicity of the signal depends upon how many protons are attached to it. The ^{13}C-^{13}C coupling is not possible while ^{1}H-^{13}C coupling is possible.

Molecular structure determination

The NMR is very useful method to structure determination for organic compounds. When the proton or carbon exhibit in the similar chemical environment it gives similar signal. In this case the chemical shift provides a clue about the environment of a particular proton or carbon, and thus allows conclusions as to the nature of functional groups. Spin–spin interactions allow conclusions as to how protons are linked with the carbon skeleton. For structure determination, the fine structure usually is the most useful information because it provides a unique criterion while chemical shifts of some groups can vary over an extended range. The structures of proteins up to a mass of about 50 kDa can be determined with biomolecular NMR spectroscopy. The development of magnets with very high field strengths (currently 900 MHz) continues to push the size limit. Heteronuclear multidimensional NMR spectra need to be recorded for the assignment of all chemical shifts (1H, 13C, 15N). For inter proton NOEs, 13C- and 15N-edited 3D NOESY spectra are required.

Magnetic resonance imaging

The NMR spectroscopy is very useful in the imaging of live samples because the proton is one of the more sensitive nuclides and is present in all biological systems abundantly. The ^{1}H NMR spectroscopy has significance role in the imaging of live samples. The most important compound in biological samples in this context is water. It is distributed differently in different tissues, but constitutes about 55% of body mass in the average human. In NMR, the resonance frequency of a particular nuclide is proportional to the strength of the applied external magnetic field. If an external magnetic field gradient is applied then a range of resonant frequencies are observed, reflecting the spatial distribution of the spinning nuclei.

Magnetic resonance imaging (MRI) can be applied to large volumes in whole living organisms and has a central role in routine clinical imaging of large-volume soft tissues. The number of spins in a particular defined spatial region gives rise to the spin density as an observable parameter. This measure can be combined with analysis of the principal relaxation times (T_1 and T_2). The imaging of flux, as either bulk flow or localized diffusion, adds considerably to the options available. In terms of whole-body scanners, the entire picture is

reconstructed from images generated in contiguous slices. MRI can be applied to

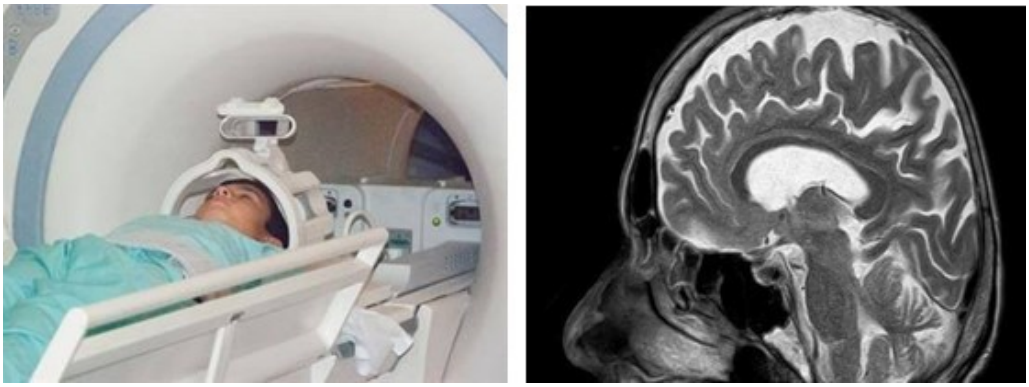


Fig. 6.8: MRI Image of brain

the whole body or specific organ investigations on head, thorax, abdomen, liver, pancreas, kidney and musculoskeletal regions (Fig. 6.8). The use of contrast agents with paramagnetic properties has enabled investigation of organ function, as well as blood flow, tissue perfusion, transport across the blood–brain barrier and vascular anatomy. Resolution and image contrast are major considerations for the technique and subject to continuing development. The resolution depends on the strength of the magnetic field and the availability of labels that yield high signal strengths.

4.8. Summary

In this unit you have learn that-

NMR spectroscopy has become must important tool without any question for the biochemistry. It is a different type of absorption spectroscopy as compare to IR and UV spectroscopy. Some of the nuclei absorb the electromagnetic radiation in the range of radio-frequency in presence of external magnetic field and governed by the characteristic of the compound. The elements which possess gyromagnetic ratio i.e. their nuclei behave like tiny bar magnets are responsible for nuclear magnetic resonance. The nuclear magnetic resonance can be compelled to: 1. Reveal their presence 2. Self-identification 3. Environment.

4.9. Terminal questions

Q.1. What do you understand for NMR? Write the application of NMR.

Answer:----------

Q.2. Write the phenomenon of NMR.

Answer:----------

Q.3. NMR work on which law. Discuss briefly.

Answer:----------

Q.4. Describe the principle and instrumentation of ^{1}HNMR .

Answer:----------

Q.5. Discuss about types of NMR

Answer:----------

Q.6. What is signal in NMR spectrum? Describe the principle of ^{13}C NMR.

Answer:----------

4.10. Further readings

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Unit-5: Atomic Adsorption Spectroscopy

Contents

5.1. Introduction

Atomic absorption spectroscopy (AAS) is a technique that involves study of absorption of electromagnetic radiations in relationship to atomic structure. Atomic absorption is a process involving the absorption of light by free atoms of an element at a wavelength specific to that element. It is a means by which the concentration of metals can be measured in their atomic state. In various phenomena of Atomic Spectroscopy (emission, absorption and fluorescence), energy is supplied to the atoms in the form of thermal, electromagnetic, chemical or electrical energy which is converted to light energy by various atomic and electronic processes before measurement. Atomic Absorption Spectrometry is useful not only for the identification but also for quantitative determination of many elements present in samples. The technique is specific and sensitive in that individual elements in each sample can be dependably identified in small amounts,. The technique is simple and reliable based on absorption of radiation by free atoms for their determination. AAS is the oldest instrumental elemental analysis principle, the origins of which go back to the work of Bunsen and Kirchhoff in the mid-19th century. AAS is based on absorption by atoms or elementary ions. The components of a sample are converted into gaseous state (gaseous atoms) or elementary ions by suitable heat treatment. The first AA Spectrometer was built by scientist Alan Walsh at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in 1954, Division of Chemical Physics, in Melbourne, Australia.

5.2. AAS Overview

Atomic absorption spectroscopy is a spectro-analytical technique. The absorption wavelength is associated with transition that requires a

minimum of energy change. The electronic transition in atom are limited by the availability of empty orbital, because one orbital can be occupied with maximum of two electrons and spin of electron need to be paired in anti parallel fashion. The analyte molar concentration is determined from the amount of absorption. The sample used in atomic absorption spectroscopy, needs to volatile by using the higher energy. Thus, the tested sample either liquid should be converted into vapor form. Atomic absorption is very technique for detecting specific metals and its concentration present in the sample at ppm.

Concentration analysis by atomic absorption spectroscopy is carried out by comparison with calibration standard. It finds extensive applications in the analysis for trace metals in biological serum and drinking water. Some examples of elements detected by AAS are as given below.

Table.1.3: Some elements detected by AAS.

S. No.	Element	Wavelength (nm)	Limit of Detection (meu g/mL
1	Au	243	0.009
2	Hg	254	0.160
3	Cu	325	0.002
4	Ag	328	0.002
5	U	358	3900
6	Ca	423	0.002
7	Na	589	0.0002
8	K	767	0.002

Atomic Structure and Spectra

To understand the concept of atomic absorption process, it is important to understand the structure of atom first. The basic processes in this spectrometry involve the outer electrons of the atom and thus its pros and cons can be well understood from the theory of atomic structure itself. According to the Niels Bohr (1913), the structure of the atom consists of central core or nucleus, made up of protons and neutrons surrounded by the electrons in orbits of differing energy. These orbits were described as energy levels which differ in energy from each other. Each orbit has a fixed energy associated with it, in general an electron has lowest energy in its ground level, and higher energy in its excited state, and can be easily removed. The electron tends to remain in its permitted energy levels but may change to another level if the amount of energy absorbed is equal to the difference between the two levels. When the electron moves to the higher energy level, such as E_1, it is said to be excited. Each atom has quantized energy levels depending upon the number of protons and electrons

present. Each element has a unique set of energy levels pertaining to the unique set of electrons and protons. It is these energies which are measured in relation to the ground state, and a particular excited state above the ground state (Fig. 2).

When the electron moves to the higher energy level, such as E_1, it is said to be excited. Each element has a unique set of energy levels pertaining to the unique set of electrons and protons. The main function of absorbed energy is to raise the molecule from the ground state energy E_0 to the higher excited state (energy E_1) that can be represented by following formula such as

$$\Delta E = E_1 - E_o$$

We know

$$\Delta E = hv$$

$$hv = hc/\lambda$$

$$v = c/\lambda$$

where h = Planck's constant

c = velocity of light

λ = wavelength of the absorbed ration.

ΔE depends upon how tightly the electrons are bound in the bonds and accordingly the absorption will occurs.

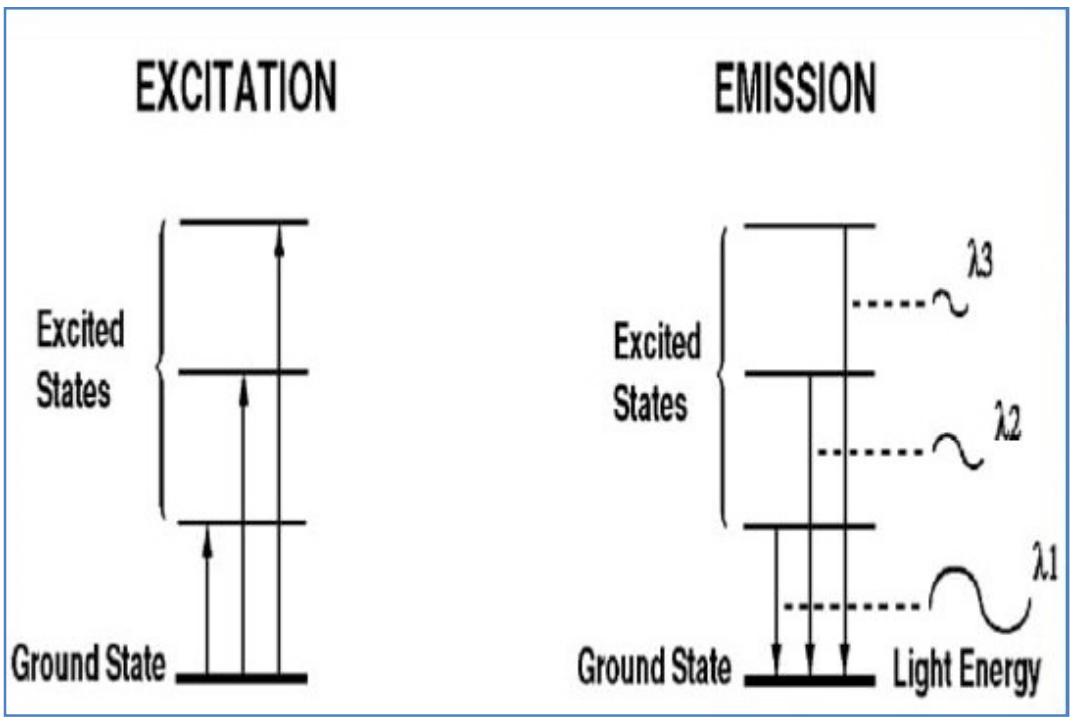
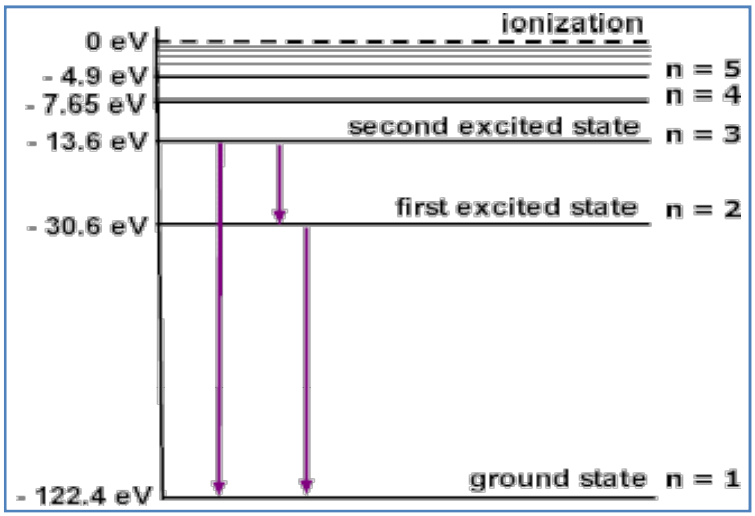


Fig: Energy level Diagram

An element can go many electronic transitions and the transition between electronic energy states is characterized by their different in energy and hence different atoms will absorb at different set of wavelengths. When a range of wavelength is surveyed a sharp energy maximum is shown by each wavelength (a spectroscopic 'line') and these lines characteristic to atom and are basis of distinguished atomic spectra. The lines originating from the ground state of the atomare called resonance lines and are the most sensitive and useful analytical lines for atomic absorption spectroscopy.



Different level Energy Diagram

5.3. Principle

The principle of AAS is measurement of the concentration of elements present in the sample through their property of absorption of light. In AAS the free atoms (gas) generated in an atomizer can absorb radiation at specific frequency. Atomized element absorbs energy of a wavelength that is peculiar to that element. In the process of atomization the hallow cathode lamp is used as a light source which emits light of wavelength that is peculiar to that element. When a beam of electromagnetic radiation of a particular wavelength is passed through the vaporized atom present in the flame, the atoms absorb the radiation and extent of radiation will be directly proportional to the number of ground state atoms presented in the flame. Atomic-absorption spectroscopy quantifies the absorption of ground state atoms in the gaseous state. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption of electromagnetic radiation. The principle is based on the Beer-Lambert's law. The absorption of radiation by the free atoms is proportional to their concentration.

$$\text{Absorption} = \log_{10} \frac{I_o}{I_t} = KCL$$

Where,

I_o = Intensity of incident radiation

I = Intensity of transmitted radiation

C = Concentration of analyte

K = Constant

L = Path length

5.4. Instrumentation

The Atomic absorption spectroscopy has simple instrumentation. Every absorption spectrometer must have the three basic component requirements (1) a light source; (2) a sample cell; and (3) a means of specific light measurement. But, unlike other spectroscopy methods, it has two additional requirements. These include a specially designed lamp to produce light of a desired wavelength and a burner to prepare the sample for absorption of light radiation. Additionally, the instrument also sprays the sample in the solution state over an atomizer (burner). This leads to evaporation of the solvent and leaves a fine dry residue. This residue has neutral atoms in the ground state. The sample of interest is aspirated and atomized into the flame. If that metal is present in the sample, its atoms will absorb some of the light, thus reducing its intensity. This decrease in intensity of the light is the process of atomic absorption. The instrument measures the change in intensity. A computer data system converts this change into an absorbance.

i. Only liquid samples can be analyzed

ii. Non metal cont be analyzed

iii. It may be used for quantitative analysis under special circumstances

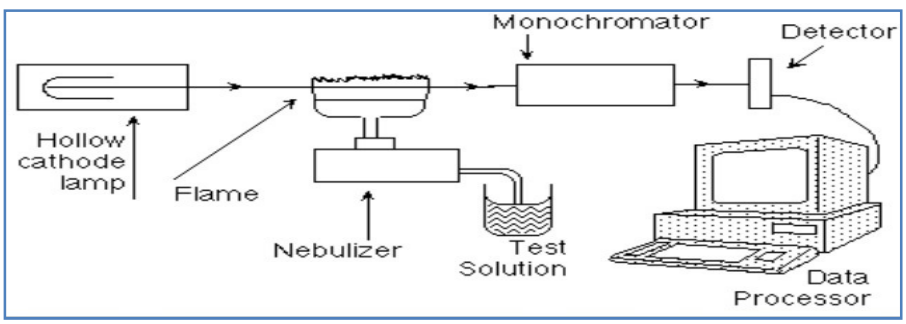


Fig.1.3: Diamagnetic representation of AA spectrophotometer:

An atom absorbs light at discrete wavelengths. So, it is necessary to use a light source, which emits the specific wavelengths which can be absorbed by the atom. The two most common light sources used in AA are the ‘‘hollow cathode lamp’’ and the ‘‘electrode less discharge lamp. The light should be stable and have sufficient intensity and should produce a narrow spectrum with little background noise. Hollow cathode lamps (HCL) are the most common radiation source in AAS. It contains a tungsten anode and a hollow cylindrical cathode made up of metal to be determined. For instance, if sodium is to be analyzed from the sample, a cathode coated with sodium is used. These are sealed in a glass tube filled with an inert gas like argon or neon which is ionized by an electric arc. The ions get attracted toward cathodes and strike it leading to excitation of metal ions. This leads to the emission of radiation with a characteristic wavelength of analyte metal. The advantage of hollow cathode lamp is that it provides radiation with a bandwidth of 0.001 to 0.01 nm. So these lamps give highly specific radiation. The disadvantage of this hollow cathode lamp is that for every metal different cathode lamp has to be employed.

Nebulizer sucks up the liquid sample at controlled rate and creates fine aerosol spray that mixes with fuel and oxidant and where it utilized by exposing them to higher thermal energy and introduce into the flame. The nebulizer uses the combustion flames to atomize and introduce the sample into the light path. More small the size of the droplets produced, more high will be the sensitivity of the element tested. Alternating, the gases form can be generated by using induced coupled plasma (ICP).

Atomization is separation of particles into individual molecules and breaking molecules into atoms. This is done by exposing the analyte to high temperatures in a flame or graphite furnace. The (spectroscopic) flames and electrothermal (graphite tube) atomizers are the two most common atomizers used nowadays. Other atomizers, such as glow-discharge atomization, hydride atomization, or cold-vapor atomization might be used for special purposes.

Flame atomizers is the oldest and most commonly used atomizers. In this atomizer air-acetylene flame with a temperature of about 2300 °C or air-nitrous oxide flame with a temperature of about 2700 °C are used. Liquid or dissolved samples are typically used with flame atomizers. In flame AAS; a steady-state signal is generated during the time period when the sample is aspirated. This technique is typically used for determinations in the mg L^{-1} range, and may be extended down to a few μg L−1 for some elements.

Electrothermal atomizer uses graphite coated furnace to vaporize the sample. The graphite tubes are heated using a high current power

supply. In ET AAS a transient signal is generated, the area of which is directly proportional to the mass of analyte (not its concentration) introduced into the graphite tube. This technique has the advantage that any kind of sample, solid, liquid or gaseous, can be analyzed directly.

Monochromator is a very important part of an AA spectrometer used to select the specific wavelength of light from the lines emitted by the Hollow cathode lamp and transmit it to the detector. It not only selects the specific analytical line, but excludes all other interfering lines in that region. The selection of specific light allows the determination of the selected elements in the presence of others.

Detector detects the intensity of radiation absorbed by the elements. The detector consists of a photomultiplier tube or simple photocell. The PMT determines the intensity of photons of the analytical line exiting the monochromator.

The processing of electrical signal is fulfilled by a signal amplifier. The signal from the PMT is converted to digital format by a transducer for read-out, or further fed into data station for printout by the requested format. The unknown concentration of the element is then calculated from the calibration curve. The absorbance of each known solution is measured and after that calibration curve of absorbance is plotted against concentration.

5.5. Application

Atomic absorption spectrometry has many uses in different areas of chemistry. AAS has both qualitative and quantitative application in different areas. The modern emission spectrophotometers allow determination of about 20 elements in biological samples, the most common being calcium, magnesium and manganese. Absorption spectrophotometers are usually more sensitive than emission instruments and can detect less than 1 p.p.m. of each of the common elements with the exception of alkali metals. The relative precision is about 1% in a working range of 20–200 times the detection limit of an element. The different methods of Atomic absorption spectrometry are very powerful for analysis elements in a solution. The instruments are simple and easy to operate. They are useful when few elements have to be determined in a large number of samples, as is the case in clinical or food analysis. Atomic absorption spectroscopy methods are of great importance compared with other methods of elemental analysis.

- **Clinical analysis:** This spectroscopy is helpful to analyze metal present in biological fluids such as blood and urine.

- **Environmental analysis:** AAS has numerous applications in monitoring our environment such as finding out the levels of various elements in rivers, seawater, drinking water, air, soil, petrol.
- **Pharmaceuticals:** This technique helpful to detect the impurities in drugs because in some pharmaceutical manufacturing processes, minute quantities of a catalyst used in the process (usually a metal) are sometimes present in the final product. By using AAS the amount of catalyst present can be determined. Now days, AAS is used to detect the amount of heavy metal present in synthetic drugs.
- **Industry:** Many raw materials are examined and AAS is widely used to check that the major elements are present and that toxic impurities are lower than specified. For example, in concrete, where calcium is a major constituent, the lead level should be low because it is toxic.
- **Mining:** Testing the concentration of valuable substances in potential mining areas can be done by using AAS, such as gold in rocks can be determined to see whether it is worth mining the rock to extract the gold.
- **Food industries:** Determination of calcium, iron and many other elements present in drinks such as wine, beer and fruit drinks and quality assurance and contamination testing for food materials can also be performed with the help of AAS.

5.6. Summary

AAS technique was introduced for analytical purpose by Walsh and Alkemade, under the designation Atomic absorption spectroscopy. It is found to be superior to other techniques as it can be used to determine elements from trace to large quantities. Atomic absorption spectroscopy (AAS) is an analytical measurement method relying on the spectroscopic processes of excitation. It is used for the qualitative and quantitative analysis of around seventy elements (metals or non-metals). In Atomic absorption spectroscopy, the absorption of resonant radiation by ground state. Atoms of the analyte are used as the analytical signal. This process is highly selective as well as very sensitive.

5.7. Terminal questions

Q.1. What do you understand for spectroscopy? Discuss types of atomic spectroscopy.

Answer:----------

Q.2. Distinguish clearly between emission and absorption spectrum.

Answer:----------

Q.3. Write the function of atomic adsorption spectroscopy.

Answer:----------

Q.4. Write the principles of Atomic adsorption spectroscopy and its applications.

Answer:----------

Q.5. Briefly discuss instrumentation of AAS.

Answer:----------

Q.6. What is the difference between atomic and molecular spectroscopy?

Answer:----------

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Unit-6: Atomic Emission Spectroscopy

Contents

6.1 Introduction

In Unit 5, you have learnt in about the fundamental principles, instrumentation and applications of Atomic Absorption Spectrophotometry (AAS). You recall that in AAS the atomic vapours in the ground state absorb the characteristic radiation of the element and the absorbed radiation and its intensity form the basis for the qualitative and quantitative applications. In contrast to that, in the current unit we take up atomic emission spectrometry (AES) that concerns with the emission of radiation by the suitably excited atomic vapours of the analyte. In atomic emission spectrometry (AES) is one of the oldest spectroscopic methods for analysis. In this, the emitted radiation and its intensity form the basis for the qualitative and quantitative applications of the technique. It is a multi-element analytical technique that can be used for the analysis of materials in gaseous state, liquid state and solid or powder state. Its high detection power and wide variety of excitation sources makes it the most extensively used method for analysis. One of the various different atomic emission spectrometric methods an inductively couple plasma (ICP) acts as an atomisation- excitation source, the technique being called ICP-AES will be discussed in next Unit-8. The number of elements that can be determined simultaneously is only limited by the availability of sufficiently sensitive interference free spectral lines.

Objectives

After studying this unit, learner will be able to:

- Explain the principle of atomic emission spectrometry
- Various excitation sources for atomic emission spectrometry
- Describe the methods of solution preparation for AES
- Describe the different types of plasma sources and nebulisers used in ICP-AES
- To draw a schematic diagram illustrating different components of an atomic emission spectrometer
- Compare and contrast the different types of instruments used for ICP-AES
- Illustrate the qualitative determination strategy of AES using suitable examples

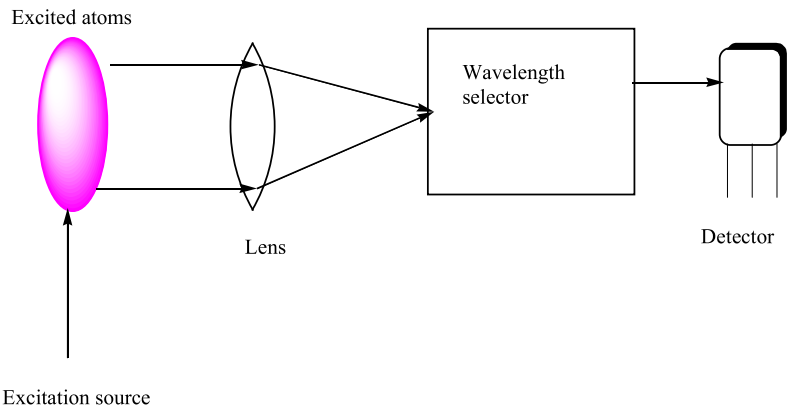
6.2 Principle of emission spectroscopy

The first observation of atomic emission dates back to at least the first where humans observed a yellow color in the flame. This color was caused by the relaxation of the 3p electron to a 3s orbital in sodium. Slightly more advanced, observations were responsible for the first development of colorful fireworks in China over 2000 years ago. A few of the more relevant discoveries for atomic spectroscopy were the first observations by Newton of the separation of white light into different colors by a prism in 1740, the development of the first spectroscope. The birth of atomic spectrometry began with the first patent of atomic absorption spectrometry by Walsh in 1955. The first atomic absorption instrument was made commercially available in 1962. Since then, there have been a series of rapid developments that are ongoing in atomic and emission spectrometry including a variety of fuels and oxidants that can be used for the flame, the replacement of prisms with grating monochromators, a variety of novel sample introduction techniques such as hydride, graphite furnace, cold vapor, and glow discharge and in advances in electronics especially microprocessors to control the instrument and for the collection and processing of data in the development of atomic fluorescence spectrometry. The basic instruments used in flame atomic absorption and emission spectrometry have improved little since the 1960s but specialty sample introduction techniques such as hydride generation and graphite furnace have greatly improved detection limits for a few elements.

An emission spectrometer can be identical to an absorption system except that no external light source is used to excite the atoms. In flame emission spectroscopy, the electrons in the analyte atoms are excited by the thermal energy in the flame. Thus the sample is the source of photon emissions through relaxation via resonance fluorescence.

Atomic emission spectroscopy (AES or OES) uses quantitative measurement of the optical emission from excited atoms to determine analyte concentration.

Analyte atoms in solution are aspirated into the excitation region where they are desolvated, vaporized and atomized by a flame, discharge, or plasma. These high-temperature atomization sources provide sufficient energy to promote the atoms into high energy levels. The atoms decay back to lower levels by emitting light. Since the transitions are between distinct atomic energy levels, the emission lines in the spectra are narrow. The spectra of multi-elemental samples can be very congested and spectral separation of nearby atomic transitions requires a high-resolution spectrometer. Since all atoms in a sample are excited simultaneously, they can be detected simultaneously and is the major advantage of AES compared to atomic-absorption (AA) spectroscopy.



Instrumentations

Quantitative analysis with plasma can be done using either an atomic or an ionic line. Ionic lines are chosen for most analyses because they are usually more intense at the temperatures of plasmas than are the atomic lines.

Sample:

Liquid samples are nebulizer and carried into the excitation source by a flowing gas. Solid samples can be introduced into the source by slurry or by laser ablation of the solid sample in a gas stream. Solids can also be directly vaporized and excited by a spark between electrodes or by a laser pulse.

Excitation:

The excitation source must desolvate, atomize, and excite the analyte atoms. A variety of excitation sources are described as – Flame, – Arc / Spark, – Plasma, –Inductively-coupled plasma (ICP), –Direct-current plasma (DCP), – Microwave-induced plasma (MIP), –Laser-induced plasma, Laser-induced breakdown (LIBS)

AES WITH ELECTRICAL DISCHARGE

AES based on Plasma Sources: Plasma is an electrical conducting gaseous mixture containing significant amounts of cations and electrons with net charge approaches zero.

1) Increased atomization/excitation

2) Wider range of elements

3) Simultaneous multi element analysis

4) Wide dynamic range

Atomic absorption is the absorption of light by free atoms. An atomic absorption spectrophotometer is an instrument that uses this principle to analyze the concentration of metals in solution. The substances in a solution are suctioned into an excited phase where they undergo vaporization, and are broken down into small fragmented atoms by discharge, flame or plasma.

Atomic Emission Spectroscopy, by exposing these atoms to such temperatures they are able to "jump" to high energy levels and in return, emit light. The versatility of atomic absorption an analytical technique has led to the development of commercial instruments. In all, a total of 68 metals can be analyzed.

Advantages of AA

Determination of about 68 metals is possible. Ability to make ppb determinations on major components of a sample Precision of measurements by flame is better than 1% rsd. There are few other instrumental methods that offer this precision so easily. AA analysis is subject to little interference. Most interference that occur have been well studied and documented. Sample preparation is simple i.e often involving only dissolution in an acid, Instrument easy to tune and operate.

Flame Emission and Atomic Absorption Spectroscopy (3 main types)

- Atomic Emission (with thermal excitation), AES
- Atomic Absorption (with optical photon unit), AAS
- Atomic Fluorescence, AFS

Three types of high-temperature plasmas

- The inductively coupled plasma (ICP).
- The direct current plasma (DCP).
- The microwave induced plasma (MIP).

The most important of these plasmas is the inductively coupled plasma (ICP).

The Direct Current Plasma Technique

The direct current plasma is created by the electronic release of the two electrodes. The samples are placed on an electrode. In the technique solid samples are placed near the discharge to encourage the emission of the sample by the converted gas atoms.

Atomic Emission Spectroscopy

Qualitative analysis is done using Atomic Emission Spectroscopy **(**AES) in the same manner in which it is done using FES. The spectrum of the analyte is obtained and compared with the atomic and ionic spectra of possible elements in the analyte. Generally an element is considered to be in the analyte if at least three intense lines can be matched with those from the spectrum of a known element.

Quantitative analysis with plasma can be done using either an atomic or an ionic line. Ionic lines are chosen for most analyses because they are usually more intense at the temperatures of plasmas than are the atomic lines.

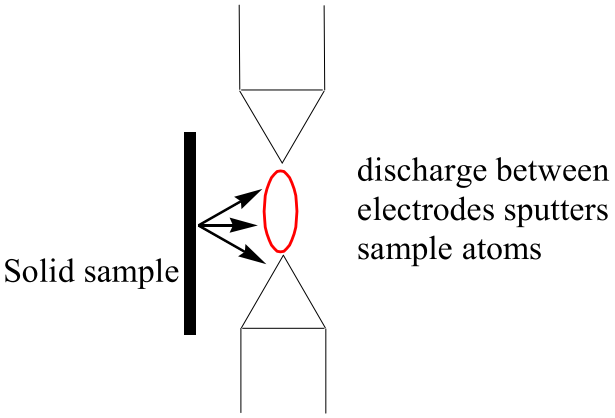
6.3 Instrumentation Emission Spectroscopy

Atomic Emission Spectroscopy with Electrical Discharges

An electrical discharge between two electrodes can be used to atomize or ionize a sample and to excite the resulting atoms or ions. The sample can be contained in or coated on one or both of the electrodes or the electrode(s) can be made from the analyte. The second electrode which does not contain the analyte is the counter electrode. Electrical discharges can be used to assay nearly all metals and metalloids. Approximately 72 elements can be determined using electrical discharges. For analyses of solutions and gases the use of plasmas is generally preferred although electrical discharge can be used. Solid samples are usually assayed with the aid of electrical discharges. Typically it is possible to assay about 30 elements in a single sample in less than half an hour using electrical discharges. To record the spectrum of a sample normally requires less than a minute.

ELECTRODES FOR AES

The electrodes that are used for the various forms of AES are usually constructed from graphite. Graphite is a good choice for an electrode material because it is conductive and does not spectrally interfere with the assay of most metals and metalloids. In special cases metallic electrodes (often copper) or electrodes that are fabricated from the analyte are used. Regardless of the type of electrodes that are used, a portion of each of the electrodes is consumed during the electrical discharge. The electrode material should be chosen so as not to spectrally interference during the analysis.



WAVELENGTH SELECTION AND DETECTION FOR AES

Arc and spark instruments normally contain non-scanning monochromators. Either a series of slits is cut in the focal plane of the monochromator and a photomultiplier tube is placed behind each slit that corresponds to the wavelength of a line that is to be measured, or one or more photographic plates or pieces of film are placed on the focal of the monochromator.

QUALITATIVE ANALYSIS WITH ARC AND SPARK AES

Qualitative analysis is performed by comparing the wavelengths of the intense lines from the sample with those for known elements. It is generally agreed that at least three intense lines of a sample must be matched within a known element in order to conclude that the sample contains the element.

QUANTITATIVE ANALYSIS WITH ARC AND SPARK AES

Regardless of the type of detection used for the assay, the precision of the results can be improved by matrix-matching the standards with the sample. Use of the internal-standard method also improves precision. Usually a working curve is prepared by plotting the ratio or logarithm of the ratio of intensity of the standard's line to the internal standard's line as a function of the logarithm of the concentration of the standard. The corresponding ratio for the analyte is obtained and the concentration determined from the working curve.

6.4 Principle of flame photometry

It is a branch of atomic spectroscopy is used for inorganic chemical analysis for determining the concentration of certain metal ions such as sodium, potassium, lithium, calcium, Cesium, etc. In the flame photometry the species such as metal ions used in the spectrum are in the form of atoms. The International Union of Pure and Applied Chemistry (IUPAC) on Spectroscopic Nomenclature have been recommended it as flame atomic emission spectrometry (FAES). The basis of working flame photometric is the species of alkali metals (Group-I) and alkaline earth metals (Group-II) metals are dissociated due to the thermal energy provided by the flame source. Due to this thermal excitation, some of the atoms are excited to a higher energy level, where they are not stable. The absorbance of light due to the electrons

excitation can be measured by using the direct absorption techniques. The subsequent loss of energy will result in the movement of excited atoms to the low ground energy state with emission of some radiations, which can be visualized in the visible region of the spectrum. The absorbance of light due to the electrons excitation can be measured by the direct absorption techniques while the emitting radiation intensity is measured by the emission techniques. The wavelength of emitted light is specific for specific elements.

Parts of a flame photometer

1. Source of flame

A burner that provides flame and can be maintained in a constant form and at a constant temperature.

2. Nebuliser and mixing chamber

Helps to transport the homogeneous solution of the substance into the flame at a steady rate.

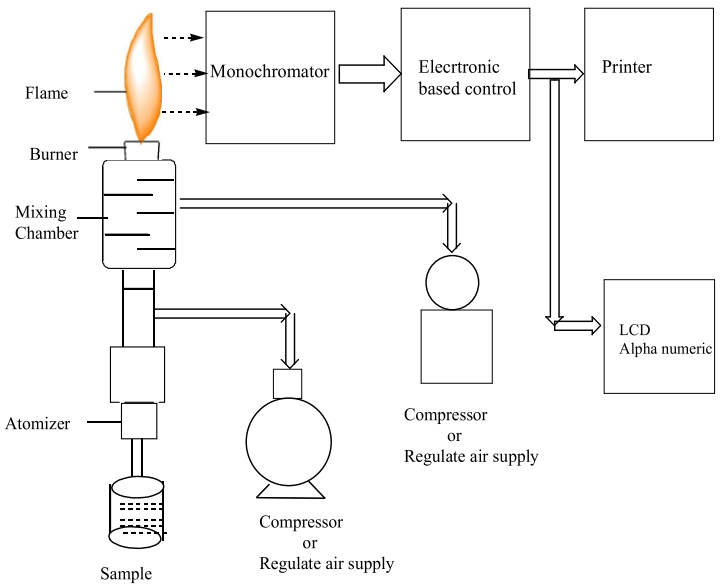
3. Optical filter

The optical system comprises three parts: convex mirror, lens and filter. The convex mirror helps to transmit light emitted from the atoms and focus the emissions to the lens. The convex lens help to focus the light on a point called slit. The reflections from the mirror pass through the slit and reach the filters. This will isolate the wavelength to be measured from that of any other extraneous emissions. Hence it acts as interfering type color filter.

4. Photo detector

Detect the emitted light and measure the intensity of radiation emitted by the flame. That is the emitted radiation is converted to an electrical signal with the help of photo-detector. The produced electrical signals are directly proportional to the intensity of light.

A schematic representation of flame photometer is shown in figure below:



A schematic representation of flame photometer

Working Mechanism:

The working of the flame photometer involves a series of steps which is discussed in the following sections.

1. **Nebulisation:**

The solution of the substance to be analyzed is first aspirated into the burner, which is then dispersed into the flame as fine spray particles.
A brief overview of the process:

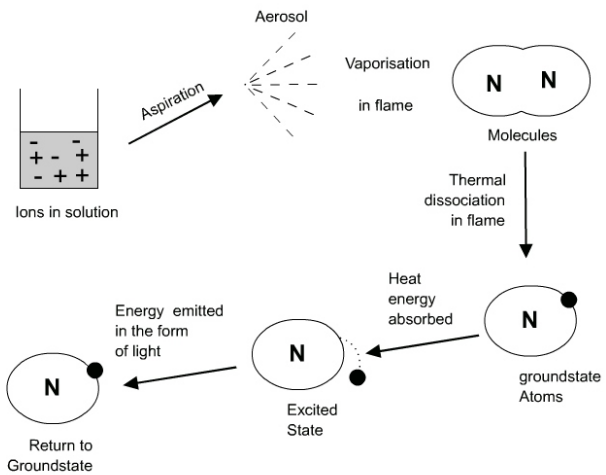
1. The solvent is first evaporated leaving fine divided solid particles.

2. This solid particles move towards the flame, where the gaseous atoms and ions are produced.

3. The ions absorb the energy from the flame and excited to high energy levels.

4. When the atoms return to the ground state radiation of the characteristic element is emitted.

5. The intensity of emitted light is related to the concentration of the element.



Brief overview of the process

Events :

Flame photometry employs a variety of fuels mainly air, oxygen or nitrous oxide (N_2O) as oxidant. The temperature of the flame depends on fuel-oxidant ratio.

The various processes in the flame are described below:

1. **Desolvation**:

The metal particles in the flame are dehydrated by the flame and hence the solvent is evaporated.

2. **Vapourisation**:

   The metal particles in the sample are dehydrated. This also led to the evaporation of the solvent.

3. **Atomization**:

   Reduction of metal ions in the solvent to metal atoms by the flame heat.

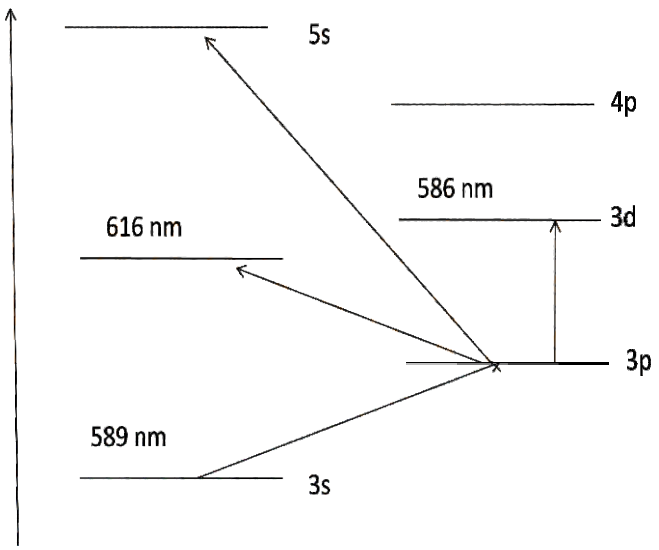
4. **Excitation**:

   The electrostatic force of attraction between the electrons and nucleus of the atom helps them to absorb a particular amount of energy. The atoms then jump to the exited energy state.

5. **Emission process**:

   Since the higher energy state is unstable the atoms jump back to the stable low energy state with the emission of energy in the form of radiation of characteristic wavelength, which is measured by the photo detector.

The energy level diagram of the sodium atom



Energy level diagram for sodium atom

The intensity of the light emitted could be described by the Scheibe-Lomakin equation:

$$I = kc^n$$

Where:

I= Intensity of emitted light

c = the concentration of the element

k = constant of proportionality

Then,

n ~1 (at the linear part of the calibration curve)

$$I = kc$$

That is the intensity of emitted light is directly related to the concentration of the sample.

The comparison of emission intensities of unknown samples to either that of standard solutions to those of an internal standard helps in the quantitative analysis of the analyte metal in the sample solution.

The flame emissions of the alkali and alkaline earth metals in terms of the emission wavelength and the characteristic color produced by each element is given in table below:

Name of the element	**Emitted wavelength range (nm)**	**Observed colour of the flame**
Potassium (K)	766	Violet 
Lithium (Li)	670	Red 
Calcium (Ca)	622	Orange 
Sodium (Na)	589	Yellow 
Barium (Ba)	554	Lime green 

Application of flame photometry

Flame photometer has both quantitative and qualitative applications. Flame photometer with monochromators emits radiations of characteristic wavelengths which help to detect the presence of a particular metal in the sample. This help to determine the availability of alkali or alkaline earth metals which are critical for soil cultivation. In field of agriculture, the fertilizers requirement of the soils is analyzed by flame test analysis of the soils. In human clinical field, Na^+ and K^+ ions in body fluids, muscles and heart can be determined by diluting the blood serum and aspiration into the flame. Analysis of soft drinks, fruit juices and alcoholic beverages can also be analyzed by using flame photometry.

6.5 Advantages of flame photometry

1. Simple quantitative analytical test based on the flame analysis.
2. It is Inexpensive and the determination of elements such as alkali and alkaline earth metals is performed easily with most reliable and convenient methods.
3. Quite quick, convenient, and selective and sensitive to even parts per million (ppm) to parts per billion (ppb) range.

5.6 Disadvantages of flame photometry

Moreover the flame photometer has a wide range of applications in the analytical chemistry, it possess many disadvantages which are explained below:

1. The concentration of the metal ion in the solution cannot be measured accurately.
2. A standard solution with known molarities is required for determining the concentration of the ions which will corresponds to the emission spectra.
3. It is difficult to obtain the accurate results of ions with higher concentration.
4. The information about the molecular structure of the compound present in the sample solution cannot be determined.
5. The elements such as carbon, hydrogen and halides cannot be detected due to its non radiating nature.

6.7 Summary

In this unit atomic emission spectrometry (AES) has been discussed as one of the oldest spectroscopic method of analysis. It is be used for the analysis of materials in gaseous state, liquid state and solid or powder state. Its high detection power and wide variety of excitation sources makes it the most extensively used method for analysis. One of the various different atomic emission spectrometric methods an inductively couple plasma (ICP) acts as an atomisation- excitation source, the technique being called ICP-AES. The number of elements that can be determined simultaneously is only limited by the availability of sufficiently sensitive interference free spectral lines.

6.8 Terminal Questions

Q.1: Give Enlist different components of ICP torch and give their function.

Answer:----------

Q.2: Explain AES based on plasma sources.

Answer:----------

Q.3: Give types of high-temperature plasmas.

Answer:----------

Q.4: Brief parts of a flame photometer.

Answer:----------

Q.5: Give color detection of a flame of following elements: K, Li, Ca, Na and Ba.

Answer:----------

Q.6: Give Scheibe-Lomakin equation.

Answer:----------

6.9.Suggested readings

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DCEBCH - 106

Spectroscopy

Block

3

Luminescence and Electron spectroscopy

DCEBCH -106

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Introduction

This is the third block of Spectroscopy. This consists of three units.

Unit-7: This unit covers the ICP-atomic emission spectroscopy: the principle and application of plasma spectroscopy discussed. The application of adsorption spectroscopy and its comparison of ICP-AES with atomic adsorption spectroscopy also discussed here.

Unit-8: This unit covers the basics of Luminescence spectroscopy the details of Luminescence and chemiluminescence briefly mentioned here. The principle of fluorescence and application of Fluorimetry also mentioned here.

Unit-9: Electron spectroscopy mentioned in these units. The principle of electron spectroscopy, electron spectroscopy for chemical analysis (ESCA) and chemical shift in ESCA also discussed in his unit.

Unit-7 : ICP-atomic emission spectroscopy

Contents

7.1. Introduction

Plasma based sources, Plasma is a high energy source which is an electrically neutral conducting gaseous mixture having a significant concentration of cations and electrons. As an electrical conductor it can be heated inductively by coupling with an oscillating magnetic field. The temperature of the plasma may be of the range of 5,000 - 8,000 Kelvin. The plasma based AES in principle, is similar to the flame photometry the only difference being that flame is replaced by much more energetic atomization-excitation processes using plasma. In emission work the argon plasma is frequently employed. The analyte sample is introduced into the centre of the plasma as an aerosol with the help of a nebuliser using argon flow. As in a flame, in plasma also the sample undergoes various transformations like desolvation, vaporisation, atomisation, ionisation and excitation. The excitation and ionisation occurs due to collisions of the analytical atoms with high energy electrons. The high temperature assures that most of samples are completely atomized. However, some molecular species e.g., N^2, N^{2+}, OH, etc. do exist in the plasma are measurable. As the energy of the plasma source is quite high it ensures the excitation of the atoms of all the elements present in the sample which then relax by emitting Electromagnetic (EM) radiation of characteristic wavelengths of different elements. Thus, it is also called a multielement technique. Depending on the composition of the analyte, the excited species consist of atoms singly charged ions and sometimes the doubly charged ions. The emission lines observed are mainly from the excited atoms and singly charged ions. The emissions from the doubly charged ions however are relatively rare. The energies of the transitions are such that the emitted radiation falls in the ultraviolet and visible region i.e. between 160-900 nm of

the spectrum. The emission lines from the atomic and ionic species are very narrow and the width being less than 5 pm.

Objective

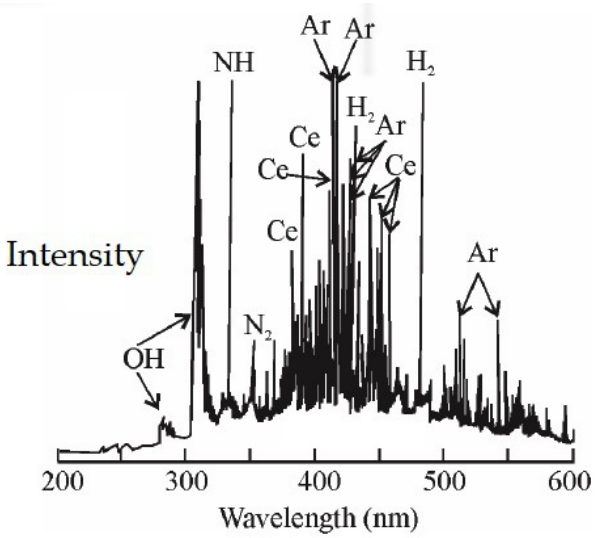
After studying this Unit learner is able to explain

- Principal of Emission spectroscopy
- Principle of plasma spectroscopy
- Application of adsorption spectroscopy
- Comparison of ICP-AES with ASS

7.2 Principle of plasma spectroscopy

ICP-AES spectrum

ICP-AES spectrum of Cerium having a concentration of 100 pm., you may note the presence of a number of lines from the analyte, the source gas -Ar and the molecular species and other background radiation. This would give you an idea of what to expect for a multi element analyte. Having learnt about the basic principles, let us learn about the instrumental aspects of atomic emission spectrometry based on plasma sources.



ICP-AES spectrum of Cerium at 100 pm

Some of the characteristics expected of an ideal atomization-excitation source. You may compare your list with the following characteristics and complete
An ideal atomization-excitation source should have the following

characteristics.

1. It should completely separate the analytes from its original matrix so as to minimize interferences.
2. It should have appropriate energy to ensure complete atomization but keep ionization to a minimum.
3. It should provide an inert environment, so as to keep the undesirable molecular species formation to a minimum.
4. It should have none / negligible background radiation.
5. It should provide for the analysis of samples in all possible forms like, solids, liquids, semi liquids/ slurries and gases.
6. Of course, it should be inexpensive, need minimal maintenance and be easy to operate.
7. As always, it is difficult to attain ideality, however, **plasma sources** are quite promising for the purpose of atomization-excitation job in atomic emission spectrometry. You have learnt above that a plasma source is an electrically neutral, highly ionized gas that consists of ions and electrons. It is sustained by absorbing energy from an electric or magnetic field.

*Three types of power sources have been employed in **argon plasma spectroscopy**.*

- One is a powerful **radio frequency generator** that powers an induction coil, the magnetic field generated from it help in establishing the plasma.
- The second is a ***dc*** **electrical source** capable of maintaining a current of several amperes between electrodes immersed in the argon plasma and third one is a **microwave frequency generator** through which the argon flows.

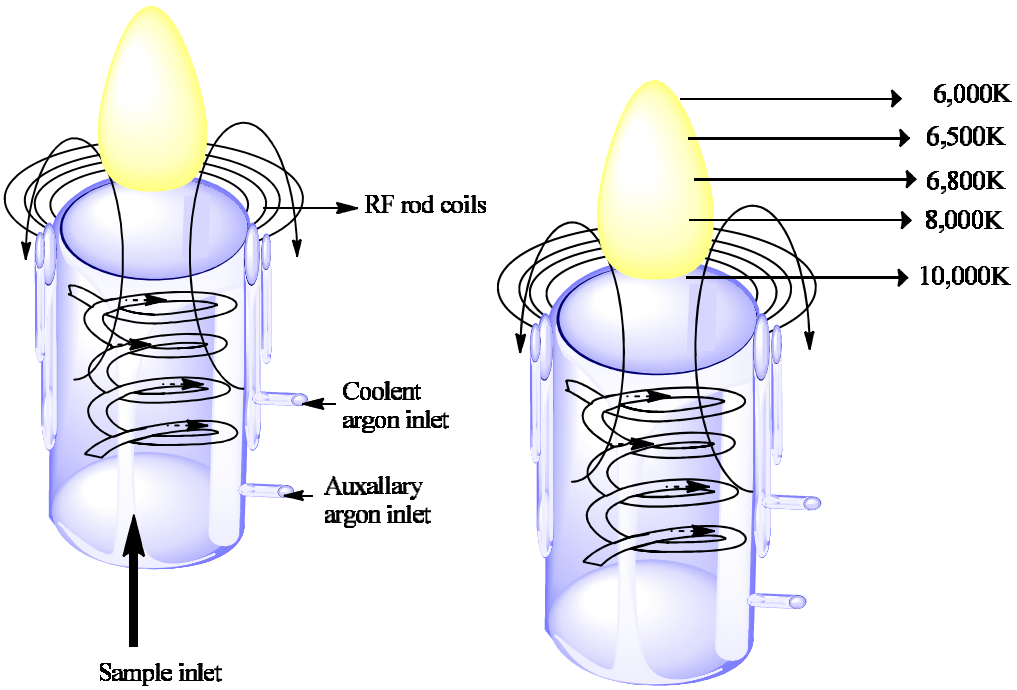
Accordingly, there are three types of plasma sources are as given below.

- Inductively coupled plasma (ICP)
- Direct current plasma (DCP)
- Microwave induced plasma (MIP)

7.3. Inductively Coupled Plasma (ICP)

The inductively coupled plasma (ICP) is plasma induced by radiofrequency. The energy transfer is mediated by an induction in that produces a magnetic field which is helps in establishing and sustaining the plasma. The energy of a high frequency generator is transferred to a gas, generally argon, flowing at atmospheric pressure. A typical inductively coupled plasma source is called a **torch**. The most common ICP torch in use today has evolved over decades of

development. It consists of a quartz tube whose upper part is surrounded by a radiofrequency work coil. The torch assembly is designed to deliver gases so that stable argon plasma is formed at the open end through which the sample aerosol can be injected. Let us learn about the different components of the ICP torch and their functions.



ICP torch and the temperature profile of a typical ICP torch

Quartz tube

The body of the torch consists of three concentric quartz tubes; the diameter of the largest tube being about 2.5 cm. The tube has three separate gas inlets. The argon gas enters the plasma through the outer channel with a tangential flow pattern at a rate of 8-20 L min^{-1}. The gas traveling up the central channel is called auxiliary gas and also has a tangential flow pattern. The innermost third inlet is connected to the nebulizer from which the gas enters the plasma along with the sample in a laminar flow.

Radio Frequency Power Generators:

The majority of radiofrequency (RF) power generators are crystal controlled radiofrequency generators. These are designed to operate within the industrial frequency bands of 27.12 or 40.68 MHz, although plasma generators have been described operating at between 5 to 100 MHz. The radio frequency at which the torch operates is an important parameter in sustaining plasma operation. At the lower frequency range it is necessary to deliver large power up to 30 kW to sustain the plasma. At a frequency of 27.12 MHz, the generators are normally designed with maximum power ratings of between 2 and 5 kW, and are often used routinely to deliver about 1 kW to the plasma. The radiofrequency power

supply is so controlled that it ensures a constant power transfer to the induction coil.

Work coil

The work coil consists of a water-cooled induction coil that is powered by a radio frequency generator. The copper induction coil is wrapped two or three times around the ICP torch and has water flowing through it for cooling purposes. The lines of force, generated by the magnetic field, are directed along the axis of the solenoid inside the tube and take the form of an ellipse on the outside.

Argon gas supply:

The conventional argon plasma torch uses between 13 and 20 liters of argon per minute, mainly to ensure that the outer quartz glass tubing adjacent to the plasma is adequately cooled. Such a high consumption of argon gas is met by installing a liquid argon supply Dewar flask rather than relying on conventional compressed gas cylinders.

Mechanism of plasma formation

The ionisation of the argon gas flowing through the crystal tube inside the solenoid is initiated by a spark from a Tesla coil. The resulting ions and the electrons are subjected to the fluctuating magnetic field produced by the induction coil. This makes them flow in the closed annular paths within the coils. The induced or eddy currents are thus produced. These electrons rapidly acquire enough energy from the oscillatory field generated by the induction coil and sustain a high degree of ionisation. This leads to the formation of ring shaped toroidal plasma. The spacing between coil and torch, the distance between individual copper coils and the concentricity of both coil and torch are important parameters in the formation of stable plasma.

Appearance of ICP plasma

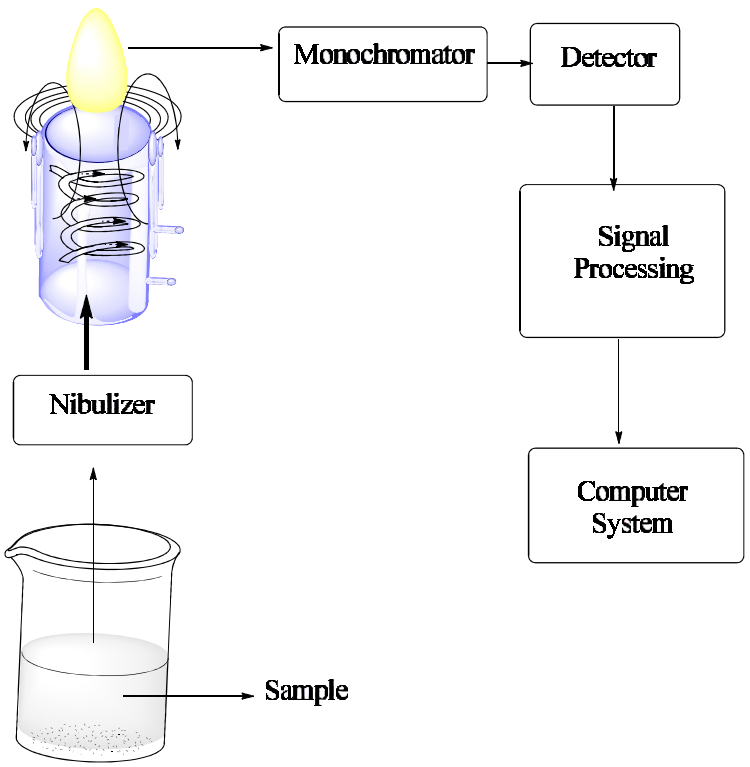
The typical plasma has a very intense, luminous white, nontransparent core topped by a flame like tail. The analytical zone has a temperature of about 5000- 8000 K and approximately 1 cm above the coils. It offers the best optical viewing area for maximum sensitivity the variation of temperature throughout the plasma. The plasma emits a continuum of background radiation that extends from the visible into the ultraviolet region. The radiation originates from electrons, Ar and Ar^+ ions, as well as various atomic and molecular species in the matrix and their recombination products. In the region 10 to 30 mm above the core, the continuum fades, and the plasma is optically transparent. The spectral observations are generally made at a height of 15 to 20 mm above the induction coil. Here the background radiation is remarkably free of argon lines and is well suited for analysis.

7.4. Instrumentation for ICP-AES

The essential components of a plasma based atomic emission spectrometer are as given below-

- **Plasma source:** an atomisation-excitation device
- **Nebuliser** : a sample introduction device
- **Monochromator**: a dispersion device
- **Detector**: a radiation detection device
- **Processing and readout device**

A schematic layout of an ICP-AES spectrometer is given in Fig. 10.6. The sample in the solution form is nebulised and injected into the plasma source with the help of a suitable nebulizer. The emitted radiation is passed through monochromator and after dispersion it is detected by a photo detector and sent to the processing unit for the processing and generating suitable output.



Sample Introduction

Inductively coupled plasma emission spectroscopy is used primarily for the qualitative and quantitative analysis of samples that are dissolved or suspended in aqueous or organic liquids. The sample is brought as a fine aerosol into the plasma, which is generally achieved by pneumatic nebulisation. These are carried into the torch by argon gas flowing at a rate of 0.3 to 1.5 dm^3 min^{-1} through the central quartz tube. The aerosol enters the hot plasma zones with a

low injection velocity so as to have a residence times of the order of ms that is required for an efficient atomisation and excitation. Thus, there are two steps in the sample introduction. These are as follows.

a) Sample preparation

b) Nebulisation

Let us learn about these.

a) Sample preparation

The solution preparation for the analysis using ICP-AES depends on the nature of the sample and the concentration of elements to be determined. There are two main types of sample preparation methods used for ICP analysis. These are explained below-

i) **Acid digestion method**

You have learnt about the acid digestion method of sample preparation. In the acid digestion method, the acids use their oxidising or reducing properties for the dissolution of the analyte. You would recall that acid digestion generally produces a clear solution of the analyte without loss of any of the elements to be determined. However, one must take care of the possible loss of volatile elements. For example in the acid digestion using HCl, there is a possibility of the loss of As, Se, Sn as their volatile chlorides. Similarly, in case of sulphuric acid, the formation of precipitates of Ca, Ba, Pb is also a potential source of error. Another aspect that needs attention of the analyst is that the quantity of acid or flux in the solution must be as low as possible so that there is minimum perturbation of the plasma. Some of the acids perturb the plasma more than the others. Accordingly, there is an order of preference of the acid to be used for sample preparation.
The decreasing order of their preference is as follows.

$$HNO_3 > HCl > HClO_4 > H_2SO_4 > H_3PO_4$$

ii) **Dry attack method**

In dry attack method, the sample preparation involves an alkaline fusion as well as high temperature calcination about 450 - 600 °C followed by acid recovery of ashes. However in this method the losses due to volatilisation and insolubilisation are not negligible. More so there is a possibility of contamination of the solution due to the reagents.

b) Nebulisation

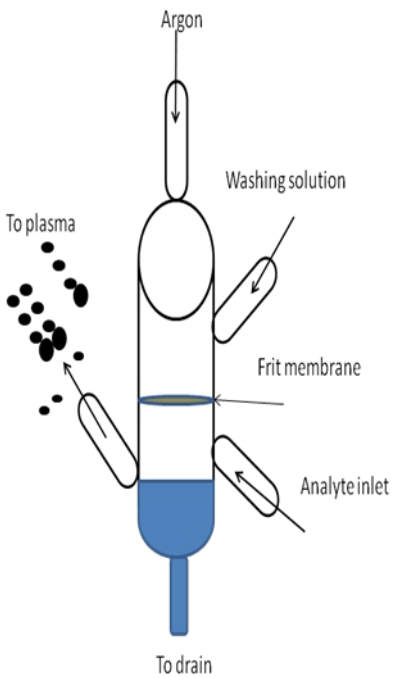
The analyte samples in all three states (solid, liquid, gas) have been successfully introduced into an ICP. For solutions, a nebuliser is used to convert the liquid stream into an aerosol consisting of particles are about 1–10

mm in diameter. Direct injection of liquids into the plasma would either extinguish the plasma or cause the atoms to be improperly desolvated. Making them excitation and emission less efficient. Let us learn about different types of nebulisers employed in ICP-AES.

Nebulisers for ICP-AES

Nebuliser is a device that converts a solution of the analyte into finely divided droplets that are carried into the atomiser. In a cross flow nebuliser, another type of pneumatic nebuliser, the aspirated sample is nebulised by a stream of argon and the resulting finely divided droplets are carried into the plasma.

In a **frit nebuliser**, the sample solution is pumped to a glass frit membrane consisting of a porous coral like synthetic material. The argon gas passes through the membrane and converts the sample into an aerosol spray then directed into the plasma. **Nebuliser**, the sample is pumped on to a piezoelectric crystal vibrating at ultrasonic frequencies about 50 kHz to 4 MHz. The vibrations of the crystal break the droplets into smaller particles which are transported to the plasma. Larger aerosol drops are drained out. A schematic diagram of the ultrasonic nebuliser used in ICP-AES is given in below-



Frit nebuliser a

Monochromators

Emission from plasma is usually polychromatic in nature which implies that it consists of a large number of radiations of different wavelengths. More so, the bandwidths of optical emission lines from the plasma are typically of the order

of 0.001 nm. These need to be suitably dispersed to analyse for the possible elements in the analyte. Resolution is important because of the complexity of ICP emission spectra, in terms of very large number of individual emission lines observed from samples. If not resolved, it would cause extensive spectral interference. There are three common devices used for the separation or dispersion of light. These are gratings, prisms and Michelson interferometers. In ICP-AES spectrometers, the resolution of the complex spectra is achieved by using ruled grating monochromators having a large number of grooves on the grating. Some instruments on the other hand employ holographic gratings.

Detectors

In molecular spectroscopic methods, there are three basic types of detector systems. These are photomultiplier tubes (PMTs), photodiode arrays (PDAs) and charge coupled devices (CCDs). Most of the instruments use photoelectric means of detection in the form of photomultiplier tubes (PMTs) or with photodiode arrays. In some cases photoplates are also used for detection purposes in AES. A number of modern instruments have now switched to solid state array detectors that use charge coupled devices (CCDs) or charge injection devices (CIDs). Processing and Readout Device Whether the detection of the analyte signal is done sequentially or simultaneously. A large amount of data needs to be handled in terms of the positions of the spectral lines and their intensities. Therefore the processing of the data needs good computers along with multichannel analog to digital converter to acquire and save the detected signals for further processing. The results of the determinations are printed out by a dedicated printer.

7.5. TYPES OF INSTRUMENTS FOR ICP-AES

The basic principle or the set up is similar for these. However, these differ on the type of dispersion method or the monochromator assembly used to disperse or resolve the large number of emission lines emerging out of the plasma and the detector device. The two types of spectrometers for ICP-AES are as given below.

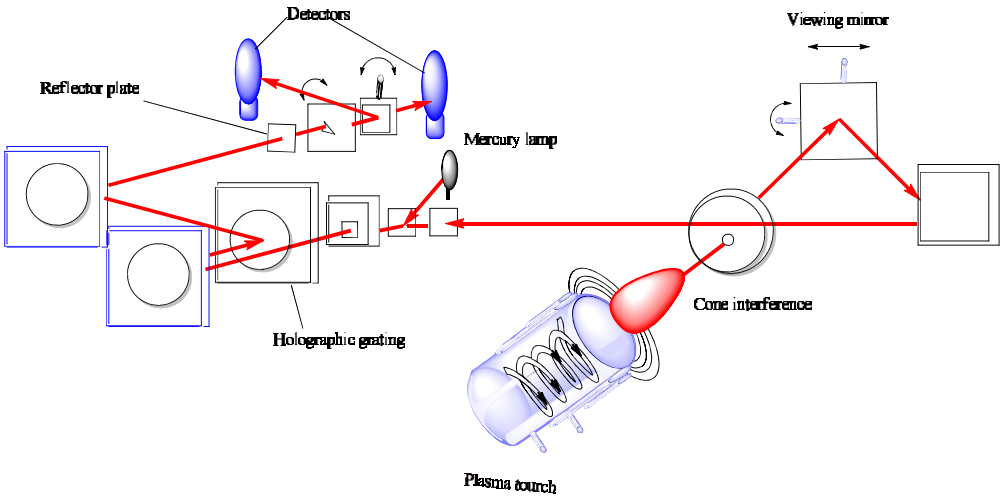
- Sequential spectrometers
- Simultaneous spectrometers

The sequential and simultaneous spectrometers are extensively used in the analytical laboratories. Of these, the sequential spectrometers are less expensive and more flexible but usually require a higher degree of operator skill and experience. On the other hand, the simultaneous spectrometers are more precise and accurate and are obviously more expensive. The simultaneous spectrometers employing solid state array detectors are of recent origin and are also capital intensive. However, these are quite sensitive, precise and accurate. Let us learn about these spectrometers in some details.

Sequential Spectrometers

These instruments use a moveable grating monochromator to select wavelengths in sequential order and use a single photomultiplier tube to detect them. The grating of the monochromator is of holographic type having a large number of grooves. The monochromator is programmed to make measurements in turn or sequentially at a preprogrammed series of peaks and background wavelengths to cover the elements of interest.

For this purpose, the grating of the monochromator is rotated in a controlled manner so as to sequentially focus a predetermined wavelength on the exit slit. Alternatively, the grating is kept fixed and the slit and the photomultiplier detector are moved to detect the desired wavelengths. As only a few components are used these instruments are economical. A schematic diagram of a sequential spectrometer is given below:



A schematic diagram of a sequential spectrometer

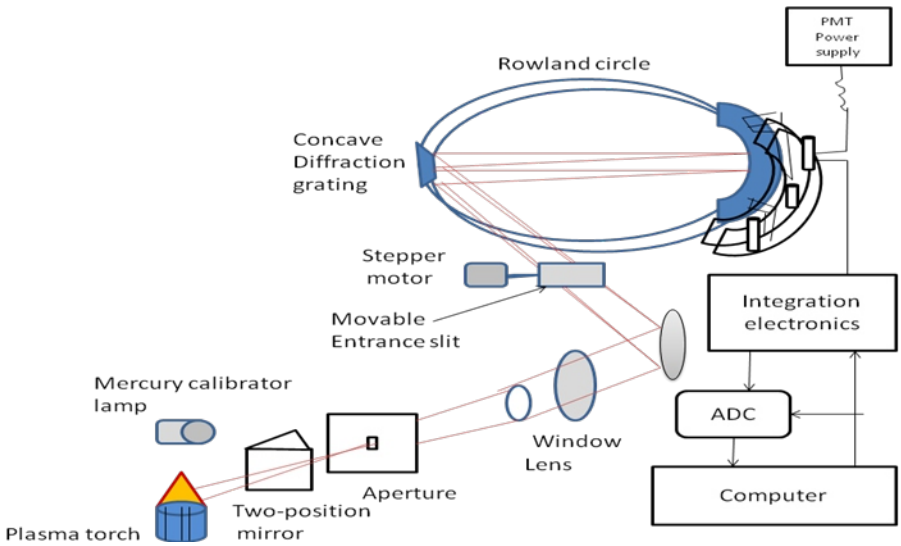
However, longer analysis times, poorer accuracy and precision are the major disadvantages of sequential instruments. In addition to these, the reproducibility of wavelength selection is another area of concern. To partly overcome the problem of longer analysis times slew scan instruments have been developed. In these instruments the monochromator or the detector and slit combination are moved in such a way that they quickly reach on to a wavelength of interest for a given analyte and then slowly scan around it in small steps. Needless to say, this can be achieved only with the help of an electronic control by a suitable computer.

Simultaneous Spectrometers

These instruments are designed to simultaneously measure the response at different wavelengths so as to overcome the drawbacks of the sequential

spectrometers. There are two types of spectrometers belonging to this category. These are given below-

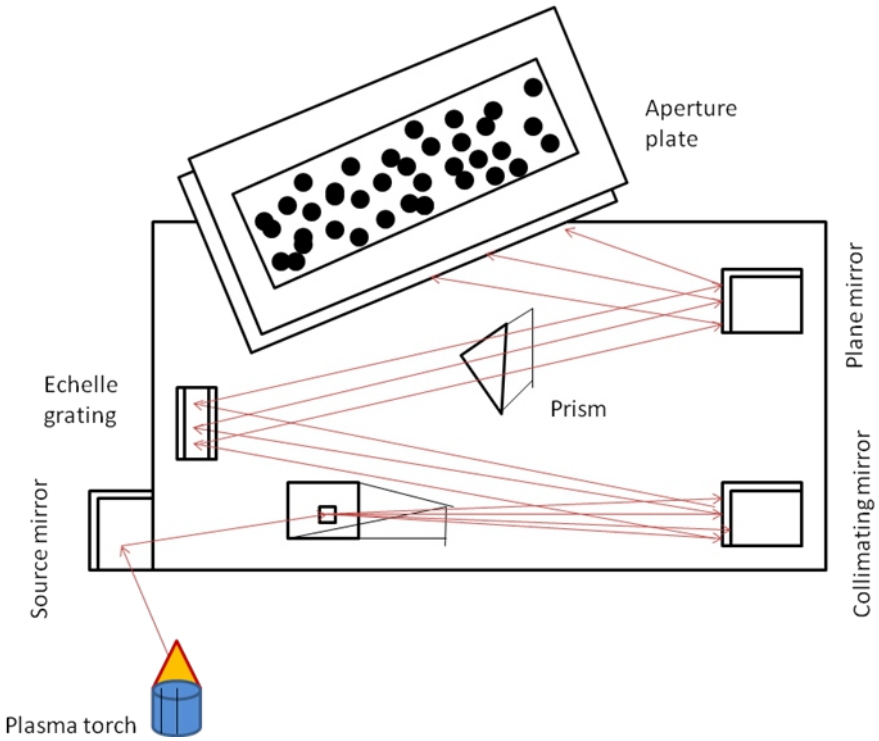
a) Polychromators b) Solid state array based spectrometers. The polychromators use multiple photodetectors whereas the solid state array base spectrometers depend on charge coupled or charge injection based devices as detectors. You have learnt in Unit 4 that the charge coupled devices are capable of simultaneous measurement. Let us learn about the two types of spectrometers.



Schematic diagram showing the layout and optical diagram of a ICP polychromator

(a) Polychromators

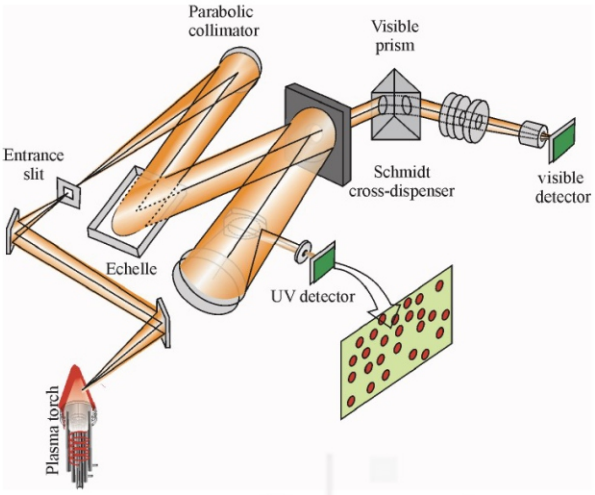
These simultaneous spectrometers are designed with a fixed diffraction grating monochromator and multiple 'exit slits' and associated photomultipliers. It means that these spectrometers have a number of photomultiplier tubes. Each of the photomultiplier is optimised to detect a specified wavelength corresponding to an element of interest. Typically, about 60 photomultiplier tubes or detectors are installed in a polychromator. Therefore, the simultaneous measurement of a prespecified range of elements becomes very rapid. A schematic diagram showing the setup of a polychromator is given in Figure. In Echelle spectrometers a special configuration of diffraction grating is used that displays the entire spectrum as a two-dimensional array, each line in the array representing a particular multiple order of diffraction. A schematic diagram of this spectrometer based on Echelle grating is shown in given figure.



The monochromators used here are very compact and offer particular advantages in simultaneous detection. These spectrometers are faster, more precise and accurate for a given combination of analytes and interferants. The major drawback of the simultaneous spectrometers or direct readers as these are generally called is related to the lack of flexibility. One has to make a detector available for each analyte of interest which makes it very expensive. Further, if a newer analyte sample is to be analysed one needs to procure new detectors for the same and augment the battery of the detectors present in the instrument. Further, if a new matrix is encountered, one or more of the analytical lines may have severe spectral interferences or background shifts. This may compromise with the precision and accuracy of the system.

(b) Solid State Array Detector Spectrometers

These spectrometers are of recent origin and as the name suggests these make use of solid state detectors. These have allowed the analysts to get over the problems of conventional sequential and simultaneous spectrometers. Recent commercial instruments have started using two dimensional detectors. These incorporate charge injection devices (CIDs) or charge coupled devices (CCDs). These devices may allow the simultaneous measurement of more than 250,000 lines and 5000 lines respectively. Schematic representation of the solid state array detector based spectrometers.



A schematic diagram showing the layout and optical diagram of spectrometer based on solid state detector

In the CCD based spectrometers the whole analytically usable part of the spectrum is measured on a series of CCDs placed in the Rowland circle of a spectrometer. The important aspect of these instruments is that these give the resolution and sensitivity virtually identical to sequential or direct reading polychromators using photomultiplier tubes. The CID based instruments on the other hand are Echelle spectrometers that incorporate the CIDs as the detectors. These instruments are expected to replace photomultiplier based direct reading ICP emission spectrophotometers in the next few years.

7.6 Application of ICP-AES

i. ANALYTICAL METHODOLOGY IN ICP-AES

The analytical determination of different elements on the basis of characteristic emission spectrum has developed a lot from the times of Bunsen and Kirchhoff. These developments have been made possible by the availability of better atomization excitation sources, dispersion and detection devices, etc. The atomic emission spectra for some elements such as Na and K are simple, consisting of only a few wavelengths, while in others, such as Fe and U, thousands of distinct reproducible wavelengths are present. Today with plasma based sources and state of the art monochromators coupled with solid state detection devices the potential of the technique in terms of the range, accuracy and the detection limits has become excellent. In principle, all metallic elements can be determined by plasma emission spectrometry and these can be measured simultaneously in a given analyte. However, for the determination of the nonmetals such as boron, phosphorus, nitrogen, sulphur, and carbon, a vacuum spectrometer is necessary. This is so because the emission lines for these elements lie at wavelengths below 180 nm where the

atmospheric gases absorb strongly. In practice, the plasma emission spectroscopy is generally limited to the determination of about 60 elements. Let us learn about the methodologies adopted in using the technique for qualitative and quantitative determinations.

Qualitative Analysis using ICP-AES

In principle it is possible to obtain qualitative information from ICP emission spectrometry by using sequential spectrometers. However, this technique has not been exploited for the purpose. This is so because the amount of sample and instrument time required are prohibitive. Similarly, as it is almost impossible to install individual photomultipliers to monitor multiple lines for each possible element of interest the direct reading spectrometers or polychromators are also not used for qualitative analysis. Yet the method has the potential and is used to some extent for qualitative purposes. The qualitative analysis is based on the principle that each element has a characteristic spectrum consisting of the most persistent lines and the characteristic lines. These are used to identify the presence of an element in the analyte. The most persistent or RU (raies ultimes) lines are the ones that persist even when the concentrations of the respective elements are progressively reduced to zero. The RU lines are not necessarily the most intense lines. For example, in manganese spectrum, a triplet at 279.8 nm is more intense at normal concentrations than the 403.3 nm triplet, but the latter are the three most persistent lines for manganese. Further each element has a set of characteristic line groupings such as doublets, triplets, groups of doublets, etc. However, for an element the characteristic line groupings do not necessarily consist of the most persistent lines. For example, the most persistent line for magnesium is the single line at 285.213 nm, while the characteristic magnesium emission occurs at 277.62782 nm. The atomic spectral lines are very narrow with respect to the whole spectral range, yet there is a possibility of encountering line coincidences. It is important to note that more than 200 000 lines in the spectral range of 200-400 nm have been tabulated and assigned to different elements of the periodic table. In addition there are a large number of lines in other regions also. Therefore, high-resolution spectrometers have to be used for the qualitative evaluation of the spectra. The unambiguous detection and identification of a single non interfered atomic spectral line of an element is sufficient to indicate its presence in the analyte sample. However, use of more than one line is necessary to conclude the presence or absence of an element. Qualitative emission spectral analysis is quite easy when the spectra are recorded on a photographic plate or with a scanning monochromator. A number of atlases are available for the photographically recorded spectra; spectra are reproduced and the most sensitive lines are indicated in these atlases. The spectrum of an analyte can then be compared with the collated spectra in the atlases to identify it. Also books with spectral

scans around the analysis lines with superimposed spectra from possible interferon's have been published, e.g. for the case of ICP-AES. These are also useful for the practising analytical chemist. In order to facilitate quantitative determinations, spectral line tables, in which the wavelengths of the spectral lines together with their excitation energy and a number indicating their relative intensity for a certain radiation source are tabulated. These are available for different sources, such as arc and spark sources, but also in a much less complete form for newer radiation sources such as inductively coupled plasmas. 10.6.2 Quantitative Analysis using ICP-AES The strength of ICP-AES lies in quantitative analysis. The intensity of an elemental atomic or ion line is used as the analytical signal in quantitative atomic emission spectrometry. In fact the spectral intensities are unequivocally related to the elemental concentration in the analyte. However, in practice, AES is a relative method and a calibration has to be performed. Once the analyte matrix is understood and the background and spectral overlap correction details have been worked out the calibration of the instrument becomes rapid and straightforward. The analyst however does not need to bother about making the calibration standards as high purity multi element plasma calibration solutions are commercially available. The calibration curves most often consist of a plot of the output current or voltage of a transducer as a function of analyte concentration. For large concentration ranges log-log plots are employed. Fig. 10.14 shows typical calibration curves for the trace elements analysis by AAS. In one of the quantitative determination strategies the sample is spiked with several elements that are not present in the sample. That is we add a kind of internal standard to the sample. The emission spectrum of the spiked sample is then monitored in terms of the relative emission intensities of several lines of the spiking elements to the magnitude of other elements present in the analyte. In this case the vertical axis of the calibration curve is the ratio or the log ratio of the detector signal for the analyte to the detector signal for the spiked element or the internal standard. This can then be used to obtain a reasonably precise and accurate determination of the concentrations of the elements present in the sample. High sensitivity and large linear range are the strengths of ICP emission spectrometry. The sensitivity in the UV region of the spectrum can be attributed to the high temperature of the plasma which makes it an efficient excitation source and to the fact that in the ultraviolet region, the plasma background is very low. As most of the elements have one or more useful emission lines in the ultraviolet region, these can be easily determined as the signal-to-background ratios are very good.

Interferences in ICP-AES

You have learnt earlier in the context of other atomic spectrometric methods that any chemical, physical or spectral process that adversely affects the measurement of the radiation of interest can be classified as interference. In

case of ICP-AES the interferences may occur in any stage of the operation; starting from the sample preparation stage to the plasma operating conditions. Let us learn about different types of interferences in ICP-AES.

Spectral Interferences Spectral interferences in ICP-AES are caused by the following.

- ✓ overlap of a spectral line from another element
- ✓ unresolved overlap of molecular band spectra
- ✓ recombination phenomena
- ✓ background emission from the source
- ✓ stray light from the line emission of high concentration elements,

A common interference involves the overlap of the spectral lines of two or more elements in the matrix emitting radiation at the same wavelength. For example, Cu emission at 515.323 nm is likely to show an overlapping with the emission from Ar at 515.139 nm. These spectral interferences can be minimized either by using a high resolution dispersion system or by using more than one analytical line for the detection of a single element. Spectral scans of samples or single element solutions in the analyte regions may indicate when alternate wavelengths are desirable because of severe spectral interference. Another common spectral interference involves the formation of undesired species (e.g.- ions, metal oxides). Some metals are extremely sensitive to small plasma fluctuations. This results in the changes in the relative amounts of neutral atom and the corresponding ions. You may note that the emission spectrum of an atom of a specific element (Fe) is quite different from that of its ions (e.g., Fe^{+} , Fe^{+2}, etc.). Similarly, the formation of metal oxides or metal carbides, are also potential source of interference and need to be evaluated on an individual basis. Background emission and stray light can usually be compensated for by subtracting the background emission determined by measurements adjacent to the analyte wavelength peak.

Physical Interferences

These interferences are associated with the processes of sample nebulisation and transport. The differences in the physical properties of the sample and calibration solutions lead to variations in the aerosol droplet size. This may influence the efficiency of the nebuliser and the sample introduction. The changes in viscosity and surface tension of the solutions can also cause significant inaccuracies. These become especially important in samples containing high amounts of dissolved solids or high acid concentrations. The physical interferences can be reduced by diluting the sample. The use of an internal standard also proves to be useful.

Chemical Interferences

The chemical interferences include molecular compound formation, ionisation effects, and solute vaporisation effects. Some of these have already been discussed under spectral and physical interferences. These effects are generally not very significant in ICP-AES. If present, these can be minimized by carefully controlling the operating conditions such as incident power, observation position, matrix matching, etc. Memory effects resulting from the presence of the analytes in a previous sample and contributing to the signals measured in a new sample are also an area of concern. These can be minimized by purging the system with a blank in between samples. The purging or the rinsing times necessary for a particular element must be suitably estimated before undertaking the analysis.

Applications of ICP-AES

The ICP-AES technique is versatile tool in the hands of analytical chemists. As many as 60 elements can be determined by it in a wide range of analyte samples such as rocks, minerals, soil, air, water, agriculture, forestry ecology, food analysis, etc. Therefore it has become an indispensable technique. Some of the important analytical applications in different areas are given below, you would learn about the applications in detail in the next unit. Agricultural science Analysis of agricultural products and foods besides soil analysis. Health sciences Determination of Al in blood, Cu in brain tissue, Se in liver, Na in breast milk. Direct determination of Ca, Fe, Cu, Mg, Na and K in serum samples. Geological sciences Presence of lanthanides and other elements in rock samples. Forensic Sciences Crime scene soil analysis. Metallurgy Analysis of trace elements in stainless steel. Environmental science Waste water analysis, determination of pollutant metals in variety of matrices. Industry Presence of metals like Cu, Fe, Ni, and Si in lubricating oils or gasoline at tracer concentration. Traces of metals like Ca, Cu, Fe, Mn, Mg, P, K and Zn in beer or wine; determination of trace elements in polymers, evaluation of catalysts, and so on. The wide applicability of ICP-AES in the quantitative analysis of various materials can be attributed to the following advantages offered by the technique. Rapid and simultaneous multi-element analysis. Lack of chemical interferences. High temperature of the excitation source: 5000 to 10000K. Low sample requirements. Absence of self absorption, the cause of non-linear calibration plots in FES. Validity of calibration curves over 4 to 6 orders of magnitude. Low detection limits: 1 to 100 ng/g or µg/l (part per billion). Good accuracy and precision: relative standard deviation of about 1 per cent. Applicability to elements that are difficult to be determined by AAS: B, C, Ce, La, Nb, Pr, S, P, Ti, Ta, V and Zr can also be measured. Let us summarise what have we learnt about atomic emission spectrometry based on ICP.

7.7 Summary

In atomic emission spectrometry (AES), a reproducible and representative amount of the sample is introduced into an atomization-excitation source where it is converted into atomic vapours of the analyte in excited state. In the atomisation-excitation source 53 Atomic Emission Spectrometry the analyte undergoes a number of processes to be atomised and then get excited. As the excited state is short lived, the excited atoms return back to the ground state accompanied by the emission of electromagnetic radiation that is characteristic of the constituents of the sample. The AES is a versatile method due to the availability of a wide range of atomisation-excitation sources. Plasma is a high energy source which is an electrically neutral conducting gaseous mixture having a significant concentration of cations and electrons. As an electrical conductor it can be heated inductively by coupling with an oscillating magnetic field. The temperature of the plasma may be of the order of 5,000 to 8,000 K. In the ICP-AES the analyte sample is introduced into the centre of the plasma as an aerosol with the help of a nebuliser using argon flow. As the energy of the plasma source is quite high it ensures the excitation of the atoms of all the elements present in the sample which then relax by emitting EM radiation of characteristic wavelengths of different elements. Thus, it is a multi element technique. Three types of power sources have been employed in argon plasma spectroscopy accordingly; there are three different types of plasmas. These are the inductively coupled plasma (ICP), the direct current plasma (DCP) and the microwave induced plasma (MIP). These plasmas use radiofrequency, direct current and microwave radiation respectively as the power sources. Argon gas is used as plasma gas in most of the plasma instruments. This is due to its inertness, whereby it does not form stable compounds with analyte elements, its simple emission spectrum that makes it optically transparent in the UV-Visible region of the spectrum, moderately low thermal conductivity, and good natural abundance. The essential components of a plasma based atomic emission spectrometer are plasma source, a nebuliser, a monochromator, a detector and a processing and readout device. In a typical AES determination the sample is prepared by using acid dissolution method or dry attack method and is inserted into the plasma source with the help of a suitable nebuliser. The spectral emission is suitably resolved, detected, processed and utilised for qualitative and quantitative determinations. The two types of spectrometers for ICP-AES are sequential spectrometers and simultaneous spectrometers. These have the capabilities of measuring the different elements present in the analyte in a sequential manner or simultaneously. Of these, the sequential spectrometers are less expensive and more flexible but usually require a higher degree of operator skill and experience. On the other hand, the simultaneous spectrometers are more precise and accurate and are obviously more expensive. The qualitative

analytical determination of different elements on the basis of characteristic emission spectrum has developed a lot. The qualitative analysis is based on the principle that each element has a characteristic spectrum consisting of the most persistent lines and the characteristic lines. On the other hand the intensity of an elemental atomic or ion line is used as the analytical signal in quantitative atomic emission spectrometry. In fact the spectral intensities are unequivocally related to the elemental concentration in the analytes. However, in practice, AES is a relative method and a calibration has to be performed. The ICP- AES spectrum is influenced by spectral, physical and chemical interferences and suitable procedures are to be followed to undertake analytical determinations using ICP-AES.

7.8 TERMINAL QUESTIONS

Q.1: Write the principle of atomic emission spectrometry based on induced coupled plasma.

Answer:----------

Q.2: Enlist different types of plasma sources used for atomic emission spectrometry and state the sources of their energies.

Answer:----------

Q.3: Explain working of a Frit nebuliser.

Answer:----------

Q.4: What property of helium makes it a potential gas to be used in a plasma source?

Answer:----------

Q.5: Why nitrogen gas is generally not used as a plasma gas in plasma based atomic emission spectrometry?

Answer:----------

Q.6: Enlist the advantages of ICP-AES method that makes it widely applicable for quantitative analysis.

Answer:----------

7.8. Further suggested readings

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Unit-8: Luminescence spectroscopy

Contents

8.1. Introduction

Florescence spectroscopy is spectroscopy techniques used widely as a tool for quantitative analysis, characterization, and quality control in biochemistry, clinical, environmental, agricultural, nanotechnology and analytical chemistry. However, there are several chemical species have special feature in which get photoluminescent by using electromagnetic radiation of same or longer wavelength. This process of photoluminescence can be classified as fluorescence or phosphorescence. The fluorescence or phosphorescence depends on the lifetime of the exited state of electron. The fluorescence from a material can be measured by fluorescence spectroscopy that detects and records the amount of fluorescence light with high sensitivity. The devices that measure the fluorescence are called fluorometers. The luminescence is another process in which emission of light by a substance is occurs spontaneous without obtaining or generating heat. This can be caused by chemical or biochemical reactions, activity of subatomic particles, radiation or stress on a crystal. For example chemiluminescence, crystalloluminescence, electroluminescence, phosphorescence, photoluminescence and fluorescence etc. The chemiluminescence is defined as the generation of light due to any chemical reaction and bioluminescence is a natural phenomenon of light emission conferred by living organisms. Chemiluminescence is currently been used as sensitive method for quantitative analysis of molecules. Chemiluminescence creates light through a chemical reaction. A wide range of chemiluminescence agents such as H_2O_2, luminol, oxalate derivatives, dioxetanes, fluorescein, and acridinium dyes are used in numerous biological and industrial applications.

Objectives

- To discuss the nature of luminescence spectroscopy
- To know about fluorescence spectroscopy and its applications
- To discuss the about chemiluminescence and their nature

8.2. About Luminescence

Luminescence is generally known as the emission of light from any specific objects. Luminescence (from Latin *luminare* = to light up or illuminate) is a collective term for different phenomena where a substance emits light without being strongly heated, i.e. the emission is not simply thermal radiation. This definition is also reflected by the term "cold light". It is thus a form of cold-body radiation. It can be caused by chemical reactions, electrical energy, subatomic motions or stress on a crystal. It is carried out by Luminometry that is used to measure luminescence, which is the emission of electromagnetic radiation in the energy range of visible light as a result of are action However, the Luminometry is not strictly speaking a spectrophotometric technique, but is included here due to its importance in the life sciences. The process of can be seen in neon and fluorescent lamps. The television, radar, and X-ray fluoroscope screens is also the example of luminescence.

Luminescence is described by light absorption and emission shown in Fig.8.1 a and b

Absorption: The process in which electron absorbs the incident photon and moves to an excited state is called absorption. The energy of incident photon must be equal to the energy difference between ground state and excited state.

Emission: The process in which the excited electron loses its energy by emission of photon and comes back to its ground state is called emission.

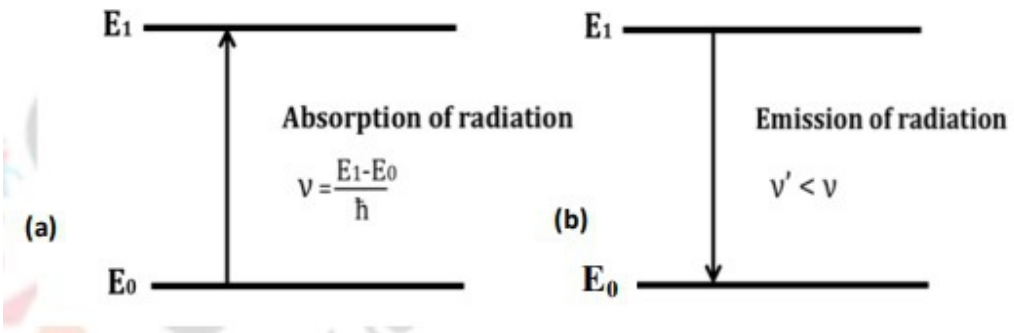


Fig.8.1. Transition of electron during (a) absorption of radiation and (b) emission of radiation

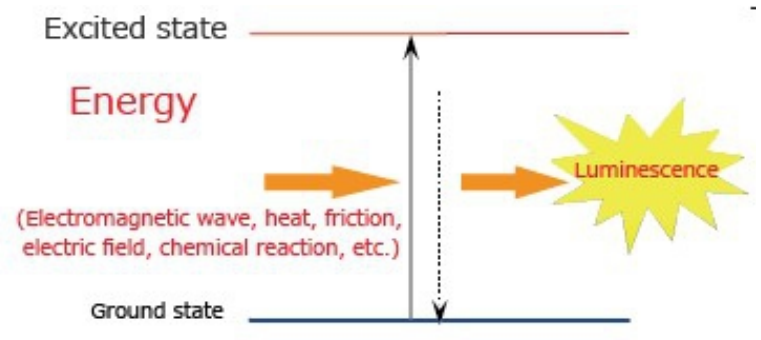


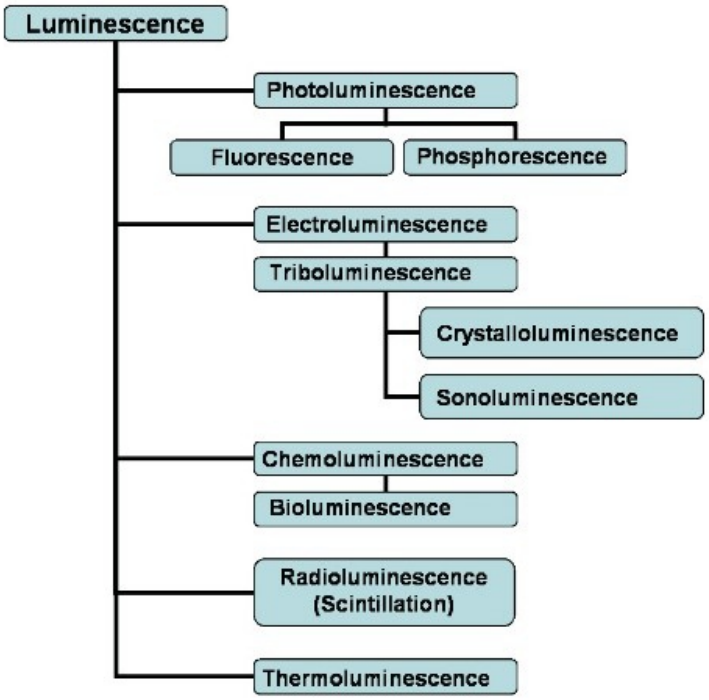
Fig. 8.2: Luminescence

The practical value of luminescent materials lies in their capacity to transform invisible forms of energy into visible light (Fig.8.2.). Luminescence emission occurs after an appropriate material has absorbed energy from a source such as ultraviolet or X-ray radiation, electron beams, chemical reactions, and so on. Luminescence efficiency depends on the degree of transformation of excitation energy into light, and there are relatively few materials that have sufficient luminescence efficiency to be of practical value. Luminescence applications are so numerous and diverse that contemporary reviews and books are unable to accommodate them all.

Fig. 8.3: Image of luminescence of substance

8.2.1. Different Kinds of Luminescence

There are several types of luminescence are found in nature such in which some are as follows;



Bioluminescence is a type of chemoluminescence that is used by living creatures. It is used to see what is going on inside of organisms through the emission of visible light. The technique enables researchers to study biological processes in real-time, *in vivo*. It has been developed over the past few decades and used primarily in small animals for the purposes of molecular imaging. Bioluminescence works by detecting visible light that is produced when an enzyme oxidizes a molecular substrate. Bioluminescence mainly comprised of two main components; a light emitting substance known as luciferin and an enzyme called as luciferase. Luciferin reacts with ATP, the energy source of the cell to create a new compound. This new compound then reacts with luciferase which adds on an oxygen atom creating a new compound that decays quickly by emitting a photon of light.

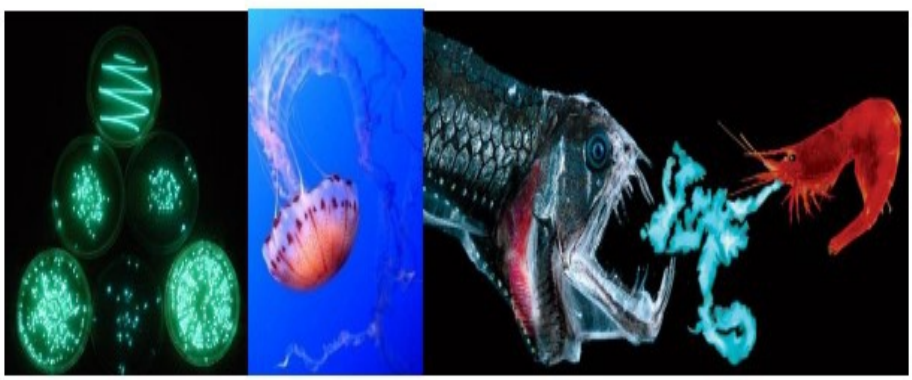


Fig. 8.4: Bioluminescence as observed in marine bacteria

Photoluminescence requires that a molecule absorb light radiation and for its electrons to become excited. When this occurs, molecules transform from the ground state to an excited state. Because the excited state is unstable, the molecule inevitably reverts back to its ground state, dissipating energy.

Electroluminescence occurs when an electromagnetic field is applied to solid or gaseous material. This excites the molecules and the substance glows. LEDs, or light-emitting diodes, are found in flat panel televisions and computer monitor.

Fig.8.5: Image of Electroluminescence of objects

Triboluminescence can occur when a material is mechanical treated, e.g. fractured or polished. It can also be observed when peeling adhesive tapes.

Chemiluminescence is light emitted during (cold) chemical reactions. Here, reaction products are generated in excited states, which decay to the ground state through luminescence.

Crystalloluminescence is a type of Luminescence generated during crystallization, used to determine the critical size of the crystal nucleus. There is a theory that the light from crystalloluminescence emerges through the micro-fracture of growing crystallites.

Luminescence is an important tool in life sciences research, particularly for visualizing molecules and biological processes. The best luminescence approach depends on the specific goals of the research. Navigating some of these tools' applications can help elucidate their relative advantages and disadvantages.

8.3. About Chemiluminescence

The chemiluminescence is the process in which light emit during chemical reaction. Chemiluminescence (CL) is the luminescence produced by chemical reactions that induce the transition of an electron from its ground state to an excited electronic state. When the excited molecule decays to the electronic

ground state, CL emission at different wavelengths occurs, from ultraviolet-visible to infrared radiation.

In chemiluminescence, the chemical energy converts into chemical reaction. In brief, given reactants **A** and **B** are transformed into an excited intermediate **I**. The decay of the excited intermediate **I** to a lower energy level is responsible for the emission of light.

[A] + [B] --> [I] --> [Products] + Light

However, chemiluminescence, not requiring an external source of light and therefore avoids the challenges associated with photo bleaching, light scattering, and auto-luminescence. As with bioluminescence, this feature of chemiluminescence is conducive to higher signal-to-noise ratios than other luminescence methods, making chemiluminescence a well-established technique with many applications. The chemical substance luminol emits blue light upon contact with the iron in haemoglobin if blood is present is example of chemoluminescence. Compared to absorbance and fluorescence assays, CL assays have lower background signal, leading to higher sensitivity.

In chemiluminescence these atoms/molecules take energy from some chemical reactions. Chemiluminescence reaction yields one of the reaction products in an electronic excited state producing light on falling to the ground state. The underlying process of light emission in chemiluminescence is similar to photoluminescence, except for the excitation process. In chemiluminescence reactions, two reagents, a substrate and an oxidant along with cofactors react to generate a product sometimes in the form of catalyst. The catalyst in the reaction is used for reducing activation energy and providing an adequate environment for producing high chemiluminescence efficiency. Indirect chemiluminescence is based on the transferring of energy to a fluorophore. All of these approaches lead to numerous practical uses of chemiluminescence. Fig. 2 shows the basic principle of chemiluminescence detection in HPLC.

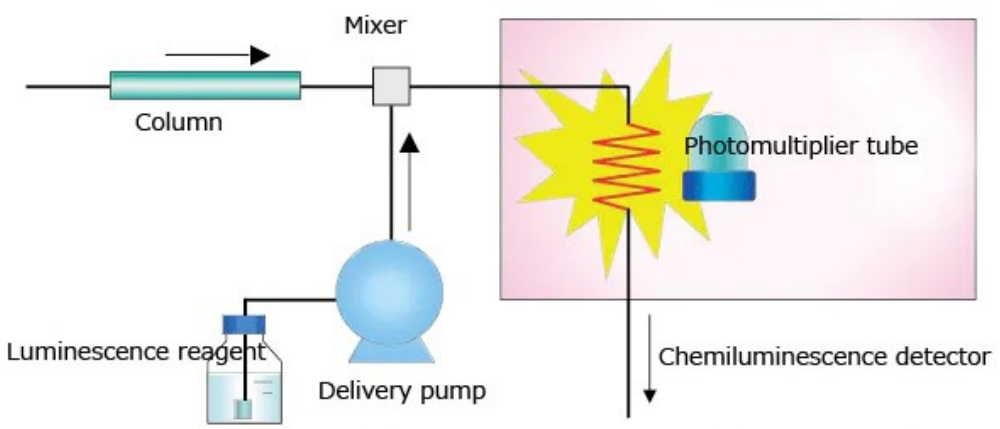


Fig.8.6: Basic principle of cheminescence

Chemiluminescence does not require a light source lamp (such as a xenon lamp) since material is excited by the energy of a chemical reaction caused by a luminescence reagent. So that there are two types of chemiluminescence reagents:

Direct luminescence reagents and indirect luminescence reagents.

Direct luminescence reagents where the excited material itself emits light. Example of direct luminescence is luminol, which has been used for many years to identify blood. Luminol emits a blue light (425 nm) in response to strong alkalinity, oxidizing agents such as hydrogen peroxide, and heat

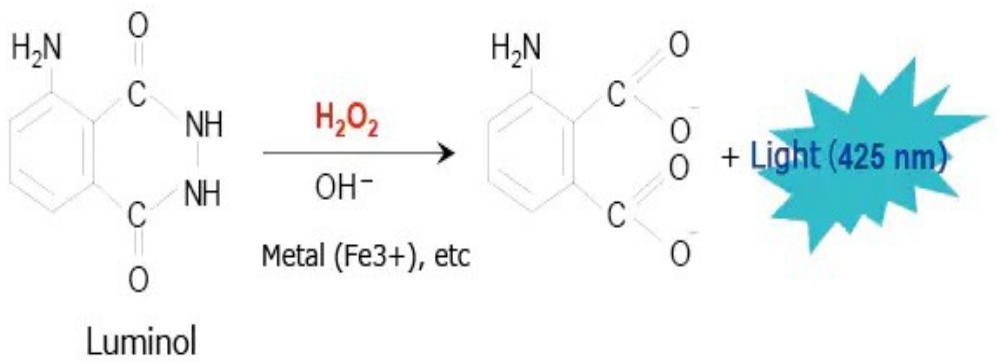


Fig.8.7: Chemiluminescence reaction caused by Luminol

The indirect luminescence is that where energy from the chemical reaction excites another material. Example of indirect luminescence is an oxalic acid diester and hydrogen peroxide. Fig. 4 shows the chemiluminescence reaction caused by oxalic acid bis(2,4,6-trichlorophenyl) (TCPO). In this reaction, the active intermediate (1,2-dioxetanedione) produced by the reaction between TCPO and hydrogen peroxide delivers excitation energy to the luminescent material when it decomposes into carbon dioxide.

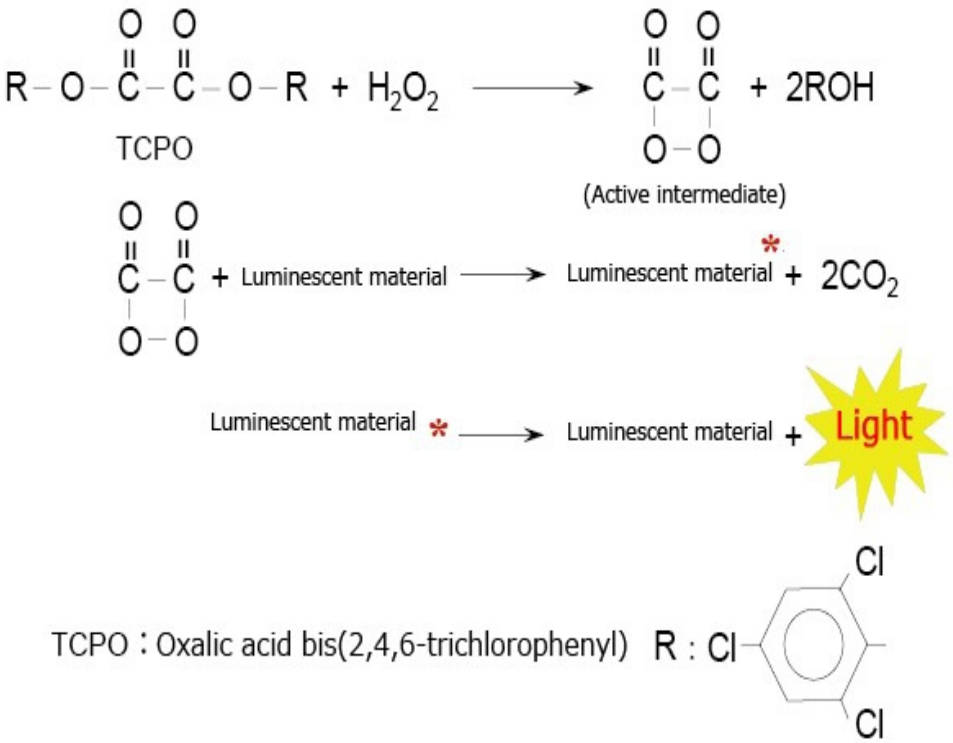


Fig.8.8: Chemiluminescence Reaction between an Oxalic Acid Diester (TCPO) and Hydrogen Peroxide

8.4. Fluorescence

Fluorescence spectroscopy is a type of electromagnetic spectroscopy which measures fluorescence emitted from a molecule. It involves a beam of light (ultraviolet light) of particular wavelength. Due to absorption of light of higher energy, the electron of material goes to higher energy level and return downs its lower energy by emission of absorbed radiation. But during this process the electron just colloid to another electron of another atom and during this process exited electron loss own energy in the form of heat and thus λ_{max} of reflected light get shifted toward higher wavelength. In this process radiation less decay electron comes down to lower energy levels and finally they jump to ground sate energy level by emission of remaining radiation. Thus process called fluorescence. The average lifetime of the electron in the excited state is only 10^{-5} -10^{-8} sec. However, the materials absorb light at a particular wavelength and emit light at longer wavelengths than the incident light. This process can also be explained as absorbance of light of electromagnetic radiation at a frequency ν and emits light at lesser frequency ν' (ν > ν').

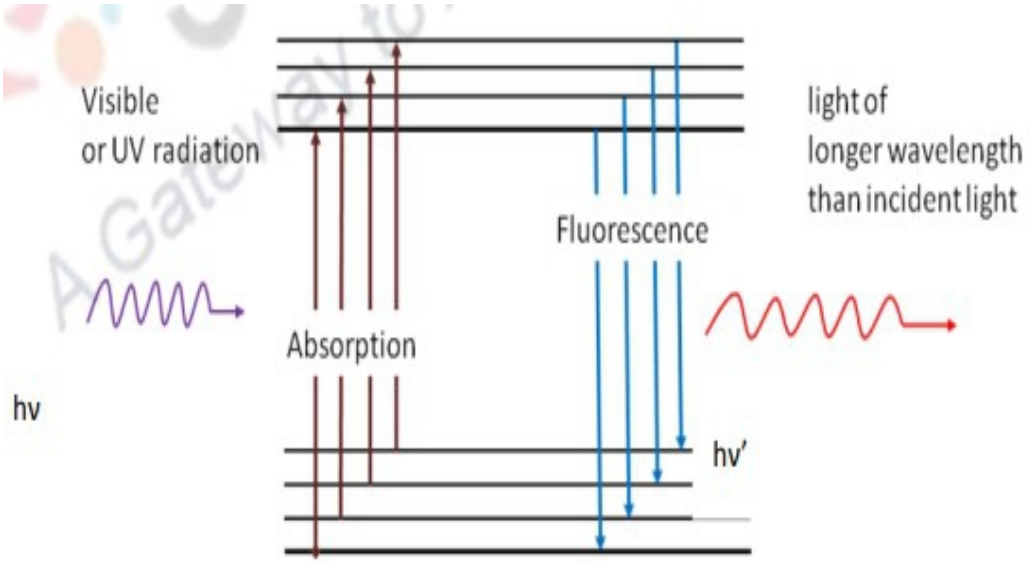


Fig.8.9: Process of absorption and emission of light in fluorescence

The fluorescence take place due to molecular relaxation of an electronic species form excited states to the ground state. Here transition occurs between vibrational and electronic states resulted fluorescence produced. This of state transitions are explained in the Jablonski diagram as illustrated in the Fig.1.

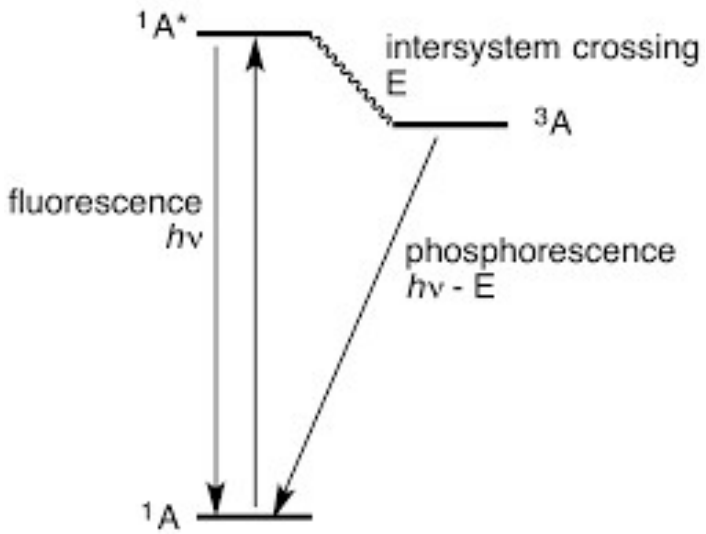


Fig.8.10: Jablonski diagram of a fluorophore excitation.

A Jablonski diagram is basically an energy diagram. It illustrates the excited states of a molecule and the radiative and non-radiative transitions that can occur between them.

The photons with high energy (UV region of the spectrum) are required to induce this type of electronic transition. Because the energy gap among the excited state and ground states is considerably higher as compared to thermal energy gap.

The designs of spectrofluorometer instrument can be varied according to applications. It commonly used light source is xenon lamp. Photomultipliers or charge coupled device cameras are used as high sensitivity photo detectors. A simple diagram of spectrofluorometer is shown in the Figure 2.

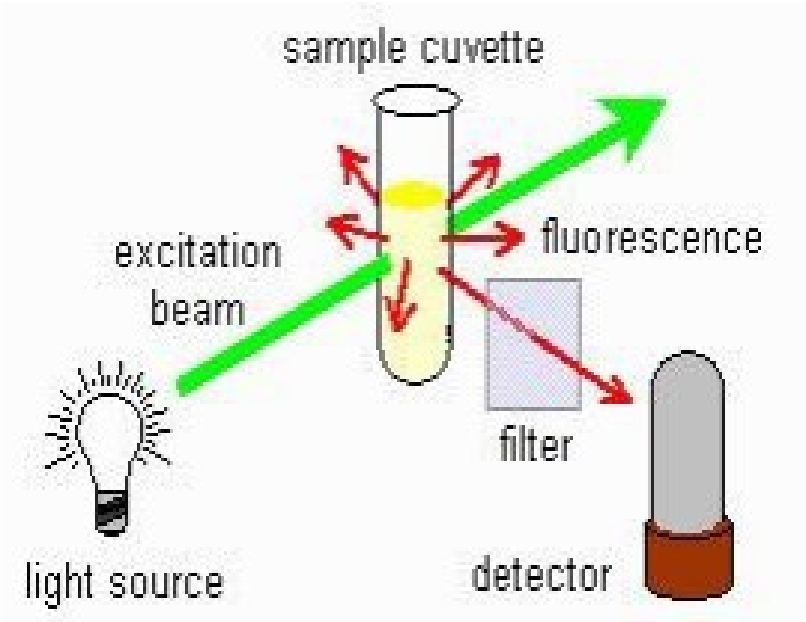


Fig.8.12: Diagram of spectrofluorometer.

The excitation (absorption spectrum) and emission spectrum of any fluorescent compound can be measured by the spectrofluorometer. The emission or fluorescence spectrum can see which wavelengths are emitted after absorbing the incoming light. The intensity of emission is depending on the amplitude of the excitation wavelength. Thus, the excitation spectrum is described as the fluorescent intensity recorded as a function of wavelength used for excitation by keeping emission wavelength constant The excitation (200-400 nm) and emission spectra (375-550 nm) in is shown in Fig (8.13).

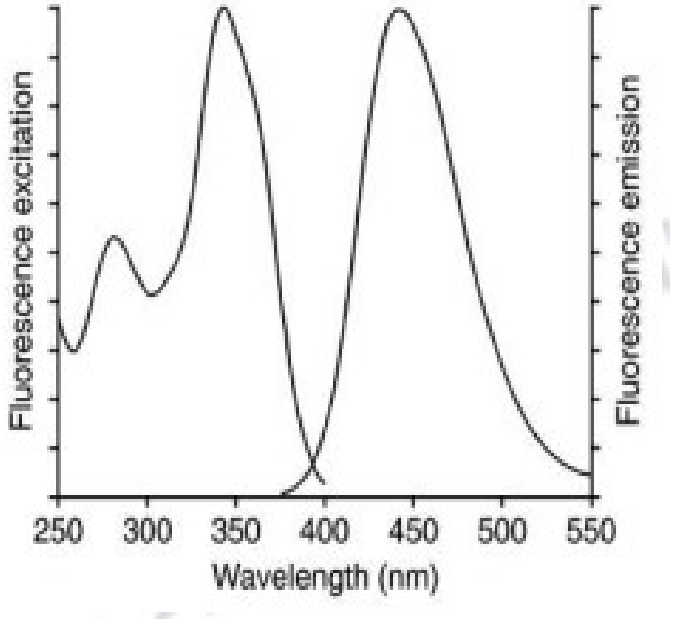


Fig.8.13: A typical fluorescence spectrum showing excitation (left) and emission (right) fluorescence

8.4.1. Fluorescence principle

Fluorescence work on the principle of absorption of light at particular wavelength by the material. The material which shows fluorescence works on the following principle such as

Franck Condon Principle: According to this principle, during electron transition the interatomic distance remain unaltered because the time required for electronic transition (order 10-16 second) us very short as compared to the time of vibration (order 10-13 sec). It is thus possible to represent the electronic transition by vertical line on a lote of potential energy verses the interatomic distance. The Frank-Condon principle says that the transition of electrons from the ground to the excited state occurs without any change in the position of the nucleus.

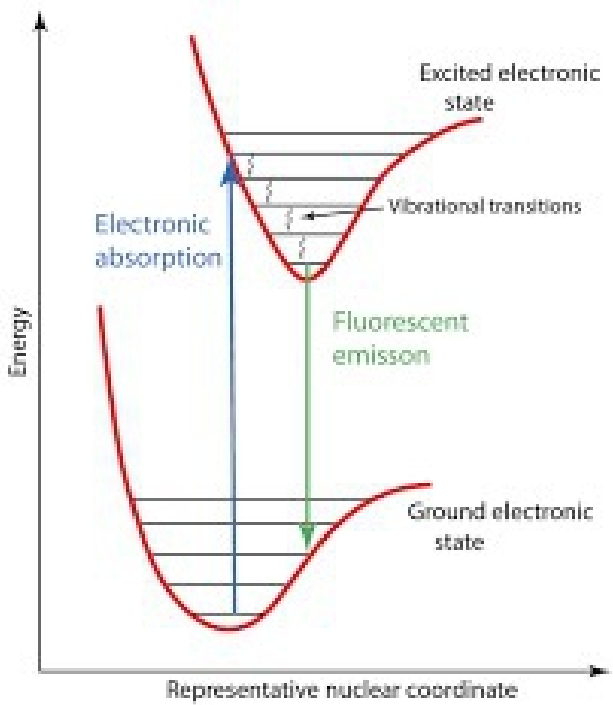


Fig.8.14. Transition between different electronic states

Stokes shift

When electron absorbed incident light it's goes to an excited state and falls back to its ground state with the emission of light having lower energy than the absorbed light. In this process emission spectra move towards the longer wavelength and a shift is observed in absorption and emission spectra. This shift in wavelength ($\Delta\lambda$) is called Stokes shift or governed by stokes law. The Stokes shift occurs due to the loss of energy to bring the excited electrons to the lowest vibrational energy level of the excited state from the highest vibrational energy levels. However, the Stokes shift is measured as the difference between the wavelengths at the maximum peak intensities in the excitation and emission spectra of a particular fluorophore. Fluorophores are organic molecules of 20-100 Daltons. Fluorescent molecules absorb the electromagnetic radiation in visible region and emit the radiation at a higher wavelength in the visible. Chromophores which exhibit the phenomenon of fluorescence are called fluors or fluorophores.

Good results are achieved with the compounds having the greater Stokes shift. Greater the Stokes shift, lesser will be the interference as the excitation and the emission spectra do not overlap.

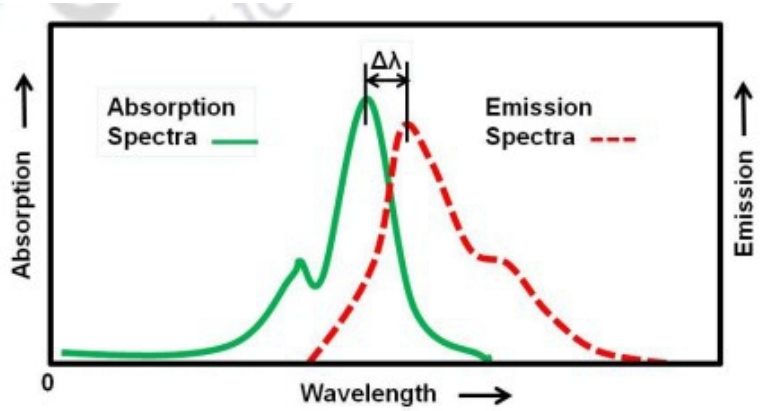


Fig.8.15: Shift in absorption and emission spectra in fluorescence spectroscopy

Mirror image rule:

The emission spectra are the mirror image of absorption spectra during the transition from the ground state (S_0) to the first excited state (S_1). Same transition occurs between the ground state and first excited state shows the similarities of vibrational levels of ground and first excited states.

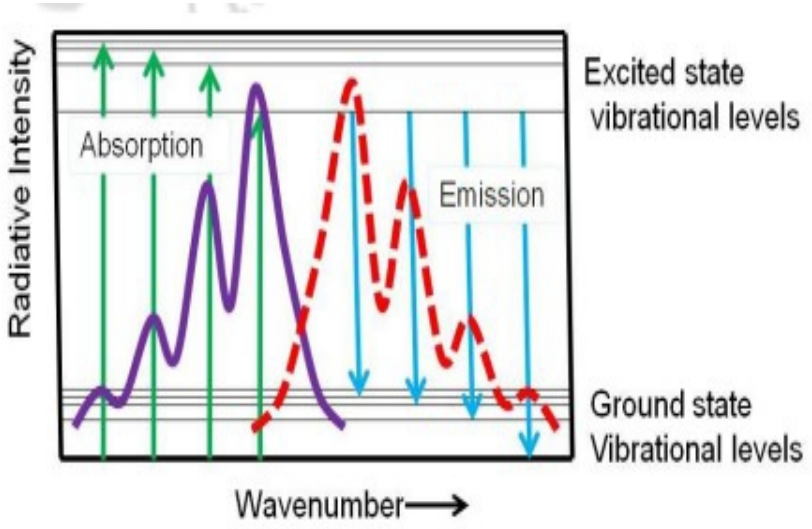


Fig.1.6: Mirror image of absorption and emission spectra

8.5. Application of Fluorimetry

The fluorescence widely used in as a scientific research tool in field medicine, forensic science and biochemistry.

In pharmaceutical industry it is useful for the investigation of medicine and also in biosensing application. Lysergic acid diethylamide can be analyzed by fluorimetry special from a sample of blood or urine.

In environmental chemistry, the carcinogens belonging to polynuclear aromatic hydrocarbons is analysed by fluorimetry. It also used full for the analysis of metals like Al, Be, Ca,Cd, Ge, Hg, Mg etc.

The use of fluorescence spectroscopy we can improve ligand binding and ligand dependent conformational changes in receptors.

In biochemistry fluorescence-based technology includes assays for biomolecules, metabolic enzymes. It is also useful in DNA sequencing, research into biomolecule dynamics, cell signaling, and adaptation. The color image of the gene and cell can me enhance by using fluorimetry. The fluorimetry is usefull for determination of structure of protein and peptide. The intrinsic fluors such tryptophan, tyrosine and phenylalanine present in the protein are responsible for the fluorescence exhibited by the proteins.

8.6. Summary

- Luminescence is described by light absorption and emission. Luminometry that is used to measure luminescence, which is the emission of electromagnetic radiation in the energy range of visible light.
- The chemiluminescence is defined as the generation of light due to any chemical reaction and bioluminescence is a natural phenomenon of light emission conferred by living organisms. Bioluminescence works by detecting visible light that is produced when an enzyme oxidizes a molecular substrate.
- Bioluminescence mainly comprised of two main components; a light emitting substance known as luciferin and an enzyme called as luciferase.
- Fluorescence spectroscopy is a widely used technique in physics, chemistry, biology and medicine. Fluorescence is the emission of light by a substance (fluor) that has absorbed light or other electromagnetic radiation.
- A spectrofluorimter is equipped with microscope which enables to observe the binding of antibody to a single bacterial cell or subcellular organelle and also helps in the identification of cancerous cells from normal cell as they express different set of protein for which fluoresces labelled specific antibody can be applied.

8.7. Self assessment Questions

Q.1. Discuss about luminescence. Who it is useful in the biochemistry.

Answer:----------

Q.2. Discus the chemiluminescence in briefly.

Answer:----------

Q.3. Short Notes:

(a)Stocks shift (b) Frank Condon Principle

Answer:----------

Q.4. Describe the bioluminescence.

Answer:----------

Q.5. Describe the significance fluorescence principle

Answer:----------

8.8. Further Readings

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Unit-9: Electron spectroscopy

Contents

9.1. Introduction

Electron spectroscopy is the method of determining the energy with which electrons are bound in chemical species by measuring the kinetic energies of the electrons. This electron emitted after bombardment of the species with X-ray or ultraviolet radiation. Photoelectron spectroscopy (PES) is an experimental technique used to determine the relative energies of electrons in atoms and molecules. It is work on ionizing samples using high-energy radiation (such as UV or x-rays) and then measuring the kinetic energies. X-ray photoelectron spectroscopy (XPS), which also known as Electron Spectroscopy for Chemical Analysis (ESCA), Electron energy loss spectroscopy (EELS), Ultraviolet photoelectron spectroscopy (UPS), and Auger electron spectroscopy (AES). XPS is useful in determination of surface chemistry of the surface layer through the bulk materials and reveal the spatial distributions of elements on the surface and inside layers of the bulk materials. The XPS spectra are quantified in terms of peak positions and peak intensities. Peak position indicates the elemental and chemical composition, and peak intensities measures quantity of materials at the surface.

Objectives

- To know about electron spectroscopy and its working principles
- To discuss how electron spectroscopy is used for chemical analysis (ESCA)
- To discuss the Chemical shift in ESCA
- to know the application of electron spectroscopy

9.2. About Electron spectroscopy

Electron spectroscopy is the method of determining the energy with which electrons are bound in chemical species by measuring the kinetic energies of the electrons emitted upon bombardment of the species with X-ray or ultraviolet radiation. It relies on the quantized nature of energy states where an electron can be excited from its initial ground state or initial excited state (hot band) and briefly exist in a higher energy excited state. Electronic transitions involve exciting an electron from one principle quantum state to another. Without incentive, an electron will not transition to a higher level. Only by absorbing energy, can an electron be excited. Electron spectroscopy gives spectra according to wavelength of visible, ultraviolet, and infrared light. The spectra can be classified as emission and adsorption spectra. An emission spectrum consists of all the radiations emitted by atoms or molecules, whereas in an absorption spectrum, portions of a continuous spectrum are missing because they have been absorbed by the medium through which the light has passed. The spectra of incandescent gases are called line spectrum that reflects the characteristic of the elements that emit the radiation. The spectrum of incandescent solids is said to be continuous because all wavelengths are present. Band spectra is the name given to groups of lines so closely spaced that each group appears to be a band, e.g., nitrogen spectrum. Auger electron spectroscopy (AES) and X-ray photoelectron spectroscopy (XPS), Electron energy loss spectroscopy and Ultraviolet photoelectron spectroscopy are the example of electron spectroscopy. The X-ray photoelectron spectroscopy (XPS), which also known as Electron Spectroscopy for Chemical Analysis (ESCA). In electron spectroscopy, depending on the technique, irradiating the sample with high-energy particles such as X-ray photons, electron beam electrons, or ultraviolet radiation photons, causes Auger electrons and photoelectrons to be emitted.

9.3. Types of electron spectroscopy

Electron spectroscopies are very surface sensitive, providing information from the uppermost 2–20 atomic layers, depending on the kinetic

energy of the emitted electrons. There are two types of electron spectroscopy: Auger electron spectroscopy (AES) and X-ray photoelectron spectroscopy (XPS). They are commonly used for the study of carbonaceous materials.

9.3.1. Auger electron spectroscopy (AES)

AES is widely used for the chemical analysis of solid surfaces of materials. AES was first discovered in 1923 by Lise Meitner and later independently discovered once again in 1925 by Pierre Auger. In this method the electron means to irradiate a sample and measures the kinetic energy of the Auger electrons produced. The Auger electron Spectroscopy is governed by allowing a high-energy electron from the incident beam to eject an electron from its inner orbit creating a vacant space or a hole in the orbit. Due to this another electron from the higher orbital jumps into and occupies the vacant state. In this process the transition of electron from higher orbit to lower orbit is occurs and resulted energy is released. This energy might eject another electron from its orbit instead of emitting a photon. This electron is called Auger electron. These transitions are characteristic transitions and are used to identify the elements by measuring the energy of the emitted Auger electrons. These transitions depend on initial energy of the incident beam and their interaction with the atom. AES is accomplished by exciting a sample's surface with a finely focused electron beam which causes Auger electrons to be emitted from the surface. AES provides quantitative elemental and chemical state information from surfaces of solid materials. The average depth of analysis for an AES measurement is approximately. Thus, nanostructures can be readily imaged along with creating 2-D maps of elemental composition of surface via Scanning Auger Microscopy (SAM). AES is a rather fast technique, despite being an expensive and sophisticated technique. Bonding or chemical state information can be obtained from the chemical shift and line shape observed in AES. However, AES is less accurate than XPS for obtaining bond information. AES has depth resolution of 5–25 Å, which makes it particularly attractive for depth profiling with simultaneous ion sputtering.

Auger is a three-electron process, wherein an electron beam (having 1 to 10 keV energy) is bombarded onto a solid surface. The impact of the beam ejects an electron from inner or core shell of the surface atom, thereby creating an ionized excited atom. An electron from an outer shell can occupy the vacancy produced in the core-shell, via a non-radioactive transition. The excess energy of the electron is removed via:

a) X-ray emission, this phenomenon forms the basis for X-ray fluorescence (XRF)/electron microprobe (EMP) analysis.
b) Emitting another electron from the atom. These atoms are termed as Auger electrons, after Pierre Auger, discoverer of this process in 1920s.

9.3.1.1. Principle of AES

The principle of Auger operates by allowing a high-energy electron from the beam to eject an electron from its orbit creating an empty hole in the orbit. In this process the fine electron beam bombards the sample and ejects an electron of the inner shell of the atom which creates vacancy that must be refilled by an electron from a higher energy level. When the higher energy electron fills the hole, the release of energy is transferred to an electron in an outer orbit electron. That electron has sufficient energy to overcome the binding energy and the work function to be ejected with a characteristic kinetic energy. In AES, the sample depth is dependent on the escape energy of the electrons.

9.3.1.2. The Auger effect

The Auger effect describes the non-radiative emission of an electron after a core vacancy is filled. A schematic of the Auger process is shown in Figure.

(a) An impinging high-energy electron collides with a core electron, leaving behind a vacancy.

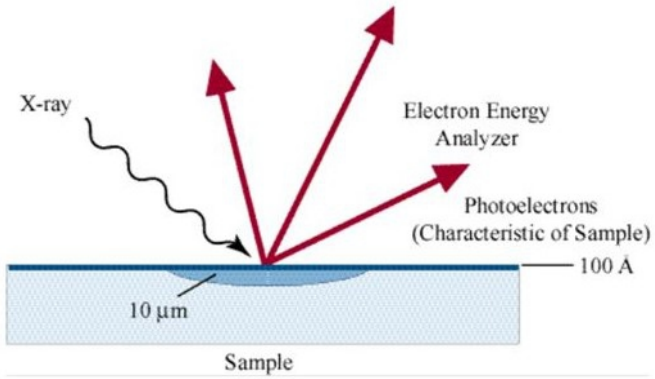
(b) The vacancy is filled by a higher-level electron. The energy released causes the emission of an Auger electron.

(c) The energy of the Auger electron is defined by the difference between the initial transition and the original energy level of the Auger electron before emission

9.3.2. X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) is used in analyzing the surface chemistry of a material. XPS can measure the chemical, electronic state and composition of the materials. XPS spectra are obtained by irradiating a solid surface with a beam of X-rays while simultaneously measuring the kinetic energy and electrons that are emitted from the surface of the material within few nanometer of depth. An XPS spectrum is recorded by counting the number of ejected electrons over a range of electron kinetic energies. The peaks in the spectrum are the characteristic energy of that particular element. The energies and intensities of the photoelectron peaks are essential for the identification and quantification of all surface elements.XPS involves irradiating the sample with low energy ((~1.5 keV) X-rays such that

photoelectric effect is induced. An electron spectrometer with high resolution records the energy spectrum of emitted photoelectrons.



9.3.2.1. Principle of XPS

It involved irradiation of the sample by low-energy x-rays and the subsequent analysis of the energy of emitted electrons. Here the x-rays such as Kα lines of Mg (1.2536 keV) and Al (1.4866 keV) is used for the analysis of material. In XPS the interaction take place between the incidents photons are the surface atoms leading to the photoelectric emission of electrons. The kinetic energy (K.E.) of the emitted electrons is expressed as:

$$K.E. = h\nu - B.E. - \varphi s$$

Where hν is the energy of the photon,

B.E. is the binding energy of the atomic orbital

φs, is the work function of spectrometer.

B.E. can also be described as the energy difference of the initial and final states when an electron is released from the atom. Electrons emitted from an ion possess different kinetic energies owing to the possibilities of different final states of the ion from each type of atom.

The XPS spectrum is analyzed by identifying the peaks and locating the shift in peak positions due to presence of elemental compositions. XPS spectra, however, are more complicated because Auger peaks may be present. An Auger peak represents the kinetic energy of an Auger electron that changes with energy of primary X-rays. Thus, an Auger peak will shift in apparent binding energy in an XPS spectrum when we change the X-ray source. For example, an Auger peak shifts by 233 eV in the XPS spectrum when we change the radiation from Mg Kα (1253.6 eV) to Al Kα (1486.6 eV). Peak positions in an XPS spectrum are likely to be affected by spectrometer

conditions and the sample surface. Before XPS peak identification, we need to calibrate the binding energy.

9.3.2.2.Chemical shifts

Chemical shifts of binding energy peaks for an element are caused by the surrounding chemical states of the element. Knowledge of the possible chemical shifts is necessary to identify the peaks accurately. We can use the features of the chemical shifts to identify the elements and chemical compounds. For example, Fig. 1 shows the XPS spectrum of poly-vinyl trifluoroacetate (PVTFA). The carbon atoms in different environments generate distinctive peaks as shown in Fig. 1a. Similarly, oxygen atoms in two different environments also generate two peaks as shown in Fig. 1b. The XPS spectra shown in Fig. 1 with defined peak positions and relative peak intensities can also be used as a fingerprint to identify PVTFA. The small shifts in binding energy are caused peak overlap as shown in Figure1b. Hence, we might need to carefully resolve the overlapped peaks with assistance from other computer software.

The chemical shifts of the C1S peak are summarized in Fig. 2. The range of the C1S shift extends over 12 eV. Quantifying the amount of chemical shifts in the spectrum can identify different carbon bonds. The binding energy difference between C - C and C - O is about 3 eV. This is extremely useful for identifying polymers by revealing various carbon bonds. Also, the chemical shifts reveal the degree of oxidation in molecular solids. Higher chemical shifts occur for larger number of electrons transferred. Chemical shifts decrease with increasing atomic number and suggests less possibility in metallic and semiconducting elements. For example, the shift range for Si2p is less than 6 eV. Chemical shifts also occur in AES spectra, and the chemical shifts can be significantly larger than the shifts in XPS. For example, the shift between metallic and oxide Al peaks AlKL2,3 (AES) is more than 5 eV and the corresponding shift for Al2p (XPS) binding energy is only about 1 eV [1, 3-4].

9.3.2.3.Applications of XPS

- XPS is a surface chemical analysis technique that can be used to analyze the surface chemistry of a material.
- XPS is used in inorganic chemistry for the analysis of metal alloys, semiconductors, polymers, paints and catalysis.
- XPS has d for most of the elements are in the parts per thousand range (1,000 ppm). Detection limits of parts per million (ppm) are possible, but require special conditions: concentration at top surface or very long collection time (5 hr).
- XPS is used to measure the elemental composition of the surface, empirical formula of pure materials and elements that contaminate a surface.

- XPS is also useful for the determination of chemical structure and chemical or electronic state of each element in the surface

9.4. Electron spectroscopy for chemical analysis (ESCA

ESCA provides unique information about chemical composition and chemical state of a surface. It is useful for biomaterials analysis, it is surface sensitive and useful in wide range of solids it has disadvantages like it is expensive, slow and poor spatial resolution. ESCA is based on the photoelectron effect. It is used a high energy X-ray photon that can ionize the atom. In this process the detecting electrons ejected from higher orbitals and producing an ejected free electron with kinetic energy KE:

$$KE = hv - BE$$

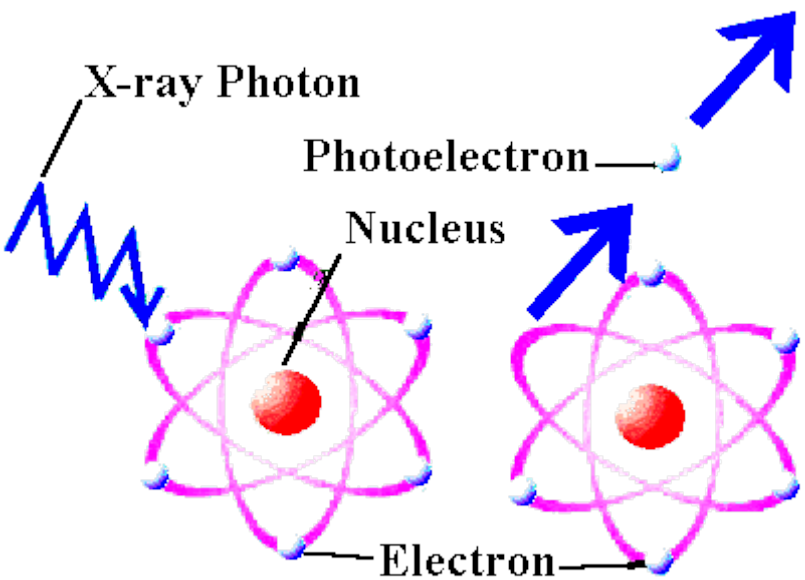
Where BE=energy necessary to remove a specific electron from an atom.

BE ≈ orbital energy

h=Planck Constant

v = frequency of light

Here BE is the binding energy of the electron to the atom concerned. This relation is the basis of ESCA: since hν is known, a measure of K determines BE. The value of BE is specific to the atom concerned; measurement of BE serves as a "fingerprint" to identify the atom. ESCA can be used to detect all elements except hydrogen and helium, with a sensitivity variation across the periodic table of only about 30. It is most useful for solids, including powders and soft materials. The qualitative and quantitative chemical state analysis capabilities, combined with extreme surface sensitivity (usually a few atomic layers) have made ESCA the most broadly applicable surface analysis technique today.



Electron Spectroscopy for Chemical Analysis, also known as X-ray Photoelectron Spectroscopy (XPS), provides information on the atomic composition and chemical bonding of a sample on the surface (top few nanometers). ESCA can detect all elements except for hydrogen and helium. ESCA is widely application for both organic and inorganic samples. In the ESCA process the X-ray beam to bombard the surface of sample resultant the core beam of electrons emitted. The X-ray beam penetrates several microns into the sample. When this occurs, core electrons from the atoms of the samples are freed, but only the electrons from the near surface region (top few nanometers) can leave the surface. The instrument collects and measures the kinetic energy of these electrons from which we can calculate the binding energy, which originally held the electron to its source atom. A typical spectrum of ESCA shows peaks as a function of binding energy such as that shown in Fig. 10.34 for PTFE. The presence of C1s and F1s peaks on a "clean" surface (b–d in Fig. 10.34) indicates that the PTFE surface is comprised of only carbon and fluorine.

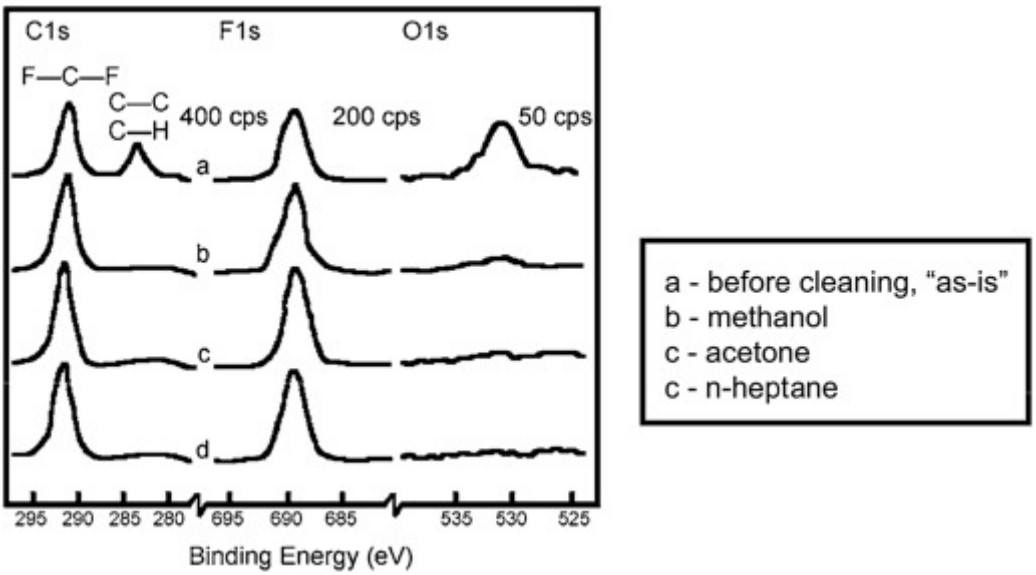


Fig. Electron spectroscopy for chemical analysis spectra for polytetrafluoroethylene before and after cleaning.

ESCA has following applications such

a. Material evaluation

- It useful in determination of oxidation states and Identification of polymeric coatings
- It also useful in determination of surface compound identification and surface composition

- Plasma treatment and Anti-reflection coatings is also determine by ESCA

b. Failure Analysis

- It useful in corrosion product
- It useful in material delamination
- It useful in discoloration of epoxy
- It useful in chemical degradation of surfaces
- It useful in catalyst poisonin

c. Quality Control

- It useful in identification of organic contamination
- It useful in breakdown of surface lubricants
- It also useful in chemical degradation of surfaces

9.5. Summary

Auger electron spectroscopy (AES) is a non-destructive electron spectroscopy technique for partial identification of elemental composition of materials. The samples can include surface, thin film, interface, etc. High surface sensitivity (analysis depth < 100 Å) and a relatively lower detection limit (~0.1 atomic percent) make AES a very popular technique for surface analyses, including industrial and technological applications.

XPS is a powerful tool for chemical analysis at the surface of the specimen materials. It identifies the presence of elements in the layer within several nanometers from the surface. Importantly, the positions of peaks in the XPS spectra are sensitive to their chemical compositions. XPS spectra, however, are more complicated because Auger peaks may be present. XPS used Anode Mg/Al (300/400W) as X-Ray Source and electron Gun with spot size < 50 m dia. It detects 110 mm radius hemispherical analyzer with 7 channeltrons.

ESCA testing and analysis, which is short for Electron Spectroscopy for Chemical Analysis, is an incredibly useful surface analysis technique with applications across several industries. The technique, which is also known as X-Ray Photoelectron Spectroscopy (XPS analysis), provides crucial information about the elemental and binding energy of a material's surfaces and interfaces, making it a fabulous product testing and quality assurance tool for manufacturers of polymers, medical devices, electronics, and more.

9.6. Terminal question

Q.1. What is electron spectroscopy? Define its use in biochemistry.

Answer:----------

Q.2. Write the application of XPS.

Answer:----------

Q.3. Discuss about principle of Auger electron spectroscopy (AES).

Answer:----------

Q.4. What is X-ray photoelectron spectroscopy ? How it is useful in biological sample analysis.

Answer:----------

Q.5. Write about Electron Spectroscopy for Chemical Analysis.

Answer:----------

Q.6. Discuss Chemical shifts in XPS

Answer:----------

9.7. Further reading

1. Y R Sharma, Elementary organic spectroscopy; S Chand publication, 5th Edition, 2013.
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