UNIT-5 SPECTROSCOPIC AND ANALYTICAL TECHNIQUES

Introduction- Electromagnetic radiation- interaction of electromagnetic radiation with matter- Beer- Lambert's law- principle, instrumentation (Block Diagram) and applications of UV-Visible spectroscopy, IR spectroscopy- colorimetry- flame photometry and Atomic absorption spectroscopy (AAS).

INTRODUCTION

Spectroscopy is one of the most powerful tool available for the study of atomic and molecular structure, and is used in the analysis of a most of the samples. Spectroscopy deals with the study of interaction of electromagnetic radiation with the matter. During the interaction, the energy is absorbed or emitted by the matter. The measurement of this radiation frequency (absorbed or emitted) is made using spectroscopy.

TYPES OF SPECTROSCOPY

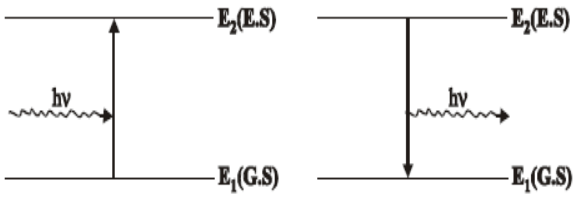
1. Atomic spectroscopy: It deals with the interaction of the electromagnetic radiation with atoms. During which the atoms absorb radiation and gets excited from the ground state electronic energy level to another.

2. Molecular spectroscopy: It deals with the interaction of electromagnetic radiation with molecules. This results in transition between rotational, vibrational and electronic energy levels.

How does a Spectrum arise?

1. **Absorption spectrum**

Consider a molecule having only two energy levels: $E1$ and $E2$ as shown in the figure.1.



(a) Absorption spectrum **(b) Emission spectrum** in **Fig. 1**

- When a beam of electromagnetic radiation is allowed to fall on a molecule in the ground state, the molecule absorbs photon of energy hv and undergoes a transition from the lower energy level to the higher energy level.
- The measurement of this decrease in the intensity of radiation is the basis of absorption spectro scopy. The spectrum thus obtained is called the ***absorption spectrum***. (Fig1. a)

2. Emission spectrum

If the molecule comes down from the excited state to the ground state with the emission of photons of energy *hv*, the spectrum obtained is called ***emission spectrum***, (Fig.1.b).

PHOTOPHYSICAL LAW

The absorption of light in the visible and near UV region by a solution is governed by a photophysical law known as Beer-Lambert's law.

Lambert's Law

Lambert's law states that, "when a beam of monochromatic radiation is passed through a **homogeneous absorbing medium** the rate of decrease of intensity of the radiation 'dI' with thickness of absorbing medium '*dx*' is Proportional to the **intensity of the incident radiation** '*I*'.

It is mathematically expressed as

$$\frac{-dI}{dx} = kI \qquad \ldots\ldots\ldots\ldots (1)$$

where, k = absorption coefficient.

On integrating the equation (1) between limits $I = I_0$ at $x = 0$ and $I = I$ at $x = x$, we get

$$\int_{I_0}^{I} \frac{dI}{I} = -\int_{0}^{x} k\,dx$$

$$\boxed{\ln \frac{I}{I_0} = -kx} \qquad \ldots\ldots\ldots\ldots (2)$$

The equation (2) is known as Lambert's law.

Beer's law (or) Beer-Lambert's Law

According to this law, "when a beam of monochromatic radiation is passed through a ***solution of an absorbing substance***, the rate of decrease of intensity of radiation '*dI*' with thickness of the absorbing solution '*dx*' is proportional to the ***intensity of incident radiation 'I'*** as well as **the concentration of the solution '*C*'.**"

It is mathematically represented as

$$\frac{-dI}{dx} = kIC \qquad \text{..... (3)}$$

where, k = proportionality constant.

On integrating the equation (3) between limits $I = I_0$ at $x = 0$ and $I = I$ at $x = x$, we get

$$\int_{I_0}^{I} \frac{dI}{I} = -\int_{0}^{x} kCdx$$

$$\ln \frac{I}{I_0} = -kCx \text{ (or) } 2.303 \log \frac{I}{I_0} = -kCx$$

$$\text{(or) } \log \frac{I_0}{I} = \frac{k}{2.303} Cx$$

$$\text{(or) } \boxed{A = \varepsilon Cx} \qquad \text{..... (4)}$$

where, $\varepsilon = \frac{k}{2.303}$ = molar absorptivity (or) molar extinction coefficient

$$\log \frac{I_0}{I} = A = \text{Absorbance (or) Optical density}$$

The equation (4) is called Beer-Lambert's law. ***Thus, the absorbance*** (***A***) ***is directly proportional to molar concentration*** (***C***) ***and thickness (or) path length*** (***x***)**.**

Application of Beer-Lambert's law: **Determination of unknown concentration**

First absorbance '*As*' of a standard solution of **known concentration** '*Cs*' is measured, then '*Au*' of a standard solution of un**known concentration** '*Cu*' is measured then according to Beer-Lambert's law

$$\therefore \boxed{C_u = \frac{A_u}{A_s} \times C_s}$$

VISIBLE AND ULTRAVIOLET (UV) SPECTROSCOPY

A

Principle

Visible and Ultraviolet (UV) spectra arise from the transition of valence electrons within a molecule or ion from a lower electronic energy level (ground state E0) to higher electronic energy level (excited state E1).

This transition occurs due to the absorption of UV (wavelength 100-400 nm) or visible (wave length 400-750 nm) region of the electronic spectrum by a molecule (or) ion.

The actual amount of energy required depends on the difference in energy between the ground state and the excited state of the electrons.

$$E1 - Eo = h\nu.$$

INSTRUMENTATION:

1. Radiations source

In visible – UV spectrometers, the most commonly used radiation sources are hydrogen (or) deuterium lamps.

2. Monochromators

The monochromator is used to disperse the radiation according to the wavelength. The essential elements of a monochromator are an entrance slit, a dispersing element and an exit slit. The dispersing element may be a prism or grating (or) a filter.

3. Cells (sample cell and reference cell)

The cells, containing samples or reference for analysis, should fulfil the following conditions

- They must be uniform in construction.
- The material of construction should be inert to solvents.
- They must transmit the light of the wavelength used.

4. Detectors

There are three common types of detectors used in visible UV spectrophotometers. They are Barrier layer cell, Photomultiplier tube, Photocell.

The detector converts the radiation, falling on which, into current. The current is directly proportional to the concentration of the solution

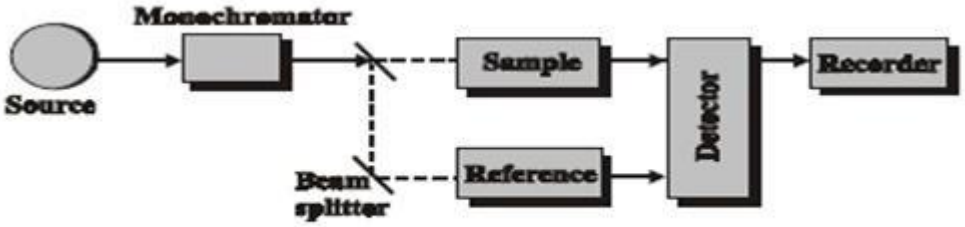
5. Recording system

The signal from the detector is finally received by the recording system. The recording is done by recorder pen.

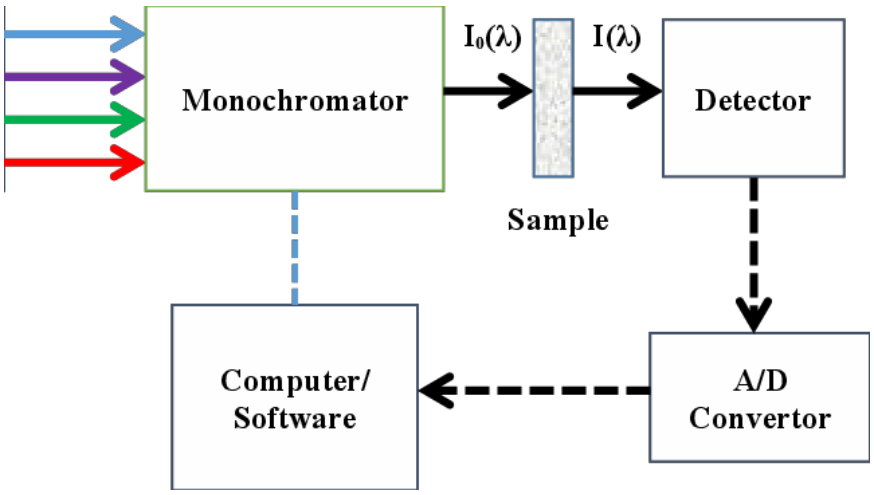
WORKING OF VISIBLE AND UV SPECTROPHOTOMETER:

- The radiation from the source is allowed to pass through the monochromator unit.
- The monochromator allows a narrow range of wavelength to pass through an exit slit.
- The beam of radiation coming out of the monochromator is split into two equal beams.
- One-half of the beams (the sample beam) is directed to pass through a transparent cell containing a solution of the compound to be analysed.

- The another half (the reference beam) is directed to pass through an identical cell that contains only the solvent. The instrument is designed in such a way that it can compare the inten sities of the two beams.
- If the compound absorbs light at a particular wavelength, then intensity of the sample beam (*I*) will be less than that of the reference beam (*Io*).The instrument gives output graph, which is a plot of wave length Vs absorbance of the light. This graph is known as an absorption spectrum.



Block diagram of visible uv spectrophotometer



Applications of UV-Visible:

- Predicting relationship between different groups
- Qualitative analysis
- Detection of impurities
- Quantitative analysis
- Determination of molecular weight

B

COLORIMETRY

Colorimetry is concerned with the visible region (400-750 nm) of the spectrum. The instrument, used for measuring absorption of radiant energy in the visible region from the substances is called **colorimeter.**

Principle:

This method is convenient for the coloured substances or coloured solutions. The intensity of colour can be easily measured by using a photo electric colorimeter, from which the concentration of coloured solution can be obtained by using Beer-Lambert's law.

If the substance is colourless, then a suitable complexing agent is added to the solution so that a coloured complex is obtained, which can absorb the light.

Example

For the estimation of cuprous ions, complexing agent, ammonium hydroxide, is added to get blue coloured solution.

Instrumentation:

Components:

1. Radiation sources

The wavelength range of visible light lies between 400-750 nm. In this region, a tungsten-filament lamp is most widely used.

2. Filter (or) monochromator

It is a instrument, which allows the light of the required wavelength to pass through, but absorbs the light of other wavelengths.

3. Slits

(a) Entrance slit: It provides a narrow source of the light.

(b) Exit slit: It selects a narrow band of dispersed spectrum for observation by the detector.

4. Cell

The cell, holding the test sample (usually a solution), should be transparent. For visible region the cell is made of colour-corrected fused glass.

5. Detector

It is used for measuring the radiant energy transmitted through the sample. Photosensitive devices are used to detect radiations. These detectors produce current, which is directly proportional to the intensity of the incident radiation.

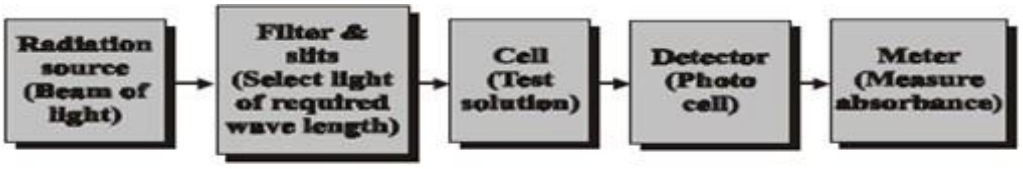
6. Meter

It is used to measure directly the fraction of light absorbed.

WORKING OF COLORIMETER:

- In a colorimeter, a narrow beam of light is passed from radiation source through the test solution (cell) towards a sensitive detector (photocell).
- Usually colorimeter is provided with the arrangement of filter and slits, which select the light of required wave length.

- The detector (photocell) generates the current, which is proportional to the amount of light transmitted by the solution.
- The amount of light transmitted depends on the depth of colour of the test solution. Thus, the current from the Photocell will be more when the light transmitted is more. This is possible only if the coloured solution is most dilute.



Block diagram of colorimeter

Current α Light transmitted α 1/Concentration

- The transmitted light is allowed to send through a meter, which is calibrated to show not the fraction of light transmitted but the fraction of light absorbed. The light absorbed is proportional to the concentration of the test solution.

Applications of colorimetry:

- Molar compositions of complexes can be determined.
- The instability constants of metal complexes are also determined.
- Dissociation constants (*Pk*) of an indicator can be determined.
- Structure of inorganic compounds, complexes (cis & trans isomer) can be determined.
- Molecular weight of a compound can also be determined using colorimetric measurements.

C

FLAME *PHOTOMETRY* (or) FLAME EMISSION SPECTROSCOPY

Flame photometry is a method in which, the intensity of the emitted light is measured, when a atomised metal is introduced into a flame. The **wavelength** of the colour tells us **what the element is,** and the **intensity** of the colour tells us **how much of the element is present.**

Theory (or) Principle:

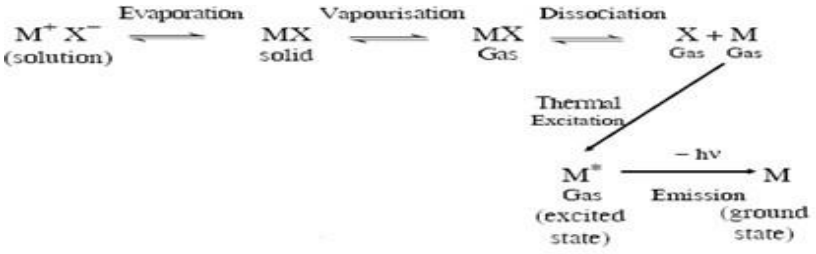
When a metallic salt solution is introduced into a flame, the following processes will occur.

(i) The solvent is evaporated leaving behind the solid salt particle.

(ii) The salt is vapourised into the gaseous state and dissociated into atoms.

(iii) Some of the atoms from the ground state are excited to higher energy state by absorbing thermal energy from the flame.

The excited atoms, which are unstable, quickly emit photons of different wave lengths and return to the lower energy state. Then the emitted radiation is passed through the filter, which permits the characteristic wavelength of the metal under examination. It is then passed into the detector, and finally into the recorder.



Instrumentation:

The various components of the flame photometer are described as follows.

1. Burner: The flame must possess the following characteristics.

- It should evaporate the solvent from the sample solution.
- It should decompose the solid into atoms.
- It should excite the atoms and cause them to emit radiant energy.

2. Mirror: The radiation from the flame is emitted in all directions in space. In order to increase the amount of radiation reaching the detector, a convex mirror is used which is set behind the burner.

3. Slits

***Entrance slits*:** It is kept between the flame and monochromator. It permits only the radiation coming from the flame and mirror.

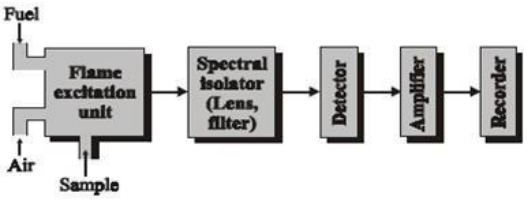
Exit slit: It is kept between the monochromator and detector. It prevents the entry of interfering lines.

4. Monochromator (or) Prism (or) Grating (or) Filter

It allows the light of the required wave length to pass through, but absorbs the light of other wavelengths.

5. Detector

The radiation coming out from the filter is allowed to fall on the detector, which measures the intensity of the radiation falling on it. Photo multiplier (or) photocell is used as detector, which converts the radiation into an electrical current.



Block diagram of a flame photometer

6. Amplifier & Recorder

The current coming out from the detector is weak, so it is amplified and recorded.

Working of Flame photometer:

- Air, at a given pressure, is passed into an atomiser. The suction so-produced draws some solution of the sample into the atomiser.
- Air + sample solution is then mixed with fuel gas in the mixing chamber. The Air + sample solution + fuel gas mixture is then burnt in the burner.
- The radiation, emitted by burner flame, is passed successively through the lens, filter, detector, amplifier and finally into a recorder.

Applications of flame photometry

1. Qualitative Analysis

- The elements of group I & II. (K, Na, Li, Ca, Mg, etc) can be detected visually from the colour of the flame. Eg; Na Brick red colour at 422nm and K red colour at 766nm.
- Non-radiating elements such as carbon, hydrogen and halides cannot be detected using this method.

2. Quantitative analysis

- The amount of the elements in group I & II (alkali & alkaline-earth metals) can be determined from the sample.
- Certain transition elements, such as Cu, Fe & Mn can also be determined using flame photometry.

3. Other applications

- The measurement of these elements is very useful in medicine, agriculture and plant science.
- Flame photometry is extensively used in the analysis of biological fluids and tissues
- In soil analysis the elements like, Na, K, Al, Ca, Fe, etc., are determined.
- Industrial and natural waters, petroleum products, cement, glass, and metallurgical products can also be analysed by this method.

Limitations of flame photometry:

- It cannot be used for the determination of all metal atoms and inert gases.
- Only liquid samples must be used.

D

ATOMIC ABSOPTION SPECTROSCOPY:

Atomic absorption spectroscopy is based on the atomization of the sample followed by absorption of characteristic radiation by the ground state gaseous atoms.

When the light of the required wavelength is allowed to pass through a flame, having atoms of the metallic species, part of that light will be absorbed and the absorption will be proportional to the concentration of the atom in the flame.

Thus, in atomic absorption spectroscopy, the amount of light absorbed is determined.

Instrumentation:

Various components

1. Radiation source

The radiation source should emit, stable, intense, characteristic radiation of the element to be determined. The hollow cathode lamp, which consists of a glass tube containing noble gases like a argon (anode) and hollow cathode, made of the analyte metal, is generally used.

2. Chopper

A rotating wheel is interposed between the hollow cathode lamp and the flame. It breaks the steady light, from the lamp, into an pulsating light (because the recorder will record only the pulsating (alternating) current).

3. Burner (or) Flame

The flame is used for converting the liquid sample into the gaseous state. It converts the molecule into atomic vapour. Two types of burners are used

1. Total consumption burner.

2 Premixed burner.

4. Nebulisation of the liquid sample

Before the liquid sample enters the burner, it is first of all converted into small droplets. This method of formation of small droplets from the liquid sample is called **nebulisation**.

5. Monochromators

The monochromators select a given absorbing line from the spectral lines emitted from the hollow cathode. The most common monochromators are

(i) Prisms.

(ii) Gratings.

6. Detectors

The photomultiplier tube is a most suitable detector. When the photon strikes the photomultiplier tube, an electric current (emf) is produced.

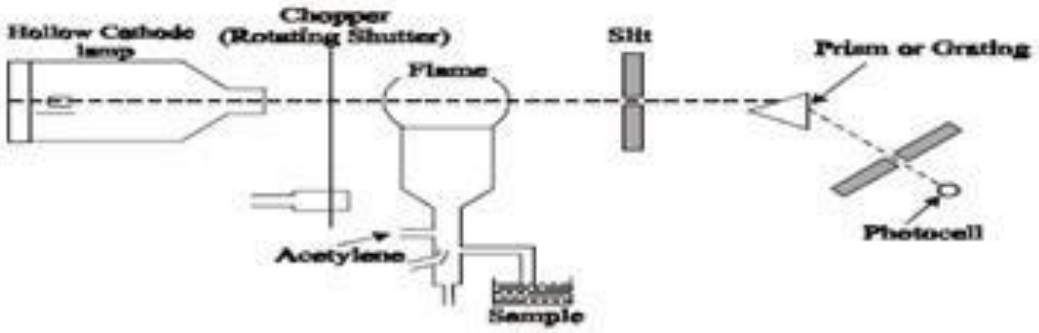
7. Amplifier

The electric current, from the photomultiplier detector, is fed into the amplifier, which amplifies the electric current many times.

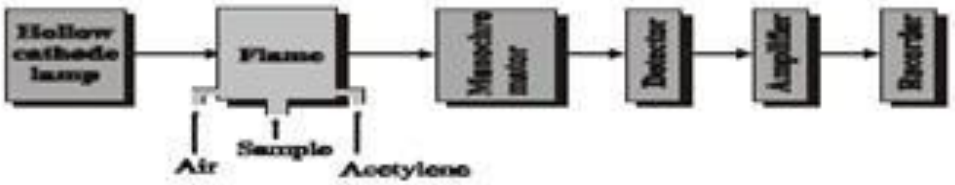
8. Read-Out Device (or) Recorder

The signal coming out from the amplifier is recorded using chart recorder (or) digital read-out devices. The characteristic radiation, obtained from the hollow cathode lamp, is passed through a flame into which the sample is aspirated. The metallic compounds are decomposed into atoms of the element to be measured.

The atoms absorb a fraction of radiation in the flame. The unabsorbed radiation from the flame is allowed to pass through a mono chromator. From the monochromator the unabsorbed radiation is led into the detector. From the detector, the output is amplified and measured on a recorder.



Atomic absorption spectrophotometer



Block diagram of atomic absorption spectroscopy

E

IR SPECTROSCOPY

Principle:

An IR spectrum is produced by the absorption of energy by a molecule in the infrared region and the transitions occur between vibrational levels. So, *IR* spectroscopy is also known as vibrational spectroscopy.

Range of Infrared Radiation

The range in the electromagnetic spectrum extending from 12500 to 50 cm− 1 (0.8 to 200 μ) is commonly referred to as the infrared. This region is further divided into three sub regions.

Range of IR radiation

Molecular Vibrations and Origin of IR Spectrum:

Since atoms in a molecule are continuously vibrating, molecules are also vibrating. There are two kinds of fundamental vibrations in the molecule.

Stretching vibrations: During stretching the distance between two atoms decreases or increases, but bond angle remains unaltered.

Bending (or) deformation vibrations: During bending bond angle increases and decreases but bond distance remains unaltered.

When *IR* light of the same frequency is incident on the molecule, energy is absorbed resulting in increase of amplitude of vibration.

When the molecule returns from the excited state to the original ground state, the absorbed energy is released as heat.

Thus every compound shows characteristic absorption bands in the *IR* region of the spectrum. Different functional groups produce easily recognisable band at definite positions in the *IR* spectral range (12500 to 50 cm− 1).

Finger Print Region: The vibrational spectral (*IR* spectra) region at 1400 − 700 cm− 1 gives very rich and intense absorption bands. This region is termed as **fingerprint region**. The region 4000 − 1430 cm− 1 is known as **Group frequency region**.

USES OF FINGERPRINT REGION:

1. IR spectra are often characterized as molecular finger prints, which detect the presence of functional groups.
2. Fingerprint region is also used to identify and characterize the molecule just as a fingerprint can be used to identify a person.

IR SPECTROSCOPY INSTRUMENTATION:

1. Radiation source

The main source of IR radiation is,

1) Nichrome wire
2) Nernst glower (filament containing oxides of Zr, Th, Ce)

When they are heated electrically at 1200 to 2000°C, they glow and produce IR radiation.

2. Monochromator

It allows the light of the required wave length to pass through, but absorbs the light of other wave length.

3. Sample Cell

The cell, holding the test sample, must be transparent to IR radiation.

4. Detector

IR detectors generally convert thermal radiant energy into electrical energy. There are so many detectors, of which the followings are important.

(a) Photoconductivity cell.
(b) Thermocouple.
(c) Pyroelectric detectors.

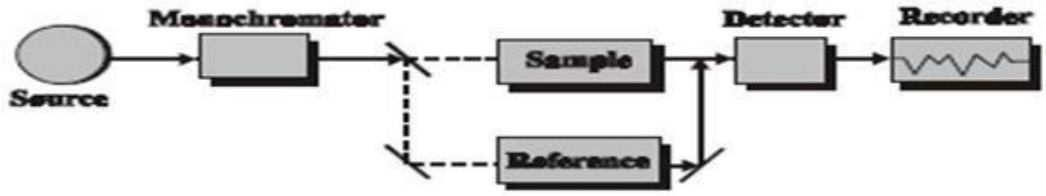
5. Recorder

The recorder records the signal coming out from the detector.

WORKING OF IR SPECTROPHOTOMETER:

The radiation emitted by the source is split into two identical beams having equal intensity. One of the beams passes through the sample and the other through the reference sample.

When the sample cell contains the sample, the half-beam travelling through it becomes less intense. When the two half beams (one coming from the reference and the other from the sample) recombine, they produce an oscillating signal, which is measured by the detector. The signal from the detector is passed to the recording unit and recorded.



Block diagram of double beam IR spectrophotometer.

Applications of IR spectroscopy

1. Identity of the compound can be established
2. Detection of functional groups:
3. Testing the purity of a sample.
4. Study of progress of a chemical reaction
5. Determination of shape (or) Symmetry of a molecule