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Review Article

A Review on UV-Visible Spectroscopy

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ABSTRACT

The main goal of the review paper is to study the all detailed information about UV visible spectroscopy & its pharmaceutical applications. UV visible spectroscopy is a very useful method which is used in industry from long time ago. This is very simple and quick method. One of the earliest instrumental techniques for analysis is UV-VIS spectroscopy. Many different types of materials can be characterised using UV-Vis spectroscopy. The UV- Vis delivers details based on the degree of absorption or transmittance of a varied wavelength of beam light and the various responses of samples. Radiant energy absorption by materials can be quantitatively described using the general law know as Beer's law. The UV- VIS spectrometer is simple to use and handle.

INTRODUCTION

Introduction to spectroscopy:

- Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation (EMR) absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state.
- This change may be from Ground State to excited state or excited state to Ground state.

•At ground state, the energy of a molecule is the sum total of rotational, Vibrational and electronic energies.

- In other words, spectroscopy measures the changes in rotational, vibrational and /or electronic energies.

a) **Atomic spectroscopy**:-The atomic spectroscopy deals with study of interaction of UV radiation with atoms.

b) **Molecular spectroscopy**:

The molecular spectroscopy deals with study of interaction of UV radiation with molecules.

Spectrophotometer is a device which are design to determine spectrum of a compound. UV spectroscopy = 200 – 400 nm

UV visible spectroscopy =400 – 800 nm.

c) **Frequency**:-frequency is the number of occurrences of repeating event per unit time.(t)

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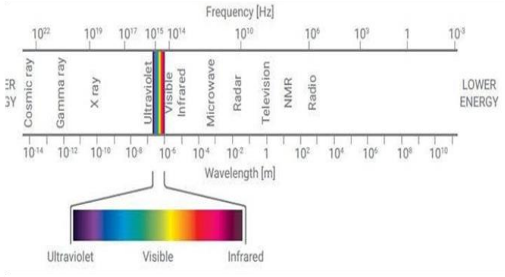
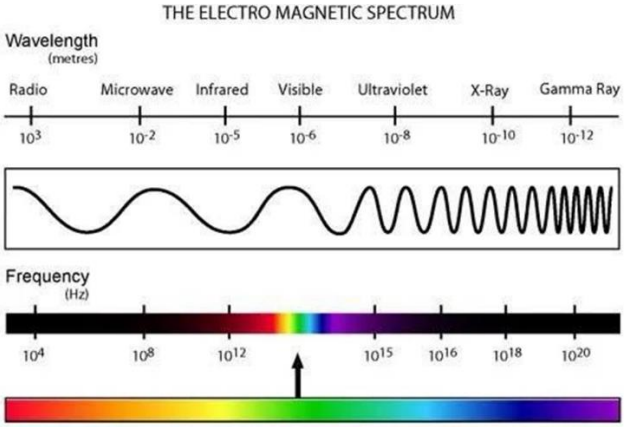
d) Wavelength:-it is distance between the identical points on adjoining waveforms longitudinal wave.



Electromagnetic Spectrum:-

The ability of electromagnetic radiation to discretely interact with atoms and molecules and produce distinctive absorption or emission profiles is essential for spectroscopic activities. The wavelength of electromagnetic radiation is the characteristic that governs the perceived colour spectrum. The visible section of the electromagnetic spectrum is that portion of the spectrum that the human eye can see. These visible wavelengths span a region between 400 and 800 nm.



The major characteristics of various spectrum regions are outlined as follows

a)-ray region: This lies between 0.02 to 1A0. The gamma rays are shortest waves emitted by atomic nuclei, involving energy changes of 10-9 to 1011 Joules / gram atom.

b)X –ray region: This lies between 1 to 10 A0. X-rays emitted or absorbed by

movement of electrons close to the nuclei of relatively heavy atoms, involve energy changes of the order thousand kilo Joules.

c)Visible and Ultraviolet Region: these are further made up of the following regions: Vacuum ultraviolet: 1 -180 nm

Ultraviolet: 80 – 400 nm Visible: 400 – 750 nm

Deviations form Beer's Law :-

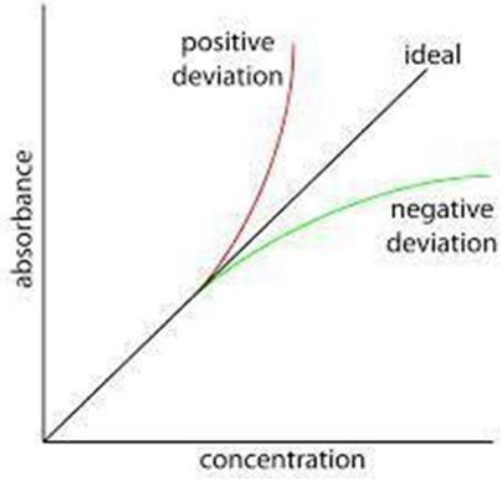
•A system is said to obey Beer’s law, when a plot of Concentration Vs absorbance gives a straight line.

•The straight line is obtained by using line of best fit or method of least squares or by joining the maximum no of points in such a way that positive and negative errors are balanced or minimised.

•The regression line can also be used for determining concentration of a solution whose absorbance is obtained using a colorimeter/spectrophotometer.

•When a straight line is not obtained, that is a non-linear curve is obtained in a plot of concentration Vs absorbance, the system is said to undergo deviation from Beer's Law.

•Such deviation can be positive deviation or negative deviation.



Several reasons for the observed deviations from Beer's law are as follows:

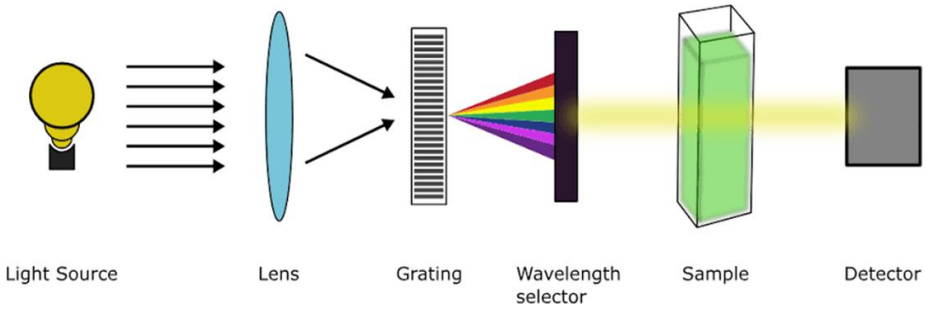
1. **Instrumental deviations**: Factors like stray radiation, improper slit width, fluctuations in single beam and when monochromatic light is not used can influence the deviation.
2. **Physicochemical changes in solution**: Factors like Association, Dissociation, ionisation (Change in pH), faulty development of colour (incompletion of reaction), refractive index at high concentrations, can influence such deviations.

Scattering and reflection can modify the absorption reported. Reaction with the solvent High concentration affects charge distribution, the average distance between ion decreasing, making particles close to each other

Components of UV-vis spectrophotometer

- Source of radiant energy.
- Collimating system.
- Monochromator system.
- Sample holder or container to hold sample
- Detector system of collecting transmitted radiation.

Limitation in Laws:-



Types of filters used in spectroscopy: -

1) Absorption filters

2) Interference filters

1) Absorption filters:

Absorption filters are simple filters which Works by selective absorption of unwanted radiation and transmits the radiation which is required.

a) **Glass filter**:

b) **Gelatine filter**:

Merits: -

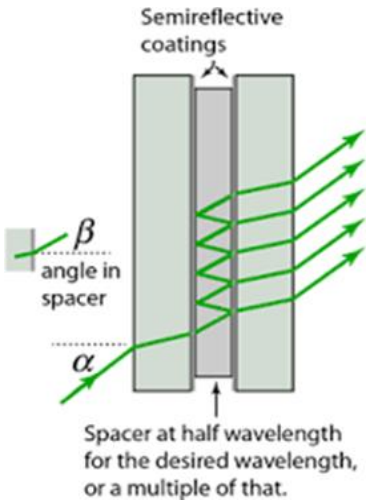
- Simple in construction
- Cheaper
- Selection of the filter is easy.

Demerits: -

- Less accurate
- Band pass (bandwidth) is more (20 -30 nm) i.e. if we have to measure at 400nm; we get radiation from 370-430nm. Hence less accurate results are obtained.

2) Interference filters:

- Works on the interference phenomenon, causes rejection of unwanted wavelength by selective reflection.
- It is constructed by using two parallel glass plates, which are silvered internally and separated by thin film of dielectric material of different (CaF2, SiO, MgF2).



refractive index. These filters have a band pass of 10-15nm with peak transmittance of 40-60%.

Merits:

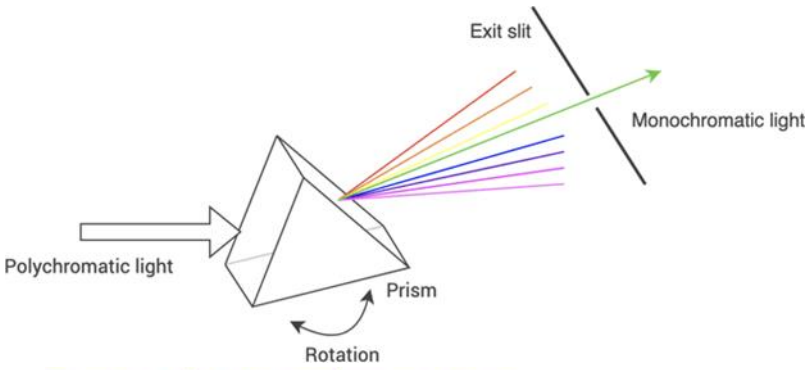
- It provides greater transmittance and narrower band pass (10 -15 nm) as compare to absorption filter.
- Greater the bandwidth definition, lower is the percentage transmittance through that filter.
- Inexpensive
- Additional filters can be used to cut off undesired wavelength.

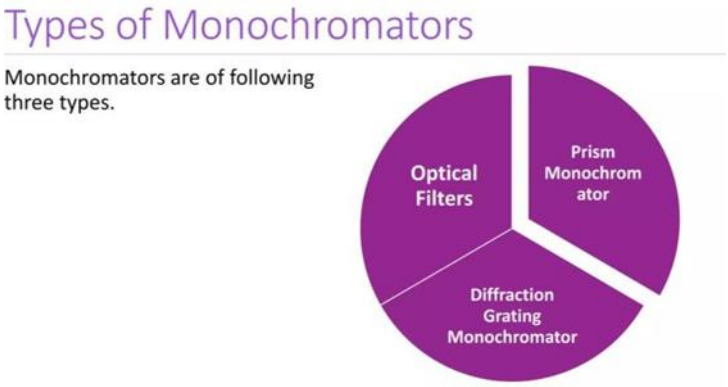
Demerits: -

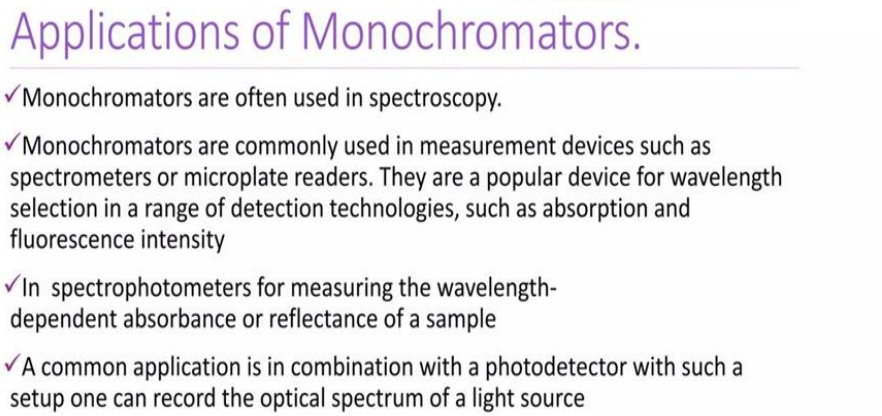
- Peak transmission is low, and becomes so when additional filters are used to cut off undesired wavelength.
- The band pass is only 10-15nm and hence higher resolution obtained with monochromator or gratings cannot be achieved.

Monochromator:

- Monochromator are better and more efficient than filters in converting polychromatic light or heterochromatic light into monochromatic light.
- Mechanical construction of monochromator for UV, visible and IR radiation is similar in that all of them employ slits, lenses, mirrors, windows, and gratings or prisms.
- It provides greater transmittance and narrower band pass (10 -15 nm) as
- Monochromator are primarily designed for spectral scanning, i.e. a process of continuously varying the radiation wavelength over a considerable range.







Types of lamps used in an uv-visible spectroscopy:

1)Deuterium Lamp:

Its wavelength range is 190nm - 370nm, and it is also known as a D2 lamp. Because of its high temperature behaviour, normal glass housing is insufficient, necessitating the use of quartz, MgF2, or other materials. A typical of about 1000 hours. In order cover the entire UV and visible light wavelength, a UV / Vis spectrophotometer will design a deuterium lamps with halogen lamps.

2)**Halogen lamp:** Halogen lamps are also known as tungsten or quartz lamps, and their wavelength range is in the visible light region, ranging from 320nm to 1100 nm. If the

instrument is only equipped with a halogen lamp, it can only measure visible light. The average nm. If the instrument is only equipped with a halogen lamp, it can only measure visible light.

3)LED Lamp:-

Because LED lamps produce a single wavelength of light, they do not require a monochromator. It has a very long life. The bandwidth of an LED light source varies little and is stable. A low-cost light source is an LED lamp.

Detectors used in uv-vis spectroscopy

Detector is defined as the device that identifies and records the signals produced by the sample components.

An ideal detector has:-

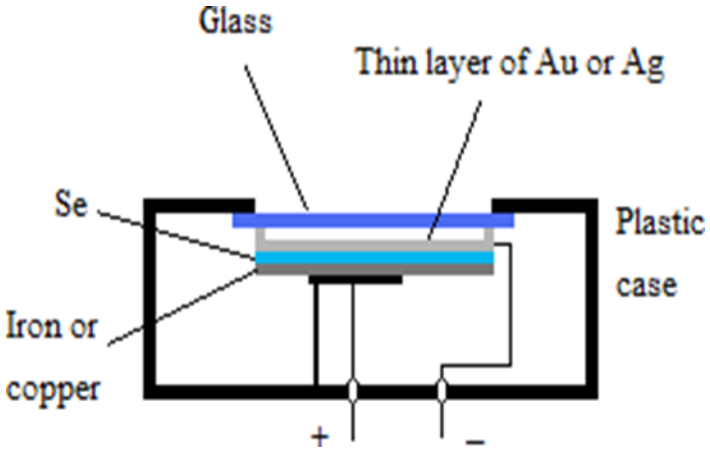
- Adequate sensitivity – range 10-18 to 10-15 g analyte/sec
- Good stability and reproducibility
- Good linearity
- A temperature range from room temperature to at least 4000C.
- A short response time that is independent of flow rate.
- High reliability and ease of use.
- Nondestructive of sample.

Detectors used in UV-Visible Spectroscopy mainly includes:-

- Barrier layer cell or photo voltaic cell
- Photo tubes or photo emissive cells
- Photo multiplier tubes and
- Photo diode array detector

Photo Voltaic Cell

Also known as barrier layer cell



Advantages:

- Simple in design and does not require any external power supply.
- Cheapest and inexpensive of all UV detectors.

Disadvantages:

- Amplification of the detector is not possible.
- Lesser response of the detector with light other than blue and red light.

Photo Emissive Cells

Also known as Photo tubes.

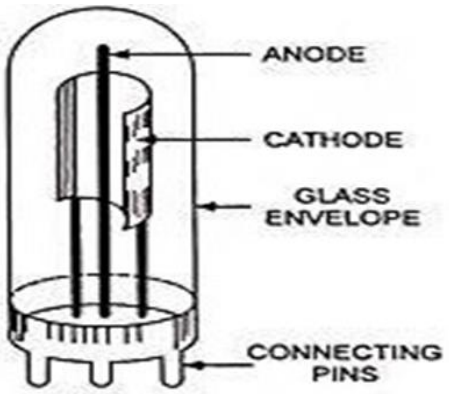


Fig. 25.46 *Photoemissive Tube*

Advantages:

• Better sensitivity than photovoltaic cell.

• Amplification of the signal is possible.

Photo Multiplier Tubes

The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons. In a vacuum tube, a primary photo-cathode is fixed which receives radiation from the sample.

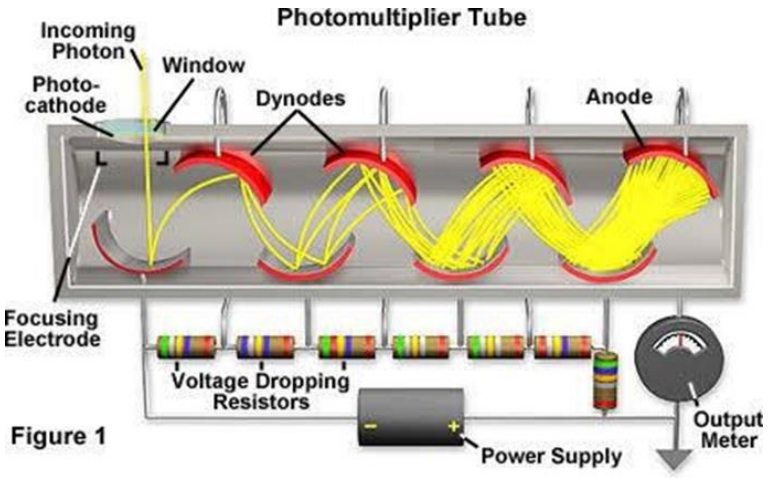


Figure 1

- Near the last dynode is fixed an anode or electron collector electrode.
- Photo-multiplier is extremely sensitive to light and is best suited where weaker lower addition is received.

Instrument Design:

•Depending upon the monochromator (filters or dispersing device) used to isolate and transmit a narrow beam of radiant energy from the incident light determines whether the instrument is classified as Photometer or a Spectrophotometer.

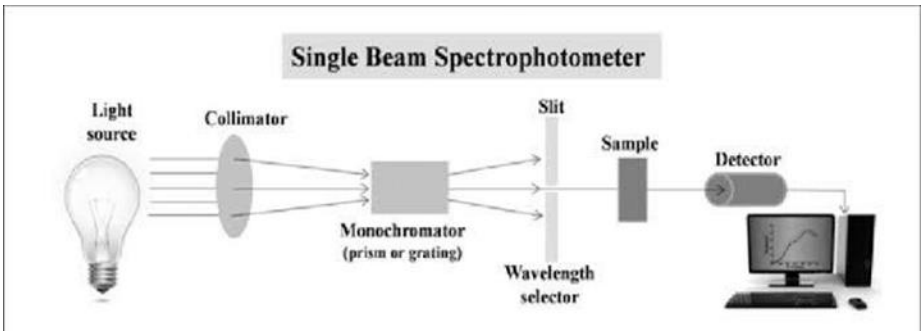
•Spectrophotometers used here detects the percentage transmittance of light radiation, when light of certain intensity and frequency range is passed through the sample.

Single Beam Spectrophotometer:

•Light from the source is carried through lens and /or through aperture to pass through a suitable filter.

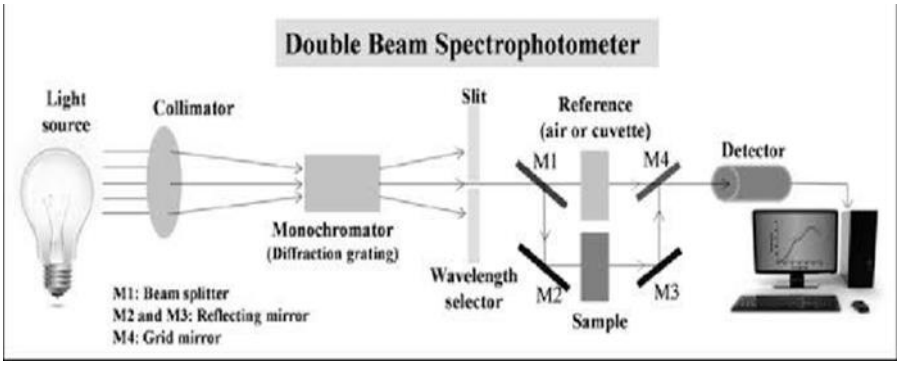
•The type of filter to be used is governed by the colour of solution.

•The sample solution to be analysed is placed in cuvette.



Double Beam UV-VIS spectrophotometer:

- Double beam instrument is the one in which two beams are formed in the space by a U-shaped mirror called as beam splitter or beam chopper.
- Single beam Chopper is a device consisting of a circular disc. One third of the disc is opaque and one third is transparent, remaining one third is mirrored.
- It splits the monochromatic beam of light into two beams of equal intensities.



Advantages: of single and double beam spectrophotometer.

Single beam: – Simple in construction, Easy to use and economical.

Double beam:• It facilitates rapid scanning over wide region.

•Fluctuations due to radiation source are minimised.

•It doesn't require adjustment of the transmittance at 0% and 100% at each wavelength.

Disadvantages:

•Any fluctuation in the intensity of radiation sources affects the absorbance.

•Continuous spectrum is not obtained.

The function of uv vis spectroscopy:-

•A UV / Vis spectrophotometer analyses the chemical structure of a substance using visible and ultraviolet light.

•A spectrophotometer is a type of spectrometer used to measure the intensity of light, which is proportional to the wavelength.

Application of uv-vis spectroscopy

1.DNA and RNA analysis

2.Pharmaceutical analysis

3.Bacterial culture

4.Beverage analysis

The advantages of UV-vis spectroscopy:-

•An Ultraviolet - Visible Light Spectrophotometer (UV-Vis spectrophotometer) has the advantage of being quick to analyse and simple to use

•An UV / Vi spectrophotometer is used in astronomy research to help scientists analyse galaxies, neutron stars, and other celestial objects.

•A UV spectrum can provide detailed information about an astronomical object's velocity and elements.

•UV / Vis spectrophotometers introduced high-tech spectral analysis capabilities to other industries.

•In the food industry, for example, the quality and safety of foods are two of the most important factors for consumers.

The Disadvantages Of Uv-Vis Spectroscopy:-

1)Stray light from UV-Vis spectrophotometers caused by faulty equipment design and other factors may influence spectra measurement accuracy of absorption in substance, because stray light reduces linearity range and thus the absorbency of substance measured.

2)Further more, the spectrometer's electronic circuit design and detector circuit quality will affect the amount of noise that is coupled into the measurement signal, affecting measurement accuracy and decreasing the instrument's sensitivity.

Example:-

•To perform the assay of Paracetamol tablet by UV spectrophotometer at specific absorbance.

Objectives:-

1.To understand the application of A 1% 1cm by spectrophotometer.

2.To develop skill of handling of UV- visible spectrophotometer.

3.To perform assay calculations.

4.To find out percentage purity of paracetamol tablet.

Procedure:-

1.Weigh and powder 20 tablets.

2.Weigh accurately a quantity of the powder equivalent to about 0.15 g of Paracetamol.

3.Take clean 200ml volumetric flask and place clean funnel on it.

4.Transfer carefully weighed quantity of powder into funnel.

5.Add 50 ml of 0.1M sodium hydroxide into funnel so as to all powder gets Transferred to volumetric Flask.

6.ilute the resulting mixture with 100 ml of distilled water and shake for 15 Minutes.

7.Finally add sufficient quantity of water to produce 200ml.

8.Filter resulting solution from Whatman Filter.

9.Take 10 mL of filtrate and dilute to 100 mL with water.

10.Take 10 mL resulting solution in 100 mL volumetric flask and add 0.1M Sodium Hydroxide.

11.Adjust the volume of solution using distilled water and mix.

12.Measure the absorbance of the resulting solution at the maximum at about 257nm.

13.Calculate the content of, $C_8H_9NO_2$ taking 715 as the value of A(1%, 1 cm) at The maximum at about 257nm.

CONCLUSION

The review paper contains all information about UV visible spectroscopy, its principle, theory, Instrumentation, advantages, Disadvantage's & its applications. The identification of impurities are carried out by using UV visible spectroscopy more accurately & UV visible spectroscopy is a very crucial spectroscopy.

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