UNIT – 1
PART – A
UV-Visible Spectroscopy

The wavelength range of UV radiation is 200 nm- 400 nm. There are mainly two types of UV region.
1. 200 nm - 400 nm that is called near ultraviolet region.
2. Below 200 nm that is called far ultraviolet region.

The wavelength of visible radiation is 400 nm- 800 nm.

Wavelength in UV and visible region is expressed in nanometers or in angstroms. Absorption is expressed in terms of wave number (cm^{-1}).

Absorption spectra arise from transition of electron or electrons within a molecule from a lower electronic energy level to a higher electronic energy level. Ultraviolet emission spectra arise from the reverse types of transition. For the radiation to cause electronic excitation, it must be in the UV region of the EMR spectrum.

Radiation in this region is of sufficient energy to cause electronic transition of outer valence electrons.

Both organic and inorganic species exhibit electronic transitions in which outermost or bonding electrons are promoted to higher energy levels. Electronic transitions are associated with vibrational as well as rotational transitions.

A compound appears coloured if it selectively absorbs light in the visible region. The main function of absorbed energy is to raise the molecule from ground energy state (E_0) to higher excited energy state (E_1). The difference is given by:

$$\Delta E = E_1 - E_0 = h\nu = hc/\lambda$$

ΔE depends upon how tightly the electrons are bound in the bonds and accordingly, absorption will occur in UV or visible range, for example; If the electrons of a molecule are tightly bound as in compounds containing sigma bonds (e.g. saturated compounds) no light of region will be absorbed. The light of UV region will only be absorbed and hence compound appears

colourless.

If the electrons of molecule are loosely bound as in unsaturated compound. Such absorption may occur in visible region and substance will appear as coloured.

Energy absorbed in the ultraviolet region produces change in the electronic energy of the molecule that is resulting from transitions of valance electrons in the molecule. There are three types of electrons in organic molecules.

a) σ (sigma) electrons- they are found in saturated systems like alkane. They require large amount of energy for their excitation and hence do not show absorption in UV region. Their absorption band is appeared in vacuum UV region. Hence, compounds containing σ- bonds do not absorb in near UV region. For example saturated hydrocarbons are transparent in near UV region and thus they can be used as solvents.

b) ∏ (pie) electrons- they are found in multiple bonds. They are generally mobile electrons. Since ∏- bonds are weak bonds, the energy produced by UV radiation can excite ∏- electrons to higher energy levels.

c) n (non-bonding) electrons- valance electrons which do not participate in chemical bonding in molecule are called as non bonding electrons or n-electrons. These are located principally in atomic orbital of N, O, S and halogens(X) as a lone pair of electrons. They can be excited by UV radiation.

Principle of UV-Visible Spectroscopy:

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. When the matter absorbs the light, it undergoes excitation and de-excitation, resulting in the production of a spectrum.

When matter absorbs ultraviolet radiation, the electrons present in it undergo excitation. This causes them to jump from a ground state (an energy state with a relatively small amount of energy associated with it) to an excited state (an energy state with a relatively large amount of energy associated with it). It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it.

Beer's and Lambert's Law:

When a light passes through absorbing medium at right angle to the plane of surface or the medium or the solution, the rate of decrease in the intensity of the transmitted light decreases exponentially as the thickness of the medium increases arithmetically.

Accordingly, Lambert's law can be stated as follows:

"When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of light."

Mathematically**,** the Lambert's law may be expressed as follows.

$$- dI / dt \; \alpha \; I$$

$$-dI / dt = KI \qquad \ldots\ldots\ldots\ldots(1)$$

Where I = intensity of incident light

t = thickness of the medium

K= proportionality constant

By integration of equation (1), and putting $I=I_0$ when t=0,

$$I_0/ I_t = kt \quad or \quad I_t= I_0 \, e^{-kt}$$

Where, I_0 = intensity of incident light

I_t = intensity of transmitted light

k = constant which depends upon wavelength and absorbing medium

used

By changing the above equation from natural log, we get,

$$I_t = I_0 \, e^{-Kt} \qquad \ldots\ldots\ldots\ldots(2)$$

Where K = k/ 2.303

So, $\quad I_t = I_0 \, e^{-0.4343 \, kt}$

$$I_t = I_0 10^{-Kt} \qquad \ldots\ldots\ldots\ldots(3)$$

Beer's law may be stated as follows:

"Intensity of incident light decreases exponentially as the concentration of absorbing medium increases arithmetically."

The above sentence is very similar to Lambert's law. So,

$$I_t = I_0\, e^{-k'c}$$

$$I_t = I_0\, 10^{-0.4343\, k'c}$$

$$I_t = I_0\, 10^{K'c} \qquad \ldots\ldots\ldots(4)$$

Where k' and K'= proportionality constants

c = concentration

By combining equation (3) and (4), we get,

$$I_t = I_0\, 10^{-act}$$

$$I_0 / I_t = 10^{act}$$

Where, K and K' = a or ε

c = concentration

t or b = thickness of the medium

$$\log I_0 / I_t = \varepsilon bc \qquad \ldots\ldots\ldots(5)$$

Where ε = absorptivity, a constant dependent upon the λ of the incident radiation and nature of absorbing material. The value of ε will depend upon the method of expression of concentration.

The ratio I_0 / I_t is termed as transmittance T, and the ratio $\log I_0 / I_t$ is termed as absorbance A. formerly, absorbance was termed as optical density D or extinction coefficient E. the ratio I_0 / I_t is termed as opacity. Thus,

$$A = \log I_0 / I_t \qquad \ldots\ldots\ldots(6)$$

From equation (5) and (6),

$$A = \varepsilon bc \qquad \ldots\ldots\ldots(7)$$

Thus, absorbance is the product of absorptivity, optical path length and the concentration of the solution.

The term $E^{1\%}_{1\,cm}$ or $A^{1\%}_{1\,cm}$ refers to the to the absorbance of 1 cm layer of the solution whose concentration is 1 % at a specified λ.

According to equation (7),

$$A = \log I_0 / I_t$$

Transmittance T is a ratio of intensity of transmitted light to that of the incident light.

$$T = I_0 / I_t$$

The more general equation can be written as follows:

$$A = \log I_0 / I_t = \log 1/ T = - \log T = abc = \varepsilon bc$$

INSTRUMENTATION:

Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;
1. Sources (UV and visible)
2. filter or monochromator
3. Sample containers or sample cells
4. Detector

1. Radiation source:

It is important that the power of the radiation source does not change abruptly over its wavelength range.
The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon
Both Deuterium and Hydrogen lamps emit radiation in the range 160 - 375 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 350 nm.

Various UV radiation sources are as follows
a. Deuterium lamp
b. Hydrogen lamp
c. Tungsten lamp
d. Xenon discharge lamp
e. Mercury arc lamp

Various Visible radiation sources are as follows
a. Tungsten lamp
b. Mercury vapour lamp
c. Carbonone lamp

2. filters or monochromators:

All monochromators contain the following component parts;
• An entrance slit
• A collimating lens
• A dispersing device (a prism or a grating)

• A focusing lens
• An exit slit
Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

3. sample containers or sample cells:
A variety of sample cells available for UV region. The choice of sample cell is based on
a) the path length, shape, size
b) the transmission characteristics at the desired wavelength
c) the relative expense

The cell holding the sample should be transparent to the wavelength region to be recorded. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm. The thickness of the cell is generally 1 cm. cells may be rectangular in shape or cylindrical with flat ends.

4. Detectors:
In order to detect radiation, three types of photosensitive devices are
a. photovoltaic cells or barrier- layer cell
b. phototubes or photoemissive tubes
c. photomultiplier tubes

Photovoltaic cell is also known as barrier layer or photronic cell. It consists of a metallic base plate like iron or aluminium which acts as one electrode. On its surface, a thin layer of a semiconductor metal like selenium is deposited. Then the surface of selenium is covered by a very thin layer of silver or gold which acts as a second collector tube.

When the radiation is incident upon the surface of selenium, electrons are generated at the selenium- silver surface and the electrons are collected by the silver. This accumulation at the silver surface creates an electric voltage difference between the silver surface and the basis of the cell.

Phototubes are also known as photo emissive cells. A phototube consists of an evacuated glass bulb. There is light sensitive cathode inside it. The inner surface of cathode is coated with light sensitive layer such as potassium oxide and silver oxide.

When radiation is incident upon a cathode, photoelectrons are emitted. These are collected by an anode. Then these are returned via external circuit. And by this process current is amplified and recorded.

The photomultiplier tube is a commonly used detector in UV spectroscopy. It consists of a photo emissive cathode (a cathode which emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them) and an anode.

A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first dynode (which is 90V more positive than the cathode). The electrons strike the first dynode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. Eventually, the electrons are collected at the anode. By this time, each original photon has produced 106 - 107 electrons. The resulting current is amplified and measured.

Photomultipliers are very sensitive to UV and visible radiation. They have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation.
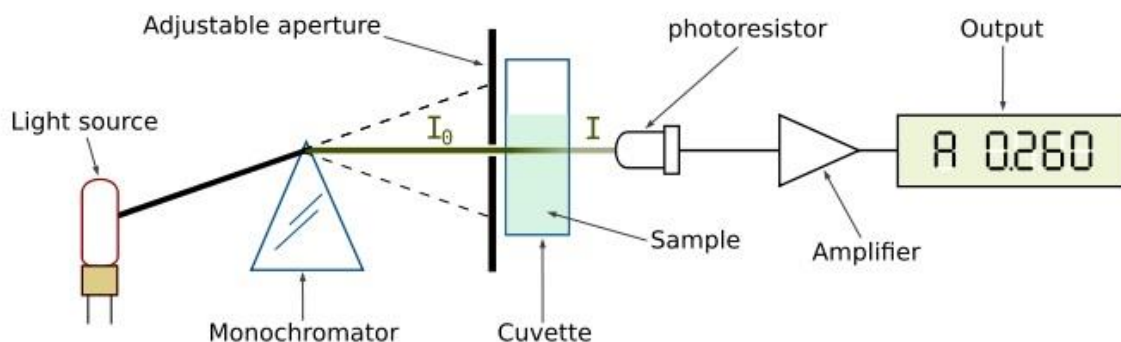
Instrumentation

Representative Diagram of a Simple UV-Visible Spectrophotometer
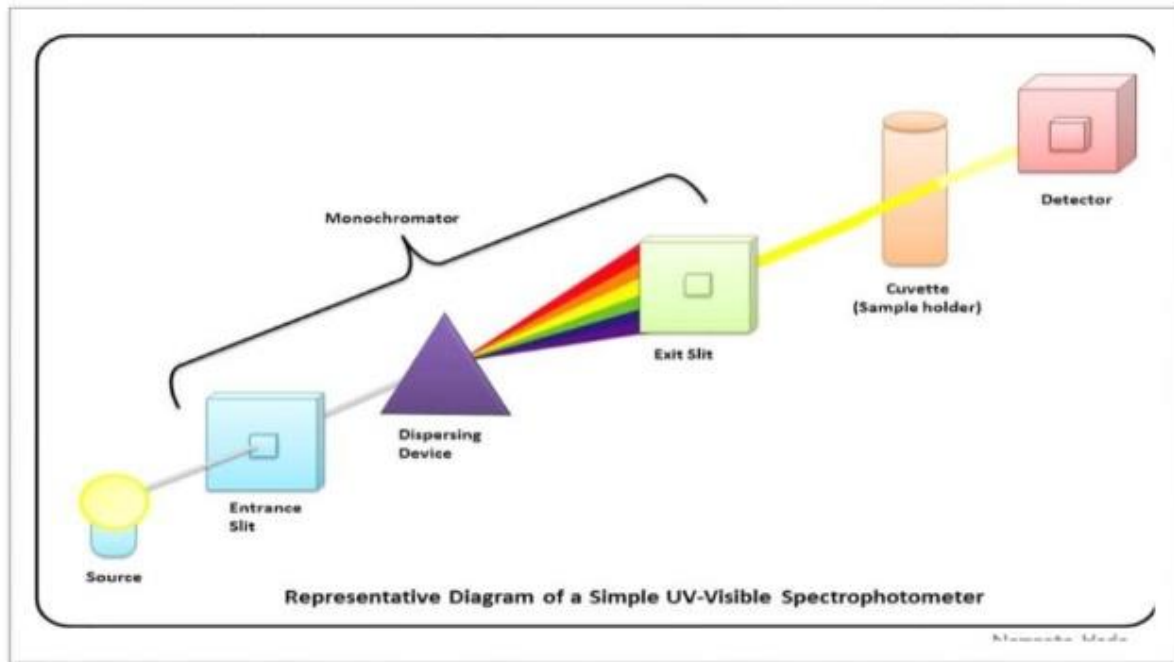
23

Single Beam Spectrophotometer:

Single beam spectrophotometer is an analytical instrument in which all the light waves coming from the light source passes through the sample. Therefore, the measurements are taken as the intensity of light before and after the light pass through the sample. These single beam spectrophotometers are more compact and optically simpler than double beam spectrophotometers. And also these instruments are less expensive.
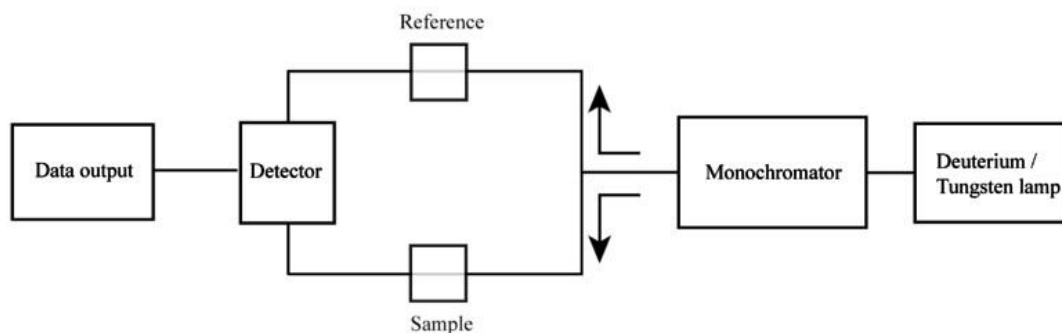

Single Beam Spectrophotometer

The sensitivity of detection of the light beam after it passes through the sample is high since it uses a non-split light beam (therefore, high energy exists throughout). Single beam spectrophotometers are available in analysis at visible and ultraviolet wavelength ranges.

A single beam spectrophotometer measures the concentration of an analyte in a sample by measuring the amount of light absorbed by that analyte. Here, the Beer Lambert Law comes into operation. This law states that the concentration of an analyte is directly proportional to the absorbance.

Double Beam Spectrophotometer:

Double beam spectrophotometer is an analytical instrument in which the light beam coming from the light source splits into two fractions. One fraction acts as the reference (the reference beam) while the other fraction passes through the sample (sample beam). As a result, the reference beam does not pass through the sample.

The Pathway of Light Beam in a Double Beam Spectrophotometer
The sample beam can measure the absorbance of the sample. The reference beam can measure the absorption (the sample beam can be compared with the reference beam). Therefore, the absorption is the ratio between the sample beam (after passing through the sample) and a reference beam. A spectrophotometer has a monochromator that isolates the desired wavelengths from a light beam. The reference beam and sample beam recombine before moving to the monochromator. Consequently, this avoids or compensate the electronic and mechanical effects on both sample and reference beams, equally.

What is the Difference Between Single Beam and Double Beam Spectrophotometer?

Single Beam vs Double Beam Spectrophotometer	
Single beam spectrophotometer is an analytical instrument in which all the light waves coming from the light source passes through the sample.	Double beam spectrophotometer is an analytical instrument in which the light beam coming from the light source splits into two fractions.
Light Beam	
Single beam spectrophotometer uses a non-split light beam.	Double beam spectrophotometer uses a light beam that is split into two fractions before passing through the sample.
Measurement	
The measurements taken from single beam spectrophotometers are less reproducible because a single light beam is used.	The measurements taken from double beam spectrophotometers are highly reproducible because electronic and mechanical effects on both sample and reference beams are equal.

Summary – Single Beam vs Double Beam Spectrophotometer:

A spectrophotometer is an instrument that analyses the components of a solution by observing the capability to absorb light. There are two main types of spectrophotometers; single beam and double beam spectrophotometer. The difference between single beam and double beam spectrophotometer is that, in single beam spectrophotometer, all the light waves pass through the sample whereas, in double beam spectrophotometer, the light beam splits into two parts and only one part passes through the sample.

Applications:

1. Detection of Impurities
- It is one of the best methods for determination of impurities in organic molecules.
- Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material.
- By also measuring the absorbance at specific wavelength, the impurities can be detected.
2. Structure elucidation of organic compounds
- It is useful in the structure elucidation of organic molecules, such as in detecting the presence or absence of unsaturation, the presence of hetero atoms.
3. UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation.
4. UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation thus used in qualitative determination of compounds. Identification is done by comparing the absorption spectrum with the spectra of known compounds.
5. This technique is used to detect the presence or absence of functional group in the compound. Absence of a band at particular wavelength regarded as an evidence for absence of particular group.
6. Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.
7. Many drugs are either in the form of raw material or in the form of formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at specific wavelength.
8. Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.
9. UV spectrophotometer may be used as a detector for HPLC.