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## 1. Learning Outcomes

After studying this module, you shall be able to:

- Understand what is electromagnetic radiation.
- Analyze the electromagnetic spectrum .
- Understand concept of UV-Visible spectroscopy.
- Learn about the possible electronic transitions.
- Learn about the working of UV-spectrophotometer.

## 2. Introduction

The most challenging task of a chemist is to determine the chemical structure of an unknown compound. There are many ways by which we can identify the unknown substance. A person can use physical methods such as boiling point, melting point, spectroscopy as well as chemical methods such as functional group testing and others to determine the structure of compounds. Spectroscopy is one of the best methods to identify a substance, which may include UV, IR, NMR, Raman and others. Here we will discuss about the various aspects of different spectroscopic techniques and more specifically about UV-spectroscopy and its uses.

## 3. Electromagnetic Radiation and Spectroscopy

Electromagnetic spectrum covers a wide range of electromagnetic radiations, ranging from γ-rays having wavelength which are the order of a fraction of an angstrom, to radio waves having wavelength in meters or kilometers. The arrangement of all types of radiations in the order of their increasing wavelength or decreasing frequencies is known as electromagnetic (EM) spectrum. The electromagnetic spectrum includes radio and TV waves, microwaves, infrared, visible light, ultraviolet, X-rays, γ-rays, and cosmic rays, as shown in the **Figure 1**.

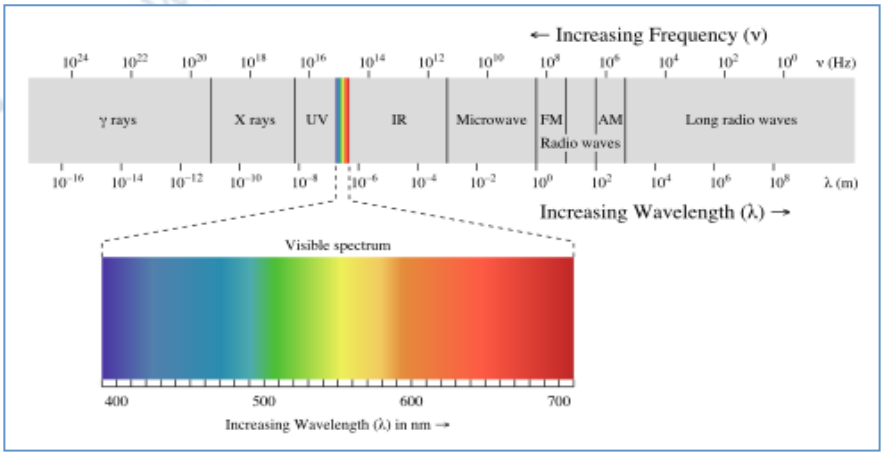


**Figure 1: Electromagnetic spectrum**

**Spectroscopy:** Spectroscopy is the study of interaction of electromagnetic radiations with matter. Electromagnetic radiations can interact with matter in various ways. Each interaction gives us insights about certain properties of the matter and use of electromagnetic radiations of different energies can give different information about the matter under study.

It is the motion of electrically charged particles that give rise to electromagnetic radiations. There are various forms of electromagnetic radiation e.g. radio waves, X-rays, gamma rays, infrared, visible, ultraviolet etc. All the types of radiations travel with the same velocity but differ from each other in terms of frequency and wavelength. They do not require any medium for their propagation and can travel through empty space as well as through air and other substances. Each type of electromagnetic radiation has a dual nature- wave like nature and particle like nature. The particle nature has been established by the fact that the energy of particular radiation occurs in discrete packets or quanta known as photons. Each photon contains a certain amount of energy. The different types of radiation are defined by the amount of energy found in the photons. The energy associated with particular electromagnetic radiation is directly proportional to its frequency. The photons with the highest energy correspond to the shortest wavelengths.

## 4. Absorption of Electromagnetic Radiations by Organic Molecules

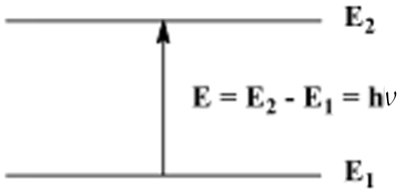
When electromagnetic radiations are passed through an organic compound, some of the part gets absorbed, while the remaining is transmitted. The absorption of energy can bring about translational, rotational or vibrational motion, electronic transition or ionization of the molecules depending upon the frequency of the electromagnetic radiation they receive (Table 1).

| Types of Radiation | Spectral method | Energy Change involve |
|---|---|---|
| Gamma ray | Gamma spectroscopy | Ionisation |
| X-rays | X-ray Spectroscopy | Inner electrons |
| Ultraviolet | UV-Spectroscopy | Middle and valence shell electrons |
| Visible | Visible Spectroscopy | Valence shell electrons |
| Infrared | IR-Spectroscopy | Molecular Vibration and rotation |
| Microwave | ESR-Spectroscopy | Molecular rotation and spin orientation |
| Radio | NMR-Spectroscopy | Spin orientation of Nuclei (NMR) |

**Table 1: Energy changes depending on the type of radiation**

The energy required for these transitions is quantized. Excited molecules are unstable and quickly come back to the ground state by releasing the energy they had received as electromagnetic radiation.

The wavelength and intensity of the electromagnetic radiation absorbed or emitted can be recorded with the help of spectrometer to get a spectrum. The energy required for the transition (E) from a state of lower energy ($E_1$) to state of higher energy ($E_2$) is exactly equivalent to the energy of electromagnetic radiation that causes transition.




$$E_1 - E_2 = E = h\nu = hc/\lambda$$

Where E is energy of electromagnetic radiation being absorbed, h is the universal Planck's constant, 6.624 x $10^{-27}$ J sec and ν is the frequency of incident light in cycles per second (cps or hertz, Hz), c is velocity of light 2.998 x $10^{10}$ cm $s^{-1}$ and λ = wavelength (cm)

Higher is the frequency, higher would be the energy and on the other side, longer is the wavelength, lower would be the energy. As we move from Gamma rays to ultraviolet region to infrared region and then radio frequencies, we are gradually moving towards regions of lower energies.

However, almost all the parts of electromagnetic spectrum are used for understanding the matter, in organic chemistry we are mainly concerned with energy absorption from only ultraviolet and visible, infrared, microwave and radiofrequency regions.

## 5. Ultraviolet–Visible Spectroscopy

UV-Visible spectroscopy deals with the study of the electronic transitions of molecules as they absorb light in the UV (190-400 nm) and visible regions (400-800 nm) of the electromagnetic spectrum. The absorption of ultraviolet or visible radiation lead to transition among electronic energy levels, hence it is also often called electronic spectroscopy.

As a rule, the energetically favored electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The resulting species is said to be in an excited state. The wavelengths at which absorption occurs, together with the degree of absorption at each wavelength is recorded by **optical spectrometer**. A spectrum is obtained as a result. It commonly provides the knowledge about π-electron systems, conjugated systems, aromatic compounds and conjugated non-bonding electron systems etc.

## 6. Types of Electronic Transitions

The ground state of an organic molecule contains valence electrons in three principal types of molecular orbitals, namely -sigma (σ) orbitals, pi (π) orbitals and filled but nonbonding orbitals (n).

Both σ and π orbitals are formed from the overlap of two atomic or hybrid orbitals. Each of these molecular orbitals therefore has an antibonding σ* or π* orbital associated with it. An orbital containing *n* electrons does not have an antibonding orbital because it is not formed from two orbitals. Sigma bonding orbitals have lower energy than π bonding orbitals, which in turn have lower energy than non-bonding orbitals. In the Electronic transitions, promotion of an electron from one of the three ground states (σ, π, or n) to one of the two excited states (σ∗, or π∗) takes place. As a result, there are six possible transitions- σ to σ∗, σ to π∗, π to π∗, π to σ∗, n to π∗, n to σ∗. The most commonly observed transitions and their relative energies are summarized in **Figure 2**. The exact energy differences between the orbitals depend on the nature of atoms present and the nature of the bonding system.

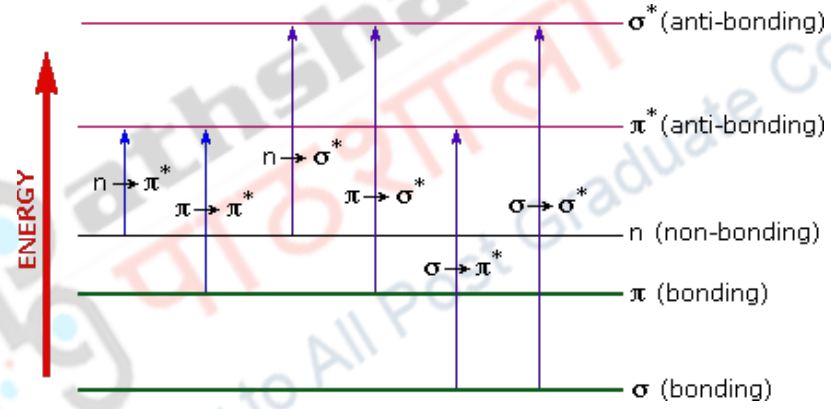


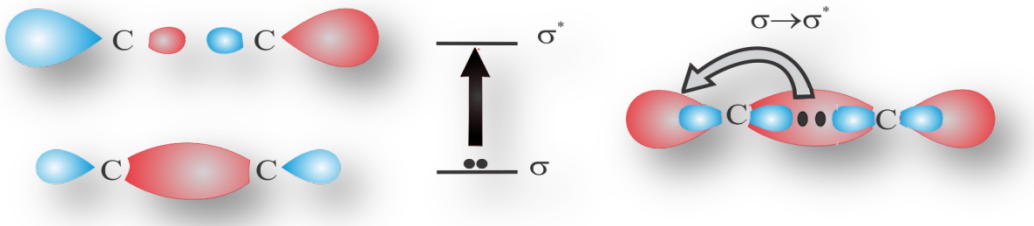

**Fig 2: Electronic Transitions in UV/VIS spectroscopy**

You can see, in each possible case, an electron is excited from a low energy, ground state orbital into a higher energy, excited state anti-bonding orbital (**Figure 2**). Each transition requires a defined amount of energy. The larger the gap between the energy levels, the greater the energy required to promote the electron to the higher energy level, resulting in electromagnetic radiation of higher frequency, and therefore shorter wavelength, being absorbed. The important modes of electronic transitions are described here.

**1) σ to σ∗**

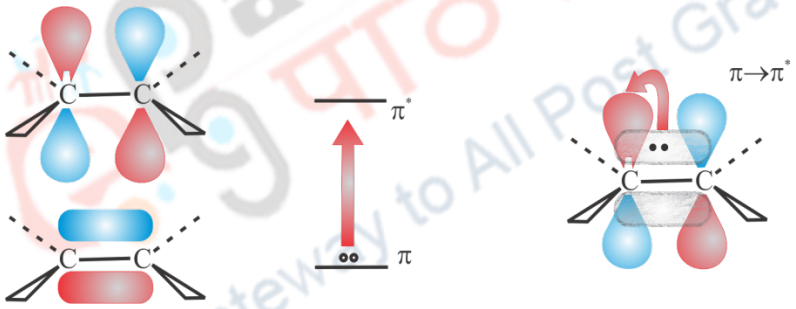
A transition of electrons from a bonding sigma orbital to the antibonding sigma orbital is designated as σ to σ∗ transition. These are high energy transitions as σ bonds are generally very strong. Thus these transitions involve very short wavelength ultraviolet light (< 150 nm) and usually fall outside the range of UV-visible spectrophotometers (200-800 nm). Alkanes can only undergo σ to σ* transitions. Methane and ethane undergo σ → σ* transitions with an absorbance maximum at 122 and 135 nm, respectively. Study of such transitions is usually done in an

evacuated spectrophotometer (< 200 nm) since oxygen present in air absorbs strongly at 200 nm and below. Similarly nitrogen absorbs at ~150 nm and below.



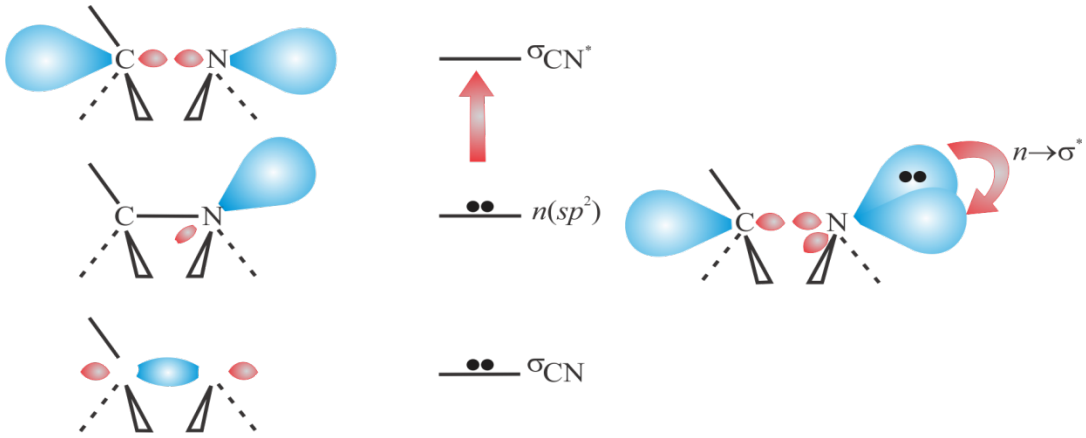

**2) π to π∗**

The transition of an electron from a π bonding orbital to a π∗ antibonding orbital is designated as π to π∗ transition. These types of transitions take place in compounds containing one or more unsaturated groups like simple alkenes, carbonyl, aromatics, nitriles, nitro etc. These transitions require lesser energy than n to σ∗ transitions. In non-conjugated alkenes, this type of transition occurs in the range 170-190 nm e.g. ethane shows absorption maxima at 171 nm. Similarly, π to π∗ transition in the range of 180-190 nm occurs in non-conjugated carbonyl compounds e.g. acetone shows absorption maxima at 188 nm.



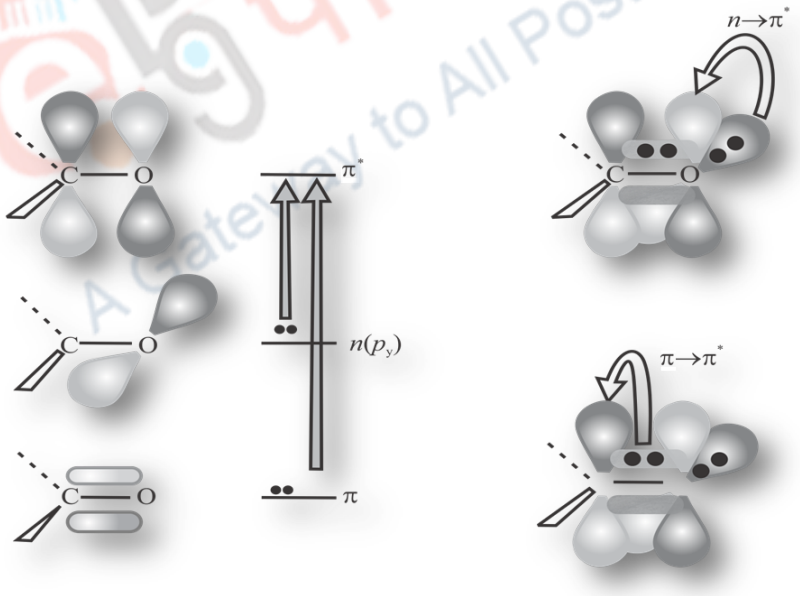

**3) n to σ∗**

The transition of an electron from a non-bonding orbital to the antibonding sigma orbital is designated as n to σ∗ transition. Saturated compounds containing atoms with lone pairs (non-bonding electrons) like saturated alcohols, amines, halides, ethers etc are capable of showing n to σ∗ transitions. Energy required for these transitions is usually less than σ to σ∗ transitions. Such compounds absorb light having wavelength in the range 150-250 nm. For example the absorption maxima for water, methyl alcohol, methyl chloride and methyl iodide are 167 nm, 174 nm, 169 nm and 258 nm, respectively.




**4) n to π∗**

The transition of an electron from a non-bonding orbital to a π∗ antibonding orbital is designated as n to π∗ transition. This transition involves the least amount of energy in comparison to all other transitions and therefore gives rise to an absorption band at longer wavelength.
Saturated carbonyl compounds show two types of transitions, low energy n to π∗ (270-300 nm ) and high energy π to π* (180-190 nm). The transition n to π∗ is of lowest energy but is of low intensity as it is symmetry forbidden. Thus the most intense band for these compounds is always due to π to π* transition.



## 7. Selection Rule

Some electronic transitions, which are otherwise theoretically possible, are generally not observed in the UV/VIS spectroscopy. Therefore there are some restrictions which govern the observable transitions.

1. Transitions, which involve change in the spin quantum number of an electron during the transitions do not occur, i.e. singlet-triplet transitions are not allowed.

2. Transitions between orbitals of different symmetry do not occur. For example, transition n to $\pi*$ is forbidden because the symmetry of n and $\pi*$ do not match.

## 8. UV-Spectrophotometer

Spectrophotometer is a kind of spectrometer, which measures the transmittance or absorbance of a sample as a function of wavelength, when light of certain intensity and frequency range is passed through the sample. Unlike a spectrometer (which is any instrument that can measure the properties of light over a range of wavelengths), a spectrophotometer measures only the *intensity* of light as a function of its wavelength. The key components of a spectrophotometer are:

- Light source
- Monochromator
- Sample area
- Detector and Recorder

### 8.1 Light Source

The most suitable sources of light are

1. Deuterium lamp which emits the light in the UV-region (160-375 nm)
2. Tungsten lamp or tungsten-halogen lamp which emits radiation in the Visible and near infrared regions (350-2500 nm)
3. Xenon arc lamp which emits radiation in the range 190-800 nm
4. Light emitting diodes (LED) which emits radiation in the visible range 400-800 nm.

The instruments automatically swap lamps when scanning between the UV and UV-VIS regions.

### 8.2 Monochromator

The main function of the monochromator is to disperse the beam of light obtained from the primary source, into its component (**Figure 3**). The principle components of monochromator are

- An entrance slit
- A collimating lens
- A dispersing device
- A focusing lens
- An exit slit

The radiation emitted from the primary source (polychromatic radiation) enters the monochromator through the entrance slit. The beam is collimated and then strikes the dispersing element (Prism or grit) at a particular angle. Two types of dispersion devices viz. prisms and holographic gratings (**Figure 4**) are commonly used in UV-visible spectrophotometers.

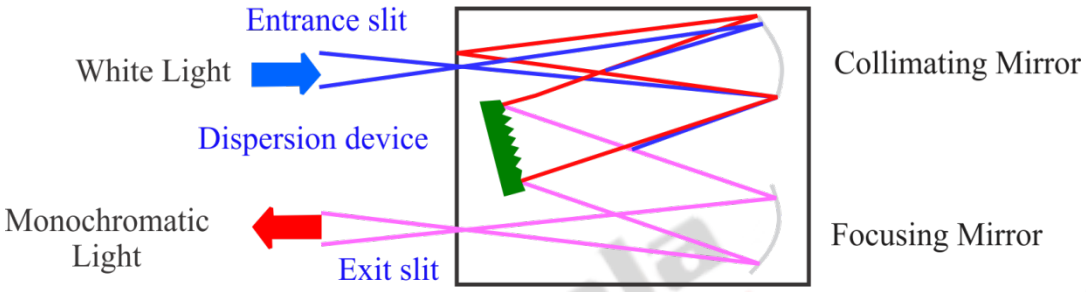



**Figure 3: Functioning of Monochromator**

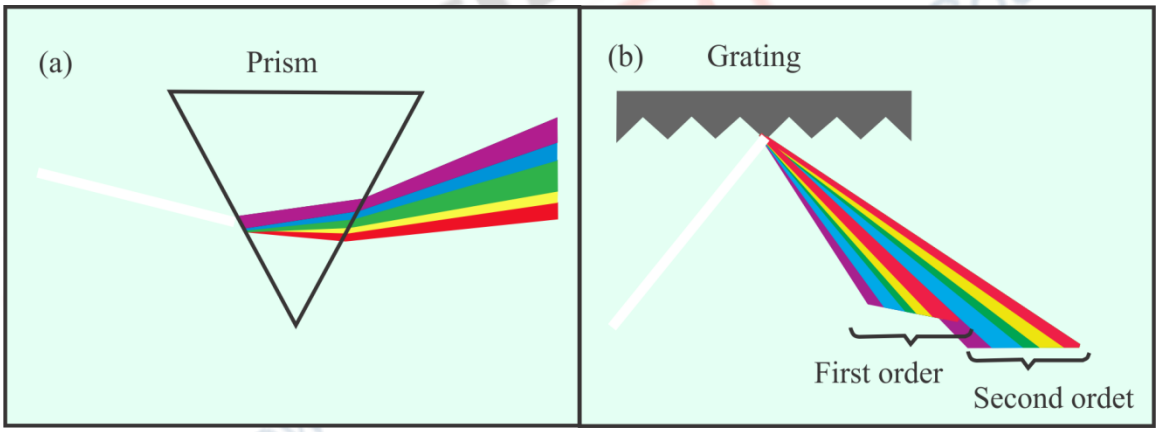



**Figure 4: Dispersion of white light through prism and grating**

Light falling on the prism is reflected at different angles, depending on the wavelength. The beam is split into its component colors. By moving the dispersing element or the exit slit, radiation of only a particular wavelength can be obtained, which leaves from the exit slit and can be used for the recording purpose (**Figure 3**). The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism, which then passes through the sample and reference solutions.

### 8.3 Sample area

One of the two divided beams is passed through the sample solution and the other beam is passed through the reference solution. Although, the samples for recording spectra are most commonly liquids, but the absorbance of gases and even of solids can be measured. Both sample and

reference solutions are placed in a transparent cell, known as cuvette. The cuvettes are rectangular in shape, and usually have an internal width of 1 cm (**Figure 5**).

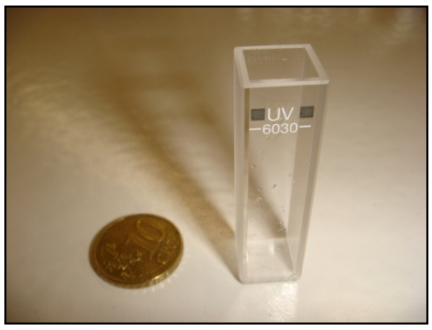



**Figure 5: A quartz cuvette**

It is important that the material of cells must be transparent to the radiation throughout the region under study. The cells are usually made of glass, plastic as well as silica or quartz. Of these, glass cells cannot be used for the UV region as they absorb light in the UV region but can be used satisfactorily in the visible region. Quartz is transparent in all (200-700 nm) ranges and is the best choice and hence can be easily used in UV as well as visible region.

### 8.4 Detector and Recorder

A detector converts a light signal into an electrical signal. After the beams are passed through the sample under study as well as the reference cell, the intensities of the respective transmitted beams are then compared over the whole wavelength range of the instrument. Generally two photocells are used as detector in UV spectrometer to record the spectra. One of the photocell receives the beam from sample cell and second detector receives the beam from the reference. The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.

Spectrophotometers consist of either a photomultiplier tube detector or a photodiode detector.

The commonly used detector in UV-Vis spectroscopy is **photomultiplier tube (Figure 6)**. It is m consists of three components:

- a cathode which emits electrons when struck by photons of radiation known as **photo emissive cathode**
- several **dynodes** which emit several electrons for each electron striking them
- an **anode**

In it's functioning, when a photon of radiation strikes the cathode, emission of several electrons take place. These emitted electrons are then accelerated towards the many dynodes. The first dynode 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. Finally all the electrons are collected at the anode. Several dynodes are arranged

between the anode and the cathode to produce an amplification effect. Each photon usually produces $10^6$-$10^7$ electrons, resulting in the amplified current that can be measured.

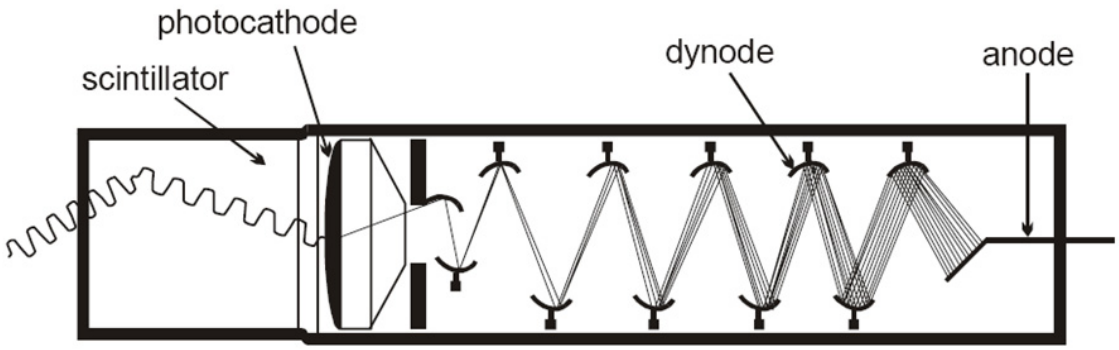



**Figure 6: A photomultiplier tube**

Photomultipliers are very sensitive to both UV and visible radiations and have fast response times. It is significant to note that Intense light may damage photomultipliers, hence they are limited to measuring low power radiation.

**Photodiodes** are increasingly being used as detectors in modern spectrophotometers. Photodiode detectors have a wider dynamic range and are more robust than photomultiplier tube detectors. In a photodiode, light falling on the semiconductor material allows electrons to flow through it, thereby depleting the charge in a capacitor connected across the material. The amount of charge that is required to recharge the capacitor at regular intervals is proportional to the intensity of the light. The limits of detection are approximately 170–1100 nm for silicon-based detectors.

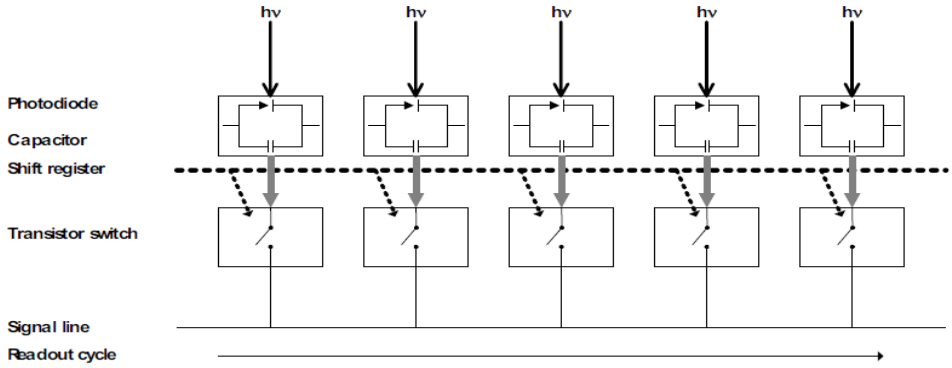



**Figure 7: An array of photodiodes**

Some modern spectrophotometers contain an array of photodiode detectors instead of a single detector (**Figure 7**). A diode array consists of a series of photodiode detectors positioned side by side on a silicon chip. Each photodiode is connected to a transistor switch via a charged capacitor When photons strike the diode, free electrical charge carriers are generated that discharge the capacitors. The capacitors are recharged at regular intervals. The amount of charge needed to recharge the capacitors is proportional to the number of photons detected by each diode, which in turn is proportional to the light intensity. The absorption spectrum is obtained by measuring the variation in light intensity over the entire wavelength range

## 9. Types of UV/VIS Spectrophotometer

There are two types of spectrophotometers, namely, single beam spectrophotometer or double beam spectrophotometer **(Figure 8**). Each has its advantages and disadvantages.

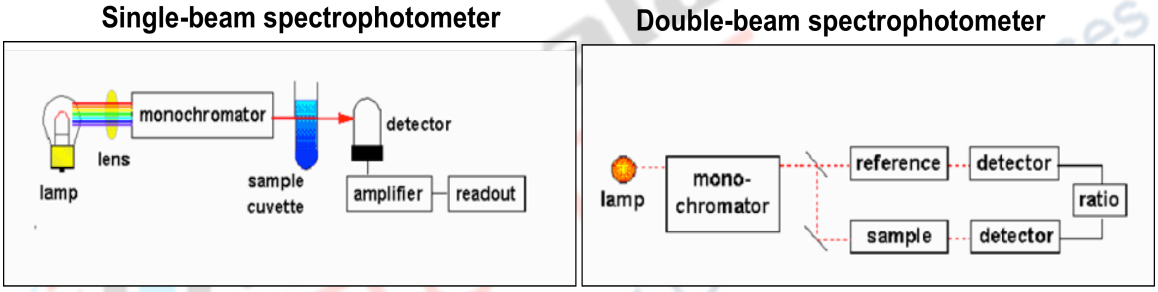



**Figure 8: Types of Spectrophotometer**

A single beam spectrophotometer has only one beam of light, which passes through the sample, therefore it requires taking reading for the reference and sampling separately.

On the other hand, in a double-beam instrument, the light is split into two beams before it reaches the sample. The two beams move simultaneously, one passing through a reference solution and the other through the sample. The reference beam intensity is taken as 100% Transmission (or 0% Absorbance), and the measurement displayed is the ratio of the two beam intensities.
Of the two types of spectrophotometer, double beam spectrophotometers are faster to operate and in their performance. They provide more reproducible results because they perform an automatic correction for the loss of light intensity as the beam passes through the sample and reference solution.

## 10. Sample Preparation

The UV-Vis spectra are usually measured in very dilute solutions. Usually 1 mg of the compound of molecular weight of 100-200 is dissolved in 100 ml of suitable solvent and only a portion is used for recording the spectra. The solvent should not absorb radiation and must be transparent over the desired range of wavelength. The solvents, which do not contain conjugated system, are most suitable for recording the UV-spectra. The solvent should also be inert to the sample. Some commonly used solvents are water, 95% ethanol, methanol and hexane.

## 11. Absorption Laws

When the radiation passes through a solution, some amount of light is absorbed. Suppose $I_0$ is the intensity of the incident radiation and I, the intensity of the transmitted radiation.
The amount of radiation absorbed can be measured by

Transmittance $T = I/I_0$

% Transmittance = $T \times 100$

Absorbance $A = \text{Log}_{10}\ I_0/I = \text{Log}_{10}\ 1/T$

If the entire light passes through a solution without any absorption, then absorbance is zero.
In this case, the percent transmittance is 100%.
If all the light is absorbed, then absorption is infinite and the percent transmittance is zero %.

**Beer-Lambert Law:** It gives a linear relationship between absorbance and concentration of an absorbing species. *This law states that the absorption is directly proportional to the concentration of absorbing substance and the length of the path of radiation through the sample*. It is independent of the intensity of the incident light and each successive unit layer absorbs an equal fraction of light incident on it.

$$\mathbf{A = \varepsilon cb}$$

$$\mathbf{\text{Log}_{10}\ I_0/I = \varepsilon cb}$$

Where **A** is the sample's Absorbance value at specific wavelength or frequency
ε is the molar absorptivity or the molar extinction coefficient of the substance
b is the path length of the sample in cm
c is the concentration of the solute in mol $L^{-1}$.
The value of ε absorptivity coefficient is constant for a particular material at a particular wavelength.

### Limitations of the Beer-Lambert Law

Under certain conditions Beer-Lambert law fails to maintain a linear relationship between absorbance and concentration of the solution.

1. Deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity.
2. Scattering of light due to particles present in the sample.
3. Chemical deviations due to the specific chemical species of the sample under investigation.
4. Non-monochromatic radiation
5. Fluorescence or phosphorescence of the sample

## 12. Summary

In this module you have learnt that:

- Spectroscopy is the study of interaction of electromagnetic radiations with the matter and is used to identify a substance, which may include UV, IR, NMR and Raman etc.
- The arrangement of all types of radiations in order of their increasing wavelength or decreasing frequencies is known as electromagnetic spectrum.
- The absorption of energy can bring about translational, electronic, rotational or vibrational motion or ionization of the molecules depending upon the frequency of the electromagnetic radiation they receive.
- Spectrophotometer is an instrument, which measures the transmittance or absorbance of a sample as a function of wavelength, when light of certain intensity and frequency range is passed through the sample.
- Although, there are six possible transitions σ to σ∗, π to σ∗, π to π∗, π to σ∗, n to π∗ and n to σ, the most commonly observed transitions in organic molecules are π to π∗, n to σ∗ and n to π∗.