CHAPTER

# 1

# Ultraviolet Visible Spectroscopy

## INTRODUCTION

The word **spectroscopy** is derived from *Latin* words '*spectrum*', which means 'image' and '*skopia*', which means 'observation'. Spectroscopy is the study of the interaction between matter and electromagnetic radiation as a function of the wavelength or frequency of the radiation. A **spectrometer** measures the wavelength and frequency of light, and used to identify and analyse the atoms in a sample.

Spectrometer is used for the study of atomic and molecular structure in analytical chemistry, pharmaceutical industry and drug research. Spectroscopic methods are advantageous over other methods because these are quick, sensitive, highly reliable, gives permanent record, very small quantity of substance is required for the determination of spectra. The spectroscopic methods are very useful for qualitative analysis, identification and structural elucidation of organic compounds.

## INSTRUMENTAL METHODS OF ANALYSIS

Instrumental analysis is a field of analytical chemistry that investigates analysts using scientific instruments. Analytical chemistry is the art and science of chemical characterization. Instrumental method of analysis is the part of analytical chemistry. These methods are based on the measurement of optical, electrical, thermal and other properties.

### Merits of Instrumental Methods of Analysis

- A small amount of a sample is required for analysis.
- Determination by instrumental method is considerably fast.
- Complex mixture can be analyzed either with or without their separation.
- Sufficient reliability and accuracy of results are obtained.
- When non-instrumental method is not possible, instrumental method is the only answer to the problem.

### Demerits of Instrumental Methods of Analysis

- Generally, instrumental methods are costly.
- The sensitivity and accuracy depend upon the type of instrument.
- Specialized training for handling instrument is required.
- There is a frequent need to check results with other methods.
- In some cases, instrumental method may not be specific.

## ULTRAVIOLET VISIBLE (UV-Vis) SPECTROSCOPY (Fig. 1.1)

In 1941, Beckman and colleagues introduce the DU UV-Vis spectrophotometer, which has higher resolution and lower stray light in the ultraviolet region than any other commercial instrument.

Ultraviolet visible (UV-Vis) spectroscopy is an important tool in analytical chemistry and also known as electronic spectroscopy. Ultraviolet visible spectroscopy is used to measure light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum. When incident light falls on matter it can either be absorbed, reflected, or transmitted. The absorbance of radiation in the UV-Vis range causes atomic excitation, which refers to the transition of molecules from a low-energy ground state to an excited state.

A UV-Vis spectrophotometer measures the intensity of light transmitted through a sample compared to a reference measurement of the incident light source. This instrument is very versatile and able to detect nearly every molecule.

**Electromagnetic radiation** (EM radiation or EMR) refers to the waves (or their quanta, photons) of the electromagnetic field, propagating (radiating) through space, carrying electromagnetic radiant energy. It includes radio waves, microwaves, infrared, visible light, ultraviolet, X-rays, and gamma rays.

### Electromagnetic Spectrum (UV Electromagnetic Radiations)

**A. UV region:** The UV radiation region extends from 10 to 400 nm (4000 Å). The UV region of the electromagnetic spectrum is subdivided into two spectral regions as follows:

i. Near ultraviolet region (ordinary ultraviolet region): This region comprises 200–400 nm (2000–4000 Å) because atmosphere is transparent in this region.

ii. Far ultraviolet (vacuum ultraviolet region): This region comprises below 2000 Å or below 200 nm.

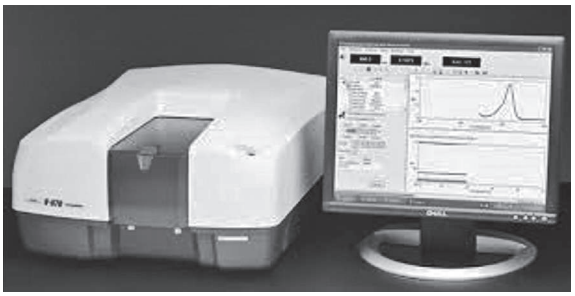


**Fig. 1.1:** UV spectroscopy instrument

**B. Visible region:** Visible region lies between 400 and 800 nm (4000–8000 Å). The wavelength is usually expressed in nanometers (nm) or angstroms (Å). Occasionally, absorption is reported in wavenumbers ($v = cm^{-1}$).

Ultraviolet and visible light range is widely applied in research, production and quality control for the classification and study of substances. UV-Vis spectroscopy is also used in analytical chemistry for the quantitative determination of different analytes such as transition metal ions, biological macromolecules and highly conjugated organic compounds. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

## PRINCIPLE

Principle of UV-Vis spectroscopy is based on Beer-Lambert law or on the absorption of ultraviolet light/visible light by chemical compounds, which results in the production of distinct spectra.

The Beer-Lambert law states that; when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of intensity of radiation decrease with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

The expression of Beer-Lambert law is

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon cl,$$

where A = absorbance

$I_0$ = intensity of light incident upon sample cell

$I$ = intensity of light leaving sample cell

$c$ = molar concentration of solute

$l$ = length of sample cell (cm)

$\varepsilon$ = molar absorptivity

From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV spectroscopy.

## THEORY

When an electromagnetic radiation in ultraviolet region is made to pass through a compound containing multiple bonds, it is observed that a part of the incident radiation is usually absorbed which causes electronic excitation. The amount of radiation absorbed depends upon the structure of the compound as well as wavelength of the radiation.

The energy of the radiation absorbed causes excitation of electron from lower energy level to higher energy level and the difference of energy is given by (Bohr's equation)

$$E = h\nu = hc/\lambda,$$

where $E$ = energy of photon, $\nu$ = frequency of the electromagnetic radiation, $h$ = the constant of proportionality is also known as the Planck constant, $\lambda$ = its wavelength, $c$ = speed of light.

Thus, the actual energy required depends upon the difference in energy between ground state ($E_0$) and the excited state ($E_1$) of the electron.

$$E_1 - E_0 = h\nu$$

## BLOCK DIAGRAM

UV-Vis spectrophotometer is made up of the following components: UV-Vis radiation source, wavelength selector, sample and reference cells, suitable detector and readout device (Fig. 1.2).

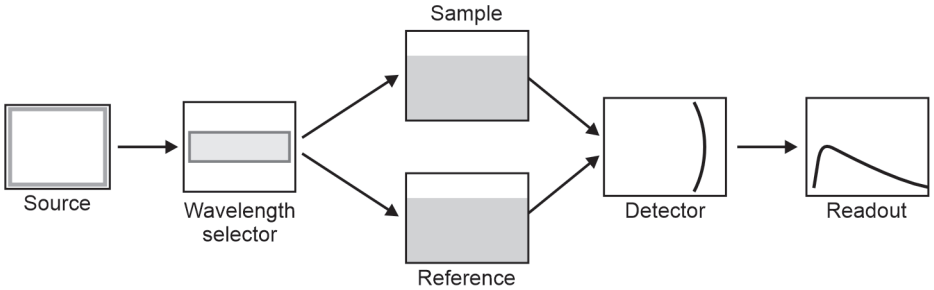



**Fig. 1.2:** Schematic block diagram of UV-Vis spectroscopy (double beam)

## ELECTRONIC TRANSITIONS

UV visible spectroscopy is also known as electronic spectroscopy. The molecules have non-bonding (η), and bonding (π or σ) or a combination of these electrons. These bonding (σ and π) and non-bonding (η) electrons absorb the characteristic radiation and undergo transition from ground state to excited state.

**Theory of electronic transitions or UV spectroscopy:** The absorption of UV or visible radiations corresponds to the excitation of outer electrons. Transitions consist of excitation of an electron from occupied molecular orbital to next higher energy orbital. Thus, the promotion of an electron from the lower energy π-orbital to higher anti-bonding π* orbital, is indicated as π–π*.

**Types of electronic transitions**

1. Transitions involving **σ, π and η** electrons, these electrons are involved in molecules.
   - σ Electrons: The electrons which form single bonds are electrons according to molecular notation. These electrons are involved in saturated bonds, such as those between carbon and hydrogen in paraffins.
   - π Electrons: The electrons which are forming double bonds. These electrons are involved in unsaturated hydrocarbons. Typical compounds with its bonds are trienes and aromatic compounds.
   - η Electrons: These are the unshared or non-bonded electrons and are not involved in the bonding between atoms in molecules. Examples are organic compounds containing nitrogen, oxygen and halogens.
2. Transitions involving charge-transfer electrons.
3. Transitions involving d and f electrons in metal complexes.

When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state. In a molecule, the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels (Fig. 1.3), which can be considered as being packed on top of each electronic level.

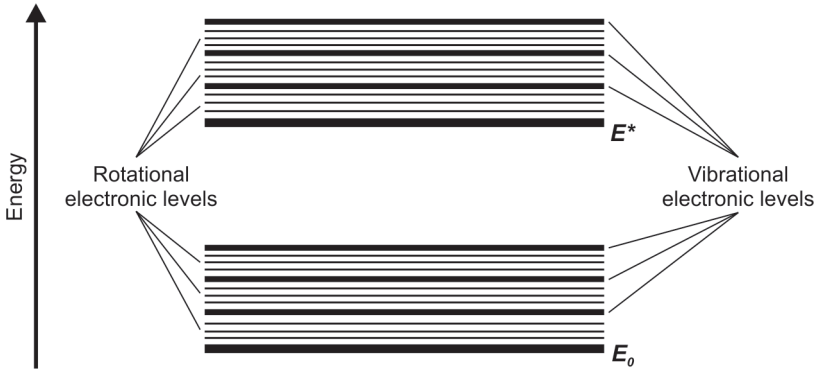



**Fig. 1.3:** Energy level diagram

### Absorbing Species Containing σ, π and η Electrons

Absorption of ultraviolet and visible radiation in organic molecules is restricted to certain functional groups (chromophores) that contain valence electrons of low excitation energy.

The spectrum of a molecule containing these chromophores is complex. This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines. This appears as a continuous absorption band.

Possible electronic transitions of **σ**, **π** and **η** electrons are shown in Fig. 1.4. It is clear that the changes in energy values for different transitions are in the following order:

$$\eta \rightarrow \pi^* < \pi \rightarrow \pi^* < \eta \rightarrow \sigma^* < \sigma \rightarrow \sigma^*$$

i. **σ→ σ* transitions:** An electron in a bonding σ orbital is excited to the corresponding antibonding σ* orbital. Such transitions occur in case of saturated hydrocarbons which do not contain lone pairs of electrons. The energy required for this type of transitions is very large and absorption band occurs in the far ultraviolet region (126 to 135 nm). For example, methane (which has only C-H bonds, and can only undergo σ→σ* transitions) shows an absorbance maximum at 125 nm. Absorption maxima due to σ→σ* transitions are not seen in typical UV-Vis spectra (200–700 nm).

ii. **η→σ* transitions:** Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of η→σ* transitions. These transitions usually need less energy than σ→σ* transitions. They can be initiated by light whose wavelength is in the range 150–250 nm. The number of organic functional groups with η→σ* peaks in the UV region is small. In case of saturated molecules which contain atoms having unshared pairs of electrons, η→σ* transition becomes be determined by commonly available spectrophotometers. For examples are alcohols and amines (containing –OH and $–NH_2$ groups) absorb between 175 and 200 nm.

iii. **η→π* and π→π* transitions:** Most absorption spectroscopy of organic compounds is based on transitions of η or  electrons to the π* excited state (η→π* and π→π*). This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200–700 nm). These transitions need an unsaturated group in the molecule to provide the π electrons.

Molar absorptivity from η→π* transitions are relatively low, and range from 10 to 100 L $mol^{-1}$ $cm^{-1}$. Such transitions are shown by unsaturated compounds which contain atoms like, N, O and S. This transition is between non-bonding orbital and anti-bonding orbital.

π→π* transitions normally give molar absorptivity between 1000 and 10,000 $Lmol^{-1}$ $cm^{-1}$. These types of transitions are related to the transition of electron from bonding π orbital to antibonding π∗ orbital.

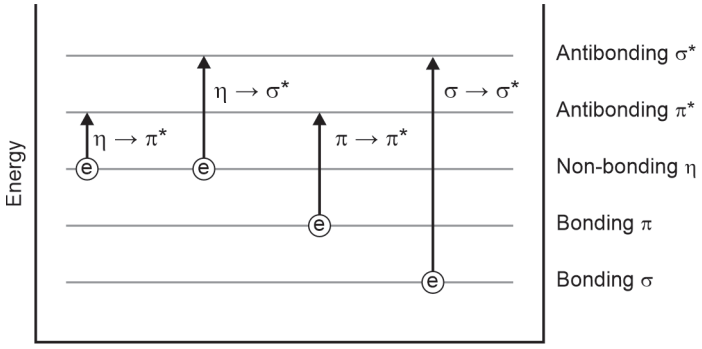



**Fig. 1.4:** Electronic transitions of σ, π and η electrons

The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species. Peaks resulting from $\eta \rightarrow \pi^*$ transitions are shifted to shorter wavelengths (blue shift) with increasing solvent polarity. This arises from increased solvation of the lone pair, which lowers the energy of the $\eta$ orbital.

Often (but not always), the reverse (i.e. red shift) is seen for $\pi \rightarrow \pi^*$ transitions. This is caused by attractive polarization forces between the solvent and the absorber, which lower the energy levels of both the excited and unexcited states. This effect is greater for the excited state, and so the energy difference between the excited and unexcited states is slightly reduced-resulting in a small red shift. This effect also influences $\eta \rightarrow \pi^*$ transitions but is overshadowed by the blue shift resulting from solvation of lone pairs.

iv. **Charge-transfer absorption:** Many inorganic species show charge-transfer absorption and are called charge-transfer complexes. For a complex to demonstrate charge-transfer behavior one of its components must have electron donating properties and another component must be able to accept electrons. Absorption of radiation then involves the transfer of an electron from the donor to an orbital associated with the acceptor. Molar absorptivity from charge-transfer absorption is large (greater than 10,000 $Lmol^{-1}$ $cm^{-1}$).

## CHROMOPHORE AND AUXOCHROME IN THE UV SPECTROSCOPY (Fig. 1.5)

### Chromophore

Chromophore derived from Greek word *chromo* = color and *pherein* (phore) = to bear. The part of molecules responsible for imparting color is called chromophore. The term chromophore was previously used to the functional group containing multiple bonds capable of absorbing radiations above 200 nm due to $\eta \rightarrow *$ and $\pi \rightarrow \pi^*$ transitions. For example: Nitro group is a chromophore because its presence in a compound gives yellow color to the compound.

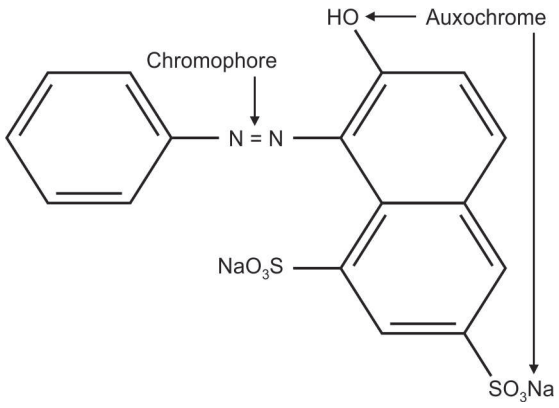



**Fig. 1.5:** Chromophore and auxochrome

But these days the term chromophore is used in a much broader sense which may be defined as any group which exhibit absorption of electromagnetic radiation in a visible or ultra-visible region it may or may not impart any color to the compound.

### Category

- **Independent chromophores:** If one chromophore is required to impart color, e.g. azo group –N=N, nitroso group $-NO^-$.
- **Dependent chromophores:** If more than one chromophore is required to impart color, e.g. acetone having one ketone group is colorless, whereas diacetyl having two ketone groups is yellow.

Covalently unsaturated groups responsible for the impairing of the colors (e.g. >C=C, >C=O). Some of the important chromophores are: Ethylene, acetylene, carbonyls, acids, esters and nitrile groups, etc. A carbonyl group is an important chromophore, although the absorption of light by an isolated group does not give rise to any color in the UV spectroscopy.

**Examples of chromophoric structures**

i. Carbonyl (⟩C=O)
ii. Azo (R–N=N–R′)
iii. Nitrite (–$NO_2$)
iv. Thioketone (⟩C=S)
v. Nitro (–N=O)
vi. Conjugated diene ($H_2C$=CH–CH=$CH_2$)
vii. Conjugated triene ($H_2C$=CH–CH=CH–CH=$CH_2$)
viii. Benzene ($C_6H_6$)

**Types of chromophores**

1. Chromophores in which the groups have π electrons undergo π–π* transitions. For examples; ethylenes, acetylenes, etc.
2. Chromophores having both π-electrons and η-electrons undergo two types of transitions, i.e. π–π* and η –π*. For examples: carbonyls, nitriles, azo and nitro compounds, etc.

### Identification of Chromophores

There is no set rule for the identification of a chromophore. The change in position as well as the intensity of the absorption depends upon a large number of factors. Following points can be considered.

1. Spectrum having a band near 300 mμ may contain two or three conjugated units.
2. Absorption bands near 270–350 mμ with very low intensity $\varepsilon_{max}$ 10–100 are because of η–π* transitions of carbonyl group.
3. Simple conjugated chromophores like dienes or α, β-unsaturated ketones have $\varepsilon_{max}$ values, i.e. from 10,000 to 20,000.
4. The absorption with $\varepsilon_{max}$ value between 1,000 and 10,000 reveals the presence of an aromatic system. If aromatic nucleus is substituted with groups which can extend the chromophore, the absorption take place at still higher value of extinction coefficients.

## AUXOCHROMES

Auxochrome derived from Greek words *auxanien* = to increase and *chrome* = color. The term auxochrome is defined as any group which itself does not act as a chromophore but when attached to a chromophore, it shifts the adsorption towards longer wavelength (red end of the spectrum) along with an increase in the intensity of absorption. An auxochrome group is called color enhancing group. The effect of the auxochrome is due to its ability to extend the conjugation of a chromophore by sharing of its non-bonding electrons. Some commonly known auxochromic groups are: –OH, –$NH_2$, –OR, –NHR, and –$NR_2$.

For example, when the auxochrome –$NH_2$ group is attached to benzene ring its absorption changes from $\lambda_{max}$ 225 ($\varepsilon_{max}$ 203) to $\lambda_{max}$ 280 ($\varepsilon_{max}$ 1430). All auxochromes have

one or more non-bonding pairs of electrons. If an auxochrome is attached to a chromophore, it helps to extending the conjugation by sharing of non-bonding pair of electrons as shown below

$$CH_2 = CH - NR_2 \rightarrow CH_2 = CH - NH_2$$

The extended conjugation has been responsible for Bathochromic effect of auxochromes.

### Interactions of Chromophores and Auxochromes with UV Radiation

When chromophores and auxochromes interact with UV radiation gives varies characteristics given below.

1. **Alkanes:** For molecules, such as alkanes, that contain nothing but single bonds and lack atoms with unshared electron pairs, the only electron transitions possible are of the $\sigma \rightarrow \sigma^*$ types.
2. **Alcohols, ethers, amines and sulfur compounds:** In saturated molecules that contain atoms bearing nonbonding pairs of electrons, transitions $\eta-\sigma^*$ of the type become important.
3. **Alkenes and alkynes:** With unsaturated molecules, $\pi-\pi^*$ transitions become possible.
4. **Carbonyl compound:** Unsaturated molecules that contain atoms such as oxygen or nitrogen may also undergo $\eta-\pi^*$ transitions.

## ABSORPTION AND INTENSITY SHIFTS IN THE UV SPECTROSCOPY (SPECTRAL SHIFTS)

Changes in chemical structure or the environment cause changes in the absorption spectrum of molecules and materials. There are four types of spectral shifts observed in the UV spectroscopy.

1. **Bathochromic effect:** This type of shift is also known as **red shift**. Bathochromic shift is an effect by virtue of which the absorption maximum is shifted towards the longer wavelength due to the presence of an auxochrome or change in solvents. The nonbonding $\eta \rightarrow \pi^*$ transition of carbonyl compounds observes bathochromic or red shift. In alkaline medium p-nitrophenol shows red shift.
2. **Hypsochromic shift**: This effect is also known as **blue shift**. Hypsochromic shift is an effect by virtue of which absorption maximum is shifted towards the shorter wavelength. Generally, it is caused due to the removal of conjugation or by changing the polarity of the solvents. Aniline shows blue shift in acidic medium, it loses conjugation.
3. **Hyperchromic effect:** Hyperchromic shift is an effect by virtue of which absorption maximum increases. The introduction of an auxochrome in the compound generally results in the hyperchromic effect.
4. **Hypochromic effect:** Hypochromic effect is defined as the effect by virtue of intensity of absorption maximum decreases. Hypochromic effect occurs due to the distortion of the geometry of the molecule with an introduction of new group (Fig. 1.6).

## SOLVENT EFFECT

- Solvent effects are the influence of a solvent on chemical reactivity. Solvents can have an effect on solubility, stability and reaction rates.
- Solvents play an important role in UV spectra.

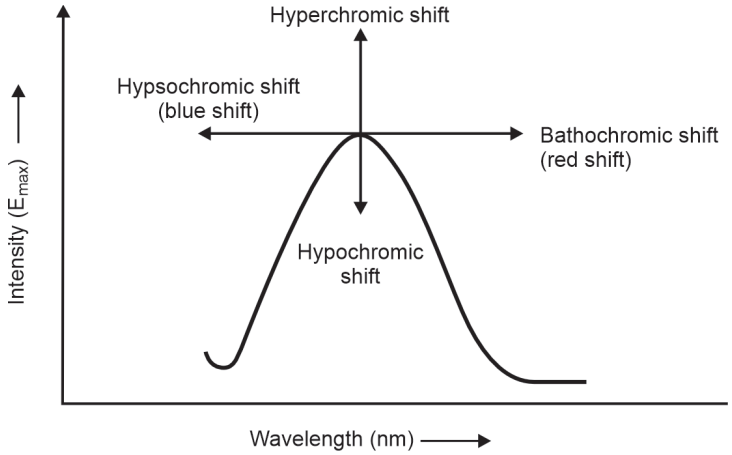



**Fig. 1.6:** Spectral shifts

- Solvent can induce significant changes in the intensity of peaks. Hyperchromic, increase in absorption intensity. Hypochromic, decrease in absorption intensity.
- Compound peak could be obscured by the solvent peak.
- So a most suitable solvent is one that does not itself get absorbed in the region under investigation.
- A solvent should be transparent in a particular region.
- A dilute solution of sample is always prepared for analysis.
- Most commonly used solvents are given in Table 1.1.

**Table 1.1:** Solvents with upper wavelength limit

| Solvent | λ of absorption |
|---|---|
| Water | 191 nm |
| Methanol | 203 nm |
| Ethanol | 204 nm |
| Ether | 215 nm |
| Tetrahydrofuran | 220 nm |
| Chloroform | 237 nm |
| Carbon tetrachloride | 265 nm |
| Benzene | 280 nm |

- The study of solvent effects on the structure and spectroscopic behavior of a solute is essential for the development of solution chemistry.
- The presence of specific and non-specific interaction between the solvent and the solute molecules are responsible for the change in the molecular geometry, electronic structure and dipolar moment of the solute.
- These solute–solvent interactions affect the solute's electronic absorption spectrum and this phenomenon is referred to as **solvatochromism**.
- Moreover, the behavior of a solute in a neat solvent is very different from the behavior in mixed binary solvent systems.

- In these kinds of systems, the solute can induce a change in the composition of the solvents in the cybotactic region compared to that in the bulk leading to preferential solvation.
- This situation commonly results from specific (hydrogen bonding) and non-specific (dielectric effects) interactions.
- The separation of specific from non-specific interactions in the interpretation of experimental measurements of absorption spectra is a difficult task.
- Quantitative measures for polarity are necessary in order to differentiate between these two effects.

### Solvent Effects on Transitions (Fig. 1.7)

- The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species.
- $\pi \rightarrow \pi^*$ transitions lead to more polar excited state that is more easily stabilized by polar solvent associations (H bonds). The $\pi^*$ state is more polar and stabilized more in polar solvent relative to nonpolar one, thus in going from nonpolar to polar solvent there is a red shift or bathochromic shift (increase in $\lambda_{max}$, decrease in $\Delta E$).
- $\eta \rightarrow \pi^*$ transition, the $\eta$-state is much more easily stabilized by polar solvent effects (H bonds and association), so in going from nonpolar to polar solvent there is a blue shift or hypsochromic shift (decrease in $\lambda_{max}$, increase in $\Delta E$).

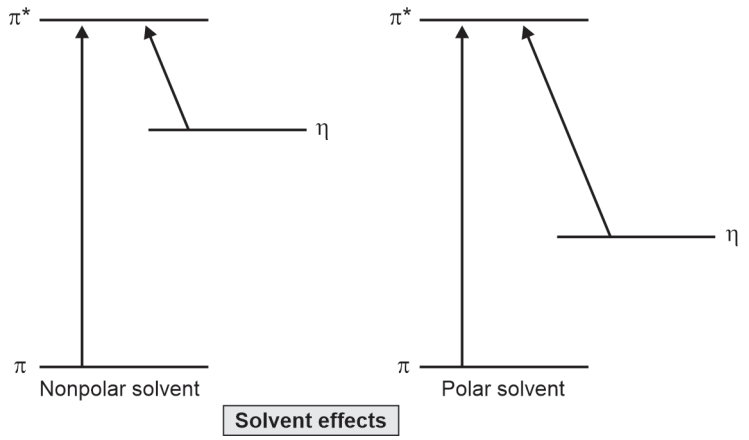



**Fig. 1.7:** Solvent effect on transitions

### Choice of a Solvent for Ultraviolet Spectroscopy

A suitable solvent for ultraviolet spectroscopy should meet the following requirements;

i. It should not itself absorb radiations in the region under investigation.

ii. It should be less polar so that it has minimum interaction with the solute molecules.

The most commonly employed solvent is 95% ethanol.

## LAMBERT-BEER LAW (LAWS OF ABSORBANCE OR LAW OF PHOTOCHEMISTRY)

1. This law also called as Lambert-Beer law, Beer's law, Beer-Lambert law, or Beer-Lambert-Bouguer law. This law relates the attenuation of light to the properties of the

material through which the light is travelling. The law is commonly applied to chemical analysis measurements and used in understanding attenuation in physical optics, for photons, neutrons, or rarefied gases.

2. **Lambert's law** stated that the loss of light intensity when it propagates in a medium is directly proportional to intensity and pathlength.
3. **Beer's law** stated that the transmittance of a solution remains constant if the product of concentration and pathlength stays constant.
4. The modern derivation of **the Lambert-Beer law** combines the two laws and correlates the absorbance. "It states that for a parallel beam of monochromatic radiation passing through a homogenous solution the absorbance of the light is proportional to the product of the concentration and pathlength."

   Absorbance (A) ∝ Concentration (c) × Pathlength (l)

   $$A \propto cl$$

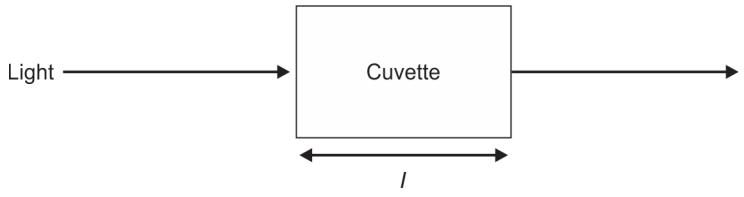
   $$A = \varepsilon cl, \text{ where } \varepsilon \text{ is molar extinction coefficient}$$
5. The relation may be used to determine the concentration of a chemical species in a solution using a colorimeter or spectrophotometer. The relation is most often used in UV visible absorption spectroscopy. Note that Beer's law is not valid at high solution concentrations (disadvantage).

### Beer-Lambert Law Equation

The absorbance of an electronic transition depends on two external factors:

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

   $$A \propto c \quad \text{(i)}$$
2. The absorbance (A) is directly proportional to the length of the light path (l), which is equal to the width of the cuvette.




   $$A \propto l \quad \text{(ii)}$$
3. The absorbance (A) can be defined via the incident intensity $I_0$ and transmitted intensity *I* by

   $$A = \log_{10}\left(\frac{I_0}{I}\right) \quad \text{(iii)}$$
4. Combining the two relationships (i) and (ii)

   $$A \propto cl \quad \text{(iv)}$$
5. This proportionality can be converted into an equation by including a constant

   $$A = \varepsilon cl, \quad \text{(v)}$$

   where, *A* is the amount of light absorbed for a particular wavelength by the sample, ε is the molar extinction coefficient

$l$ is the distance covered by the light through the solution (pathlength),
$c$ is the concentration of the absorbing species.

6. This formula is common form of the Beer-Lambert law, although it is also written in terms of intensities

$$A = \log_{10}\left(\frac{I_0}{I}\right) = \varepsilon c l \qquad \text{(vi)}$$

7. This formula equation (vi) is known as the Lambert-Beer law, and the constant ε is called molar absorptivity or molar extinction coefficient and is a measure of the probability of the electronic transition. The larger the molar absorptivity, the more probable the electronic transition.
8. Modern absorption instruments can usually display the data as transmittance (T), percent transmittance (%T), or absorbance (A). An unknown concentration of an analyte can be determined by measuring the amount of light that a sample absorbs and applying Beer's law.

### Derivation of Lambert-Beer Law

1. The amount of light passing through a substance is called transmittance, T or percent transmittance (%T), and is defined by the following equation:

$$T = \frac{I}{I_0} \qquad \text{(i)}$$

$$\%T = \frac{I}{I_0} \times 100 \qquad \text{(ii)}$$

where $I_0$ = the intensity of the incident light and
$I$ = the intensity of the absorbed light.

2. The amount of light of a specified wavelength absorbed by the substance depends on the length of the light path through the substance. The negative logarithm of the transmittance, the absorbance (A), is directly proportional to the amount of light absorbed and to the length of the light path and is described by the Lambert's law, which is expressed as follows:

$$-\log T = -\log \frac{I}{I_0} = A = K_1 l \qquad \text{(iii)}$$

$$A = K_1 l \qquad \text{(iv)}$$

Here l is the length of the medium usually a solution in a cell and $K_1$ is a constant.

3. A comparison of the scales for percent transmittance and absorbance may be used to convert percent transmittance into absorbance.

The negative logarithm of the transmittance is also directly proportional to the concentration of the absorbing substance c and is expressed by Beer's law as follows:

$$-\log I/I_0 = -\log T = A = K_2 c \qquad \text{(v)}$$

$$A = K_2 c \qquad \text{(vi)}$$

4. Combining e.q. (iv) Lambert's law and (vi) Beer's law, it means combining the two laws as the Lambert-Beer law gives the equation:

$$-\log I/I_0 = -\log T = A = \varepsilon c l$$

5. Where ε is a constant called the extinction coefficient incorporating $K_1$ and $K_2$ (also called molar absorbance). The extinction coefficient is dependent on the wavelength of the light passing through the substance and on the chemical nature of the substance; $l$ is the pathlength (cm) and $c$ is the concentration of the substance.

### Beer-Lambert Law Applications

**Applications in spectroscopy:** Beer-Lambert law is applied to the analysis of a mixture by spectrophotometry, without the need for extensive pre-processing of the sample.

1. **Application in determination of bilirubin count (medical applications):** Bilirubin count in a blood plasma sample can be determined by using a spectrophotometer. The spectrum of pure bilirubin is known thus the molar absorbance is known.
2. **Applications in determining the concentration of colored solution:** Colorimetry is a scientific technique that is used to determine the concentration of colored compounds in solutions by the application of the Beer-Lambert law, which states that the concentration of a solute is proportional to the absorbance.
3. **Applications in analytical chemistry:** This analysis mainly concentrates on the separation, quantification, and identification of matter by spectrophotometry. There is no involvement of extensive pre-processing of the sample to get the results.
4. **Applications in atmosphere:** Solar or stellar radiation in the atmosphere can be described using Lambert-Beer law.
5. **Applications in UV-Vis spectroscopy:** UV-Vis spectroscopy can be used to determine the concentration of the absorber in a solution using Lambert-Beer law.

### Importance

- Beer-Lambert absorbance law is a universally accepted relationship which helps calculation of concentration of an absorbing species from measured absorbance values.
- Beer-Lambert law's linearity of absorbance with concentration.
- This law is especially important in the fields of chemistry, physics, and meteorology.
- This law is used in chemistry to measure the concentration of chemical solutions, to analyze oxidation, and to measure polymer degradation.
- The law also describes the attenuation of radiation through the Earth's atmosphere.
- While normally applied to light, the law also helps scientists understand the attenuation of particle beams, such as neutrons.
- In theoretical physics, the Beer-Lambert law is a solution to the Bhatnagar-Gross-Krook (BGK) operator, which is used in the Boltzmann's equation for computational fluid dynamics.

### Limitations

The linearity of the Lambert-Beer law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- Deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity.
- Scattering of light due to particulates in the sample.
- Fluorescence or phosphorescence of the sample.

- Changes in refractive index at high analyte concentration.
- Shifts in chemical equilibria as a function of concentration.
- Non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band.
- High stray light frequently leads to deviations from the Beer-Lambert law, with subsequent inaccuracies in sample photometric values.

### Deviations

- Beer-Lambert law fails at higher concentrations because the linearity of the law is limited to chemical and instrumental factors. We observe deviation from linearity and thereby deviation in Beer-Lambert law (Fig. 1.8).
- Real deviations are most usually encountered in relatively concentrated solutions of the absorbing compound. These deviations are due to interactions between the absorbing species and to alterations of the refractive index of the medium.
- Under ideal conditions absorbance versus concentration plot is a straight line passing through the origin. However, under real situations a curvature in the plot is observed beyond a particular concentration. The curvature can be either positive or negative depending on the nature of absorbing species and experimental conditions.
- Positive deviations are observed when the measured absorbance values are more than the expected theoretical values and negative deviations result when the measured values are lower than the theoretical values.

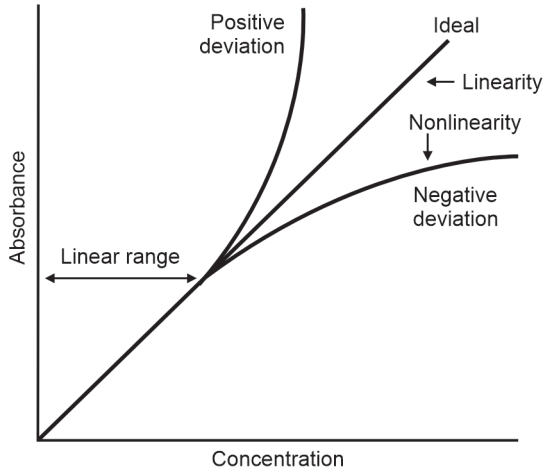



**Fig. 1.8:** Lambert-Beer law deviation

### Reasons for Deviation from Beer-Lambert Law

This relationship is a linear for the most part. However, under certain circumstances the Beer's relationship gives a non-linear relationship. These deviations from the Beer-Lambert law can be classified into three categories.

- **Real/True deviation:** These are fundamental deviations due to the limitations of the law itself. True deviations are related to the concentration of the absorbing substance observed at higher concentrations of analyte. Beer's law holds good only for dilute solutions.

- **Chemical deviation (Fig. 1.9):** Chemical deviations arise if the absorbing species undergo chemical changes such as association, complex formation, dissociation, hydrogen bonding, hydrolysis, ionization or polymerization. Analyte at low concentration may be inert but at high concentration it may undergo association or even dissociation. Incomplete reaction of substance, change in pH, faulty development of color, presence of impurities and concentration ratio also cause deviation from the Beer-Lambert law.

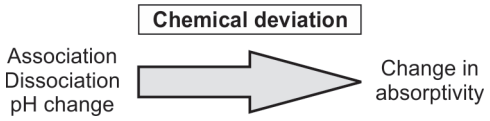



**Fig. 1.9:** Chemical deviation

- **Instrumental deviation:** This deviation is also known as spectral deviation. This deviation is mainly related to the instrument. Any fluctuations in intensity of light, change in the sensitivity of detector, improper slit width can lead to deviation from Beer-Lambert law. If we irradiate the sample with polychromatic radiation, simply deviation from Beer-Lambert law is produced.

### Types of UV-Vis Spectrophotometer

The measurement of a UV-Vis spectrum is recorded with a UV-Vis spectrometer. **Two types** of instruments can be used in Table 1.2.

1. **Single beam spectrophotometer:** A single-beam instrument (Fig. 1.10) uses only single beam of radiation through a single cell. The reference cell is used to set the absorbance scale at zero for the wavelength to be studied. It is then replaced by sample cell to determine the absorbance of the sample at that wavelength. A detector analyzes the intensity of light reaching it.

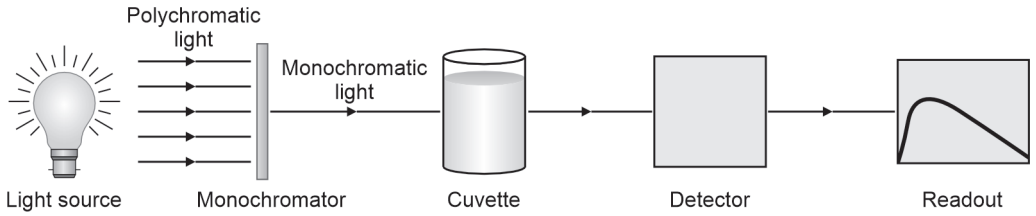



**Fig. 1.10:** Single beam instrumentation

**Table 1.2:** Difference between single beam and double beam spectrophotometer

| Single beam spectrophotometer | Double beam spectrophotometer |
|---|---|
| It is an analytical instrument in which all the radiation waves coming from the light source passes through the sample | It is an analytical instrument in which the radiation beam coming from the light source splits into two fractions |
| This instrument uses a non-split light beam | This instrument uses a light beam that is split into two fractions before passing through the sample |
| 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 |

2. **Double beam spectrophotometer:** A double beam instrument divides the radiation into two beams of equal intensity which is passed through two separate cells. One of the two cells contains the sample solution, while other, called the reference cell, contains either the pure solvent or a blank solution. Since the absorption by the sample is automatically corrected for absorption occurring in the solvent, the readout from the instrument is the difference between amounts of the radiations absorbed in the two cells.

## INSTRUMENTATION

**Double beam UV spectrophotometer instrumentation:** Basic components of UV spectrophotometer are source of radiant energy, collimating system, monochromator system, sample holder or container to hold sample, detector system of collecting transmitted radiation, suitable amplifier and readout device (Fig. 1.11).

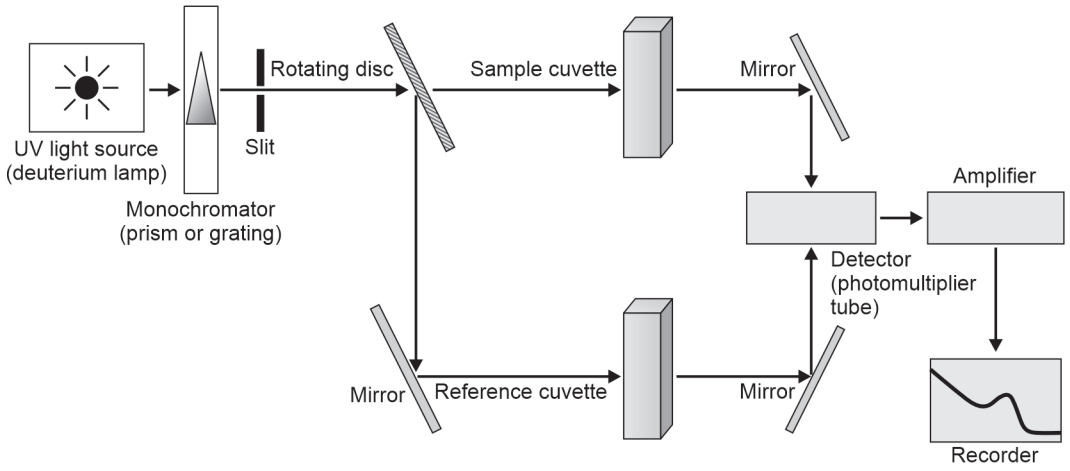



**Fig. 1.11:** Double beam UV spectrophotometer instrumentation

### A. Light Source (Fig. 1.12)

UV-Vis spectroscopy requires a continuous source, or one that emits radiation over a broad range of wavelengths. In general, UV-Vis experiments are conducted at wavelengths ranging between 180 and 780 nm.

**Radiation source:** The power of radiation source must not change abruptly over its wavelength range. To get a light source to cover the entire UV and visible range, a combination of two lamps has to be used, a deuterium lamp for the 190–400 nm part of the UV spectrum, and a tungsten lamp 350–2500 nm for the visible part.

- **Various UV radiation sources** are deuterium lamp, hydrogen lamp, tungsten lamp, xenon discharge lamp, mercury arc lamp.
- **Various visible radiation sources** are tungsten lamp, mercury vapor lamp, carbon arc lamp.

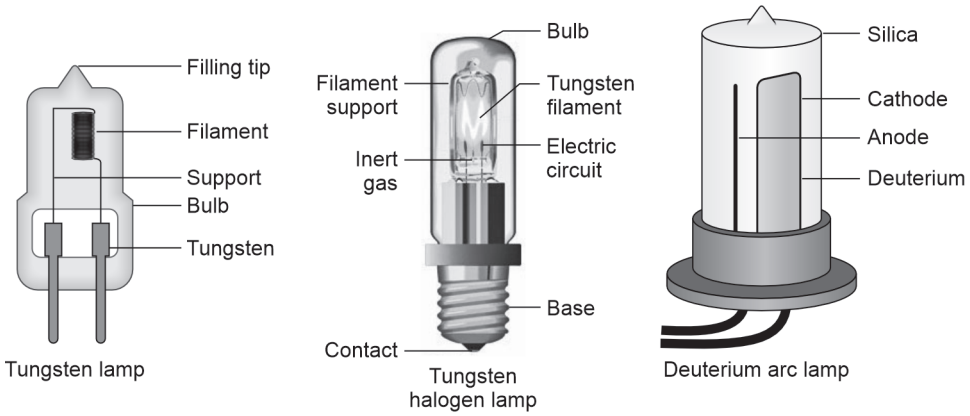



**Fig. 1.12:** Radiation sources (lamps)

**i. Tungsten lamp:** The tungsten filament lamp is commonly employed as a source of visible light. This is the conventional incandescent lamp. The filament is enclosed in a bulb to protect the filament from oxidation. Current is supplied to the filament by terminals or wires embedded in the glass. The spectral output is black-body radiation and wavelength range is about 350–3000 nm. The bulb is constructed of glass and the wavelength and intensity depend on temperature.

An inexpensive, stable light intensity can be obtained with a simple power supply. However, the glass bulb darkens because of evaporation of tungsten from the filament. This results in loss of energy, noisy display and eventually, complete lamp failure.

**ii. Tungsten halogen lamp:** Its construction is similar to a household lamp. The bulb contains a filament of tungsten fixed in evacuated condition and then filled with inert gas. Tungsten halogen lamps, also called quartz halogen lamps, contain a small amount of iodine within the quartz envelope that houses the filament. Sublimated form of tungsten reacts with iodine to form tungsten–iodine complex.

Quartz allows the filament to be operated at a temperature of about 3500 K, leading to higher intensities and extending the range of the lamp well into the UV. The lifetime of a tungsten halogen lamp is more than double that of an ordinary tungsten lamp.

**iii. Deuterium arc lamp:** A deuterium lamp is a gas discharge lamp and is often used as a UV source. A deuterium lamp consists of a tungsten filament and a Ni plate anode filled with deuterium gas under low pressure. When subjected to a high voltage it produces a continuous spectrum in the UV region due to formation of excited molecular species. It emits radiation in the range of 160–375 nm. The lamp envelope is made of optical grade silica or quartz and exhibits excellent ultraviolet transmissions.

An arc is struck between the anode and cathode. The cathode is first heated and begins to emit electrons. A high voltage is then applied across the electrodes and the arc is established. This produces enough heat to excite molecular deuterium and main continuous emission. The heating current can then be switched off.

**iv. Mercury arc lamp or mercury vapor lamp:** In mercury arc lamp, mercury vapor is stored under high pressure and excitation of mercury atoms is done by electric discharge. Mercury lamps emit strong UV and visible radiation, with strong spectral lines in the ultraviolet superimposed over continuous spectra. Not suitable for continuous spectral studies because it does not give continuous radiations.

### B. Wavelength Selectors

There are multiple types of wavelength selectors depending on the nature of the given instrument and desired form of measurement.

**Monochromators (Fig. 1.13):** The name is monochromator comes from the Greek words '*mono*' means 'single' and '*chroma*' means 'color', and the Latin suffix '*ator*' denoting an 'agent'. It means they change polychromatic light to monochromatic light. In UV spectroscopy, monochromator is used to isolate the radiation of the desired wavelength from wavelength of the continuous spectra, e.g. filters, prism, gratings.


**Fig. 1.13:** Monochromator

**Following types of monochromatic devices are used**

**I. Filters:** Absorption and interference filter (Fig. 1.14).

**i. Absorption filters:** Works by selective absorption of unwanted radiation and transmits the radiation which is required. Examples; glass and gelatin filters.

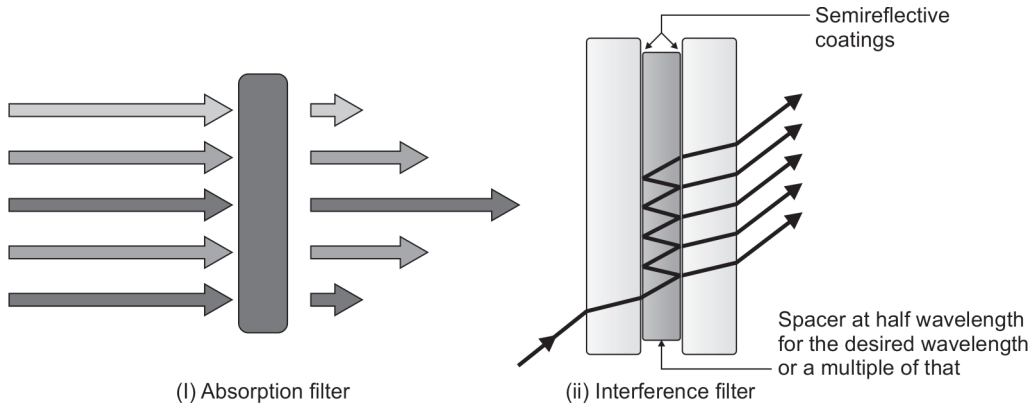



**Fig. 1.14:** Absorption filter and interference filter

a. An absorption **glass filter** is made of solid sheet of glass that has been colored by pigments which is dissolved or dispersed in the glass. The color in the glass filters are produced by incorporating metal oxides like of vanadium (v), chromium (Cr), iron (Fe), nickel (Ni), copper (Cu).

b. **Gelatin filter** is an example of absorption filter prepared by adding organic pigments; here instead of solid glass sheets, thin gelatin sheets are used. Gelatin filters are not use nowadays because it tends to deteriorate with time and gets affected by the heat and moisture. The color of the dye gets bleached.

**ii. Interference filter:** 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 refractive index. These filters have a band pass of 10–15 nm with peak transmittance of 40–60%.

**II. Prism:** Prism bends the monochromatic light. Amount of deviation depends on wavelength. Quartz prism used in UV region and glass prism used in visible region spectrum. They produce nonlinear dispersion (Fig. 1.15).

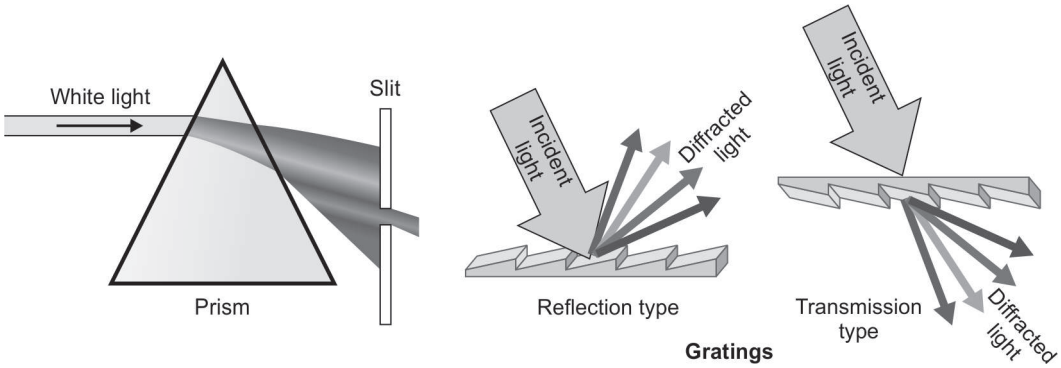



**Fig. 1.15:** Prism and gratings

**III. Gratings:** Gratings are most effective one in converting a polychromatic light to monochromatic light. As a resolution of +/– 0.1 nm could be achieved by using gratings, e.g. diffraction and transmission.

**Gratings are of two types**

i. ***Diffraction grating*:** More refined dispersion of light is obtained by means of diffraction gratings. These consist of large number of parallel lines (grooves) about 15000–30000/ inch is ruled on highly polished surface of aluminum. These gratings are replica made from master gratings by coating the original master grating with a epoxy resin and are removed after setting. To make the surface reflective, a deposit of aluminum is made on the surface. In order to minimize to greater amounts of scattered radiation and appearance of unwanted radiation of other spectral orders, the gratings are blazed to concentrate the radiation into a single order.

ii. ***Transmission grating:*** It is similar to diffraction grating but refraction takes place instead of reflection. Refraction produces reinforcement. This occurs when radiation transmitted through grating reinforces with the partially refracted radiation.

### C. Sample Cells/Sample Holders

**Cuvette:** The containers are used for the liquid sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. The cell may either be rectangular or cylindrical in nature. Most common cell length in the UV region is 1 cm (10 mm).

**Reference:** The reference, also known as the blank, is exposed to the same radiation as the sample and allows the instrument to measure a baseline in which there is none of the given sample present. The reference will generally be the solvent used to dissolve the sample in order to eliminate any absorption effects from the solvent. Both the reference and the sample are placed in matching cuvettes.

**Sample:** The sample is what is actually being measured in the experiment. It is generally set up so that the molecule of interest is kept in solution. Both aqueous and organic solvents are commonly used in UV-Vis spectroscopy.

**Solvent and solution:** Solvent must be transparent to UV-Vis radiation.

*Common solvents are*

1. Polar solvents: Methanol, ethanol and water.
2. Nonpolar solvents: Hexane, cyclohexane.
3. Intermediate polarity: Ether and dioxane.

**D. Detectors** (Fig. 1.16)

Detectors are the device, which converts light energy into electrical signals that are displayed on readout devices. The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample. Generally, two photocells serve the purpose of detector in UV spectroscopy. 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. Detectors used for UV and visible regions are photovoltaic cells, photomultiplier tubes, phototubes, and silicon photodiodes.

**Requirements of an ideal detector**

- It should give quantitative response.
- It should have a short response time.
- It should have high sensitivity and low noise level.
- It should provide signal or response quantitative to wide spectrum of radiation received.

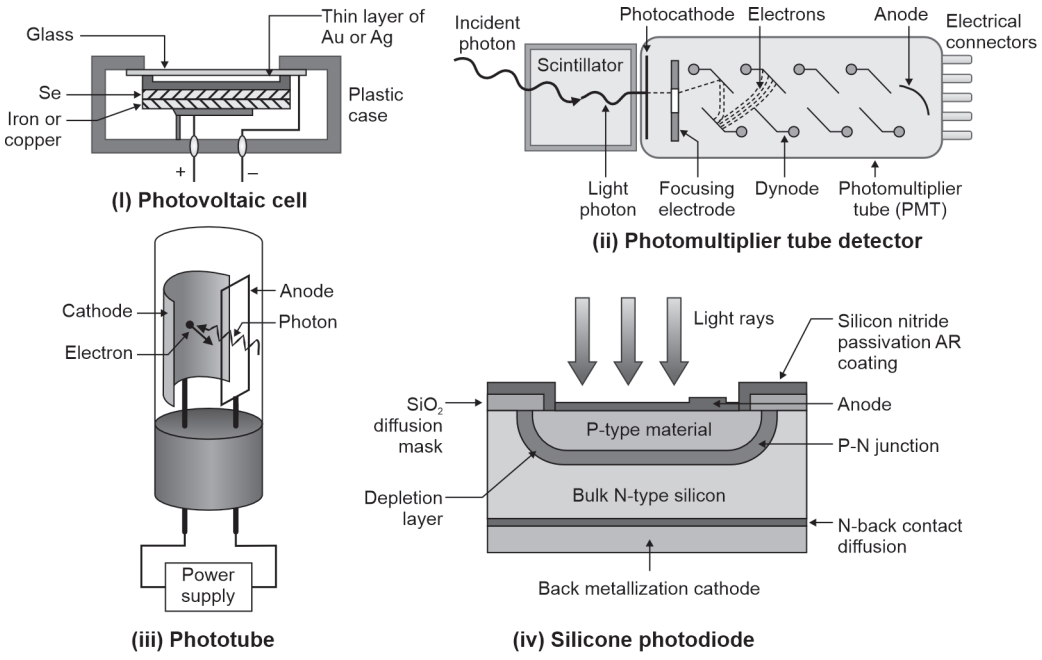



**Fig. 1.16:** Detectors used in UV-Vis spectroscopy

**In order to detect radiation and photosensitive devices**

**i. Photovoltaic cell/barrier layer cell:** It consists of a metallic base plate like iron or aluminum 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 light of radiation falls on the surface of selenium, electrons become mobile and generated at the selenium-silver surface, 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. When it is connected to galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light falling on it.

**ii. The photomultiplier tube (PMT)**: The photomultiplier tube is a commonly used detector in UV spectroscopy. Photomultiplier is extremely sensitive to light and is best suited where weaker or low radiation is received. It consists of a photoemissive 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 90 V 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 toward the second dynode, to produce more electrons which are accelerated towards third dynode 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.

**iii. Phototubes/photoemissive cells:** A phototube consists of an evacuated glass tube or 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 fall 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.

**iv. Silicon photodiode detector:** Silicon photodiodes are semiconductor devices used for the detection of light in ultraviolet, visible and infrared spectral regions. Because of their small size, low noise, high speed and good spectral response, silicon photodiodes are being used for both civilian and defense related applications.

The diode array detector is a multichannel detector capable of simultaneous measurement of all wavelengths of dispersed radiation. It comprises an array of silicon photodiodes on a single silicon chip. The individual diodes are subsequently scanned for response. The diode array detector is less sensitive than the photomultiplier tube but offers the advantage of simultaneous measurement of different wavelengths.

**v. Amplifier:** The alternating current generated in the photocells is transferred to the amplifier. Generally, current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.

**vi. Recording devices:** Most of the time amplifier is coupled to a pen recorder which is connected to the computer. Computer stores all the data generated and produces the spectrum of the desired compound.

## PROCEDURE

Following steps are involved in this double beam spectroscopy:

1. An appropriate source of radiation can be taken.

2. The common solvent used for preparing sample to be analyzed is either ethyl alcohol or hexane.
3. The UV radiation emits from the source of light.
4. Radiations pass through a monochromator unit via a mirror.
5. The radiation of narrow range of wavelengths coming out of the monochromator is received by the rotator system which divides the beam into two identical beams, on passing through a sample cell and other through reference cell and focused on detector.
6. The output from the detector is connected to phase sensitive amplifier.
7. The signal transmitted by the amplifier is transmitted and recorded which is connected to recorder.
8. Absorbance and transmittance of the sample are recorded as a function of wavelength.

## APPLICATION OF UV SPECTROSCOPY

Important applications of UV spectroscopy in pharmaceutical analysis are given below.

1. **Qualitative analysis:** UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds. UV absorption spectroscopy is generally used for characterising aromatic compounds and aromatic olefins.
2. **Quantitative analysis of pharmaceutical substances:** UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is generally based on Beer's law; which states that absorption is directly proportional to concentration of sample in the solution. For this, we produce calibration curve for different standard concentrations of the sample solution. Then unknown sample concentration can be determined by comparing the values in standard graph.

   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. Diazepam tablet can be analyzed by 0.5% $H_2SO_4$ in methanol at the wavelength 284 nm.
3. **Detection of impurities:** UV absorption spectroscopy 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. Benzene appears as a common impurity in cyclohexane. Its presence can be easily detected by its absorption at 255 nm.
4. **Detection of functional groups:** UV spectroscopy is used to detect the presence or absence of chromophore in the compound. This technique is not useful for the detection of chromophore in complex compounds. The absence of a band at a particular band can be seen as an evidence for the absence of a particular group. If the spectrum of a compound comes out to be transparent above 200 nm than it confirms the absence of: (a) Conjugation, (b) a carbonyl group, (c) benzene or aromatic compound, and (d) bromo or iodo atoms.
5. **Detection of extent of conjugation:** The extent of conjugation in the polyenes can be detected with the help of UV spectroscopy. With the increase in double bonds the absorption shifts towards the longer wavelength.

6. **Identification of an unknown compound:** An unknown compound can be identified with the help of UV spectroscopy. The spectrum of unknown compound is compared with the spectrum of a reference compound and if both the spectrums coincide then it confirms the identification of the unknown substance.
7. **Determination of configurations of geometrical isomers:** It is observed that *cis*-alkenes absorb at different wavelength than the *trans*-alkenes. The two isomers can be distinguished with each other when one of the isomers has non-coplanar structure due to steric hindrances. The *cis*-isomer suffers distortion and absorbs at lower wavelength as compared to *trans*-isomer.
8. **Determination of the purity of a substance:** Purity of a substance can also be determined with the help of UV spectroscopy. The absorption of the sample solution is compared with the absorption of the reference solution. The intensity of the absorption can be used for the relative calculation of the purity of the sample substance.
9. **Structure elucidation of organic compounds:** UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms. From the location of peaks and combination of peaks, it can be concluded that whether the compound is saturated or unsaturated, hetero atoms are present or not, etc.
10. **Study of chemical kinetics:** 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.
11. **Molecular weight determination:** Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds. For example, if we want to determine the molecular weight of amine then it is converted in to **amine picrate**.
12. **Examination of polynuclear hydrocarbons:** Benzene and polynuclear hydrocarbons have characteristic spectra in ultraviolet and visible region. Thus, identification of polynuclear hydrocarbons can be made by comparison with the spectra of known polynuclear compounds. Polynuclear hydrocarbons are the hydrocarbon molecule with two or more closed rings.
13. **Application as a HPLC detector:** A UV-Vis spectrophotometer may be used as a detector for HPLC.
14. **Applications in astronomy research:** In astronomy research, an UV-Vis spectrophotometer helps the scientists to analyze the galaxies, neutron stars, and other celestial objects.

### Advantages

- Its instrumentation is simple and inexpensive.
- UV is a fast medium of analysis and can give high accuracy, precision.
- It has quick analysis ability and easy to use.
- It provides robust operation.
- Small sample volume required.
- Generally, most organic molecules absorb UV-Vis light.
- UV-Vis spectrophotometer also brought the high-tech spectral analysis possibilities.
- It can be utilized in the qualitative and quantitative analysis
- Due to the advantage of fast and easy analysis ability, it is also widely used in the researches.

### Disadvantages

- Only those molecules are analyzed which have chromophores.
- Only liquid samples are possible to analyze.
- The results of the absorption can be affected by pH, temperature, contaminants, and impurities.
- It takes time to get ready to use it.
- Cuvette handling can affect the reading of the sample.
- It does not work with compounds that do not absorb light at this wavelength region.
- Mixture of molecule can be a problem due to overlap (routinely requires significant sample preparations).
- The stray light and electronic noise decrease linearity range and reduce the absorbency of substance it measures.
- Design and quality of the detector will affect the measurement accuracy and reduce the sensitivity of the instrument.

## SPECTROPHOTOMETRIC TITRATIONS

- The titration in which titrant, analyte or reaction products absorbs. The absorbance of a solution can be monitored during a titration.
- If the analyte ($A_t$), the titrant (T), or the reaction product (P) absorbs radiation in the UV-Vis regions, absorbance measurements at fixed wavelength can be used to locate the end point of the titration:

$$\text{Analyte } (A_t) + \text{Titrant (T)} \rightarrow \text{Product (P)}$$

- The absorbance of the analyte solution is measured after each addition of the titrant and the end point is located from the plot of the absorbance reading the volume of the titrant added (titration curve). (Titrant is the compound in the titration burette, mostly its concentration is exactly known.)
- A spectrophotometric titration is one in which the equivalence point of the titration is determined or indicated by a rapid change in solution.
- If at least one species in a complexation titration absorbs electromagnetic radiation.
- We can identify the end point by monitoring the titrand's absorbance at a carefully selected wavelength. (Titrand is a substance which is being analyzed.)
- For example, we can identify the end point for a titration of $Cu^{2+}$ with EDTA, in the presence of $NH_3$ by monitoring the titrand's absorbance at a wavelength of 745 nm, where the Cu $(NH_3)_4^{2+}$ complex absorbs strongly.
- At the beginning of the titration the absorbance is at a maximum.
- As we add EDTA the concentration of $Cu(NH_3)_4^{2+}$, and thus the absorbance, decreases as EDTA displaces $NH_3$.
- After the equivalence point the absorbance remains essentially unchanged.
- The resulting spectrophotometric titration is shown below in panel (i).
- It is noted that the titration curve's y-axis is not the actual absorbance, A, but a corrected absorbance, $A_{corr}$

$$A_{corr} = \frac{A \times (V_{EDTA} + V_{Cu})}{V_{Cu}}$$

where $V_{EDTA}$ = the volume of EDTA and
$V_{Cu}$ = the volume of Cu

- Correcting the absorbance for the titrand's dilution ensures that the spectrophotometric titration curve consists of linear segments that we can extrapolate to find the end point.
- Other common spectrophotometric titration curves can be seen in panels (ii) to (vi).
- Examples of the shapes of spectrophotometric titration curves are (Fig. 1.17):
  i. Only the titrand absorbs
  ii. Only the titrant absorbs
  iii. Only the product of the titration reaction absorbs
  iv. Both the titrand and the titrant absorb
  v. Both the titration reaction's product and the titrant absorb
  vi. Only the indicator absorbs. The red arrows indicate the end points for each titration curve.

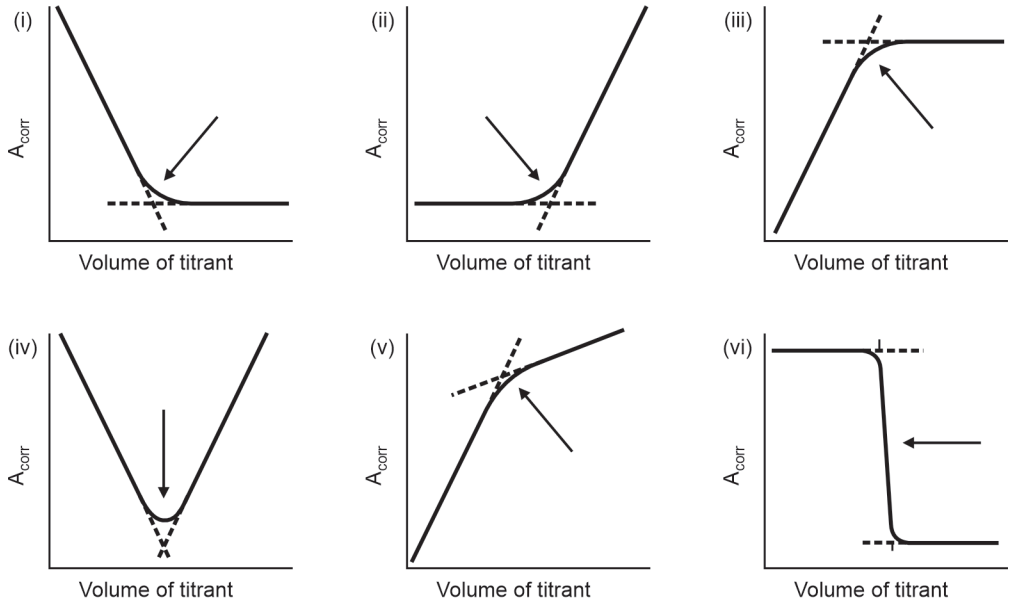



**Fig. 1.17:** Spectrophotometric titration curves

## SINGLE COMPONENT ANALYSIS

The concentration of a drug or absorbing substance in a given sample can be easily analyzed by measuring the absorbance of the solution prepared in a transparent solvent in a UV spectrophotometer.

The **quantitative** UV spectroscopy is applicable to determine the concentration of a single component in the given sample and to determine the concentration of different components in mixture.

### *Types of Single Component Analysis*

a. **Direct analysis:** All compounds containing conjugated double bond or aromatic rings, and many inorganic species absorb light in the UV visible regions. In these techniques the substance to be determined is dissolved in suitable solvent and diluted to the required concentration by appropriate dilutions and absorbance is measured.
b. **Indirect analysis:** Indirect analysis is an analysis after addition of some reagent. Indirect analysis is based on the conversion of the analyte by a chemical reagent that has different spectral properties.

**Chemical derivatization may be adopted for any of the several reasons**

1. If the analyte absorbed weakly in the UV region.
2. The interference forms irrelevant absorption may be avoided by converting the analyte to a derivative, which absorbs in the visible region, where irrelevant absorption is negligible.
3. This technique can be used to improve the selectivity of the assay in presence of other UV radiation absorbing substance.
4. Most important reason is cost, it should be inexpensive.

### Methods of Calculation

Methods of calculating concentration in single component analysis are:

1. **Standard absorptivity value method:** In this method the concentration of the unknown compound can be determined by using the measured absorbance and standard absorptivity value. Absorbance can be determined by the following equation:
   $A = acl$, where $A$ is absorbance of solution, $a$ is absorptivity value, $l$ is path length of the solution, $c$ is concentration of unknown to be determined.
2. **Calibration graph/curve method:** In this method the absorbance of a number of standard solutions of the reference substance at concentration encompassing the sample concentrations are measured and calibration graph is constructed.
3. **Single or double point stardardization method:** In this method the absorbance of a sample solution and a standard solution of the reference substance will be measured in single point procedure. The standard and sample solution should be prepared in the same manner, where the concentration of the standard solution should be close to the sample solution. The concentration of the substance in the sample is calculated from the proportion relationship that exists between absorbance and concentration

$$C_{test} = \frac{(A_{test} \times C_{std})}{A_{std}}$$

$C_{test}$ and $C_{std}$ = Concentration of test and standard solution

$A_{test}$ and $A_{std}$ = Absorbance of test and standard solution.

### Multi-component Analysis

In **multi-component** systems, specific analytical methods are required to determine the concentrations of individual components in the presence of interfering substances. Ultraviolet visible spectrophotometry is the most widely used spectrophotometric technique for the **quantitative analysis** of chemical substances as pure materials and as components of dosage forms.

By adopting multi-component analysis the actual amount of sample under investigation can be easily calculated after removing the irrelevant absorption. Application of

spectrophotometric multi-component analysis can be done where the spectra of the drugs overlap. In such cases of overlapping spectra, simultaneous equation can be framed to achieve the concentration of individual components otherwise multi-component analysis can be applied on any degree of spectral overlap provided that two or more spectra do not remain similar exactly.

**Different UV visible spectrophotometric methods for multi-component analysis**

a. Simultaneous equation method
b. Absorbance ratio method
c. Geometric correction method
d. Orthogonal polynomial method
e. Derivative spectrophotometry
f. Difference spectrophotometry

**a. Simultaneous equation method:** If a sample contains two absorbing drugs (X and Y) each of which absorbs at the $\lambda_{max}$ of the other ($\lambda_1$ and $\lambda_2$), it may be possible to determine both drugs by the simultaneous equations method.

**b. Absorbance ratio method:** The absorbance ratio method is a modification of the simultaneous equations procedure. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbance is measured at two wavelengths, one being the $\lambda_{max}$ of one of the components ($\lambda_2$) and other being a wavelength of equal absorptivity of two components ($\lambda_1$), i.e. an iso-absorptive point.

**c. Geometric correction method:** A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest of this procedure is the three point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected.

**d. Orthogonal polynomial method:** The technique of orthogonal polynomials is another mathematical correction procedure, which involves more complex calculations than the three-point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions.

**e. Derivative spectroscopy:** For the purpose of spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy. Derivative spectrophotometry involves the conversions of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order, or $D_0$ spectrum.

**f. Difference spectroscopy:** Difference spectroscopy provides a sensitive method for detecting small changes in the environment of a chromophore or it can be used to demonstrate ionization of a chromophore leading to identification and quantitation of various components in a mixture.

## WOODWARD-FIESER RULES

In 1945, Robert Burns Woodward gave certain rules for correlating $\lambda_{max}$ with molecular structure. In 1959, Louis Frederick Fieser modified these rules with more experimental

data, and the modified rule is known as Woodward-Fieser rules. It is used to calculate the position and $\lambda_{max}$ for a given structure by relating the position and degree of substitution of chromophore.

**Woodward-Fieser rules** (Fig. 1.18)**:** Each type of diene or triene system is having a certain fixed value at which absorption takes place; this constitutes the base value or parent value. The contribution made by various alkyl substituents or ring residue, double bond extending conjugation and polar groups such as –Cl, –Br, etc. are added to the basic value to obtain $\lambda_{max}$ for a particular compound. According to Woodward's rules the $\lambda_{max}$ of the molecule can be calculated using a formula:

$$\lambda_{max} = \text{Base value} + \Sigma \text{ Substituent contribution} + \Sigma \text{ Other contributions}$$

### Advantages

- It is used to calculate the position and maximum wavelength for a given structure.
- By relating the position and degree of substitution of chromophore.

**There are three sets of rules**

1. Woodward-Fieser rule for conjugated dienes and polyenes.
2. For unsaturated carbonyl compounds.
3. For aromatic compounds or benzoyl derivatives.

**1. Woodward-Fieser rules for conjugated dienes:** Conjugated dienes and polyenes are found in most organic compounds. Longer the conjugated system, greater the wavelength of absorption maximum. According to Woodward's rules the $\lambda_{max}$ of the molecule can be calculated using a formula:

$$\lambda_{max} = \text{Base value} + \Sigma \text{ Substituent contribution} + \Sigma \text{ Other contributions}$$

- Homoannular diene: Cyclic diene having conjugated double bonds in same ring.
- Heteroannular diene: Cyclic diene having conjugated double bonds in different rings.
- Endocyclic double bond: Double bond present in a ring.
- Exocyclic double bond: Double bond in which one of the doubly bonded atoms is a part of a ring system. Here ring A has one exocyclic and endocyclic double bond. Ring B has only one endocyclic double bond.

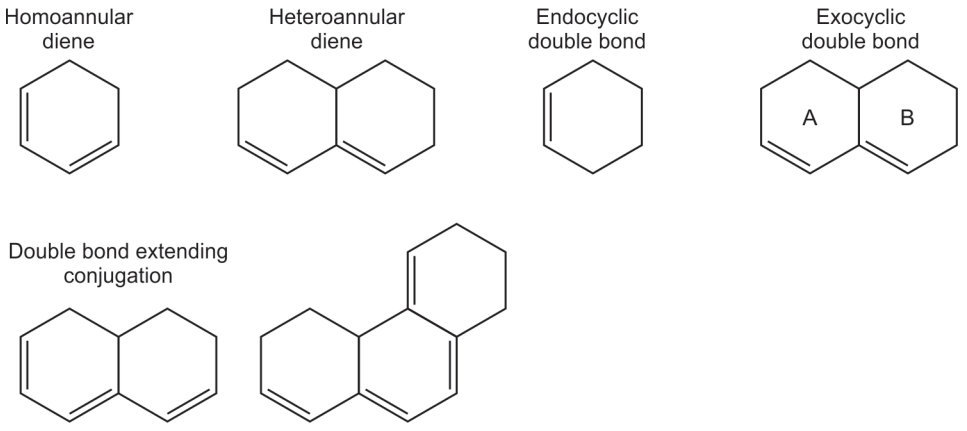
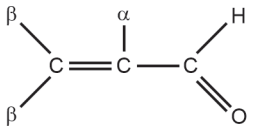



**Fig. 1.18:** Conjugated dienes

**2. Woodward-Fieser rule for α, β-unsaturated carbonyl compounds:** Woodward-Fieser rules can be extended to calculate the $\lambda_{max}$ of α, β-unsaturated carbonyl compounds. In a similar manner to Woodward rules for dienes discussed previously, there is base value to which the substituent effects can be added and the $\lambda_{max}$ can be calculated using the formula:

$$\lambda_{max} = \text{Base value} + \Sigma \text{ Substituent contributions} + \Sigma \text{ Other contributions}$$




| | |
|---|---|
| Parent | 208 nm |
| With α or β alkyl groups | 220 nm |
| With α, β or β, β alkyl groups | 230 nm |
| With α, β, β alkyl groups | 242 nm |

**Fig. 1.19:** Parent value

**α, β-Unsaturated aldehydes, acids, and esters:** α, β-Unsaturated aldehydes generally follow the same rules as enone (or alkenone) except that their absorptions are displaced by about 5 to 8 nm toward shorter wavelength than those of the corresponding ketones. Nielsen developed a set of rules for α, β-unsaturated acids and esters that are similar to those for enones (an organic compound containing both alkene and ketone functional groups) (Fig. 1.19 and Table 1.3).

**Empirical rules:** Empirical rules for the unsaturated aldehyde.

**Table 1.3:** Parent value and increments

| | |
|---|---|
| **Parent value** | |
| X = alkyl/ring residure, ArCOR | 246 nm |
| X = H, Ar CHO | 250 nm |
| X = OH/O-alkyl, $ArCO_2H$, $ArCO_2R$ | 230 nm |
| **Increments** | |
| R = alkyl/ring resiude | o, m = 3 nm |
| | p = 10 nm |
| R = OH/O-alkyl | o, m = 7 nm |
| | p = 25 nm |
| R = $NH_2$ | o, m = 23 nm |
| | p = 58 nm |

**3. Woodward-Fieser rule for aromatic compounds or benzoyl derivatives:** The absorption that result from transitions within the benzene chromophore can be quite complex. The ultraviolet spectrum contains three absorption bands, which sometimes contain a great deal of fine structure. The electronic transitions are basically of the $\pi \rightarrow \pi^*$ type.

## SPECTROPHOTOMETER VS SPECTROMETER

- A spectrophotometer is a spectrometer that only measures the intensity of electromagnetic radiation (light) and is distinct from other spectrometers such as mass spectrometers.
- A spectrometer measures the change over a range of incident wavelengths (or at a specific wavelength).
- A spectrophotometer is a color measurement device that is used to capture and evaluate color on just about anything, including liquids, plastics, paper, metal and fabrics.
- A spectrometer is a device used to detect spectra, which are specific wavelengths of electromagnetic radiation.
- All spectrophotometers contain spectrometers.

## Questions

### Very Short Questions

1. What do you understand by UV visible spectroscopy?
2. What is electronic transition in UV visible spectroscopy?
3. Define any two
   a. Chromophore
   b. Auxochrome
   c. Spectral shifts
   d. Solvatochromism
   e. Spectroscopy
   f. Monochromator
   g. Sample cuvette
4. Define blue shift and red shift?
5. Define Beer-Lambert law.
6. What do you understand by the term deviations in spectroscopy?
7. Draw a block diagram of UV spectroscopy?

### Short Answer Type Questions

1. Discuss in detail UV visible spectroscopy and its theory.
2. Write the concept of electronic transitions in UV visible spectroscopy.
3. How does solvent affect the absorption spectra?
4. Write in detail the principle and instrumentation of UV visible spectroscopy.
5. What are the factors that affect deviation in UV visible spectroscopy?
6. Write a note on light sources used in UV visible spectroscopy.
7. Write a note on wavelength selectors used in UV visible spectroscopy.
8. Write a note on different types of detectors used in UV visible spectroscopy.
9. Write the applications of UV visible spectroscopy.
10. Discuss in detail spectrophotometric titrations.
11. Define Beer-Lambert law. Write its limitation, deviation and applications.
12. Derivation of Beer-Lambert law. What are the reasons for deviation from Beer-Lambert law? Importance of this law.
13. Merits and demerits of instrumental methods of analysis
14. Short note on single component and multi-component analysis?
15. Short note on Woodward-Fieser rules.

### Long Answer Type Questions

1. Explain in detail UV visible spectroscopy. Write its various applications.
2. Explain in detail the principle, instrumentation and advantages of UV visible spectroscopy.
3. Discuss the principle, theory and applications of UV visible spectroscopy.
4. Write in detail the derivation and deviation in UV visible spectroscopy.