CHAPTER 1

UV Visible Spectroscopy

INTRODUCTION

Spectroscopy is the branch of science that deals with studying the **interaction of matter with electromagnetic radiation**. Due to this interaction, energy is either absorbed or emitted by the matter in discrete amounts called quanta. The measurement of this absorbed or emitted radiation by the matter forms the basis of spectroscopic techniques. Spectroscopic techniques are helpful in the study of atomic and molecular structures. Spectroscopy is one of the most powerful tools available for the qualitative and quantitative analysis of different samples.

Spectroscopy can be studied under two major headings

1. **Atomic spectroscopy** – deals with the interaction of electromagnetic radiation with atoms in the ground energy level (lowest energy).
   E.g., Atomic absorption spectroscopy, Atomic emission spectroscopy, Flame emission spectroscopy
2. **Molecular spectroscopy** – deals with the interaction of electromagnetic radiation with molecules.
   E.g., IR spectroscopy, Raman spectroscopy.

Spectroscopy can also be classified based on the measured mode of interaction of matter with electromagnetic radiation, which is given in the Table 1.1.

Table 1.1 Instrumental Methods based on measurement of property

Absorption of radiation	Emission of radiation	Scattering
Absorption spectroscopy: X-ray, UV, IR, Colorimetry, Atomic absorption, Nuclear magnetic resonance, and electron spin resonance spectroscopy	**Emission spectroscopy:** Flame emission photometry, Fluorescence spectroscopy	Turbidimetry, Nephelometry, Raman spectroscopy.

PROPERTIES OF ELECTROMAGNETIC RADIATION

Electromagnetic radiation is an energy form that travels with enormous velocity in space. Electromagnetic radiation (EMR) possesses both wave and particle nature. These properties are a distinct feature of EMR, and they are inseparable.

Wave properties of Electromagnetic radiation

Electromagnetic radiation has an electrical component and a magnetic component oscillating in perpendicular planes and also perpendicular to the direction of propagation. The wave nature of EMR shows properties like refraction, reflection, constructive and destructive interferences.

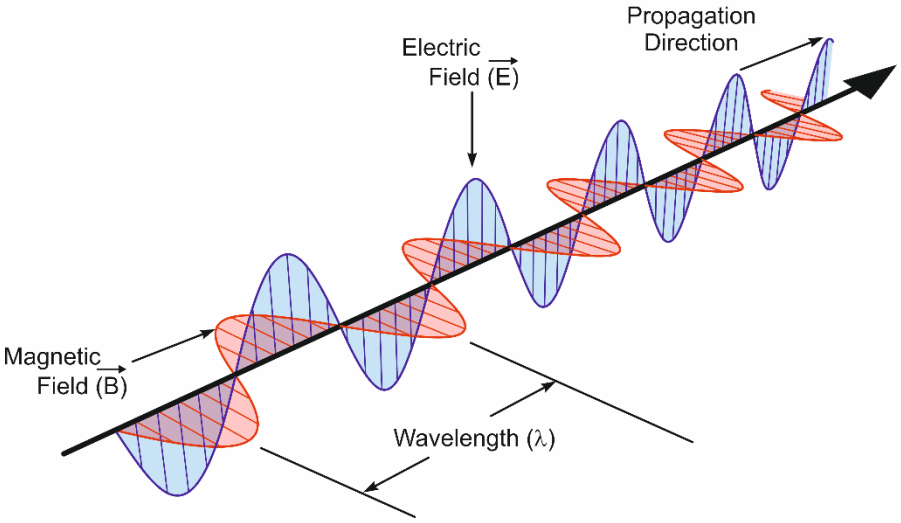


Fig. 1.1 Electromagnetic wave with its components.

The electromagnetic wave parameters are

(a) Wave length, λ: Is the **distance between two successive maxima or minima in an electromagnetic wave**. It is denoted by the Greek letter lambda. Units of wavelength are meters (m), centimeter (cm), millimeter (mm), micrometers (μm), nanometers (nm) and angstrom (Å).

$$1Å = 10^{-8}\ cm = 0.1nm = 10^{-10}m$$

A beam of radiation with only one wavelength is called **monochromatic radiation,** and the beam consisting of several wavelengths is called **polychromatic or heterochromatic radiation.**

(b) Frequency, υ: Is the **number of wavelength units passing through a given point in unit time.** It is denoted by the Greek letter υ.Unit is cycles per second or Hertz.

(c) Wave number $\bar{\upsilon}$: Frequency is usually a large number making its use more difficult in regular practice. To overcome this problem, frequency is usually expressed as wave number. **The wave number is the number of waves in vacuum**. The symbol is $\bar{\upsilon}$**.** Unit for wave number is cm^{-1}

$$\bar{\upsilon} = 1/\lambda$$

Particle properties of electromagnetic radiation

When electromagnetic radiation hits a material, it emits electrons called photoelectrons. This **photoelectric effect** is due to the particle properties of EMR. To explain this property, it is assumed that EMR consists of a stream of discrete packets (particles) of energy called photons

or quanta. The energy of the photon is proportional to the frequency of the radiation and is given by the relationship

$$E = h\upsilon, \text{ where,}$$

E is the energy of a photon in ergs, υ is the frequency of the EMR in cycles per second, and h is called Planck's constant (6.624×10^{-34} joules/second).

EMR of longer wavelength (low frequency) has lower energy than the shorter wavelength (high frequency).

Electromagnetic spectrum

The entire range of EMR is called as EMR spectrum.

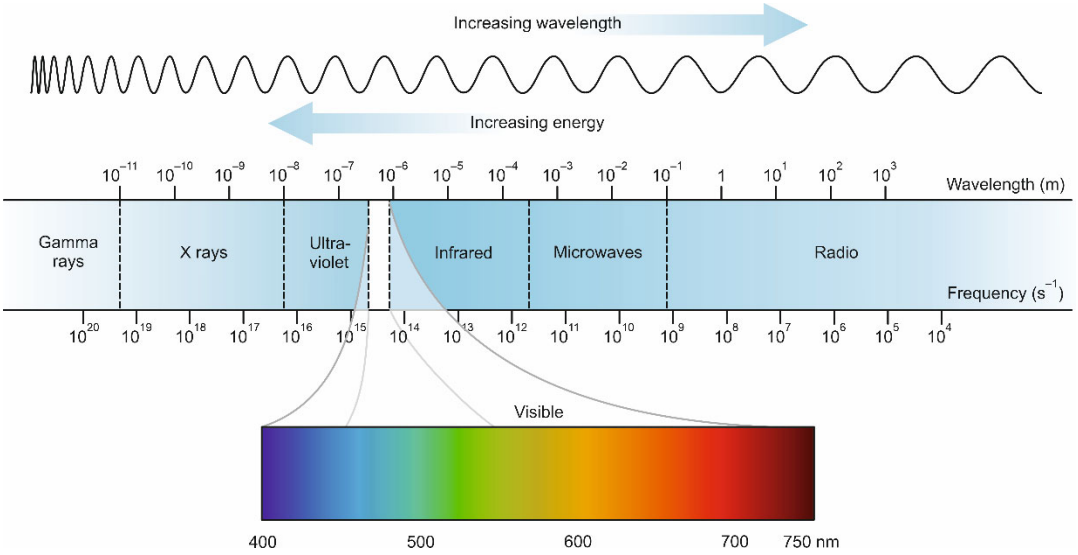


Fig. 1.2 Electromagnetic spectrum showing different regions of EMR.

The various spectral regions are

γ(Gamma) ray region – lies between 0.02 to 1 angstrom. These are the shortest waves.

X-ray region –This region lies between 1 to 10 angstrom. These rays cause core electron excitation.

Visible and Ultra violet region – These rays cause valence electron excitation. This region consists of three parts

Vacuum UV : 1 - 180 nm
Ultraviolet : 180 - 400 nm
Visible : 400 - 800 nm

Infrared region – This region is subdivided into three regions

Near IR : 0.7 - 2.5 μm
Mid IR : 2.5 - 15 μm
Far IR : 15 - 200 μm

Microwave region – 0.1 mm – 1 cm
Radiofrequency region – 100m – 1 cm

INTERACTION OF ELECTROMAGNETIC RADIATION WITH MATTER

When electromagnetic radiation passes through matter, a variety of events may occur. Some of them are

1. **Absorption of radiation:** If the radiation of appropriate energy is used, they may be absorbed by the matter resulting in electronic, vibrational, and rotational changes or a combination of these changes. After absorption, the molecules become excited and go to a high energy level (low stability). They lose energy in the form of heat or re-emit EMR to come back to the ground state (high stability).
   The total energy of the molecule at the ground state is given as
   $$E_0 = E_{electronic} + E_{vibrational} + E_{rotational}$$
   The schematic energy level diagram for a simple diatomic molecule is shown in the figure.

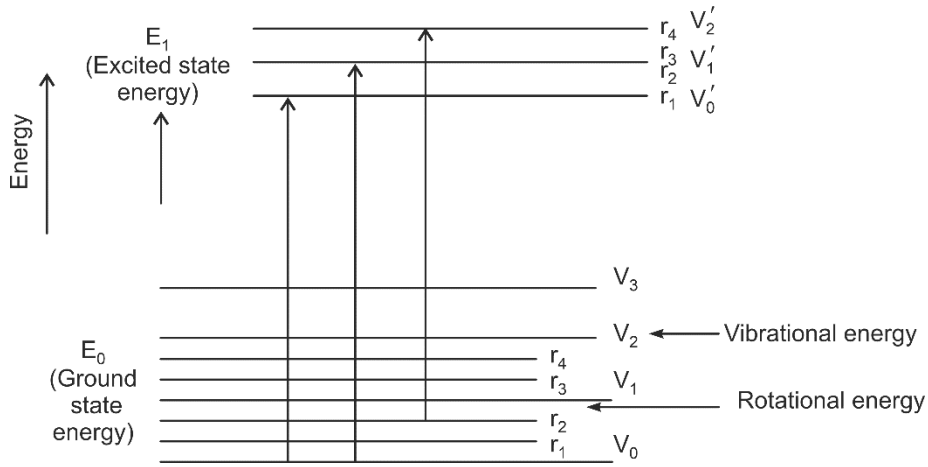


Fig. 1.3 Energy level diagram of a molecule showing vibrational and rotational energy level.

   An electronic transition due to absorption of light may include vibrational or rotational changes.
2. **Scattering**: Sometimes, the radiation is not absorbed completely by the matter, and a portion of it may undergo scattering or reflection
3. **Emission:** In some cases, the molecules become excited after absorbing the radiation, and they lose energy by re-emitting radiation either instantaneously or with a time delay.

Franck–Condon principle

Franck–Condon principle gives the interaction between the electronic and vibrational motions. It states that the timescale of electronic transitions are so rapid when compared with the nuclear

motion and the mass of the nuclei are heavy compared with electrons. An electronic transition is therefore considered to be a vertical transition. The light is absorbed in femtoseconds to nanoseconds, and within this timescale, electrons move, but the nuclei cannot. The heavier atomic nuclei cannot adjust during the absorption but readjust after the absorption process, creating vibrations. Simultaneous occurrence of electronic and vibrational transitions is called vibronic transitions, which give rise to the vibrational structure of the electronic bands.

The quantum mechanical formulation of this principle is that the **intensity of a vibronic transition is proportional to the square of the overlap integral between the vibrational wave functions of the two states that are involved in the transition.**

The Figure illustrates the Franck–Condon principle for vibronic transitions in a molecule. Potential energy functions in both the ground and excited electronic states are shown. In the low temperature, the molecule in the $v = 0$ vibrational level of the ground electronic state absorbs a photon of the necessary energy, makes a transition to the excited electronic state. The electron configuration of the excited state results in a shift of the equilibrium position of the nuclei of the molecule. In the figure, this shift in nuclear coordinates between the ground and the first excited state is labeled as q_{01}. In a simple case of a diatomic molecule, the nuclear coordinate axis refers to the internuclear separation. The vibronic transition is indicated by a vertical arrow, assuming constant nuclear coordinates during the transition. In the electronic excited state, molecules quickly relax to the lowest vibrational level of the lowest electronic excitation state (Kasha's rule). From there, they decay to the electronic ground state through photon emission. The Franck–Condon principle is applied equally to absorption as well as fluorescence.

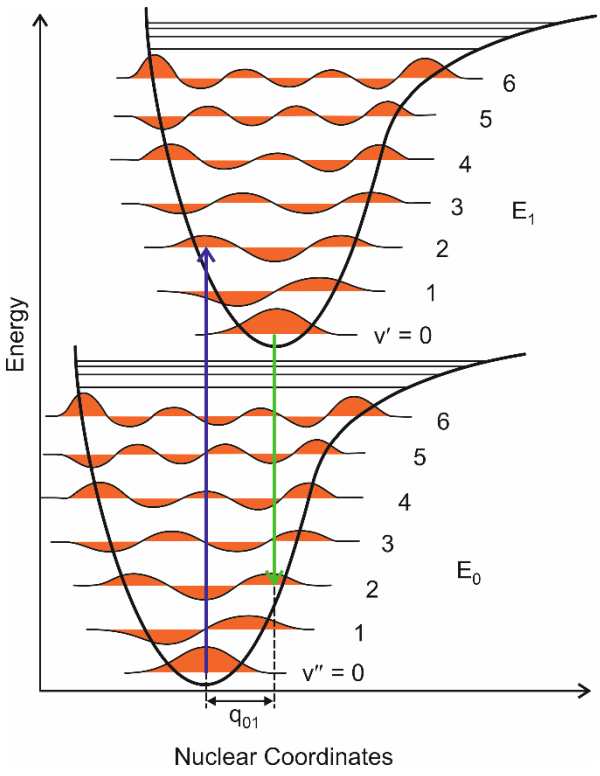


Fig. 1.4 Franck-condon principle for vibronic transitions in a molecule.

1.1 VISIBLE SPECTROPHOTOMETRY AND COLORIMETRY

UV-Visible Spectroscopy is one of the important absorption spectroscopic techniques, and it is most commonly employed for quantitative and qualitative analysis. It is also playing a role in the structural elucidation of organic compounds.

The wavelength range of visible light is 800-400 nm (8000-4000 Angstrom). This range of visible radiation is used for colorimetric analysis. Determination of concentration of coloured compounds in solution is called colorimetry. A colorimeter is a device used to test the concentration of a solution by measuring its absorbance at a specific wave length of light.

Coloured substances absorb in the visible region, and the colorless substances absorb in the UV region. The amount of light absorbed differs with the wavelength, and a plot of absorbance vs. wavelength is called an absorption spectrum**. In the absorption spectrum, the wavelength at which maximum absorption occurs is called λ_{max}.** This λ_{max} is characteristic or unique for every substance and is used to identify the substances (qualitative analysis). **λ_{max}** is **independent of concentration, meaning that the λ_{max} will not change** even if the concentration is changed.

A calibration graph is a plot of concentration vs. absorbance. This graph helps to determine the concentration/amount of the drug present in the given sample solution (quantitative analysis).

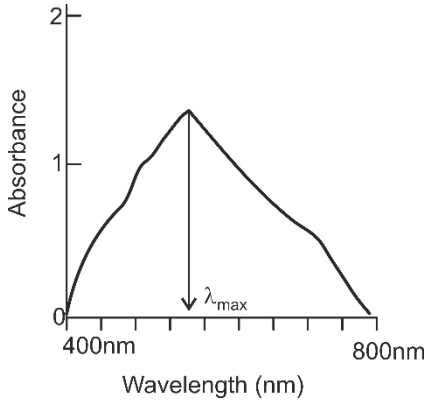


Fig. 1.5 Absorption spectrum.

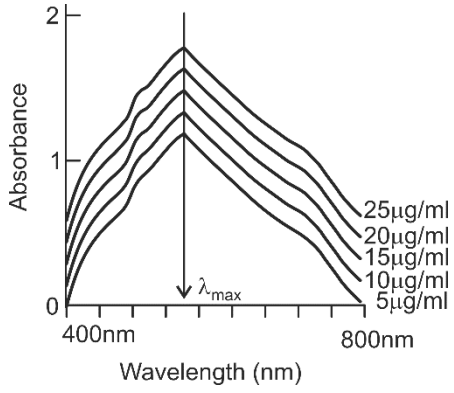


Fig. 1.6 Absorption spectrum of a substance in different concentrations.

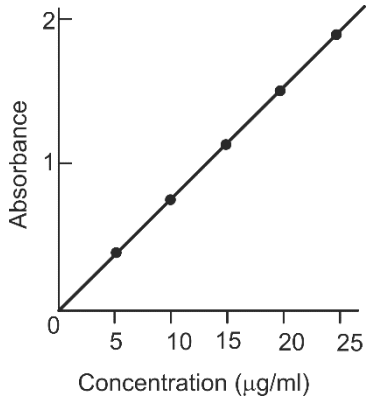


Fig. 1.7 Calibration curve.

THEORY OF SPECTROPHOTOMETRY & COLORIMETRY (ABSORPTION LAWS)

When light is incident upon a homogenous medium, a part of the incident light is reflected, a part is absorbed, and the remainder is transmitted so

$$\mathbf{I_0 = I_a + I_t + I_r} \quad \text{.....(1.1)}$$

Where $\mathbf{I_0}$ – incident light; $\mathbf{I_a}$ – absorbed light; $\mathbf{I_t}$ – transmitted light and $\mathbf{I_r}$ – is reflected light. The value of I_r is minimal (about 4%), and it is eliminated for air-glass interfaces if a comparison cell is used. So equation (1.1) becomes as

$$I_0 = I_a + I_t \quad \text{.....(1.2)}$$

The two laws governing the absorption of light are:

1. **Beer's law** (concentration-dependent)
2. **Lambert's law** (Thickness/path length dependent absorbance)

Lambert's law states that when a beam of monochromatic light is allowed to pass through a transparent medium, **the rate of decrease of intensity with the thickness of the medium is directly proportional to the intensity of incident light.**

$$-\frac{dI}{dt} \alpha\, I$$

$$-\frac{dI}{dt} = k\, I \quad \text{.....(1.3)}$$

$$-\frac{dI}{I} = kdt \qquad \text{(rearranging the equation)}$$

On integration

$$-\ln I_t = kt + b \quad \text{(ln – natural logarithm)} \quad \text{.....(1.4)}$$

where b is the integration constant

When thickness is zero, there is no absorbance. So $I_t = I_0$

Substituting this in equation 1.4

$$- \ln I_0 = k \times 0 + b$$

$$- \ln I_0 = b$$

Substituting this value of b in equation 1.4

$$- \ln I_t = kt - \ln I_0$$

On rearranging

$$\ln I_0 - \ln I_t = kt$$

$$\ln \frac{I_0}{I_t} = kt$$

Removing natural logarithm

$$I_0/I_t = e^{kt}$$

Taking inverse on both sides

$$I_t/I_0 = e^{-kt}$$

$$\mathbf{I_t = I_0.e^{-kt}} \qquad \text{.....(1.5)}$$

This equation is called as Lambert's law where I_0 is intensity of incident radiation, I_t is transmitted radiation and k is a constant.

Lambert's law shows the logarithmic relationship between transmittance and the optical path length

Beer's law: Beer observed a similar relationship between transmittance and the concentration of a solution. Beer's law states that the **intensity of a beam of monochromatic light decreases** exponentially with an **increase in the concentration of absorbing species arithmetically**.

$$-\frac{dI}{dc} \alpha I$$

$$-\frac{dI}{dc} = k'I \qquad \text{.....(1.6)}$$

Rearranging the equation

$$-\frac{dI}{I} = k'dc$$

On integration

$$- \ln I_t = k'c + b \text{ (ln – natural logarithm)} \qquad \text{.....(1.7)}$$

where b is the integration constant

When concentration is zero, there is no absorbance. So $I_t = I_0$

Substituting this in equation 1.7

$$- \ln I_0 = k' \times 0 + b$$

$$- \ln I_0 = b$$

Substituting this value of b in equation 1.7

$$-\ln I_t = k'c - \ln I_0$$

On rearranging

$$\ln I_0 - \ln I_t = k'c$$

$$\ln \frac{I_0}{I_t} = k'c$$

Removing natural logarithm

$$I_0/I_t = e^{k'c}$$

Taking inverse on both sides

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

$$\mathbf{I_t = I_0.e^{-k'c}} \quad \text{.....(1.8)}$$

This equation is called as Beer's law equation where I_0 is intensity of incident radiation, I_t is intensity of transmitted radiation and k is a constant.

On combining equations 1.5 and 1.8, we get

$$\mathbf{I_t = I_0.e^{-Kct}}$$

on changing from natural logarithms to base 10,

$$\mathbf{I_t = I_0.10^{-Kct}} \text{ where } K = k \times 0.4343$$

Rearranging terms

$$I_t/I_0 = 10^{-Kct}$$

Taking inverse on both sides

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

Taking log on both sides

$$\log I_0/I_t = Kct \quad \text{.....(1.9)}$$

Transmittance $T = I_t/I_0$, Absorbance $A = \log 1/T$

Substituting for T in absorbance A, the equation becomes

$$A = \log I_0/I_t \quad \text{.....(1.10)}$$

From equations 1.9 and 1.10

$$A = Kct.$$

If a is used instead of K, then

$$A = act$$

$$\mathbf{\log I_0/I_t = act}$$

is the mathematical expression of Beer Lambert's law,

Where A= absorbance, a = absorption coefficient or absorptivity, c= concentration of the substance and t = path length in cm.

Absorptivity reflects the sensitivity of the procedure. Sensitivity increases with an increase in absorptivity.

If the concentration is expressed in moles/liter, then a is replaced with ε.Then the equation becomes

$$\mathbf{A = \varepsilon ct} \qquad \text{.....(1.11)}$$

where

A = absorbance, ε = molar absorption coefficient or Molar absorptivity, c = concentration of the substance in mol/lit, t = path length in cm.

A = ε, when the concentration is one mole per liter and the path length is one centimeter.

Thus **Molar absorption coefficient** is defined as the absorption of a one molar solution when the path length is one cm. The molar absorption coefficient is otherwise called molar absorptivity.

The value of **molar absorptivity is constant** even if the concentration of the solution and the thickness of the container change. But it **differs with different wavelengths**.

Absorbance also varies with wavelength. For this reason, only monochromatic light is used in spectrophotometry and colorimetry.

Molar absorption coefficient **ε** is expressed as

$$\boldsymbol{\varepsilon} = A^{1\%}_{1cm} \times \frac{\text{Molecular weight}}{10}$$

Where, $A^{1\%}_{1cm}$ is the **specific absorbance which is the absorbance of 1% w/v solution, using a path length of 1cm**. It is a constant for each drug at the given wavelength. This specific absorbance can be used for the quantitative estimation of formulations. $\boldsymbol{\varepsilon_{max}}$ is the value of ε at $\boldsymbol{\lambda_{max}}$.

DEVIATIONS FROM BEER'S LAW

According to Beer's law, the plot of concentration Vs. absorbance should give a straight line passing through the origin. The straight line can also be obtained by using the line of best fit or method of least squares or by joining the maximum number of points so that the positive and negative errors are balanced or minimized. The regression line obtained can also be used for determining the concentration of a solution. When a nonlinear curve (not a straight line) is obtained in a plot of concentration vs. absorbance, the system is said to have deviations from Beer's law.

Beer's law is normally obeyed only in a certain concentration range, and above or below it may exhibit deviation.

The reasons for the deviations may be classified as

1. Real Deviations
2. Instrumental Deviations
3. Chemical Deviations
4. Incomplete reaction

1. **Real Deviations:** There are two types of real deviations from Beer's law
   (i) **Positive deviation (concave upwards):** It occurs when a small change in concentration produces a great change in absorbance.
   (ii) **Negative deviation (concave downwards):** It occurs when a large change in concentration produces a small change in absorbance.

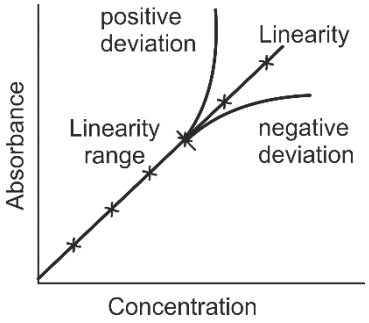


Fig. 1.8 Real deviations

2. **Instrumental deviations:** Factors like stray radiation, improper slit width, fluctuation in a single beam, and the type of light used can influence the deviation.
   - If monochromatic light is not used, deviation may occur.
   - If the width of the slit is not proper and if it allows undesirable radiations to fall on the detector, these undesirable radiations might be absorbed by the impurities present in the sample leading to changes in absorbance.
   - The possibility of error due to monochromatic radiation may be minimized by selecting a spectral region, where the change in absorptivity with a change in wavelength is very small.
   - This dictates that wavelength selection should be from a broad band (close to λ_{max}) rather than a sharply rising or sharply falling section of the absorption curve.
   - It is necessary to prepare a calibrated curve for the absorbance concentration relationship at the chosen wavelength to avoid errors.
3. **Chemical deviations:**
   Physicochemical changes in solution: Factors like association, dissociation, ionization (change in pH), faulty development of colour (incompletion of reaction), and refractive index at high concentration can influence such deviation.
   - **Association:** Methylene blue at a concentration of 10^{-5} M exists as monomer and has λ_{max} of 660nm. But methylene blue at a concentration above 10^{-4} M exist as a dimer or trimer and has a λ_{max} of 600. If the shift of this λ_{max} is not considered during absorbance measurements, then deviations occur.
   - **Dissociation**: Benzyl alcohol in chloroform exists in a polymeric equilibrium;
     $4C_6H_5CH_2OH \leftrightarrow (C_6H_5CH_2OH)_4$.
     Dissociation of the polymer increases with dilution. The monomer absorbs at 2.750 to 2.765 μ, where as the polymer absorbs at 3.0 μ. Hence absorption at 2.75 micron shows a negative deviation whereas at 3.0 μ gives a positive deviation.
     Potassium dichromate at high concentration exists as an orange solution (λ_{max} of 450nm). But on dilution, dichromate ions are dissociated into chromate ions which is yellow in color (λ_{max} of 410nm)
     $Cr_2O_7^{2-}$ (orange) + $H_2O \rightarrow 2H^+ + 2CrO_4^{2-}$ (yellow).

If 450 nm is used for absorbance measurement for dilute solutions, then it leads to deviations from Beer's law.

- **Coloured solute ionization or dissociation** in solution leads to deviation in Beer's law. Colour change of dichromate ion on dilution can be given as an example.
- The deviation may also occur due to the **presence of impurities** that fluoresce or absorb at the absorption wavelength. This interference introduces an error in the measurement of absorption of the sample.
- If the solute undergoes **polymerization**, Ex Benzyl alcohol in carbon tetrachloride in high concentration exists in polymeric form. Dissociation of this polymer increases with dilution, which causes changes in absorbance, causing deviation.
- Beer's law can't be applied for suspensions, but colorimetry can be used with different known concentrations to prepare the reference curve.

4. **Incomplete reaction:**
   - Sufficient time should be allowed before taking absorbance for the reaction to complete so that the colour will be formed completely.
   - If the readings are taken after the colour fades away (instability), deviations can occur. Ex: Limit test for iron: reaction of iron with thioglycolic acid.

INSTRUMENTATION

Table 1.2 Summary of instrumentation

Source of light	Filters and Monochromators	Sample cells	Detectors
Tungsten lamp Carbon arc lamp	Absorption filters. Interference filters Prisms Grating	Glass cuvettes Polystyrene cells for aqueous solutions	1. Barrier layer cell or Photo voltaic cell 2. Photo tubes or Photo emissive cells 3. Photomultiplier tubes.

The common instruments used for measuring emission or absorption of radiant energy are

(i) Photometer (ii) Spectrophotometer

Photometer: These are inexpensive and less accurate instruments. Filters are employed to isolate a wave length region, and a photocell or photo tube is used as a detector. A commercial photometer with filters is called **colorimeter**. It measures either absorbance or transmittance. The working range of wavelength for these instruments is 400-700nm.

Spectrophotometer: These are a little more expensive than colorimeters. They are used for wider wavelength regions, i.e., 360 nm- 900nm or 1000nm. Since these instruments employ **grating monochromators** for scanning and **photomultipliers** as detectors, they are highly accurate. The modern spectrophotometers are microprocessors or computer-based for easy handling of data.

The basic components of both types of instruments are

(a) Source of light
(b) Filters or monochromators
(c) Sample cells
(d) Detectors

Now we will discuss these components

(a) Source of light /radiation sources: The visible region extends from 400-800nm

The requirements of the radiation sources are

1. It must be stable and no fluctuations should be there.
2. It should provide continuous radiation from 400-800nm.
3. The intensity should be adequate.

The common radiation sources used in colorimetry (visible region) are

1. Tungsten lamp: Most widely used. The lamp consists of a tungsten filament in a vacuum bulb. It provides sufficient intensity.

Advantages

(i) Stable, robust and easy to use.
(ii) The Emission intensity varies with wavelength.

Disadvantages

(i) The intensity at the shorter wavelength is less
(ii) Difficult to maintain a constant intensity

2. Carbon arc lamp: This lamp is used for high intensity. It provides an entire range of the visible spectrum.

(b) Filters and Monochromators: The source emits continuous spectra from 400-800nm. This is called Polychromatic light consisting of several wavelengths. A colorimeter or spectrophotometer works only with a monochromatic light consisting of a single wavelength. A filter or a monochromator is used in these instruments to convert the polychromatic light into monochromatic light.

(a) Filters: There are two types of Filters

1. Absorption filters.
2. Interference filters

1. Absorption filters: These filters are made of a solid sheet of glass coloured by dissolved or dispersed pigments. Dyed gelatin is also used as an absorption filter.

The selection of filters is based on this color wheel. If the solution is red coloured, then the green filter is used. For the green solution red filter is used (complementary colors).

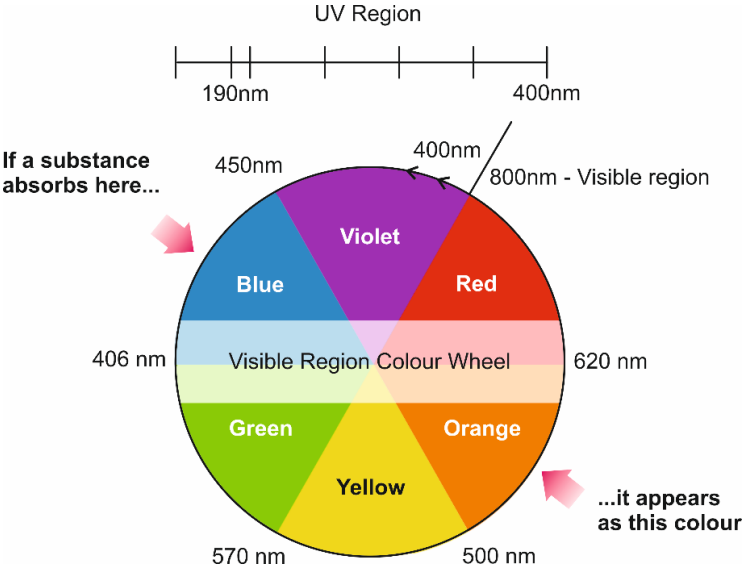


Fig. 1.9 Color wheel showing complimentary colors.

Absorption filters are further classified as **Cut off filters and band pass filters.**

Advantages:

1. Simple construction
2. Cheaper.
3. Easy Filter selection.

Disadvantages:

1. Accuracy is less because of broad band pass (± 30 nm).
2. Absorption by filters lead to less intensity of radiation

2. Interference Filters

1. This filter consists of a dielectric spacer film made up of CaF_2, MgF_2, or SiO, between two reflecting parallel silver films.
2. The thickness of the dielectric film may vary to give 1/2λ (1st order), 2λ/2 (2nd order), 3λ/2 (3rd order spectra), etc.
3. The principle of working is that the radiation reflected by the 2nd film and the incident radiation undergo constructive interference giving monochromatic radiation. The wavelength of the monochromatic radiation depends on the formula

$$\lambda = 2\eta b/m$$

Where λ = wavelength produced
η = dielectric constant of the film material
b = layer thickness
m = order no (1st,2nd, 3rd, etc)

4. Band pass is 10-15 nm. Transmission is maximum 40%

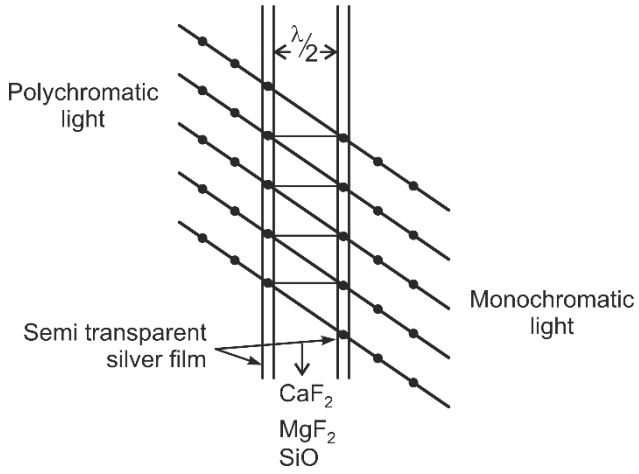


Fig. 1.10 Interference filter – FABRY PEROT.

Advantages:

1. Inexpensive
2. More accurate than absorption filters because of lower band pass.
3. Additional filters can be used to cut off undesired wavelengths.

Disadvantages:

1. Transmission is low
2. Because of the narrow band pass, higher resolution is not obtained.

(b) Monochromators: The **monochromators** are more efficient in converting the **polychromatic light to monochromatic light** when compared to filters. The parts of a monochromator are

1. Entrance slit (to get a narrow source of light)
2. Collimator (to make the light parallel)
3. Grating or prism (to disperse the light)
4. Collimator (to reform the monochromatic image of entrance slit)
5. Exit slit (to allow the light to fall on sample cell)

There are two types of monochromators

1. Prisms
2. Grating

1. Prisms: The prisms disperse the light into individual wavelengths or colors. They are made of glass and used in inexpensive instruments. Its **resolution is better than filters** because the band width is lower. The resolution of the prism is determined by the size and refractive index of the prism.

There are two types of the prism

I. Refractive type: In this type, the light from the source falls on the collimator through the entrance slit. The parallel rays of light from the collimator enter the prism, which disperses the light into component colors or wavelengths. The second collimator receives the dispersed light and reforms the images of the

entrance slit. The reformed images are the colors of the VIBGYOR (Violet, Indigo, Blue, Green, Yellow, Orange, and Red). The required wavelength can be selected by rotating the prism or by moving the exit slit and the selected wavelength passes through the exit slit to the sample.

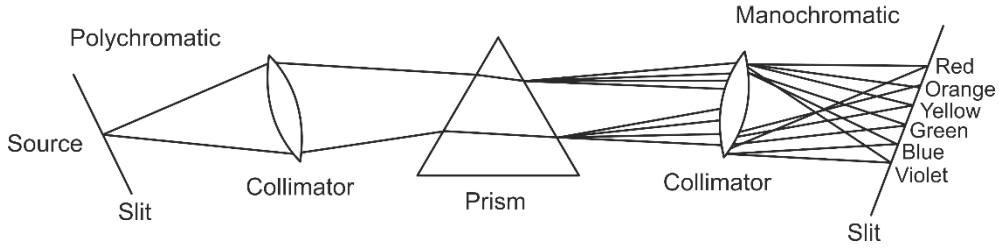


Fig. 1.11 Prism monochromator-dispersive type

II. Reflective type (Littrow mounting): This type has a reflective surface present on one side of the prism. The dispersed radiation gets reflected on the same side of the source and is collected on the same side. The working principle is the same as the refractive type.

The advantage of prisms:

1. Dispersed wavelengths do not overlap

Disadvantages:

1. Non-linear dispersion
2. Temperature sensitive

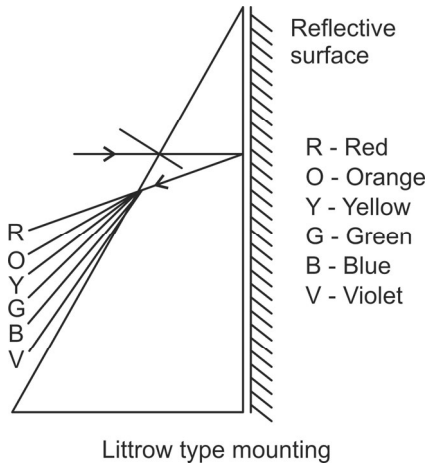


Fig. 1.12 Prism monochromator-reflective type (LITTROW MOUNTING)

2. Gratings: Gratings are **more efficient** in the conversion of polychromatic light to monochromatic light—a **resolution of ±0.1 nm** can be achieved with gratings, and hence they are used in spectrophotometers.

There are two types of Gratings

1. Diffraction grating
2. Transmission grating

1. Diffraction grating: A grating is made up of a large number of parallel lines (grooves) ruled on highly polished surfaces like glass, quartz, alumina, and alkyl halides based on the type of the instrument (visible/UV/IR spectrophotometer). Generally 3600 grooves or more per mm are drawn for ultraviolet and visible regions, and 20 grooves per mm are drawn for the IR spectrophotometer.

These are replica gratings made from the master grating. Replica gratings are prepared by coating the original master grating with epoxy resin and removed after setting. Aluminizing its surface makes the surface of the replica grating reflective.

The principle is **diffraction causes interference**. The incident rays on the grating, get constructive interference with the reflected rays and the resulting radiation wavelength is given by the equation

$$m\lambda = b (\sin i \pm \sin r)$$

Where λ = wavelength of produced light
b = grating spacing
i = angle of incidence
r = angle of reflection
m = order (0, 1, 2, 3, etc).

There are two different types of diffraction grating – the ruled grating and the holographic grating.

Gratings are preferred over prisms because of their efficiency.

2. **Transmission grating:** In these types of gratings, refraction takes place. The principle is **refraction produce interferences**. The radiation transmitted through the grating reinforces the partially refracted radiation leading to constructive interferences.

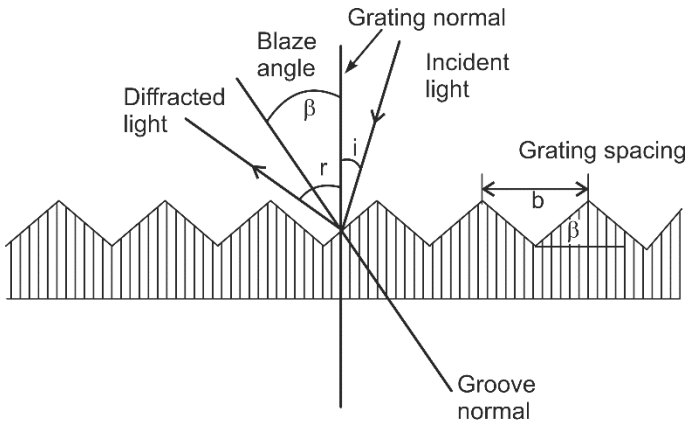


Fig. 1.13 Diffraction grating.

Transmission Grating

The wavelength produced can be calculated using the formula

$$\lambda = \frac{d \sin\theta}{m}$$

Where λ = wavelength of radiation produced.
d = 1/lines per cm
m = order no (0, 1, 2, 3, etc)
θ = angle of deflection/diffraction

Hence, the required light radiation (λ) can be produced either by moving grating and keeping the slit fixed or vice versa.

Advantage:

1. They give linear dispersion.
2. High resolution

Disadvantage:

1. Overlap of spectral orders.

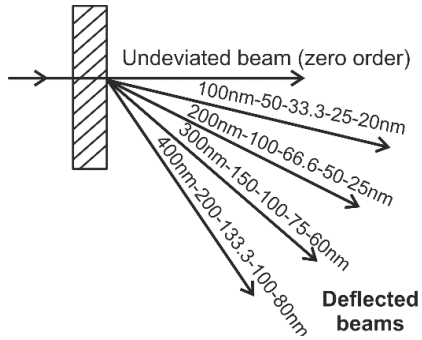


Fig. 1.14 Transmission Grating

Slits: The two slits are the **entrance slit and the exit slit**. The width of the monochromatic image of the entrance slit is the same as the width of the exit slit to pass through the exit slit.

(c) Sample cells: The sample cells, known as cuvettes, are used to hold the sample.

The construction material of the cuvette varies with the instrument and the sample nature.

The material of the cuvette should not absorb in the working wavelength.

Material for **visible region: Color corrected fused glass.**

For aqueous solutions, Polystyrene cells are available.

For UV region: Quartz cuvettes are used because **glass absorbs UV radiation**.

The cells are available in different shapes like cylindrical or rectangle.

Small volume cells (0.5ml or less) and large volume cells (5-10ml) are available depending on their capacity to hold sample volume

The common path length (internal distance) is 1cm. Long path length cells with 10cm and short path length cells with 1 or 2mm are also available.

(d) Detectors: The radiation from the source through the monochromator passes through the sample. The sample absorbs part of the radiation, and the rest is being transmitted. This transmitted radiation falls on the detector, and the intensity of the absorbed radiation is determined by the detector and displayed.

Detectors used in UV Visible spectrophotometers are called photometric detectors. They convert the **light energy to an electrical signal** which is recorded.

Three types of detectors are

1. Barrier layer cell or Photo voltaic cell
2. Photo tubes or Photo emissive cells
3. Photomultiplier tubes.

1. Barrier layer cell or Photo voltaic cell: The detector consists of a metal base like iron or aluminum acting as one electrode. A thin layer of semiconductor material like selenium is deposited on its surface. The surface of the selenium is covered by a very thin layer of silver or gold which acts as a second collector electrode.

The selenium layer separates the two electrodes, and it has extremely low electrical conductivity. When radiation falls on the selenium layer, the electrons are generated at the selenium silver interface. The silver layer collects these electrons. The collection of electrons on the silver surface creates a potential difference between the two electrodes. If the resistance in the external circuit is small, the current flows through the system. The **current produced is directly proportional to the intensity of the incident radiation** on the detector.

Advantages:

(i) Simple in design and rugged

(ii) No external power supply is required.

Disadvantages:

(i) Less sensitive to blue region; use is limited to the visible region

(ii) Amplification of the signal is not possible because the resistance of the external circuit should be low.

(iii) Fatigue effects.

2. **Photo tubes or Photo emissive cells:** This detector consists of an evacuated glass tube containing a photo cathode and an anode as a collector electrode. The photo cathode is coated with a light-sensitive layer like Cesium, potassium or silver oxide.

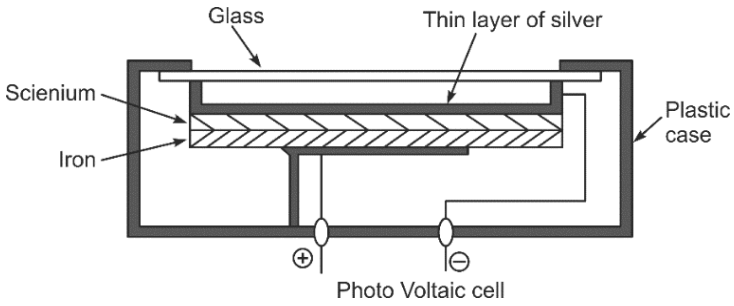


Fig. 1.15 Barrier layer cell.

When the transmitted radiation falls on the photo emissive cathode, it emits photoelectrons. The electron flow towards the anode causes the **current flow, which is proportional to the intensity of radiation falling on the detector.**

Advantages:

1. A signal from the detector can be amplified
2. More sensitive than Photovoltaic cells.
3. Composite coatings containing Cesium, cesium oxide, or silver oxide can be used on the cathode to increase the sensitivity and workable wavelength range.

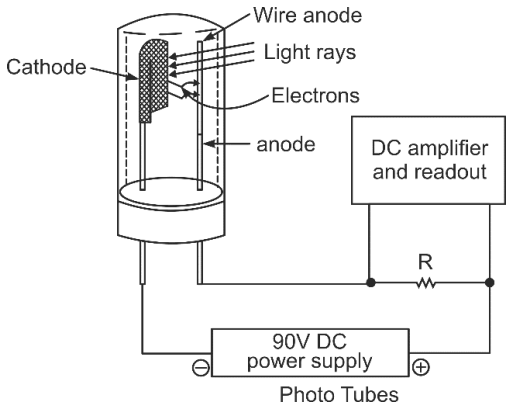


Fig. 1.16 Photo tubes.

3. **Photomultiplier tubes (PMT):** Though expensive, this detector is employed in sophisticated instruments due to its sensitivity.

   The working principle of this detector is the **multiplication of photo electrons by secondary emission of electrons.** PMT detector consists of a photo cathode and a series of anodes called dynodes. Each dynode is maintained at a successively higher potential 75-100V higher than the preceding dynode. Up to 10 dynodes are used in the series. When the radiation hits the cathode surface, electrons are emitted. When these electrons hit the dynodes, at each stage, the electrons are multiplied by a factor of 4 or 5 because of the secondary emission. An overall amplification factor of about 10^8 is achieved.

   Advantages:

   1. Most sensitive
   2. Can detect very weak signals
   3. Faster response time
   4. It can be used in fluorescence measurements

   Disadvantages:

   1. Expensive
   2. It should be shielded from stray light.

   Advantages of Colorimetry

   1. Specific to one chemical species.
   2. Inexpensive per analysis.
   3. Even non-chemistry personnel can perform the experiment.

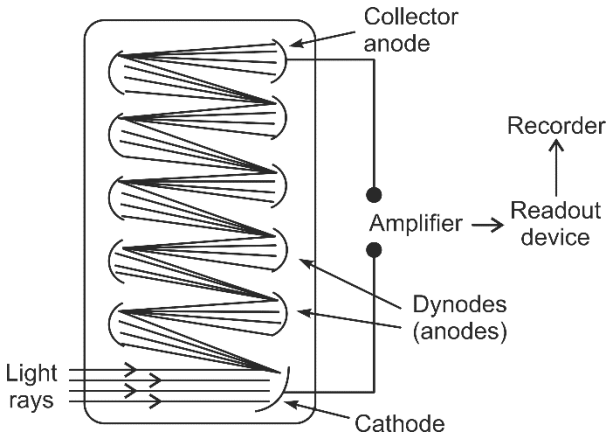


Fig. 1.17 Photo multiplier tube.

Disadvantages

1. Similar colour from interfering substances can produce errors in results
2. May not be suitable for more precise analysis.
3. Matrix interferences produce bad results in controlled conditions.

Types of instruments

The various types of instruments used in colorimetry are Colorimeters, Spectrocolorimeters, and Spectrophotometers. They differ in their construction, cost, sophistication, and automation.

Colorimeters: These instruments contain a tungsten lamp, absorption filters and photovoltaic cells. They read either transmittance or absorbance. Colorimeters are single beam instruments. Wavelength accuracy is normally ± 30nm.

Spectrocolorimeters: Contain prism monochromators instead of filters and either photovoltaic cell or phototube as a detector. These are also single beam instruments measuring either transmittance or absorbance. Wavelength accuracy usually is ± 5nm.

Spectrophotometers: These types of instruments are expensive and more sophisticated. They can read either transmittance or absorbance. They can record the absorption spectrum using a recorder. Spectrophotometers are double beam instruments, where the absorbance of sample and reference solution can be measured simultaneously. The automated devices possess various options like storage of spectrum, comparison of spectra, quantitative techniques, rapid wavelength scanning, data manipulation and derivative spectral mode. Most of them are software-driven instruments. Wavelength accuracy is normally ± 0.1 nm.

These instruments are more accurate and reliable.

The schematic diagram and the working of the instruments are given below

Single beam colorimeter

The source of light here is the tungsten lamp. A concave mirror focuses the light onto the slit. The slit allows the light to pass through the absorption filter, which selects the required

wavelength and makes it fall on the sample cell containing the analyte solution. The analyte absorbs the part of the radiation, and the remaining light is transmitted. The intensity of this transmitted radiation is determined by a photo voltaic cell. The diagram of single beam colorimeter is shown in fig 1.18.

Advantages:

1. Simple in construction
2. Less cost
3. Easy operation

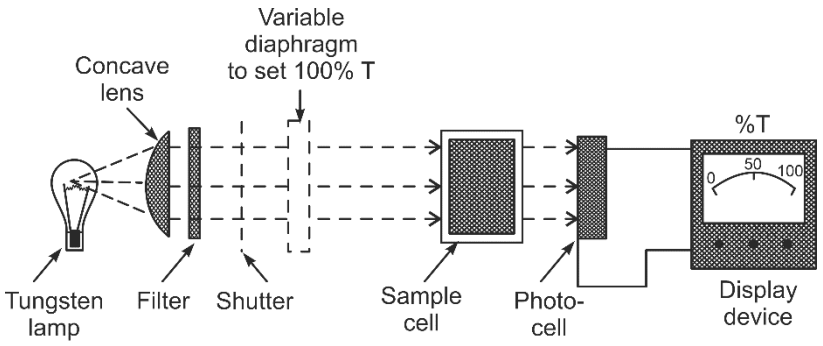


Fig. 1.18 Single beam colorimeter.

Disadvantages:

1. Fluctuations in the intensity of the source affect the absorbance readings.
2. Time-consuming because zero adjustment is to be done in every wavelength.
3. No recorder is available.

Double beam colorimeter: The construction of a double beam colorimeter is the same as a single beam except that the light beam, after passing through the filter or monochromator, is split into sample and reference beams by a beam splitter. Two detectors are used to detect the reference beam and the sample beam individually.

Applications of Colorimetry

1. **Purity determination:** Colorimetry is used to detect the presence of impurities in the sample. Coloured impurities absorb the radiation and give additional peaks in the spectrum.

   E.g., Vitamin B_{12} (cyano cobalamin) absorbs in three wavelengths 278,361 and 550 nm.

   The ratio of absorbances 278/361= 0.57

   550/361 = 0.3

   We can identify the presence of impurities by the change in the absorbance ratios.

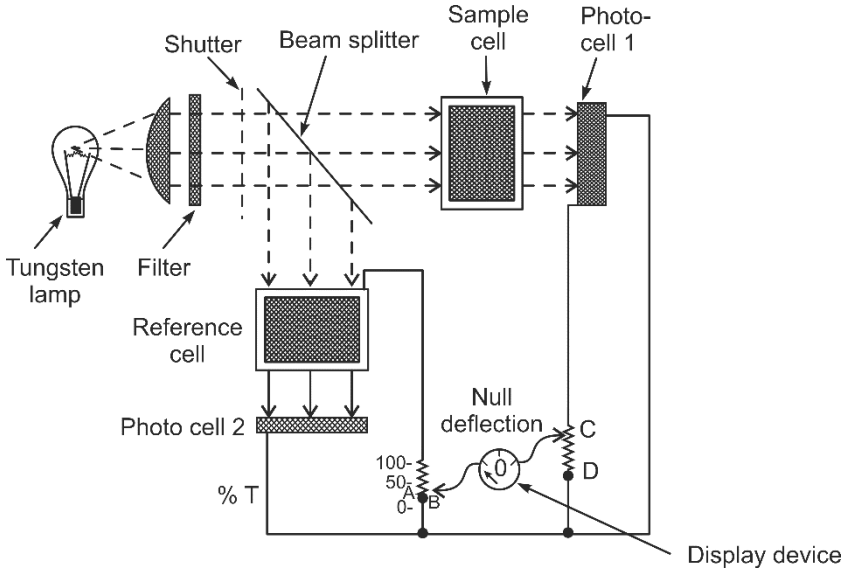


Fig. 1.19 Double beam colorimeter.

2. **Quantitative analysis:** Determination of concentration and amount of the substance present in the given sample is called Quantitative estimation. Using these values, percentage purity can be determined. The quantitative estimation of drugs by colorimetry can be performed by knowing the parameters like λ_{max}, Beer's law concentration range for that substance, solvent, reagents and other conditions.

   The types of quantitative estimation are

   (a) Using $A^{1\%}_{1cm}$ value (specific absorbance): This method is useful in the estimations of raw material as well as formulations when the reference standard is not available. $A^{1\%}_{1cm}$ value can be obtained from Pharmacopoeia, Text books or Journals

   (b) Reference standard available: If a reference standard is available, specific absorbance can be found experimentally and the above method can be used. Other methods available are

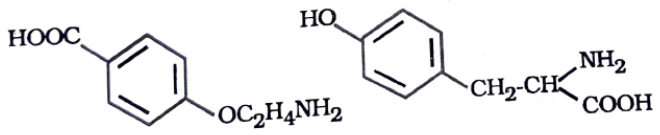
   (i) Direct comparison method (single standard): The absorbance of a standard solution of known concentration is compared with the absorbance of the sample solution and the concentration of the sample solution is calculated. From the concentration, the amount and the % purity can be calculated.

   (ii) Calibration curve method or multiple standard method: The calibration graph method is used to minimize the error introduced in the preparation of solutions or absorbance measurements.

   In this method, a calibration curve is plotted with concentration vs. absorbance measured with five or more concentrations of the reference or standard. A straight line passing through the origin is obtained. If a straight line is not obtained, a straight line is obtained through the maximum number of points or by regression analysis leading to a line of best fit. The unknown concentration of the sample can be obtained using the absorbance of the sample and the calibration

graph. The extrapolation of the graph gives the unknown concentration and amount, and the % purity of the sample can be calculated.

3. **Determination of Ligand/Metal ratio in metallic complexes:** Some organic compounds form metallic complexes with some metals, and the ratio of the metal complexes can be determined using the calibration graph method.
4. **Structural elucidation of organic compounds:** The structure of the unknown compound can be elucidated by comparing the absorption spectrum with that of the known compound. For e.g.,



I II

These are the structures proposed for Tyrosine. When the absorption spectrum of Tyrosine was obtained, it was found to be similar to the absorption spectrum of Phenol. Hence the structure II is proposed for Tyrosine.

5. **Determination of pK_a of indicators:** Determination of dissociation of acid-base indicators like Methyl orange, Methyl red can be determined by the formula

$$pK_a = pH - \log [ionized]/[unionized]$$

The plot of absorbance vs. concentration at different pH is recorded and pK_a is calculated using the equation.

6. **Determination of molecular weights:** Molecular weight is determined using the formula

$$\text{Mol. wt} = \varepsilon / a = \varepsilon \times ct/A$$

where A = absorbance, c = concentration, t = pathlength and a = absorptivity

7. **Determination of functional groups and elements:** Even low concentrations of ions, elements or functional groups can be determined by using chromogenic (colour producing) reagents**.**

E.g., Determination of Iron using 1,10 phenanthroline, Ammonia using Nessler's reagent and amino acids using Ninhydrin.

8. **Determination of organic compounds and Pharmaceutical substances:** The pharmaceuticals are also determined by colorimetry using chromogenic reagents for producing colors.

E.g., Phenols determined using Ferric chloride, Betamethasone using Phenyl hydrazine.

1.2 UV SPECTROSCOPY

INTRODUCTION

The coloured compounds absorb in the visible region, most of the colorless compounds absorb in the UV region. The UV radiation wavelength starts from the blue end of the visible light

(4000Å) and extends up to 2000 Å. The UV region is divided into two regions

1. Near UV region - 2000 Å -4000 Å (200-400nm)
2. Far or vacuum UV region – below 2000 Å (200nm).

THEORY/PRINCIPLE OF UV SPECTRA

UV Spectroscopy is the study of the absorption of UV radiation by atoms or molecules. Both visible and UV radiations excite only the **valence electrons.** The molecules absorb UV radiation, and they undergo the transition from the ground state to the excited state. Thus, **electronic excitation** is the principle involved in UV spectroscopy. Because of this, UV spectroscopy is also called **Electronic spectroscopy**.

UV absorption is characteristic for each substance, and it depends on the type of electrons present in the molecule. The absorption intensity follows Beer–Lamberts law, and it is proportional to concentration and path length.

Types of electrons in molecules

Three types of electrons are present in molecules. They are

1. **σ electrons** present in saturated compounds. These electrons show absorption in **vacuum UV** region (<200nm)
2. **π electrons** present in unsaturated compounds. (e.g., double or Triple bonds).
3. **n electrons** are called non bonded electrons and are not taking part in bonding between atoms. e.g., organic compounds containing sulphur, nitrogen, oxygen, or halogens.

All molecules having π electrons, **n electrons,** or a combination of both electrons absorb in the characteristic wavelength region and undergo the transition from the ground state to the excited state. This characteristic absorption helps in the structural elucidation of molecules by identifying the nature of electrons present.

ELECTRONIC TRANSITIONS

When an atom or molecule **absorbs energy**, **electrons are promoted** from their ground state to an excited state. When a molecule is **electronically excited**, the electrons move from **bonding to antibonding orbitals**. The π electrons are excited to π* antibonding orbitals, and n electrons are excited to either σ* or π* anti bonding orbitals.

The amount of **energy** required to excite **σ electrons** is very high and hence they do not absorb in the **UV** region. The molecules which possess **π electrons (unsaturated compounds**) and **n electrons (lone pair**) absorb energy in the **UV** and **visible region** and these electrons get excited to the higher energy levels. Thus, the possible transitions are **π-π*, n-π*** transitions.

The possible electronic transitions are shown diagrammatically

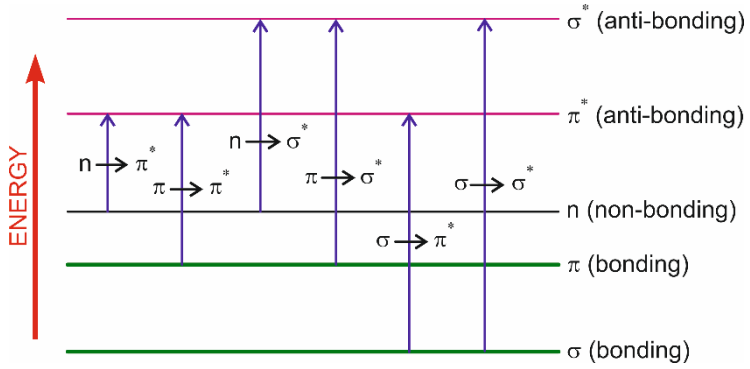


Fig. 1.20 Different types of electronic transitions.

TYPES OF ELECTRONIC TRANSITIONS

The electronic transitions are caused by the absorption of UV light by the molecules leading to the excitation of valence electrons from the ground state to the excited state. The various transitions are **π-π*, n-π*, n-σ* and σ-σ*.** The energy requirements for these transitions are given in increasing order n-π*< π-π*< n-σ*< σ-σ*.

Types of transition: It is not necessary that the exposure of the compound to UV radiation will always result in an electronic transition. The probable occurrence of the transition is dependent on the value of the molar extinction coefficient (λ_{max}) and certain other factors. Thus, the transitions can be divided into two types

(i) Allowed transitions
(ii) Forbidden transitions

(i) Allowed transitions: Transitions having ε_{max} value 10^4 or more are allowed transitions. They arise due to π-π* transitions.

E.g., In 1,3 butadiene π-π* transition has ε_{max} value of 21,000 at 217 nm. Thus, it is an allowed transition.

(ii) Forbidden transitions: Transitions having ε_{max} value less than 10^4 are forbidden transitions. They arise due to n-π* transitions.

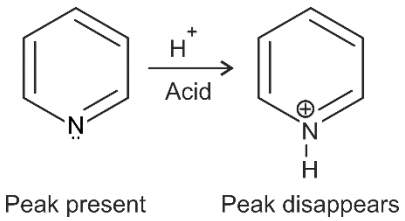
E.g., n-π* transition of saturated aldehyde or ketone shows weak absorption near 290 nm and its ε_{max} value less than 100. So, it is a forbidden transition.

On application of selection rules, σ-σ*, n-σ*,π-π* are allowed transitions, and n-π* transition is forbidden.

DIFFERENT ELECTRONIC TRANSITIONS

1. **n-π*transitions:** This transition requires the lowest energy (longer wavelength) of all other transitions. The compounds containing n electrons (present in S, O, N, or halogens) and double or triple bonds (E.g., aldehydes and ketones, nitro compounds) exhibit these transitions. The peaks of these transitions are also called R –bands.

Aldehydes and ketones (RCHO, RCOR) absorption peak,
Without double or triple bonds occurs at 270-300 nm.
With double or triple bonds separated by two or more single bonds occurs at 300-350nm
Identification of n-π*transition can be done by recording the UV spectrum of the substance in an acid medium. The peak due to n-π* transition disappears in acid solution. The presence of other hetero atoms can be identified by comparing the spectrum of a similar compound without hetero atom.



2. **π-π* transitions:** This transition gives B, E and K bands
B bands are benzenoid bands due to aromatic and hetero aromatic systems. E bands are ethylenic bands due to aromatic systems. K bands (π-π*) are due to conjugated systems. The energy required for this transition lies between n-σ* and n-π*. **The alkyl substitutions and extended conjugations** (addition of alternate double or triple bonds) shift the λ_{max} towards longer wavelength (bathochromic shift). The trans isomer of the alkene absorbs at a longer wavelength with more intensity than the cis isomer (bathochromic shift with hyperchromic effect). Extended conjugation and alkyl substitution shift λ_{max} in such a way that the λ_{max} moves to the colorimetric region. E.g., plant pigments like β carotene, lycopene, etc.
The λ_{max} of ethylenic (C=C) chromophore is 174nm
The λ_{max} of acetylenic (C≡C) chromophore is 178 nm
3. **n-σ* transition:**
Saturated compounds with nonbonding electrons (presence of heteroatoms) exhibit this transition. The energy requirement is less than σ-σ* transition. The wavelength range for this transition is 180-250nm. Transition needs more energy that is shorter wavelength because the lone pair of electrons on the hetero atoms form hydrogen bonds. **Hydrogen bonding shifts UV absorption to a lower wavelength** E.g., of compounds with n-σ* transition Methylene chloride (173nm), water (191nm), Methanol (203nm), Ethanol (204 nm), ether (215 nm) etc.
4. **σ-σ* transitions:**
This transition requires the highest energy. This transition occurs in saturated compounds (more importantly, hydrocarbons). The absorption peaks appear in the vacuum UV region. 125-135nm. E.g., Methane (122nm), Ethane (135 nm). The commercial UV spectrophotometers operate above 200nm, saturated hydrocarbons like cyclohexane (195 nm) can be used as nonpolar solvents because the solvent peak does not appear.

Laws of absorption

The two laws related to UV Visible spectroscopy are

1. Beer's law (concentration-dependent)
2. Lambert's law (Thickness/path length dependent absorbance)

Lambert's law states that when a beam of monochromatic light is allowed to pass through a transparent medium, the rate of **decrease of intensity** with the **thickness** of the medium is **directly proportional** to the intensity of incident light.

Beer's law: Beer observed a similar relationship between transmittance and the concentration of a solution. Beer's law states that the intensity of a beam of monochromatic light decreases exponentially with an increase in the concentration of absorbing species arithmetically.

$$A = act$$

$$\textbf{Log } I_0/I_t = \textbf{act}$$

is the mathematical expression of Beer Lambert's law,

Where A = absorbance, a = absorption coefficient or absorptivity, c = concentration of the substance and t = path length in cm.

The derivation of Beer lambert's law is given in colorimetry (chapter-1 pg. no. 9-11).

CHROMOPHORES

Any group which exhibits absorption of electromagnetic radiation in the visible or UV region is called as chromophore. It may or may not impart colour to the compound. Example of important chromophores is ethylene, acetylene, carbonyls, acids, esters, and nitrile group.

Eg: NO_2, N=N, C=O, C=C, N=O, C=N, C=C, C=S

Types of Chromophores

1. **Chromophores with π electrons**

   These chromophores undergo $\pi \rightarrow \pi^*$ transitions. E.g., ethylenes and acetylenes.

2. **Chromophores with both π electrons and n (non-bonding) electrons**

   These chromophores undergo two types of transitions.

   $\pi \rightarrow \pi^*$ **and** $n \rightarrow \pi^*$**,** E.g., carbonyls, nitriles, azo compounds and nitro compounds

Examples of some chromophores

1. Nonconjugated alkenes show intense absorption below 200 nm and are inaccessible to UV spectrophotometer. ($\pi \rightarrow \pi^*$)
2. Non-conjugated carbonyl group compounds give a weak absorption band in the 200-300nm region. ($n \rightarrow \pi^*$).
3. Conjugation of C=C and C=O groups shifts the λ_{max} of both groups to longer wavelengths.

Some simple chromophore groups are given in the Table 1.3.

Table 1.3 Some simple examples of chromophores

Chromophore	Transition	λ_{max}
C=C	$\pi \rightarrow \pi^*$	175
C≡C	$\pi \rightarrow \pi^*$	175
C=O	$n \rightarrow \pi^*$. $\pi \rightarrow \pi^*$	285 180
N=O	$n \rightarrow \pi^*$. $\pi \rightarrow \pi^*$	275 200
C-X X=BR,I	$n \rightarrow \sigma^*$ $n \rightarrow \sigma^*$	205 255

AUXOCHROME

It is a group that itself **does not act as a chromophore. Still,** when **attached to** a chromophore, it shifts the **absorption maximum towards a longer wavelength with an increase in the intensity of absorption**.

Ex: The auxochrome NH_2, when attached to a benzene ring, shifts its absorption maximum (λ_{max}) from 255 to 280nm.

Other Eg: OH, NH_2, OR, NHR and NR_2

All auxochromes have one or more nonbonding pair of electrons.

The auxochrome, when attached to a chromophore, extends the conjugation of chromophores by sharing nonbonding pair of electrons. Ex: $CH_2=CH-\ddot{N}H_2 \leftrightarrow \ddot{:}^{-}CH_2 - CH=NR_2^{+}$

SPECTRAL SHIFTS

Isolated chromophores such as >C=C < and C≡C absorb in the far UV region, which cannot be observed in normal spectrophotometers. The position of absorption maximum and the intensity of absorption can be modified by some structural changes or changes of solvent. These kind of changes of λ_{max} and intensity of absorption are called spectral shifts. The different kinds of spectral shifts are explained below.

BATHOCHROMIC SHIFT OR RED SHIFT

It is the shift of absorption maximum **(λ_{max}) towards longer wavelength** because of the presence of groups like OH and NH_2 (auxochrome) or by change of solvent.

Decreasing the polarity of the solvent causes a redshift in the carbonyl compounds (n-π^* transitions)

When two or more chromophores are present in conjugation in a molecule, redshift is observed.

Eg: ethylene π-π^* transitions – 170nm

1, 3 butadiene (two double bonds in conjugation) – 217nm

HYPSOCHROMIC SHIFT OR BLUE SHIFT

It is the shift of absorption maximum towards **shorter wavelength**. This may be caused due to the removal of conjugation in a system or by the change of the solvent. The absorption shift towards a shorter wavelength is also called a **blue shift**.

E.g., Aniline absorption is maximum at 280 nm because the pair of electrons on the nitrogen atom is in conjugation with π bond system of the benzene ring.

In acidic solutions, the absorption shifts to a shorter wavelength (blue shift 200nm). Anilinium ion is formed in acidic solution, and the electron pair is no longer present, and hence conjugation is removed.

HYPER CHROMIC EFFECT

It is the **increase in the intensity of absorption** and is brought about by the introduction of an auxochrome.

E.g.: Introduction of methyl group in 2nd position of Pyridine increases ε_{max} (λ_{max} 262nm) from 2750 to 3560 (λ_{max} 262nm) for π-π*.

HYPO CHROMIC EFFECT

It is the **decrease in the intensity of absorption** and is brought about by groups that are able to distort the geometry of the molecule.

E.g., When a methyl group is introduced in position 2 of the biphenyl group, there is a decrease in intensity because of distortion caused by the methyl group.

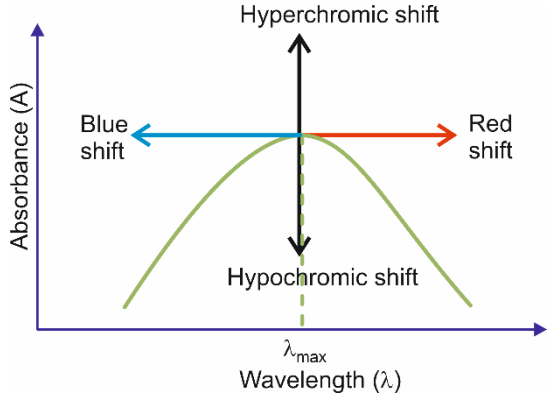


Fig. 1.21 Different types of spectral shifts.

SOLVENT EFFECT ON ABSORPTION SPECTRA

The most commonly used solvent is 95% ethanol. Other solvents which can be used in UV spectroscopy are n-hexane, methyl alcohol, cyclohexane, acetonitrile, diethyl ether, water, etc.

The λ_{max} and the intensity of absorption get shifted for a chromophore by changing the polarity of the solvent. In general, the absorption maximum for the nonpolar compounds is usually shifted with the change in polarity of the solvents.

1. **Effect of solvent on n-π* transitions:** The absorption **shifts to a shorter wavelength** (blue shift) when the **polarity of solvent** is **increased**. In n-π* transitions, (e.g., carbonyl), the ground state is more polar than the excited state. When the polarity of the solvent is increased, the nonbonding electrons in the ground state are more stabilized than the excited state by hydrogen bonding. So, more energy is required to cause excitation, and thus absorption shifts to a shorter wavelength. E.g., absorption maximum of acetone is 279 nm in hexane but it is 264 nm in water.

   Thus, an increase in polarity of solvent generally shifts n-π* and n-σ* transitions to a shorter wavelength.
2. **Effect of solvent on π-π*transitions:** The absorption shifts to a **longer wavelength (red shift)** when the **polarity of solvent is increased.** The π*orbitals get more stabilized by hydrogen bonding with polar solvents like ethanol and water. The polarity of π* orbital is more than the π orbital. When the polarity of the solvent is increased, the dipole-dipole interactions with the solvent molecules lower the energy of the excited state than the ground state. Thus, the energy required for π-π* transitions decreases, leading to shifting of absorption maximum to longer wavelength (red shift).

   Thus, an increase in polarity of solvent generally shifts π-π*transitions to longer wavelength.

   Generally,

1. The absorption maximum will be shifted to a shorter wavelength for a group which is more polar (carbonyl) in the ground state than the excited state (n-π*)
2. The absorption maximum will be shifted to a longer wavelength for a group which is more polar in the excited state than the ground state (π-π*).

CHOICE OF SOLVENT

The solvent for the UV spectroscopy should have the following requirements

(i) It should not absorb in the region of absorbance measurement of the sample (should be transparent). It should not affect the absorption of the sample.

(ii) It should be less polar so that its interaction with the solute molecules is minimum.

A commonly used solvent is 95% ethanol. It is cheap, has good solvating power, and is transparent above 210nm.

Example of other solvents transparent above 210 nm is n-hexane, cyclohexane, methanol, water, and ether. The other solvents like benzene, chloroform, and carbon tetrachloride cannot be used because they absorb in the 240-280 nm range.

The solvents like hexane and other hydrocarbons are preferred to polar solvents due to their minimum interactions with the solute molecules.

Some common solvents used in UV spectroscopy and their absorption wavelengths are given in the following Table 1.4.

Table 1.4 Common UV solvents and their absorption wavelengths

Solvent	Wavelength
Water	205nm
Methanol	210nm
ethanol	210nm
Ether	210nm
Chloroform	245nm
Carbon tetra chloride	265nm
Cyclohexene	210nm
Dichloroethane	220nm

INSTRUMENTATION

The common instruments used for measuring the emission or absorption of radiant energy in the UV region are UV spectrophotometers.

Table 1.5 Summary of Instrumentation in UV spectrophotometers

Source of light	Filters and Monochromators	Sample cells	Detectors
Hydrogen discharge lamp Deuterium lamp Xenon Discharge lamp Mercury arc lamp	Absorption filters. Interference filters **Prisms** Refractive type Reflective type **Grating** Diffraction grating Transmission grating	Quartz cuvettes are used because **glass absorbs UV radiation**.	1. Barrier layer cell or Photovoltaic cell 2. Photo tubes or Photo emissive cells 3. Photomultiplier tubes. 4. Photo diode array detector

The essential components of instrument are

(a) Source of light
(b) Filters or monochromators
(c) Sample cells
(d) Detectors
(e) Display or readout devices
(f) Computers and recorders

Now we will discuss these components

(a) Source of light /radiation sources: The Ultraviolet (UV) region extends from 200-400nm

The requirements of the radiation sources are

1. It must be stable, and no fluctuations should be there.

2. It should provide continuous radiation from 200-400nm.
3. Its intensity should be adequate.

(i) Hydrogen discharge lamp: Hydrogen gas is stored under high pressure in these lamps. When an electric discharge is passed through the gas, excited hydrogen molecules are produced, which emit UV radiations. The high pressure of hydrogen gives a continuous spectrum. Hydrogen lamp gives radiation from 120 -350 nm.

Advantages:

1. Stable, robust and widely used
2. Emits broadband (continuous spectrum)

(ii) Deuterium lamp: In this lamp, deuterium is used in the place of hydrogen, leading to 3 to 5 times increase in the intensity of emitted radiation.

Advantages:

1. More intensity

Disadvantage

1. Expensive

(iii) Xenon Discharge lamp: Xenon gas is stored under 10-30 atmosphere pressure in this lamp. It has got two tungsten electrodes separated by a distance of 8 mm. When a low voltage is applied, an intense arc is produced between the electrodes producing UV radiation. The intensity produced is greater than the hydrogen discharge lamp.

(iv) Mercury arc lamp: In this lamp, mercury vapour is used under high pressure, and the mercury atoms are excited by electrical discharge. The spectrum produced is not continuous and hence not used widely.

(b) Filters and Monochromators: The source emits continuous spectra from 200-400nm. This is called polychromatic light consisting of several wavelengths. A colorimeter or spectrophotometer works only with a monochromatic light consisting of a single wavelength. A filter or a monochromator is used in these instruments to convert the polychromatic light into monochromatic light.

(i) Filters: There are two types of filters

1. Absorption filters.
2. Interference filters

1. Absorption filters: These filters are made of a solid sheet of glass coloured by dissolved or dispersed pigments. Dyed gelatin is also used as an absorption filter.

Absorption filters are further classified as Cut off filters and bandpass filters.

Advantages:

1. Simple construction
2. Cheaper.
3. Easy Filter selection.

Disadvantages:

1. Accuracy is less because of broad bandpass (± 30 nm).
2. Absorption by filters lead to less intensity of radiation

2. Interference Filters

1. This filter consists of a dielectric spacer film made up of CaF_2, MgF_2, or SiO, between two reflecting parallel silver films.
2. The thickness of the dielectric film may vary to give $1/2\lambda$ (1^{st} order),$2\lambda/2$ (2^{nd} order),$3\lambda/2$ (3^{rd} order), etc.

   The principle of working is that the radiation reflected by the 2^{nd} film and the incident radiation undergo constructive interference to give monochromatic radiation. The wavelength of the monochromatic radiation depends on the formula

   $$\lambda = 2\eta b/m$$

   Where λ = wavelength produced
   η = dielectric constant of the film material
   b = layer thickness
   m = order no(1^{st}, 2^{nd}, 3^{rd}, etc)
3. Bandpass is 10-15 nm. Transmission is maximum 40%

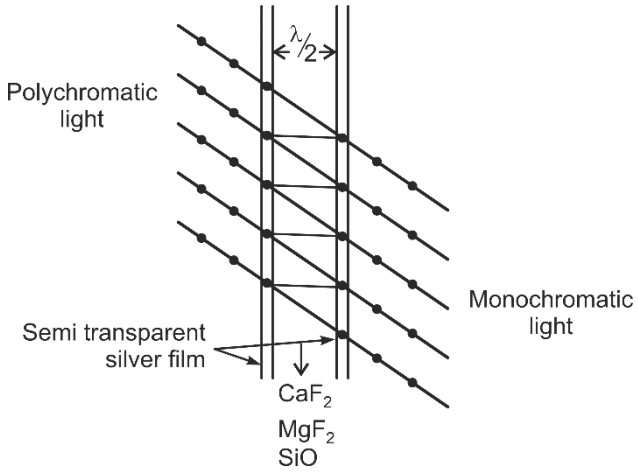


Fig. 1.22 Interference filter FABRY PEROT.

Advantages:

1. Inexpensive
2. More accurate than absorption filters because of the lower bandpass.
3. Additional filters can be used to cut off undesired wavelengths.

Disadvantages:

1. Transmission is low
2. Because of the narrow bandpass, higher resolution is not obtained.

(ii) Monochromators: The **monochromators** are more efficient in converting the **polychromatic light to monochromatic light** when compared to filters. The parts of a monochromator are

1. Entrance slit (to get a narrow source of light)
2. Collimator (to make the light parallel)
3. Grating or prism (to disperse the light)
4. Collimator (to reform the monochromatic image of entrance slit)
5. Exit slit (to allow the light to fall on sample cell)

There are two types of monochromators

1. Prisms
2. Grating

1. Prisms: The prisms disperse the light into individual wavelengths or colours. They are made of glass and used in inexpensive instruments. Its resolution is better than filters because the bandwidth is lower. The resolution of the prism is determined by the size and refractive index of the prism.

There are two types of the prism

I. Refractive type: In this type, the light from the source falls on the collimator through the entrance slit. The parallel rays of light from the collimator enter the prism, which disperses the light into component colours or wavelengths. The second collimator receives the dispersed light and reforms the images of the entrance slit. The reformed images are the colours of the VIBGYOR (Violet, Indigo, Blue, Green, Yellow, Orange, and Red). The required wavelength can be selected by rotating the prism or by moving the exit slit and the selected wavelength passes through the exit slit to the sample.

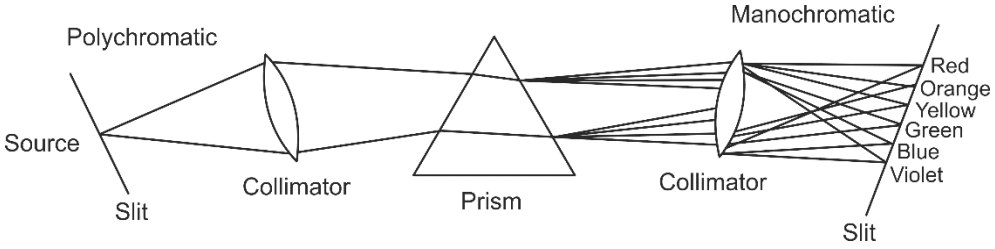


Fig. 1.23 Prism monochromator-Dispersive type.

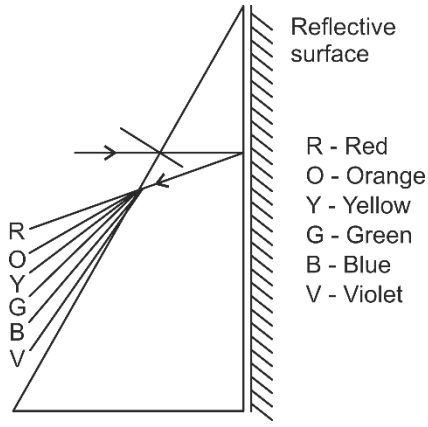


Fig. 1.24 Prism monochromator-Reflective type (Littrow mounting).

II. Reflective type (Littrow mounting): This type has a reflective surface present on one side of the prism. The dispersed radiation gets reflected on the same side of the source and is collected on the same side. The working principle is the same as the refractive type.

The advantage of prisms:

1. Dispersed wavelengths do not overlap

Disadvantages:

1. Non-linear dispersion
2. Temperature sensitive

2. Gratings: Gratings are more efficient in the conversion of polychromatic light to monochromatic light. A resolution of ±0.1 nm can be achieved with gratings, and hence they are used in spectrophotometers.

There are two types of Gratings

I. Diffraction grating
II. Transmission grating

I. Diffraction grating: A grating is made up of a large number of parallel lines(grooves) ruled on highly polished surfaces like glass, quartz, alumina and alkyl halides based on the type of the instrument (visible/UV/IR spectrophotometer. Generally, 3600 grooves or more per mm are drawn for ultraviolet and visible regions, and 20 grooves per mm are drawn for the IR spectrophotometer.

These are replica gratings made from the master grating. These are prepared by coating the original master grating with epoxy resin and removed after setting. Aluminizing its surface makes the surface of the replica grating reflective.

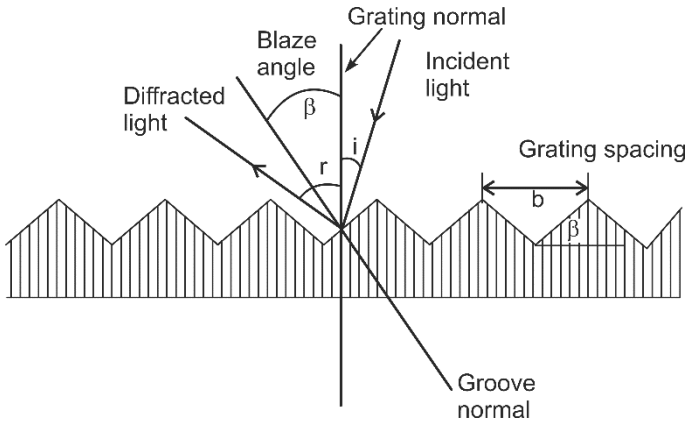


Fig. 1.25 Diffraction grating.

The principle is diffraction causing reinforcement. The incident rays on the grating, get reinforced with the reflected rays and the resulting radiation wavelength is given by the equation

$$m\lambda = b (\sin i \pm \sin r)$$

Where λ = wavelength of produced light
b = grating spacing
i = angle of incidence
r = angle of reflection
m = order (0, 1, 2, 3, etc).

Gratings are preferred over prisms because of their efficiency.

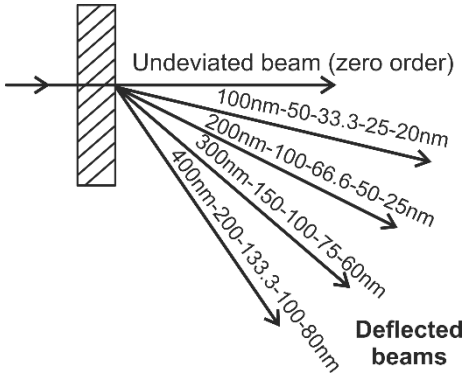


Fig. 1.26 Transmission grating.

II. **Transmission grating:** In these types of gratings, refraction takes place. The principle is refraction produces reinforcement. The radiation transmitted through the grating reinforces with the partially refracted radiation leading to reinforcements.

Transmission grating: The wavelength produced can be calculated using the formula

$$\lambda = \frac{d \sin\theta}{m}$$

Where λ = wavelength of radiation produced.
d = 1/lines per cm
m = order no (0, 1, 2, 3, etc)
θ = angle of deflection/diffraction

Hence, the required light radiation (λ) can be produced either by moving grating and keeping the slit fixed or vice versa.

Advantage:

1. They give linear dispersion.
2. High resolution

Disadvantage:

1. Overlap of spectral orders.

Slits: The two slits are the **entrance slit and the exit slit**. The width of the monochromatic image of the entrance slit is the same as the width of the exit slit so that it can pass through the exit slit.

(c) Sample cells:

- The sample cells, known as cuvettes, are used to hold the sample.
- The construction material of the cuvette varies with the instrument and the sample nature.
- The material of the cuvette should not absorb in the working wavelength.
- Material for **visible region**:
  Colour corrected fused glass.
  For aqueous solutions, Polystyrene cells are available.
  For UV region: Quartz cuvettes are used because **glass absorbs UV radiation**.
- The cells are available in different shapes like cylindrical or rectangle.
- Small volume cells (0.5ml or less) and large volume cells (5-10ml) are available depending on their capacity to hold sample volume
- The common path length (internal distance) is 1cm. Long path length cells with 10cm and short path length cells with 1 or 2mm are also available.

(d) Detectors: The radiation from the source through the monochromator passes through the sample. The sample absorbs part of the radiation, and the rest is being transmitted. This transmitted radiation falls on the detector, and the intensity of the absorbed radiation is determined by the detector and displayed.

Detectors used in UV Visible spectrophotometers are called as photometric detectors. They convert the **light energy to an electrical signal** which is recorded.

Three types of detectors are

1. Barrier layer cell or Photovoltaic cell
2. Photo tubes or Photo emissive cells
3. Photomultiplier tubes.

1. **Barrier layer cell or Photo voltaic cell:** The detector consists of a metal base like iron or aluminium acting as one electrode. A thin layer of semiconductor material like selenium is deposited on its surface. The surface of the selenium is covered by a very thin layer of silver or gold which acts as a second collector electrode.
   The selenium layer separates the two electrodes, and it has extremely low electrical conductivity. When radiation falls on the selenium layer, the electrons are generated at the selenium silver interface. The silver layer collects these electrons. The collection of electrons on the silver surface creates a potential difference between the two electrodes. If the resistance in the external circuit is small, the current flows through the system. The **current produced is directly proportional to the intensity of the incident radiation** on the detector.

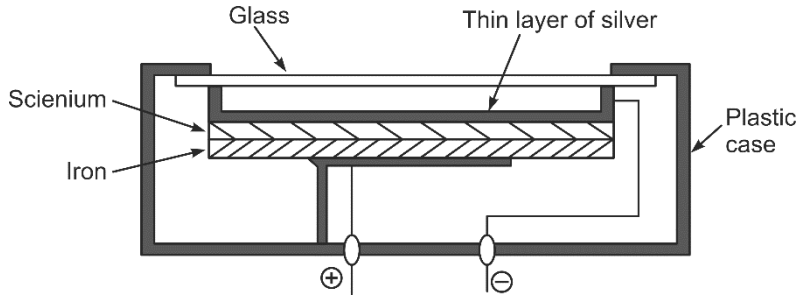


Fig. 1.27 Barrier layer cell.

Advantages:

1. Simple in design and rugged
2. No external power supply is required.

Disadvantages:

1. Less sensitive to blue region. use is limited to the visible region
2. Amplification of the signal is not possible because the resistance of the external circuit should be low.
3. Fatigue effects.

1. **Photo tubes or Photo emissive cells:** This detector consists of an evacuated glass tube containing a photocathode and an anode which acts as a collector electrode. The photocathode is coated with a light-sensitive layer like Cesium, potassium, or silver oxide. When the transmitted radiation falls on the photoemissive cathode, it emits photoelectrons. The electron flow towards the anode causes the **flow of the current, which is proportional to the intensity of radiation falling on the detector.**

   Advantages:

   1. The signal from the detector can be amplified
   2. More sensitive than Photovoltaic cells.
   3. Composite coatings containing Cesium, caesium oxide, or silver oxide can be used as the cathode to increase the sensitivity and workable wavelength range.

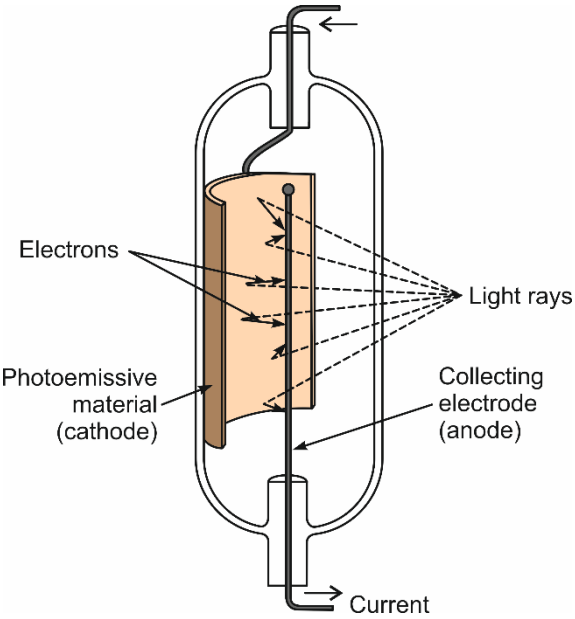


Fig. 1.28 Photo tube.

2. **Photomultiplier tubes (PMT):** Though expensive, this detector is employed in sophisticated instruments due to its sensitivity.
   The working principle of this detector is the **multiplication of photoelectrons by secondary emission of electrons.** PMT detector consists of a photocathode and a series of anodes called dynodes. Each dynode is maintained at a successively higher potential 75-100v higher than the preceding dynode. Up to 10 dynodes are used in the series.
   When the radiation hits the cathode surface, electrons are emitted. When these electrons hit the dynodes, at each stage, the electrons are multiplied by a factor of 4 or 5 because of the secondary emission. An overall amplification factor of about 10^8 is achieved.

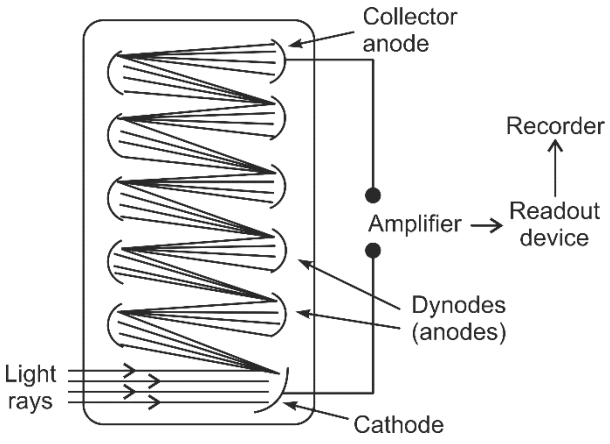


Fig. 1.29 Photo multiplier tube (PMT).

Advantages:

1. Most sensitive
2. Can detect very weak signals
3. Faster response time
4. It can be used in fluorescence measurements

Disadvantages:

1. Expensive
2. It should be shielded from stray light.

4. Silicone photodiode detector: (PDA detector):

Photodiodes are made up of photosensitive semiconductor materials like silicon, gallium, arsenide, etc. They absorb light in the wavelength range 250 to 1100 nm, characteristic of silicon. These detectors can measure light at different wavelengths simultaneously.

A photodiode is one type of light detector used to convert the light into current.

It comprises of optical filters, built-in lenses and also surface areas. These diodes have a slow response time when the surface area of the photodiode increases.

But they are less sensitive than PMTs.

Working of Photodiode

The working principle of a photodiode is, when a photon of ample energy strikes the diode, it makes a couple of electron-hole. This mechanism is also called the inner photoelectric effect. If the absorption arises in the depletion region (junction), then the carriers are removed from the junction by the inbuilt electric field of the depletion region. Therefore, holes (positive charge where the electrons are removed) in the region move toward the anode, electrons move toward the cathode, and a photocurrent will be generated.

A photodiode continually operates in a reverse bias mode. The current produced is directly proportional to light incident on the Photodiode layer

PDA detector/Diode Array Detectors (DAD)

It is an assembly of individual photodiodes on a chip.

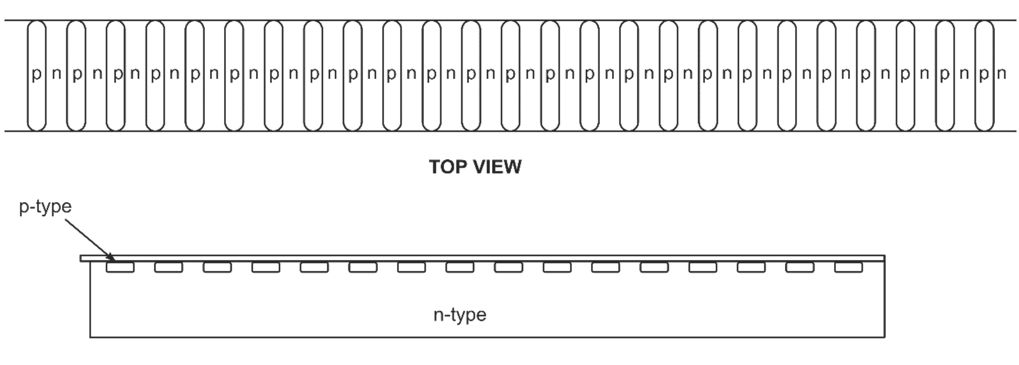


Fig. 1.30(a) Assembly of individual photodiodes on a chip.

Each diode can be addressed individually

Monochromator disperses light across PDA, with a small no of diodes /wavelength.

Allow simultaneous collection of all wavelengths.

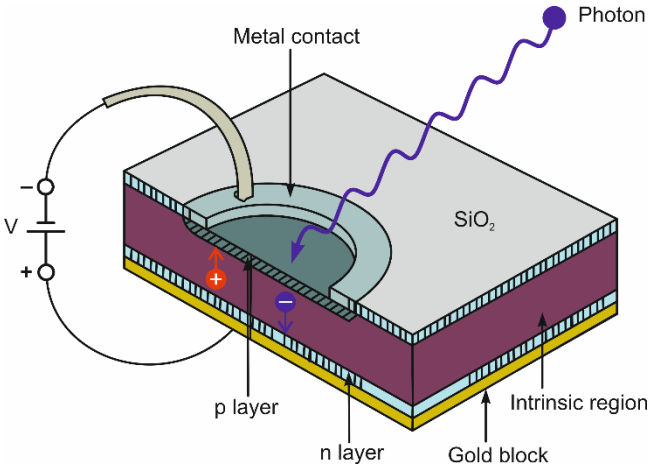


Fig. 1.30(b) Photo diode array (PDA) detector.

DADs differ from UV-VIS detectors in that **light from the lamps is shone directly onto the flow** cell, light that passes through the flow cell is **dispersed by the diffraction grating**, and the amount of the dispersed light is estimated for each wavelength in the photodiode arrays

Advantages of PDA detector

1. Determination of absorbance in all wavelengths in one run.
2. Absorbance maximum can be calculated for each peak
3. Peak purity can be determined
4. Rapid scanning.
5. Used for kinetic studies
6. Used as a detector in LC

Disadvantages

1. Large noise because the amount of light is small
2. Lamp fluctuations

(e) **Display or Readout Devices:** The electrical energy from the detector is displayed in a read-out system such as LCD. They have faster responses and are easier to read.

(f) **Computers and Recorders:** Computers are helpful in storing and processing of the data received from the detector. The recorders are for recording the absorption spectrum or absorbance of the analyte of interest.

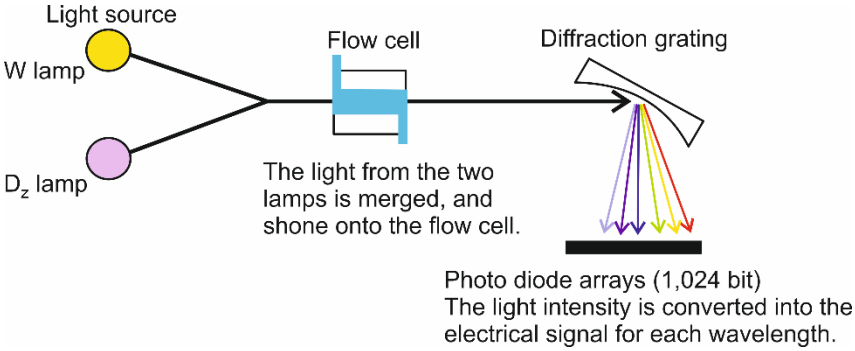


Fig. 1.31 Working of photo diode array (PDA) detector.

The different types of instruments used in UV spectroscopy are

1. Single Beam UV spectrophotometer
2. Double beam UV spectrophotometer

1. **Single beam spectrophotometer:** The construction and working of a single-beam spectrophotometer are the same as a single beam colorimeter, except the source and the detectors used are different here. The schematic diagram is given below

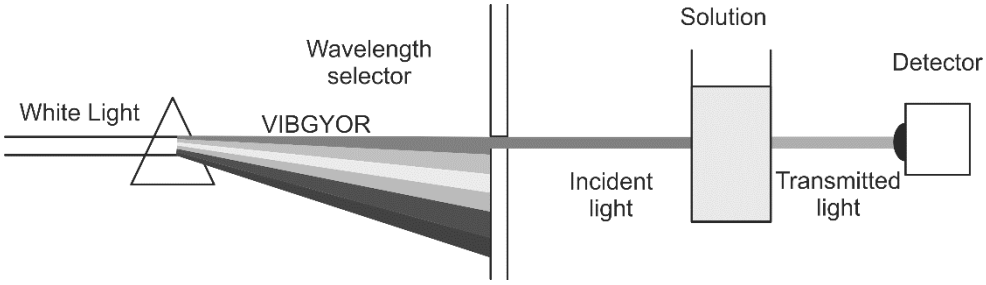


Fig. 1.32 Single beam spectrophotometer.

2. **Double beam UV Spectrophotometer:** The construction and working of the double beam are the same as that of the double beam colourimeter. The difference is that instead of a filter or prism, here grating monochromator is used, and photomultiplier detectors are used in the place of photo tube detectors. These are the widely used instruments.

   Working:

   1. The radiation from the source passes via the mirror system to the monochromator.
   2. The monochromator allows a narrow range of wavelengths to pass through the exit slit.
   3. The radiation from the monochromator through the exit slit is received by the optical chopper, which divides the beam into two beams; one passes through the reference and the other through the sample cell.
   4. The beams, after passing through reference and sample cells, are passed to the detector.

5. The output of the detector is connected to the amplifier, which responds to the change in transmission through the sample and reference.
6. Then the signal is transferred to the recorder, where the absorbance or transmittance is recorded as a function of wavelength.

Advantages:

1. Zero adjusting can be done for all wavelengths simultaneously and need not be repeated for every wavelength.
2. In double beam spectrophotometers, the ratio of sample and reference beams is used for measurement, minimizing the errors due to variation in the intensity of the source and detector fluctuation.
3. Rapid scanning over a wide wavelength region.
4. High accuracy, sensitivity, reliability, and repeatability.

Disadvantages:

1. Complicated
2. Expensive

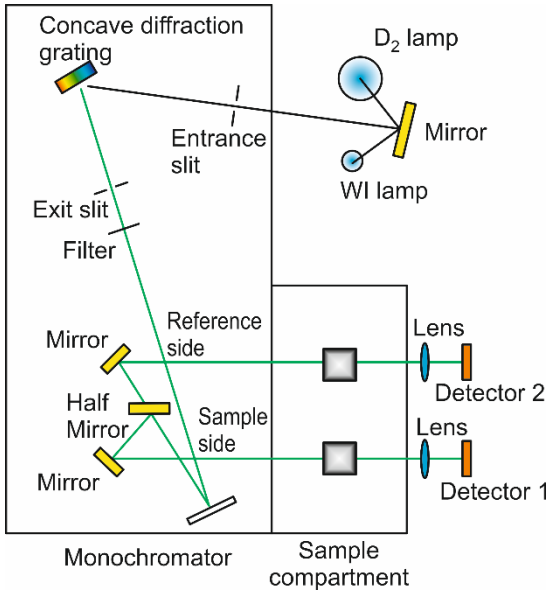


Fig. 1.33 Double beam UV Visible spectrophotometer.

APPLICATIONS

1. **Spectrophotometric titrations:** In these titrations, the absorbance of the solution is measured after each addition of the titrant, and the **absorbance is plotted against the volume of the titrant**. The endpoint is determined from the graph. The titrant or the titrated substance can be the absorbing species. The endpoint is determined by the

intersection of lines of the titration curve. If there is a curvature without intersection due to incomplete reaction, the lines can be extrapolated to give the endpoint

The first curve (a) is the example where the **titrant only absorbs**. In this titration, only the titrant that is bromine is absorbing, and its absorption is plotted against the volume of the titrant. So, the absorbance will not change till all the arsenic is consumed. Once all the arsenic is consumed by the titrant, only bromine will be there, causing an increase in absorption.

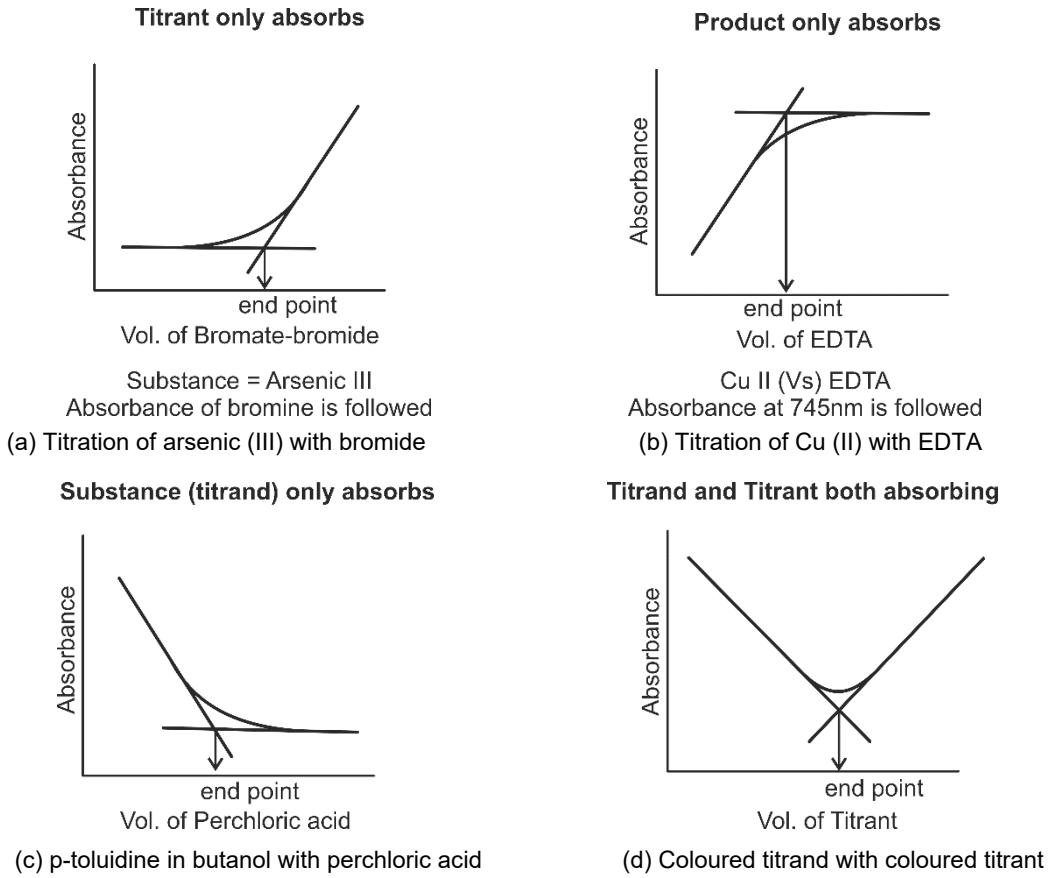


Fig. 1.34 Types of spectrophotometric titrations.

The second curve (b)is the example of the case where **only the product is absorbing**. E.g., is the titration of Cu II ions with Ethylene diamine tetracetic acid (EDTA). There is an increase in absorbance as the product is formed, and once the reaction is complete, no new product is formed, and the absorbance becomes constant with no further increase.

In the third case, (c) **only the substance titrated (titrand) absorbs.** Eg is the titration of p-toluidine in butanol with perchloric acid. As p-toluidine is being consumed by the perchloric acid, there is a decrease in absorbance, and once all the p-toluidine is

consumed, there is no change in absorbance. The graph becomes horizontal with no further change in absorbance.

In the fourth case (d), coloured substance reacts with coloured titrant to give a colourless product. So, **both the titrand and the titrant will be absorbing and the product is not absorbing**. Initially, there is a sharp decrease in absorbance due to the formation of non-absorbing product formation. After the endpoint, the concentration of the absorbing titrant increases, leading to an increase in absorbance.

Conditions to be followed:

(a) Titrand, titrant, or product should absorb in the selected wavelength.
(b) The concentration should be in the Beer's law range
(c) Titrant must be stronger so that dilution error can be minimized (absorbance is proportional to concentration).

Applications

1. **Acid-base titrations:** Eg: titration of phenols with sodium hydroxide. Absorbance due to phenolate ion is recorded.
2. **Oxidation-reduction titrations:** Eg: Ce (III) titration with Co (III).Ce(IV) formation is followed.
3. **Complexometric Titrations:** Eg: Cu (II) is titrated with EDTA. Cu-EDTA complex formation is followed.
4. **Precipitation Titrations:** Eg: SO_4^{2-} ions titrated with Ba (II) ions, and the turbidity appearance is followed.

Advantages

1. As only one absorbing species is needed, these titrations can be applied to a large no. of non –absorbing substances.
2. No interference from other absorbing species, as only the change in absorbance is important
3. The method is applicable to highly coloured solutions where visual indicators cannot be used.
4. The endpoint can be determined even if the reaction is incomplete
5. Accurate and precise.
6. No need to work only at the absorption maximum (λ_{max}). So wavelength choice is there.
7. Dissociation constants can be determined from these graphs.
8. Convenient, rapid.

2. Single component analysis: Determination of concentration and amount of the substance present in the given sample is called Quantitative estimation. Using these values, percentage purity can be determined. The quantitative estimation of drugs by UV-visible spectrophotometry can be performed by knowing the parameters like λ_{max}, Beer's law concentration range for that substance, solvent, reagents and other conditions.

The types of quantitative estimation are

1. **Using $A^{1\%}_{1cm}$ value (specific absorbance):** This method is useful in the estimations of raw material as well as formulations when the reference standard is not available. $A^{1\%}_{1cm}$ value can be obtained from Pharmacopoeia, Textbooks or Journals
2. **Reference standard available:** If reference standard is available, specific absorbance can be found experimentally and the above method can be used.
3. **Direct comparison method (single standard):** The absorbance of the standard solution of known concentration is compared with the absorbance of sample solution and the concentration of sample solution is calculated. From the concentration, the amount and the % purity can be calculated.
4. **Calibration curve method or multiple standard Method:** The calibration graph method is used to minimize the error introduced in the preparation of solutions or absorbance measurements.

   In this method, a calibration curve is plotted with concentration vs. absorbance measured with five or more concentrations of the reference or standard. A straight line passing through the origin is obtained. If a straight line is not obtained, a straight line is obtained through the maximum no of points or by regression analysis leading to a line of best fit. The unknown concentration of the sample can be obtained using the absorbance of the sample and the calibration graph. The extrapolation of the graph gives the unknown concentration and amount and the % purity of the sample can be calculated.
5. **Multi-component analysis:** Simultaneous analysis of numerous components in a sample is called multi-component analysis. Several methods are available in UV spectroscopy for multi-component analysis. In these methods, the absorption spectra is recorded and mathematically processed.

The basis of all the Spectrophotometric techniques for multi-component samples is "At all wavelengths, the absorbance of a solution is the sum of absorbance of the individual components"

or

The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference cell.

The excipients present in the formulation should not be absorbed at the wavelength of the experiment.

The different UV spectrophotometric multi component analysis methods are

1. Simultaneous equation method (Vierdott's method)
2. Absorbance ratio method (Q-Absorbance method)
3. Derivative Spectrophotometric method
4. Multi wavelength UV-spectrophotometry
5. Dual wavelength method
6. Difference spectroscopy
7. Geometric correction method
8. Orthogonal poly nominal method

9. Solvent extraction method
10. H-point standard addition method
11. Least square approximation method

1. Simultaneous equation method (Vierdott's method): If a sample contains two absorbing drugs (X & Y) with λ_{max} at λ_1 and λ_2 respectively, then it is possible to determine the concentration of both the drugs by simultaneous equation method if certain criteria are satisfied.

Criteria for using the simultaneous equation method are

The criteria are that the ratios,

$$\frac{A_2 / A_1}{ax_2 / ax_1} \quad \text{and} \quad \frac{ay_2 / ay_1}{A_2 / A_1}$$

should lie outside the range of 0.1-2

This criteria is satisfied only when the λ_{max} of the two components are reasonably dissimilar.

The second criterion is that the two components should not interact chemically with each other.

Example: Validated spectrophotometric methods for simultaneous′ estimation of Telmisartan and Indapamide in the pharmaceutical dosage form. λ_{max}: TEL 296 nm IND 242 nm

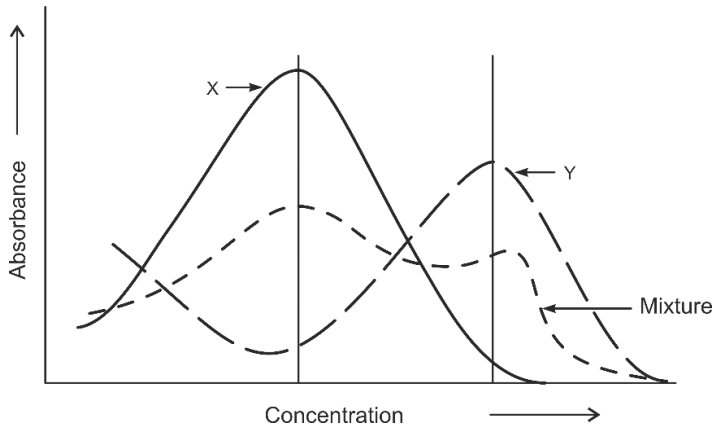


Fig. 1.35 Simultaneous estimation of Telmisartan and Indapamide in pharmaceutical dosage forms.

At λ_1 and λ_2, the absorbance of the mixture is the sum of the individual absorbance of X & Y. Two equations are constructed using this fact.

At λ_1, $A_1 = a_{x1}\, bc_x + ay_1\, bc_y$ .....(1)

At λ_2, $A_2 = a_{x2}\, bc_x + ay_2\, bc_y$ .....(2)

Where,

The absorptivity of X at λ_1 is ax_1, and the absorptivity of X at λ_2 is ax_2

The absorptivity of Y at λ_1 is ay_1 and at λ_2 is ay_2.

The absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

The concentration of X & Y are C_x & C_y respectively, in the diluted sample.

Rearrange eq. (2).

$$c_y = A_2 - ax_2cx / ay_2$$

Substituting for c_y in eq. (1). and rearranging gives

$$\mathbf{C_x = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2}$$

$$\mathbf{C_y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2}$$

3. **Absorbance ratio method:** The absorbance ratio method is a modification of the simultaneous equations method. It depends on the property that, for a substance, which obeys Beer's law at all wavelengths, the ratio of absorbances at any two wavelengths is a constant value and is independent of concentration or path length.

   In the quantitative estimation of two components in a mixture by the absorbance ratio method, absorbance is measured at two wavelengths, λ_{max} one of the components (λ_2) and wavelength of equal absorptivity of two components (λ_1), i.e. an iso-absorptive point.

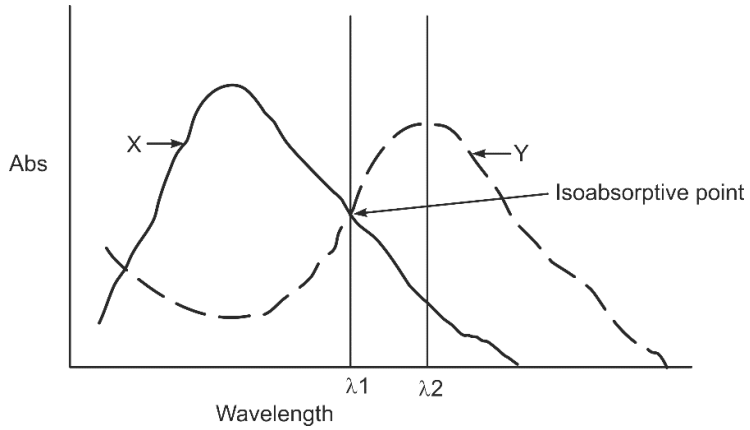


Fig. 1.36 Absorbance ratio method of Atrovastatin and Niacin in tablet dosage forms.

The concentration of the components x and y can be determined by the equation

$$C_X = (Q_m\text{-}Q_y).\ A_1 / (Q_x\text{-}Q_y).\ ax_1$$

$$C_Y = (Q_m\text{-}Q_x).\ A_1 / (Q_y\text{-}Q_x).\ ay_1$$

Where $Q_m = A_2/ A_1$ $Q_x = ax_2/ ax_1$ $Q_y = ay_2/ay_1$

A_2 =Absorbance at λ_2; A_1 =Absorbance at λ_1

ax_1 = Absorptivity of Drug X at λ_1

ay_1 = Absorptivity of Drug Y at λ_1

ax_2 = Absorptivity of Drug X at λ_2

ay_2 = Absorptivity of Drug Y at λ_2

E.g., Spectrophotometric method for absorbance ratio method of Atorvastatin and Niacin in the tablet dosage form. λ_{max}: ATR: 246nm NIA: 262nm. The iso-absorptive point at 258nm in Methanol.

4. **Derivative spectrophotometric method:** When the mixture has got interfering absorption, the derivative spectroscopy method is employed.

   Derivative spectroscopy involves the conversion of a normal spectrum to its first, second or higher derivative spectrum.

   The normal spectrum is known as fundamental, zero-order, or D^0 spectra. The plot of the rate of change of absorbance with wavelength against wavelength is called the first derivative spectrum (D1)

   i.e. plot of $\Delta A/\Delta\lambda$ vs λ

   The second derivative spectrum is a plot of $\Delta 2A/\ \Delta\lambda 2$ vs λ

   The quantitative estimations in derivative spectroscopy give accurate results with second derivative spectroscopy.

   For the quantitative estimation of binary mixtures(A&B) using the derivative spectroscopy, the Zero Crossing Points (ZCP) for both the components (A and B) are noted.

   The ZCP for A and B is selected in such a way that at that particular ZCP, the other component shows remarkable absorbance.

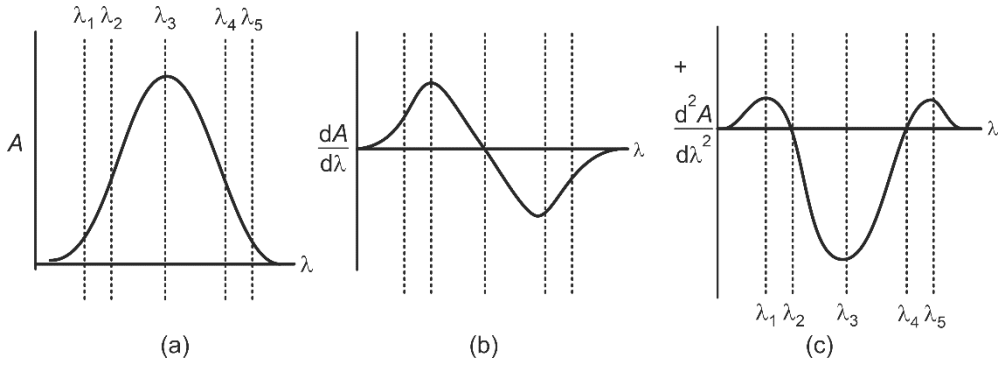


Fig. 1.37 Zeroth (a), First (b), Second (c) derivative spectra

The calibration curve of A is constructed at the ZCP of B and B at the ZCP of A. Unknown concentrations can be found from the calibration graph by extrapolation.

E.g., Simultaneous Spectrophotometric estimation of Ofloxacin and Satranidazole in the tablet dosage form.

5. **Multi wavelength UV-spectrophotometry:** This method helps in the determination of the composition of a binary mixture with overlapping spectra without determining molar absorptivities. The method requires standard solution absorbance of each component and the unknown mixture absorbance

All the standard solutions can be scanned over the range of 200-400nm in the multi-component mode, using two sampling wavelengths. The overlay spectra of standard solutions can be used to determine the concentrations of the drugs in a sample solution.

E.g., Simultaneous Estimation & Validation of Paracetamol, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in Tablets by Spectrophotometric Method:

6. **Dual wavelength method:** The dual-wavelength method is also known as the two wavelengths method. In this method, one of the drugs is considered as analyte and the other drug is considered as an interfering component and vice-versa. The two wavelengths are selected in such a way, that in one wavelength, the interfering component shows the same absorbance (ΔA equals zero), and the analyte shows a significant difference in absorbance with a concentration in the other wavelength. The difference in absorbance (ΔA) between two points on the mixture sample spectra is directly proportional to the concentration of the analyte independent of the interfering component

   E.g., Simultaneous determination of atenolol and indapamide

7. **Difference spectroscopy:** Quantitative estimation of the substance in the presence of absorbing interferents can be carried out by this method. The essential feature of this method is that the measured value is the absorbance difference (ΔA) between two equimolar solutions in the same wavelength.

   Two equimolar solutions of the analyte in different chemical forms will exhibit different spectral characteristics. The difference in absorption in the selected wavelength is measured.

   The alteration in the spectral properties of the analyte is mainly done by the adjustment of the pH using acids, alkalies, or buffers.

   Eg: Difference in absorbance of Ibuprofen in 0.1N HCl and 0.1 N NaOH is used in determination of ibuprofen

8. **Geometric correction method:** It is the three-point geometric procedure, the simplest of mathematical correction procedures and it is applied if the interfering absorption is linear at the three wavelengths selected.

   If the wavelengths λ_1, λ_2 and λ_3 are selected to that the background absorbances B_1, B_2 and B_3 are linear, then the corrected absorbance D of the drug may be calculated from the three absorbances A_1, A_2 and A_3 of the sample solution at λ_1, λ_2 and λ_3 respectively as follows

   The general formula is

$$D = y(A_2 - A_3) + z(A_2 - A_1) / y\,(1 - w) + z(1 - v)$$

   Where

   A_1, A_2 and A_3 – three absorbances of the sample

   y and z –wave length intervals $(\lambda_2 - \lambda_1)$ and $(\lambda_3 - \lambda_2)$

   v and w - absorbance ratios vD/D and wD/D respectively

   Let vD and wD be the absorbance of the drug alone in the sample solution at λ_1 and λ_3 respectively,

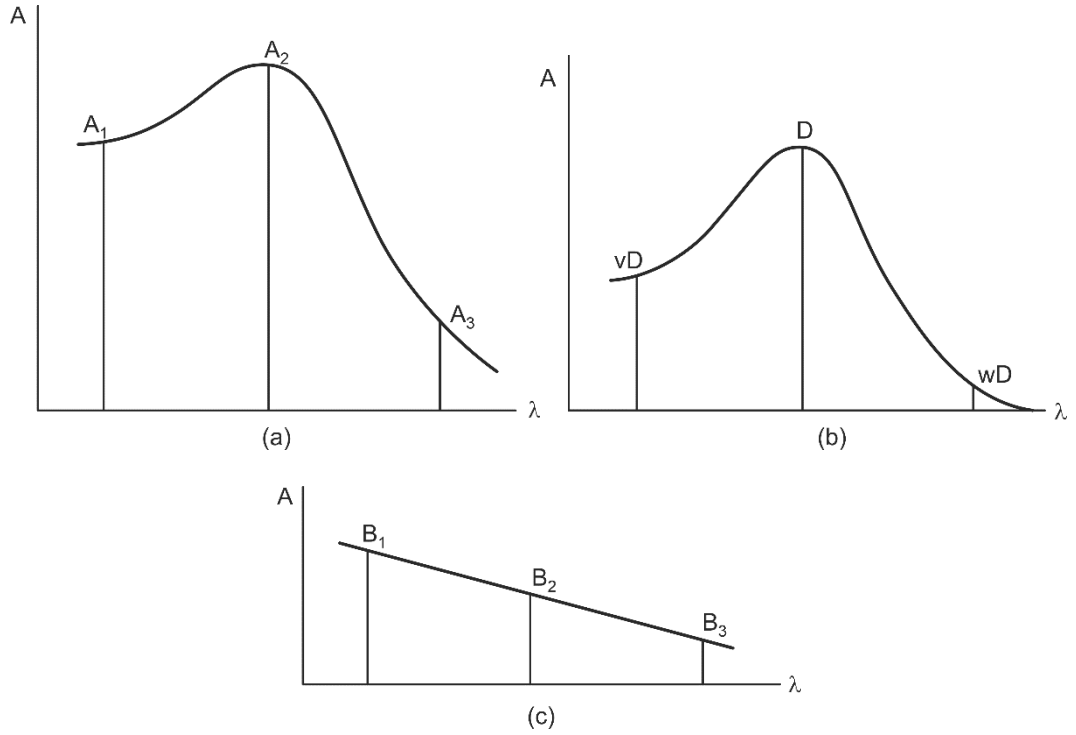


Fig. 1.38 An absorption spectrum (a), the spectrum of analyte (b), background absorption (c).

9. **Orthogonal polynomial method:** It is another mathematical correction procedure, which involves more complex calculations.
   In this method, an absorption spectrum is represented in terms of orthogonal functions like
   $$A(\lambda) = p\ P(\lambda) + p1\ P1(\lambda) + p2\ P2(\lambda) \ldots\ pn\ Pn(\lambda)$$
   Where A denotes the absorbance at wavelength λ. A set of n+1 equally spaced wavelengths are used at which the orthogonal polynomials, P (λ), P1 (λ), P2 (λ) ….. Pn (λ) are defined
10. **Solvent extraction method:** In this method, different solvents are selected based on the solubility of the components of the mixture and the components are extracted individually. Then the extracted components are analyzed separately.
11. **H-point standard addition method:** The H-point standard addition method (HPSAM) is a modification of the standard addition method. The method is used for resolving strongly overlapping spectra of two analytes and errors produced by the matrix of the sample can also be corrected directly. The technique is a combination of dual-wavelength spectrophotometry and the standard addition method.
12. **Least square approximation:** Experimental measurements are always subject to random errors. To correct these errors and to obtain an accurate result, more number of experimental data is used. That is, the analysis of a binary mixture is carried out in three or four wavelengths instead of at two wavelengths,

The method of least squares is a standard approach to the approximate solution of overdetermined systems, i.e., sets of equations in which there are more equations than unknowns. "Least squares" means that the overall solution minimizes the sum of the squares of the errors made in the results of every single equation.

Other Applications of UV spectroscopy

1. **Qualitative analysis:** Identification of the compounds is done by comparing the absorption spectrum with the spectra of standard known compounds.
2. **Detection of Impurities:** UV spectroscopy is one of the best methods for the detection of impurities in organic compounds. When comparing the absorption spectrum of the sample with a standard absorption spectrum, the presence of additional peaks indicates the presence of impurities. Impurities can also be detected by measuring in specific wavelengths characteristic for them

   E.g., The impurity, cyclohexane, in benzene can be detected by measuring the absorption at 255nm.
3. **Structural elucidation of Organic compounds:** Structural elucidation like the presence/absence of unsaturation, presence of heteroatoms like S, O, N, or halogens can be determined using UV spectroscopy. The general absorptions of these features are given

   E.g. Saturated compound σ - σ^* appears below 200nm (vacuum uv)

   The other three possibilities are given below, and they appear between 200-400 nm.

   Presence of heteroatoms (S, N, O) with saturation n - σ^*

   Unsaturated compounds π - π^*

   Hetero atom and double bond n-π^*

   Thus the location of peaks in the absorption spectrum gives an idea about the structural features.
4. **Structural analysis of organic compounds:** The effect of conjugation, cross conjugation, and alkyl substitution are discussed here

   (i) Detection of conjugation: It helps to know the presence of different groups with respect to conjugation, e.g., The conjugation maybe

   Between two or more C=C (or triple) bonds

   Between C=C and C=O

   Between double bonds and aromatic rings.

   The presence of aromatic rings can be identified and the no and positions of attachment to the carbons of the conjugated system can also be identified.

   Extension of the conjugation gives **bathochromic** shift or redshift by shifting the λ_{max} to longer wavelength.

   Reduction or saturation of double bonds leads to **hypsochromic** shift or blue shift by shifting the λ_{max} to the shorter wavelength.

(ii) Detection of Geometrical Isomerism: The **trans isomer** of the geometrical isomers absorb at a **longer wavelength** and have a **larger extinction coefficient** than cis isomers.

Eg: Calciferol- λ_{max} 265 nm (cis)

Iso vitD_2-λ_{max} 287 nm (trans)

Conversion of Cis – trans-isomer shows Bathochromic shift & Hyperchromic effect

Conversion of Trans to cis exhibits Hyposochromic shift and Hypochromic effect

(iii) Cross conjugation: The cross conjugation has got no effect on λ_{max}.

(iv) Alkyl substitution: Bathochromic shift occurs with alkyl substitution. More the no of alkyl substitution more is the shift.

(v) No of rings: Bathochromic shift occurs with the addition of rings.