CHAPTER 1 UV VISIBLE SPECTROSCOPY

1.1 INTRODUCTION

The term spectroscopy is derived from the word's spectrum, which implies a curve of distinct colours caused by wavelength differences, and skopin, which means examination or evaluation. Spectroscopy is thus the discipline of science concerned with the analysis or examination of spectrum.

The term "absorption spectroscopy" refers to spectroscopic methods that assess how much radiation interacts with a material and is absorbed as a function of frequency or wavelength. The sample takes in photons, or energy, from the emitting field. The absorption spectrum is the variation in absorption intensity as a function of frequency. Throughout the electromagnetic spectrum, absorption spectroscopy is used. When the molecule, atom, or ion in the sample changes from one energy state to another and the change in rotational, vibrational, and/or electronic energies is detected, electromagnetic radiation (EMR) is absorbed or emitted.

1.2 THEORY

The wavelength range of UV radiation is 200 nm- 400 nm. There are mainly two types of UV region.

1. 200 nm-400 nm that is called near ultraviolet region.
2. Below 200 nm that is called far ultraviolet region.
3. Visible light has a wavelength between 400 and 800 nm.

In the UV and visible spectrum, wavelength is measured in nanometres or angstroms. Wavenumber is used to express absorption (cm^{-1}).

The transfer of an electron or electrons within a molecule from one electronic energy level to another produces absorption spectra. The reversible kinds of transition give rise to ultraviolet emission spectra. The UV portion of the EMR spectrum is required for radiation to cause electronic excitation.

The energy of the radiation in this area is high enough to cause the outer valence electrons to undergo an electronic transition.

If a substance preferentially absorbs light in the visible spectrum, it seems to be coloured.

The primary purpose of absorbed energy is to elevate the molecule from its ground state (E_0) to a more highly excited state (E_1). The distinction is explained by:

$$\Delta E = E_1 - E_0 = h_v = h_c/\lambda$$

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

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

(a) σ (sigma) electrons: In saturated systems like alkane, a) σ (sigma) electrons are present. They don't exhibit UV absorption since their activation requires a significant amount of energy. In the vacuum UV area, their absorption band may be seen. Consequently, substances containing σ- bonds do not absorb in the range of near UV. For instance, saturated hydrocarbons can be utilised as solvents since they are transparent in the vicinity of the UV spectrum.

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

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

Electronic Transition

The σ, π, and n electrons are present in molecule and can be excited from the ground state to excited state by the absorption of UV radiation. The various transitions are $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$, & $\sigma \rightarrow \sigma^*$.

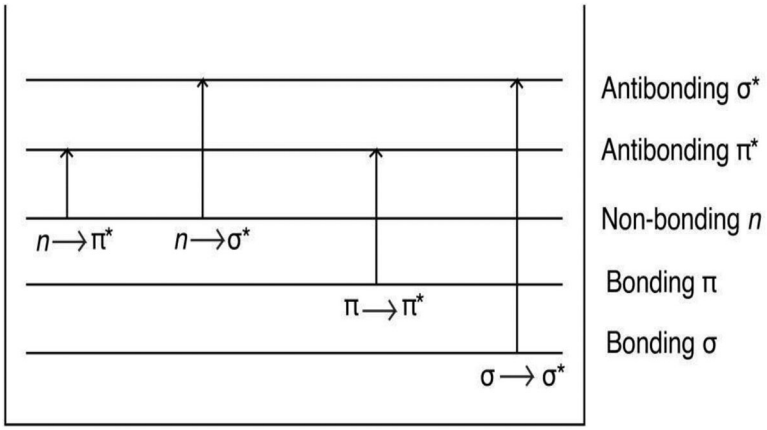


Figure 1.1: Shows the energy requirements for different electronic transitions.

The energy requirement order for excitation for different transitions is as follows. $n \rightarrow \pi^* < \pi \rightarrow \pi^* < n \rightarrow \sigma^* < \sigma \rightarrow \sigma^*$ $n \rightarrow \pi^*$ transition requires lowest energy while $\sigma \rightarrow \sigma^*$ requires highest amount of energy.

(1) *n* → π* transition: $n \rightarrow \pi^*$ transition requires lowest energy due to longer wavelength. So, they are forbidden and corresponding bands are characterized by low molar absorptivity. $\varepsilon_{max} < 100$. It is also known as R- band. They are further characterized by hypochromic shift or blue shift observed with an increase in solvent polarity.

(2) π → π* transition: It is due to the promotion of an electron from a bonding π orbital to an anti-bonding π * orbital. Energy requirement is between
$n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$. But the extended conjugation and alkyl substituents shift the λ_{max} towards longer wavelength (Bathochromic shift). It is also called K band.

(3) *n* → σ* transition: Saturated compounds with lone pair of electrons undergo $n \rightarrow \sigma^*$ transition in addition to $\sigma \rightarrow \sigma^*$ transition. Corresponding absorption bands appear at longer wavelengths in near UV region.

(4) σ → σ* transition: These transitions can occur in such compounds in which all the electrons are involved in single bonds and there is no lone pair of electrons.

Energy required for $\sigma \rightarrow \sigma^*$ transition is very large so the absorption band occurs in the far UV region. So, this transition can't normally be observed.

1.3 PRINCIPLE (LAMBERT BEER LAW)

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

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

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

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

$$-dI / dt \propto I$$

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

Where,

I = intensity of incident light

t = thickness of the medium

K = proportionality constant

By integration of equation (1), and Putting $I = I_0$ when $t = 0$, $I_0/I_t = kt$ or $I_t = I_0 e^{-kt}$

Where,

I_0 = intensity of incident light

I_t = intensity of transmitted light

k = constant which depends upon wavelength and absorbing medium used.

By changing the above equation from natural log.

We get, $I_t = I_0 e^{-Kt}$ …(2)

Where

$K = k/2.303$

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

$I_t = I_0 10^{-Kt}$ …(3)

Beer's law may be stated as follows:

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

The above sentence is very similar to Lambert's law. So, $I_t = I_0 e^{-k'c}$

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

$I_t = I_0\ 10^{K'c}$ …(4)

Where

k' and K' = proportionality constants

c = concentration by combining equation (3) and (4), we get,

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

$I_0/I_t = 10^{act}$

Where

K and K' = a or εc = concentration

t or b = thickness of the medium log I_0 / I_t = εbc …(5)

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

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

$A = \log I_0/I_t$ …(6)

From equation (5) and (6),

A = εbc …(7)

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

The term $E^{1\%}_{1cm}$ or A refers to the to the absorbance of 1 cm layer of the solution whose concentration 1 % at a specified λ. According to equation (7), $A = \log I_0/I_t$. Transmittance T is a ratio of intensity of transmitted light to that of the incident light.

$T = I_0/I_t$

The more general equation can be written as follows:

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

1.3.1 Deviation of Beer-Lambert Law

Beer-Lambert's law proves a direct correlation between the absorbance (A) of a molecule to the concentration (c) and the path length (b) of the sample as has been observed in the article for the Derivation of Beer Lambert Law. This relationship is a linear for the most part. However, under certain circumstances the Beer Lambert relationship breaks down and gives a non-linear relationship. These deviations from the Beer Lambert law can be classified into three categories:

1. Real Deviations– These are fundamental deviations due to the limitations of the law itself.
2. Chemical Deviations– These are deviations observed due to specific chemical species of the sample which is being analyzed.
3. Instrument Deviations– These are deviations which occur due to how the absorbance measurements are made.

1.3.2 Real Limitation and Deviation of Beer-Lambert Law

Beer law and Lambert law is capable of describing absorption behavior of solutions containing relatively low amounts of solutes dissolved in it (<10mM). When the concentration of the analyte in the solution is high (>10mM), the analyte begins to behave differently due to interactions with the solvent and other solute molecules and at times even due to hydrogen bonding interactions.

1.3.2.1 At high concentrations, solute molecules can cause different charge distribution on their neighboring species in the solution. Since UV-visible absorption is an electronic phenomenon, high concentrations would possibly result in a shift in the absorption wavelength of the analyte. At times, even electrolyte concentrations (such as those present in buffers) play an important role in altering the charge distributions and affecting UV-visible absorbance. Some large ions or molecules show deviations even at very low concentrations. For e.g. methylene blue absorptivity at 436 nm fails to observe Beer Lambert law even at concentrations as low as 10μM.

1.3.2.2 High analyte concentrations can also possibly alter the refractive index (η) of the solution which in turn could affect the absorbance obtained. If the addition of solute causes a significant change in the refractive index of the solution a correction to the Beer Lambert formula can be placed as:

$$A = \varepsilon bc\ (\eta^2 + 2)^2$$

1.3.3 Chemical Deviations and Limitations to Beer-Lambert Law

Chemical deviations happen as a result of analyte molecule connection, dissociation, and contact with the solvent, which results in a product with various absorption properties. For instance, the transition of phenol red from its acidic form (yellow) to its basic form involves a resonance transformation (red). The electron distribution of a molecule's bonds varies with the pH of the solvent in which it is dissolved as a result of this resonance. Since UV-visible spectroscopy is an electron-related phenomena, the pH of the solvent affects how the sample's absorption spectrum varies.

1.3.4 Instrumental Deviations and Limitations to Beer-Lambert Law

A. Due to Polychromatic Radiation (Also the reason why absorbance measurements are taken at the wavelength of maximum absorbance λ_{max})

When a monochromatic radiation source is present, Beer-Lambert law is carefully adhered to. To generate a monochromatic beam from a polychromatic source of radiation with a continuous distribution of wavelengths, however, a filter or grating unit (monochromators) is typically used.

When the molar absorptivities at both wavelengths are equal (i.e. ε' = ε"'), Beer-Lambert rule is used to create a straight line to represent the connection between absorbance and concentration. However, the deviations from linearity also rise as the gap between ε' and ε" increases.

Beer-Lambert law is seldom violated if the spectrometer's band of wavelength is chosen in a way that the analyte's molar absorptivities are almost constant. However, the analyte's absorbance will deviate from Beer-Lambert law if a band is selected so that the analyte's molar absorptivity at these wavelengths changes significantly. When the wavelength being examined is at its maximum, it is found that variations in absorbance over wavelengths are at their lowest. This is why measurements of absorption are made at certain wavelengths.

B. Due to Presence of Stray Radiation

Radiation from the device that is beyond the chosen nominal wavelength range is referred to as stray radiation or dispersed radiation. The wavelength of stray radiation typically differs much from the chosen wavelength band. It is well known that the radiation that emerges from a monochromator frequently contains very small amounts of dispersed or stray radiation. Usually, the surfaces of lenses, mirrors, gratings, filters, and windows reflect and scatter this radiation. Similar to the deviation brought on by polychromatic radiation, the Beer-Lambert equation deviates if the analyte absorbs at the wavelength of the stray radiation.

C. Due to Mismatched Cells or Cuvettes

It stands to reason that the Beer-Lambert equation would deviate if the cells containing the analyte and the blank solutions had different path lengths or different optical properties. In such cases when a plot of absorbance versus concentration is made, the curve will have an intercept k and the equation will be defined as: $A = \varepsilon bc + k$

In today's instrument this problem is generally not observed, however if it is present, appropriate linear regression to quantify this deviation must be made.

1.4 INSTRUMENTATION

Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;

1. Sources (UV and visible)
2. Filter or monochromator
3. Sample containers or sample cells
4. Detector

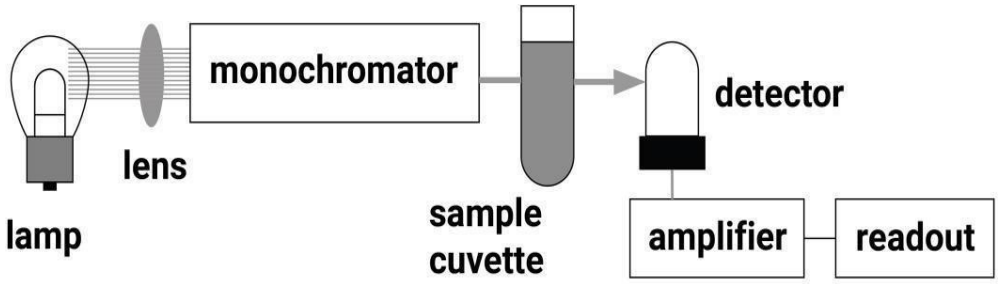


Figure 1.2: Line diagram of UV Spectrophotometer.

Radiation source

It is crucial that the radiation source's power does not fluctuate drastically along the spectrum of its wavelengths. A continuous UV spectrum is produced when deuterium or hydrogen is electrically excited at low pressure. The process that causes this requires the production of an excited molecular species, which splits into two atomic species and an ultraviolet photon. Lamps made of deuterium and hydrogen both produces radiation between 160 and 375 nm. These lamps must have quartz cuvettes and windows since glass absorbs light with wavelengths less than 350 nm.

The characteristics of Ideal source of radiation:

1. It should be stable and should not allow fluctuation.
2. It should emit light of continuous spectrum of high and uniform intensity over the entire wave length region in which it is used.
3. It should provide incident light of sufficient intensity for the transmitted energy to be detected at the end of optic path.
4. It should not show fatigue on continuous use.

Various UV radiation sources are as follows: -

a. Deuterium lamp
b. Hydrogen lamp
c. Tungsten lamp
d. Xenon discharge lamp
e. Mercury arc lamp

Various Visible radiation sources are as follows: -

a. Tungsten lamp

b. Mercury vapour lamp

- **Tungsten lamp**
  - Its construction is similar to a house hold lamp.
  - The bulb contains a filament of Tungsten fixed in evacuated conditions and then filled with inert gas.
  - The filament can be heated up to 3000k, beyond this temperature the Tungsten starts sublimating.
  - It is used when polychromatic light is required. To prevent this with inert gas some amount of halogen is introduced (Preferably Iodine).
  - Sublimated form of Tungsten reacts with iodine to form Tungsten-iodine complex. Which migrates back to the hot filament where it gets decompose and Tungsten get deposited. Demerit- it emits the major portion of its radiation energy in near IR region of spectrum.
- **Hydrogen lamp**
  - In hydrogen discharge lamp pair of electrodes is enclosed in a glass tube (provided with silica or quartz window for UV radiation to pass through) filled with hydrogen gas.
  - When current is passed through these electrodes maintained at high voltage, discharge of electrons occurs which excites the hydrogen molecule which in turn causes emission of UV radiation in near UV region.
  - They are stable and robust.
- **Xenon discharge lamp**
  - It possesses two tungsten electrode separated by some distance.
  - These are enclosed in a glass tube with quartz or fused silica and xenon gas is filled under pressure.

- An intense arc is formed between electrodes by applying high voltage. This is good source of continuous plus additional intense radiation. Its intensity is higher than The hydrogen discharge lamp.
- Demerit- the lamp since operates at high voltage becomes very hot during operation and hence needs thermal insulation.

- **Mercury Arc lamp**
  - In mercury arc lamp, mercury is filled under high pressure and excitation of mercury atoms is done by electric discharge.
  - Demerit-not suitable for continuous spectral studies because it does not give continuous radiation.

- **Filters or monochromators**

  All monochromators contain the following component parts;
  - An entrance slit
  - A collimating lens
  - Dispersing device (a prism or a grating)
  - A focusing lens
  - An exit slit

- **Collimating lens**

  The radiation emitted by the source is collimated (made parallel) by lens, mirrors and slits.
  - Material used for the lenses must be transparent to the radiation being used.
  - Ordinary silicate glass transmits between 350 to 3000 nm and is suitable for visible and IR region.
  - Quartz or fused silica is used as a material for lenses to work below 300 nm.

- **Mirrors**
  - These are used to reflect, focus or collimate light beams in spectrophotometer.
  - To minimize the light loss, mirrors are aluminized on their front surfaces.
- **Slits**
  - Slit is important device in resolving polychromatic radiation into monochromatic radiation.
  - To achieve this, entrance slit and exit slit is used.
  - The width of slit plays an important role in resolution of polychromatic radiation.
- **Monochromators**

  It is a device used to isolate the radiation of the desired wavelength from the continuous spectra. Following types of monochromatic devices are used.

  1. Filters
  2. Prisms
  3. Gratings
- **Filters**

  Selection of filters is usually done on a compromise between peak transmittance and band pass width; the former should be as high as possible and later as narrow as possible.
- **Sample containers or sample cells**

  A variety of sample cells available for UV region. The choice of sample cell is based on:-

  (a) The path length, shape, size

  (b) The transmission characteristics at the desired wavelength

  (c) The relative expense

The sample-holding cell needs to be transparent to the wavelength range being measured.

For UV spectroscopy, quartz or fused silica cuvettes are necessary. For usage between 350 and 2000 nm, cuvettes can be made from silicate glasses. In most cases, a cell is 1 cm thick. Cells might be cylindrical with flat ends or rectangular in form.

- **Detectors**

In order to detect radiation, three types of photosensitive devices are:-

(a) Photovoltaic cells or barrier-layer cell

(b) Phototubes or photo emissive tubes

(c) Photomultiplier tubes

a. A barrier layer or photronic cell is another name for a **photovoltaic cell**. One electrode is a metallic base plate made of iron or aluminium that makes up the device. A tiny coating of a semiconductor metal, such as selenium, is placed on its surface. Then, a very thin coating of silver or gold is applied to the surface of the selenium, serving as a second collection tube.

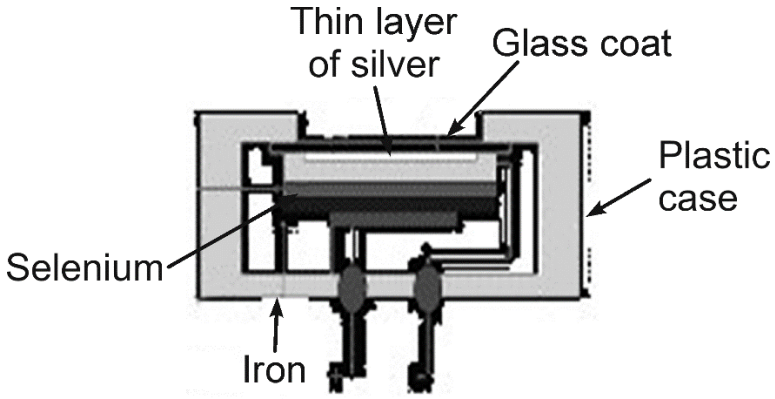


Figure 1.3: Photovoltaic Cell.

When radiation strikes the surface of selenium, electrons are produced at the selenium-silver interface, and the silver absorbs them. Between the silver surface and the cell's foundation, this accumulation at the silver surface generates an electric voltage differential.

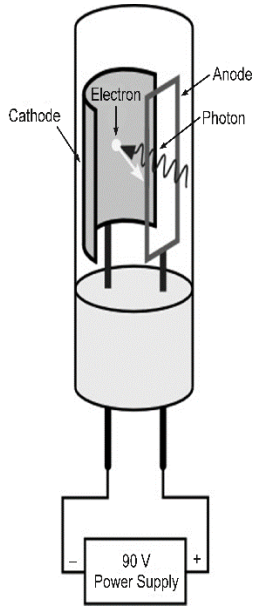


Figure 1.4: Phototubes.

b.Photo emissive cells are another name for **phototubes**. A glass bulb that has been emptied makes up a phototube. It contains a light-sensitive cathode. Silver oxide and potassium oxide are two light-sensitive materials that are applied to the inner surface of the cathode. Photoelectrons are released when radiation strikes a cathode. By using an anode, they are gathered. These are then sent back through an external circuit. This procedure also amplifies the current and records it.
c. One popular detector in UV spectroscopy is the photomultiplier tube. It is made up of an anode, many dynos, which emit multiple electrons for each photon of radiation that strikes them, a photo emissive cathode, which emits electrons when impacted by photons of radiation.

When a photon of radiation enters the tube and hits the cathode, many electrons are released. The first dynode is where these electrons are accelerated (which is 90V more positive than the cathode). For every electron that strikes the initial dynode, numerous more electrons are released. These electrons are subsequently accelerated in the direction of the second dynode,

creating more electrons that are accelerated in the direction of the third dynode and so on. The anode is where the electrons are eventually gathered. By this time, each original photon has produced 106-107 electrons. The resulting current is amplified and measured.

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

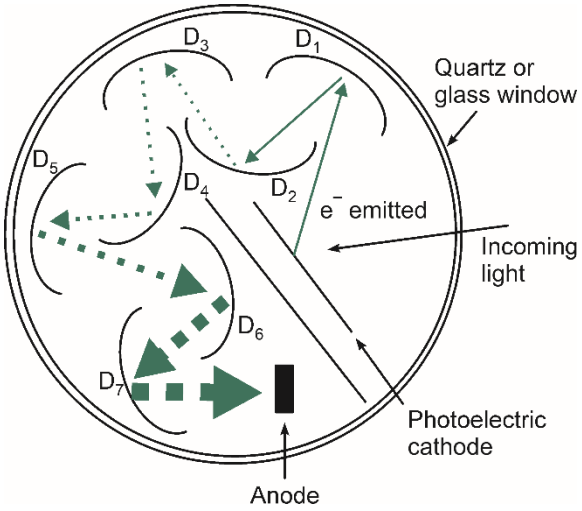


Figure 1.5: The photomultiplier tube.

1.5 TYPES OF UV SPECTROPHOTOMETER

A. UV Spectrophotometer Single Beam System

The source emits UV light in a single beam system. The radiation beam is gathered and focused on the inlet slit using a convex lens. The inlet slit lets source light through while blocking stray radiation. The monochromator is where the light is separated into its constituent wavelengths as it reaches it. The exit light is placed such that it may let through the necessary wavelength of light. All other wavelengths of radiation are suppressed. The detector gauges the radiation's strength as it receives the chosen radiation through the sample cell. By comparing the intensity of radiation before and after it passes through the sample, it is possible to measure how much radiation is

absorbed by the sample at the particular wavelength used. The output of the detector is usually recorded on graph paper.

One problem with the single beam system is that it measures the total amount of light reaching the detector, rather than the percentage absorbed.

Another issue is that the detector's reaction considerably changes depending on the wavelength of radiation it is exposed to. By adopting a twofold beam system, the instrument variation issue may be significantly solved.

B. UV Spectrophotometer Double Beam UV System

The basic layout of a double beam ultraviolet spectrophotometer is shown in figure 1.6.

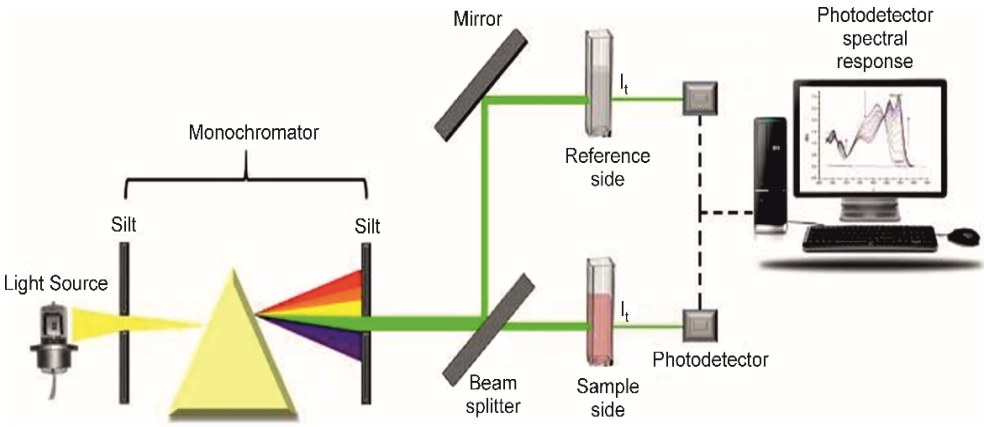


Figure 1.6: Double Beam UV Spectrophotometer.

A. The radiation from the source is allowed to pass via mirror system to the monochromator unit. The function of the monochromator is to allow a narrow range of wavelength to pass through an exit slit.

B. The radiation coming out of the monochromator through the exit slit is received by the rotating sector which divides the beam into two beams, one passing through the reference and the other through the sample cell.

C. After passing through the sample and references cells, the light beams are focused onto the detector.

D. The output of the detector is connected to phase sensitive amplifier which responds to any change in transmission through sample and reference.

E. The phase sensitive amplifier transmits the signals to the recorder which is followed by the movement of the pen on chart. The chart drive is coupled to the prism and thus the absorbance or transmittance of the sample is recorded as a function of wavelength.

1.6 ADVANTAGE OF DOUBLE BEAM SPECTROPHOTOMETER

The double beam instruments provide the following benefits while being more complicated and expensive:

- It is necessary to continually replace the blank with the sample or to zero adjust at each wave length as in the single beam units.
- The ratio of the powers of the sample and reference beams is constantly obtained and used. Any error due to variation in the intensity of the source and fluctuation in the detector is minimized.
- Because of the previous two factors, the double beam spectrophotometer lends itself to Rapid scanning over a wide wavelength region and to the use of a recorder or digital read out.

1.7 EFFECTS IN UV TRANSITIONS

Different transitions between the bonding and anti-bonding electronic states when light energy is absorbed in UV-Visible Spectroscopy.

When a sample is exposed to light energy that matches the energy difference between possible electronic transitions within the molecule, a fraction of the light energy would be absorbed by the molecule and the electrons would be promoted to the higher energy state orbital. A spectrometer records the degree of absorption by a sample at different wavelengths and the resulting plot of absorbance (A) versus wavelength (λ) is known as a spectrum. The wavelength at which the sample absorbs the maximum amount of light is known as λ_{max}. For example, shown below is the spectrum of isoprene. Isoprene is colorless as it does not absorb light in the visible spectrum, and has a λ_{max} of 222nm.

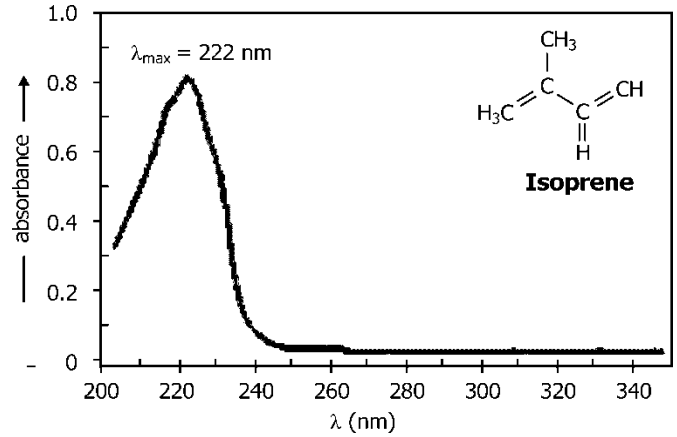


Figure 1.7: UV-visible spectrum of isoprene showing maximum absorption at 222 nm.

1.8 CHROMOPHORE

Due to the electron arrangement in the functional group, certain chemical groups or entities are prone to absorb light. These groups are referred to as chromophores. The table below, for instance, lists frequently occurring chromophores along with their estimated absorbances.

Chromophore	Example	Excitation	λ_{max} (nm)	Solvent
C = C	Ethene	$\pi \rightarrow \pi^*$	171	Hexanes
C = O	Ethanal	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	180 290	Hexane
N = O	Nitro methane	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	200 275	Hexane

1.8.1 Effect of Conjugation

Conjugation of π-electrons affects the energy levels of the π-electrons. Four molecular orbitals are created when two double bonds are conjugated by their electrons (i.e. two bonding and two anti-bonding). See the illustration below the lowest unoccupied molecular orbital (LUMO) is at a lower energy level as a result, and the highest occupied molecular orbital (HOMO) is at a higher energy state. Therefore, less energy would be needed to excite the electrons from the HOMO to the LUMO in order to excite this system. This

decrease in energy levels causes the wavelength at which conjugated molecules are absorbed to grow.

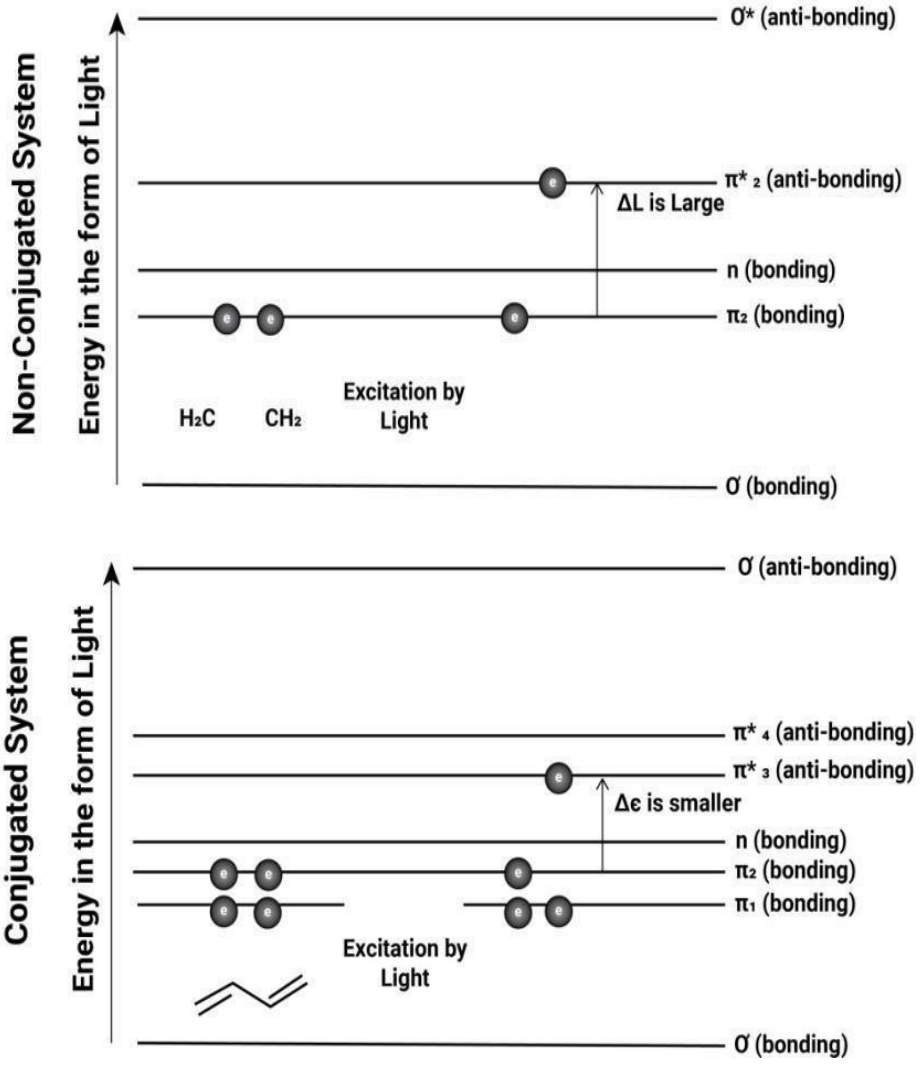


Figure 1.8: Diagram shows how a non-conjugated system would require more energy for absorption as compared to conjugated system.

The vibrational energy levels produced by vibrational changes within the system are located between the various electronic energy levels. It should be kept in mind that UV-visible light can also stimulate some chemical vibrational frequencies. This phenomenon causes the UV-Visible spectra to

produce a smooth curve-shaped peak for absorption rather than a single sharp peak, as will be seen in various cases.

1.9 PHARMACEUTICAL APPLICATIONS

1) Detection of Impurities

UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected. Benzene appears as a common impurity in cyclohexane. Its presence can be easily detected by its absorption at 255 nm.

2) Structure elucidation of organic compounds

UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms. From the location of peaks and combination of peaks, it can be concluded that whether the compound is saturated or unsaturated, hetero atoms are present or not etc.

3) Quantitative analysis

UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is based on Beer's law which is as follows.

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

Where ε is extinction co-efficient, c is concentration, and b is the length of the cell that is used in UV spectrophotometer. Other methods for quantitative analysis are as follows.

a. calibration curve method

b. simultaneous multicomponent method

c. difference spectrophotometric method

d. derivative spectrophotometric method

4) Qualitative Analysis

UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the

absorption spectrum with the spectra of known compounds.

UV absorption spectroscopy is generally used for characterizing aromatic compounds and aromatic olefins.

5) Dissociation Constants of Acids and Bases

$$PH = PKa + \log [A^-]/[HA]$$

From the above equation, the PKa value can be calculated if the ratio of $[A^-]/[HA]$ is known at a particular PH. and the ratio of $[A^-]/[HA]$ can be determined spectrophotometrically from the graph plotted between absorbance and wavelength at different PH values.

6) Chemical kinetics

Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

7) Quantitative analysis of pharmaceutical substances

Many drugs are either in the form of raw material or in the form of formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at specific wavelength. *Diazepam tablet can be analyzed by 0.5% H_2SO_4 in methanol at the wavelength 284 nm.

8) Molecular weight determination

Molecular weights of compounds can be measured spectrophoto-metrically by preparing the suitable derivatives of these compounds.

For example, if we want to determine the molecular weight of amine then it is converted in to amine picrate. Then known concentration of amine picrate is dissolved in a litre of solution and its optical density is measured at λ_{max} 380 nm. After this the concentration of the solution in gm moles per litre can be calculated by using the following formula.

$$C = \frac{\log 1_0/1_1}{\varepsilon_{max} \times 1}$$

"**c**" can be calculated using above equation, the weight "**w**" of amine picrate is known. From "**c**" and "**w**", molecular weight of amine picrate can be calculated. And the molecular weight of picrate can be calculated using the molecular weight of amine picrate.

9) As HPLC detector

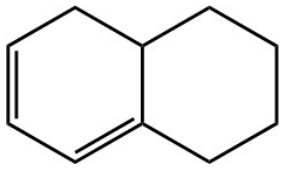
A UV/V is spectrophotometer may be used as a detector for HPLC. The presence of an analyte gives a response which can be assumed to be proportional to the concentration. For more accurate results, the instrument's response to the analyte in the unknown should be compared with the response to a standard; as in the case of calibration curve.

1.10 WOODWARD-FIESER RULES FOR CALCULATING ABSORPTION MAXIMA

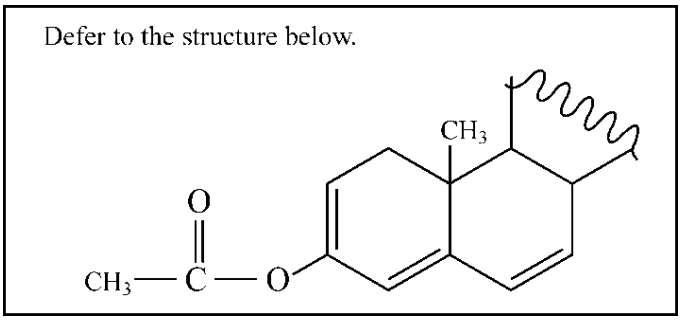
Form the study of the ultraviolet absorption spectra of a large number of compounds, Woodward gave certain rules for correcting λ_{max} with molecular structure. Since then these rules have been modified by Scott and Fisher because of more experimental data. The modified rules known as Woodward-Fisher rules can be used to calculate the position of λ_{max} for a given structure by relating the position of λ_{max} with the position and degree of substitution of chromophore.

A. Woodward: Fieser rules for calculating λmax in conjugated dienes, trienes and polyenes

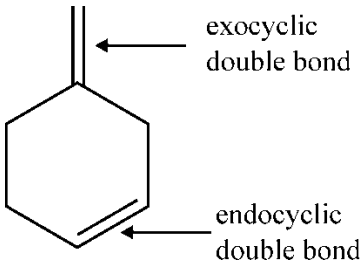
1. Homoannular diene: It is a cyclic diene having conjugated double bond in the same ring. Example are-



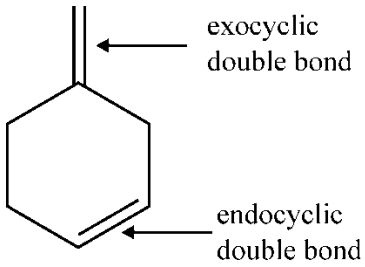
2. **Heteroannular diene:** It is a cyclic diene in which double bonds in conjugation are present in different rings. Example are-



I. **Endocyclic double bond.** It is a double bond present in a ring as shown in example



II. **Exocyclic double bond.** It is a double bond in which one of the doubly bonded atoms is a part of a ring system as shown in example



Woodward-Fieser rules conjugated dienes, trienes and polyenes

According to these rules, each type of diene or triene system is having a certain fixed value at which absorption takes place. This makes the basic value or parent value. The values of certain alkyl substituents or ring residue, double bond extending conjugation and polar groups such as chloride, bromide and –OR are added to the basic value to obtain λMAX for a particular compound.

Table 1.1: Parent values and increments for different substituents/groups**.**

1	**Parent Values**	Acyclic conjugated diene and heteroannular conjugated diene	215 nm
		Homuncular Conjugated diene	253 nm
		Acyclic triene	245 nm
2	**Increments**	Each alkyl substituent or ring residue	5 nm
		Exocyclic double bond	5 nm
		Double bond extending conjugation	30 nm
3	**Auxochromes**	-OR	6 nm
		-SR	30 nm
		-Cl,-Br	5 nm
		NR2	60 nm
		$-OCOCH_3$	0 nm

B. Woodward-fieser rules for α, β-unsaturated carbonyl compounds

Woodward fieser rules for calculating λ_{max} for α, β-unsaturated carbonyl compounds modified by Scott which is shown in tabulated form.

Table 1.2: Parent values and increments for different substituents/groups.

1.	Parent Values	α, β-unsaturated acyclic or six membered ring lactones	215 nm		
		α, β-unsaturated aldehyde	207 nm		
2.	Increments	Each alkyl substituent or ring residue	At α position 10 nm	At β position 12 nm	At Ɣ and higher position 18 nm

Table 1.2 contd....

		Exocyclic double bond	5 nm		
		Double bond extending conjugation	30 nm		
		Homoannular conjugated diene	39 nm		
3.	Auxochromes		At α position	At β position	At Ɣ position
		-OH	35	30	50
		-OR	35	30	17
		-SR	-	85	-
		-Cl	15	12	-
		-Br	25	30	-
		NR_2	-	95	-
		$-OCOCH_3$	6	6	6

In these compounds, the actual spectra obtained are affected considerably by the nature of the solvent employed. Hence a solvent correction is applied to the calculated value to get the value for that particular solvent.