आईएफटीएम विश्वविद्यालय, मुरादाबाद, उत्तर प्रदेश

IFTM University, Moradabad, Uttar Pradesh

NAAC ACCREDITED

E-Content

IFTM University, Moradabad

ULTRAVIOLET
SPECTROSCOPY

Ultraviolet Spectroscopy

- **Introduction**
  - The ultraviolet region is divided into two spectral regions-
  - The region between **2000 Å-4000 Å** is known as **near ultraviolet region**.
  - The region **below 2000 Å** is called **far or vacuum ultraviolet region.**
  - The UV radiations have sufficient energy to excite **valence electrons** in many atoms or molecules; consequently, UV is involved with electronic excitation.

Note:

A **valence electron** is an outer shell **electron** that is associated with an atom, and that can participate in the formation of a chemical bond if the outer shell is not closed; in a single covalent bond, both atoms in the bond contribute one **valence electron** in order to form a shared pair.

Origin and Theory of Ultraviolet Spectra

- **Ultraviolet absorption spectra** arises from transition of electron or electrons within a molecule from a **lower to a higher electronic energy level** and the **ultraviolet emission spectra** arises from the **reverse type of transition.**
- For radiation to cause **electronic transitions**, it must be in the **UV region of electromagnetic spectrum.**

Important Terms

*The **electromagnetic spectrum** is the range of frequencies (the **spectrum**) of **electromagnetic** radiation and their respective wavelengths.

***Electromagnetic Radiations-** A kind of radiation including UV light, visible light, radio waves, gamma rays, and X-rays, in which electric and magnetic fields vary simultaneously.

*A **wavelength** is a measure of distance between two identical peaks.

Electric field is defined as the **electric** force per unit charge.

A **magnetic field** is a vector **field** that describes the **magnetic** influence on an electric charge of other moving charges or magnetized materials.

- When a molecule absorbs ultraviolet radiation of frequency $v\ \sec^{-1}$, the electron in that molecule undergoes transition from a **lower to a higher energy level,** the energy difference is given by

E=*hv erg*……………….*(Eq – 1)*

- The actual amount of energy required depends on the difference in energy between the ground state E_0 and the excited state E_1 of the electrons.

  Equation-1 becomes as-:

$$E_1 - E_0 = hv \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (Eq\text{-}2)$$

- We know that **the total energy of a molecule** is equal to the sum of **electronic, vibrational and rotational energy**.

Important Terms

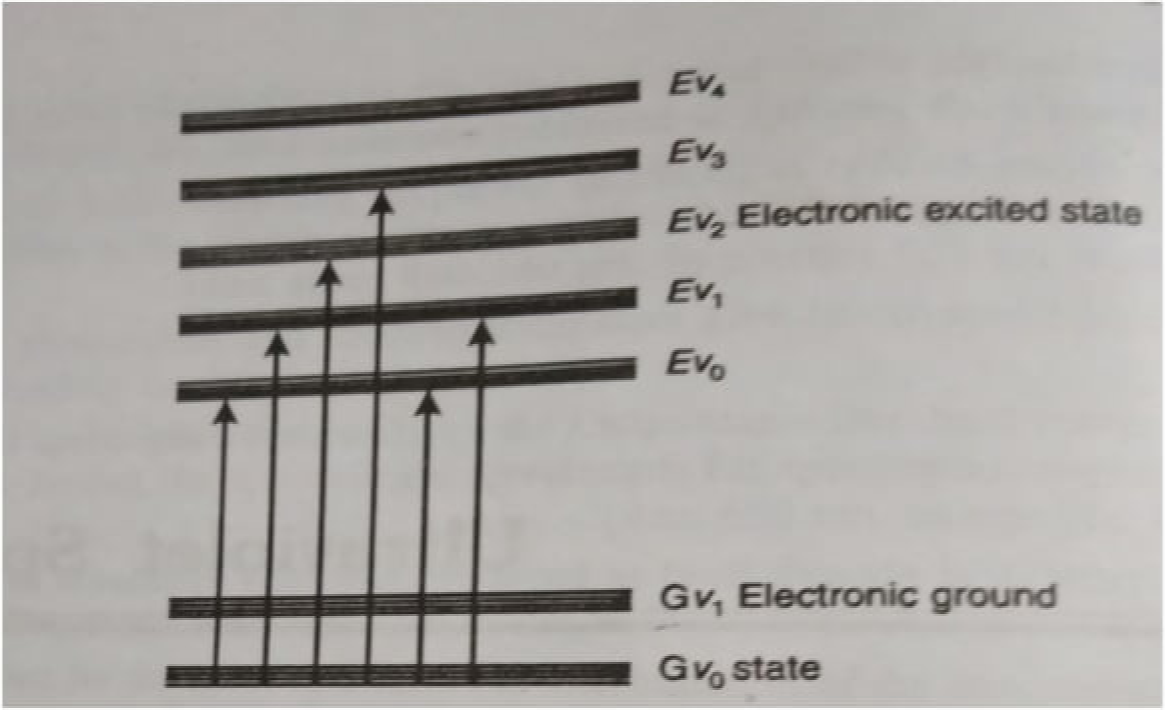
***Vibrational Energy** -The **energy** in a vibrating system, otherwise at rest; especially that in a molecule due to the vibrations of its atoms.

*The **electronic energy** is that generated by electron movement within the molecular bonds.

***Rotational energy** concerns with the **rotation** of the molecule.

*h = Planck's Constant

*h= 6.626×10^{-34} Joules second



Energy Level Diagram of a Diatomic molecule

- Energy absorbed in the ultraviolet region produces changes in the electronic energy of the molecule resulting from transition of valence electrons in the molecule.
- Three different types of electrons are involved in the organic molecules.
- **σ-electrons:** These electrons are involved in **saturated bonds**, such as those between carbon and hydrogen in paraffins. These bonds are called **σ-bonds.**
- **π–electrons:** These electrons are involved in unsaturated hydrocarbons. Typical compounds with **π bonds** are **trienes and aromatic compounds.**
- ***n* – electrons:** These are the electrons which are not involved in the bonding between atoms in molecules. Examples are organic compounds containing **nitrogen, oxygen , halogen.**

Note:

Trienes- Three separate carbon-carbon double bonds.

- Types of Transitions in Organic Molecules
  - Energy absorbed in the ultraviolet region by complex organic molecules causes transition of valence electrons in the molecules.
  - These transitions are $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$, and $\pi \rightarrow \pi^*$
  - The energy level scheme for a molecule is shown in figure given below-

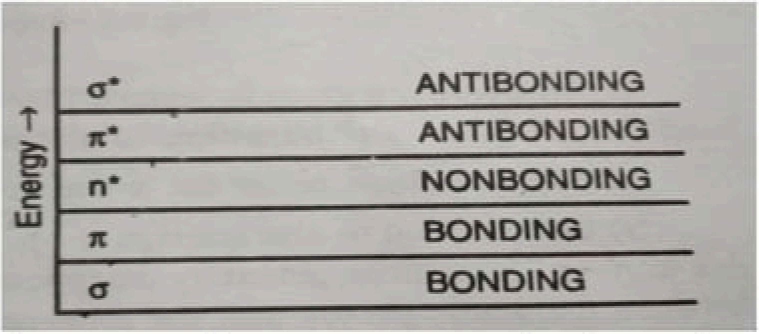
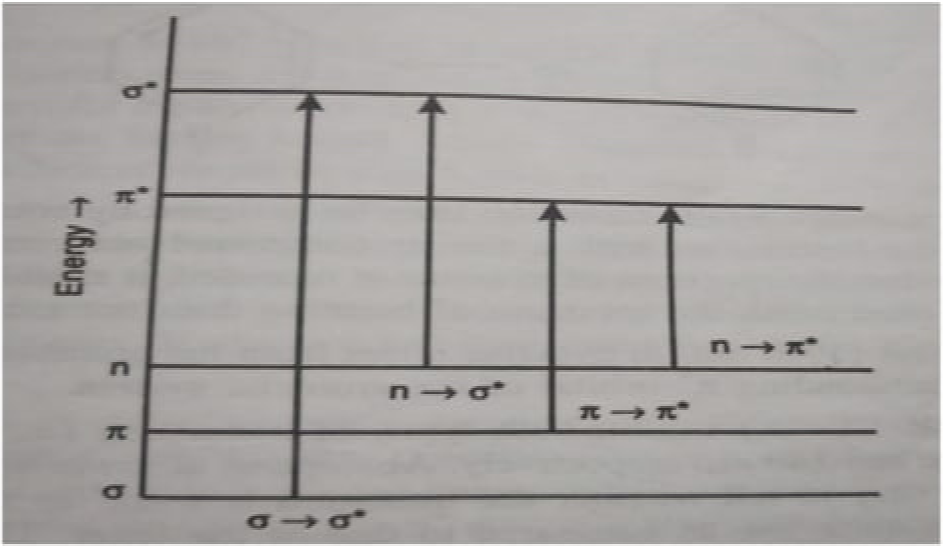


Figure – Various Energy Levels of a molecule

Note:
A non-bonding orbital (NBMO) is a molecular orbital for which the addition or removal of an electron does not change the energy of the molecule.

- From the figure given below it is clear that the change in energy values for different transitions are in following order -:
- $n \rightarrow \pi^* < \pi \rightarrow \pi^* < n \rightarrow \sigma^* < \sigma \rightarrow \sigma^*$



Electronic Excitation Energies

- The main features of $n \rightarrow \pi^*, \sigma \rightarrow \sigma^*, n \rightarrow \sigma^*, \pi \rightarrow \pi^*$ transitions are discussed below -:

1. $n \rightarrow \pi^*$ Transitions -: These type of transitions are shown by **unsaturated molecules** which contain atoms such as **oxygen, nitrogen and sulphur.**
2. $\sigma \rightarrow \sigma^*$ Transitions -: These transitions can occur in such compounds in which all the electrons are involved in **single bonds** and there are **no lone pairs of electrons.** Examples involving such transitions are **saturated hydrocarbons.**
3. $n \rightarrow \sigma^*$ Transitions -: Saturated compounds with lone pair (non-bonding) electrons undergo $n \rightarrow \sigma^*$ transitions.

   The energy required for an $n \rightarrow \sigma^*$ transition is generally less than that required for a $\sigma \rightarrow \sigma^*$ transition.
4. $\pi \rightarrow \pi^*$ Transitions -: A $\pi \rightarrow \pi^*$ transition corresponds to the promotion of an electron from a **bonding π orbital to an antibonding π* orbital** . This transition can occur in **any molecule having a π electron system.**

Chromophore and Related Terms

- **Chromophore –** The term chromophore was previously used **to denote a functional group or some other structural feature the presence of which gives a color to a compound.** For example, **nitro group is a chromophore** because its presence in a compound gives **yellow colour to the compound.** But these days the term chromophore is used in a much broader sense which may be defined **as any group which exhibits absorption of electromagnetic radiations in the visible or ultraviolet region.** It may or may not impart any color to the compound.

Two types of chromophores are known -:

- Chromophores in which the group is having **π electrons** undergo $\pi \rightarrow \pi^*$ transitions. Examples of such compounds are **ethylenes, acetylenes.**
- Chromophores having both π electrons and *n* (non-bonding) electrons undergo two types of transitions , *i.e.,* $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$. Examples of this type include **carbonyls, nitriles, azo compounds.**

- **Auxochrome –** It is a group which itself does not act as a chromophore but when attached to the chromophore it shifts the absorption maximum towards the **longer wavelength** along with an increase in the **intensity of absorption.**
- Some commonly known auxochromic groups are –OH, -NH_2, -OR -NHR .
- For example -NH_2 group is attached to benzene ring , its absorption changes from λ_{max} 255 (ε_{max} 203) to λ_{max} 280 (ε_{max} 1480).

- **Bathochromic Shift or Red Shift-** It involves the shift of **absorption maximum** towards longer wavelength because of the presence of certain groups such as **OH and NH_2 called auxochromes** or by change of solvent. For example, decreasing the polarity of solvent causes a red shift in the absorption of carbonyl compounds.
- **Hypsochromic Shift or Blue Shift –** It involves the shift of absorption maximum towards the shorter wavelength and may be caused **by removal of conjugation** in a system or by change of solvent. The absorption shift towards shorter wavelength is also called **blue Shift.**
- **Hyperchromic Shift –** This effect involves an increase in the **intensity of absorption** and is usually brought about by introduction of an **auxochrome.**
- **Hypochromic Shift –** It involves **a decrease in the intensity of absorption** and is brought about by groups which are **able to distort the geometry of the molecule.**
- For example when a **methyl group** is introduced in **position 2** of biphenyl group **hypochromic effect** occurs **because of distortion** caused by **methyl group.**

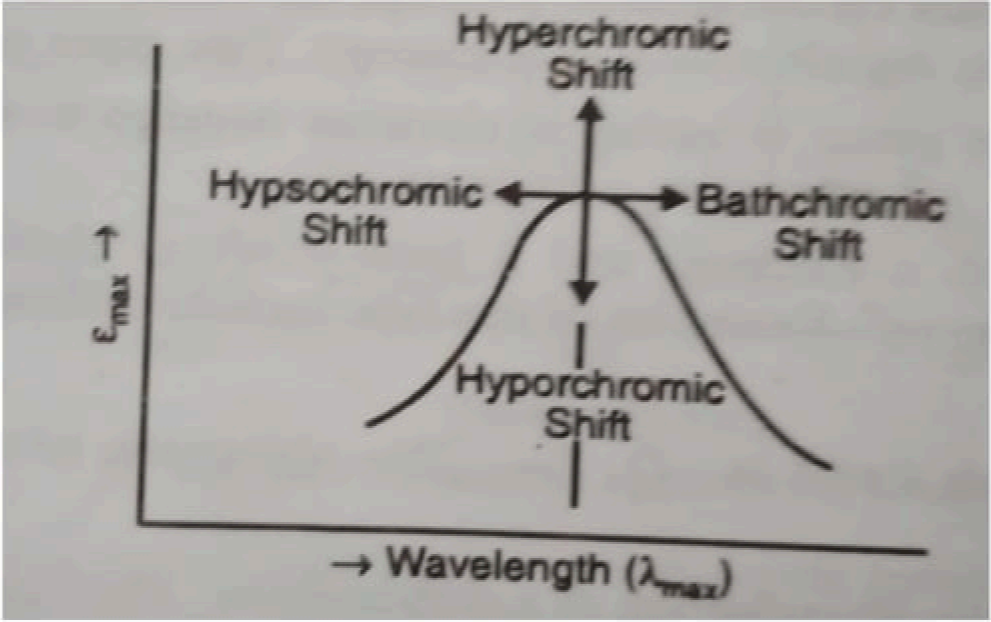


Figure - Absorption and Intensity Shifts

Important Terms

Conjugates system - A system with delocalized electrons in the molecule, which in general lowers the overall energy of the molecule and increases stability.

Intensity – Strength of Absorption.

- Solvent Effects/Choice of Solvent
  - A most suitable solvent is one that does not itself absorb in the region under investigation.
  - A dilute sample of solution is always prepared for spectral analysis.
  - Most commonly used solvent is **95% ethanol.**
  - Ethanol is the best solvent as it is cheap and it is transparent down to **210 nm.**
  - Some solvents with their upper wave-length limit of absorption are given in table below-:

Solvent	λ of absorption (nm)
Ethanol	210
Hexane	210
Methanol	210
Water	205
Benzene	280
Chloroform	245
Carbon tetrachloride	265

- Hexane and other hydrocarbons can be used because these are less polar and have least interactions with the molecule under investigation.
- For ultraviolet spectroscopy ethanol and water serve the purpose best.

Woodward-Feiser Rules for Calculating Absorption Maxima

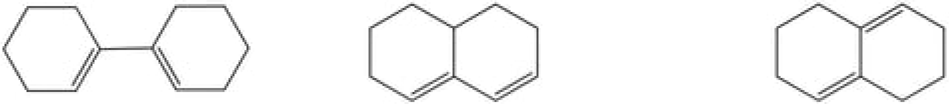
- From the study of the ultraviolet absorption spectra of a large number of compounds, Woodward (1941) gave some rules for correlating λ_{max} with molecular structure.
- Since then these rules have been modified by Scott and Feiser.
- The modified rules known as Woodward–Feiser rules can be used to calculate the position of λ_{max} for a given structure.

Woodward-Feiser Rules for Calculating λ_{max} in Conjugated Dienes, trienes and polyenes

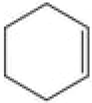
- Before we consider the rules for dienes, trienes, etc., it will be useful to explain some terms involved in discussing the rules

1. **Homoannular Diene –** It is a cyclic diene having conjugated double bond in the same ring. For example

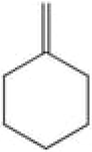
2. **Heteroannular Diene –** It is a cyclic diene in which double bond in conjugation are present in different rings. For example



- **Endocyclic Double Bond** – It is a double bond present in a ring.

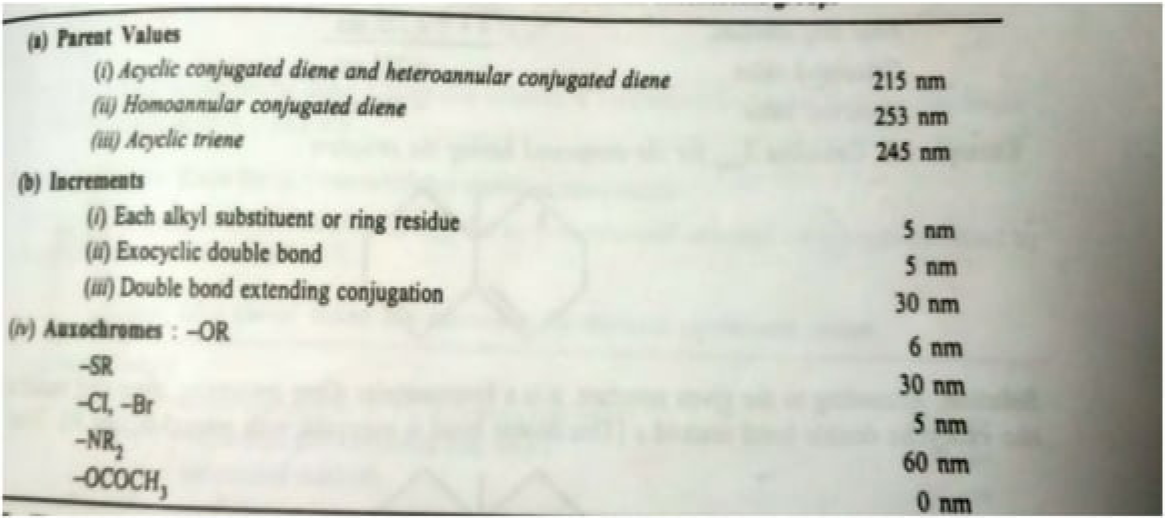


- **Exocyclic Double Bond** – It is a double bond in which one of the doubly bonded atoms is a part of ring system.



- According to these rules, each type of diene or triene systems is having a **certain fixed value** at which absorption takes place. This constitutes the **basic value or parent value.**
- The contributions made by various **alkyl substituents or ring residues, double bonds extending conjugation and polar groups such as –Cl, -Br, -OR** are added to the basic value to obtain λ_{max} for a particular compound.

- The parent values and contributions of different substituents/groups are given in the following figure-:

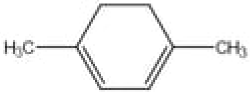


(a) Parent Values	
(i) Acyclic conjugated diene and heteroannular conjugated diene	215 nm
(ii) Homoannular conjugated diene	253 nm
(iii) Acyclic triene	245 nm
(b) Increments	
(i) Each alkyl substituent or ring residue	5 nm
(ii) Exocyclic double bond	5 nm
(iii) Double bond extending conjugation	30 nm
(iv) **Auxochromes** : –OR	6 nm
–SR	30 nm
–Cl, –Br	5 nm
$-NR_2$	60 nm
$-OCOCH_3$	0 nm

Figure: Parent Values and increments for different substituents/groups

Examples

- Calculate the λ_{max} for 1,4 – dimethylcyclohex-1,3-diene.

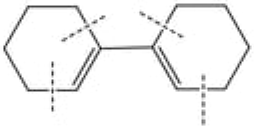


- **Solution –** The given compound is a homoannular diene which is having two alkyl substituents and two ring residues (as depicted by dotted lines).

- Therefore, Parent value for homoannular diene = 253 nm
- Two alkyl substituents 2×5 = 10 nm
- Two ring residues 2×5 = 10 nm
- Calculated Value = 273 nm

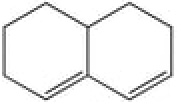
- Calculate the λ_{max} for the compound

- **Solution** - The given compound is a heteroannular diene. It is having 4 ring residues as shown below-:

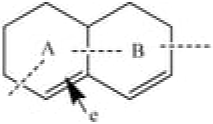


- Parent Value of Heteroannular diene = 215 nm
- Four Ring Residues 4×5 = 20 nm
- Calculated Value = 235 nm

- Calculate the λ_{max} for the compound



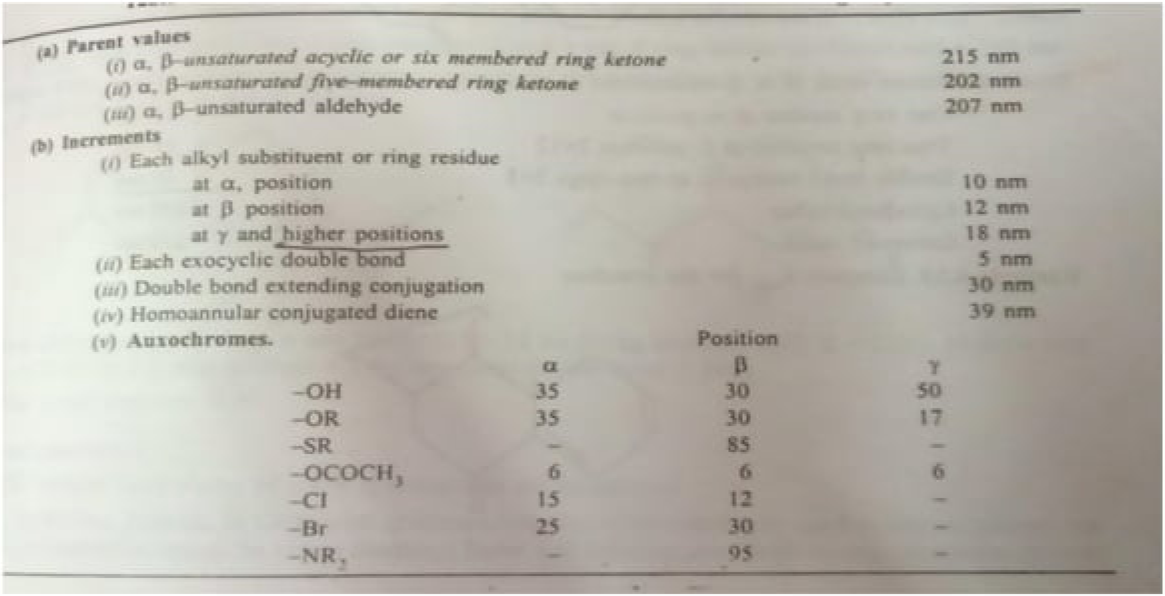
- **Solution -** According to the given structure, it is a heteroannular diene possessing three ring residues and one exocylic double bond marked e (This double bond is exocyclic with respect to ring B).



- Parent Value for heteroannular diene = 215 nm
- Three ring residues 3×5 = 15 nm
- One exocyclic double bond = 5 nm
- Calculated Value = 235 nm

Woodward – Feiser Rules for α,β-unsaturated carbonyl compounds

- Woodward – Feiser Rules for calculating λ_{max} for α,β- unsaturated carbonyl compounds modified by Scott may be summed up in the figure given below -



(a) Parent values

(i) α, β–*unsaturated acyclic or six membered ring ketone*	215 nm
(ii) α, β–*unsaturated five–membered ring ketone*	202 nm
(iii) α, β–unsaturated aldehyde	207 nm

(b) Increments

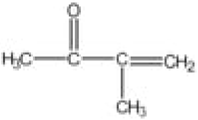
(i) Each alkyl substituent or ring residue	
at α, position	10 nm
at β position	12 nm
at γ and higher positions	18 nm
(ii) Each exocyclic double bond	5 nm
(iii) Double bond extending conjugation	30 nm
(iv) Homoannular conjugated diene	39 nm

(v) Auxochromes.

	Position		
	α	β	γ
$-OH$	35	30	50
$-OR$	35	30	17
$-SR$	–	85	–
$-OCOCH_3$	6	6	6
$-Cl$	15	12	–
$-Br$	25	30	–
$-NR_2$	–	95	–

Examples

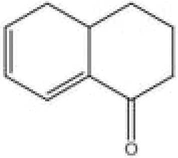
- Calculate the λ_{max} for the compound



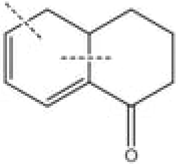
- Solution – The given compound is an **α,β-unsaturated acyclic ketone which is having alkyl substituent to α position**

- **Therefore** Parent Value for **α,β-unsaturated acyclic ketone = 215 nm**
- **One alkyl substituent in α position = 10 nm**
- **Calculated Value = 225 nm**

- Calculate the λ_{max} for the compound

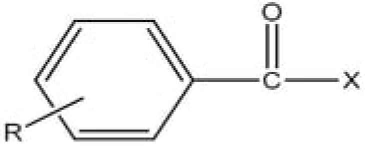


- **Solution** - The given structure represents an **α,β- unsaturated six membered ring ketone having a ring residue at α position and another ring residue at δ position.** It is having an exocyclic double bond, a double bond extending conjugation and a homoannular diene.



- Parent Value = 215 nm
- One α ring residue = 10 nm
- One δ ring residue = 18 nm
- One exocyclic double bond = 5 nm
- One double bond extending Conjugation = 5 nm
- One Homoannular Conjugated Diene = 30 nm
- Calculated Value = 317 nm

Woodward-Feiser Rules for Calculating absorption maximum for benzoyl derivatives



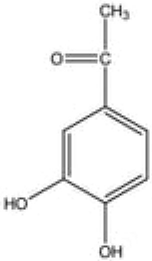
- The base value for the above system is **246 nm**, when X is an alkyl group.
- The base value for above system will be **250 nm**, if X is a hydrogen or halogen atom (aromatic acid chloride, aromatic aldehydes)
- The base value become **230 nm**, if X is OH or OR (Aromatic Carboxylic acids/esters)

- The incremental contribution of substituents to be added to any of above mentioned base value are illustrated in table given below-

S.No	Substituent R	Increment in nm (Position of the Substituent)		
		Ortho	Meta	Para
1.	Alkyl	+3	+3	+10
2.	OH, OR	+7	+7	+25
3.	Cl	0	0	+10
4.	Br	+2	+2	+15
5.	NH_2	+13	+13	+58
6.	NR_2	+20	+20	+85
7.	NHAc	+20	+20	+45

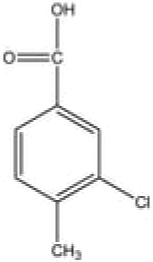
Examples

- Calculate the λ_{max} for the following compound



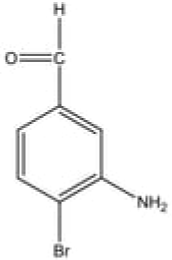
- Base Value of Parent Compound = 246 nm
- OH substituent at meta position = 7 nm
- OH substituent at para position = 25 nm
- Calculated Value = 278 nm

- Calculate the λ_{max} for the following compound



- Base Value of Parent Compound = 230 nm
- Cl substituent at meta position = 0 nm
- CH_3 substituent at para position = 10 nm
- Calculated Value = 240 nm

- Calculate the λ_{max} for the following compound



- Base Value of Parent Compound = 250 nm
- Br substituent at para position = 15 nm
- NH_2 substituent at meta position = 13 nm
- Calculated Value = 278 nm

Instrumentation

- The various components of a UV spectrometer are as follows -:

1. **Radiation Source –** In ultraviolet spectrometers the most commonly used radiation sources are **hydrogen or deuterium lamps, the xenon discharge lamps and mercury arcs.**

- In all sources, excitation is done by passing electrons through a gas and these collisions between electrons and gas molecules result in electronic, vibrational and rotational excitation in the gas molecules.

Note:

H OR D; also known as heavy hydrogen nucleus of deutrium atoms called as deutron contains one proton and one neutron.

Xenon-Colouless, dense, odourless noble gas of atomic number 54.

Tungsten-Hard Steel-Grey Metal

- When the pressure of the gas is low, only line spectra are omitted. But, if the pressure of gas is high, band spectra and continuous spectra will be obtained.
- The following are the requirements of a radiation source-:
  - It must be stable.
  - It must be of sufficient intensity for the transmitted energy to be detected at the end of optical path.
  - It must supply continuous radiation over the entire wavelength region in which it is used.

I. **Tungsten Lamp –** The tungsten lamp is similar in functioning to an electric light bulb. It is a tungsten filament heated electrically to white heat. These lamps are generally stable, robust and easy to use.

II. **Hydrogen discharge lamp** – In these lamps, hydrogen gas is stored under relatively high pressure. When an electric discharge is passed through the lamp, excited hydrogen molecules will be produced which emit UV radiations. The high pressure in the hydrogen lamps causes the hydrogen to emit a continuum rather than a simple hydrogen spectrum. Hydrogen lamps cover the range 1200 to 3500 Å. These lamps are stable, robust and widely used.

Note:

White Heat- The temperature or state of something that is so hot that it emits white light.
Continuum- A continuous sequence
Robust - Strong

III. **Deuterium Lamps-** If deuterium is used in place of hydrogen, the intensity of radiation emitted is 3 to 5 times the intensity of a hydrogen lamp of comparable design.

IV. **Xenon Discharge Lamp** – In these lamps, xenon gas is stored under pressure in the range of 10-30 atmospheres. The xenon lamp possesses two tungsten electrodes separated by about 8 mm. When an intense arc is formed between two tungsten electrodes by applying a low voltage, the ultraviolet light is produced. The intensity of ultraviolet radiation produced by xenon discharge lamp is much greater than that of hydrogen lamp.

V. **Mercury Arc** – In this, mercury vapour is under high pressure, and the excitation of mercury atoms is done by electric discharge.

Note:
Xenon-Colorless, dense, odorless noble gas of atomic number 54.
Tungsten-Hard Steel-Grey Metal
Electric arcs – Electrical breakdown of a gas that produces a prolonged electrical discharge.

2. Monochromators –

- The Monochromator is used to disperse the radiation according to the wavelength. The essential elements of a monochromator are an entrance slit, a dispersing element and an exit slit.
- The entrance slit sharply defines the incoming beam of **heterochromatic radiation.**
- The dispersing element disperses the heterochromatic radiation into its component wavelengths whereas exit slit isolates the desired spectra bands.
- The dispersing element may be a **prism or grating.** The prisms are generally made of **glass, quartz or fused silica.**

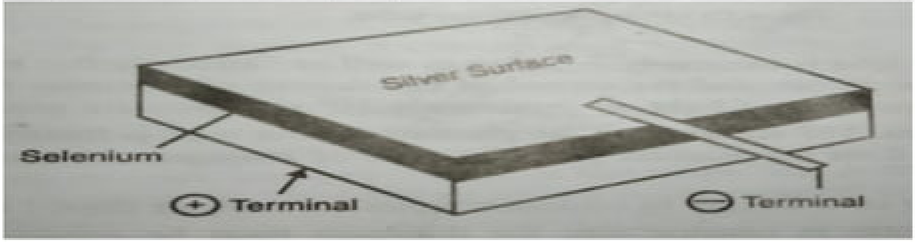
Note:
Heterochromatic radiation- Consisting of or concerned with different frequencies or wavelengths.

- Glass has the highest resolving power but it is not transparent to radiations having the wavelength between **2000 and 3000 Å** because glass absorbs strongly in this region.
- **Quartz and fused silica prisms** which are transparent throughout the entire UV range are widely used in **UV spectrophotometers.**
- **A grating** is any regularly spaced collection of essentially identical, parallel, elongated elements which **diffracts (bending of light)** the component of beam of incident light resulting in separation of various components.

Resolving Power – The ability of an optical instrument to distinguish small or closely adjacent images.

3. **Detectors –** There are **three common types of detectors** which are widely used In UV Spectrophotometer. These are as follows-:

I. **Barrier Layer Cell –** This cell is also known as **Photovoltaic Cell.** A typical barrier cell is shown in figure given below-



Barrier Layer Cell

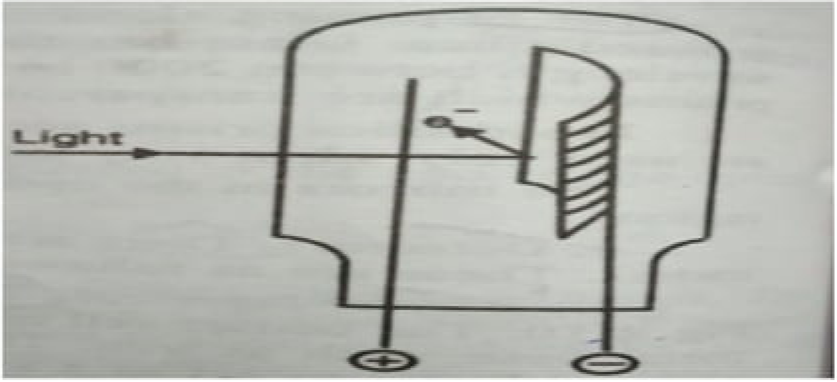
- The barrier cell consists of a semiconductor, **such as selenium**, which is deposited on a strong metal base, **such as iron.** Then a very thin layer of **silver or gold** is sputtered over the surface of the semiconductor to act as a **second collector electrode.**
- The radiation falling on the surface produces electrons at the **selenium-silver interface.** A barrier exists between the selenium and iron which prevents the electrons from flowing into iron. The electrons are therefore accumulated on the silver surface.

- The accumulation of electrons on the silver surface produces an **electrical voltage difference** between the silver surface and the base of cell.
- If the external circuit has a low resistance, a photocurrent will flow which is directly proportional to the intensity of incident radiation beam.
- **Photovoltaic Cell** is simple in design. It does not require any external power supply. However it can be hooked directly to a micrometer or galvanometer to read its output.

Note:
Galvanometer, instrument for measuring a small electrical current.

II. **Photocell** – It consists of high sensitive cathode in the form of a half cylinder of metal which is contained in an evacuated tube. The anode is also present in the tube which is fixed more or less along the axis of the tube.

The inside surface of a photocell is coated with a light sensitive layer as shown in figure given below-:



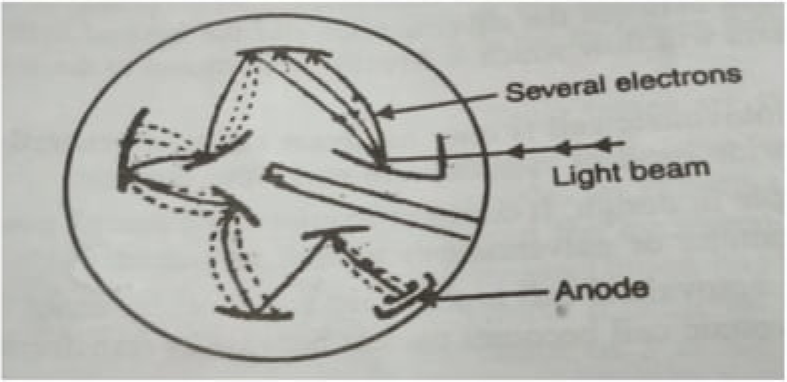
Photocell

When the light is incident upon a photocell, the surface coating emits electrons. These are attracted and collected by an anode. The current, which is created between the cathode and anode is regarded as a measure of radiation falling on the detector.

Note:

Cathode Material – Cesium Oxide, Potassium Oxide, Silver Oxide

iii. **Photomultiplier Tube** – A photomultiplier tube is generally used as a detector in UV spectrophotometers. A typical photomultiplier is shown in figure given below-:



Photomultiplier Tube

- A photomultiplier tube is a **combination of a photodiode** and an **electron-multiplying amplifier.** A photomultiplier tube consists of an **evacuated tube** which contains one photo-cathode and 9-16 electrodes known as dynodes.

- When radiation falls on a metal surface of the **photocathode** it emits electrons. The electrons are attracted towards the **first dynode**.
- When the electrons strike the first dynode, more electrons are emitted by the surface of dynode, these emitted electrons are then attracted by a second dynode where similar type of electron emission takes place.
- The process is repeated over all the dynodes present in the **photomultiplier tube** until a shower of electrons reaches a **collector.**
- The number of electrons reaching the collector is a **measure of intensity of light falling on the detector.**
- The dynodes are operated at an optimum voltage that gives a steady signal.
- A photomultiplier tube is extremely sensitive as well as extremely fast in response.

4. **Recording System –** The signal from the photomultiplier tube is finally received by the **recording system.** The recording is done by recording pen. The type of arrangement is only done in **recording UV spectrophotometers.**
5. **Sample Cells –** The cells that contain samples for analysis should fulfill three main conditions -:
   - They must be **uniform in construction**, the thickness must be **constant** and surfaces facing the incident light must be **optically flat.**
   - The material of construction should be **inert to solvents.**
   - They must transmit light of the wavelength used.

   The most commonly cells are made of **Quartz or fused silica.** These are readily available even in **matched pairs where sample cell is almost identical to the reference cell.**

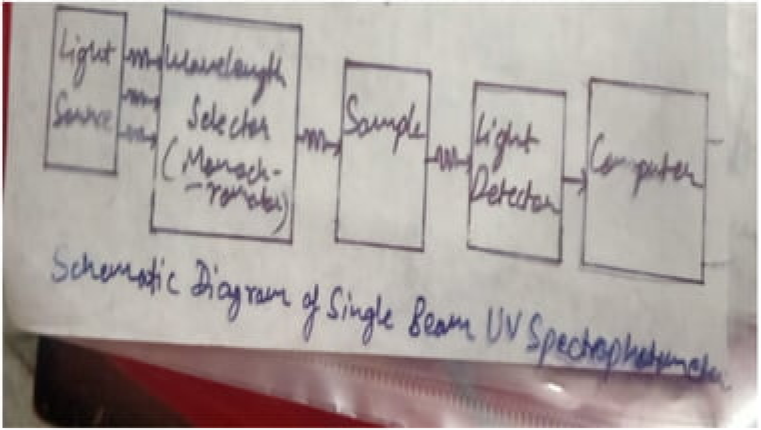
6. **Matched Cells -** When double – beam instrumentation is used, two cells are needed **one for the reference** and **one of the sample.** For most accurate work, matched cells are used. These are cell in which the absorption of each one is equal to or very nearly equal to the absorption of the other. A large number of these cells are manufactured at one time and there respective **absorptivities** measured . Those with very **similar absorptivities** are put together and designated as **matched cells.**
7. **Power Supply-** The power serves following function - :
   - It decrease the line voltage to the instruments operating level with a transformers.
   - It converts A.C. to D.C. with a rectifier if direct current is required by the instrument.

Note:
Absorptivity – Measure of how strongly a species absorb light at a given wavelength.

Description of UV Spectrophotometer

- **Single Beam System –**
  - In the single beam system, UV radiation is given off by the source. A convex lens gathers the beam of radiation and focuses it on the inlet slit.
  - The inlet slit permits light form the source to pass, but blocks out stray radiations. The light then reaches the monochromator, which splits it up according to wavelength.
  - The exit slit is positioned to allow light of the required wavelength to pass through. Radiation at all other wavelengths is blocked out.

- **The selected radiation passes through the sample cell to the detector, which measures the intensity of the radiation reaching it.**
- 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 computer.



Schematic Diagram of Single Beam UV Spectrophotometer

- Advantages
  - Simple in Construction
  - Easy to use
  - Economical
- Disadvantages
  - Absorbance is affected by any fluctuation in the intensity of radiation source.
  - Continuous spectrum is not obtained.

- Double Beam System

- The basic layout of the double beam ultravoilet spectrophotometer is shown in figure given below-:
- The description of a double-beam ultraviolet spectrophotometer is as follows
  - The radiation from the source is allowed to pass via a mirror system to the monochromator unit . The function of monochromator is to allow a narrow range of wavelengths to pass through an exit slit.
  - The radiation coming out of the monochromator through the exit slit is received by the **rotating sector** which divided the beam **into two beams,** one passing through the **reference** and the other through the **sample cell.**
  - After passing though the **sample and reference cells**, the light beams are focused onto the **detector.**

- The output of the detector is connected to a phase sensitive amplifier.
- The phase senstive amplifier transmits the signal to the recorder.
- Thus the absorbance of the sample is recorded as a function of wavelength.

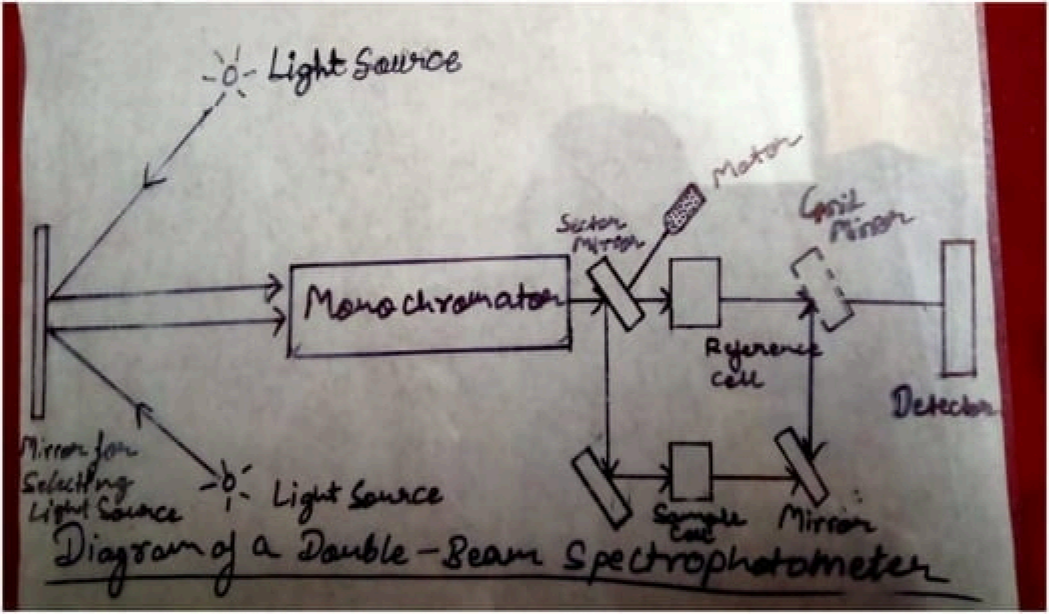


Diagram of a Double-Beam Spectrophotometer

Advantage of Double-Beam Instruments

- **Advantages**
  - It facilitates rapid scanning over wide wavelength region.
  - Possible fluctuations due to radiation sources are minimized.
- **Disadvantages**
  - Instrument is more expensive than single beam instruments. This is not a major issue considering potential applicability of the method to routine analysis.

Applications of Ultraviolet Spectroscopy

- **Detection of Impurities**
  - UV absorption spectroscopy is a useful method for **detection of impurities in organic molecules.** The absorbance scan can be compared with that of the standard raw material and any additional peaks observed may be attributable to the presence of **various impurities in the sample**.

Example

- Presence of Benzene impurity in cyclohexane can be conveniently detected by absorption band at 255 nm as pure cyclohexane will not absorb in this region.

Cyclohexane – 210 nm

- **Structure Elucidation of Organic Compounds**
- UV spectroscopy is useful in structure elucidation of organic compounds, presence or absence of unsaturation and presence of heteroatoms.
  - Effect of Conjugation
  - Extended conjugation causes a shift in absorption maximum (λ_{max}) towards longer wavelength (Bathochromic Shift).
  - On the other hand saturation of double bonds or reduction of compound gives opposite effect.

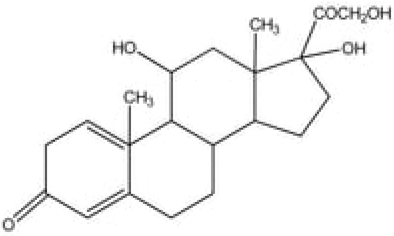
S.NO.	Substance	Number of Double Bonds	λ_{max} (nm)
1	Ethylene	1	174
2	1,3-Butadiene	2	217
3	1,3,5-Hexatriene	3	267
4	Vitamin A_1	5	326
5	Vitamin A_2	6	351

- **Effect of Geometrical Isomerism**
  - Geometrical isomerism has a significant effect on UV absorption (example Cis and Trans isomerism).
  - The trans isomers generally absorb at longer wavelength compared to Cis isomer. For example Calceferol (Cis isomer) has λ_{max} of 265 nm whereas Vitamin D_2 (Trans isomer) has a λ_{max} of 287 nm.

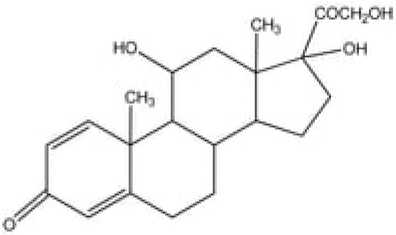
- **Effect of Number of Rings**
  - Fusion of two or more benzene rings result in bathochromic shift. For example Benzene has a absorption maximum at 255 nm , Naphthalene having two rings has absorption maximum at 312 nm and Anthracene having three rings has absorption maximum at 375 nm.
- **Effect of Substituents**
  - As the number of alkyl substituents in the compound increases it leads to bathochromic shift. For example, Crotonaldehyde (having one alkyl substitution) gives absorption at 217 nm and α-ionine (having two alkyl substitution) gives absorption at 228 nm.

- **Qualitative Analysis**
  - UV absorption spectroscopy characterize all those compounds which are capable of absorbing UV radiation. It is useful for the detection of certain chromophoric functional groups.
  - Carbonyl groups show a weak absorption band at 280-290 nm.
  - Bathochromic shift is observed from 326 nm in Vitamin A_1 and 351 nm in vitamin A_2.

- Intense UV absorption of many ketosteroids is entirely due to the presence of conjugated enone system. Thus λ_{max} is same for all the compounds which possess any extended conjugation.



Prednisolone
λ_{max} =241 nm



Prednisone
λ_{max} =241 nm

- **Quantitative Analysis**

  - Compounds which absorb UV radiation can be quantitavely determined by UV absorption spectroscopy based on their **intensity of absorption at a selected wavelength.**

  - Qualitative analysis establishes the **chemical identity of the species** in the sample whereas quantitative analysis **determines or indicates the amount of each substance or analyte in a sample.**

- **Direct Comparison Method**
  - This method involves comparison of absorbance value of the sample with those of a known concentration of the standard substance.
  - If A_{std} and A_{sample} are the absorbances of the standard and the sample solutions, C_1 and C_2 represent there respective concentrations and b is the path length, then, the concentration of the unknown (i.e., C_2) can be calculated as:
  - $A_{std} = \varepsilon C_1 b$ Eq1
  - $A_{sample} = \varepsilon C_2 b$ Eq2
  - On dividing the two equations, we get
  - $A_{std} / A_{sample} = \varepsilon C_1 b / \varepsilon C_2 b$
  - $A_{std} / A_{sample} = C_1/C_2$
  - Since $C_{1.}$ A_{std} and A_{sample} are known, C_2 can be calculated.

For example: Estimation of Diclofenac sodium

- Sample absorbance(A_{sample})= 0.329
- Standard absorbance($A_{standard}$)= 0.413
- Concentration of Standard (C_1)= 10 µg/ml
- Concentration of Sample (C_2) = ?
- So, $C_2 = C_1 \times A_{sample} / A_{standard}$
- $C_2 = 10 \times 0.329/0.413 = 7.96$ µg/ml

- **Indirect Methods**
  - These may be applicable to the substances which are themselves **non-absorbing in UV or visible regions but can be reacted selectively with certain reagents to yield products which are strong chromophores.**
  - Organic reagents include *o*- phenanthroline for determination of iron, *N,N*-dimethylgloxime for determination of nickel, diethyldithiocarbamate for copper determination and diphenyldithiocarbazone for estimation of lead.

THANK YOU