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CHEMISTRY	PAPER No. 12: ORGANIC SPECTROSCOPY
	MODULE No. 2: Nature of electronic transitions and factors affecting it

1. Learning Outcomes

After studying this module, you will learn about

- Woodward Fieser rule and its Applications
- Calculation of wavelength for absorption maximum of different functionalities
- Application of UV-Vis spectroscopy

2. Introduction

As we know that the presence of auxochrome or extension of chromophore may cause the change in wavelength and the intensity of the absorption maximum. Woodward and Fieser formulated a set of empirical rules, which could be used to predict absorption maxima of conjugated systems like dienes, enones, aromatic systems (bezene and its derivatives), benzoyl compounds etc.

3. Woodward-Fieser Rule

In 1945 Robert Burns Woodward gave certain rules for correlating λ_{max} with molecular structure. In 1959 Louis Frederick Fieser modified these rules with more experimental data, and the modified rule is known as Woodward-Feiser Rules. It is used to calculate the position and λ_{max} for a given structure by relating the type of chromophores present, the substituents on the chromophores, and shifts due to the solvent.

According to Woodward's rules the λ_{max} of the molecule can be calculated using a formula:

$$\lambda_{max} = \textit{Base value} + \Sigma \textit{ Substituent Contributions} + \Sigma \textit{ Other Contributions}$$

4. Woodward-Fieser Rule for conjugated dienes or polyenes

Each type of diene or triene system is having a certain fixed value of λ_{max} which is known as the **Base value or Parent value**. The base value depends upon whether the diene is a linear or heteroannular or transoid diene, or whether it is a cyclic or homoannular diene. Substituents such as alkyl substituents or ring residue, double bond extending conjugation and polar groups such as –Cl, -Br etc also contribute some numeral values which are added to the base value to obtain λ_{max} for a particular compound. A diene can be acyclic diene, homoannular conjugated diene, heteroannular diene.

1. Acyclic dienes

An acyclic diene exists mostly in s-trans (transoid) conformation as shown in the case of butadiene. As acyclic diene can rotate about their single bond to give either cisoid or transoid, therefore they exist in trans-form due to the high stability of the later. When this diene becomes a part of a ring system as in 1,3 cyclohexadiene, it is forced into a cisoid configuration. The wavelength of absorption is also shifted to longer wavelength and intensity is lowered in

comparison with acyclic dienes. The absorption maxima for 1,3 cyclohexadiene is 256 nm, whereas 1,3-butadiene shows λ_{max} at 217 nm.

S-trans (97.5%)
λ_{max} = 217 nm

S-cis (2.5%)

1,3-Cyclohexadiene
S-cis
λ_{max} = 256 nm
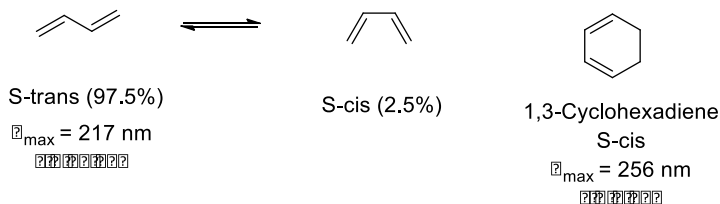
2. Cyclic dienes: Cyclic dienes can be classified into two classes depending upon whether the double bonds are in the same ring or in different rings.

a) Homo-annular conjugated dienes: Both conjugated double bonds are in the same ring and are having s-cis (cisoid) configuration. The s-cis configuration causes strain which raises the energy of ground state, while the energy of the excited state is relatively unchanged. Thus the transition energy is lowered resulting absorption at longer wavelength.

b) Hetero-annular dienes: Conjugated double bonds are present in different rings and have S-trans configuration.

The homo-annular diene resembles with 1,3 cyclohexadiene, whereas hetero-annular diene with butadiene.
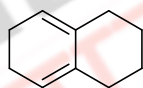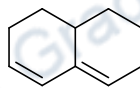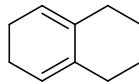

Homoannular diene
S-cis or Cisoid
λ_{max} = 273 nm

Heteroannular diene
S-trans or Transoid
λ_{max} = 234 nm

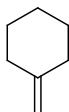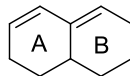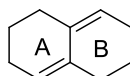**3. Endocyclic double bond:** Double bond/s is present inside the ring.

4. Exocyclic double bond: Double bond/s projecting outside the ring is called as exocyclic double bonds.
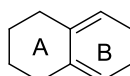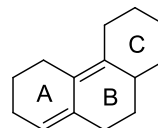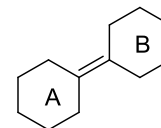

I   II   III   IV   V   VI

In example II, the double bond present within ring B is exocyclic to ring A, whereas endocyclic to ring B.

In example III, double bond present in ring A is exocyclic to ring B, similarly double bond present in ring B is exocyclic to ring A.

In example IV, both double bonds are present within ring B making them endocyclic to ring B, while both the double bonds are exocyclic to ring A.

In example V, the double bond present in the ring B is exocyclic to ring A and ring C. Similarly the double bond present in the ring A is exocyclic to ring B.

In example VI, the double bond is exocyclic to two different rings at a time. In such cases, the contribution would be 2 times.

Special Note: Double bond which is common to two rings is considered endocyclic because at any given time the double bond will only belong to one ring.
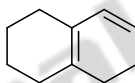
5. Empirical rules for dienes

Base value for homoannular (cisoid) diene = 253 nm

Base value for heteroannular (transoid) diene = 214 nm

Increment for

a) Alkyl substituent or Ring residue attached to the parent diene = 5 nm

b) Double bond extending conjugation = 30 nm

c) Exocyclic double bonds = 5 nm

d) Polar substituents

I) -OCOCH$_3$ = +0

II) -OR = +6

III) -SR = +30

IV) -Cl, -Br = +5

V) -NR$_2$ = +60
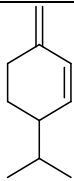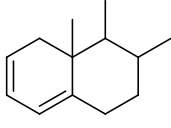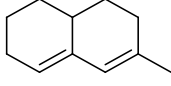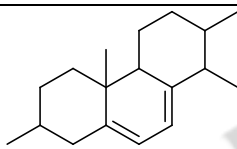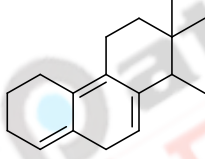
With the help of these rules the UV absorption maximum (λ_{max}) can be predicted for any alkene compound.

Important Note:

1. While calculating λ_{max} only those alkyl substituents or ring residues are counted which are directly attached to the carbon atoms making conjugated system within the compound.

2. If a molecule has both **homoannular diene and heteroannular diene** (Entry 5) then always use the **homoannular diene** as the **core chromophore**.

Let us take some examples to explain the calculation for λ_{max}

Entry	Structures	Calculated λ_{max}
1		Base value=214 2 Alkyl groups=2X5=10 1 exoyclic bond=1X5=5 Thus λ_{max} = 229 nm
2		Base value=253 (homoannular) 3 ring residues =3X5=15 1 exoyclic bond=1X5=5 Thus λ_{max} = 273 nm
3		Base value=214 (heteroannular) 4 ring residues =4X5=20 1 exoyclic bond=1X5=5 Thus λ_{max} = 239 nm
4		Base value=253 (homoannular) 4 ring residues =4X5=20 2 exoyclic bond=2X5=10 Thus λ_{max} = 283 nm
5		Base value = 253 (homoannular) 6 ring residues =6X5=30 2 exoyclic bond=2X5=10 1 double bond exocyclic to two rings= 2X5=10 1 double bond extending conjugation=1X30=30 Thus λ_{max} = 333 nm **Note:** Both homoannular as well as heteroannular systems are present, but we have to choose longer wavelength i.e. homoannular system

For polyenes with more than 4 conjugated double bonds, Fieser-Kuhn rule is used to calculate λ_{max}

Fieser-Kuhn derived the following equation to calculate the wavelength of maximum absorption λ_{max} and also maximum absorptivity ε_{max}

$$\lambda_{max} = 114 + 5x + y (48.0 - 1.7 y) - 16.5 R_{endo} - 10 R_{exo}$$

Where,
λ_{max} is the wavelength of maximum absorption
x is the number of alkyl substituents / ring residues in the conjugated system
y is the number of conjugated double bonds

R_{endo} is the number of rings with endocyclic double bonds in the conjugated system

R_{exo} is the number of rings with exocyclic double bonds in the conjugated system

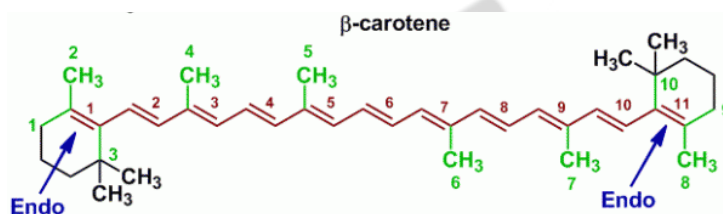$$\varepsilon_{max} = (1.74 \times 10^4)\, y$$

Where,

ε_{max} is the maximum absorptivity

y is the number of conjugated double bonds

We can use this equation to calculate wavelength of maximum absorbance (λ_{max}) and the maximum absorptivity (ε_{max}) for polyene such as β-carotene, Lycophene etc.

Let us take β-carotene as an example. The observed λ_{max} of β-carotene is 452 nm, while the observed ε_{max} is 15.2×10^4.

x (number of alkyl substituents) = 10

y (number of conjugated double bonds) = 11

R_{endo} (number of endocyclic double bonds) = 2

R_{exo} (number of exocyclic double bonds) = 0

Thus $\lambda_{max} = 114 + 5x + y\,(48.0 - 1.7\,y) - 16.5\,R_{endo} - 10\,R_{exo}$

$= 114 + 5(10) + 11\,(48.0 - 1.7(11)) - 16.5\,(2) - 10\,(0)$

$= 114 + 50 + 11\,(29.3) - 33 - 0$

$= 114 + 50 + 322.3 - 33$

$= 453.30$ nm

$\varepsilon_{max} = (1.74 \times 10^4)\, y$

$= (1.74 \times 10^4)\, 11$

$= 19.14 \times 10^4$

5. Woodward-Fieser Rule for α,β-unsaturated carbonyl compounds

Carbonyl compounds have two principal UV radiations, the allowed π to π^* transitions and the forbidden n to π^* transitions. In amides, acids, esters or acid halides, the substituents viz. NR_2, OH, OR, or –X on carbonyl group show pronounced hypsochromic effect on the n to π^* transitions. The hypsochromic effect is due to inductive effect of nitrogen, oxygen or halogen atoms. The heteroatom withdraws electrons from carbonyl carbon and makes carbonyl oxygen

lone pair of electrons more stabilized due to its involvement in increasing C=O bond order. As a result, the n to π^* transition of these compounds is shifted to 200-215 nm range relative to 270 nm in aldehydes and ketones. Conjugation of the carbonyl group with double bond shifts both n to π^* and π to π^* transitions to longer wavelengths. The effect on π to π^* band is more pronounced.

Woodward formulated rules to predict the position of an absorption maximum in an unknown enone. These rules have been summarized below

Base value for

a) Acyclic α, β unsaturated ketones = 215 nm

b) 6 membered cyclic α, β unsaturated ketones = 215 nm

c) 5 membered cyclic α, β unsaturated ketones = 202 nm

d) α, β unsaturated aldehydes = 210 nm

e) α, β unsaturated carboxylic acids and esters = 195 nm

Increment for

ii) Each double bond extending conjugation = 30 nm

iii) Exocyclic double bonds = 5 nm

iv) Homoannular diene component = 39 nm

Increment for substituents
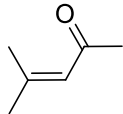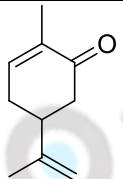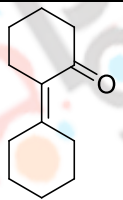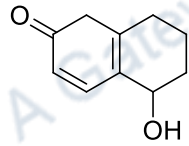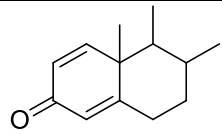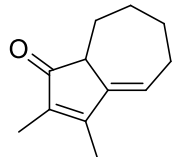
Substituents	α position	β position	γ position	δ position
Each alkyl substituent or Ring residue	10 nm	12 nm	18 nm	18 nm
–OH	35 nm	30 nm	50 nm	50 nm
–OAc	6 nm	6 nm	6 nm	6 nm
–OMe	35 nm	30 nm	17 nm	31 nm
–Cl	15 nm	12 nm	12 nm	12 nm
–Br	25 nm	30 nm	25 nm	25 nm
–SR	-	85 nm	-	-
–NR$_2$	-	95 nm	-	-
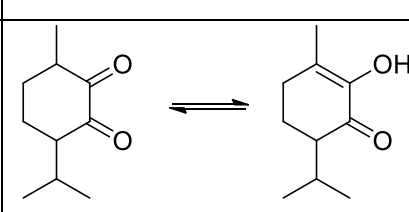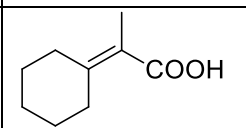
Since carbonyl compounds are polar in nature, the positions of absorption bands depend on the solvent used. Hence solvent corrections are required to calculate the λ_{max} values. All the above corrections are taken in ethanol as solvent. For calculating λ_{max} in other solvents, one must include solvent corrections.

Water	−8	
Methanol	0	
Chloroform	+1	
Dioxane	+5	
Ether	= +7	
Hexane	+11	

Note: In cross conjugated system, λ_{max} is calculated by using highly substituted conjugated system as parent system (Entry 5).

Let us see few typical examples to elaborate the above rules:

Entry	Structures	Calculated λ_{max}
1		Base value=215 2 β-alkyl substituents= 2X12=24 Calc. λ_{max} = 239
2		Base value=215 1 α-substituent=1X10=10 1 β-alkyl substituents=1X12=12 Calc. λ_{max} = 237
3		Base value=215 1 α-substituent=1X10=10 2 β-alkyl substituents= 2X12=24 1 double bond exocyclic to 2 rings=2X5=10 Calc. λ_{max} = 259
4		Base value=215 1 γ-substituent=1X18=18 2 δ-alkyl substituents= 2X18=36 1 double bond extending conjugation=1X30=30 1 homoannular diene system=1X30=39 Calc. λ_{max} = 338
5		Base value=215 2 β-ring residues=2X12=24 1 exocyclic double bond =1X5=5 Calc. λ_{max} = 244
6		Base value=202 1 α-substituent=1X10=10 1 β-alkyl substituents= 1X12=12 1 γ- ring residues =1X18=18 1 δ- ring residues = 1X18=18 1 double bond extending conjugation=1X30=30

7		1 exocyclic double bond =1X5=5 Calc. λ_{max} = 295 nm
7		Base value=215 1 β-alkyl substituents= 1X12=12 1 β-ring residues=1X12=12 1 α-OH substituent=1X35=35 Calc. λ_{max} = 274
8		Base value=195 1 α-substituent=1X10=10 2 β-ring residues=2X12=24 1 exocyclic double bond =1X5=5 Calc. λ_{max} = 234 nm

6. Woodward-Fieser Rule for benzene and its derivatives

Benzene is the classic example of a molecule with an extended π electron system. Electronic transitions in benzene could be complex due to closely spaced π orbitals. Three absorption bands are observed in benzene due to π-π* transitions. Two absorption bands at 184 (ε = 47,000) and 202 (ε = 7400) nm are called as primary bands and a band at 255 nm (ε = 200) is secondary (fine structure) band. The most intense peak at 184 nm is an allowed transition and is not observed under usual experimental conditions because the absorption at this wavelength lies in the vacuum ultraviolet region of the spectrum. In case of polycyclic aromatic systems, this band is often shifted to longer wavelength. The other two low intensity bands at 202 nm and 255 nm arise from forbidden transitions. The secondary band at 255 nm is also known as benzenoid band (B-band) and is usually easily identifiable. The B-band of benzene or its homologues has fine structure, when the spectrum is recorded in the vapour phase or in non-polar solvents. The fine structure is caused by the interaction of the electronic energy levels with vibrational modes. This fine structure is lost if the spectrum is determined in polar solvent or if a single functional group is attached with the benzene ring. In such cases, the secondary peak appears as broad peak.

Substitution on the benzene ring can cause bathochromic and hypsochromic shifts. Unlike dienes and unsaturated ketones, the effects of various substituents on the benzene ring are not predictable. However, qualitative understanding of the effects of substituents on the characteristics of UV-Vis spectrum can be considered by classifying the substituents into different classes described below.

(i) Effect of alkyl substituents: When benzene is substituted with alkyl groups, the absorptions bands are shifted slightly to longer wavelength and the fine structure remains intact. The bathochromic shift is attributed to the hyperconjugation effect of alkyl group. The introduction of a second alkyl group at the para position is most effective in causing bathochromic shift. The para isomer absorb at the longest wavelength whereas ortho isomer has the lowest wavelength. This

effect is due to the steric interaction between the ortho substituents, which effectively reduce hyperconjugation.

(ii) Effect of substituents with non-bonding electrons: The non-bonding electrons increase the length of π-system through resonance and can cause the shifting of the primary and secondary absorption bands to longer wavelength. The fine structure of the B-band is diminished. More is the availability of the non-bonding electrons greater will be the shifting of wavelength towards longer wavelength.
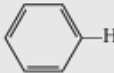
This effect can be easily seen if we compare the absorption spectrum of benzene and phenol. In going from benzene to phenol, the primary absorption band at 203.5 nm shifts to 210.5 nm and secondary absorption band at 254 nm shifts to 270 nm. Further, in negatively charged phenoxide ion, the primary band shifts from 203.5 to 235 nm and secondary band shifts from 254 nm to 287 nm. Both the bands also show hyperchromic effect (increase in intensity). In phenoxide ion there are more non-bonding electrons and they are more easily available for interaction with the aromatic π-system.

Similarly from benzene to aniline, the primary band shifts from 203.5 to 230 nm and the secondary band shifts from 254 to 280 nm. In case of anilinium cation, there are no non-bonding electrons for interaction and hence the absorption properties are quite close to benzene.

(iii) Effect of π-Conjugation: If an unsaturated group is directly attached to the benzene ring then because of π-π conjugation, a strong bathochromic shift is observed. The primary band at 203.5 nm shifts to longer wavelength more effectively than the secondary band at 254 nm. In some cases, the primary band overtakes the secondary band. For example, benzoic acid shows primary band at 250 nm and secondary band at 273 nm, but cinnamic acid that has longer chromophore exhibits primary band at 273 nm and secondary band remains merged with it. Similarly, in benzaldehyde, the primary band appears at 242 nm and secondary band at 282 nm but in case of cinnamaldehyde, primary band appears at 281 nm and remains merged with secondary band.

(iv) Effect of Electron-withdrawing and Electron-releasing Groups: The primary absorption band shifts to longer wavelength regardless the electronic effect of the substituents. But as far as secondary absorption band is concerned, electron-withdrawing substituents such as NH_3^+, SO_2NH_2, CN, COOH, $COCH_3$, CHO and NO_2 etc have no effect on the position of secondary absorption band unless the electron-withdrawing group is also capable of acting as a chromophore. However, electron-donating groups such as $-CH_3$, -Cl, -Br, -OH, $-OCH_3$, $-NH_2$ etc increase both the wavelength and intensity of the secondary absorption band. The table given below shows the effect of the both types of substituents, electron releasing as well as electron withdrawing.

ULTRAVIOLET MAXIMA FOR VARIOUS AROMATIC COMPOUNDS

Substituent	Primary		Secondary	
	λ (nm)	ε	λ (nm)	ε
(benzene ring)—H	203.5	7,400	254	204
—CH₃	206.5	7,000	261	225
—Cl	209.5	7,400	263.5	190
—Br	210	7,900	261	192
—OH	210.5	6,200	270	1,450
—OCH₃	217	6,400	269	1,480
—NH₂	230	8,600	280	1,430
—CN	224	13,000	271	1,000
—COOH	230	11,600	273	970
—COCH₃	245.5	9,800		
—CHO	249.5	11,400		
—NO₂	268.5	7,800		

Electron-releasing substituents: —CH₃, —Cl, —Br, —OH, —OCH₃, —NH₂
Electron-withdrawing substituents: —CN, —COOH, —COCH₃, —CHO, —NO₂

(v) Disubstituted benzene derivatives: In case of disubstituted benzene derivatives, it is essential to consider the effect of both the substituents.

a) Para-substituted benzenes: If both the groups are electron-donating or electron withdrawing then they exert similar effect as observed in mono-substituted benzenes. The group with stronger effect determines the extent of shifting of primary absorption band.

If one group is electron-donating and the other is electron-withdrawing then the magnitude of the shift of the primary band is greater than the sum of the shifts due to the individual groups. This is attributed to the increased electron shifting from electron-donating group to the electron-withdrawing group through π-bond of benzene ring. For example aniline shows secondary absorption band at 285 nm which is shifted to 367 nm with a significant increase in absorptivity in *p*-nitro aniline due to presence of electron-withdrawing *p*-nitro substituent.
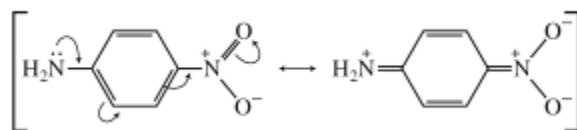
b) Ortho or meta-substituted benzenes
If two groups are placed at ortho- or meta-positions to each other, the magnitude of the observed shift is approximately equal to the sum of the shifts caused by the individual groups.

Like Woodward-Fieser rules, Scott formulated a set of rules for calculating the absorption maximum of the primary absorption band of aromatic aldehydes, ketones, carboxylic acids and esters. In the absence of steric hindrance to co-planarity, the calculated values are within $\pm$ 5 nm of the observed value.
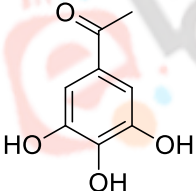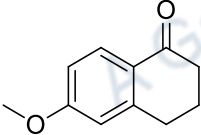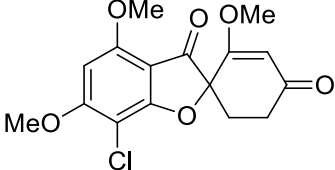
1) Base values for

a) ArCOR = 246 nm

b) ArCHO = 250 nm

c) $ArCO_2H$ = 230 nm

d) $ArCO_2R$ = 230 nm

2) Increment for substituents:

Substituents	ortho	meta	Para
Alkyl group or ring residue	3 nm	3 nm	10 nm
–OH, –OCH₃, –OAlkyl	7 nm	7 nm	25 nm
–O (oxonium)	11 nm	20 nm	78 nm
–Cl	0 nm	0 nm	10 nm
–Br	2 nm	2 nm	15 nm
–NH₂	13 nm	13 nm	58 nm
–NHCOCH₃	20 nm	20 nm	45 nm
–NHCH₃			73 nm
–N(CH₃)₂	20 nm	20 nm	85 nm

Let us take some examples showing the applicability of this rule

	Base value=246 2 m-OH=2X7=14 1 p-OH=1X25=25 Calc. λ_{max} = 285 nm
	Base value=246 Ring residue at ortho position =1X3=3 1 p-OCH₃=1X25=25 Calc. λ_{max} = 274 nm
	Base value=246 2 o-O Alkyl=2X7=14 1 m-Cl=1X0=0 1 p-OCH₃=1X25=25 Calc. λ_{max} = 285 nm

(vi) Heteroaromatic Compounds
In general, the UV-spectra of hetero aromatic compounds are similar to that of their corresponding hydrocarbons. Thus the absorption of five membered heteroaromatics are compared with that of cyclopentadiene which absorb near 200 nm ϵmax= 10,000 and near 238

nm, ϵ_{max}=3400. Pyrrole displays a comparable absorption at 211 nm ϵ_{max}=15,000 and 240 nm, ϵ_{max}=300. The absorption at 240 nm has fine structure as observed in the B-band of benzene. The presence of an auxochrome or chromophore substituents on the five membered ring causes a bathochromic shift and an increase in the intensity of the bands of the parent molecule.

Similarly, the spectrum of six membered aromatic heterocycle is comparable with that of benzene, but the B-band of pyridine is more intense and has less distinct fine structure. This transition (π to π*) resulting in the B-band is allowed in pyridine because of the lower symmetry introduced by nitrogen and hence is more intense than that in benzene. An increase in the solvent polarity does not affect either the position or intensity of B-band of benzene but produces a hyperchromic effect on the B-band of pyridine and its homologues.

(vii) Polycyclic Aromatic Compounds: In case of polycyclic aromatic hydrocarbons, due to extended conjugation, both primary and secondary bands are shifted to longer wavelength. These spectra are usually complicated but are characteristic of parent compound. The primary band at 184 nm in benzene shifts to 220 nm in case of naphthalene and 260 nm in case of anthracene. Similarly, the structured secondary band which appears as broad band around 255 nm in benzene is shifted to 270 nm and 340 nm respectively in case of naphthalene and anthracene molecules.

7. Applications of UV-Vis Spectroscopy

UV-visible spectroscopy is a technique that readily allows one to determine the concentrations of substances and therefore enables scientists to study the rates of reactions, and determine rate equations for reactions, from which a mechanism can be proposed. As such UV spectroscopy is used extensively in teaching, research and analytical laboratories for the quantitative analysis of all molecules that absorb ultraviolet and visible electromagnetic radiation.

1. Qualitative analysis: UV-Vis spectroscopy has been exploited for the structure elucidation of organic compounds, detection of different organic compounds present in a mixture and their separation by several analytical techniques such as thin layer chromatography.

a) Structure elucidation of organic compounds

UV spectroscopy is useful in the structure elucidation of organic compounds. The presence or absence of a particular absorption band at a particular wavelength may be regarded as an evidence for the presence or absence of a particular chromophore in the compound.

b) Determination of impurities: TLC is a very simple technique and is commonly used for the qualitative analysis of the reaction mixture. Many organic compounds absorb UV light of various wavelengths. This can be used for the determination of impurities in organic compounds. A small spot is kept on the TLC plate which is then allowed to run in appropriate solvent media. Different compounds travel different distance based on their polarity which can be visualized on the TLC plate under a UV lamp. Extra spots on TLC plate can be observed due to impurities in the sample and it can be compared with the spot of standard.

A concept of UV/Vis spectroscopy may be used as a detector for HPLC (High-performance liquid chromatography formerly referred to as high-pressure liquid chromatography). UV detectors are usually variable wavelength and can be used to detect the molecules with absorption maxima above 210 nm by measuring the absorbance of the eluent. It provides very fast detection and

separation of a reaction mixture. When a compound is eluted from the HPLC column, it absorbs UV radiation at the appropriate wavelength. The amount of UV radiation absorbed is directly proportional to the amount of a particular compound that is passing through the column at the time.

2. Quantitative analysis

a) Determination of concentration of the compound

UV absorption spectroscopy can be used for the quantitative determination of compounds which absorb UV radiation. This determination is based on Beer's law which relates the absorbance A of a substance at a particular wavelength to the concentration, c as follows.

$$A = \log_{10} I_0 / I = \log 1/ T = - \log T = \varepsilon bc$$

Where,

ε is extinction coefficient
c is concentration
b is the length of the cell that is used in UV spectrophotometer.
I_0 is the intensity of the incident radiation
I intensity of transmitted radiation

The use of UV-spectroscopy in quantitative analysis can be understood by a simple example of the estimation of a mixture of anthracene and naphthalene. The UV spectrum of ethanolic solution of anthracene shows λmax at 375 nm, whereas naphthalene does not absorb in this region. Thus from a mixture of anthracene-naphthalene, we can calculate the amount of anthracene by using Lambert Beer's law. Measure the absorption of the mixture at 375 nm wavelength and thus by using the equation A= εbc, we can calculate the concentration of pure anthracene and hence the proportion of anthracene in the mixture.

b) UV-Vis spectroscopy has been used extensively in industrial analytical organic chemistry. The percentage of Vit A1 and Vit A2 in natural fats or oils can be estimated by the measurement of the intensities of peaks at 325 nm and at 351 nm, respectively. Thus by comparing the intensities of the solution of these two vitamins with the intensities of the known solutions of these two enables the concentration to be calculated. A similar procedure may be used for the estimation of ergosterol in fats, anthracene in benzene, carbon disulphide in carbon tetrachloride, chlorophyll in plant material etc.

b) Chemical kinetics: Study of chemical reactions

Ultraviolet/visible spectroscopy can also be used to study reaction rates. Spectrometric rate determination involves the measurement of absorption of either the reactants or products at a fixed wavelength, when a reaction occurs. If a reactant or reagent or product of the reaction absorbs radiation at a particular wavelength, the spectrometer can be set to measure the absorption at that wavelength as a function of time and hence the rate of a reaction can be measured.

To explain this let us a take an example which shows the removal of proton from nitroethane with the help of hydroxide ion. Here only the anion of nitroethane absorbs in the UV region at 240 nm, while other reactants or products do not show any significant absorbance at this wavelength. In

order to measure the rate at which hydroxide ion removes a proton from nitroethane (i.e., the rate at which the nitroethane anion is formed), the UV spectrophotometer is adjusted to measure absorbance at 240 nm as a function of time. Nitroethane is taken in a cuvette containing a basic solution, and the rate of the reaction is determined by monitoring the increase in absorbance at 240 nm.
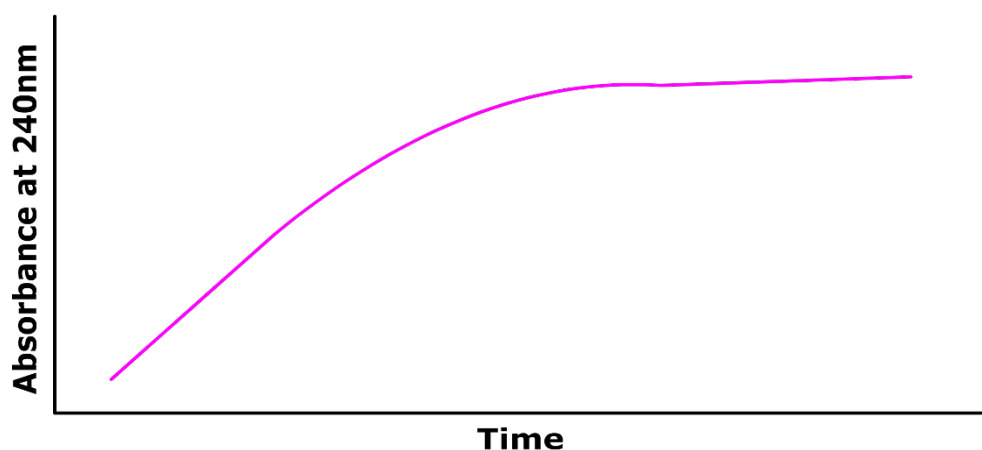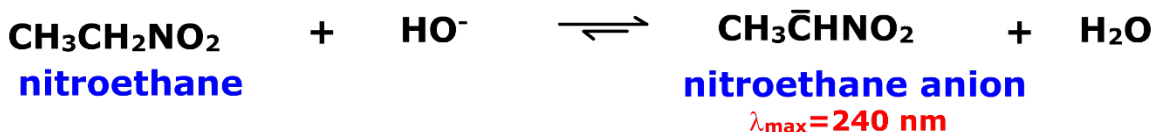
$$CH_3CH_2NO_2 \quad + \quad HO^- \quad \rightleftharpoons \quad CH_3\bar{C}HNO_2 \quad + \quad H_2O$$

nitroethane   **nitroethane anion**
$$\lambda_{max} = 240 \text{ nm}$$

Figure: The rate at which a proton is removed from nitroethane is determined by monitoring the increase in absorbance at 240 nm

The most widely used application of spectrometric rate measurements is for study of enzymes (proteins that are present in all living tissue). Enzymes cannot be measured directly but their catalytic properties allow their estimation from the speed of the reactions which they catalyse. Enzymes have many uses as reagents or as labels that can be attached to other molecules to permit their indirect detection and measurement. The widest use in the field of clinical diagnostics is as an indicator of tissue damage. When cells are damaged by disease, enzymes leak into the bloodstream and the amount present indicates the severity of the tissue damage. The relative proportions of different enzymes can be used to diagnose disease, say of the liver, pancreas or other organs which otherwise exhibit similar symptoms.

The most common reaction used in these clinical assays is the reduction of nicotinamide adenine dinucleotide (NAD) to $NADH_2$. The spectra of NAD and $NADH_2$ are shown in Figure given below. It is clear that if the absorbance is measured at 340 nm, the readings will increase as the reaction progresses towards the formation of $NADH_2$.
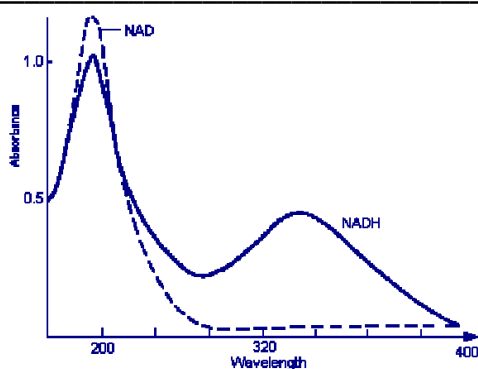
Figure 12 Absorption spectra of NAD and NADH

Similarly, the enzyme lactate dehydrogenase catalyzes the reduction of pyruvate by NADH to form lactate. Here, NADH is the only species in the reaction mixture which absorbs light at 340 nm hence by measuring the decrease in absorbance at 340 nm, the rate of reaction can be determined.
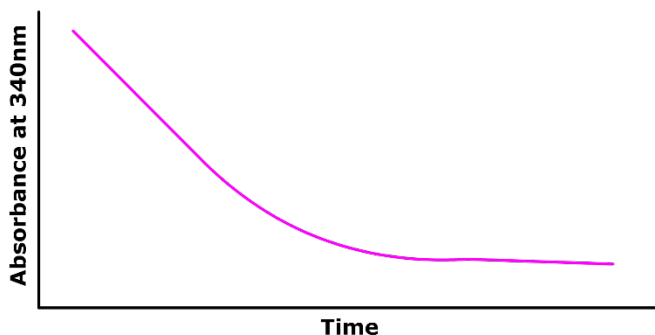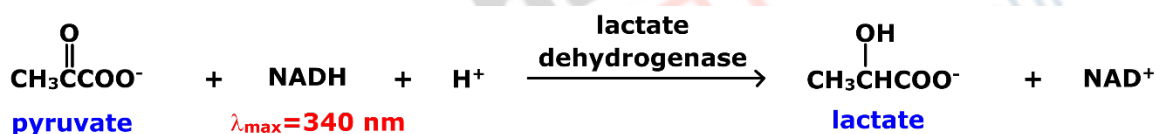

Figure: The rate of reduction of pyruvate by NADH is measured by monitoring the decrease in absorbance at 340 nm

c) Dissociation constants of acids and bases

The pKa of a compound can be determined by UV/Vis spectroscopy if either the acidic form or the basic form of the compound absorbs UV or visible light. For example, the phenoxide ion has a λ_{max} at 287 nm. If the absorbance at 287 nm is determined as a function of pH, the pKa of phenol can be calculated by using Henderson–Hasselbalch equation

PH = PKa + log [A⁻] / [HA]

By plotting graph between absorbance and wavelength at different PH values, the ratio of [A⁻] / [HA] can be determined and hence the pK_a value of the compounds can be calculated.

d) In the quantification and thermal denaturation of DNA

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UV spectroscopy can also be used to estimate the nucleotide composition of DNA. The two strands of DNA are held together by both A–T base pairs and G–C base pairs. When DNA is heated, the double stranded DNA breaks down. Single-stranded DNA has a greater molar absorptivity at 260 nm than does double-stranded DNA. The melting temperature (Tm) of DNA is the midpoint of an absorbance versus temperature curve. For double-stranded DNA, Tm increases with increasing numbers of G–C base pairs because they are held together by three hydrogen bonds, whereas A–T base pairs are held together by only two hydrogen bonds. And hence can be used to estimate the number of G–C base pairs.
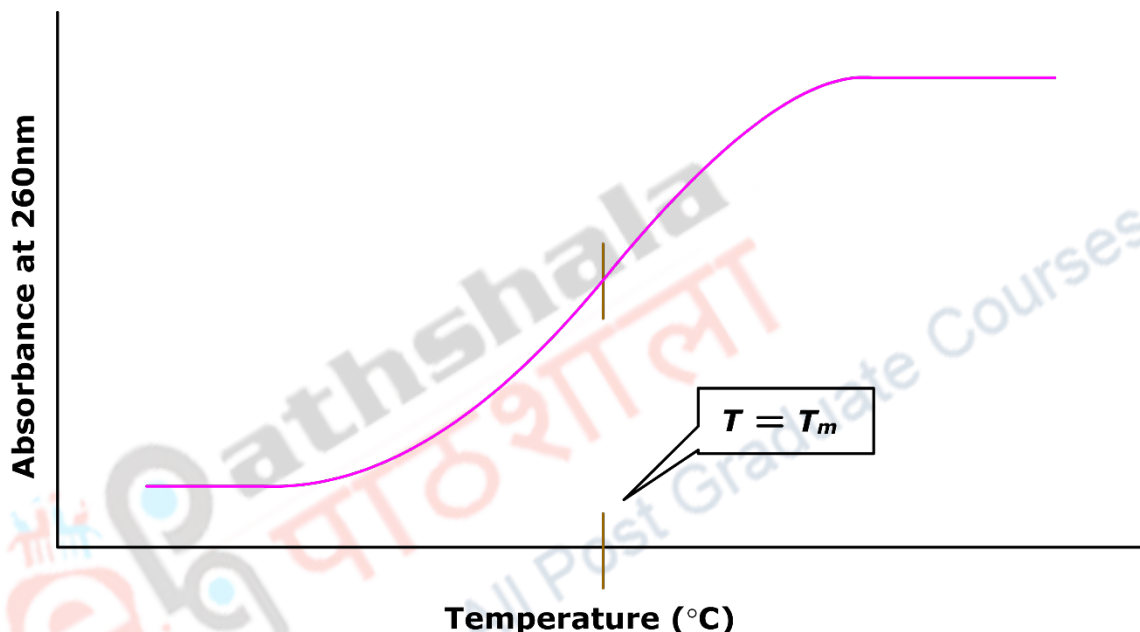

Figure: The absorbance of a solution of DNA as a function of temperature

3. Study of charge transfer complexes

The formation of charge-transfer complex occurs between molecules which, when mixed, allow the transfer of electronic charge through space from an electron rich molecule to an electron deficient molecule with molecular orbitals of suitable energy and symmetry.
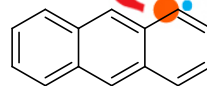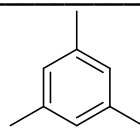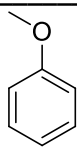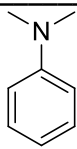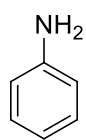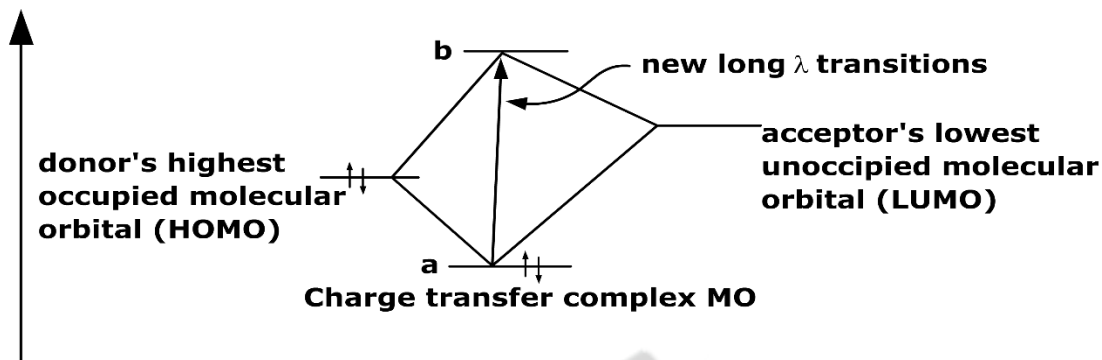
**Charge transfer acceptors**
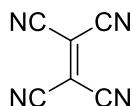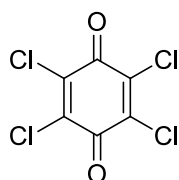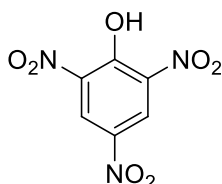
Charge transfer donors

The filled π-orbitals in the donor molecule overlap with the depleted orbital in the acceptor molecule and generate two new molecular orbitals. Thus transition between these newly formed orbitals are responsible for the new absorption bands observed in the charge transfer complexes. The two new molecular orbitals formed and the transitions between them are shown in the figure given below.

The brown colour of iodine in benzene or the appearance of deep blue colour when tetracyanoethylene is added to a chloroform solution of aniline may be explained due to the

Electronic transitions for charge-transfer complexes. Donor and acceptor orbitals combine to form two new orbitals (a and b) for the complex. New electronic transitions of long   are then possible between a and b.

formation of charge transfer complex. The structure of most charge transfer complexes can be visualized as a face to face association on a 1:1, donor: acceptor basis to provide maximum overlapping of π-orbitals of benzene ring.
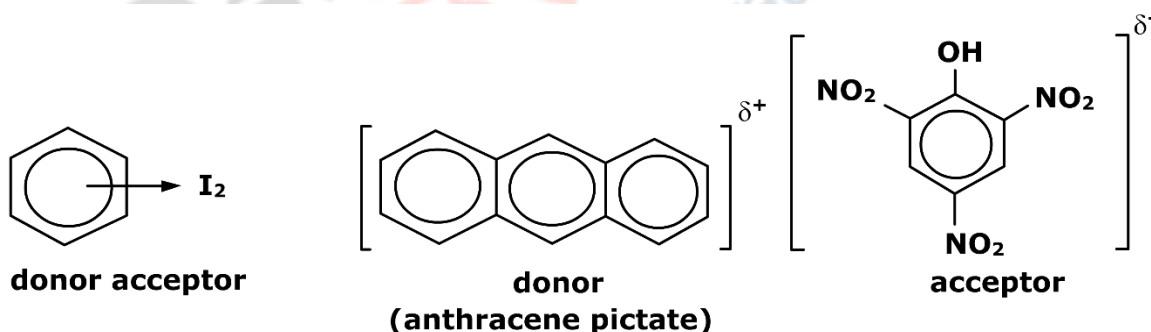
The λmax of benzene is 255 nm while for iodine in hexane is 500 nm. The charge transfer complex (benzene-iodine) displays an intense additional band at 290 nm. Similarly in the aniline-tetracyanoethylene complex, λmax for aniline and tetracyanoethylene are 280 nm and 300 nm, respectively, while the deep blue complex has λmax at 600 nm.

4. Other uses

UV-visible spectroscopy is also used in the quality control in the development and production of dyeing reagents, inks and paints and the analysis of intermediate dyeing reagents. In environmental and agricultural fields the quantification of organic materials and heavy metals in fresh water can be carried out using UV-visible spectroscopy. A special type of UV-watermark is kept on many sensitive documents such as credit cards, driving licenses, passports to prevent forgery. The watermark can only be seen in UV light. The optical whiteners absorb ultraviolet light and re-emit it in the visible range. This feature is used in washing powders. Optical whiteners are also added to many toothpastes and detergent powders.

8. Summary

1. In this module we have seen that the UV-vis spectroscopy have become the most important analytical tool in the modern day laboratory due to its simplicity, versatility, speed, accuracy and cost-effectiveness.

2. We came to know about the Woodward Fieser rule and its applicability in calculating the absorption maxima for several functional groups.

3. How the substituents affect the absorption pattern in benzene and other mono or di-substituted benzenes.

4. We have seen different applications of UV spectroscopy: qualitatively as well as quantitatively.