15

Ultraviolet Spectrophotometry

15.1 General

UV radiation is that portion of the electromagnetic spectrum (EMS) which bridges the gap between the longest wavelength X-rays and the shortest wavelength visible light as shown in the chart below (Fig. 15.1)

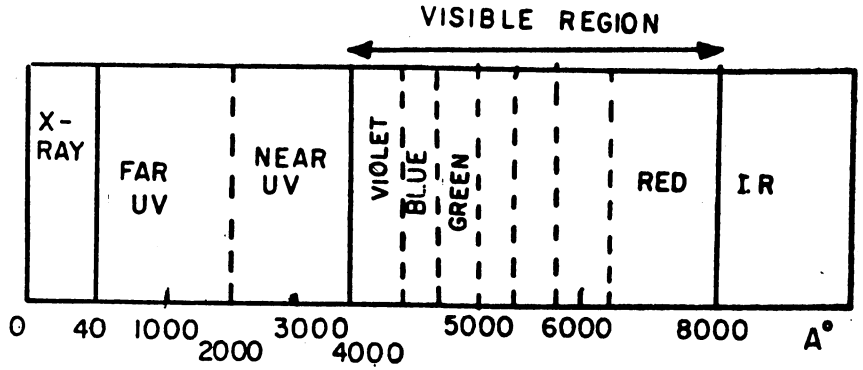


Fig. 15.1 UV region of EMS.

The UV region extends from 40 — 4000 A° and can be divided into *near* (2000 – 4000 A°) and *far* (40 – 2000 A°) regions. The far UV radiation is absorbed by air and also produces electronic transitions in atmospheric O_2, N_2 and CO_2. Therefore one must use vacuum apparatus to study far UV radiation. It is called the Vacuum UV. Further, glass absorbs radiation of wavelength less than 3000 A°. The instruments to study far UV must use quartz optics and quartz sample holders. Therefore, the region between 2000 – 3000 A° is called *quartz* region.

It takes radiation of higher energy to effect electronic transitions than to effect rotational and vibrational transitions. Electronic states in a molecule are thus always associated with a large number of

vibrational and rotational states leading to a broad UV peak (Fig. 15.2). The electronic levels E_1 and E_2 of two electronic states (Fig. 15.2) are shown to be composed of a series of vibrational states V_o, V_1 V_2 etc and corresponding series of rotational states r_0, r_1, r_2 etc.

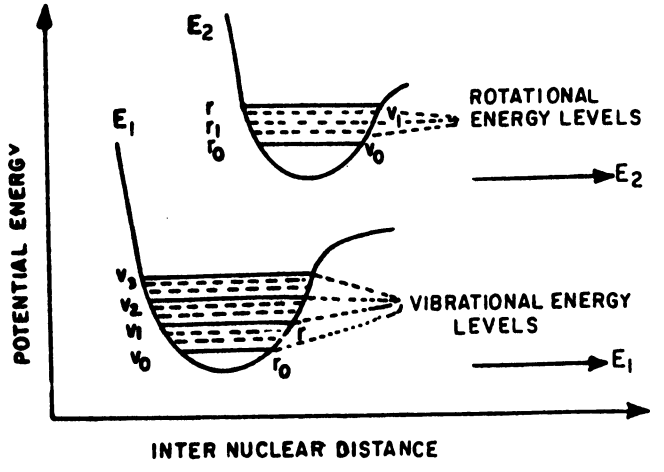


Fig. 15.2 Potential energy diagram.

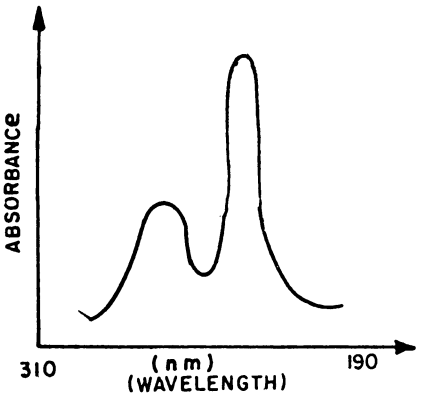


Fig. 15.3 UV absorption Spectrum.

The energy associated with each photon in UV radiation is powerful in the range of 6.62×10^{-12} ergs whereas in case of microwaves energy per photon is 6.62×10^{-18} erg and can bring about only rotational transitions.

UV spectrum is a plot of *absorption intensity* (ordinate) VS wavelength in A° or nm (abscissa). Absorption intensity is plotted as ε or log ε and reported as ε_{max} meaning *maximum* molar extinction coefficient. The wavelength of absorption is reported as λ_{max} meaning maximum absorption.

The quantitative aspects of absorption spectrophotometry has already been dealth with in Chapter 14 and the same are applicable to the quantitive UV.

In case of an unknown substance where molecular weight (M) and hence molar concentration can be determined, the absorption intensity of 1 % solution of the substance is determined in a 1 cm cell. This is reported as $E|_{\%}^{m}$ where E is the numerical value of the absorbance in a 1 unit cell of solution containing unit volume solution. E is called specific extinction coefficient, its relation to ε is

$$E|_{\%}^{m} = \frac{10 \times \varepsilon}{\text{molecular weight}}$$

or $\varepsilon = E|_{\%}^{m} \times 0.1M$

15.2 Instrumentation

As already stated in the previous chapter sophisticated types of spectrophotometers for studies in UV and visible are commercially available alongwith operational details. There main components are : (i) a source of radiation (ii) a monochromator (iii) a dectector and (iv) a read out system. The properties and other requirement as well as design aspects have already been described . A schematic optical diagram of a double beam UV - Visible spectrophotometer is shown below (Fig. 15.4)

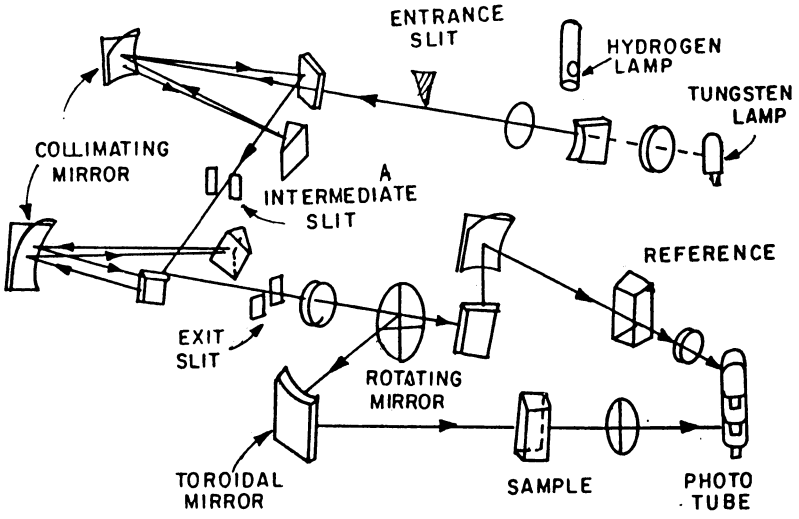


Fig. 15.4 Schematic optical diagram of double beam-in-time spectrophotometer.

In the operation of a spectrophotometer called double beam in time, a single beam of energy leaves the prism or grating monochromator and is alternately switched between the reference and sample paths to provide a double beam system within the sample compartment. The two beams are then recombined to fall on a single detector. The output of the detector is an alternating signal whose amplitude is proportional to the difference in intensities in the two channels. A slit servo continuously adjusts the slit opening of the monochromator to keep the energy through the reference cell constant at a 100% setting when the reference solution is in the light beam. A rotating half-sector mirror system is used to switch the reference and the sample paths. It is placed either before the entrance slit or after the exit slit of the monochromator.

In case of automatic recording spectrophotometers the measuring sequence is carried out automatically to provide the recorded data of the analysis. The sample - beam energy is compared automatically with reference beam energy and the ratio is the transmittance of the sample. Thus time consuming adjustments are eliminated and fast and accurate spectrograms are obtained.

15.3 Selection of Solvent

Pure water and ethanol shift the position and tend to broaden the bands. Highly pure non-polar solvents e.g. saturated hydrocarbons do not affect absorption bands. The solvents should have the ability to transmit radiation in the region of wavelength under study. They should not absorb in the same region as the solute.

The concentration of solutions should be in the range of $10^{-2} - 10^{-5}$ moles. The quantity of substance available for analysis should be in the range of 0.1 to 100 mg. The % transmittance should be between 20-65%, at high concentration the transmittance is low.

15.4 Absorption by Organic Molecules

It has been recognized that many substances appear coloured because they contain functional groups which are capable of absorbing radiation of certain wavelengths when ordinary light shines on them. Such functional groups which confer colour on substances are known as *chromophores*. They generally contain

unsaturated bonds like C = C, C = O, N = N etc. The functional groups like — OH – NH_2, – Cl, – Br etc do not confer colour but have the ability to increase the colouring power of a chromophore and are called *auxochromes*. In other words the auxochromes do not absorb radiation longer than 200 nm, but when attached to a chromophore cause a shift in the absorption to a longer wavelength and also increase the degree of absorption; e.g. benzene has λ_{max} at 254 nm whereas in case of phenol it shifts to 270 nm with ε_{max} at 1450 nm. A shift in the absorption peak of a chromophore from a standard position to longer wavelength is called *bathochromic shift* (blue shift) whereas to a short wavelength is called *hypsochromic shift* (red shift). When the degree of intensity is increased, it is called *hyperchromic shift* and when the intensity is decreased or lowered, it is called *hypochromic shift.*

(i) **Saturated Hydrocarbons**: In order to understand as to how substances absorb UV radiation one has to recall the fundamentals such as that the valence electrons are of three types namely : σ, π and non-bonding (n), and that the bond formation involves overlap of two orbitals each containing an electron. When two atomic orbitals overlap, the nature of the molecular orbital depends on whether the overlap occurs between two regions having the same sign or opposite sign. When the former occurs, the result is bonding orbital, whereas when the latter occurs the result is an anti-bonding orbital. In bonding orbitals, the electron charge is concentrated in the region between the two nuclei thereby holding the two nuclei together. On the other hand in anti-bonding orbitals electron charge is withdrawn from the region between the two nuclei thereby resulting in increased repulsion between the two nuclei. This is represented by the energy diagram (Fig. 15.5) below :

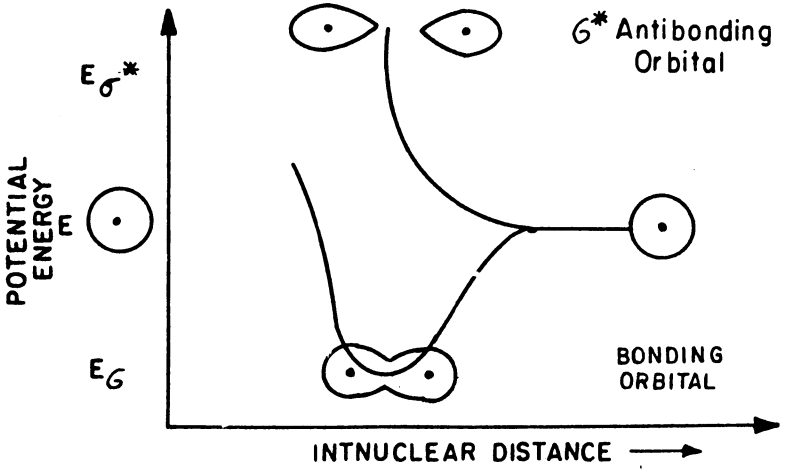


Fig. 15.5. Sigma (σ) bond in saturated hydrocarbons.

In the electronic state called ground state, both the electrons in the bonding orbital have opposite spin ($\uparrow\downarrow$) and no electrons are contained in the antibonding orbital. When energy absorbed equal to ΔE ($\sigma \longrightarrow \sigma^*$), takes place, the electronic transition from bonding to antibonding state occurs with retention of spins — this excited state is called *singlet state*. Since $\sigma \longrightarrow \sigma^*$ energy change is very high, it is found in vacuum UV region. Saturated hydrocarbons have C – C or C – H single bonds only (i.e. σ bonds) therefore they do not absorb in UV–Vis regions. However, the exception being cyclopropane, alicyclic compounds with small ring strain absorb at slightly longer wavelength at 190 nm.

15.4.1 Effect of Auxochromes on the Position of U.V. Bands

The functional groups like –ÖH, NH_2 and halogens possess a pair of non-bonded (n) electrons in addition to some sigma electrons. The n - electrons can be excited to σ^* energy level and since they are already at a higher energy level than the σ electrons in the molecule, the energy required for the $n \rightarrow \sigma^*$ transition is less than that needed for $\sigma \rightarrow \sigma^*$ i.e. $\Delta E_{(n \rightarrow \sigma)} < \Delta E_{(n \rightarrow \sigma)}$. This means that $n \rightarrow \sigma^*$ transition occurs at longer wavelength than $\sigma \rightarrow \sigma^*$ (Fig. 15.6).

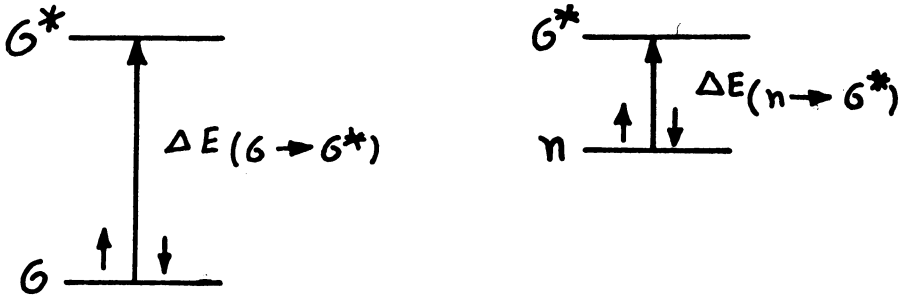


Fig. 15.6. $\sigma \rightarrow \sigma^*$ and $n \rightarrow \sigma^*$ *transitions.*

For example, saturated alcohols or epoxides absorb UV radiation at 180 — 185 nm ($\log \varepsilon = 2.5$) involving $n \rightarrow \sigma^*$ transition. Similarly saturated amines absorb at 190 – 200 nm ($\log \varepsilon = 3.4$). The saturated chlorides and bromides have λ_{max} of 170 – 175 nm ($\log \varepsilon = 2.5$) and 200 – 210 nm ($\log \varepsilon = 2.6$) respectively. The $n \rightarrow \sigma^*$ transition will depend on the ease with which n - electrons can be excited. If, in the ground state the n-electrons are stabilized by hydrogen bonding with solvents or by other means, the absorption will be shifted to lower wavelength i.e. a hypsochromic shift will occur.

15.4.2 Olefinic and Other Chromophoric Groups

Let us consider the case of a simple olefin like ethylene. Ethylene consists of one σ - bond and one π– bond. The bond formation in ethylene is represented by the energy diagram in Fig. 15.7.

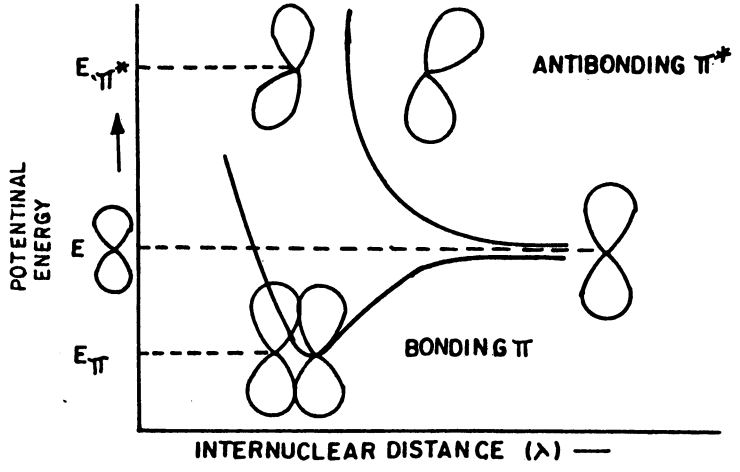
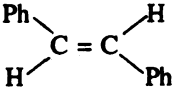


Fig. 15.7. π-Bond in ethylene.

The π bond is weaker than σ bond i.e. π -electrons are at higher energy level than σ electrons. consequently $\pi \longrightarrow \pi^*$ transition takes place at a longer wavelength than $\sigma \longrightarrow \sigma^*$ i.e. $\Delta E_{(\pi \longrightarrow \pi^*)} < \Delta E_{(\sigma \longrightarrow \sigma^*)}$. Thus substances like ethylene, propylene, 1-octene etc which contain *isolated* double bonds undergo $\pi \longrightarrow \pi^*$ transition at 170 – 200 nm. The $\pi \longrightarrow \sigma^*$ transition can also take place but for these transitions $\Delta E_{(\pi \longrightarrow \sigma^*)}$ is >> $\Delta E_{(\pi \longrightarrow \pi^*)}$ and therefore they are observed in far UV regions. Ethylene normally shows two peaks of unequal intensity, a stong peak at λ_{max} = 165 nm (ε_{max} = 10,000) and a weak peak at λ_{max} = 210 nm ($\varepsilon_{max} \cong 1000$). Both these peaks are due to $\pi \longrightarrow \pi^*$ transitions. However, if auxochromes are substituted for the ethylene protons (e.g. NR_2 to vinyl carbon) a bathochromic shift to 250 nm takes place. This shift is due to resonance interaction. Similarly, differences in the absorption spectra of cis and trans isomers of disubstituted olefins occurs. The values for λ_{max} and E_{max} of the trans isomer are generally greater than those of cis isomer e.g. in case of diphenylethylene



the trans isomer shows a $\pi \longrightarrow \pi^*$ transition at 2 59 nm (ε_{max} 27,000) while the cis isomer has a lower value at λ_{max} = 280 nm (ε_{max} = 13,500).

Thus we have learnt that transitions occur in molecules which contain isolated auxochromes or π or σ bonds All these transitions take place below 200 nm which is below the region for which commercial instruments are designed for laboratory work.

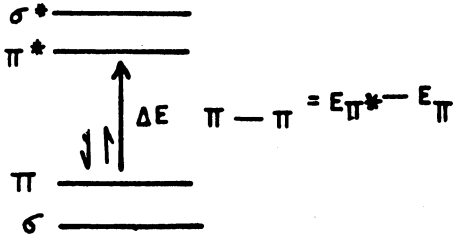


Fig. 15.8. $\pi \longrightarrow \pi^*$ transitions

Now, let us consider chromophoric groups like C = O, C = S, –N = N– in which σ, π and n - electrons are all present. The various types of transitions that can take place in these chemical moieties are : (i) $\sigma \longrightarrow \sigma^*$,(ii) $\sigma \longrightarrow \pi^*$, (iii) $n \longrightarrow \sigma^*$, (iv) $\pi \longrightarrow \pi^*$ (K– band). (v) $n \rightarrow p^*$ (R-band) and (vi) $\pi \rightarrow \sigma^*$. These transitions are shown by means of an energy diagram in the Fig. 15.9. Of these various transitions only $n \longrightarrow \pi^*$ takes place in the region above 200 nm (with a low ε) e.g. in the case of acetaldehyde (CH_3—C(=O)—H) $n \rightarrow \pi^*$ transitions takes place at 294 nm (log ε = 1.08) and $\pi \rightarrow \pi^*$ transition occurs at 190 nm (log ε = 2.0) So in general, an absorption at 275 – 294 nm region is a positive indication of an aldehyde or ketonic carbonyl function. The $n \longrightarrow \pi^*$ transition is also affected by the solvent and this will depend upon the nature of the chromophore itself, e.g. carbonyl group can be represented by its resonance or hybrid forms as :

$$>C=O \longleftrightarrow C^+ - O^- = C^{\delta+} \cdot O^{\delta-}$$

Here, the degree of contribution to the hybrid by the polar form will depend on the nature of the substitution on the carbonyl carbon e.g. The electron releasing groups will make the hybrid more polar while the electron withdrawing groups will make it less polar. The hybrid will be stabilized by hydrogen bonding or non-bonding interactions of C = O with the solvent if its ground state structure is more polar than its structure in the excited state. Therefore, hypsochromic shift will occur in a polar solvent, e.g.

acetone absorbs at 279 nm due to n → π* transition when the absorption is measured in a non-polar solvent like hexane. The value shifts to 264.5 nm when the absorption is measured in a polar solvent like water.

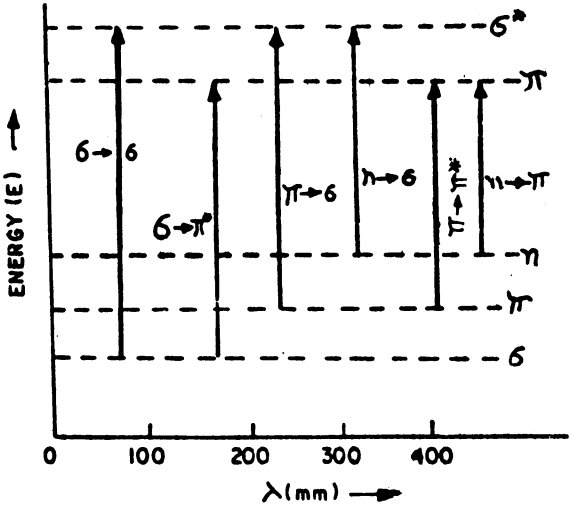


Fig. 15.9. Different type of electronic transitions in UV region.

15.4.3 Effect of Conjugation on the position of UV Bands

We have already learnt that 6 types of electronic transition are possible for various chromophoric groups and out of these n → π* transition is within the usual range detectable in the laboratory instruments. Normally simple olefin undergoes π → π* transition at 165 – 200 nm. When there are two double bonds, absorption will change depending on how they are located in the molecule. If two double bonds are isolated π → π* transition will still be at 165 – 200 nm, when they are conjugated, absorption will occur at longer wavelength i.e. a bathochromic shift will be produced due to conjugation. It also means that conjugation lowers the energy needed for the excitation of electrons. One of the good examples of a system containing two conjugated double bonds is 1, 3-butadiene having a structure $H_2C = CH - CH = CH_2$. 1, 3-Butadiene is a diolefin and can be represented by its resonance structures as : $H_2C^{\delta+} - CH = CH - C^{\delta-}H_2 \longleftrightarrow H_2C^{\delta-} - CH = CH - C^{\delta+}H_2$. The energy requirement for 1, 3-butadiene can be explained by means of molecular orbital theory. In butadiene, all the 4 carbon atoms are sp^2 hybridzed, and one pure p orbital is associated with each carbon atom. Due to resonance $C_1 = C_4$ and $C_2 = C_3$. All the p orbitals can be treated as forming a molecular orbital (M.O.) covering all the four carbon atoms. The bonds produced are delocalized bonds. The wave equation for butadiene can be written as $C_1\Psi_1 + C_2\Psi_2 + C_3\Psi_3 + C_4\Psi_4$. The molecular energy diagram for ethylene and 1, 3-butadiene is shown in Fig. 15.10.

Thus in butadiene, the easiest π ⟶ π* transition involves excitation of electron from Ψ_3 to Ψ_3. The energy required is $E_{\Psi_3} - E_{\Psi_2} = \Delta E_2$ which is less than in ethylene. Therefore, π ⟶ π* transition in conjugated system is at longer wavelength than in isolated olefin. 1, 3-Butadiene shows absorption at 220 nm in a non-polar solvent.

In an isolated carbonyl (C = O) group the π → π* transition absorbs at 170 nm and n → π* at 290 nm (e.g. acetaldehyde) but in crotonaldehyde which is a conjugated system the $\psi_2 \longrightarrow \psi_3$* transition occurs at 218 nm (ε_{max} = 18,000) and n ⟶ ψ_3* transition takes place at 320 nm (ε_{max} = 30 in ethanol) as shown in Fig. 15.11.

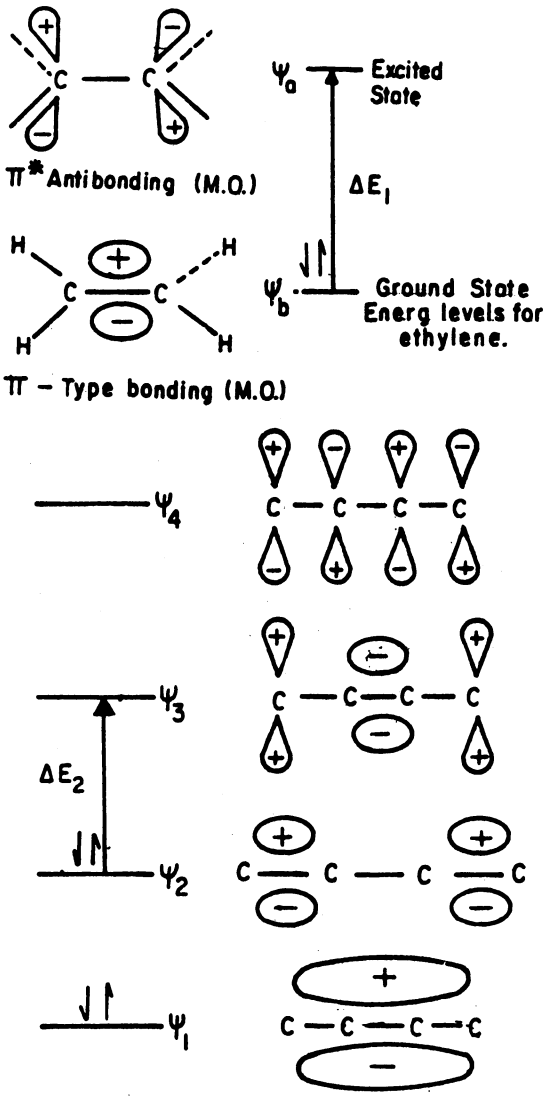


Fig. 15.10. Molecular orbital energy diagram for ethylene and 1, 3-butadiene.

15.5 Applications

The various applications of UV spectrophotometry in chemical and pharmaceutical analysis are enumerated below :

(i) Determination of structure of drug substances by using qualitative rules and comparing them with model compounds.

(ii) Quantitative estimations of drug substances and relating it to structure e.g. assay of alkaloids, antibiotics and other drugs.

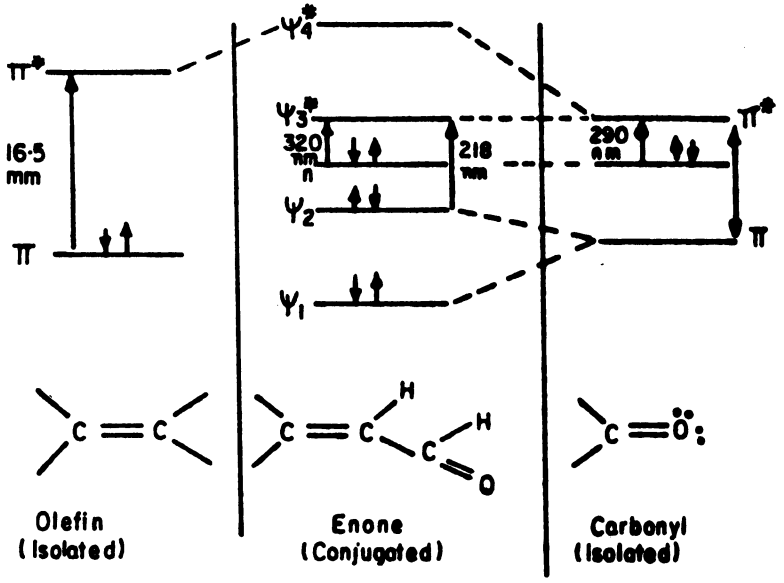


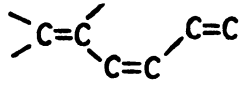
Fig. 15.11. Energy level diagram for C = C , C = C — C = O and C = O.

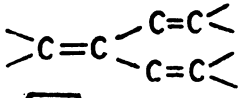
(iii) Determinations of configurations like *cis* and *trans* isomers and steric interactions between neighbouring atoms e.g. trans isomer has greater λ_{max} and ε_{max} than the cis isomer.

(iv) Determination of hydrogen bonding i.e. association of a compound with the solvent and its effect on the absorption can be established e.g. carbonyl compounds in H_2O.

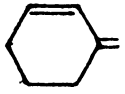
(v) Detection of ions and free radicals in reaction mechanisms.

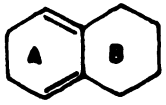
In order to be able to make use of various types of qualitative rules for solving the problems of structure elucidation of chemical and drug substances, various forms of chemical systems may be recognised and these are shown below :

(i)  Linear Conjugation (1, 3, 5-hexatriene)

(ii)  Cross conjugation

(iii)  Cyclic diene

(iv)  Semicyclic diene. (exocyclic double bond)

(v) 

Homoannular diene (Two double bonds are conjugated

(vi) 

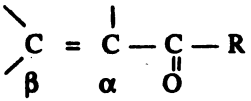
and are in the same ring)
Heteroannular diene
(Two double bonds belong
to two different rings)

Further various types of rules have been framed for calculating the theoretical (expected) values for chromophores incorporating above type of conjugated system and can then be compared with the observed values. These rules are titled as Woodward and Fieser rules for dienes and Nielson's rules for α, β - unsaturated ketones. Practice problems are available in the text books, However, a detailed correlation chart for solving such types of problems is incorporated in this Chapter.

15.8 The Interpretation of Ultraviolet Spectra

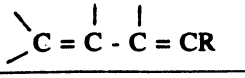
Empirical Rules for Calculating the Ultraviolet Absorption Maxima of Aliphatic and Alicyclic Compounds

I. α, β — Unsaturated ketones

Parent system  λ_{max} = 215 mμ

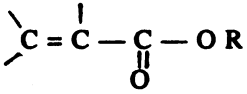
Structural variation	increment in λ_{max}
α — alkyl substituent	+ 10 mμ
β — alkyl substituent	+ 12 mμ
exocylic C = C	+ 5 mμ
cyclopentenone system	− 11 mμ
C = C extending conjugation	+ 30 mμ
each γ — or σ —alkyl substituent	+ 18 mμ
presence of a homoannular diene	+ 39 mμ

II. Conjugated Dienes

Parent system  λ_{max} = 214 mμ

structural variation	increment in λ_{max}
alkyl substituent	+ 5
exocyclic C = C	+ 5
presence of homoannular diene	+ 39
C = C extending conjugation	+ 30

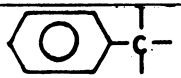
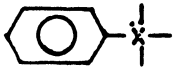
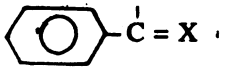
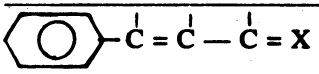
III. α, β — Unsaturated Acids and Esters

parent system  λ_{max} — 200 mμ

where R = H or alkyl

structural variation	increment in λ_{max}
alkyl substituent	+ 8
exocyclic C — C	+ 5

Empirical Rules for calculating the Ultraviolet Absorption Maxima of Benzene Derivative

Structure	λ_{max}
	250—270 mμ (100—500)
 x = atom with at least one unshared electron pair	260–280 mμ (500–2000)
 X = C, O or N	240 — 250 mμ (10,000)
 X = C, O or N	280—290 mμ (10,000 — 30,000)

The Ultraviolet Absorption Spectra of Some Representative Compounds

Compounds	λ_{max} (ε)
isolated double bonds	
Acetaldehyde	293 mμ (ε 12)
acetaldehyde	204 mμ (ε 60)
acetone	271 mμ (ε 16)
acetonitrile	160 mμ
ethylene	193 mμ (Σ 10,000)

olefins (R = alkyl)	at 210 mμ (not max)
$RCH = CHR$ or $R_2C = CH_2$	ε 200 — 1000
$R_2C = CHR$ or $R_2C = CHR$	ε 1400 — 5600
$R_2C = CR_2$	ε 4400 — 10500
aliphatic and alicyclic conjugated systems	
butadiene	217 mμ (ε 20,900)
trans—$CH_3CH = CHCOCH_3$	224 mμ (ε 9.750), 314 mμ (ε 38)
cis — $CH_3CH = C(CH_3)COCH_3$	235.5 mμ (ε 4,570)
trans — $CH_3CH = C(CH_3)COCH_3$	230 mμ (ε 12,800)
Benzene derivatives	
aniline	230 mμ (ε 7,000), 280 mμ (ε 1470)
anisole	265 mμ (ε 2300)
benzene	256 mμ (ε 230)
phenol	273 mμ (ε 2050)
toluene	262 mμ (ε 270)
acetophenone	244 mμ (ε 13000), 278 mμ (ε 1100)
benzaldehyde	244 mμ (ε 15800), 280 mμ (ε 1500)
benzoic acid	230 mμ (ε 10000), 270 mμ (ε 800)
benzonitrile	230 mμ (ε 12500), 270 mμ (ε 650)
nitrobenzene	252 mμ (ε 10000), 280 mμ (ε 1000) 330 mμ (ε 130)
styrene	244 mμ (ε 12000), 282 mμ (ε 450)
2, 4 — dinitrophenylhydrazones	
benzaldehyde 2, 4 — dinitrophenylhydrazone	378 mμ (ε 29,200)
propionaldehyde 2, 4 — dinitrophenylhydrazone	359 mμ (ε 21,800)
heterocycles	
furan	200 mμ (ε 10,000), 252 mm (ε 1)
pyridine	195 mμ (ε 7,500), 250 mμ (ε 2000)
pyrrole	210 mμ (ε 16,000), 340 mμ (ε 300)
thiophene	235 mμ (ε 4.500)
aromatic systems with extended conjugation	
trans — benzalcetone	220 mμ (ε 12,000), 86 mμ (ε 23,500)

cis — benzalacetophenone	250 mμ (ε 13,700), 292 mμ (ε 8,800)
trans — benzalacetophenone	228 mμ (ε 9,800), 308 (ε 24,300)
benzophenone	252 mμ (ε 20,000), 330 mμ (ε 180)
diphenyl	252 mμ (ε 18,000)
naphthalene	220 mμ (ε 110,000), 275 mμ (ε 5,600), 314) mμ (320)
1 — phenylbutadiene	223 mμ (ε 12,000), 280 mμ (ε 28,000)
cis — Stilbene	230 mμ (ε 24,000), 285 (ε 12,000)
trans — stilbene	230 mμ (ε 13,000), 295 mμ (ε 25,000)

EXERCISE 1:

(a) Verification of Beers — Lambert Law with Benzoic acid.

(b) Find the concentration of an unknown solution.

PROCEDURE

(i) Prepare 500 ml of N/1000 benzoic acid as a stock solution.

(ii) From the stock solution, prepare 100 ml each benzoic acid solution of 2, 4, 6, 8 and 10 percent respectively. Determine the absorbance for each solution at 250 nm. Plot a graph between concentration versus absorbance and show that Beers—Lambert Law is obeyed.

(iii) From the stock solution, prepare another solution of unknown concentration. Determine the absorbance of this solution. Find the concentration of the solution from the graph obtained in step ii.

EXERCISE 2 : Determine the λ_{max} for benzene, phenol and aniline, using UV spectrophotometer and explain the shift in λ_{max} for phenol and aniline with respect to benzene. Also determine the λ_{max} for phenol in alkaline medium and aniline in acid medium. What change do you get in λ_{max} and why ?

Substance	λ_{max} E — Band	λ_{max} B — Band	Medium
C_6H_6			Neutral
C_6H_5OH			Neutral
C_6H_5OH			Alkaline (pH = 13)
$C_6H_5NH_2$			Neutral
$C_6H_5NH_2$			Acidic (pH = 3)

EXERCISE 3 : Determine in grammes per litre the quantity of sulphanilamide and sulphathiazole in a given mixture, using UV spectrophotometer.

Theory : When a solution contains several solutes the absorbances are additive, provided that there is no reaction between the components of the mixture.

Thus,

$$A_1 = A_{1,a} \cdot 1.C_a + A_{1,b} \cdot 1.C_b$$

and $$A_2 = A_{2,a} \cdot 1.C_a + A_{2,b} 1.C_b$$

where $A_{1,a}$ and $A_{1,b}$ are the optical densities of a and b at λ_1; those at λ_2 are $A_{2,a}$ and $A_{2,b}$; C_a and C_b are the molar concentrations of the components in the solutions. The solution of these simultaneous equations is obtained by the method of determinants or successive approximation.

Apparatus : Spectrophotometer (ultra-violet range); 1.0 cm quartz cells; solutions of sulphanilamide and sulphathiazole in water (4 mg per litre each); mixed solution of sulphanilamide and sulphathiazole containing 3.2 (x) and 0.8 (y) mg per litre of water, respectively.

Procedure : Find the absorption peaks of the pure substance at 25°C. Measure the optical densities of the solutions of the pure components and of the mixed solution at the peaks thus found. Multiply each density by 250, in order to obtain the result in grammes per litre, and set up the two simultaneous equations.

Observation : The absorption curves show peaks at 260 mμ for sulphanilamide and 286 mμ for sulphathiazole. The optical densities found are shown in the table below :

Substance	Optical density	
	260 mμ	286 mμ
Sulphanilamide	0.403	0.127
Sulphathiazole	0.272	0.315
Mixture	0.392	0.176

Calculations

$0.392 = 0.403 \times 250x + 0.272 \times 250y$

$0.176 = 0.127 \times 250x + 0.315 \times 250y$

Hence, $x = 0.0033$ and $y = 0.0009$ grammes per litre.

Prepare a similar table for given unknown mixture.

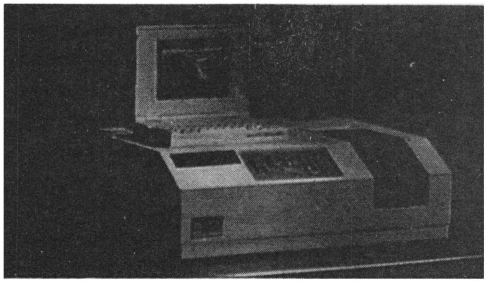


Fig. 15.12 UV-VIS Lambda 12/14 Spectrophotometer (Courtesy of Perkin Elmer)