Chapter 1

UV-Visible Spectroscopy

Spectroscopy and **spectrography** are the techniques based on the measurement of radiation intensity (absorbed or emitted or scattered by the sample or analyte) as a function of wavelength. Spectral measurement instruments are more commonly referred as spectrometers, spectrophotometers, spectrographs or spectral analyzers. These instruments measure the light intensity or resultant light from sample, (emitted or transmitted or scattered). Absorption is the measure of difference between incident and transmitted light (Figure 1.1).

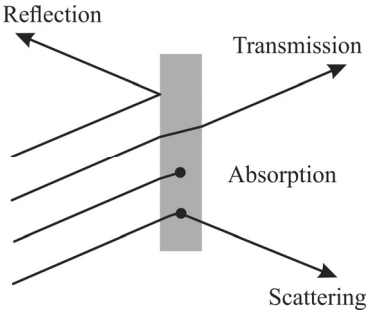


Figure 1.1 Various interaction of effect of light with matter (Analyte).

The output of scan measurement of sample from a range of wavelength is represented as 2D or 3D spectrum. In general X axis (Input) will be wavelength, Y axis (output) will be light absorbed (UV-Visible, IR, NMR or any absorption spectroscopy, Light emitted (fluorescence or any emission spectroscopy) or scattered (intensity of scatted light Raman spectroscopy). There are three types of Spectrum produced by spectroscopy

1. Continuous spectrum
2. Emission lines
3. Absorption lines

Selection Region based on Sample Nature

The difference between UV and Visible spectroscopy is the wavelength region used in measurement. UV region (190-380 nm) for colorless sample and visible region 380-790 nm for coloured sample. As result of scanning of sample using the light, the spectrum obtained is characteristic of sample / molecule nature, its highly depends on double bonds (pi electrons) configuration of the structure.

Absorption/Emission of Light by a Chemical Molecule

The mechanism behind the absorption of light by a molecule (analyte) or / and emission of light from the molecule (Analyte) is depends on the phenomena of electronic excitation / transition (Figure 1.2). The amount (probability) or type of the electron (sigma, pi, n) involved in the excitation determine the characteristic photon energy absorbed or emitted, will determine the shape and pattern of UV-Visible Spectrum.

As no two chemical molecules will have same number of electrons or configuration, the UV spectrum will be different for different compounds.

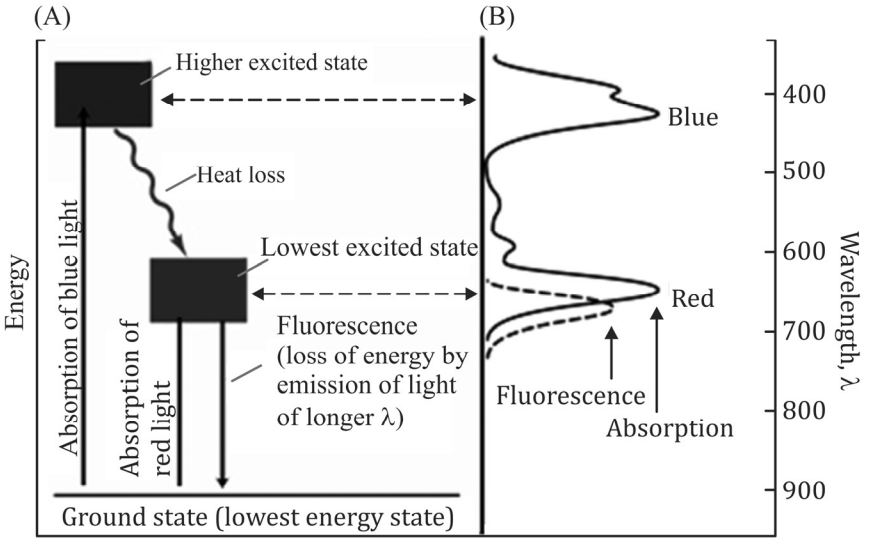


Figure 1.2 Absorption and emission process of matter (Analyte).

- The above figure shows the electronic excitation process and relaxation process of electron of molecule when UV-Visible light is irradiated on sample.

- Blue light and red light is indicated to understand the wavelength comparison.
- *NOTE*: sample should be low concentration, pure and highly transparent for any qualitative and quantitative analysis

Types of Electronic Transitions

- There are different types of electronic transition take place in the molecule, and each molecule undergoes more than one electronic transition, and requires different energy (Figure 1.3).
- Sigma transitions are higher takes in very low wavelength of UV region or in Vacuum UV region (< 190 nm).
- The below diagram indicate the comparison of different electronic transition and energy in which n' electron transition require less energy (absorbs higher wavelength)

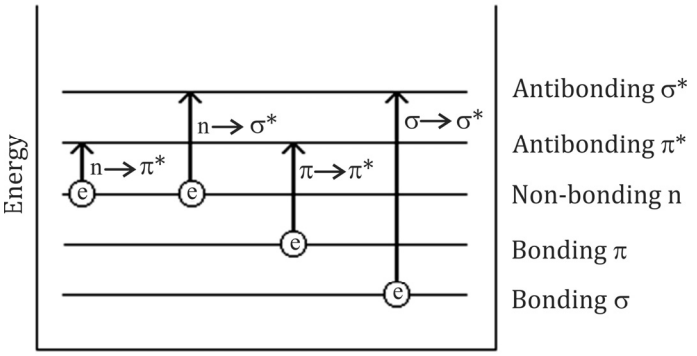


Figure 1.3 Types of electronic transition in a organic molecules upon UV-Visible light exposure.

From above figure, we can observe that the energy required for sigma transition (alkane) is the highest which is not available in UV region, so sigma transition may not be a practically observed. sigma transition may be possible at vacuum UV region (<190 nm).

How to predict the possible electronic transition for the given structure?

To predict the possible transition, we need to know the chemical structure and type of electrons available.

Table 1.1 Transition and Chemical structures

Chemical class	Types electron	Possible transitions (theoretical)
Alkane	Sigma electron only	Sigma to sigma*
Alkene (C=C), Benzene, Alkynes	Sigma and Pi electrons	Sigma to sigma* Pi to Pi*
Aldehyde, ketones, heterocyclic, acids, esters, (all compounds containing C=O, C=S	Sigma, pi electrons n' electrons	All type of transitions
Complexes	n' electrons	Charge transfer mechanism

Schematic Procedure in Measuring Absorbance

- In the instrumentation, always a monochromatic light will be used to measure an absorbance (in quantitative analysis).
- The monochromatic light to be used has to be selected from the UV spectrum (spectrum as a result of scan for entire UV-Visible range).
- Sample cell need to be quartz in UV light measurement, because glass can absorb UV light. But for Visible light measurement (colorimetric) glass cuvettes can be used.
- The sample concentration should be low, and transparent to avoid scattering and beers law deviations.

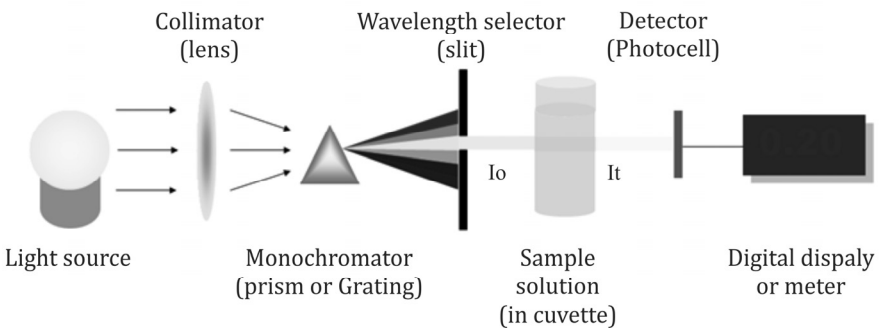


Figure 1.4 Basic Instrumentation of UV-Visible Spectrophotometer.

- Io is the intensity of incident light (monochromatic), $I_t$is the intensity of transmitted light.
- If Io = I_t sample inactive and indicate no absorption, and molecule structure may not have n and pi electrons.

- If Io is greater than I_t - indicate the reduction of intensity of transmitted light due to absorption of photon energy by the molecular electrons, by the process of electronic excitation.
- Hence Absorbance A = log (Io/I_t) or A = –log T; (where T = It/Io).

The UV Spectrum

For example (2D- UV spectrum) the following UV spectra shows absorption in UV region (190 – 380 nm) indicate the sample is colorless (Figure 1.5).

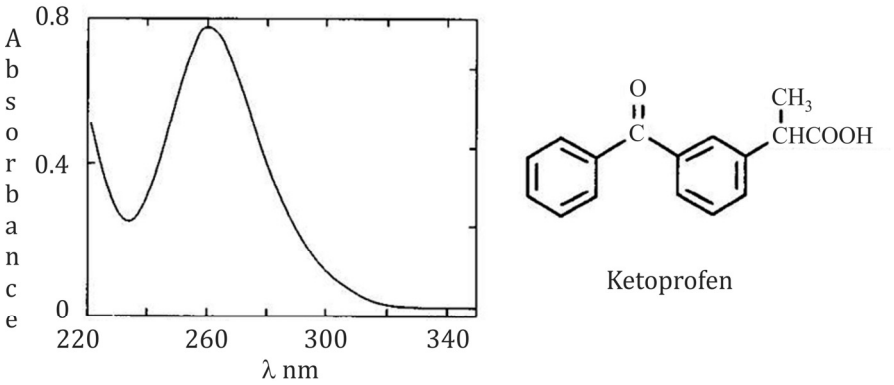


Figure 1.5 UV – Spectrum and UVmax (Lambda max) : Colourless Compound.

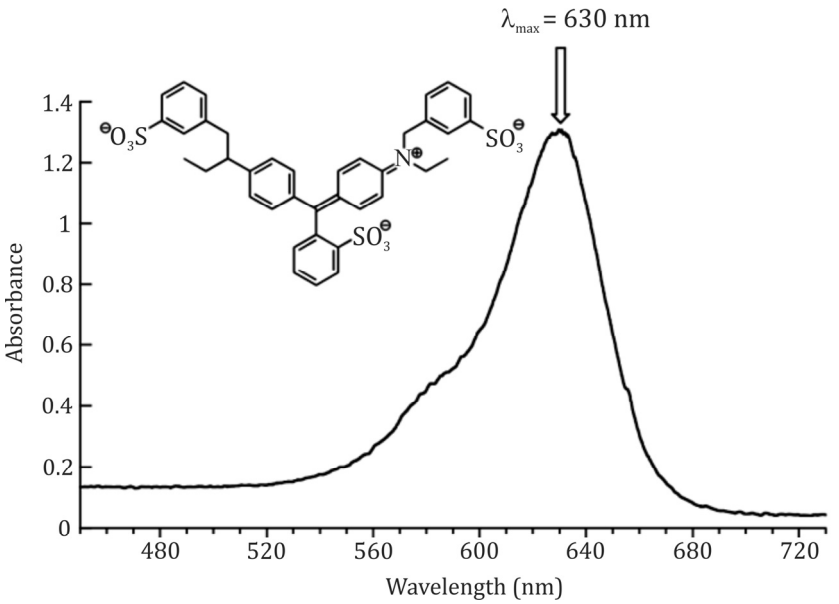


Figure 1.6 UV – Spectrum and absorption maximum: Coloured Compound.

The above UV spectrum is for Colour less sample shows a cut-off wavelength around 340 nm and lambda max of 260 nm. Thus the spectrum shows no absorbance in visible region

- In the same way coloured compound showed absorption maximum at 630 nm, but showed no absorbance in UV region (Figure 1.6).
- Visible spectrum of Coloured sample. Indicate the absorption characteristics of sample in the visible region (400-780 nm).
- The spectrum (Figure 1.7) shows that a spectrum characteristic does not affected by Concentration.

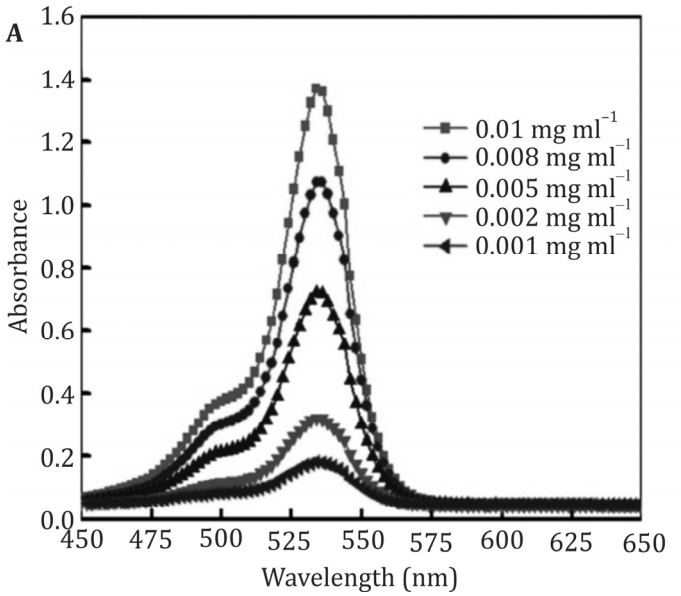
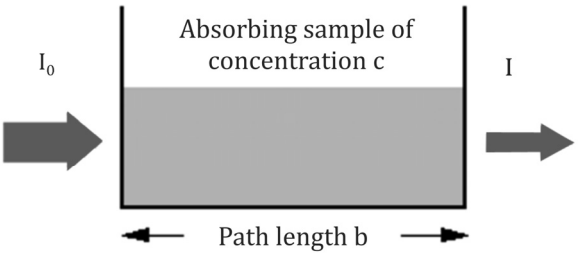


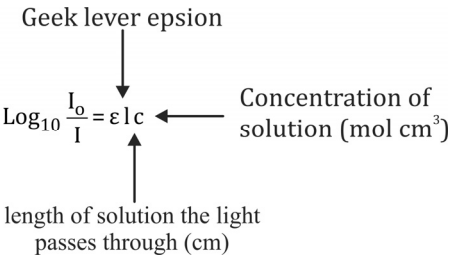
Figure 1.7 UV – Spectrum Vs Concentration (Shows that there is no change in spcetrum chracteristics).

Beer-Lambert Law

- The absorption and Concentration is related by Beer-Lambert law.



The Beer-Lambert equation shall be expressed as following,



(Or)

$$A = A^{1\%}_{cm}.\ b.\ c$$

Where

- A = Absorbance, (log (Io/I)
- b or l-Path length and c is concentration (g/100 ml),
- A1%cm- the specific absorbance of compound which is constant (it is the absorbance of 1 % w/v solution in 1cm path length).

The above formula is recommended by many pharmacopoeias in quantification of drug in pharmaceutical dosage form, when standard substance is not available.

- The relation between concentration and absorbance is expressed as linearity cure or Beer-Lambert curve. This curve can be used for quantification if both standard and sample available (Figure 1.8).

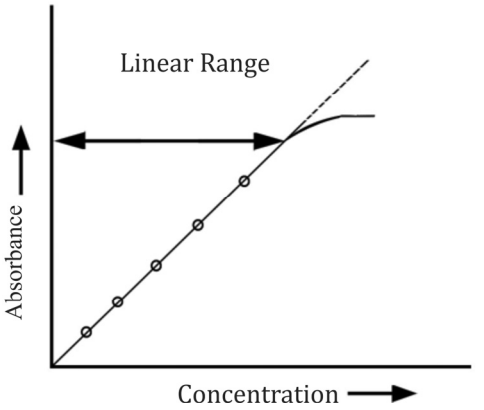


Figure 1.8 Beer-Lambert Plot – Shows the linear relationship between concentration and absorbance to certain limit.

3D UV Spectrum

It is more commonly employed in Chromatography as a function of detection using Photo-diode array detection (PDA). X-axis (wavelength), Y-axis (absorbance), Z axis (time), which can be more conveniently used for kinetic studies. PDA Detection is more commonly employed in stability and degradation of analyte. This detection can give peak purity when coupled with chromatography. This can also predict the response of analyte at different wavelength.

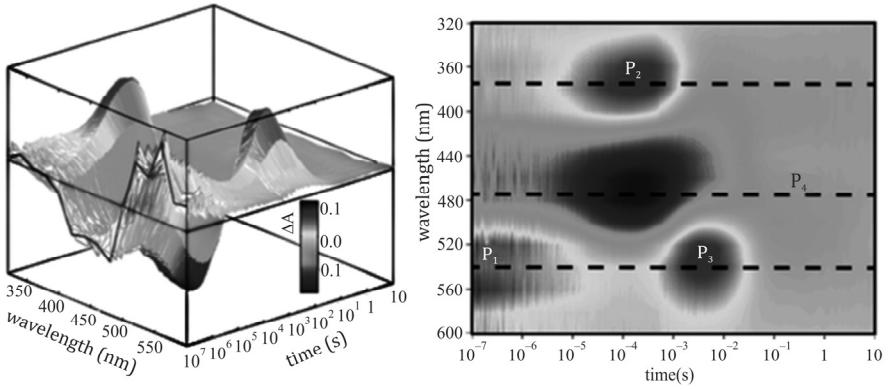


Figure 1.9 3D – UV Visible Spectrum (Example – PDA).

The comparison of absorption spectrum and emission spectrum

- The molecule which possesses more conjugation and exhibit tendency to emit light are called fluorescence. This molecule will have both absorption characteristic spectrum (excitation) and emission characteristic spectrum (emission).

- But emission spectrum will be bathochromic (red shift), it means the spectrum will shift to higher wavelength. The spectrum (Figure 1.9) shows that fluorescence wavelength shifted to higher wavelength than absorption wavelength.
- Maxima of absorption spectrum is excitation wavelength and maxima of emission spectrum is emission wavelength (In fluorimetry)
- If the wavelength shifts to lower wavelength it's called hypsochromic shift. The hyper and hypo chromic shift are used to indicate the absorption to higher and lower value respectively (Figure 1.10). These effects are called auxochrome effect (UV Spectrum nomenclature).

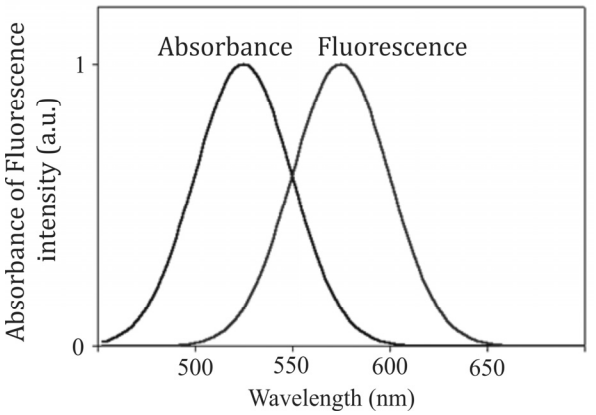


Figure 1.10 Absorption and Emission (Fluorescence) spectrum for an analyte (Stokes shift).

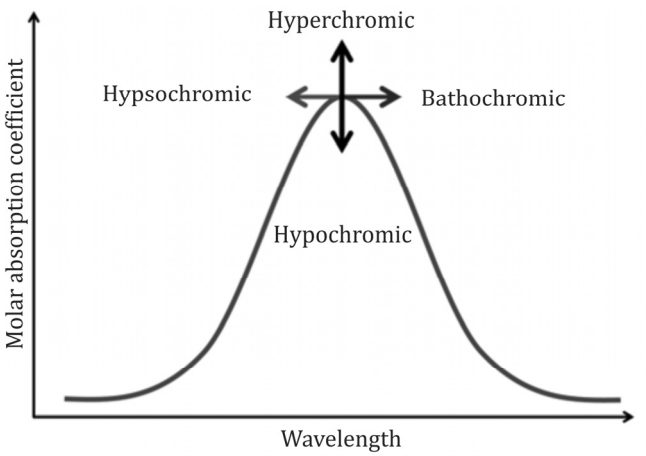


Figure 1.11 Various effect of auxochrome on Absorption maximum (UV-Spectra nomenclature).

All these four effect is seen (Figure 1.10), when there is any change in the following,

- pH,
- solvent,
- scan speed,
- chemical decomposition,
- change in structure,
- isomerism,
- tautomer's

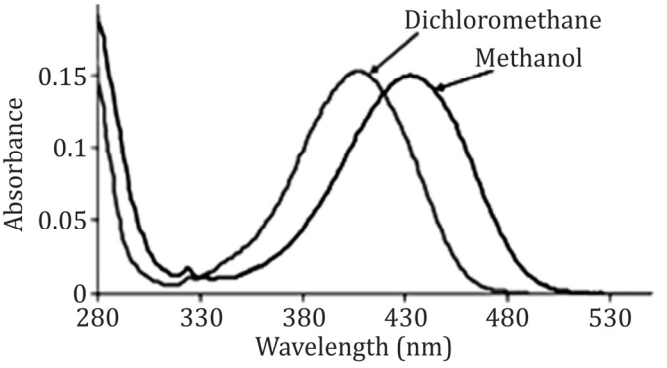


Figure 1.12 Effect of solvent on light absorption of an analyte.

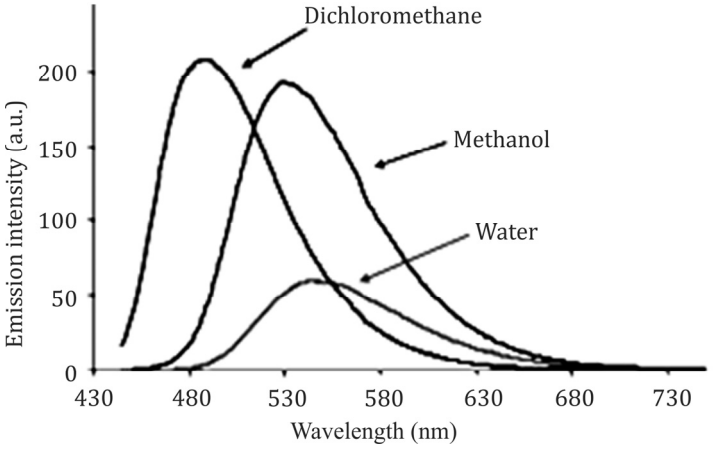


Figure 1.13 Effect of solvent on emission intensity of analyte (Fluorescence).

- The above spectrum (Figure 1.11) shows that there is a bathochromic shift for a molecule when polarity of solvent is increased. (Methanol is polar and dichloromethane is non-polar).

- The relation between polarity of solvent and shift of wavelength depends on the type electronic excitation (nothing but depends on structure and electron involved).
- Usually n- electrons excitation more affected by polar solvent (Hypochromic effect with increase in polarity) and vice versa for pi electrons (Hence, both "positive and negative solvatochromism" is possible)
- In the same way, solvent also affect the emission intensity of an analyte (Figure 1.12).

Solvent Effect and Absorption

Solvato chromism is the ability of a chemical substance to change color due to a change in solvent polarity. Negative solvate chromism corresponds to hypsochromic shift (or blue shift) with increasing solvent polarity. The corresponding bathochromic shift (or red) is termed positive solvatochromism.

In the UV spectrum the absorption from 200 nm to 220 nm,(sliding slope) is due to solvent or , due to both solvent and solute, hence, the wavelength should not be chosen in quantitative analysis. Furthermore, the absorption characteristics also changes in between 200 – 220 nm up on change in solvents. The various solvents and cut-off wavelength are listed in Table 1.2.

So it is always advisable to choose characteristic wavelength beyond the solvent absorption (I.e. above 230 nm). The following overlay spectrum shows the significant difference in the absorbance as well in wavelength for a same compound but in different solvents. It's all due various interactions exist between solute and solvent that ultimately affect the electron transition energies and transition probability. In the below UV-Visible spectrum dotted line (…….) is due to solvent absorption. Solid lines are spectrum of sample with solvent / without solvent effect.

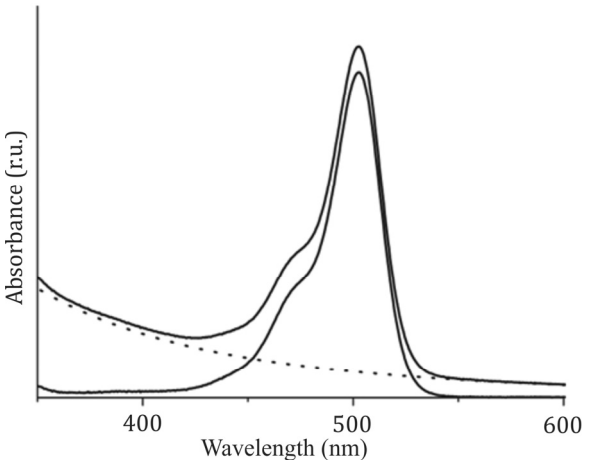


Figure 1.14 Solvent effect on UV-Visible light absorption.

The transparency limit of various UV solvent is given in the below Table 1.2.

Table 1.2 Transparency limit of commonly used Solvent in UV-Visible spectroscopy

Solvent	Cutoff Point (nm)	Solvent	Cutoff Point (nm)[a]
Water	200	Dichloromethane	233
Ethanol (95%)	205	Butyl ether	235
Acetonitrile	210	Chloroform	245
Cyclohexane	210	Ethyl proprionate	255
Cyclopentane	210	Methyl formate	260
Heptane	210	Carbon tetrachloride	265
Hexane	210	*N*,*N*-Dimethylformamide	270
Methanol	210	Benzene	280
Pentane	210	Toluene	285
Isopropyl alcohol	210	*m*-Xylene	290
Isooctane	215	Pyridine	305
Dioxane	220	Acetone	330
Diethyl ether	220	Bromoform	360
Glycerol	220	Carbon disulfide	380
1,2-Dichloroethane	230	Nitromethane	380

[a]Wavelength at which the absorbance is unity for a 1-cm cell, with water as the reference.

Structure and Spectrum Characteristics

- The absorption spectrum shape and pattern is depends on the structure, so that UV spectrum is considered as one of the identification tools in modern analytical chemistry.

- The electrons and bonds and their position (electronic configuration) determine the spectrum characteristics.

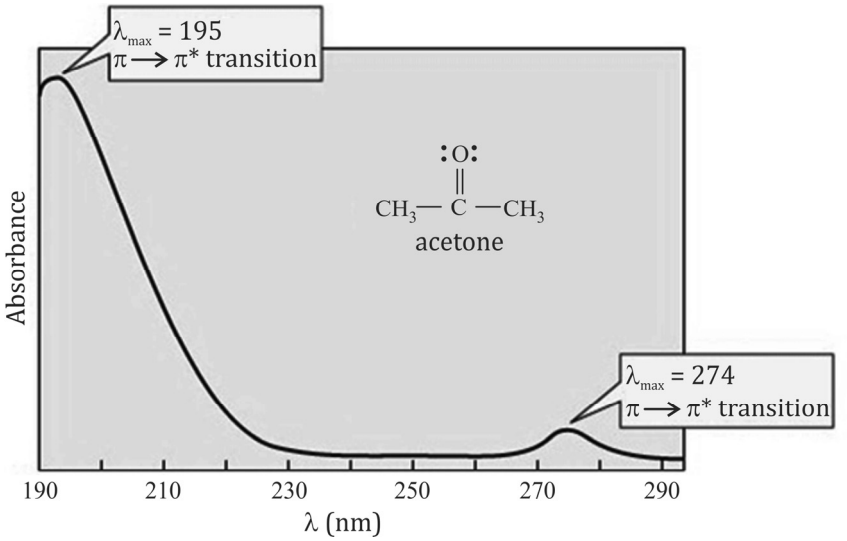


Figure 1.15 UV-Spectrum of Acetone.

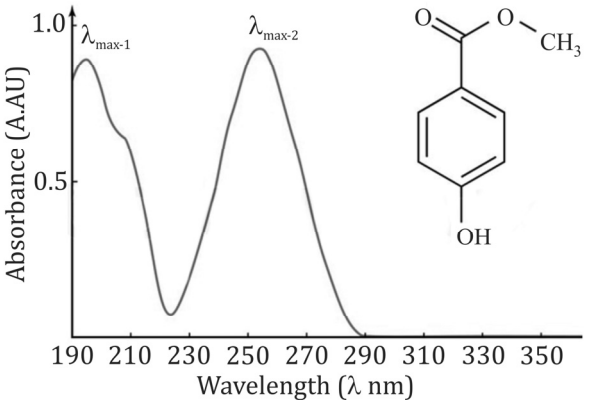


Figure 1.16 UV-Spectrum of aromatic ester.

1. For example above two spectrum are not similar indicate the compounds are different
2. Acetone UV spectrum (Figure 1.12) two maxima, one at 195 nm (for pi electron transition) and one at 274 nm (n' electron transitions), but the intensity for pi' (195 nm) is more than n' electron (274 nm). It indicate that the transition probability for pi is more in the structure when compare to n' electron. As a

result, the molar absorptivity (Epsilon value) will be more for Pi' and less for n' electron).

3. When look into the second spectrum (Figure 1.13), the intensity of peak absorption is increased due to conjugation. Furthermore, lambda max increases due to conjugation.
4. Hence the conjugation in structure will have greater effect on transition energy as well in wavelength absorbed.
5. The following UV spectrum is one more example for different transition in a molecule and its effect on UV spectrum. Where Pi to Pi is stronger and n' to pi is weaker. So always pi to pi transition yield greater absorbance than n' to pi transitions.
6. Pi to pi transitions always occur in low wavelength when compare to n' electrons due to the fact the energy of electron is in the order of Sigma > pi > n' electrons.

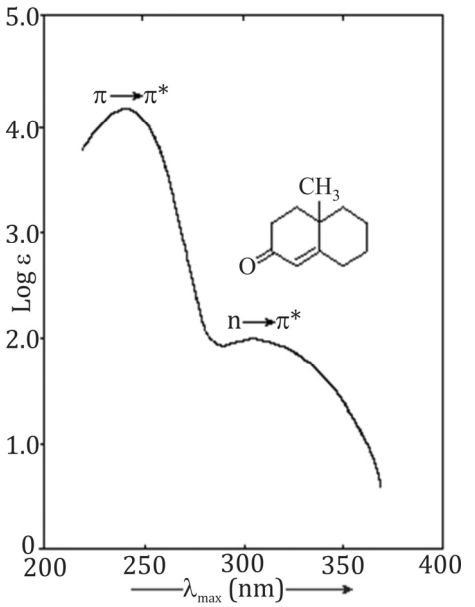


Figure 1.17 UV-Spectrum of Naphthalenone hexahydro methyl.

1. The following overlay UV- Visible spectrum shows the effect of conjugation on UV-spectrum and number maxima in spectrum characteristics. As the conjugation increase in the structure the spectrum moves towards higher wavelength (bathochromic effect).
2. Number of maxima is also increases as number conjugation increases.

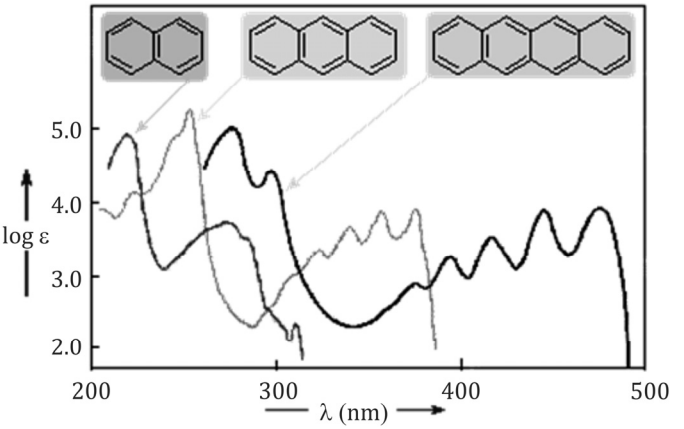
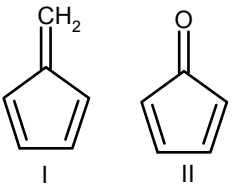


Figure 1.18 Effect of Conjugation on UV-Visible spectrum Characteristics.

1. **Cross Conjugation:** The below two structures are similar but differ in the electronic transition pattern as well in UV spectrum due to the n' electron contribute addition transition. Below structure also an example for cross conjugation.



Auxochrome effect: The below spectrum of quinones, shows the comparison of similar structures with same chromophoric system (conjugated system responsible for light absorption), but differ in substitution at NH2 group. Thus the substitution on chromophoric system has effect on both lambda max and absorbance (Figure 1.14 and Figure 1.15)

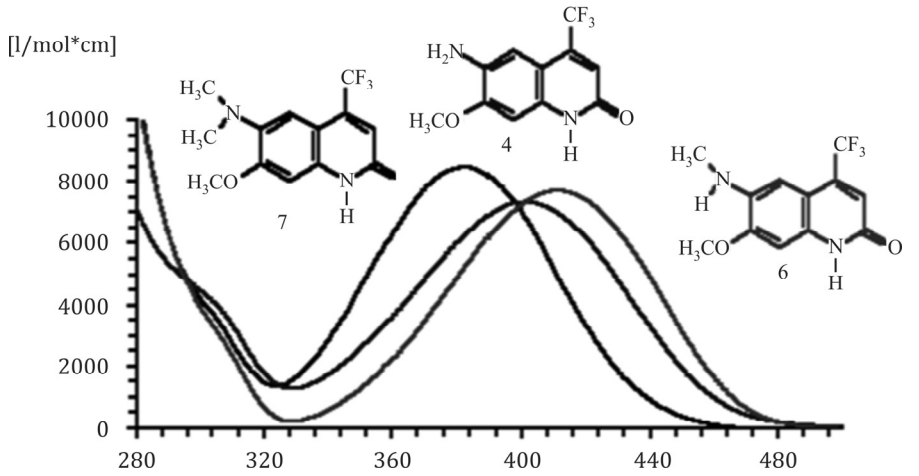


Figure 1.19 Auxochrome effect of UV-Visible spectrum Characteristics (Solvent: DMSO).

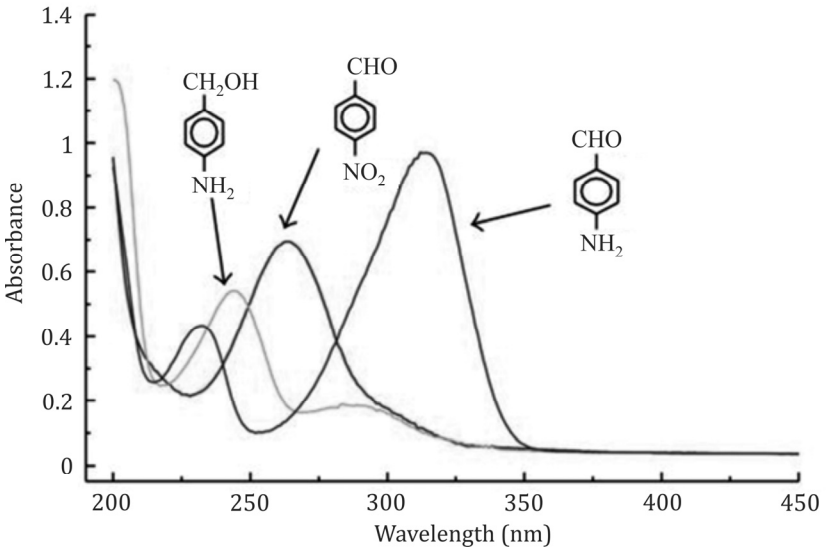


Figure 1.20 Auxochrome effect of UV-Visible spectrum Characteristics.

Order of Conjugation: The imidazole carboxylic acid (Figure 1.16) represents the order of conjugation in UV spectrum characteristics. The UV spectrum move higher wavelength when there is more conjugation. You can observe the change in the double bond position in imidazole nucleus of the structure.

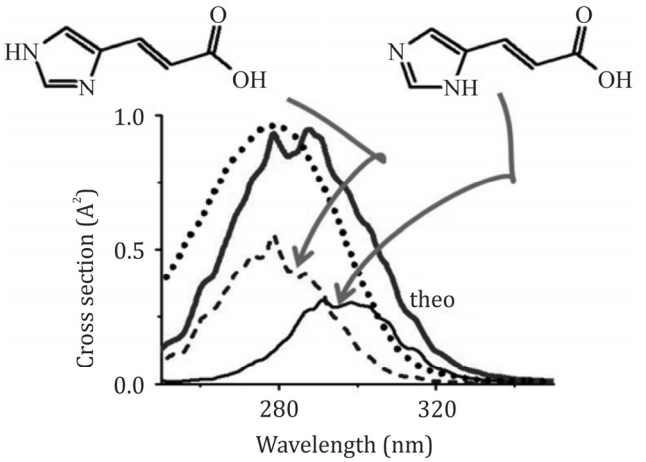


Figure 1.21 Effect of Order of Conjugation in UV-Visible spectrum Characteristics.

Position of Auxochrome in Chromophore: The below structure represent the importance of Auxochrome in benzene and their UV light absorption pattern. The presence of amine group may have significant effect on Lambda max and light absorption intensity. The ortho position in the below structure may contribute intra molecular hydrogen bonding and produce a addition cyclic residue to the structure as well as it also produce, inductive effect.

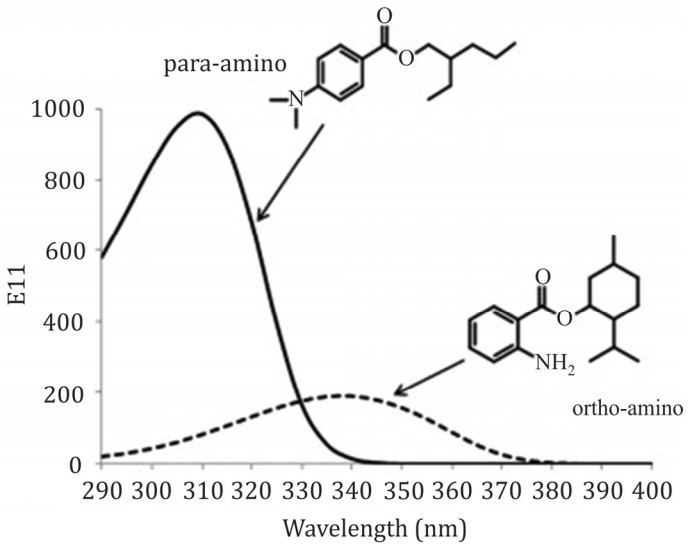
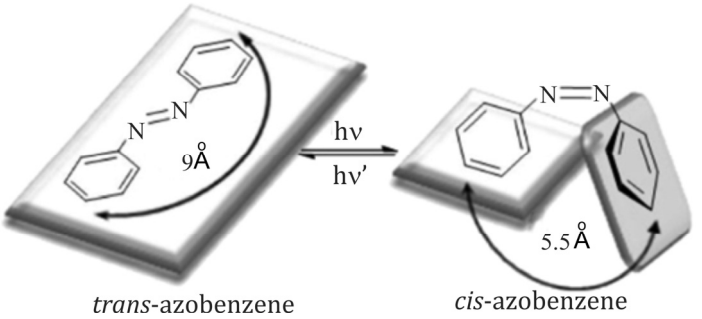


Figure 1.22 Effect of Auxochrome position on UV-Visible spectrum Characteristics.

Cis - Trans Isomerism and UV spectrum: The azobenzene is the best example of conformation transformation due to UV light and both are inter-convertible.



Like a C=C double bond, the azobenzenes have two geometric isomers (*Z*/*E*) around the N=N double bond, the *trans* isomer (*E*) is ~12 kcal·mol^{-1} more stable than the *cis* isomer (*Z*). The energy barrier of the photoexcited state is ~23 kcal·mol^{-1}, such that the *trans* isomer is predominant in the dark at room temperature. This energy barrier make different UV spectrum for trans (high intense) and Cis isomer.

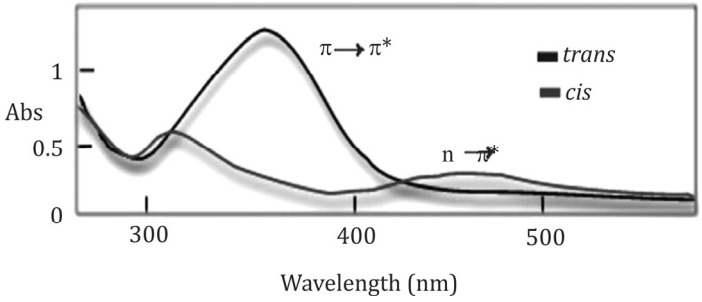


Figure 1.23 Effect of *Cis* /*Trans* isomer on UV-Visible spectrum Characteristics.

Keto-enol tautomer's in UV spectrum: The following figure shows the UV spectrum for same structure but exist in different tautomerism. B is Keto' form. A and C are enol form. For enol' structure spectrum is moved to higher wavelength due to induction of conjugation and extension of conjugation. Where as in structure B' both benzene is isolated not in conjugation.

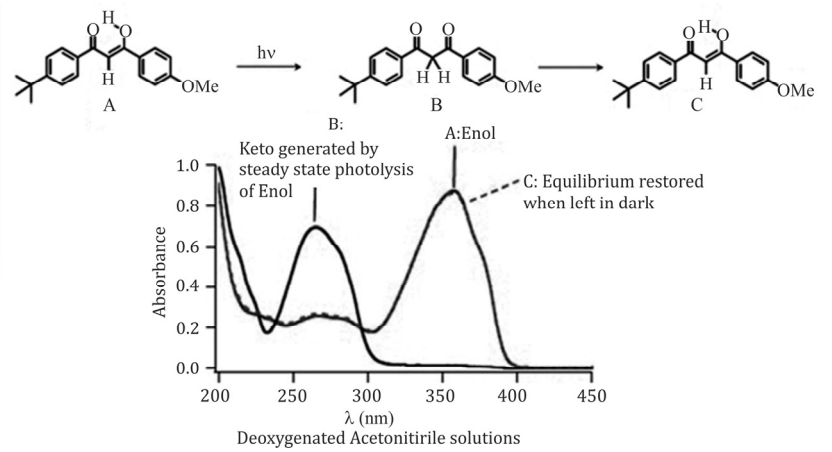


Figure 1.24 Effect of tautomerism on UV-Visible spectrum Characteristics.

Identification of Compound by UV Spectrum

For example: Ibuprofen, the following figure 1.19, shows the three UV spectra overlapped indicated that three (a, b, c) are same, as they have same UV spectrum and fingerprint matching of absorption characteristics.

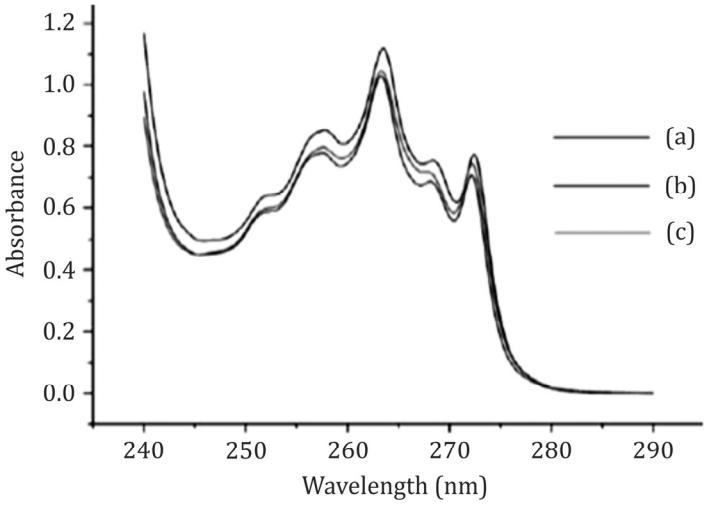


Figure 1.25 Overlay UV- Spectra of three different batch Ibuprofen samples.

The following UV spectrum (Figure 1.20) of four chrysene derivatives indicates that substitution (Auxochrome) will have impact on UV spectrum fingerprint (pattern of absorption), so no two derivatives of same chemical class would have same spectrum

Identification of degradation by UV spectrum

The degradation by UV spectrum can be identified by three ways

1. **Change in absorption pattern and fingerprint (shift of maxima, appearance and disappearance of maxima, change in cut off wavelength):** The following UV spectrum (Figure 1.21) shows the change in absorption pattern of compound after 90 minutes.

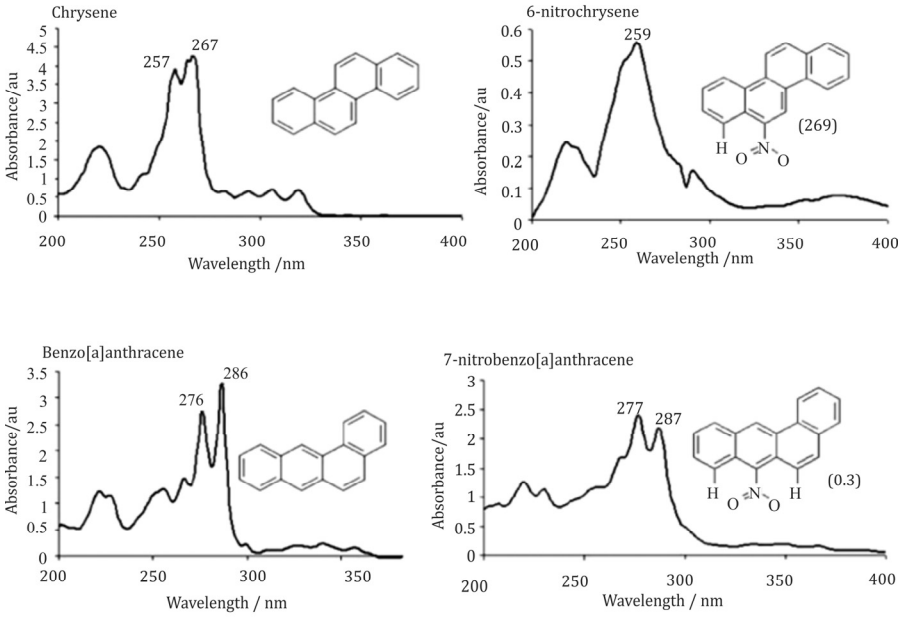


Figure 1.26 Overlay UV- Spectra of four derivatives of chrysene.

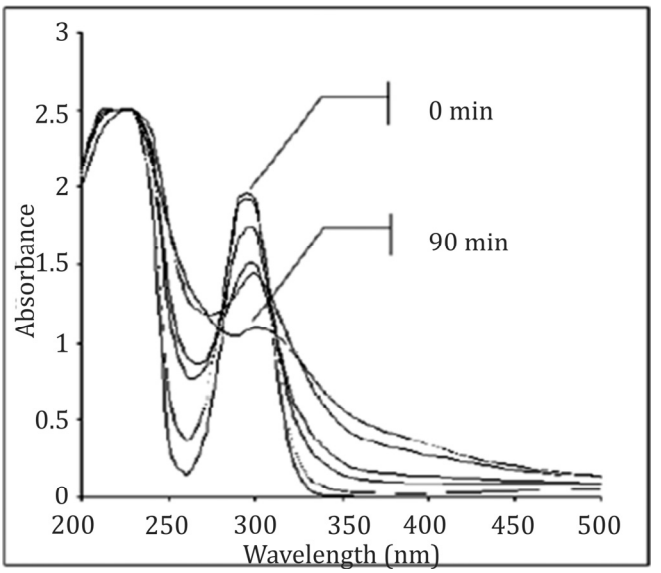


Figure 1.27 Overlay UV- Spectra of decomposed product at different time intervals.

2. **Change in absorption (at maxima and valley):** The UV spectrum (Figure 1.21) shows that there is an absolute shift of UVmax and valley point with respect to time.
3. **Change in shape of the spectrum and AUC of the UV spectrum:** The UV spectrum (Figure 1.22) shows the change in AUC and shape of UV spectrum up on degradation.

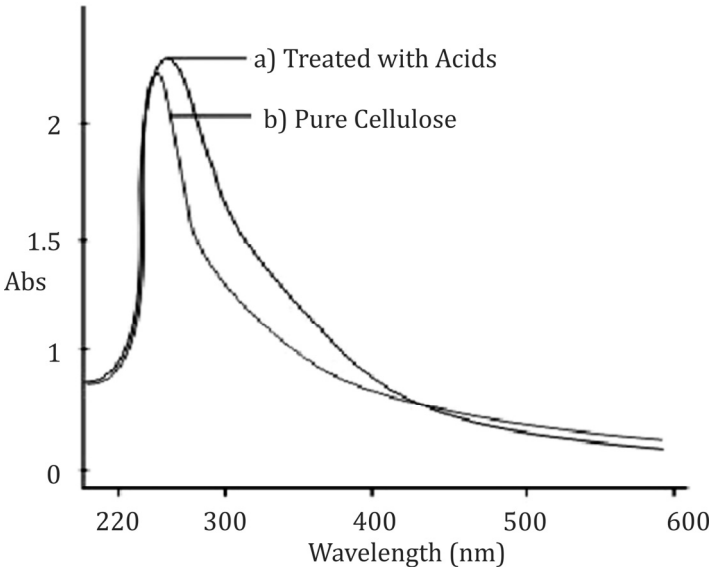


Figure 1.28 Overlay UV- Spectra of decomposed cellulose (Acid treated) and Pure Cellulose.

Differentiation of benzenoid and quinonoid by UV spectrum: It is more common with indication, at different pH the indicator colour will be different so the UV-visible spectrum also different for same indicator at different pH. The following is the example (Figure 1.22) of Phenol red indicator; we can observe the shift of spectrum between acid form and base form.

Isosbestic point: However, there is a different absorption pattern for acidic /basic or ionized or unionized form of a chemical species, there a wavelength in which two compounds or different forms will have same absorbance. This particular wavelength is called as isosbestic point (Figure 1.22). The isosbestic point and its relevant absorbance are very import in simultaneous or multicomponent analysis (Q-analysis or absorbance ratio method).

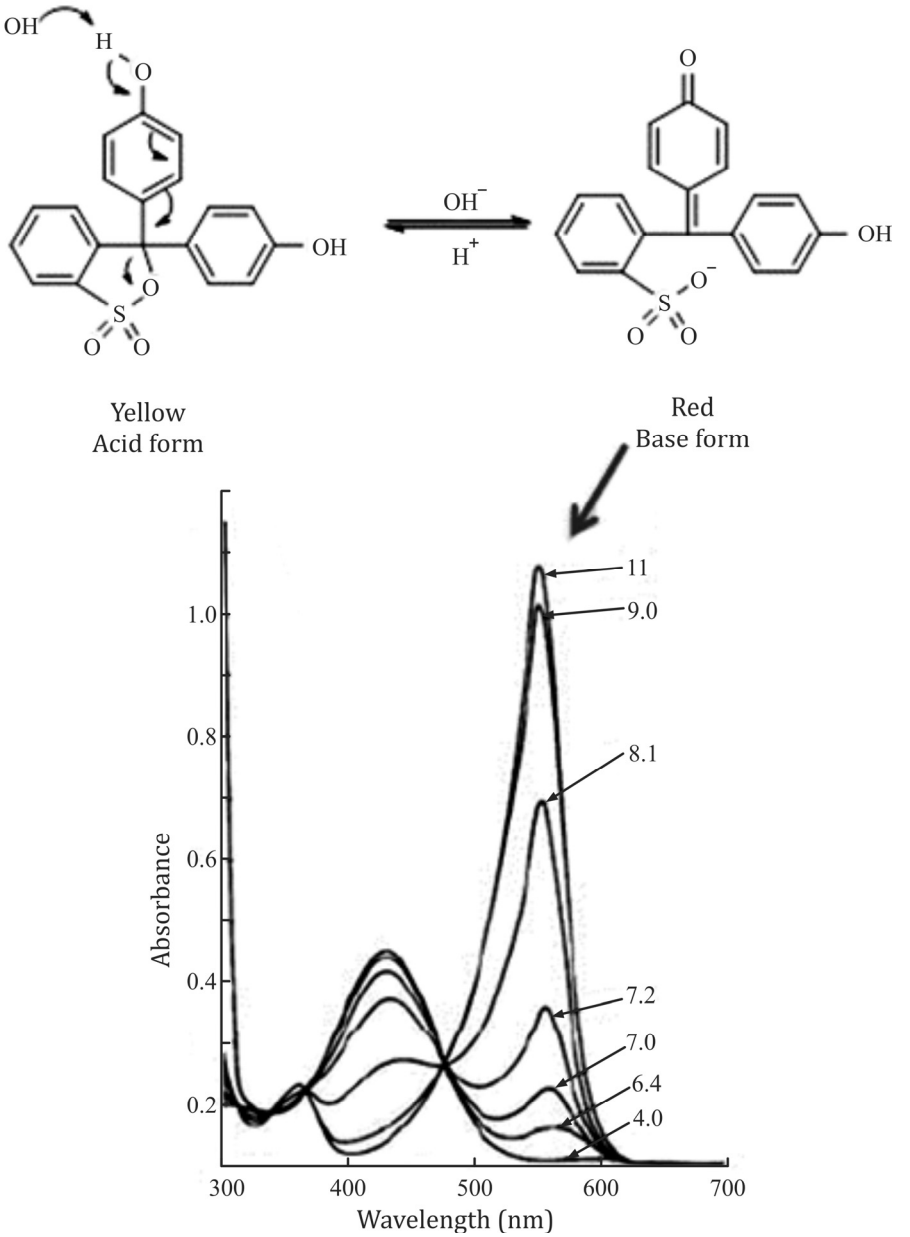


Figure 1.29 Overlay UV - Spectra of Phenol red indicators (at different pH).

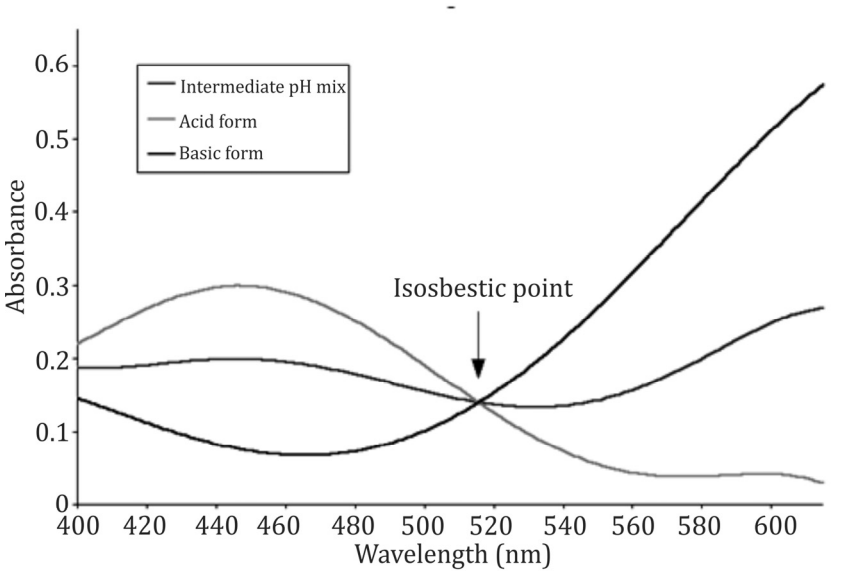


Figure 1.30 Overlay UV - Spectra of a compound at different pH (Isosbestic point).

Differentiation of ionized and unionized form of compounds (ex. Barbiturates): The following UV spectrum (Figure 1.23) represents the example of ionized and unionized spectrum of Barbiturate. It can be noticed that maxima was absent unionized form of barbiturate (A), Where Spectrum B' and C' are other ionized form of barbiturate. So, pH of solvent in quantitative analysis is very important factor for certain drugs.

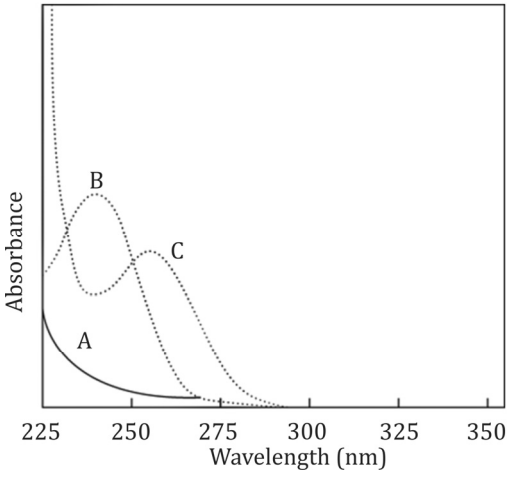


Figure 1.31 Overlay UV- Spectra of Barbiturate (A: unionized; B &C are ionized at different proportions).

Determination of pKa by UV-spectrum

When there is a pH dependent absorbance changes takes place for a molecule, it is possible to determine the pKa by UV spectroscopy. The following figure 1.32 shows, the change absorbance with respect to change in pH values for isolapachol. The pKa was found to be 5.75 for isopachol

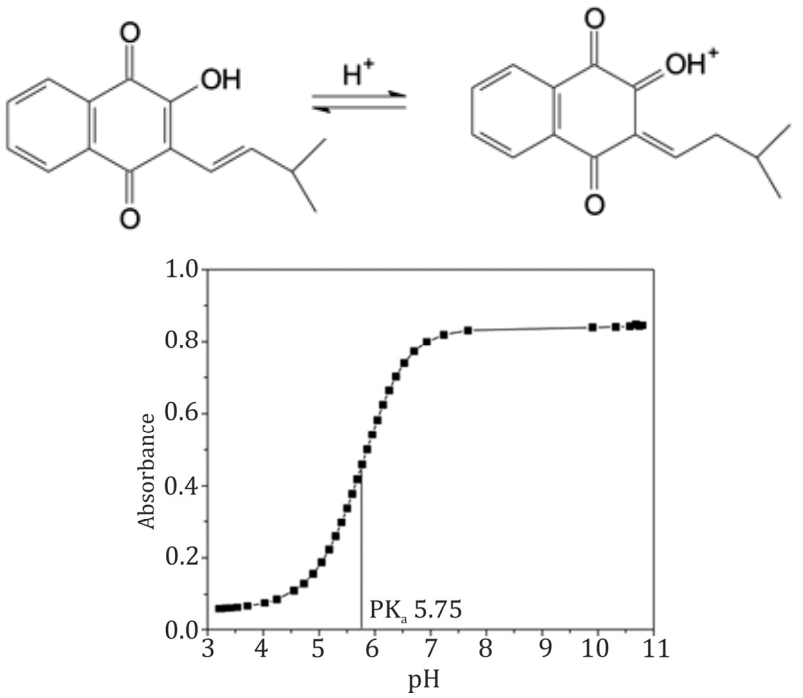


Figure 1.32 Effect of pH on UV- Visible light absorbance.

pKa is the pH in which both ionized and unionized forms are equal. That exhibit direct relation in absorbance value.

Use of UV spectrum in Immune mediated complex: The following spectrum (Figure 1.33) shows the assay relies upon gold nanoparticles (AuNPs) based complex in the immunoreactions of antigen and antibody that can induce the aggregation of antibody-functionalized AuNPs. The UV spectrum shows significant shift in absorbance as well in wavelength to indicate the presence or absence of complex.

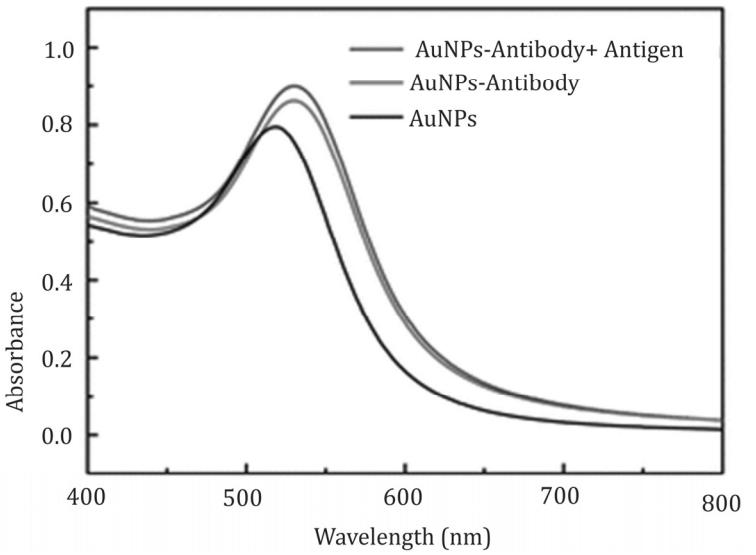


Figure 1.33 Overlay UV spectra in Immune mediated recation and its complex.

Identification of natural pigments: The UV spectrum is the good indicator to identify the natural pigments. The following figure 1.34 shows the UV- visible spectrum of several natural compounds which are distinct in their absorption characteristics.

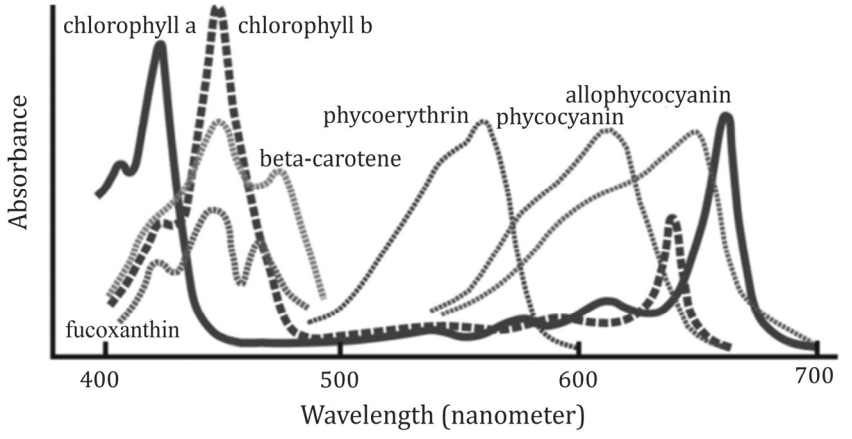


Figure 1.34 Overly UV spectra of natural pigments.

Detection of impurity by UV spectrum: There is a chance for predicting the presence of impurity (preferably at moderate level of concentration). The following UV spectrum (Figure 1.35) shows considerable shift of 2 nm for both maxima (275 nm) and Valley (248 nm) along with the maxima effect below 246 nm. This may be because of the presence of potential dissolved impurities.

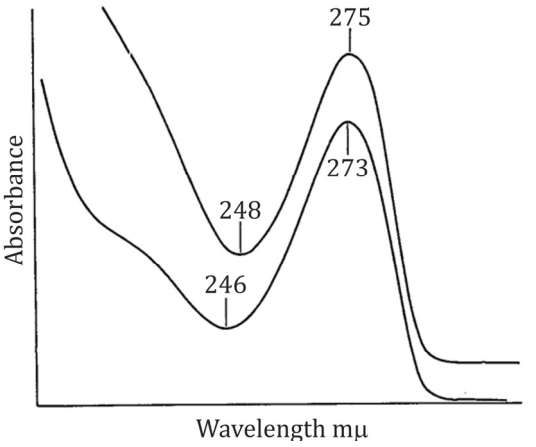


Figure 1.35 Detection of impurity by UV spectra.

Identification of Active pharmaceutical ingredients in Dosage form: It is done by comparison of Sample UV spectrum with reference UV spectrum (finger print matching; Figure 1.36). The excipient effects in UV absorption below 250 nm have to be considered or placebo correction may be required. The spectra A and C are sample, B is standard.

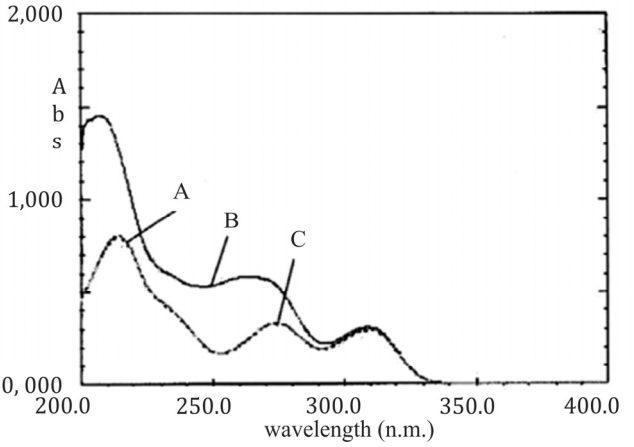


Figure 1.36 Identification of API in dosage form by UV spectrum.

Identification mixture using standards: If a UV spectrum is considered as mixture of two drugs (A and B), then the overlay spectra of pure drugs A and B and the mixture C can give us the information regarding authentication of drugs in the mixture. In the below figure 1.37, A and B are UV spectrum of pure drugs, where C (dotted line) is the mixture. The spectrum of C can be matched with spectrum of both A and B.

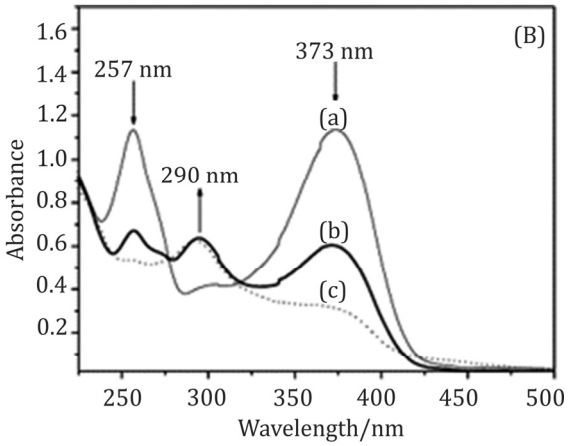


Figure 1.37 Identification of mixture using standard by UV spectra overlay.

WOODWARD RULES (Rules in calculation of UV max (λ max) for chemical compounds)

"As lambda (**λ**) max increases that indicate the compound structure has more conjugation, with increase in pi-electron density. This lambda **(λ)** max vary from structure to structure (Figure 1.38 and 1.39) even with same number of double bond and conjugation, Lambda max (**λ**) is depends on position of the double bond rather than degree and number of double bond"

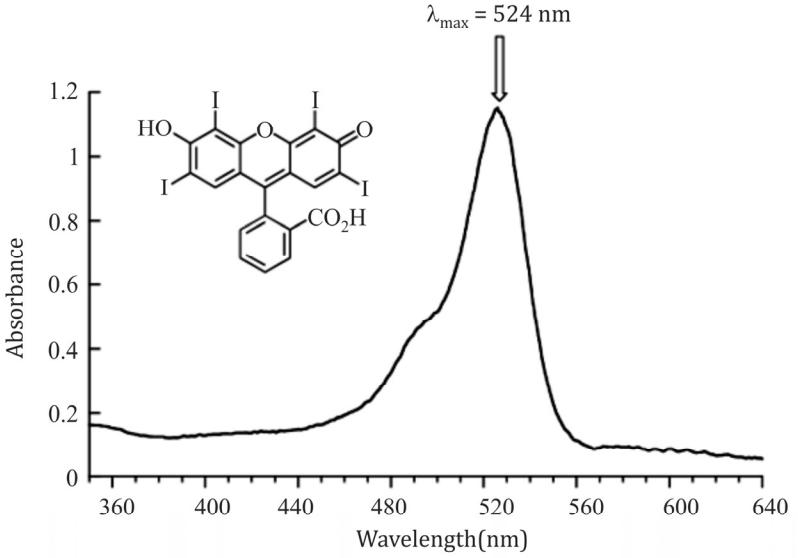


Figure 1.38 Determination of lambda (**λ**) max in UV Spectrum.

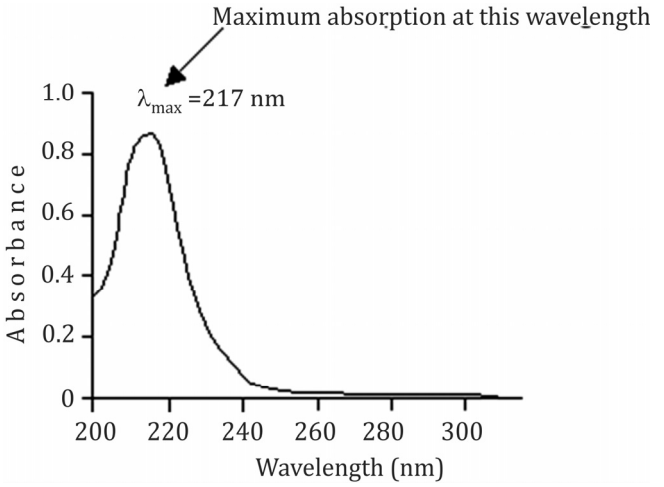
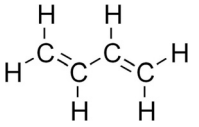


Figure 1.39 Maximum Absorption (λ max) in UV absorption of diene compounds.

'WOODWARD- FIESER RULES: Each type of diene or triene system is having a certain fixed value at which absorption takes place; this constitutes the *Base value or Parent value*. The contribution made by various alkyl substituents or ring residue, double bond extending conjugation and polar groups such as –Cl, -Br etc are added to the basic value to obtain λ_{max} for a particular compound'.

Descriptors used in (λ max) calculations of 1, 3 butadiene chromophore



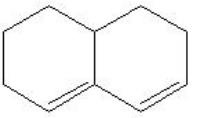
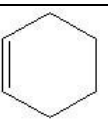
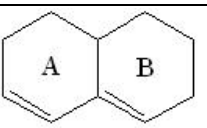
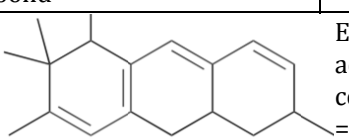
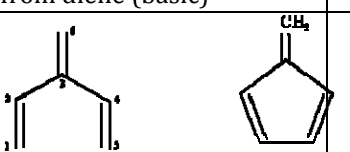
Core chromophore

NOTE: *Only conjugated dienes are considered to account for woodward rules for prediction* (**λ** *max)*.1, 3 *–butadiene system is essentioal to consider the structure for woodward rule.*

Table 1.3 Basic understanding in Woodward rule for calculation of λ max

Descriptors	Definition	Structure	Value
Homoannular Diene	Cyclic diene having conjugated double bonds in same ring		253 nm

Table 1.3 ***contd…***

Descriptors	Definition	Structure	Value
Hetero annular Diene	Cyclic diene having conjugated double bonds in different rings (A and B)		215 nm
Endocyclic double bond	Double bond present in a ring		Not considered. It's a part of basic value
Exocyclic double bond	Double bond in which one of the doubly bonded atoms is a part of a ring system.	 Here Ring A has one exocyclic and endocyclic double bond. Ring B has only one endocyclicdouble bond	5 nm
Addition Conjugation	Addition one double to the basic Chromophor e (diene)	 This molecule has two addition conjugation apart from diene (basic)	Each additional conjugation = 30 nm
Cross conjugation	Addition conjugation which is branched	 I II The above structure the additional conjugation not considered as addition conjugation.	0 nm

(A) Calculation of λ max for the given 1, 3 butadiene structures using Woodward Fieser Rule

Table 1.4 Calculation of λ max for the given 1, 3 butadiene structures using Woodward Fieser Rule

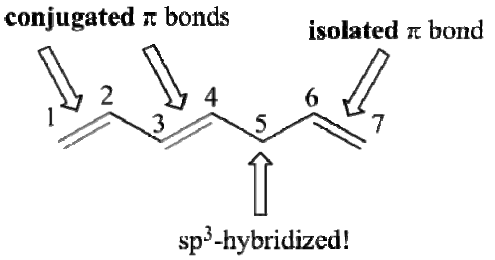
Descriptors	Value to be considered in calculation (in nm) – Substituent effect (Auxochrome)
Homoannular (cisoid)	253 nm
Heteroannular (transoid)	215 nm
Acyclic diene	217 nm
Double bond extending conjugation	30 nm
Alkyl substituent or ring residue	5 nm
Exocyclic double bond	5 nm
-OC(O)CH_3	0 nm
-OR	6 nm
-Cl, -Br	5 nm
-NR_2	60 nm
-SR	30 nm
Phenyl ring	75 nm

λ max = BAERS (B+A+E+R+S)

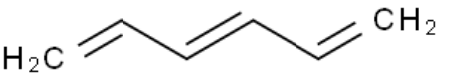
- *B*- Basic value (such as Hetero annular or Homoannular or acyclic)
- *A* – additional conjugation
- *E* – exocyclic double bond
- *R* – Ring residues
- *S* – substitution on the conjugated chain (not on other part of structure)

Additional Conjugation

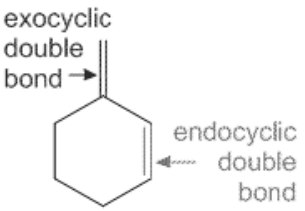
Bonds in alternative position are considered to be extending conjugation. In the below structure the bond between C6 and C7 is isolated from diene, so it cannot be considered as extending conjugation or addition conjugation. **Thus it has 0 (zero) value in calculation.**

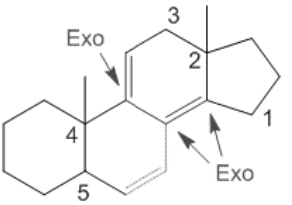


In the below structure, there is a ONE extended or additional conjugation to diene. So (1 × 30 nm) 30 nm has to be added in the calculation.

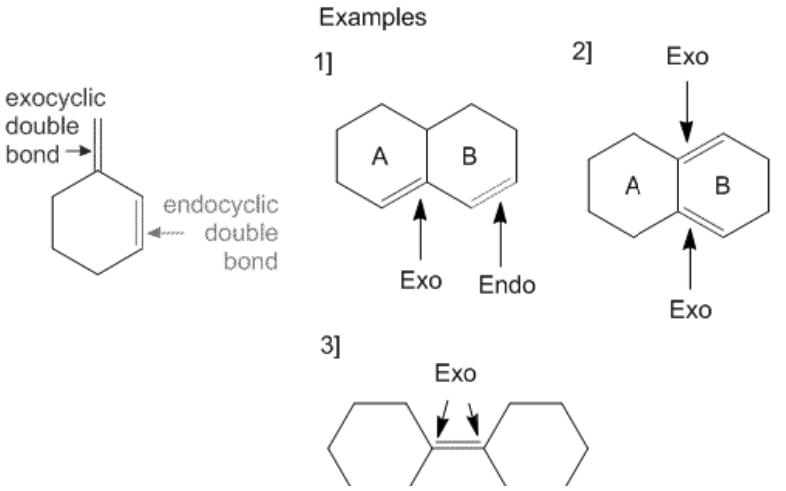


Exocyclic Double Bond





- The above structure has both heteroannulardiene and homoannulardiene. But basic value has be based on homoannulardiene.
- The exocyclic double bond has to be calculated based on the position, thus above structure has THREE exocyclic double bond (3 × 5 = 15 nm)

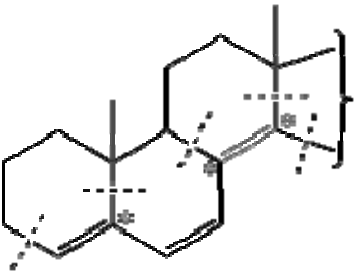


The above figure differentiates between exocyclic (shown in red) and endocyclic (shown in green) double bonds.

1. In example 1, the double bond present within ring A is exocyclic to ring B as it is attached to an atom which is shared between ring A and ring B, while the double bond present in ring B is not connected to any ring A atoms and is within just one ring, hence making it endocyclic.
2. In example 2, both double bonds are present within ring B with connections to shared carbon atoms with ring A, making both the double bonds exocyclic.
3. In example 3, there is a single double bond which is exocyclic at two points to two different rings. In such a case, the influence would be 2 times + 5 nm (i.e + 10 nm).

"***Solvent effects:*** *Since the conjugated diene base is relatively non-polar, contribution due to different solvents is very minor and can be ignored in most cases".*

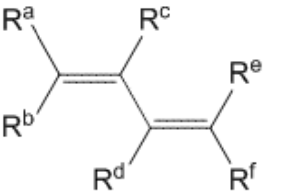
Ring Residue



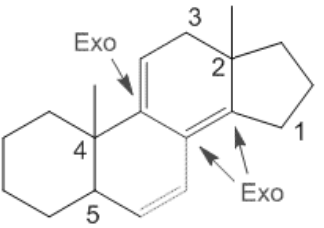
It is the number of bond cleavage to be done on the ring structure to isolate the entire conjugated chain, thus above structure has five ring residue (5 × 5 = 25 nm) and 25 nm has to added in the calculation

Substitution on the Structure

In the below structure all substitution (R) are attached with the carbon which involved in the conjugation, hence value has to added (Auxochrome effect) in the calculation.

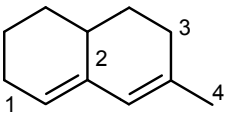


In the below steroidal compounds, substitutions ar C2 and C4 are not considered, as they are not a part of conjugated system, thus the below structure has no substitution value in calculation. But has ring residue, exocyclic and basic values.

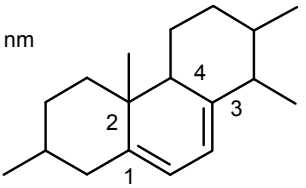


Example of Calculation

Absorption maximum : 214 + 20 + 5 = 239 nm

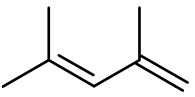


Heteroannular diene : 214
alkyl substituents 4 × 5 = 20
exocyclic double bond : 5



Homoannular diene : 253
alkyl substituents : 4 × 5
exocyclic double bond : 2 × 5
Absorption maximum : 253 + 20 + 10 = 283 nm

Example 1:



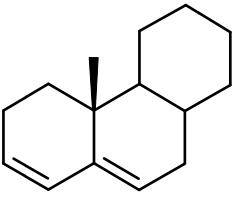
Basic value (acyclic)	Acyclic diene	217
Additional extended conjugation	--	0
Exocyclic double bond	--	0
Ring residue	Not a cyclic compound	0
Substitution	3 alkyl (3 × 5)	15
TOTAL		**232 nm**

Example 2:

 Basic value (acyclic)	Homoannular	253
Additional extended conjugation	--	0
Exocyclic double bond	--	0
Ring residue	2 residues (2×5)	10 nm
Substitution	--	0
TOTAL		**263 nm**

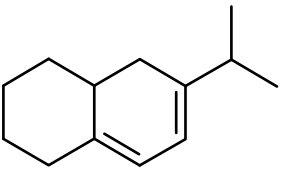
Example 3:

Table 1.5 λ max for structural futures



Basic value (acyclic)	Heteroannular	215
Additional extended conjugation	--	0
Exocyclic double bond	One (1 × 5)	5
Ring residue	3 residues (3 × 5)	15 nm
Substitution	--	0
TOTAL		**235 nm**

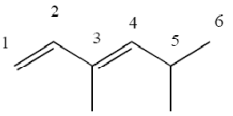
Example 4:



Basic value (acyclic)	Homoannular	253
Additional extended conjugation	--	0
Exocyclic double bond	One (1 × 5)	5
Ring residue	3 residues (3 × 5)	15 nm
Substitution	1 (isopropyl) alkyl group	5 nm
TOTAL		**278 nm**

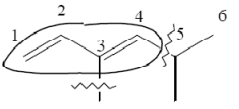
Other examples:

Diene Example #1:

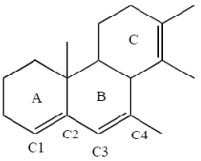


Calc. λ_{max} = 214 (acyclic base) + 5 (alkyl auxochrome at C_3) + 5 (alkyl auxochrome at C_4) = 224 nm

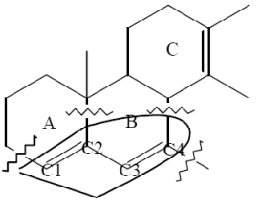
One way to identify an auxochrome is to draw a loop around the entire conjugated system (including extending olefins) and then add hash marks across all bonds attached to the loop. The hash marks define auxochrome attachments.



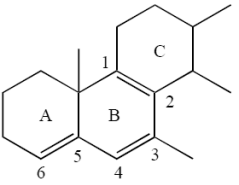
Diene Example #2:



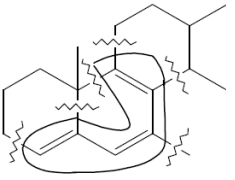
Calc. λ_{max} = 214 (heteroannular since two pi bonds are not in the same ring) + 20 (5 + 5 + 5 + 5 – 20, for each of the alkyl or ring auxochromes attached to C_1, C_2, C_4, and C_4) + 5 (pi bond of C_1-C_2 is exocyclic to ring B) = 239 nm



Diene Example #3:



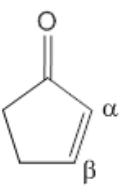
Calc. λ_{max} = 253 (choose diene with highest base value, pi bonds C_{1-2} and C_{3-4} are within same ring, so homoannular base should be selected) + 30 (C_{5-6} pi bond is conjugated to diene and is therefore an extending diene) + 5 (C_{5-6} is exocyclic to ring B) + 30 (5 + 5 + 5 + 5 + 5 + 5 = 30, for the alkyl or ring auxochromes at C_1, C_1, C_2, C_3, C_5, and C_6) = 318 nm



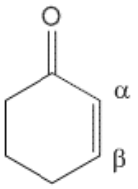
(b) Calculation of λ max for unsaturated carbonyl compounds

The unsaturated carbonyl compounds (C=O) still considered as conjugated system, so the same method of calculation can be used, with little modification in basic value and residue and substitution values.

Cyclic Unsaturated Carbonyl System



Cyclopentenone

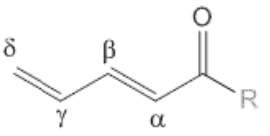


Cyclohexenone

Base value:

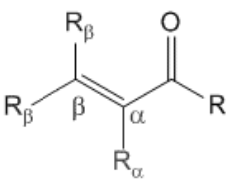
(a)	Acyclic α, β unsaturated ketones	=	214 nm
(b)	6 membered cyclic α, β unsaturated ketones	=	215 nm
(c)	5 membered cyclic α, β unsaturated ketones	=	202 nm
(d)	α, β unsaturated aldehydes	=	210 nm
(e)	α, β unsaturated carboxylic acids & esters	=	195 nm

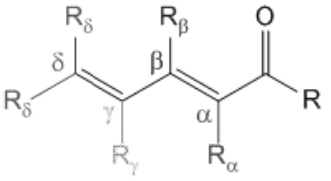
Acyclic unsaturated carbonyl system



Extended Conjugation

R = H = Aldehyde = 240 nm
R = Alkyl = Ketone = 245 nm
R = OAlkyl = Ester = 225 nm





Auxochrome value to be added to basic value of unsaturated carbonyl system in calculation of λ max.

Auxochrome	Alpha	Beta	Gamma	delta
OH or	35	30	--	50
OR (-OCH3)	35	30	17	31
Br	25	30	--	--
Cl	15	12	--	--

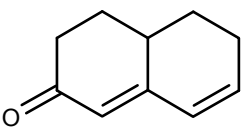
CH3 or Any alkyl group	10	12	18	18
Ring residue	10	12	18	18
-OCOR	6	6	6	6
NR2	--	95	--	--

NOTE: Alpha, beta, delta position is assigned only to conjugated carbon system.

Formula

λ max = BAERS (B+A+E+R+S)

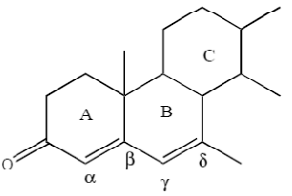
- *B* - Basic value (such as acyclic or cyclopentanone or hexanone)
- *A* – additional conjugation
- *E* – exocyclic double bond
- *R* – Ring residues(different value based on the position like alpha, beta etc)
- *S* – substitution on the conjugated chain (not on other part of structure)



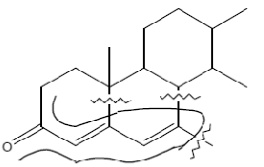
Basic value (acyclic)	**Cyclohexanone**	**215**
Additional extended conjugation	01	30
Exocyclic double bond	One (1 × 5)	5
Ring residue	12 (beta) + 18 (delta)	30 nm
Substitution	--	0 nm
TOTAL		**280 nm**

Other illustrated Examples

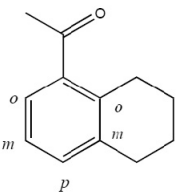
Enone Example #1:



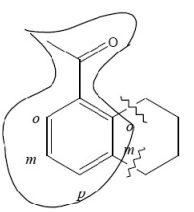
Calc. λ_{max} = 215 (cyclohexenone base) + 30 (extending conjugation) + 5 (α,β olefin is exocyclic to ring B) + 12 (β auxochrome) + 36 (2 δ auxochromes) = 298 nm



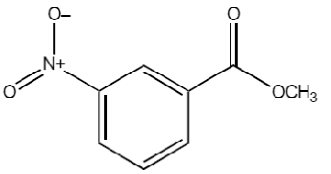
Benzoyl Example #1:



Calc. λ_{max} = 246 (Benzoyl Base, where Z is the aliphatic methyl group) + 3 (*o* auxochrome) + 3 (*m* auxochrome) = 252 nm

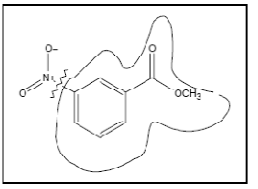


Benzoyl Example # 2:

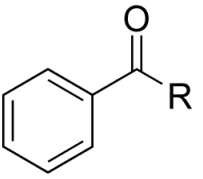


Calc. λ_{max} = 230 (Benzoyl Base, where *Z* is O-R in this ester functionality) + ?
(There is no value listed for a *meta* nitro group.)

> **230 nm**. The rules are not perfect. They do not allow you to make predictions for all compounds, not even all the simple ones. The best you can predict in this situation is that the observed λ_{max} should be greater than 230 nm. After all, the nitro group contains a π bond that extends the length of the conjugated π system. And if nothing else, you know that greater conjugation means longer wavelength λ_{max}.



(C) λ max Calculation for Aromatic (benzoyl system) compounds



λ max = Basic value + Substitution effect (ortho or meta or para)

Basic values:

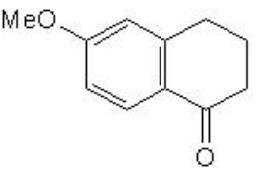
- (a) Ar-COR (ketone) = 246 nm
- (b) Ar-CHO (Aldehyde) = 250 nm
- (c) Ar-CO_2H (Acid) = 230 nm
- (d) Ar-CO_2R (Ester) = 230 nm

Value (in nm) for Substitution effect (ortho or meta or para)

Table 1.6 λ max for structural futures

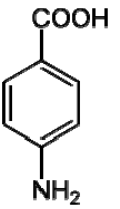
Auxochrome	ortho	meta	para
-NR2 (amine)	20	20	85
-NH2	13	13	58
-NHCH3	-	-	73
-NHCOCH3	20	20	45
-OH , -OR	7	7	25
Alipahtic (- R)	3	3	10
-Br	2	2	15
-Cl	0	0	10
-O+ (oxonium)	11	20	78

Example:



λ max = 230 nm (base value) + 58 nm (-NH2 at para position)

= 288 nm



λ max = 246 nm (base value) + 3 nm (alkyl ring residue) + 25 nm (OCH3 at para position)

= 274 nm

Exercise: *calculate the □ max for the following structure ?*