ULTRAVIOLET- VISIBLE SPECTROSCOPY

Absorption of light in the UV/Visible part of the spectrum 200-800 nm.

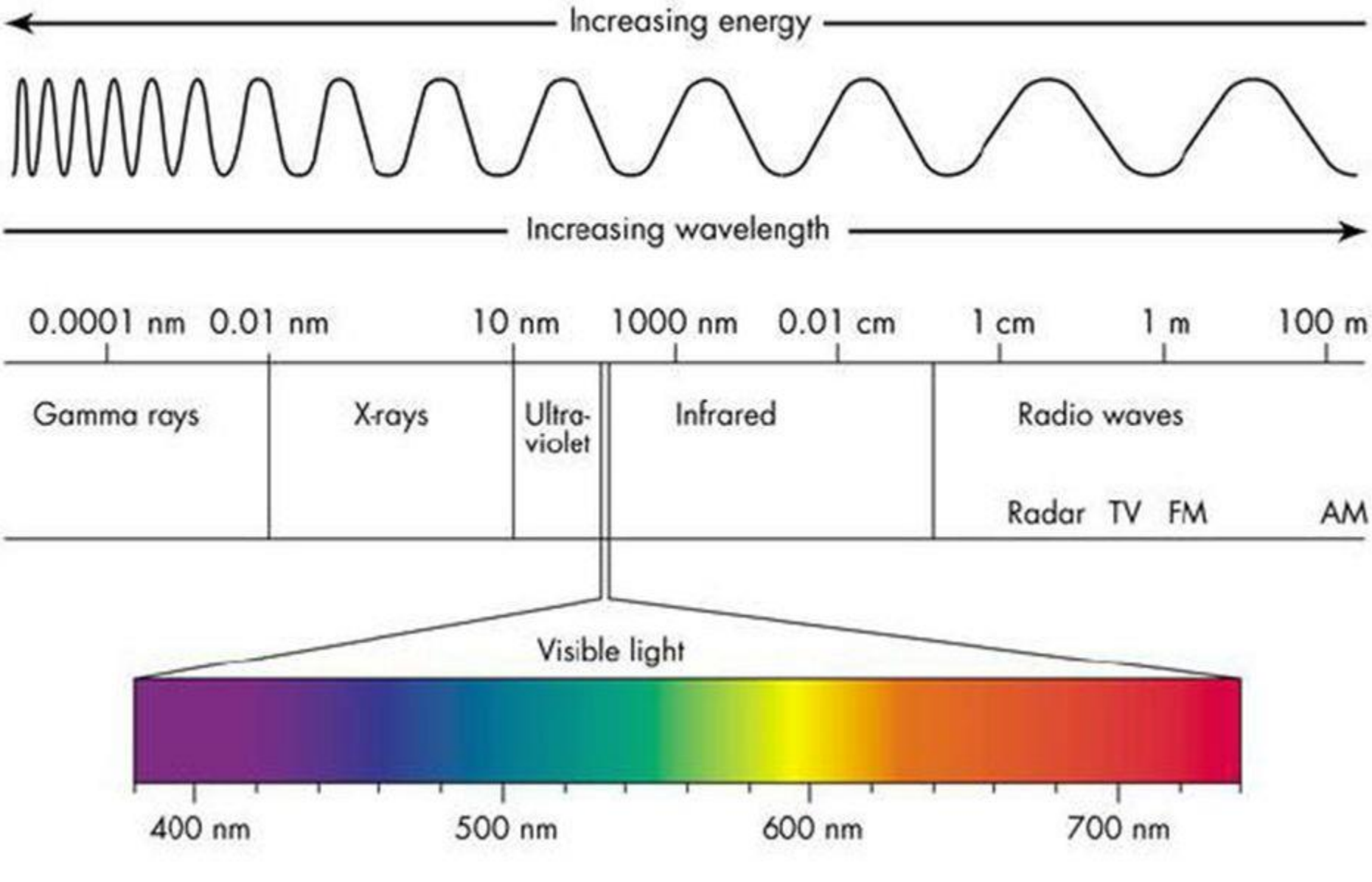
The result of the absorption of electromagnetic radiation in this region of the spectrum are transitions between electronic energy levels. Absorption of visible and ultraviolet (UV) radiation is associated with excitation of electrons, in both atoms and molecules, from lower to higher energy levels.

*The type and degree of conjugation in the molecule is determined.

10-200 nm ⇨ Vacuum UV (Far UV)
200-400 nm ⇨ UV region
400-800 nm ⇨ Visible region

} Electronic Spectroscopy

Electromagnetic Waves



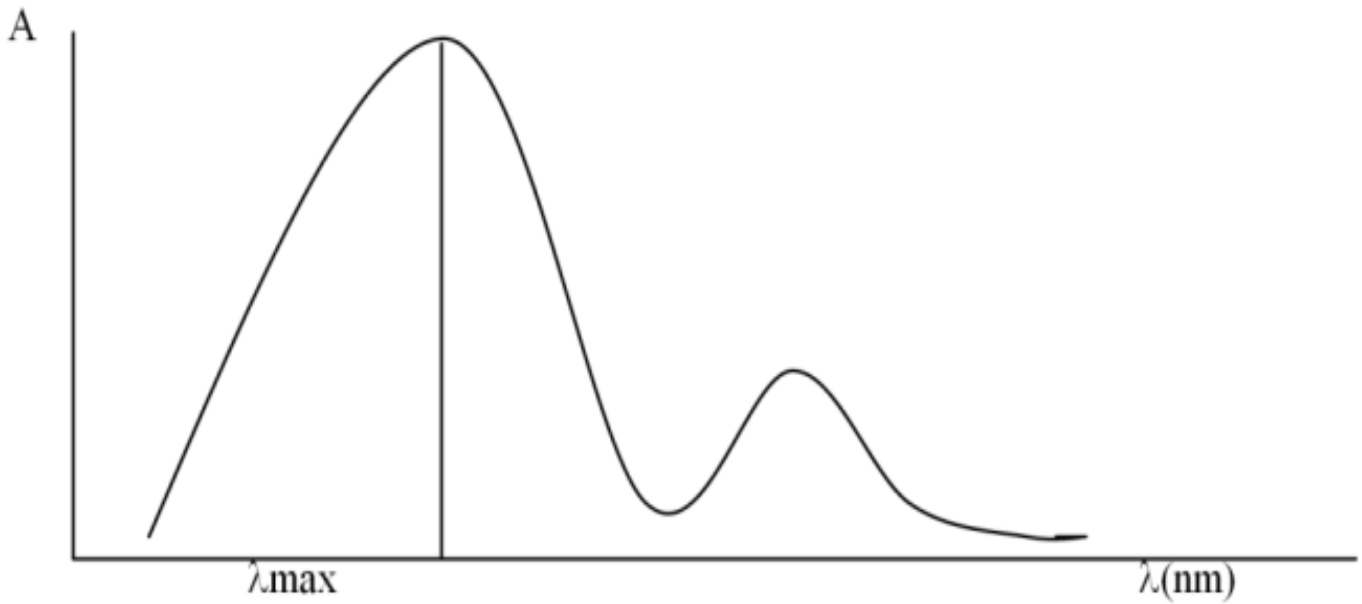
- The area of the electromagnetic radiation that contains the rays of the wavelength of 200-400 nm is the UV (Ultraviolet / Ultraviolet) region. Since the rays between 10-200 nm are absorbed by the oxygen in the air, this area is called Vacuum UV (Far UV) and it is operated in this area with the help of special mechanisms and in the air-free environment (vacuum).

- The area used in the rays at 200-400 nm is called the ultraviolet (UV) region. However, since the rays in this area are absorbed by ordinary glass, the glasses used in UV spectroscopy are made of quartz glass.

- The portion of the electromagnetic spectrum lying between 400 and 800 nm is the visible region. Light waves with wavelengths between these limits, appear colored to the human eye.

- Different types of light sources are used for UV regions to obtain radiation at different wavelengths. Advanced spectrophotometers have both types of radiation source and change the frequency properly and automatically. The resulting radiation or radiation beams absorbed through the transmitted beam are sensitively detected by the device and transferred to the spectrum (as absorbance band). The graph of the relationship between the amount of absorption and wavelength or frequency of light is called the absorption spectrum. (in other words, the graph of the relationship between absorbance and transmittance is called absorption spectrum)

Absorption Spectrum



Absorption of Organic Substances in UV and Visible Regions

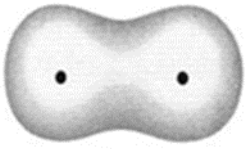
- The electrons or bond electrons of outer layer of matter absorb the beam energy or electromagnetic energy. When they absorb the light rays, they rise to higher energy levels as previously mentioned. Some of the bonds have low energies and absorb low-energy (long wavelength) rays. Some of the bonds have larger energies, which absorb higher energy (short wavelength) rays. This difference, the absorption of rays of different wavelengths by different bonds gives us the opportunity to analyze. For example, a molecule that does not carry a single bond and a functional group, absorb a very short wavelength and a high energy, but since the same beams are also absorbed by other molecules in the air, it is necessary to work in a vacuum without air. Since the electrons in the molecules bearing functional group can more readily reach their upper energy levels, the lower energy (wavelength longer) rays may be sufficient to stimulate them.

Theory of Absorption and Spectrum Explanation

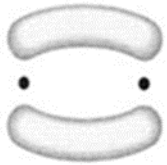
- In addition to some other theories on the subject, the main theory used in explaining the formation of absorption and spectra is the MOLECULAR ORBITAL THEORY. The theory is based on the presence of molecular bond orbitals, anti-bond orbitals and electrons responsible for absorption in the molecules analyzed.

- Molecular Orbital: The electron cloud of bonding electrons which is not localised between two atoms or in other words, overlapping atomic orbitals are called molecular orbital.

- Molecular Bond Orbital: It is the orbital which is formed by the overlaping of two atom orbitals and has a lower energy level than the energy levels of the orbitals of these atoms in total. It can occur in two ways:
- The orbitale, in which the electrons make a mono-valence (single) bond, is called the sigma molecular orbital and is indicated by (σ). (single bonds are also referred to as σ bonds of sigma electrons).
- The orbitale in which the bonds make double bonds, is called the pi molecular orbital and indicated by (π). (double bonds are also referred to as π bonds of π electrons).



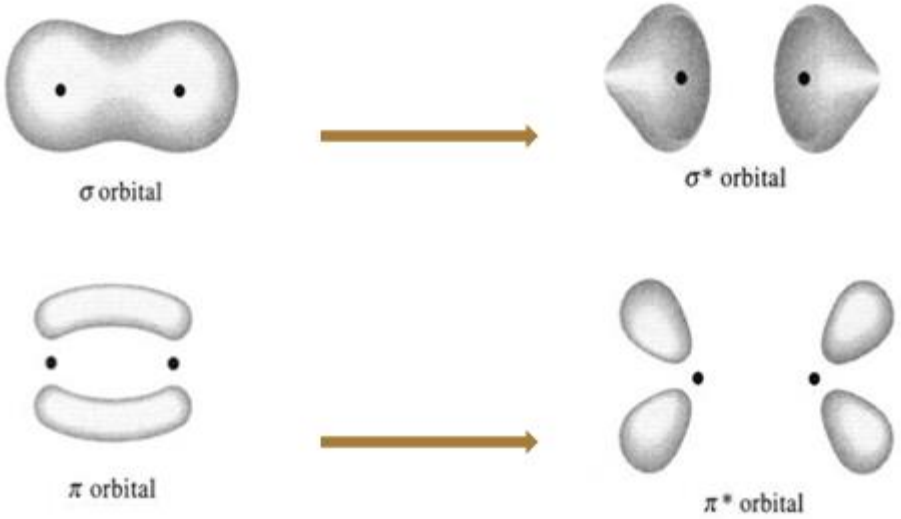
σ orbital



π orbital

- Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals

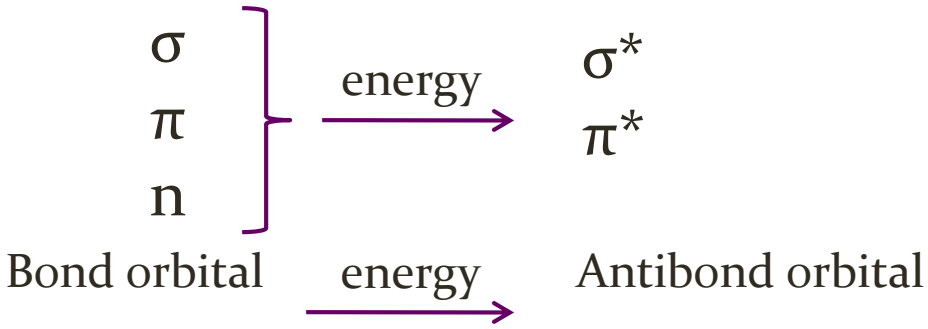
- Antibonding Orbital: It is the orbital which is formed by the overlaping of two atom orbitals and has a higher energy level than the energy levels of the orbitals of these atoms in total. It is indicated by (σ^*) and (π^*).



Absorption and Electrons Responses for Absorption: Electrons that are responsible for absorption (also referred to as outer layer electrons) can be listed as follows;

- Electrons connecting the atoms in the molecule
- Single bond, (σ) electrons
- Double bond, (π) electrons
- The unconjugated (n) electrons on hetero atoms (such as O, S, N and X) of molecule

The electrons listed above, if they encounter with the energy of the appropriate level of their energy, they absorb them. the absorption takes place by transferring from the initial bond-orbital energy level (σ), (π) and (n) to the anti-bond energy levels (σ^*) and (π^*).



Absorption and Chromophore Groups:

- Although the absorption of ultraviolet radiation results from the excitation of electrons from ground to excited states, the nuclei that the electrons hold together in bonds play an important role in determining which wavelengths of radiation are absorbed. The nuclei determine the strength with which the electrons are bound and thus influence the energy spacing between ground and excited states. Hence, the characteristic energy of a transition and the wavelength of radiation absorbed are properties of a group of atoms rather than of electrons themselves. The group of atoms producing such an absorption is called a **chromophore**.
- Groups in a molecule which consist of alternating single and double bonds (conjugation) and absorb visible light are known as chromophores.
- As structural changes occur in chromophore, the exact energy and intensity of the absorption are expected to change accordingly.

- The attachment of substituent groups in place of hydrogen on a basic chromophore structure changes the position and intensity of an absorption band of the chromophore. The substituent groups may not give rise to the absorption of the ultraviolet radiation themselves, but their presence modifies the absorption of the principal chromophore. Substituents that increase the intensity of the absorption, and possibly the wavelength, are called **auxochromes.** Typical auxochromes include methyl, hydroxyl, alkoxy, halogen, and amino groups.

- Other substituents may have any of four kinds of effects on the absorption:
  Bathochromic shift (red shift)—a shift to lower energy or longer wavelength.
  Hypsochromic shift (blue shift)—a shift to higher energy or shorter wavelength.

  Hyperchromic effect—an increase in intensity.
  Hypochromic effect—a decrease in intensity

- Chromophore ⟶ a group of atoms responsible for UV/VIS absorption of the molecule
- e.g. -C=C-, -NO2 , -N=O, -C=O, -C=S

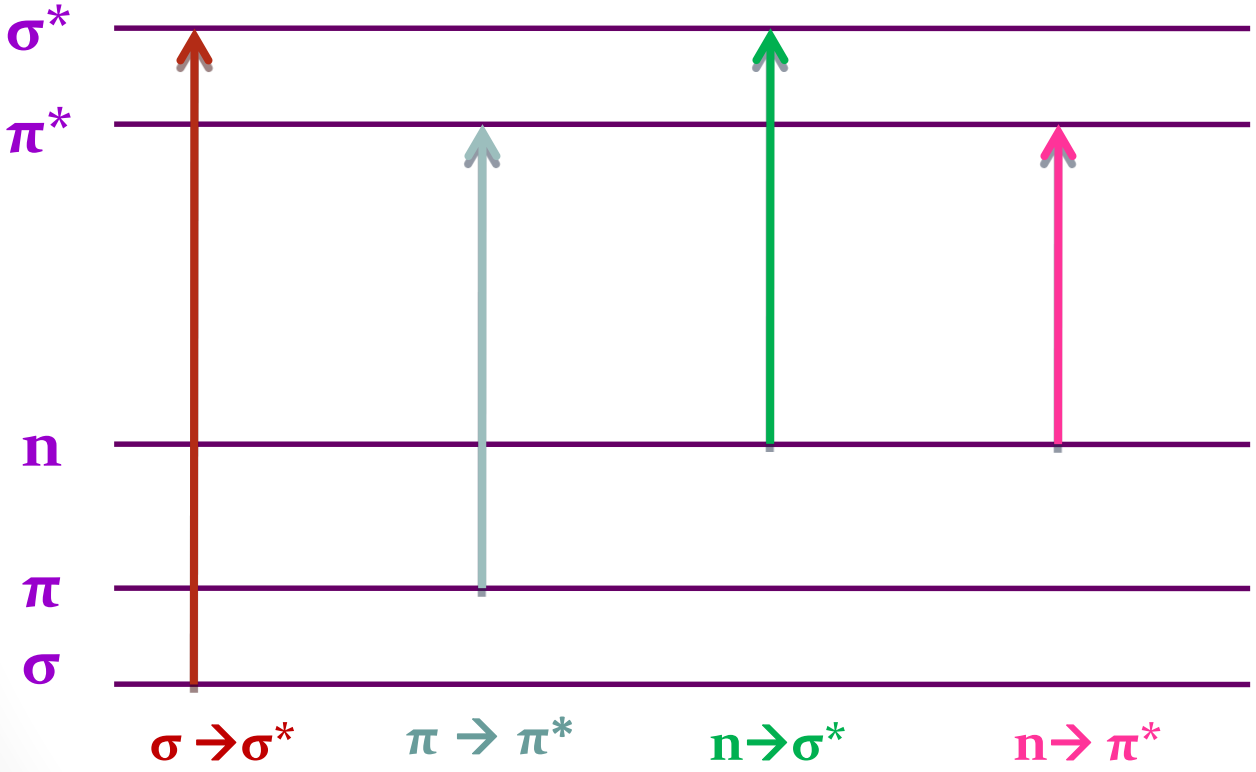
TYPICAL ABSORPTIONS OF SIMPLE ISOLATED CHROMOPHORES

Class	Transition	λ_{max} (nm)	Class	Transition	λ_{max} (nm)
R−OH	$n \rightarrow \sigma^*$	180	$R-NO_2$	$n \rightarrow \pi^*$	271
R−O−R	$n \rightarrow \sigma^*$	180	R−CHO	$\pi \rightarrow \pi^*$	190
$R-NH_2$	$n \rightarrow \sigma^*$	190		$n \rightarrow \pi^*$	290
R−SH	$n \rightarrow \sigma^*$	210	R_2CO	$\pi \rightarrow \pi^*$	180
$R_2C{=}CR_2$	$\pi \rightarrow \pi^*$	175		$n \rightarrow \pi^*$	280
R−C≡C−R	$\pi \rightarrow \pi^*$	170	RCOOH	$n \rightarrow \pi^*$	205
R−C≡N	$n \rightarrow \pi^*$	160	RCOOR′	$n \rightarrow \pi^*$	205
R−N=N−R	$n \rightarrow \pi^*$	340	$RCONH_2$	$n \rightarrow \pi^*$	210

- Oxychrome ⟶ a substituent that changes the wavelength or intensity of absorption when bound to a chromophore.
- e.g. -OH, -NH2, -Cl , alkyl, -OR

Molecular Orbital Energy Levels and Transitions Between Levels

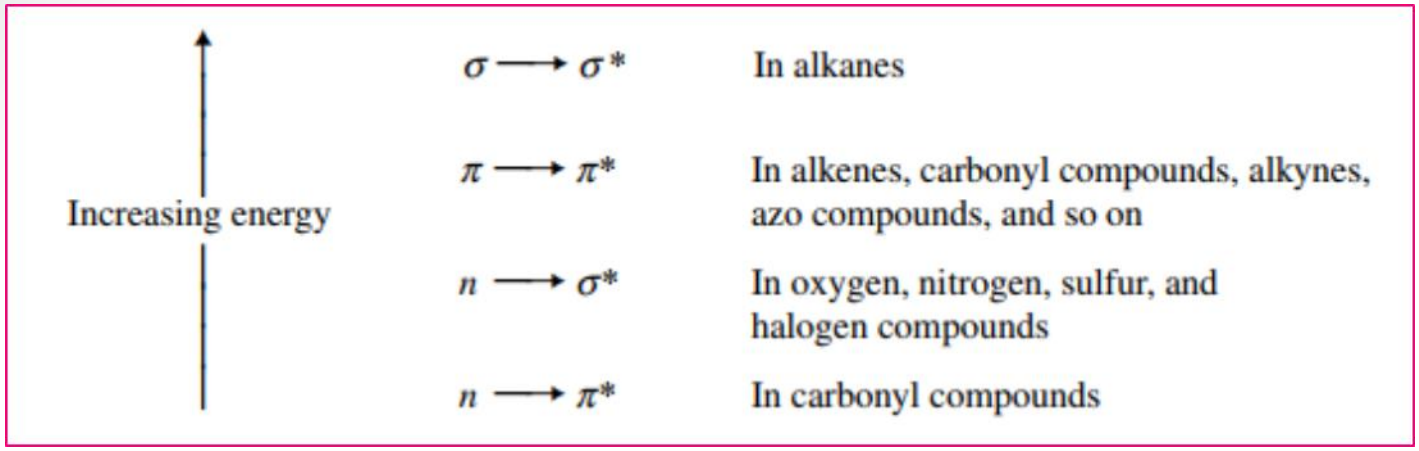
$\Delta E\ \sigma \rightarrow \sigma^* > \Delta E\ n \rightarrow \sigma^* \sim \Delta E\ \pi \rightarrow \pi^* > \Delta E\ n \rightarrow \pi^*$



Antibonding orbital

Nonbonding orbital

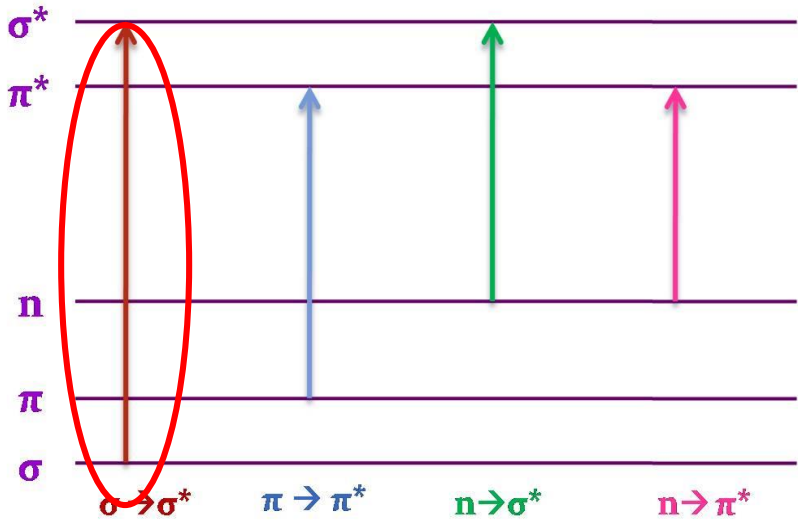
Bonding orbital



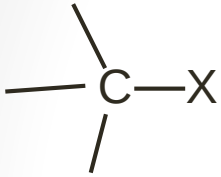
- ***σ→σ* transition:*** (125-150 nm) The energy of this transition is very high and is observed in the far ultraviolet region. The σ electrons of the C-C and C-H bonds in alkanes make this type of transition, thus σ→σ* transition has occurred.

  Alkanes do not absorb in the ultraviolet-visible region.

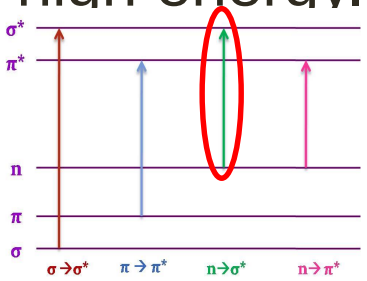
- An electron at the C-H bond can be stimulated with a beam at a wavelength of 125 nm to remove at σ*. An electron at the C-C bond can be stimulated with a beam at a wavelength of 135 nm. For example, when looking at the spectra of methane and ethane, methane has one peak at 125 nm and ethane has two peaks at 125-135 nm.


σ*
π*
n
π
σ
σ→σ*
π → π*
n→σ*
n→ π*

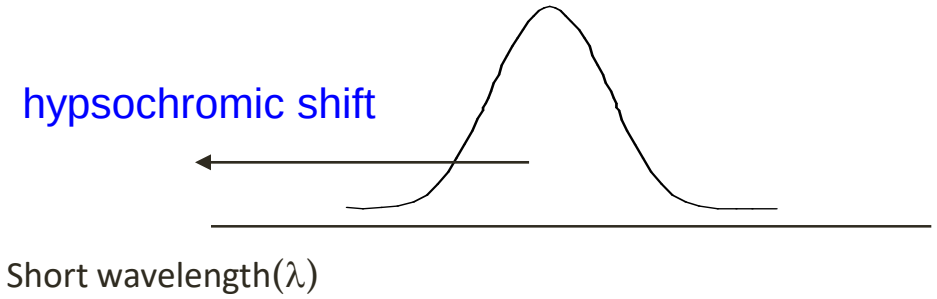
- ***n→σ* transition :*** (150-250 nm) These transitions are seen on compounds that contain unconjugated free electron pairs. These transitions require high energy.



X= Atom with unconjugated electron pairs
(F, Cl, Br, I, S, N, O)

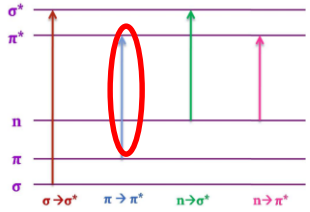


- As the polarity of the solvent increases, the maximum absorption shifts to the shorter wavelength. This shift is called hypsochromic shift or blue shift. It is very difficult to detect, should work in vacuum.

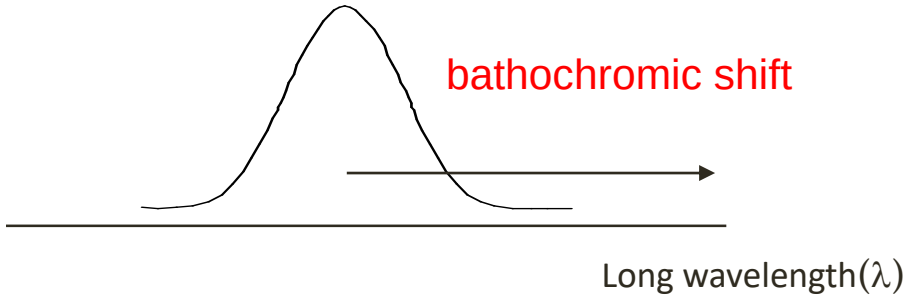


- ***π →π* transition :*** (200-700 nm) It is one of the most common transitions. These transitions can be performed with less energy rays with a wavelength of 200-700 nm. It is observed in unsaturated compounds.

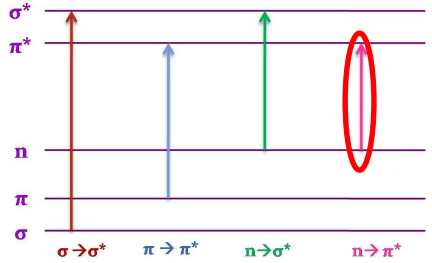
  C=C, C≡C, C=O, C=N, C=S, C≡N



- In such transitions, as the polarity of the solvent increases, the absorption peak shifts to the longer wavelength. This shift, which occurs depending on the solvent, is called a bathochromic shift or red shift.

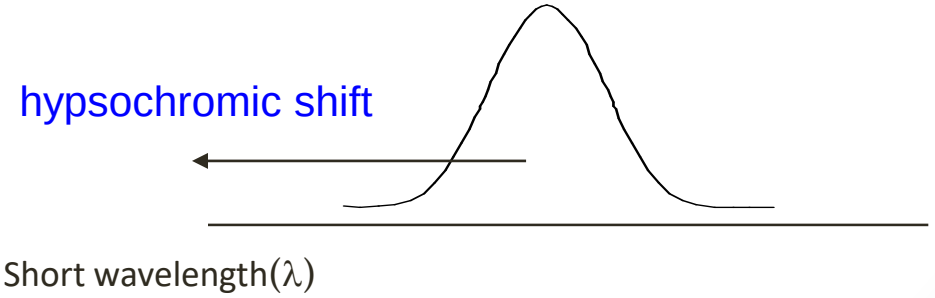


- ***n→π* transition :*** (200-700 nm) The peaks in the UV region are obtained most from n→π* transitions, are mostly observed on visible region. In the compounds which form this transition, there must be both non-bonded n electrons and π electrons.



C=Ö C=N̈ C=S̈ C≡N̈

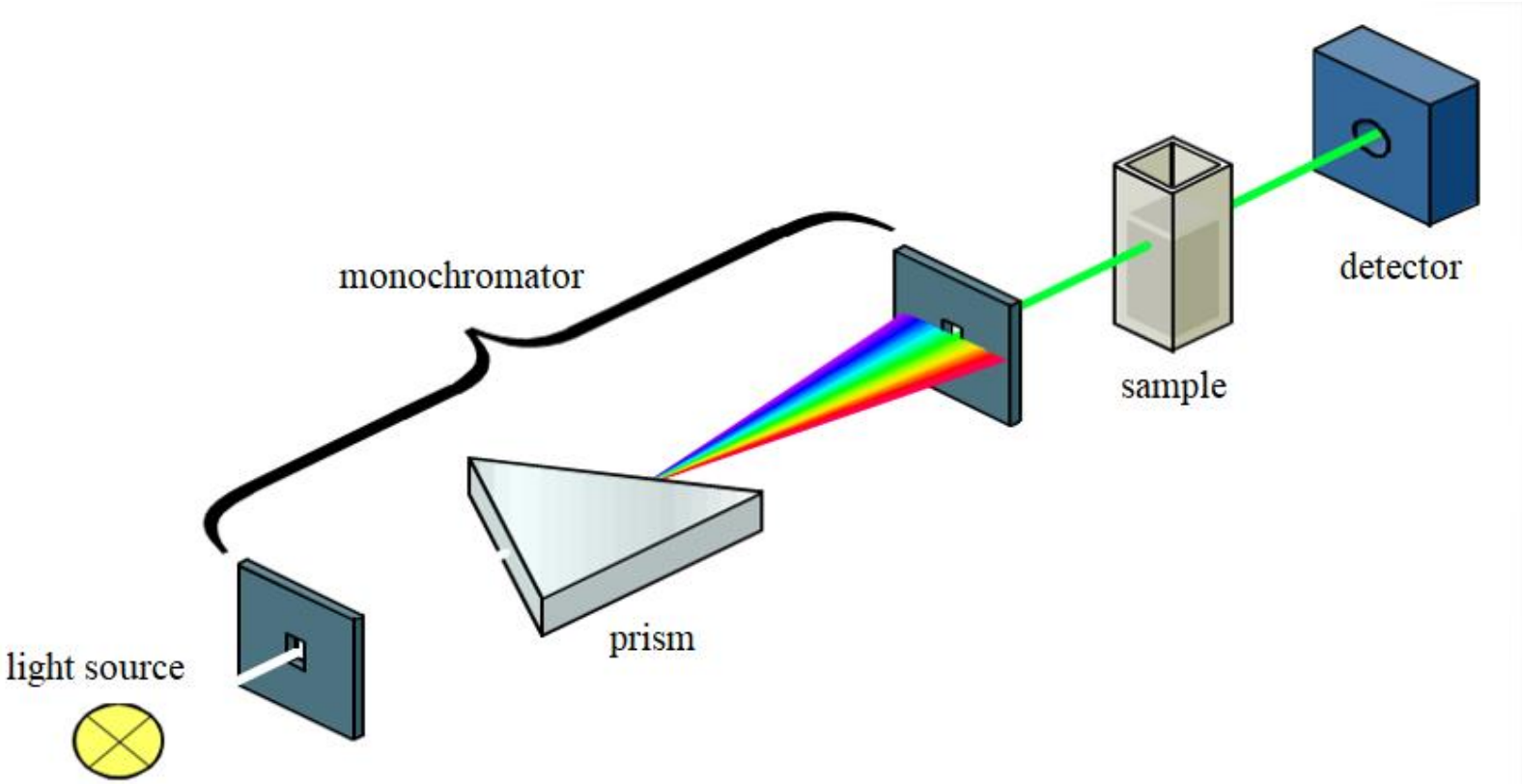
- As the polarity of the solvent increases, the absorption peak shifts to the shorter wavelength. (hypsochromic shift or blue shift). Blue shift may be up to 30 nm sometimes . The reason for this shift is the hydrogen bonding of the unconjugated electron pairs of the molecule with the -OH groups present in the polar solvents.



INSTRUMENTATION (UV and Visible Spectrophotometers)

The main parts of the devices are:

- Light source
- The part separating the rays from the light source according to the wavelength
- Transparent containers (sample cell) for solution and solvent
- The part or detector that converts the energy of the ray into electrical energy
- A signaling device (printer)


monochromator
detector
sample
prism
light source

- The typical ultraviolet–visible spectrophotometer consists of a **light source,** a **monochromator,** and a **detector.**

- The light source is usually a deuterium lamp, which emits electromagnetic radiation in the ultraviolet region of the spectrum. Tungsten lamp, which gives all the beams between 320-2500 nm, is generally used for visible works.

- Devices separating the ray to wavelength:

-Filters

With the filters, beam at certain intervals are obtained. There are many wavelengths in these beams.

-Monochromator

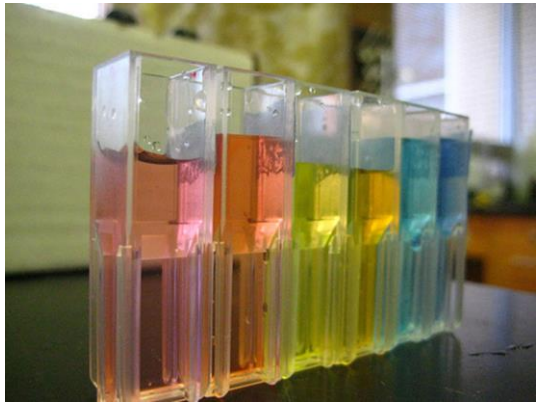
Monochromators are used to convert a beam of various wavelengths into single-wave beam. A glass prism is used for the visible region and quartz (silica) is used for the ultraviolet. They consist of three main parts.

*Input and output splits of beam

*Lens System

*Prism

- The sample cell must be constructed of a material that is transparent to the electromagnetic radiation being used in the experiment. For spectra in the visible range of the spectrum, cells composed of glass or plastic are generally suitable. For measurements in the ultraviolet region of the spectrum, however, glass and plastic cannot be used because they absorb ultraviolet radiation. Instead, cells made of quartz must be used since quartz does not absorb radiation in this region.



- Detectors are devices that convert light energy into electrical energy. A good detector;

-must be equally sensitive to all wavelengths,

-should be sensitive to low energy beam,

-should be convert the energy of the light immediately and completely to the electrical energy and during this it should not be vibrated.

-Signals should be proportional to the intensity of the beam falling on the detector

UV Sampling Techniques

- In UV spectroscopy we can measure between 190-780 nm. When performing the analysis, a solution of the appropriate concentration is prepared and the spectrum is generally taken up in cells of 1 cm thickness (preferably quartz sample cell). The baths can take up to 3-4 ml of solution.
- The spectrum is obtained by evaluating the signals given by the interaction of molecules with the light rays.
- Molar absorption is the absorbance of the solution (1 mol/l concentration) in the cell (1 cm thick) and is used to compare the spectra obtained at different concentrations.
- The sample cells should be filled by pipette.
- Water and ethanol are sufficient for cleaning.

- The choice of the solvent to be used in ultraviolet spectroscopy is quite important. The first criterion for a good solvent is that it should not absorb ultraviolet radiation in the same region as the substance whose spectrum is being determined. Usually solvents that do not contain conjugated systems are most suitable for this purpose, although they vary regarding the shortest wavelength at which they remain transparent to ultraviolet radiation.
- The selected solvent should be pure and not interact with the assayed substance.
- The most useful solvents are n-hexane, cyclohexane, chloroform and carbon tetrachloride.
- Polar solvents (water, diethyl ether, ethanol, methanol, etc.) are not used for this purpose unless very necessary.

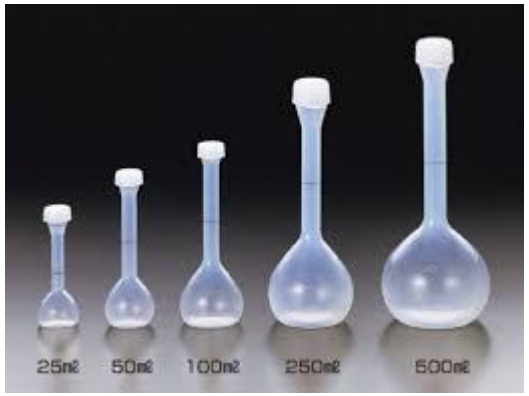
SOLVENT CUTOFFS

Acetonitrile	190 nm	*n*-Hexane	201 nm
Chloroform	240	Methanol	205
Cyclohexane	195	Isooctane	195
1,4-Dioxane	215	Water	190
95% Ethanol	205	Trimethyl phosphate	210

- There are two major classes of devices: single beam and double beam. A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample. A single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted. Double-beam instruments are easier and more stable.

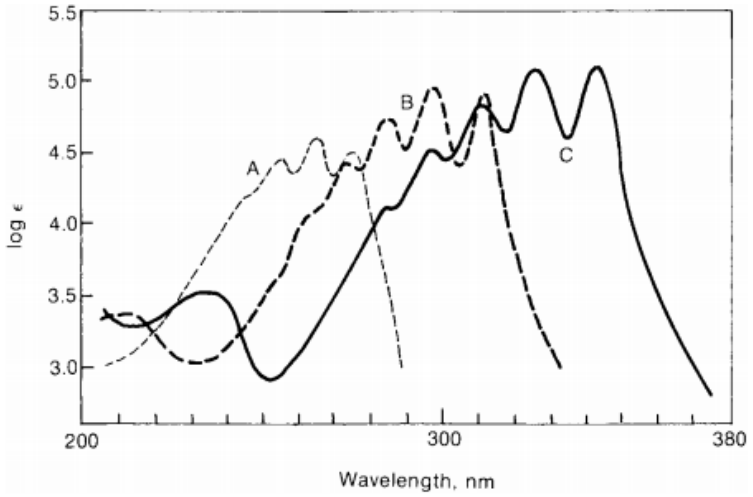
Preparation of solutions

- The sample of the substance to be analyzed is dissolved in a balloon by a suitable solvent and completed with the same solvent to the marking line. Suitable dilutions from this solution provide the desired concentrations. The sample cell is washed thoroughly and several times with the solution and then dried.

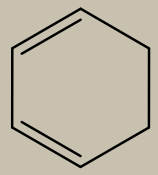
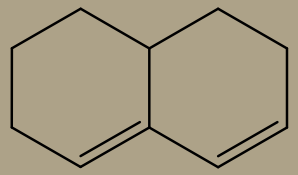


THE EFFECT OF CONJUGATION

- One of the best ways to bring about a bathochromic shift is to increase the extent of conjugation in a double-bonded system. In the presence of conjugated double bonds, the electronic energy levels of a chromophore move closer together. As a result, the energy required to produce a transition from an occupied electronic energy level to an unoccupied level decreases, and the wavelength of the light absorbed becomes longer.
- Conjugation of two chromophores not only results in a bathochromic shift but increases the intensity of the absorption. These two effects are of prime importance in the use and interpretation of electronic spectra of organic molecules because conjugation shifts the selective light absorption of isolated chromophores from a region of the spectrum that is not readily accessible to a region that is easily studied with commercially available spectrophotometers. The exact position and intensity of the absorption band of the conjugated system can be correlated with the extent of conjugation in the system.



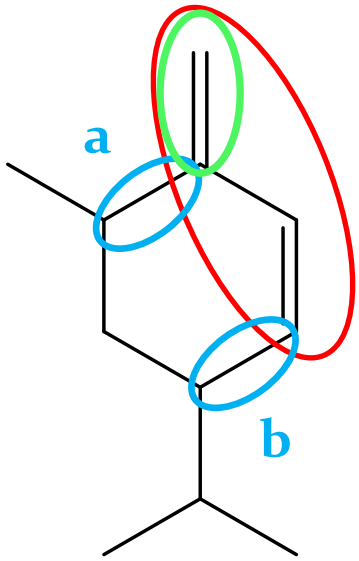
THE WOODWARD–FIESER RULES FOR DIENES

Temel Değerler		
Diene		217 nm
Homoannular diene (cisoid or *s-cis*)		253 nm
Heteroannular diene (transoid or *s-trans*)		214 nm

- In cyclic dienes, where the central bond is a part of the ring system, the diene chromophore is usually held rigidly in either the *s-trans* (transoid) or the *s-cis* (cisoid) orientation.
- By studying a vast number of dienes of each type, Woodward and Fieser devised an empirical correlation of structural variations that enables us to predict the wavelength at which a conjugated diene will absorb.

EMPIRICAL RULES FOR DIENES	
Alkyl substituent or ring residue	5 nm
Exocyclic double bond	5 nm
Double-bond-extending conjugation	30 nm
-OR	6 nm
-SR	30 nm
$-NH_2$ veya $-NR_2$	60 nm
-X	17 nm

Example 1:



Diene= 217 nm

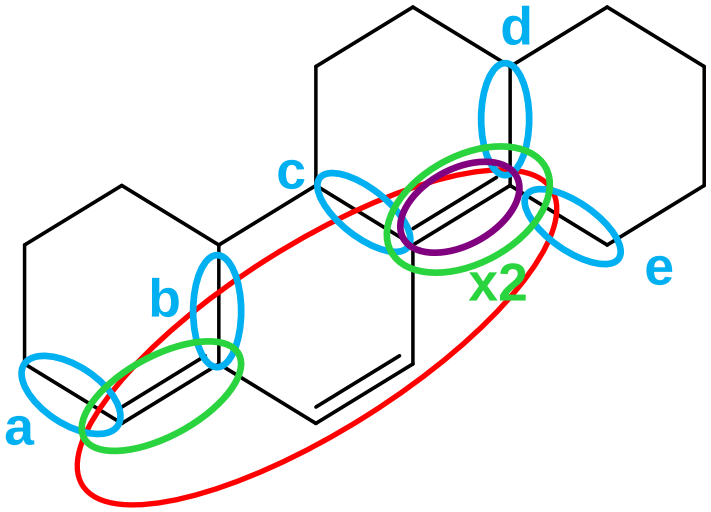
2 R (a and b) = 2x5 = 10 nm

Exocyclic double bond= 5 nm

\+

232 nm

Example 2:



Heteroannular diene = 214 nm

5 R (a, b,c, d, e) = 5x5 = 25 nm

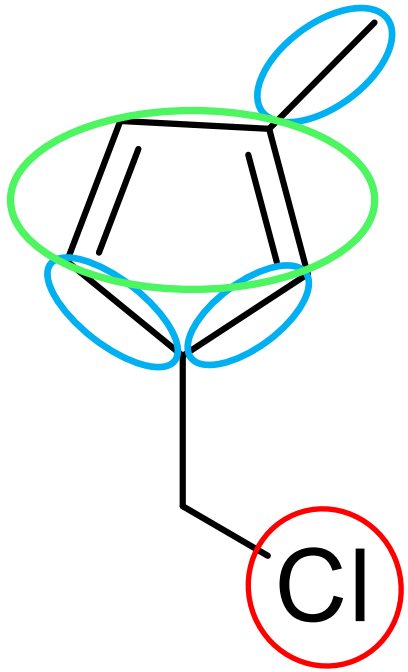
Double-bond-extending conjugation= 30 nm

Exocyclic double bond= 3x5 = 15 nm

+

284 nm

Example 3 :



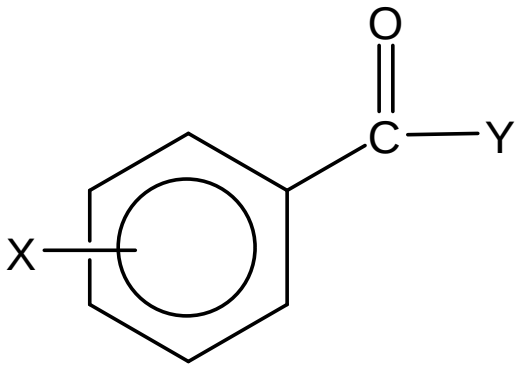
Homoannular diene= 253 nm

3 (R)= 3*5= 15 nm

\+ X= 17 nm

285 nm

If acetophenone is taken as the main system for aromatic compounds, the absorption rule can give a numerical correlation to α, β-unsaturation.

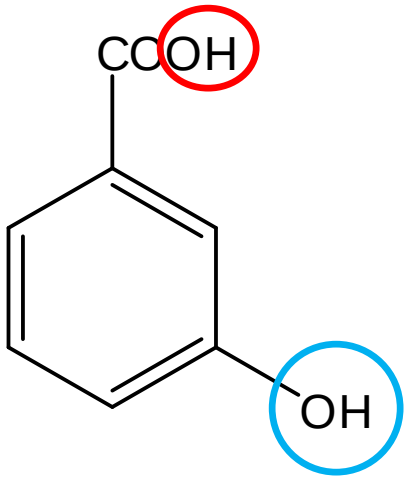


Y	λmax
R (Ar)	246 nm
H	250 nm
OH	230 nm

X		λmax
R(Ar)	-o	3 nm
	-m	3 nm
	-p	10 nm
OH	-o	7 nm
	-m	7 nm
	-p	25 nm
Cl	-o	0 nm
	-m	0 nm
	-p	10 nm
NH	-o	15 nm
	-m	15 nm
	-p	58 nm

Example 4:

m-hydroxy benzoic acid



Value= 230 nm

m-OH = 7 nm

\+

237 nm

WHAT TO LOOK FOR IN AN ULTRAVIOLET SPECTRUM: A PRACTICAL GUIDE

- Very often, the ultraviolet spectra of several members of a particular class of compounds are very similar. Unless you are thoroughly familiar with the spectroscopic properties of each member of the class of compounds, it is very difficult to distinguish the substitution patterns of individual molecules by their ultraviolet spectra. You can, however, determine the gross nature of the chromophore of an unknown substance by this method.
- Since the absorption peak of some compounds, eg, mono-functional alkanes, alkenes, alkynes, acids, esters, amides and oximes are outside the UV range, UV cannot be utilized in their analysis.
- It is often difficult to extract a great deal of information from a UV spectrum used by itself. It should be clear by now that a UV spectrum is most useful when at least a general idea of the structure is already known; in this way, the various empirical rules can be applied. Nevertheless, several generalizations can serve to guide our use of UV data. These generalizations are a good deal more meaningful when combined with infrared and NMR data—which can, for instance, definitely identify carbonyl groups, double bonds, aromatic systems, nitro groups, nitriles, enones, and other important chromophores. In the absence of infrared or NMR data, the following observations should be taken only as guidelines:

- *A single band of low-to-medium intensity (*e = *100 to 10,000) at wavelengths less than 220 nm* usually indicates an $n \rightarrow \sigma^*$ transition. Amines, alcohols, ethers, and thiols are possibilities, provided the nonbonded electrons are not included in a conjugated system. An exception to this generalization is that the $n \rightarrow \pi^*$ transition of cyano groups (-C≡N :) appears in this region. However, this is a weak transition (ε < 100), and the cyano group is easily identified in the infrared. Do not neglect to look for N-H, O-H, C-O, and S-H bands in the infrared spectrum.
- *A single band of low intensity (ε = 10 to 100) in the region 250 to 360 nm, with no majör absorption at shorter wavelengths (200 to 250 nm)*, usually indicates an $n \rightarrow \pi^*$ transition. Since the absorption does not occur at long wavelength, a simple, or unconjugated, chromophore is indicated, generally one that contains an O, N, or S atom. Examples of this may include C=O, C=N, N=N, -NO2, -COOR, -COOH, or -CONH2. Once again, infrared and NMR spectra should help a great deal.

- *Two bands of medium intensity (ε = 1,000 to 10,000), both with λmax above 200 nm*, generally indicate the presence of an aromatic system. If an aromatic system is present, there may be a good deal of fine structure in the longer-wavelength band (in nonpolar solvents only). Substitution on the aromatic rings increases the molar absorptivity above 10,000, particularly if the substituent increases the length of the conjugated system.
- *Bands of high intensity (ε = 10,000 to 20,000) that appear above 210 nm* generally represent either an α,β-unsaturated ketone (check the infrared spectrum), a diene, or a polyene. The greater the length of the conjugated system, the longer the observed wavelength. For dienes, the λmax may be calculated using the Woodward–Fieser Rules.
- *Simple ketones, acids, esters, amides, and other compounds containing both π systems and unshared electron pairs show two absorptions:* an $n \rightarrow \pi^*$ transition at longer wavelengths (>300 nm, low intensity) and a $\pi \rightarrow \pi^*$ transition at shorter wavelengths (<250 nm, high intensity). With conjugation (enones), the λmax of the $\pi \rightarrow \pi^*$ band moves to longer wavelengths and can be predicted by Woodward's Rules. The e value usually rises above 10,000 with conjugation, and as it is very intense, it may obscure or bury the weaker $n \rightarrow \pi^*$ transition.

- *Compounds that are highly colored* (have absorption in the visible region) are likely to contain a long-chain conjugated system or a polycyclic aromatic chromophore. Benzenoid compounds may be colored if they have enough conjugating substituents. For nonaromatic systems, usually a minimum of four to five conjugated chromophores are required to produce absorption in the visible region. However, some simple nitro, azo, nitroso, α-diketo, polybromo, and polyiodo compounds may also exhibit color, as may many compounds with quinoid structures.

Characteristic Absorbtion of Organic Substances :

Ethylene Chromophore :

- $\pi \rightarrow \pi^*$ transition

- Alkyl substitution or heteroatom substitution with unconjugated electrons } Bathochromic shift

Characteristic Absorbtion of Organic Substances :

Carbonyl Chromophore :

- Aldehydes and Ketones

150 nm ⟹ $\pi \rightarrow \pi^*$

190 nm ⟹ $n \rightarrow \sigma^*$

} Far UV

270-300 nm ⟹ $n \rightarrow \pi^*$ } UV ⟹ R band

The R band is caused by $n \rightarrow \pi^*$ transitions in a single chromophore group such as carbonyl, nitro.

- Substitution of carbonyl chromophore with an auxochrome ⟹ shifts to the shorter wavelength

- Spectra of substances containing a carbonyl conjugated ethyl group ⟹ 215-250 nm strong K band
  310-330 nm weak R band

The K band is derived from $\pi \rightarrow \pi^*$ transitions of molecules with chromophoric substitution, such as benzaldehyde, acetophenone.

- Spectra of substances containing an ethylenic group conjugated with a carbonyl group give a strong K band between 215 and 250 nm and a weak R band between 310-330 nm.
- With the increasing polarity of the solvent, R bands of carbonyl compounds show hipsochromic shift, K bands show batochromic shift.

- Nitriles and Azo Compounds: They do not show absorption in near ultraviolet.
- The $\pi \rightarrow \pi^*$ transmission of the azo group gives the absorption band in far distant UV ,
- The n $\rightarrow \pi^*$ transmission of aliphatic azo compounds gives the absorption band at 350 nm.

- Nitro, Nitrozo, Nitrate and Nitrite Derivatives: They show a weak absorption due to n $\rightarrow \pi^*$ transmission at near UV.

- Organic Sulfur Compounds: Aliphatic sulfones do not absorb near UV. The absorbtion of α,β-unsaturation shifts the absorbtion near UV.

- Aromatic Compounds: Absorption bands vary depending on the structure of the molecule. Benzene has three absorption bands, including , 184 nm, severe E bands at 204 nm and B band at 256 nm.

APPLICATIONS OF UV AND VISIBLE SPECTROSCOPY

- In qualitative analysis
- In quantitative analysis
- Purity control
- Spectrophotometric titration
- Determination of molecular weight
- Determination of acid and base constants
- Investigating the stereochemistry of molecules
- In determining the configuration of geometric isomers

Applications of Drug Molecules in Visible Region

- The portion of the electromagnetic spectrum lying between about 400 and 750 nm is the **visible** region.
- The Lambert-Beer Law applies in this measurement.

- The absorbance of the substance at the selected wavelength is measured and the amount of matter is calculated by the following methods:

- The substance to be tested is compared with the reference standard under the same conditions (e.g. wavelength, solvent). The absorbances of the solutions prepared from both are measured and the amount of the substance is calculated using the following formula:

$$\frac{C_x}{C_s} = \frac{A_x}{A_s} \qquad C_x = \frac{A_x}{A_s} \times C_s$$

Cx: The concentration of the amount unknown substance
Cs: Concentration of the standard substance
Ax: Absorbance of an amount unknown substance
As: Absorbance of standard substance

- Different concentration solutions of the reference standard are prepared and their absorbance at the selected wavelength is recorded.
- Calibration curve is drawn with concentration at x-axis and absorbance at y-axis.
- Then the absorbance of the solution prepared from the unknown sample is measured and marked on the y-axis.
- From this point, a perpendicular line is drawn to the calibration curve. The percentage of purity is calculated.