

**FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA
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COURSE CODE: CHM323 COURSE UNIT: 2
COURSE TITLE: SPECTROSCOPY**

INTRODUCTION

Spectroscopy is the study of the interaction of light and matter. Many types of spectroscopy rely on the ability of atoms and molecules to absorb or emit electromagnetic (EM) radiation. **Spectroscopy** technique uses radiation to obtain information on the structure and properties of matter. The basic principle shared by all spectroscopic techniques is to shine a beam of electromagnetic radiation onto a sample and observe how it responds to such a stimulus. The absorption or emission of different forms of EM radiation is related to different types of transitions. Absorption in the ultraviolet and visible regions of the electromagnetic spectrum corresponds to transitions between electronic energy levels and provides useful analytical information for both organic and inorganic samples.

Absorption Spectroscopy

The visible and ultraviolet spectra of organic compounds are associated with transition between electronic energy levels. The transitions are generally between a bonding or lone-pair orbital and an unfilled non- bonding or anti-bonding orbital. The absorption of UV/Visible radiation occurs through excitation of electrons within the molecular structure to a higher energy state. Radiation is a form of energy and we are constantly reminded of its presence via our sense of sight and ability to feel radiant heat. Radiation can be considered either as a continuous wave travelling through space, or as discrete photons of the same energy. The wave approach is more useful for many spectrometric approaches. It may be considered in terms of a wave motion where the wavelength, λ , is the distance between two successive peaks (Figure 1). The frequency, v , is the number of peaks passing a given point per second. These terms are related in the equation below:

where c is the velocity of light in a vacuum.

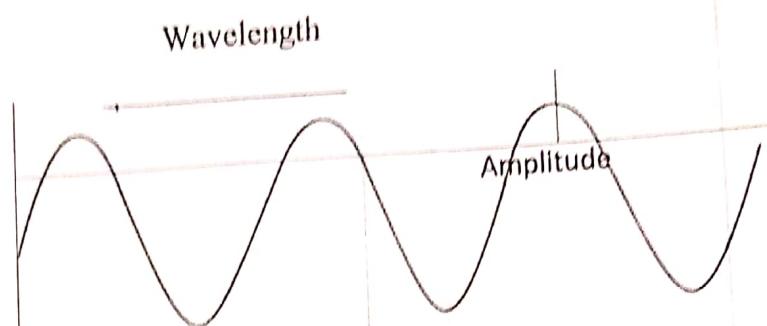


Fig. 1.: The Wavelength and Amplitude of a Wave

The standard unit of wavelength is expressed in nanometers. Other units like Angstrom and the millimicron ($m\mu$) may have been countered, but which their uses are now being discouraged. In some cases, it is more convenient to consider light as a stream of particles called photons. Photons are characterised by their energy, E . The energy of a photon is related to the frequency of light by the equation below:

Where E is the energy in joules (J), h is Planck's constant, 6.626×10^{-34} Js, and v is the frequency in inverse seconds (Hz). From equations (1) and (2) we can deduce that:

(2) we can deduce that: (3)

(2) we can deduce that: $E = hc/\lambda$ (3)
 From the equations above, we can see that the energy of electromagnetic radiation is directly proportional to its frequency and inversely proportional to its wavelength. Electromagnetic radiation ranges from very low energy (long wavelength, low frequency) radiation, like radio waves and microwaves, to very high energy (short wave-spectrum of interest to us as analytical chemists are shown in Figure 2. It is clear from this figure that the electromagnetic spectrum, to which the human eye responds, is only a very small portion of all radiant energy.

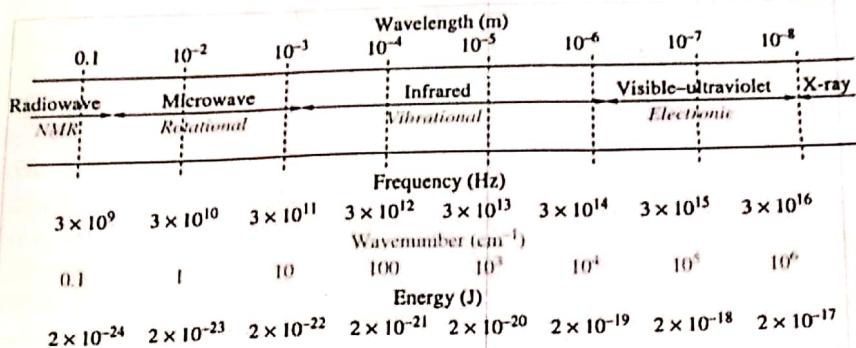


Figure 2: Electromagnetic Spectrum showing Wave-Length, Frequency and Energy

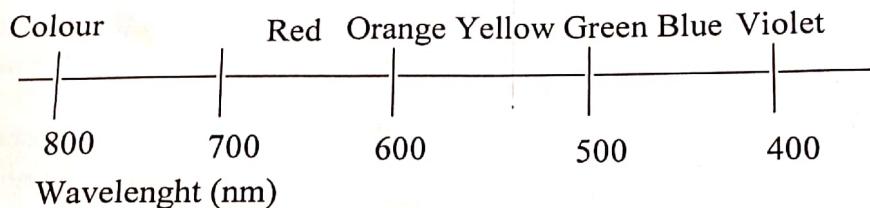


Fig. 2.1: Electromagnetic Spectrum

The ultraviolet (UV) and visible region of the electromagnetic radiation covers the wavelength range from about 100 nm to about 800 nm. The vacuum ultraviolet region, which has the shortest wavelengths and highest energies (100-200 nm) is difficult to make measurements in and is relatively uninformative. Useful ultraviolet and visible absorption spectra are produced by the absorption of electromagnetic radiation with wavelengths in the 200-400 nm (UV) and 400-800 nm (Visible) regions of the electromagnetic radiation.

An atom consists of a nucleus surrounded by electrons. Every element has a unique number of electrons, equal to its atomic number for a neutral atom of that element. The electrons are located in atomic orbital of various types and energies and the electronic energy states of atoms are quantised. The lowest energy, most stable electron configuration of an element is its ground state. The ground state is the normal electron configuration predicted from the „rules“ for filling a many-electron atom. These rules are based on the location of the atom in the periodic

table, the Aufbau principle, the Pauli Exclusion Principle and Hund's rule. For example, the ground state electronic configuration for sodium, atomic number 11, is $1s^2 2s^2 p^6 3s^1$ based on its position in the third row, first group of the periodic table and the requirement to account for 11 electrons. If energy of the right magnitude is provided to an atom, the energy may be absorbed and an outer (valence) electron promoted from the ground state orbital it is in, to a higher energy orbital. The atom is now in a higher energy, less stable, excited state. The electron will return spontaneously to the ground state, because the excited state is less stable than the ground state. In the process, the atom will emit energy; the energy will be equivalent in magnitude to the difference in energy levels between the ground and excited states.

The energy states associated with molecules, like those of atoms are also quantised. When atoms combine to form molecules, the individual atomic orbitals combine to form a new set of molecular orbitals. Molecular orbital with electron density in the plane of the bonded nuclei, that is, along the axis connecting the bonded nuclei, are called sigma (σ) orbital. The molecular orbitals with electron density above and below the plane of the bonded nuclei are called pi (π) orbitals. Sigma and pi orbitals may be of two types, bonding or antibonding orbitals. As an example, the atomic orbitals of carbon, hydrogen and oxygen combine in the molecule of propanone, C_3H_6O (Figure 3), so the three carbon atoms are linked in a chain by single (σ) bonds, the two outer carbons are each linked by σ bonds to three hydrogen atoms, while the central carbon atom is linked by a double bond to the oxygen atom, that is by both a σ bond and π bond. Additionally, the oxygen still has unpaired or nonbonded n electrons. This results in a set of bonding and corresponding antibonding electronic orbitals or energy levels. Bonding energies are lower in energy than the corresponding antibonding orbitals. Transitions may occur selectively between these levels, for example between π and π^* levels.

Under normal conditions of temperature and pressure, the electrons in the molecule are in the ground state configuration, filling the lowest energy molecular orbitals available. Absorption of the appropriate radiant energy may cause an outer electron to be promoted to a higher energy excited state. **As was the case with atoms, the radiant energy required to cause electronic transitions in molecules lies in the visible and UV regions.** The excited state of a molecule is less stable than the ground state as with atoms, the molecules will spontaneously revert (relax) to the ground state emitting UV or visible radiant energy. Unlike atoms, the energy states in molecules have rotational and vibrational sublevels, so when a molecule is excited electronically, there is often a simultaneous change in the vibrational and rotational energies. The total energy change is the sum of the electronic rotational and vibrational energy changes. However, in the condensed states of solid and

liquid, rotation is restricted. Organic molecules contain carbon-carbon bonds, and bonds between carbon and other elements such as hydrogen, oxygen, nitrogen, sulphur, phosphorus and the halogens. Single bonds correspond to the bonding σ orbital, which has an associated antibonding σ^* orbital. Multiple bonds may also be formed and correspond to the π bonding and π^* antibonding orbitals. Bonding orbitals have lower energy, while antibonding orbitals have higher energy. Lone pair of electrons on atoms such as oxygen is little changed in energy. Thus, a molecule such as propanone (acetone) has the structure below (Figure 3).



Fig. 3: Structure of Propanone

The $\pi-\pi^*$ transitions are very important, as they occur in all molecules with multiple bonds and with conjugated structures, such as aromatic compounds. The transitions occur around 200 nm, but the **greater the extent of the conjugation, the closer the energy levels and the higher the observed absorption wavelength.** Transitions involving the lone pairs on heteroatom such as oxygen or nitrogen may be $n-\sigma^*$, which occur around 200 nm, or $n-\pi^*$, which occur near 300 nm. **These values are considerably altered by the specific structure and the presence of substituent (auxochromes) in the molecules.**

The single C-H and C-C bond relate to σ orbitals, the carbonyl double bond to the π orbitals and the unpaired electrons on the oxygen to the non- bonding n -levels. The energy levels may be grouped approximately as shown in Figure 4. Transitions between σ and σ^* levels and between π and π^* are favoured and those of the n electrons to the higher levels also occur.

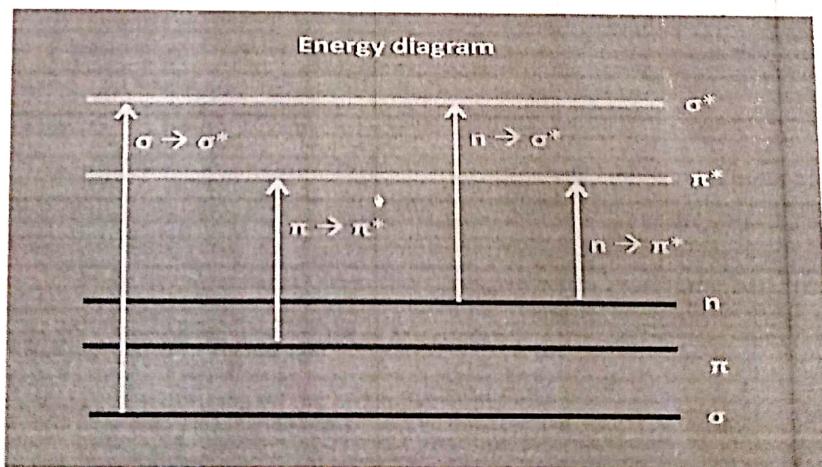


Fig. 4: Typical Transitions for Organic Molecules

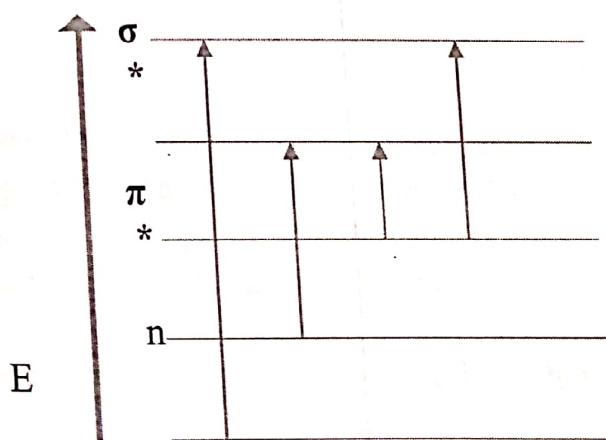
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Fig. 1.3: Structure of Propanone

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π σ **Fig. 4: Typical Transitions for Organic Molecules**

UV-Vis spectroscopy

UV-Vis spectroscopy is a quantitative laboratory technique used for the measurement of the absorbance of light by a chemical compound. It is performed through measurement of the intensity of light, which passes through a sample with respect to the intensity of light through a reference. It is used particularly in ultraviolet and visible regions. The obtained light intensity versus wavelength is called the spectrum.

The energy levels involved in transitions in the UV/visible region are the electronic levels of atoms and molecules. For example; although, light atoms have widely spaced energy levels, some heavy atoms have their outer orbitals close enough together to give transitions in the visible region. This accounts for the colours of iodides. Transition metals, having partly occupied d or f orbitals, often show absorption bands in the visible region and these are affected by the bonding of ligands e.g. Iron (III) reacts with thiocyanate ion to produce an intense red colour due to the iron(III) thiocyanate complex, which may be used to determine iron(III) in the presence of iron(II).

Instrumentation

The components include (Figure 5):

- i. **The light sources** – a deuterium lamp for the UV region from 90 nm to 350 nm and a quartz halogen or tungsten lamp for the visible region from 350 nm to 900 nm.

- i. **The monochromator** – used to disperse the light into its component wavelengths, which are further selected by the slit. The monochromator is rotated so that a range of wavelengths is passed through the sample as the instrument scans across the spectrum.

- ii. **The optics** – may be designed to split the light beam so that the beam passes

through two sample compartments, and in such a double-beam instrument, a blank solution can then be used in one compartment to correct the reading or spectrum of the sample. The blank is most commonly the solvent in which the sample is dissolved.

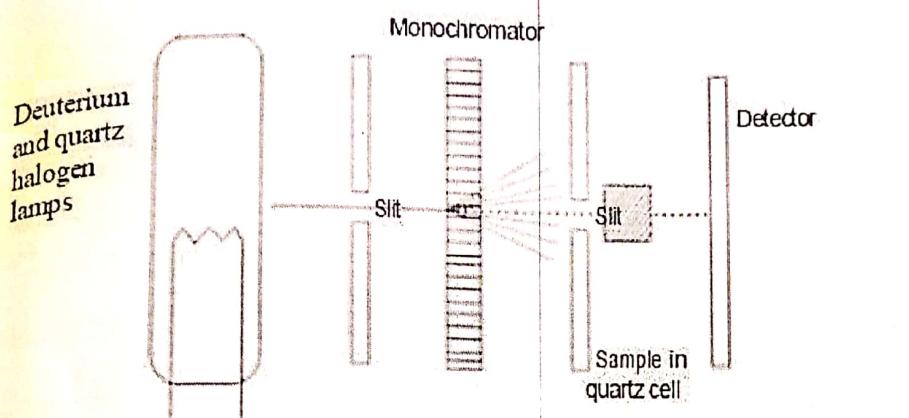


Fig. 1.5: Schematic Diagram of a UV/Visible Spectrophotometer (Reproduced from David G. Watson. (2005). Pharmaceutical Analysis, Elsevier Churchill Livingstone, UK)

The UV spectrum is usually computer generated in modern UV spectrophotometers and it is characterised by wavy lines with a peak absorbance. Absorbance is plotted on the y axis and wavelength on the x axis (Figure 6).

Sometimes, the spectrum obtained from the UV-Vis spectrophotometer need not be the same every time. The result may be erroneous. Reproducibility is missing if any parameters like temperature, pH, concentration are changed. Hence, it is essential to study the factors influencing UV-Vis spectroscopy, so that a clear picture will be obtained for careful measurement of the spectrum. This blog takes you to the study of variables, which affect the spectrum obtained from UV-Vis spectroscopy.

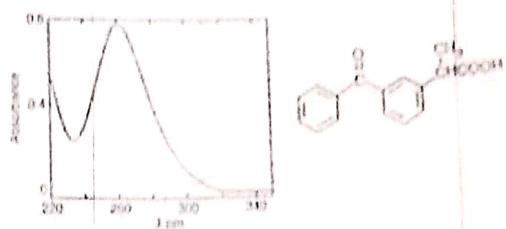


Fig. 6: A Representative UV Spectrum

Factors Governing Absorption of Radiation in the UV/Visible Region

Factors affecting UV-Vis Spectroscopy

For measurement of absorbance and studies, there are numerous factors, which impact the results in ultraviolet visible spectroscopy. The factors are as follows:

- i. Effect of sample temperature
- ii. Effect of sample concentration
- iii. Effect of sample pH
- iv. Effect of solvent
- v. Effect of steric hindrance
- vi. Effect of conjugation

Effect of Sample Temperature

The change in temperature of the sample plays a role in the spectrum.

- With the decrease in temperature, the sharpness of absorption bands increases.
- With the decrease in temperature, the position of the peak (absorption maximum) moves very little towards the longer wavelength side.
- However, total absorption intensity is independent of the temperature.
- Simple thermal expansion of the solution can change the intensity of the absorption band.
- Rotational and vibrational energy states depend on the temperature.

- When the temperature is decreased, rotational and vibrational energy states of the molecules also get lowered.
- Fine absorption bands will be produced when absorption occurs at lower temperatures due to the smaller distribution of excited states.
- The position of the band maximum does not shift much with a decrease in temperature.
- Hence, for obtaining more accurate results, the spectrum needs to be taken at a constant or particular temperature.

Effect of Sample Concentration

The concentration of sample present is directly proportional to the intensity of light absorption, thus influencing the spectrum.

- At a high concentration of solvent, molecular interactions occur, which causes changes in the shape and position of absorption bands. For qualitative work, this effect needs to be identified and taken into consideration.
- The type of solvent used also affects the fineness of the absorption band in the UV spectra. Polar solvents provide broader bands, but non-polar solvents give better resolution. Removing the solvent gives the best resolution. These effects are all due to solvent-solute interactions.
- There are stronger solvent-solute interactions if the dielectric constant of the solvent is high.
- Water and ethanol, which are polar solvents, exhibit a stronger binding to solute through induced dipole-dipole interactions or hydrogen bonding.
- Through London interactions in non-polar solvents, ground state and excited state will change and the frequency of absorbed photons can be changed. This leads to overlap of different transition energies in spectra, which broaden the absorption band.

Effect of Sample pH

The change of pH of the solution and the aquatic environment has an influence on the spectrum obtained from UV-Vis spectroscopy.

- If the pH of the solution is changed, absorption spectra of aromatic compounds like amines and phenols also change. Upon addition of a base, acidic compounds like phenols and substituted phenols undergo a change in absorption spectra. On removal of the phenolic proton, phenoxide ion is obtained, which increases the conjugation. This leads to a decrease in energy difference between LUMO and HOMO orbitals, resulting in a shift to a longer wavelength along with an increase in absorption intensity.
- If an aromatic amine gets protonated in an acidic medium, there is a disturbance of the conjugation system. The shift of peak towards shorter wavelength happens and a decrease in intensity also occurs.
- The acid-base indicators have an application due to their absorptions in the visible region of the UV-Vis spectrum.
- A minute change in the chemical structure of the indicator causes a change in the chromophore, which absorbs wavelength maximum at different values resulting in color change at different pH.
- An example is a phenolphthalein. It is a weak acid, dissociating in water to give anions, which adds a negative charge to the oxygen atom, contributing a shift in absorption maximum to a longer wavelength. The anion of phenolphthalein is orange, while non-ionized phenolphthalein is colorless. At neutral and acidic pH, the equilibrium shifts towards the left and there will be a lesser concentration of anions and pink color is not observed. But at basic pH, equilibrium shifts towards the right, leading to a higher concentration of anions, and pink color is observed.
- Hence, to maintain pH at a constant value, the UV-Vis spectrum should be measured in an appropriate buffer solution. Over the wavelength range of measurement, the buffer requires it to be transparent. The absorbance value is higher if the buffer solution also absorbs the light.

Effect of Solvent

To an extent, the choice of solvent also affects the spectrum.

- The absorption spectrum is also dependent on the solvent in which the absorption molecule gets dissolved. The option of choosing a solvent can shift the absorption peak to longer or shorter wavelengths. It is based on the interaction of solvent with the chromophore of the desired molecule.

CHM 323

- When compared to hexane solution, ethanol gives absorption maximum at longer wavelengths.
- Alcohols and water can form hydrogen bonding with the substance, which shifts the absorption bands of polar molecules. As the polarities of ground and excited states of chromophore are different, a change in solvent polarity causes a change in the energy gap between the two states.
- Highly pure and non-polar solvents do not interact with the solute molecules either in the ground state or excited state. However, polar solvents impact the molecular orbitals at the ground state of the excited state.
- Hence, the spectrum recorded in non-polar solvent differs from that one recorded in the polar solvent.

Effect of Steric Hindrance

The configuration of molecules also has a say in the spectrum.

- When a molecule is planar in conjugation, electronic conjugation works well. The position of the absorption peak is dependent on the effectiveness and length of the conjugative system. If autochrome is there, it prevents the molecule to exist in a planar configuration and shift towards longer or shorter wavelengths depending on the distortion. Distortion of chromophore causes the absorption peak to shift, due to loss of conjugation.
- Steric hindrance can also be seen in geometric isomerism. Trans isomers exhibit absorption peaks at longer wavelengths and molar absorptivity is higher than the cis counterpart. Due to the steric effect, trans-stilbene absorbs with greater intensity at longer wavelengths.

Effect of Conjugation

Molecular conjugation plays a role in determination of spectrum.

The absorption peak is shifted to a shorter frequency or longer wavelength when two or more chromophores are conjugated. Conjugation enhances the energy of the highest occupied molecular orbital and mitigates the energy of the lowest unoccupied molecular orbital. Hence, lesser energy is needed for an electronic transition to take place in a conjugated system. If the number of conjugated bands increases, the value of the absorption peak also increases. An increase in the double

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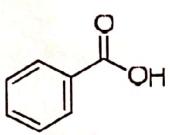
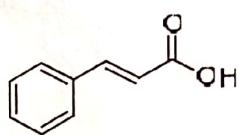
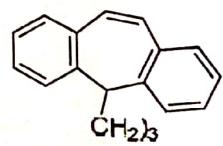
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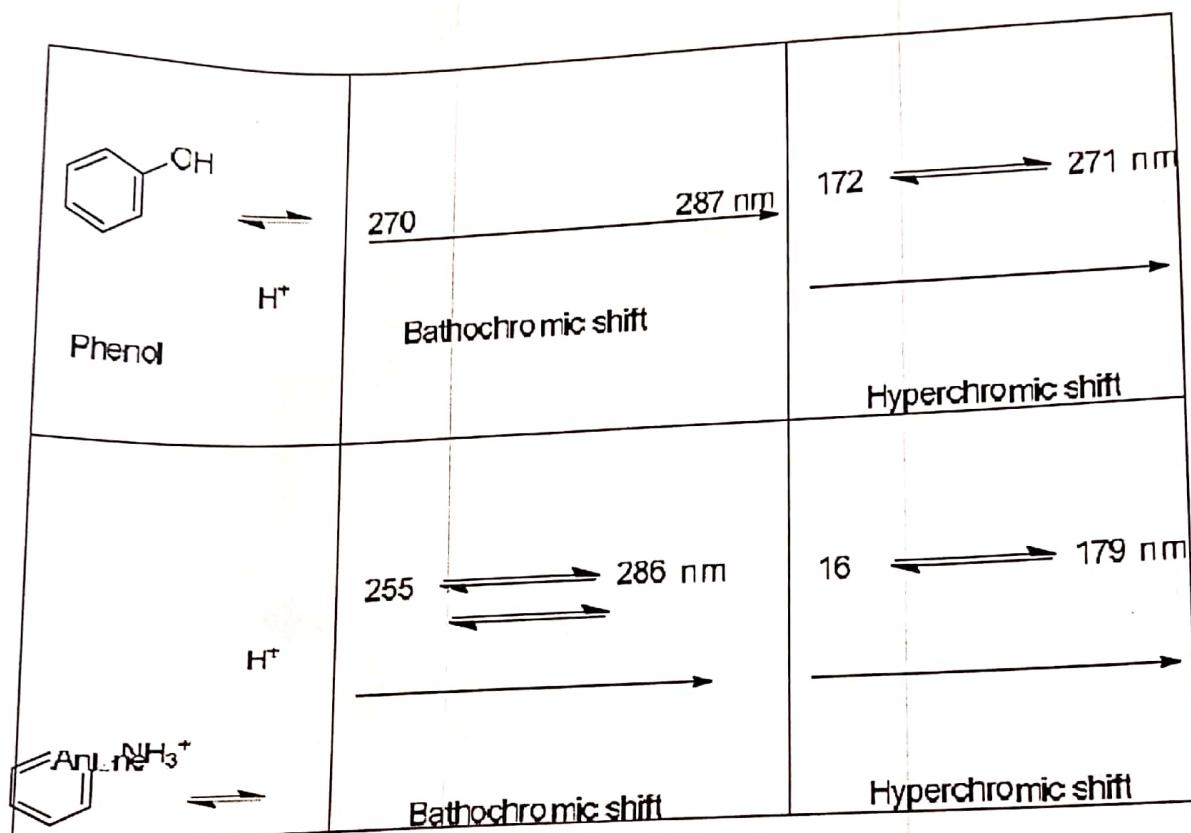
- The absorption peak is shifted to a shorter frequency or longer wavelength when two or more chromophores are conjugated. Conjugation enhances the energy of the highest occupied molecular orbital and mitigates the energy of the lowest unoccupied molecular orbital. Hence, lesser energy is needed for an electronic transition to take place in a conjugated system. If the number of conjugated bands increases, the value of the absorption peak also increases. An increase in the double

bonds of a conjugation leads to lesser energy needed for electronic transition. Conjugation of two chromophores also leads to an increase in molar absorptivity and intensity.

- An increase in the number of conjugated bonds leads to absorption of visible light and compounds will be colored. An example is beta carotene, which is a precursor compound of vitamin A. It has 11 conjugated bonds and the absorption peak is shifted from the UV region to the visible region (blue), giving it an orange color.

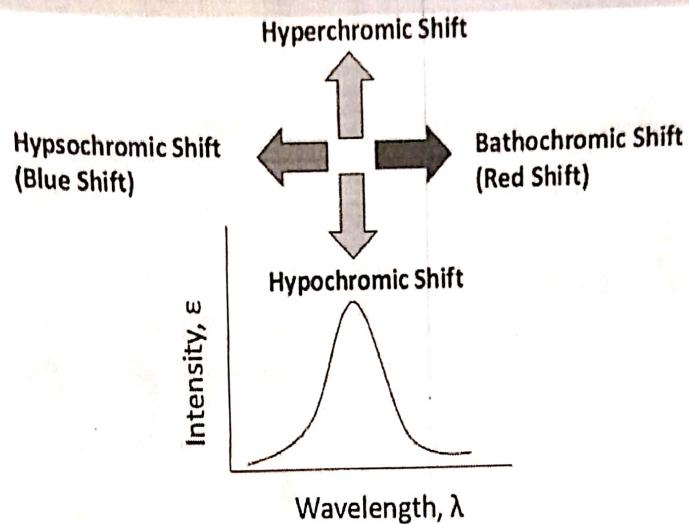
Table 1.2: The UV Absorption Characteristics of some Chromophores based on the Benzene Ring

Chromophore	Longest wavelength λ_{max} (nm)	A (1%, 1cm)
 Benzene	255	28
 Benzoic acid	273	85
 Cinnamic acid	273	1420
 Protropine	290	530



C Hm 3 2

Absorption Shifts:



Substituents when present may cause shift in the position of absorption spectrum of any chromophore.

1. **Bathochromic Shift:** An shift to **longer wavelength** (red shift).
2. **Hypsochromic Shift:** An shift to **shorter wavelength** (blue shift).
3. **Hyperchromic Shift:** An increase in intensity of absorption.
4. **Hypochromic Shift:** An decrease in intensity of absorption.



Bathochromic Shift:

- Also known as red shift.
- Shift in the position of absorption maximum to the longer wavelength.
- Change of solvent polarity (low)/ auxochromes/conjugation may cause bathochromic shift.
- Example; $\pi-\pi^*$ in ethylene : $\lambda_{\max} = 165 \text{ nm}$
 $\pi-\pi^*$ in 1,3-butadiene : $\lambda_{\max} = 217 \text{ nm}$

Hyperchromic Shift:

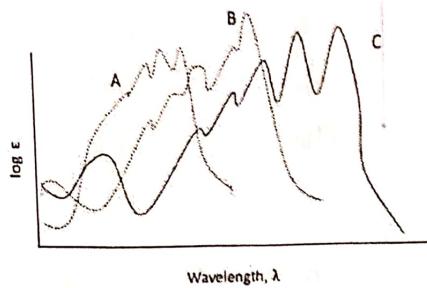
- Shift due to increase in intensity; ϵ_{\max} increases.
- Due to introduction of auxochrome.
- Example; Pyridine : $\lambda = 257 \text{ nm}, \epsilon_{\max} = 2750$
2-methyl Pyridine : $\lambda = 262 \text{ nm}, \epsilon_{\max} = 3560$

Hypsochromic Shift:

- Also known as blue shift.
- Shift in the position of absorption maximum to the shorter wavelength.
- Change of solvent polarity (high)/ removal of conjugation may cause bathochromic shift.
- Example; Aniline (lone pair in conjugation with ring) : $\lambda_{\max} = 280 \text{ nm}$
In acidic solution, $C_6H_5NH_3^+$ (lone pair not present) : $\lambda_{\max} = 203 \text{ nm}$

Effect of conjugation:

- In presence of conjugation, electronic energy levels of a chromophore move closer together and energy required to cause transition decreases, and the wavelength of the light absorbed becomes longer, and bathochromic shift is observed.



$\text{CH}_3-(\text{CH}=\text{CH})_n-\text{CH}_3$ UV spectra of dimethyl polyenes. (a) n=3 (b) n=4 (c) n=5



5- Chromophore:

It is unsaturated group responsible for electronic absorption e.g. $\text{C}=\text{C}$, $\text{C}=\text{O}$, NO_2 .

6- Auxochrome:

It is a saturated group, which when attached to a chromophore alter both of wavelength λ and the intensity of the maximum absorption.

These groups are generally characterized by having unshared electron pairs e.g. $-\text{OH}$, $-\text{NH}_2$, $-\text{Cl}$.