



Spectroscopic Techniques

are non-destructive and generally require small amounts of sample.

Four common spectroscopic techniques used to determine structure:

Ultraviolet Spectroscopy
Observes electronic transitions
Provides information on the electronic bonding in a molecule.

electronic UV-Vis

Infrared spectroscopy:
– Used to determine the functional groups present in a molecule

vibrational infrared

Mass spectrometry
Breaks molecule into fragments
Analysis of the masses of the fragments gives MW and clues to the structure of the molecule

Nuclear Magnetic Resonance Spectroscopy
– Observes the chemical environment of the hydrogen (or carbon) atoms in the molecule
• Helps provide evidence for the structure of the carbon skeleton and/or the alkyl groups present

nuclear spin radiofrequency

UV - VIS Molecular Spectroscopy



This absorption spectroscopy uses electromagnetic radiations between 190 nm to 800 nm and is divided into the ultraviolet (UV, 190-400 nm) and visible (VIS, 400-800 nm) regions.

- Since the absorption of ultraviolet or visible radiation by a molecule leads transition among electronic energy levels of the molecule, it is also often called as electronic spectroscopy.
- The information provided by this spectroscopy when combined with the information provided by NMR and IR spectral data leads to valuable structural proposals.

Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface.

Absorption measurements can be at a single wavelength or over an extended spectral range.

The region beyond red is called infra-red while that beyond violet is called as ultra-violet. The wavelength range of uv radiation starts at blue end of visible light(4000Å) & ends at 2000A.

UV RADIATION

PRINCIPLE OF UV-VIS SPECTROMETRY

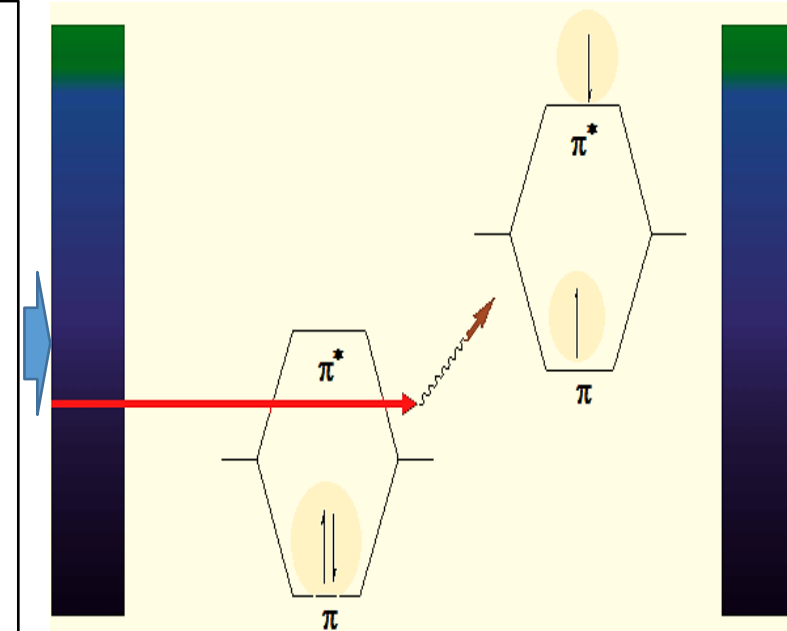


- A UV-Vis spectrophotometer measures the amount of light absorbed at each wavelength of the UV and visible regions of the electromagnetic spectrum.
- A UV or visible spectrophotometer has the same basic design as an infrared spectrophotometer.
- In a standard UV-Vis spectrophotometer, a beam of light is split; one half of the beam (the sample beam) is directed through a transparent cell containing a solution of the compound being analyzed, and one half (the reference beam) is directed through an identical cell that does not contain the compound but contains the solvent.

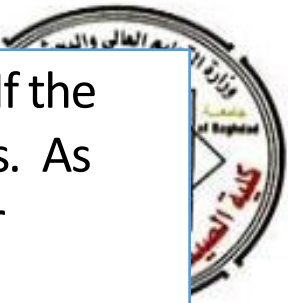
Solvents are chosen to be transparent in the region of the spectrum being used for analysis.

So; the Spectroscopic Process

1. In UV spectroscopy, the sample is irradiated with the broad spectrum of the UV radiation
2. a particular electronic transition matches the energy of a certain band of UV, it will be absorbed
3. The remaining UV light passes through the sample and is observed
4. From this residual radiation a spectrum is obtained with "gaps" at these discrete energies – this is called an **absorption spectrum**



The electronic excitations take place in molecules containing pi electrons or non-bonding electrons. If the electrons of molecules in the sample can easily be excited, the sample can absorb longer wavelengths. As a result, the electrons in pi bonds or non-bonding orbitals can absorb energy from light waves in UV or visible range.

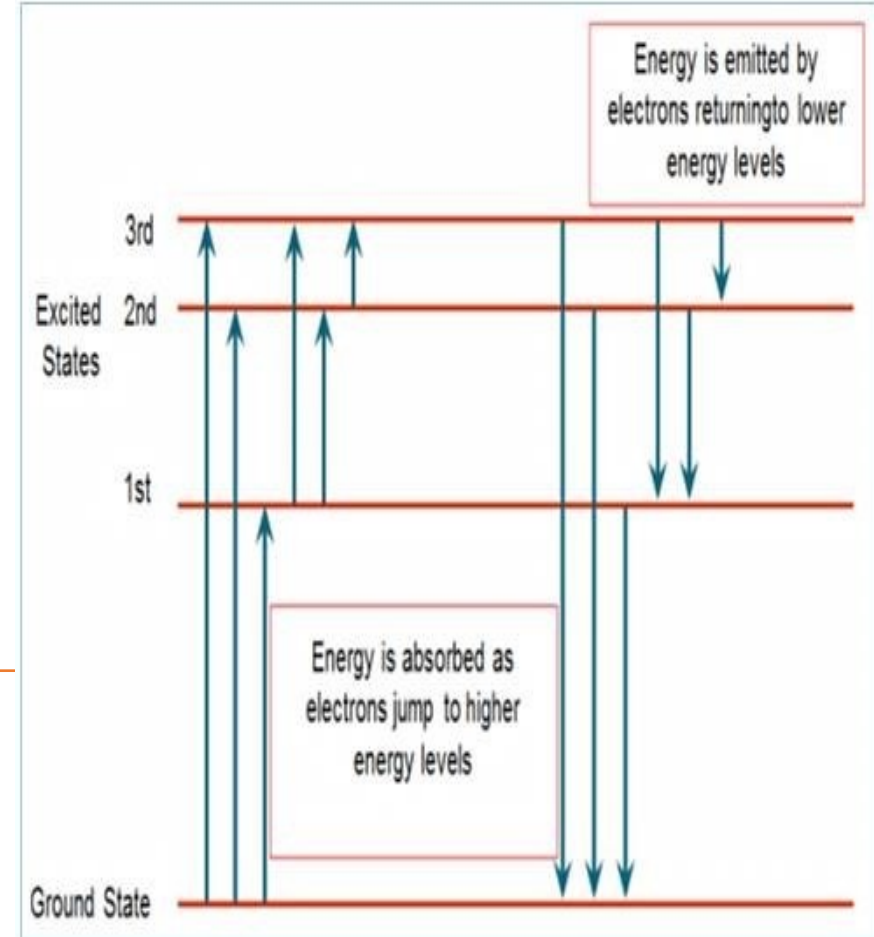


➤ The Spectroscopic Techniques are based on the fact that (**Absorption**) is **directly** proportional to the **Concentration** of the absorbing component .

➤ Utilises the **Absorption** and **Emission** of electromagnetic radiation by atoms. **Absorption:**

Low energy electrons absorb energy to move to **higher energy level**

Emission: Excited electrons return to **lower energy states**.



- The UV radiation region extends from 10 nm to 400 nm
- the visible radiation region extends from 400 nm to 800 nm.
- Near UV Region: 200 nm to 400 nm
- Far UV Region: below 200 nm

Observed electronic transitions

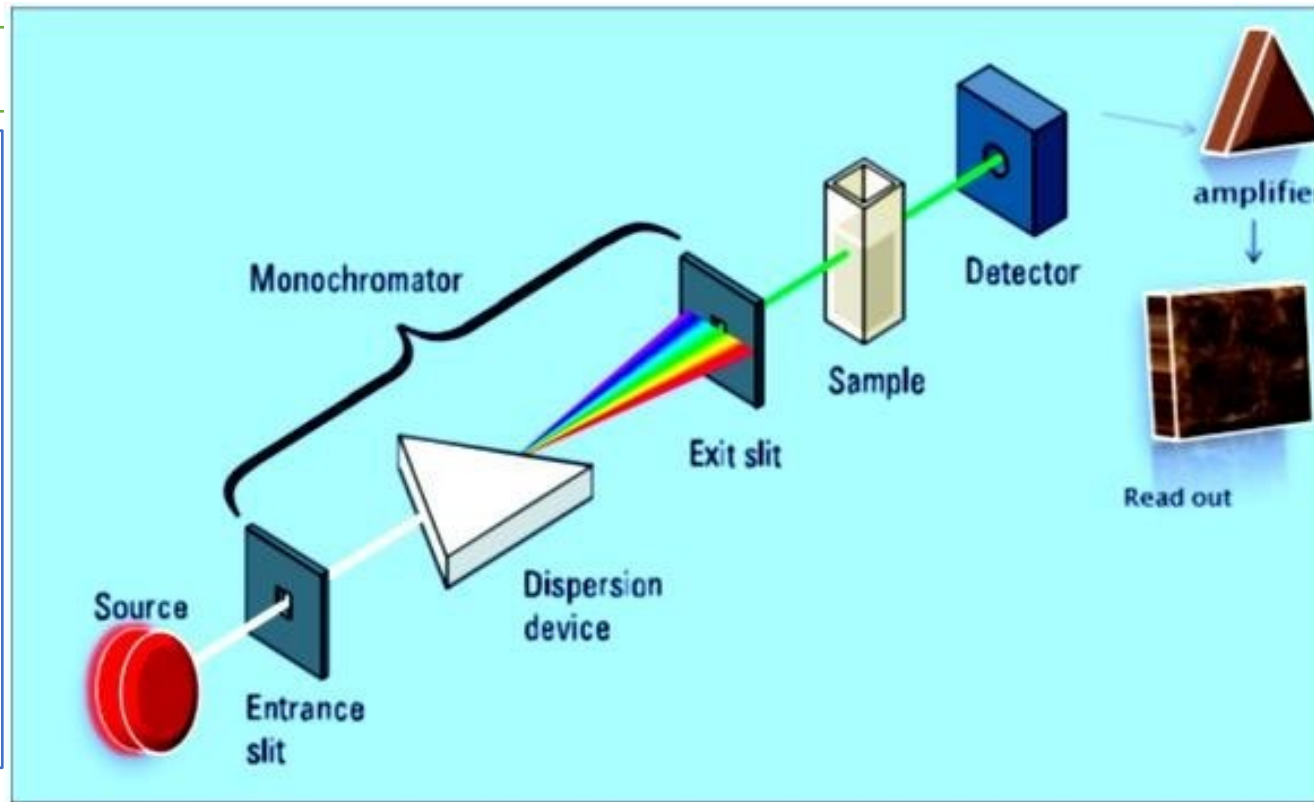
- The lowest energy transition (and most often obs. by UV) is typically that of an electron in the Highest Occupied Molecular Orbital (HOMO) to the Lowest Unoccupied Molecular Orbital (LUMO)
 - For any bond (pair of electrons) in a molecule, the molecular orbitals are a mixture of the two contributing atomic orbitals; for every bonding orbital "created" from this mixing (s, p), there is a corresponding anti-bonding orbital of symmetrically higher energy (s^* , p^*)
 - The lowest energy occupied orbitals are typically the s; likewise, the corresponding anti-bonding s^* orbital is of the highest energy while p-orbitals are of somewhat higher energy, and their complementary anti-bonding orbital somewhat lower in energy than s^* .
 - Unshared pairs lie at the energy of the original atomic orbital, most often this energy is higher than p or s (since no bond is formed, there is no benefit in energy)
- The absorption of UV-visible radiation generally results from excitation of bonding electrons.
- Can be used for quantitative and qualitative analysis

- **Molecular orbital** is the nonlocalized fields between atoms that are occupied by bonding electrons. (when two atom orbitals combine, either a low-energy bonding molecular orbital or a high energy antibonding molecular orbital results.)
- **Sigma (σ) orbital**
The molecular orbital associated with single bonds in organic compounds
- **Pi (π) orbital**
The molecular orbital associated with parallel overlap of atomic P orbital.
- **n electrons**
No bonding electrons

INSTRUMENTATION

Components of spectrophotometer

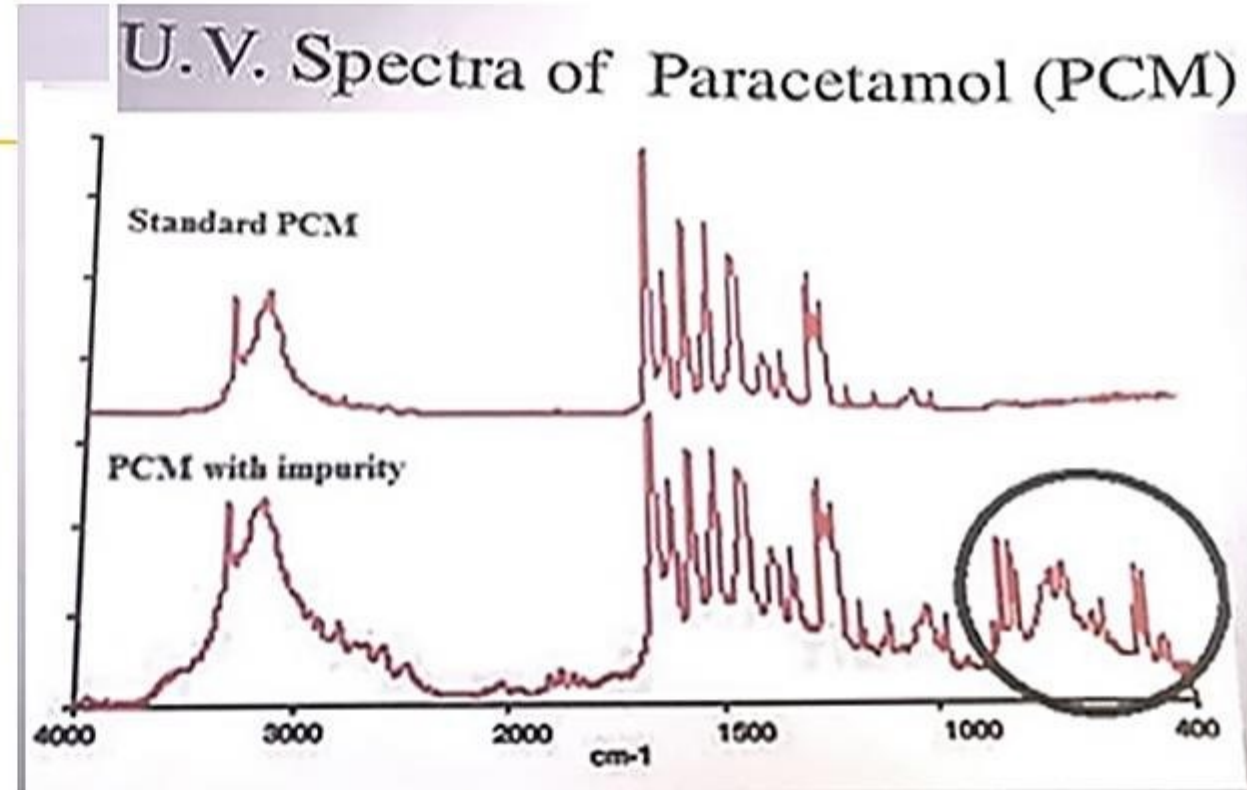
- Source
- Monochromator
- Sample compartment
- Detector
- Recorder



- Most spectrophotometers are **double-beam instruments**.
The primary source of light is split into two beams, one of which passes through a cell containing the sample solution and the other of which passes through a cell containing the reference solvent.
- The spectrophotometer electronically subtracts the absorption of the solvent in the reference beam from the absorption of the solution in the sample beam. Thus, effects owing to absorption of light by the solvent are minimized.

Why we use UV spectroscopy?

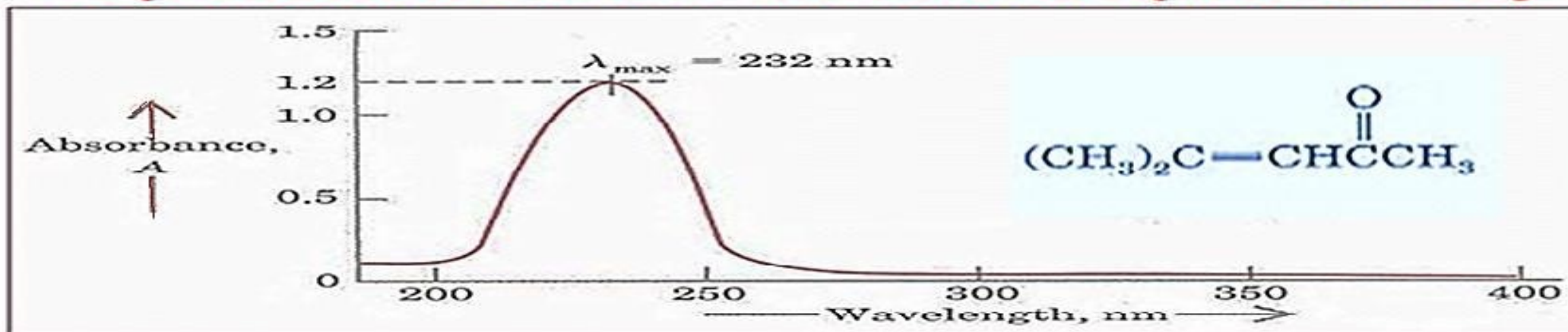
- Detection of functional groups.
- Detection of impurities. →
- Qualitative analysis.
- Quantitative analysis.
- Single compound without chromophore .
- Drugs with chromophoric reagent
- It is helps to show the relationship between different groups, it is useful to detect the conjugation of the compounds





- ❑ In order to obtain useful information from the ultraviolet or visible spectrum of a compound, both of the followings must be measured accurately.
 - the wavelength of maximum absorption (λ_{\max}) &
 - ϵ_{\max} (The intensity of maximum absorption) constant (defined, no units)

Expressions Used in Ultraviolet Spectrometry



- ❑ The spectrum shows that the scan is from 200-400 nm.
- ✓ Because absorption by atmospheric carbon dioxide becomes significant below 200 nm, the 100-200 nm region is usually not scanned unless special air-free techniques are employed.
- The technique of using an evacuated spectrophotometer enables the range below 200 nm to be studied. This region is frequently called the *vacuum ultraviolet region*.



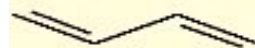
- ❑ The wavelength of absorption is usually reported as λ_{\max} which represents the wavelength at the highest point of the curve.
- ❑ The absorption of energy is reported as absorbance (not transmittance as in infrared spectra).



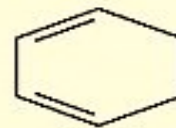
Partial structures :

❖ Strong UV ($\epsilon > 1000$):-

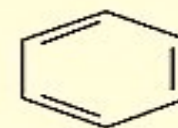
Conjugated π systems give $\epsilon > 1000$.



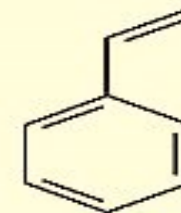
$\epsilon = 20,900$



$\epsilon = 4,580$



$\epsilon = 8,000$



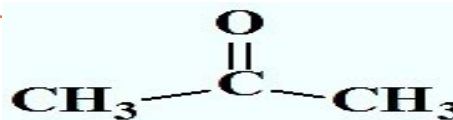
$\epsilon = 12,000$

Q// If $\epsilon > 1000$, what kind of partial structure is indicated?

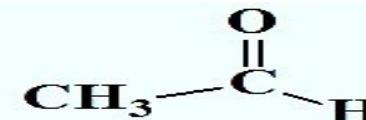
- ✓ The compound **MUST CONTAIN** a **conjugated π system**.
- ✓ It might be **aromatic** (like benzene).
- ✓ It might be a **conjugated polyene** (like 1,3-butadiene).

❖ Weak UV ($\epsilon < 100$):

-Aldehydes or Ketones give $\epsilon < 100$



acetone $\epsilon = 19$



acetaldehyde $\epsilon = 12$

Q// If $\epsilon < 100$, what kind of partial structure is indicated?

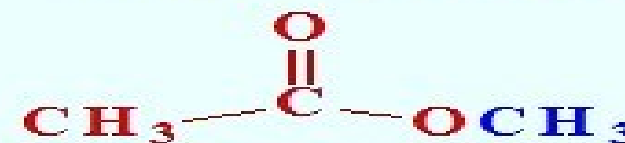
- ✓ The compound must have an aldehyde or ketone carbonyl.
- ✓ There is **no heteroatom next to the carbonyl carbon** (only C or H).

❖ UV End Absorption (No ϵ) **Esters give end absorption**

Q// If **UV = end absorption**, what partial structure is indicated?

- ✓ A carbonyl group with an **Oxygen atom next to the carbonyl carbon** is indicated.

methyl acetate





- ❑ The wavelength of UV or visible light absorbed depends on the ease of electron promotion.
- ❑ Molecules that require more energy for electron promotion absorb at shorter wavelengths.

absorption at 100 nm (UV) \longrightarrow 750 nm (visible)
increasing ease of electronic transition \longrightarrow

- ❑ The absorbance at a particular wavelength is defined by the equation:

$$A = \log \frac{I_0}{I}$$

where A = absorbance

I_0 = intensity of the reference beam

I = intensity of the sample beam

- ❑ The absorbance by a compound at a particular wavelength increases with an increasing number of molecules undergoing transitions.

Therefore, the absorbance depends on:

- the electronic structure of the compound.
- the concentration of the sample -
- the length of the sample cell.

- a longer path length, l through the sample will cause more UV light to be absorbed – linear effect
- the greater the concentration, C of the sample, the more UV light will be absorbed – linear effect



These effects are combined into the Beer-Lambert Law: $A = \epsilon c l$

for most UV spectrometers, (l) would remain constant (standard cells are typically 1 cm in path length)

concentration is typically varied depending on the strength of absorption observed or expected – typically dilute – sub .001 M

molar absorptivities vary by orders as:

- values of (10^4 - 10^6) → are termed high intensity absorptions.
- values of (10^3 - 10^4) → are termed low intensity absorptions.
- values of (0 to 10^3) → are the absorptions of forbidden transitions.

A is unitless, so the units for ϵ are $\text{cm}^{-1} \cdot \text{M}^{-1}$ and are rarely expressed

Since path length and concentration effects can be easily factored out, absorbance simply becomes proportional to ϵ , and the y-axis is expressed as ϵ directly or as the logarithm of ϵ

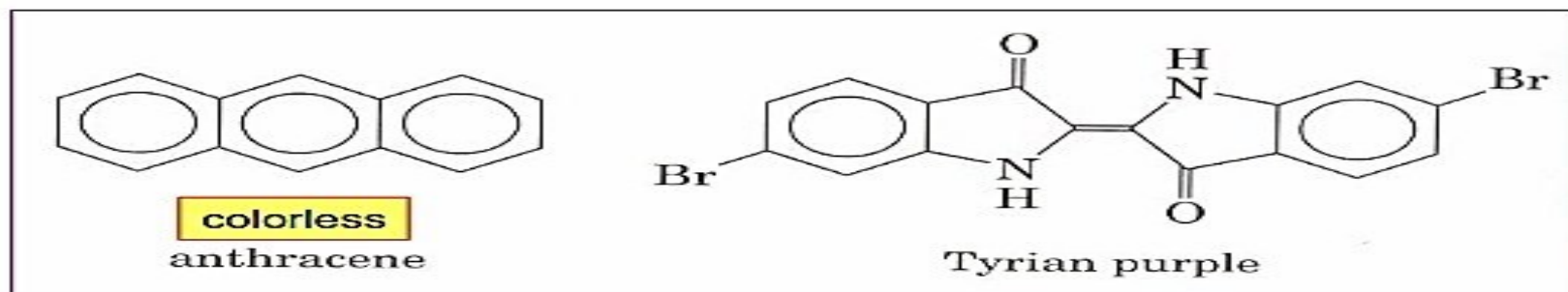
- Ultraviolet absorption spectra arise from transition of electron within a molecule from a lower level to a higher level.

$$E_{\text{total}} = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}}$$

The energies decrease in the following order:

Electronic \gg Vibrational \gg Rotational

- ❑ Compounds that absorb light in the visible region (that is colored compounds) have more-easily promoted electrons than compounds that absorb at shorter UV wavelengths.



- ❑ Although the energy absorption by a molecule is quantized, a UV or visible spectrum consists of not a spectrum of lines or sharp peaks but rather of broad absorption bands over a wide range of wavelength.
- ❑ The reason for the broad absorption is that the energy levels of both the ground state and the excited state of a molecule are subdivided into rotational and vibrational sublevels.

Types of Electron Transitions

- The ground state of an organic molecule contains valence electrons in three principal types of molecular orbitals: sigma (σ) orbitals; pi (π) orbitals; and filled but nonbonded orbitals (n).

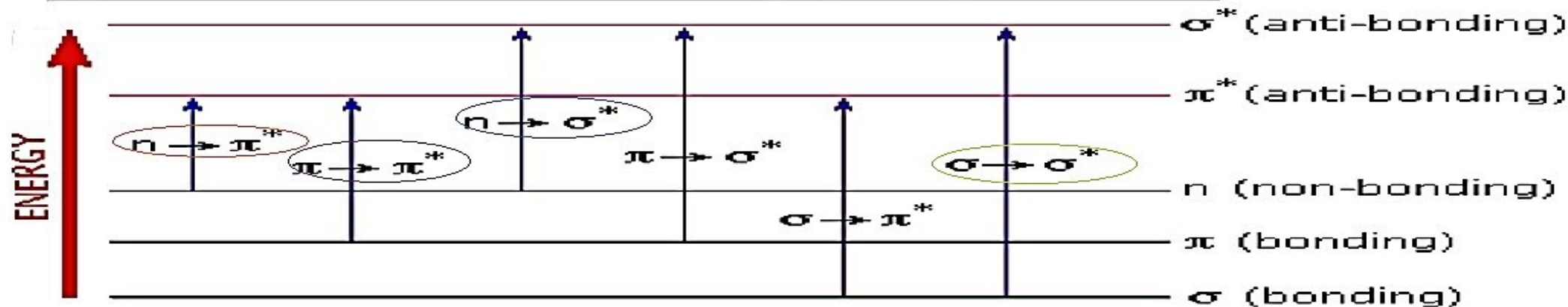


- Both σ and π orbitals are formed from the overlap of two atomic or hybrid orbitals. Each of these molecular orbitals therefore has an antibonding σ^* or π^* orbital associated with it.
- An orbital containing n electrons does not have an antibonding orbital (because it was not formed from two orbitals).

- Electron transitions involve the promotion of an electron from one of the three ground states (σ , π , or n) to one of the two excited states (σ^* , or π^*).
- There are six possible transitions; the four important transitions and their relative energies are:

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The possible electronic transitions can graphically shown as:





- ❑ The most useful region of the UV spectrum is at wavelengths longer than 200 nm.
- ❑ The following transitions give rise to absorption in the nonuseful 100-200 nm range:
 - $\pi \rightarrow \pi^*$ for an isolated double bond, and
 - $\sigma \rightarrow \sigma^*$ for an ordinary carbon-carbon bond.
- ❑ The useful transitions (200 nm-400 nm) are $\pi \rightarrow \pi^*$ for compounds with conjugated double bonds, and some $n \rightarrow \sigma^*$ and some $n \rightarrow \pi^*$ transitions.
- ❑ Alkenes and nonconjugated dienes usually have absorption maxima below 200 nm.

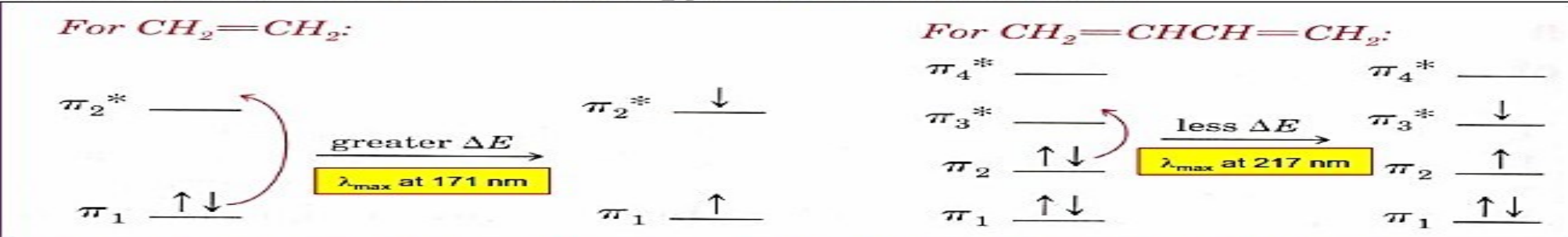
➤ **Example:**

- Ethene gives an absorption maximum at 171 nm,
- 1,4-pentadiene gives an absorption maximum at 178 nm.

highest occupied molecular orbital (HOMO)
lowest unoccupied molecular orbital (LUMO)

➤ Absorption by Polyenes

- ❑ Compounds whose molecules contain conjugated multiple bonds have absorption maxima at wavelengths longer than 200 nm.
- ❑ For example, less energy is required to promote a π electron of 1,3-butadiene than is needed to promote a π electron of ethylene.
- ❑ The reason is that the energy gap between the HOMO and the LUMO for conjugated double bonds is less than the energy difference for an isolated double bond.
- ❑ Resonance-stabilization of the excited state of a conjugated diene is one factor that decreases the energy of the excited state.



Because less energy is needed for a $\pi \rightarrow \pi^*$ transition of 1,3-butadiene, this diene absorbs UV radiation of longer wavelengths than does ethylene.

The possible electronic transitions are

1	• $\sigma \rightarrow \sigma^*$ transition
2	• $\pi \rightarrow \pi^*$ transition
3	• $n \rightarrow \sigma^*$ transition
4	• $n \rightarrow \pi^*$ transition
5	• $\sigma \rightarrow \pi^*$ transition
6	• $\pi \rightarrow \sigma^*$ transition

1 • $\sigma \rightarrow \sigma^*$ transition

- σ electron from orbital is excited to corresponding anti-bonding orbital σ^* .
- The energy required is large for this transition.
- e.g. Methane (CH_4) has C-H bond only and can undergo $\sigma \rightarrow \sigma^*$ transition and shows absorbance maxima at 125 nm.

Q. The energy required is the highest one for $\sigma \rightarrow \sigma^*$ transition ?

ANS. All saturated alkane need highest energy, so they show shortset wave length, with an exeption Cyclopropane because of ring strength which give normal UV region above 200 nm.



2

• $\pi \rightarrow \pi^*$ transition

- π electron in a bonding orbital is excited to corresponding anti-bonding orbital π^* .
- Compounds containing multiple bonds like alkenes, alkynes, carbonyl, nitriles, aromatic compounds, etc undergo $\pi \rightarrow \pi^*$ transitions.
- e.g. Alkenes generally absorb in the region 170 to 205 nm.



3

• $n \rightarrow \sigma^*$ transition

- Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of $n \rightarrow \sigma^*$ transition.
- These transitions usually requires less energy than $\sigma \rightarrow \sigma^*$ transitions.
- The number of organic functional groups with $n \rightarrow \sigma^*$ peaks in UV region is small (150 – 250 nm).

4

• $n \rightarrow \pi^*$ transition

- An electron from non-bonding orbital is promoted to anti-bonding π^* orbital.
- Compounds containing double bond involving hetero atoms (C=O, C≡N, N=O) undergo such transitions.
- $n \rightarrow \pi^*$ transitions require minimum energy and show absorption at longer wavelength around 300 nm.



5

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

&

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

6

- These electronic transitions are forbidden transitions & are only theoretically possible.
- Thus, $n \rightarrow \pi^*$ & $\pi \rightarrow \pi^*$ electronic transitions show absorption in region above 200 nm which is accessible to UV-visible spectrophotometer.
- The UV spectrum is of only a few broad of absorption.

Note:

Energy



δ to δ^*
 n to δ^*
 π to π^*
 n to π^*



λ max
Wave Strength

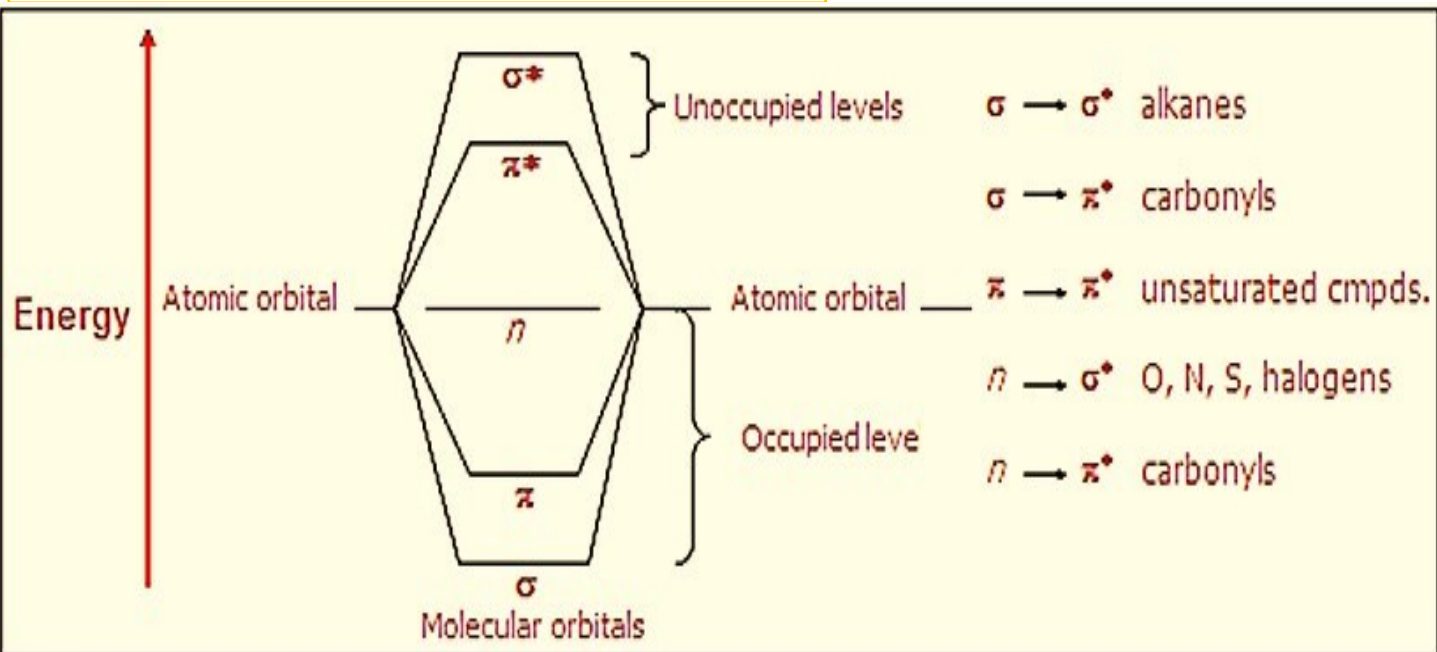
Band Structure

In molecules, when a bulk sample of molecules is observed, not all bonds (read – pairs of electrons) are in the same vibrational or rotational energy states



This effect will impact the wavelength at which a transition is observed – very similar to the effect of H-bonding on the O-H vibrational energy levels .

Observed electronic



Electronic Transitions Involving n , σ , and π Molecular Orbitals

Transition	Wavelength Range	Examples
$\sigma \rightarrow \sigma^*$	<200 nm	C-C, C-H
$n \rightarrow \sigma^*$	160-260 nm	H ₂ O, CH ₃ OH, CH ₃ Cl
$\pi \rightarrow \pi^*$	200-500 nm	C=C, C=O, C=N, C≡C
$n \rightarrow \pi^*$	250-600 nm	C=O, C=N, N=N, N=O

- $\sigma \rightarrow \sigma^*$

High energy required, vacuum UV range

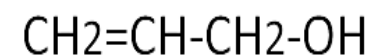
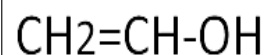
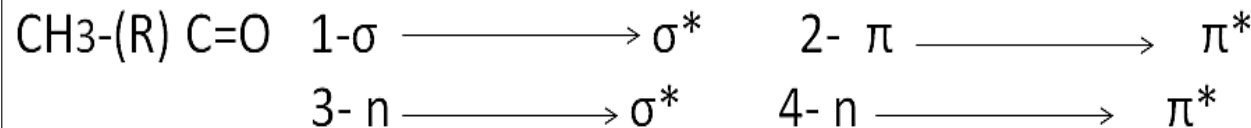
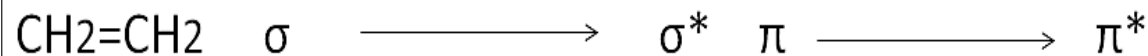
CH₄: $\lambda = 125$ nm

- $n \rightarrow \sigma^*$

Saturated compounds, CH₃OH etc ($\lambda = 150 - 250$ nm)

- $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$

Mostly used! $\lambda = 200 - 700$ nm



As the above example (1-4)

As the above example (1-3) no 4 x

Q.// $\pi-\pi^*$ Transition is the most convenient and useful transition in UV-Vis Spectroscopy. Why?

In $\sigma-\sigma^*$ transitions :

- The high energy required can cause rupture of the σ bonds and breakdown of the molecule.
- Air components absorb in vacuum UV which limits the application of the method ,working in vacuum UV requires special training
- Special sources and detectors.
- All solvents contain σ bonds

In $n-\sigma^*$ transitions:

- The absorption wavelength for a $n-\sigma^*$ transition occurs at about 185 nm where, unfortunately, most solvents absorb. For example, water which has two pairs of nonbonding electrons that will strongly absorb as a result of the $n-\sigma^*$.(H₂O and other solvents with nonbonding electrons).
- in polar solvents the energy required for the $n-\sigma^*$ increases and thus the probability for the transition decreases.

In $n-\pi^*$ transitions:

- requires very little energy , However, unfortunately, the absorptivity of this transition is very small which precludes its use for sensitive quantitative analysis.
- using polar solvents increases the energy required for this transition, thus decreasing its probability.

In $\pi-\pi^*$ transitions : The most frequently used transition is the $\pi-\pi^*$ transition for the following reasons:

- The molar absorptivity for the $\pi-\pi^*$ transition is high allowing sensitive determinations.
- The energy required is moderate, far less than dissociation energy.
- In presence of the most convenient solvent (water), the energy required for a $\pi-\pi^*$ transition is usually smaller.