Al-Mustaqbal University
College of Science
Forensic Evidence Department

كلية العلوم
قسم الادلة الجنائية

المحاضرة الثالثة

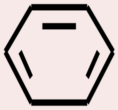
Analytical Chemistry

المادة : كيمياء تحليلية
المرحلة : الثانية
اسم الاستاذ: م.د. كرار مجيد عبيد

UV-Visible Spectroscopy

Principles and instrumentation

Spectra - Structure Correlation

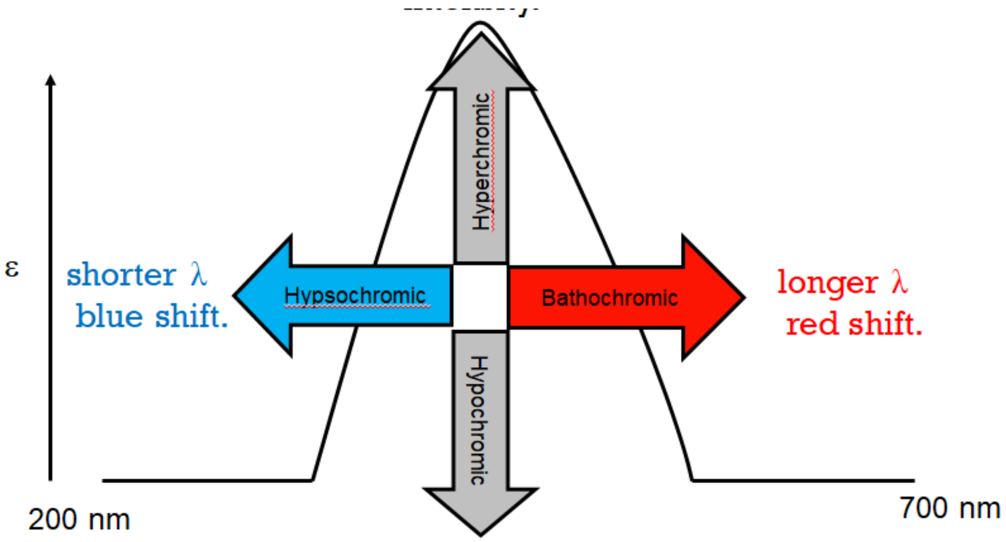
Group	Structure	λ_{max}
Carbonyl	> C = O	280
Azo	-N = N-	262
Nitro	-N=O	270
Thioketone	-C =S	330
Nitrite	-NO2	230
Conjugated Diene	-C=C-C=C-	233
Conjugated Triene	-C=C-C=C-C=C-	268
Conjugated Tetraene	-C=C-C=C-C=C-C=C-	315
Benzene		261

Chromophores: Unsaturated organic functional groups that absorb in the ultraviolet or visible regions.

Chromophores: Unsaturated organic functional groups that absorb in the ultraviolet or visible regions.

Auxochromes: substituents with unshared pairs like OH, NH, SH ..., when attached to π chromophore they generally move the absorption max. to longer λ.

Increase in absorption intensity.



Decrease in absorption intensity.

Bathochromic shift (red shift) : shift of absorption to longer wavelength due to substitution or solvent effect.

Hypthochromic shift (blue shift) : shift of absorption to shorter wavelength due to substitution .

Hyperchromic effect : increase in absorption intensity .

Hypochromic effect : decrease in absorption intensity .

Monochromatic beam : single beam of radiation .

Polychromatic beam : multiple beam of radiation .

Why we use UV-Visible spectroscopy?

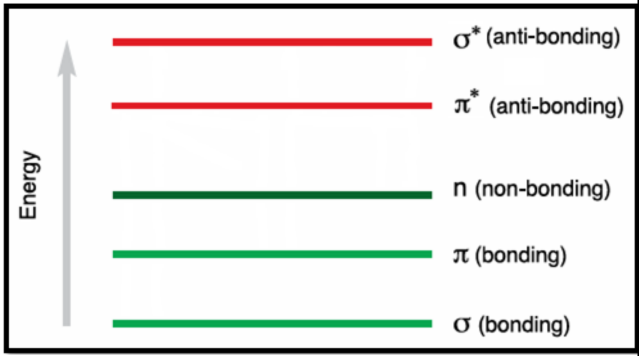
- ✓ Detection of functional groups
- ✓ Detection of impurities
- ✓ Qualitative and quantitative analysis
- ✓ Its helpful to show the relationship between different groups, and detect the conjugation of compounds.

Types of electrons

σ electrons: in saturated compounds

π electrons: in unsaturated compounds

n electrons: in non bonded electrons



Types of Electronic Transitions

σ → σ* transition

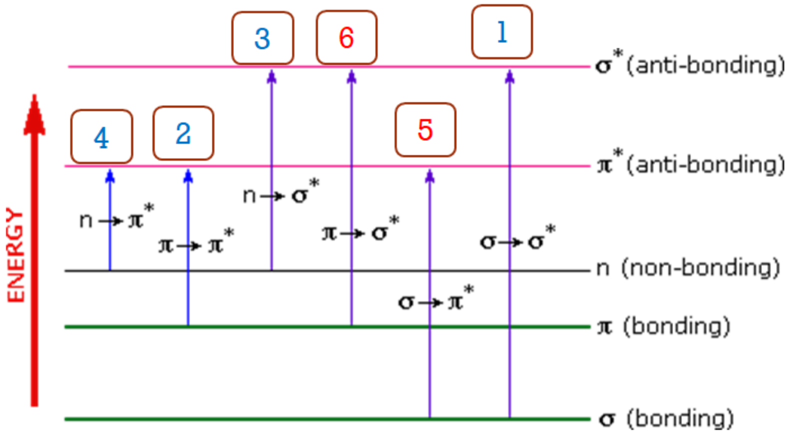
π → π* transition

n → σ* transition

n → π* transition

σ → π* transition

π → σ* transition



n → π*	n → σ*	π → π*	σ → σ*

High energy (E)
Low wavelength (𝝀)

σ →

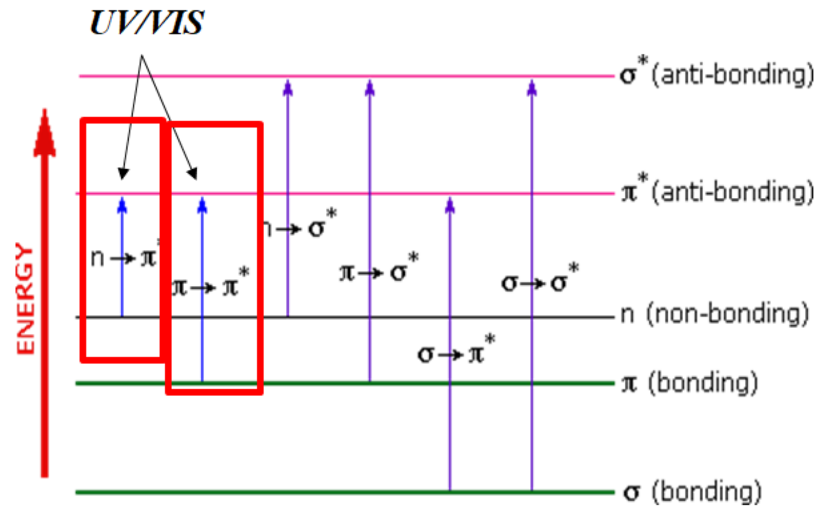
- ❖ An electron in a bonding σ orbital is excited to the corresponding antibonding orbital. The energy required is large.
- ❖ For example, methane (CH_4) (which has only C-H bonds, and can only undergo σ → σ* transitions) shows an absorbance maximum at 125 nm.
- ❖ Absorption maxima due to σ → σ* transitions are not seen in typical UV-VIS spectra (200 - 700 nm)

n → σ* transition

- Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of *n*→ *σ** transitions.
- These transitions usually need less energy than s→ σ * transitions. They can be initiated by light whose wavelength is in the range 150 - 250 nm.
- The number of organic functional groups with *n* → σ * peaks in the UV region is small.

n → π* transition **π → π* transition**

- Most absorption spectroscopy of organic compounds is based on transitions of *n* or p electrons to the **π*** excited state.
- These transitions fall in an experimentally convenient region of the spectrum (200 - 700 nm).
- These transitions need an unsaturated group in the molecule to provide the **π** electrons.
- *n* → **π*** have low (∈) (10-100 $L.mol^{-1}.cm^{-1}$)
- **π → π*** have high (∈) (1000-10000 $L.mol^{-1}.cm^{-1}$)
- With highly polar solvent, (n → **π***) are shifted to lower (λ) (blue shift), due to unpaired electrons.
- With highly polar solvent, (**π → π***) are shifted to higher (λ) (red shift), because of the attractive polarisation forces between solvent and absorbent.



(π → π*) transition is the most convenient and useful transition in uv-vis spectroscopy. why?

π–π* transition is the most frequently used transition for the following reasons:

- The Є for the π–π* 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 π–π* transition is usually smaller.

Chromophore	Excitation	λ_{max}, nm	Solvent
C=C	π→π*	171	hexane
C=O	n→π* π→π*	290 180	hexane hexane
N=O	n→π* π→π*	275 200	ethanol ethanol
C-X X=Br, I	n→σ* n→σ*	205 255	hexane hexane

Factors influencing uv-vis absorption:

1. **Type of solvent**

- ❑ This depends on the nature of the interaction of the particular solvent with the environment of the chromophore in the molecule under study.

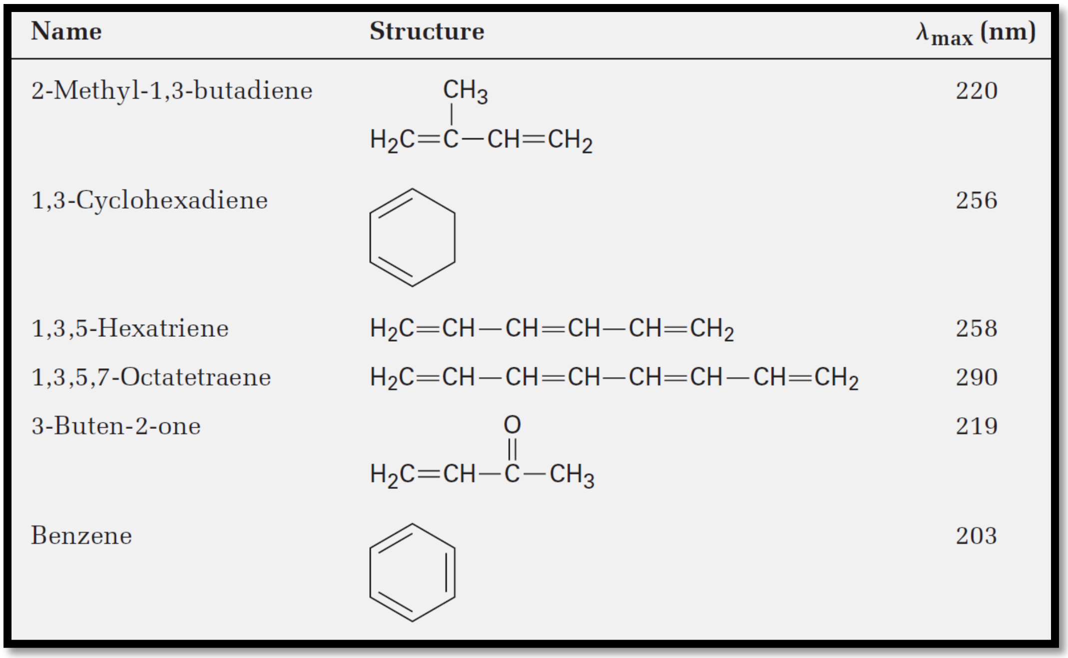
- ❑ It is usually observed that ethanol solutions give absorption maxima at longer λ than hexane solutions.

- ❑ Changes in the polarity of the solvent can influence shifts to longer or shorter λ, by changing in the energy gap between these electronic states.

- ❑ **Non-polar solvents** (saturated hydrocarbons) **do not** interact with solute molecules either in the ground or excited state.

2. **pH of the solution**
    - The buffer though needs to be transparent over the wavelength range of the measurements. If the buffer absorbs radiation, absorbance readings attributed to the analyte may be higher than they should because the buffer and analyte absorptions will add together at each wavelength.
    - If the optimum pH buffer solution is suitable with analyte, The absorbance spectra will show clear peak of the analyte, and vice versa.
3. **Temperature**
4. **Concentration**
5. **Conjugation**

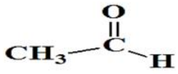
- Wavelength of UV radiation that causes π–π* excitation in a conjugated molecule ultimately depends on the nature of the conjugated system
- Degree of conjugation has a significant influence on the wavelength and molar absorptivity.



Name	Structure	λ_{max} (nm)
2-Methyl-1,3-butadiene	$H_2C{=}\overset{CH_3}{\overset{\vert}{C}}{-}CH{=}CH_2$	220
1,3-Cyclohexadiene		256
1,3,5-Hexatriene	$H_2C{=}CH{-}CH{=}CH{-}CH{=}CH_2$	258
1,3,5,7-Octatetraene	$H_2C{=}CH{-}CH{=}CH{-}CH{=}CH{-}CH{=}CH_2$	290
3-Buten-2-one	$H_2C{=}CH{-}\overset{O}{\overset{\Vert}{C}}{-}CH_3$	219
Benzene		203

$CH_3{-}\overset{O}{\overset{\Vert}{C}}{-}CH_3$

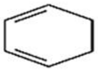
ε = 19



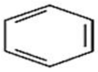
$CH_3{-}\overset{O}{\overset{\Vert}{C}}{-}H$

ε = 12

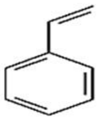
ε = 20, 900



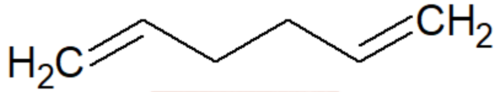
ε = 4,580



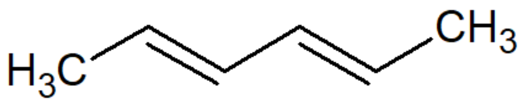
ε = 8,000



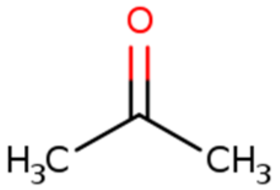
ε = 12,000

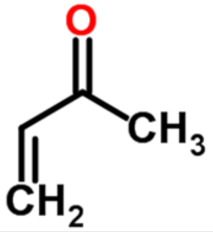


λ_{max}= 178 nm



λ_{max}= 227 nm





n → π*	λ_{max}= 274 nm (ϵ_{max}= 13.6)	λ_{max}= 331 nm (ϵ_{max}= 25)
π → π*	λ_{max}= 195 nm (ϵ_{max}= 9000)	λ_{max}= 203 nm (ϵ_{max}= 9600)

Note: more conjugated double bonds means less (*E*)is required for the electronic transition, and therefore longer ***λ*** at which the electronic transition occurs.

Absorption Spectra

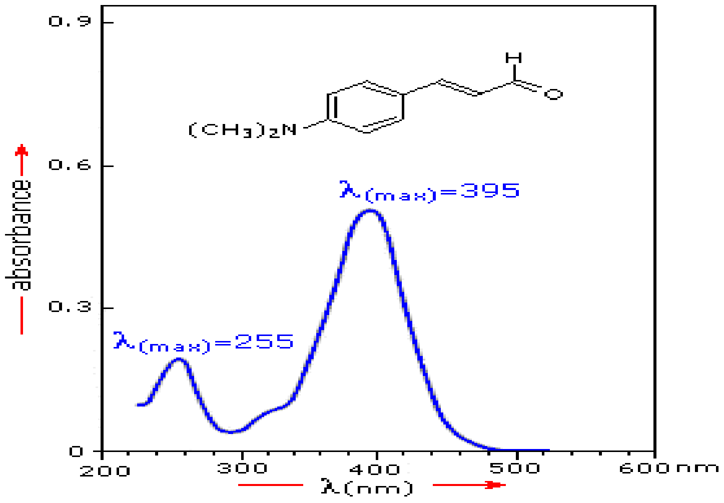
Absorption spectrum is a plot of absorption of radiation vs wavelength . According to the absorbed radiation we can classified the absorption spectra into:

1 - UV - Spectrum

2 - Visible Spectrum

3 - IR - Spectrum

4 - NMR -Spectrum

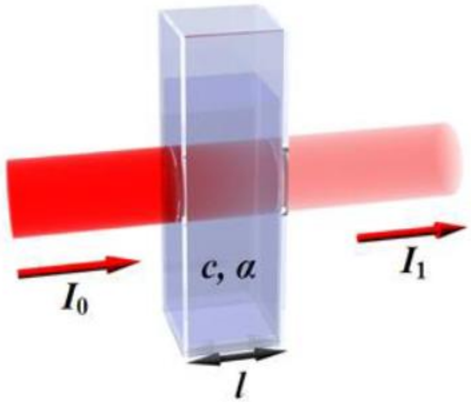


Experimental measurements are usually made in terms of transmittance (T), which is defined as:

$$T = \frac{P}{P_0}$$

where P is the power of light after it passes through the sample and Po is the initial light power. **The relation between A and T is:**

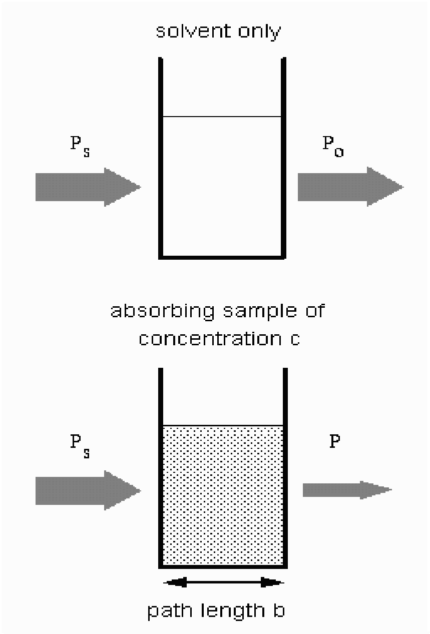
$$A = -\log(T) = -\log\left(\frac{P}{P_0}\right)$$



Beer-Lambert Law

% Transmittance = (I / I^0) x 100

where:



I = intensity of transmitted light

I^0 = intensity of incident light

$$\boxed{A = a\ b\ c}$$

where A : absorbnce

a (Є): absorpitivity,

b : thickness cm

c : concentration g/L

The value of "a" will depend upon the method of expression of the concentration :

If C is expressed in mole/liter , then "a" is given the symbol Ɛ (L.mol^{-}1cm^{-1})and is called ***molar absorbitivity***

$A = Ɛ\ b\ c$

When c is in gm/100ml , b = 1 cm we get

$A = ab\ c$

Deviation From Beer,S Law

- Real Deviation
- Instrumental Deviation
- Chemical Deviation

a - Real Deviation :

-dilution effect - molecular interaction

-complex formation

b - Instrumental Deviation (Errors)

1 - Irregular Deviation

-unmatched cells

-unclean optics (cells - lenses - mirrors - lamps)

2 - Regular Deviation

- error in wavelength scale

- slit-width

-stray light

3 - Non linear response of the phototubes

- Radio and TV interference's

- Voltage fluctuations

c- Chemical Deviations

•pH effect - solvent effect - temperature effect - photodegradation

• time factor (oxidation - reduction - hydrolysis - polymerization)