

# MOLECULAR ABSORPTION METHODS

Absorption spectroscopy refers to spectroscopic techniques that measure the absorption of radiation, as a function of wavelength ( absorption spectrum ) , due to its interaction with sample molecules in solution . The intensity of the absorption is direct proportional to the sample concentration . Absorption spectroscopy is performed across the electromagnetic spectrum . However molecular absorption spectroscopy in the UV/Vis region is applied for the qualitative and quantitative analysis of organic and inorganic compounds and it will be the subject of this unit.

Absorption methods require two power measurements: one before a beam has passed through the medium that contains the analyte ( $P_0$ ) and the other after the sample ( $P$ ). Two terms, which are widely used in absorption spectrometry and are related to the ratio of  $P_0$  and  $P$ , are **transmittance** and **absorbance**.

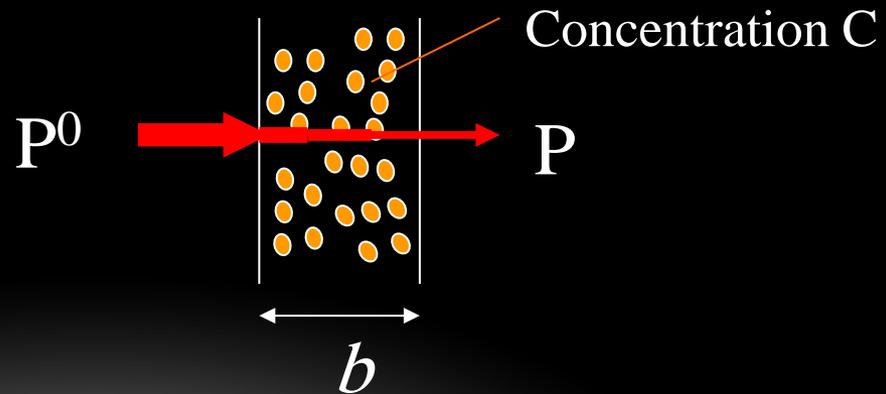
# Beer's Law

The relative amount of a certain wavelength of light absorbed ( $A$ ) that passes through a sample is dependent on:

distance the light must pass through the *sample (cell path length -  $b$ )*

*amount of absorbing chemicals in the sample (analyte concentration -  $c$ )*

*ability of the sample to absorb light (molar absorptivity -  $\epsilon$ )*



## Beer's Law

The relative amount of light making it through the sample ( $P/P_0$ ) is known as the **transmittance (T)**

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

**Percent transmittance**

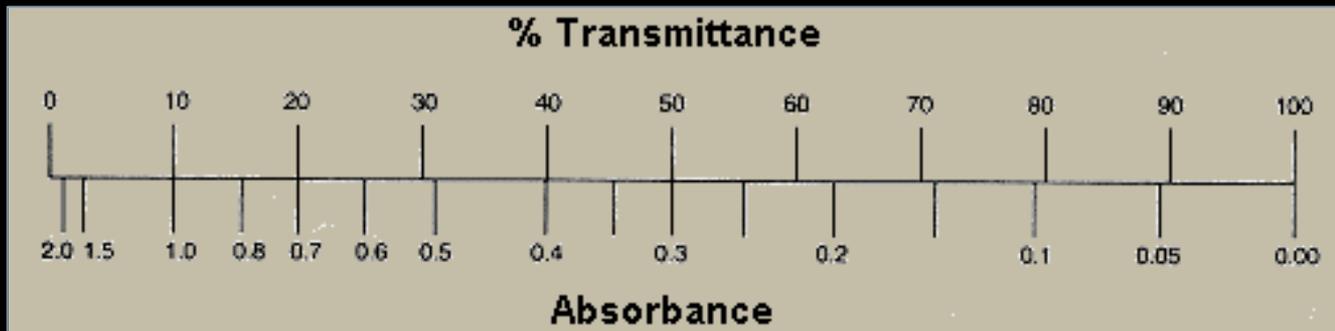
$$\%T = 100 \times \left( \frac{P}{P_0} \right)$$

## Beer's Law

Absorbance (A) is the relative amount of light absorbed by the sample and is related to transmittance ( $T$ )

$$A = -\log\left(\frac{P}{P_o}\right) = -\log(T) = -\log(\%T / 100)$$

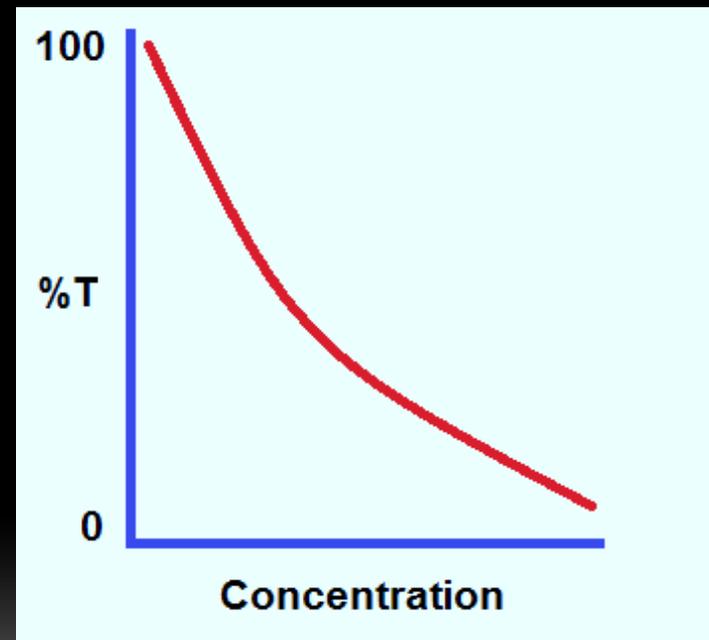
$T$  has a range of 0 to 1 ,  $\%T$  has a range of 0 to 100%



$A$  has a range of 0 to infinity

# Beer's Law

Spectrophotometers measure transmittance but transmittance is not linear when plotted against concentration . Therefore, we convert transmittance to absorbance because the relationship between absorbance and concentration is linear as we will see shortly .



## Beer's Law

Absorbance is directly related to the analyte concentration , cell path length and molar absorptivity . This relationship is known as Beer's Law

$$A = abx$$

$$A = \epsilon bc$$

where:

$A$  = absorbance (no units)

$\epsilon$  = molar absorptivity (L/mole-cm)

$b$  = cell pathlength (cm)

$c$  = concentration of analyte (mol/L)

$x$  = Concentration in any unit rather than mol/L .

$a$  = absorptivity using  $x$  unit .

## The Molar Absorptivity $\epsilon$

It is clear that  $\epsilon$  is an important element in Beer's law expression. It is an indicator of sensitivity. As  $\epsilon$  increases, it becomes easier to determine lower concentrations of analytes.

The molar absorptivity has large values for  $\pi \rightarrow \pi^*$  transitions ranging from 1000 to 10000 L cm<sup>-1</sup> mol<sup>-1</sup>. For  $n \rightarrow \pi^*$  transitions,  $\epsilon$  ranges from 10 - 100 L cm<sup>-1</sup> mol<sup>-1</sup>. This means that the most important transition in UV-Vis is the  $\pi \rightarrow \pi^*$  transition and, therefore, will be subjected to further studies in different solvents as we will see shortly .

## Calibration

To measure the absorbance of a sample, it is necessary to measure  $P_0$  and  $P$  ratio

$P_0$  is measured with a blank cell

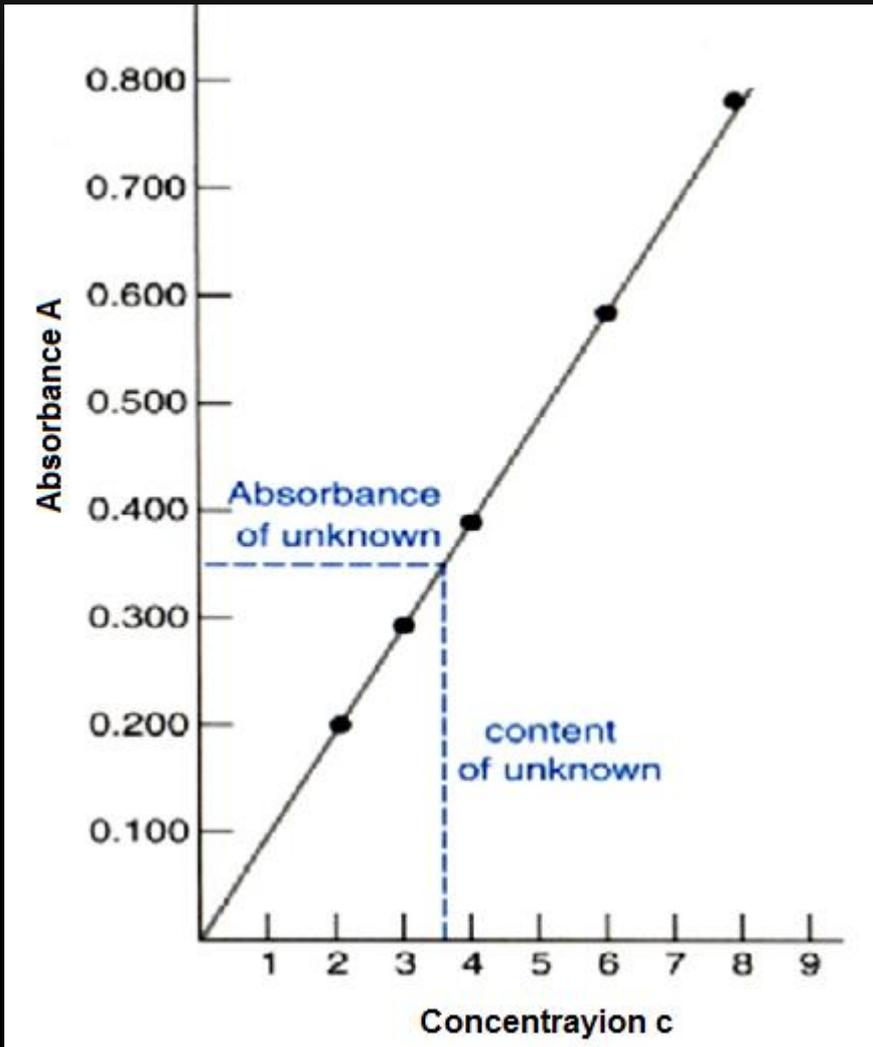
A blank cell contains all components in the sample solution **except** the analyte of interest

$P$  is measured by placing the sample in the cell .

To accurately measure an unknown concentration, obtain a calibration curve using a range of known concentrations for the analyte.

The greater the absorbance of a compound at a given wavelength (high  $\epsilon$ ), the easier it will be detected at low concentrations .

The relationship between the analyte concentration and the absorbance is linear .



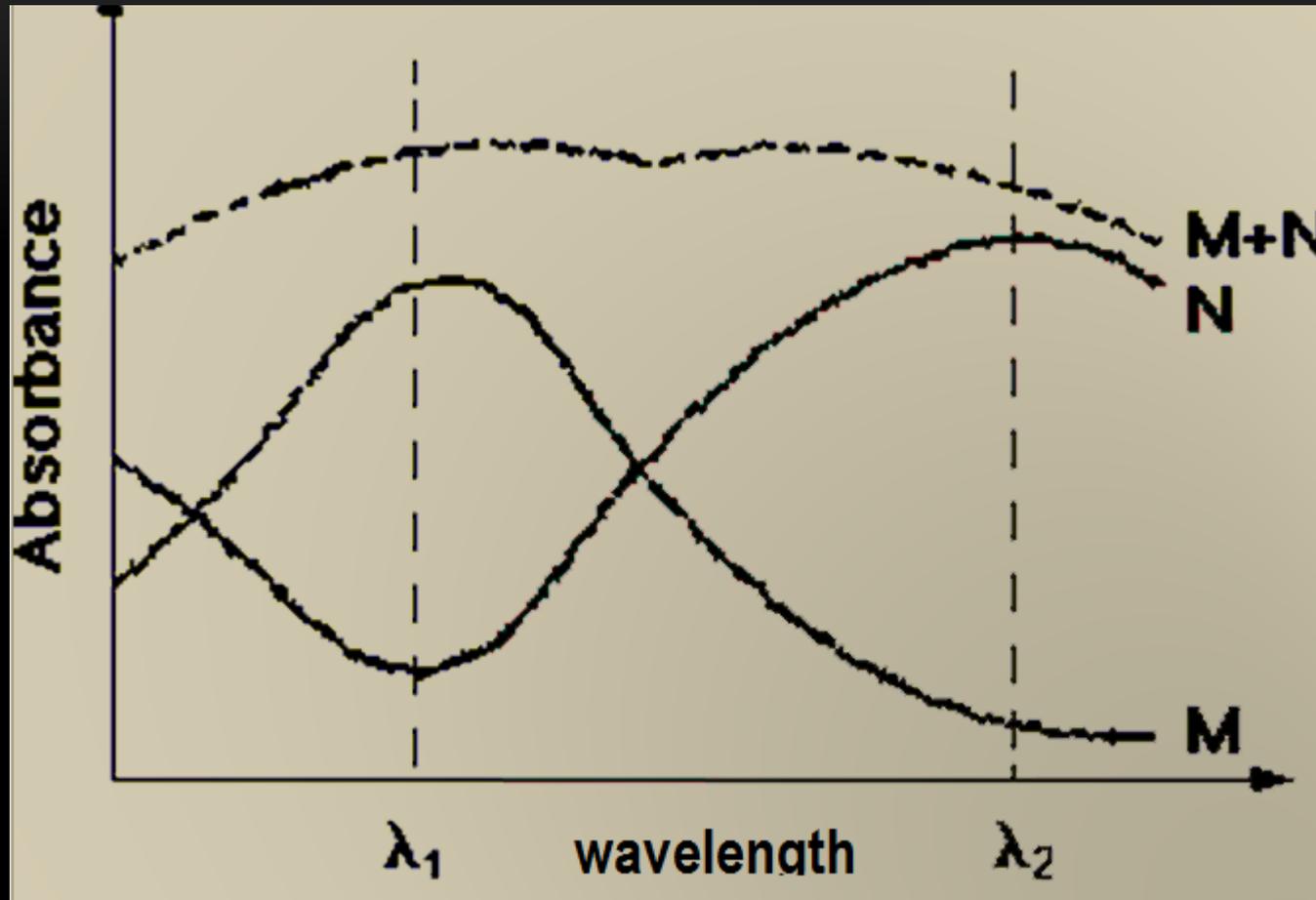
Standard solutions



Blank – is solution identical to sample but without analyte .

$$\epsilon = \Delta A / \Delta C \quad \text{when } b = 1$$

# Analysis of a mixture containing two compounds M and N



The above spectrum of the mixture shows that there is obviously no wavelength at which the absorbance of this mixture is due simply to one of the components; thus an analysis for either M or N by a single measurement is not possible. However, the absorbance of the mixture at the wavelengths  $\lambda_1$  and  $\lambda_2$  may be expressed as follows:

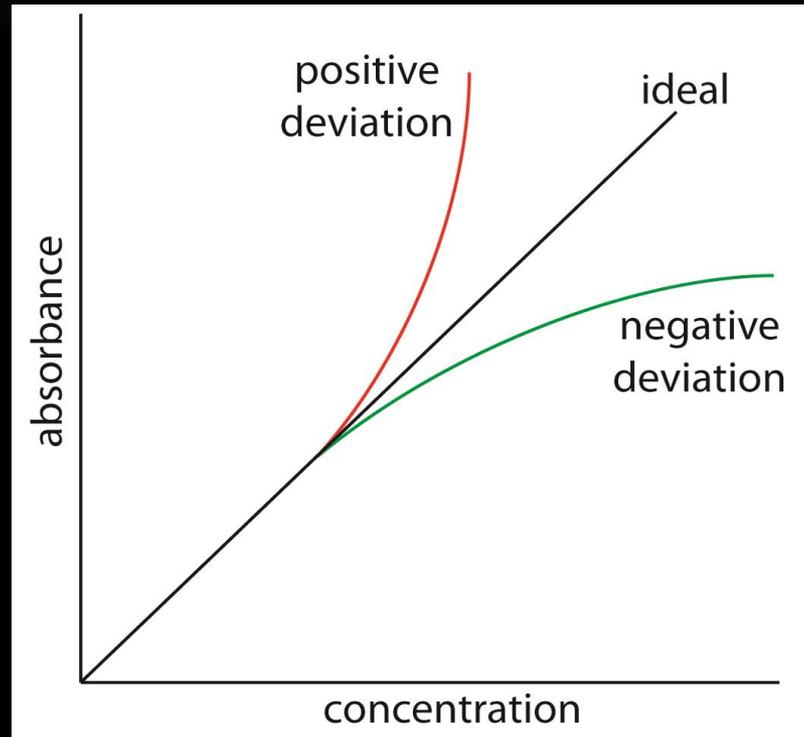
$$\text{At } \lambda_1 : A^1 = \epsilon_M^1 bc_M + \epsilon_N^1 bc_N$$

$$\text{At } \lambda_2 : A^2 = \epsilon_M^2 bc_M + \epsilon_N^2 bc_N$$

The four molar absorptivities  $\epsilon_M^1$ ,  $\epsilon_M^2$ ,  $\epsilon_N^1$ , and  $\epsilon_N^2$  can be evaluated from standard solutions containing only M or N. Then, if the absorbance of the mixture are measured at  $\lambda_1$  and  $\lambda_2$ , the concentration of the individual components can be calculated by solving the two equations given above simultaneously. The best accuracy in an analysis of this sort is obtained by choosing wavelengths at which the differences in molar absorptivities between the two ions is large.

# Limitations in Beer's Law

Beer's law suggests direct proportionality between Absorbance and concentration and that a straight line relationship should be obtained. However, some of the following factors can lead to different behavior :



## 1. Concentration

It is observed that only solutions that are less than 0.01 M can result in successful application of Beer's law. High concentrations of analyte lead to interactions between neighboring molecules resulting in a change in the absorbance characteristics of molecules. i.e. shifts in chemical equilibria as a function of concentration.

## 2. Monochromatic Light

Beer's law is valid only when a monochromatic radiation is used .

## 3. Stray Radiation

Fluorescence or phosphorescence of the sample or scattering of light due to particulates in the sample solution .

#### 4. Refractive Index

The application of Beer's law requires the measurement of the absorbance of different concentrations. Since  $\epsilon$  is dependent on refractive index, it is expected that solutions for which the refractive index changes a lot with concentration will show a deviation from Beer's law.

#### 5. Chemical Reactions or Transformations

If an analyte reacts with any species or dissociates forming species of different absorption characteristics, the absorbance may not necessarily be as predicted by Beer's law.

### Example:

A  $3.96 \times 10^{-4}$  M solution of compound A exhibited an absorbance of 0.624 at 238 nm in a 1.000 cm cuvette. A blank had an absorbance of 0.029. The absorbance of an unknown solution of compound A was 0.375.

Find the concentration of A in the unknown ?

### Solution :

$$\text{Absorbance due to standard} = 0.624 - 0.029 = 0.595$$

$$\text{Absorbance due to unknown} = 0.375 - 0.029 = 0.346$$

$$\epsilon = A / bc = 0.595 / ( 1 \times 3.96 \times 10^{-4} ) = 1502.5$$

$$c = A / \epsilon b = 0.346 / ( 1 \times 1502.5 ) = 2.3 \times 10^{-4} \text{ M}$$

## Example :

A 0.0450 M solution of X had an absorbance of 0.844 at 267 nm in a 1.00 cm cuvette, and an absorbance of 0.034 at 240 nm. A 0.0366 M solution of Y had absorbances of 0.010 and 0.755 at 267 and 240 nm, respectively. A mixture of X and Y had absorbances of 0.552 and 0.403 at 267 and 240 nm, respectively. Calculate the concentration of X and Y in the mixture ?

## Solution :

For a mixture analysis using Beer's Law, the general rule would be:

$$A = \epsilon b [X] + \epsilon b [Y] \quad , \quad \text{at a given wavelength.}$$

That is, the absorbance is equal to each individual component. You can find both concentrations if you know the molar absorptivity of all components involved. So let's find the molar absorptivity:

$$\epsilon = A/b[\ ] \quad , \quad \text{with } b = 1.00 \text{ cm, so } \epsilon = A/[\ ]$$

$\epsilon$  at 240 nm:

$$\text{X: } \epsilon = 0.844 / 0.045 = 18.76$$

$$\text{Y: } \epsilon = 0.010 / 0.0366 = 0.2732$$

$\epsilon$  at 267 nm:

$$\text{X: } \epsilon = 0.034 / 0.045 = 0.7556$$

$$\text{Y: } \epsilon = 0.755 / 0.0366 = 20.63$$

You will now have 2 equations:

$$A = \epsilon [X] + \epsilon [Y] \text{ at } 240 \text{ nm}$$

$$A = \epsilon [X] + \epsilon [Y] \text{ at } 267 \text{ nm}$$

Plug in the molar absorptivity and the mixture absorbances the A at their respective wavelengths. This will give you 2 equations with 2 unknowns.

$$0.267 = 18.76C[X] + 0.2732C[Y]$$

$$0.403 = 0.7556C[X] + 20.63C[Y]$$

Solve the linear system of equations and you get:

$$[X] = 0.0140 \text{ M and } [Y] = 0.0190 \text{ M}$$

So the mixture contains 0.0140 M of X and 0.0190 M of Y .

# SPECTROPHOTOMETER : SEE UNIT 3

## 1- Light source

**Tungsten lamp:** VIS.(320 nm~2500 nm)

**Deuterium lamp:** UV (200~400 nm)

## 2- Monochromator consists :

- lenses and mirrors: focus the radiation.
- entrance and exit slits: restrict unwanted and control the spectral purity of radiation.
- dispersing medium: separate the  $\lambda_{\max}$  from polychromatic radiation of the source. (a) **prism** and (b) **diffraction grating** .

**3- Sample Cell** : sample container of fixed length.  
Usually round or square cell .  
Made of material that does not absorb light in the  
wavelength range of interest

- Glass – visible region
- Quartz – ultraviolet



## 4- Detector

Convert radiant energy (photons) into an electrical current .  
Ideal detector should has high sensitivity , high signal/noise ratio , constant response for  $\lambda$ s, and fast response time.

The most commonly used detector is **photomultiplier tube** which is very sensitive .

# Types of Spectrophotometers

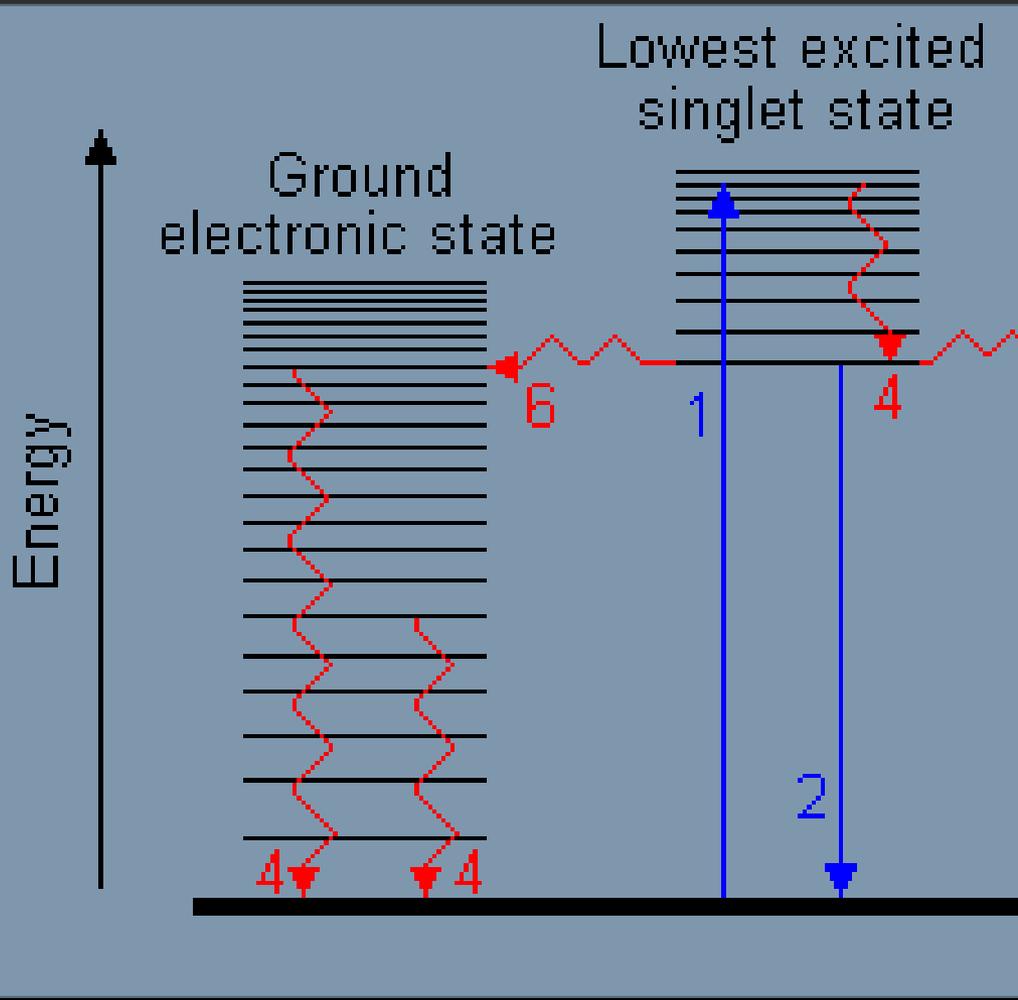
1- Single-Beam Instrument

2- Double-Beam Instrument which has many advantages over single – beam where it continuously compares sample and blank Automatically and corrects for changes in electronic signal or light intensity of source. For more details see unit 3 .

## What Happens When a Molecule Absorbs Light?

- Combined electronic, vibrational and rotational Transitions .
- Absorption of radiation by a molecule will cause electronic, vibrational and rotational transitions .
- There are multiple vibrational and rotational energy levels associated with each electronic state.

Therefore, transition between electronic states or within one electronic state can occur between different vibrational and rotational states .



*Vibrational and rotational states associated with an electronic state*

*Energy: Electronic >> Vibrational > Rotational*

## Absorption Spectrum

Different chemicals have different energy levels ( electronic , vibrational and rotational levels). Therefore they will have different abilities to absorb light at any given wavelength i.e. different absorption spectrum . plot of absorbance (or  $\epsilon$ ) vs. wavelength for a compound is its spectrum . For more details see unit 2 .

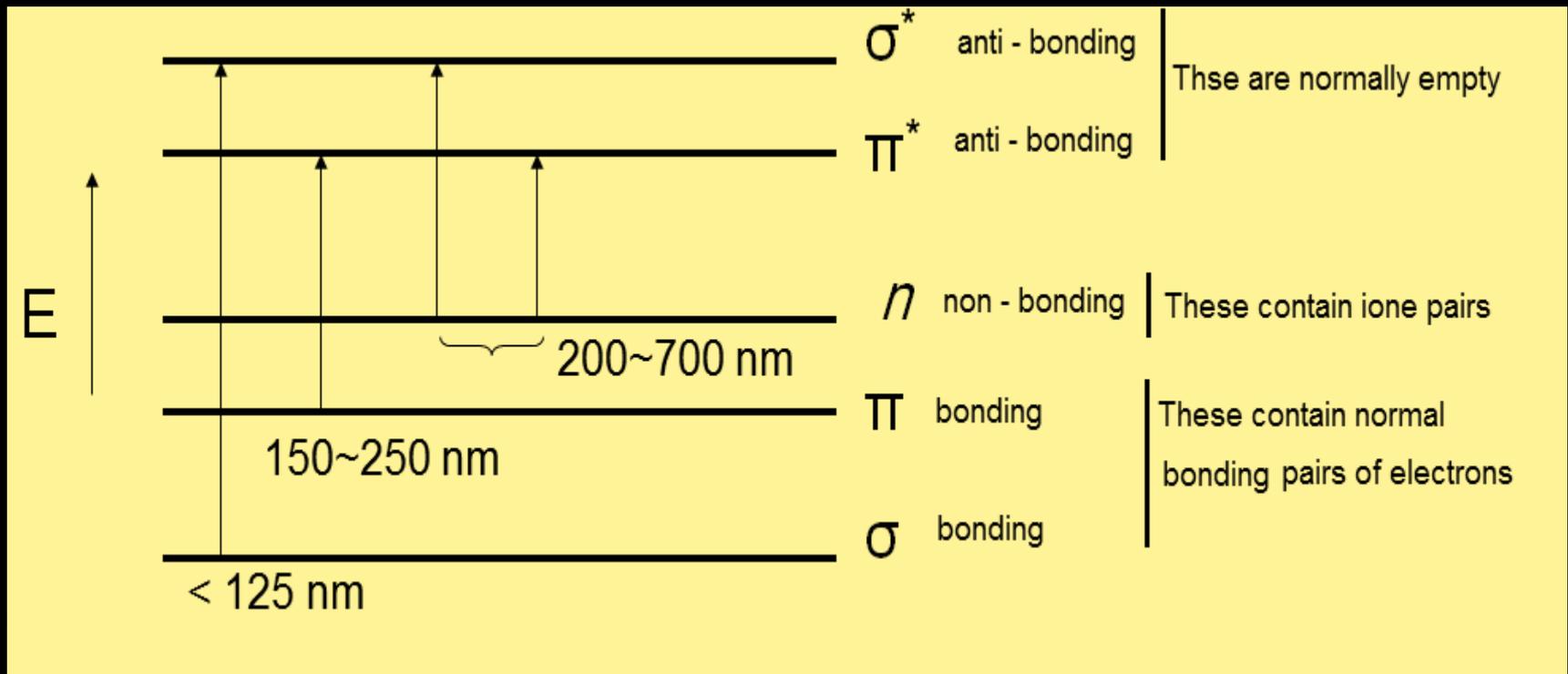
## chromophores

When atoms combined to form a molecule three types of molecular orbitals will be formed,  $\sigma$  ( C-C ),  $\pi$  ( C=C or C $\equiv$ C ) and n ( N,O,S, X ). Normally electrons are in these orbitals but when the molecule absorb 200 – 800 nm radiation, these electrons will move to higher excited molecular orbitals  $\sigma^*$  or  $\pi^*$ . Groups in a molecule which absorb light are known as chromophores e.g C = C, C  $\equiv$  C, C = O, N = O. See the following Table.

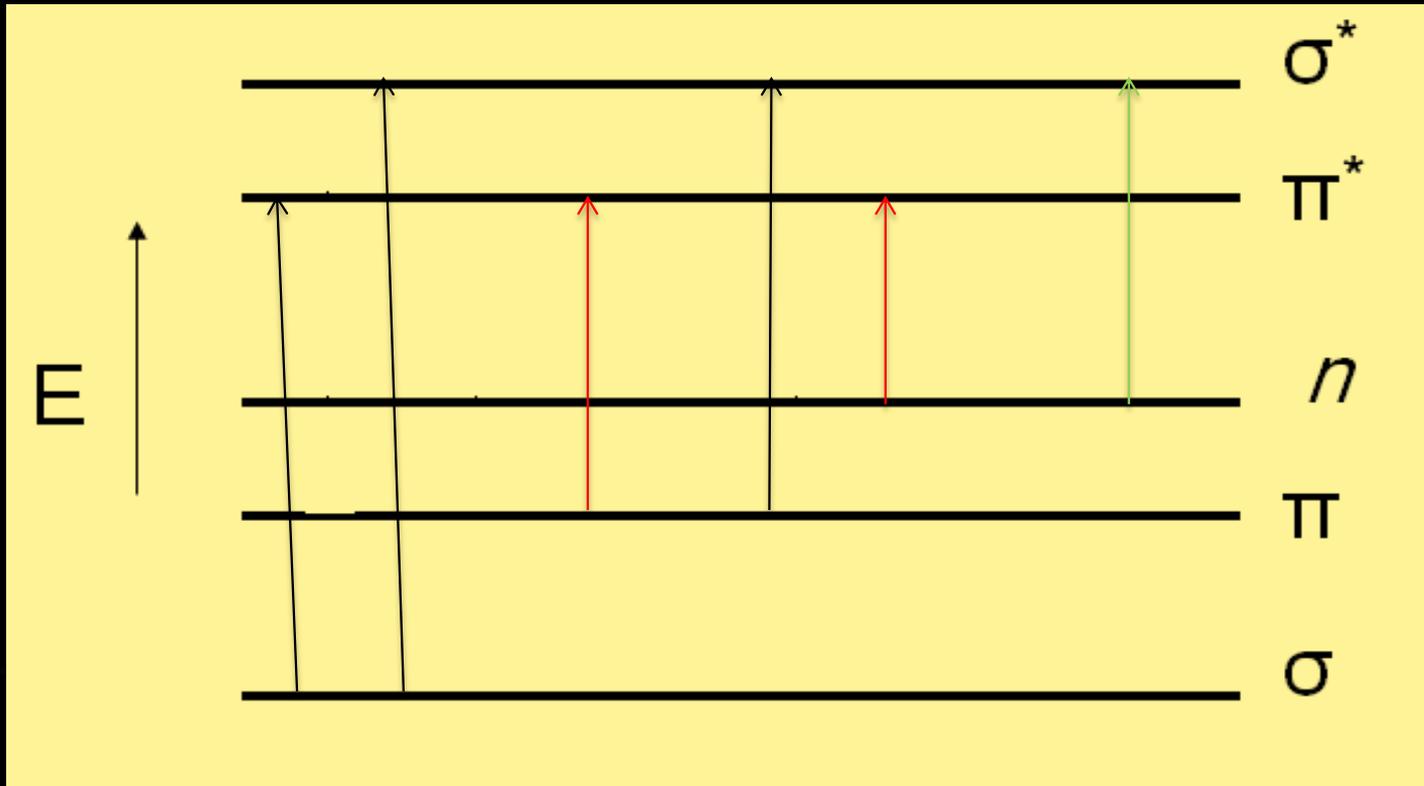
Molecules having unsaturated bonds ( double or triple bonds ) or free nonbonding electrons ( n free electrons ) can absorb radiation. Examples include alkenes, alkynes, ketones, aldehydes, phenyl and other aromatic species.

We will explain what happens when organic compounds absorb UV or visible light, and why the wavelength of light absorbed varies from compound to compound .

When a molecule absorbs EM radiation , four types of electronic transitions may occur :



The important jumps are shown in red, and a less important one in green. The black arrows show jumps which absorb light outside the region of the spectrum that we are working in ( UV – Vis ).



Chromophore	Example	Excitation	$\lambda_{\max}$ , nm	$\epsilon$	Solvent
C=C	Ethene	$\pi \rightarrow \pi^*$	171	15000	hexane
C $\equiv$ C	1-Hexyne	$\pi \rightarrow \pi^*$	180	10000	hexane
C=O	Formaldehyde	$n \rightarrow \pi^*$	290	15	hexane
		$\pi \rightarrow \pi^*$	180	10000	hexane
N=O	Nitromethane	$n \rightarrow \pi^*$	275	17	ethanol
		$\pi \rightarrow \pi^*$	200	5000	ethanol
C-X X = I or Br	Methyl bromide	$n \rightarrow \sigma^*$	205	200	hexane
	Methyl iodide	$n \rightarrow \sigma^*$	255	360	hexane
C - C	Saturated compds (Solvents) ?	$\sigma \rightarrow \sigma^*$	Shorter $\lambda$ than uv-vis region < 200 nm		

$\pi \rightarrow \pi^*$  allowed

$n \rightarrow \pi^*$  Not allowed by the selection rules but sometimes it happens

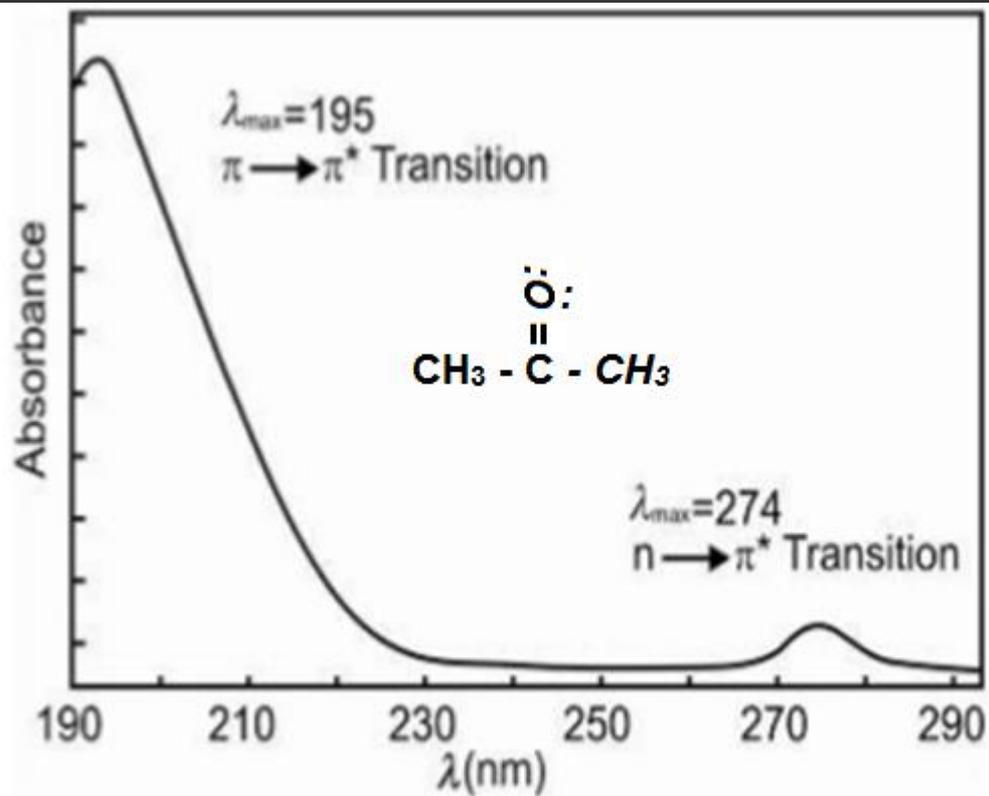


An electron in a bonding  $\sigma$  orbital of a molecule is excited to the corresponding anti-bonding orbital  $\sigma^*$  as a result of its absorption of radiation. To induce  $\sigma \rightarrow \sigma^*$  transitions it required a larger energy (  $< 190 \text{ nm}$  ) than uv or vis.

Example : Methane contain only single C-H bonds that undergo only  $\sigma \rightarrow \sigma^*$  transition , it gives absorption maximum at  $125 \text{ nm}$  .

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

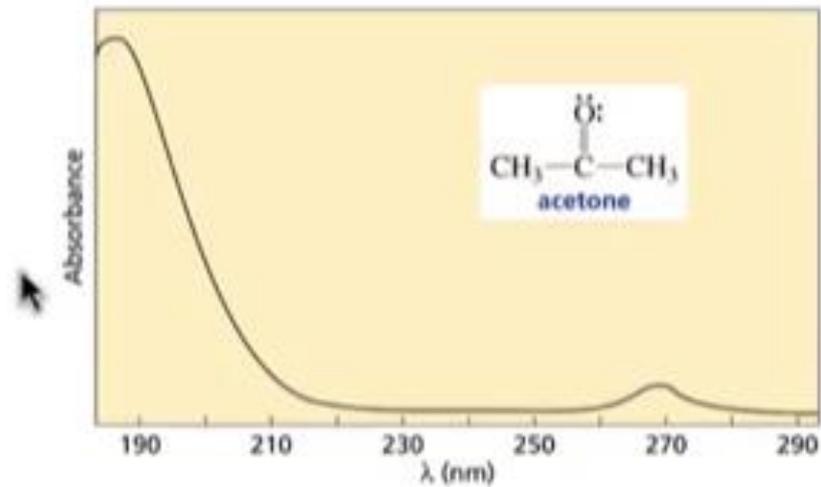
Most organic compounds undergo  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions.  $\pi \rightarrow \pi^*$  transitions are generally intense and more important while  $n \rightarrow \pi^*$  transitions are weak. For example acetone exhibits a high  $\pi \rightarrow \pi^*$  transition peak at 195 nm and a weak  $n \rightarrow \pi^*$  transition peak at 274 nm. See the following spectrum. The energy required for these transitions can be obtained from sources operating in the UV-Vis range.



spectrum of acetone showing the  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions

$n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions require unsaturated functional groups ( double bonds , triple bonds and N or O or halides atoms ) , most commonly used in uv/vis absorption .

# UV Visible Spectral Transitions



## **n $\rightarrow$ $\sigma^*$ transitions**

In this type saturated compounds containing atoms with unshared electrons pairs ( N,O,S and halogens atoms ) undergo n  $\rightarrow$   $\sigma^*$  transitions . Example : Methyl chloride  $\text{CH}_3\text{Cl}$  . It requires less energy than the  $\sigma \rightarrow \sigma^*$  type . Most of the absorption peaks appear below 200 nm which is out side our range ( 200 – 800 nm ).

The absorption intensity is indication of the probability of the transition i.e. whether the transition is allowed or not by the selection rules .

# The importance of conjugation and delocalization

Consider these three molecules:

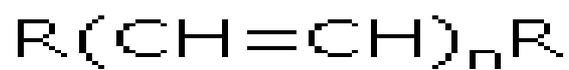
$\text{CH}_2 = \text{CH}_2$	$\text{CH}_2 = \text{CH} - \text{CH} = \text{CH}_2$	$\text{CH} = \text{CH} - \text{CH} = \text{CH} - \text{CH} = \text{CH}_2$
ethen	buta – 1,3 – diene	hexa – 1,3,5 - triene
$\lambda_{\text{max}} = 171 \text{ nm}$	$\lambda_{\text{max}} = 258 \text{ nm}$	$\lambda_{\text{max}} = 271 \text{ nm}$

Ethene contains a simple one isolated carbon-carbon double bond, but the other two have conjugated double bonds. In these cases, there is delocalization ( stability ) of the  $\pi$  bonding orbitals over the whole molecule. Now look at the wavelengths of the light which each of these molecules absorbs.

All of the molecules give similar UV-visible absorption spectra the only difference being that the absorptions move to longer and longer wavelengths as the amount of delocalization in the molecule increases .

As conjugation is increased in a molecule, more delocalization (stability) of the  $\pi$  electrons results. The effect of this delocalization is to decrease the  $\pi^*$  molecular orbital. The result is a decrease in transition energy from  $\pi - \pi^*$  and thus a bathochromic shift. The molar absorptivity will increase in this case and better quantitative analysis will be achieved . See the following figure .





100,000

$\epsilon$

50,000

$n = 5$

$n = 4$

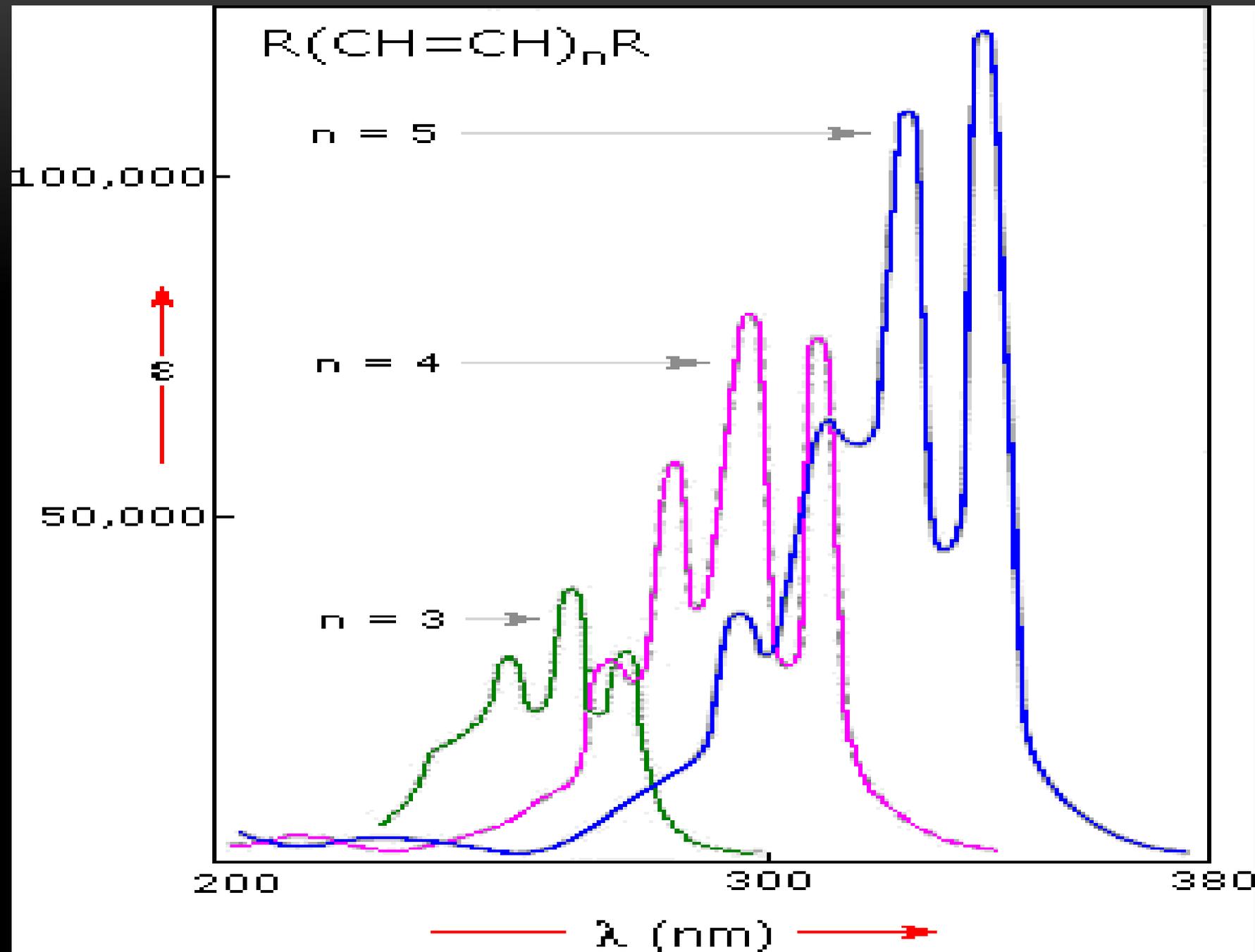
$n = 3$

200

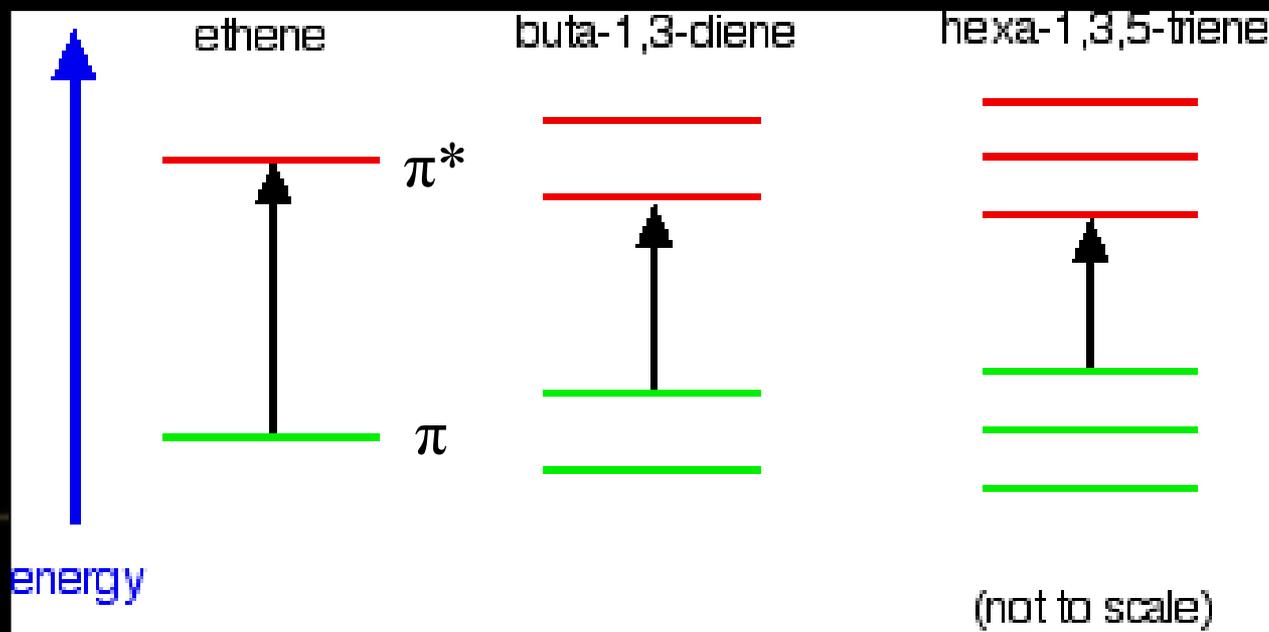
300

380

$\lambda$  (nm)



The highest occupied molecular orbital is often referred to as the HOMO - in these cases, it is a  $\pi$  bonding orbital. The lowest unoccupied molecular orbital (the LUMO) is a  $\pi^*$  anti-bonding orbital. Notice that the gap between these has fallen. It takes less energy to excite an electron in the buta-1,3-diene case than with ethene. In the hexa-1,3,5-triene case, it is less still .



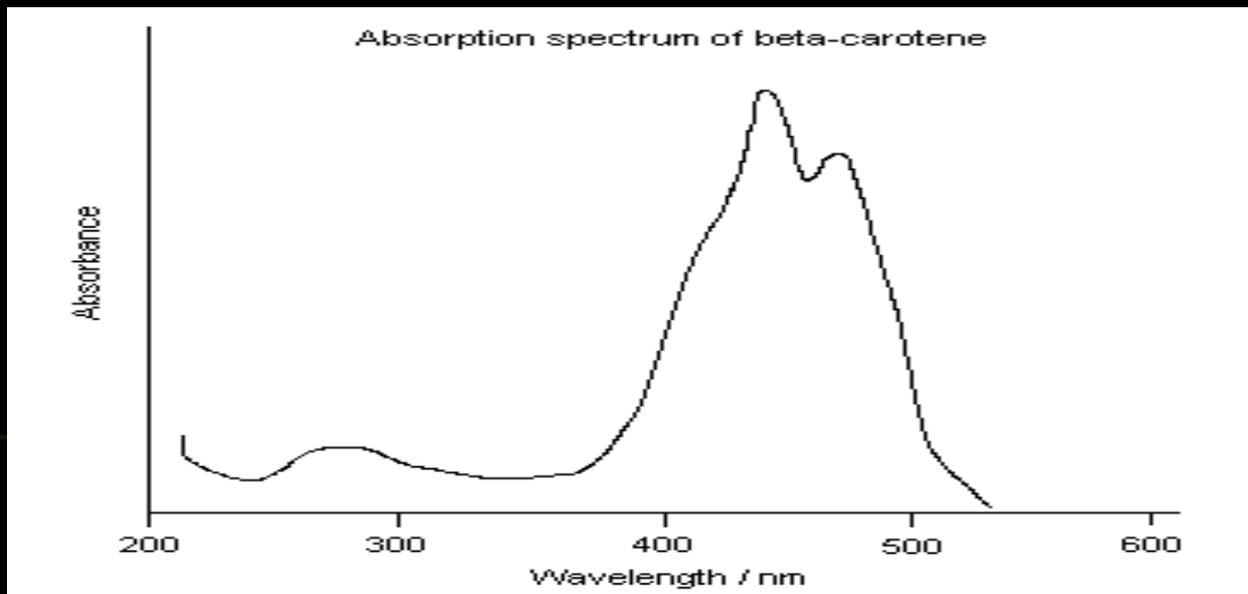
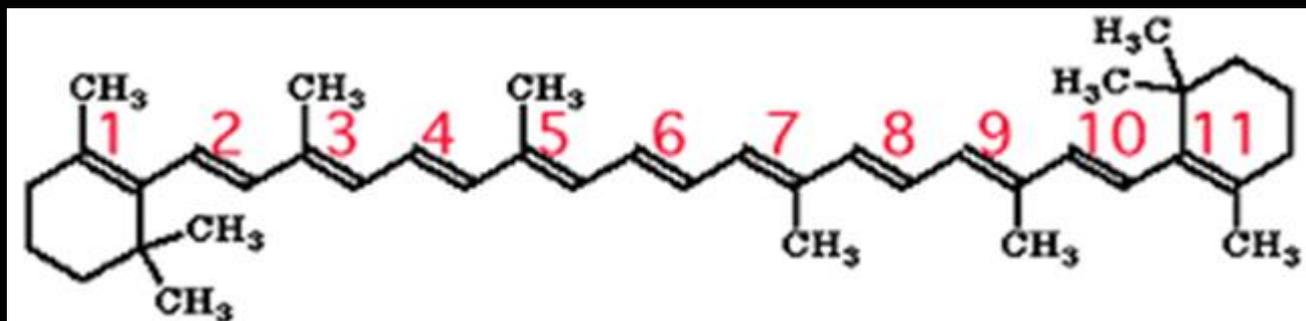
The maximum absorption is moving to longer wavelengths as the amount of delocalization increases.

Therefore maximum absorption is moving to shorter frequencies as the amount of delocalization increases.

Therefore absorption needs less energy as the amount of delocalization increases.

Therefore there must be less energy gap between the bonding and anti-bonding orbitals as the amount of delocalization increases.

If you extend this to compounds with really massive delocalization, the wavelength absorbed will eventually be long enough to be in the visible region of the spectrum, and the compound will then be seen as colored. A good example of this is the beta-carotene present in carrots :



# Pi Delocalization and Color



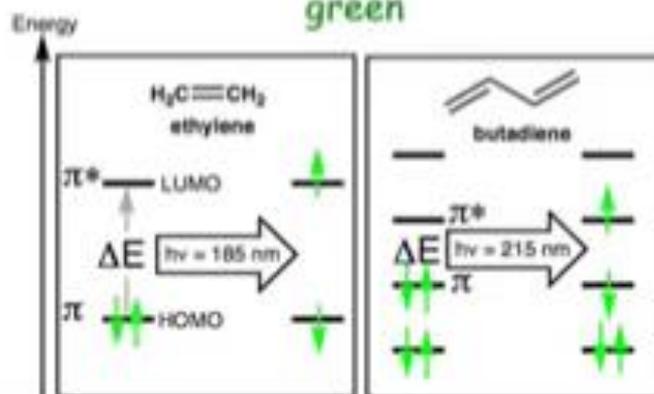
## 1. Color

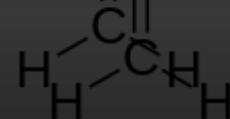
Colored compounds have electronic absorptions in the visible region of the electromagnetic spectrum. Color is usually associated with extended conjugation. The color we see is the complement of the light wavelength absorbed.

Wavelength of Light (nm)	Color	Complementary Color
400-430	violet	green-yellow
430-480	blue	yellow
480-490	green-blue	orange
490-510	green	purple
510-530	yellow-green	violet
570-580	yellow	blue
580-600	orange	green-blue
600-680	red	blue-green
680-750	purple	green

### Origins of color

- (a) extended conjugation
- (b) charge
- (c) low lying  $\pi^*$  orbitals

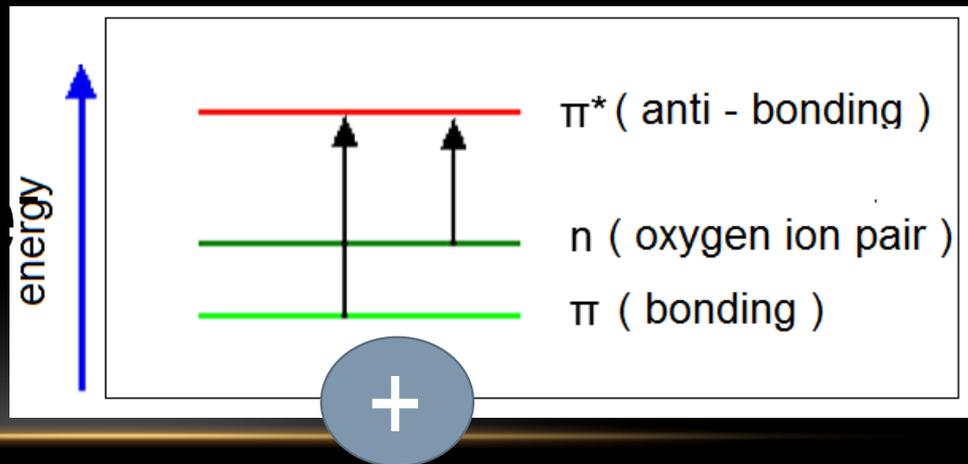
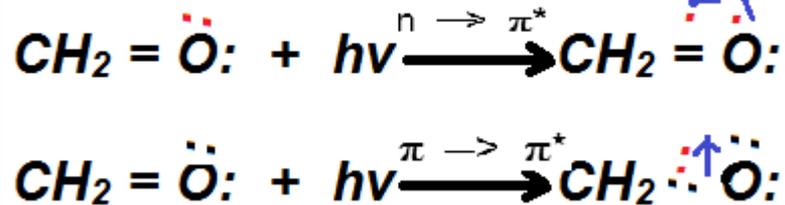




## A chromophore producing two peaks

A chromophore such as the carbon-oxygen double bond in formaldehyde, for example, obviously has  $\pi$  electrons as a part of the double bond, but also has lone pairs on the oxygen atom. That means that both of the important absorptions from the last energy diagram are possible. You can get an electron excited from a  $\pi$  bonding to a  $\pi^*$  anti-bonding orbital, or you can get one excited from an oxygen lone pair (a non-bonding orbital) into a  $\pi^*$  anti-bonding orbital.

formaldehyde



The non-bonding orbital  $n$  has a higher energy than a  $\pi$  bonding orbital. That means, the jump from an oxygen lone pair into a  $\pi^*$  anti-bonding orbital needs less energy. That means also it absorbs light of a lower frequency and therefore a longer wavelength. formaldehyde can therefore absorb light of two different wavelengths:

the  $\pi$  bonding to  $\pi^*$  anti-bonding absorption peaks at 180 nm;  
the non-bonding  $n$  to  $\pi^*$  anti-bonding absorption peaks at 290 nm.  
Both of these absorptions are in the ultra-violet, but most spectrometers won't pick up the one at 180 nm because they work in the range from 200 - 800 nm

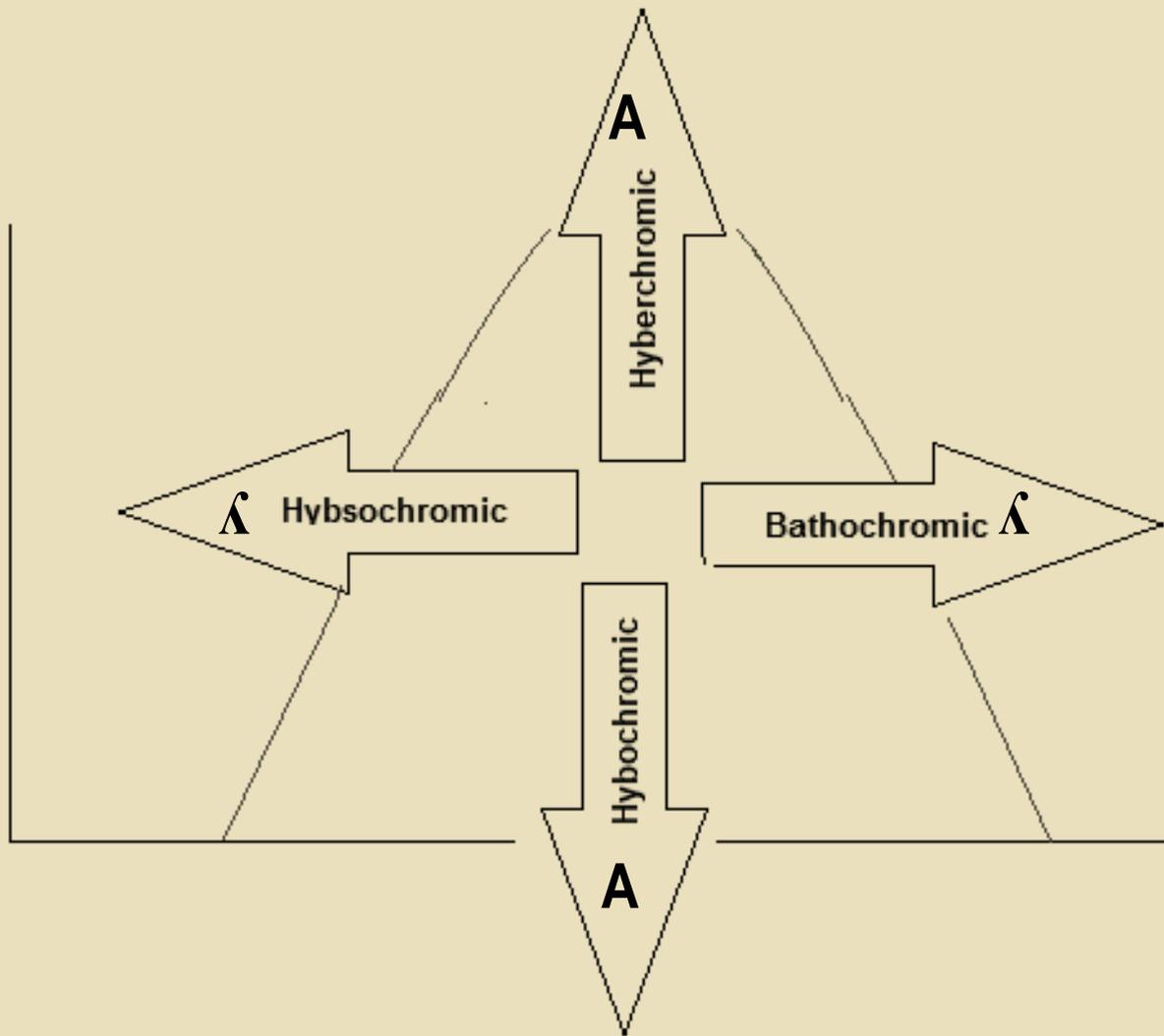
## Auxochrome

It is the group which itself does not act as a chromophore but when attached to chromophore it shifts the absorption maximum towards longer wavelength with an increase in intensity of absorption .

Example - OH , NH<sub>2</sub> , - OR groups .For example when the auxochrome -NH<sub>2</sub> is attached to the benzene ring , it's absorption changes from  $\lambda_{\max} = 255 \text{ nm}$  to 280 nm .

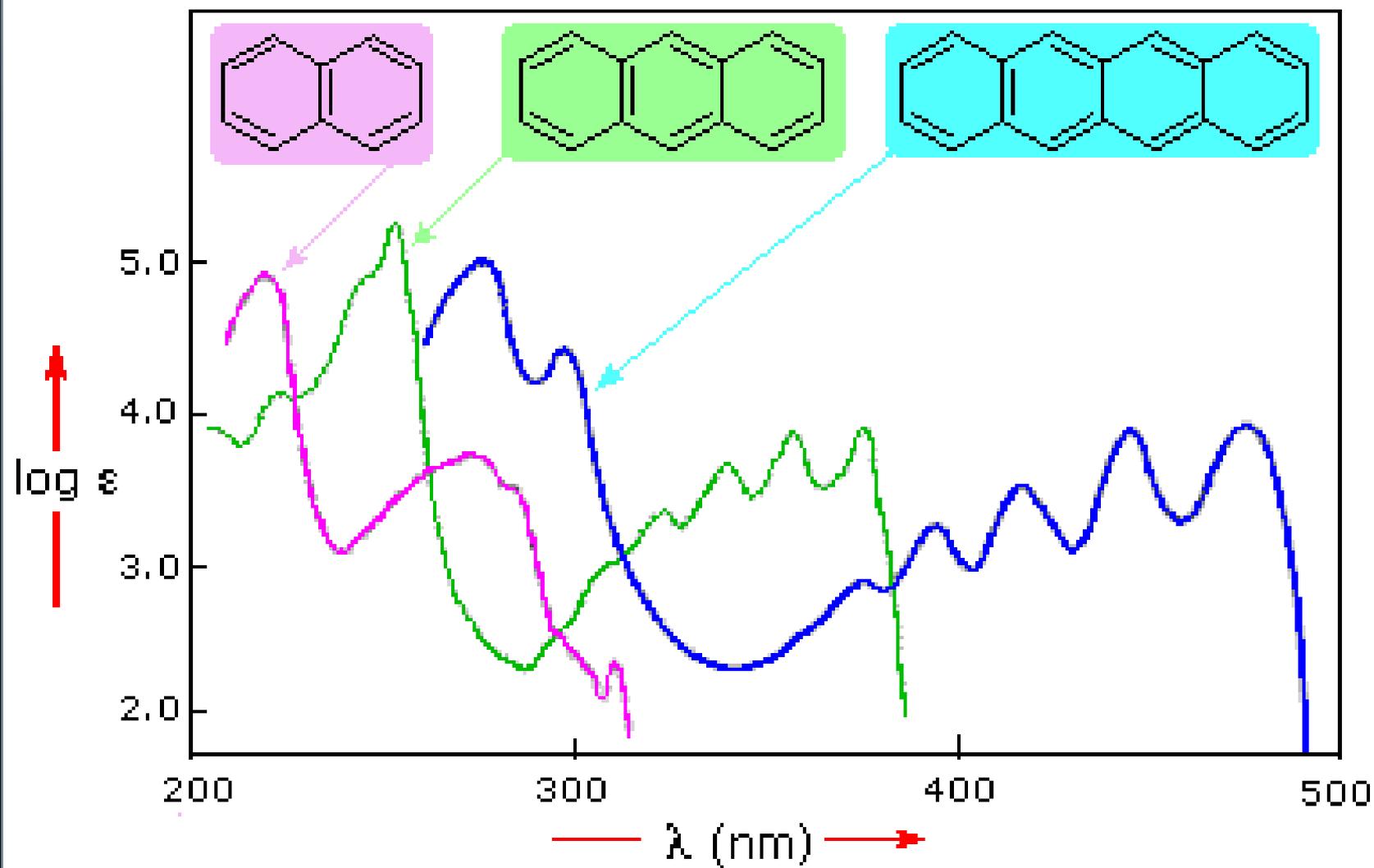
## Terminology for Absorption Shifts :

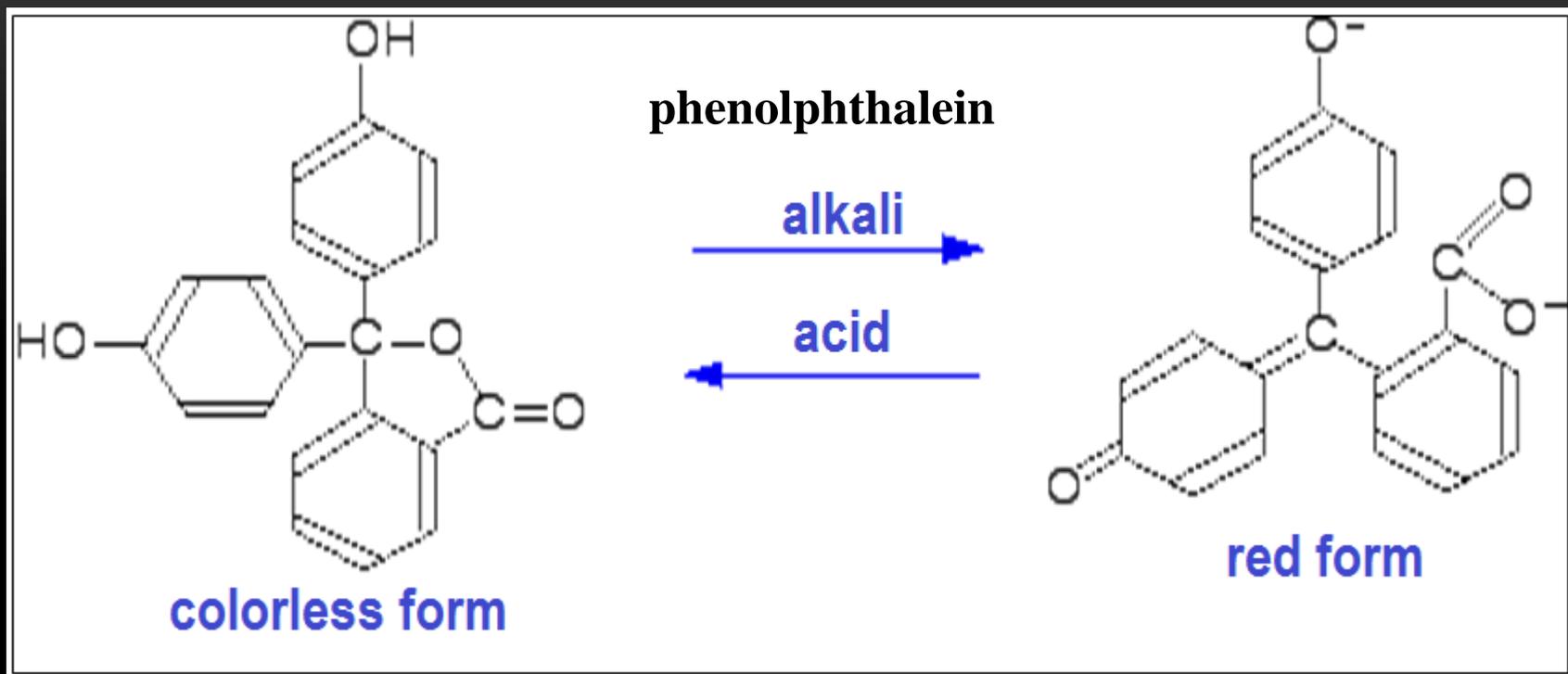
Nature of Shift	Descriptive Term
To Longer Wavelength	Bathochromic
To Shorter Wavelength	Hypsochromic
To Greater Absorbance	Hyperchromic
To Lower Absorbance	Hypochromic



## Effect of aromaticity

Benzene exhibits very strong light absorption near 180 nm ( $\epsilon > 65,000$ ), weaker absorption at 200 nm ( $\epsilon = 8,000$ ) and a group of much weaker bands at 254 nm ( $\epsilon = 240$ ). The added conjugation in naphthalene, anthracene and tetracene causes bathochromic shifts of these absorption bands, as displayed in the figure below. As might be expected from their spectra, naphthalene and anthracene are colorless, tetracene is orange.





The delocalization is broken around the carbon, and so it doesn't cover the whole molecule.

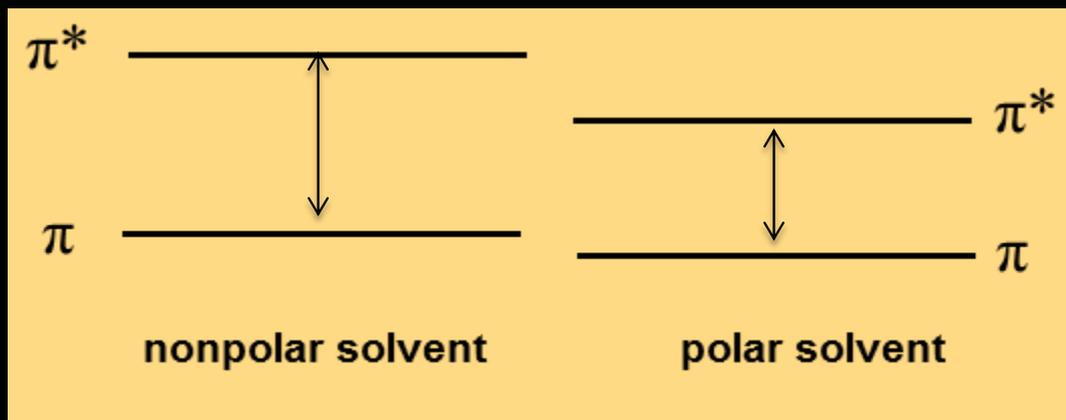
The delocalization covers absolutely *Everything in the ion formed*.

This greater delocalization of red form lowers the energy gap between the highest occupied molecular orbital and the lowest unoccupied pi anti-bonding orbital. It needs less energy to make the jump and so a longer wavelength of light is absorbed.

Aromaticity results in extraordinarily high degree of delocalization of electrons and thus stabilization of the  $\pi^*$ . If we assume a molar absorptivity of about  $10,000 \text{ L mol}^{-1} \text{ cm}^{-1}$  for each double bond, we expect the sum of the three double bonds in benzene to be just above  $30,000 \text{ L mol}^{-1} \text{ cm}^{-1}$  (at 185 nm) but actually the value is about  $60,000 \text{ L mol}^{-1} \text{ cm}^{-1}$  due to increased delocalization as a result of aromaticity. It is therefore advantageous to use UV-Vis absorption spectroscopy for determination of compounds having aromatic character .

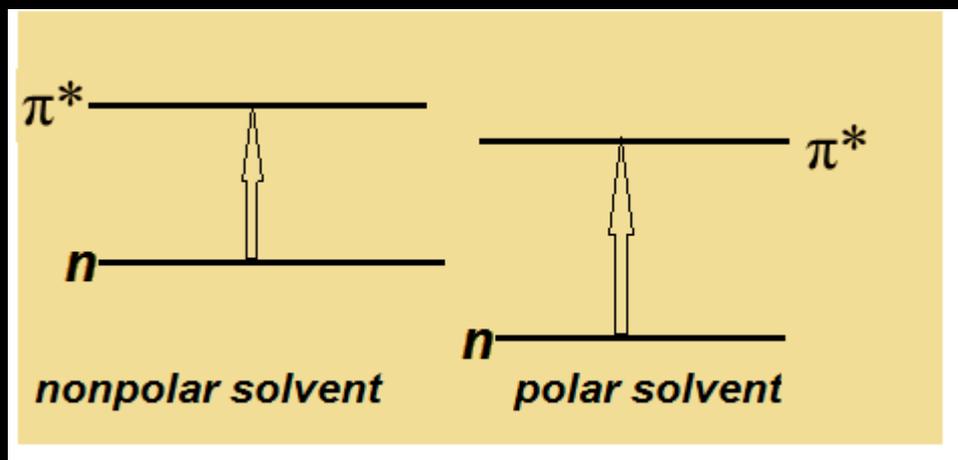
## Effect of solvent polarity on absorption wavelength

Solvent polarity is an important factor in the definition of the energy required to cause a  $\pi - \pi^*$  transition. Figure 3 shows the effect of polar solvents on the energy of this transition



$\pi^*$  is more polar than  $\pi$  and is stabilized more than  $\pi^*$  in polar solvents

The energy required for  $\pi \rightarrow \pi^*$  transition in polar solvents is thus reduced and the wavelength of incident radiation increases. This is referred to as bathochromic shift or red shift. A  $n - \pi^*$  transition is affected in an opposite way since the  $n$  electrons are stabilized more than the  $\pi^*$  leading to increased energy and shorter wavelength. This is referred to as hypsochromic shift or blue shift.



## Solvents for the Ultraviolet and Visible Regions

<b>Solvent</b>	<b>Lower Wavelength Limit, nm</b>	<b>Solvent</b>	<b>Lower Wavelength Limit, nm</b>
Water	180	Diethyl ether	210
Ethanol	220	Acetone	330
Hexane	200	Dioxane	320
Cyclohexane	200	Cellosolve	320
Carbon tetrachloride	260		

## Absorption by Inorganic Groups

Inorganic groups containing double bonds absorb in the UV-Vis region. The most transitions are a result of  $n - \pi^*$  transitions as in nitrate (313 nm), carbonate (217 nm) and nitrite (280 and 360 nm) .

## Absorption Involving d Orbitals

Many transition metals have colored solutions. The transition metals have some of their  $d$  orbitals empty where a  $d-d$  transition can occur. The  $d-d$  transitions require excitation energy in the UV-Vis region

## Charge Transfer Absorption

When a ligand permanently donates an electron to a metal ion, a charge transfer is said to take place. The net outcome of the process is an oxidation reduction phenomenon occurring within the complex. An example is the reaction of  $\text{Fe}^{3+}$  with thiocyanate where the product is an excited species with neutral thiocyanate and  $\text{Fe}^{2+}$ .

In less common situations, the transfer of electrons can take place from the metal ion to the ligand. An example is the  $\text{Fe}^{2+}$  complex with 1,10-phenanthroline where  $\text{Fe}^{2+}$  metal ions donate electrons to 1,10-phenanthroline. The complex will then have  $\text{Fe}^{3+}$  ion. Charge transfer complexes are of special interest, their molar absorptivities are usually high; allowing very sensitive determinations.

# DESIGN OF SPECTROMETRIC METHODS

1- The analyte absorbs at a unique wavelength (not very common) e.g.  $\text{MnO}_4^-$ .

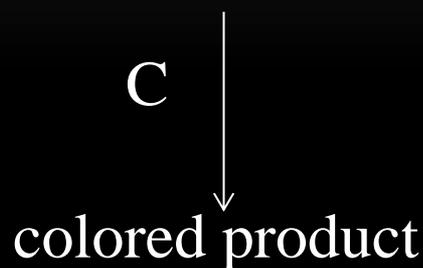
2- The analyte reacts with a reagent to produce an adduct that absorbs at a unique wavelength (a chromophore) e.g. a reaction of a metal with a chelating agent . Example , the determination of iron(II) with 1,10- phenanthroline (phen ) :



orange – red complex

The absorbance of the complex is direct proportional to the concentration of iron(II) .

3- The analyte X ( a catalyst or an inhibitor ) is involved in a reaction that produces a colored compound :



If E or D is colored , its absorbance is direct proportional to the catalyst ( or the decrease in absorbance is direct proportional to the inhibitor ) , otherwise we add a reagent C to produce a colored compound with any of the products E or D .

The first step of an analytical procedure in UV-Vis spectroscopy is to find the wavelength that yields maximum absorbance. This is done by scanning through the UV or Vis range, depending on the characteristics of the absorbing species. The spectrum is plotted with absorbance on the Y-axis and the wavelength on the X-axis. Then the

# Applications

## Qualitative Analysis

The broad band absorption spectra obtained in UV-Vis absorption spectroscopy usually lacks details that can be used in qualitative analysis. Therefore, this technique is mainly a quantitative technique.

## Quantitative Analysis

The basis for quantitative analysis in the UV-Vis relies on Beer's law. Several characteristics of quantitative measurements using UV-Vis absorption spectroscopy can be rationalized:

1. Applicability to all types of analytes that absorb in the UV-Vis region.
2. Moderate sensitivities in the range from  $10^{-4}$  to  $10^{-6}$  M with possibility to extend this range under certain conditions

3. The relative standard deviation occurs within 1-3% which reflects good precision.
  4. Easy to perform and convenient.
  - 5- Can be used for quantitative analysis in liquid chromatographic separations and in flow injection analysis.
  6. Non absorbing species can also be determined if they are derivatized with an absorbing species as the case of metal ions when complexed to ligands.
-

## Calibration Curves

Usually, a plot of the absorbance of a series of standards is plotted versus the concentration. The absorbance of the unknown is then measured and the prepared calibration plot is used for the determination of the analyte concentration( see the previous curve on slide 10 ). If the absorbance of the analyte was located outside the calibration plot, more standards should be made or the analyte concentration must be adjusted to occur on the calibration plot.

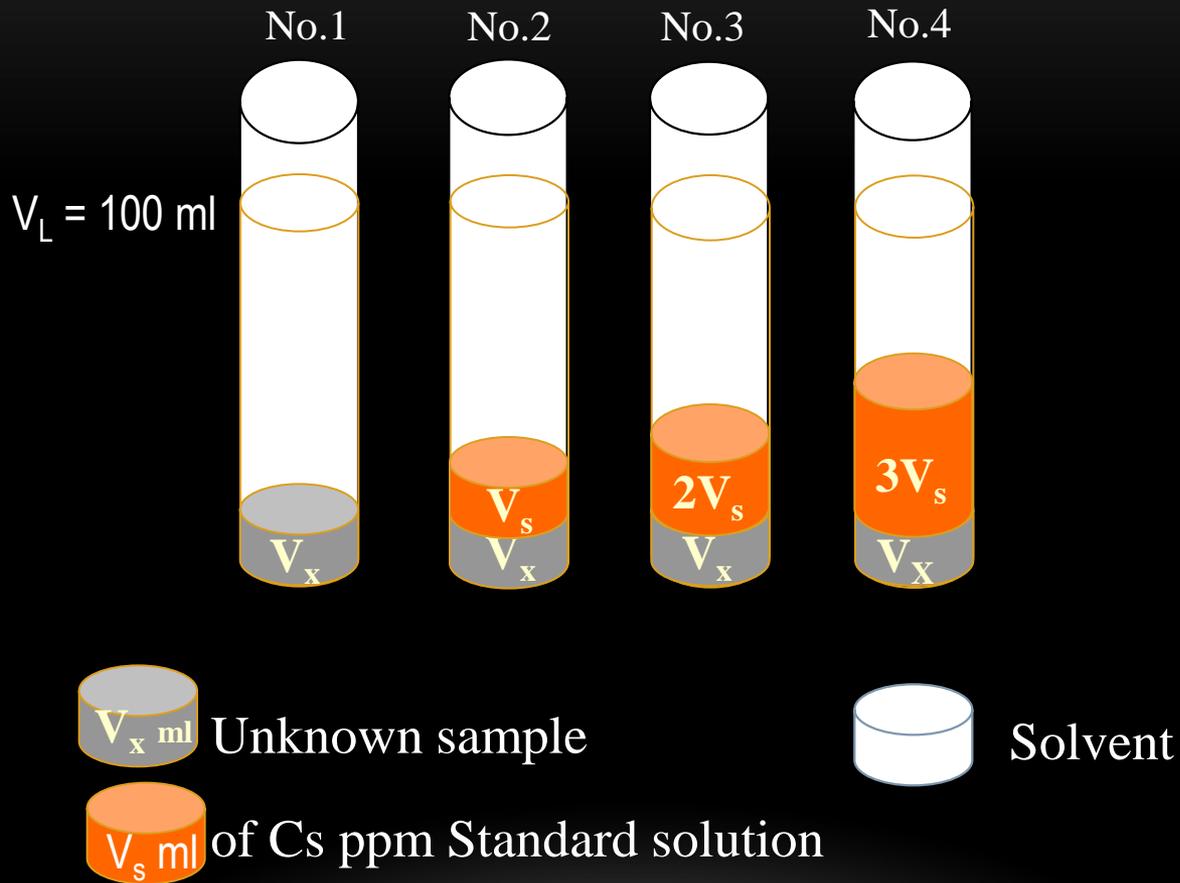
It is not allowed to theoretically extrapolate or interpolate a calibration plot. It should also be appreciated that the composition of standard solutions must approximate that of the sample solution. The slope of the linear calibration plot is the molar absorptivity when the path length is 1.00 cm. Larger slopes mean higher sensitivities

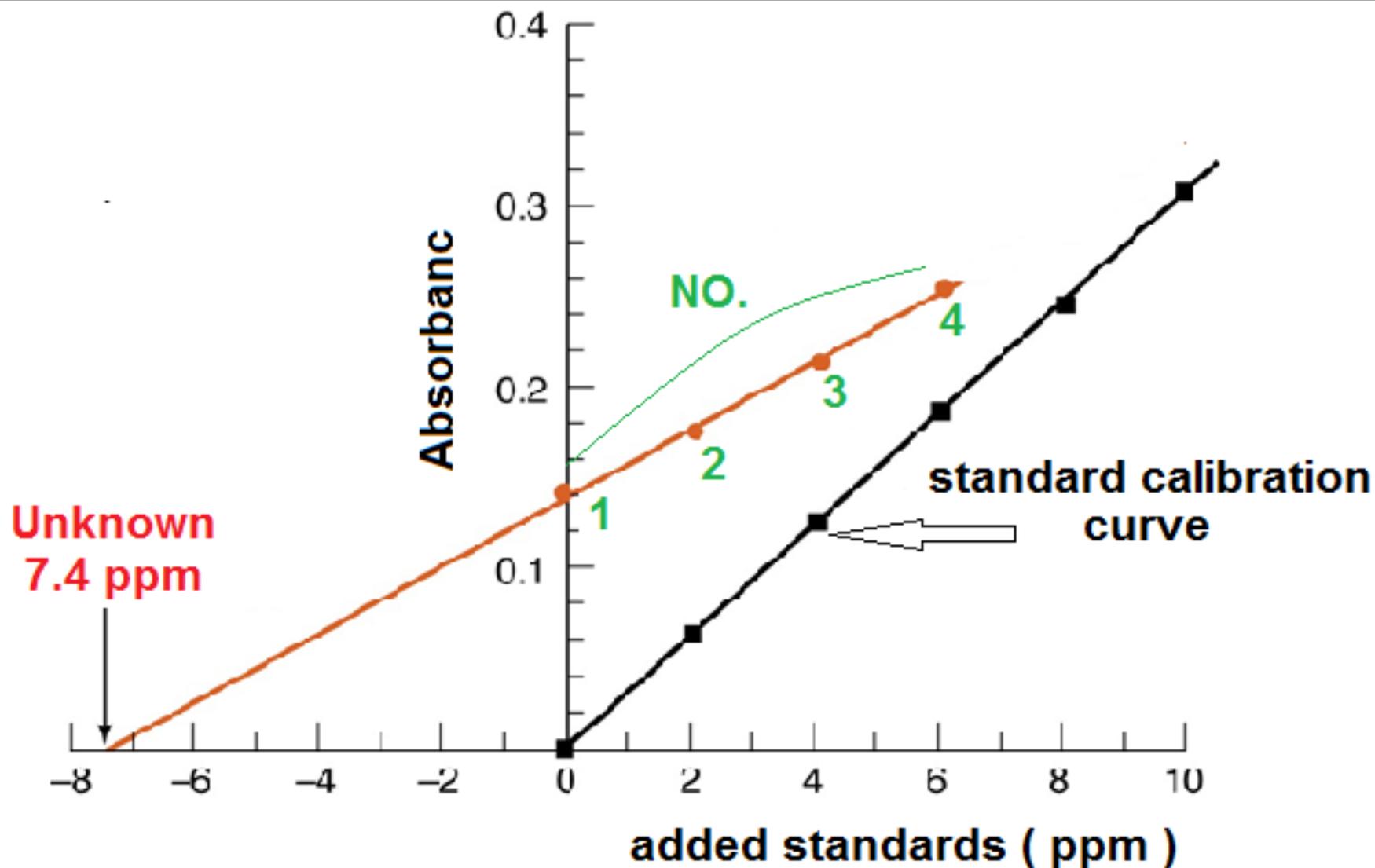
In cases where the sample composition is not known i.e. standards solutions can not approximate the sample solution , the method of standard addition should be used..

## Standard Addition method

The standard addition technique is used for non-repetitive analyses or to correct for matrix effects that cannot be accommodated in the calibration standards. In this method a known amount of the standard solution of known increasing concentrations of the analyte is added to a number of aliquots of the sample solution. A resulting solution is diluted to the similar last volume and their absorbances are measured. A graph is drawn between the absorbance and the added concentrations of the analyte. It is then extrapolated to the concentration axis to acquire the concentration of sample solution. If the plot is nonlinear then extrapolation is not probable. It is necessary to perform blank correction within such a case. The calibration plot acquired through using standard addition method is display in the following Figure.

# Standard Addition Method





## The Method Of Standard Additions

No.	Sample Added ml	Standard Added ml	Concentr.of standard mg/L	Last Concentration mg/L
1	$V_x$	0	$C_s$	0
2		$V_s$		$V_s \cdot C_s / V_L$
3		$2V_s$		$2 V_s \cdot C_s / V_L$
4		$3V_s$		$3 V_s \cdot C_s / V_L$

## Determination of a Ligand to Metal ratio

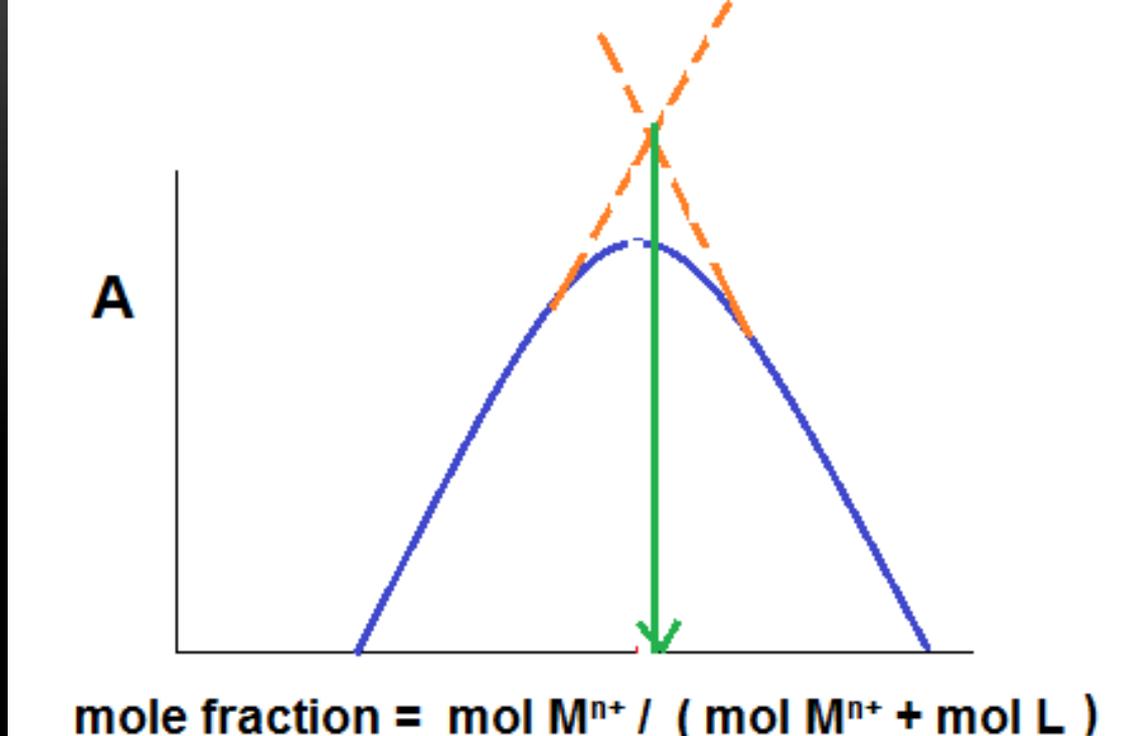
UV-Vis spectroscopy is very useful in determining the ratio between a ligand and a metal in a complex .



Usually, complexation of ligands with metals result in different spectroscopic characteristics for both. The most pronounced situation is the formation of a colored complex. Two widely used methods to find this ratio :

### a. The Method of Continuous Variation (Job's Method)

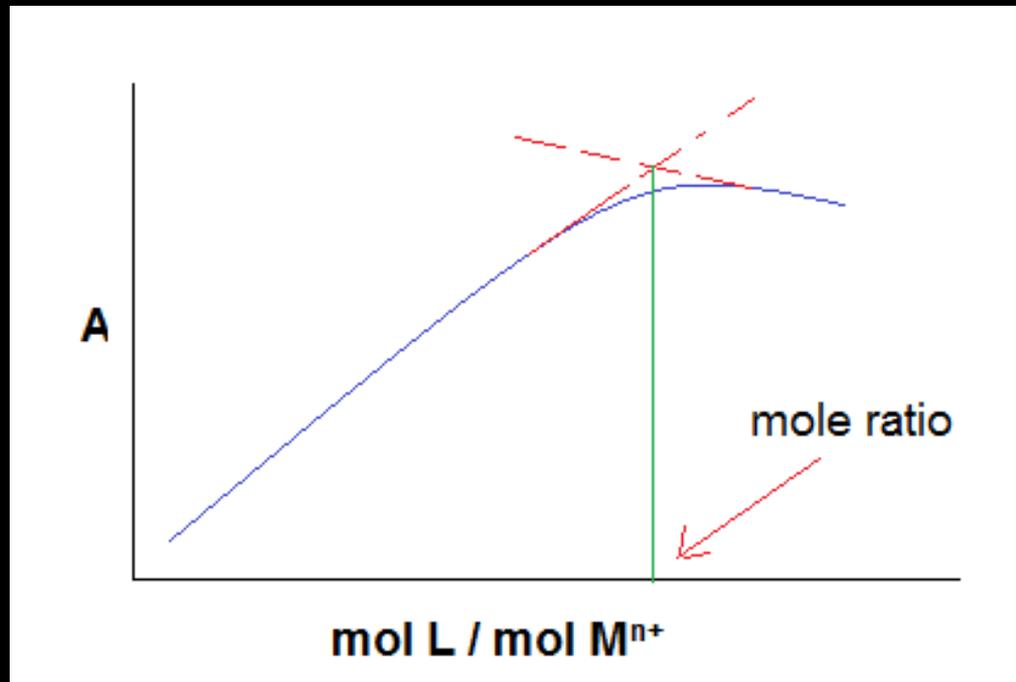
In this method, the mole fraction of either the metal or the ligand is plotted against absorbance. This yields a result similar to that shown in the following Figure.



If the mole fraction of the metal is 0.5 then it is a 1:1 complex and if it is 0.33 then it is a 1:2 complex, etc. The method of continuous variation is excellent for complexes that are 1:1 but if the ratio is more than 1:2 there will be some considerable uncertainty and the mole ratio method is preferred.

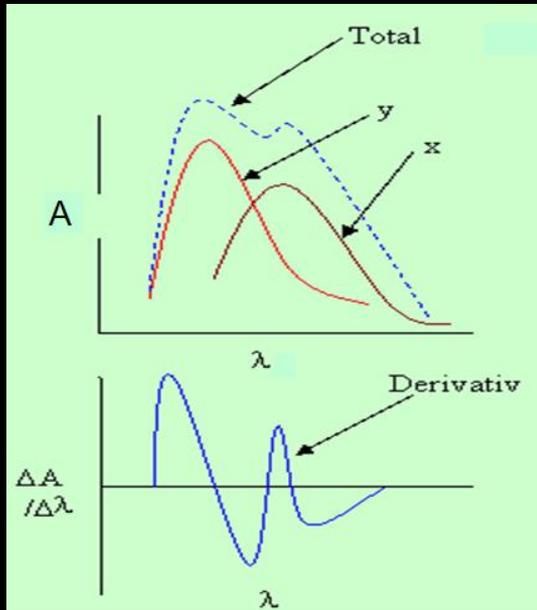
## b. Mole Ratio method

The concentration of the metal ion is usually kept constant and a variable amount of the complexing agent is added. The mole ratio of the metal ion to the ligand is plotted versus absorbance and a result as shown in the following Figure is obtained



# Derivative UV-Vis Absorption Spectroscopy

In derivative spectroscopy,  $DA/D\lambda$  is plotted against the wavelength. The derivative spectrum contains sharper peaks where better location of peaks and wavelengths maxima can be achieved. Not only the first derivative of the absorbance spectrum can be obtained but up to the fourth derivative is possible.



Derivative spectroscopy is excellent for determination of multi components in a sample, if they can be resolved.

## Other applications :

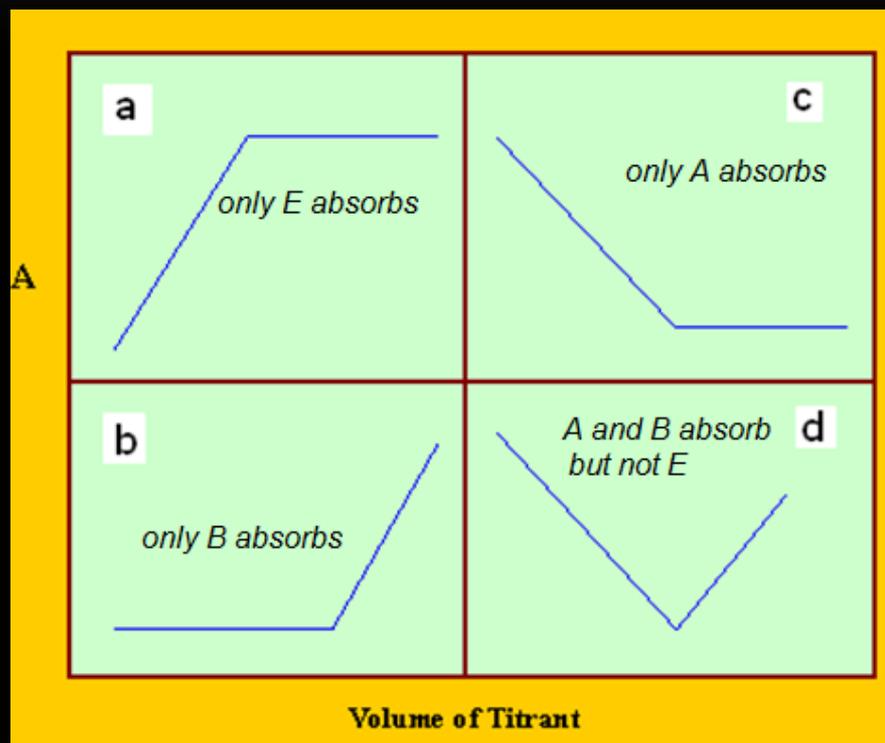
- Detection of impurities in organic compounds .
- Can be used to study the kinetics of reactions .
- Molecular weight of compounds can be measured by spectrometry .
- It is used as a detector in HPLC , flow injection analysis and other techniques .
- Determination of composition of complex , mole ratio method ,
- UV/Vis spectrophotometry is probably more widely used in laboratories throughout the world than any other single method .
- *Moderate to high selectivity.*
- Good accuracy the relative error encountered in concentration lie in the range from 1% to 3%

## spectrophotometric Titrations

In cases where an analyte reacts with a reagent so that the analyte, the reagent or the product absorbs UV-Vis radiation, the technique can be used for determination of the analyte by a spectrophotometric titration reaction. spectrophotometric titrations are similar to conventional visual titrations but following the course of a spectrophotometric titration occurs with the aid of a UV-Vis detector, rather than the naked eye.

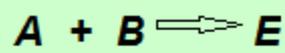
A plot of absorbance versus titrant volume is called a spectrophotometric titration curve. The titration curve is supposed to consist of two linear lines intersecting in a point corresponding to the end point of the reaction. The absorbance reading should be corrected for volume where as a titrant is added to the reaction mixture the absorbance will change.

Several shapes of photometric titration curves can be identified depending on the reaction. Consider addition of analyte A to a reagent B so that a product E is formed according to the reaction:

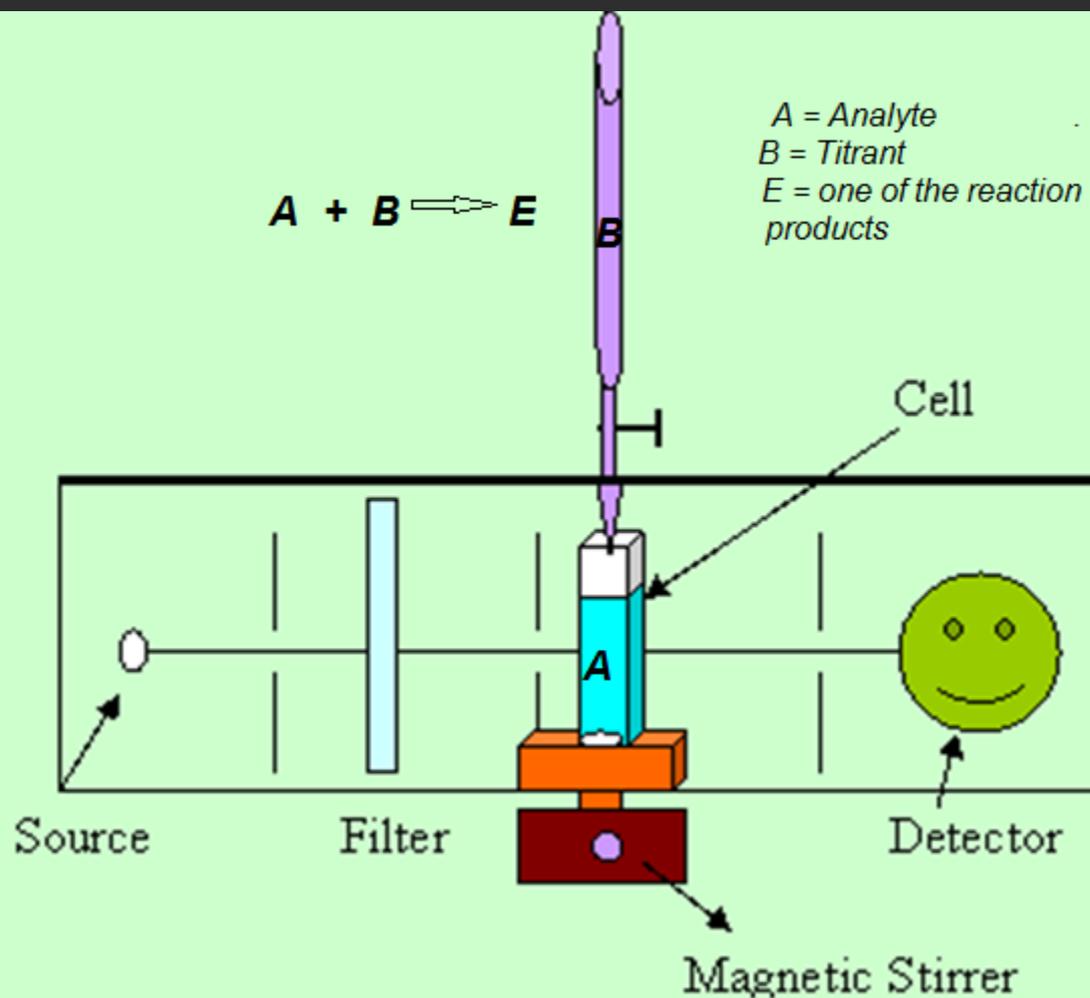


It should be pointed out here that the lines shown are what is supposed to be. However, as the equivalence point is approached concentrations become smaller that the reaction is not fast enough and the end point is usually not sharp. This is not a problem as extrapolation of the linear portions will result in a very sharp intersection indicating the end point.

A conventional photometer or spectrophotometer can be adapted to performing spectrophotometric titrations where the analyte is placed in the sample cell which contains a small magnet and is located on the top of a magnetic stirrer. The wavelength is selected and the titrant is added, from a dark burette, gradually and the absorbance is recorded



*A = Analyte  
B = Titrant  
E = one of the reaction products*



# Advantages and Applications of Photometric Titrations

1. Usually, photometric titrations are more accurate than visual titrations.
2. Photometric titrations are faster than visual titrations as only few points at the beginning and end of the titration is necessary. Extrapolation of the straight lines will intersect at the end point.
3. Titration reactions that are slow at the end point can not be performed by visual titrations but are well suited for spectrophotometric titrations. Only few points at the beginning and end of the titration, well away from the equivalence point where the reaction is slow, are necessary. Extrapolation of the straight lines will intersect at the end point. Therefore, dilute solutions or weak acids and bases can be also titrated spectrophotometrically.

LAB WORK  
SPECTROPHOTOMETRIC  
DETERMINATION OF IRON USING 1,10-  
PHENANTHROLINE

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# GOALS AND OBJECTIVES

## Goals

To become familiar with basic methods in UV-Visible molecular absorption spectrophotometry for quantitative chemical analysis

## Objectives

Obtain absorption spectrum of the tris(1,10-phenanthroline)iron(II) complex and determine the analytical wavelength

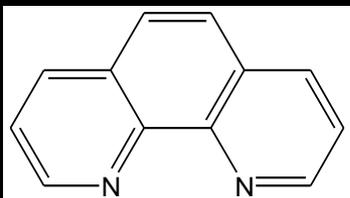
Determine percent iron in a solid sample

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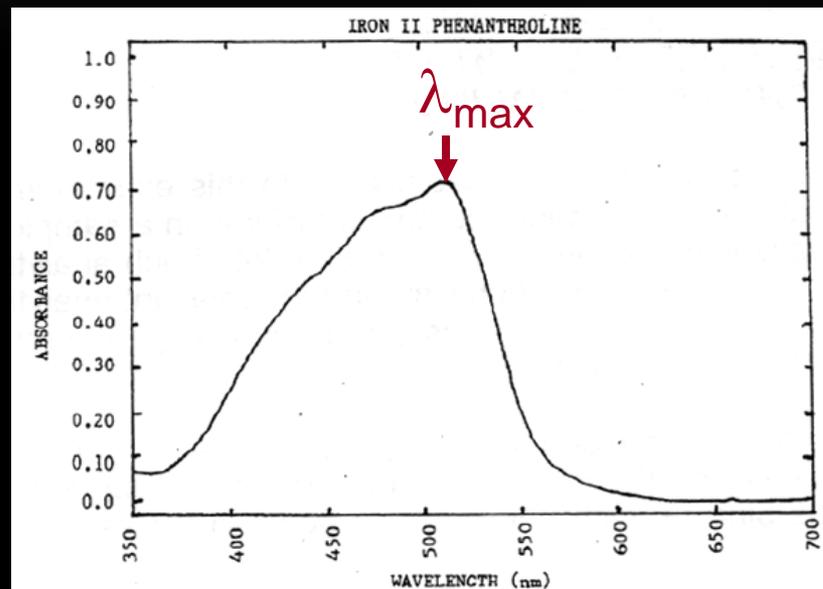
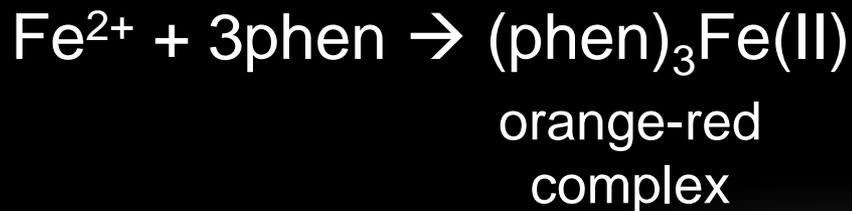
The first step of an analytical procedure in UV-Vis spectroscopy is to find the wavelength that yields maximum absorbance. This is done by scanning through the UV or Vis range, depending on the characteristics of the absorbing species. The spectrum is plotted with absorbance on the Y-axis and the wavelength on the X-axis. Then the wavelength that yields maximum absorbance is chosen for further work. This also gives maximum molar absorptivity.

# TRIS(1,10-PHENANTHROLINE)IRON(II)

$$A = \epsilon b c$$



1,10-Phenanthroline



Visible spectrum of  $(\text{phen})_3\text{Fe(II)}$

# PROCEDURE

Obtain absorption spectrum of complex and determine  $\lambda_{\max}$

Prepare working stock solutions

Standard Fe from pure  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  (Mohr's salt)

Stock unknown solution

Weigh 0.2 g solid unknown, dissolve in dilute acid and dilute to 500 mL

Prepare standard and unknown solutions.

To 100-mL volumetric flasks add

5 mL pH 5 buffer

5 mL 10%  $\text{NH}_2\text{OH}\cdot\text{HCl}$

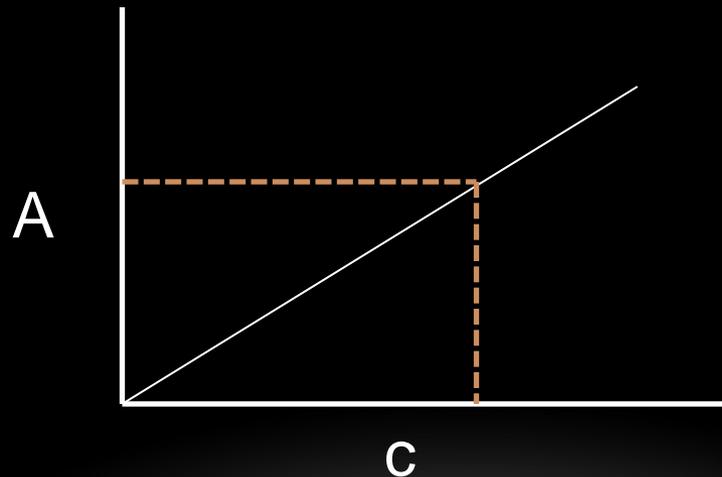
Aliquots of standard Fe or unknown

include a blank solution with no Fe

10 mL of 0.02 M 1,10-phenanthroline

Dilute to volume and measure absorbance at  $\lambda_{\text{max}}$

Construct Beer's Law Plot (A vs c) and calculate %Fe in original solid sample.



# CALCULATIONS

You want to report %Fe in your solid sample, so you need to find the mass of Fe in your weighed sample. Here is a general outline of the approach.

Preparation of your stock sample solution.

You weighed  $x.xxxx$  g of your solid unknown.

You dissolved it in dilute acid and diluted it to  $yyy.y$  mL to prepare your stock sample solution.

Your stock sample solution contains all of your solid sample. If you can determine the molar concentration of Fe in this solution, you can calculate the mass of Fe in your sample.

Your calculated concentrations are based on the absorbances of your prepared standards and your prepared unknown.

Prepared standards.

Based on the mass of pure  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ , and the dilutions you did, you compute the concentrations of Fe (mol/L) in your calibration standards.

You measure the absorbance of each standard and construct a calibration curve.

Prepared unknown (the one for which you measured the absorbance)

From the calibration curve, you determine the concentration of Fe (mol/L) in your prepared unknown.

Based on the concentration of Fe in your prepared unknown, and the dilutions(s) of the stock sample solution you performed to prepare that standard, you compute the molar concentration of Fe in your stock sample solution.

Now you can calculate the amount of Fe in your unknown sample

على الراغبين في الاستماع الى محاضرة عن موضوع هذه الوحدة باللغة العربية الضغط على كل من الروابط التالية :

[Part 10 : UV/Vis molecular absorption spectrometry](#)

[Part 11 : UV/Vis molecular absorption spectrometry](#)

[Part 12 : UV/Vis molecular absorption spectrometry](#)

[Part 13 : UV/Vis molecular absorption spectrometry](#)

[Part 14 : UV/Vis molecular absorption spectrometry](#)

[Part 15 : UV/Vis molecular absorption spectrometry](#)

[Part 16 : UV/Vis molecular absorption spectrometry](#)

[Part 17 : UV/Vis molecular absorption spectrometry](#)