Ultraviolet and visible Spectroscopy

The absorption of ultraviolet, visible, and infrared radiation is widely used to identify and determine many inorganic, organic, and biochemical species.1 Ultraviolet and visible molecular absorption spectroscopy is used primarily for quantitative analysis and is probably applied more extensively in chemical and clinical laboratories than any other single technique. Several types of molecular species absorb ultraviolet and visible radiation. Molecular absorption by these species can be used for qualitative and quantitative analyses. UV-visible absorption is also used to monitor titrations and to study the composition of complex ions.

The wavelength of absorption of an organic molecule depends on how tightly its electrons are bound. The shared electrons in carbon-carbon or carbon-hydrogen single bonds are so firmly held that their excitation requires energies corresponding to wavelengths in the vacuum ultraviolet region below 180 nm. Single-bond spectra have not been widely exploited for analytical purposes because of the experimental difficulties of working in this region. These difficulties occur because both quartz and atmospheric components absorb in this region, which requires that evacuated spectrophotometers with lithium fluoride optics be used. Electrons in double and triple bonds of organic molecules are not as strongly held and are therefore more easily excited by electromagnetic radiation. Thus, species with unsaturated bonds generally exhibit useful absorption bands. Unsaturated organic functional groups that absorb in the ultraviolet or visible regions are known as **chromophores**. The wavelength and peak intensity data are only rough guides since both are influenced by solvent effects as well as the structural details of the molecule. In addition, conjugation between two or more **chromophores**

Chromophores are unsaturated organic functional groups that absorb in the ultraviolet or visible region.

An auxochrome is a functional group of atoms with one or more lone pairs of electrons when attached to a chromophore, alters both the wavelength and intensity of absorption. If these groups are in direct conjugation with the pi-system of the chromophore, they may increase the wavelength at which the light is absorbed and as a result, intensify the absorption. A feature of these auxochromes is the presence of at least one lone pair of electrons which can be viewed as extending the conjugated system by resonance. They themselves fail to produce the color, but instead intensify the color of the chromogen when present along with the chromophores in an organic compound. Examples include the hydroxyl (−OH), amino ($-NH_2$), aldehyde (−CHO), and methyl mercaptan groups $-SCH_3$)

UV-VIS Spectroscopy Theory

When the interaction between incident radiation and the electron cloud in a chromophore results in an electronic transition involving the promotion of one or more of the outer shell or the bonding electrons from a ground state into a higher energy state, ultraviolet-visible (UV-Vis)

spectra are derived. Generally, the UV and visible spectral bands of substances are large. And may not exhibit a high degree of compound recognition accuracy. Nonetheless, they are sufficient for quantitative assays and are useful as an alternate means of detection for several substances. The radiation from typical hot solids consists of several wavelengths and depends primarily on the temperature of the solid and is predictable from the principle of chance, the energy released at each given wavelength.

More recently, using a version of this-the tungsten-halogen lamp has become standard practice. Radiation is transmitted deep into the UV zone through the quartz envelope. The most popular source is the deuterium lamp for the UV region itself, and a UV-visible spectrometer would normally have all types of lamps to fill the whole wavelength spectrum.

Most molecules and ions absorb energy in the ultraviolet or visible range, i.e., they are chromophores. The absorbed photon excites an electron in the chromophore to higher energy molecular orbitals, giving rise to an excited state. For organic chromophores, four possible types of transitions are assumed: π–π*, n–π*, σ–σ*, and n–σ*. Transition metal complexes are often colored (i.e., absorb visible light) owing to the presence of multiple electronic states associated with incompletely filled d orbitals .

Effects on chromophore

It increases the colour of any organic compound. For example, benzene does not display colour as it does not have a chromophore; but nitrobenzene is pale yellow colour because of the presence of a nitro group (−NO2) which acts as a chromophore. But p-hydroxy nitrobenzene exhibits a deep yellow colour, in which the −OH group acts as an auxochrome. Here the auxochrome (−OH) is conjugated with the chromophore $-NO_2$. Similar behavior is seen in azobenzene which has a red colour, but p-hydroxyazobenzene is dark red in colour. The presence of an auxochrome in a chromogen is essential to make a dye. However, if an auxochrome is present in the meta position to the chromophore, it does not affect the colour. An auxochrome is known as a functional group that produces a bathochromic shift, also known as redshift because it increases the wavelength of absorption, therefore moving closer to infrared light.

There are mainly two types of auxochromes:

- Acidic: $-COOH$, $-OH$, $-SO_3H$
- Basic: $-NH_2$, $-NHR$, $-NR_2$

What is the effect of the solvent on absorptions π → π*, n ← π* ?

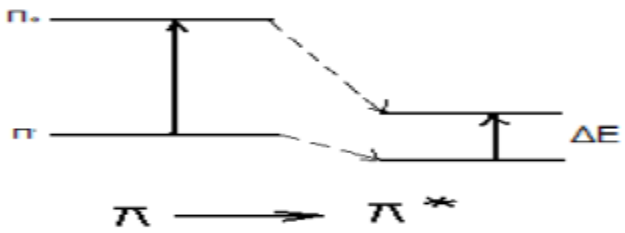
The solvent effect plays a crucial role in determining the energy and intensity of electronic transitions in molecules. In particular, the solvent effect on π-π* and n-π* transitions in a one-one system is of great significance. Let's discuss this in detail.

1. π-π* Transition: The π-π* transition involves the excitation of an electron from a filled π orbital to an empty π* orbital. This transition is commonly observed in conjugated systems containing double or triple bonds. The solvent effect on π-π* transitions can be explained as follows: -

A- The polarity of Solvent: Polar solvents, such as water or alcohol, can strongly interact with the solute molecules through dipole-dipole interactions or hydrogen bonding. This interaction can lead to the solvation of the solute, which affects the energy of the transition.

B-Dielectric Constant: The dielectric constant of a solvent measures its ability to reduce the electrostatic forces between charged or polar species. A solvent with a high dielectric constant can stabilize the charged or polar transition state, leading to a decrease in the energy of the transition.

C-Solvatochromism: The phenomenon Solvatochromism refers to where the absorption wavelength of a molecule changes with the polarity of the solvent. In the case of π-π* transitions, the absorption wavelength usually shifts to longer wavelengths (redshift) in polar solvents due to solvation effects.

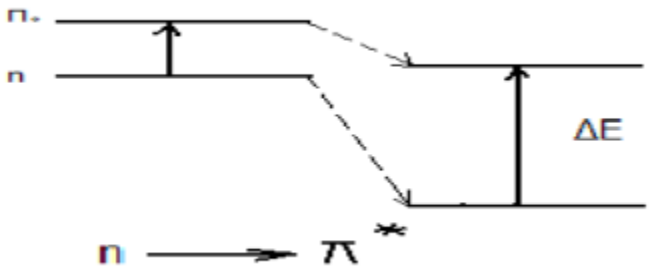


2-n-π* Transition:The n-π* transition involves the excitation of an electron from a non-bonding lone pair (n) orbital to an empty π* orbital. This type of transition is often observed in molecules containing functional groups such as carbonyl (C=O) or nitro (NO2) groups. The solvent effect on n-π* transitions can be explained as follows: -

A-Solvent Polarity: Similar to π-π* transitions, the polarity of the solvent influences the energy of n-π* transitions. Polar solvents can stabilize the transition state, resulting in a decrease in the energy of the transition. -

B-Hydrogen Bonding: Solvents capable of forming hydrogen bonds, such as alcohols or amines, can interact with the lone pair of electrons involved in the n-π* transition. This interaction can affect the energy and intensity of the transition.

C-Solvent Donor or Acceptor: Depending on the nature of the solute and solvent, the solvent can act as a donor or acceptor in the n-π* transition. For example, in the case of hydrogen-bonding solvents, the solvent can donate a hydrogen bond to the solute or accept a hydrogen bond from the solute, leading to changes in the transition energy.



In conclusion, the solvent effect on π-π* and n-π* transitions in a system is determined by the polarity, dielectric constant, solvatochromism, hydrogen bonding, and solvent-solute interactions. These factors can significantly influence the energy and intensity of the transitions, leading to shifts in absorption wavelengths and changes in electronic properties.

Charge-Transfer Absorption

Charge-transfer absorption is particularly important for quantitative analysis because molar absorptivities are unusually large (e > 10,000 L mol1 cm1), which leads to high sensitivity. Many inorganic and organic complexes exhibit this type of absorption and are therefore called charge-transfer complexes. A charge-transfer complex consists of an electron-donor group bonded to an electron acceptor. When this product absorbs radiation, an electron from the donor is transferred to an orbital that is largely associated with the acceptor. The excited state is thus the product of a kind of internal oxidation/reduction process. This behavior differs from that of an organic chromophore in which the excited electron is in a molecular orbital that is shared by two or more atoms. Familiar example of charge-transfer complexes include the phenolic complex of iron(III), the 1,10-phenanthroline complex of iron(II), the iodide complex of molecular iodine, and the ferro/ferricyanide complex responsible for the color of Prussian blue.

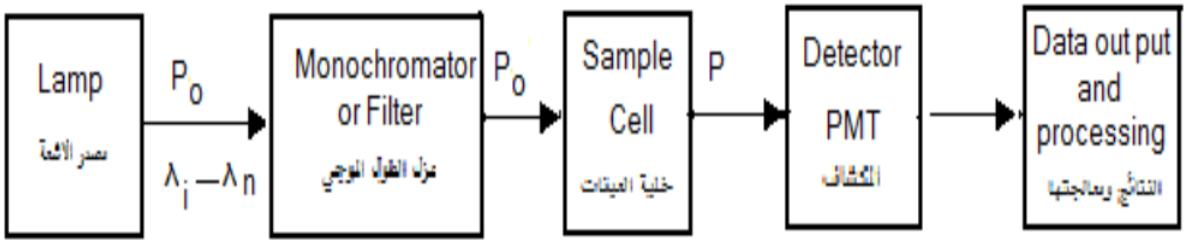
$$Cl^{-}(H_2O)_4 \rightarrow Cl(H_2O)_4^{-}$$

$$Fe^{3+}(OH)^{-} \rightarrow Fe^{2+}(OH)$$

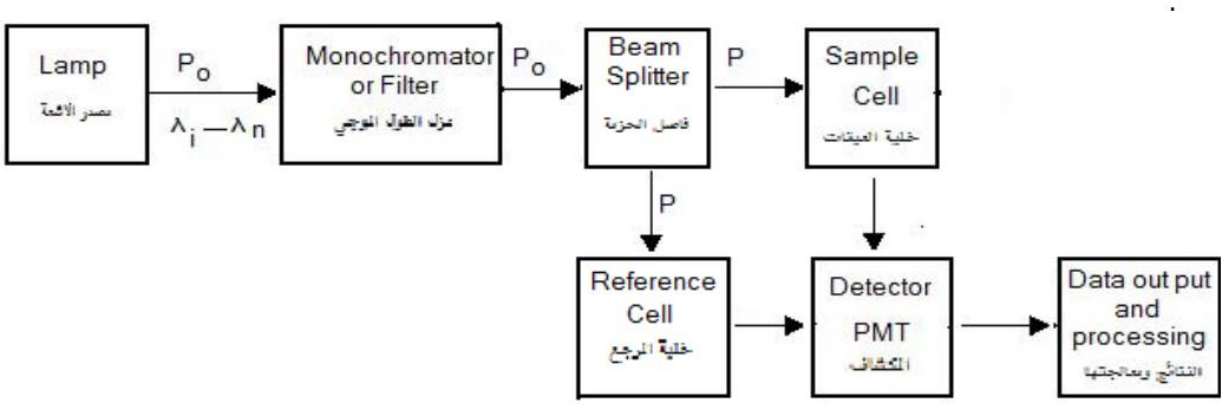
Ultraviolet–visible spectrophotometer

The optical components of spectrometer can be combined in various ways to produce two Types of instruments for absorbance measurements.

1-Spectrophotometer Single – beam



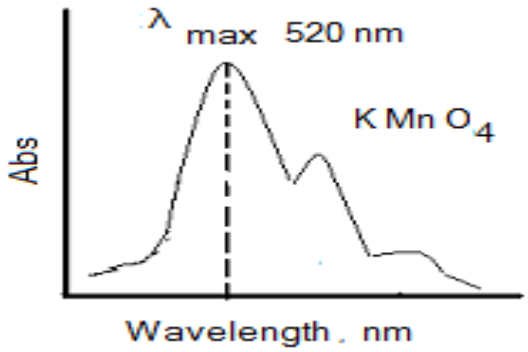
2-Spectrophotometer Double – beam



In the first type, the single beam is more sensitive, less accurate and less expensive. The double beam spectrometer is less sensitive, more accurate, and more expensive because its parts are more. The single beam spectrometer requires changing the sample solution and the blank solution for each wavelength and performing a new zeroing and calibration. As for the double beam spectrometer, the beam is divided or split using a beam splitter, which is a semi-transparent mirror that allows the first part to fall on the sample cell and the second part to fall on the blank solution.

Absorption spectrum:

The UV-Vis absorption spectrum is represented by two axes, the horizontal axe represents the wavelength in nm units and the vertical axe represents the absorbance A or transmittance, % T and is taken as the absorbance value at the wavelength of maximum absorption λmax in which the solution absorbs the highest energy and represents the highest sensitivity in the spectrum.



Beer-Lambert law

The absorption law, also known as the Beer-Lambert law or just Beer's law, tells us quantitatively how the amount of attenuation depends on the concentration of the absorbing molecules and the path length over which absorption occurs. As light traverses a medium containing an absorbing analyte, the intensity decreases as the analyte becomes excited. For an analyte solution of a given concentration, the longer the length of the medium through which the light passes (path length of light), the more absorbers are in the path, and the greater the attenuation. Similarly, for a given path length of light, the higher the concentration of absorbers, the stronger the attenuation. The attenuation of a parallel beam of monochromatic radiation as it

passes through an absorbing solution of thickness b cm and concentration c moles per liter. Because of interactions between the photons and absorbing particles, the radiant power of the beam decreases from P^0 to P. The transmittance T of the solution is the fraction of incident radiation transmitted by the solution, as shown in the Equation below. **Transmittance** is often expressed as a percentage and called the percent transmittance

$$T = P/P_0$$

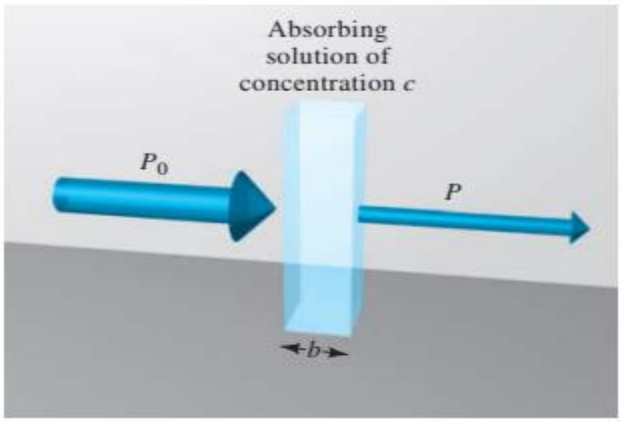
$$A = -\log T = -\log \frac{P}{P_0} = \log \frac{P_0}{P}$$

$$\text{Percent transmittance} = \%T = \frac{P}{P_0} \times 100\%.$$

Absorbance can be calculated from percent transmittance as follows:

$$T = \frac{\%T}{100\%}$$

$$A = -\log T$$
$$= -\log \%T + \log 100$$
$$= 2 - \log \%T$$



Attenuation of a beam of radiation by an absorbing solution. The larger arrow on the incident beam signifies a higher radiant power P^0 than that transmitted by the solution *P*. The path length of the absorbing solution is *b* and the concentration is *c*.

The beam's power transmitted through a cell containing the analyte solution is compared with one that traverses an identical cell containing only the solvent, or a reagent blank. An experimental absorbance that closely approximates the true absorbance for the solution is thus obtained, that is, Because of this close approximation, the terms P^0 and P will henceforth refer to the power of a beam that has passed through cells containing the solvent (or blank) and the analyte solution, respectively.

Beer's Law

According to Beer's law, absorbance is directly proportional to the concentration of the bsorbing species, c, and to the path length, b, of the absorbing medium as expressed by Equation below :

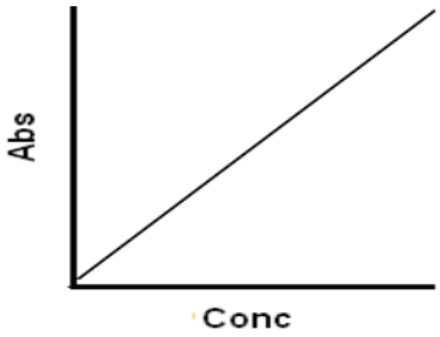
$$A = \log(P_0/P) = abc$$

a is a proportionality constant called the absorptivity. Because absorbance is a unitless quantity, the absorptivity must have units that cancel the units of b and c. If, for example, c .has the units of g. L^{-1} and b has the units of cm, absorptivity has the units of L. g^{-1} cm^{-1}. When we express the concentration in Equation below in moles per liter and b in cm, the proportionality constant is called the molar absorptivity and is given the symbol e. Thus,

$$A = \varepsilon bc$$

where € has the units of L mol^{-1} cm^{-1}.

The molar absorptivity of (€) a species at an absorption maximum is characteristic of that species. Peak molar absorptivities for many organic compounds range from 10 or less to 10,000 or more. Some transition metal complexes have molar absorptivity's of 10,000 to 50,000. High molar absorptivity's are desirable for quantitative analysis because they lead to high analytical sensitivity.



Applying Beer's Law to Mixtures

Beer's law also applies to solutions containing more than one kind of absorbing substance. Provided that there is no interaction among the various species, the total absorbance for a multicomponent system at a single wavelength is the sum of the individual absorbances. In other words,

$$A_{\text{total}} = A_1 + A_2 + \cdots + A_n = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \cdots + \varepsilon_n bc_n$$

where the subscripts refer to absorbing components 1, 2, . . . , n.

If the band of wavelengths selected for spectrophotometric measurements corresponds to a region of the absorption spectrum in which the molar absorptivity of the analyte is essentially constant, departures from Beer's law will be minimal. Many molecular bands in the UV/visible region of the spectrum fit this description. Beer's law is obeyed for these bands, as demonstrated

by Band A in Figure below. On the other hand, some absorption bands in the UV-visible region and many in the IR region are very narrow, and departures from Beer's law are common To avoid such deviations, it is best to select a wavelength

Let's practice to solve Question

Q1/ solution containing the complex formed between Hi(III) and thiourea has a molar absorptivity of 9.32 X 10^3 L .mol^{-1} .cm^{-1} at 470 nm.

(a) What is the absorbance of a 3.79 x 10^{-5} M solution of the complex at 470 nm in a I.00 cm cell?

(b) What is the percent transmittance of the solution described in (a)?

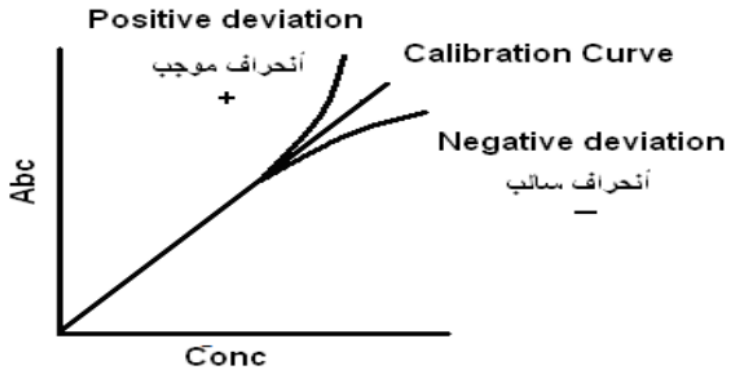
(c) What is the molar concentration of the complex in a solution that has the absorbance described in when measured at 470 nm in a 2.50 cm cell?

Deviations from Beer's law

with polychromatic radiation. The absorber has the indicated molar absorptivities at the two wavelengths $\lambda 1$ and λ 2.

NOTE:

- High-quality spectrophotometers produce narrow bands of radiation and are less likely to suffer deviations from Beer's law due to polychromatic radiation than low-quality instruments.
- Polychromatic light, literally multicolored light, is light of many wavelengths, such as that from a tungsten light bulb. Light that is essentially monochromatic can be produced by filtering, diffracting, or refracting polychromatic light

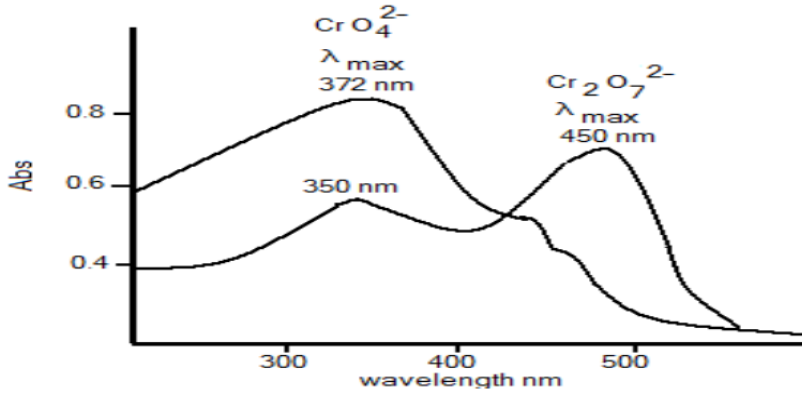


1- Chemical effects, including:

Interaction between the solute molecules with the solvent to form new compounds Different from the original compound, for example, the orange color of the potassium dichromate solution

$K_2Cr_2O_7$ changes to yellow when diluted with water and turns into potassium chromate K_2CrO_4. The chromate $CrO4^{-2}$ gives a single absorption peak, while the dichromate $Cr_2O_7^{-2}$ gives two peaks with less absorption intensity and linearity than the chromate

$$\underset{\text{orange}}{Cr_2O_7^{2-}} + H_2O \rightarrow \underset{\text{yallow}}{2\ CrO_4^{2-}} + 2\ H^+$$



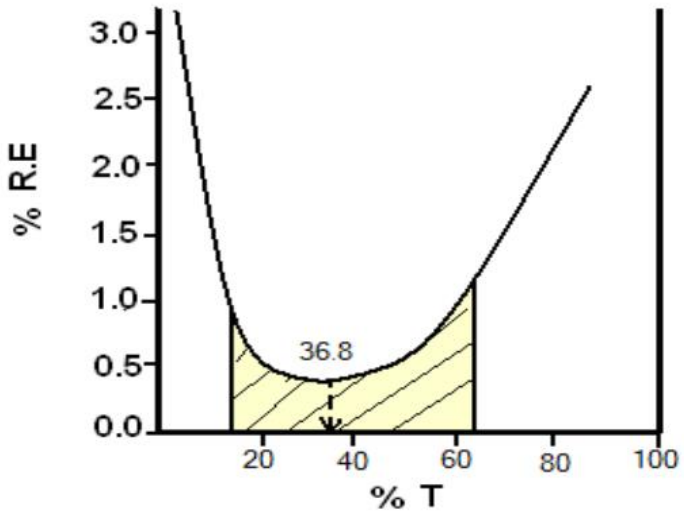
2- The interaction or influence of the solute molecules with each other when the concentration increases to form new compounds that differ in absorption capacity from the primary molecules such as polymers, and the absorbance decreases and a negative deviation occurs, and a deviation occurs when the solute molecules are ionized.

3- Automatic error:

a- Using an impure beam, i.e. not of a single wavelength, which causes a negative deviation. b - A group of reasons including the instability of the light source or voltage , the non-linear response of the detector-amplifier system and finally stray rays, which are rays that pass through the sample and go to the detector due to the presence of dust or scratches, and these reasons are removed by using a double -beam spectrometer.

Photometric Accuracy

To know which concentration is used to give the best accuracy in spectral measurements, samples with different concentrations were prepared and spectral measurements were performed to calculate the concentration. After conducting statistical analysis to calculate the relative error % R.E, it was found that the best accuracy is achieved when measuring a solution with a concentration that gives an absorbance at A = 0.4343 or a transmittance of T % = 36.8 because the value of the relative error % Relative Error is the lowest value. This is evident from the figure that shows the relationship between the transmittance %T and the relative error %R.E. The amount of the relative error % R.E is acceptable at a transmittance rate %T between 15-65%, i.e. an absorbance rate A between 0.8 - 0.2 because the value of the relative error %R.E is the smallest possible.

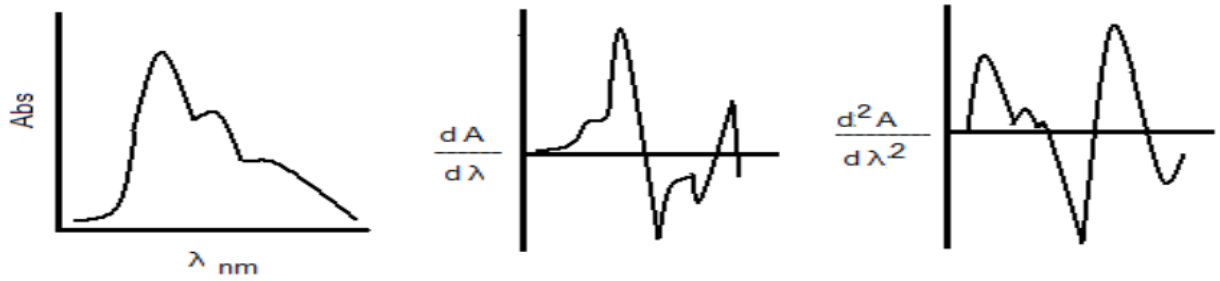


Qualitative & Quantitative Analysis

Qualitative Analysis

1-spectra of standard materials

UV-Vis spectra are used in the qualitative analysis of unknown samples by comparing them with the spectra of standard materials. Spectra plot are usually drawn between absorbance or transmittance values versus wavelength. The transmittance spectrum has a range of 0-100% of concentration where the intensity is proportional to the concentration and tends to lose sharpness in shape at low transmittance values. Absorption spectra give absorption bands that are directly proportional to the concentration and can vary slightly with concentration. This can be avoided by plotting the logarithm of the absorbance (Log A) versus wavelength. More spectral details can be obtained by using the first derivative d A / d λ or the second derivative d2A / d λ2 of the absorption spectrum, where we get more useful information from the original spectra. However, the drawbacks here are the extreme sensitivity of the shape of the derivative plots with concentration.



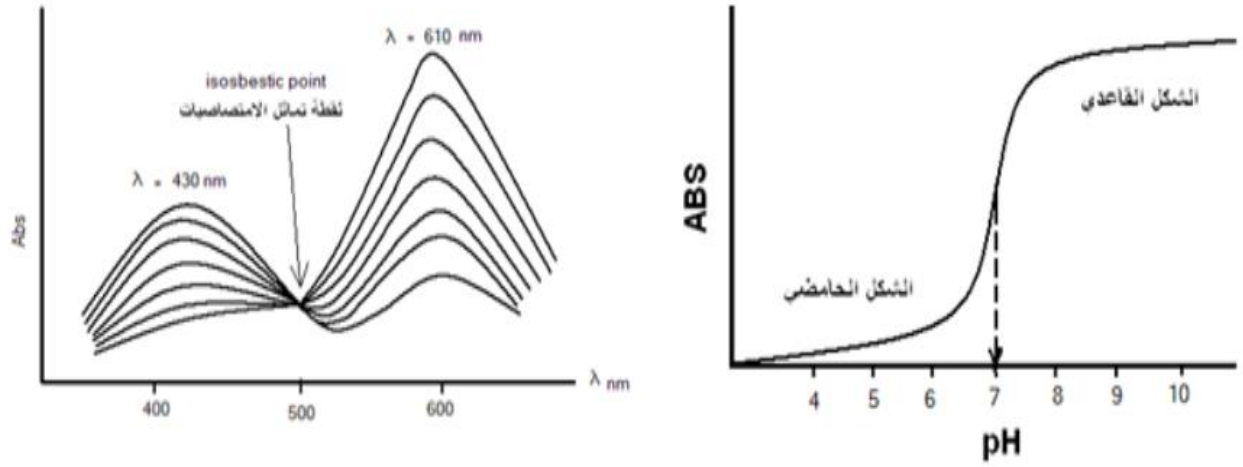
2- pK value of chemical indicators:

Chemical indicators are weak organic acids or bases that have a color in their acidic molecular formula and another color in their basic ionic formula. The change in the shape of the absorption

spectrum of an acid-base indicator when the pH value changes is an excellent method for estimating the pK value in which the ratio of the acidic molecular form and the basic ionic form of the indicator is equal, i.e. half of the indicator is in the basic form and the other half is in the acidic form.

$$\underset{\text{الشكل الحامضي (الجزيئي)}}{Hind} + H_2O \leftrightarrow \underset{\text{الشكل القاعدي (الايوني)}}{ind^-} + H_3O^+$$

For example, the phenol red indicator gives two absorption peaks, the first at 430 nm and the second at 610 nm. Solutions of constant concentration are prepared but at different pH values, and the absorption spectrum is taken. It is noted that with increasing pH of the solution, the absorption increases at 610 nm, while the absorption decreases at 430 nm. The multiple curves intersect at a specific point at a wavelength of 500 nm, called the **isoabsorption point** or **isosbestic point**. It is a distinctive feature of the indicator, in which the chemical concentration between the acidic molecular indicator and the basic ionic indicator forms is equal and balanced. To calculate pKind, absorbance readings A are taken at a wavelength of 610 nm. It is more sensitive to indicator solutions of fixed concentration and variable pH values and is plotted against the pH values of these solutions. We have a curve in the shape of the letter S in the middle and at the inflection point the value of pH = pKind. For the indicator phenol red, pH = 7.



yellow (pH = 6) to red (pH = 8). If the chemical indicator shows two **isosbestic points**, the indicator has three molecular structures in equilibrium and gives two other colors in the other region at a different pH range.

2- Determination The ratio of ligand to metal in the complex M : L :-

The complexes consist of two parts, the first is the metal M and the other is the ligand: L

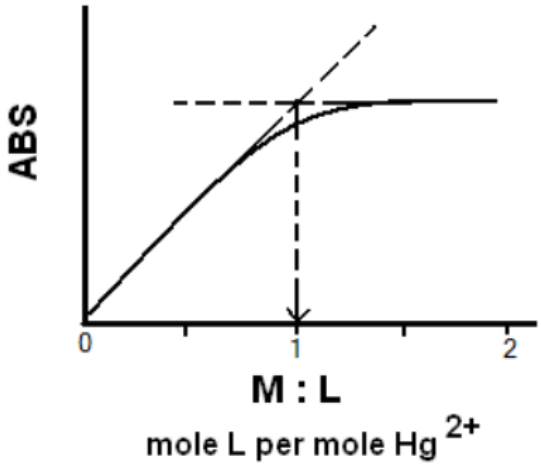
Complex = Metal : Ligand M:L

$[Cu(NH3)_4]^{2+} = Cu^{2+} : 4NH_3$

- Organometallic complexes show absorption in the UV-Vis region This technique is used to estimate the composition of the elemental union M:L in one of two ways

A- Mole – Ratio Method

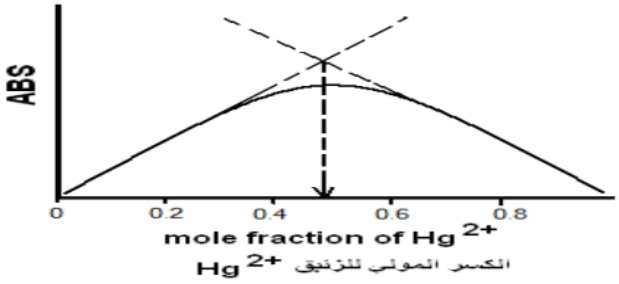
For example, a complex of diphenyl carbazone mercury ion - Hg^{2+} - diphenyl carbazone. A number of solutions are prepared containing a fixed concentration of the metal M or the chelate L with a variable concentration of the chelate (or the metal if the chelate concentration is constant), i.e. one of them has a fixed concentration and the other has a variable concentration. The absorbance of these solutions is measured at a specific wavelength of 520 nm and the absorbance values are plotted against the molar ratio M: L moles of metal to moles of chelate. A sloping curve appears indicating the presence of equivalent quantities of both the metal M and the chelate L. This is an indication of the formation of the complex. The absorbance values increase with the increase in the chelate or metal if the chelate is the constant or vice versa until the absorbance reading is fixed and the line becomes horizontal, which indicates that increasing the concentration of the added chelate L does not give any increase in the formation of the two complexes. The concentration of the metal M, i.e. Hg^{2+}, has been consumed. The value of the molar ratio M: L is calculated from the drawing directly from the point of intersection of the straight line extension of the two sides of the curve and is equal to 1 for the complex.



B- Continuous Variation Method (Job Method) :

A series of solutions containing varying concentrations of components M and L are prepared provided that the sum of the concentrations is constant. An example is the diphenylcarbazone mercury ion complex.

Hg^{2+}	0	0.25	0.5	1	1.25	0.5	1.75	2.0	ml x 10^{-4} M
L	2	1.75	1.5	1	0.75	0.5	0.25	0	ml x 10^{-4} M



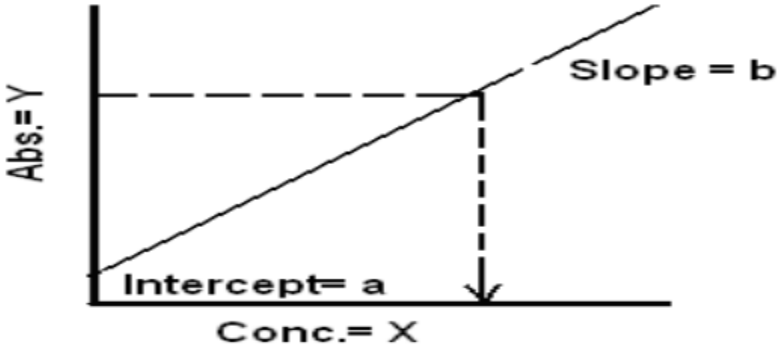
NOTE: One of the disadvantages of this method is the possibility of forming more than one stable complex for both the metal and the chelate.

Quantitative Analysis

UV-Vis spectroscopy is often used in quantitative estimation due to its ease, sensitivity, accuracy, cheapness of equipment and low cost of analysis. The analysis is done in two ways:

1- Direct method : This method is used when the sample contains a chromophore group, where the absorbance is measured directly at the maximum wavelength, max λ, and a standard material is prepared to draw the calibration curve, after which the appropriate statistical treatments are used.

2- Indirect method : This method of analysis is used when the substance does not contain a chromophore so the substance reacts with a chromophore molecule or detector to give a product with a chromophore that can absorb radiation**. In quantitative analysis, Beer's law is applied.**



$$Y = bX \pm a$$

$$X = \frac{Y \pm a}{b}$$

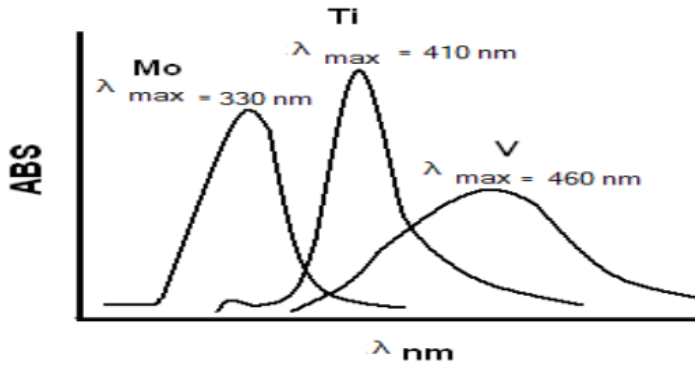
3-Additively of Absorbances : If a beam passes through a solution containing ions, the absorbance is directly proportional to the number of molecules or concentration. However, if the solution contains ions or particles of more than one substance, the particles or molecules of the substances will absorb part of the beam, and the absorbance is proportional to the concentration of the substance. The sum of the absorbances is called the additivity of absorbances. Thus, two or more elements can be identified in a mixture of them, provided that there is no overlap in the maximum absorption wavelength max λ for each substance in the solution components, i.e. the spectra of the substances are separate, where equations are formulated that can be solved and the concentration of each substance in the mixture can be found and can be expressed in a mathematical way

$$A_i = a_i\ b_i\ C_i$$

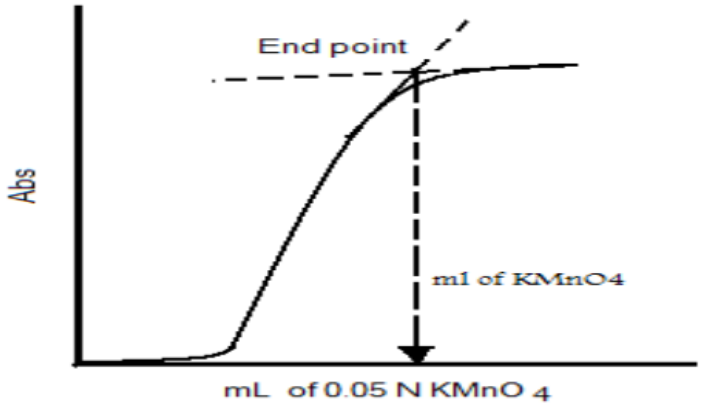
$$A_i = b \sum a_i\ C_1 + ai\ C_2 + ai\ C_3$$

.

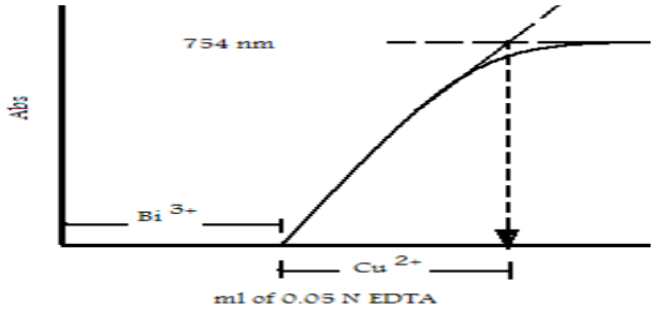
Example: Elements V, Ti and Mo in standard solutions containing 0.4 mg/L were treated with a mixture of dilute H_2O_2 and H_2SO_4. The complexes of these three elements gave the maximum absorption max λ at wavelengths, Mo = 330nm, T = 410nm and, V = 460nm, and b = 1. It can be estimated in the unknown mixture.



4- Spectrophotometric Titration : Spectral titration involves adding the standard substance to the unknown sample and measuring the absorbance after each addition, such as: Estimating the amount of ferrous ion Fe^{+2} in a sample of a copper sulfate solution, $CuSO_4$. In normal titration in an aqueous medium, the solution is titrated with a standard solution of potassium permanganate $KMnO_4$ and we note the end point of titration, but the presence of $CuSO_4$ hinders the appearance of the color. In spectral titration, the spectrometer is used to measure the absorbance of the sample at the maximum absorption wavelength max λ after each addition of the standard $KMnO_4$ solution and we continue adding until the absorbance reading is fixed. From drawing the relationship between the measured absorbance and the volume of the standard potassium permanganate solution $KMnO_4$, we can find the concentration.



Example: A sample contains a mixture of bismuth ions: Bi^{3+} and copper ions, Cu^{2+}. To find their concentration, they are irrigated with a standard solution of EDTA, and the absorbance is measured after each addition of the standard EDTA at the maximum absorption wavelength (λ max) of 754 nm.



The curves show two end point of titration, the first is the end point of titration of the Bi^{3+} ion which does not give absorbance with EDTA, and the second is the end point of titration of the Cu^{2+} ion which gives a colored complex. The addition of EDTA increases the volume and therefore the concentration and absorbance decreases. To delete the effect of dilution, a high concentration of EDTA solution is prepared so that the small added volume does not affect the concentration or a correction is made for the absorbance value A by multiplying it by the **dilution factor**, which is the sum of the original and added volumes divided by the original volume.