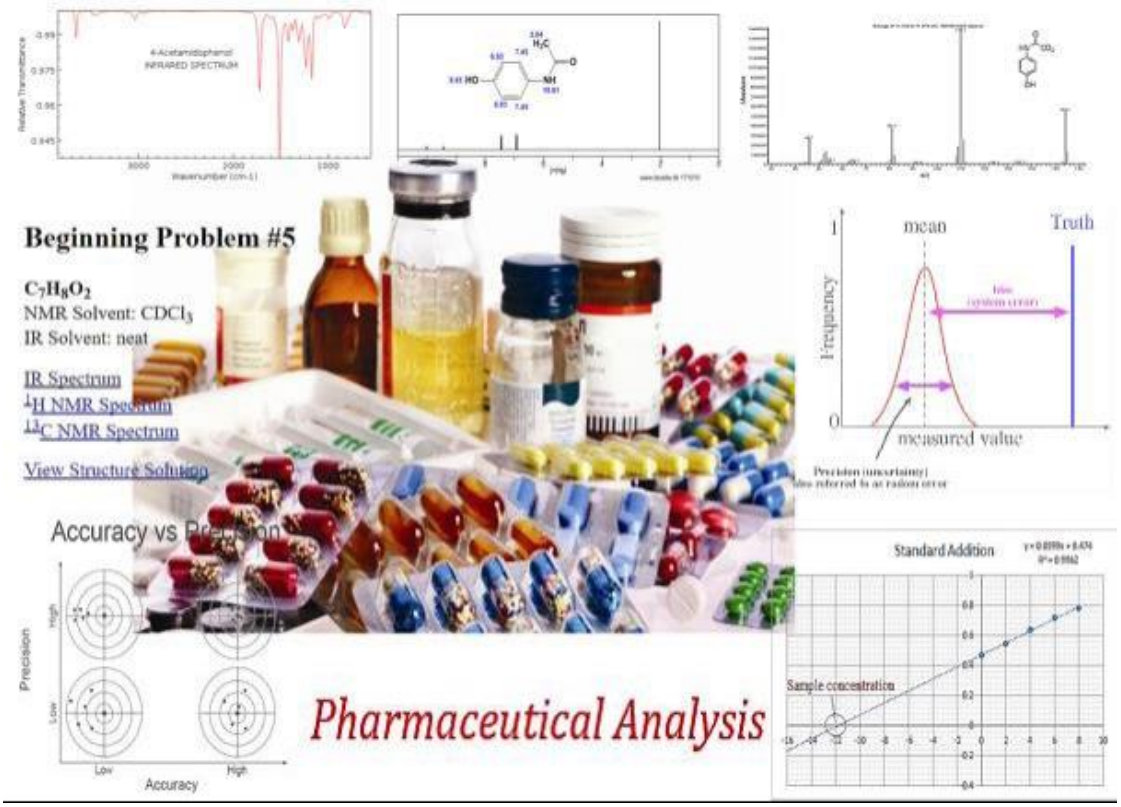
College of Pharmacy

Department

Of Pharmaceutical Chemistry

Advanced Pharmaceutical Analysis laboratory

Fifth Year Pharmacy Students



ULTRAVIOLET - VISIBLE SPECTROSCOPY

UV-Vis spectroscopy is one of the most important quantitative spectroscopic techniques. The wavelength range extends from about 190 nm to 750 nm which corresponds to electronic transitions of different origins. The energy level diagram can simply be represented as shown in Figure 1.

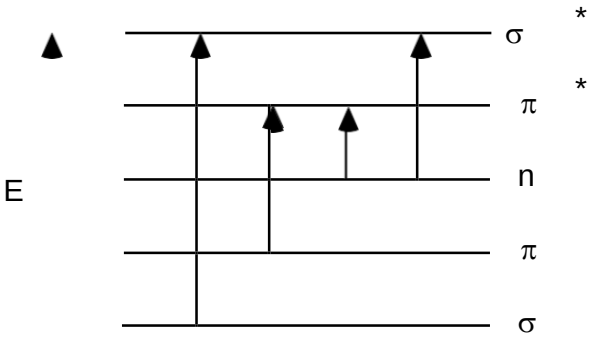


Figure 1: Energy level diagram representing electronic transitions.

Four transitions are shown in the figure, which correspondto:

a. ⋆ ⋆ ⋆ *Transitions

This type of transition requires large energy which may result in breakdown of chemical bonds. It is not important from the analytical point of view for reasons to be discussed later. The radiation wavelength which should be used to enforce this type of transition is below 190 nm .

b. ⋆ ⋆ ⋆ *Transitions

This is the most analytically useful type of transitions where radiation of certain wavelength cause electrons present in the π bonds to be excited to the higher energy π* state. The energy required is moderate and can be obtained from sources operating in the UV-Vis range.

c. n - ⋆ * and n - ⋆ *Transitions

Molecules which contain lone pair(s) of electrons exhibit some special characteristics. Electrons which do not participate in chemical bonds can absorb energy and are excited either to the π* (if the molecule has πbonds) or to the σ*state. Energy required for a n - π* transition is small while a n - σ*transition requires more energy. Factors to be mentioned later eliminate the use of these transitions in analytical work.

Beer's Law

It is logical that the amount of energy absorbed by molecules depends on the number of the absorbing species and, therefore, on concentration. A quantitative relationship was derived by Beer, Lambert and Bouger separately. Therefore, the relationship can be referred to as Beer's, lambert or Bouger law or some combination of these names.

The law states that:

A = εbC, where

A is the absorbance.
b is the cell length in cm
ε is the molar absorptivity in L cm^{-1} mol^{-1}
C is the concentration in mol/L
Away from mathematical derivation, the law can be simply derived as follows.

It is logical to assume that A α C since as C increases, the number of absorbing species increases and thus A increases.
Also A α b since as b increases more molecules are encountered by radiation and therefore possibility of absorbance increases.

This can yield the relation
A αbC or
A = εbC, where ε is a proportionality constant and is called molar absorptivity. Old conventions used the expression "extinction coefficient" or "optical density" for the molar absorptivity. However, the last expression is the internationally accepted one.

In Beer's law, ε is the most important sensitivity indicator. When b is 1 cm and A is plotted as a function of the concentration, a straight line relationship is obtained as in Figure2.

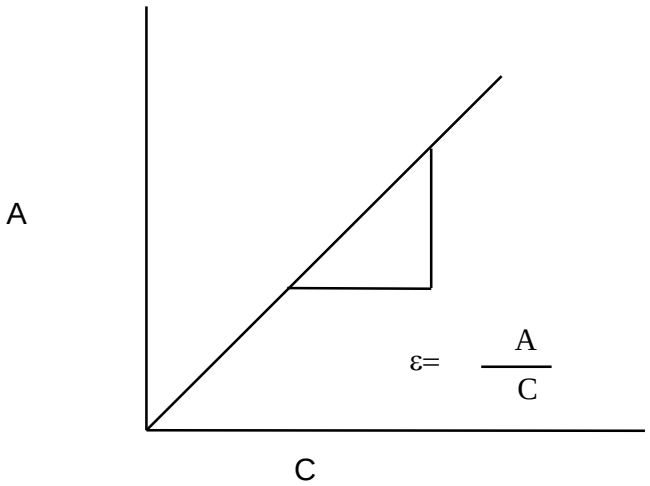


Figure 2: A straight line relationship between absorbance (A) and concentration (C). Molar absorptivity is the slope when b equals 1 cm.

When the concentration is expressed in mol/L and is plotted on the X-axis, ε is the slope and its value is an indication of the sensitivity of the method.

Deviations from Beer's Law

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

1- Real limitations:

This law obey for dilute solution .At high concentrations (i.e. >0.02M),

- Linearity is observed in the low concentration ranges (<0.01), but may not be at higher concentrations.

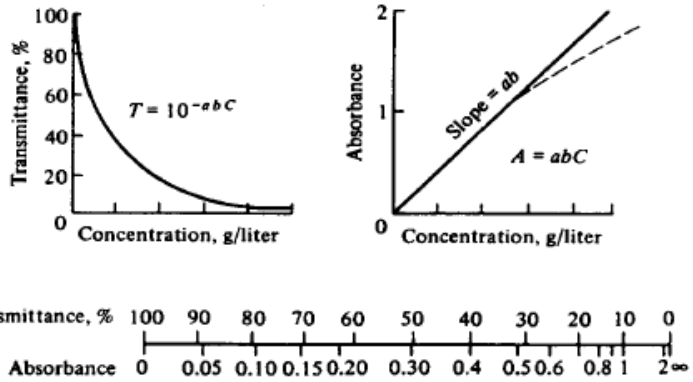


FIGURE 3-1 Representation of Beer's law and comparison between scales in absorbance and transmittance.

- This deviation at higher concentrations is due to intermolecular interactions.
- As the concentration increases, the strength of interaction increases and causes deviations from linearity.
- The absorptivity not really constant and independent of concentration but is related to the refractive index (η) of the solution by the expression:

$$\varepsilon_{true} \bullet \frac{\eta}{(\eta + 2)^2}$$

- If the addition of solute causes a significant change in the refractive index of the solution a correction to the Beer Lambert formula can be placed as:

$$A = \varepsilon bc\ (\eta^2 + 2)^2$$

- This correction is normally not required below concentrations of 10mM.
- At low concentrations the refractive index is essentially constant, so ε constant and linearity is observed.

2- Chemical Deviation:

Occur when any chemical reaction that can alter the conc. of an absorbing species causing a deviation from Beer's Law.

a- If the conc. is decreased because of chemical reaction & the product does not absorb radiation at the λ_{max} at which the measurement is made , a negative deviation occurs.

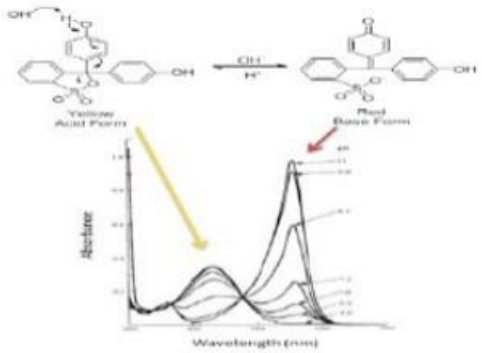
b- If the product of the chemical reaction absorbs more strongly than the assayed substance . a positive deviation occurs.

What are the types of chemical reaction can lead to deviation from Beer's Law?

1- Association –dissociation reactions
2- Acid-base reactions
3- Polymerization reaction
4- Reactions with the solvent.

Examples

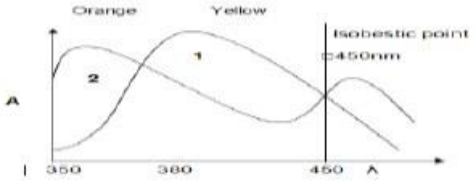
phenol red undergoes a resonance transformation when moving from the acidic form (yellow) to the basic form (red). Due to this resonance, the electron distribution of the bonds of molecule changes with the pH of the solvent in which it is dissolved. Since UV-visible spectroscopy is an electron-related phenomenon, the absorption spectrum of the sample changes with the change in pH of the solvent.



Acid and Base forms of phenol red along with their UV spectra at different pH demonstrates chemical deviations of Beer-Lambert law in UV-Visible spectroscopy

(a) Acid- base reaction: e.g. $K_2Cr_2O_7$ Unbuffered solution

$$Cr_2O_7^{-2} + H_2O = 2HCrO_4^- = 2H^+ + 2CrO_4^-$$



(1) $K_2Cr_2O_7$ in 0.05 N NaOH
(2) $K_2Cr_2O_7$ in 3.5 N H_2SO_4

We can show from above figure that the (ε) and the absorption spectrum for $Cr_2O_7^{-2}$ (orange) is also different compared to CrO_4^- (yellow).

We can show from above figure that the (ε) and the absorption spectrum for $Cr_2O_7^{-2}$ (orange) is also different compared to CrO_4^- (yellow).

But if we made measurement at λ = 445 nm, no deviation from Beer's Law is observed. Here the values of absorbances are equal for $Cr_2O_7^{-2}$, CrO_4^- at the point known as an isoabsorptive λ or isobestic point.

Isobestic point: A wavelength at which the absorbance spectra of two species cross each other. The appearance of isobestic points in a solution in which chemical reaction is occurring is evidence that there are two components present, with a constant total concentration.

(C) Polymerization reaction: (dimerization, trimerization...etc) are common with certain type of compounds such as basic dyes.Hence, the variation in the absorptivities of the various forms at a fixed wavelength causes a deviation from Beer's Law. For MeIB, Beer's Law is obeyed only at conc. below about $1x10^{-5}$M, when the monomer is the only polymeric form in solution.

3-Instrumental Deviation: These are ,

A-Non-monochromatic light –(i.e polychromatic) consider that real spectrometers provide a range of wavelengths (Δλ) λ1 λ2

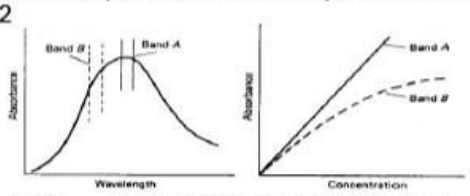


FIGURE The effect of polychromatic radiation upon the Beer's law relationship. Band *A* shows little deviation, because ε does not change greatly throughout the band. Band *B* shows marked deviations because ε undergoes significant changes in this region.

B- Stray light:

* Some light that's not within Δλ a reach the detector
* Stray light does not interact with the sample as does Δλ but it cause a ΔAbs

Note : Instrumental limitation are always NEGATIVE

Δλ should be ≤ 0.01 of the band ½-width for <0.5% abs. error

This stray light can be minimized or eliminated by painting the cell compartment with flat black paint & carefully sealing the door of the compartment so that the radiation from outside the compartment is prevented from the entering.

C- Inaccurate response of the detector:

Error occurs if the intensity of the EMR that strikes the detector is either **very large** or **Very small**

The Molar Absorptivity

From the above argument, it is clear that ε is an important element in Beer's law expression. It is an indicator of sensitivity which means that the value of ε is very important in characterizing a system for quantitative capacity. As ε increases, it becomes easier to determine lower concentrations ofanalytes.

The molar absorptivity has large values for π π transitions ranging from 1000 to 10000 L cm^{-1} mol^{-1}. For n - π^* transitions, ε ranges from 10 - 100 L cm^{-1} mol^{-1}. This means that the most important trasition in UV-Vis is the π π^* transition and, therefore, will be subjected to further studies in differentsolvents.

Additivity of Absorbances

When two absorbing species are present in solution, the absorbance value measured will represent bothspecies

$A = A_1 + A_2$

However, measuring absorbance at two different wavelengths can yeild the exact concentration of eachcomponent

$A_1 = \varepsilon_1 b C_1 + \varepsilon_2' b C_2$ $_2$ at λ_1

$A_2 = \varepsilon_1' b C_1 + \varepsilon_2 b C_2$ at λ_2

$\varepsilon_1, \varepsilon_2, \varepsilon'_1$, and ε'_2 are constants and can be determined experimentally.

Only C_1 and C_2 are not known which can be determined by solving the twoequations.

Determination of a Ligand to Metal ratio

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

$$M^{n+} + m\ L = (ML_m)^{n+}$$

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 experimental methods will be discussed:

a. The Method of Continuous Variation (Job'sMethod)

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 Figure4.

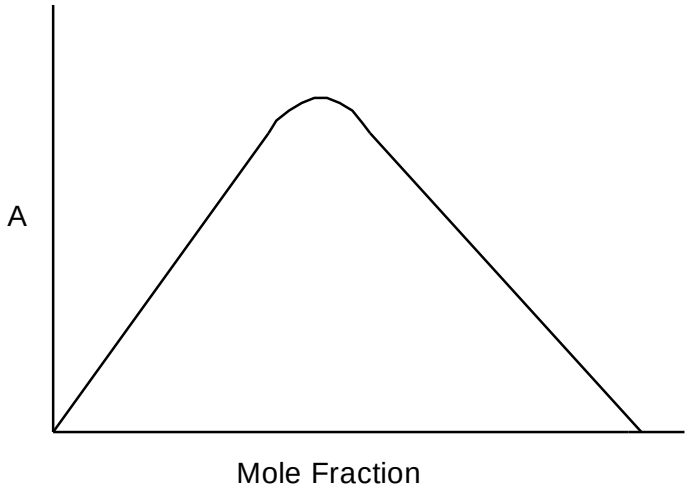


Figure 4 : A plot of absorbance versus mole fraction.

$$\text{Mole Fraction} = \frac{\text{mol}M^{n+}}{\text{mol}M^{n+} + \text{mol L}}$$

Tangents are drawn on both side of the maximum obtained and a perpendicular line is drawn to the axis representing the mole fraction. This gives the mole fraction of the metal. If the value 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 ispreferred.

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 Figure 5 is obtained

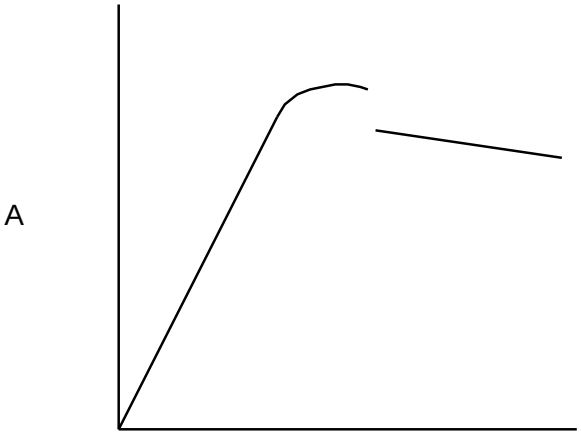


Figure 5: A plot of absorbance versus mole ratio.

Tangents are drawn and a perpendicular line is drawn to the mole ratio axis showing the exactratio.

Instrumentation.

In this section, only a brief description of instrumental features will be mentioned. This is important since you may be required to perform some experiments in UV-Vis spectroscopy without enough background.
Two types of instruments are available according to the wavelength selector used.

FilterPhotometer

This uses filters for the selection of working wavelengths. Photometers are cheap machines that are widely used in most primitive analytical laboratories. The optical system and instrumental components can be represented by Figure6.

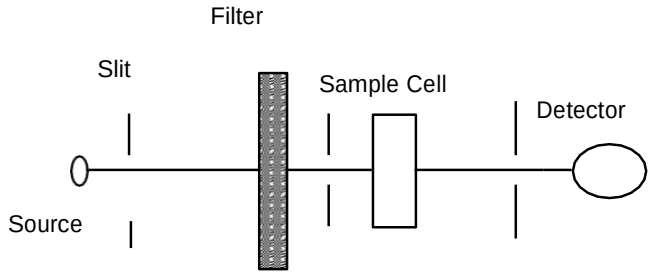


Figure 6: Schematic diagram of a photometer

As can be seen from the figure, light is emitted from the source passing through a suitable filter for wavelength selection. Part of the light at the selected wavelength is absorbed by the sample and the transmitted light hits the phototube detector resulting in a signal that is displayed by the instrument asabsorbance.

a. DispersiveSpectrophotometers

These use either prisms or gratings for wavelength selection. Prisms and gratings are excellent wavelength selectors where a very narrow band of light at specific wavelength can be chosen especially with good gratings. Dispersive instruments are divided into two types:

1. Single BeamSpectrophotometers

This is similar to the photometer design but the wavelength selector is either a prism or grating instead of the filter. Usually, single beam instruments are of moderate price and require adjustment to zero using a blank before sample measurement. As the instrument is kept in the operational mode, multiple zero adjustments should be undertakenbecausethereisalwayssomedriftinresponsewithtime.

2. Double Beam Spectrophotometers

These incorporate places for two cells one for the blank and the other for the sample. The instrument automatically subtracts the

absorbance of the blank or reference from that of the sample. The different optical components of the instrument can be seen in Figure 7.

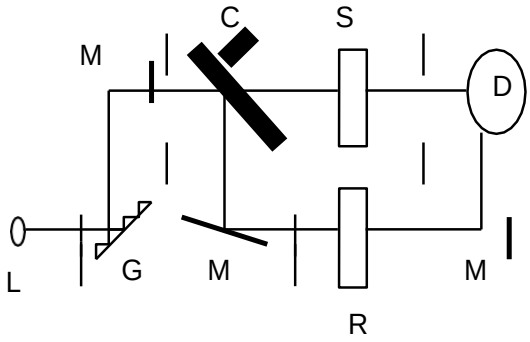


Figure 7: Schematic diagram of a double beam spectrophotometer: L,source; G, grating; M, mirror; C, chopper; S, sample; R, reference; and D, detector.

The chopper (C) splits the incident beam into two halves, one passes through the sample and the other passes through the reference. The detector automatically records the difference which is displayed asabsorbance.

Sources

The most commonly used sources are deuterium lamps in the ultraviolet region and tungsten - halogen lamps in the visible region. Make sure not to look at the deuterium lamp while in the operational mode since UV light is damaging to your eyes.

Cells

Remember that glass absorbs UV light, therefore make sure to use quartz cells when working in the UV region. Glass cells are adequate for measurement of absorbance in the visible region while quartz cells are adequate through the whole UV-Vis range.

Routine Methodology in Spectrophotometric Analysis

The first step of an analytical procedure in UV-Vis spectroscopy is to find the wavelength that yeilds 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 yeilds maximum absorbance is chosen for further work. This also gives maximum molar absorptivity.

When the problem involves the determination of an unknown analyte concentration, standard analyte is used to construct a calibration curve at the preselected wavelength and the unknown absorbance is measured which can be correlated with concentration from the curve.

Why *Pharmaceutical Analysis* is considered so important?

1. Is the identity of the drug in the formulated product correct?
2. What is the percentage of the stated content of a drug present in formulation?
3. Does this formulation contain solely the active ingredient or are additional impurities present?
4. What is the stability of the drug in the formulation and hence what shelf-life of the product?
5. At what rate is the drug released from its formulation so that it can be absorbed by the body?
6. Do the identity and the purity of a pure drug substance to be used in preparation of a formulation meet specification?
7. What are the concentration of specified impurities in the pure drug substance?
8. What is the concentration of drug in sample of tissue or biological fluid?
9. What are the pKa value(s), partition coefficients, solubilities and stability of a drug substance under development?