CHAPTER - I

THEORY OF SPECTROPHOTOMETRY

C H A P T E R - I

THEORY OF SPECTROPHOTOMETRY

INTRODUCTION

Spectrophotometric method is often preferred for the determination of trace amount of metal ions. New analytical methods are based on instrumental technique. Atomic emission, flame photometry, polarography potentiometry, radiochemical techniques etc. are commonly used. Spectophotometric techniques are partially useful when insuffecient sample is present for gravimetric and volumetric methods. In colorimetric or spectrophotometric, trace analysis depends on sensitivity of the colour reaction. The sensitivity of the colour reaction may be defined as the smallest weight of substance that can be detected in a column of solution of unit cross section. The sensitivity is based on law of Lambert-Beer. The spectrophotometic technique is highly selective, sensitive and rapid for analysis of variety of materials. The absorption of Ultra-violet and visible radiation is measured in spectophotometric determination. The upper limit of the method is, in general, the determination of constituents which are present in the quantities of less than 1 or 2 percent.

The variation of the colour of the system with change in concentration of some component forms the bases of colorimetric analysis. The colour is usually due to the formation of a coloured compound by the addition of an appropriate reagent, or it may be inherent in the disired constituent itself. The intensity of the colour may then be compared with that obtained by treating a known amount of substance in same manner.

Colorimetry is concerned with the determination of the concentration of substance by measurment of the relative absorption of light with respect to a known concentration of the substance. In visual colorimetry, natural or artificial white light is generally used as a light source, and determinations are usually made with a simple instrument termed a colorimeter or colour comparator. When the eye is replaced by a photoelectric cell the instrument is termed as photoelectic colorimeter. In spectrophotometric analysis light of definite wavelength extending to the Ultra-violet region of the spectrum constitutes the source of light and thus necessitates the use of a more complicated and consequently more expensive instrument, the instrument has been named a spectrophotometer; or photoelectric spectrophotometer. Spectrophotometer is an instrument for measuring the intensity of the light of various wavelengths transmitted (or reflected) by a solution (or other medium). It consists essentially of a device (usually a monochromator or a diffraction gratting) for furnishing "monochromatic light", one or more absorption cells for holding

the sample and reference solutions and a photometric arrangement for the subjective or objective measurment of the intensity of transmitted light.

Higher precision can be obtained in photoelectric spectrophotometry than in ordinary colorimetry. More over, when many deteminations are to be made, objective spectrophotometric methods are more rapid and less fatiguing than the subjective colorimetric methods.

In spectrochemical analysis we make use of spectrum of electromagnetic radiation to analyse chemical species and study their interactions with electromagnetic radiation. Electromagnetic radiation is a form of energy that can be described in terms of its wavelike properties. In contrast to sound waves, electromagnetic waves travel at extreme velocities and do not require the existence of some supporting medium for propagation. Radiant energy is the energy associated with electromagnetic waves of different wavelengths. It consists of minute units of energy called quanta, or photons. The relationship between the energy of a photon and the frequency appropriate for the description of its propagation is

$$E = h\nu$$

where, E = represents energy in ergs

ν = represents frequency in cycles, per second and

h= Planck's constant

Radiant energy also can be thought of a continuous wave motion in which λ. represents the interval between nodes in the wave pattern. The wavelength λ of a beam of elecromagnetic radiation is the linear distance travelled by one complete wave cycle. The frequency ν. is the number of cycles occuring per second. The relationship between wavelength and frequency is

$$\nu = C/\lambda \text{ where,}$$

λ = the wavelength in centimeter

C = the velocity of the radiant energy in centimeter per second

The equivalent expression invoving wave length is

$$E = \frac{hc}{\lambda}$$

visible light, a very small part of the electromagnetic spectrum is generally concered to extend from 380 to 780 nm. The ultraviolet region of the electromagnetic spectrum is frequently subdivided into the far or vacuum ultraviolet region approximately 10-200nm and the near ultraviolet region which extends from 200-380nm. To state that a solution coloured means that of all wavelengths of white light incident upon the solution, only selected wavelengths are absorbed depending on the colour of the solution the remaining wavelegths are transmitted. A red solution for example appears red because it absorbs the shorter wavelength of the visible region and transmits the larger wavelengths, therefore, colour is

attributed to the selective absorption of incident radiant energy of certain wavelengths.

Absorption in the ultra-violet region of the spectrum has been related to the presence of unsaturation and polarizability in ultraviolet absorbing materials. Saturated compounds are transparent in the ultraviolet region.

When an electromagnetic wave of a specific wavelength impinges upon a substance the energy associated with that wave may be altered by reflection, refraction, absorption and transmission processes. Reflection and refraction effects are generally negligible in the spectrophotometric analysis of solutions. Each of these transitions require a definite quntity of energy, the probability of occurence for a perticular transition is greatest when the photon absorbed supplies precisely this quantity of energy.

The energy is required for these transitions. In general, promotion of electrons to higher levels requires greater energies than those needed to bring about vibrational changes. Alternation in rotational mode are likely to have the lowest energy requirements of all. Thus, absorptions observed in the microwave and far infrared regions will be due to shifts in rotational level, since the energy of the radiation is insufficient to cause other type of transition. Changes in vibrational levels are responsible for absorptions in the near infrared and visible regions. Absorption due to promotion of an

electron to some higher energy level takes place in the visible, ultraviolet, and X-ray regions of the spectrum.

The absorption of radiation by a system can be described by means of a plot of absorption as a function of wavelength. Such a graph is called an absorption spectrum. In as much as energies required for various processes responsible for absorption are unique for agiven species, its absorption spectrum is also unique as a consequence absorption spectra are often helpful for qualitative identification purposes.

Irrespective of amount of energy absorbed an excited species tends spontaneously to return to its unexcited or ground state. To accomplish this the energy of absorbed photon must somehow be given up, and this is ordinarily dissipated in the form of heat. In some instances, however transition to another excited state proceeds to return the ground state

Theory of Spectophotometry and Colorimetry :

When light (monochromatic or heterogeneous) falls upon a homogeneous medium, a portion of incident light is reflected, a portion is absorbed within the medium and the remainder is transmitted. If the intensity of the incident light is expressed by Io, that of the absorbed light by Ia, and that of the transmitted light by I_t and that of the reflected light by I_r, then ;

$$I_o = I_a + I_t + I_r$$

I_r is usually eliminated by the use of a control such as comparison cell, hence :

$$I_o = I_a + I_t$$

Lambert (1760) investigated the relation between I_o and I_t. Beer (1852) extended the experiments to solutions. Spectrophotometry and colorimetry are based upon Lambert's and Beer's laws.

Lambert's law : This law states that when monochromatic light passes through a transparent medium, the rate of decrease in intensity with thickness of the medium is proportional to the intensity of the light. This is equivalent to stating that the intensity of the emitted light decreases exponentially as the thickness of the absorbing medium increases arithmetically, or that any layer of given thickness of the medium absorbs the same fraction of the light incident upon it. We may express the law by the differential equation

$$-\frac{dI}{dl} = kI$$

Where I = Intensity of the incident light of wave length λ

l = thickness of the medium

k = proportionality factor

Integrating the above equation and putting

$$I = I_o \text{ when, } \quad l = 0$$

$$l \quad \frac{I_o}{It} = kl$$

or in other terms

$$I_t = I_o.e^{-kl}$$

Where, I_o = intensity of the incident light falling up on an absorbing medium of thickness l

I_t = = Intensity of the transmitted light

k = cosnstant for the wave length and the absorbing medium used.

By changing from natural to common logarithms we obtain

$$I_t = I_o.10^{-0.4343kl}$$

$$= I_o.10^{-k}$$

Where, $K = k_{/2.3026}$ and is usually termed the absorption coeffcient or extinction coefficient. The absorption coefficient is generally defined as the reciprocal of the thickness (1 cm) required to reduce the light to $1_{/10}$ of it's intensity.

$$I_{t/I_o} = 0.1 = 10^{-kl}$$

or, $Kl = 1$ and $K = 1/l$

The ratio I_t/I_o is the fraction of the incident light transmitted by a thickness l of the medium and is termed the

transmittance T. Its reciprocal I_o/I_t is the opacity and the optical density D of the medium, sometimes designated the extinction E or absorbance A, is given by

$$D = \log \frac{I_o}{I_t}$$

Thus medium with optical density 1 for a given wavelength transmits 10 percent of the incident light at the wavelength in question.

Beer's law :

Beer (1852) studied the effect of the concentration of the coloured constituent in solution upon the light transmission or absorption. He found the same relation between transmission and concentration as Lambert had discovered between transmission and thickness of the layer, i.e. the intensity of a beam of monochromatic light decreases exponentially as the concentration of the absorbing substance increases arithmetically. This may be written in the form :

$$I_t = I_o.e^{-k'c}$$

$$= I_o.10^{-0.4343k'c}$$

$$= I_o.10^{-K'c}$$

where c = concentration

k' and K' are constants

Combining equations for Lambert's and Beer's law, we have

$$I_t = I_o.10^{-\varepsilon cl}$$

or

$$\log \frac{I_o}{I_t} = acl$$

This is the fundamental equation of colorimetry and spectro photometry, and is often spoken of as the 'Beer-Lambert law'.

The value of 'a' will clearly depend upon the method of expression of the concentration. If C is expressed in gram mole per litre and l in centimeters, then 'a' is given the symbol ε and is called the molar absorption coefficient or molar absorptivity (formarly molar extinction coefficient) or molar absorbancy index. The later is equal to the reciprocal value of the thickness in centimeters of a molar solution (c=1) at which

$$I_t = 0.1 \times I_o$$

Since $I_t = I_o.1^{-}0^{\varepsilon}$ when t = 1 and C = 1, The specific extinction coefficient Es may be defined as the extinction or optical density per unit thickness and unit cocentration.

Where the molecular weight of substance is not definitely known, it is obviously not possible to write dawn the molecular extiction coefficient, and in such cases it is

usual to write the unit of concentration as a subscript and the unit of length as subscript. The optical density D, also called the extinction E or the absorbancy A, of a medium is the logarithmic ratio of the intensity of the incident light to that of the emergent light, i.e.

$$D = E = A = \log (I_o/I_t)$$

or $$I_t = I_o \times 10^{-D}$$

D is obviously related in a simple way to the molecular extinction coefficient,

Since : $D = \varepsilon c l$

The molecular extinction coefficient is therefore the optical density when the layer of solution is 1 cm thick and the concentration of the absorbing substance is 1 gram mole per litre.

The terrm transmittancy Ts, is defined as the ratio of the transmittance of a cell containing the coloured solution to that of an identical cell containing the solvent or a blank solution.

The optical density D may therefore be put equal to the logarithm of the reciprocal of the transmission.

$$D = \log (1/T) = -\log T$$

The scales of spectrophotometers are often calibrated to read directly in optical densities, and frequently also in

percentage transmittance. It may be mentioned that for colorimetric measurments Io is usually understood as the intensity of the light transmitted by the pure solvent or the intensity of the light entering the solution. It is the intensity of the light emerging from the solution or transmitted by the solution. It will be noted that : the extinction coefficient is the optical density for unit path length.

$$k = \frac{D}{t}$$

or

$$I_t = I_o \cdot 10^{kl}$$

the specific extinction coefficient is the optical density per unit path length and unit concentration

$$E_s = \frac{D}{cl}$$

or

$$I_t = I_o \cdot 10^{-Escl}$$

The molar extiction coefficient is the specific extinction coeffecient for a concentration of 1 gm mole per litre and a path length of 1 cm.

$$\varepsilon = {}^{D}/_{Cl}$$

<u>Application of Beer's Law</u> :-

Let us consider the case of two solutions of a

coloured substance with concentrations C_1 and C_2. These are placed in an instrument in which the thickness of the layers can be altered and measured easily, and which also allows a comparison of the transmitted light when the two layers have to the same colour intensity

$$I_{t1} = I_o.10^{-\varepsilon l_1 C_1}$$

$$I_{t2} = I_o.10^{-\varepsilon l_2 C_2}$$

Here l_1 and l_2 = lengths of the coloumn of solutions with concetration C_1 and C_2 respectively.

when the system is optically balanced Hence under these conditions and when Beer's law holds;

$$l_1 C_1 = l_2 C_2$$

A colorimeter can therefore be employed in a dual capacity:

a) to investigate the validity of Beer's law by varying C_1 and C_2 and noting whether above equation applies and

b) for the determination of an uknown concentration C_2 of a coloured solution by comparison with a solution of known concentation C1. It must be emphasised that the equation is valid only if Beer's law obeyed over the concentration range employed and the instrument has no optical defects.

When a spectrophotometer is used it is unnecessary to make

comparison with solutions of known concentration. With such an instrument the intensity of the transmitted light or, better the ratio I_t/I_o (the transmittance) is found directly at a known thickness l. By varying l and c the validity of the Lombert-Beer law, equation can be tested and the value of ε may be evaluated. When the latter is known the concentration C_x of an unknown solution can be calculated from the formula

$$C_x = \frac{\log I_o/I_t}{\varepsilon l}$$

Attention is directed to the fact that the extinction coefficient ε depends upon the wavelength of the incident light, the temperature and the solvent employed. In general, it is best to work with light of wavelength approximating to that of for which the solution exhibits a maximum selective absorption (or minimum selective transmittance) : the maximum sensitivity is thus attained. For matched cells (i.e. l constant) the Lambert-Beer law may be written

$$C = A \log \frac{I_o}{I_t}$$

$$= A \log \frac{l}{T}$$

or $$C = B \log T$$

where A and B are constants : the concentration is therefore, proportional to the logarithm of its transmittance. Hence by

plotting log 1/T (ordinates) against concentration (abscissa) a straight line will be obtained, and this will pass through the point C= 0, T= 100 percent. This calibration line may then be used to determine unknown concentrations of solutions of the same material after measurement of absorbances.

Deviation from Beer's Law :

Beer's law will generally hold over a wide range of concentration if the structure of the coloured ion or of the coloured non-electrolyte in the dissolved state does not change with concentration. Small amounts of electrolytes which do not react chemically with the coloured compounds, do not usually affect the light absorption, large amount of the electrolytes may result in a shift of the maximum absorption and may also change the value of the extinction coefficient. Discrepancies are usually found when the coloured solute ionises, dissociates or associates in solution, since the nature of species in solution will vary with the concentration. The law does not hold when the coloured solute forms complexes, the composition of which depends upon the concentrtion. Also discrepancies may occur when monochromatic light is not used. The behaviour of a substance can always be tested by plotting log I_o/I_t or log T against the concentration : a straight line passing through the origin indicates conformity to the law.

For solutions which do not follow Beer's law, it is best to prepare a calibration curve using a series of standards of

known concentration. Instrumental readings are plotted as ordinates against concentration in, say, mg per 100 ml or 1000 ml as abscissae. For the most precise work each calibration curve should cover the dilution range likely to be met with in the actual comparison.

Instrumentation :

A spectrophotometer, as the name implies, is an instrument composed of two units viz. spectrometer and photometer. One which produces a light from spectrum of definite wavelength and photometer a device to measure the intensity of the transmitted or absorbed light. Thus this serves as a device for the measurement of relative energy, whether energy is emitted, transmitted or reflected, as the function of wavelength. The chief advantage of using spectrophotometer over simple photometer is that we are in position to obtain a selected wavelength from white light, suitably dispersed either by prism or by diffraction gratings and slits. So more or less monochromatic light is obtained, whereas in filter photometer an attempt is made to obtain light of known waveband and not known wavelength by passing through filters of different color which possess characteristics of allowing the light of particular wavelength to pass through. In photometer with filters, light source arrangement is such that it is not possible to get light exactly of particular wavelength but it will be varying between 30-40 nm. However, in spectrophotometer it is possible to

obtain light of selected waveband by suitably dispersing it through prism and slit or diffraction gratings.

Ultra-violet spectrophotometer consists of (1) an intense source of radiant energy in the 200 to 700 nm region, (2) a monochromator to isolate the wavelength region to be used in irradiating the solution, (3) an absorption cell assembly which provides for alternate examination of the reference solution and simple solution (4) a photometer, comprised of a photo-electric detector which converts radiant energy to electrical energy, and a meter to indicate the resulting electric current. The distinguishing feature of a spectrophotometer is the use of monochromator to select specific monochromatic radiant energy.

A spectrophotometer is composed of source for continuous visible spectrum, a device for obtaining monochromatic light, absorption cells for sample and blank solution and a means of measuring difference of absorption between the sample and blank or reference.

Source :

The first essential component of a spectrophotometer is a source of continuous radiant energy at constant and sufficient intensity for the region of the spectrum in which the characteristic absorption bands on the sample are found. A tungsten filament lamp provides sufficient intensity for the region and is widely used for the visible and near infrared region (350-

3000 nm). Hydrogen or deuterium discharge lamps with quartz windows provide a continuous spectrum of radiant energy in the 185 to 370 nm region. Measurements below 195 nm require purging of the optical system with dry nitrogen to eliminate absorption due to oxygen and water vapour. Most commercial spectrophotometers are designed so that the ultraviolet and the tungsten lamp for visible spectrometry, can be rapidly interchanged.

Monochromators :

The monochromator, the second essential component of a spectrophotometr, permits the selection of radiant energy of the desired wavelength. Light admitted through an entrance slit is collimated with a lens or mirror. The monochromator consists of entrance and exit slits and a dispersive device, either a prism or grating, so arranged that radiant energy of a relatively narrow spectral bandwidth is obtained. Dispersion in a prism arises from the phenomenon of refraction. Because its velocity depends upon the medium through which it travels, light is frequently refracted or bent, as it passes from one medium to another.

The capability of either a prism or a grating to separate two adjacent wavelength is termed resolving power R. The resolving power of a prism is dependent on its effective thickness t and the slope of the dispersion plot of its optical material :

$$R = t\ (\delta n/\delta\lambda) \simeq \lambda/\Delta\lambda$$

For a grating, the resolving power depends on the number of rulings N and the order of the spectrum m. R = mN. Most monochromators used in commercial spectrophotometers have equal width entrance and exit slits so that the energy distribution of the transmitted beam is triangular. The slits control the spectral slit width (SSW) of the emerging beam, thereby causing the nominal wavelngth to be slightly contaminated with radiant energy of slightly higher and lower wavelengths.

Absorption Cells :

The third essential component of a spectrophotometer is the assembly in which absorption cells are held and positioned in a reproducible manner. Absorption cells are constructed of glass for measurement in the visible region, while quartz or fused silica cells are used for measurement in the ultraviolet region. The sample and reference absorption cells should be matched with respect to optical path length and transmittance at specific wavelengths. It is especially important to have matched cells, since the transmittance characteristics in the ultraviolet region may be different, even though two cells might have identical lengths. Absorption cells in 1 cm internal optical path lengths are used extensively. Cells are also available with path lengths of 0.1, 0.5, 2, 5 and 10 cm.

Photometers :

The most essential component of the photometer is the detector of radiant energy which produces a signal proportional to the radiant power of the beam. There are three types of photo sensitive detectors commonly used :

1. Barrier layer cells
2. Photoemissive cells

and

3. Electron multiplier phototubes.

The barrier or photovoltaic cell consists of a conductor in close contact with a semiconductor is irradiated. The electric current produced is proportional to the radiant power of the incident beam and to the area of the photosensitive surface being irradiated. The spectral response of this detector is somewhat similar to the eye and is suitable for photometric measurements in the visible region.

The photoemissive cell consists of a photosensitive cathode containing an alkali metal oxide, and an anode mounted in a glass envelop, which is evacuated. The spectral response of this cell depends on the material used for the photocathode surface. Therefore, by proper selection of a photocathode the spectral response can correspond to the specific ranges of the visible and ultraviolet region.

The electron multiplier phototube has a series of photo-sensitive surfaces each changed at a successively higher potential, and the photoelectrons emitted by the first photo-cathode surface are accelerated from one dynode to the next, with the current being increased in each step by the secondary emission of electrons.

Working of Spectrophotometer :

The overall operation of spectrophotometer is very briefly explained. Keep the reference solution i.e. blank in the first cell and keep the analytical solution in second cell compart-ment. Then select a proper photocell (200-650 nm or 650-1100 nm) for covering required wavelength region. With photocell compartment closed, zero the galvonometer by means of dark current switch, select a proper wavelength, open photocell and keep reference blank in beam of light and zero the galvanometer by sensitivity knob and also slit. With transmittance dial set it to 100%. Then keep cuvette containing analytical solution in the path of beam of light and zero the galvanometer by setting of absorbance scale and read absorbance reading directly.

Preperation of the Chemical system :

The preparation of a suitable colored or absorbed system for spectrophotometric measurements requires considerable care. First, the resultant system should be stable and capable of being prepared in a reproducible manner. Second, the absorbance

of the system should be sensitive to small changes in concentration. For these conditions to be realized it is necessary to control solution variable such as pH, concentration of reagents and time and temperature required for development of maximum absorbance. The following suggestions should be helpful in preparing a sample for spectrophotometric measurement.

1 Know the selectivity and specificity of the reagents used for the preparation of a light absorptive system.

2 Determine the proper order of addition of reagents and allow time for completion of chemical reactions.

3 If the sample solution exibits an absorbance exceeding 0.8, use a smaller sample size.

4 If the solution exibits low absorbance reading less than 0.2, use a larger sample size.

5 Absorption cells should be scrupulously cleaned, matched optically, and properly positioned in the optical beam of the instrument.

6 Solution should be free of gas bubbles.

Measurement of the absorptive system :

After preperation of a suitable ultraviolet or visible light absorptive system, the next step in the general analytical procedure is to measure the absorptive capacity or

absorbance at specific wavelength. The selection of an appropriate wavelength for measurement should be based on a thorough knowledge of the chemical system being measured and the instrument being used. The avoidance of interferences due to another absorbing species at the sample absorbance maximum can be attained by selecting a wavelength where the interferer does not absorb, but the desired constituents has appreciable absorptivity.

Analytical applications of Spectrophotometry :

Among the various applications of spectrophotometry, is the determination of empirical formula of complexes formed by metal ions and ligands. There are three spectrophotometric methods used for the determination of the composition of complexes.

1. Mole-ratio method
2. Slope ratio metod and
3. Continuous variations method

Mole ratio method :

Consider the complex MxLy, which has a characteristics absorption maximum; the ligand to metal ratio can be determined spectrophotometrically by the mole ratio method provided that the complex has a sufficiently large formation constant K_f. In practical application, a series of solutions is prepared in which the metal ion concentration C_M is constant and the

concentration of the ligand CL is varied. Aplot of ligand to metal ratio, C_L/C_M, versus absorbance shows an intersecting of gradually increasing line and a virtually horizontal line indicating attainment of maximum absorbance of the complex. The intersection of these two lines is indicative of the composition of the complex.

Slope Ratio Method :

In this method two series of solutions are prepared. The first series contains a constant concentration of legand C_L which corresponds to a sufficient excess to minimize any appreciable dissotiation of the complex. Concentration of the metal ion C_M are varied so that the absorbance of the complex can be assumed to be propotional to the metal ion concentration C_M.

$$C_{Mx}Ly = C_M/x$$

A plot of absorbance Vs C_M is linear with slope equal to 1/x. A second series pf solutions is prepared containing a constant concentration of metal ion and various concentration of the ligand . The following relationship is obtained.

$$C_{Mx}Ly = C_L/y$$

The slope is obtained from a plot of the absorbance Vs C_L and equal to 1/y. The ratio of the slopes for the two plots is equal to the ligand to metal ratio y/x.

Continuous variations Method :

This method helps to determine the formula and formation constants of complex ions in solution. The method is based on plotting measured absorbances, corrected for absorbances of reactants assuming number complex ion versdus the mole fraction of either the ligand or metal. The method requires the preparation of a series of solutions in which the molar concentrations of metal and ligand are varied ,but their total molar concentration, $C_M + C_L$ remains constant. The absorbance Vs mole fraction plot gives a characteristics triangular plot. The mole fraction of the maximum of this plot, the apex of the triangle, indicates the composition of the complex.

Determination of P^k Values :

The P^k value of an acid base indicator can be determined spectrophotometrically. A series of solution of known pH containing a constant total concentration of indicator is prepared. These solutions should be of known and constant ionic strength. The absorbance is messured at the wavelength of the absorption maximum and a plot of abaorbance Vs. pH is obtained. The inflexion point of this point, (A max - A min)/2, corresponds to the $p^k \pm 2$ range of the indicator. The wavelength at which all solutions have an identical absorbance is designated on the "isobestic point". At this point, the absorbance for both molecular forms of the indicator is the same and only dependent on the concentration of the indicator.

Analysis of Metals :

The spectrophotometric method for the determination of metals is one of the most extensively employed analytical technique because it is sensitive, specific, rapid. and the instrumentation required is expensive. Organic reagents which form colored metal chelates are frequently used for metal analysis. Often the sensitivity of these spectrophotometric methods can be increased by extracting the metal chelate into a relatively small volume of an organic phase. Sensitivities, selectivities, and sample size for spectrophotometric methods vary according to the individual procedure and cannot be generally characterised.

Analysis of Non-metals :

Spectrophotometry in the determination of non-metals involves a variety of chemical reactions. Non-metalic elements such as phosphrous and silicon form yellow heteropoly complexes which can be measured photometrically, or these complexes can be reduced heteropoly blues which have unusually high absorptivity values.

Analysis of Organic Compounds :

Ultraviolet spectrophotometry has been applied extensively to the determination and identification of organic substances such as aromatic hydrocarbons, vitamins, steroids,

heterocyclic, and conjugated aliphatics. U.V. absorption spectra are used often for the identification of degradation products and to test for purity in biochemical and pharmaceutical research. In qualitative analysis the correlation of U.V. absorption bands with specific structures is made chiefly by analogy.

Ultraviolet absorption is used extensively in the determination of molecular structure. Some of the more comman applications include :

1) Determination of a particular group;
2) Determination of the position in the molecule of a particular group,
3) Studies of steric effects,
4) Determination of cis-trans isomers
5) Choice of correct structure among several possibilities and
6) Quantitative determination of tautomarization equilibra

There are some special spectrophotometric techniques such as :

1) Differential spectrophotometry
2) Multicomponent analysis
3) Indirect techniques and
4) Photometric titration