CHAPTER-I

Theory of Spectrophotometry

C H A P T E R - I

THEORY OF SPECTROPHOTOMETRY

Spectrophotometry is the Science which deals with the measurement of the relative capacity of Chemical systems to absorb incident radiant energy at specific wavelengths. The development of ultraviolet and visible absorption spectrometry as a discipline in modern analytical chemistry can be attributed primarily to the inherent utility of spectrophotometric methodology and the availability of reliable and inexpensive commercial spectrophotometers.

The colour is an important criteria for the identification of objects. The variation of the colour of a system component forms the basis of what the Chemist commonly terms 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 desired constituent itself. The intensity of the colour may then be compared with that obtained by treating a known amount of the substance in the same manner.

Colorimetry is concerned with the determination of the concentration of a substance by measurement 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 simple instrument termed a colorimeter or colour comparator. When the eye is replaced by a photoelectric cell, the instrument is termed a photoelectric 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.

The chief advantage of colorimetric and spectrophotometric methods is that they provide a simple means for determining minute quantities of substances. The upper limit of colorimetric methods is in general, the determination of constituents which are present in quantities of less than 1 or 2 percent.

In spectrochemical analysis we make use of spectrum of electromagnetic radiation to analyse chemical species and study their interaction 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 is 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 electromagnetic radiation is the linear distance traversed by one complete wave cycle. The frequency ν is the number of cycles occuring per second. The relationship between wavelength and frequency is -

$$\nu = c/\lambda \quad \text{where}$$

λ - is the wavelength in centimeters and

C - is the velocity of the radiant energy in centimeters per second.

The equivalent expression involving wavelength is

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

Visible light, a very small part of the electromagnetic spectrum, is generally concerned 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, 10-200 nm, and the near ultraviolet region which extends from 200-380 nm.

To state that a solution is colored 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 wavelengths 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, color is attributed to the selective absorption of incident radiant energy of certain wavelengths.

Absorption in the ultraviolet 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 quantity of energy, the probability of occurence for a particular transition is greatest when the photon absorbed supplies precisely this quantity of energy.

The energy requirements for these transitions vary considerably. 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, ultra-violet, 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 the energies required for the various processes responsible for absorption are unique for a given species, its absorption spectrum is also unique; as a consequence absorption spectra are often helpful for qualitative identification purposes.

Irrespective of the amount of energy absorbed, an excited species tends spontaneously to return to its unexcited or ground, state. To accomplish this, the energy of the 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 preceds return to the ground state.

Laws of Absorption :

When monochromatic light falls upon a homogeneous medium, a portion of the 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, that of the transmitted light byIt, and that of the reflected light by Ir, then :

$$Io = Ia + It + Ir$$

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

$$Io = Ia + It$$

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

Lambert's Law :

This law states that when monochromatic light passes through a transparent medium, the rate of decrease in intensity with the 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 differential equation :

$$-\frac{dI}{dt} = KI$$

where,

I is the intensity of the incident light of wavelength

t is the thickness of the medium, and K is a proportionality factor.

Integrating the above equation and putting

$$I = I_o \text{ when } t = o$$

$$\ln = \frac{I_o}{I_t} = Kt$$

or in other terms $I_t = I_o\, e^{-Kt}$

Where I_o is the intensity of the incident light falling upon an absorbing medium of thickness t, I_t is the intensity of the transmitted light, and K is a constant, the absorption coefficient for the wavelength and the absorbing medium used. By changing from natural to Briggsian logarithms we obtain :

$$I_t = I_o.10^{-0.4343\,kt} = I_o.10^{-Kt}$$

where $K = k/2.3026$ and is usually termed the extinction coefficient. The extinction coefficient is generally defined as the reciprocal of the thickness (t cm). required to reduce the light to $\frac{1}{10}$ of its intensity.

$$\frac{I_t}{I_o} = 0.1 = 10^{-Kt} \quad \text{or} \quad Kt = 1 \text{ and } K = 1/t$$

The ratio I_t/I_o is the fraction of the incident light transmitted by a thickness t of the medium, and is termed the transmission and transmittance I. 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 :

$D = \log I_o/I_t$.

Thus a 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 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 :

$$I_t = I_o \cdot e^{K'C}$$
$$= I_o \cdot 10^{-0.4343K'C}$$
$$= I_o \cdot 10^{-K'C}$$

Where,

C is the concentration ,and K' and K are constants.

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

$$I_t = I_o \cdot 10^{-\epsilon Ct}$$

or

$$\log \frac{I_o}{I_t} = \epsilon Ct$$

This is the fundamental equation of colorimetry and spectrophotometry, and is often spoken of as the " Beer-Lambert Law." The value of ϵ will clearly depend upon the method of expression of the concentration. If C is expressed in grams mols per litre and t in centimeters then ϵ is the molecular extinction coefficient (also termed molar absorptivity or molar absorbancy index). The later is equal to the reciprocal value of the thickness in centimeters of a 1 molar solution (C = 1) at which,

$$I_t = 0.1 \times I_o$$

since $I_t = I_o . 10^{-\epsilon}$ 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 concentration.

Where the molecular weight of a substance is not definitely known, it is obviously not possible to write down the molecular extinction 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.

$$D = E = A = \log (I_o/I_t) \text{ or } I_t = I_o \times 10^{-D}$$

D is obviously related in a simple way to the molecular extinction coefficient , since : $D = \epsilon ct$.

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 1g.mol per litre.

The transmission T is defined as the ratio of the intensity of the transmitted to that of the incident light thus $T = I_t/I_o$.

The term transmittancy T_s, 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 measurements I_o 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,

$$K = D/t \quad \text{or} \quad I_t = I_o.10^{Kt}$$

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

$$E_s = D/cl \qquad \text{or} \qquad I_t = I_o.10^{-Escl}$$

the molar extinction coefficient is the specific extinction

coefficient for a concentration of 1g mol per litre and a path length of 1 cm.

$$\epsilon = D/Ct \qquad (1.21)$$

Limitations to the Applicability of Beer's Law :

The linear relationship between absorbance and path length at a fixed concentration of absorbing substance is a generalization for which no exceptions have been found. On the other hand, deviations from the direct proportionality between measured absorbance and concentration are quite frequently encountered. Some of these deviations are of such a fundamental nature that they represent a real limitations of the law; others however occur as consequence of the manner in which the absorbance measurements are made or as a result of chemical changes associated with concentration changes; the latter two are sometimes known, respectively as instrumental and chemical deviations.

Real Limitations to Beer's Law :

Beer's law is successful in describing the absorption behaviour of dilute solutions only, in this sense it is a limiting law. At high concentrations the average distance between the solute molecules is diminished to the point where each affects the charge distribution of its neighbors. This interaction in turn, can alter their ability to absorb a given wavelength of radiation. Because the degree of interaction is dependent upon concentration, the occurence of this phenomenon causes deviations from the linear relationship between absorbance and concentration.

A second fundamental limitation arises from alterations in the refractive index of the solution as a result of high solute concentrations.

Chemical Deviations :

Apparant deviations from Beer's law are frequently encountered as a consequence of association, dissociation,or reaction of the absorbing species with the solvent. Deviations in the absorbance of this system from Beer's law are more apparent than real,because they result from the shifts, in chemical equilibria.

Instrumental Deviations :

Strict adherence of an absorbing system to Beer's law is observed only when monochromatic radiation is employed. Use of a monochromatic beam for absorbance measurements is seldom practical, however, and polychromatic radiation may lead to departures from Beer's 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 caligraph curve should cover the dilution range likely to be met with in the actual comparison.

Whether or not Beer's law is obeyed, Ringbom showed that the ratio between relative error in concentration $\triangle C/C$ and photometric error $\triangle P$ can be visualized by constructing a plot of absorbance (= 1 - transmittance) or % absorbance (= 100 - % T)against the logarithm of the concentration of the absorbing substance,such a plot is known as a Ringbom plot.

Such a plot has a sigmoid shape with a virtually linear segment at intermediate absorbance or concentration values. This segment, which is also the steepest portion of the plot, represents the optimum range of concentrations.

When Beer's law is obeyed,

$$\frac{\Delta C/C}{\Delta P} = -\frac{2.30}{P / \Delta \log C}$$

where $\Delta P / \Delta \log C$ is the slope of the Ringbom plot.

For a photometric error of 1 % (P = 0.01),

$$100 \times \left(\frac{\Delta C}{C}\right) = \frac{230}{\text{slope}} = \frac{\text{\% relative error}}{\text{1 \% photometric error}}$$

When Beer's law is not obeyed, the Ringbom plot will be the general shape, but it will have a different slope and the optimum range corresponding to its intermediate linear portion may be quite different from that for the case where Beer's law is obeyed.

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 spectrophotometers 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 photoelectric 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 a 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.

Sources :

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 spectrophotometer, 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 harrow 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 adjecent 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 :

$$R = t \left(\partial n / \partial \lambda \right) \geq \lambda / \Delta\lambda$$

For a grating, the resolving power depends on the number of rulings N and the order of the spectrum m. R = m N. 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 wavelength 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 1cm 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 photosensitive detectors commonly used :

1 Barrier layer cells

2 Photoemissive cells

and

3 Electron multiplier phototubes

The barrier or photovoltalic 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 succesively higher potential, and the photoelectrons emitted by the first photocathode 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 compartment. Then select a proper photocell (200-650 nm or 650-1100 nm) for covering required wavelength region. With photocell compartment closed, zero the galvondmeter 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%. Thenkeep 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.

Preparation of the Chemical system :

The preparation of a suitable colored or absorption 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 exhibits an absorbance exceeding 0.8, use a smaller sample size.

4 If the solution exhibits low absorbance readings 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 preparation 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 appreeiable 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 method and
3. Continuous variations method

Mole ratio method :

Consider the complex MxLy, which has a characteristic 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 Kf. 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. A plot 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 ligand C_L which corresponds to a sufficient excess to minimize any appreciable dissociation of the complex. Concentration of the metal ion C_M are varied so that the absorbance of the complex can be assumed to be proportional to the metal ion concentration C_M.

$$C_{M_x}L_y = C_M / \mathfrak{a}$$

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

$$C_{M_x}L_y = C_L/y$$

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 formulas and formation constants of complex ions in solution. The method is based on plotting measured absorbances, corrected for absorbances of reactants assuming no complexation Vs. 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 characteristic 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 measured at the wavelength of the absorption maximum and a plot of absorbance Vs. pH is obtained. The inflection point of this point, (A max - Amin)/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.

Analyais of Non-Metals :

Spectrophotometry in the determination of non-metals involves a variety of chemical reactions. Non-metallic elements such as phosphorus and silicon form yellow heteropoly complexes which can be measured photometrically, or these complexes can be reduced to produce heteropoly blues which have unusally 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, heterocyclics,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 structures. Some of the more common 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 equilibria

There are some special spectrophotometric techniques such as :

1) Differential spectrophotometry
2) Multicomponent Analysis.
3) Indirect Techniques and
4) Photometric Titration