CHAPTER II

THEORY

Ultraviolet spectroscopy is an widely used technique for the qualitative and quantitative analysis of organic and inorganic systems. This technique is based on the measurement of absorption or emission of electromagnetic radiation. The absorption or emission process is a result of an electronic rearrangement in atoms or molecules. In absorption process the atom or molecule is first in a ground electronic state, on absorbing the incident radiation, it rises to a higher energy electronic excited state. In emission process, a molecule already in an excited state reverts to the ground state by emission of energy in form of electromagnetic radiation. Only the absorption process will be considered in this thesis. The energy absorbed is proportional to the frequency of the electromagnetic radiation, it obeys the Planck condition.

$$E = h\nu \qquad (1)$$

Where E is the energy absorbed in an electronic transition in a molecule from a low-energy state (ground state) to a high energy state (excited state), h is Planck's constant (6.626×10^{-34} Joule.sec), and ν is the frequency of the radiation in sec^{-1} (Hertz).

The frequency of radiation is seldom measured by the relationship between the frequency (ν), wavelength (λ) and the speed of light (C).

$$\lambda = \frac{C}{\nu} \qquad (2)$$

Combining the eq.(1) and (2)

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

Changes in the electron configuration and energy of molecules produce spectra in ultraviolet region of the electromagnetic spectrum. The ultraviolet region is indicated in Figure 1. Wavelengths in the ultraviolet region are usually expressed in nanometers (1 nm = 10^{-9}m) or angstorms, Å(1Å = 10^{-10}m). In the near ultraviolet (quartz)

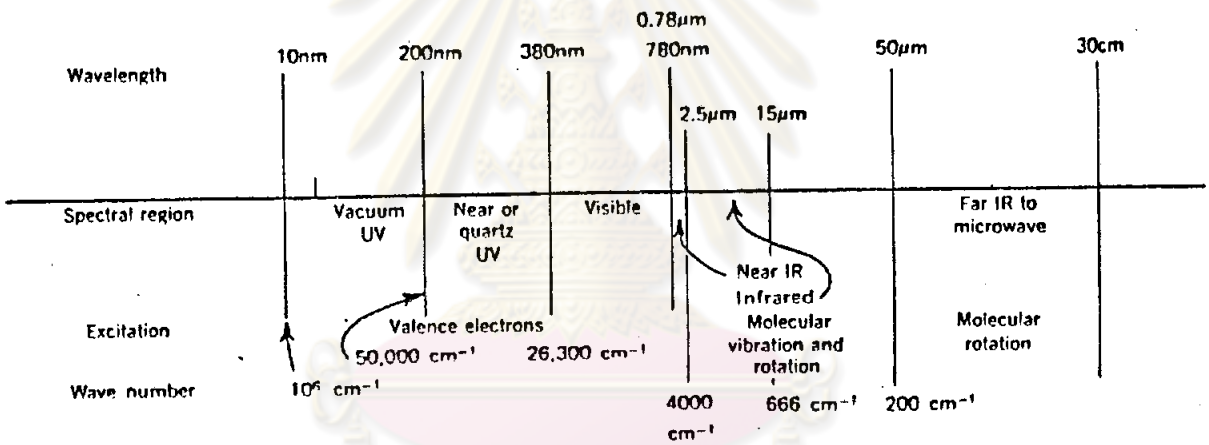

Figure 1. Electromagnetic spectrum (58)

region extending from 200 to 380 nm, the atmosphere is transparent in this region which is readily accessible with quartz optics. Atmospheric absorption starts near 200 nm and extends into the shorter wavelength region which is accessible through vacuum ultraviolet spectroscopy.

An absorption spectrum is obtained by the spectroscopic analysis of the light transmitted by an absorbing medium. An ultraviolet spectrum is a plot of the wavelength or frequency of absorption versus the

absorption intensity (transmittance or absorbance), as shown in Figure 2. Absorption measurements involve in the determination of the reduction in power suffered by a beam of radiation as a consequence of passing through the absorbing medium. The fundamental laws governing the absorption intensity are Lambert's law, Beer's law or Lambert-Beer law.

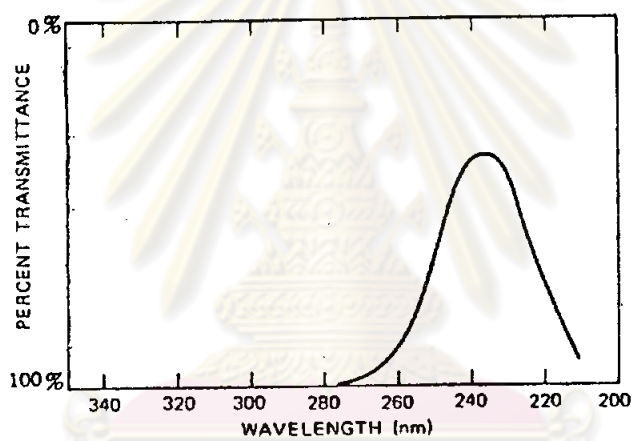

Figure 2. Ultraviolet spectrum of mesityl oxide in 95 % ethanol, concentration of 6.29×10^{-5} mole per liter (58)

2.1 Lambert's law

When monochromatic light (i.e. light of a single wavelength) 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. Any thickness of medium absorbs the same fraction of the light incident on it. Expressed mathematically (59).

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

Where I = intensity of the incident light of wavelength

t = thickness of the layer

k = constant

Integrating (I=Io when t=ó) above equation gives

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

or $$I_t = I_o e^{-kt}$$

Where I_t = intensity of the transmitted light

I_o = intensity of the incident light

k = constant (absorption co-efficient)

Changing from natural logarithms

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

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

Where $K = k/2.3036$ (K : extinction coefficient)

The extinction coefficient is generally defined as the reciprocal of the thickness (t cm.) required to reduce light to 1/10 of its intensity.

I_t/I_o is the fraction of incident light transmitted by thickness t, and is usually termed transmission or transmittance, (T).

$$T = \frac{I_t}{I_o} \qquad (3)$$

2.2 Beer's law

Beer studied the effect of concentration of a compound in solution upon the intensity of the light. He found the same relation between concentration and transmittance as Lambert had discovered for thickness of the layer and transmittance, defined by (59)

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

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

where c = concentration of solute

Combining the two laws :

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

or $$\log \frac{I_o}{I_t} = \epsilon ct \qquad (4)$$

where ϵ = molecular extinction coefficient or molar absorptivity.

The value of ϵ will depend on the method of expressing concentration.

If c = gram moles per liter

and t = centimeters

From (3), (4)

$$\log \frac{1}{T} = \epsilon c t$$

$$-\log T = \epsilon c t$$

$$A = \epsilon c t$$

or $$A = \epsilon c b \qquad (5)$$

where A = Absorbance = - log T

b = path length through the sample (centimeters)

This is usually refered to as the Lambert - Beer law, Bouguer-Beer law or most commonly, as Beer's law.

Equation 5 shows that the absorbance of a solution is directly proportional to the concentration of absorbing species when the length of light path is fixed, or is directly proportional to the light path when the concentration is fixed; a quantitative analysis based on the absorption of radiation makes use of one or the other of these relationships.

Beer's law applies to a solution containing more than one kind of absorbing substance, provide there is no interaction among the various species. Thus for a multicomponent system, defined by

$$A_{total} = \epsilon_1 bc_1 + \epsilon_2 bc_2 + \epsilon_3 bc_3 \ldots\ldots\ldots$$

From equation 5, if the concentration of solute (c) is defined as g/liter, the equation becomes :

$$A = abc$$

where a is the aborptivity of the component of interest in solution. The absorptivity is a constant dependent on the wavelength of the radiation and the nature of absorbing material. The absorptivity is related to the molar absorptivity by

$$\epsilon = aM$$

where M = molecular weight of the solute

The intensity of an absorption band in the ultraviolet spectrum is usually expressed as the molar absorptivity at maximum absorption ϵ_{max} or log ϵ_{max}. If the molecular weight of the substance is not known, the expression $E_1^{1\%}$ cm is often used. This is related to ϵ by the equation

$$E_{1\ cm}^{1\ \%} = \frac{\epsilon \times 10}{\text{Molecular weight}}$$

Thus, when the constitution of an absorbing material is unknown, the intensity of absorption may be expressed as

$$E_{1\ cm}^{1\ \%} = \frac{A}{cb}$$

where c = concentration in grams per 100 milliliter

A plot absorbance versus concentration will be a straigh line passing through the origin as shown in Figure 3. This is much more convenient than the relationship between transmittance and concentration. For this reason readout meters on spectrophotometers are also calibrated to read absorbance, although the instrument actually measures the light that is transmitted.

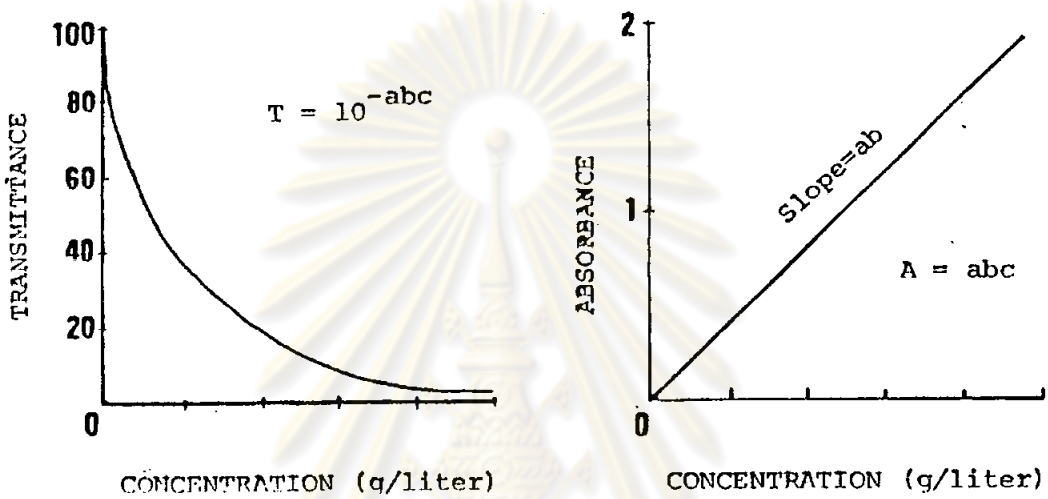

Figure 3. Representation of Beer's law and comparison between scale in absorbance and transmittance (60)

2.3 Sources of Error

In most analyses where the absorption band is completely resolved, there will be a linear relationship between the measured absorbance and the concentration. In analyses where the absorption band is not completely resolved, or where the state of the absorbing component changes with concentration, the relationship between absorbance and concentration may be nonlinear. Such a curve is still useful in quantitative analysis, but the concentration must be read from a standard curve that must be verified at frequent intervals.

Beer's law is successful in describing the absorption behavior of dilute solution only. At high concentrations, the average distance between solute molecules (or ions) is dimished because each molecule affects the charge distribution of its neighbors. This interaction can turn alter their ability to absorb the radiation. Because the degree of interaction is dependent upon the concentration, thus it causes deviation from the relationship between absorbance and concentration.

Deviations from Beer's law also because ϵ is dependent upon the refractive index of the solution. Thus, concentration changes cause alterations in the refractive index (n). A correction for this effect can be made by substitution of the quantity $\epsilon n/(n^2 + 2)^2$ for ϵ in Equation 5. This correction is not significant at concentrations less than 10^{-3} M.

When the absorbing solute dissociates or associates in solution or reacts with solvent, discrepanices are usually found, because the nature of the species in solution will vary with the concentration. Indicator systems of weak acids or bases are observed with unbuffer potassium dichromate solution which the dichromate ion and the two chromate species involve.

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

The absorbance due to these ions remains directly proportional to it molar concentration. Thus deviations in the absorbance are more apparent than real because they result from shifts in chemical equilibria. These deviations can be predicted from the equilibrium constant for the reactions and the molar absorptivities of the ions.

In the deviation of Beer's law the use of a beam of monochromatic radiation is implied. The wider the bandwidth of radiation passes by dispensing device, the greater will be the apparent deviation from adherence to Beer's law. On absorbance versus concentration plot, the deviation becomes apparent at higher concentrations when the curve bends towards the concentration axis. This departure arises because in all photometers it is the radiant power of the component wavelengths which are additive.

Temperature often shifts ionic equilibria and, in addition, an increase in temperature exerts a bathochromic effect on ions in solution; i.e., the absorption bands are shifted to longer wavelengths.

2.4 Instrumentation

Instruments employed for measuring the transmittance or absorbance of solutions consist of five sections or areas : (1) radiation source, (2) monochromator, (3) photometer, (4) sample area, and (5) detector area. The optical layout of a typical double-beam instrument is presented in Figure 4.

2.4.1 Radiation Source : There is a source of continuous radiation. The radiation source for the ultraviolet region of the spectrum is a hydrogen discharge tube. The hydrogen discharge tube can be replaced by a tangsten incandescent lamp when absorption in the visible region is to be determined. The hydrogen discharge tube consists of a pair of electrodes in a glass envelope with a quartz or fused silica window. This tube contains hydrogen or deuterium at a reduced pressure.

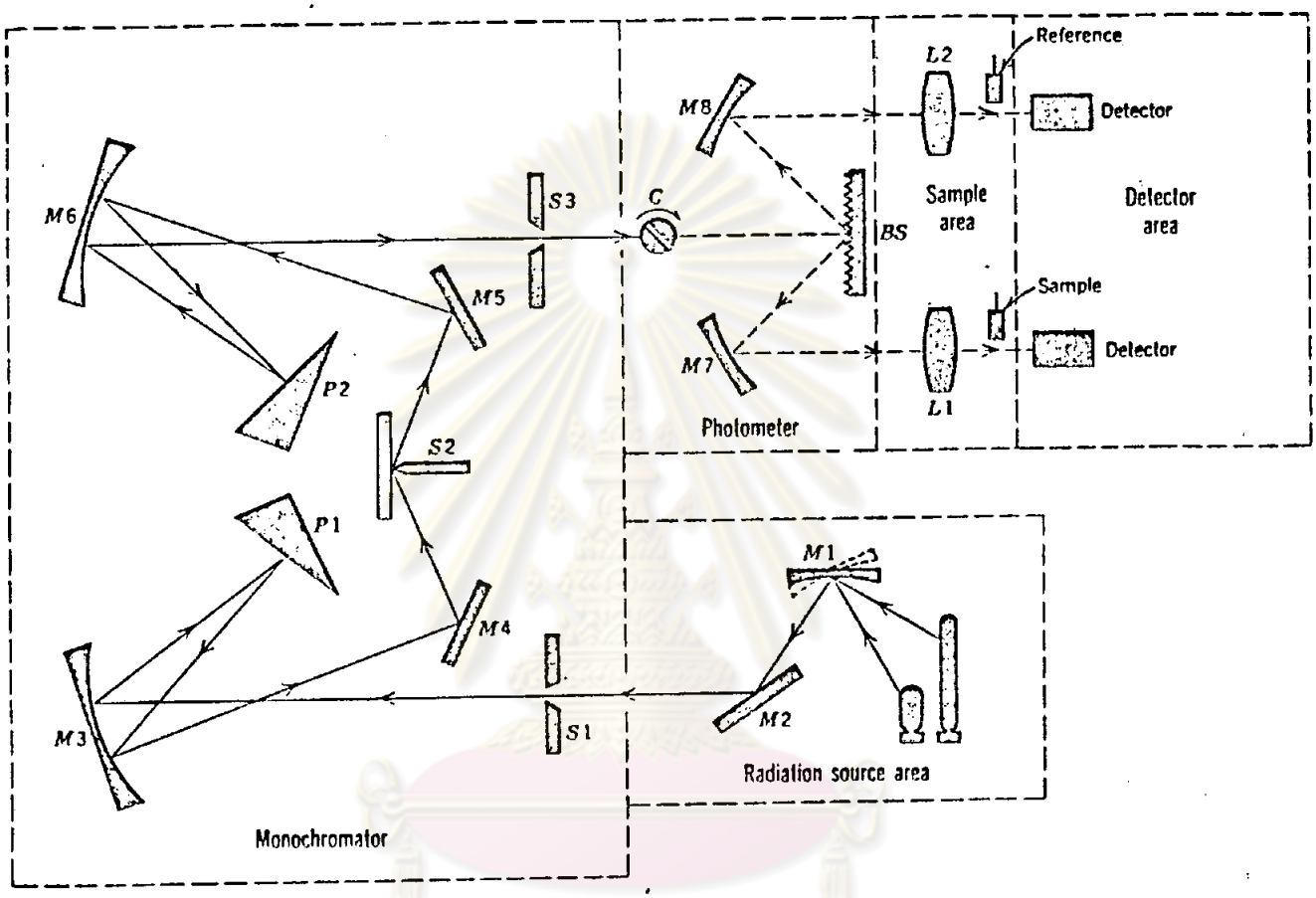

Figure 4. Optical layout of a double-beam ultraviolet spectrophotometer (58)

M : Mirror

S : Slit

P : Prism

C : Chopper

BS : Beam splitter

L : Lense

2.4.2 Monochromator : is device that isolate a beam of high spectral purity. Monochromator selects a certain part of the continuous radiation to be passed through the sample.

2.4.3 Photometer : the monochromatic light is pulsed by chopper and split into sample and reference beams by beam splitter. The sample and reference beams are reflected to the sample area. Optics for the transmission of ultraviolet radiation are made of quartz.

2.4.4 Sample area : the beams entering the sample area become more concentrated by lense. They pass through the area toward the detectors. Cells (or cuvettes) are usually placed in the region nearest the detector area. Quartz or fused silica cells are required for work in the Ultraviolet region.

2.4.5 Detector area : The radiation beams that pass into the detector area are focused on separate photomultiplier tubes that generate a voltage proportional to the energy that strikes the detectors. The voltage can be amplified and the amplified current is read on a meter. Many instruments have scales reading directly in absorbance.

2.5 Quantitative Absorption Spectroscopy

The basic principle of most quantitative absorption methods consists in comparing the absorbances of a sample and a standard solution at a suitable wavelength, usually maximum wavelength (λ max.), selected from the absorption curve.

In a spectrophotometric analysis, absorbance measurements are made at a wavelength corresponding to an absorption peak. At this wavelength (λ max.), the change in absorbance per unit of concentration

is greatest and the maximum sensitivity is realized. In addition, the absorption curve is often flat in this region ; thus, good adherence to Beer's law is to be expected and measurement will be less sensitive to uncertainties arising from failure to reproduce precisely the wavelength setting of the instrument. In order to avoid interference from other absorbing substances, a wavelength other than a peak may be appropriate for analysis. In this event, the region selected should be one in which the change in absorptivity with wavelength is not too great.

Common variables that influence the absorption of a substance include the nature of the solvent, the temperature, the pH of the solution, and the presence of interfering substances. These variables must be known and a set of analytical conditions chosen will not be influenced by uncontrolled variables.

The compound should be dissolved in some suitable that does not itself absorb light in the region under investigation. The most commonly used solvent for ultraviolet spectral determinations is 95 % ethyl alcohol. Water and hexane are also commonly used. The positions of the absorption peaks of a compound may be shifted if different solvents are used.

The solution must be placed in some suitable container that is transparent to light in the region being studied. Although ordinary glass is satisfactory for work in the visible region, glass absorbs ultraviolet light strongly ; hence, quartz cells must be used. The most commonly used cells have 1.0 cm path length.

Having decided upon a set of conditions for the analysis, it is necessary to prepare a calibration curve from a series of standard solutions. These standards should approximate the overall composition of the samples and should cover a range of concentration with respect to the species being determined. Seldom, it is safe to assume adherence Beer's law. Concentration of a sample is defined by comparing the absorbance of the sample and the standard solutions at calibration curve.