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# UV-VISIBLE SPECTROSCOPY- A REVIEW

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## ABSTRACT

Ultraviolet and visible spectrometers have been in general use for the last 35 years and over this period have become the most important analytical instrument in the modern day laboratory.. It is intended purely as a brief introduction to the technique and it is Thermo Spectronic's policy to continually add to this range of documentation for further details, as they become available.Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The pharmaceutical analysis comprises the procedures necessary to determine the “identity, strength, quality and purity” of such compounds. It also includes the analysis of raw material and intermediates during manufacturing process of drugs. It is well known that the dissociation constant is most important parameter in development and optimization of new compound for effective formulation development.

## INTRODUCTION

- **Ultra Violet Spectroscopy :-**

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near infrared ranges. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration.[1] Ultraviolet and visible spectrometers have been in general use for the last 35 years and over this period have become the most important analytical instrument in the modern day laboratory. In many applications other techniques could be employed but none rival UV-Visible spectrometry for its simplicity, versatility, speed, accuracy and cost-effectiveness. This description outlines the basic principles for those new to UV-Visible spectrometry. It is intended purely as a brief introduction to the technique and it is Thermo Spectronic's policy to continually add to this range of documentation for further details, as they become available.[2]

**Principle:-**

A molecule or ion will exhibit absorption in the visible or ultraviolet region when radiation causes an electronic transition within its structure. Thus, the absorption of light by a sample in the ultraviolet or visible region is accompanied by a change in the electronic state of the molecules in the sample. The energy supplied by the light will promote electrons from their ground state orbitals to higher energy, excited state orbitals or antibonding orbitals.

Potentially, three types of ground state orbitals may be involved:

1. σ (bonding) molecular
2. π (bonding) molecular orbital
3. n (non-bonding) atomic orbital

In addition, two types of antibonding orbitals may be involved in the transition:

i) σ* (sigma star) orbital

ii) π* (pi star) orbital

(There is no such thing as an n* antibonding orbital as the n electrons do not form bonds).

A transition in which a bonding s electron is excited to an antibonding σ orbital is referred to as σ to σ* transition. In the same way π to π* represents the transition of one electron of a lone pair (non-bonding electron pair) to an antibonding π orbital. Thus the following electronic transitions can occur by the absorption of ultraviolet and visible light:

σ to σ*,

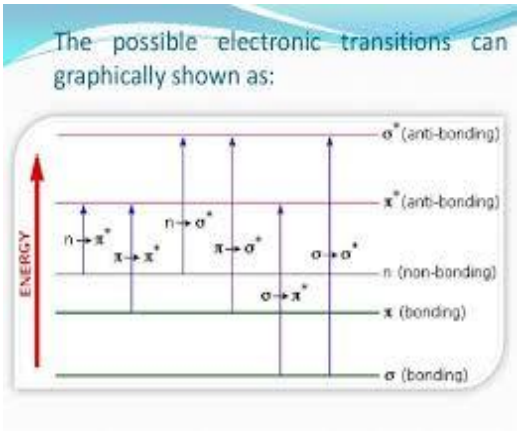
n to σ*

n to π*

π to π*.

Both s to σ* and n to σ* transitions require a great deal of energy and therefore occur in the far ultraviolet region or weakly in the region 180-240nm. Consequently, saturated groups do not exhibit strong absorption in the ordinary ultraviolet region. Transitions of the n to π* and π to π* type occur in molecules with unsaturated centers; they require less energy and occur at longer wavelengths than transitions to σ* antibonding orbitals.

It will be seen presently that the wavelength of maximum absorption and the intensity of absorption are determined by molecular structure. Transitions to π* antibonding orbitals which occur in the ultraviolet region for a particular molecule may well take place in the visible region if the molecular structure is modified. Many inorganic compounds in solution also show absorption in the visible region. These include salts of elements with incomplete inner electron shells (mainly transition metals) whose ions are complexed by hydration e.g. [Cu(H204)]2+. Such absorptions arise from a charge transfer process, where electrons are moved from one part of the system to another by the energy provided by the visible light.[3]




**Ultraviolet Absorption Spectrophotometry:-**

Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The photometric methods of analysis are based on the Bouger-Lambert-Beer's law, which establishes that the absorbance of a solution is directly proportional to the concentration of the analyte. The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval

of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer. Ultraviolet-visible spectroscopy is used to obtain the absorbance spectra of a compound in solution or as a solid. What is actually being observed spectroscopically is the absorbance of light energy or electromagnetic radiation, which excites electrons from the ground state to the first singlet excited state of the compound or material. The UV-visible region of energy for the electromagnetic spectrum covers 1.5 - 6.2 eV which relates to a wavelength range of 800 - 200 nm.

[4,5] The Beer-Lambert Law (Equation1) is the principle behind absorbance spectroscopy. For a single wavelength, A is absorbance (unit less, usually seen as arb. units or arbitrary units), is the molar absorptivity of the compound or molecule in solution (M-1cm-1), b is the path length of the cuvette or sample holder (usually 1 cm), and c is the concentration of the solution (M).

$$\mathbf{A = a\ b\ c}$$

Where, A = Absorbance,

a = absorptivity,

b = path length,

c = concentration.

$$\mathbf{C = A\ /a\ b}$$

There are three types of absorbance instruments used to collect UV-Visible spectra:

1. Single beam spectrometer.
2. Double beam spectrometer.
3. Simultaneous spectrometer.

All of these instruments have a light source (usually a deuterium or tungsten lamp), a sample holder and a detector, but some have a filter for selecting one wavelength at a time. The single beam instrument (Figure 2) has a filter or a monochromator between the source and the sample to analyze one wavelength at a time. The double beam instrument (Figure 3) has a single source and a monochromator and then there is a splitter and a series of mirrors to get the beam to a reference sample and the sample to be analyzed, this allows for more accurate monochromator between the sample and the source; instead, it has a diode array detector that allows the instrument to simultaneously detect the absorbance at all wavelengths. The simultaneous instrument is usually much faster and more efficient, but all of these types of spectrometers work well (Figure4).

**Figure 2. Illustration of a single beam UV-Visible instrument**

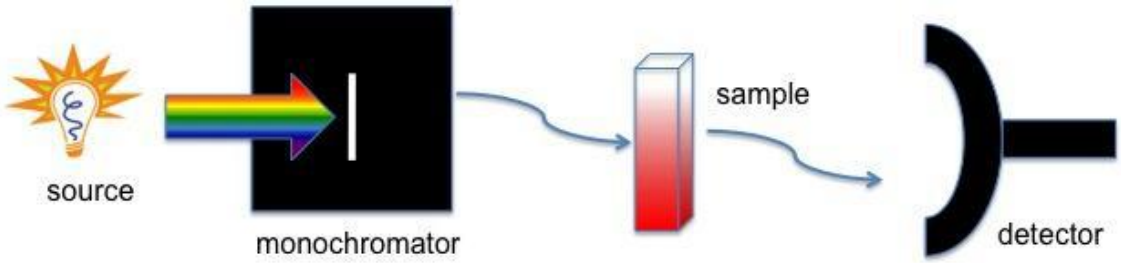



**Figure 3. Illustration of a double beam UV-Visible instrument**

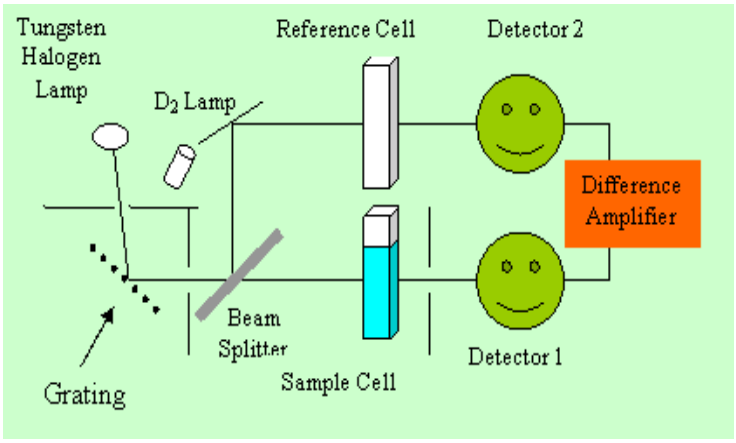
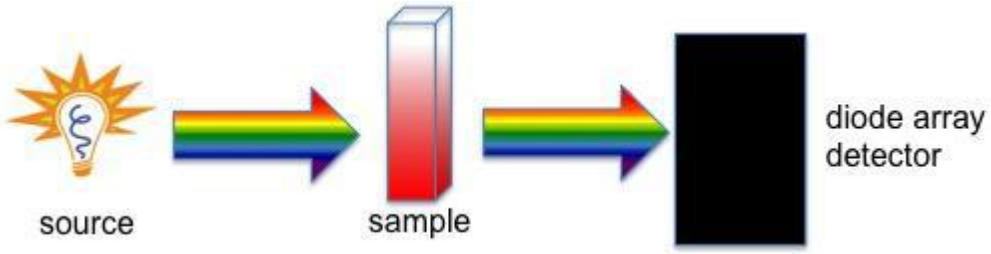
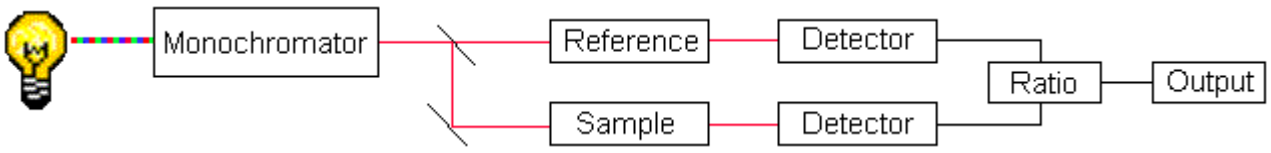



**Figure 4. Illustration of a simultaneous UV-Visible instrument**




**Instrumentation:-**

Have a look at this schematic diagram of a double-beam UV-Vis. spectrophotometer;



Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;

1. Sources (UV and visible)
2. Wavelength selector (monochromator)
3. Sample containers
4. Detector
5. Signal processor and readout

Each of these components will be considered in turn.

**Instrumental components**

- **Sources of UV radiation**

It is important that the power of the radiation source does not change abruptly over it's wavelength range.

The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon. This can be shown as;

$D_2$ + electrical energy □ $D_2^*$ □ D' + D'' + $h\nu$

Both deuterium and hydrogen lamps emit radiation in the range 160 - 375 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 350 nm.

- **Sources of visible radiation**

The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of 350 - 2500 nm. The energy emitted by a tungsten filament lamp is proportional to the fourth power of the operating voltage. This means that for the energy output to be stable, the voltage to the lamp must be *very* stable indeed. Electronic voltage regulators or constant-voltage transformers are used to ensure this stability.

Tungsten/halogen lamps contain a small amount of iodine in a quartz "envelope" which also contains the tungsten filament. The iodine reacts with gaseous tungsten, formed by sublimation, producing the volatile compound $WI_2$. When molecules of $WI_2$ hit the filament they decompose, redepositing tungsten back on the filament. The lifetime of a tungsten/halogen lamp is approximately double that of an ordinary tungsten filament lamp. Tungsten/halogen lamps are very efficient, and their output extends well into the ultra-violet. They are used in many modern spectrophotometers.

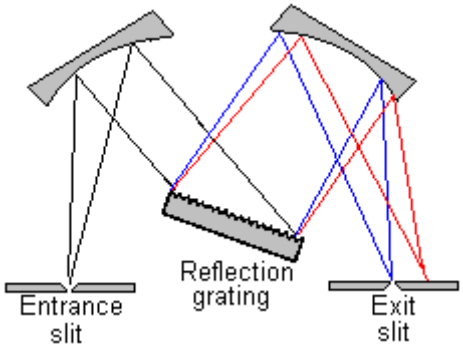
- **Wavelength selector (monochromator)**

All monochromators contain the following component parts;

- An entrance slit
- A collimating lens
- A dispersing device (usually a prism or a grating)
- A focusing lens
- An exit slit

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

**Czerney-Turner grating monochromator**




**Cuvettes**

The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.

**Detectors**

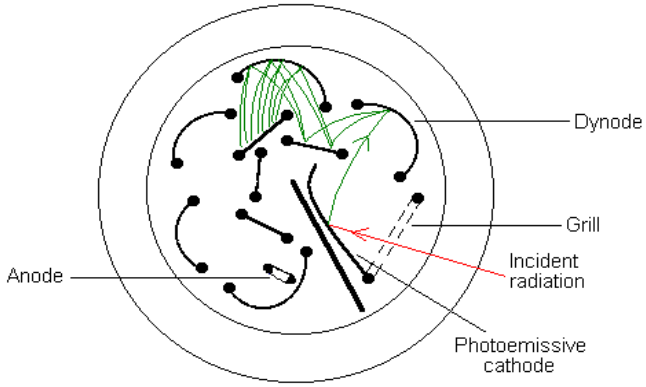
**The photomultiplier tube** is a commonly used detector in UV-Vis spectroscopy. It consists of a *photoemissive cathode* (a cathode which emits electrons when struck by photons of radiation), several *dynodes* (which emit several electrons for each electron striking them) and an *anode*.

A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first dynode (which is 90V more positive than the cathode). The electrons strike the first dynode, causing the emission of

several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. Eventually, the electrons are collected at the anode. By this time, each original photon has produced $10^6$ - $10^7$ electrons. The resulting current is amplified and measured.

Photomultipliers are very sensitive to UV and visible radiation. They have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation.

**Cross section of a photomultiplier tube**

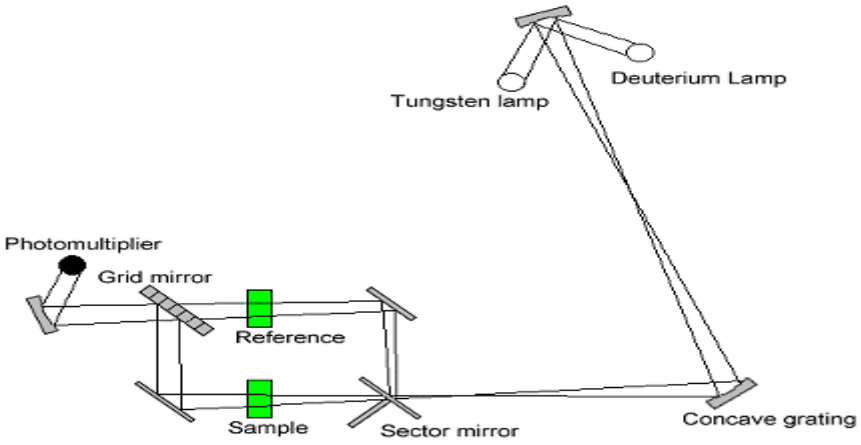



**The linear photodiode array** is an example of a *multichannel photon detector*. These detectors are capable of measuring all elements of a beam of dispersed radiation simultaneously.

A linear photodiode array comprises many small silicon photodiodes formed on a single silicon chip. There can be between 64 to 4096 sensor elements on a chip, the most common being 1024 photodiodes. For each diode, there is also a storage capacitor and a switch. The individual diode-capacitor circuits can be sequentially scanned.

In use, the photodiode array is positioned at the focal plane of the monochromator (after the dispersing element) such that the spectrum falls on the diode array. They are useful for recording UV-Vis. absorption spectra of samples that are rapidly passing through a sample flow cell, such as in an HPLC detector.

**Charge-Coupled Devices (CCDs)** are similar to diode array detectors, but instead of diodes, they consist of an array of photocapacitors.[6]




- **General Chemical Origins:-**

When white light falls upon a sample, the light may be totally reflected, in which case the substance appears white or the light may be totally absorbed, in which case the substance will appear black. If, however, only a portion of the light is absorbed and the balance is reflected, the color of the sample is determined by the reflected light. Thus, if violet is absorbed, the sample appears yellow-green and if yellow is absorbed, the sample appears blue. The colors are described as complementary. However, many substances which appear colorless do have absorption spectra. In this instance, the absorption will take place in the infra-red or ultraviolet and not in the visible region. A close relationship exists between the color of a substance and its electronic structure. **[7]**

**Table 1 : illustrates the relationship between light absorption and color.**

**Color absorbed Color observed Absorbed radiation (nm):-**

| Color Absorbed | Color Absorbed | Absorbed Radiation(nm) |
|---|---|---|
| Violet | Yellow-green | 400-435 |
| Blue | Yellow | 435-480 |
| Green-blue | Orange | 480-490 |
| Blue-green | Red | 490-500 |
| Green | Purple | 500-560 |
| Yellow-green | Violet | 560-580 |
| Yellow | Blue | 580-595 |
| Orange | Green-blue | 595-605 |
| Red | Blue-green | 605-750 |

***Correlation of Molecular Structure and Spectra Conjugation:-***

π to π * transitions, when occurring in isolated groups in a molecule, give rise to absorptions of fairly low intensity. However, conjugation of unsaturated groups in a molecule produces a remarkable effect upon the absorption spectrum. The wavelength of maximum absorption moves to a longer wavelength and the absorption intensity may often increase. The same effect occurs when groups containing n electrons are conjugated with a π electron group.

In general, the greater the length of a conjugated system in a molecule, the nearer the λmax comes to the visible region. Thus, the characteristic energy of a transition and hence the wavelength of absorption is a property of a group of atoms rather than the electrons themselves. When such absorption occurs, two types of groups can influence the resulting absorption spectrum of the molecule: chromophores and auxochromes. [8]

- **Chromophores:-**

Many organic molecules absorb ultraviolet/visible radiation and this is usually because of the presence of a particular functional group. The groups that actually absorb the radiation are called chromophores. Mathematical treatments of the energy levels of orbital systems suggest that some electronic transitions are statistically probable (said to be 'allowed', and these absorptions are strong, and tend to have ε values in excess of 10 000). Other transitions have a probability of zero – they are not expected to occur at all – and are said to be 'forbidden' but they frequently do occur, to give weak bands with ε values that rarely exceed 1 000. Some particularly useful forbidden transitions are: d→d absorptions of transition metals; the n→π * absorption of carbonyl groups at ca 280 nm; and the π→π* absorption of aromatic compounds at ca 230–330 nm, depending on the substituents on the benzene ring. [9]

- ***Auxochromes:-***

The color of a molecule may be intensified by groups called auxochromes which generally do not absorb significantly in the 200-800nm region, but will affect the spectrum of the chromophore to which it is attached. The most important auxochromic groups are OH, NH2, CH3 and NO2 and their properties are acidic (phenolic) or basic. The actual effect of an auxochrome on a chromophore depends on the polarity of the auxochrome, e.g. groups like CH3-, In general it should be possible to predict the effect of non-polar or weakly polar auxochromes, but the effect of strongly polar auxochromes is difficult to predict. In addition, the availability of non-bonding electrons which may enter into transitions also contributes greatly to the effect of an auxochrome. CH3CH2- and Cl- have very little effect, usually a

small red shift of 5-10nm. Other groups such as -NH2 and -NO2 are very popular and completely alter the spectra of chromophores.

***Solvents :-***

The effect on the absorption spectrum of a compound when diluted in a solvent will vary depending on the chemical structures involved. Generally speaking, non-polar solvents and non-polar molecules show least effect. However, polar molecules exhibit quite dramatic differences when interacted with a polar solvent. Interaction between solute and solvent leads to absorption band broadening and a consequent reduction in structural resolution and ε max. Ionic forms may also be created in acidic or basic conditions. Thus care must be taken to avoid an interaction between the solute and the solvent. Commercially available solvents of 'spectroscopic purity' are accompanied by their cut-off wavelengths, based on a 10mm pathlength. Water and 0.1N solutions of hydrochloric acid and sodium hydroxide are commonly used solvents for absorption spectrometry. Again care has to be taken to avoid interaction. Where methodology requires buffering, solutions have to be non-absorbing and generally both the composition and pH will be specified. However, if this information is not available lists can be found in the literature. For reactions in the 4.2 to 8.8 pH region, mixtures of 0.1N dihydrogen sodium phosphate and 0.1N hydrogen disodium phosphate are generally used. [10]

**Table 2: Commonly used solvents and their 'cut-off' wavelengths**

| Solvent | Cut-off (nm) |
|---|---|
| Is-octane | 202 |
| Ethyl alcohol | 205 |
| Cyclohexane | 200 |
| Acetone | 325 |
| Tetrachloroethylene | 290 |
| Benzene | 280 |
| Carbon tetrachloride | 265 |
| Chloroform | 245 |
| Ethyl ether | 220 |
| Isopropyl alcohol | 210 |
| Methyl alcohol | 210 |

**AREA UNDER CURVE STUDIES:-**

The principle for Area under curve method is "the area under two points on the mixture spectra is directly proportional to the concentration of the compound of interest".AUC is particularly suitable for the compounds where there is no sharp peak or broad spectra are obtained, which is shown in the figure 5.AUC method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths ƛ1 and ƛ2.Area calculation processing item caliculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated.The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration.[11]

- **Lambert's (or Bouguer's) Law :-**

Lambert's Law states that each layer of equal thickness of an absorbing medium absorbs an equal fraction of the radiant energy that traverses it. The fraction of radiant energy transmitted by a given thickness of the absorbing medium is independent of the intensity of the incident radiation, provided that the radiation does not alter the physical or chemical state of the medium. If the intensity of the incident radiation is *Io* and that of the transmitted light is *l*, then the fraction transmitted is:

***l*/*lo* = T**

The percentage transmission is

**%T = *l*/*lo* x 100**

If a series of colored glass plates of equal thickness are placed in parallel, each sheet of which absorbs one quarter of the light incident upon it, then the amount of the original radiation passed by the first sheet is:

**(1 - 1/4)/1 x 100 = 75%**

and by the second sheet is 56.25%, i.e. 75% of 75%, and by the third sheet is 42.19%, i.e. 75% of 56.25%, and by the nth sheet is **(0.75)^n x 100%.**

Now imagine a container with parallel glass walls 10mm apart filled with an absorbing solution. If monochromatic light is incident on one face and 75% of the light is transmitted, Lambert's Law states that if a similar cell is put next to the first the light transmitted will be reduced to 56.25%. If the contents of the two containers are evaporated to half their volume, thereby doubling their concentration, and then measured in a single container, it will be found that the transmission will again be reduced to 56.25%. It can be immediately seen that to determine the concentration of an unknown sample the percentage transmittance of a series of

solutions of known concentration or 'standards' can be plotted and the concentration or the unknown read from the graph. It will be found that the graph is an exponential function which is obviously inconvenient for easy interpolation.

- **The Beer-Lambert Law :-**

The Beer-Lambert Law states that the concentration of a substance in solution is directly proportional to the 'absorbance ', A, of the solution.

**Absorbance A = constant x concentration x cell length**

The law is only true for monochromatic light, that is light of a single wavelength or narrow band of wavelengths, and provided that the physical or chemical state of the substance does not change with concentration. When monochromatic radiation passes through a homogeneous solution in a cell, the intensity of the emitted radiation depends upon the thickness (l) and the concentration (C) of the solution. *I*o is the intensity of the incident radiation and *I* is the intensity of the transmitted radiation. The ratio *I*/*I*o is called transmittance. This is sometimes expressed as a percentage and referred to as %transmittance. Mathematically, absorbance is related to percentage transmittance T by the expression:

**A = log10(*I*o/*I*) = log10(100/T) = kcL**

where L is the length of the radiation path through the sample, c is the concentration of absorbing molecules in that path, and k is the extinction coefficient - a constant dependent only on the nature of the molecule and the wavelength of the radiation. Now, in the example above, the transmittance of our sample fell from 75 to 56.25% when the concentration doubled. What happens to the absorbance in the same circumstance?

**A = log10(100/T) = log10(100) - log10 (T) = 2 - log10 (T)**

**When T = 75%, A = 2 - 1.875 = 0.125**

**When T = 56.25% A = 2 - 1.750 = 0.250**

Quite clearly as the absorbance doubles for twice the concentration, it is far more convenient to work in absorbance than transmittance for the purposes of quantitative analysis. It is useful to remember that

**0%T = ∞ A**

**0.1% = 3.0A**

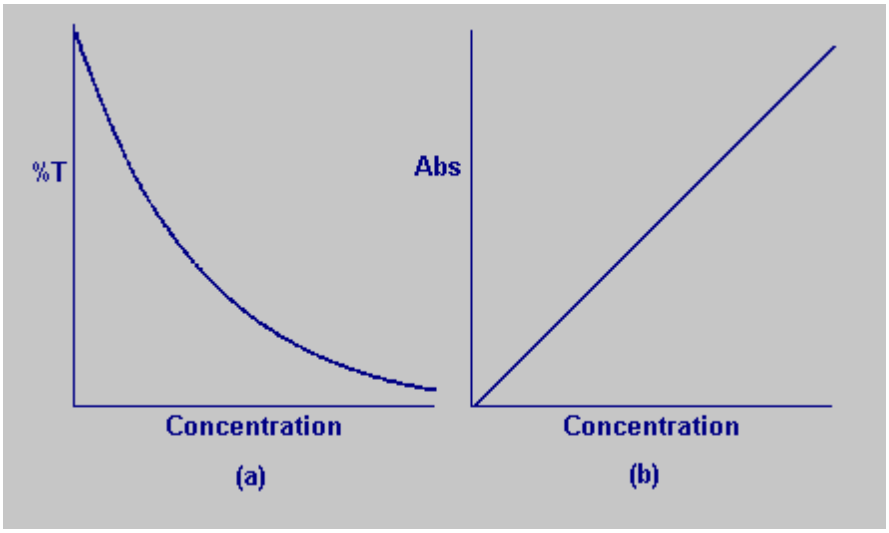
**1.0%T = 2.0A**

**10%T = 1.0A**

**100% = 0A**

Absorbance in older literature is sometimes referred to as 'extinction' or 'optical density' (OD

**Figure 11 (a) %T vs concentration (b) Absorbance vs concentration**




If, in the expression A = kcl, c is expressed in molar-1 and l in m, then k is replaced by the symbol τ and is called the molar absorption coefficient. The units of τ are mol-1m2. τ was formerly called the molar extinction coefficient and concentrations were often expressed as mol l-1, mol dm-3 or M and the cell length in cm to give units mol-1lcm-1, mol-1dm3cm-1 and M-1cm-1 respectively. Alternatively, if the relative molecular mass (molecular weight) of the substance is unknown, then a 1% w/v solution is prepared and the absorbance is measured in a 1 cm cell. In this case, k is replaced by E1% Sometimes the wavelength is included:

**E1%(325 nm).**

C Sometimes is expressed in g dm-3(gl-1) and l in cm. In this case, k is replaced by A (sometimes E). A is known as the specific absorption coefficient.[12]

***Applications of ultraviolet/visible spectroscopy:-***

In research, ultraviolet/visible spectroscopy is used more extensively in assaying than in identification. The trace metal content of an alloy, such as manganese in steel, can be determined by firstly reacting the sample to get the metal into solution as an ion. The ion is then complexed or made to react so that it is in a form that can be measured – eg manganese as the manganate(VII) ion. When the spectrum is recorded, the most useful piece of information is the absorbance because if the absorption coefficient of the chromophore is known the concentration of the solution can be calculated, and hence the mass of the metal in the sample. The same principle can be applied to drug metabolites. Samples are taken from various sites around the body and their solutions are analysed to determine the amount of

drug reaching those parts of the body. A useful feature of this type of analysis is the ability to calculate very small concentrations (of the order 0.0001 mol dm-3) with extreme accuracy. It is important that the absorbance of the solution remains below two for quantitative measurements because of limitations of the instrument and solute-solute interactions that can cause deviations from the Beer-Lambert law. The absorption of ultraviolet light is a feature of optical whiteners put into washing powders. The whitener absorbs radiation in the near ultraviolet and re-emits it in the visible range. Optical whiteners are also added to many toothpastes and detergent powders. They are often evident in discos that have ultraviolet lighting. White shirts and blouses frequently appear purple/blue, and people with false teeth should not smile! Other applications include adding ultraviolet absorbing inks to water marks on paper so that they show up under an ultraviolet lamp; postcoding of household valuables with ultraviolet sensitive ink; and using invisible (but ultraviolet fluorescent) inks for signatures in building society savings books[13].Having looked, albeit very briefly, at the theory and origins of UV-Visible spectra, let us now investigate how we can apply the technique to chemical analysis starting with consideration of sample state. With solid sample it is usually found that the material is in a condition unsuitable for direct spectrometry. The refractive index of the material is high and a large proportion of the radiation may be lost by random reflection or refraction at the surface or in the mass. Unless the sample can be easily made as an homogenous polished block or film, it is usual to eliminate these interfaces by dissolving it in a transparent solvent. Liquids may be contained in a vessel made of transparent material such as silica, glass or plastic, known as a cell or cuvette. The faces of these cells through which the radiation passes are highly polished to keep reflection and scatter losses to a minimum. Gases may be contained in similar cells which are sealed or stoppered to make them gas tight. With the sample now ready for measurement, the *I*o (incident intensity) can be set by moving the sample out of the beam and letting the light fall directly on the detector. On today's modern instrumentation, *I*o setting is generally accomplished by an 'autozero' command. In practice, such a method does not account for the proportion of radiation which is reflected or scattered at the cell faces. It also does not account for the radiation which is absorbed by any solvent and thus does not effectively pass through the sample. Therefore it is usual to employ a reference or blank cell, identical to that containing the sample, but filled only with solvent and to measure the light transmitted by this reference as a true or practical *I*o. Having established the *I*o or reference position, the procedure adopted for the analysis will depend on the analytical information required. In

general terms there are two major measurement techniques; how much analyte is in the sample (quantitative analysis) and which analyte is in the sample (qualitative analysis).[14]

## CONCLUSION

UV-Visible Spectroscopy is based on a firm theoretical basis, more selective, efficient, fast and reproducible analytical methods can be developed. In general terms there are two major measurement techniques; how much analyte is in the sample (quantitative analysis) and which analyte is in the sample (qualitative analysis).Area under curve method is "the area under two points on the mixture spectra is directly proportional to the concentration of the compound of interest" particularly suitable for the compounds where there is no sharp peak or broad spectra are obtained. ). The pharmaceutical analysis by UV-Visible Spectroscopy comprises the procedures necessary to determine the "identity, strength, quality and purity" of compounds.

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