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UV-VISIBLE SPECTROSCOPY: A COMPREHENSIVE REVIEW ON INSTRUMENTATION

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ABSTRACT

The first spectroscopic scientist was Johannes Marcus Marci. Spectroscopy is a branch of science that commence with Isaac Newton splitting light with a prism and was called optics. It involves the determination of Electromagnetic Radiation which is absorbed or emitted when molecules or atoms or ions of the sample move from one energy state to another energy state. Principle involved in Ultraviolet-Visible spectroscopy is electronic transition which describes the excitation of an electron from ground state to the excited state. For the measurements in spectrophotometers, we need to use a narrow band of wavelength of light. For these filters and monochromator are used. A filter is a device which transmit radiation of some wavelength, but absorbs wholly or partially other wavelengths. The monochromator is used to convert the polychromatic light into desirable monochromatic light. Converting the light source into electrical signal is the main function of the detector. Detectors are instrument that are used for measurement and to convert transmitted or reflected light from the sample to signal.

KEYWORDS: UV- Visible Spectroscopy; Electronic transition, Beer's, and Lambert's Law; Instrumentation; Photomultiplier tube.

INTRODUCTION

The first spectroscopic scientist was Johannes Marcus Marci of Kronland (1595-1667) in Eastern Bohemia. He showed interest in phenomenon of rainbow and performed experiments to explain it. He also published a book in 1648 called The Book of Thaumas, about the Heavenly Rainbow and the Nature of the Colors That Appear and about Its Origin and Thereof. Isaac Newton showed that the white light from the sun could be dispersed into continuous series of colors to which he coined the term "Spectrum".[1] Beckman in 1941 introduced the first commercial spectrophotometer Beckman DU. First application is to measure vitamin A and D which is used for the determination of vitamin content from several sources. In 1953, the Beckman DR, which is next generation has introduced. In 1960, UV-VISIBLE Spectrophotometers is used to perform kinetic assays on multiple samples. Computers were introduced in 1970s, which allows researchers and technicians to identify and quantify chemical and biological species. In 1980s, photodiode array detectors, were introduced. In several microbiology and biochemistry laboratories, UV-VIS Spectrophotometry is a crucial analytical tool. In solution, it is used for the determination of protein levels, in pharmaceutical products, it is used for the determination of enzyme kinetics and for the identification of microbes.[2] Beckman is one of pioneers in Analytical Instrument, dominating this field of area for about 50 years, has come up with latest "Beckman DU series 60 spectrophotometer, which makes use of two different source of light namely: (a) H2 or D2 Lamp-for the measurement in visible region, which permits measurements from 190nm-1000nm. A PC-based system has been also provided to enable the automatic spectrochemical measurements and perform calculations simultaneously. Double beam spectrophotometers are manufactured by several manufacturers such as: SHIMADZU, VARIAN, CECIL, BECKMAN, PERKIN ELMER; etc. These are mostly PC-controlled devices with built-in recorder to enable faster speed and greater operating stability.[3] Spectroscopy is a branch of science that commence with Isaac Newton splitting light with a prism and was called optics. Spectroscopy is study of visible light which we usually call as color that later under the James Clerk Maxwell's studies include the entire electromagnetic spectrum.[4] Spectroscopy is a branch of science that commence with ISAAC NEWTON splitting light with a prism and was called optics. UV-VIS spectroscopy involves the study of interaction of electromagnetic radiation with matter. The energy is either absorbed or emitted by matter in distinct amount called quanta.[5] It is one of the earliest instrumental techniques for the analysis and determination of micro and semi micro quantities of analyte in a sample which is based on atomic and molecular spectroscopy.[6] It is used for analysis of different types of solvents and

substances such as inorganic, organic, and biomolecules and is preferred mostly for its simplicity, versatility, accuracy, speed and cost effectiveness and maintenance is easy. It is used to determine the identity, strength, quality, and purity of several compounds.[6, 7] It is a measuring device which is used for quantitative analysis generally used for chemical substance by determining amount of light that is partially absorbed by analyte present in the solution. It can be classified according to the spectral region, such as UV (From 190nm to 380nm), VIS (From 380nm to 750nm), and near infrared (From 800nm to 2500nm).[8] The basic principle involves the absorption of visible and UV radiation (200-400nm) is associated with excitation of electrons from low to high energy levels.[9] It involves observation of electrons and is also known as electron spectroscopy.[10] It involves the determination of Electromagnetic Radiation (EMR) which is absorbed or emitted when molecules or atoms or ions of the sample move from one energy state to another energy state. It may be from ground state to excited state or from excited state to ground state.[11] It is a physical technique which utilizes light in region of visible, ultraviolet and near infrared areas.[12] In biochemistry labs, for e.g., enzyme assays are often performed to determine the presence of colored compound to determine enzyme efficacy, which is the basis of Enzyme-Linked Immunosorbent Assay (ELISA), which colored compound is produced when enzyme interact with targeted analyte.[13] For many years, UV-VIS Spectroscopy was used in clinical laboratory. The dependence on UV has been reducing over the years. In market, several multichannel clinical analyzers and readers have been available.[14] Chromophore is an area where electronic transitions take place. When a radiation is passed through a transparent substance, certain amount of radiation may be absorbed. If it happens, the residual radiations, when passed through a prism, yields spectrum with gaps in it, known as absorption spectrum. The atoms (or) molecules move from a state of lower energy (Ground state) to a state of higher energy (Excited state).[15] In the instrumentation, the main area of focus is the Light source, Monochromator, Sample cells, Filters, and Detectors. As far as we know, there are not many references that consists all the information about the components of the UV-VIS Spectroscopy.[16] Apart from the wavelength, the other parameters of interest are absorbance (A), transmittance (%T), and reflectance (%R), as well as their variations over time.[17] There are three types of electrons involved in organic molecules. They are

i. **σ-electrons:** These are saturated bonds, such as those between carbon and hydrogen in paraffin. For the excitation of σ-electron, energy produced by UV light is not enough and needed much more energy than that of the UV. If the compounds have σ bonds, they do not absorb UV radiation.

ii. **π-electrons:** These are unsaturated hydrocarbons. The compounds with π bonds are trienes and aromatic compounds.

iii. **n-electrons:** These are not involved in bonding between atoms in a molecule. For example: Organic compounds containing Nitrogen, Oxygen (or) Halogens. n electrons are excited by UV radiations. If any compounds have atoms like Nitrogen, Oxygen, Sulphur, Halogen, it may absorb UV radiation.[18]

Principle

UV/VISIBLE Spectroscopy involves the study of how a sample responds to light. When a monochromatic beam of light passes through sample, certain amount of light may be absorbedand the remaining is transmitted through the sample.[14] It is a technique based on the measurement on depletion of electromagnetic radiation by absorbing substance. The depletion electromagnetic radiation is resulted from the reflection, scattering, absorption, or interference. The spectral range of this radiation is approximately around 190-800nm.[16] A distinct spectrum is obtained by the absorption of ultraviolet or visible light by chemical compounds. The basis of spectroscopy involves the interaction of light with matter. When a matter absorbs light, it may experience excitation and de-excitation, which may result in the formation of spectrum. Electromagnetic wave strikes a material resulting in various phenomenon such as transmission, absorption, reflection, as well as scattering. Principle involved in Ultraviolet-Visible spectroscopy is electronic transition which describes the excitation of an electron from ground state to the excited state.[17] When a monochromatic light passes through the sample, it may absorb light in ultraviolet or visible range. The molecules present in sample may experience change in their electronic state. The electrons will be promoted from ground state orbital (Lower energy) to excited state orbital (Higher energy) by obtaining the energy from the light.

There are three types of ground state orbitals (Lower energy) may be involved:

i. σ (bonding) molecular orbital

ii. π (bonding) molecular orbital

iii. n (bonding) atomic orbital

There are two types of anti-bonding orbitals may be involved:

i. σ^* (sigma star) orbital π^* (pi star) orbital

There is no n* anti-bonding orbital as n electrons do not form bonds. The electronic transition occurring by absorption of ultraviolet and visible light are as follows:

i. σ to σ^*
ii. π to π^*
iii. n to σ^*
iv. n to π^*

σ to σ^* and n to σ^* transition requires high energy which takes place in far ultraviolet area or in 180nm-240nm range.[4]

Terminologies

1. Radiant power (P)

It is the rate at which energy is transported in a beam of radiant energy, i.e., radiant flux.

2. Transmittance (T)

It is the ratio of the radiant power transmitted by the sample (p) to the radiant power incident on the sample (p_0), both being measured at the same slit width. Thus, transmittance T is defined by P/P_0. The obsolete or alternate of transmittance is the name **transmission**.

3. Absorbance (A)

It is the logarithm to the base 10 of the reciprocal of the transmittance. A = log 10 (1/T) = log T. It can also be expressed as A = log P_0/P. The obsolete or alternate of this term is the name **extinction or optical density (D).**

4. Absorptivity (a)

It is the ratio of the absorbing to the product of concentration and length of optical path. It is a constant characteristic of (a = A/bc) substance and wavelength. The obsolete or alternate of this term is **extinction coefficient or absorbance index.**

5. Molar absorptivity (ε)

The absorptivity expressed in units of litre / (mole cm) is called **molar absorptivity.** Here the concentration is in mole per litre and cell length in centimeters. Thus, **molar absorptivity** ε =AM/bc. The obsolete or alternate term for molar absorptivity is **molar extinction coefficient or absorbancy index.**

6. Path length (b)

It is the internal cell length expressed in centimeters.

7. Millimicron (mμ)

It is a unit of length equal to one thousandth of a micron. One micron $=10^{-6}$ metre

8. Spectral bandwidth

The range of wavelengths of radiant energy emerging from the exit slit of the monochromator.

9. Visible or White light

Radiant energy which is perceived by the normal human eye (approx...380-780mμ).

10. Slit width

The **slit width (SW)** is the mechanical distance (mm) between the sides of the narrow aperture which permits radiant energy to enter and to leave the monochromator. **Spectral slit width (SSW)** is the width of the image of the exit slit along the wavelength scale. The **effective slit width (ESW)** is the width of the image at which the intensity is half of the maximum. **Both SSW and ESW are generally expressed inmμ.**

The absorption laws

There are two types of absorption laws involved. They are,

i. Lambert's law

ii. Beer's law.

i. **Lambert's law**

The **Lambert-Bouguer's law** states that "When a monochromatic light passes through a transparent medium, layers of equal thickness of that homogeneous absorbing medium absorbs equal proportions of incident radiation."[19]

Mathematically, the law can be expressed as

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

Where,I = intensity of radiation after passing through a thickness x, of the medium.

dI = extremely small decrease of intensity of radiation on passing through extremely small thickness, dx of the medium

k = proportionality constant or absorption coefficient. Its value depends upon the nature of the absorbing medium.

Let I_0 be the intensity of radiation before entering the absorbing medium (x = 0)

Then$I$, the intensity of radiation after passing through any thickness, say x of the medium can be calculated as:

$$\int_{I_0}^{I} \frac{dI}{I} = -\int_{x=0}^{x=x} k\,dx$$

Or $ln\frac{I}{I_0} = -kx$

Or $\frac{I}{I_0} = e^{-kx}$

$I = I_0 e^{-kx}$

The intensity of the radiation absorbed, I_{abs} is given by:

$I_{abs} = I_0 - I = I_0(1 - e^{-kx})$

The above Lambert's law equation can also be written by changing the natural logarithm to the base 10.

$I = I_0 10^{-ax}$

Where a = extinction coefficient of the absorbing medium

$\left(a = \frac{k}{2.303}\right)$.[20]

Beer's law

The Beer's law states that "The fraction of the monochromatic radiant energy absorbed on passing through a solution is directly proportional to the concentration of the absorber." It is a relationship between the light absorptive capacity and the concentration of the absorber in solution.[19] Mathematically, this law is stated as

$$-\frac{dI}{dx} = k' Ic$$

Where c = concentration of the solution in moles litre^{-1}.

k' = molar absorption coefficient and its value depend upon the nature of the absorbing substance.

Suppose I_0 be the intensity of the radiation before entering the absorbing solution. (When x =0), then the intensity of radiation, I after passing through the thickness x, of the medium can be calculated as:

$$\int_{I_0}^{I} \frac{dI}{I} = -\int_{x=0}^{x=x} k' \, cdx$$

$I = I_0 e^{-k' cx}$

Or $I = I_0 e^{-a' cx}$

Here $\frac{k'}{2.303} = a'$

Where a' = molar extinction coefficient of the absorbing solution.

Alternative expression

A = a.b.c

Where A = Absorbance, a = molar absorptivity, b = path length of the sample (usually 1 cm), c = Concentration of solution in moles litre^{-1}.[20]

Deviations from beer's and lambert's law

Beer and Lambert's law is found to be obeyed by a system, if a straight line passing through origin is obtained when a graph is plotted between absorbance and concentration. But there is always a deviation from the linear relationship between the absorbance and concentration particularly at higher concentration and hence the absorption curve changes with changes in concentration of solution. The deviation may be positive or negative.

If the resulting curve is concave upwards it is called **positive deviation.** If the resulting curve is concave downwards it is called **negative deviation.**

The reasons for deviation of Beer's law are

1. The law does not hold if the substance ionizes, dissociates, or associates in solution, since the nature of the ionized species in solution vary with the concentration.

Example 1: Benzoic acid in benzene associate to form dimer and hence deviation in absorption curve is expected.

Example 2: Molecules of methylene blue associate to form dimers with increase in concentration and hence the deviation in absorption curve is expected.

2. The law does not hold if a solute forms complexes, the composition, and extent of complexation depends upon the concentration.
3. Large number of electrolytes may shift the λ_{max} and may also change the extinction coefficient.
4. If change in concentration cause significant alterations in the refractive index, then deviations from Beer's law are observed.
5. Changes in pH with change in concentration of solute may also cause deviation.
6. Change of color due to dilution with solvent is an important chemical deviation.

Example: Orange color dichromate ion on dilution with water becomes yellow color.

The effect can be represented by the following equilibrium.

7. The presence of impurities that fluoresce or absorb at the required absorption wavelength, may also cause deviation from the law.
8. If slit width of the monochromator is not proper then deviations are expected to occur because improper slit width may allow undesirable radiations to fall on to the detector.[21]

Instrumentation

The essential components of UV-VIS Spectrophotometer are as follows:

- A Source of radiant energy

- A filter or monochromator to isolate the desired wavelength
- A pair of cuvettes one for the colored, and other for blank or reference solution.
- A detector

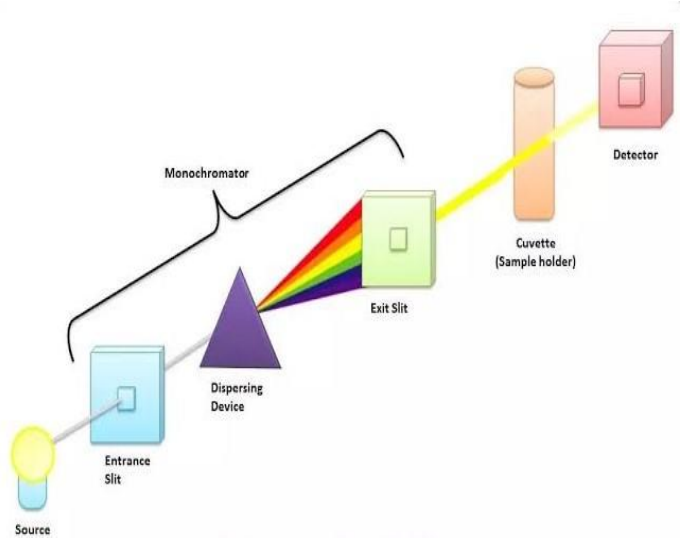

Fig. 1: (UV-VIS Spectroscopy).

Light sources

Hydrogen (or) deuterium lamps, Xenon discharge lamps, Mercury arcs are most frequently used radiation sources. By passing the electrons through gas; collision occurs between gas molecules and electrons resulting in electronic, vibrational, and rotational excitation in the gas molecules. If the pressure of gas is high, band spectra and continuous spectra will be obtained and if the pressure of the gas is low, line spectra will be obtained. The requirements of the radiation source are as follows:

i. It should be stable.
ii. It should have sufficient intensity for transmitted energy to be detected at the end of the optical path.
iii. It should supply continuous radiation over the entire wavelength region in which it is used.[18]

A. Lasers

Lasers are used in trace analysis by fluorescence measurement or laser-induced fluorescence (LIF). It can be of gas, solid, or dye type. In modern semiconductor lasers, pressure and temperature can also be used to detune the emission wavelength by 20-30nm.[10]

B. Led lamp

LED lamp does not require a monochromator because they produce a single wavelength of light. LED lamp has very long life and cost is low.[17]

C. Deuterium lamps

It is also called as D2 lamp with wavelength range 190nm-370nm. If deuterium is replaced by hydrogen, the intensity of emitted radiation is 3 to 5 times than the intensity of hydrogen lamp. Due to its high temperature, quartz is used for it. These are expensive than the hydrogen lamp, but it is used when high intensity is necessary. The lifespan of deuterium lamp is 1000hours.

D. Tungsten lamp

It is also called as halogens (or) quartz lamps, whose wavelength range is 320nm-1100nm. Hydrogen discharge lamps and tungsten lamps are the two most frequently used radiation sources. The function of the tungsten lamp is same as an electric light bulb. The lamp is stable, robust, and easy to use. The lifespan of the lamp is around 2000hours.

E. Xenon discharge lamp

In Xenon lamp, steady state can be reached in a short period of time whose wavelength range from 190nm-1100nm in UV and VISIBLE Spectrums. In the lamp, xenon gas is stored under pressure in range of 10-30 atmosphere. It consists of two tungsten electrode which are separated by about 8mm. When a low voltage is supplied, an intense arc is formed between two tungsten electrodes which produces ultraviolet light. The intensity of the ultraviolet produced is greater than the hydrogen lamp. When compared to deuterium (or) halogen lamp, xenon lamp has longer life span and it is expensive.[17, 18]

F. Hydrogen discharge lamp

In hydrogen discharge lamp, hydrogen gas is stored under relatively high pressure. When an electric discharge is passed through the lamp, excited hydrogen molecules will be produced that emits UV radiation. In hydrogen lamp, the high pressure causes the hydrogen to emit continuum rather than a simple hydrogen spectrum. The lamps are stable, robust, and used widely.

G. Mercury arc

In the lamp, mercury vapour is stored under high pressure. By passing an electric discharge, the mercury atoms are excited. The lamps are not preferred for continuous spectral studies due to the presence of sharp lines, while the less pressure mercury arc is very useful for calibration.[18]

Wavelength selectors

For the measurements in spectrophotometers, we need to use a narrow band of wavelength of light. Filters are used in less expensive instrument to isolate the radiant energy and to provide a broad band of wavelength. In many cases, we need to vary the wavelength continuously over a defined range which can be achieved by using monochromator that employ a prism or diffraction grating as the dispersion medium.[6]

Filters

There are three important reasons for using a radiation that is restricted to limited wavelength region in quantitative techniques. These are:

i. The probability of adherence of the absorbing system to Beer's law is greatly increased.
ii. The substance which absorbs in other wavelength region are less likely to interfere, therefore a greater selectivity is assured.
iii. A greater sensitivity is attained, because greater change in absorbance as increase of concentration is observed when wavelengths which are strongly absorbed are used.

A filter is a device which transmit radiation of some wavelength, but absorbs wholly or partially other wavelengths. Among the several devices that produce limited bands of radiation, filters are the simplest and least expensive and the filters are of two types:

1. Absorption filters
2. Interference filters

1. Absorption filters

Absorption filters offers limited radiation by absorbing certain proportion of spectra and have effective band width of 30-250mμ. Absorption filters comprises of colored glass or dyed gelatin sandwiched between glass plates. The glass filters which have transmittance maxima over the entire visible region are commercially available. The colored glass filter offers the advantage of greater thermal stability.[19] The narrow bandwidth filtersabsorbs the radiation striking them, allowing only a small fraction to pass. Filters may also require air cooling to prevent overheating.[22]

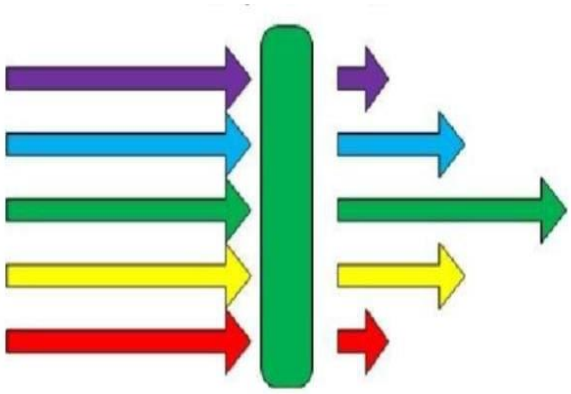

Fig. 2: (Absorption filter).

2. Interference filters

It comprises of transparent calcium fluoride or magnesium fluoride solution that occupies space between the two semitransparent metallic films coated on the inside surface of two glass plates. The thickness of dielectric layer of calcium fluoride or magnesium fluoride is carefully controlled, as it determines the wavelength of transmitted radiation. When compared to absorption filter, interference filter generally provides significantly narrower bandwidth (as low as 10mm) and greater transmittances of desired wavelength. The interference filter can be constructed by depositing semitransparent film of silver on a glass plate. Upon the silver a very thin layer of transparent material such as magnesium fluoride and again on the magnesium fluoride another film of silver is placed. In commercially available interference filters thin layers are covered with a second glass plate for providing protection. The selection of filter is based on the relationship between absorption and transmission spectra. The color of the filter should be complementary to solution color, if spectral characteristics of sample solution are not known. The choice of filter is such that the filter would give maximum absorption for change in concentration.[19] Interference filters relyon optical interference to produce a narrow band of wavelengths. They are more efficient as well as more expensive. The light radiationthat passes through the first film is partially reflected at the second film. In turn, this reflected beam is partially reflected by the first film which produces interference effects.[22]

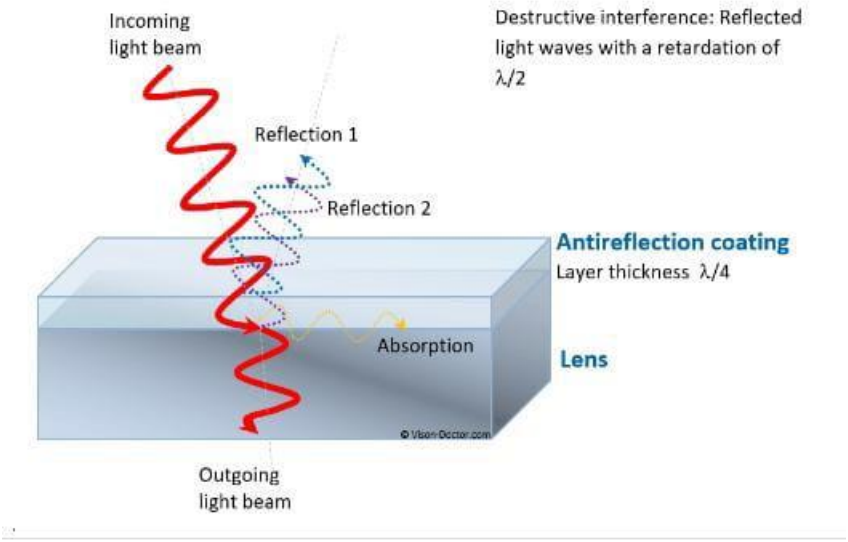

Fig. 3: (Interference filter).

Monochromators

It can be achieved by filtering out the undesirable wavelength from radiation source.[4] The monochromator is used to convert the polychromatic light into desirable monochromatic light. The main function of the monochromator is to disperse the beam of light obtained from the primary source into its components. The monochromator comprises of the following components:

i. Focusing lens;
ii. An entrance slit;
iii. An exit slit;
iv. A dispersing device.[19]

Through the entrance slit, polychromatic light is entered the monochromator and followed by collimation, beam is directed at an angle towards the dispersion component. By the use of grating or prism the polychromatic light is converted into monochromatic light and specific wavelength exits the monochromator through the exit slit. It includes the following types:

1. Prism monochromator
2. Grating monochromator

1. Prism

In recent years, the use of prism monochromator has been decreased as the spectrum is non-linear and the resolution is not as good as with gratings. However, many prism monochromators are still in use. As the light source enters the entrance slit, it is collimated by the first lens, and it is directed to a glass prism which is refracted. Wavelengths that are

shorter are refracted more strongly than the longer wavelength (i.e., blue light is bent more than red). The dispersed light is then focused by the second lens onto the exit slit. Different wavelengths can be focused by rotating the prism.[22] They are mechanically very complex and therefore it is very expensive. Prism are mostly made up of quartz for use in the UV region, since glass absorbs wavelength shorter than about 330nm. Glass prism are usually preferred for the VISIBLE region of the spectrum.[23]

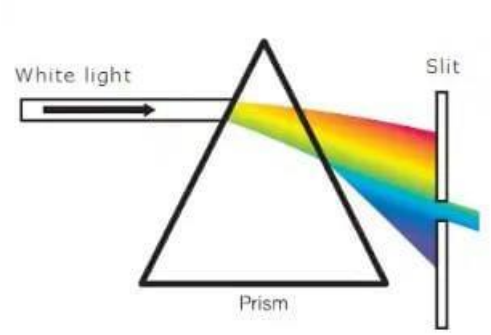

Fig. 4: (Prism monochromator).

2.Grating

In recent years, diffraction gratings have become less expensive and of improved quality. It can be used for either reflection grating or transmission grating. The dispersion of the grating is almost linear.[10] A transmission grating comprises of series of parallel and closely spaced grooves ruled on a piece of glass or other transparent material. Reflection grating are obtained by ruling polished metal surface or by evaporating a thin film of aluminium onto the surface of a replica grating. A concave grating can be produced by ruling a spherical reflecting surface. Such diffraction element focuses the radiation to the exit slit and eliminates the use of the lens. The major drawback is that they give more than one order of diffraction. Stray radiation can be eliminated by using two gratings or by using filters in front of entrance slit.[19] There are number of different classic design for grating monochromator. The most frequently used grating monochromator is Czerny-Turner comprises an entrance slit and concave collimating mirror on one side of the normal to the grating and focusing mirror (which is identical to collimating mirror) and exit slit on the other side. Ebert grating monochromator is similar to the Czerny-Turner, but the two collimating mirrors is replaced by a single, large mirror. The Littrow grating monochromator is that the size of the monochromator is reduced by the use of single mirror to collimate and focus the radiations. The instruments often place the entrance and exit slits at 90° to each other and use small

plane mirror to direct the dispersed light onto the exit slit.[22] It comprises of a very large number of equispaced lines (200-2000 per mm) ruled on glass blank which is coated with thin film of aluminium. Gratings are now produced by modern holographic techniques. Rotation of the grating permits suitable wavelength of spectrum to emerge from the exit slit of the monochromator. For convenience in scanning, plane gratings are most frequently used which can be made to cover all the spectral regions from 180nm-15mμ.[23]

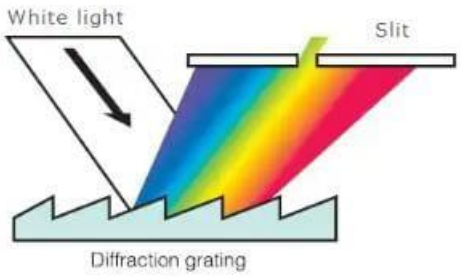

Fig. 5: (Grating monochromator).

Sample containers

Cuvette or cell must satisfy the following two main requirements:

i. Sample present in the cell or cuvette must be made of substance which are transparent in the spectral region.

ii. They are designed in such a way that their path length may be easily determined.

The cuvette must be constructed with quartz, if the work has to be done in the UV region and for Visible region, they are made up of color corrected fused glass. The cuvette thickness depends upon whether the absorption is strong or weak. It may be either rectangular or cylindrical or cylindrical with flat ends. It is recommended that the entrance and the exit slit of the cell or cuvette should be plane parallel surface for best results. If cylindrical cells are used, the entrance and exit sides are curved and the thickness of the cuvette or cell are of 1cm, 2cm, and 5cm.[19] Alternate to the glass and quartz cuvette, several manufacturers offer disposable cuvettes which are usually made up from various plastics that includes polystyrene and acrylic. These offers main advantage that it does not require cleaning between samples. The disadvantage is that they have a restricted wavelength range.[14]

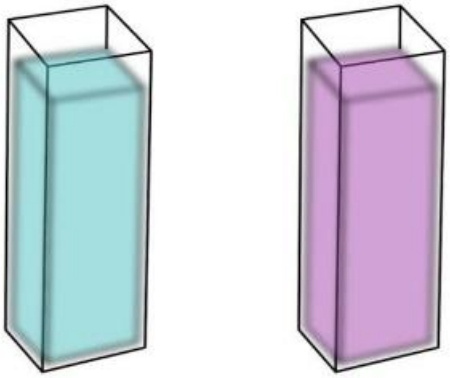

Fig. 6: (Sample Containers).

Detector

When the transmitted radiation strikes the detector, it allows the detector in determining the amount of radiation absorbed by the sample.[4] The detector is placed in a position such that they receive the resulting monochromatic radiation after passed through sample and reference substance which is stored in quartz cuvettes.[8] Detectors are usually responding with high sensitivity with low noise having a linear response range, fast response, and low consumption of sample.[16] Converting the light source into electrical signal is the main function of the detector. Detectors are instrument that are used for measurement and to convert transmitted or reflected light from the sample to signal.[17] Monochromatic light after passed through sample and reference cell, intensities of respective transmitted beams are then compared over whole wavelength range of the instrument. In UV spectrometer there are generally two detectors to record the spectra. One receives beam from sample cell and second receives beam from reference. Reference cell's intensity radiation is stronger when compared to beam of sample cell. Due to an excellent responsibility over the entire UV -VIS- NIR range; Si detectors are present in almost all detector that operate in UV-VISIBLE region. Most portable UV-VIS spectrometers today use diode array in the complementary metal oxide semiconductor (CMOS) or change coupled device (CCD).[24] In early instruments for the measurement of absorption of radiation visual or photographic methods for detection is required which are now completely replaced by photoelectric photometers and spectrophotometers. Ideal characteristics of the detector are as follows:

1) It should respond to radiant energy over or broad wavelength range.
2) It should be sensitive to low levels of radiant power.
3) It should rapidly respond to the radiation and produce an electrical signal that can be rapidly amplified.

4) It should have a relatively low noise level (for stability)
5) The signal produced is directly proportional to power of beam striking it.

There are three types of detectors which have been used for radiation detection. There are:

a. Photovoltaic cell/Barrier layer cell
b. Phototubes
c. Photomultiplier tube

Photovoltaic cell/Barrier layer cell

The photovoltaic cell or Barrier layer cell are primarily used for the measurement and detection of radiation in visible region. The maximum sensitivity of this cell is about 550nm. It consists of a flat copper or iron electrode upon which a layer of semiconducting material such as selenium copper oxide is deposited. Transparent metallic film of silver, gold, or lead present on the surface of semiconductor acts as the second or collector electrode. The interface between the metal film and the selenium acts as barrier to the passage of electrons. Electrons in the oxide layer have sufficient energy to overcome this barrier, and electrons flow from semiconductor to metal film, if the film is connected via external circuit to the plate on the other side of semiconducting layer, and if resistance is very small, a flow of electrons takes place. By using galvanometer or microammeter the current caused by flow of electrons can be measured.

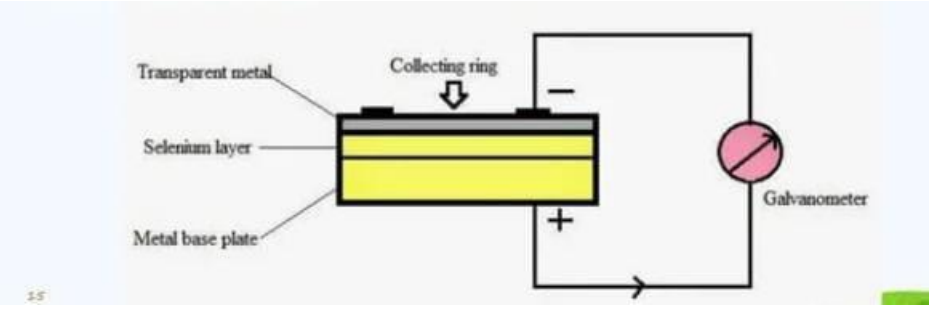

Fig. 7: (Photovoltaic cell/Barrier layer cell).

Photo emissive (vacuum) tube

It consists of semi cylindrical cathode and a wire anode which is sealed inside a clear transparent envelope. A layer of Photo emissive material is supported on the concave surface of the cathode which emits electrons when irradiated with light. Electrons are emitted when a potential is applied across the electrode. These emitted electrons flow to wire anode and a photocurrent result. The number of electrons emitted or ejected from the photo emissive

surface is directly proportional to the radiant power of the beam striking the surface. If the potential applied across two electrodes is increased, emitted electrons reaching the anode also increases. When saturation is attained all the electrons are collected at the anode. They are generally operated at a potential 90V. The photo emissive cathode surface generally consists of alkali metals or alkali metal oxides alone or combined with other metals or oxides. It can be divided into two groups:

I. High vacuum type.
II. Gas filled type.

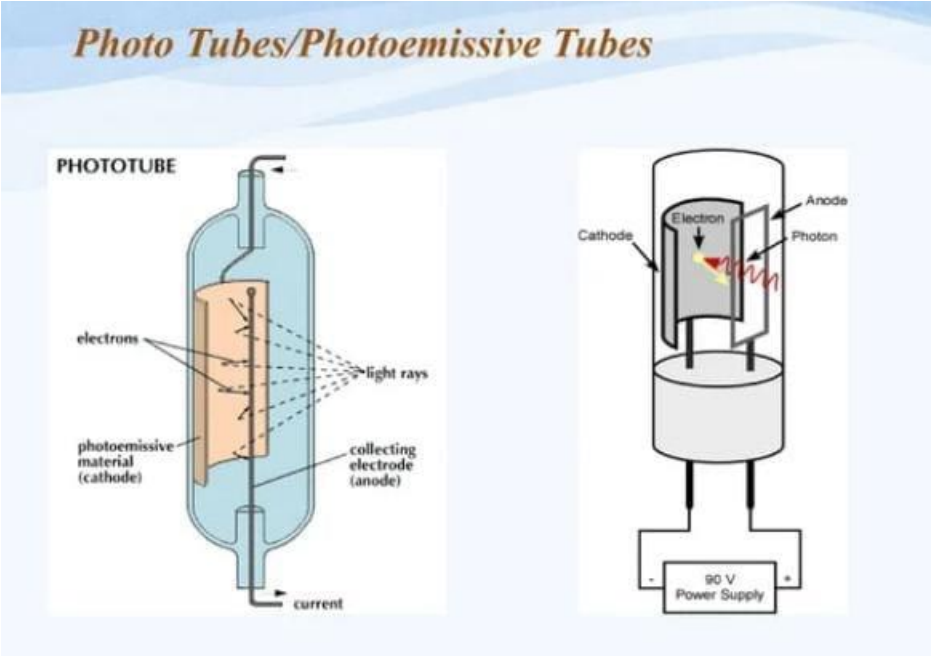

Fig. 8: (Photo emissive tube).

Photomultiplier tube

The commonly used detector is the Photomultiplier tube. It consists of three components:

i. Photo emissive cathode
ii. Several dynodes which emit several electrons for each striking them.
iii. An anode

It consists of an electrode covered with photo emissive material and series of positively charged plates, each charged at a successively higher potential. The electrons are emitted upon expose to radiation. It also consists of additional electrodes, known as dynodes. Dynode 1 is maintained at a potential of 90V which is more positive than cathode and the emitted electrons are accelerated towards it. On striking the dynode, electrons cause ejection of several additional electrons which are accelerated towards dynode 2, which is 90V more

positive than that of the dynode 1. Again, on striking the dynode, electrons are emitted as several additional electrons. 10^6-10^7 electrons are formed if the process is repeated for nine times (If the tube contains 9 dynodes). The electrons are finally collected at the anode which is passed through readout device for amplication and measurement. Photomultiplier tube is very sensitive for both UV and VISIBLE radiation and have fastresponse. The intense light may damage the photomultiplier tube; hence they are limited to measuring low power radiation.[19]

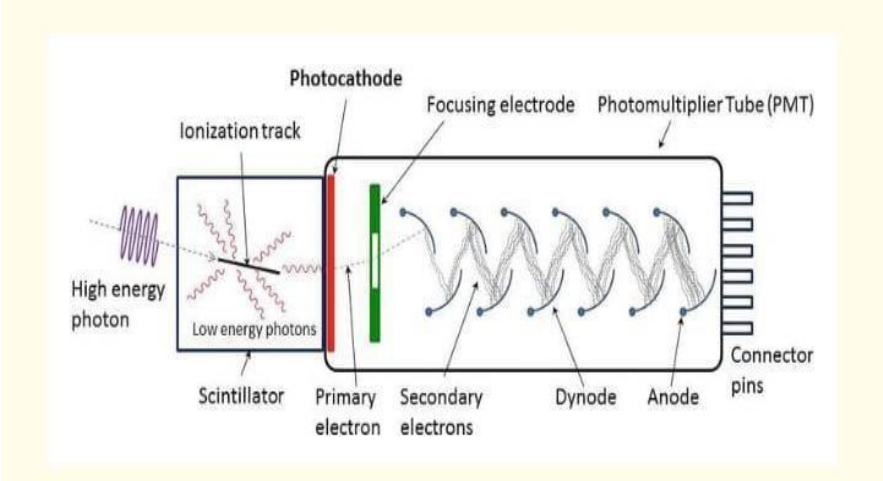

Fig. 9: (Photomultiplier tube).

Photodiode detector array

In modern spectrophotometers photodiode detector is used. The operation of photodiode detector array is based on fact that each diode is reverse-based. They have wide dynamic range and more powerful than the photomultiplier tube. The diodes are completely charged before exposing the detector to light reaches the photodiode charge carrier will be generated. These carriers will neutralize the stored charge which have opposite polarity. The amount of charge that is required to recharge the capacitor at regular interval is proportional to intensity of light. It can monitor at all wavelength. They are widely used as a multichannel detector. They can record a complete spectrum in very short period. The limits of detection are approximately 170nm- 110nm for silicon-based detectors.[16]

Thermocouples

It is widely used in the infrared region which operates on the heat detection.[19] It comprises of elements of two different type of metals which is joined together at one end, the other end is attached to a sensitive galvanometer.[23]

Bolometers

Bolometers is a miniature resistance thermometer which contains of tint platinum wire as sensing element.[19] It makes use of increase in resistance of a metal with increase in temperature. Bolometers also used in the infrared region.[23]

DISCUSSION AND CONCLUTION

Ultraviolet-Visible spectroscopy is a cost-effective method for the determination of identity, strength, quality, purity of the compounds. It comprises two majorsteps to measurements of qualitative and quantitative analysis for the sample. UV-Visible spectroscopy is used in wastewater treatment, kinetic and monitoring studies which can be conducted to ensure that specific dyes or dye by-products have been effectively eliminated from the wastewater by comparing their spectra over time. UV Visible Spectroscopy is also extremely useful in the investigation of food authenticity and the monitoring of air quality. Ultra violet/visible spectroscopy is an analytical technique that is used to determine qualitatively and quantitativelyfor the estimation of different ions. This technique may also be used in many other industries. For example, measuring a color index is useful for monitoring transformer oil as a preventative measure to ensure electric power is being delivered safely. From the above given information, we can conclude that UV/VIS Spectroscopy is a great method which is used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules.

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