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A Review on Instrumentation of UV Visible Spectroscopy

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Abstract: *Isaac Newton invented spectroscopy as a branch of science, which he called optics, by splitting light with a prism. Thus, under James Clerk Maxwell's research, the study of visible light which we refer to as color eventually expanded to encompass the full electromagnetic spectrum. The scientific field that examines how electromagnetic radiation interacts with matter is called spectroscopy. The matter absorbs or emits energy in definite amounts known as quanta, which is the most significant effect of this interaction*

Keywords: UV Visible Spectroscopy, Instrumentation of UV Spectroscopy, Applications

I. INTRODUCTION

Is that matter absorbs or emits energy in discrete amounts known as quanta. From the gamma area (nuclear resonance absorption or the Mossbauer effect) to the radio region (nuclear magnetic resonance), the absorption or emission processes are understood throughout the electromagnetic spectrum. An experimental measurement of radiation frequency yields a value representing the energy shift involved, from which a set of potential discrete energy levels of the matter can be inferred. Spectroscopy is the experimental process of measuring the frequency of radiation (emitted or absorbed) and determining the energy levels from thesemeasurements.

Spectroscopy :

Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation (EMR) that a sample's molecules, atoms, or ions release and absorb when they transition between different energy states.

UV – Visible Spectroscopy :

Based on the principle that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length, UV spectroscopy is a physical technique of optical spectroscopy that uses light in the visible, ultraviolet, and near- infrared ranges. As a result, it can be used to find the absorber concentration in a solution fora specific path length. Knowing how quickly absorbance varies with concentration is essential. UV-VIS spectroscopy has been widely used for the past 37 years, during which time it has emerged as the most significant analytical instrument is the modern day laboratory.

Electromagnetic spectrum :

Spectrum of Electromagnetics For spectroscopic operations to take place, electromagnetic radiation must be able to interact with atoms and molecules in a defined manner and generate characteristic absorption or emission profiles. The property of electromagnetic radiation that controls the perceived color spectrum is its wavelength. The electromagnetic spectrum's visible region is the area that is visible to the human eye. The range of these visible wavelengths is 400–800 nm. When measured using a particular visible light wavelength or color, the optical density relates to When measured using spectrophotometers, the optical density correlates to a particular visible light wavelength or color.

This light disappears and is absorbed, turning it invisible. Figure illustrates the roughly complementary relationship between the transmitted and absorbed light wavelengths. For example, a blue substance would absorb orange, which is the complimentary color of light, intensely.

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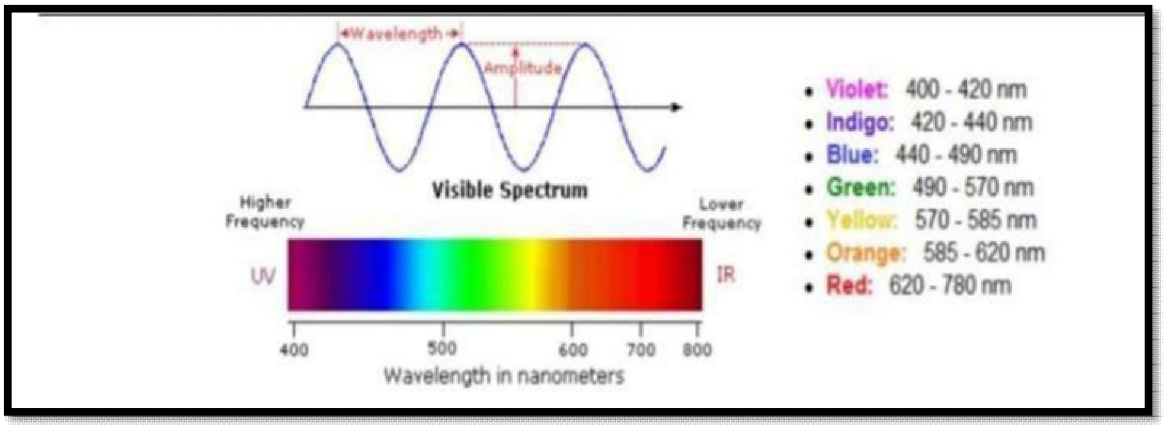

Figure 1: Electromagnetic radiation and spectrum represented as sine waves The wavelength is the separation between adjacent peaks or troughs. The wavelength of EMR as a function of its frequency, v, and the speed of light, c, may be found using the simple equation $\nu = c/\lambda$ (1).The dual nature of light, or EMR, exhibits both particle and wave behavior. The equation E hc/ λ *109 (2) describes the relationship between the energy and wavelength of an EMR particle, or photon. where c is the Planck's constant (6.63 x 10-34 Js), E is the speed of lightin a vacuum (2.998 x 108 ms-1), and is the wavelength is nm

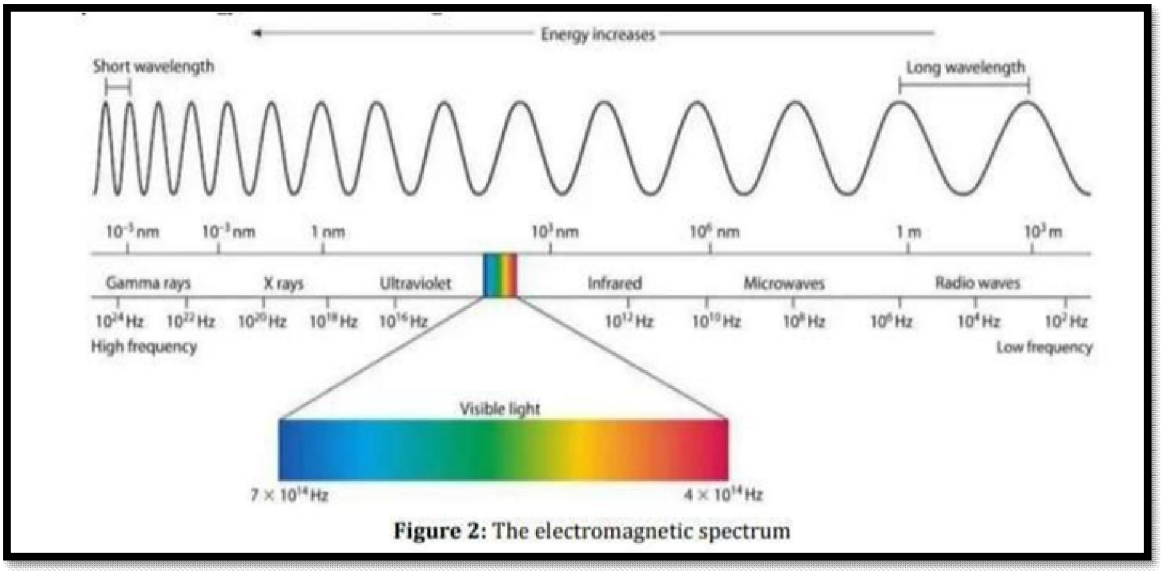

Figure 2: The electromagnetic spectrum

Principle of UV-Vis Spectroscopy: 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 changein the electronic state of the molecules in the sample. The energy supplied by the light will promote electrons from their ground state orbital to higher energy, excited state orbital or anti-bonding orbital. Potentially, three types of ground state orbitals may be involved.

σ (Bonding) molecular

π (Bonding) molecular orbital

n (non-Bonding) atomic orbital.

In addition, two types of anti-bonding orbitals may be involved in the transition.

σ* (sigma star) orbital.

π* (pi star) orbital.

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There is no such thing as an n* anti-bonding orbital as the n electrons do not form bonds).
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 σ 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 from then 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* anti-bonding orbital. It will be seenpresently that the wavelength of maximum absorption and the intensity of absorption are determined by molecular structure. Transitions to π* anti-bonding orbital which occurs 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. 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.

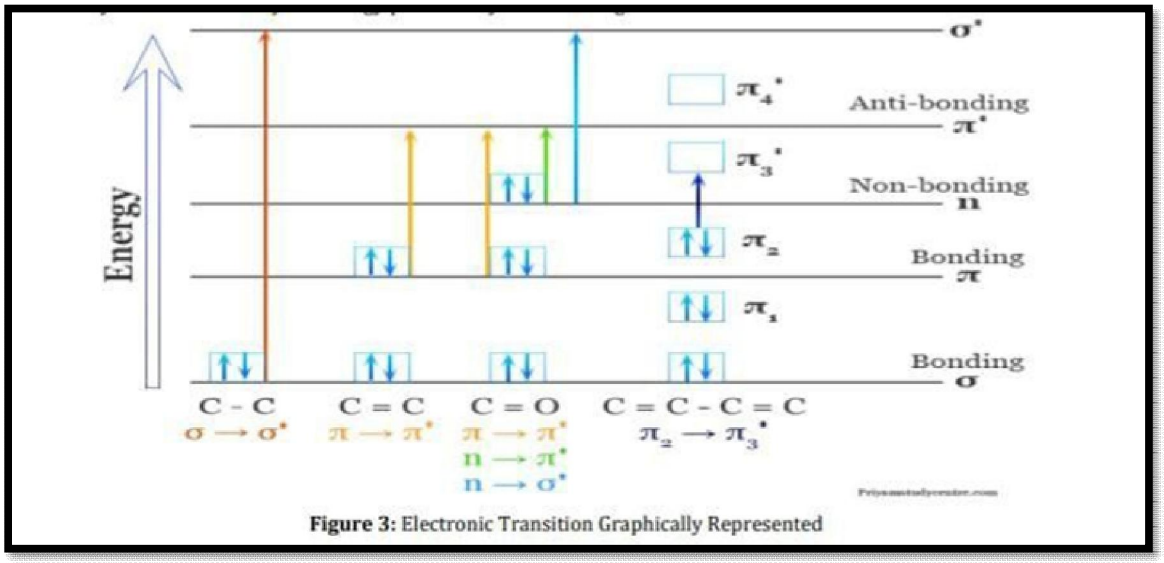

Figure 3: Electronic Transition Graphically Represented

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 the spectrum (200-400nm). 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 6.2EV which relates to a wavelength range of 800 - 200 nm. The Beer-Lambert Law is the principle behind absorbance spectroscopy.
A = a bcWhere,
A = Absorbance,a = absorptivity,b = path length, c = concentration.

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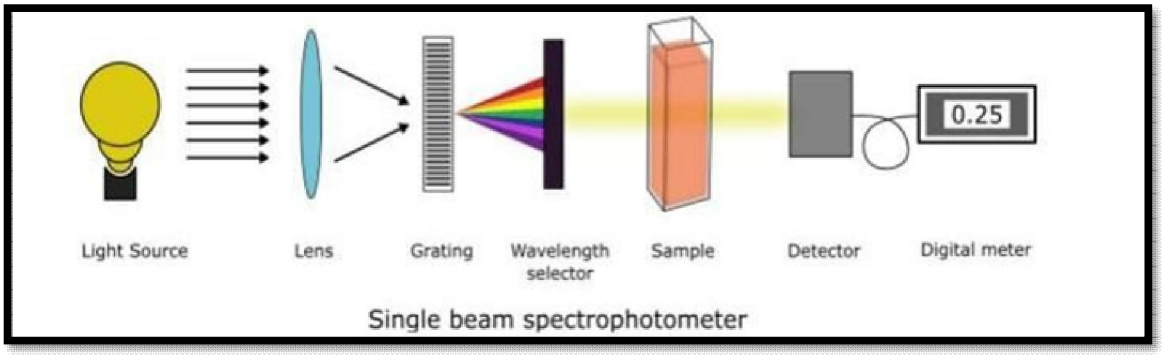
Limitation in Laws: Scattering and reflection can modify the absorption reported.
Reaction with the solvent
High concentration affects charge distribution, the average distance between ion decreasing, making particles close to each other.
Presence of stray light.
There are two types of absorbance instruments used to collect UV-Visible spectra:
Single beam UV-Vis spectrometer
Two-beam UV-Vis spectrometer

SINGLE BEAM UV-VISIBLE SPECTROPHOTOMETER

A single beam UV-Vis spectrophotometer has a single beam, as the name suggests. The light from the source is directed through the monochromator so that the incoming monochromatic light passes through the slit. It then passes through the test solution. Part of the incident light is absorbed by the sample, while part is emitted. The detector detects the emitted light. The detected light is then amplified, stored and then displayed on a suitable readout. The spectrumis plotted and λ max is located. The single-beam UV-vis spectrophotometer consists of light source

- Lens
- Gratings
- Wavelength selector
- Sample container/cuvette
- Detector
- Digital meter/recorder



DOUBLE BEAM UV-VISIBLE SPECTROPHOTOMETER

The instrumentation of single- and double -beam spectrophotometers is almost the same. The main difference with a single-beam UV-Vis spectrophotometer is that the incoming light beam passes through the sample and reference cells simultaneously. The incoming light is split and directed into both reference and sample cuvettes. Detectors detect a refracted or transmitted beam. A two-ray UV-vis spectrophotometer requires two detectors that detect the ratio of electrons to measure or calculate the absorbance of the sample being studied. It also requires a stabilized voltage supply.

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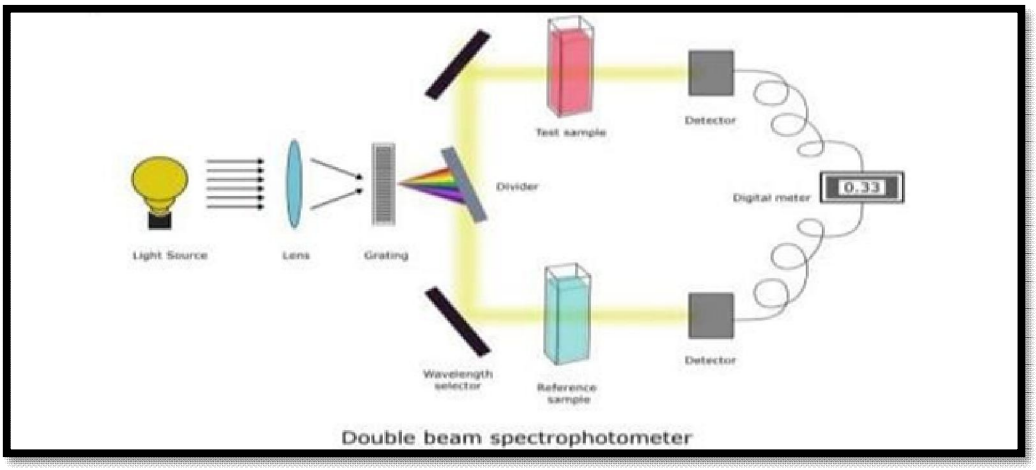

Instruments for measuring the absorption of U.V. or visible radiation are made up of thefollowing components

- Source
- Monochromator
- Sample Cell
- Detector
- Readout system
- Amplifier
- Display

Instrumentation: At this schematic diagram of a double-beam UV-Visible Spectrophotometer.

Fig.6 UV Vis Spectrophotometer

Sources of UV radiation:-

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

The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves the formation of an excited molecular species, which breaks up to give two atomic species and an UV photon. This can be shown as; D2 + electrical energy D2* D' + D''+hv

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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 glassabsorbs 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.Assorted UV radiation sources include the following:

Deuterium Lamp:
Its wavelength range is 190nm - 370nm, and it is also known as a D2 lamp. Because of its high temperature behavior, normal glass housing is insufficient, necessitating the use of quartz, MgF2, or other materials. A typical deuterium lamp has a lifespan of about 1000 hours. In order to cover the entire UV and visible light wavelength, a UV / Vis spectrophotometer will design a deuterium lamp with halogen lamps.
A gas discharge lamp called a deuterium lamp is frequently employed as a UV source. It emits radiation in the 160–450nm range. It costs more than a hydrogen lamp.

Halogen Lamp:
Halogen lamps are also known as tungsten or quartz lamps, and their wavelength range is in the visible light region, ranging from 320nm to 1100 nm. If the instrument is only equipped with a halogen lamp, it can only measure visible light. The average halogen lamp life is around 2000 hours or more.

Xenon Lamp:
A Xenon lamp is a high-energy light source that can reach a steady state in a short period of time. Its light ranges from 190nm to 1100nm in the UV and visible spectrums. A xenon lamp flashes at a frequency of 80Hz, giving it a longer life than a deuterium or halogen lamp. A xenon lamp, on the other hand, is more expensive. A xenon lamp is a discharge light source that contains xenon gas inside a bulb. Radiation from xenon ranges from 250 to 600 nm.

LED Lamp:
Because LED lamps produce a single wavelength of light, they do not require a monochromator.
It has a very long life. The bandwidth of an LED light source varies little and is stable. A low- cost light source is an LED lamp.

Hydrogen lamp:
Hydrogen lamps are reliable, steady, and continuously emit radiation between 160 and 380 nm. It consists of hydrogen gas at high pressure, which causes an electrical discharge. The excited hydrogen molecules produce radiation.

Tungsten lamp:
The most typical light source utilized in spectrophotometers is the tungsten lamp. With a wavelength range of roughly 330 to 900 nm, it comprises of a tungsten filament encased in a glass envelope and is utilized for the visible spectrum.

B. The Monochromator (Wavelength selector):
Types of monochromators:
1) Prism monochromator
2) Grating monochromator
All Monochromator contain the following component parts;
An entrance slit
A collimating lens
A dispersing device (usually a prism or a grating)
A focusing lens

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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.

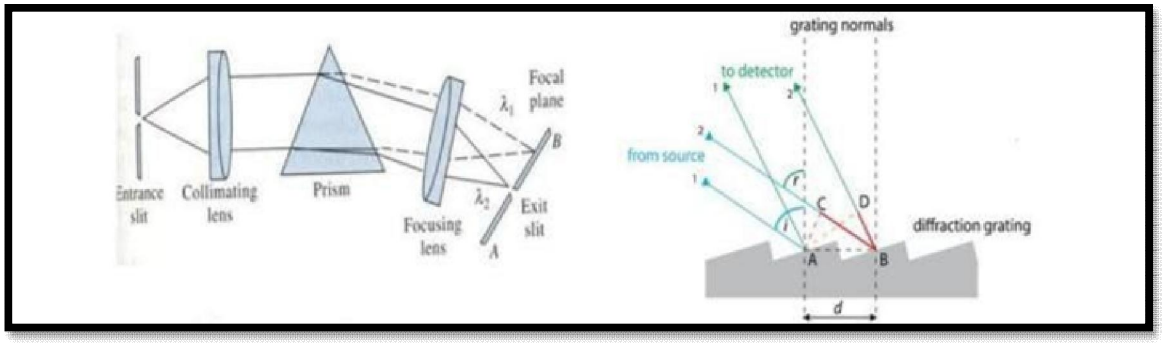

Fig. 7 Monochromator

C. Sample Cell:

Cuvettes are sample containers that are transparent to all wavelengths of light flowing through them and are used to hold samples for spectroscopic measurements. The cuvette is composed of quartz, is square in shape, has a 1 cm route length, and may be utilized forwavelengths between 190 and 200 nm.

Fig.8 UV Vis Spectrophotometer

Light energy is converted by detectors into electrical impulses that are read out by readout devices. The transmitted radiation strikes the detector, determining the amount of radiation absorbed by the sample. The absorption spectrophotometer's apparatus uses the following types of detectors.

Types of Detectors:

1. Barrier layer cell/Photovoltaic cell
2. Phototubes/ Photo emissive tube
3. Photomultiplier tube
4. Barrier layer cell / Photovolatic cell

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1. Barrier layer cell / Photovolatic cell :

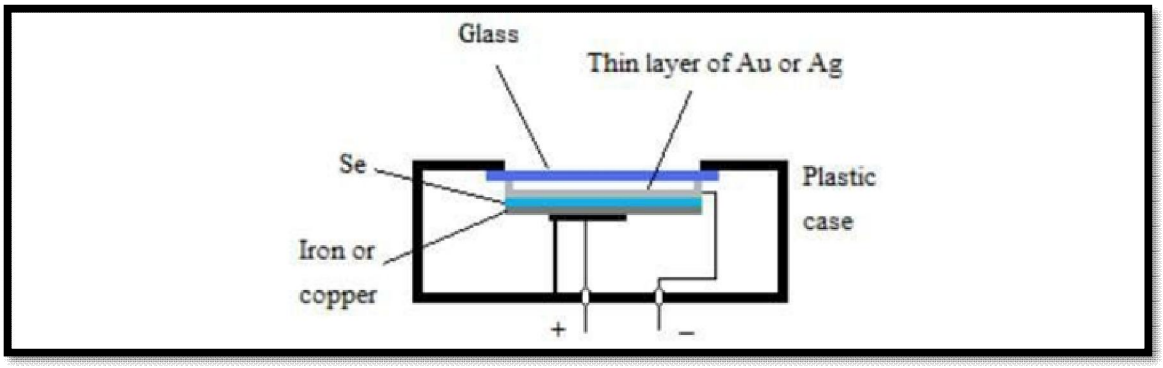

Fig.9 Barrier layer cell / Photovolatic cell

Construction:

It consists of a thin film metallic layer coated with silver or gold and acts as an electrode. It also has a metal base plate made up of iron which acts as an electrode. These two layers are separated by a semiconductor layer of selenium.

Working:

When light radiation falls on selenium layer, electrons become mobile and are taken up by transparent metal layer. This creates a potential difference between two electrodes and causes the flow of current. When it is connected to galvanometer, a flow of current observed whichis proportional to the intensity and wavelength of light falling on it.

Advantages:

It is simple, sturdy and does not require external power supply

At low level of illumination it produces photocurrent proportional to radiant powerreceived on it.

Phototubes/ Photo emissive tube

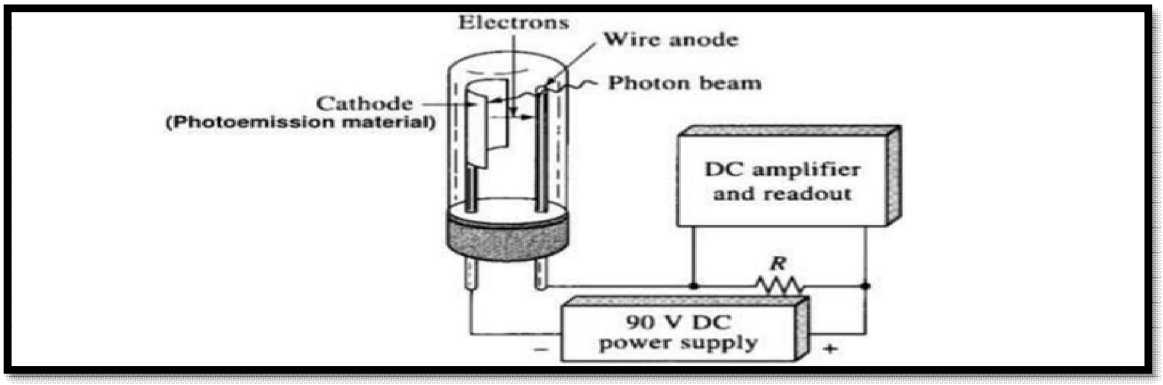

Fig.10 Phototubes/ Photo emissive tube

Construction:

It consists of spherical shaped vacuum bulb containing photo emissive cathode and anode. The inner surface of cathode mounted inside bulb is coated with photosensitive material like cesium oxide, potassium oxide or silver oxide. It has anode to attract the electrons.

Working:

When the radiant energy falls on photosensitive cathode, electrons are emitted which are attracted towards anode causing current to flow. It is more sensitive as compared to barrier layer cell and hence widely used.

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Disadvantage:

It produces dark current which attributed due to scattered electron emission from photosensitive cathode due to stray radiation striking its surface

Photomultipller tube detector

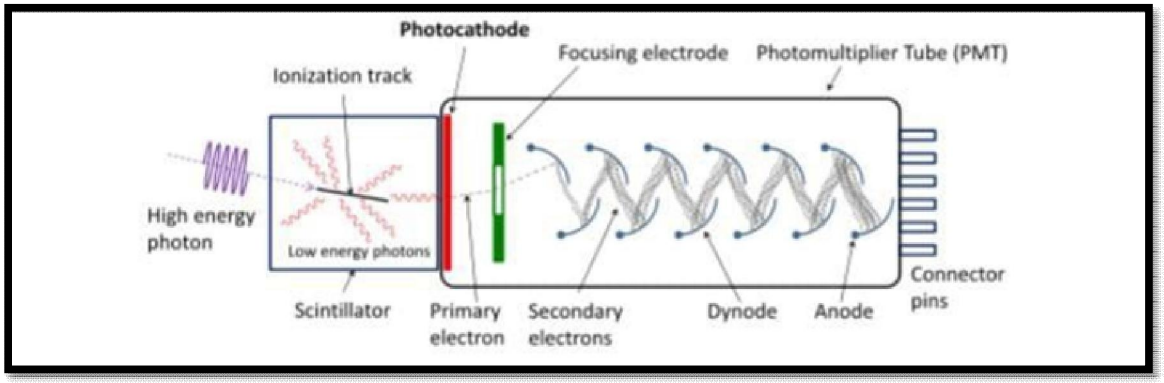

Fig.11 Photomultipller Tube Detector

Principle:

The principle employed in the detector is that multiplication of photoelectrons by secondary emission of electrons.

Construction:

It consists of a vacuum tube in which a primary photocathode coated with photosensitive materials like cesium oxide, potassium oxide or silver oxide is fixed which receives radiation from the sample.

Some eight to ten dyanodes are fixed each with increasing potential of 75-100 V higher than preceding one which acts as electron active surfaces. Near the last dyanode is fixed an anode which acts as electron collector electrode.

Working:

When the radiant energy falls on photocathode coated with photosensitive material, electrons arereleased. These released electrons when comes in contact with another electron active surface i.e. dyanode, generate more and more electrons and large current is generated by photomultiplication.

Photodiode detector:

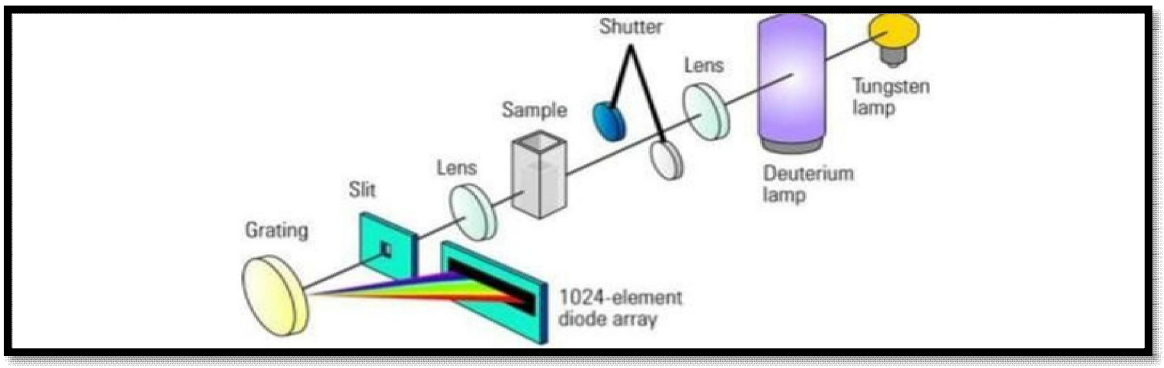

Fig.12 Photodiode detector

It 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

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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.

II. CONCLUSION

UV-visible spectroscopy is a dependable, easy-to-use, and reasonably priced method for determining the concentration of absorbing species when applied to pure compounds and utilised with the appropriate standard curve. UV-Vis spectroscopy is a vital technique for examining the optical properties of PMCs. steps required to ascertain the "identity, strength, quality and purity" of substances are included in the UV-visible spectroscopy pharmaceutical examination.Derivative techniques in spectroscopy often offer a powerful tool for a resolution enhancement, when signal overlaps or interference exists. Several specific signals were singled out for the components in the spectra of different derivative orders but the first- order derivative spectra seemed to be generally the most suitable for analytical aim. A derivative spectrum shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the max of the individual bands. Secondly, DS discriminates in favor of substances of narrow spectral bandwidth against broad bandwidth substances. All the amplitudes in the derivative spectrum are proportional to the concentration of the analyte provided that Beer's law is obeyed by the fundamental spectrum

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