



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

UV VISIBLE SPECTROSCOPY

Yogita B Ade¹ Ramkrashna S Chavan² Rushikesh S Garad³ DR. Santosh Jain⁴ Akhil A

Students¹ Student² Guide³ Principal⁴ Student⁵

ADITYA INSTITUTE OF PHARMACEUTICA BEED

DR. BABASAHEB AMBEDKAR TECHNOLOGICAL EDUCATION, LONERA

Abstract: Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. This article uses the term UV-Vis spectroscopy to include a variety of absorption, transmittance, and reflectance measurements in the ultraviolet (UV), visible, and near-infrared (NIR) spectral regions. These measurements can be at a single wavelength or over an extended spectral range. This article provides an overview of the technique and does not attempt to provide a comprehensive review of the many applications of UV-Vis spectroscopy in materials research. In this regard, many of the references were chosen to illustrate the diversity of applications rather than to comprehensively survey the uses of UV-Vis spectroscopy. Rapid and easy analytical methods are needed due to increasing number of multicomponent formulation bio therapeutic product and sample of complex matrix Number of ultraviolet spectrophotometer method use for these purposes. Ultraviolet and visible absorption Spectroscopy is the measurements of the attenuation of a beam of light after reflection from a sample surface

Keywords : *Ultraviolet-Visible-near Infrared (UV-VIS-NIR), Ultraviolet-Visible (UV-VIS), Light emitting diode (LED), Transmittance*

1) INTRODUCTION :

What is UV-Vis spectroscopy? UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. Ultraviolet-visible (UV-visible) spectrophotometry is primarily a quantitative analytical technique concerned with the absorption of near-UV (180-390 nm) or visible (390-780 nm) radiation by chemical species in solution. Spectroscopy in the ultraviolet (UV), visible (Vis) and near infrared (NIR) region of the electromagnetic spectrum • The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. • Spectroscopic investigations of solutions, gas phase crystals usually take place in transmission, but it is very difficult to obtain transparent films of powders (e.g., heterogeneous catalysts), making transmission experiments almost impossible. Alternatively, diffuse reflected light can be collected and this technique has been named diffuse reflectance spectroscopy (DRS) [2]. One of the advantages of DRS is that the obtained information is directly chemical in nature since outer shell electrons of the ions are probed. This provides information about state and coordination environment of ions in catalytic solids. The same holds for the nature species and different hydrocarbon species can be investigated. Furthermore, DRS is quantitative and can be in-situ conditions. The main disadvantage of the technique is that DRS spectra are complex, and usually encompass several broad and overlapping bands. In order to spectral analysis, techniques need to. This is especially important for in-situ time-resolved DRS studies because of the extensive to be handled. This chapter starts with a short overview of the principles of DRS. Theoretical as well as practical aspects will be discussed. The next section focuses on three examples in order to illustrate the potential and limitations of in situ NIR spectroscopy. UV visible spectroscopy is the classical and the most reliable technique for qualitative and quantitative analysis of

organic compounds visible spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample.

2) PRINCIPLE OF UV VISIBLE SPECTROSCOPY:-

When radiation in the wavelength range 10 to 780 nm passes through the solution of the compound electron get excited from lower energy level to higher energy level during this process it absorption some energy to the solution. The difference between the energies of ground state and higher excited state is called as ΔE and this energy is equal to the amount of UV radiation absorb by the molecule. UV visible spectroscopy is the most important techniques for qualitative or quantitative analysis it is also called as electronic spectroscopy. The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. When the matter absorbs the light, it undergoes excitation and de-excitation. resulting in the production of a spectrum. When matter absorbs ultraviolet radiation, the electrons present in it undergo excitation. This causes them to jump from a ground state (an energy state with a relatively small amount of energy associated with it) to an excited state (an energy state with a relatively large amount of energy associated with it). It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it. Spectrophotometry is a procedure for determining how much light is reflected by a chemical material by measuring the strength of light as a light beam travels through the sample solution. The fundamental theory is that light is absorbed or emitted over a certain wavelength spectrum by each compound. Spectroscopy is known as the measurement and interpretation of electromagnetic radiation emitted or absorbed, when ions, atoms or molecules of a sample move from one energy state to another energy state. Ultraviolet-Visible spectroscopy UV-Vis spectroscopy is a type of absorption spectroscopy, which utilizes the radiation in the UV range and adjacent visible range of the electromagnetic radiation spectrum. The absorption wavelength mainly ranges from 100-700 nm

3) ELECTRONIC TRANSITION:-

The absorption of ultraviolet or visible radiation generally results from excitation of bonding electron as a consequence, the wavelength of absorption peaks can be correlated with the types of bonds that exist in the species under study UV visible spectroscopy involve transition of electrons to from lower energy level to higher energy level. When any molecule absorb UV radiation their outermost electrons absorb the radiation and get excited to higher energy level.

● THREE TYPES OF ELECTRON :-

1. π ELECTRON

2. SIGMA ELECTRON

3. N-ELECTRON

1. π ELECTRON :- Compounds having double and triple bonds undergo this type of transition electron. Example -- Benzene. The bonding molecular orbital component of a pi bond. The orbital of ethylene's carbon-carbon pi bond has two orbital lobes, one above the plane of the atoms, and another below the plane. This is a bonding molecular orbital. The plane containing the atoms is also the pi orbital's one node.

2. SIGMA ELECTRON :- These electrons present in all single bonded compounds. example-methane, propane. Sigma bonds are the strongest type of covalent chemical bond. They are formed head-on overlapping between atomic orbitals. Sigma bonding is most simply defined for diatomic molecules using the language and tools of symmetry groups.

3. N ELECTRON (non bonding electron) :- These electrons are not involve in bond formation. They are absolutely free or easily available. Example nitrogen oxygen sulfur. Non-bonding orbitals are often designated by the letter n in molecular orbital diagrams and electron transition notations.

FOUR TYPES OF ELECTRON TRANSITION :-**1) SIGMA TO SIGMA STAR****2) N TO SIGMA STAR****3) π TO π STAR****4) N TO π STAR**

1. SIGMA TO SIGMA STAR :- Saturated compounds undergo this type of transition. Example - methane, propane, ethane. Single bonded compounds. An electronic in a bonding Sigma orbital of a molecule is excited to the corresponding anti- bonding orbital by the absorption of radiation. In ethane, electrons of C-C bond appear to be involved. Because the strength of the C-C bond is less than that of the C-H bond, less energy is required for excitation thus the absorption peaks occurs at a longer wavelengths.

2. N- TO SIGMA STAR :-

This type of transition involves compound having at least one hetero atom with lone pair of electron. It is less than sigma to sigma star N to sigma star transition ($n \rightarrow \sigma^*$) involves saturated compounds with one hetero atom like oxygen, nitrogen, fluorine, chlorine, etc. Normally, saturated halides, alcohols, ethers, aldehyde, ketones, and amines participate in this type of transition. Absorption maxima for the formation of the n. sigma star state tend to shift to shorter wavelengths in the presence of polar solvents such as water or ethanol

3. N- TO π STAR :- Compounds having double and triple bonds undergo this type of transition. All aromatic compounds. Example - Benzene. These transitions involve moving an electron from a bonding a orbital to an antibonding **

orbital. They tend to have molar absorptivity on the order of 10,000 and undergo a red shift with solvent interactions (a shift to lower energy and longer wavelengths).

4. N TO π STAR :-

It involves very less energy as compare to other transition. Compounds having double and triple bonds at least one hetero atom undergo this type of transition. The "n" electrons (or the nonbonding electrons) are the ones located on the oxygen of the carbonyl group of tetraphenyclopentadienone.

4) CHROMOPHORE AND AUXOCHROME :

CHROMOPHORE :- This term used for any group which gives colour to the compound any group which absorb UV and visible radiation and shows pic in this spectrum is called as chromophore Both organic and inorganic molecules may exhibit absorption and emission of UV-VIS radiation. Molecular groups that absorb visible or UV light are called chromophores. A chromophore group is a functional group, not conjugated with another group, which exhibit a characteristic absorption spectrum in the ultraviolet or visible region. The chromophore is a region in the molecule where the energy difference between two separate molecular orbitals falls within the range of the visible spectrum. Visible light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state.

A chromophore is the section of a molecule that causes us to see color. The chromophore portion of the molecule will have alternating double bonds, or conjugated double bonds. For example, beta-carotene, the molecule responsible for the color in carrots, has many double bonds.

AUXOCHROMES:- Any group which dose not act as chromophore but it's presence shift the absorption maxima towards higher wavelengths. The colour of molecules may be interested by the groups called as auxochromes which generally do not absorb significantly in the 200-800 nm region, but will affect the spectrum of the chromophore to which it is attached. The most important auxochromes groups are OH, NH₂, CH₃ and NO₂ and their properties are acidic or basic An auxochrome is a functional group of atoms with one or more lone pairs of electrons when attached to a chromophore, alters both the wavelength and intensity of absorption.

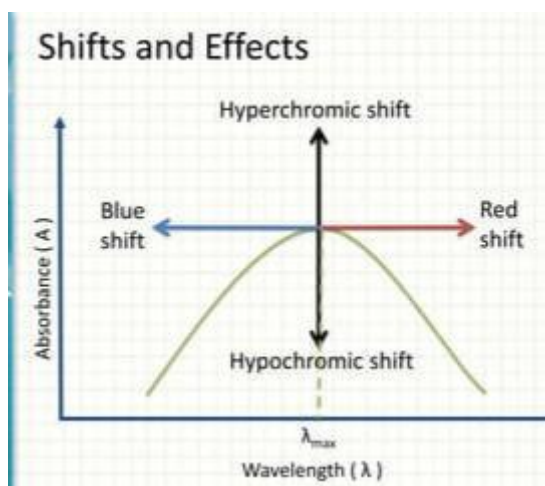


FIG.1 Shifting of absorption bond

FOUR SHIFTING OF CHROMOPHORE :-

- 1) **BATHOCHROMIC SHIFT**
- 2) **HYPISOCHROMIC SHIFT**
- 3) **HYPERCHROMIC SHIF**
- 4) **HYPOCHROMIC SHIFT**

1. **BATHOCHROMIC SHIFT (RED SHIFT) :-**

Shifting of absorption maxima towards higher wavelengths due to addition of auxochromes groups is called as **BATHOCHROMIC** shift and also called as red shift. Bathochromic shift is a change of spectral band position in the absorption, reflectance, transmittance, or emission spectrum of a molecule to a longer wavelength. Because the red color in the visible spectrum has a longer wavelength than most other colors, the effect is also commonly called a red shift. Due to the presence of an auxochrome, or solvent effect is called a bathochromic shift or red shift. For example, benzene shows A_{max} 256 nm and aniline shows max 280nm.

2) **HYPISOCHROMIC SHIFT (BLUE SHIFT) :-** • Shifting of absorption maxima towards lower wavelengths due to removal of auxo group is called as hypsochromic shift and also called as blueshift. Hypochromic shift is a change of spectral band position in the absorption, reflectance, transmittance, or emission spectrum of a molecule to a shorter wavelength. Because the blue color in the visible spectrum has a shorter wavelength than most other colors, this effect is also commonly called a blue shift.

• A hypsochromic shift is the shift of a peak or signal to shorter wavelength (higher energy). Also called a blue shift. For an absorption peak starting at $A_{max} = 550$ nm, a shift to higher wavelength such as 650 nm is bathochromic, whereas a shift to lower wavelength such as 450 nm is hypsochromic

2. **HYPERCHROMIC SHIFT:-** Shifting in the intensity of absorption maximum towards higher values of absorbance is called as hyperchromic shift. An increase in the absorption of ultraviolet light by a solution of DNA as these molecules are subjected to heat, alkaline conditions, etc. The shift is caused by the disruption of the hydrogen bonds of each DNA duplex to yield single-stranded structures. • The phenomenon of UV absorbance increasing as DNA is denatured is known as the hyperchromic shift. The purine and pyrimidine bases in DNA strongly absorb ultraviolet light. Double-stranded DNA absorbs less strongly than denatured DNA due to the stacking interactions between the bases.

4. **HYPOCHROMIC SHIFT:-**

Shift in the intensity of absorption maxima towards lower absorbance is called as hypochromic shift. The Hypochromic Effect describes the decrease in the absorbance of ultraviolet light in a double stranded DNA compared to its single stranded counterpart. Compared to a single stranded DNA, a double stranded DNA consists of stacked bases that contribute to the stability and the hypochromicity of the DNA.

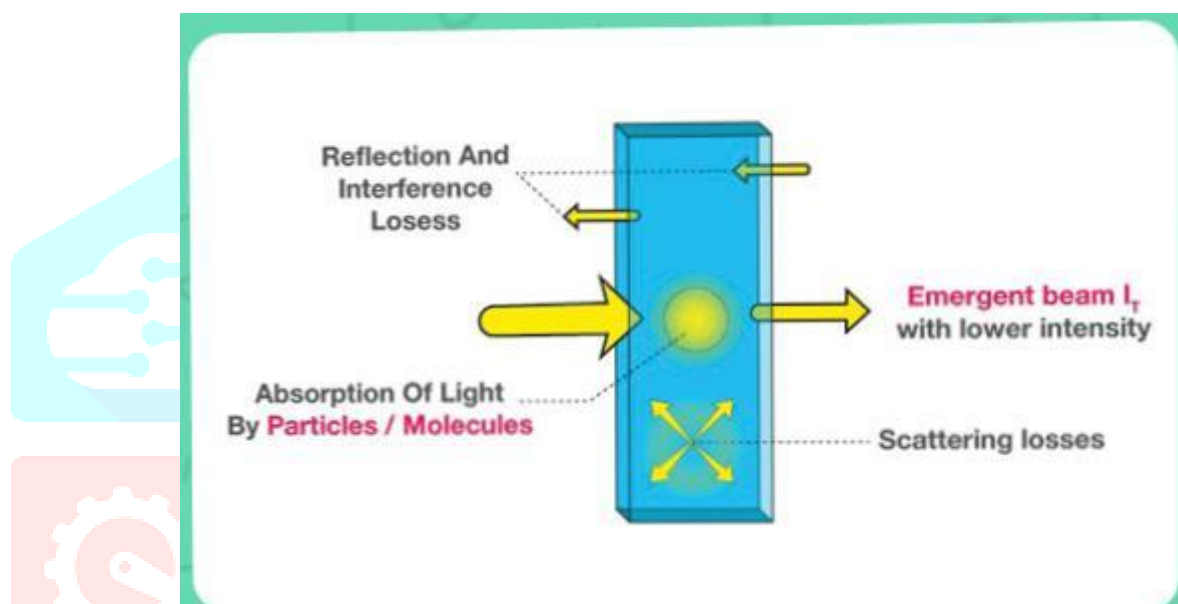
5) BEER-LAMBERT LAW :-

Beers Lamberts law states that the absorption 'A' of a substance in solution is directly proportional to the concentration of the solution whereas Lamberts law states that each layer of equal thickness of an absorbing medium absorbs an equal fraction of the radiant energy.

a) When passing through a transparent cuvette filled with sample solution, the light intensity is reduced proportional to the sample solution concentration. In other words, a higher concentrated sample solution will absorb more light. In addition the reduction the light intensity is also proportional to the length of the cuvette, a longer cuvette will be to a higher absorption of light.

b) The Beer-Lambert law states that there is a linear relationship between the concentration and the absorbance of the solution, which enables the concentration of a solution to be calculated by measuring its absorbance.

FIG NO 2: BEER-LAMBERT LAW



FORMULA-

$A = a b c$ Where,

Where,

A = Absorbance

a = Molecular absorbing coefficient of the species.

b = Absorbing layer (path length) and

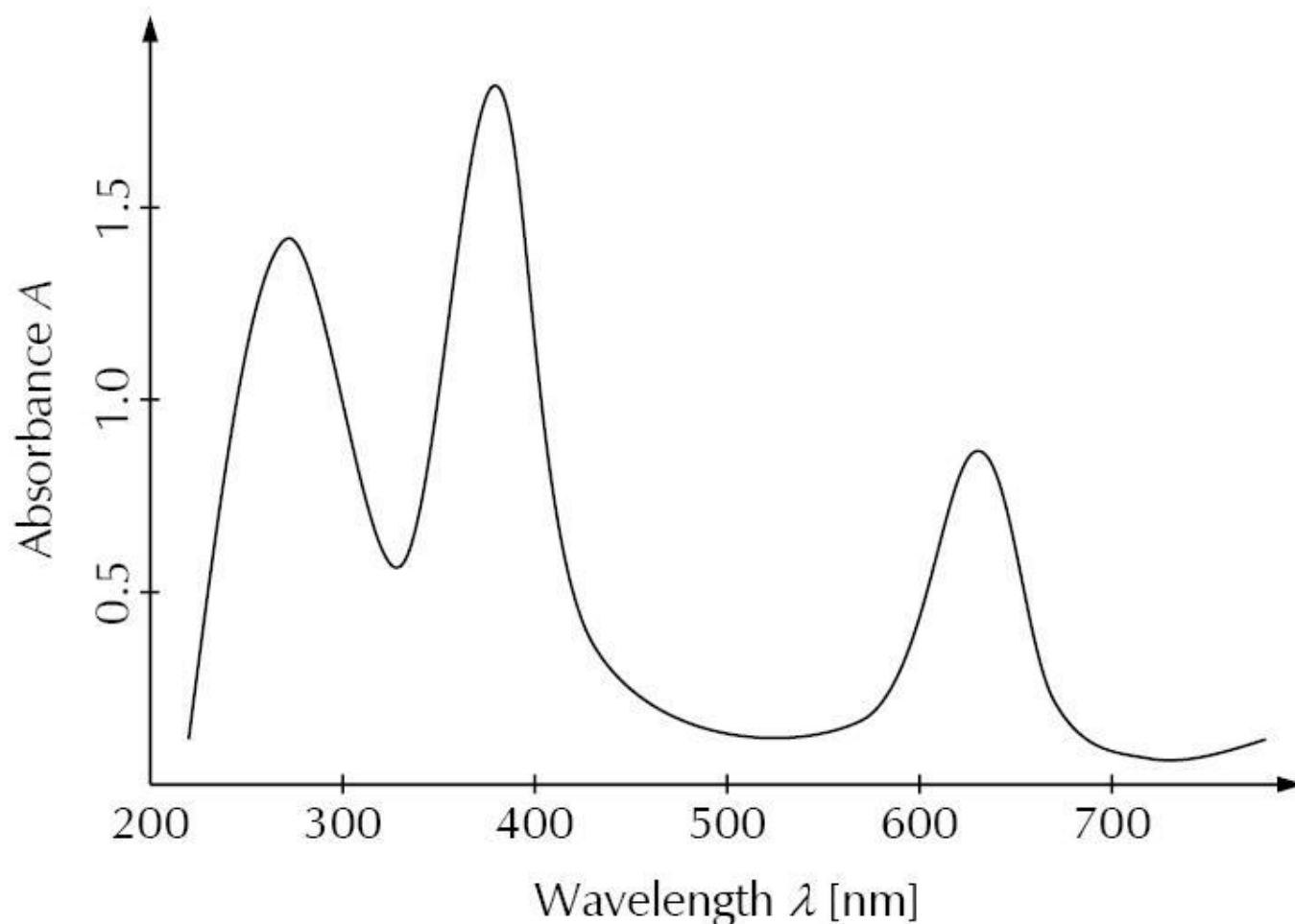
C = Molecular concentration of the absorbing species.

6) SPECTROSCOPY:- When an electromagnetic radiation is incident on a matter, phenomena like reflection, transmission, absorption are occurring. Spectroscopy is the study of interaction of electromagnetic

radiation with matter based on the Bohr-Einstein Frequency relationship $E = h\nu$ here h is the proportionality constant called planks constant and ν is frequency. Measurements of radiation intensity as a function of wavelengths is described by spectroscopy

FIG NO 03: SPECTROSCOPY

Spectroscopy is the field of study that measures and interprets the electromagnetic spectra that result from the



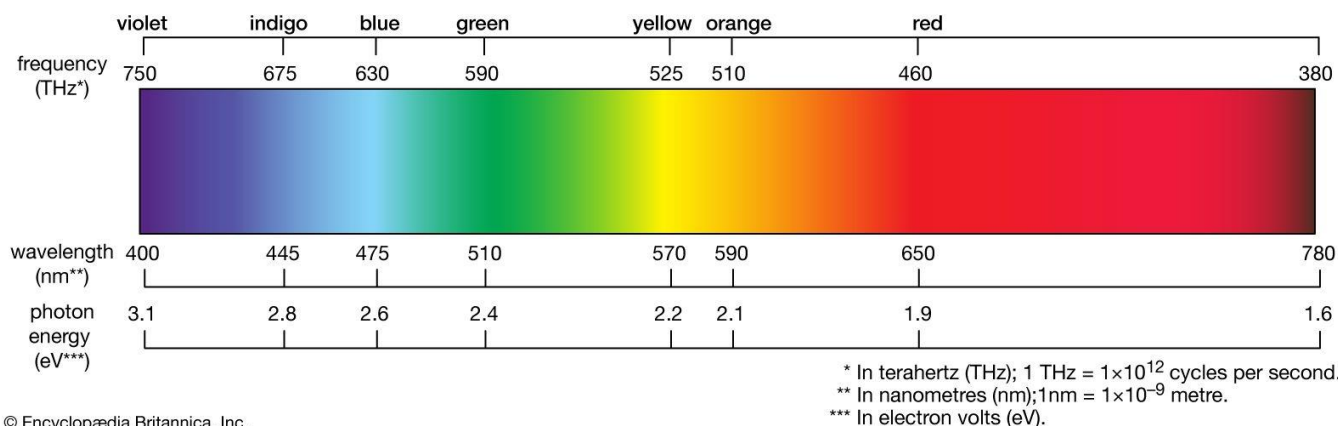
interaction between electromagnetic radiation and matter as a function of the wavelength or frequency of the radiation. Spectroscopy is used in physical and analytical chemistry to detect, determine, or quantify the molecular and/or structural composition sample. Each type of molecule and atom will reflect, absorb, or emit electromagnetic radiation in its own characteristic way.

Spectroscopy is the study of the absorption and emission of light and other radiation by matter. It involves the splitting of light (or more precisely electromagnetic radiation) into its constituent wavelengths (a spectrum), which is done in much the same way as a prism splits light into a rainbow of colours.

SPECTRUM :- The spectrum is formed by electromagnetic waves and the wavelength is varies. Plural spectra spectra or spectrums.: a continuum of color formed when a beam of white light is dispersed (as by passage through a prism) so that its component wavelengths are arranged in order. Autism is known as a "spectrum" disorder because there is wide variation in the type and ● severity of symptoms people experience.

• See figure –

Light, the visible spectrum



© Encyclopædia Britannica, Inc.

FIG.04 ELECTROMAGNETIC SPECTRUM

. When a narrow beam of light is allowed to pass through a prism, grating it is dispersed into seven color from red, violets and band is called spectrum.

Fig.5 : Glass prism dispersion.

When white light is passed through a glass prism it splits into its spectrum of colours (in order violet, indigo, blue, green, yellow, orange and red) and this process of white light splitting into its constituent colours is termed as dispersion.

A word was first used scientifically in optics to describe the rainbow of colours invisible light after passing through a prism as scientific understanding of light advanced it came to apply to the entire electromagnetic spectrum

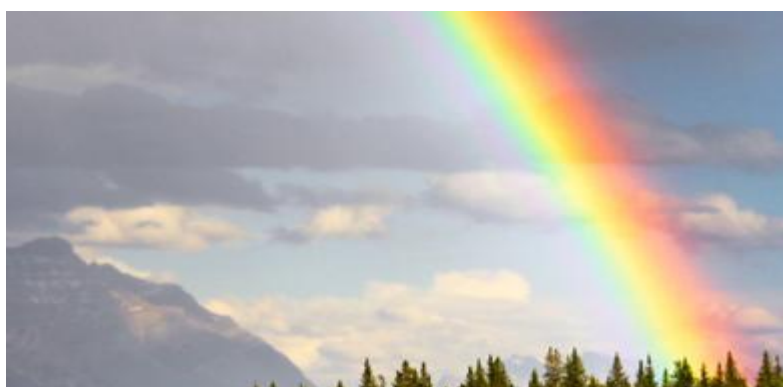
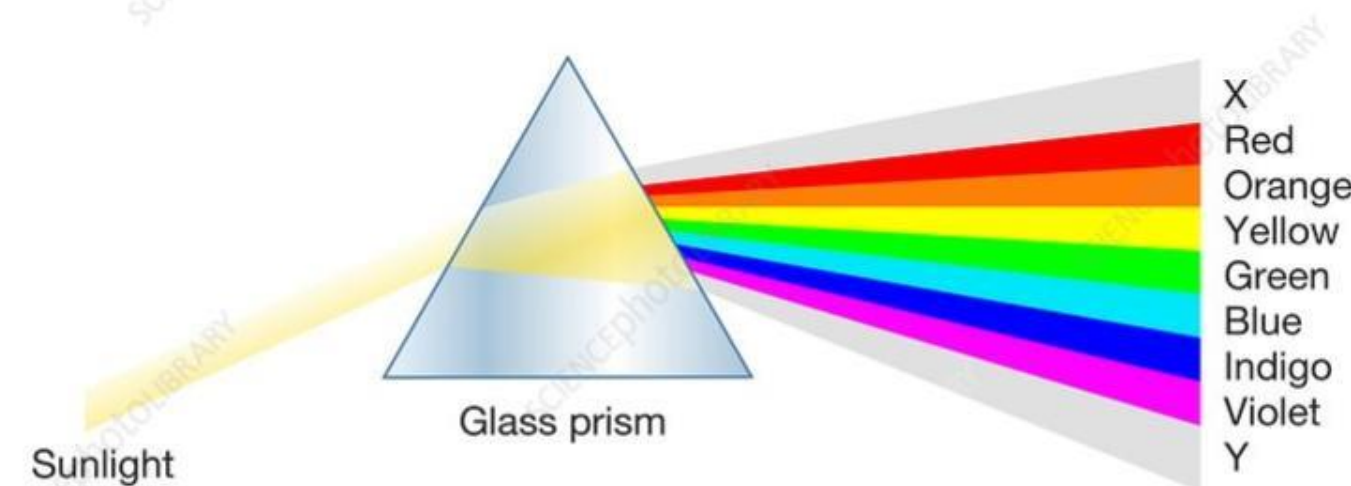


FIG . 06 RAINBOW

Colors A rainbow shows up as a spectrum of light: a band of familiar colors that include red, orange, yellow, green, blue, and violet. The name "Roy G. Biv" is an easy way to remember the colors of the rainbow, and the order in which they appear: red, orange, yellow, green, blue, indigo, and violet.

This is the order of the wavelengths of visible light starting with red (the longest) and ending with violet (the shortest). It is also the order that colors appear in a rainbow!

Rainbows appear when sunlight shines through water droplets suspended in the atmosphere.

This is the order of the wavelengths of visible light starting with red (the longest) and ending with violet (the shortest). It is also the order that colors appear in a rainbow! Rainbows appear when sunlight shines through water droplets suspended in the atmosphere.

The only difference between UV and IR light versus visible light is the wavelength.

UV-VISIBLE SPECTROSCOPY:-

Ultraviolet visible spectrum - can be generated when ultraviolet light and visible light (200-900) are absorbed by materials. The spectrum can be used to analyze the composition and the structure of the material. For a particular wavelength in the ultraviolet visible ranges, the absorption degree is proportional to the components of the material.

UV spectroscopy or UV-visible spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum.

Ultraviolet-visible (UV/Vis) spectroscopy is based on the absorption of the electromagnetic radiation in UV/Vis region, with the wavelength ranges of 200-400 nm, called 'ultraviolet spectroscopy, and 400-800 nm, called 'visible spectroscopy

UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration.

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. Ultraviolet-visible (UV-Vis) spectroscopy is a widely used technique in many areas of science ranging from bacterial culturing, drug identification and nucleic acid purity checks and quantitation, to quality control in the beverage industry and chemical research

UV spectroscopy or UV-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum.

6) USES OF UV VISIBLE SPECTROSCOPY:-

1. It is used to study aromatic conjugation within molecules also used to differentiate whether the system is aromatic or aliphatic.

2. Used to major multiple bonds within compounds.

3. Used to differentiate alpha, beta, unsaturated compounds.

4. Ultraviolet-visible (UV-Vis) spectroscopy is a widely used technique in many areas of science ranging from bacterial culturing, drug identification and nucleic acid purity checks and quantitation, to quality control in the beverage industry and chemical research.

5. The advantage of an Ultraviolet Visible Light Spectrophotometer (UV-Vis spectrophotometer) is its quick analysis ability and easy to use. In astronomy research. an UV/Vis spectrophotometer helps the scientists to analyze the galaxies, neutron stars, and other celestial objects.

6. For semiconductors, UV-vis spectroscopy offers a convenient method of estimating the optical band gap, since it probes electronic transitions between the valence band and the conduction band.

7. While interaction with infrared light causes molecules to undergo vibrational transitions, the shorter wavelength, higher energy radiation in the UV (200-400 nm) and visible (400-700 nm) range of the electromagnetic spectrum causes many organic molecules to undergo electronic transitions.

8. What this means is that when the energy from UV or visible light is absorbed by a molecule, one of its electrons jumps from a lower energy to a higher energy molecular orbital.

9. UV-Vis spectrophotometers are used in almost every laboratory in the world due to their measurement versatility. This guide is a snapshot of the main spectrophotometer uses, with links to application notes by measurement technique and industry type.

10. UV/VIS spectroscopy is used for the quantitative determination of different substances.

8) INSTRUMENTATION :-

1) SOURCES OF LIGHT

2) MONOCHROMATOR 3) SAMPLE SOLUTION IN CUVETTE

4) PHOTO DETECTOR

5) READOUT DEVICES

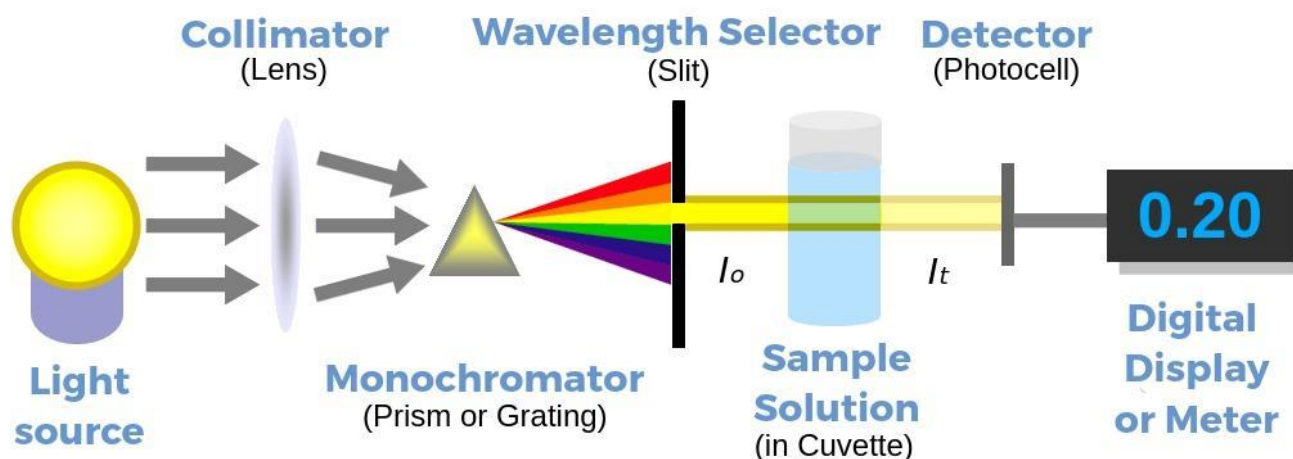


FIG NO. 07 COMPONENTS OF SPECTROMETER

A spectrophotometer is an analytical instrument used for the objective calculation of visible light. UV light, or infrared light emission or reflection. Spectrophotometers measure intensity as a function of the wavelength of the light source.

A spectrophotometer measures the number of photons emitted to estimate the intensity of light spectra absorbed and transmitted by a sample. This provides information on the amount of a compound in the sample. Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance.

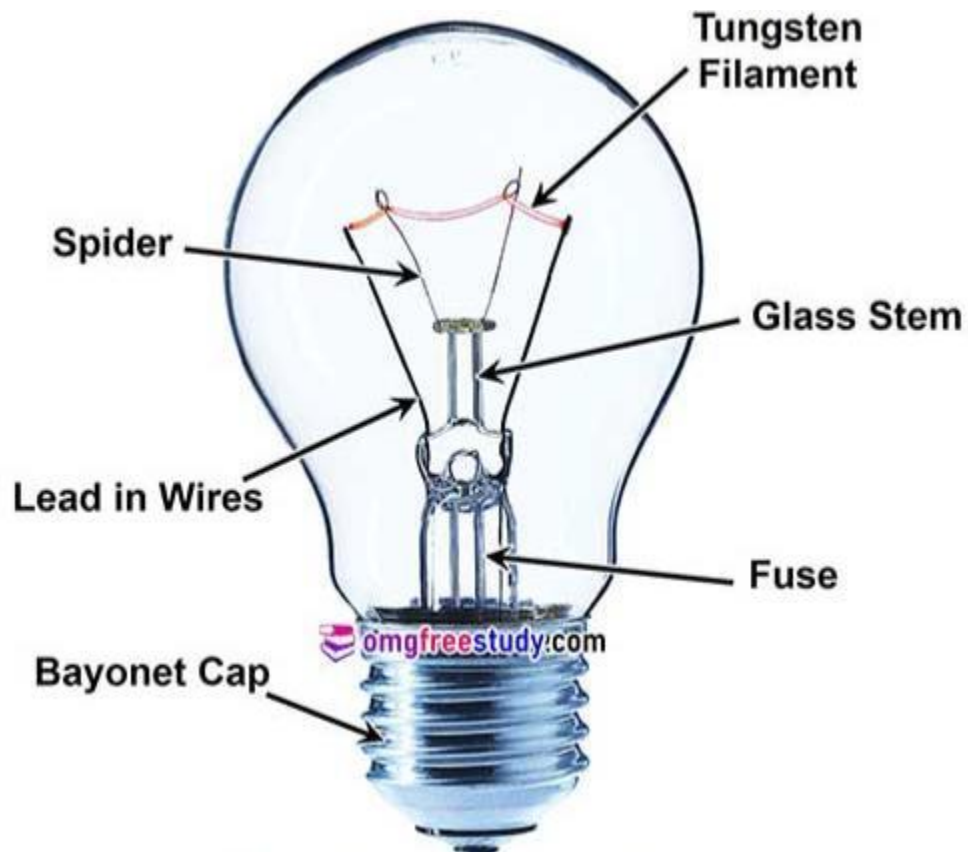
A spectrophotometer is an analytical instrument used for the objective calculation of visible light. UV light, or infrared light emission or reflection. Spectrophotometers measure intensity as a function of the wavelength of the light source.

A spectrophotometer measures the number of photons emitted to estimate the intensity of light spectra absorbed and transmitted by a sample. This provides information on the amount of a compound in the sample. Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance.

• SOURCES OF LIGHT :-

Radiation sources should be economic. It should be long lasting should be stable over time. Example - Tungsten lamp, hydrogen discharge lamp, Deuterium lamp, xenon discharge lamp, mercury are

● Part of the UV and visible radiation source is Tungsten lamp. A light source is anything that makes light, whether natural and artificial. Natural light sources include the Sun and stars. Artificial light sources include lamp posts and televisions.

**FIG NO. 07 TUNGSTEN LAMP**

Tungsten filament incandescent lamps, particularly tungsten halogen lamps, are often used in illumination systems. UV radiation sources is Deuterium or hydrogen lamp. Range of wavelengths 200-400 nm. A deuterium are lamp is a low-pressure gas-discharge light source often used in spectroscopy when a continuous spectrum in the ultraviolet region is needed. Plasma "are" or discharge lamps using hydrogen are notable for their high output in the ultraviolet, with comparatively little output in the visible and infrared.

**FIG NO 08 : DEUTERIUM LAMP**

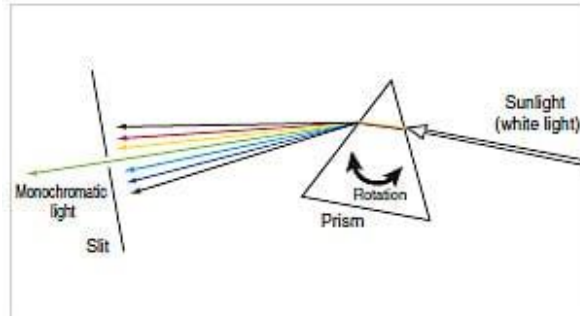
MONOCHROMATOR :-

It is a device that breaks the polychromatic radiation into components wavelengths. It converts polychromatic light into monochromatic light. A monochromator is an optical device that transmits a mechanically selectable narrow band

of wavelengths of light or other radiation chosen from a wider range of wavelengths available at the input.

See figure-

FIG NO 09: MONOCHROMATOR



A monochromator is an optical instrument which measures the light spectrum. Light is focused in the input slit and diffracted by a grating. In this way, only one color is transmitted through the output slit at a given time. Spectra are then recorded wavelength by wavelength, rotating the grating. There are modified types of monochromator, for example the Fastie-Ebert monochromator with a common collimator/refocusing mirror, and devices with two gratings for better resolution. The quality of the diffraction grating can be important for the performance: Its diffraction efficiency determines the power losses.

- Monochromator is a mechanism that emits monochromatic light from a light source. A dispersive element, generally a prism or diffraction grating, is used to create the monochromatic light.

- There are two types of monochromator: prisms and grating systems.

The most common materials for laboratory X-ray monochromator are pyrolytic graphite for broad band use and either silicon, germanium, or quartz for narrow band use.

THE MONOCHROMATOR UNIT CONSISTS OF:-

- **ENTRANCE SLIT :-** Definition narrow beam of radiation from source provide a narrow optical image of the radiation source

A thin slit in an opaque screen by which light enters a spectrometer. The spectrum thus formed is the image of this slit in each wavelength of light present.

- **COLLIMATING MIRROR :-**

A collimator is a device which narrows a beam of particles or waves. To narrow can mean either to cause the directions of motion to become more aligned in a specific direction, or to cause the spatial cross section of the beam to become smaller. ● In optics, a collimator may consist of a curved mirror or lens with some type of light source and/or an image at its focus.

DIFFRACTION GRATING OR PRISM :-

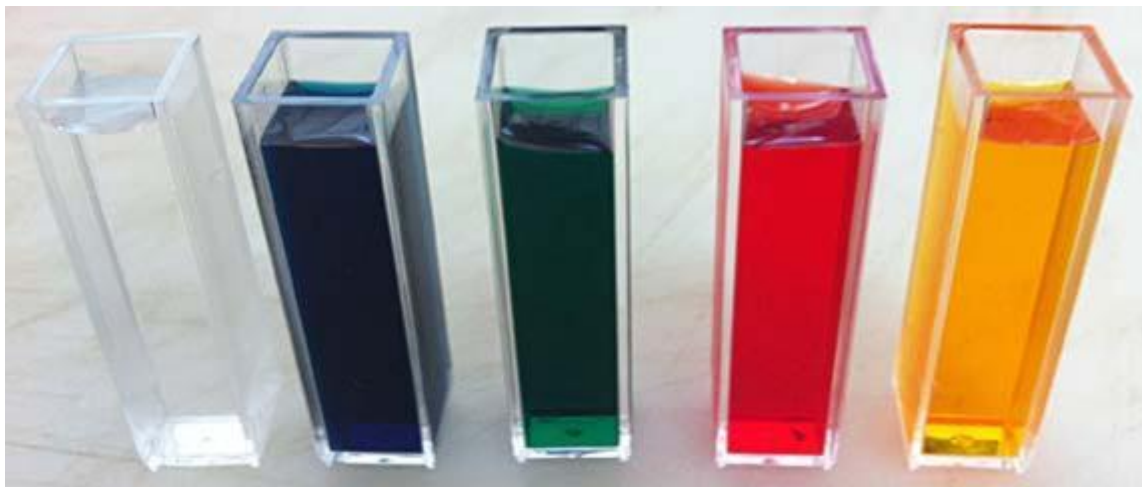
- Made of quartz disperses the light into specific wavelengths. The prism achieves dispersion due to the difference in the material refractive index according to the wavelength. However, the diffraction grating uses the difference in diffraction direction for each wavelength due to interference.

FOCUSING MIRROR :-

- Capture the dispersed light and sharpens the same to the sample via exit slit.
- A focusing mirror re-form the image of the entrance slit and focuses it onto the exit slit.
- Focusing mirrors (concave mirrors) are characterized by one concave surface with a high reflection coating. The reflective coating allows focusing of a light beam.
- When parallel light rays fall on the surface of a concave mirror, all the rays after reflection converge (meet) at a single point (focus). Hence, a concave mirror is also called a converging mirror.

SAMPLE SOLUTION IN CUVETTE :-

- Liquid sample is usually contained in a cell called as cuvette. Fingerprints are droplets of water disrupt light rays during measurements.
- Cuvette from Quartz can be used in UV as well as in visible spectroscopy. Cuvette from glass is suitable for visible but not for UV spectroscopy because it absorb UV radiation.
- See figure-

**FIG NO. 10 : sample solution in cuvette.**

Often the sample is a solution, with the substance of interest dissolved within. The sample is placed in a cuvette and the cuvette is placed in a spectrophotometer for testing. The cuvette can be made of any material that is transparent in the range of wavelengths used in the test.

A cuvette is a type of sample holder for liquid samples. Often, they are made of plastic, borosilicate glass, or quartz. Stellar Net offers glass cuvettes for experiments in the visible or NIR ranges and quartz cuvettes for experiments in the UV range. Cuvettes also come with two or four polished sides.

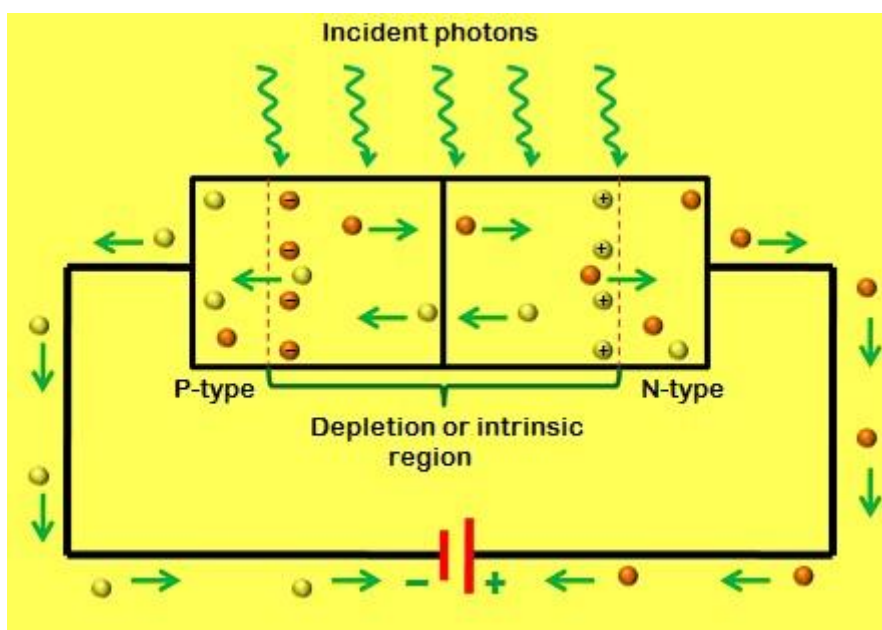
When you rinse the cuvettes with water, the water dilutes the sample (concentration changes). Therefore, you must rinse the cuvettes with the sample (rather than water) so you can avoid changing the concentration of your sample.

PHOTO DETECTOR :

A photo detector is a semiconductor device which converts light energy to electrical energy. It consists of a sample P-N junction diode and is designed to work in reverse biased condition. The photons approaching the diode are absorbed by the photodiode and current is generated.

Photodetectors, also called photo sensors, are sensors of light or other electromagnetic radiation. There is a wide variety of photodetectors which may be classified by mechanism of detection, such as photoelectric or photochemical effects, or by various performance metrics, such as spectral response.

seen fig of photodiode

**FIG NO 11 photodiode**

Photodetectors are sensors that can convert the photon energy of light into electrical signal. The photodetector is also called an optical receiver. It converts the variation in optical power into a corresponding variation in the electric current. As compare to the optical transmitter the design of optical receiver is more complicated just because the receiver must detect weak, distorted signals and then make decisions on what type of data was sent based on an amplified version of this totally distorted signal.

TYPES OF DETECTOR :-

1. BARRIER LAYER CELL DETECTOR
2. PHOTO TUBE DETECTOR
3. PHOTO MULTIPLIER TUBE DETECTOR
4. SILICON PHOTODIODE DETECTOR

1. BARRIER LAYER CELL DETECTOR :-

● **Barrier layer** cell detector is also called as photo volatile cell. It consists of semiconductor (selenium) which is deposited on strong base ion A very thin layer of silver or gold is placed over the surface of semiconductor.

• To act as collector electro radiation when falls on the surface electron on produce. Electron are produce and their it is converted into electric current. To dose not required power supply

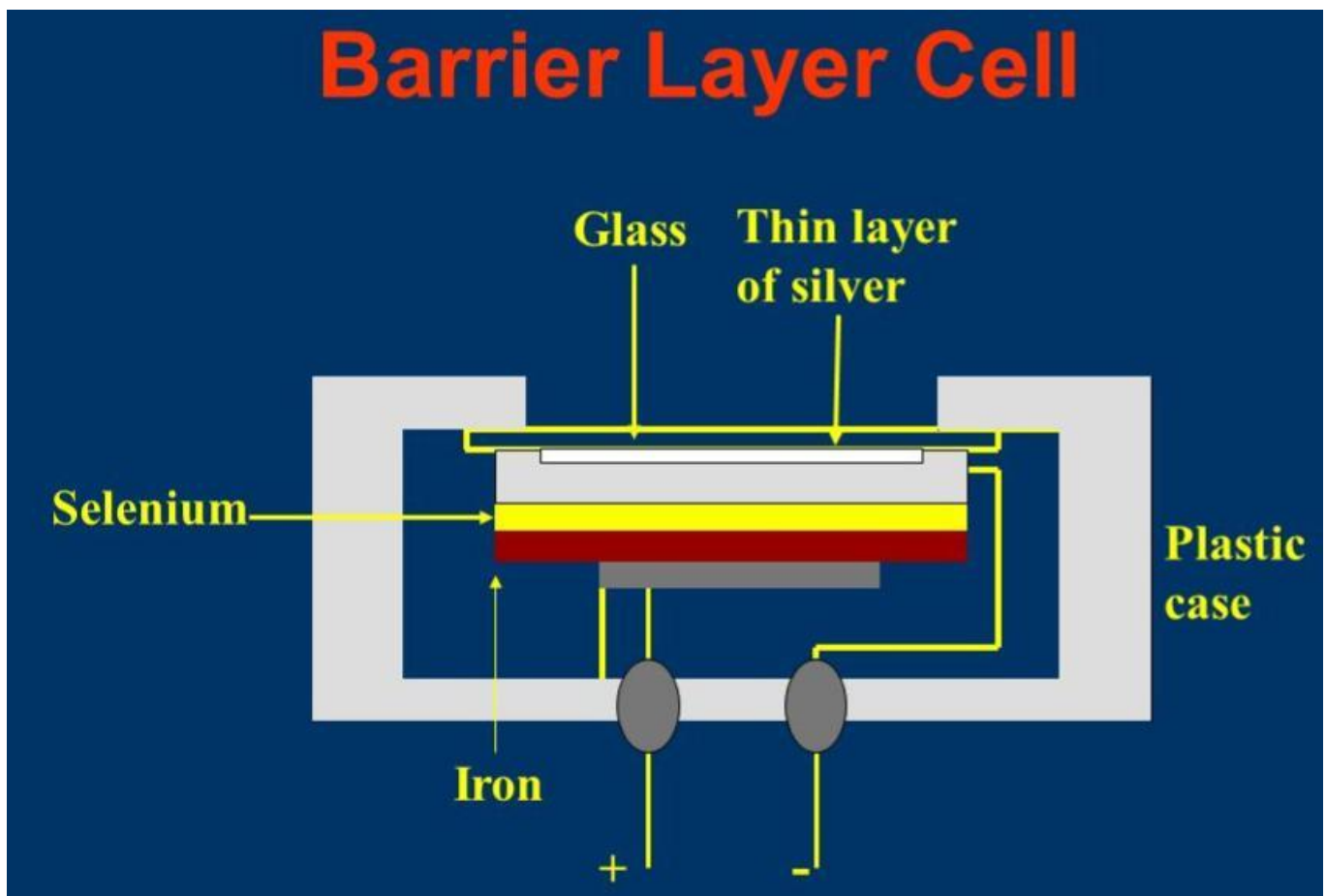


FIG NO. 12 BARRIER LAYER CELL

A photoelectric detector which is made of iron coated with a semiconductor film when light from 250-750nm hits this cell, you get a current; this is a cell which is mainly good for intense light sources, because there is not a huge signal enhancement. Also known as a self-generating barrier layer cell. A photoelectric detector that converts radiant flux directly into electrical current. Generally, it consists of a thin silver film on a semiconductor layer deposited on an iron substrate.

2. PHOTO TUBE DETECTOR :-

UV Visible Spectroscopy

- Photo tube detector is also called as photo electric cell detector. When the light is incident upon the photo cell it get converted into electric current.
- The current which is created in between cathode and anode is regarded as a measure of radiation on the detector.

• A phototube or photoelectric cell is a type of gas-filled or vacuum tube that is sensitive to light. Such a tube is more correctly called a 'photo emissive cell' to distinguish it from photovoltaic or photoconductive cells.

5) READOUT DEVICES:-

● The signal from detector is received by recording system. Recording is done by recording pen. The earliest instruments were simple and directly connected the amplifier detector signal to a chart recorder. Nowadays, all experimental settings are controlled by a computer and detector signal are digitized processed and stored. The device used to accept the signal transmitted from the analyzer and display it for use in process operation or other decision making. Displayed as a measured property or concentration in the accepted units of that property or concentration.

• Several types of readout devices are used in modern instruments. These devices include Digital Meters, Recorders, Cathode-Ray Tubes, LCD panels, and Computer Displays. This image shows an example of what a readout will look like from the signal processed. It is the expected output for the determination of lead. The process of removing information from an automatic device (such as a computer or sensor) and displaying it in an understandable form. A digital readout (DRO) is a numeric display, usually with an integrated keyboard and some means of numeric representation. Its integral computer reads signals generated by linear encoders or (less frequently) rotary encoders installed to track machine axes, using these measures to keep track of and display to a machine operator the work piece position (e.g. milling machines), or tool position (lathes, grinders, etc.) in space.

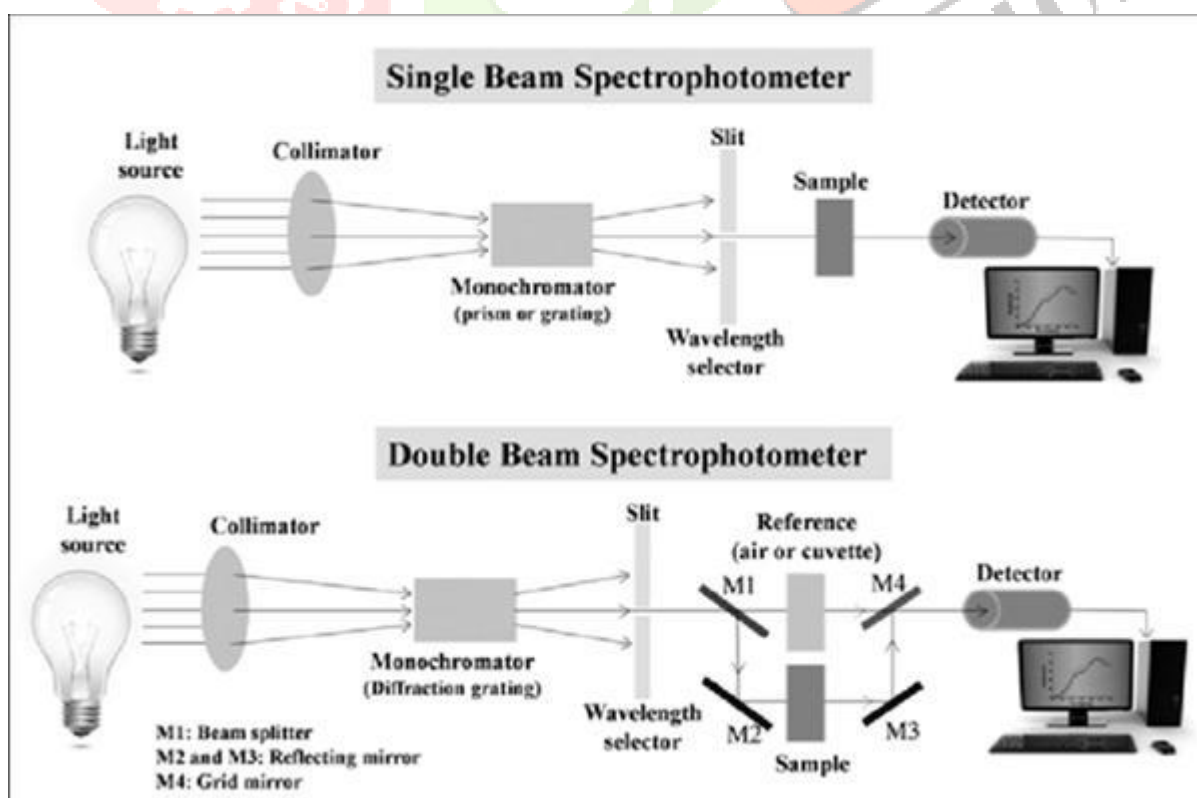
Digital screen to record an UV spectrograph with absorbance against the wavelengths. Definitions of recording system. Audio system for recording sound. Type of audio system, sound system. A system of electronic equipment for recording or reproducing sound.

Signal. Many measuring instruments also record the quantities they measure; see Category: Measuring instruments. Digital recording is the process of storing binary data in the form of a two-level magnetization pattern. Digital recording is also referred to as saturation recording since channel bits are recorded by saturating the media either in the positive or negative direction.

9) TYPES OF SPECTROPHOTOMETER :-

A spectrophotometer is an analytical instrument used for the objective calculation of visible light, UV light, or infrared light emission or reflection. Spectrophotometers measure intensity as a function of the wavelength of the light source. A spectrophotometer measures the number of photons emitted to estimate the intensity of light spectra absorbed and transmitted by a sample. This provides information on the amount of a compound in the sample.

FIG.NO 13: TYPES OF SPECTROPHOTOMETER



There are generally two types of spectrophotometers: a single beam, and double beam. Single beam spectrophotometers use a single beam of light - visible or UV- which passes through a sample in a cuvette. According to different wavelengths and application fields, spectrophotometers can be divided into visible spectrophotometer, ultraviolet visible spectrophotometer, infrared spectrophotometer, fluorescence spectrophotometer and atomic absorption spectrophotometer.

SINGLE BEAM SPECTROPHOTOMETER :

Single beam spectrophotometers determine color by measuring the intensity of the light sources before versus after a test sample is inserted. This light source is modulated (turned on and off) to differentiate the light coming from the light source versus the light coming from the flame. A UV-Vis spectrophotometer is used to determine the absorption of light from a sample and can be used as a detector for HPLC. A sample is placed in the UV/VIS beam and absorbance versus wavelength is measured UV-visible spectrophotometers have five main components: the light source, monochromator, sample holder, detector, and interpreter. The standard light source consists of a deuterium arc (190-330 nm) and a tungsten filament lamp (330-800 nm), which together generates a light beam across the 190-800 nm spectral range.

DOUBLE BEAM SPECTROPHOTOMETER :-

A double beam spectrophotometer is an instrument that determines the absorption of light in liquid or gas samples in graduated cylinders. Its components are: Monochromator, Detector, Light source..

For a double-beam UV/Vis Spectrophotometer: A beam of light from a visible and/or UV light source is separated into its component wavelengths by diffraction grating or monochromator. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device.

- An important advantage of a double-beam spectrophotometer over a single-beam spectrophotometer is that a double beam instrument permits compensation for source power fluctuations greatly improving S/N and extension to dilute solution samples and measurements with gases.

A typical double beam UV-visible spectrophotometer consists of: A light or energy source, which is typically a lamp. A filter or a monochromator that is attached to the device for the selection of the wavelength of light.

A place for cuvettes to read the measurements.

10) APPLICATION OF UV VISIBLE SPECTROSCOPY:-

Used to determine impurities

Determine double bond and triple bonds.

Determine aromatic and aliphatic conjugation

Alpha and beta unsaturation.

Quantitative and qualitative analysis.

Pharmacokinetic properties.

Structural elucidation in compound.

Ultraviolet-visible (UV-Vis) spectroscopy is a widely used technique in many areas of science ranging from bacterial culturing, drug identification and nucleic acid purity checks and quantitation, to quality control in the beverage industry and chemical research.

Growth of metal nanoparticles in polymeric network or growth of polymeric network around metal nanoparticle core can be studied by using UV/Vis spectroscopy. This technique can also be used for investigation of various applications of hybrid materials in catalysis, photonics, and sensing.

Spectrophotometry is used for the quantitative determination of a great variety of substances in solution. These range from water and waste water analysis, pharmaceutical quality control and food analysis. ● UV/Vis molecular absorption is routinely used for the analysis of narcotics and for drug testing. The presence of these drugs can be confirmed from their absorption maxima i.e. λ_{max} or by comparing the UV/visible spectra of these drugs with spectra of authentic sample.

Atomic absorption spectroscopy is utilized across many industries and is instrumental in the detection of metals within a sample. As such, this process is commonly utilized in pharmacology, archaeology, manufacturing, mining, and forensics.

Besides chemical analysis, there are many physical methods performed to characterize purity and determine the mixture of substances. Even though there are many techniques such as determination of melting point, refractive index, and density. Ultraviolet and Visible light spectroscopy is widely applied in a market segment, research areas, production, and quality control for the classification and study of substances.

11) CONCLUSION :

UV-visible spectroscopy is a valid, simple and cost effective method for determining the concentration of absorbing species if applied to pure compounds, and used with the appropriate standard curve. Ultra violet/visible spectroscopy is an analytical technique that is used to determine qualitatively and quantitatively for the estimation of different ions. It is a powerful technique for resolution enhancement when signal overlaps interference occurs. This technique may also be used in many other industries. For example, measuring a colour index is useful for monitoring transformer oil as a preventative measure to ensure electric power is being delivered safely.

12) REFERENCE :-

- 1) Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Belmont, CA: Thomson Brooks/Cole; 2007. Pp. 169-173.
- 2) Leong YS, Ker PJ, Jamaludin MZ, Nomanbhay SM, Ismail A, Abdullah F, et al. UV-vis spectroscopy: A new approach for assessing the color index of transformer insulating oil. Sensors. 2018;18(7):2175
- 3) Ilev I, Waynant R. Ultraviolet Spectroscopy and UV Lasers. New York: Marcel Dekker; 2002
Wavelength Accuracy in UV/VIS Spectrophotometry [Internet]. Available from <https://www.mt.com/ch/en/home/library/white-papers/lab-analytical->
- 5) Metha A. Principle. PharmaXChange.info [Internet]. 2011. Available from: <http://pharmaxchange.info/press/2011/12/ultraviolet-visible-uv-vis-spectroscopy-principle/> [Accessed: 8 September 2021]
- 6) Atole DM, Rajput HH. Ultraviolet spectroscopy and its pharmaceutical applications-Abrief review. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(2):59-66
- 7) Metha A. Derivation of Beer-Lambert Law. Pharma XChange.info [Internet]. 2012. Available from: <http://pharmaxchange.info/press/2012/04/ultraviolet-visible-av-vis-spectroscopy-derivation-of-beer-lambert-law/> [Accessed: 8 September 2021]
- 8) Reserved M-TII All Rights. Spectrophotometry Applications and Fundamentals.
Atole D, Rajput H. Ultraviolet spectroscopy and its pharmaceutical applications-A brief review. Asian Journal of Pharmaceutical and Clinical Research, 2018;11:59.
- 11) Chatwal GR. Instrumental methods of chemical analysis. Pg. no. 2.116-2.122.
- 12) Sharma YR. Elementary Organic analysis, Principles and chemical applications. Pg. no.12 -14

