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A REVIEW ARTICLE ON VISIBLE SPECTROSCOPY

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Abstract:

This study delves into the application of visible spectroscopy as a powerful analytical tool in the pharmaceutical field. Visible spectroscopy, leveraging the interaction of light with matter, provides valuable insights into the electronic transitions within compounds. In this research, we focus on its use in drug analysis, with a particular emphasis on Drug gliclazide The spectral fingerprints obtained through visible spectroscopy allow for precise characterization and quantification of Drug gliclazide showcasing the technique's potential for quality control and formulation optimization in the pharmaceutical industry. One of the earliest instrumental techniques for analysis is visible spectroscopy. Many different types of materials can be characterized using Visible spectroscopy. The delivered details based on the degree of absorption or transmittance of a varied wavelength of beam light and the various responses of samples. Radiant energy absorption by materials can be quantitatively described using the general law known as Beer's law.

KEYWORDS: *Visible spectroscopy, Colorimetry, Spectrophotometers, Accuracy, Precision, Robust.*

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INTRODUCTION VISIBLE SPECTROSCOPY:

Spectroscopy is a measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample moves from one energy state to another energy state. This change may be from ground state to excited state or excited state to ground state. At ground state the energy of a molecule is the some total of rotational, vibrational and electronic energies. In other words spectroscopy measures the changes in rotational vibrational and or electronic energies. Electromagnetic radiation is made up of discrete particles called photons. EMR has got both wave characteristics as well as particle characteristics. This means that it can travel in vacuum also. The different types of EMR are Visible radiation, IR Radiation, Micro waves, Radio waves, X-Rays, Gamma Rays or Cosmic Rays. As these radiations have different wave length or frequency or energy, they are conveniently named.

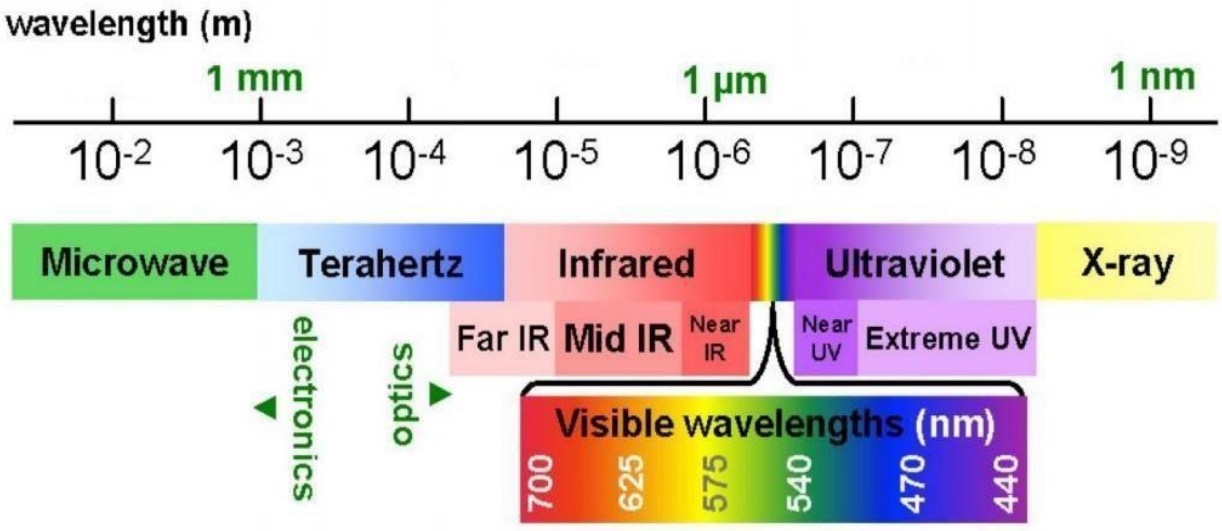


Fig:1 Wavelength

Correct instrument calibration procedure together with a good instrument and a skill full operator gives accurate and precise determination of an unknown sample concentration. For any instrument used for process analysis in the industry, the precision and accuracy of such instrument must be determined so as to give credibility to the response obtained from it especially in a newly installed instrument using a known sample such as Potassium permanganate (kMnO4) which must be of spectra grade standard (100.00+02).

Visible spectrophotometer investigates the interaction of light radiation with matter in the ultraviolet (200-400) and visible (400-800) range. Potassium permanganate absorbs strongly in the visible range of wavelength between 500 and 550nm on different visible spectrophotometers, it has been reported as having its wavelength of maximum absorption (λ max) of normal wavelength as 525nm using spectronic 20, 522nm and Robert Bohman reported his work as 520nm using perkins-elmer. C

$$\Delta E = h\nu$$

Where; **ΔE-** is the energy difference between two quantum levels and

v-is the frequency of photon which can result In the electronic excitation.

h is the Planck's constant (6.63 x 10-34 Js),

Definition

Visible spectroscopy is a scientific technique that involves the study of the interaction between electromagnetic radiation within the visible region of the electromagnetic spectrum (typically 400 to 700nm in wavelength) and matter. This method is employed to analyse and characterize substances based on their absorption, transmission, or reflection of light in the visible range. By measuring the intensity of light at different wavelengths, visible spectroscopy provides valuable insights into the electronic structure and composition of materials, making it a widely used tool in fields such as chemistry, physics, biology, and environmental science. Visible spectroscopy is an analytical technique that measures the number of discrete wavelengths of 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. Since this spectroscopy technique relies on the use of light, let's first consider the properties of light.

Electromagnetic Spectrum

The ability of electromagnetic radiation to discretely interact with atoms and molecules and produce distinctive Absorption or emission profiles is essential for spectroscopic activities. The wavelength of electromagnetic Radiation is the characteristic that governs the perceived color spectrum. The visible section of the electromagnetic Spectrum is that portion of the spectrum that the human eye can see. These visible wavelengths span a region Between 400 and 800 nm.

A specific wavelength or colour of visible light corresponds to the optical density when it is measured with Spectrophotometers. This light is absorbed, vanishes, and becomes invisible. The approximate complimentary Connection between the light wavelengths that are absorbed and those that are transmitted is shown in Figure. For Instance, the complimentary colour of light, orange, would be strongly absorbed by a blue substance.

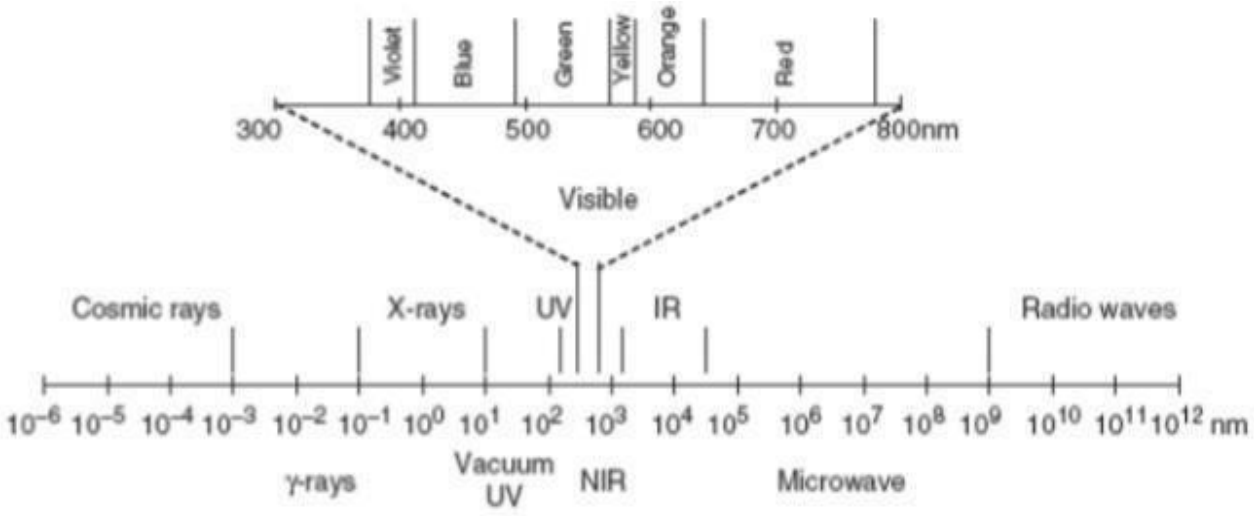


Fig2:The electromagnetic spectrum

The distance between neighboring peaks or troughs is known as the wavelength. The following straightforward equation can be used to define the wavelength, of EMR as a function of its frequency v, and the speed of light c

$$v = c \quad (1)$$

EMR has both particle and wave behaviour (the dual nature of light), and the relationship between energy and the wavelength of such a particle, a photon, is given by the equation

$$\mathbf{E = hc/\lambda \ *109 \quad (2)}$$

Where; **c** is the speed of light in a vacuum (2.998 x 108 ms-1),

h is the Planck's constant (6.63 x 10-34 Js),

E is the photon's energy, and is the wavelength in nm

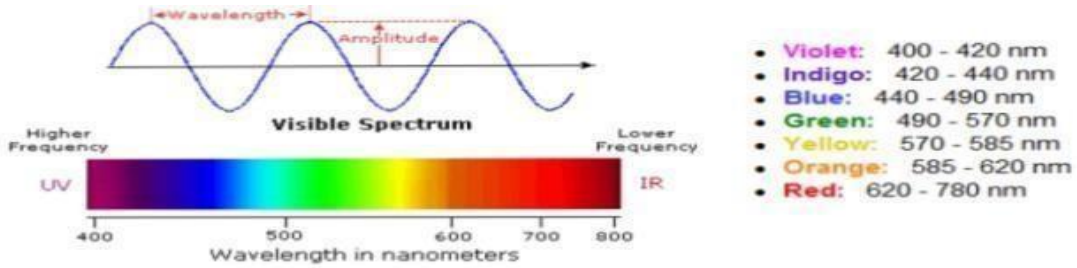


Fig3:sin wave representation of electromagnetic radiation and electromagnetic spectrum Beer's Law:

Beer's Law, also known as the Beer-Lambert Law, is a fundamental principle in analytical chemistry that describes the relationship between the absorption of light by a substance and its concentration in a solution. This law is named after two scientists, August Beer and Lambert, who independently contributed to its development in the 19th century. Beer's Law is essential for quantitative analysis using spectrophotometry, a technique widely employed in various scientific disciplines.

The intensity of the incident radiation (Io) will be higher than the emerging radiation when a beam of Electromagnetic radiation passes through an absorbing material (I). The general rule known as Beer's law can be Used to quantitatively describe how radiant energy is absorbed by materials.

According to Beer's law, the amount of radiation that is absorbed (absorbance, A) or transmitted by a solution or Medium is inversely related to the amount of the absorbing substance that is present, c (moles per litre), and the Length of the radiation's passage through the sample, b. (cm).

Therefore, a plot of absorbance against concentration should result in a line that is straight and has a slope equal to Єb, passing through the origin.

A = - log (*I*/*Io*) = Єbc

Where ; **Є = k/2.303**

The molar absorptivity is a constant ϵ that is unaffected by concentration or path length. If the route length and molar absorptivity are known, Beer's law equation can be used to calculate the Concentration of an organic molecule by locating its highest absorbance in the Vis absorption spectrum.

Lambert's law

Beer's Law, also known as the Beer-Lambert Law, is a fundamental principle in analytical chemistry that describes the relationship between the absorption of light by a substance and its concentration in a solution. This law is named after two scientists, August Beer and Lambert, who independently contributed to its development in the 19th century. Beer's Law is essential for quantitative analysis using spectrophotometry, a technique widely employed in various scientific disciplines.

The rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light.

i.e.,**-dI/dt ∝ I**

This equation can be simplified similar to equation 2 to get the following equation (by replacing C with t.)

1 = lo e-kt

(Equation (3)) Equation 2 & 3 can be combined to get I=lo e-kct

I=lo 10-kct(converting natural logarithm to base 10 & k=k×0.4343)

I/Io = 10-kct (rearranging terms) Io/I=10kct (inverse on both sides)

Log Io/I=kct (taking log on both sides)

e quation (4)

It can be learned that transmittance (T)=I/Io & absorbance (A)=log I/T

Hence, A=log I/I/Io A=log Io/I&_(5)

Using equation 4 &5 since A=log Io/I & log Io/I=kct we can infer that

A=kct (instead of k we can use ε)

A =εct

(Mathematical equation for beers-lambert's law)

Where, A=absorbance or optical density or extinction co-efficient .

ε=molecular extinction co-efficient .

C=concentration of drug (mmol/lit). t=path length (normally 10mm of or 1cm).

$$\varepsilon = \frac{E^{1\%}_{1cm}}{Molecular\ weight} \times 10$$

ε can also be expressed as follows

Where, $E^{1\%}$ means the absorbance of 1% w/v solution using a path length of 1cm.

at a wavelength is a constant value for each drug and can be seen in Pharmacopoeias and standard books on the subject. This value is useful in determining the concentration of drugs in sample formulations or in solutions. Emax is the value at λmax.

DEVIATIONS FROM BEER'S LAMBERT'S LAW

A system is said to obey beer's law, when a plot of concentration Vs absorbance gives a straight line. The straight line is obtained by using line of best fit on method of least squares or by joining the maximum number of points in such a way that positive and negative errors are balanced or minimised the regression line can also be used for determining concentration of a solution whose

absorbance is obtained by using a colorimeter or spectrophotometer.

When a straight line is not obtained i.e., a non-linear curve is obtained in a plot of concentration Vs absorbance, the system is said to undergo deviation from beer's law such deviation can be positive deviation or negative deviation. Positive deviation results when a small change in concentration produces a greater change in absorbance negative deviation results when a large change in concentration produces smaller change in absorbance it is normally seen that several system obey beer's law only in a concentration range, above which may show deviation 10-50 µg/ml, it may obey, but may exhibit deviation above 50 µg/ml.

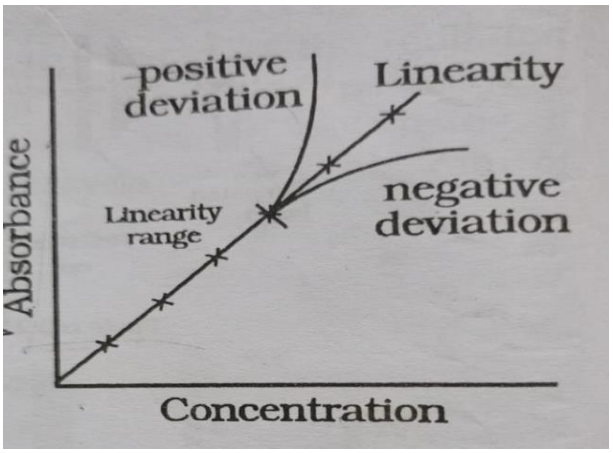


Fig4:A plot of conc. Vs Absorbance

Several reasons for the absorbed deviations from beer's law are as followsInstrumental deviations

- Physicochemical changes in solutions

Factors like stray radiation, improper slit with, fluctuations in single beam and when monochromatic light is not used can influence the deviation.

Physicochemical changes in solutions

Factors like Association, Dissociation, Ionisation, (change in pH), faulty development of colour refractive index at high concentration, can influence such deviations.

Examples: Association, Dissociation incomplete reaction.

PRINCIPLE IN COLORIMETRY

Colorimetry is concerned with the study of absorption of visible radiation whose wavelength ranges from 400nm-800nm. Any coloured substance will absorb radiation in this wavelength region. Coloured substance, absorbed light of different wavelength in different manner and hence we get an absorption curve in a unique pattern for every coloured solution. In this absorption curve, the wavelength at which maximum absorption of radiation takes place is called as λ max. This λmax is characteristic or unique for every coloured substance and this is a qualitative aspect useful in identifying the substance. λ max is not usually affected by concentration of the substance. The absorbance of a solution increases with concentration of a substance but there is no change in mix when concentration changes. When we plot a graph of concentration [Vs] absorbance, we get a calibration curve or standard curve. This calibration curve is useful in determining the concentration of amount of a drug substance in a given sample solution or a formulation by extrapolation or intrapolation and calculation

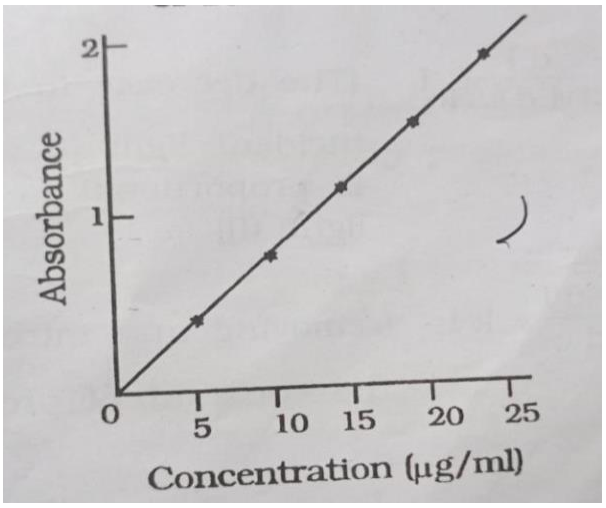


Fig5:Calibration curve

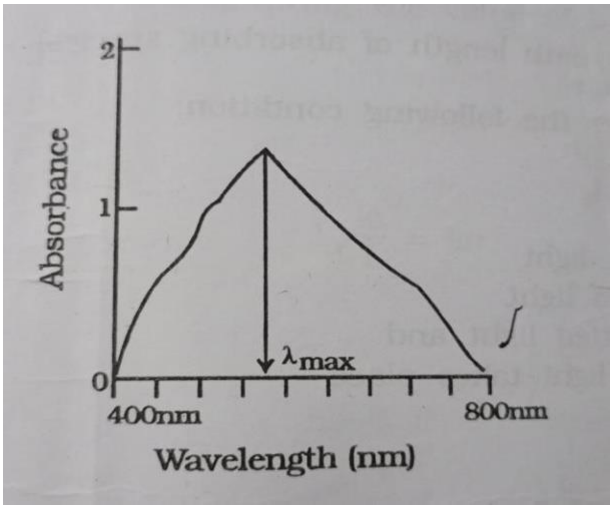


Fig6: Wavelength absorption curve

The principle is based on the measurement of spectrum of a sample containing atoms /molecules. Spectrum is a graph of intensity of absorbed or emitted radiation by sample verses frequency (v) or wavelength. Spectrometer is an instrument design to measure the spectrum of a compound.

Types:

- Absorption Spectroscopy
- Emission Spectroscopy
- Atomic spectroscopy
- Molecular Spectroscopy

Absorption Spectroscopy

An analytical technique which concerns with the measurement of absorption of electromagnetic radiation.
Example: UV (185 400 nm) / Visible (400-800 nm Spectroscopy, IR Spectroscopy (0.76-15µm).

Emission Spectroscopy

An analytical technique in which emission of a particle or radiation is dispersed according to me property of the emission & the amount of dispersion is assured.
E.g. Mass Spectroscopy

Atomic spectroscopy

This deals with the interaction of electromagnetic radiations with atom which are most commonly in their lowest Energy state called the ground state. The electronic absorption of electromagnetic radiation can occur only if the Photon has an energy which is equal to the energy difference between two quantized energy levels, i.e., Spectroscopy is one of the most powerful tools available for the study of atomic and molecules.

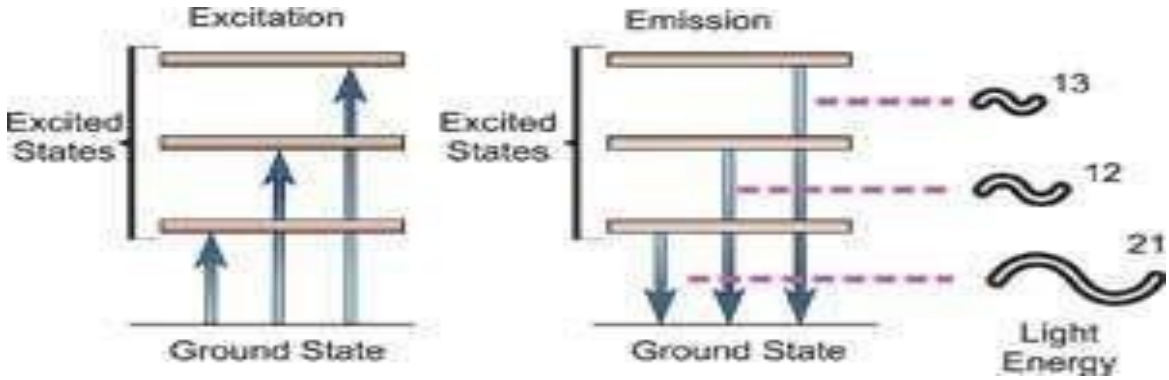


Fig7:Atomic spectroscopy

Molecular Spectroscopy

This deals with the interaction of electromagnetic radiation with molecules. This results in transitions between Rotational and vibrational energy levels in addition to electronic transitions. As a result, the spectra of molecules Are much more complicated than those of atoms. Molecular spectra extend from the visible through infrared into The microwave region. Current interest in molecular spectroscopy is very great because the number of known Molecules are extremely large as compared with free atoms. The various types of spectra given by molecular species, the regions in which these spectra lie and the energy Changes that takes place in the molecules on absorption of radiation, are listed below

- Rotational (Microwave) Spectra
- Vibrational and Vibrational-Rotational (Infrared) Spectra
- Raman Spectra
- Electronic Spectra
- Nuclear magnetic resonance
- Mossbauer Spectra

SPECTROMETERS

Spectrophotometers are expensive and more sophisticated and are designed to read % Transmittance or Absorbance, record the absorption spectrum using a plotter or recorder, are of double beam type where we can use sample and reference solution at time. Storage of spectrum, comparison of spectra, quantitative techniques, rapid wavelength scanning. data manipulation, derivative spectral mode, etc can also be present as options in more automated instruments. They can be either microprocessor based, or software driven using computer. Wavelength accuracy of such instruments is 10.1nm. These instruments are hence more accurate and reliable than other types.
The following pages describe different types of instruments.

- Single beam colorimeter
- Double beam colorimeter

Single beam colorimeter

This consists of a tungsten lamp as source of light. This light radiation is focused on to a slit by using a concave mirror. This light passes through a simple absorption filter where only the required wavelength of light passes through it and falls on the sample cell where the solution to be analysed is present. The sample or standard solution absorbs a part of the radiation and the rest is transmitted. The intensity of the transmitted or the unabsorbed radiation is determined using a photo voltaic cell using a digital display or an analogue display (Galvanometer type).

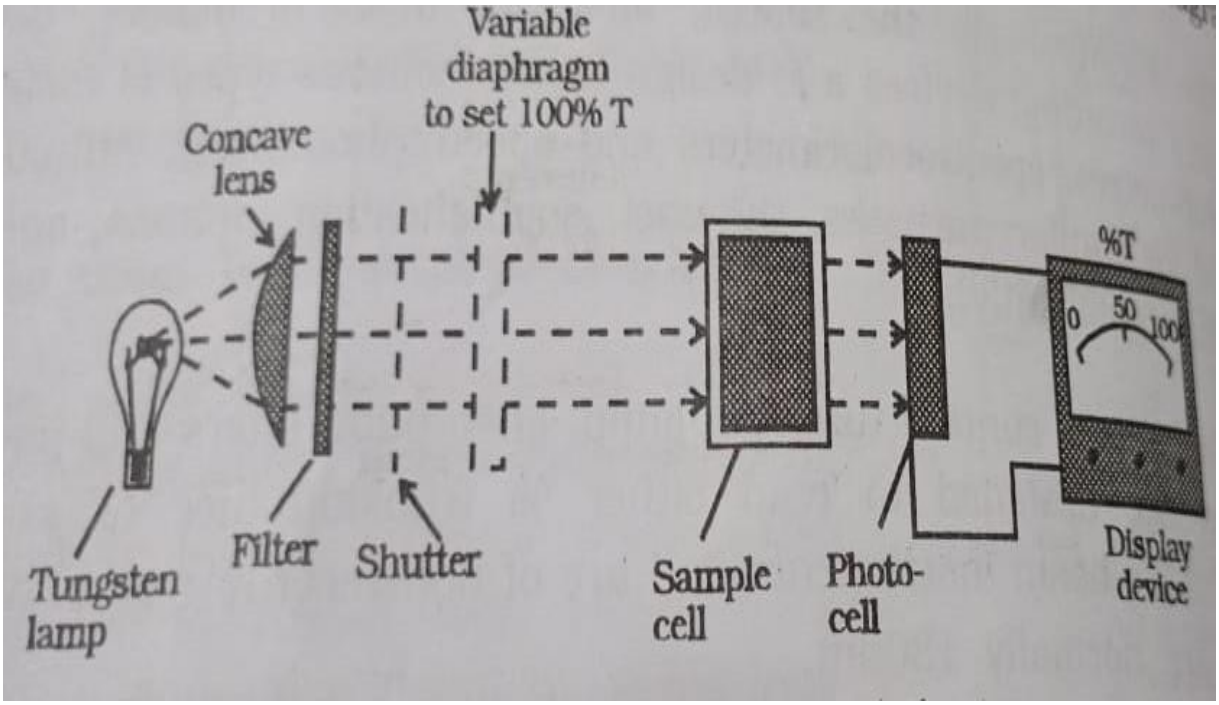


Fig8:Single beam colorimeter

Merits

1. Simple in construction 2 Inexpensive
3. Easy to operate

Demerits

1. The readings are affected by fluctuations in the intensity of source.

2. Rapid scanning to get a spectrum is not possible, since 0%T and Sotatable 100T has to be adjusted at every

wavelength. 3. Recorder cannot be used with single beam type.

Double beam colorimeter

Double beam colorimeter is similar to that of single beam instrument. Here the light beam after passing through a filter or monochromater is split into sample beam and reference am by using a beam splitter. These beam pass through sample and reference solutions and fall on two detectors

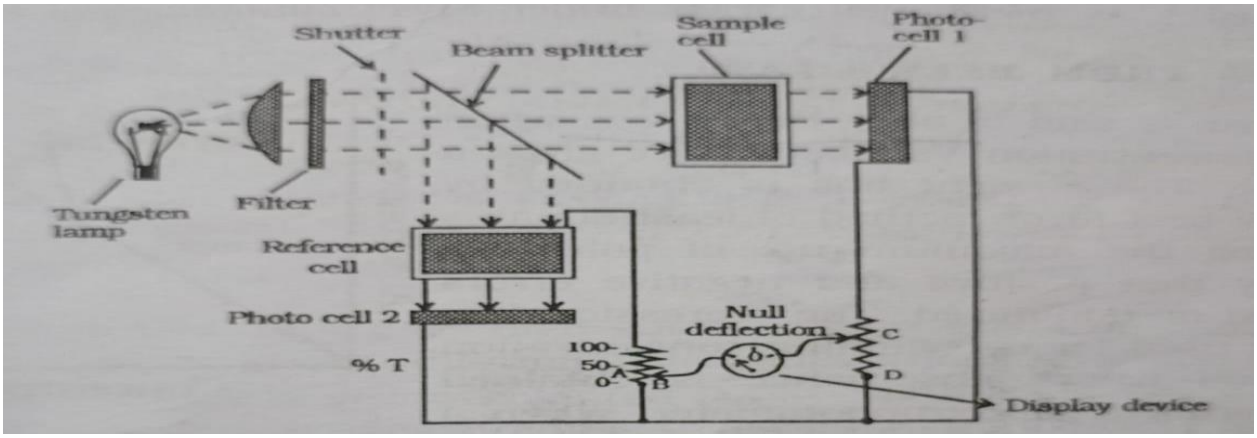


Fig9: Double beam colorimeter

separately. The final read out is in Absorbance or Transmittance, obtained after electronic manipulation of the above 2 detectors.

Double Beam spectrophotometers

The method of construction and principle of operation is similar to double beam colorimeter except that grating monochromator and photomultiplier tube are used in the place of filter/prism monochromator and phototube respectively.

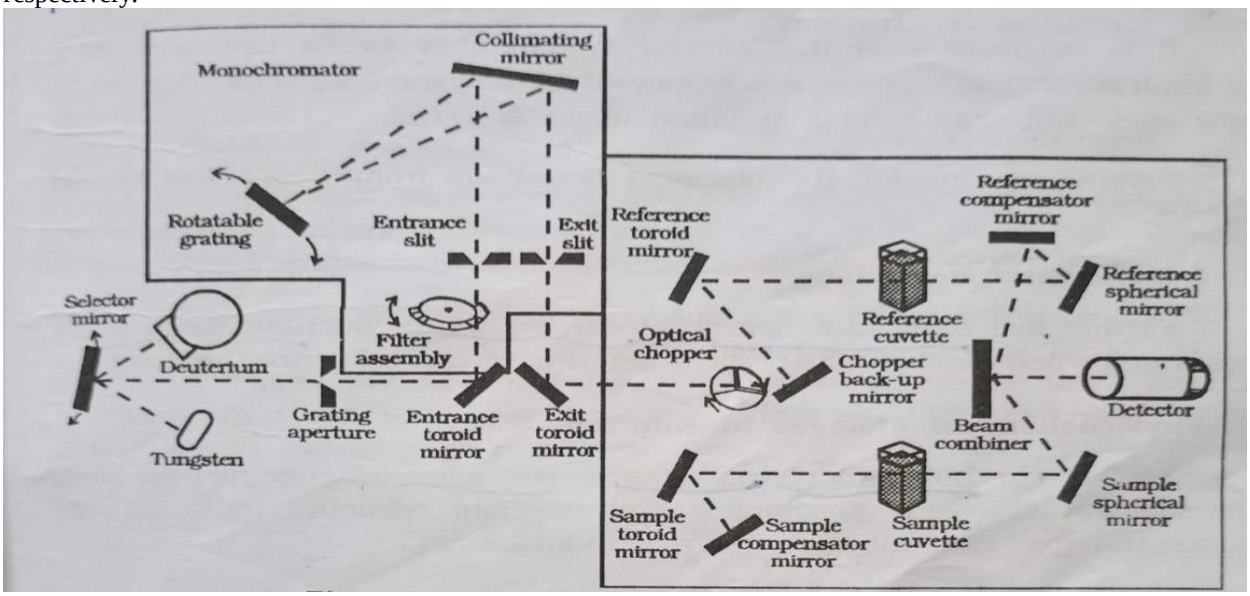


Fig10:Double Beam spectrophotometers

Nowadays only double beam spectrophotometers are used widely than other types because of their high accuracy sensitivity range reliability and repeatability moreover they have several features as mentioned earlier under spectrophotometers.

Theory of Visible spectroscopy

When radiation induces an electronic transition in a molecule or ion's structure, the object will exhibit absorption in the visible or ultraviolet range. As a result, when a sample absorbs light in the ultraviolet or visible range, the molecules inside the sample experience a change in their electronic state. Electrons will be promoted from their ground state orbital to a higher energy, excited state orbital by the energy from the light. or anti-bonding orbital. Potentially, three types of ground state orbitals may be involved.

- σ (bonding) molecular
- π (bonding) molecular orbital
- n (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

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 π*
- π to π*

Due to their high energy requirements, the σ to σ* and n to σ* transitions both take place in the far ultraviolet area or sporadically in the range of 180–240 nm. Saturated groups consequently do not show high absorption in the common UV range. In contrast to transitions to the π* anti-bonding orbital, transitions from then to the π* and to π* type occur in molecules with unsaturated centers.

They need less energy and take place at longer wavelengths. It will soon be clear that molecule structure controls both the absorption's maximum wavelength and its intensity. If a molecule's chemical structure is changed, transitions to the π* anti-bonding orbital that normally take place in the UV range could very well occur in the visible region. 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.

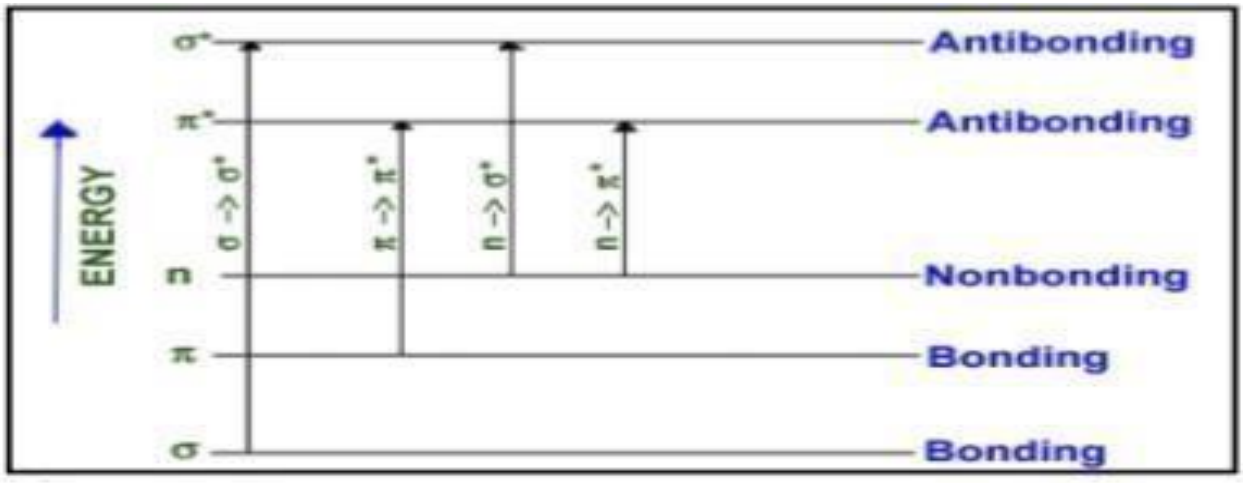
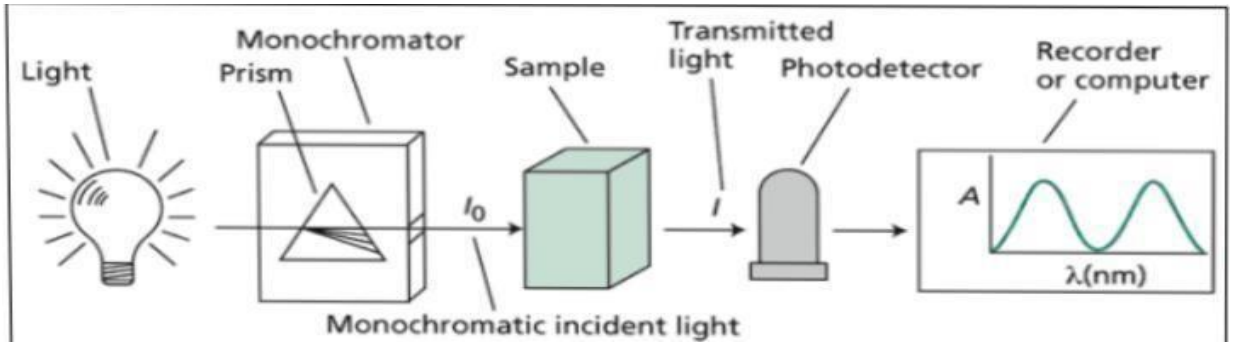


Fig11: Spectral transitions

INSTRUMENTATION

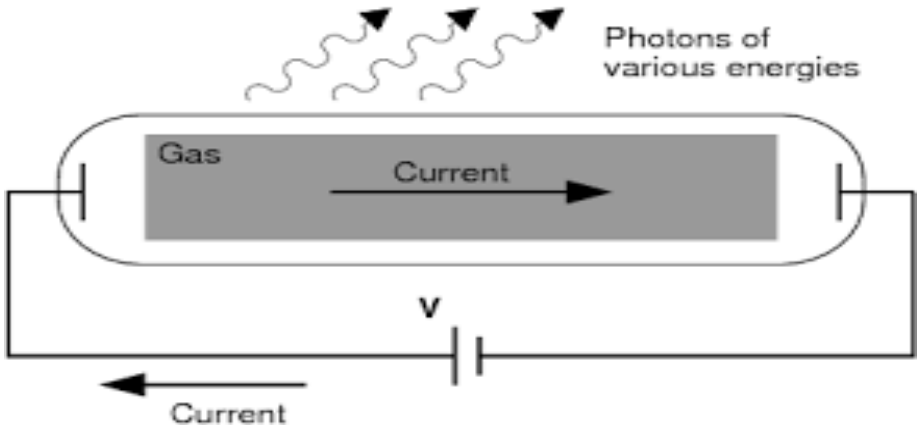
The Essential components of Visible Spectrophotometer are as follows

- Sources
- Monochromator
- Sample containers (Cuvette)
- Detector
- Amplifier and recorder



SOURCES:
A continuous source, or one that produces radiation at a variety of wavelengths, is necessary for Visible Spectroscopy. Assorted visible radiation sources include the following:
Hydrogen lamp:

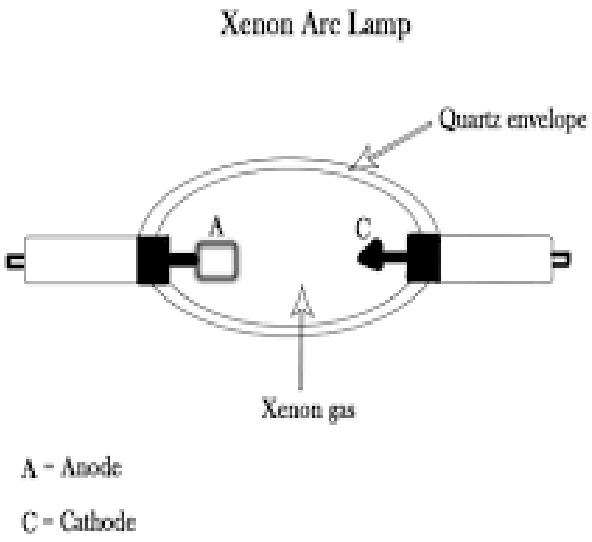
Hydrogen lamps are reliable, steady, and continuously emit radiation between 160 and 380 nm. It consists of hydrogen gas at high pressure, which causes an electrical discharge. The excited hydrogen molecules produce radiation.



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.
Xenon discharge lamp:

A xenon lamp is a discharge light source that contains xenon gas inside a bulb. Radiation from xenon ranges from 250 to 600 nm.



MONOCHROMATOR

By filtering out undesirable wavelengths from the radiation source light, a monochromator creates monochromatic Light. Through the entrance slit, multi-wavelength polychromatic light enters the monochromator. Following Collimation, the beam is directed at an angle toward the dispersion component. The grating or prism separates the Beam's wavelengths into their individual components. Only radiation of a specific wavelength exits the Monochromator through the exit slit when the dispersing element or the exit slit are moved.
Types of monochromators:

- Prism monochromator
- Grating monochromator All Monochromator contain the following

component parts:

□ An entrance slit

□ A collimating lens □ A dispersing device □ A focusing lens
□ An exit slit

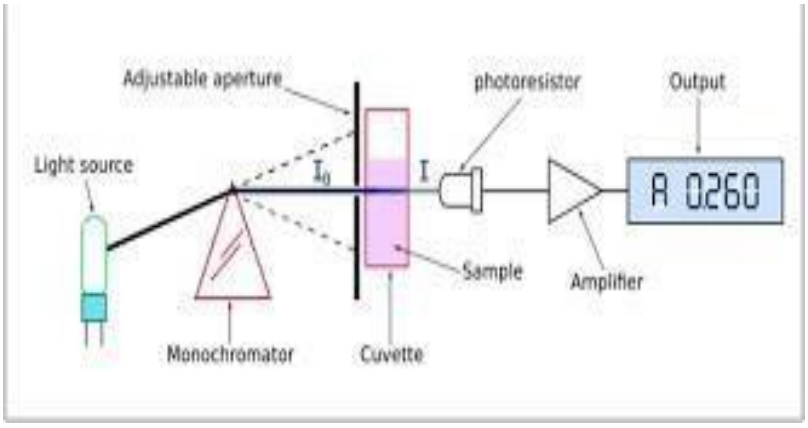


Fig12: Monochromators

Radiation with many wavelengths, or polychromatic radiation, enters the monochromator through the entrance Slit. After being collimated, the beam angles toward the dispersing component. The grating or prism separates the Beam's wavelengths into their individual components. Only radiation of a specific wavelength exits the Monochromator through the exit slit by changing the dispersing element or the exit slit.

SAMPLE CONTAINERS (CUVETTE):

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 1Cm route length, and may be utilized for wavelengths between 190 and 200 nm.

DETECTORS:

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:

- Barrier layer cell/Photovoltaic cell
- Phototubes/ Photo emissive tube
- Photomultiplier tube
- Silicon photo diodes

Barrier layer cell/Photovoltaic cell

These cells are the cheapest and are used in inexpensive instruments. Like filter type colorimeters. Fluorimeters and nephelo turbidimeters. The following Figure shows the construction and working of the detector. The detector has a thin metallic layer coated with silver or gold and acts as an

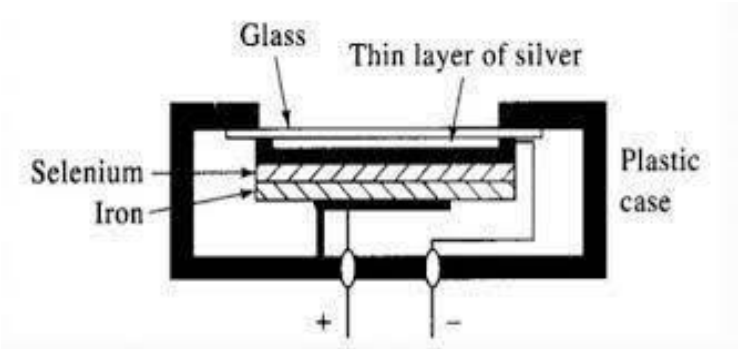


Fig13: Barrier layer cell/Photovoltaic cell

or gold and acts as an electrode. It Also has a metal base Plate which acts as Another electrode. These two layers are Separated by a Semiconductor layer of selenium Selenium has extremely low Electrical conductivity and hence the electrons are not mobile. When light radiation falls on the selenium layer, these electrons become mobile and are taken up by the transparent metal layer. This creates a potential difference between the two electrodes and causes the flow of current, when the resistance in the external circuit is small. This flow of current causes deflection of the galvanometer needle, which depends on the wavelength and intensity of radiation. The sensitivity of the instrument is similar to that of human eye. The disadvantages of this detector are the amplification of the signal is not possible, because the resistance of the external circuit has to be low, fatigue effects and the lesser response of the detector with light other than blue and red light.

Photo tubes (or) Photoemissive cells

This detector composed of an evacuated glass tube, which consists of a photo cathode and a collector anode. The photo cathode is coated with elements of high atomic volume like Caesium, Potassium or Silver oxide, which can liberate electrons, when light radiation falls on it.

This flow of electrons towards anode produces a current proportional to the intensity of light radiation.

Composite coatings like Caestum / Caesium oxide / Silver oxide can also be used, which increases the sensitivity and range of wavelength in which the detector can be used (UV/visible region).

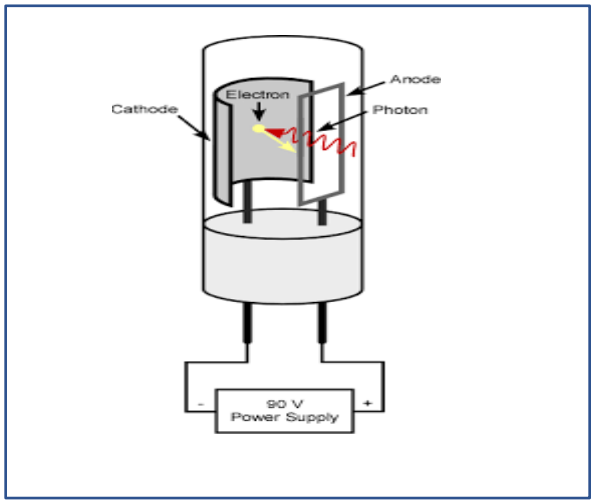


Fig14: Photo tubes (or) Photoemissive cells

The signal from the detector can also be amplified using an amplifier circuit. Photo tubes have better sensitivity when compared to Photo voltaic cell and hence are more widely used

Photo Multiplier Tubes (PMT)

This type of detector is the most sensitive of all the detectors, expensive and used in sophisticated instruments. The principle employed in this detector is that multiplication of photoelectrons by secondary emission of electrons.

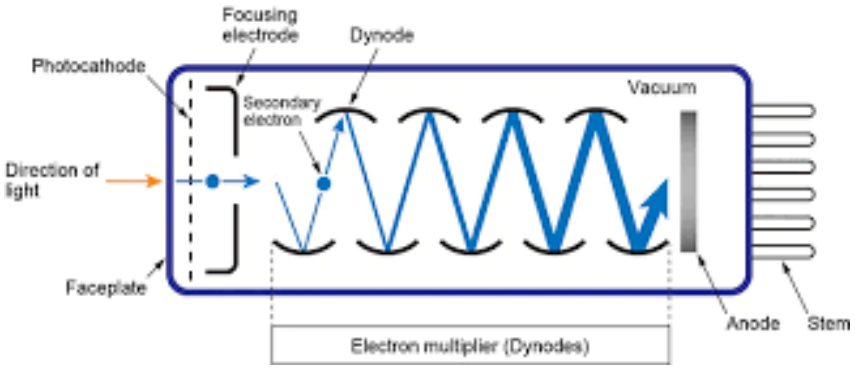


Fig15:Photo Multiplier Tubes (PMT)

This is achieved by using a photo cathode and a series of anodes (dynodes). Up to 10 dynodes are used.Each dynode maintained at 75-100V higher than the preceding one. At each stage, the electron emission is multiplied by a factor of 4 or 5 due to secondary emission of electrons and hence an overall factor of 106 is achieved.

PMT can detect very weak signals, even 200 times weaker than that could be done using Photovoltaic cell. Hence it is useful in fluorescence measurements. PMT should be shielded from stray light in order to have Accurate results.

TITRATIONS

It is the titrations in which the absorption of a reactant or product or both are followed as a function of added titrant. The advantages are

a) The end point is sharp. b)no interference from other absorbing species, c)incompleteness at the end point does not affect end point.

CONDITIONS TO BE OBSERVED:

A. Either the substance, titrant or product should absorb.
B. Beer's law must be obeyed under experimental conditions.
C. Titrant must be stronger as the error due to volume change must be minimised.
D. Absorbance is monitored at λmax of titrant, reactant of product.

Examples of titration curves:

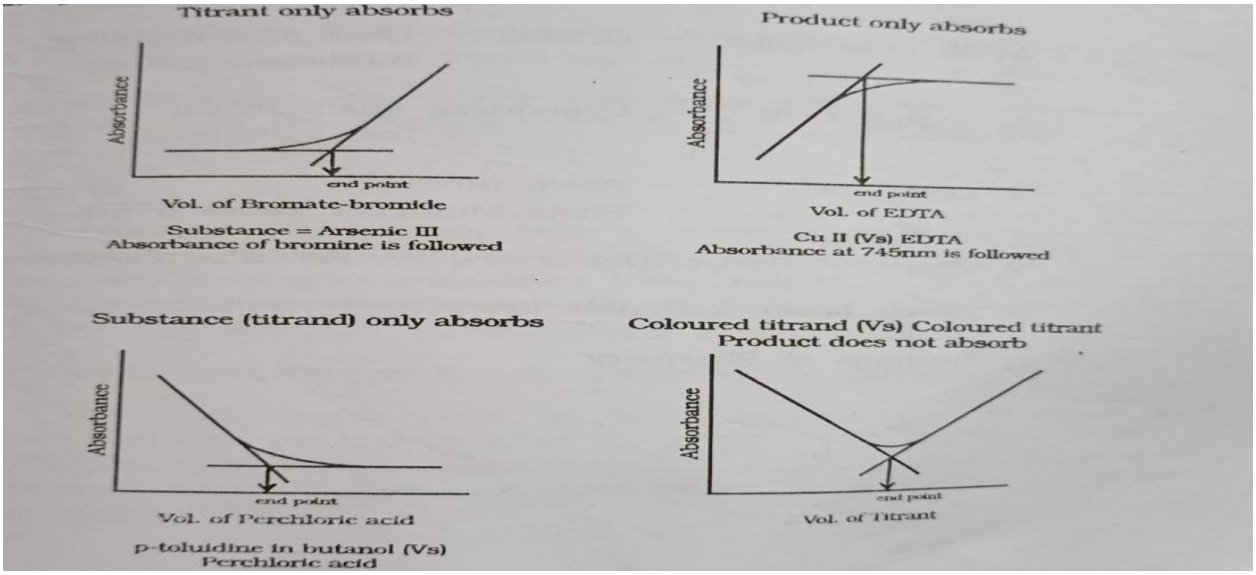


Fig16:Titrations

ADVANTAGES:

 The core advantage is the accuracy of the VIS spectrophotometer. The VIS spectrometer is easy to handle and use.
 Provide robust operation.

 VIS spectroscopy is simple to operate. Cost effective instrument.
 Cover the entire of ultraviolet and visible.

 It can be utilized in the qualitative and quantitative analysis.

 The Derivative graph can be obtained by VIS spectrophotometer. Only possible for the analytes which have a chromophore

DISADVANTAGES:

 Only those molecules are analyzed which have chromophores

 The results of the absorption can be affected by pH, temperature, contaminants, and impurities. Only liquid samples are possible to analyse
 It takes time to get ready to use it

 Cuvette handling can affect the reading of the sample

APPLICATIONS:

1. **Quality control of purity**
   Colorimetry can be used to detect the impurities, because of their irrelevant absorption. Coloured impurities present in a sample gives rise to additional peaks or more absorption at particular wavelength.
   Example: Cyanocobalamine (Vitamin B12) Absorbs at 278nm, 361nm and 550nm. The ratio of

absorbance at 278nm /371nm=0.57 and the ratio of absorbance at 550nm/361nm=0.3. If impurities are present, the ratio of absorbance change and can be known.

2. **Quantitative analysis**

This aims to determine the concentration and amount of drugs in sample solution and thus the percentage purity can be determined.

3. **Determination of ligand or metal ratio in metallic complexes**

Metals with some organic compounds from metallic complexes, the ratio of which can be determined. Example: 1:1, 2:1 or 1:2 etc can be determined by plotting curves using different ratios and absorbing the linearity of the curve.

4. **Structure elucidation of organic compounds**

The absorption spectrum of an unknown compound can be compare with known compounds so that the most probably structure may be obtained.

Since compounds of similar structure have analogous spectra. It was observed that absorption spectra of compound was similar to phenol [C6H5OH].

5. **Determination of Pka value of indicators**

Determination of dissociation of an acid and base indicator. Eg: Methyl orange, Methyl red, etc.

$$pKa = pH - \log \frac{[ionisation]}{[unionised]}$$

The value of $\log \frac{[ionisation]}{[unionised]}$ can be determined spectrophotometrically, i.e., concentration (Vs) Absorbance at different pH and from the equation pKa can be calculated.

6. **Determination of molecular weight of amines**

A known weight of amine is taken and converted to amine picrate and the absorbance of solution is found. By using the equation, the molecular weight of the amine can be Can be calculated. We know already

$$A = act,\ a = \frac{A}{ct}$$

$$\varepsilon = a \times \text{mol.wt therefore, Mol wt} = \frac{\varepsilon}{a}$$

for most amine drugs ε of amine picrates at 380nm is 13400.

$$\text{Mol wt} = \frac{13400}{A} = 13400 \times \frac{ct}{A}$$

Where,

A = absorbance of a solution concentration 'c' using pathlength 't'

7. **Determination of elements, ions are functional groups**

Several elements, ions of functional groups can be determined even at low concentration by using specific or non-specific colour producing reagents some of the examples are tabulated.

8. Quantification of nucleic acids, studying transition metals, and visualizing organic compounds.
9. **Biochemical Analysis:**
   Protein and Nucleic Acid Analysis: Visible spectroscopy is widely employed in studying biomolecules such as proteins and nucleic acids, aiding in structural and functional analysis.
10. **Food Industry**: Color Analysis: Visible spectroscopy is used for evaluating and controlling the color of food products.
    Quality Control: It aids in assessing the quality and composition of food items.

11. **Pharmaceutical industry:** Drug assay: Visible spectroscopy is employed in pharmaceutical analysis for drud quantification and quality control.

Substance	Reagent	Wavelength[nm]	Concentration Range [ppm]
Iron	1,10-Phenanthroline	510	0.5-5
Magnesium	8-quinolonine	400	1-5
Manganese	Periodate	515	1-25
Cobalt	Nitroso-R-Salt	425	0.1-1
Ammonia	Nessler's reagent	580	2-25
Urea	Diacetyl monoxime	480	8-22
Glycine	Ninhydrin	565	0-0.04
Phenols	Ferric Chloride	530	0.1-9

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