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# PAPER-IV

# INSTRUMENTAL METHOD OF ANALYSIS-I

## UNIT-I

## SPECTROSCOPIC METHODS -I

### UV-visible spectroscopy:-

#### Principle of ultra voilet spectroscopy:-

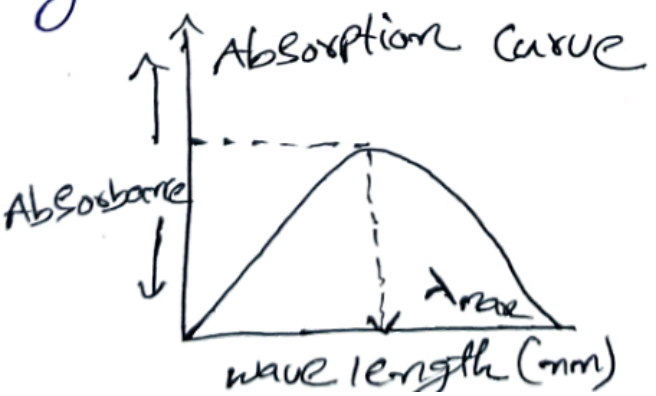
Ultra violet spectroscopy is used for the study of absorption of uv-radiation which range from 200nm to 400nm. In uv-spectroscopy electrons are transition in a molecule from ground state to excited state. Any molecule has either $\pi, \pi$ (or) are combination of these electrons. The bonding ($\sigma$ & $\pi$) and non-bonding ($n$) electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks the Nature of the electrons present and hence the molecular structure can be elucidated.

#### Principle of visible spectroscopy (Colourimetri):-

Colourimetri is considered with the study of absorption of visible radiation and wave length range from 400nm to 800nm. Any coloured substance will absorb radiation, in

this wavelength region. Coloured substance, absorb light of different wavelength in different manner and hence we get an absorption curve (Absorbance Vs wavelength). In a unique pattern for every coloured solution in this absorption curve, the wavelength at which maximum absorbance of radiation takes place is called as the "$\lambda_{max}$". This $\lambda_{max}$ is characteristic for every coloured substance and this is qualitative Aspect and useful identify in the substance.

$\lambda_{max}$ is not useful effected by concentration of the substance the absorbance of a solution increases with concentration of a substance. But there is no change in $\lambda_{max}$, when concentration changes, when we draw graph between concentration Vs Absorbance, we get a calibration (or) a standard curve. This calibration curve is useful determining the concentration (or) amount of a drug substance in the given sample solution



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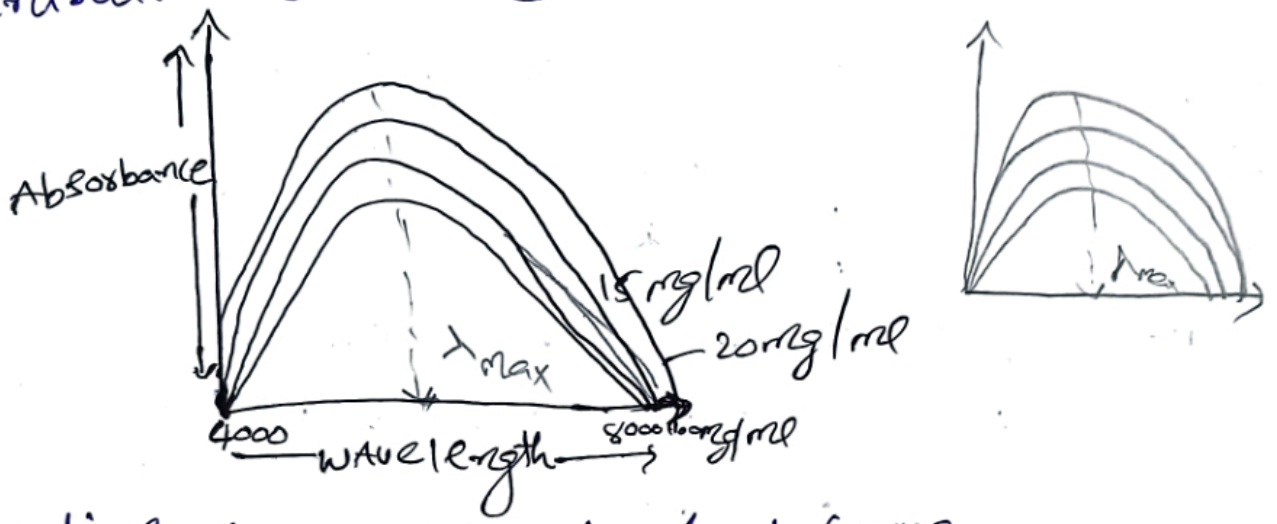
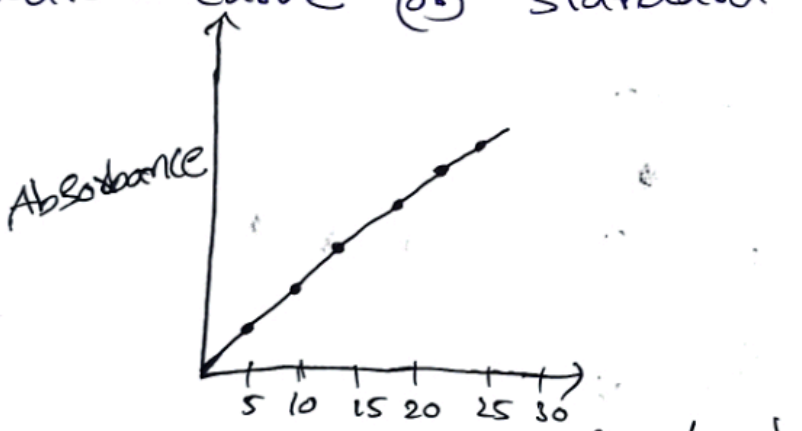
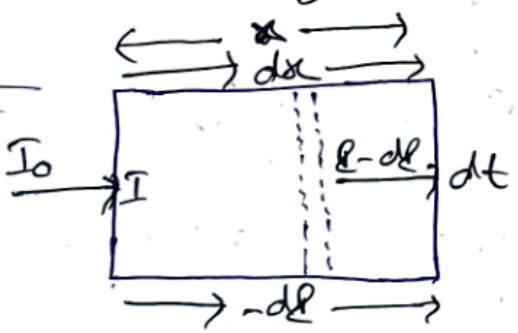
Absorption curve by using different Concentration of same sample solution.




Calibration curve (or) Standard curve.




Lambert's law :-




where, $I_0$ → radiation intensity before entering the medium

$I$ → radiation intensity after entering the medium

$x$ → Thickness of the medium

$-dI$ → decreasing radiation intensity

definition: When monochromatic light is passes through a homogeneous medium. On this the decreasing radiation intensity is proportional to thickness of medium.

$$-I \propto x$$

Here, $x$ is thickness of medium.
$I$ is radiation intensity with negative deviation

$$-\frac{dI}{dx} \propto I$$

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

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

$$\frac{dI}{I} = -k\,dx$$

Integrating on both sides

$$\int \frac{dI}{I} = -k \int dx$$

$$\log I = -kx + c \longrightarrow (1)$$

Here 'c' is integral constant.
Let consider $I = I_0$ when $x = 0$, then the above equation can be written as

$$\log I_0 = -k(0) + c$$

$$\log I_0 = c$$

the value of $c$ is substitute in equation (1)

(3)

From eqn (1)

$$\log I = -kx + \log I_0$$

$$\Rightarrow \log I - \log I_0 = -kx \qquad \left(\because \log a - \log b = \log\left(\frac{a}{b}\right)\right)$$

$$\log\left(\frac{I}{I_0}\right) = -kx$$

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

$$\boxed{I = I_0 \cdot e^{-kx}}$$

we know that the absorbance,

$$I_{abs} = I_0 - I$$

$$= I_0 - I_0 \cdot e^{-kx} \qquad \left(\because I = I_0 \cdot e^{-kx}\right)$$

$$\boxed{I_{abs} = I_0\left(1 - e^{-kx}\right)}$$

Beer's law :- When monochromatic light is passes through a homogeneous solution. In these radiation intensity is proportional to concentration of solution

$$\boxed{-I \propto C}$$

C - Concentration of solution

-I - -ve deviation in radiation intensity

derivation :-

$$\frac{-dI}{dc} \propto CI$$

$$\Rightarrow \frac{-dI}{dc} = kI$$

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

$$\frac{dI}{I} = -k\,dc$$

Integrating on both sides

$$\int \frac{dI}{I} = -k \int dc$$

$$\boxed{\log I = -kC + C_0} \longrightarrow (1)$$

Here, $C_0$ is integral constant

Let consider $I = I_0$, when $C = 0$ then the above equation can be written as

$$\log I_0 = -k(0) + C_0$$

$$\boxed{\log I_0 = C_0}$$

The value of $C_0$ is substituting in eqn (1)

From eqn (1) $\log I = -kC + \log I_0$

$$\log I - \log I_0 = -kC \qquad \left(\because \log a - \log b = \log \frac{a}{b}\right)$$

$$\log\left(\frac{I}{I_0}\right) = -kC$$

$$\frac{I}{I_0} = e^{-kC}$$

$$\boxed{I = I_0 \cdot e^{-kC}}$$

we know that the absorbance,

$$I_{abs} = I_0 - I$$

(4)

$$= I_0 - I_0 \cdot e^{-kc} \quad (\because I = I_0 e^{-kc})$$

$$\boxed{I_{abs} = I_0 (1 - e^{-kc})}$$

**Beer's lambert's law :-**

According to Beers lambert's law when monochromatic light is passes through a homogeneous medium and homogeneous solution. In this, the radiation intensity is proportional to thickness of medium and concentration of solutions. According to Beer's law $I_{abs} = I_0 (1 - e^{-kc})$ —— (1)

According to lambert's law

$$I_{abs} = I_0 (1 - e^{-kx}) \quad \text{——} (2)$$

From eqn (1) & (2)

$$\boxed{I_{abs} = I_0 (1 - e^{-kcx})}$$

* **Simultaneous determination of Mn and Cr in a mixture :-** The absorbance of additive, it is provided their is no relation between two solutes we can determine the concentration of two species like Mn (VII) and Cr (VI) simultaneously because their is no interaction between them.

Therefore both are oxidants

Generally we can determined

$$A\lambda_1 = A_1\lambda_1 + A_2\lambda_1$$

$$A\lambda_2 = A_1\lambda_2 + A_2\lambda_2$$

where $A\lambda_1$ and $A\lambda_2$ are the measured absorbance at two wavelengths $\lambda_1$ & $\lambda_2$.

The above two components are having different wavelengths.

The absorption spectra of two substance should not overlap applicable, so that the substance absorb strongly at the substance one. At wavelength $\lambda_1$ and weakly wavelength $\lambda_2$.

Similarily the substance absorb strongly at the substance to at wavelength $\lambda_2$, and weakly absorb wavelength $\lambda_1$.

we know that Beer's lambert's law

$A = kct$ (instead of k we can use $\epsilon$)

$\therefore \boxed{A = \epsilon ct}$

where, A is absorbance (or) optical density (or) extinction co-efficient.

$\epsilon$ molecular extention co-efficient.

c Concentration of drug (m.moles/lit)

t Path length (1cm moles (or) 1cm)

(5) Therefore,

$$A = \epsilon c$$

Ily $A_1 = \epsilon_1 c_1$

$$A_2 = \epsilon_2 c_2$$

Now, the above two systems can be written as

$$A\lambda_1 = \epsilon_1 c_1 \lambda_1 + \epsilon_2 c_2 \lambda_1$$

$$A\lambda_2 = \epsilon_1 c_1 \lambda_2 + \epsilon_2 c_2 \lambda_2$$

By solving, the above simultaneous equations can be obtain concentrations of two substance $c_1$ and $c_2$. $Mn(VII)$ & $Cr(VI)$ mixture :-

$$A_{total} = A_{Mn}(VII)\lambda_1 + A\,Cr(VI)\lambda_1$$

$$A_{total} = A_{Mn}(VII)\lambda_2 + A\,Cr(VI)\lambda_2$$

Dichromate absorb strongly 440nm and Permongate absorb strongly 545nm

According to Beer's law

$$A = \epsilon c t$$

If $t = 1$

$$A = \epsilon c$$

$$A_{total}^{(440nm)} = \epsilon_{Mn(VII)}^{440nm} + \epsilon_{Cr(VI)}^{440nm} \quad \text{—— (1)}$$

$$A_{total}\,(545nm) = \epsilon_{Mn(VII)}^{545nm} + \epsilon_{Cr(VI)}^{545nm} \quad \text{—— (2)}$$

$\epsilon^{440nm}_{Mn(VII)}$ and $\epsilon^{545nm}_{Mn(VII)}$ can be obtained by nothing the substance of standard Mn(VII) at 440nm, 545nm respectively.

therefore,

$$\epsilon^{545nm}_{Mn(VII)} = \frac{A}{Mn(VII)t} \quad \left(\because \epsilon = \frac{A}{Ct}\right)$$

$$\text{lly } \epsilon^{440nm}_{Mn(VII)} = \frac{A}{Mn(VII)t}$$

Similarily

$$\epsilon^{545nm}_{Cr(VI)} = \frac{A}{Cr(VI)t}$$

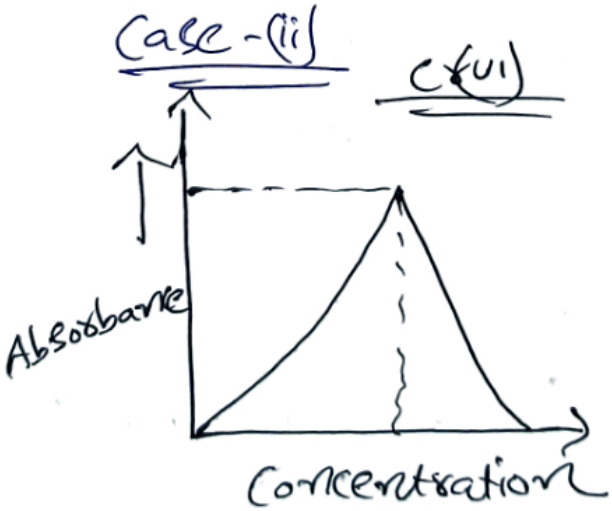
$$\text{lly } \epsilon^{440nm}_{Cr(VI)} = \frac{A}{Cr(VI)t}$$

By substituting the above $\epsilon$ values in eqn ① & ② we gets Mn(VII) and Cr(VI)

Case-(i):


$\lambda max = 545nm$

Case-(ii)




$\lambda max = 440nm$

(6)

## Spectrophotometric titrations :-

Spectrophotometric method is used for determination of end point of different samples.

It is the titration in which the Absorption of a reactant (or) Product both are followed as a function of added titrant.

The Advantages are sharp end point, and no interference from other absorbing species. Incompleteness at the endpoint doesnot effect endpoint.

In such titrations were the colour change is gradually increase (or) decrease by the addition of ~~titration~~ titrant. In a titration process, if the end point may be difficult to be identification then it is overcome by useing Phometric (or) spectrophotometric titrations.

In a spectrophotometric titration, the equivalance point is determined by useing a spectrophotometer.

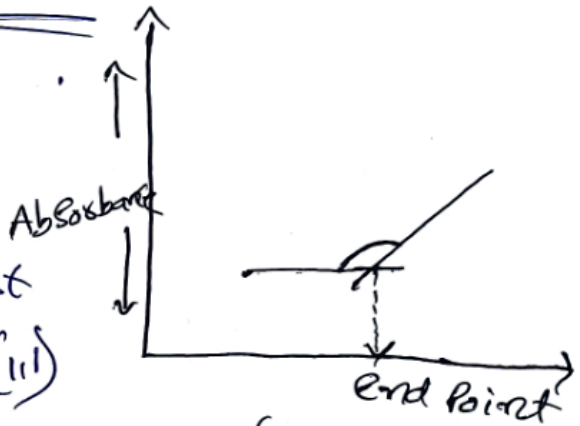
In this technique the titration vessel is kept directly in the light path of the instrument.

Than the absorbance of the solution is determine after adding the titrant.

The graph draw between absorbance as a function of volume of titrant is preferred.

If the titration reaction is complete, the titration curve will consist of two straight lines at intersecting equalence point similarily Conductometric titrations.

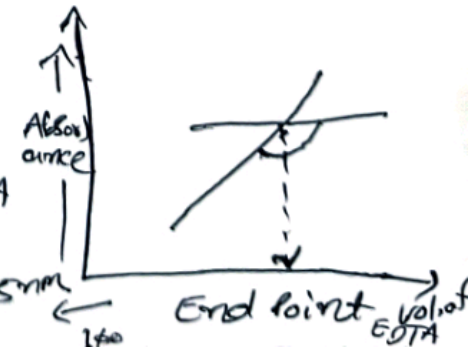
Examples of titration curves :-




Bromide curve (a)

The above curve (a) is characteristic of a case where only the titrant absorb. The most important application of Arsenic (III) Vs Bromate & Bromide. The Bromate & Bromide is taken in a Burette. The absorbance reading is taken at the wavelength the liberation of bromine. As(III) remains in the solution the absorbance will not be change because the product does not absorb in that region. As soon as As(III) is consumed by the titrant, the absorbance will be increase due to the colour of the titrant (Bromide)

2) The curve (b) is characteristic of a case where only the product of reaction absorb. Cu(II), The absorbance 745nm

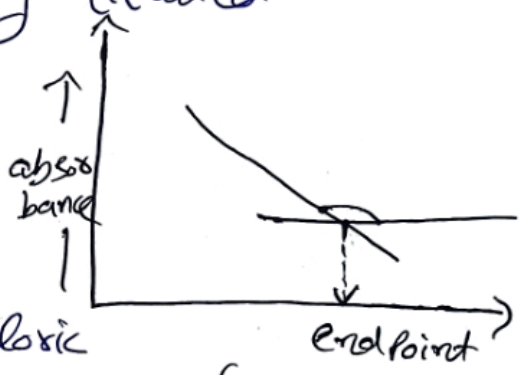



curve (b)

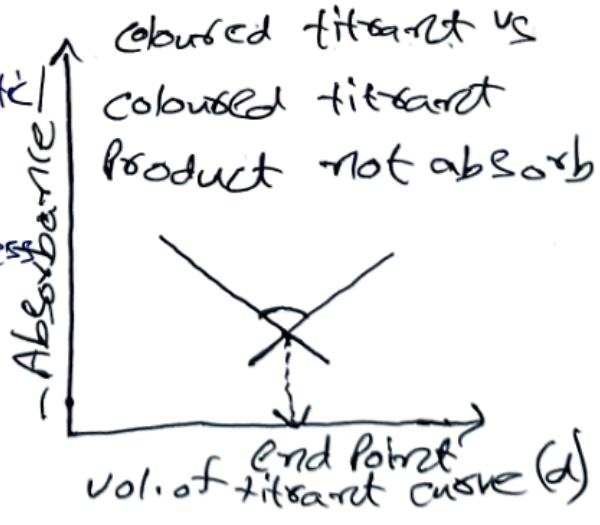
(7)

The most important example of this titration is Cu(II) Vs EDTA. & the titration is carried out at a wavelength 745nm. This is a selected the formation of complex Cu-EDTA. The complex absorbance is greater than absorbance compared to Cu(II) solution.

3) The curve (c) is characteristic of a case, where the substance being titrated absorb. The titrant and product do not absorb. The most imp eg is the titration of Para Toluidine in butanol, Vs Perchloric acid at 290nm. This wavelength is selected because Para Toluidine Vs Perchloric acid sharply absorb at this wavelength. where as the titranol Perchloric acid no absorb the radiation. & Perchloric acid is react with Para Toluidine the absorbance will be constant after the equalence point.

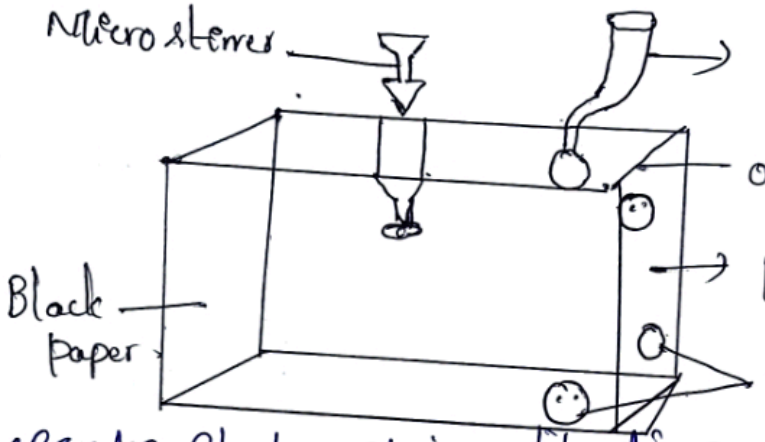



4) The curve (d) is Characteristic of a case where the coloured analyte is converted into a colourless titrant, when titrant is add it the colour of the analyte



decolourise due to the formation of colourless product but after equalence point. The absorbance again increase due to colour of the titrant alone

Apparatus :-




spectrophotometric titration are done in a 5-100ml capacity cell is employed. It is first in the compartment of the spectrophotometer.

The cell is made up of perspects sheet. As the material perspects is opaque to uv light opening (or) made in the cell and analysied to sample. The beam of monochromatic light enter into photoelectric cell. The cell has small openings one for tip of microburette and another one is micro stirrer. Except the quartz window the whole cell is covered with black paper.

Technique :-

⇒ the experimental technique is simple.

⇒ the solution to be titrated taken in the cell then the cell kept in the light path of a spectrophotometer.

(8)

⇒ The spectrophotometer is exited to the wavelength at which experiment is to be carried out and the instrument is first solid to zero. (if the reaction is colourless)

⇒ Some other colour samples are having different absorbance. After this is a known volume of titrant is add it to the stirrer in solution which taken in a cell and the absorbance is read again.

⇒ this is repeated at several points until get concurrent values are obtained.

⇒ Finally the absorbance is flat against the volume of titrant added. from this graph the equivalence point is obtained.

Applications:-

Acid-Base method:- eg:- phenols can be titrated with NaOH absorbance due to the formation of phenol ion is follows.

oxidation-Reduction method:- eg: Ce(III) can be titrated with Co(III)

Absorbance due to the formation of Ce(IV) is follows.

Complexometric titration :- eg: Cu(II) is titrated with EDTA.

Absorbance due to the formation of Cu-EDTA Complex.

Precipitation Method :- eg: Sulphate ($SO_4^{2-}$) can be titrated with Barium ($Ba^{+2}$).

## Instrumentation Spectrophotometer :-


## A Block diagram of spectrophotometer :-

Spectrophotometer is most important technique for analysis of different samples.

⇒ Spectrophotometry is used for accuracy analysed of a sample with a short period of time.

⇒ Spectrophotometry is a sensitive and accuracy when compared to colourometry.

⇒ In Spectrophotometry to maintain radiation intensity lies between 390-900nm. Sometimes it increase upto 1000nm.

Various components are used in Spectrophotometric techniques. These are

1) Source
2) Filters
3) mono chromators
4) sample cell
5) Detector

1. Source :- In spectrophotometry different sources are used for analysis of samples. mostly tungsto lamp and Carbon arc is used as a source. The sample is analysed at higher intensity Carbon arc used as a source.

2. Filters and Monochromators :-

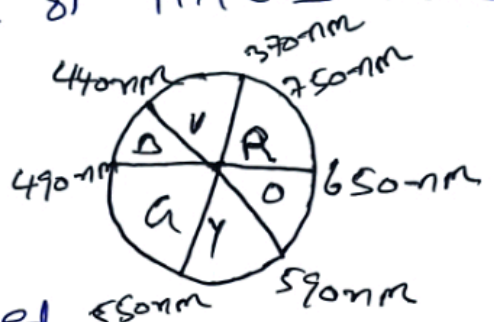
Different filters and monochromators are used in spectrophotometric technique, for absorbing unwanted radiation and convertion of polychromatic to monochromatic radiation.

1 Filters :- In spectrophotometric technique absorption Filter is used. It is made up of glass and coated with pigments (or) it is also made up of dyed gelatin they absorb unwanted radiation and transmit the rest of the radiation which is required for colourometry. The Filters can be selecte according to the Procedure.

⇒ Draw a filter wheel
⇒ write the colours (VIBGYOR) in clockwise (or) anticlockwise.

⇒ If the colour of the solution is red, we have to use green filter and if the colour of the solution is green we have to use red, Filter. (The colour of the filter is opposite to the colour of the solution).

⇒ sometimes this type of filters used in Colourimetric method.




Absorption Filters

Merits :-

1) It is easily constructed
2) It has low cost
3) use of selected Filter.

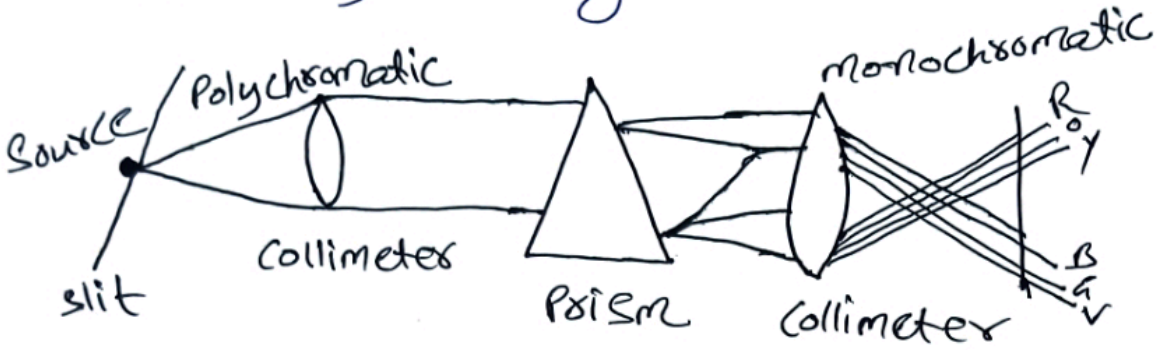
demerits :- Intensity of radiation becomes less due to absorption by Filters.

Mono chromators :- Monochromator is used for to get a single wavelength radiation.

Prism mono chromators is used in spectrophotometer method. In a prism monochromators source of light is passes into collimeter through slits. The Parallel radiation from collimeter are dispersed into different colours (or) wavelengths. By useing another collimeter the image of enterance slits are reformed. The reformed one will be either violet, blue, green, yellow, orange (or) red.

(10)

The required radiation on exit slit can be selected by rotating the prism.



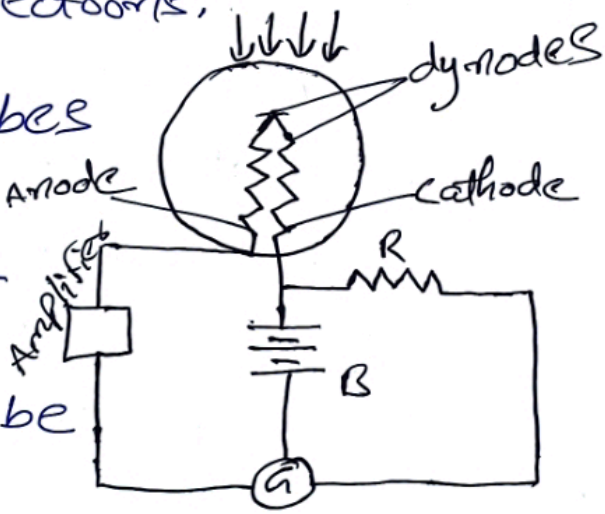

* Prism monochromator *
(dispersive type)

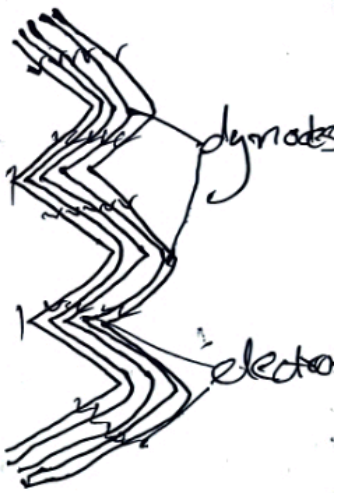
Sample cells :- The sample cell are cuvetts are used to hold a solution. The volume of sample is 0.5ml (small sample) and 5-10ml higher sample (large) and two types of sample cells are used in spectrophotometric technique: one is cylindrical and another one is rectangular, and the path length is 1cm (Normally) and 10cm (long path length) and internal distance is 1mm (or) 2mm. Sample cells are made up of glass, quartz and polystyrene. Polystyrene sample cells are preferred as Aqueous solvents and not for organic solvents.


Detectors :-

i) Photomultiplier tube :- Photomultiplier tubes used as a detector for accurate analysis of sample in uv-visible spectroscopy. This type of detector is the

most sensitive of all the detectors, and it is more expensive instrument. The principle employed in this detector is that, multiplication of photo electrons by secondary emission of electrons. This is acheived by useing a photo cathode and a series of anodes (dinodes), upto 10 dynodes are used. Each dynode contained at 75-100 volts higher than priceeding. At each stage the electron emission is multiplied by a factor of secondary emission of electrons.



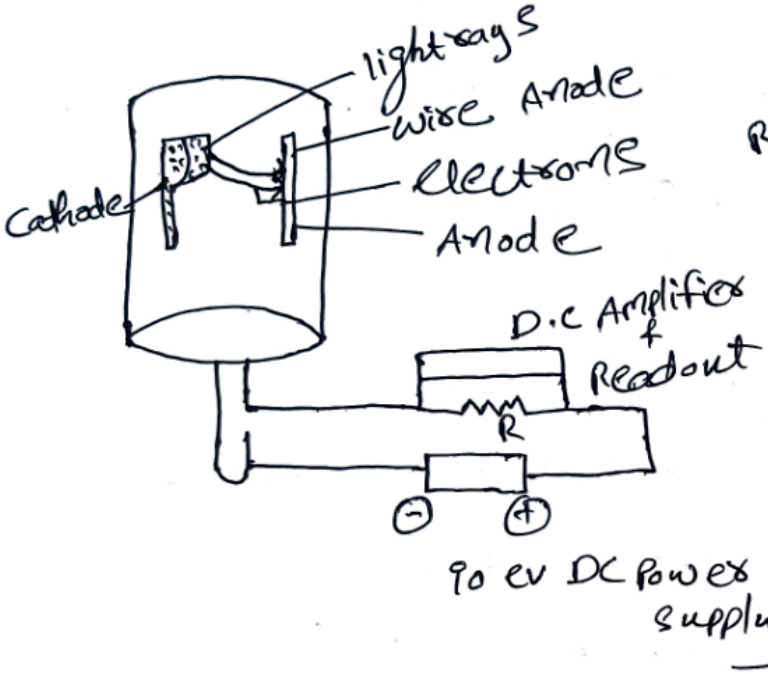



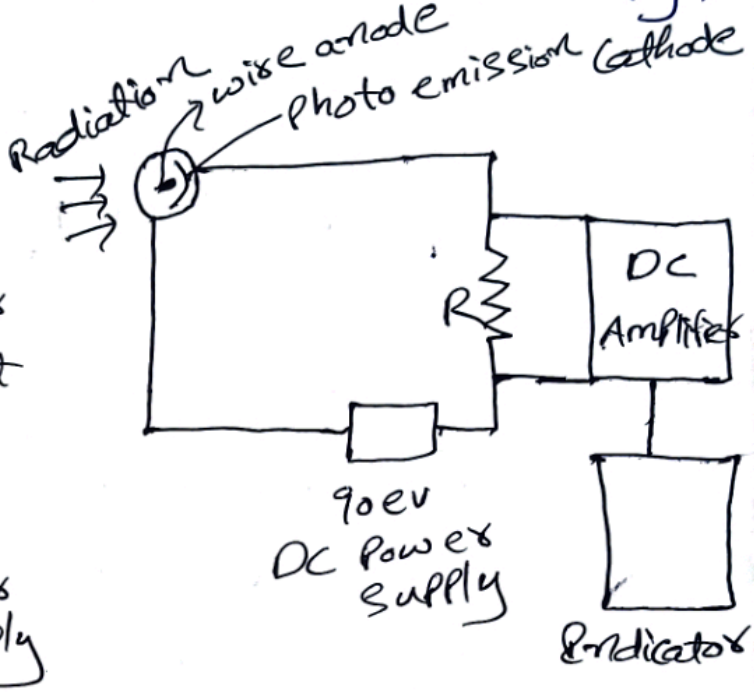

Photomultiplier tubes can detect very weak signals, even 200 times weaker than that should be done by useing photovoltic cell. Hence it is useful in flourescence measurments, photomultiplied tubes should be sealded from user light in order to have accurate results.

(ii) Photo emissive tubes (or) Photo tubes (or) vaccum tubes:-

In a detector a glasstube is used which is containing a electronic equipments.

(11) The detector is widely used for detecting of no. of samples, in uv-visible spectroscopy.




In a detector photo emissive cathode is used for producing of electrons and anode is used collecting of electrons produced by cathode. The cathode is coated with cesium ($Cs$), Potassium and silver oxides. The coated elements are used for increasing sensitivity and wavelength. The intensity of is proportional to electrons reached to anode. The signals from detector is amplified by using a D.C Amplifier. photo emissive detector is most useful than photo voltic cell.

law of Absorption :- when monochromatic (or) Polychromatic light is passes through a sample. In these a part of radiation is absorb, a part of radiation is transmitted and some of the radiation is reflected.

Now we calculate the total incident radiation is a combination of reflected radiation, transmitted radiation and absorbance radiation.

$$I_0 = I_r + I_t + I_a$$

Here,

$I_0$ - Incident radiation

$I_r$ - A part of reflected radiation

$I_t$ - A part of transmitted radiation

$I_a$ - A part of Absorbance radiation.

Reflected radiation is a smaller when compare to transmitted and Absorbance radiation, so it can be negleted. $I_0 = I_t + I_a$

The uv-visible spectroscopy absorbance place an important role, so it deals with the absorption laws.

Deviation from Beer's law :-

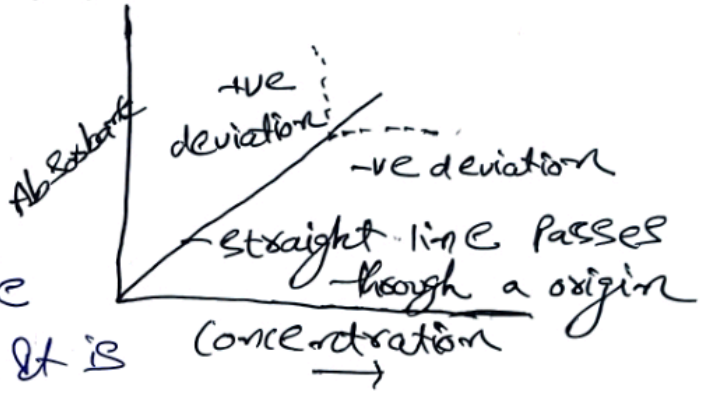
Deviations occurs due to several ~~regions~~ reasons among those two many place an important role, they are

1) chemical deviations

2) Instrumental deviations

Among these two, chemical deviations are minimised where as instrumental deviations are not.

Generally, deviations in beer's law is represented in a plot draw between concentration & Absorbance, that these are called +ve and -ve deviations.




**Chemical deviations:-**

⇒ Beer's law is not valide for concentrated solution. It is only applicable for dilute solutions.

⇒ Beer's law is invalid for non-homogenous solutions. Homogenous solutions obey Beer's law.

⇒ In NON-homogeneous solutions, they gets coagulated (or) forms turbidity which causes stray radiation is occurred.

⇒ This law is also not obey by suspension.

⇒ chemical deviation occurs due to the solution undergo Polymerisation.

⇒ **Instrumental deviation:**

⇒ due to fluctuation in a current

⇒ Stray radiation is developed due to instrumental

in perfection. this is occurs as a result of scattering and refluxtion of the surface of gratings, lenses, Filters and wall of windows,
⇒ sensitivity of instrument must be high.

determination of certain metal ions by useing ligands :-

$Fe^{2+}$ :- (Iron) 1,10-phenanthroline method :-

Discussion :- Iron(II) reacts with 1,10-phenanthroline to form an orange-red Complex. The colour intensity is independent of the acidity in the pH range 2-9, and as stable for long periods.

Reagents :- Prepare the following solutions
1,10-phenanthroline 0.25 percent solution of the monohydrate in water Sodium acetate → 0.2M and 2M hydroxylammonium chloride ⇒ 10 percent Ag. salt (or) benzene 1,4 diol (quinol), 1 percent solution in an acetic acid buffer of pH (4.5) of 0.1M. acetic acid and 35ml of 0.1M Sodium acetate solution. Prepare when required.

Procedure :- Take an Aliquot Portion of the unknown slightly acid solution containing 0.1-0.5 mg iron

and transfer it to a 50ml graduated flash. determine, by the use of a similar Aliquot Portion Containing a few drops of bromophenol blue, the volume of Sodium acetate solution required to bring the pH to 3.5 ±1.0 Add the same volume of acetate solution to the original aliquot part and then 4ml each of the quinol and 1,10-phenanthroline solutions. make up to the mark with distilled water, mix well, and allow to stand for 1 hour to complete the reduction of the iron. Compare the intensity of the colour produced with standards similarly prepared, in any convenient way. if a Calorimeter is employed use, a filter showing maximum transmission at 480-520 nm. for a spectrophometer, use a wavelength of 515 nm.

The iron may also be added with hydroxylammonium Chloride Add 5ml of to lopescent hydroxylammonium Solution, Adjust the pH of the slightly acid solution to 3-6 with Sodium acetate, then add 4ml of the 1,10-phenanthroline Solution dilute to 5ml, mix and measure the absorbance after 5-10 minutes.

Fe 3rt :-

Reagents :- Prepare the following solutions.

Standard solution of iron (III) use method (a) (b) (or) (c).

a) dissolve 0.7022g ammonium iron (II) sulphate in 100ml water, Add 5ml of 1:5 sulphuric acid, and run in a dilute solution of Potassium permanganate ($2gL^{-1}$) until a slight pink colouration remains after stirring well. Dilute to 1L and mix throughly 1ml ≡ 0.1mg of Fe.

b) dissolve 0.864g ammonium iron sulphate in water, add 10ml Concentrated hydrochloric acid and dilute to 1L, 1ml ≡ 0.1mg of Fe.

c) Dissolve 0.1000g of electrolyte iron of Pure iron wire in 50ml 1:3 Nitric acid, boil to expel oxids of Nitrogen, and dilute to 1L with de-ionised water,

Potassium thiocyanate solution dissolve 20g Potassium thiocyanate in 100ml water, the sol$^{n}$ is ca 2M.

Procedure :- Dissolve a weighed Portion of the substance in which the amount of iron to be determine in a suitable acid, and evaporate nearly, to dryness to expel excess of acid.

Dilute slightly with water, oxidise the iron to the iron(III) state with dilute Potassium permanganate solution (or) with a little bromine water, and make up the liquid to 500ml (or) other suitable volume. Take 40ml of this solution & place in a 50ml graduated flask, add 5ml of the thiocyanate solution & 3ml of 4M nitric acid. Add deionised water to dilute to the mark. Prepare blank using the sample quantities of reagents measure the absorbance of the sample solution in a spectrophotometer at 480nm. determine the concentration of this solution by comparison with values on a reference curve obtained in the same way from different concentrations of the standard iron solution.

Al$^{3+}$ :- [Aluminium]

Reagents:- Eriochrome cyanine R solution, dissolve 0.1g of the solid reagent in water, dilute to 100ml and filter through a whatmann No.1 filter filter paper of necessary. This solution should be prepared daily.

Standard aluminium solution dissolve 1.319g aluminium potassium sulphate in water, and dilute to 1L in flask, 1ml = 75 µg Al.

Buffer Solution, Concentrated dissolve 27.5g aluminium acetate & 11.0g hydrated sodium acetate in 100ml water add 1.0ml glacial acetic acid & mix well.

Buffer solution dilute To one volume of concentrated buffer solution, add five volumes water and adjust the pH to 6.1 by adding acetic acid (or) Sodium hydroxide solution.

Procedure:- Transfer an aliquot of the solution containing 2-70 µg Al and free from interfering elements to a 250ml beaker, add 5ml of five volume hydrogen peroxide & mix well. Adjust the pH of the solution to 6.0, add 5ml of eriochrome cyanine R solution, and mix. Introduce 50ml of the dilute buffer solution & dilute without delay to 100ml in a graduated flask. Measure the absorbance after 30 minutes with a spectrophotometer at 535nm against a reagent blank in a 5mm cell for an absorptiometer, use a yellow-green filter and 1cm cells.

Construct the Calibration curve useing 0, 1, 2, 3, 4, 5 of the standard aluminium solution.

(15)

## $NH_4^+$ :- (Ammonia)

**Reagents:-** Nessler's reagent is prepared as follows. dissolve 35g Potassium iodide in 100ml water and add 4 Percent mercury (II) chloride solution, with stirring (or) shaking until a slight red precipitate, then introduce with stirring a solution of 120g Sodium hydroxide in 250ml water & make up to 1L with distilled water Add more mercury(II) Chloride solution until there is a permanent turbidity. Allow the mixture to stand to one day and decant from the sediment. Keep the solution stoppered in a dark-coloured bottle.

The following is an alternative method of Preparation. dissolve 100g mercury (II) iodide add 70g Potassium iodide in 100ml.

Ammonia-free water Add slowly, and with stirring, to a cooled solution of 160g Sodium hydroxide pellets in 700ml ammonia, free water and dilute to 1L with ammonia free distilled water. Allow the precipitate to settle, preferably for a few days, before using the Pale yellow supernatant liquid.

Procedure:- For Practice in this determination employ either a very dilute ammonium chloride solution (or) ordinary distilled water which usually contains sufficient ammonia for the exercise.

Prepare a standard ammonium chloride solution as follows. dissolve 3.141g ammonium chloride dried at 100°c, in ammonia-free water & dilute to 1L with the same water. This stock solution is too concentrated for most purposes. A standard solution is made by diluting 10ml of this solution to 1L with ammonia-free water 1ml contains 0.01mg of identified.

Chromium:- [$Cr^{3+}$, $Cr^{6+}$]

discussion:- Small amount of chromium may be determined colorimetrically in Alkaline solution as chromate, uranium and cerium interfere, but vanadium has little influence. the transmittance of the solution is measured at 300-370nm (or) with the aid of a filter having maximum transmission in the violet portion of spectrum. The standards may be Prepared from analytical grade potassium chromate.

A more sensitive method is to employ 1,5-diphenylcartazide $Co(NH.NHC_6H_5)_2$, in acid soln (ca 0.2M), chromate gives a soluble violet compound with the reagent.

Molybdenium (VI), Vanadium (V), mercury, and iron interfere permanganates, if present, may be removed by boiling with a little ethanol, if the ratio of vanadium to chromium does not exceed 10:1, nearly, correct results may be obtained. Chromate remains in the Aqueous solution. Vanadium as well as iron can be precipitated in acid solution with Cupferron and thus separated from chromium (III).

Procedure:-

Prepare 0.05 percent solution of diphenyl-Carbazide in 50 percent acetone as required. The solution may contain from 0.2 to 0.5 part per million of chromate. To about 15ml of this solution add sufficient 3M sulphuric acid to make concn... about 0.1M when subsiquently diluted to 25ml, Add 1ml of the diphenylcarbazide reagent and make upto 25ml with water match the colour produced against standards prepared from 0.0002M potassium di-chromate solution. A green filter having the

the transmission maximum at about 540nm, be used.

## Anions :-

### Nitrite [$NO_2^-$] :-

**Reagents :-** Sulphanilamide solution

a) dissolve 0.5g sulphanilamide in 100ml of 20percent v/v hydrochloric acid.

N - (1-napthyl) - ethylenediamine dihydrochloride solution (B) dissolve 0.3g of the solid reagent in 100ml of 1 Percent v/v hydrochloric acid.

**Procedure :-** To 100ml of the neutral sample solution add 20ml of solution A and, after 5min 2.0 ml of solution B. The pH at this point should be about 1:5. measure the absorbance after 10min in the wavelength region of 550nm in a spectrophotometer against a blank solution prepared in the same manner. Calculate the concentration of the Nitrite to a calibration plot prepared of a series of standard Nitrite solutions.

### Phosphate :- [$PO_4^{3-}$]

**Reagents :-** Ammonium vanadium solution dissolve 2.5g ammonium vanadate ($NH_4VO_3$) in 500ml hot water.

Add 20ml Concentrated Nitric acid and dilute with water to 1ml in a graduated flask.

Ammonium molybdate solution, dissolve 50g ammonium molybdate $(NH_4)_6Mo_7O_{24}, 4H_2O$ to warm water and dilute to 1l in a graduated flask. Filter the solution before use.

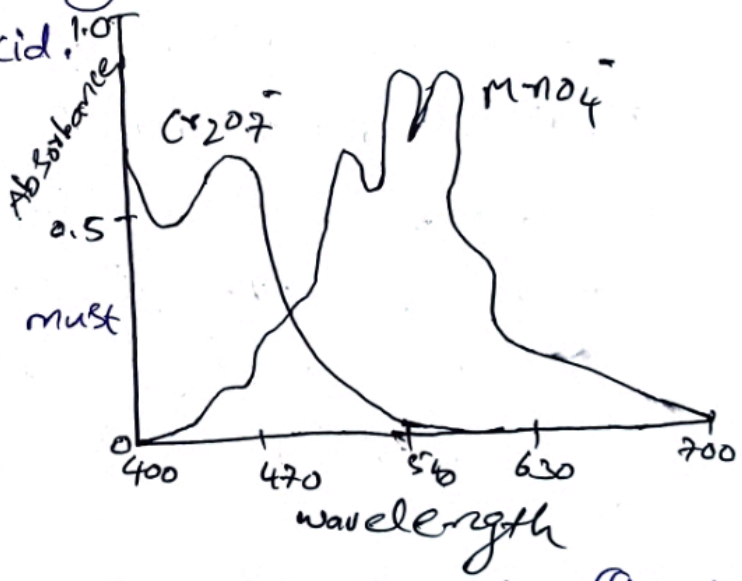
Procedure :- Dissolve 0.4g of the phosphate sample in 2.5 ml Nitric acid to give 1L in a graduated flask. Place a 10ml aliquot of this solution in a 100ml graduated flask, add 50ml water, 10ml of ammonium vanadate solution. 10ml of the ammonium molybdate solution & dilute to the mark. determine the absorbance of this solution at 465nm against a blank prepared in the same manner, useing 1cm cells.

Prepare a series of standards from Potassium dihydrogen phosphate covering the range 0.2mg phosphorus per 100ml and containing the same con. of acid, ammonium vanadate, and ammonium molybdate as the previous solution. Construct a calibration curve and use at to calculate the concentration of phosphorus in the sample.

## Simultaneous determination of dichromate and Permanganate in a mixture:- {chromium and manganese}

**Reagents:-** Potassium dichromate 0.002M, 0.001M, and 0.0005M in 1M Sulphuric acid and 0.7M Phosphoric (v) acid, Prepared from the analytical grade reagent.

Potassium Permanganate 0.002M, 0.001M, and 0.0005M in 1M Sulphuric acid. 0.7M Phosphoric(v) acid, Prepared. from the analytical grade reagents All flasks must be scrupulously clean.




**Procedure:-**

determination of Molar absorption Co-efficients and verification of additivity of absorbance.

The molar absorption Coefficients must be determined for the particular set of the Spectro-photometer, employed for the present Purpose we may write.

$$A = \varepsilon c l$$

where $\varepsilon$ is the molar absorption Coefficient, c is the concentration (mol $L^{-1}$) and $l$ is the cell thickness

(or) length.

Measure the absorbance A of the above three solutions of Potassium dichromate and of Potassium permanganate, each solution separately, at both 440nm and 545nm in 1cm cells. Calculate $\varepsilon$ in each case and record. The mean values for $Cr_2O_7^{2-}$ and $MnO_4^-$ at the too wavelengths.

mix 0.001M Potassium dichromate and 0.0005 M Potassium permanganate ~~and~~ in the following amounts and prepare a set of results. Similar to those in table 17.5, which is a set of typical results included for guidance only measure the absorbance.

Each of the mixture at 440nm calculate the absorbance of the mixtures, from

$$A_{440} = 440\,\varepsilon_{Cr} \cdot C_{Cr} + 440\,\varepsilon_{Mn} C_{Mn}$$

dichromate:-

estimation of $Cr^{++}$ in $K_2Cr_2O_7$:-

orange coloured $Cr^{+++}$ in acid mucle acts as a strong oxidising agent.

$$Cr_2O_4^{2-} + 14H^+ + 6e^{\ominus} \longrightarrow 2Cr^{3+} + 7H_2O \quad \epsilon^\circ = 1.33v$$

It oxides ferrous ion to Ferricion.

$$Fe^{2+} + e^- \longrightarrow Fe^{3+} \text{ (Pale shug)}$$

Standard ferrous ammonium solution is prepared. Similarily $K_2Cr_2O_7$ solution of known concentration is prepared.

They are mixed in the colour shown in the following table and water is added to make the total volume of 25ml. The amount of $Cr^{+++}$ ions in each tube is calculated and noted in the Test Column.

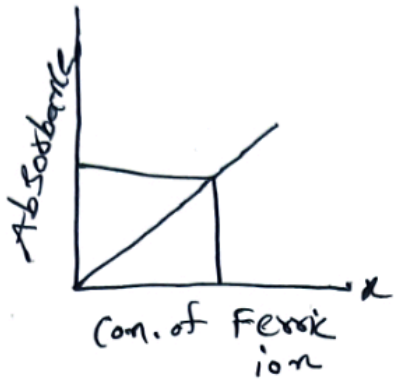
Then the mixture is observed under spectro relsonde (or) Calorimeter and noted the absorption.

A group of down bottom the absorbance and concentrate of $Cr^{+++}$ this the Concentration of $Cr^{+++}$ in the given solution is calculates.

| Volume of $K_2Cr_2O_7$ | Sol$^n$ of Ammonium ferrous sulphate | Total | Con. of $Cr^{+++}$ of the sol$^n$ |
|---|---|---|---|
| 10 | 5 | 25 | |
| 5 | 5 | 25 | |
| 4.5 | 5 | 25 | |
| 4 | 5 | 25 | |
| 3.5 | 5 | 25 | |
| 3 | 5 | 25 | |
| 2.5 | 5 | 25 | |
| | 5 | | |

(19)

graph




Permanganate:

Estimation of manganese in MnSO4 (or) steel:

Certain amount of steel (underestimation) with manganese content is selected of 1%. The sample is accurately weighed (0.1 to 0.2gm) and dissolved in moderate dilute nitric acid by boiling for a few minutes. After the oxides of nitrogen is removed completely about 1gm of ammonium persulphate is added and boiled for about 10-13 min. To make the solution free from any pink colour, few drops of pure sodium sulphate are added. It is again boiled to expel $SO_2$ completely. The mixture is cooled, diluted upto 100ml. 10ml of AR phosphoric is added and then 0.5gm of Pot Sodate. The solution is made up to the mark in a 250 ml flask.

Now this solution is taken into spectrophotometer and exposed to a source of 525nm radiation and the absorbance is determined.

this value is compared with the standard values and absorbance calibration curve is drawn. From this, the percentage of manganese in the given sample of steel is estimated.

# PART-(B)

## Spectrofluorimetry :-

When UV (or) Visible radiation is passed through a Molecule it is absorb the radiation and electrons and transferred from ground state to excited state. In excited state no molecule is stable due to loss of energy and electrons are transfered from exited state to ground state by the emission of radiation.

To measured emission of radiation when electrons are transferred from singlet excited state to singlet ground state. Similarly in a phosphorscence the emission of radiation is measured when electron transfere from triplet state to ground state

The electron states are explain as below.

1. **Singlet Ground State :-**

A State in which electrons are paired in a Molecule

i.e, ↑↓ Paired

2. **Doublet state :-** A State in which electrons are unpaired in a Molecule.

Ex:- free radical

i.e, ↑ (or) ↓

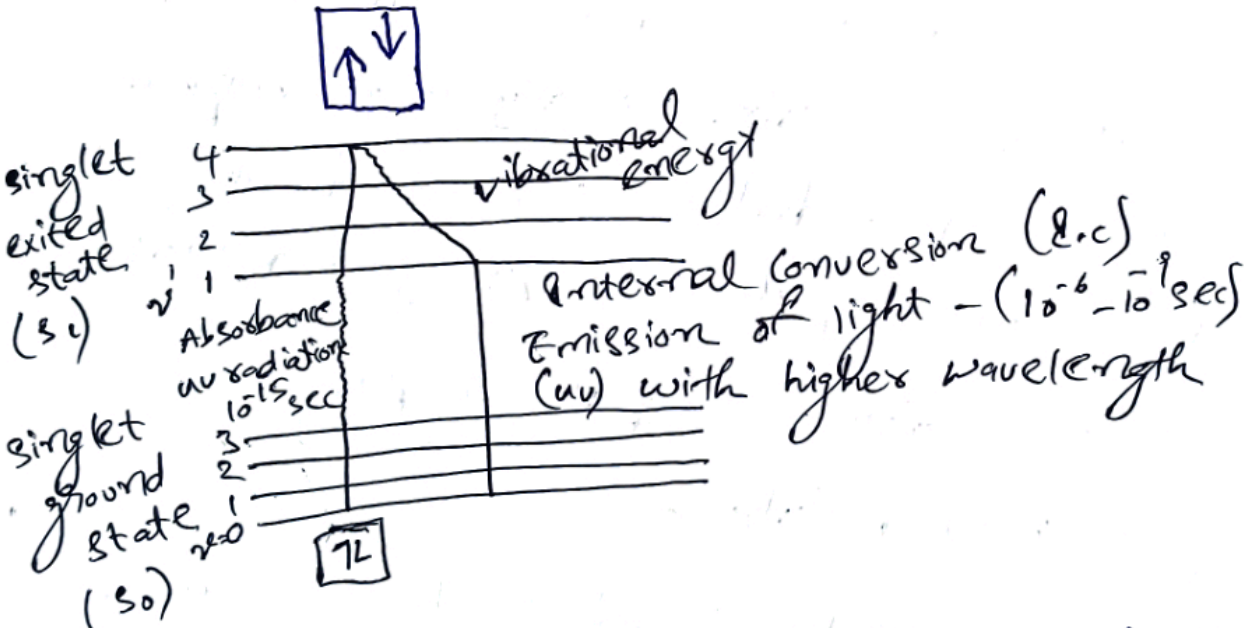
3. Triplet state :- A state in which unpaired electrons and same spin of electrons present in a molecule.

i.e, ↑↑

4. Singlet excited state :- The electrons are unpaired and opposite spin in a molecule

i.e, ↑↓

* Fluorescence :-




when uv (or) visible radiation is passes through a fluorescence material. It is absorb the radiation and electrons are transferred from ground state to exited state

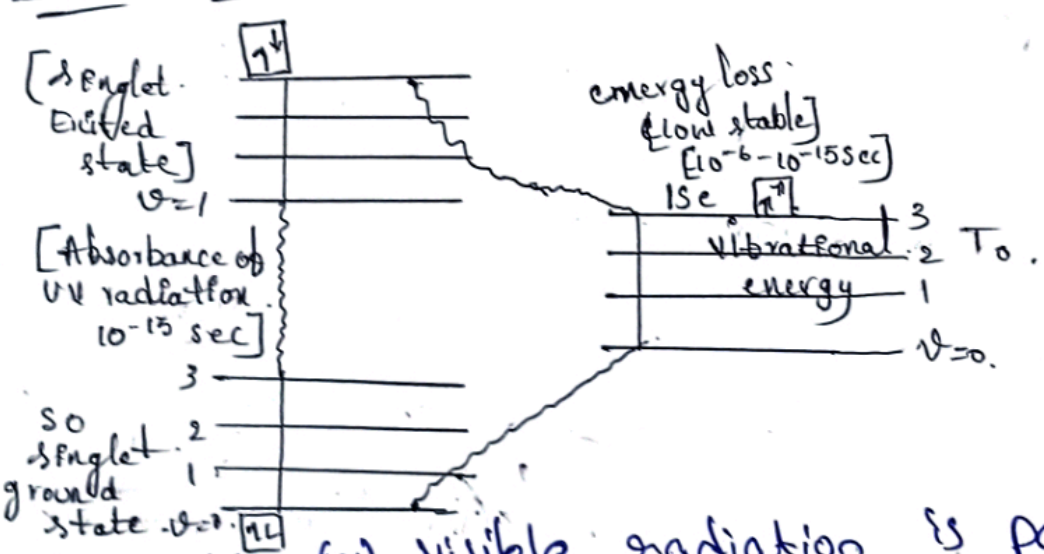
i.e; $S_0 \longrightarrow S_1$

(21)

In exited state no molecule is stable due to loss of energy and to take vibrational and electrons are transferred from exited state to ground state. It is known as internal conversion (I.C) and electron transition is denote $S_1 \longrightarrow S_0$ The electrons are comes to ground state with a short time and higher wavelength is known as fluorescence.

Ex:- Na, Hg vapours.

* Phosphorescence :-




When UV (or) visible radiation is passed through a molecule it is absorb and electrons are transfer from ground state to exited state.

i.e $\boxed{S_0 \longrightarrow S_1}$

By the loss of energy electrons are transferred

from singlet exited state to Triplet state

i.e, $S_1 \longrightarrow T_0$

In the triplet state the Molecule has unstable and vibration transition is done and electrons are transferred from triplet state to singlet ground state by the emission of radiation.

i.e., $T_0 \longrightarrow S_0$

It is known as intersystem crossing. electrons are transferred from triplet state to ground state with lower wavelength and to take higher time is known as phosphorescence.

Eg:- Zink sulphites, IIA group elements.

## * Theory of fluorescence:-

For every Molecule contain various exited states i.e, $E_1, E_2, \ldots$ etc and their wavelengths are considered $\lambda_1, \lambda_2 \ldots$ etc. The transition occurs in these state and loss of energy and due to electron transition in different states.

These Mainly following two types of relaxation.

1. Non Radiative relaxation.
2. Fluorescent relaxation.

(22) Non Radiative relaxation:-

The relaxation is generally occurs in vibrational level. The Non radiative relaxation is the electrons in exited energy transferred of electrons from higher vibrational level to lower level. In this released radiation in the form of heat. In the process electrons are transferred singlet exited state to single ground state is called internal conversion If electrons are transfer from triplet state to ground state is called inter system crossing. The lifetime is Milli sec.

2. Fluorosent relaxation:-

After exitation of molecule. then it is release radiation and electrons are transferred in different levels to ground state this is explained by two rules.

1. Frank conden principle.
2. Kash rule.

Frank conden principle:-

According to frank conden principles the intensity of radiation is changes then

the changing of vibrational energy and no appricable change between energy and intensity and electrons are transferred in different states from ground state to exited state and changing of intensity electrons are transferred from exited state to ground state.

Kash rule:-

For any poly atom molecule the radiative process uses taken place from the lowest singlet state or in the triplet state the photochemical reactions are done from a electrons are transferred in a different states.

Note:-1

1. The wave length of emission radiation is greater than wave length of absorbed radiation is known as stocks fluoresence.
2. The wavelength of emission radiation is smallerthan wave length of absorbed radiation is known as Anti stocks fluoresence.
3. The wave length of emission radiation is equal to wave length of absorbed radiation is known as resonance fluoresence.

"fluorescence."

Note-2

Collisional deactivation:- In which the entire energy is loss due to collisional deactivation and no radiation is emission.

* Effect of factors on fluorescence:-

1. Conjugation (or) nature of sample (or) nature of solvent:-

A Molecule must have unsaturation ($\pi e^-$ s) that is conjugation) so that UV (or) visible radiation can be observed. If their is no absorbtion of radiation there will not be flourescence. The intensity of exiting line is proptional to the concentration of fluorescence Material.

2. Nature of Substituent groups:-

1. Electrons donating groups like amino ($NH_2$), Hydroxyl (-OH), Methyl groups ($CH_3$) have higher fluorescence intensity

2. Electron withdrawing groups like chloride, Bromide, sodide, carboxylic groups are having lower fluorescence intensity.

3. Some examples of relative intensity of molecules is

1. Benzene – 10
2. Toulene – 17
3. phenol – 18
4. Aniline – 20
5. chlorobenzene – 7
6. Bromobenzene – 5

The group like Ammonium ion ($NH_4^+$) have no effect on fluorescence intensity

3. Effect of Temperature :–

Increase in temperature leads to increase in collision of molecule therefore deviation, then the decreasing fluorescence intensity similarly decrease in temperature leads to decrease in collision of molecule then increase in fluorescence intensity

4. Viscosity :– Increase in viscosity leads to decreased collisions of molecule and increasing of fluorescence intensity. similarly decrease in viscosity it leads to increasing collision of molecule then decreasing fluorescence intensity.

5. Effect of $p^H$ :- The effect of $p^H$ depends on chemical structure of the molecule.

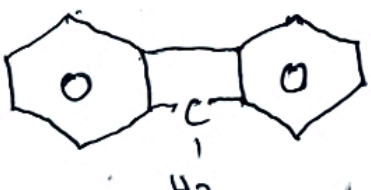
1. Aniline in neutral or alkaline medium gives visible fluorescence but in acidic condition gives fluorescence in UV region only.

2. Phenols in acidic condition (or) undissociated and donot gives fluorescence, but in Alkaline medium, they are dissociated and gives fluorescence

6. Structural Rigidity :-

Experimentally found that rigid molecules may fluorescence. According to quantum efficiency the fluorescence is measured. under similar condition the fluorescence of Biphenyl is 0.2 respectively the quantum efficiency of fluorine 1.0.

The difference in the values of fluorescence is to bridging methyl groups in fluorine and it is favour of increasing rigidity.

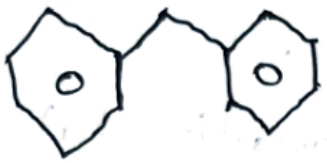



Flourene


Biphenyl

(Quantum efficiency $\phi = 1.0$) (Quantum efficiency $\phi = 0.2$)

Rigidity of a Molecule also influence the increasing fluorescence of zink complex of 8-Hydroxy quinoline than compained to 8-Hydroxy quinoline.



* Relation between intensity of fluorescence radiation & concentration :-

According to spectro fluorometry, less no. of ions present in a Molecule it is absorb less radiation as well as less amount of emission radiation similarly more no. of ions present in a Molecule it absorb higher radiation as well as higher amount of radiation is emission.

From Beer's - lamberts law,

$I_0$ - Intensity of incidant radiation

$I_t$ - Intensity of transfer radiation

F - Intensity of fluorescence radiation

$I_0 - I_t$ = Intensity of Absorbance radiation.

$$\therefore \boxed{I_0 - I_t = I_{absorbance}} \quad \text{---} \quad (1)$$

According to Beer's - lambert's law

$$\boxed{I_t = I_0 \cdot e^{-a.c.l}} \quad \text{---} \quad (2)$$

(∵ c is concentration of soln l is thickness of medium)

The value (2) is substitute in eq (1)

From eq (1), $I_0 - I_0 \cdot e^{-acl} = I_{abs}$

$$I_{abs} = I_0 - I_0 e^{-acl} \qquad (\because I_t = I_0 \cdot e^{-acl})$$

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

$$\left(e^{-acl} = 1 - \frac{acl}{1!} + \frac{(acl)^2}{2!} + \cdots + \frac{(acl)^n}{n!}\right)$$

$$\therefore I_{abs} = I_0 \left[1 - \left(1 - \frac{acl}{1!}\right)\right]$$

$$\boxed{I_{abs} = I_0 \cdot acl}$$

Now fluorescence intensity

$$F = I_{abs} \times q$$

$$= I_0 \cdot acl \times q \qquad (\because I_{abs} = I_0 acl)$$

$$= \underline{I_0 a.l.q} \times c$$

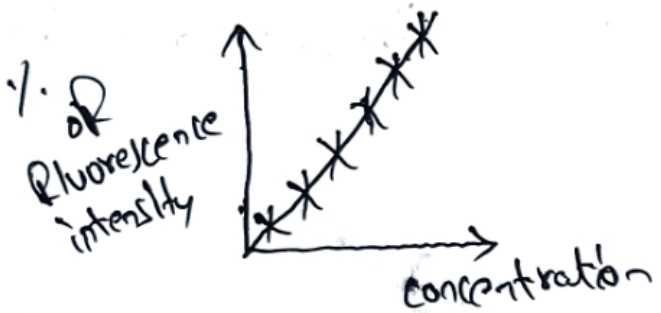
$$F = kc \qquad (\because k = I_0 \cdot a \cdot l q)$$

$$\boxed{F \propto c}$$

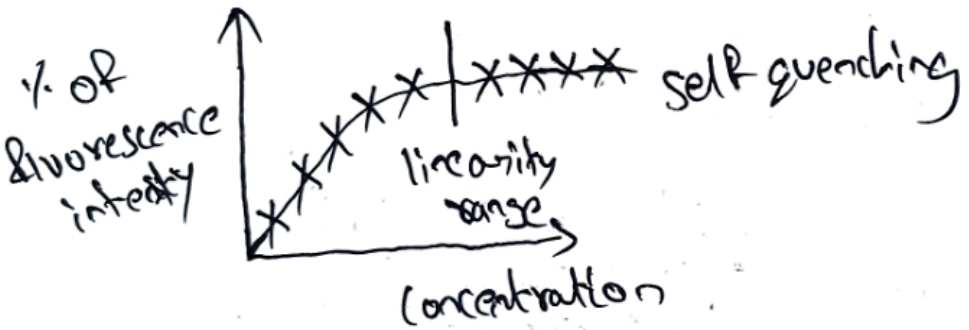
Fluorescence intensity is always proportional to concentration of solution

Graph :-

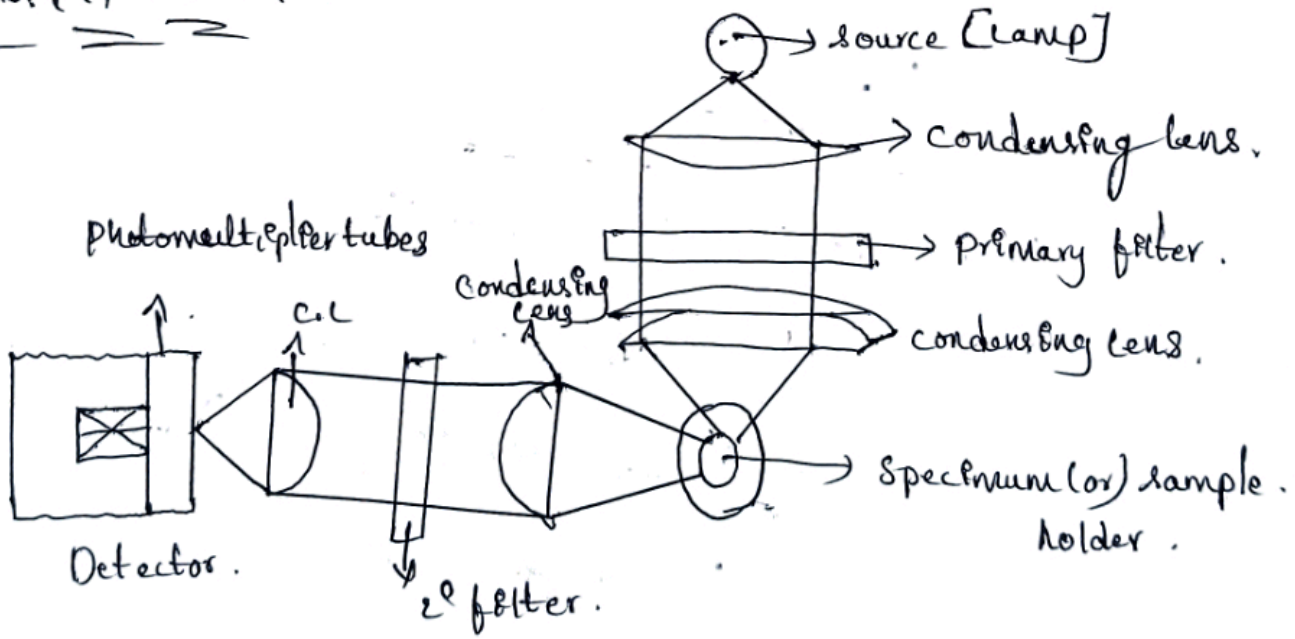
1. Calibration curve at low concentration.




2. Calibration curve at high concentration




* Instrumentation :-



## Principle :-

When UV (or) visible radiation is passed through a molecule it is absorb the radiation and electrons are transferred from ground state to exited state. In exited state no molecule is stable due to loss of energy and electrons are transferred from exited state to ground state by the emission of radiation.

To measured emission of radiation when electrons are transferred from singlet exited state to singlet ground state, similarly in a phosphorescence the emission of radiation is measured when electron transferred from triplet state to ground state.

The electron states are explain as below.

1. **Singlet Ground State :-**

A State in which electrons are paired in a molecule.

i.e., ⇅ -> paired

2. **Doublet State :-**

A State in which electrons are paired in a molecule

Exi- free radical

i.e., ↑ (or) ↓

3. Triplet State:-

A State in which unpaired electrons and Same spin of electrons present in a molecule.

i.e, 

4. Singlet exited State:-

The electrons are unpaired and opposite spin in a molecule.

i.e, 

Spectro fluorometry is used for measurement of intensity of emission radiation after transferred motion of electrons in a sample.

Working:-

A light from the lamp is passes through a condensing lens and 1° filter through double condensing lens into sample which taken in a sample holder.

In spectrofluorometry Uv (or) visible radiation is used as source for analysis of samples condensing lens are used for absorb the radiation and converted to single rays into 1° filter. 1° filter is used for convertion of polychromatic to monochromatic light

(2) i.e, It is used for passing of single wave length radiation in spectrofluorometry quartz filters are prefered for convertion of polychromatic to monochromatic light. A single wavelength radiation is passes through condensing lens into sample. the sample is absorb UV (or) visible radiation and producing of no. of electrons as well as some of the radiation is emission. The emission radiation is passes through condensing lens and 2° filters into detector. In spectrofluorometry 2° filters is also used for single wave length radiation. In these spectroscopy commonly used detectors are photomultiplier detectors. The detectors is used for detecting of intensity of emission radiation

Application's :-

1. It is used for determination of trace elments by Uranium, platinum and cadmium ($C_d$). cadmium can be determined by precipitation.
2. It is used for Analysis of sample in ~~qualities~~ qualitatively and quantitatively.
3. It can be used for determination of different samples and different components like food substance in -

Pharmaceutial, different chemicals in chemical industries and natural products.

4. It can be used for the determination of vitamins like Thiamine ($B_1$) and Riboflavin ($B_2$) etc....

## * Determination of thiamine ($B_1$) :-

Thiamin is non-fluorescent. It is determined after oxidation by using a suitable oxidising agent. The thiochrome (fluorescent as a blue colour) determined by using spectrofluorometry. Vitamin $B_1$ determined in food sample by the addition of phosphate and hydrolysis then to from phosphate ester of thiamine in the sample.

the solution on filteration and remove phosphate and other insoluble meter. Then the filterate is dilute to known volume and to take equal portion for analysis of one sample and second one is blank. To both portion equal quantity sodium hydroxide and also butyl alcohol are added

The first oxidising agent is add it like potassium ferricyanide and mix well the alcoholic solution

(28) It is seperate from the Aqueous solution. Then the alcoholic solution is determined by using fluorometry

The whole procedure including to blank and repeated the process by standard thiamin solution.

the determination value of thiamin in a sample is compare with standard values.

## * Determination of Riboflavin ($B_2$) :-

A fluorescence method is used for determination of vitamin $B_2$ in a given food sample. in fluorescence, method, fluorescense powder is taken it is depend upon nature of the sample and impurities generally a standard method is used for detamination of Riboflavin in a given sample.

### Procedure:-

The food sample (acedic character) is treated with different reagents to form precipitation various interfering ions, the solution is oxidised with dilute permongenate solution then the recedued fluorescence is measured as a blank.

Now, a digit accesies of sodium dithionate

($Na_2S_2O_4$) is add it. the fluorescence is again determine then a known volume of standard is added and fluorescence is measured.

Finally the results as follows:-

| Fluorescence Solution | Designation |
|---|---|
| 10ml of oxidised solution + 1ml water | '$F_A$' |
| 10ml of oxidised sample + Dithionyl solution | '$F_B$' |
| 10ml of oxidised sample + 1ml Standard solution | '$F_C$' |

From the above three results the concentration of fluorescence material can be calculated by using the following formula.

Formula:-

$$\frac{F_B - F_A}{F_C - F_A} = \frac{M_x}{M_x + M_s}$$

Here; $M_x$ is mass of sample.
$M_s$ is mass of standard.