Adikavi Nannaya University :: Rajahmundry
Department of Chemistry
M.Sc. (Final) Chemistry Syllabus for III-Semester
Specialization: *Analytical Chemistry*
Paper – IV: INSTRUMENTAL METHODS OF ANALYSIS - I

(Effective from 2016-17 Admitted batch)

Unit – I : Spectroscopic Methods - 1

(a) UV-Visible Spectroscopy: laws of absorption, deviation from Beer's law, single and double beam spectrophotometers-instrumentation, sources of radiation, detectors, qualitative analysis by absorption measurements, general precautions in colorimetric determinations, determination of certain metal ions by using ligands – Fe^{2+}, Fe^{3+}, Al^{3+}, NH_4^+, Cr^{3+}, Cr^{6+}, Co^{3+}, Cu^{2+}, Ni^{2+} and anions – NO_2^-, PO_4^{3-} using suitable reagents, simultaneous determinations of dichromate and permanganate in a mixture, spectrophotomeric titrations, principle of diode array spectrophotometers.

(b) Spectrofluorimetry: Theory of fluorescence, phosphorescence, factors affecting the above, quenching, relation between intensity of fluorescence and concentration, instrumentation, application with reference to Al^{3+}, chromium salts, fluorescence, thiamin (B1) and riboflavin (B2) in drug samples.

Unit – II : Spectroscopic Methods - 2

(a) ***Infrared spectroscopy:*** units of frequency, wavelength and wave number molecular vibrations, factors influencing vibrational frequencies, instrumentation, sampling techniques, detectors, characteristic frequencies of organic molecules, qualitative and quantitative analysis with reference to (petroleum refinery and polymer industry), selected molecules like CO, CO_2, non-destructive IR method for the analysis of CO and other organic compounds, principles of Fourier transform IR.

(b) ***Raman Spectroscopy:*** Raman effect and spectra, differences between Raman spectra and IR spectra, instrumentation, Raman spectra of CO, CO_2, N_2O, H_2O.

Unit – III : Spectroscopic Methods -3

(a) NMR Spectroscopy: resonance condition, origin of NMR spectra, instrumentation, chemical shift, factors affecting chemical shift, shielding, spin-spin splitting, mechanism for spin-spin coupling, interpretation of NMR spectra of typical organic compounds, factors influencing NMR spectra, fast chemical reactions, magnitude of I, nuclei with quadrupole moments, FT NMR, study of isotopes other than proton-^{13}C, ^{15}N, ^{19}F, ^{31}P, ^{11}B, double resonance, spin tickling, shift reagents, applications.

(b) ESR Spectroscopy: principle, g value, hyper fine splitting, qualitative analysis, Krammers degeneracy, fine splitting, instrumentation, introduction to double resonance technique, difference between ESR and NMR spectra, quantitative analysis, application to study of free radicals and other analytical applications.

Unit – IV : Spectroscopic Methods -4

*(a) Mass Spectroscopy: P*rinciple, basic instrumentation, energetics of ion formation, types of peaks observed, resolution, qualitative analysis, molecular weight determination, quantitative analysis, advantages

(b) X-ray Spectroscopy (XRF): chemical analysis by X-ray spectrometers, energy dispersive and wavelength dispersive techniques, evaluation methods, instrumentation, matrix effects, applications.

Text Books:

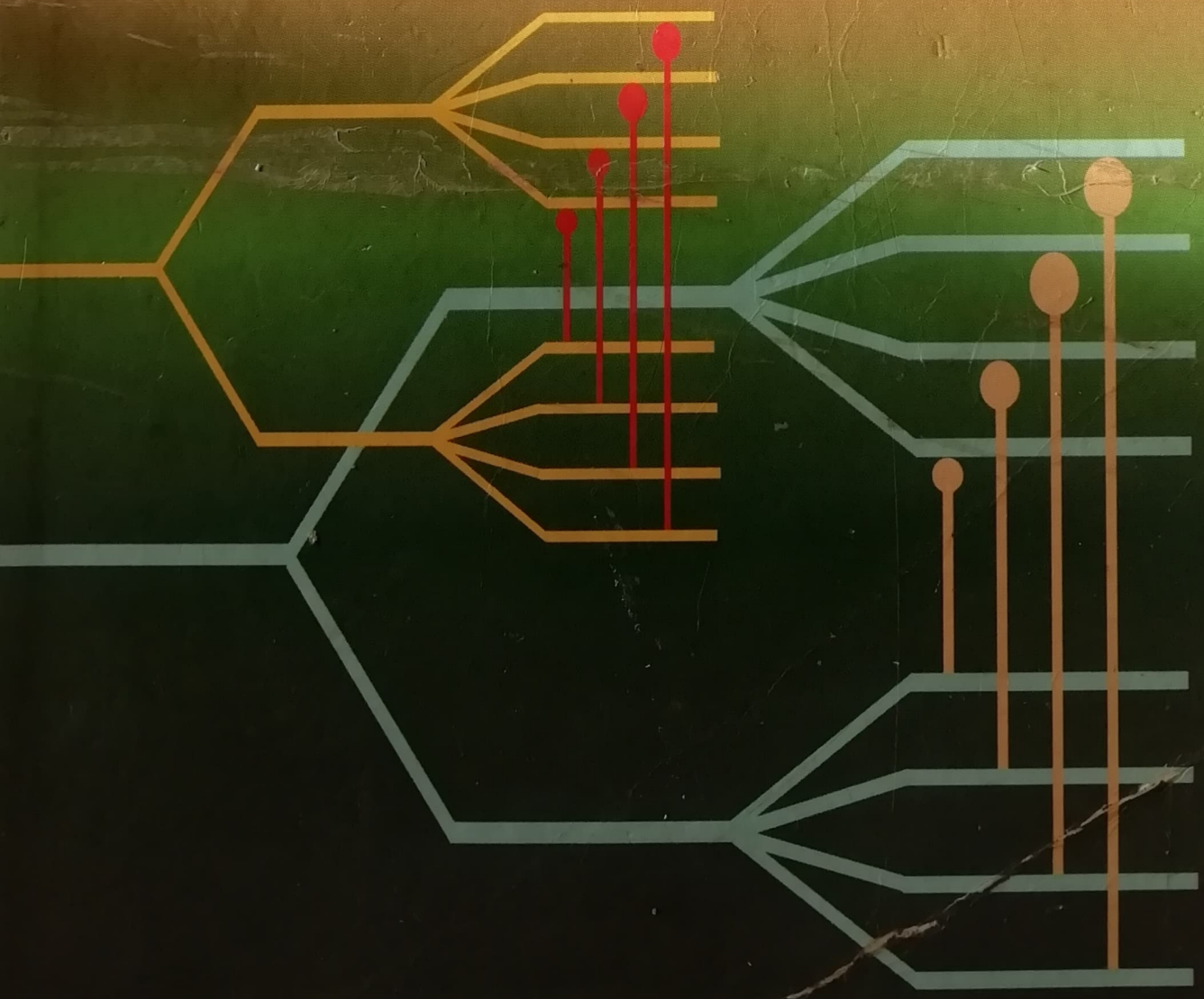
1. Instrumental methods of analysis – H.H Willard, Meritt Jr. and J.A Dean
2. Principles of instrumental analysis – Skoog and West
3. Vogels Textbook of Quantitative Inorganic analysis – J. Basset, R.C Denney, G.H Jefferey and J.Madhan
4. Instrumental methods of analysis – B.K Sarma, Goel Publishing House, Meerut
5. Instrumental methods of Analysis – Chatwal and Anand
6. Instrumental methods of Analysis – Ewing
7. Handbook of ICP
8. The ICP – Bogdain B.

Reference Books:

1. Applications of ICP-MS, A.R Date and A.L Glay, London (Eds), Blackie, London
2. A. Moutaser and D.W Gologhtly (Eds), ICP in Analytical Atomic Spectrometry, VeH Publishers, New York
3. G.I Moore, Introduction to ICP emission Spectrometry in Analytical Spectroscopy, Elsevier, Amsterdam

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS

GURDEEP R. CHATWAL
SHAM K. ANAND



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Fundamentals of

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INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS

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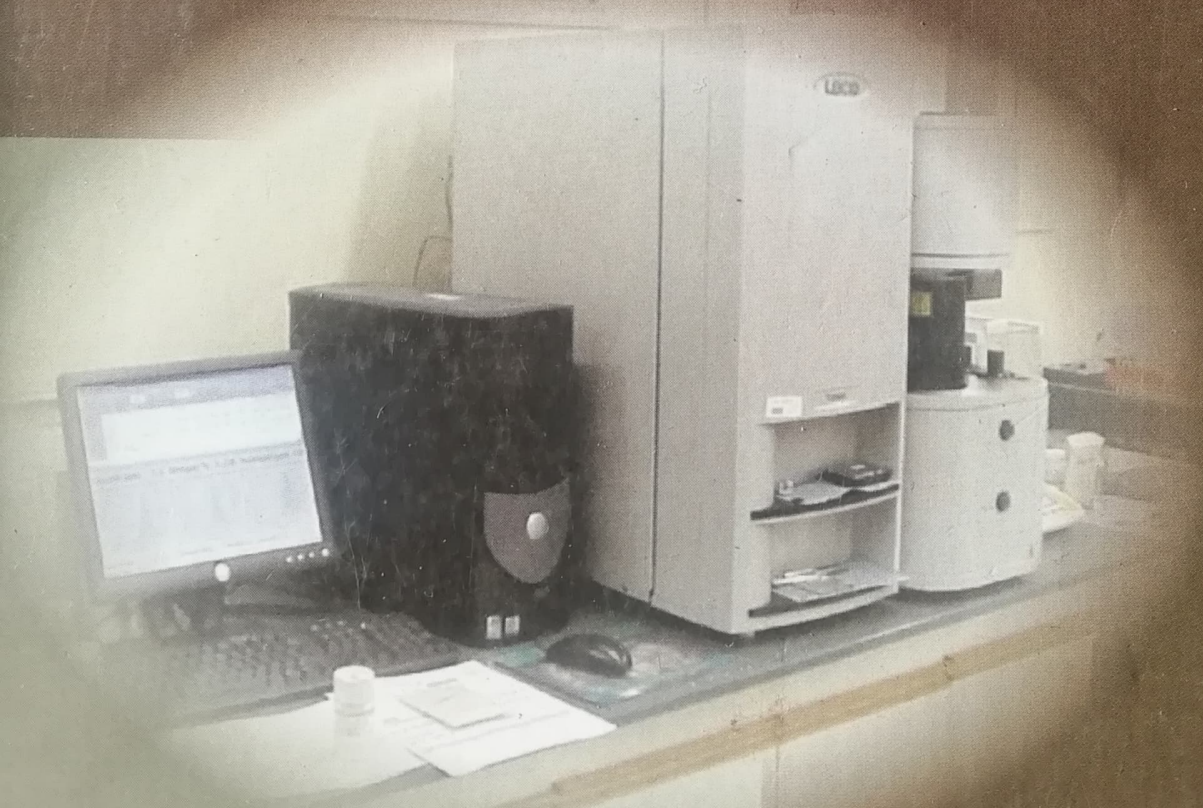

A Pragati Edition

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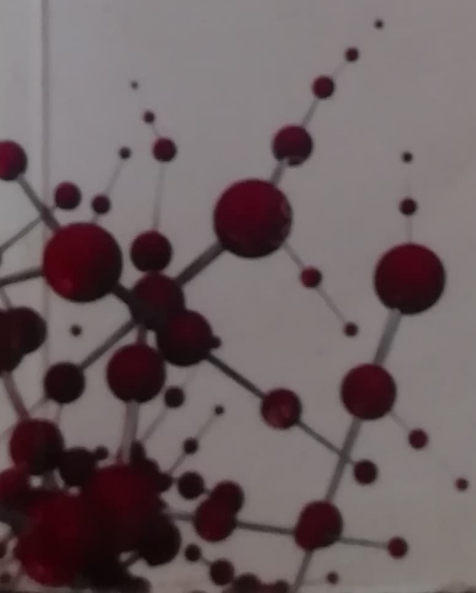
instrumental methods of chemical analysis

As per UGC Syllabus

B. K. Sharma



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August-2020

M.Sc. Analytical chemistry

semester – III; paper – 4: Instrumental Methods of Analysis – I

Syllabus:

Unit – I: Spectroscopic methods – I

(a) UV – Visible spectroscopy

(b) Spectrofluorimetry

Unit – II: Spectroscopic methods – II

(a) Infrared spectroscopy

(b) Raman spectroscopy

Unit – III: Spectroscopic methods – III

(a) NMR spectroscopy

(b) ESR spectroscopy

Unit – IV: Spectroscopic methods – IV

(a) Mass spectrometry

(b) X-ray spectroscopy (XRF)

Ref. books:

① Instrumental methods of Analysis
– H.H. Willard, Meritt Jr, J.A. Dean

② principles of Instrumental Analysis
– Skoog and West

③ Instrumental methods of Analysis
– Gurdeep Raj Chatwal and Sham K Anand.

•Introduction:

Some instrumental methods are most sensitive than classical techniques but others aenot. Inorder to solve an Analytical problem a chemist has many instrumental methods. However, his choice will be depend upon the amount of the time spent on the analytical work and the quality of the result. It is also based on the complexity of the material to be analysed and the concentration of species of the industry.

The practise in chemist choice will also depend upon the knowledge of the basic priciples underlying the various methods availble to him, And thus their strengths and limitations.

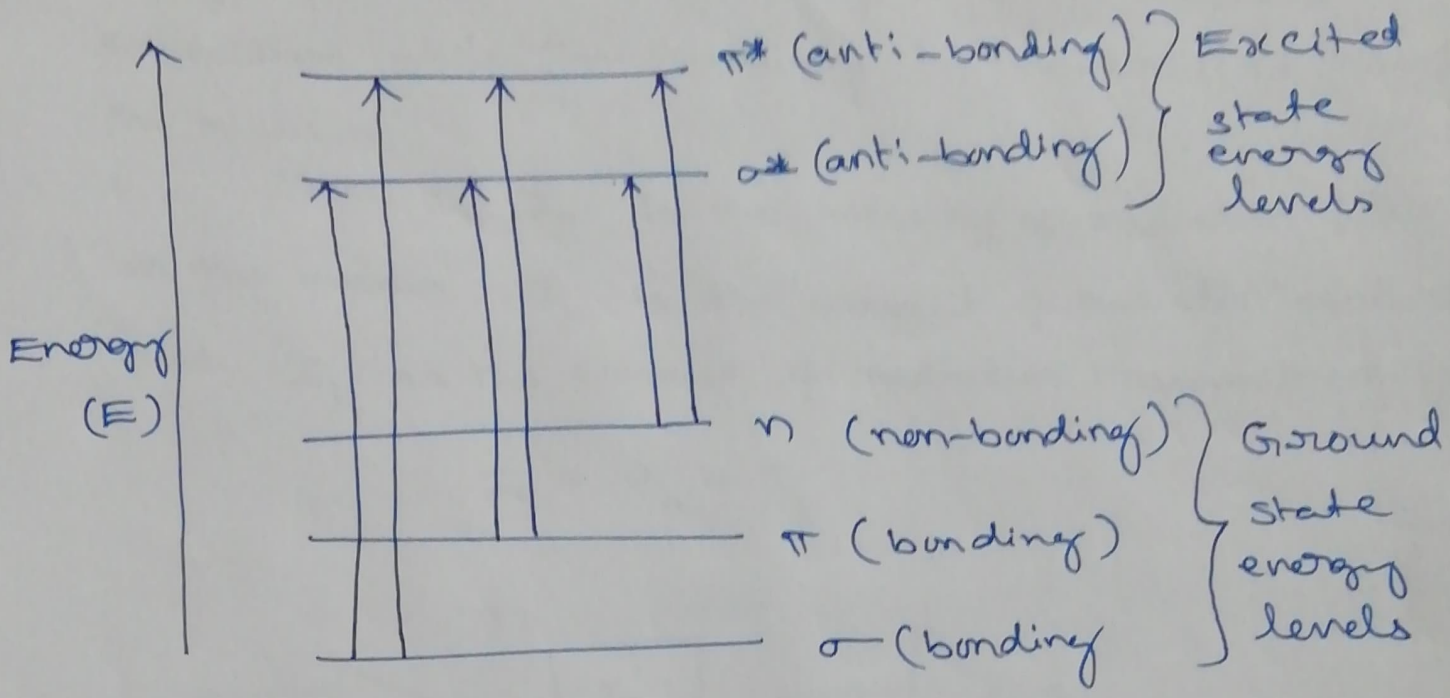
The most important methods of quantitative molecular analysis includes MS, NMR, IR, UV, x-ray diffraction and thermal analysis etc.

• Electromagnetic radiation spectrum: ..... EMR ....
• Spectroscopy; types < Absorption spectroscopics ...
Emission spectroscopies ....

Ultraviolet (UV) - Visible spectroscopy:

UV-visible spectroscopy is a type of absorption spectroscopy in which UV-visible light (210-900 nm) is absorbed by the molecule, which results in the excitation of the electrons from lower to higher energy levels. So, UV-visible spectroscopy is also called as Electronic spectroscopy.
Electronic transitions takes place from ground state to excited state by absorbing some radiation in the UV-visible region of EMR. substance absorbing the energy from visible region, will appear coloured to human eye. [VIBGYOR]
violet: 380-450 nm; Blue: 450-495 nm; Green: 496-570 nm; yellow: 570-590 nm; orange: 590-620 nm; Red: 620-750 nm.

- The possible electronic transitions that light might cause are,



- UV-visible spectrometers can be used to measure the absorbance of ultraviolet and/or visible light by a sample, either at a single wavelength or perform a scan over a range in the spectrum. The UV region from 190 nm to 400 nm and the visible region from 400 to 800 nm.
- The technique can be used both qualitatively and quantitatively.
- In qualitative analysis :-

  Advantage : It is particularly useful for identifying functional groups and the structures of molecules containing unsaturated bonds (π e compounds)

  Limitation : It doesnot indicate molecular weight and saturated bonds (σ bonded compounds)
- In quantitative analysis :-

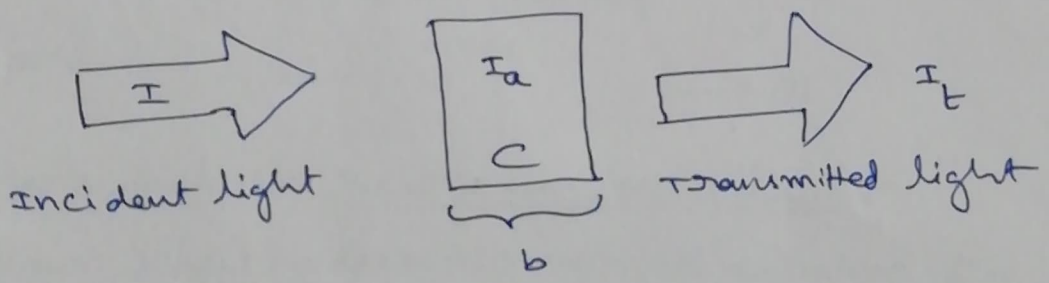
  UV, absorption is used routinely for the quantitative determination of unsaturated compounds. It is particularly useful at present level.

· Laws of absorption:

When a beam of light falls on a solution (or) homogeneous media, a potion of light is absorbed within the medium while the remaining is transmitted through the medium.

Thus, If I_o is the intensity of radiation falling on the media, I_a is the amount of radiation absorbed and I_t as the amount of radiation transmitted then,

$$I_o = I_a + I_t$$



Where I = Intensity of incident light

I_a = Intensity of absorbed light

I_t = Intensity of transmitted light

C = molar concentration of sample

b = Length or thickness of the sample cell (cm)

· Lambert's law:

When a monochromatic light passes through an absorbing medium at right angles to the plane of surface of medium & solution, the rate of decrease in intensity with thickness of the medium (b) is proportional to the intensity of incident light.

In other words the intensity of transmitted light decreases exponentially as the thickness of medium increases arithmetically.

i.e. $\frac{dI}{db} = kI \longrightarrow (1)$

where, I = intensity of incident light of λ wavelength.
b = Thickness of the medium.
k = proportionality constant.

Now, Integrating the eqn., and putting $I = I_0$ when $b = 0$

$$\ln\left(\frac{I_0}{I_t}\right) = kb \text{ (or) } I_t = I_0 \cdot e^{-kb} \longrightarrow (2)$$

where, I_0 = Intensity of Incident light falling on thickness 'b'
I_t = Intensity of transmitted light
k = Absorption coefficient for the given wavelength and medium

converting from natural logarithms to base 10 logarithms

$$\Rightarrow \quad I_t = I_0 \cdot 10^{-kb} \longrightarrow (3)$$

- **Beer's law (or) Beer–Lambert's law :-**

When light passes through a solution of a given thickness the fraction of Incident light absorbed is dependent not only on the intensity I of light but also on the concentration C of the solution. This is known as Beer's law.

$$\Rightarrow \quad -\frac{dI}{dt} = k' C I$$

$$\Rightarrow \quad \int_{I_0}^{I_t} \frac{dI}{I} = -k'c \int dt$$

$$\Rightarrow \ln \frac{I_t}{I_0} = -k'ct$$

$$\Rightarrow I_t = I_0 \cdot e^{-k'ct}$$

converting from natural logarithms to base 10 logarithms

$$\Rightarrow I_t = I_0 \cdot 10^{k'ct} \text{ (or) } I_t = I_0 \cdot 10^{\epsilon ct}$$

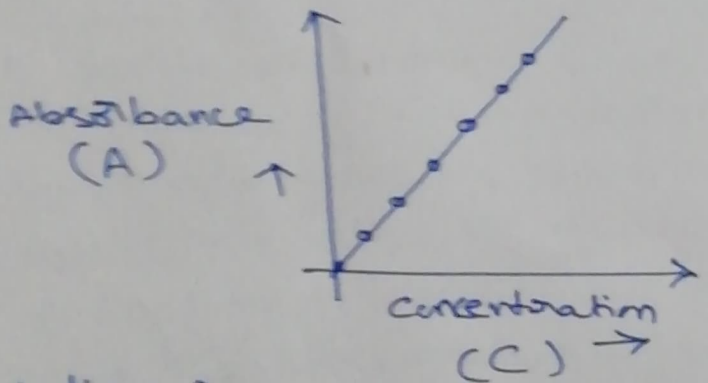
But $\log \frac{I_t}{I_0} = A$, $\frac{I_0}{I_t} = T$ (or) $\log \frac{1}{T} = A$

$$\Rightarrow \boxed{A = \epsilon ct} \text{ (or) } \boxed{A = -\log T}$$

- The mathematical statement $\log \frac{I_o}{I_t} = \epsilon c t$ is termed as Beer-Lambert's law. This is also the fundamental eqn of colorimetry and spectrophotometry.
- It is important to remark here that there exists a relationship between absorbance A, the transmittance T and molar absorption coefficient ϵ. ie.

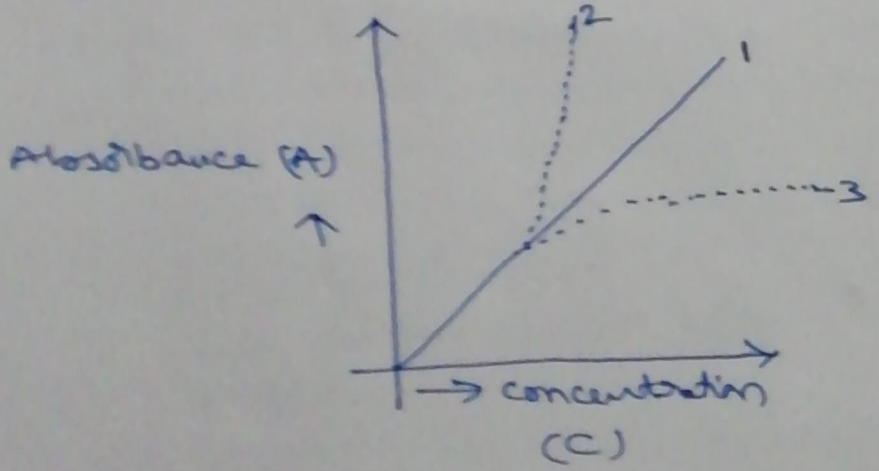
$$A = \epsilon c t = \log \frac{I_o}{I_t} = \log \frac{1}{T} = -\log T$$

- From Beer's law, it follows that, if we plot absorbance A against concentration, a straight line passing through the origin should be obtained.



Deviations from Beer's law :-

There is usually a deviation from a linear relationship between concentration and absorbance (Either positive or negative) is shown in the figure.



relationship between absorbance and concentration in the fig..

1: Law is valid, 2: positive deviations, 3: negative deviation

- Under certain conditions Beer-Lambert's law fails to maintain a linear relationship between attenuation and concentration of analyte. These deviations are classified into three categories. They are,

① Real - fundamental deviations due to the limitations of the law itself.

② chemical - deviations observed due to specific chemical species of the sample which is being analised.

③ Instrument - deviations wich occour due to how the attenuation measurements are made.

UV-Visible spectrophotometer :-

A spectrophotometer is an instrument that measures the amount of light absorbed by a sample. The use of spectrophotometer is based on Beer-Lambert's law which relates the amount of light transmitted through a solution to be a function of the length of lightpath and the concentration of the absorbing media.

spectrophotometer techniques are used to:

① Measure the concentration of a solution by measuring the amount of light absorbed by the solution.

② Measure light intensity as a function of wavelength. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the result as a graph on the detector and then the display device.

③ Identify organic compounds by detecting the absorption maxima.

④ Colour determination within the spectral range of 380 to 700 nm.

- A spectrometer is made up basically of :

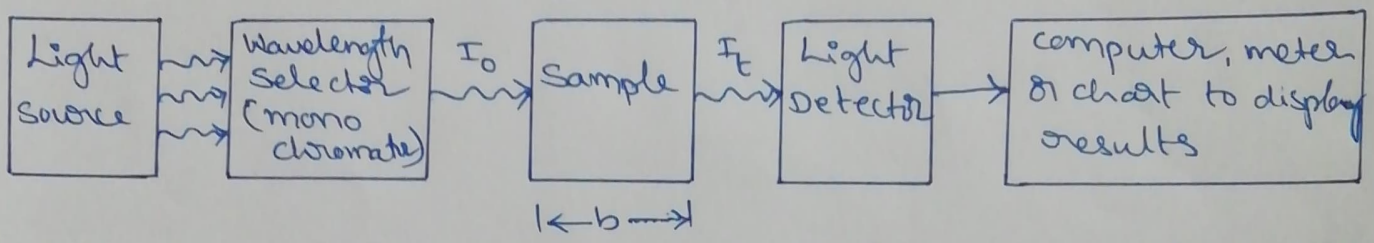
① Light source ② Monochromator ③ sample cell ④ Detector

- Types of UV-visible spectrophotometers :-

spectrophotometers are basically of 2 types - single beam and double beam.

→ In single beam spectrophotometers, a single beam of light passes through the sample holder. The instrument is standardized by placing a reference in the sample holder, and the resulting value is subtracted from subsequent sample measurements to remove effects from the solvent and the cell. This was the earliest design, but still in common use in both teaching and Industrial Labs.

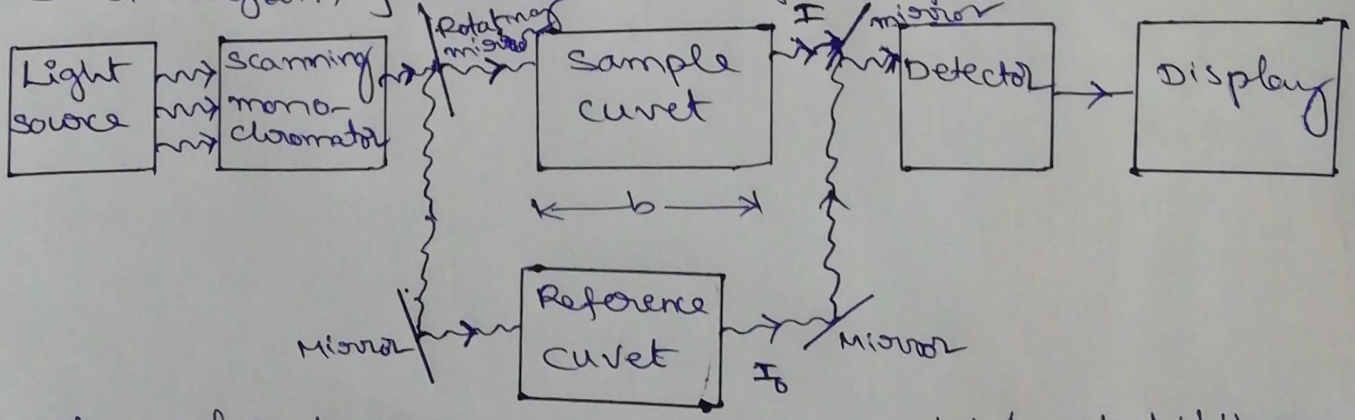
Block diagram of single beam spectrophotometer :-



- The advantages of the single beam design are, low cost, high sensitivity, because the optical system is simple while the disadvantage is that an appreciable amount of time elapses between taking the reference I and making the sample measurement I_0 such that there can be problems with drift.

→ In double beam spectrophotometer the light is split into two beams before it reaches the sample. one beam is used as the reference ; the other beam passes through the sample. The reference beam intensity is taken as 100% transmission (or 0% absorbance) and the measurement displayed is the ratio of the two beam intensities.

Block diagram of double beam spectrophotometer :-

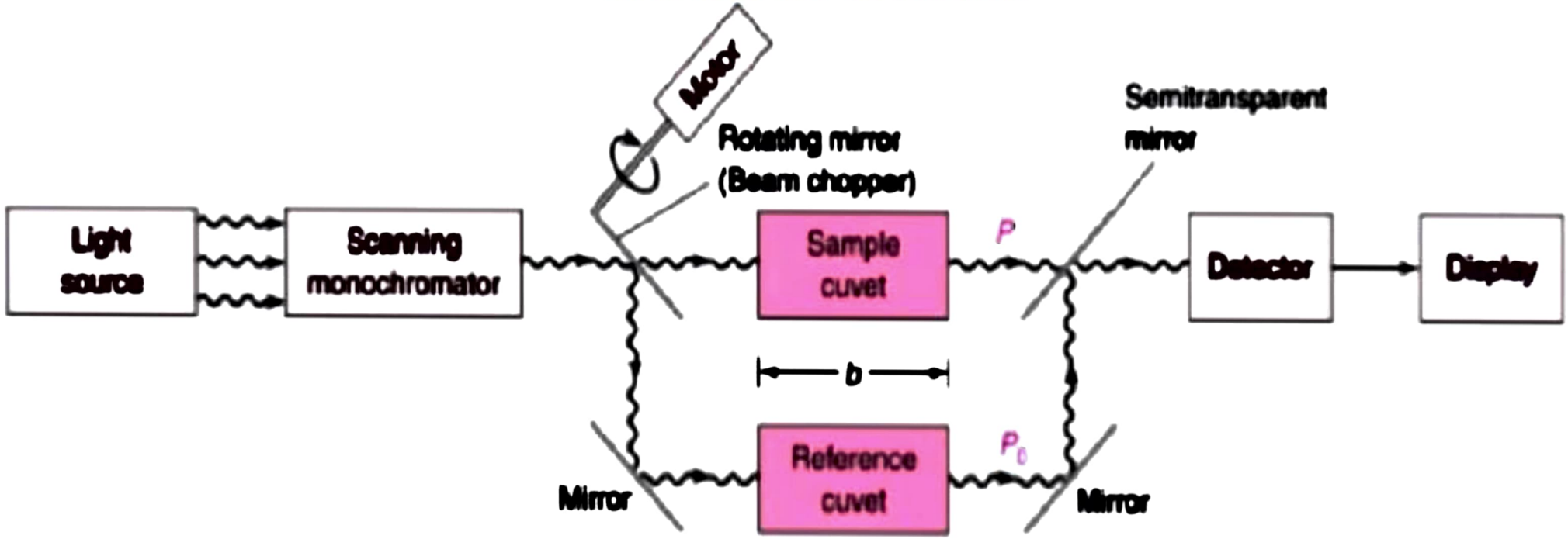


- The advantage of double beam is high stability because reference and sample are measured virtually at the same time while the disadvantages are higher cost, lower sensitivity due to complex optics and lower reliability.

The Spectrophotometer

Single-beam

Double-beam



Instrumentation:

All photometers, colourimeters and spectrophotometers have the following basic components.

(a) **Source:** A continuous source of radiant energy converting the region of spectrum in which the instrument is designed to work.

(b) **Filter** (or) **Monochromator:** Both filter and monochromator allow the light of the required wavelength to pass through but absorb the light of other wave lengths.

(c) **Sample cells:** All instruments must contain a container for the sample.

(d) **Detector:** It is used for measuring the radiant energy transmitted through the sample. In some instruments, detectors will be associated with a read-out system for the presentation of the detector response.

Radiation sources:

Requirements:
- It should be stable
- It should provide continuous radiation
- It must be the sufficient intensity for the transmitted energy to be detected at the end of the optical path.

→ The Tungsten filament lamp is the most common source of visible radiation (4000 A° to 7500 A°). Its construction is similar to the household lamp. tungstone is the most satisfactory material for lamp filaments but the 'carbon arc' is used when a more intense source of visible light is required.

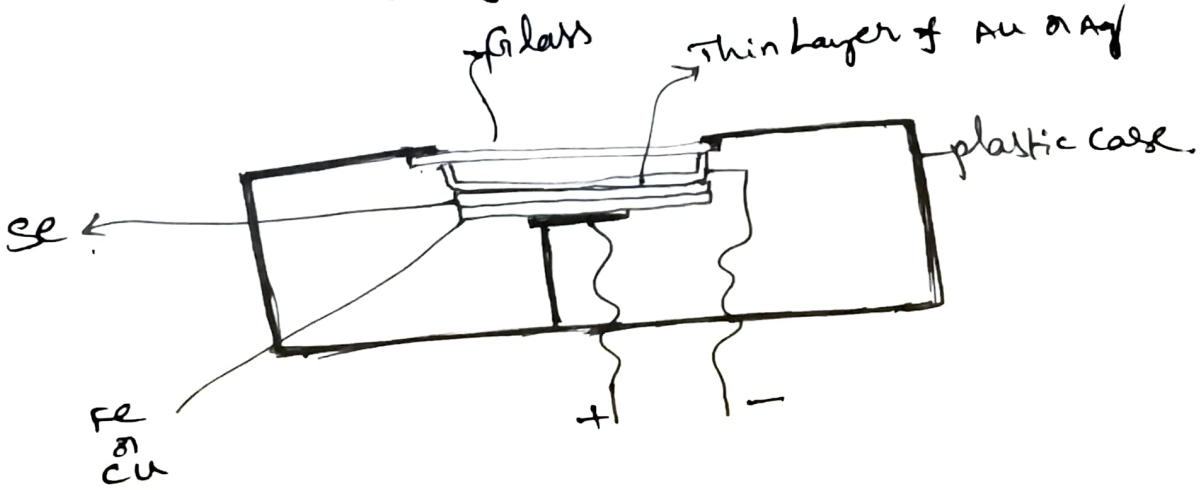
→ sources for UV radiation are: Hydrogen discharge lamp, xenon discharge lamp, Mercury arc lamp etc..

Detectors: A device which converts light energy into electrical signals, that are displayed on readout systems, and the transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample.

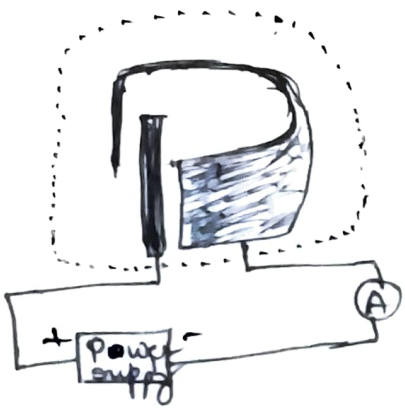
- The following types of detectors are employed in the instrumention of absorption spectrophotometers:
① photovoltaic cell ② photo tubes / photo emissive tubes
③ photomultiplier tubes.

Requirements of an Ideal detector :
- It should give quatitative response.
- It should have high sensitivity and low noise level.
- It should have a short response time.
- It should provide signal or response quantitative to wide spectrums of radiation received.

① photovoltaic cell / Barrier layer cell :
- The detector has a thin film metalic layer coated with silver or Gold and acts as an electrode.
- It is also has a metal base plate which acts as another electrode.
- These two layers are separated by a semiconductor layer of selenium
- when light radiation falls on selenium layer, electrons become mobile and are takenup by transparent metal layer. This coreases a potential difference between two electrodes & causes the flow of current.
- when it is connected to Galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light on it.



② photo-tube / photo emissive tubes :
- photoemissive tubes consists of a evaculated glass tube with a photocathode and a collector anode.



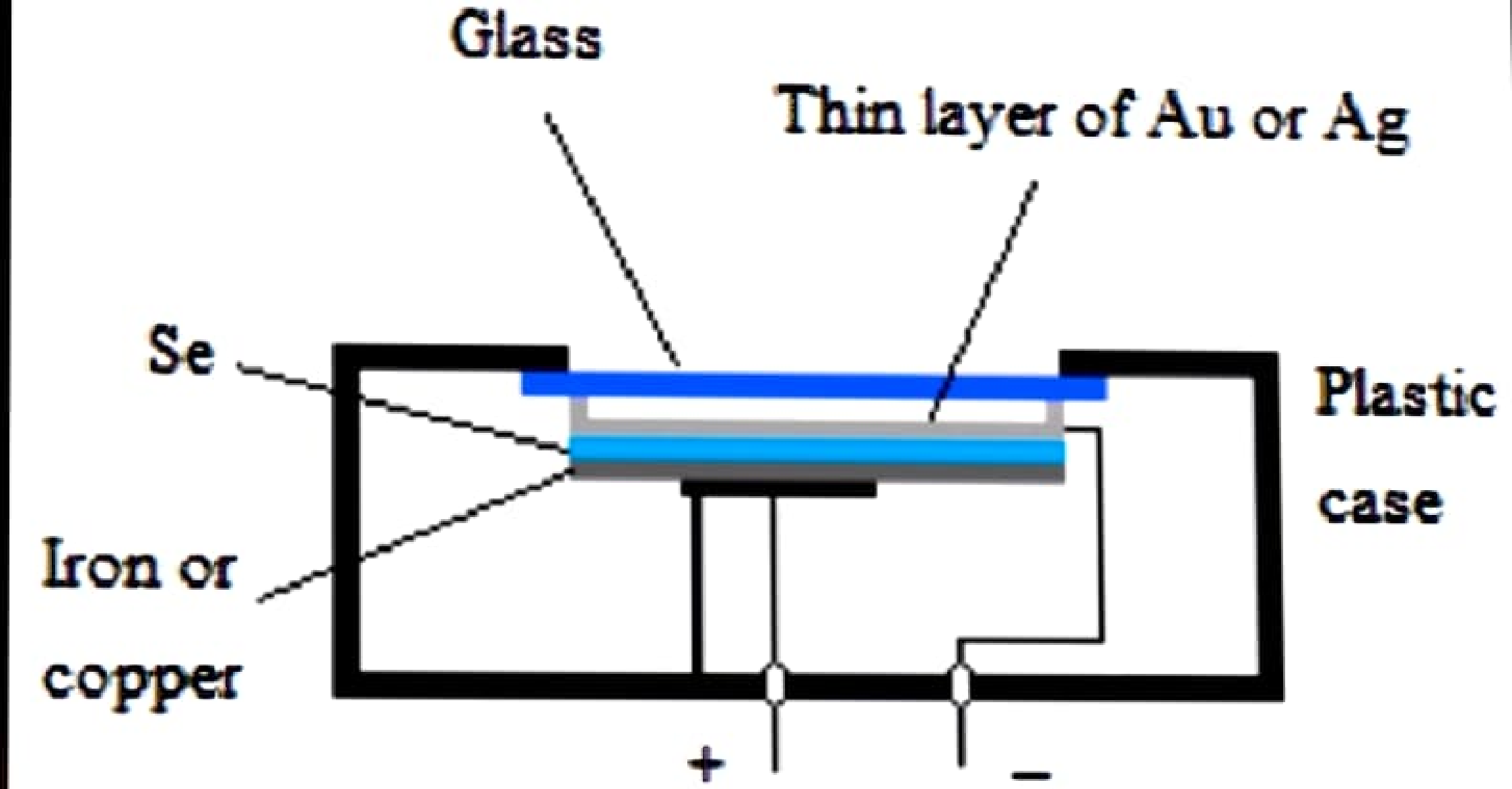
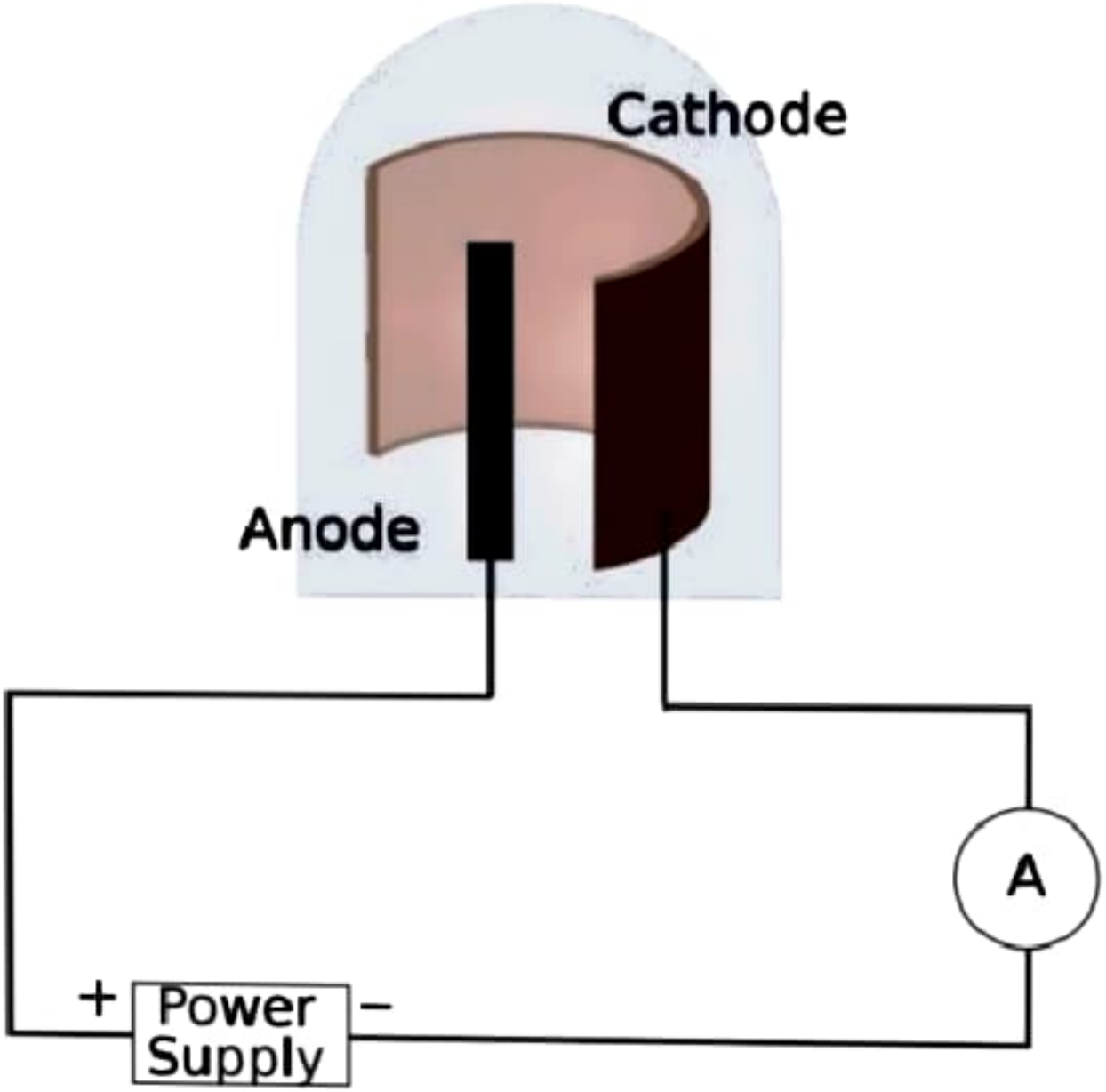
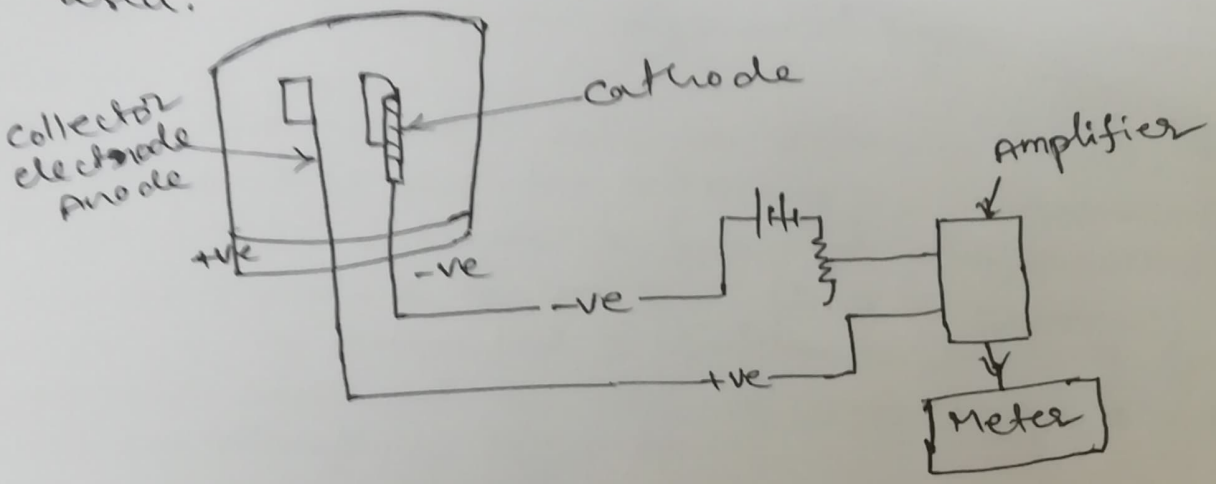

Glass
Thin layer of Au or Ag
Se
Plastic case
Iron or copper
+
–

Photo-emmiSSive tube

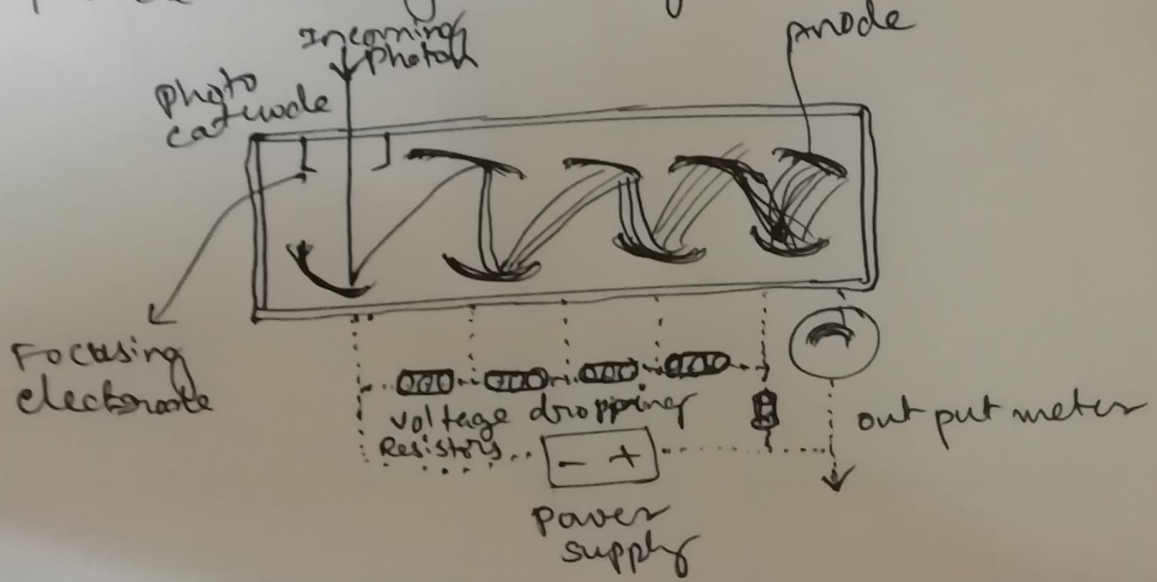


- The surface of photocathode is coated with a layer of elements like cesium, silver oxide or mixture of them.
- when radiant energy is falls on photosensitive cathode, electrons are emitted, which are attracted to anode causing current to flow.
- These photoemissive tubes (phototubes) are more sensitive compared to photovoltaic cells and therefore widely used.



③ Photo Multiplier tubes:

- The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons.



- In vacuum tube, a primary photocathode is fixed which receives radiation from sample. Some 8 to 10 dynodes are fixed each with increasing potential of 75-100 V higher than preceding one, near the last dynode is fixed an anode or electron collector electrode.
- photomultiplier tubes are extremely sensitive to light and is best suited where lower or weaker radiation is received.

Photo tubes

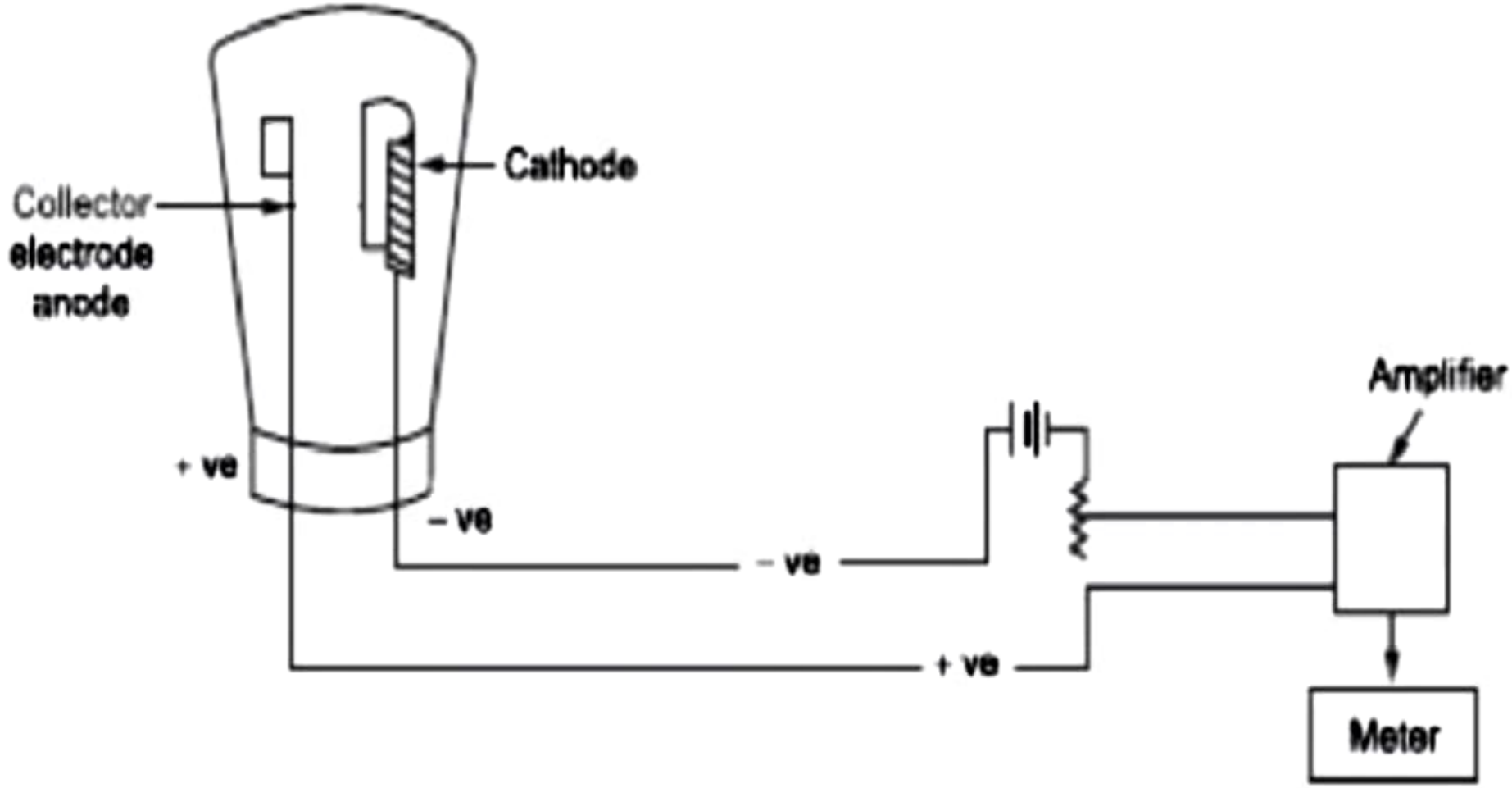

Collector
electrode
anode
Cathode
+ ve
– ve
– ve
+ ve
Amplifier
Meter

Photo Multiplier Tubes

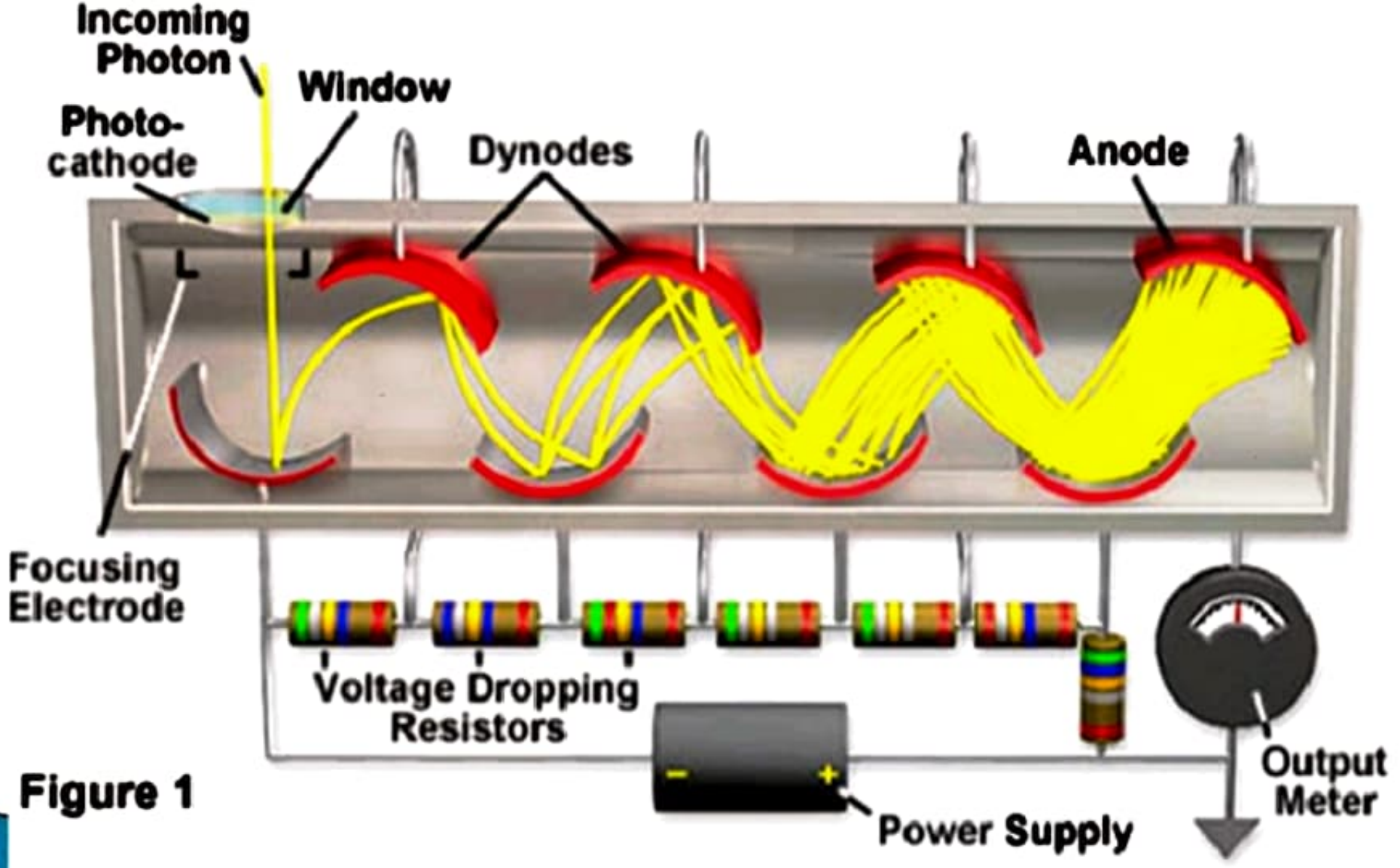
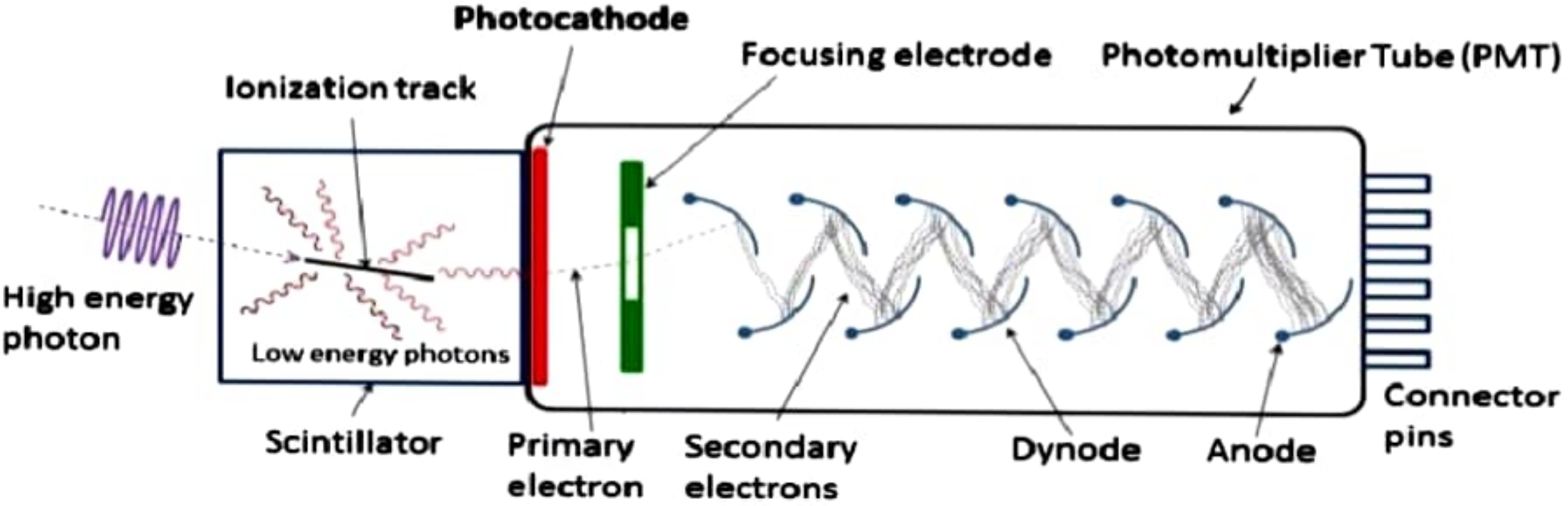
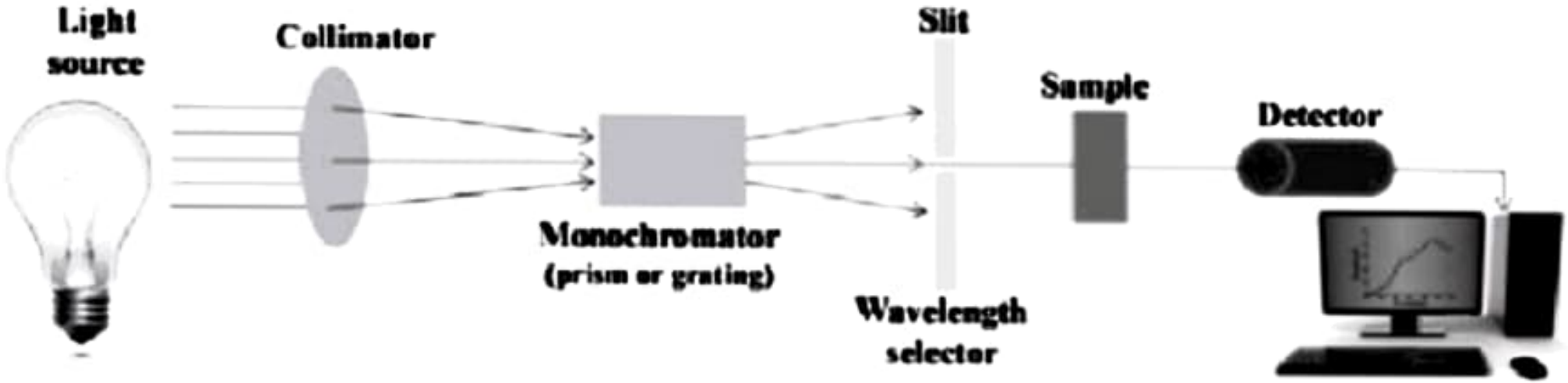


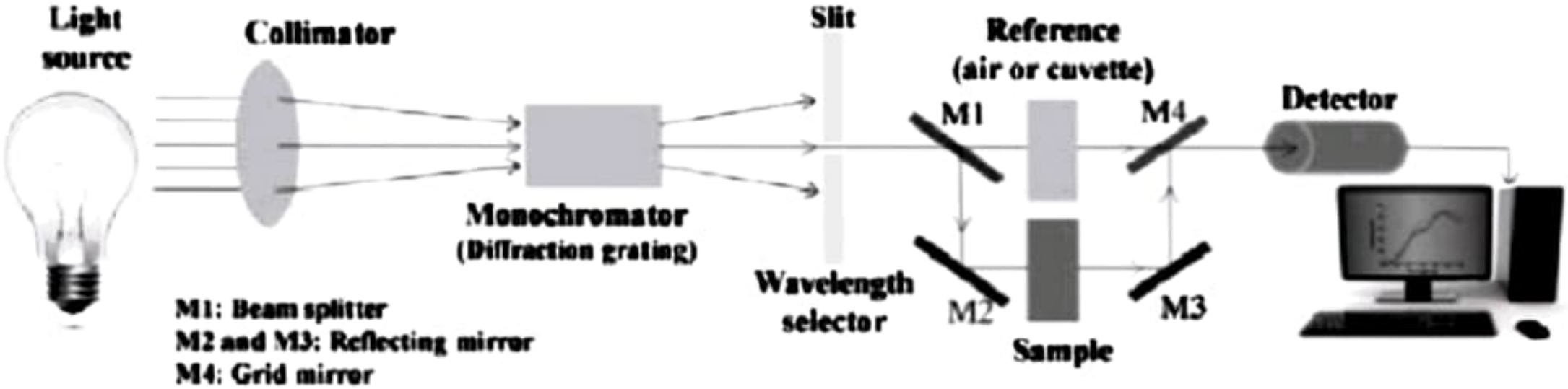
Figure 1


Photocathode
Focusing electrode
Photomultiplier Tube (PMT)
Ionization track
High energy photon
Low energy photons
Scintillator
Primary electron
Secondary electrons
Dynode
Anode
Connector pins

Single Beam Spectrophotometer



Double Beam Spectrophotometer



M1: Beam splitter
M2 and M3: Reflecting mirror
M4: Grid mirror

Qualitative analysis by absorption measurements :-

- ① Detection of Impurities :-

UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected.

- ② structure elucidation of organic compounds :-

UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms.

- From the location of peaks and combination of peaks, it can be calculated that wether the compound is saturated or unsaturated, hetero atoms are present or not etc..

- ③ Qualitative analysis :-

UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds.

- ④ In chemical kinetics
- ⑤ In detection of functional groups
- ⑥ In Molecular weight determination
- ⑦ In Quantitative analysis also.....


Thermo

General precautions in colourimetric determinations

⇒ The choice of colourimetry procedure for the determination of substance will depends upon some concentrations as the following

1. A colourimetric method will often give more accurate results at low concentrations than the corresponding other analytical procedures
2. A colourimetric method may frequently employed under conditions where no satisfactory other procedures exits. The general precautions are taking in colourimetric determinations

(i) very few reactions by using appropriate colouring agent the same may converted into a coloured compound. for colourimetric determination

(ii) For visual colourimeters it is important to maintain that the colour intensity should be increase linearly with the conc. of substances to be determined.

(iii) The colour produced should be sufficiently stable to permit an accurate reading to be taken thus, the experimental conditions must be known for development of standard colour

(iv) the colorimetric procedure must give reproducable result under specific experimental conditions. the reaction need not necessarily represent a stichometri -cally quantitative chemical change

(v) the colour 'sol' must be free from precipitate turbidity may scatters as well as absorbing the light

(vi) selection of solvent is also important for the preparation of clear coloured sample & reference soln.

Simultaneous spectroscopic determination of chromium and manganese:-

⇒ The absorption of a soln containing two solute are additive, if there is no reaction between the two solutes

⇒ we may write absorbance at λ_1 & λ_2 is

i.e, $A_{\lambda_1} = \lambda_1 A_1 + \lambda_1 A_2$

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

where $A\lambda_1$ & $A\lambda_2$ are measured absorbances at two wavelengths λ_1 & λ_2.

The subscripts ① & ② refers solute 1 & solute 2; subscripts λ_1 and λ_2 refers two different wavelengths

⇒ The wavelengths are selected to coincide with the absorption maxima of the two solutes the absorption spectra of two solutes should not overlap appreciably. Therefore substance sub-1 absorbs strongly at λ_1 & weakly at λ_2 similarly substance-2 weakly at λ_1 & strongly at λ_2.

⇒ Now $A = \epsilon c t$

$A\lambda_1 = \lambda_1 \epsilon_1 c_1 + \lambda_2 \epsilon_2 c_2$ ---①

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

Now solving the simultaneous eqns

The solution is

$$c_1 = \frac{\lambda_2 \epsilon_2 A\lambda_1 - \lambda_2 \epsilon_2 A\lambda_2}{\lambda_1 \epsilon_1 \lambda_2 \epsilon_2 - \lambda_1 \epsilon_2 \lambda_2 \epsilon_1} \rightarrow ②$$

$$c_2 = \frac{\lambda_1 \epsilon_1 A\lambda_2 - \lambda_2 \epsilon_1 A\lambda_1}{-\lambda_1 \epsilon_1 \lambda_2 \epsilon_2 - \lambda_1 \epsilon_2 \lambda_2 \epsilon_1}$$

The values of ϵ_1 and ϵ_2 can be deduced from measurements of the absorbance of pure solution of the substances 1 and 2 by measuring the absorbance of mixtures at λ_1 and λ_2 then the concentration of the two components can be calculated individually.

The above process can be illustrated by simultaneous determination of manganese and chromium in steel. The absorption spectra of 0.001 M permanganate and dichromate in 1 M H_2SO_4 are plotted against blanks.

For permanganent λ_{max} = 545nm (and a small) dichromate λ max - 440 nm

a small correction must be applied at dichromate, at which permanganate absorbs weakly.

Determination of Cr & Mn in an alloy :-

545nm, $\epsilon_{Mn} = 2.35$

$\epsilon_{Cr} = 0.011$

and

440 nm $\epsilon_{Mn} = 0.095$

$\epsilon_{Cr} = 0.0369$

Mn percentage $= \frac{0.00549V(0.426 A_{545} - 0.013 A_{440})}{W}$

and

Cr percentage $= \frac{0.0104 V}{W} (2.71 A_{440} - 0.110 A_{545})$

where W is the weight of the sample and V is volume of the sample.

* Spectrophotometric titrations:-

The spectrophotometric titrations the end point is evaluated from absorbance data of the soln. In this case the Beer-lambert's law may be written as $A = \log \frac{I_0}{I_t} = \epsilon \cdot c \cdot t$

where I_0 = intensity of incident light

I_t = intensity of transmitted light

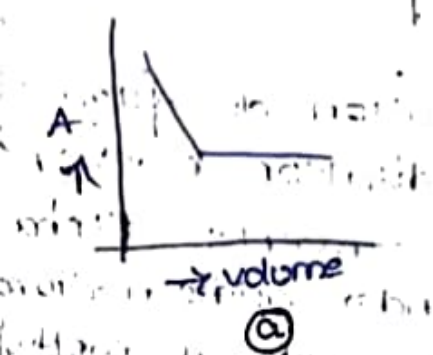
ϵ = molar absorption coefficient

c = conc. of the absorbing (soln) species

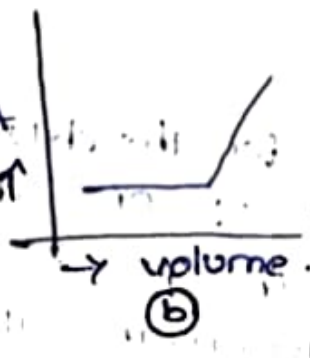
t = thickness of the absorbing medium

Since photometric titrations are carried out in a vessel for which the light path (thickness) is constant, the absorbance is propotional to the concentration of absorbing species. Thus, the plot of absorbance Vs volume of titrant added gives two straight lines intersecting at the end point.

The shape of the photometric titration curve will depends on the optical properties (and) of the reactant & titrant & product of the reqn & of the reqn of the wavelength used.



(a)



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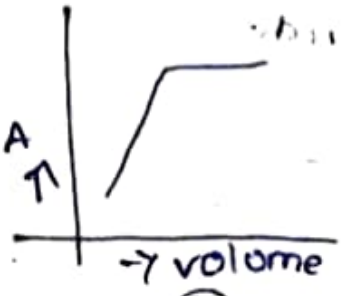
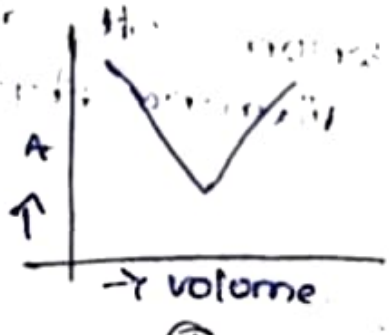


(c)



(d)

⇒ For example if a substance titrated is converted to a non absorbing product with titrant, A tipical plot is shown in diagram (a).
⇒ similarly diag(b) is obtained in which titrant alone absorbs
⇒ If titrant & reactants are colourless and the product is colour the resultant spectrophotometric titration curve is shown in dig. (c)

⇒ diag. (d) is obtained when a colour reactant is converted to a colourless product by a colour titrant.

⇒ Because of linear response of absorbance to concentration an appreciable break will often be obtained in photometric titrations.

Advantages of photometric titrations

⇒ photometric titrations have several advantages over direct colorimetric analysis

1. The presence of other absorbing substances at the same wavelength does not necessarily cause interference since only the change in absorbance is significant

2.

Nitrate:

General procedure for the determination of nitrate is usually based up on diazodisation reaction

In this case the nitrate ion converts sulpha-nilamide to diazonium salts under acidic medium then diazonium salt, at coupled with n-naphthethelene diamine dihydro chloride

Colourometric determination of phosphate: (PO_4^{-3})

Two methods are commonly used for the determination of phosphate.

1. Holybdenum Blue method
2. phosphovanado molybdate method

1. Molybdenum blue method:-

orthophosphate ions and molybedate ions condense in acidic solution give phospotholbedic acid which upon selective reduction with hydrosoniom sulphate. produces a blue colour due to molebdenum blue of uncertain compounds composition.

The intensity of blue colour is directly proportional to the amount of phosphate present in the test solution.

The colour compound absorbs at 820 to 830nm

2. phosphovanado molybdate method:-

It is slightly less sensitive than the molybdenum blue method but it has been particularly useful for phosphate determination.

It requires ammoniom vanadate solution and ammoniom molybdate solution

Spectro fluorimetry :- [Fluoresence spectroscopy]

Fluoresena spectroscopy is extensively used optical spectroscopic technique for sample analysis and various other research due to its high sensitivity dynamic range, convi. nient Instrumentation & low cost of operation

Principle

Fluorescence spectroscopy (or) spectrofluorimetry is an analytical method that involves use of uv-light to excite molecules of a given sample, which up on excitation emit light of different wavelength than the incident light. In analysis a fluorescene from the sample molecules have different vibrational & rotational energies in excited electronic states collision occurs blw different molecules at that state causing exciting molecules to loose their vibrational energy until they reach zero vibrational state of excited electronic state molecules come back to one of the vibrational level of ground electronic state again emitting photon with diff energies finaly analysis of diff frequencys of light emitted [emission spectrum] as well as their relative intensity is done to determine the structure of the diff vibrational levels fluorescene spectra obtained is in the form of band.

Fluorescence

It is a luminiscence i.e, caused by the absorption of radiation at one wavelength following by re-radiation usualy at different

wavelength molecules absorbs photons at one wavelength of colour and become excited molecule change slightly as they store the energy of the photons as they change they leak some of the energy to surrounding molecules using the remaining energy molecules emitt new photons in different colours this creates a fluroscent glow.

Instrumentation

main components of spectro-florimetry are. mercury (or) xenon arc, 2 - monochromators and a detectors, source.

- spectro fluorimetry.

Theory of fluorescence and phosphorescence :-

Luminscence : < fluorescence def :- Eg.. explanation
phosphorescence def :- Eg.. explanation

Singlet :- $S_0, S_1, S_2 \cdots S_n$

Triplet :- $T_1, T_2, T_3 \cdots T_n$

Excited states in molecules : Jablonski diagram

Factors affecting, Fluorescence and phosphorescences

1. Nature of molecules :- π-bonded and π^* conjugated double bonds.
2. Nature of substituents :- NH_2, -OH, alkyl group enhance dimensions
3. Effect of concentration :- $-NO_2$, -COOH.

fluorescence is proportional to concentration.

4. Adsorption :- , ⑤ Light ⑥ methods of illumination ⑦ oxygen , ⑧ pH , ⑨ photo dissociation, ⑩ temp and viscosity..

Relation blw fluorescence Intensity & concentration

We know that Beer - lamberts law

$$F \propto I_0 - I \quad (8)$$

$$F = K(I_0 - I)$$

F = Intensity of Fluorescent radiation

K = Proportionality constant

I_0 = Intensity of incident light

I = Intensity of transmitted light

Acc. Beer - lamberts law , $I = I_0 \cdot 10^{-abc}$

$$F = K I_0 (1 - 10^{-abc})$$

$$\log \frac{KI_0}{KI_0 - F} = abc$$

$$F = 2.303 K I_0 abc \quad (6) \quad \boxed{F = K'c}$$

from the above eqn it follows that, "The flurescent intensity is practically proportional to the concentration of the flurescent substance"

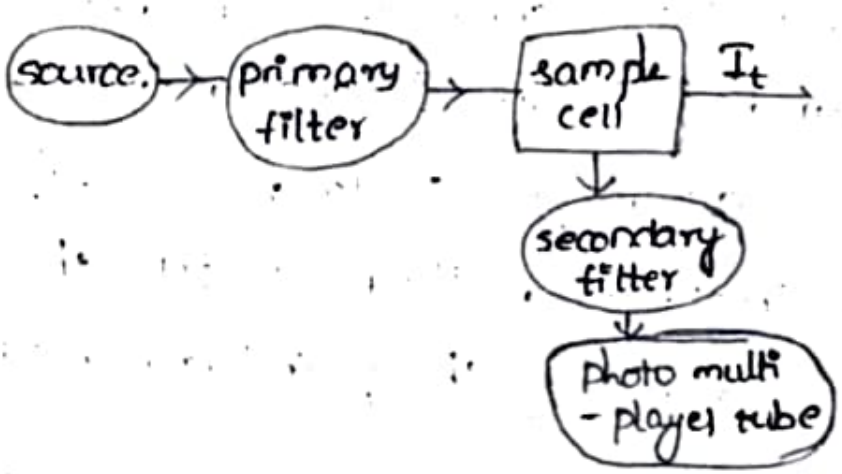
Relation blw phosphorescence intensity & concentration

(Same) similarly in the fluroscence, phosphorescence intensity 'P' is related to the concentration 'c' as

$$\log \frac{k' I_0}{k' I_0 - P} = abc$$

k' depends on Instrument used

P - phosphorescence intensity



But we will describe some of the following is

· An intersting example is the determination of uranium salts by florimetry this is used extensivly field of nuclear research

· In general inorganic ions do not exhibit flurescence however some of these inorganic ions form flurosent chelates with non flurescent organic molecules this have provide the basic for every sensitive analysis of many elements including most of the transition elements

An intresting example is the determination of Aluminium.

→ In alloys Aluminium (III) forms a complex with dipenta chrome blue black at pH 4.8 with flurosence strongly one can determined aluminium in the range of 0.2 to 25 milligrams in vol. of 50 ml with a sensitivity 1 part in 10^8

Note.

Aluminium Al^{+3} is identified by fluorimetric reagent Alizarin garnet B.

The determination chromium manganese selenium in environment biological system is are determinate accuracy (ultrarise level) in spectro fluorimetric methods using the ligand. PTQA (i.e, 2 α pyridyl thioquinilodinamide).

Ex-3 Determination of vitamin B_1 (thiamine)

Vitamin B_1 is non flurascent where as its oxidation product thiochrom flurosis with blue colour. property is used for the determination vitamin-B_1 in the food samples like meat, cereal, etc

Determination of vitamin B_2 (Riboflavian)

Determination of vitamin B_2 is done by a fluroscence method because the fluroscent power depends upon the exact condition and upon the nature amount of impurities

Generally, a method of standard increment is used in this method we can measure the flurescence actually Riboflavin is fluroses and on oxidation gives a non fluorescence substance Based on this method we can measure fluroscene and determine amount of Riboflavin.