Photometry

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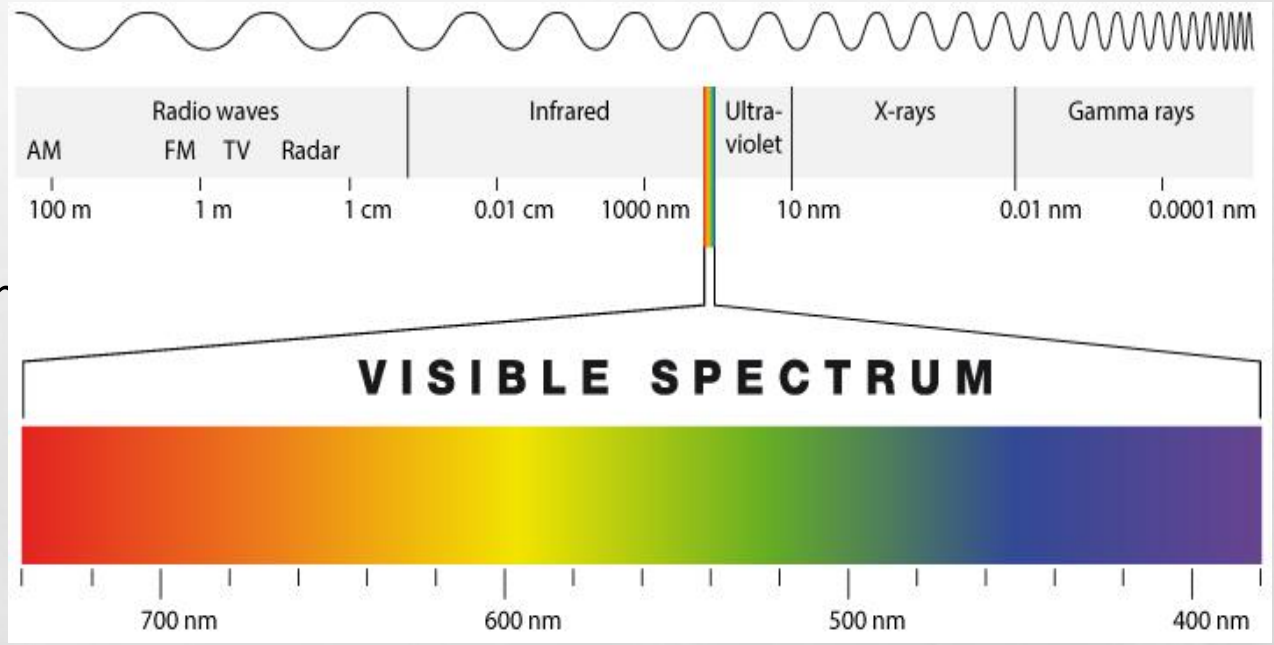
Spectrum

Light consists of electromagnetic radiations which travel in a wavelike motion called electromagnetic waves. These are measured in terms of wavelength, represented by symbol λ .The analysis of Electromagnetic radiations in terms of bands of wavelength is called Spectrum.

Types-

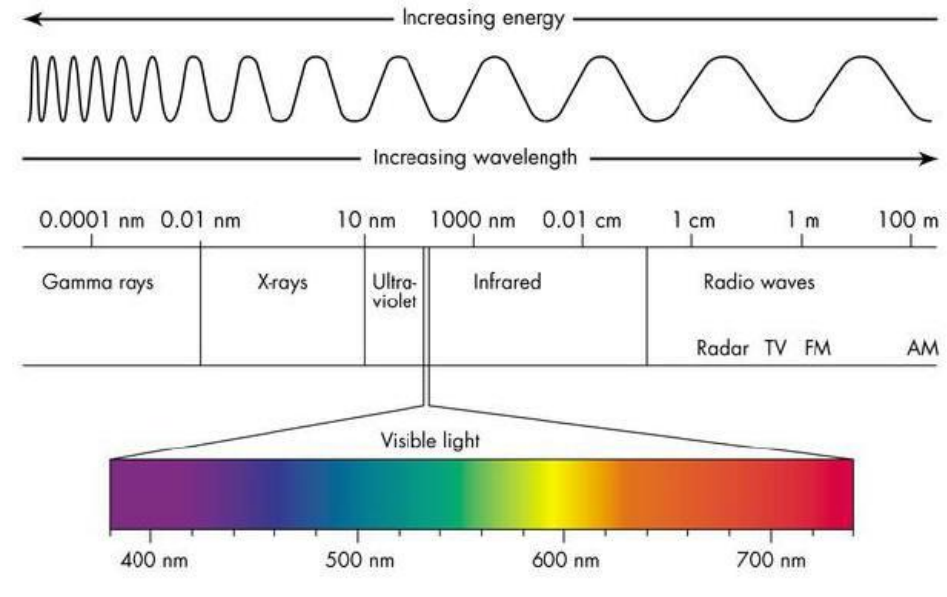
- UV (Colorless)
- Visible (colored)
- Infrared Spectrum



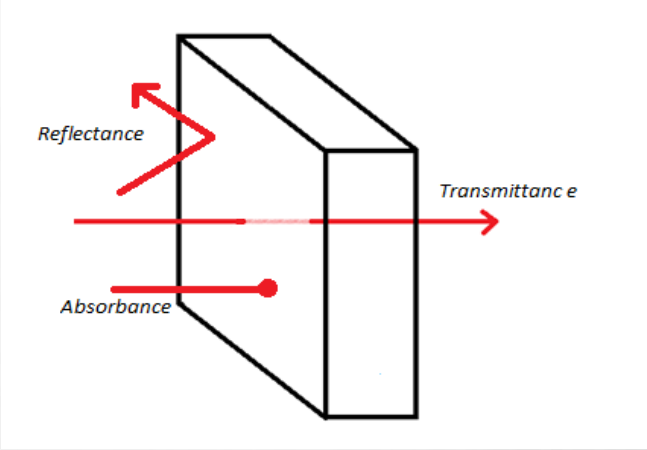
Visible Spectrum

These wave lengths are associated with colors.

Color	Wavelength (nm)
Violet	380-450
Blue	450-475
Cyan	476-495
Green	495-570
Yellow	570-590
Orange	590-620
Red	620-750

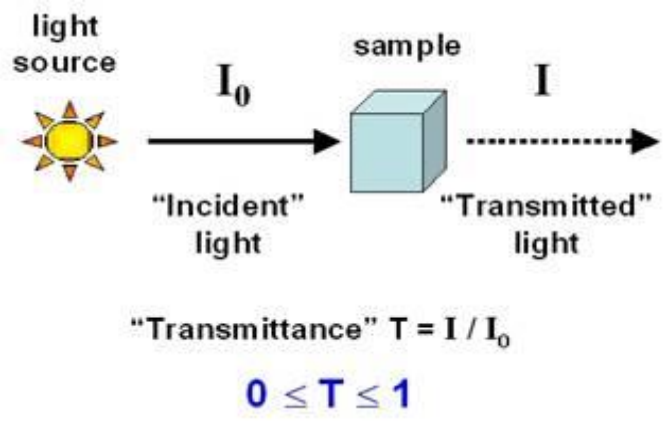


Transmittance



- Transmittance (T) is the fraction of incident light which is transmitted. In other words, it's the amount of light that "successfully" passes through the substance and comes out the other side.

- It is defined as T = I/Io, where I = transmitted light ("output") and Io = incident light ("input").
- Percent Transmittance (%T) = (I/Io) x 100.



Absorbance

- It is a measure of the capacity of a substance to absorb light of a specified wavelength.
- Absorbance (A) is equal to the logarithm of the reciprocal of the transmittance (T)

  $A = \log_{10} (I_o/I). = \log_{10} (1/T)$

Optical density

Optical density(OD) is a measurement of a refractive medium or optical component's ability to slow or delay the transmission of light. It measures the speed of light through a substance, affected primarily by the wavelength of a given light wave. The slower that light is able to travel through a given medium, the higher the optical density of the medium.

- It is often said to be identical with the absorbance. It is a logarithmic ratio of the incident light to the transmitted light through a material.
- For a given wavelength, the expression of optical density is expressed as: Log_{10} (1/T), Where T is transmittance.

Few things to note:

- The higher the optical density, the lower the transmittance.
- The ten times of this density is equal to transmission loss expressed in decibels. For example, the density of 0.3 corresponds to a transmission loss of 3db.

Absorption maxima

- The wavelength at which a substance shows **maximum absorbance** is called **absorption maxima** or **λmax**.
- It acts as a single quantitative parameter to compare the absorption range of different molecules**.**
- It can be determined by noting the optical density of that particular substance in solution at different wavelengths.

Photometry

The word Photometry is composed of the greek word photo-“light” and metry – “measure”.
It is also known as Absorptiometry.

Definition of Photometry:

Photometry is an instrumental technique used for determining the conc. of a substance by measuring the amount of light transmitted or absorbed by the substance present in the solution.

Laws of Photometry :

The amount of light absorbed or transmitted by a colored solution is in accordance with two laws:

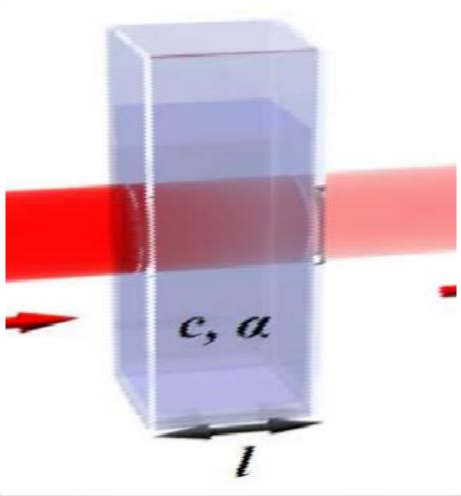
- Beer’s law
- Lambert’s law

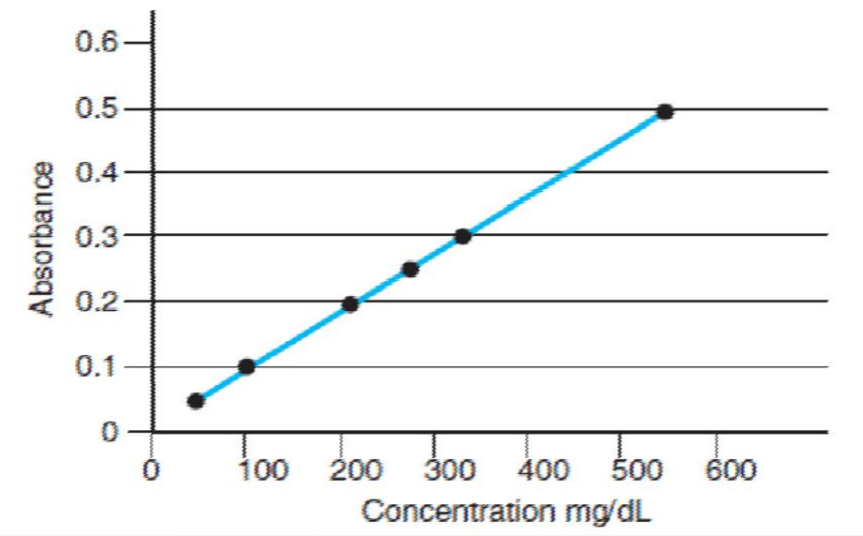
Beer's Law

"When a monochromatic light passes through a colored solution, amount of light transmitted decreases exponentially with increase in concentration of colored substance.
This means the amount of light absorbed by a colored solution is directly proportional to the conc. of substance in the colored solution."

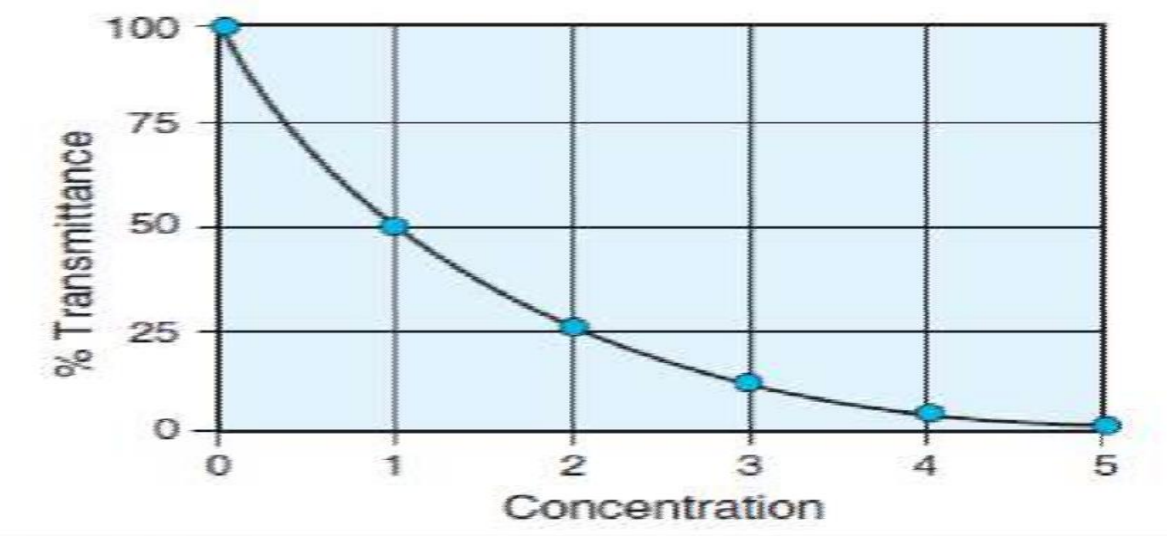
- Amount of light absorbed is measured by Optical Density.

$A \alpha C$





Beer's Law



Limitation of Beer's Law:

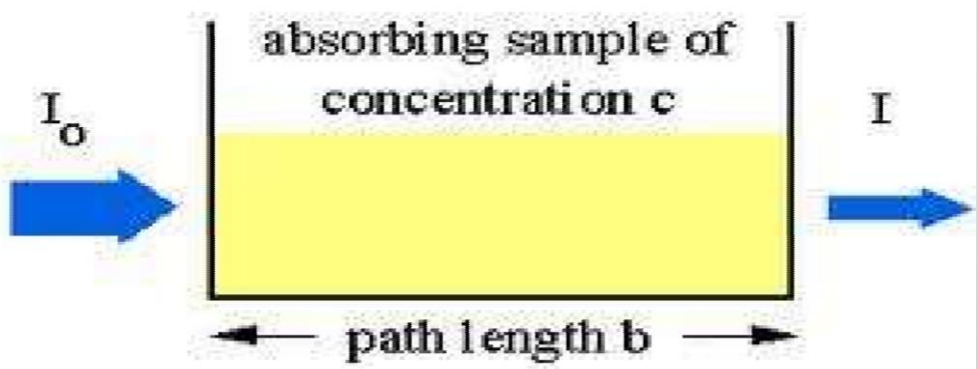
After a certain concentration optical density fails to rise with the conc. of substance so a very high concentration, absorbance can not be measured.

Lambert's Law

- When monochromatic light passes through a colored solution, the amount of light transmitted decreases exponentially with increase in the thickness of the layer through which light passes

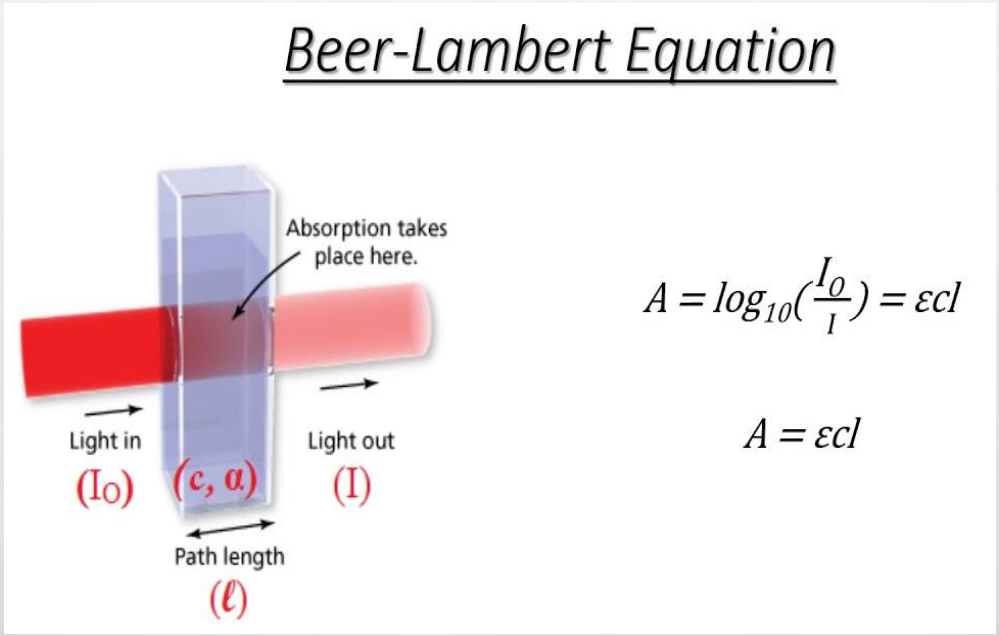
In other words absorbance of light by a colored solution is directly proportional to the

thickness of the layer through which light passes.



Beer -Lambert's Law

- Beer's law & Lambert's law together known as Beer-Lambert's Law.
- It means absorbance of light by solution is directly proportional to conc. Of light absorbing material present and path length through which light has to pass.



a= absorbance
ε= molar extinction coefficient
(characteristic of the substance being investigated)
c= concentration of the substance
l= thickness of medium through which light passes

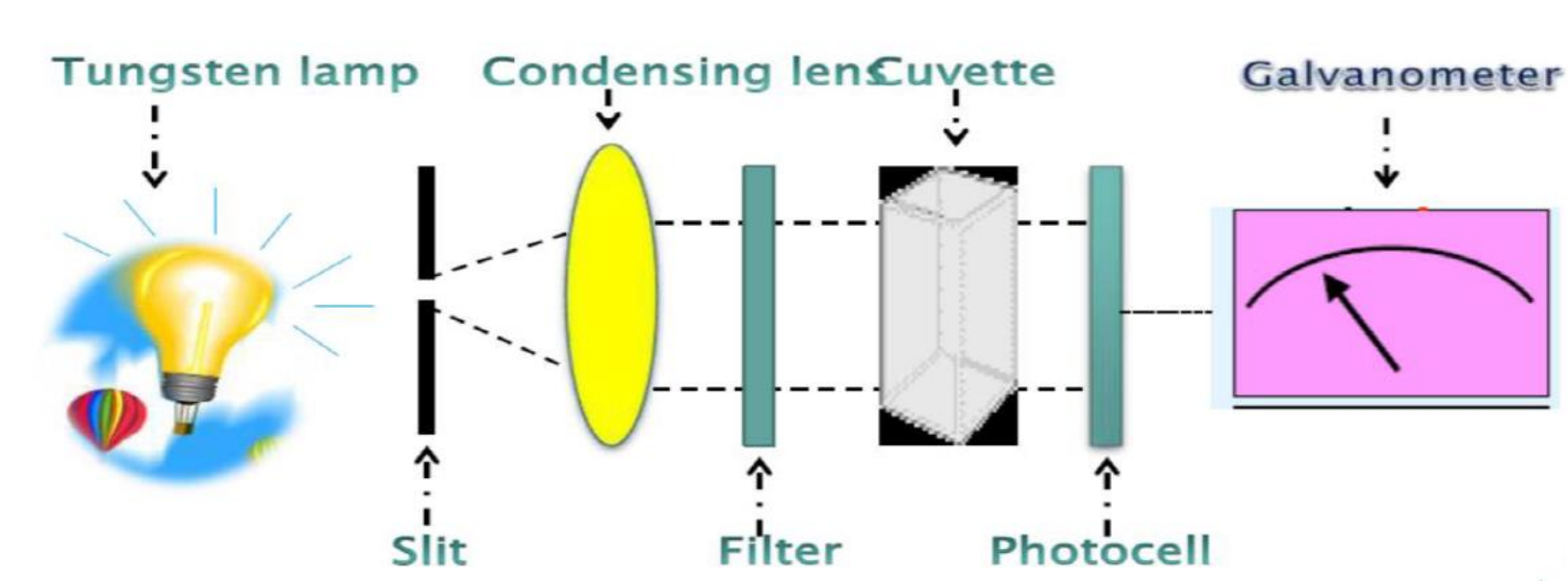
Instruments for Photometry

- A variety of instruments are available.
- The simple ones which are used for measuring colored solutions are called photometers , absorptiometers or photo electric colorimeters.
- The components of most photoelectric colorimeters are basically the same and the basic method of operation is also similar for all the instruments.
- The more advanced instruments which measure colored as well as colourless solution at a particular wavelength or varying wavelengths are called spectrophotometers.
- On the basis of photocells used in the instrument Photometers may be:

1-Single Cell or single beam filter : relatively simple, cheaper, suitable for only routine work

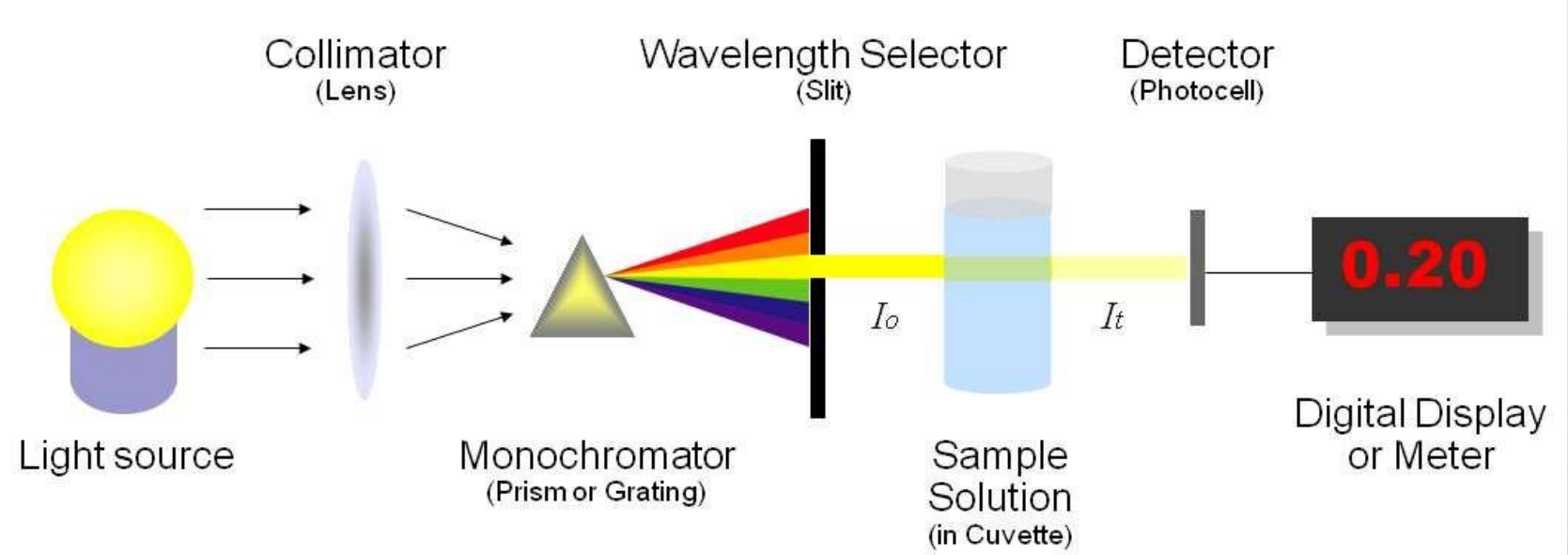
2-Double Cell or Double beam filter photometer: relatively expensive but are preferred for more study and accurate results.

Instruments for Photometry-
Parts of Photometer



Simple photometer or Photoelectric colorimeter

Instruments for Photometry-
Parts of Photometer



Spectrophotometer

Instruments for Photometry-
Parts of Photometer

1-Light source
2-Associated optical system
3-Device for the isolation of desired wavelengths of light
4-Sample holder
4-Photo sensitive detectors
5-Read out devices

Instruments for Photometry-
Parts of Photometer

1-Light source

The light source is usually a Tungsten lamp, for wavelength in the visible range (320 – 700nm) and a deutarium or hydrogen lamps for ultraviolet light (below 350nm).

a)	Tungsten lamp	-Visible range
b)	Deutarium/hydrogen lamp (preferred)	-UV Rays
c)	Black body radiators (Nerst glower)	-Infrared radiations

Instruments for Photometry-

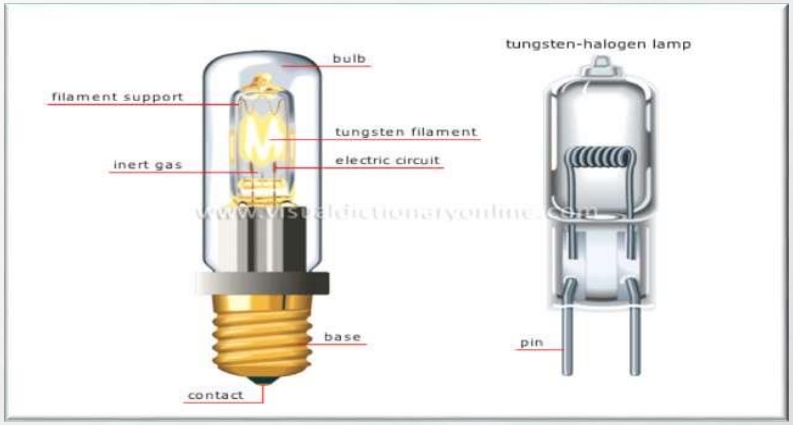
Parts of Photometer

1-Light source

Tungsten Lamp-

Filament mode of tungsten sealed in a glass envelope filled with inert gas.

Life time is limited due to gaseous tungsten formed by sublimation.



Carbon arc lamp-

If sufficient intensity of light is not obtained from tungsten lamp then carbon arc lamp can be use as a source for color measurement.

Instruments for Photometry-
Parts of Photometer

2-Associated Optical System

Lenses and mirrors which are used for providing a parallel beam of filtered light for passage through the solution (cuvette)

2-Device for the isolation of desired wavelengths of light
Mochromators/ Filters

- Used for selecting desired wavelength.

Instruments for Photometry-
Parts of Photometer

3-Device for the isolation of desired wavelengths of light

Filters-

- Absorbs light of unwanted wavelength and allow only monochromatic light to pass through.
  - –E.g.: a green filter absorbs all color, except green light which is allowed to pass through.
- Choice of appropriate filter depends upon absorption maxima of the substance to be measured.
- Used in colorimeters

Types of filters:

1-Absorption filter

Ex: Glass filter, Gelatin filter

2-Interference filter

Instruments for Photometry-
Parts of Photometer

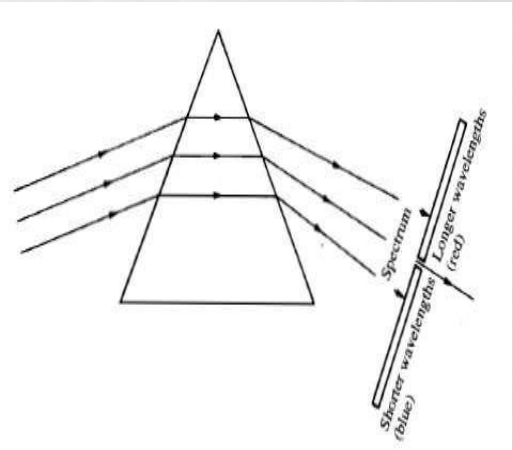
3-Device for the isolation of desired wavelengths of light

Monochromators-

A grating/prism disperses radiant energy from the source lamp into a spectrum from which the desired wavelength is isolated by mechanical slits.

Prism - Nonlinear dispersion

Grating - Linear dispersion



Prisms:

a) Less linear over lower wavelength over 550nm
b) Give only 1 order of emerging spectrum thus provide higher optical efficiency
a) Therefore 3 wavelength checks are required

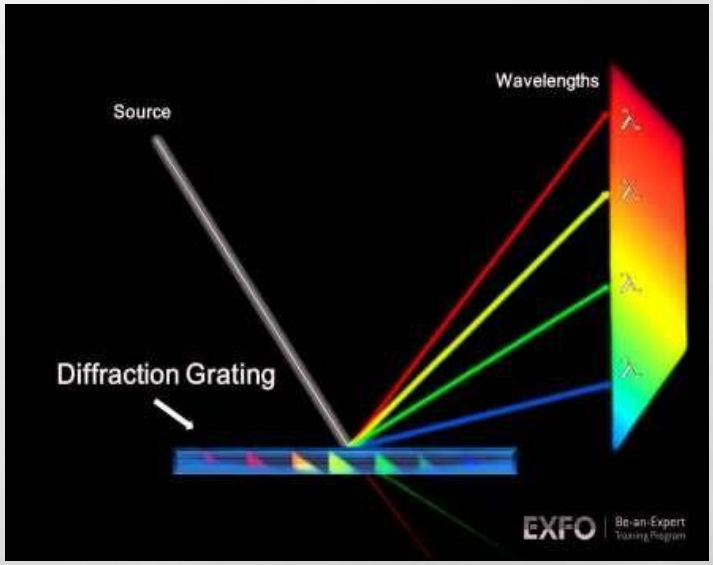
Instruments for Photometry-
Parts of Photometer

3-Device for the isolation of desired wavelengths of light

Monochromators-

Gratings:

- Linear dispersion
- Therefore only 2 wavelength checks required to certify accuracy



Instruments for Photometry-
Parts of Photometer

4-Sample Holder (Cuvette)

In a manual colorimeter the cuvettes are inserted and removed by hand. An automated colorimeter(as used in an Auto Analyzer) is fitted with a flow cell through which solution flows continuously.

- Cuvettes are rectangular cell , square cell or circular one.
- Made up of optical glass for visible wavelength, quartz or fused silica for UV.
- Common one is square, rectangular to avoid refraction artifacts.
- Optical path (length) of cuvette is always 1cm.
- Capacity may be 3ml/2ml/1ml depending upon the thickness of the wall of the cuvette
- Cuvette must be transparent, clean, devoid of scratches and no bubble on the inner side.

Instruments for Photometry-
Parts of Photometer

4-Photosensitive Detectors

Detectors are the transducers, which convert light energy to electrical energygery.

A detector should be possess following characteristics:

1-Should be sensitive

2-Should have linear response

3-Its noise level Should be low

4-Should have short response time

5-Should stable.

Different detectors used are:

1-Barrier layer cells (photocells) – simplest

2-Photoemmisive cells

3-Photomultiplier tube (for low intensity lights)

4-Photoconductive cells (photodiodes) – newest.

Instruments for Photometry-
Parts of Photometer

4-Read out devices

The detector response can be measured by any of the following devices:

a) Galvanometer

b) Ammeter

c) Recorder

d) Digital readout.

The output from a photometer may be displayed by an analogue or digital meter and may be shown as transmittance(a linear scale from 0-100%) or as absorbance (a logarithmic scale from zero to infinity). The useful range of the absorbance scale is from 0-2 but it is desirable to keep within the range 0-1 because, above1, the results become unreliable due to scattering of light.

In addition, the output may be sent to a chart recorder, data logger, or computer.

Colors & complimentary colors of visible spectrum

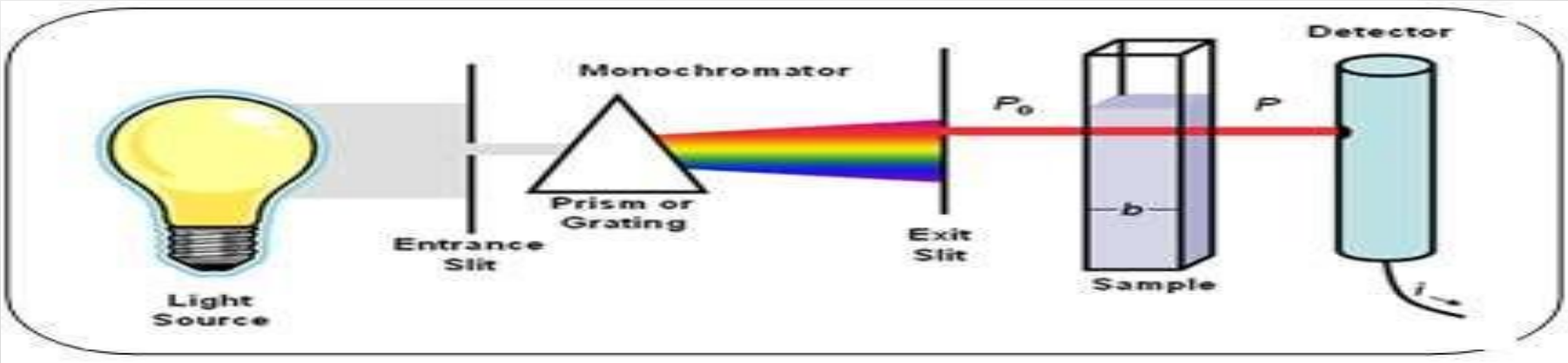
Color of the solution/ solution color transmitted	Filter used/ color absorbed	Wavelength (nm)
Yellow blue	Violet	380 – 430
Yellow	Blue	430 – 475
Orange	Green blue	475 – 495
Red	Blue green	495 – 505
Purple	Green	505 – 555
Violet	Yellow green	555 – 575
Blue	yellow	575 – 600
Green blue	Orange	600 – 650
Blue green	Red	650 - 750

Spectrophotometer

If a monochromater (prism/grating) is used as a wavelength selector, the instrument can provide monochromatic light over a continous range of wavelengths & is called spectrophotometer.

Types of spectrophotometer

1. Single beam spectrophotometer
2. Double beam in space spectrophotometer
3. Double beam in time spectrophotometer
4. Multichannel



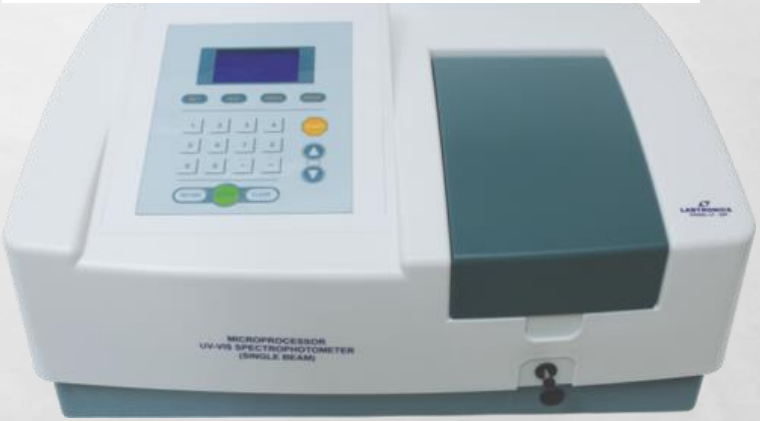
Difference between Colorimeter & Spectrophotometer

Colorimeter	Spectrophotometer
It can only be used in the visible spectra.	It can be used for UV, visible and IR regions
Filter is used as wavelength selector	Monochromater (prism/grating) is used as a wavelength selector
Can choose only a bandwidth of wavelength Only coloured solutions measured	Can choose exact wavelength, Colourless solution can also be measured
Absorbance-less accurate	Absorbance –more accurate
Cheap	Expensive
Cuvettes made of glass are used	Cuvettes made of Quartz or silica are used

Difference between Colorimeter & Spectrophotometer

Colorimeter	Spectrophotometer
Larger volume of sample is needed	Smaller volume of sample is needed. Samples of solids & gases may be used in this.
Uses- Estimation of biochemical compounds in blood, plasma, serum, CSF, urine, etc.: Glucose, Creatinine, Bilirubin, Lipids, Total Proteins etc. Enzymes [e.g. ALT, AST, ALP], Minerals [Calcium, Phosphorus etc.] etc....	Uses Detection of concentration of substances Detection of impurities Structure elucidation of organic compounds Monitoring dissolved oxygen content in freshwater and marine ecosystems Characterization of proteins Detection of functional groups Respiratory gas analysis in hospitals Molecular weight determination of compounds The visible and UV spectrophotometer may be used to identify classes of

Difference between Colorimeter & Spectrophotometer



Colorimeter

Spectrophotometer

Measurement of substance

Preparation of Solution

03 solutions are prepared-

1-Blank (B)

2-Standard (S)

3-Test or sample (T)

1-BLANK (B)

It contains all the reagents used in the procedure except the substance to be measured.

The use of blank solution compensates for non specific colour produced by the reagents and when the absorbance of blank is subtracted from the absorbance of the standard or test solution, the actual absorbance of the substance to be measured is obtained. So the zero of the instrument scale is set against reagent blank.

If the absorbance of the blank is to be considered in the calculation formula, the zero is set against water blank.

It should be noted that the solution to be measured must be clear. The cloudiness or turbidity greatly increases the absorbance value.

Measurement of substance

Preparation of Solution

2-Standard (S)

Standard contains solution of known concentration of the substance.
Absorbance of standard solution is measured.

2-Test or Sample (T)

Test solution is made by treating a specific volume of the test sample with reagents.
Absorbance of standard solution is measured.

Procedure

As given in the literature provided in related kit.

Calculation formula

Because of linear relationship between absorbance and concentration, it is possible to relate unknown concentration to single standard by a simple proportional equation;

$$\frac{As}{At} = \frac{KxCsxl}{KxCtxl} = \frac{Cs}{Ct}$$

$$Ct = \frac{At}{As} xCs$$

As= Absorbance of Standard

At= Absorbance of Test or Sample

Cs= Concentration of standard

Ct= Concentration of Test or Sample

Calculation formula

$$Ct = \frac{At}{As} xCs$$

Conc. of Substance = $\frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard}\ x\ conc.\ of\ standard$

If the result is expressed per 100 ml of blood or other body fluids, then the formula is-

Conc. of Sub. Per 100ml =

$$\frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard}\ x\ Quantity\ of\ Standard\ x\ \frac{100ml}{vol.\ of\ sample*\ used\ for\ absorbance\ of\ test}$$

$sample*$ = serum/blood or other body fluid

Calculation formula

Conc. of Substance/100ml =

$$\frac{T-B}{S-B} x\ Quantity\ of\ Standard\ x \frac{100ml}{vol.of\ sample*\ used\ for\ absorbance\ of\ test}$$

$sample*$ = serum/blood or other body fluid

B = Absorbance (OD) of Blank

S = Absorbance (OD) of Standard

T = Absorbance (OD) of Test or Sample

It should be noted that the calculation formula given for a method is valid and applicable only till the absorbance is directly proportional to the conc. of substance (Beer's Law).

This range of concentration is usually mentioned with each method.

Calculation formula

If the conc. is higher than this range, the color is diluted so that the absorbance reads with in the range and the formula is used to calculate the results and then the result obtained is multiplied by dilution factor to get the actual value or more accurately the test should be repeated with smaller amount of serum/blood.

Conc. of Substance/100ml =

$$\frac{T-B}{S-B}\ x\ Quantity\ of\ Standard\ x\ \frac{100ml}{vol.of\ sample*\ used\ for\ absorbance\ of\ test}$$ x d(dilution factor)

$sample*$ = serum/blood or other body fluid

B = Absorbance (OD) of Blank

S = Absorbance (OD) of Standard

T = Absorbance (OD) of Test or Sample

d = Dilution factor

If the system does not obey the Beer's Law or obeys in small range of conc. Calibration curve of different conc. Vs Absorbance is prepared and the values are determined by this curve.

Uses of Photometry

- Photometry is used in various industries like Chemicals, soils, agriculture,
  pharmaceuticals, glass, and ceramics, in plant materials and water, and in Biological
  and microbiological laboratories.
- It is used in determination of biochemical compounds in blood/plasma/ serum/ CSF/urine, etc.: Glucose, creatinine, bilirubin, lipids, total proteins etc. Enzymes [e.g.-ALT, AST etc.), Minerals [calcium, phosphorus etc.] etc….
- Analysis of industrial water, natural water
- Determining elements responsible for hard water

Types of Photometry

Photometric principles are applied to the several kinds of analytical techniques:

(a) Where absorbed or transmitted light is measured:

- Colorimetry
- Spectrophotometry
- Atomic absorption spectrophotometry
- Turbidimetry

(b) Where scattered light is measured

Nephelometry

(c) Where emitted light is measured:

- Flame emission photometry or flame emission spectrophotometry
- Fluorometry

Colorimetry

- Colorimetry is for the estimation of colored compounds only .
- Colorimetric methods are applicable to dilute solutions.
- For a colorimetric method to be quantitative, it must from a compound with definite color characteristics.
- Color amount must be directly proportional to the concentration.
- Colored compound must obey beer's law and lambert's law.

Colorimetry is done by colorimeters. The principal Colorimeters are:

1- Visual Colorimeter

2- Photo electric Colorimeter

Colorimetry

Visual Calorimeter-

In this the length of the column through which light passes is changed with the help of plungers so that the intensity of transmitted light of both the standards and the test as seen through the eye piece is equal.

Photoelectric Colorimeter

No color matching is done in it.

A photo cell and galvanometer are used for measuring the actual intensity of the transmitted light.

Applications of Colorimetry

It is used in the estimation of various biochemical compounds, enzymes and minerals in the various body fluids (Blood/serum/plasma or urine or CSF)

Spectrophotometry

This is for estimation of coloured and colourless compounds both.
In this, estimation may be done in ultraviolet, visible and infra red regions.

Instruments used:
Spectrophotometers

- UV Spectrophotometers work at wavelength below 400nm. This is more useful in clinical laboratory in the estimation of many dehydrogenase enzymes which needs the extinction to be taken at about 340nm and at the extinction at about 260nm, the purines and pyrimidines of nucleic acids are estimated.
- Infrared spectrometers work at the wavelength above 800nm.

Spectrophotometry: Applications

Main applications are:

- Qualitative analysis : used to identify classes of compound in both pure state and in biological preparations.
- Detection of concentration of substances in body fluids.
- Denaturation of double standard DNA.
- Enzyme assay and kinetic studies : the quantitative assay of enzyme activity is carried out.
- Molecular weight determination : the molecular weight of amines, sugars and
- many aldehyde and ketone compounds can be determined .
- Control purity : impurity in compound can be detected easily by spectrometric studies.
- Protein folding: protein or protein– nucleic acid interaction can be studied.

Atomic absorption spectrophotometry (AAS)

Atomic absorption spectrophotometry (AAS)

Basic principle

The technique uses basically the principle that free atoms (gas) generated in anatomizer can absorb radiation at specific frequency.

- It is a very common technique for detecting metals & metalloids in samples.
- Useful for determining trace metals in liquid or biological samples.
- Highly sensitive-can detect metal in conc. Lower than 1ppm.
- It can analyse over 62 elements.
- It is very reliable and easy to use.

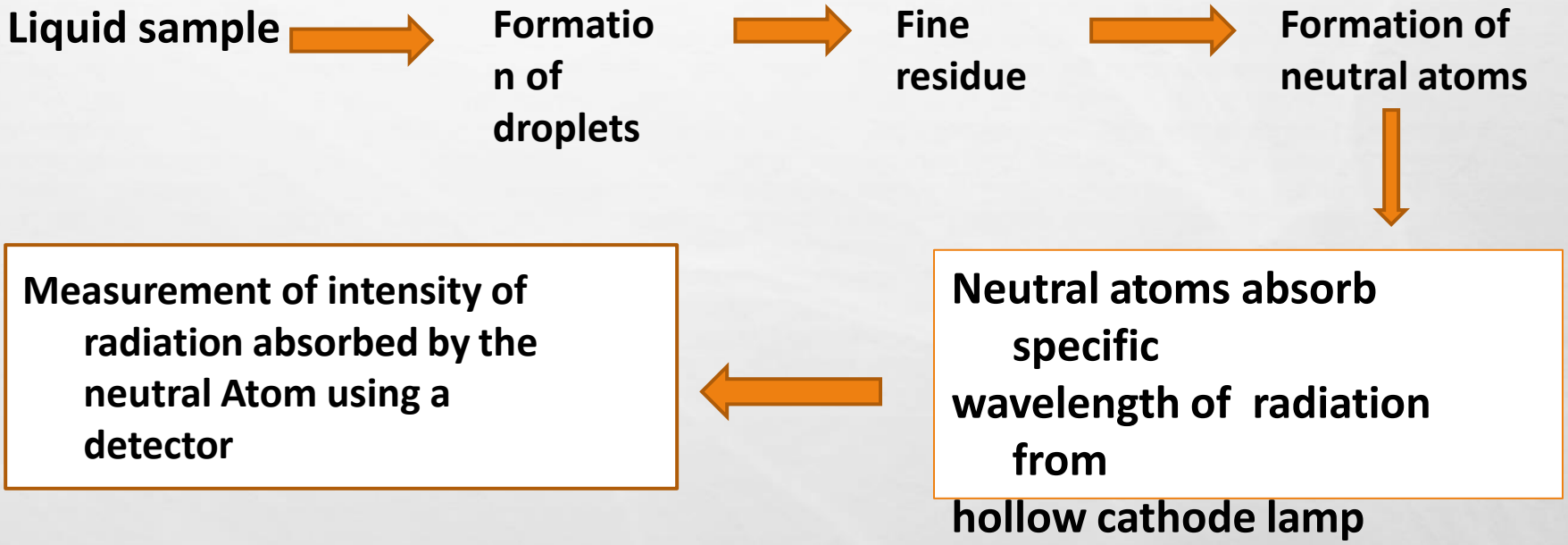
Atomic absorption spectrophotometry (AAS)- Principle

- **A**AS deals with the absorption of specific wavelength of radiation by neutral atoms in the ground state.
- When a solution of a metallic salt is aspirated into a flame, metal atoms in gaseous state are obtained.
- In flame only small fraction of atoms are thermally excited.
- When a beam of light is made to pass through the flame the dispersed atoms in the ground state absorb a part of the incident radiation much like a solution absorbing radiation passing through it.

Atomic absorption spectrophotometry (AAS)- Principle

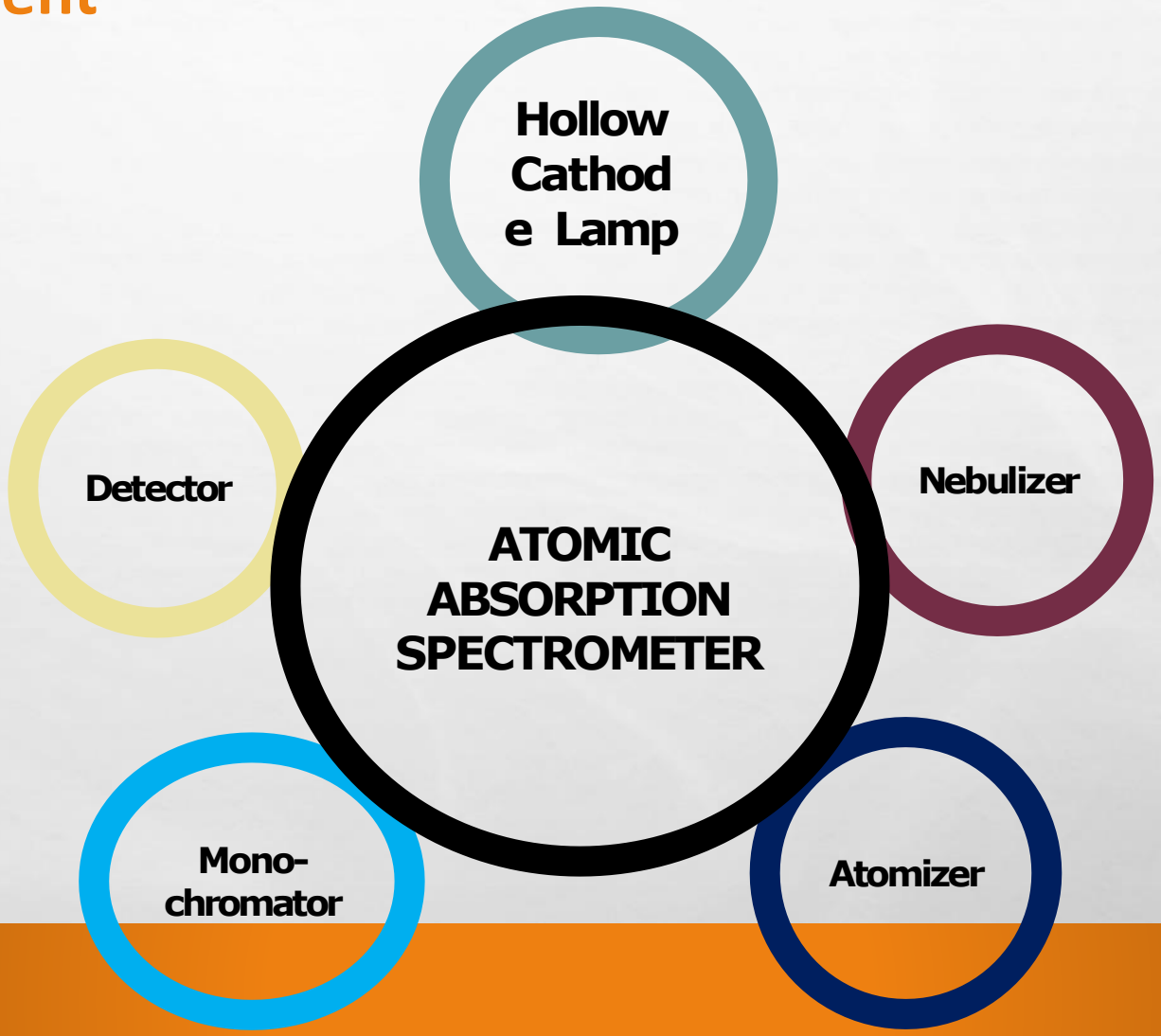
- Each element absorbs radiation which is characteristic of the element. Thus if the sample solution contains sodium salt then the source of light must be sodium metal.
- The absorption of radiation by atoms also follows Beer-Lamberts law i.e absorbance is directly proportional to the concentration of atoms in the flame and to the path length in the flame.
- Each element absorbs radiation that is characteristic of the element, therefore a separate lamp source is needed for each element.
- Most commonly used source of light is hollow cathode lamp

Atomic absorption spectrophotometry (AAS)- Principle

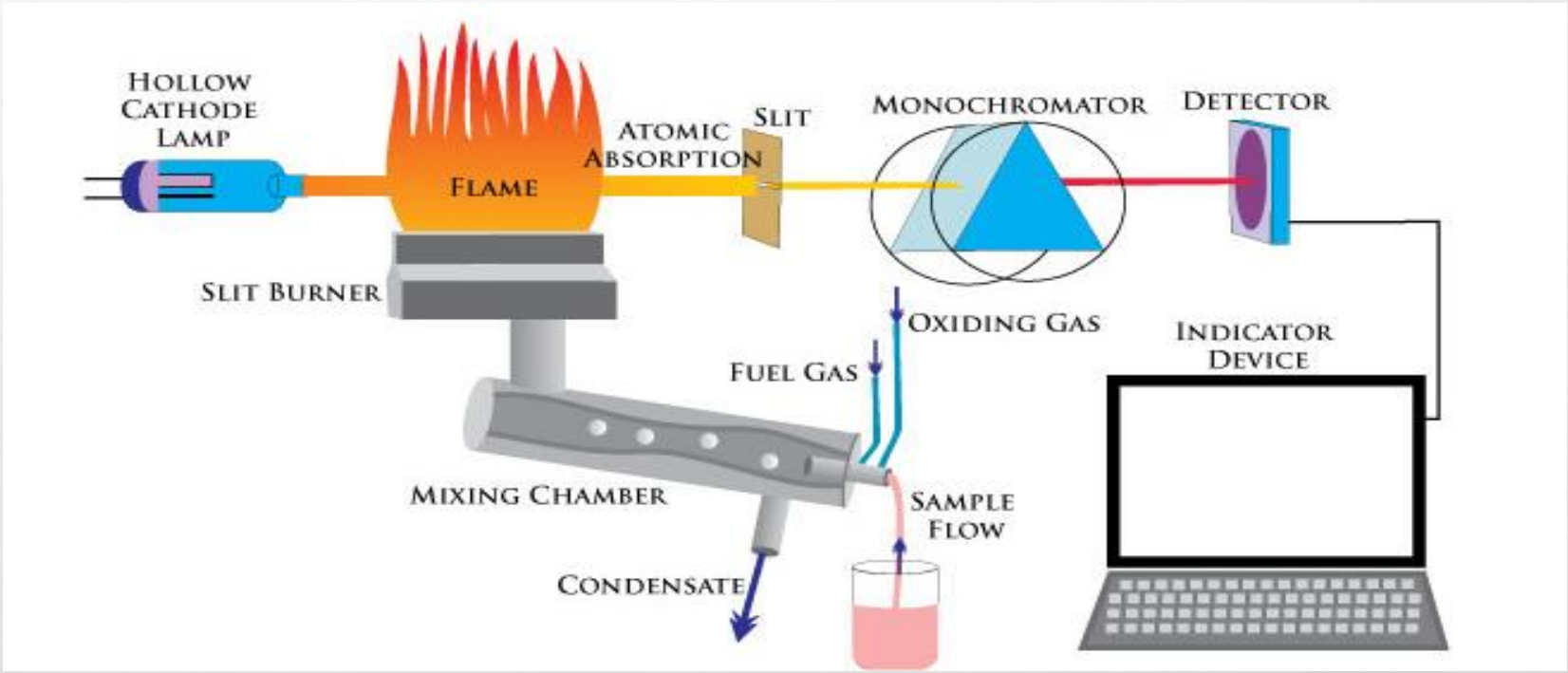


Atomic absorption spectrophotometry (AAS)

Instrument



Atomic absorption spectrophotometry (AAS)

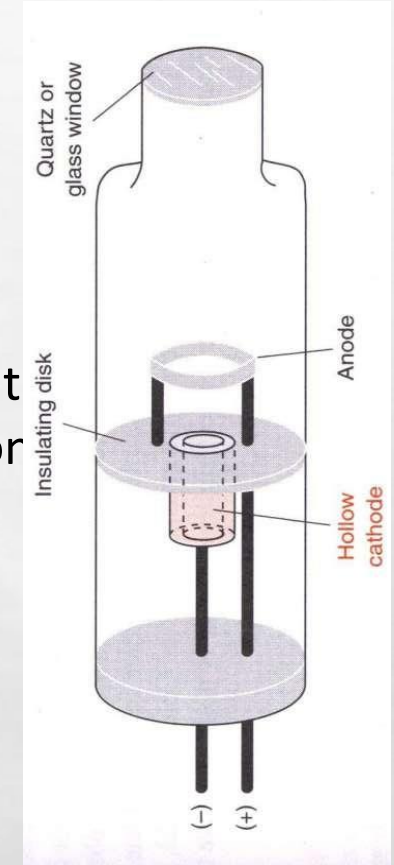


Atomic Absorption Spectrophotometer

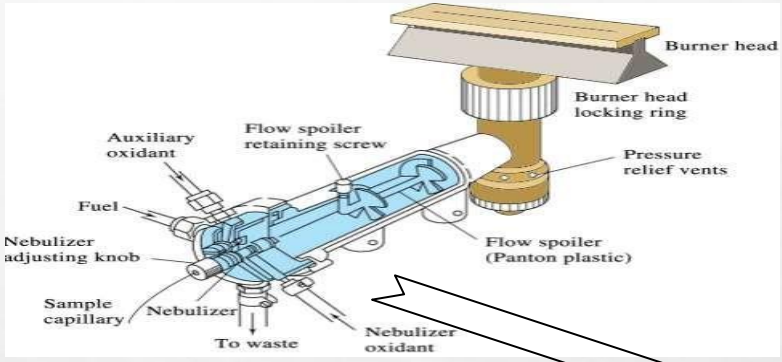
Atomic absorption spectrophotometry (AAS)

1-Hollow Cathode lamp(HCL)-

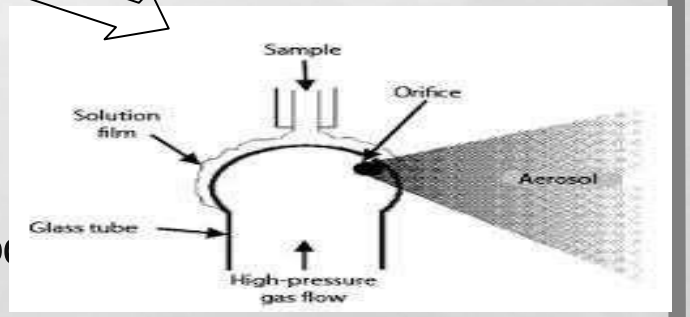
- It consists of a tungsten anode and a hollow cylindrical cat sealed in a glass tube containing an inert gas such as argor neon at a low pressure.
- The cathode is made of the same metal as the one under consideration.
- When a high potential is applied across the electrode the inert gas is ionised.
- The ions collide with the cathode surface and dislodge metal atoms from the surface.
- Some of the metal atoms are in sufficiently excited state to emit their characteristic radiation.
- This appears as a glow inside the hollow cathode space.



Atomic absorption spectrophotometry (AAS)



2-Nebulizer(Burner with fuel & oxidant)



Suck up liquid samples at controlled rate.

Create a fine aerosol spray for introduction into flame.

Mix the aerosol and fuel and oxidant thoroughly forintro into flame.

Atomic absorption spectrophotometry (AAS)

3-Atomizer

Elements to be analyzed needs to be in atomic state.

Atomization is separation of particles into individual molecules and breaking molecules into atoms.

It is done by exposing the analyte to high temperatures in a flame or graphite furnace.

Flame atomizer

To create flame, we need to mix an oxidant gas and a fuel gas.

In most of the cases air-acetylene flame or nitrous acetylene flame is used.

Liquid or dissolved samples are typically used with flame atomizer.

Electrothermal atomizer (Graphite tube)

Uses a graphite coated furnace to vaporize the sample.

Samples are deposited in a small Graphite coated tube which can then be heated to vaporize and atomize the analyte.

The graphite tubes are heated using a high current power supply.

Atomic absorption spectrophotometry (AAS)

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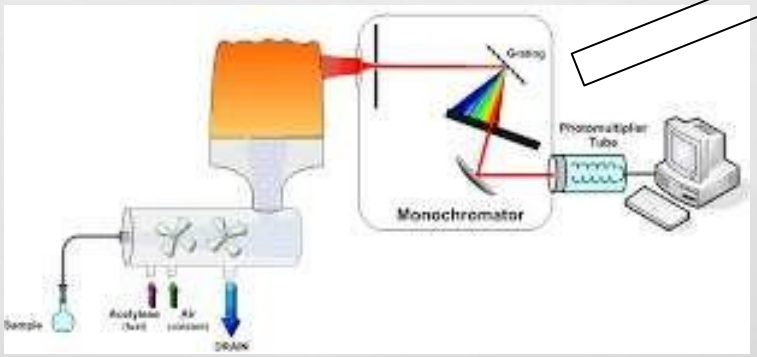
Atomic absorption spectrophotometry (AAS)

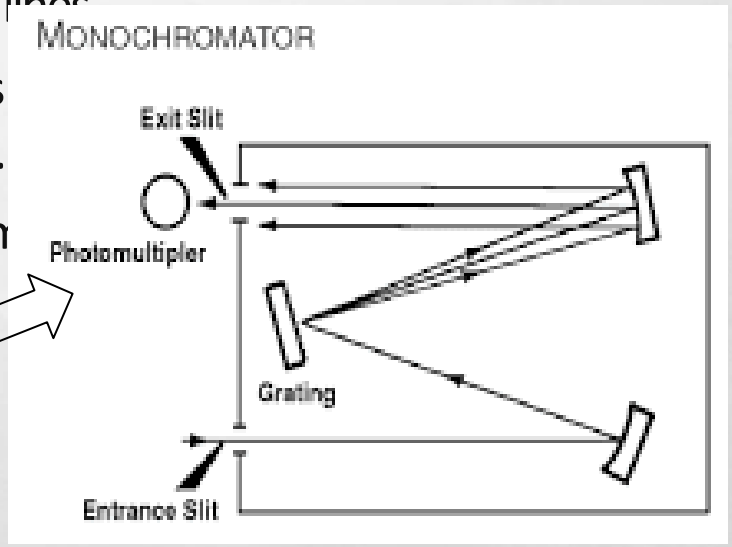
4-monochromator-

This is used to separate out all of the thousands of lines

It will select a specific wavelength of light which is by the sample, and to exclude other wavelengths.

The selection of the specific light allows the determ of the selected element in the presence of others.





Atomic absorption spectrophotometry (AAS)

5-Detector-

- It is a detector that is typically a photomultiplier tube, whose function is to convert the light signal into an electrical signal proportional to the light intensity.
- The processing of electrical signal is fulfilled by a signal amplifier.
- The signal could be displayed for readout, or further fed on to a data station for printout by the requested format.

Atomic absorption spectrophotometry (AAS)

LET'S REVIEW SOME CONCEPTS OF AAS

Hollow Cathode Lamp

conduction at a lower voltage and with more current

Nebulizer

Mix the aerosol and fuel and oxidant thorughly for introduction into flame.

Atomizer

Atomization is separation of particles into individual molecules and breaking molecules into atoms.

Monochromator

It will select a specific wavelength of light which is absorbed by the sample, and to exclude other wavelengths.

Detector

to convert the light signal into an electrical signal proportional to the light intensity.

Atomic absorption spectrophotometry (AAS): Applications

Clinical analysis: analyzing metals in biological fluids such as blood and Urine.

Environmental analysis: monitoring our environment –e.g. finding out the levels of various elements in rivers, seawater, drinking water, air, petrol and drinks such as wine, beer and fruit drinks.

Pharmaceuticals: In some pharmaceutical manufacturing processes, minute quantities of a catalyst used in the process (usually a metal) are sometimes present in the final product. By using AAS the amount of catalyst present can be determined.

Industry: many raw materials are examined and AAS is widely used to check that the major elements are present and that toxic impurities are lower than specified –e.g. in concrete, where calcium is a major constituent, the lead level should be low because it is toxic.

Mining: by using AAS the amount of metals such as gold in rocks can be determined to see whether it is worth mining the rocks to extract the gold.

Atomic absorption spectrophotometry (AAS): Advantages

- Technique is specific because the atom of particular element can only absorb radiation of their own characteristic wavelength.
- Is independent of flame temperature.
- Solutions, slurries and solid samples can be analysed.
- Much more efficient atomization.
- Greater sensitivity.
- Smaller quantities of samples (typically 5 – 50 µl) are required

Atomic absorption spectrophotometry (AAS): Disadvantages

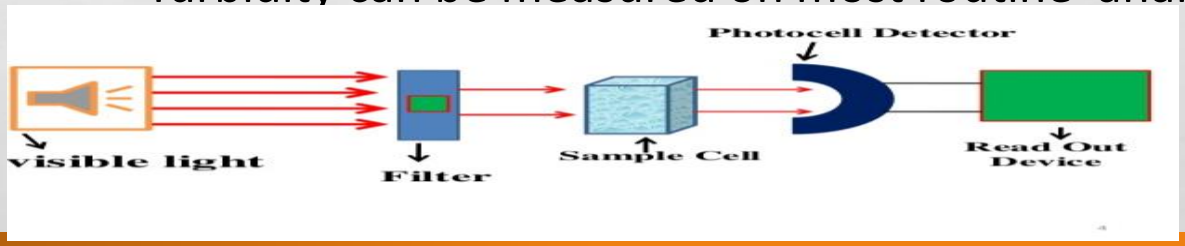
- Separate lamp for each element to be determined is required.
- Technique cannot be used successfully for estimation of element like Mo, Si etc because these element give rise to oxide in the flame.
- Predominant anion affect the signal to negotiable degree.
- Expensive
- Low precision
- Low sample throughput
- Requires high level of operator skill
- Sample must be in solution or at least volatile

Turbidimetry: Principle

- In Turbidimetry the intensity of light transmitted through the medium, is measured.
- Turbidometric measurements are made at 180^{o} from the incident light beam.
- "It is measurement of intensity of the transmitted light (It) as a function of concentration(C) of the suspended particles in a suspension."

$$It \quad 1/C$$

- Turbidity can be measured on most routine analysers by a



- High concentrated suspensions are measured.

Turbidimetry : Applications

- Analysis of water: clarity, conc. of ions, turbidity.
- Determination of Co2
- Determination of inorganic substances-: Sulphuric-barium chloride, Ammonia-nessler's reagent, Phosphorus-strychine molybedate
- In biochemical analysis: an important application of turbidimetry is to measure the amount of growth of a test bacterium in a liquid nutrient medium. It is also used to find out the amount of amino acid, vitamins and antibiotics and also used for Ag-Ab reaction, Immunocomplex reactions etc.
- Determination of Molecular Weight of high polymers.
- In Organic analysis: clarity of citrus juices, benzene in alcohol.
- In drug development
- Turbidimetric titration
- In atmospheric pollution-smokes & fog's.
- Miscellaneous-:water treatment in plant's, in sewage work, refineries, paper industries.

Turbidimetry

Advantages-

- Very rapid procedure
- Simplicity in measurement
- They are accurate

Disadvantages-

- High cost
- Easily damaged
- They require high power supply
- Turbidimetry is used for higher concentrations

Nephelometry Principle

- In Nephelometry the intensity of the scattered light is measured.
- In this decreased concentration, uniform scattering
- Nephelometric measurements are made at 90^{0}.
- "Intensity of the scattered light(Is) Is directly proportional to the conc. of the suspended particles (C)" i.e. **$Is \propto C$**

Is= Intensity of the scattered light C= Conc. of the suspended particles

- The intensity of scattered light is normally measured by Nephelometer.

Nephelometry

Light scattering is the physical phenomenon resulting from the interaction of light with a particles in solution.
Light scattering is dependent on :

- Particle size
- Wavelength
- Distance of observation,
- Concentration of particles
- MW of particles

Nephelometry

Tyndall Effect

The tyndall effect is the effect of light scattering in many directions in colloidal dispersion, while showing no light in a true solution.

This effect is used to determine whether a mixture is a true solution or a colloid.

Under the tyndall effect, the longer-wavelength light is more transmitted while the shorter-wavelength light is more scattered.

Nephelometry

Nephelometer:

Parts-

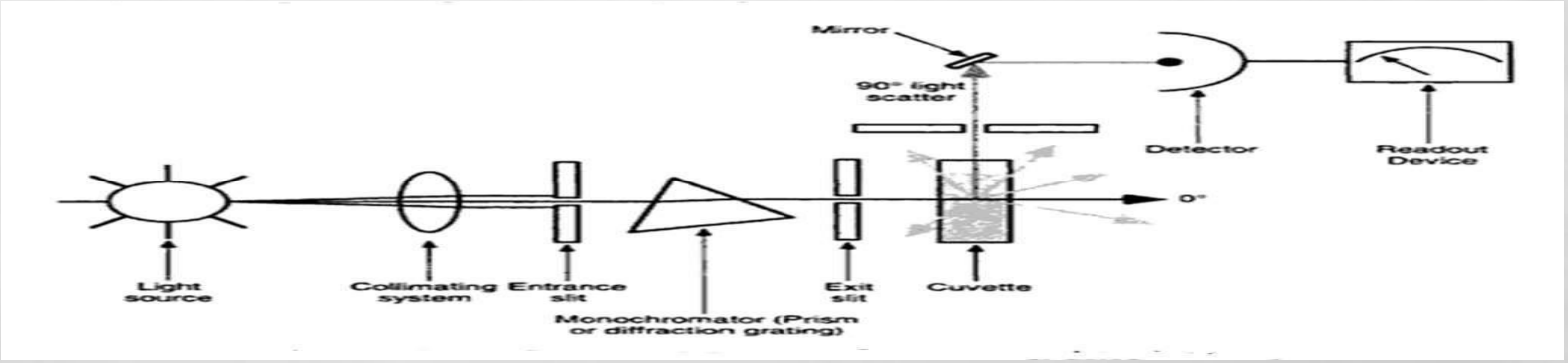
1-Light Source

2-Collimating optics

3-Monochromators

4-Sample cells

5-Detectors



Nephelometry

Nephelometer:

Parts-

1-Light Source : May be Quartz halogen lamp/ Mercury arc lamps/ Xenon lamps/ Lasers.

Lasers: stable, collimated intense light beams, reduces stray light, background scatter

2-Collimating optics: Collimator collimates the light from the entrance slit.

3-Monochromators: Consist of Prism or gratings, entrance & exit slit.

4-Sample Cells: These are called cuvettes. In Nephelometry method cuvettes made of glass

are used.

5-Detectors: these are devices which converts the light energy into

electrical signals that are displayed in the readout devices.

Photomultiplier tube detectors are mainly used in the nephelometry.

Nephelometry : Applications

- Analysis of water: clarity, conc. of ions.
- Determination of Co2
- Determination of inorganic substances-: Sulphuric-barium chloride, Ammonia-nessler's reagent, Phosphorus-strychine molybedate
- In biochemical analysis: used for Ag-Ab reaction, Immunocomplex reactions etc.
- Determination of Molecular Weight of high polymers.
- In Organic analysis: clarity of citrus juices, benzene in alcohol.
- In drug development
- In atmospheric pollution-smokes & fog's.
- Miscellaneous-:water treatment in plant's, in sewage work, refineries, paper industries.

Nephelometry

Advantages-

- Very rapid procedure
- Simplicity in measurement
- They are accurate

Disadvantages-

- High cost,
- Easily damaged
- They require high power supply
- Nephelometry is used for lower concentrations

Difference between Nephelometry & Turbidimetry

	Nephelometry	Turbidimetry
Principle	The measurement of the intensity of scattered light at right angles to the direction of the incident light as a function of the concentration of the dispersed phase. It is most sensitive for very dilute suspensions (100mg/L).	Light passing through a medium with dispersed particles, so the intensity of light transmitted is measured.
Type of light measured	Scattered Light	Transmitted Light
Conc. measured	Lower Concentrations	Higher concentrations
Instrument used	Nephelometer	Spectrophotometer
Arrangement of photometer	Measurement of light scattered at right angle to the direction of the propagation of light from the source.	Measurement is made in the same direction(180 degree) as the propagation of the light from the source.
Clinical uses	Ag-Ab rxn, Immunocomplex reactions, ppts(precipitates), lipoprotein	Ag-Ab reactions, Immunocomplex rxn, ppts, liver diseases, Estimation of proteins in urine

Flame Photometry or Flame Emission Spectrometry

- Flame photometry is also known as flame emission spectroscopy.
- Neutral atoms are involved in emission of radiation when introduced into flame.
- Atoms are simplest & pure form of matter.
- Atoms exhibit electronic transitions when absorb energy.
- Such discrete transitions are quantised & line spectra is observed.
- Atoms in the form of atomic vapour are produced in higher energy level.
- It returns to ground energy state by emitting photons & generating sharp line emission spectra.
- Flame photometry is based on the measurement of intensity of light emitted when a metal is introduced into flame.
- Wavelength of light emitted indicates the type of element present.
- Intensity of light indicates the quantity of element.

Flame Photometry or Flame Emission Spectrometry

Method

When a liquid sample containing a metallic salt is introduced into the flame:

Solvent vaporises leaving particles of solid salt.

Salt vaporises into gaseous state.

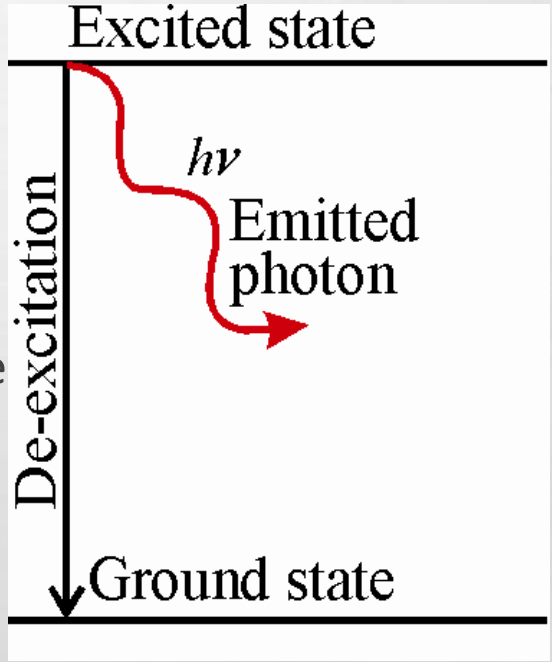
Gaseous molecules dissociated to neutral atoms.

Neutral atoms are excited by thermal energy of flame

Excited unstable atoms quickly emit photons and returns to ground state.

Emitted radiation intensity is measured.

Permitted energy levels of all atoms can be represented diagrammatically in grotrian chart.

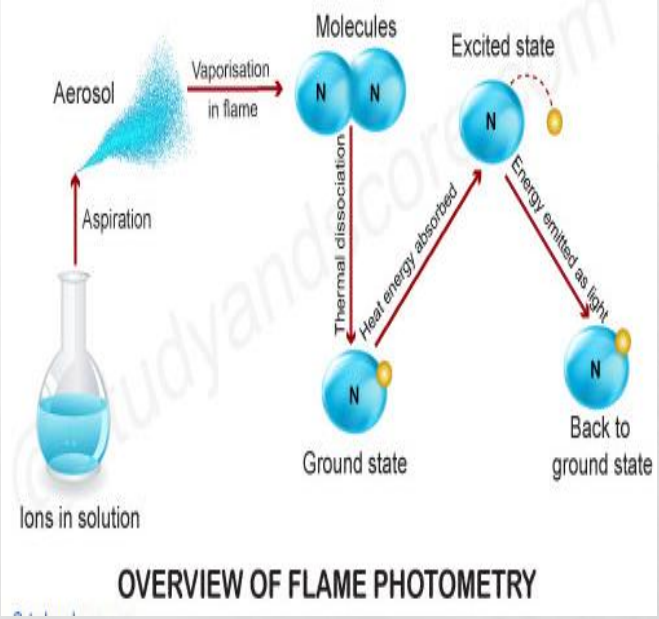


Flame Photometry or Flame Emission Spectrometry

In flame spectroscopy source of excitation energy is a flame.
It is a low energy source.
Emission spectrum produced is simple.
It has few emission lines.
Quantitative determinations are made by aspirating sample into flame.
Intensity of characteristic radiation emitted by flame for individual elements is
correlated with concentration of element in sample.
Specific wavelength emitted by elements appear as spectral lines in uv & visible regions

Flame Photometry or Flame Emission Spectrophotometry: Principle

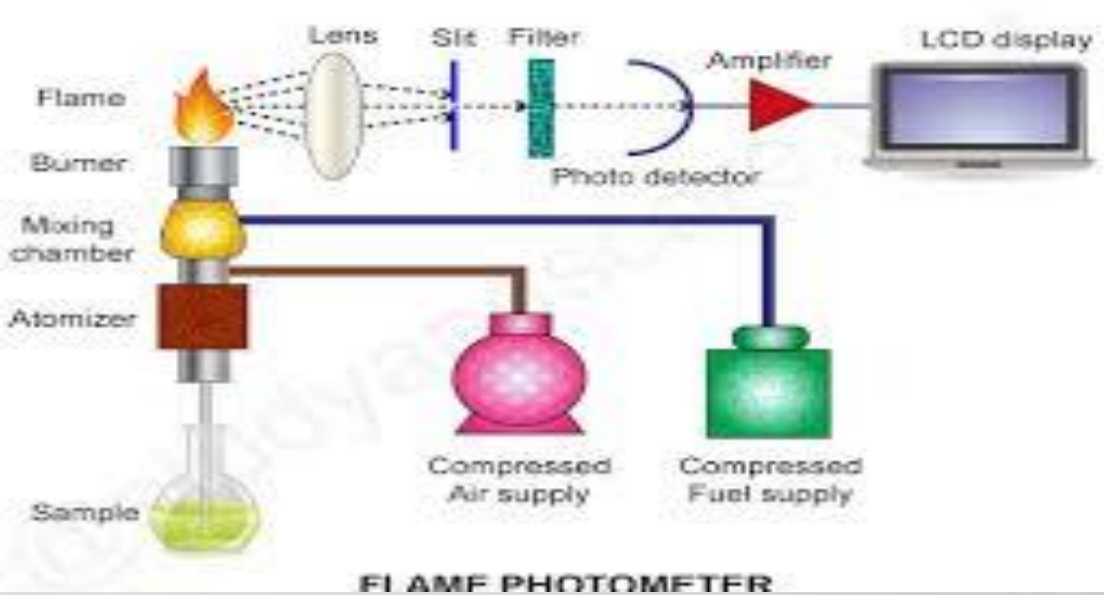
"Atoms of some metals, When given sufficient heat energy (hot flame) become excited & reemit this energy at wavelengths characteristic of the element.
The intensity of radiant energy of characteristic
wavelength produced by the atoms in the flame is directly proportional to the number of atoms excited in the flame , which in turn is directly proportional to
the concentration of the alkali metal in the sample.''



Flame Photometry or Flame Emission Spectrometry

Instrument

The instrumentation of flame emission spectroscopy is the same as that of atomic absorption, but without the presence of a Radiation source .



Flame Photometry or Flame Emission Spectrometry

Instrument

Flame : The source of energy in Flame Emission could be a flame like the one used in atomic absorption, or an inductively coupled plasma (ICP) .
The flame (1700 – 3150 oC) is most useful for elements with relatively low excitation energies like sodium, potassium and calcium.
The ICP (6000 – 8000 oC) has a very high temperature and is useful for elements of high excitation energies.
Atomizer : In atomizer the sample is atomized and the analyte atoms are excited to higher energy levels.
The excited atoms decay back to lower levels by emitting light.
Emissions are passed through monochromators or filters prior to detection by photomultiplier tubes.
Alkali metals are easy to excite by flame: Li- red emission, Na – yellow emission, K- red violet emission, Rubidium- red emission, Mg- blue emission.

Flame Photometry or Flame Emission Spectrometry: Applications

- To estimate sodium, potassium, calcium, lithium etc. level in sample of serum, urine, CSF and other body fluids.
- Flame photometry is useful for the determination of alkali and alkaline earth metals.
- Used in determination of lead in petrol.
- Used in the study of equilibrium constants involving in ion exchange resins.
- Used in determination of calcium and magnesium in cement.
- In agriculture, the fertilizer requirement of the soil is analyzed by flame test analysis of the soil.
- Analysis of soft drinks, fruit juices and alcoholic beverages can also be analyzed by using flame photometry

Flame Photometry or Flame Emission Spectrometry: Advantages

- Lower interelement interference because of higher temperature.
- Emission spectra are obtained under a single set of excitation conditions and several elements can be recorded simultaneously.
- Multiple elements can be analyzed from very small sample.
- Quite, convenient, selective and sensitive to even parts per million (ppm) to parts per billion (ppb) range.

Flame Photometry or Flame Emission Spectrometry: Disadvantages

- More expensive equipment's needed.
- Procedures are somewhat complicated than absorption method.
- More operating cost than absorption technique.
- As natural gas & air flame is used for excitation, temperature is not high enough to excite transition metals.So this cannot be used for transition metals or other metals which requires significant energy.
- Relatively low energy available from the flame leads to low intensity of radiation.
- Fuel rich oxy acetylene flame generates intense radiation bands at shorter wave length
- (300-200nm).
- Ion emission lines can be detected.
- If the flame is oxygen rich they operate at same temperature, but ion emission lines are not observed.

Flame Photometry or Flame Emission Spectrometry: Disadvantages

- Nitrous oxide-Acetylene flame gives higher temperature. Useful for oxides of Aluminium, Titanium etc, but high temperature ionises alkali metals.
- Low temperature makes this method susceptible to interference, stability of flame,
- aspiration conditions.
- Identical conditions are necessary for measuring emission of standard & unknown
  solutions.
- Preparation of liquid samples involves lengthy steps & time consuming

Comparison between Atomic absorption & Flame Emission spectrophotometry

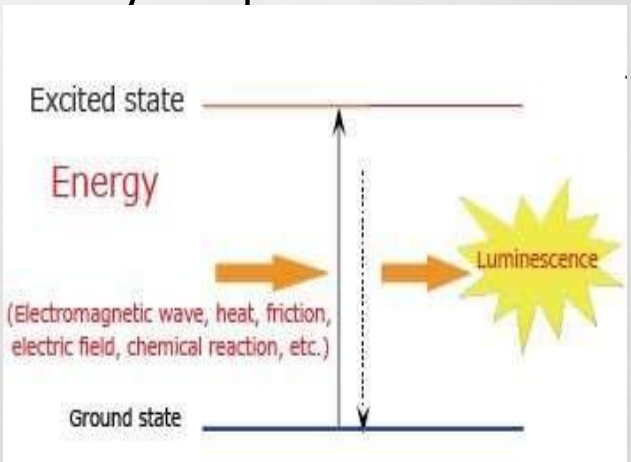
Absorption	Emission
• Measure trace metal concentrations in complex matrices .	• Measure trace metal concentrations in complex matrices .
• Atomic absorption depends upon the number of ground state atoms	• Atomic emission depends upon the number of excited atoms
• It measures the radiation absorbed by the ground state atoms.	
• Presence of a light source (HCL).	• Absence of the light source.
• The temperature in the atomizer is adjusted to atomize the analyte atoms in the ground state only.	• The temperature in the atomizer is big enough to atomize the analyte atoms and excite them to a higher energy level.

Fluorometry

Fluorimetry is defined as the measurement of the emitted fluorescence light.
The fluorometric technique allows measurement of concentrations as low as 1ng/l in suitable cases.

The emission of Electromagnetic radiation is shared by the process of fluorescence, phosphorescence and luminescen

Luminescence is the emission of light by a substance. It occurs when an electron returns to the electronic ground state from an excited state and loses its excess energy as a photon.



Luminescence

Fluorometry

Fluorescence - When the luminescence stops within 10^-8 to 10^-4 sec after the source of excitation is removed, it is called fluorescence. The substances showing this phenomenon are known as fluorescent substances.

Phosphorescence: When the luminescence continues for a slightly longer period of time (10^-4 to 10 sec) after the source of excitation is removed, it is called phosphorescence.

Only Fluorometric technique is considered for routine clinical/biochemical work.

Fluorometry: Principle

- Fluorescence is the phenomenon of emission of radiation when there is transition from singlet excited state to singlet ground state.
- Wavelength of absorbed – Excitation wavelength.
- Wavelength of emitted – Emission wavelength.
- These two are specific for a given substance under ideal condition.

Various Electronic States in Fluorometry

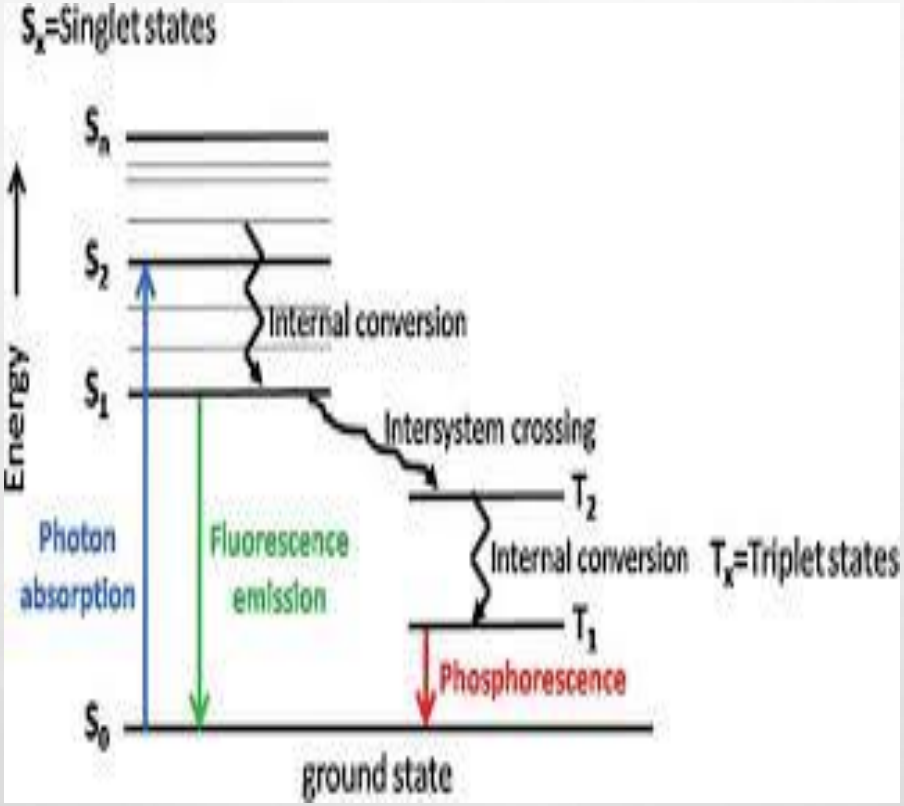
- **Singlet ground state** : A state in which all electrons in a molecule are paired.
- **Doublet state** : A state in which unpaired electrons is present.
- **Triplet state** : A state in which unpaired electrons of same spins present.
- **Singlet excited state**: A state in which electrons are unpaired out of opposite spin.

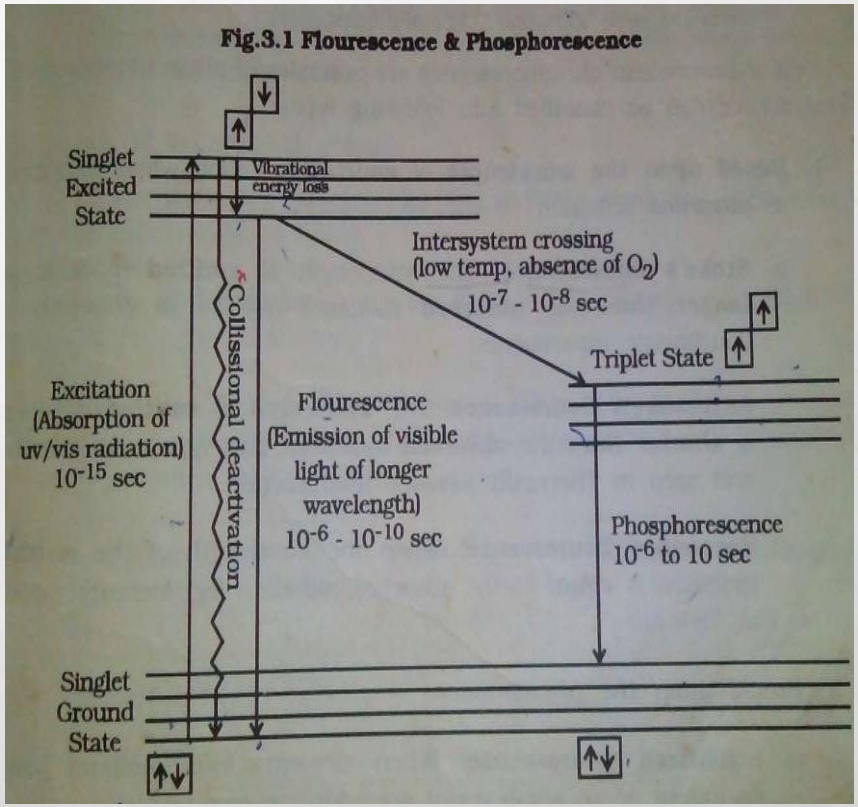
Fluorometry: Principle

To achieve transition from excited state to ground state there are the possibilities:

- **Collisional deactivation** : in which the entire energy is lost due to collisional deactivation and no radiation is emitted.
- **Fluorescence** : Apart of energy is lost due to vibrational transition and the remaining energy is emitted as UV/visible radiation of longer wavelength .
- **Phosphorescence : T**he emission of radiation when electrons undergo transition from triplet state to ground state.
- **Intersystem crossing** : At favourable conditions like low temperature and absence of oxygen there is transition from excited state to triplet state.

Fluorometry: Principle





Fluorometry

Fluorescent Substances

- Organic solutions such anthracene or stilbene dissolved in benzene or toluene
- Calcite, Rubies, Emeralds, Diamonds. willemite, esperite, uranium, uranyl cation, autunite or andersonite, and hyalite opal, ruby, europium.
- Atmospheric aurora.

Common materials that fluoresce

- Vitamin B2 fluoresces yellow.
- Tonic water fluoresces blue due to the presence of quinine.
- Highlighter ink is often fluorescent due to the presence of pyranine.
- Banknotes, postage stamps and credit cards often have fluorescent security features.

Fluorometry

Instrument-

Fluorometer-

Two types depending upon method of wavel selection

A- Filter Fuolometers: primary wavelength is made by one or more wavelength filters

- Single beam (filter) fluorimeter
- Double beam (filter) fluorimeter

B-Spectrofluorimeter:

The wavelength selection by monochromato

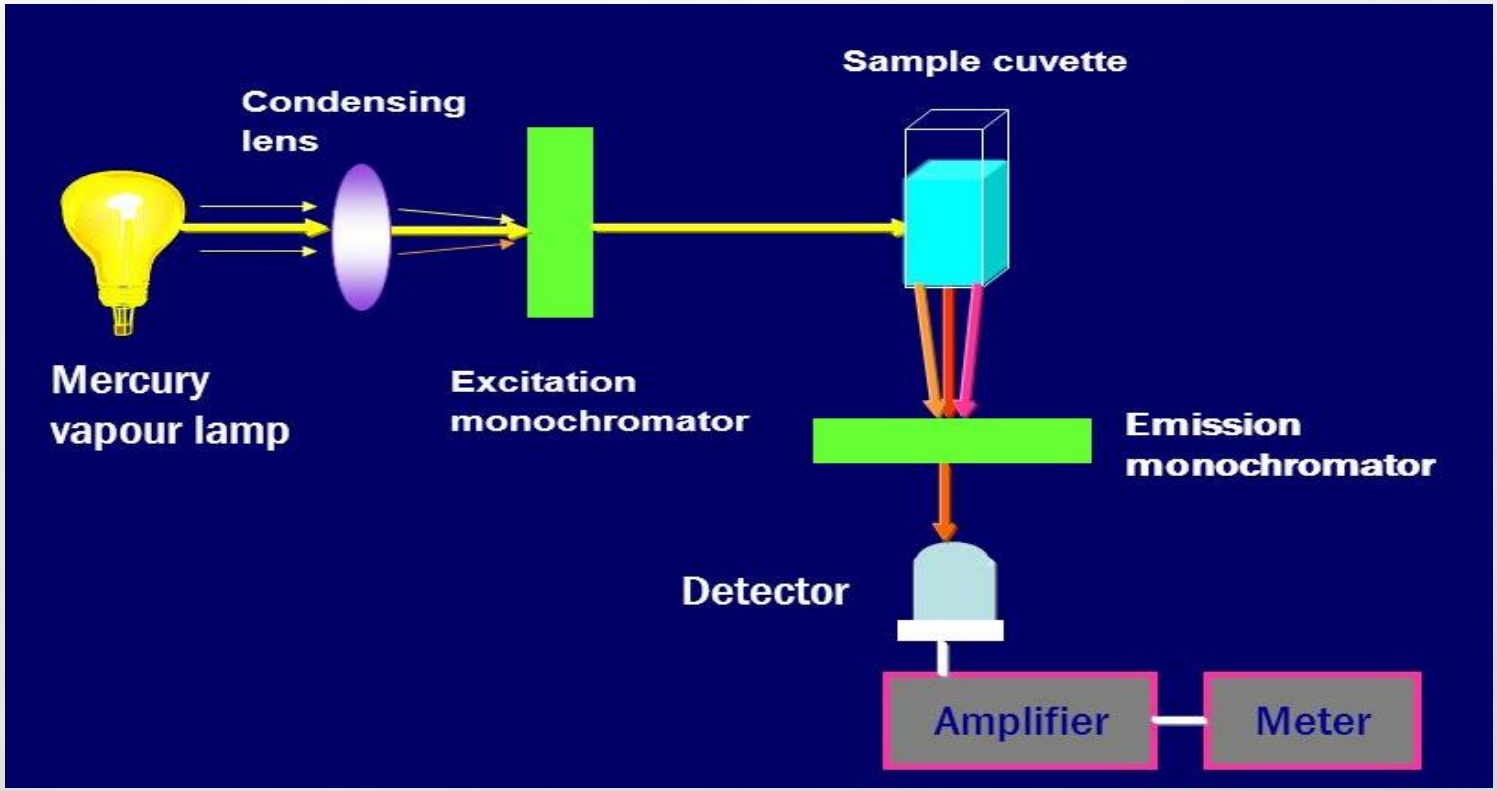
Spectrofluorimeter


Single beam fluorimeter

Double beam fluorimeter

Fluorometry

Instrument- Fluorometer- Parts



Fluorometry: Applications

Environmental :

- To detect environmental pollutants such as polycyclic aromatic hydrocarbons: pyrene, benzopyrene, organothiophosphorous pesticides, carbamate insecticides.
- Generally used to carry out qualitative as well as quantitative analysis for a great aromatic compounds present in cigarette smoking, air pollutant concentrates & automobile exhausts.

Geology:

- Many types of calcite and amber will fluoresce under shortwave UV. Rubies, emeralds, and the Diamond exhibit red fluorescence under short-wave UV light; diamonds also emit light under X ray radiation.

Fluorometry: Applications

Analytical Chemistry :

To detect compounds from HPLC flow.

TLC plates can be visualized if the compounds or a coloring reagent is fluorescent.

Plant pigments, steroids, proteins, naphthol etc. can be determined at low concentrations.

Biochemistry:

Used generally as a non-destructive way of tracking or analysis of biological molecules (proteins).

Possible direct or indirect analysis aromatic amino acids (phenylalanine- tyrosine-tryptophan).

Fingerprints can be visualized with fluorescent compounds such as ninhydrin.

Fluorometry: Applications

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Nuclear Research:

- Field determination of uranium salts.

Fluorometry: Applications

Medicine:

- Blood and other substances are sometimes detected by fluorescent reagents, particularly where their location was not previously known.
- There has also been a report of its use in differentiating malignant, bashful skin tumors from benign.

Pharmacy:

- Possible direct or indirect analysis drugs such as: vitamins (vitamin A ,vitamin B1, B2 , vitamin B6, vitamin B12, vitamin E, Folic acid), Catecholamines (dopamine, norepinephrine)
- Other drugs (quinine, salicylic acid, morphine, barbiturates, lysergic acid diethylamide (LSD))
- To measure the amount of impurities present in the sample.

Determination of Inorganic Substances:

- Determination of ruthenium ions in presence of other platinum metals.
- Determination of aluminum (III) in alloys.
- Determination of boron in steel by complex formed with benzoin.
- Estimation of cadmium with 2-(2 hydroxyphenyl) benzoxazole in presence of tartarate.

Fluorometry

Fluorescent indicators:

Intensity and color of the fluorescence of many substances depend upon the pH of solutions. These are called as fluorescent indicators and are generally used in acid base titrations.

E.g.:

Eosin: pH 3.0-4.0 – colorless to green. Fluorescein: pH4.0-6.0 – colorless to green.

Quinine sulphate: blue-violet.

Acridine: green-violet.

Fluorometry

Advantages

Sensitivity :

It is more sensitive as concentration is low as µg/ml or ng/ml.

Precision :

Upto 1 % can be achieved.

Specificity :

More specific than absorption method where absorption maxima may be same for two compounds.

Range of application :

Even non fluorescent compounds can also be converted to fluorescent compounds by
chemical compounds.

Disdvantages

Not useful for identification.

Not all compounds fluorescence.

Contamination can quench the fluorescence and hence give false or no results


thank you