Advanced analytical tech
Mphil zoology, 1st smstr

Spectroscopic Methods of Analysis

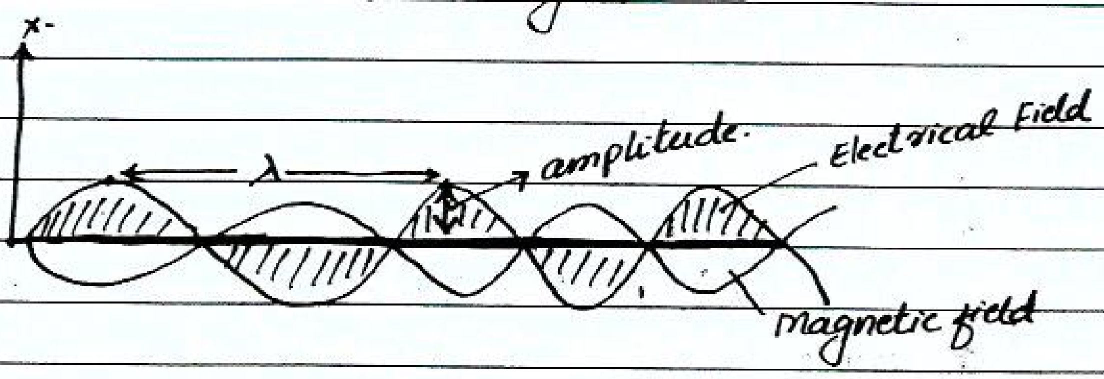
⇒ Introduction to spectroscopy:-

Spectroscopy is the general term used for the science that deals with the interactions of various types of electromagnetic radiations with matter. However, now Spectroscopy has been broadened to include interactions between matter and other forms of energy such as acoustic waves and beams of particles such as electrons, ions etc.

Spectroscopic methods have proved to be very useful for studying the properties of molecules such as molecular symmetry, bond distances, bond angles, electronic distribution, bond Strength, intra and intermolecular forces etc.

⇒ Electromagnetic Radiation Spectrum:-

When an object moves up and down or vibrates continuously, energy in the form of wave is sent or propagated by the vibrating object to a distant place. The wave produced by a vibrating object can be represented by a wavy curve as shown below. The tops of wavy curve are called Crests and the bottoms are called troughs.



J.C Maxwell (1864) used the term electromagnetic waves or electromagnetic radiations for these

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waves because they consist of oscillating electric and magnetic fields directed perpendicularly to each other and to the direction of propagation of the wave. Quantum mechanics suggests that electromagnetic radiation has a dual character. It possesses some properties which are characteristic of waves and other properties which are characteristic of particles - like discrete packets of energy called photons/quanta. An electromagnetic radiation is characterized by several fundamental properties such as wave-length, frequency, wave-number, amplitude, velocity, energy, phase angle, polarization etc.

i. Wavelength (λ)

The distance b/w two successive crests or two successive troughs of a wave is called its wavelength. It is represented by lambda (λ) and is measured in terms of meter, centimeter, micrometer, nanometer and Angstrom ($\mathring{A}$) units.

$$1\mathring{A} = 1\ 10^{-10} m = 10^{-8} cm$$

$$1 \mu m = 10^{-6} m$$

$$1\ nm = 10^{-9} m$$

ii. Frequency (ν)

The frequency is defined as the number of waves or cycles which pass a given point in one second. It is represented by symbol ν called nu and is measured in units of per second (sec^{-1}) or Hertz (Hz). It has been found that frequency of a wave is inversely proportional to its wavelength.

$$\nu \propto \frac{1}{\lambda}$$

$$\nu = c/\lambda$$

C = constant called velocity of light = 3×10^{8} m/sec

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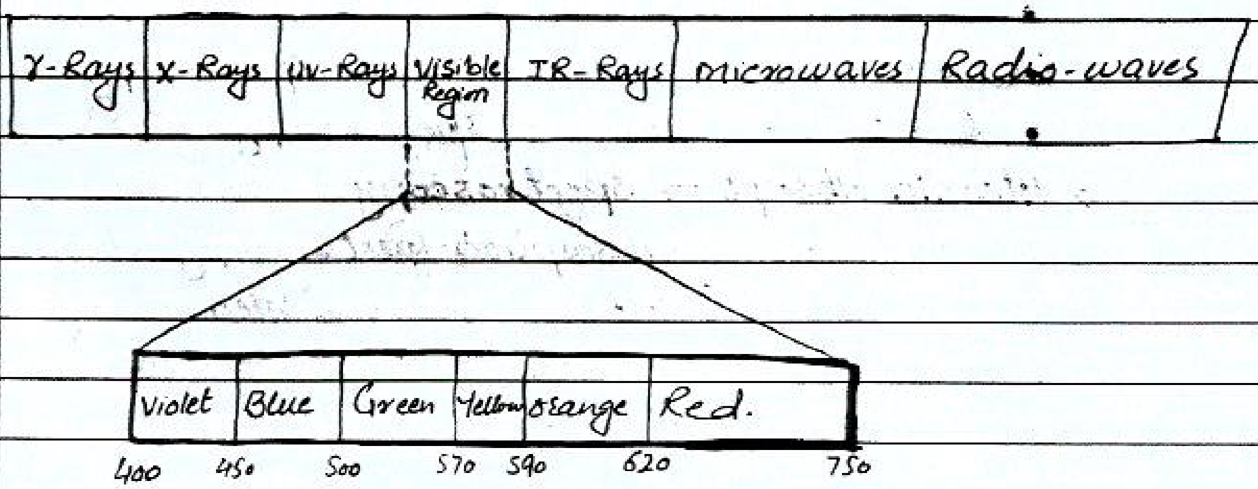
iii. Wave number ($\bar{\nu}$)

It is the number of waves per unit length. It is represented by $\bar{\nu}$ (nu bar). The unit most commonly used for wave number is per cm (cm^{-1}).

The arrangement of all types of electromagnetic radiation in order of their increasing wavelengths or decreasing frequencies is known as **electromagnetic spectrum**.

This electromagnetic spectrum comprises of various regions which include



The absorption of these radiations causes various types of transitions in the atoms or molecules present in the sample i.e

γ-Ray	Change Nuclear configurations
X-Rays	Eject core-level electrons
UV-Rays	Excitation of valence electrons in molecules
Visible Rays	" " " " in molecules.
IR-Rays	Molecular vibrations, Rotations, Bending of Bonds
Microwaves	Change spin of electrons in magnetic field.
Radiowaves	" " " nuclei " " "

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Classification of Spectroscopic Methods

The spectroscopic techniques are classified into various categories on different bases.

1. On the basis of species involved.

a) Atomic spectroscopy

⇒ Atomic Emission spectroscopy
- Flame atomic Emission spectroscopy (FAES)
- Inductively coupled Argon plasma AES (ICP-AES)
- Microwave induced plasma Atomic Emission Spectroscopy
- Glow discharg atomic emission spectroscopy.
- Electric Arc Atomic Emission Spectroscopy
- Laser Atomic Emission spectroscopy.

⇒ Atomic Absorption Spectroscopy
- Flame atomic absorption spectroscopy (FAAS)
- Electrothermal atomic absorption spectroscopy
- Glow discharge AAS.
- Hydride Generation AAS.
- cold vapour AAS. • Graphite Furnace AAS.

⇒ Atomic Florescence Spectroscopy

⇒ Atomic Mass Spectrometry
- Inductively coupled plasma mass spectrometry (ICPMS)
- Direct current plasma mass spectrometry (DCPMS)
- Microwave induced plasma MS (MIPMS)
- Spark source mass spectrometry (SSMS)
- Thermal ionization MS
- Glow discharge MS
- Laser Microprobe MS
- Secondary Ion MS

b) Molecular Spectroscopy

Friends

2. **On The Basis of Radiations used.**

- γ-Rays spectroscopy
- X-Rays spectroscopy
- UV-Visible spectroscopy
- IR-spectroscopy
- Microwave spectroscopy
- Radiowaves spectroscopy

3. **On the Basis of phenomenon occuring**

- Emission spectroscopy
- Absorption spectroscopy
- Florescence Spectroscopy
- phosphorescence spectroscopy
- Scattering Spectroscopy
- Electron Spin Resonance Spectroscopy

However spectroscopic methods are named according to the region of electromagnetic spectrum used and its interaction with the matter. Examples are listed below.

Type of Radiations	phenomenon	Spectroscopic Technique
• γ-Rays	Emission	γ-Rays emission spectroscopy
• γ-Rays	Absorption	Mossbauer Spectroscopy
• X-Rays	Absorption	X-Rays absorption "
	Emission	X-Rays emission "
	Florescence	X-Rays florescence "
	Diffracton	XRD
• UV-Visible	Absorption	• Molecular UV-Visible absorption "
		• Atomic absorption "
	Emission	• Molecular UV-Visible emission "
		• Atomic Emission "
	Florescence	• Molecular Florescence "
		• Phosphorescence "
		• Atomic florescence "

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	Refraction	• Refractometry
	Scattering	• Nephelometry
		• Turbidimetry
	Dispersion	• Optical Rotary dispersi
• IR Rays	Absorption	• IR- Absorption spectroscop
	Scattering	• Raman Spectroscopy
• Radiowaves	Absorption	• Electron Spin Rasonance Spect
		• NMR Spectroscopy
• Microwaves	Absorption	• Microwave Absorption "

Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectroscopy (AAS) can be discussed by following headings.

⇒ Introduction
⇒ Basic principle
⇒ Instrumentation
- A Radiation Source
- An atom cell (the atomizer)
- A monochromator
- A detector
- Signal processor and Readout device

⇒ Advantages of AAS
⇒ Disadvantages of AAS

Introduction :-

In analytical chemistry, atomic absorption spectroscopy is a technique used for the determination of concentration of almost all the metals and metalloids in a natural or artificial sample.
G. Kirchoff and Robert Bunsen first used atomic atomic absorption spectroscopy — alongwith atomic emission in 1859 & 1860 — as a means for identifying atoms in flames and hot gases. However, modern atomic spectroscopy has its beginnings in 1955 as a result of independent work of A.C Walsh and C.T.J a Alkemade.

Basic Principle :-

In AAS, the sample is introduced into the spectrometer as solution by means of a

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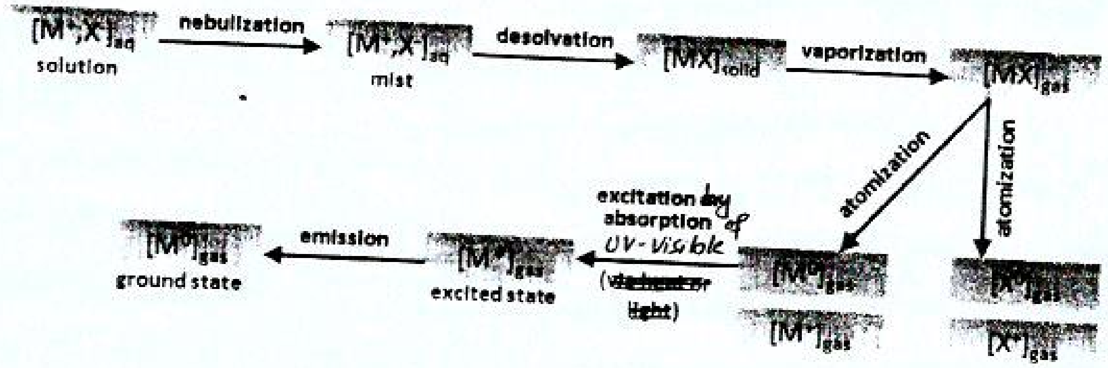
Nebulizer which introduces the solution to an atomizer that converts the analyte into free gaseous atoms. For Flame, Graphite furnace, Glow discharge etc is used as atomizer. Three steps are involved in turning a liquid sample into an atomic gas i.e
• Desolvation • vapourization & • Volatilization

The Atomization Process



These gaseous atoms in ground state absorb UV- or Visible radiations from the radiation source and undergo transitions to higher electronic energy levels. This absorption is directly related to the concentration of atoms as proposed in Beer-Lambert's law. The concentration is then determined by calibrating the instrument with standards of known concentration.

Instrumentation :-

An atomic absorption spectrometer comprises of following components.

i. **Radiation Source :-**

A sharp line source is required for excitation of gaseous atoms in ~~atomic~~ AAS. The two most

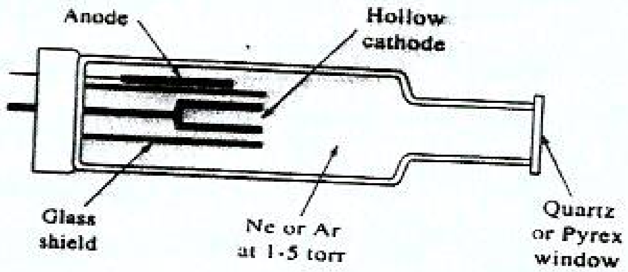
Common sources are

i. Hollow Cathode Lamp (HCL)

ii. Electrodeless discharge Lamp (EDL)

Hollow Cathode Lamp (HCL).

The Hollow Cathode Lamp is consists of a hollow cathode made up of element to be determined or an alloy of it and a tungsten anode. These are enclosed in a glass tube usually with a quartz window that allow the UV-radiations to pass through it. The tube is filled with an inert gas such as Ne or Ar at low pressure (1-5 torr).



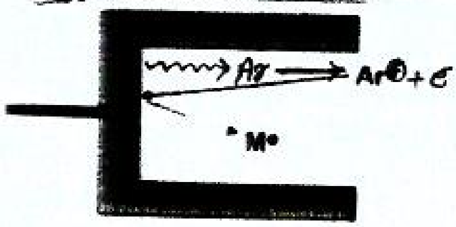
When a high potential is applied across the electrodes, the Ne/Ar atoms are ionized due to emission of cathode rays. These positive ions, bombard the cathode with high energy and sputter off the metal atoms. These metal atoms are excited to higher electronic levels by continuous collisions with high energy gas ions. When the electrons return to the ground state, the characteristic rasonance line is emitted alongwith some other lines. This characteristic rasonance line is absorbed by test element and its absorption is measured.

$\bar{e}$ (Cathode Ray from Cathode) + Ar $\longrightarrow Ar^{\oplus} + 2\bar{e}$

$Ar^{\oplus}$ + Cathode $\longrightarrow$ Ar + M (metal atom)

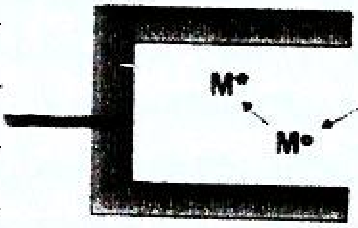
$M + Ar^{\oplus} \xrightarrow{\text{collision}} M^{*}$ (excited atoms of metal)

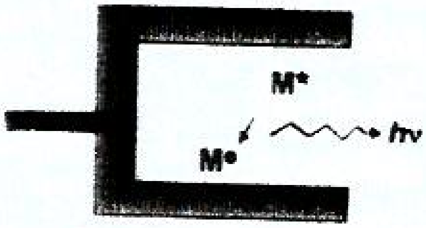
$M^{*} \longrightarrow M^{\circ} + h\nu$



Metal atoms are than sputtered off the surface of the cathode

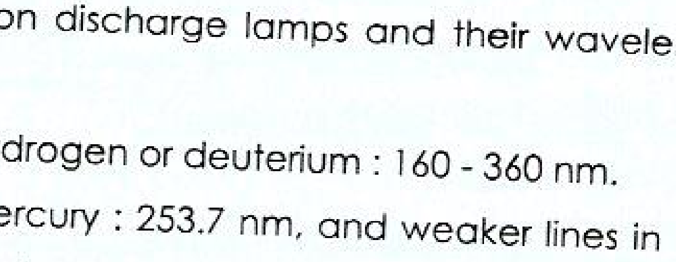
$M^* \dashrightarrow M^0 + h\nu$





Electrodeless Discharge Lamp (EDL).

A typical EDL is constructed from a sealed quartz tube containing a few torr of an inert gas (Ar, Ne) and a small quantity of the metal or its salt whose spectrum is of interest. The lamp contains no electrode but instead is energized by an intense field of radiofrequency or microwaves. Ionization of Argon occurs to give ions that are accelerated by high radiofrequency or microwaves untill they gain sufficient energy to excite the atoms of the metal whose spectrum sought.



- Common discharge lamps and their wavelength ranges are:
  - hydrogen or deuterium : 160 - 360 nm.
  - mercury : 253.7 nm, and weaker lines in the near-uv and visible.
  - Ne, Ar, Kr, Xe discharge lamps : many sharp lines throughout the near-uv to near-IR.
  - xenon arc : 300 - 1300 nm

ii. **The Atomizer :-**

The process of converting metal atoms in analyte sample & solution to free gaseous atoms is called **atomization**. There are various methods of atomization used in AAS. These include

- Flame atomization
- Electrothermal/Graphite furnace atomization
- Glow discharge atomization
- Hydride Generation atomization
- Cold vapour atomization.

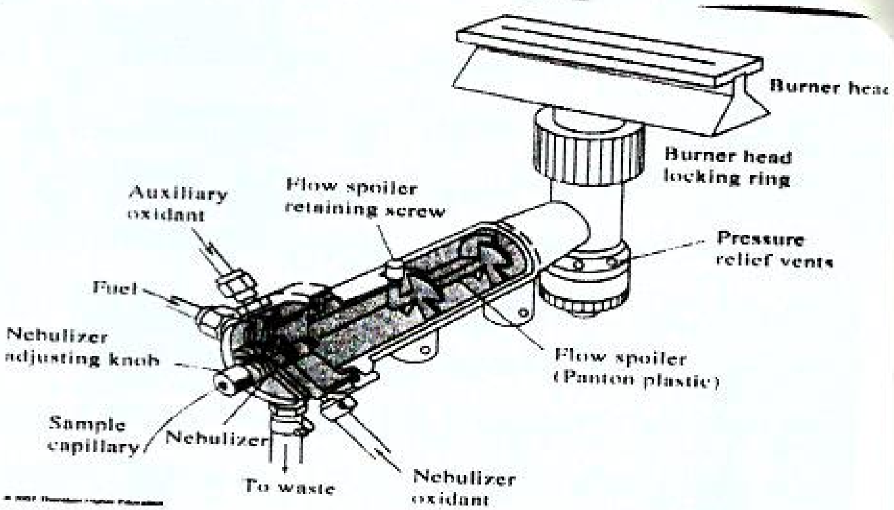
Flame Atomization :-

In a flame atomizer, a solution of the sample is nebulized by a flow of gaseous oxidant, mixed with a gaseous fuel and carried into a flame where atomization occurs. The following processes then occur in the flame.

⇒ Desolvation (produces a solid molecular aerosol)
⇒ Dissociation (leads to an atomic gas)
⇒ Ionization (to give cations and electrons)
⇒ Excitation (giving atomic, ionic and molecular emissions).

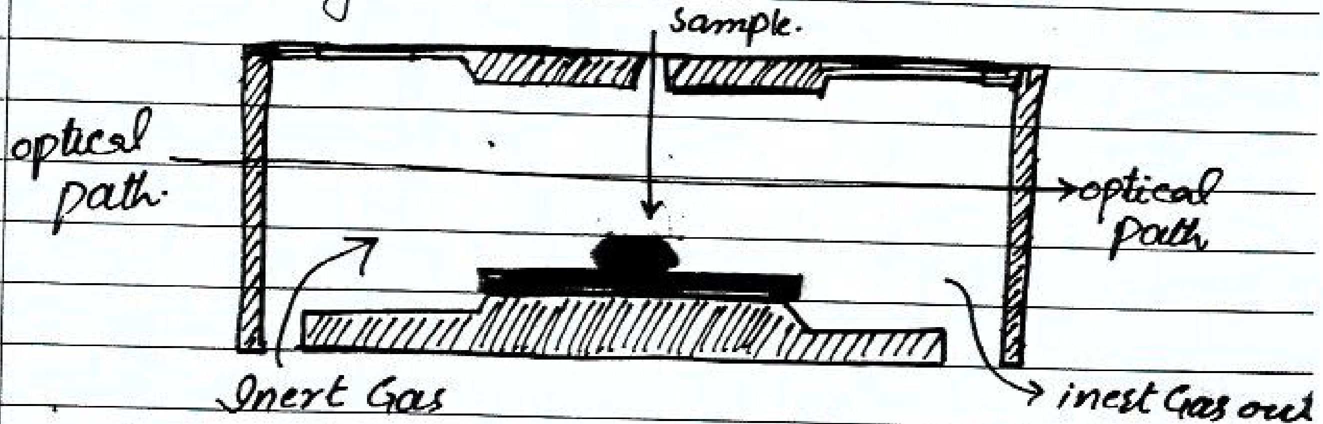
several common fuels and oxidants can be employed in flame spectroscopy depending on temperature needed. Temperatures of 1700°C to 2400°C are obtained with various fuels when air serves as the oxidant. At these temperatures, only easily decomposed samples are atomized. For more refractory samples, O_2 gas or Nitrous oxide must be employed as the oxidant. With common fuels, these oxidants produce temperatures of 2500°C – 3100°C.

Fuel	Oxidant	Temperature, °C
Natural gas	Air	1700–1900
Natural gas	Oxygen	2700–2800
Hydrogen	Air	2000–2100
Hydrogen	Oxygen	2550–2700
Acetylene	Air	2100–2400
Acetylene	Oxygen	3050–3150
Acetylene	Nitrous oxide	2600–2800



Electrothermal / Graphite Furnace Atomization.

A typical electrothermal atomizer, also known as Graphite Furnace consists of a cylindrical graphite tube approximately 1-3cm in length and 3-8 mm in diameter. There is a central hole in the tube for introduction of sample by means of a micropippette. The graphite tube is continuously flushed with an inert gas (Ar) to prevent the formation of non-absorbing metal oxides.



Samples of between 5-50 μL are injected into the graphite tube through a small hole at the top of tube. Atomization is achieved in three stages. In the first stage, the sample is dried to a solid residue using a current that raises the temperature of tube to about 110°C. In the **Frien** second stage which is called ashing, the temperature increased to between 350-1200°c. In the final stage the sample is atomized by rapidly increasing the temperature to b/w 2000 - 3000°c.

iii. **The Monochromator :-**

A monochromator is used in an AA spectrometer to select the specific wavelength of light which is absorbed by the sample and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of other.

iv. **The Detector :-**

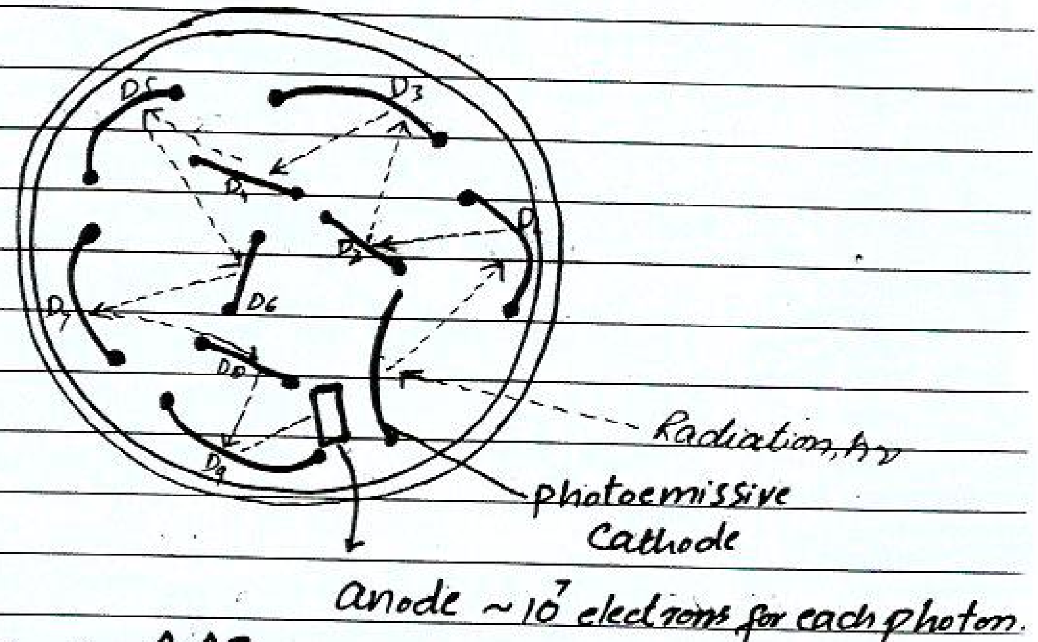
The role of a detector is to convert a light signal into an electrical signal. Since in AAS, UV-visible radiations are involved, therefore following detectors could by employed.

- photomultiplier tube
- phototube
- photocell
- silicon diode
- charge transfer detector.

However all modern AA spectrometers use photo-multiplier tube (PMT) as the detector.

Photomultiplier Tube (PMT):

The PMT contains a photoemissive cathode and a series of 9 additional electrodes called dynodes. ~~The~~ when photon emitted from sample strikes the cathode, electron(s) are ejected. The number of electrons emitted from the cathode is directly proportion to the intensity of light beam. Electrons emitted from Cathode are accelerated to the first dynode (D1) by a 90V potential where the electron impact dislodges several additional electrons which are accelerated to the next dynode by an additional 90V potential. After nine dynodes, the number of electrons finally reaching the anode is in the order of ten million for each incident photon. The current measured at the anode collector is still proportional to the intensity of the light but it has been amplified over a million times.



Advantages of A.A.S

- Widespread applications to metal for quantification in trace amounts in diverse samples.
- High Sensitivity
- No ~~interferences~~. or poor interferences.

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Disadvantages of A.A.S.

- Only one element at a time is measured.
- Used for analysis of metals only.
- cannot be used for solid or gas (must dissolve first.)

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