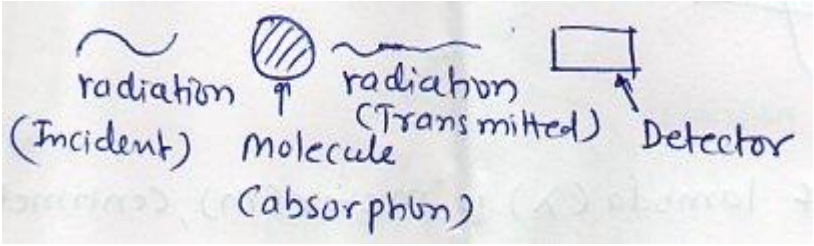
Module No.5 Modern method of analysis

Spectroscopy is basically consequence / effect / outcome of interaction of radiation and matter.

Spectro = Radiations

Scopy = Measurement

i. e. measurement of radiation means spectroscopy

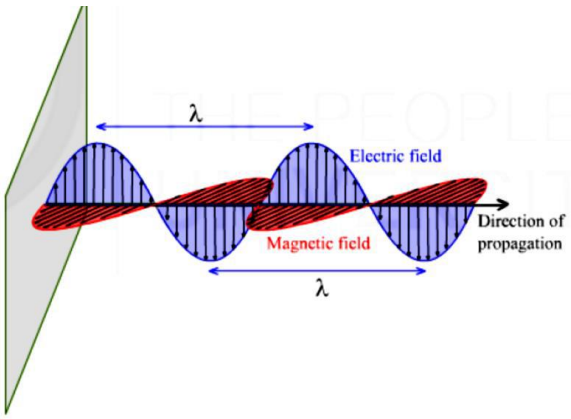


Interaction of radiation (Incident) occurs with molecules some of the radiation absorbed by molecules, some other will be transmitted. This transmitted radiation will be detect by Detector present in Spectro photometer.

Thus spectroscopy is branch of science that deals with interaction of EMR with matter.

What is EMR?

An electromagnetic radiation may be defined as the radiant energy which is transmitted through space at enormous velocities.

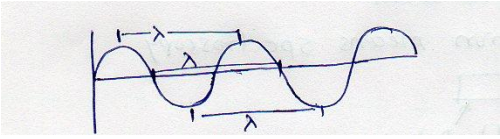


1. It consist of Oscillatory electric and magnetic field travelling in the plane perpendicular to each other and also to direction of propagation.
2. It has dual nature (wave nature & particle nature i.e photon)
3. It has velocity 3 X 10^8 ms $^{-1}$ in vacuum.
4. It does not requires medium for transmission.

Characteristic of electric magnetic Radiation (EMR)

Considering either electric or magnetic field. EM radiations are characterised by following parameter

1)Wavelength : (λ) : Distance between two consecutive crest/trough



2) Frequency : **nu (ϑ)** : Number of cycles/waves passing through stationary point in one second.

Unit is Hz or second invert. It is represented by greek letter nu (ϑ)

Frequency & wavelength relater as

$\vartheta = \frac{c}{\lambda}$ c = speed of light 3×10^{8} ms $^{-1}$ = constant

$\therefore \vartheta \propto \frac{l}{\lambda}$

3) Wave number (ῡ) nu bar : It is number of waves per centimetre/ unit distance.

Unit is cm $^{-1}$ or m $^{-1}$

$\vartheta = \frac{c}{\lambda}$

$\vartheta \propto \frac{l}{\lambda}$

$\therefore \vartheta = c\,\bar{\upsilon}$

$\therefore \vartheta \propto \bar{\upsilon}$

But $\vartheta \propto \frac{1}{\lambda}$

$\vartheta \propto \bar{\upsilon} \propto 1/\lambda$ i.e. wavelength increases but $\vartheta \propto \bar{\upsilon}$ decreases

4) Amplitude (A) : It is a measure of the radiant power & the intensity of the radiation.

Radiant power refers to the energy of the radiant striking at the given area per unit time. Denoted by P & it is square of Amplitude.

$\therefore$ **4)Amplitude(A): maximum height to which wave oscillate.**

5) Velocity (V): It is linear distance travelled by the wave in one second.

6) Energy: (E) Energy of electromagnetic radiation depends. On its wavelength and frequency

E = h ϑ $E = h\frac{c}{\lambda}$ or E = h c $\bar{\upsilon}$ or $E \propto \vartheta \propto \bar{\upsilon} \propto \frac{l}{\lambda}$ $\bar{\upsilon}$ h έ ε ά λ

Where h = planks constant = 6.626×10^{-34} Js

C = velocity of light = 3×10^{-8} m s $^{-1}$

Numerical:

1) A laser emits light of frequency 4.74×10^{14} sec $^{-1}$. what is the wavelength of the light in nm ?

$\lambda = \frac{c}{\vartheta}$ =(2.998 X 10^{8} m/s) X (1 s/ 4.74 X 10^{14}) X (1nm/10^{-9}m) = 6.32×10^{2} nm.

2) A certain electromagnetic wave has a wavelength of 625 nm then

a) What is the frequency of the wave

$\vartheta = \frac{c}{\lambda} = \frac{2.998\times 10^{8}\ \text{m/s}}{6.25\times 10^{-7} m} = 4.8 \times 10^{14}$ s Since 625 nm =$625 \times \frac{10^{9} m}{1\ nm}$ i.e= 6.25×10^{-7} m

b) What is the energy of the wave ?

E = h $\vartheta = 6.626 \times 10^{-34}$ JS $\times 4.8 \times 10^{14}\ s^{-1} = 3.18 \times 10^{-19}$ J

3) The blue color of the sky results from the scattering & sun light by air molecules. The blue light has a frequency of about 7.5×10^{14} H_Z. Then

a) calculate the wavelength, in nm associated with this radiation.

1 $H_Z = 1S^{-1}$

$\lambda = \frac{c}{\vartheta} = \frac{2.998\times 10^{-8}\ \text{m}}{7.5\times 10^{14} s^{-1}} \times \frac{1\ nm}{10^{-9}\ m} = 4.0 \times 10^{2}\ nm$

b) calculate the energy, in joules, of a single photon associated with this frequency.

$E = h\vartheta = 6.626 \times 10^{-34}$ JS $\times 7.5 \times 10^{14}$ S^{-1} = 5.0 X 10^{-19} J

4) Calculate the wave number for 2.5 μm wavelength ?

λ = 2.5 μm = 2.5 X 10^{-4} cm

$\tilde{\upsilon} = \frac{1}{\lambda} = \frac{1}{2.5\times 10-4\ cm}$

$= \frac{10}{25} \times 10^4 = 4 \times 10^3 = 4000\ cm^{-1}$

5) Calculate the frequency for 3800 A^0 wavelength.

$\vartheta = \frac{c}{\lambda} = \frac{3\times10^{10}\ \text{cm sec}^{-1}}{3800\times 10-8\ cm} = \frac{3\times10^{10}\times10^8}{3800}\ \text{sec}^{-1} = \frac{3\times10^{18}}{3800}\ \text{sec}^{-1} = \frac{3\times10^{14}}{0.38} = 7.8 \times 10^{14}\ \text{sec}^{-1}$ i. e H_Z.

6) Calculate the energy of 3800 A^0 wavelength.

$E = h\vartheta = 6.627 \times 10^{-27}\ Erg/Sec \times \left(\frac{c}{\lambda} = \frac{3\times10^8\ \text{m sec}^{-1}}{3800\times 10^{-8}\ cm/sec}\right)$

$= \frac{19.878\times10^{-17}}{3.8\times 10-8} = 5.23 \times 10^{-9}$ Erg

7) what is Δ E in joules for an atom that releases a photon with a wavelength of 3.2×10^{-7} meters ?

$\Delta E_{atom} = E_{photon} = h\vartheta = \frac{hc}{\lambda}$

$\therefore\ \Delta E = \frac{6.626\times10^{-34}\ \text{JS}\times2.998\times10^8\text{m/s}}{3.2\times10^{-7}m} = 6.2 \times 10\text{-}^{19}$ J

8) If an electron has a velocity of 5.0 $\times 10^5$ m/s, what is its wavelength in m ?

Given mass of electron = 9.109$\times 10^{-28}$ gm, h = 6.626 $\times 10^{-34}$ JS

lJ = 1 kg. m^2 / s^2, $\lambda = \frac{h}{mu}$

$\therefore$ h = 6.626 $\times 10^{-34}$ JS $\times \frac{1\ kg.\text{m2}}{s2}$ = 6.626 $\times 10^{-34}$ Kg m^2/s

m= 9.109 $\times 10^{-28}$ g X 1Kg/10^3= 9.109 $\times 10^{-31}$ kg

$\therefore \lambda = \frac{6.626\times10^{-34}\ \text{kg.m2 / s}}{9.109\times10-31\ \text{kg}\times5.0\times10^5\ m/s}$

= 1.5 $\times 10^{-9}$ m

9) The laser used to read information from a compact disk has a wavelength of 780 nm. What is the energy associated with one photon of this radiation ?

E photon = $\frac{hc}{\lambda} = \frac{6.626\times10^{-34}\ \text{JS}\times2.998\times10^8\text{m/s}}{780\times10^{-9}\ nm} = 2.55\ X10^{-19}$J

10) Radiation corresponding to green light has wavelength of 535 nm. Calculate energy of photon of green light.

$\lambda = 535 \times 10^{-9}$ m E = h ϑ = h $\frac{c}{\lambda}$ = 6.626 $\times 10^{-34}$ JS $\times$ 3 X10^8m/ 535 X10^{-9}m = 3.72 $\times 10^{-19}$ J

1 cm = 0.0l m — l m = 100 cm

1 mm = 0.00 l m = 0.1 cm — l m = 1000 mm

1 πm = 0.00000 l m = 0.001 cm — l cm = 10 mm

1 nm = 0.00 l μ m — l m = 1000 mm — l mm = 100 πm

I μm = 1000 nm

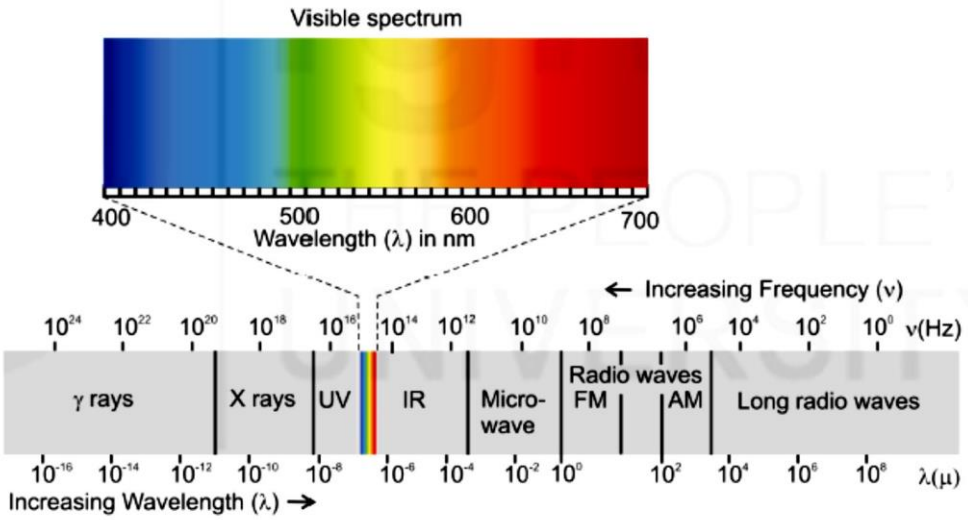
11) A microwave of radiation has frequency 1.2 GH H_Z calculate it's energy

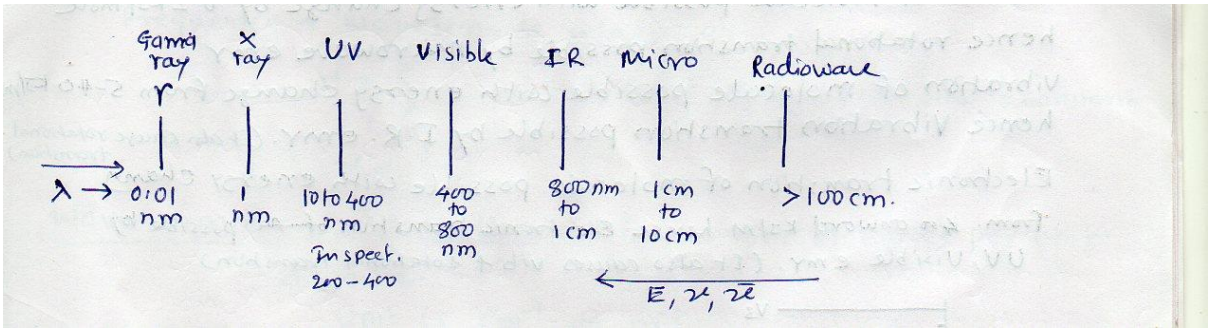
E = h ϑ

E= 6.626×10^{-34} JS $\times$ 1.2X 10^{10} s^{-1}

= 7.95×10^{-24} J

EM spectrum: Arrangement of EM radiation in increasing order of wavelength or decrease order of frequency is called EM spectrum.





1. **Gama ray** with small wavelength and high frequency and energy cause **nuclear interaction**.
2. **X-ray** cause **Inner electron transition**
3. **UV** cause **Outer electronic transition**
4. **Visible** cause **Outer electronic transition**
5. **IR cause molecular Vibration.**
6. **Microwaves causes molecular rotation**.

UV-VISIBLE SPECTROMETRY

Principle: It involves interaction of UV (190 to400nm) and visible (400 to 800nm) radiation with sample under test/characterisation. From intensity of transmitted radiation the qualitative and quantitative analysis is possible.

THEORY OF SPECTROPHOTOMETRY AND COLORIMETRY

When light (monochromatic or heterogeneous) falls upon a homogeneous medium, a portion of the incident light is **reflected**, a portion is **absorbed** within the medium, and the remainder is **transmitted**. If the intensity of the incident light is expressed by Io, that of the absorbed light by Ia that of the transmitted light by It, and that of the reflected light by Ir, then:

$$Io = Ir + Ia + It$$

For air-glass interfaces arising from the use of glass cells, it may be stated that about 4 per cent of the incident light is reflected. l, is usually eliminated by the use of a control, such as a comparison cell, hence:

$$Io = Ia + It$$

Lambert's Law: This law States that when monochromatic light passes through a homogeneous absorbing medium, the rate of decrease in intensity with the thickness of the medium is proportional to the intensity of the light. i.e decrease in intensity of incident light is directly proportional to intensity of incident light and thickness of absorbing medium.

Beer's law: This law States that when monochromatic light passes through a homogeneous absorbing medium having uniform thickness, the rate of decrease in intensity of the medium is proportional to the intensity of the light and concentration of absorbing medium.

Beer's – Lambert's law: This law States that when monochromatic light passes through a homogeneous absorbing medium having uniform thickness the rate of decrease of intensity of light with thickness of the absorbing solution is proportional to the intensity of incident light as well as to the concentration of the solution."

The ratio It/Io is the fraction of the incident light transmitted by a thickness l of the medium and is termed the transmittance T.

Transmittance (T) = It/Io

Its reciprocal Io/It is the opacity

Opacity = 1/T = Io/ It

Absorbance A of the medium is logarithmic ratio of intensity of incident to transmitted light

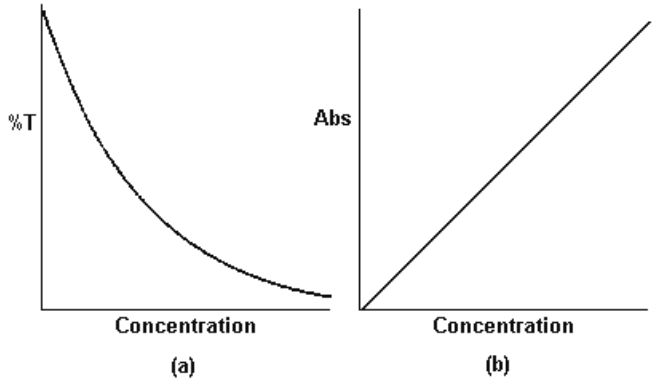
Absorbance (A) = log Io/It

Absorption coefficient: The absorption coefficient (or extinction coefficient) is the absorbance for unit path length

Specific absorption coefficient: The specific absorption coefficient is the absorbance per unit path length and unit concentration

Molar absorption coefficient: The molar absorption coefficient is the specific absorption coefficient for a concentration of 1 mol / L and a path length of 1 cm.

By plotting ***A*** [or log(l/T)] as ordinate, against concentration as abscissa, a straight line will be obtained and this will pass through the point c = O, ***A*** = O (T = 100 per cent). This calibration line may then be used to determine unknown concentrations of solutions of the same material after measurement of absorbances

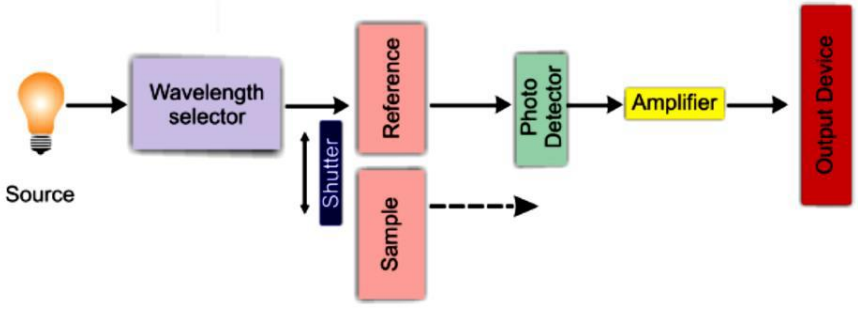


TYPES OF UV-VISIBLE SPECTROMETERS

Single Beam Spectrometers

As the name suggests, these instruments contain a single beam of light. The same beam is used for reading the absorption of the sample as well as the reference.

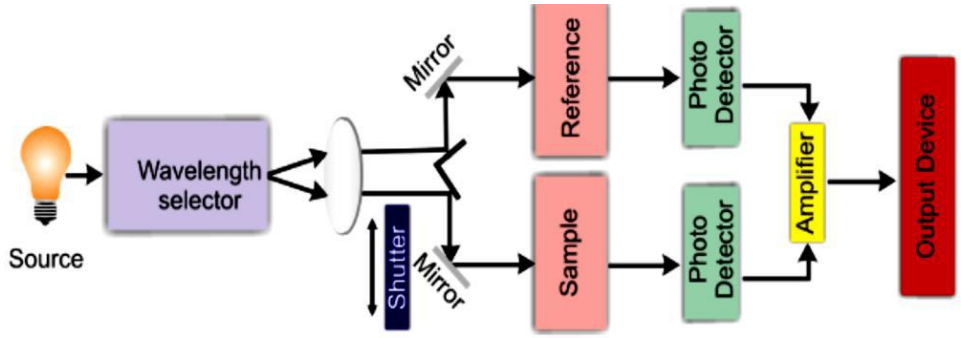
The schematic diagram of a typical single beam UV- Visible spectrometer is given in



The radiation from the source is passed through a filter or a suitable monochromator to get a band or a monochromatic radiation. It is then passed through the sample (or the reference) and the transmitted radiation is detected by the photo detector. The signal so obtained is sent as a read out or is recorded.

Double Beam Spectrometers

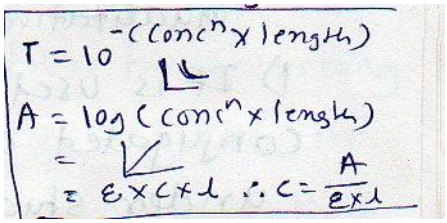
In a double beam spectrometer, the radiation coming from the monochromator is split into two beams with the help of a beam splitter. These are passed simultaneously through the reference and the sample cell. The transmitted radiations are detected by the detectors and the difference in the signal at all the wavelengths is suitably amplified and sent for the output. The general arrangement of a double beam spectrometer is shown in fig



Components

1. Radiation source a) H_2-D_2 lamp convers 200 to 375 nm, after that intensity of light decrease
   b) Tungsten filament lamp : 375 nm to 800 nm in UV region. (Complete range is 350 to 7500 nm) there are continuum source H_2 ionizes gives radiation $H_2 \longrightarrow H + H$, Tungsten works on thermal heat.
2. Monochromator : Radiation source is polychromatic light & it is converted in to monochromic light. (filter gives wide range of absorbance) component of monochromator

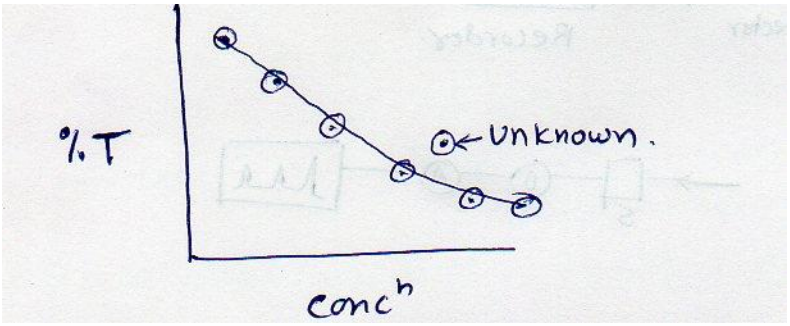
a) Entrance slit : slit can pass only specified wave length
b) Collimator (concave) lenses : it reflect light in specific direction.
c) Grating / Prism : Converts source of light in to monochromatize light
d) Reflecting lenses : Reflect light towards Exit slit
e) Exit Slit : If passes specific wavelength for analysis.
3) Chopper : S; It specific wavelength in to two equal wavelength travel at different direction.
4) Cuvette Cell : Quartz (for UV-visible) Glass (Visible)
5) Detector : Photomultiplier tube : Output device



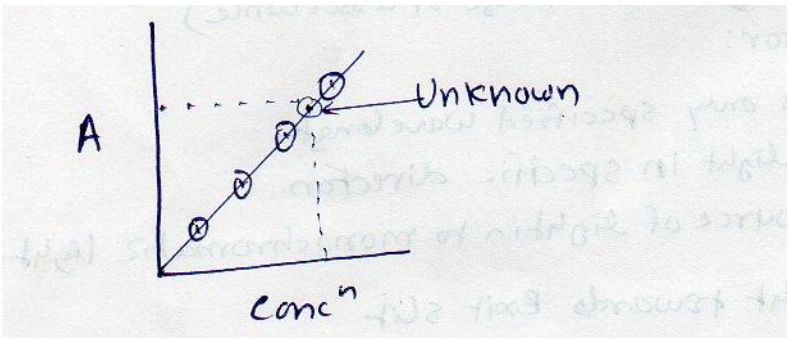
Working :- The light from the source is condensed by mirror in a such a way that beam fall on the prism / grating. Dispersed beam again reflected & allow passing through sample.

In single beam that light / radiation allow to pass through reference sample placed in curette cell position respectively.

In double beam reference & sample are placed on their position while chopper split light / radiation equally & passes through both simultaneously. Detector detects transmitted light i. e. Transmittance $T = \frac{It}{Io}$ or $10^{-(\text{concn X length})}$ for both reference & sample, Difference is amplified when graph of % T Vs Conc ^{n} plotted them curve line obtained where Transmittance decrease with increase Conc ^{n}



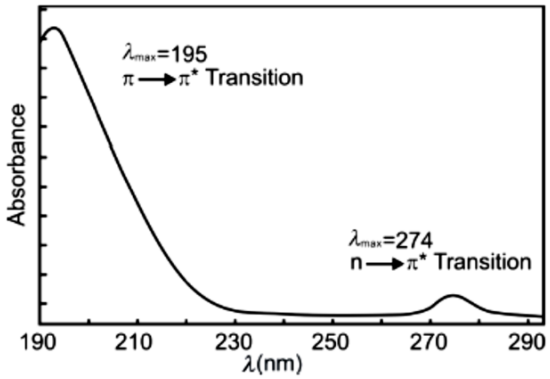
When unknown Conc ^{n} & their % not lying on that curve line it is not possible to detect Conc ^{n} of unknown analysts to overcome this difficulty log ratio of $10^{-(\text{concn X length})}$ i. e. Absorbance A = - log (Io / It) = $\frac{I}{T}$ = ∈ Ct is Calculated which is straight line passing through origin.



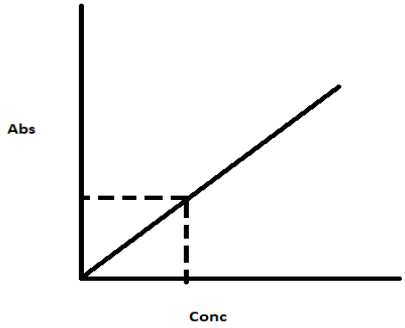
Calibration:

1) **Standard solution method:**

The standard solution of analyte under detection are prepared with regular increment in concentration are prepared with regular increment in concertation i.e. 50, 100, 150, 200 microgramin 50 cc or 100cc solution. The absorbance of each solution is recorded at λmax (most absorbed radiation by a sample) & calibration curve is proposed which is essential with electronic transition.



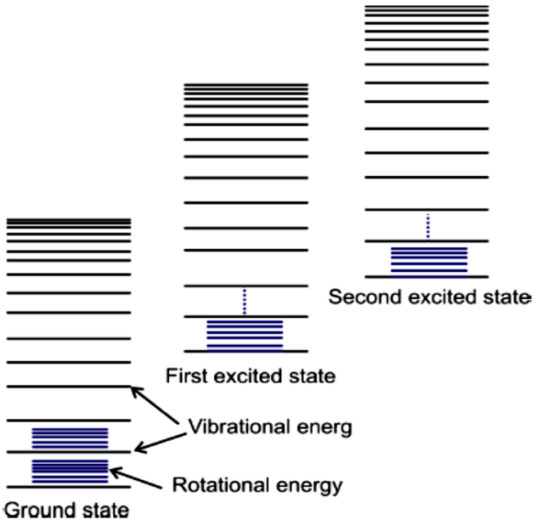
When graph is plotted between Absorbance (Y-axis) Vs Concentration for standard solution, it is straight line passing through origin & the absorbance of dilute analyte solution is recorded at λmax from calibration curve. Concentration of analyte can be indirectly determine graphically as shown in fig.



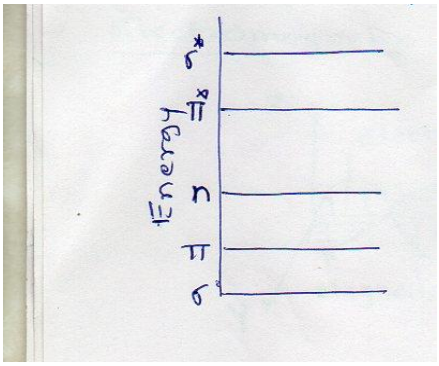
Qualitative analysis:

1.Organic molecule: The absorptions of UV-VIS radiation will depends on the electronic structure of the molecule/ absorbing species.

When energy in the form of photon(lambda) interact with molecule then it causes electronic transition from lower to higher energy level provided that if energy of photon will coincide with energy difference between two electronic state of molecules.



In organic molecules the constituent atoms are bonded through **sigma** and **Pi** bonds. In addition, these have nonbonding electrons(loan pair of electrons) on the atoms like, N,O,S and halogens etc. There are a number of transitions possible involving the bonding and the nonbonding electrons.



Sigma to Sigma*, Sigma to Pi*, Pi to Pi*, Pi to Sigma*, n to Pi^, n to Sigma*

The order of transition is as follows with declare in energy for transition

$\sigma \longrightarrow \sigma^* > n \longrightarrow \sigma^* > \pi \longrightarrow \pi^* > n \longrightarrow \pi^*$

Sigma to Sigma* C-C & C-H

n to Sigma* H_2O, CH_3OH,CH_3Cl

Pi to Pi* C=C, C=O

n to Pi* C=N, N=N

it is useful to predicts nature of Pi electron conjugate, unsaturation, aromatic nature etc.

the abrupt absorption by certain bonds/ fictional groups at particular lamda in UV-Visible range is called Chromophore.

-C=C- i.e C6H13CH=CH2 @ 177nm

-C+=C- i.e C5H11C=C-CH3 @178nm

>C=O, i.e. CH3CHO @180nm

Chromophore	Example	λ_{max}	Type of Transition
>C = C<	C_6H_{13} CH = CH_2	177	$\pi \rightarrow \pi^*$
–C ≡ C–	C_5H_{11} C ≡ C – CH_3	178	$\pi \rightarrow \pi^*$
>C = O	CH_3CO CH_3	186 280	$n \rightarrow \sigma^*$ $n \rightarrow \pi^*$
	CH_3CHO	180 293	$n \rightarrow \sigma^*$ $n \rightarrow \pi^*$
–COOH	CH_3COOH	204	$n \rightarrow \pi^*$
$–CONH_2$	CH_3CONH_2	214	$n \rightarrow \pi^*$
–N = N–	$CH_3N = NCH_3$	339	$n \rightarrow \pi^*$
$–NO_2$	CH_3NO_2	280	$n \rightarrow \pi^*$
–N = O	C_4H_9 NO	300 665	- $n \rightarrow \pi^*$

2. Charge transfer complex: When the two species bind to each other (one is electron donor and another is electron acceptor since these are colouless and not showing absorbance in UV-Visible region), the resulting species is intensely coloured. This is due to the formation of a complex between the two species. Such a complex is called **charge transfer complex**. For example, the blood red color of the complex ion, thiocyanatoiron (III) ion, Fe (SCN) 2+ is due to the formation of a charge transfer complex.

3. Inorganic species: Large number of inorganic salts containing atoms with electrons in *d*-orbitals give weak absorption bands in the visible range. Complex formation of these ions causes electronic transitions from the lower energy *d* orbitals to higher energy *d* orbitals (provide colour). These transitions are called ***d-d* transitions**.

Applications –

1. Analytical determination of metals and non-metals
2. Analytical determination of organic compounds
3. Determination of dissociation constants of organic acids and dyes
4. Determination of metal-ligand formation constants
5. Determination of kinetic stability of complexes
6. Analytical determination of environmental samples, water, waste water
7. Fluoride and iron content in water

Atomic Absorption Spectroscopy (AAS)

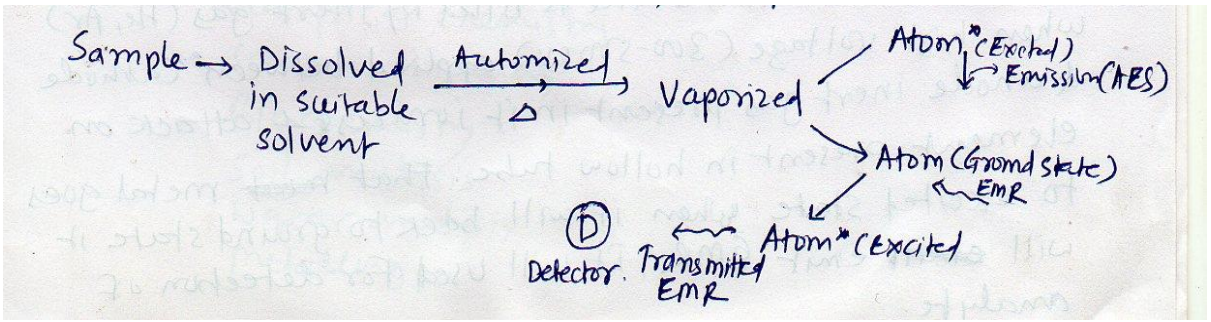
This method is variable & widely used because using this method approximate 60 to 70 element from trace to higher concentration can be detected.

This method is applicable to determine single element in presence of other elements because wavelength of particular metal is fixed / specified e. g. Na – 589 nm along with K, Ca, Ba, etc when we choose radiation source 589 nm, then Na shows absorption in 589 nm.

Mechanism / what happen in AAS .

Take sample, dissolved in solvent then heating will be there in atomizer (Atomizer evaporate sample & concerts sample into vapour state atom) at that stage some atom will be excited state & some atom will be in the grounds state, when we analysis excited by atom when they jump from excited state to ground state called Atomic emission Spectroscopy.

When ground state atom analyses called AAS where radiation source will be supply to ground state atom. The grounds state atom undergoes excitation by absorbing supplied EMR. Due to excitation there is decrease in intensity of supplied EMR, Thus measuring supplied EMR in presence of sample atom & in absence of sample atom is the basic principle of AAS



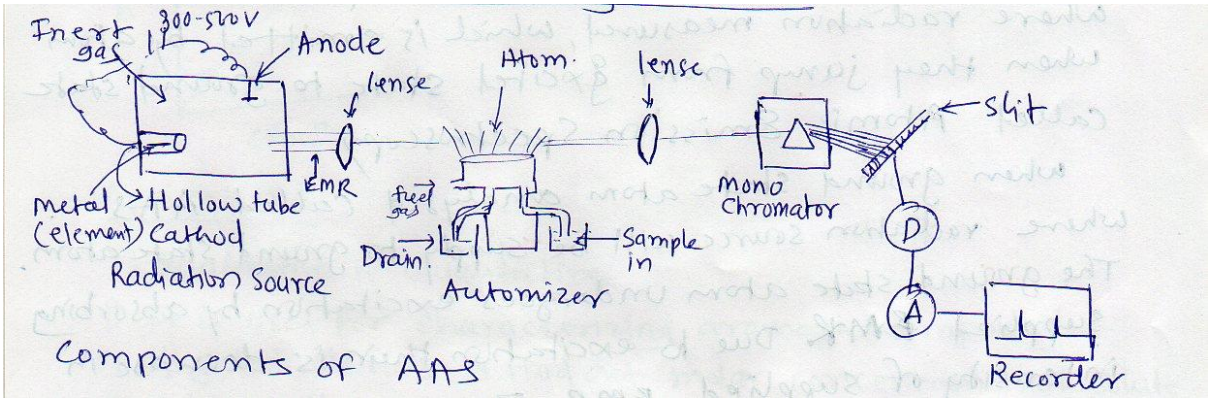
Requirements: 1. Sample must be in the form of atom. 2. Line source for an element to be analysed, which is characteristics radiation for concerned element.

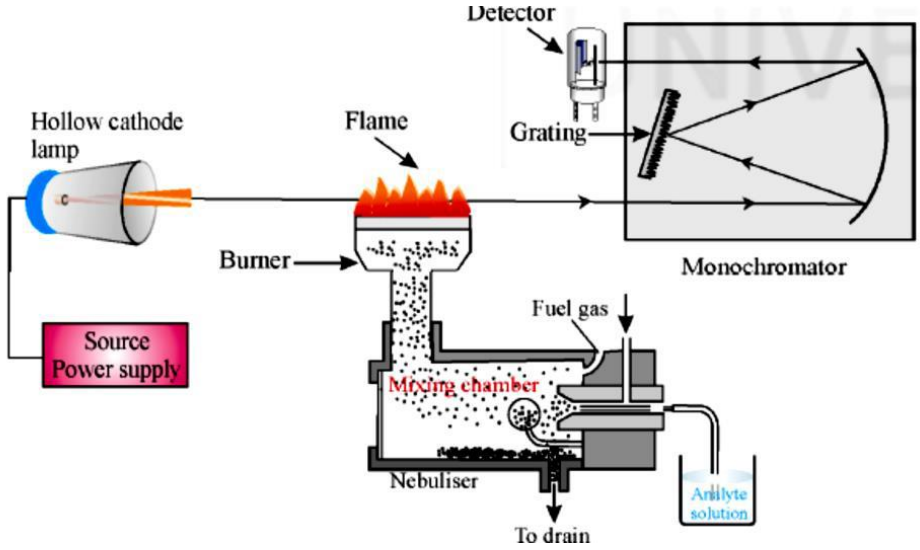
Principle : when a beam of EMR of a particular wavelength is passed through the vaporized (ground state) atoms present in the flame, then atom absorbs the radiation and decrease in the intensity of the radiation will be directly proportional to the atoms present in the ground state

Selected examples

Sr. No.	Element	wavelength	limit of detection
1	Au	243	0.009 µg / ml
2	Hg	254	0.160
3	Cu	325	0.002
4	Ag	328	0.002
5	U	358	0.002
6	Ca	423	0.02
7	Na	589	0.0002
8	K	767	0.0002

Instrumentation & workings of AAS





Components of AAS

1) Radiation Source : It consist of hollow cathode tube in which metal under test is kept. Anode is made up of w (tungsten) Radiation source is filled by inert gas (He, Ar) when high voltage (300-500 ev) applied between cathode & anode inert gas present in it ionizes & attract on element present in hollow tube. That metal goes to excited state. When it will back to ground state it will emit EMR. It will used for detection of analytic.

2) Lance : Focusing EMR towards the flame

3) Automizer :- It converts Liquid sample in presence of heat of flame (Acetylene only gas which provide temper of flame up to 3000^0 K) Un vaporized liquid will be collected at other end in Automizer

4) Monochromator :- Prism acts as monochromator

5) Slit : - Buy using slit select specified wavelength.

6) Detector :- Photomultiplier tube used as Detector

Working :- Metal under analysis is kept at hallow cathode tube. Radiation source is filled with inert gas. Sample is kept at automizer. Automizer starts with acetylene oxygen flame. Electric supply will be provided to anode and cathode of radiation source. Radiation source will emits particular wavelength that will be focus on atoms of analyte present in flame. These atom absorb this radiation according to Conc ^{n} & reaming radiation along with radiation coming from flame is focused on prism. The specific / particular radiation photon are selected by sliding slit & allow to pass through Detector containing photomultipliers.

Advantages of AAS:

1. Simple method for quantitative analysis of element (i.e. elemental composition of various material e.g. cement, all types of alloys, multimineral tablets, thin film etc)
2. Detection level is up to ppb i.e suitable to trace level analysis.
3. Moderate cost of analysis.
4. Equipment is easy to use.

Disadvantages of AAS: 1. Only one element is determine at a time 2. Separate line source is essential for each element 3. Nebulizer and burner assembly is required to be cleaned after each analysis 4. Flammable gas is required.

Used in Analytical chemistry: Trace and ultra trace analysis of complex matrices of geological, biological, environmental, industrial, glass, cement, marine sediment, pharmaceutical, engine oil or any other kind of samples. (Ceramic industry ,Bio Chemistry, Soil analysis, Water analysis, Metallurgy)

CHRIMATOGRAPHY

Introduction: The term Chromatography (In Greek : chromates meaning colors and graphos meaning writing i. e. color writing) and its principles were first discovered by Mikhail Tweet in the year 1906. He passed the plant extract over a column of calcium carbonate and washed it with petroleum ether, when the plant pigments were separated into a number of horizontal colored bands. Since the separated pigments were colored, the method was called chromatography meaning color writing. Though the term is misnomer today, it has been retained.

Definition: Chromatography is defined as the separation of components of the mixture by a continues distribution of the components between the stationary and the mobile phases, where component of mixture separated from one another either due to difference in adsorption coefficient or due to difference in partition coefficient.

Thus Chromatography is a method for separating the components of a mixture by differential adsorption between a stationary phase and a mobile (moving) phase
There are a number of chromatography techniques including:
1.Paper Chromatography 2.Thin Layer Chromatography (TLC) 3.Gel Permeation Chromatography 4.Ion Exchange Chromatography 5.High Performance Liquid Chromatography 6.Gas Chromatography (GC).

Types of chromatography:

Molecular Characteristic	Physical property	Separation Technique
Polarity	Volatility	Gas-liquid chromatography
	Solubility	Liquid-liquid chromatography
	Adsorptivity	Liquid-solid chromatography
Ionic	Charge	Ion-exchange chromatography
		Electrophoresis
Size (mass)	Diffusion	Gel permeation chromatography
		Dialysis
Shape	Sedimentation	Ultracentrifugation
	Liquid binding	Affinity chromatography

Gas-Liquid Chromatography: (GLC)

Gas chromatography is one of the most versatile for analysis of complex mixture of substances.

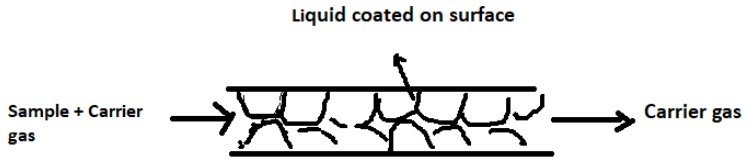
In gas chromatography, the different components of a mixture migrate at different speeds while carried along by an inert gas through the columns.

The mobile phase (i.e. inert gas like N_2,He,Ne, Ar,CO_2 is used in pure &dry form) which carries the sample molecules over the stationary phase is called a carrier gas.

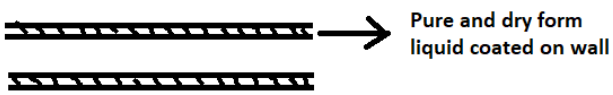
The stationary phase is a non-volatile liquid film spread on the surface of a solid support. The stationary phase is usually accommodated in a column which is a long narrow tube and is referred as packed column. Sometime the stationary liquid phase is supported by inner wall of a single tube of small diameter (capillary). These capillaries are referred as open tubular columns.

There are different types of columns: 1. Packed column 2. Wall coated column 3. Fused silica coated column.

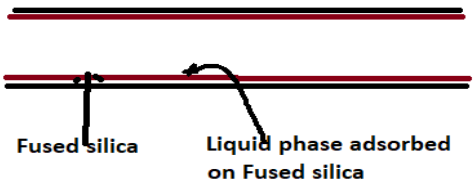
1. Packed column: Inert solid material (Diatomaceous earth)



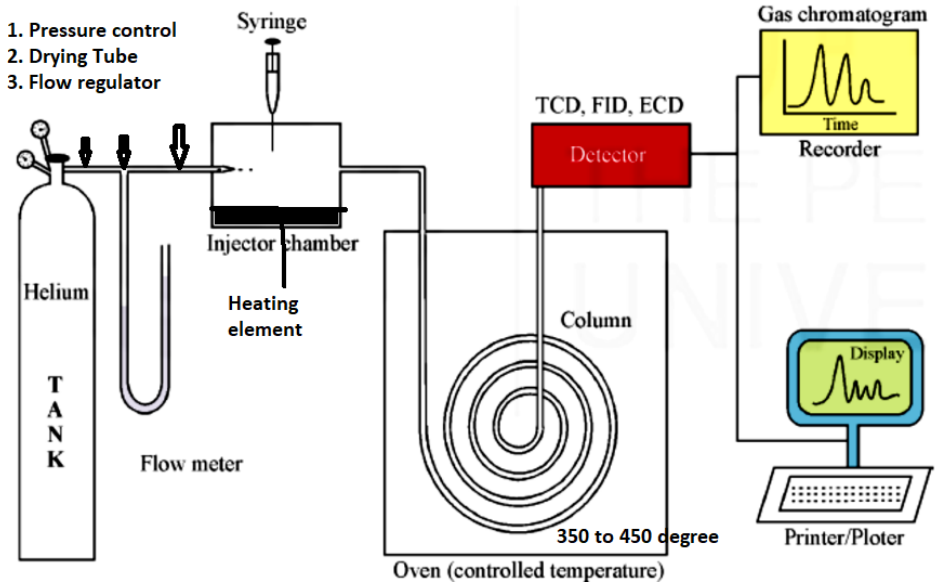
2. Wall coated column:



3. Fused silica coated column:



Instrumentation flow diagram GLC/ General outline of GLC apparatus:



TCD: Thermal conductivity Detector FID: Flame ionizable Detector ECD: Electron Capture Detector

The important components of the **apparatus are 1. Pressure & flow regulator:** Adjustment of the flow rate is essential for proper functioning of the detector unit. Flow rate is maintained between 50 & 100 ml/mm **2. Sample injection system:** The arrangement used to introduce the sample into the mobile phase is called injection system. Syringe serum cap method is used to induce small sample **3. Preparation of columns:** It is made up of glass, metal like copper, stainless steel etc. or plastic. **4. Column thermostats:** It is maintain at suitable elevated temperature. **5. The detectors:** The detector is a device which indicates the presence & measures the amount of the components of a mixture in the effluent of the gas chromatography.

Method of separation/ Working: A well dried carrier gas (nitrogen, hydrogen, helium, argon or a mixture of any two of them) under suitable pressure is passed through a packed column at an appropriate flow-rate. The column is maintained at a requisite (high) temperature by thermostatic arrangement. The sample is then injected & vaporized at the head of the column in as much little time as possible. A small quantity is injected shortly thereafter. The vaporized solutes are carried into the column by the moving phase carrier gas. In the column the different components of the mixture migrates with different speeds depending upon their distribution coefficient values. With continued flow of the carrier gas, each component leaves the column separately in a small volume of the gas & is detected by a detector system which then records the elution peaks & the time since injection. The resulting chromatogram is obtained on recorder. After passing through the detector the gas stream may be passed through a sample collector for identification.

Advantages: 1. Very small amount of sample is required. 2. Very small time is required for analysis

Disadvantages: 1. Used only for volatile components. 2. Dirty sample clog the column, hence before use column is cleaned by running inert gas.

Factor influencing separation of components:

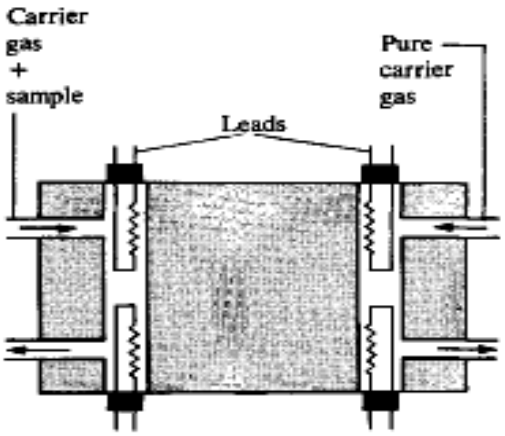
1. Boiling point: High B.P. more is retention time easy to separation. B.P must be lesser than 300 ^{0}C
2. Column temperature: Less column temp. will not sure conversion of all component in to vapour form.
3. Column length: Higher is the column length, better is the separation as sample interact more with stationary phase.
4. Sample size: A very small sample size range (1 to 10 microml) is sufficient for effective separation.
5. Carrier gas flow rate: For effective separation moderate flow rate is desirable.

Detectors : TCD: Thermal conductive detector

Working of TCD based on the comparison of two gas one containing only carrier gas and other containing carrier gas and compound of two filaments. It works on principle that a hot body lose heat at a rate which depends on

composition of surrounding gas. Thus rate of heat loss is measure of composition of surrounding gas. The detector consist of metal filaments with high coefficient of resistance. Through one channel of filaments a pure carrier gas is passed. Other channel is connected to column. The thermal conductivity of pure gas is compared with the gas coming out of column.

The temperature difference between the reference and the sample cell filaments is monitored by a Wheatstone bridge.



Applications: 1. Because of the speed with which it operates, the smallness of the sample size required. 2. High resolution power & high efficiency of separation 3. It is applicable to the separation of all classes of organic components. 4. It becomes a routine analytical tool in all kinds of laboratories. 5. It is also used for product control, for analysis of commercial products in different industry like petroleum, pharmaceutical, cosmetic, perfume etc. & forensic chemistry for chemical analysis.

Detection of components in polluted air water

Detection of different component in medicinal formations

Residue analysis of food stuff and agricultural products.

Separation of constituents of various types of complex mixture.