D.N.R.COLLEGE (AUTONOMOUS): BHIMAVARM

DEPARTMENT OF PG CHEMISTRY

SEPARATION METHODS –I

III SEMESTER

Presented By

B. Madhavi

DNR COLLEGE BHIMAVARAM

Unit-I

Chromatography-I

*Chromatography is a separation technique in which chromo means colour, graphy means graphical representation of separation of components.

*It is a Greak word

*This method was first demonstrated experimentally in 1903 by Michael Tswette.

Chromatography can be defined as a method of separation a mixture of components into individual components through equilibrium distribution between two phases.

*difeerences in the rate of moments of the components due to difference in the absorption of the components on the stationary phase (or) solubility of the molecules in the mobile phase

*separation of the components depends on the nature of the mobile phase, nature of the components.

Nature of stationary phase:

Stationary phase which gives stability to the molecules.

*Adsorption of the components take place in the stationary phase.

Ex. Charcoals caco3 Mgco3,Si,Alumina,solids,fluids also acts as a stationary phase

Nature of mobile phase:

*mobile phase gives mobility to the molecules,that means stability of the components take place in the mobile phase.

Ex. Ethanol, water, acetonitrile, cyclohexane,chloroform,carbon tetra chloride... etc acts as a mobile phase.

*in gas chromatographic technique inert gas acts as mobile phase (He,Ne,Ar,Kr,Xe)

*nitrogen acts as mobile phase depends on electronic configuration.

Chromatography technique involves dynamic equilibrium. Initially the components dissolve in mobile phase and passes through stationary phase then adsorption of the components takes place in stationary phase

Separation pattern:

Mobile phase+(component A)+Mobile phase

Mobile phase+(component B)+Mobile phase

Mobile phase+(component C)+Mobile phase

Mobile phase+(component D)+Mobile phase

*separation of the components also depends on the KD values.

K_D=concentration(A)S.P/concentration(A)M.P98

Types of chromatography

*adsorption chromatography

*partition chromatography

*Ion exchange chromatography

*size exclusion chromatography

1.Adsorption chromatography

The main principle involve in adsorption chromatography “physical surface attractive forces”

Physical surface attractive forces are responsible for the mixer of components separated into individual components here S.P-SOLID, M.P-LIQUID

2.partition chromatography

In partition chromatography partition of the components between two phases responsible for the separated components here S.P-LIQUID,M.P-LIQUID

This partition chromatography are classified in to two types

1.Normal phase chromatography

Here stationary phase –polar

Mobile phase-non polar

2. Reverse phase chromatography

Here stationary phase –non polar

Mobile phase-polar

3. Ion exchange chromatography

In exchange chromatography

Here stationary phase –ion exchange

Mobile phase –liquid

In ion exchange chromatography the ion exchange mechanism different for different ion in the separation .

Ion exchange chromatography are classified into 2 types

*cation exchange chromatography

$R^-H^+ + Na^+ - Cl^- \rightarrow R^- Na^+ + H^+ Cl^-$

*Anion exchange chromatography

$R^+ - OH^- + Na^+ - Cl^- \rightarrow R^+ Cl^- + NaOH$

4.Gel exclusion chromatography

In this chromatography the components separation based on the size and geometry of the molecule.

Here stationary phases –molecular sieves

Mobile phase-liquid

Base line:

Base line is the any path in the chromatogram. where only emerging phase from the column.

Retention time:

Time taken for the complete elution of the component from the sample injection point it is denoted by R_t.

Peak maximum:

Highest point of the Gaussion peak in chromatogram. Peak maximum indicated the complete elution of the component.

Resolution (R):

The extent of separation between any two peaks in the chromatogram.

R=RT2-RT1/W1+W2/2

R=ΔRT/W1+W2

*Peak width :The distance between two tangents drawn at the base of the peak.

R=RT2-RT1/W1+W2/2

R=ΔRT/W1+W2

Case(i) $R \propto \Delta RT$

The retention time of the component increase consequently the resolution of the component also increase

Case(ii) $R \propto 1/w1 + w2$

The peak width of the component increase consequently the resolution of the component decreases.

**Retardation factor Rf:*

Retardation factor defined as the ration of the distance travelled by the solute to the distance travelled by the mobile phase

R_f=Distance travelled by solute/ Distance travelled mobile phase

R_f is the main principle in paper chromatography technique. In paper chromatography initially we take a rectangular whatmann paper.

A horizontal line is drawn on the whatmann paper by using pencil.After sample and reference compound introduced on the line by using micro capillary as micro spots.

Paper introduced into the mobile phase. The flow of M.P is against the gravitational force in the ascending technique

R_f factor is “Qualitative factor”.

**Development techniques in chromatography:*

1.Elution development method

2.Gradient elution development

3.Displacement development method

4.frontal analysis

1.Elution development method:

Elution development method most widely used in all chromatography techniques.

A small portion of the sample is introduced on the top of the column. Pure solvent acts as mobile phase in the elution development method. It is allowed through the system. Initially column filled with micro sixe stationary phase.

Sample mixer introduced in up stream end of the stationary phase. when the sample dissolved in mobile phase. The dissolved component are passed to the stationary phase. This leads to differential migration of the solute in the mobile phase.

Assuming a fixed flow rate of mobile phase in elution developing technique. The time at which each component in the sample will emerge from the column depends on the individual

adsorption (or) partition coefficient. If there are sufficiently different for the mixture will split into separate bonds the migrate at differ their distance from one another.

2.Gradient elution technique:

In ordinary elution development technique there is only a small change of retention volume.

The partition coefficient must be large in elution development technique so the component sufficiently eluted early not pushed out the column has an un dissociated series of bond.

Generally the gradient elution development perfect for closed partition coefficient or adsorption of the mobile phase is responsible for the mixture of components separated in to individual component. In this technique mixture of solvents taken as mobile phase or polarity of the solvent or may be change or concentration or PH of mobile phase will be change in excess of solvent.

Ex.For M.P method +H2O,Methol+Cyclo hexane Acetonitrile +Water Benzene+cyclohexane

3.Displacement development:

Displace the components present in the stationary phase is called “Displacement reagent”.

Displacing reagent has more adsorption nature on the stationary phase than the components or mixture displacing reagent will enter from the column after all the component will be eluted from the column. In displacement development technique pure solvent acts as mobile phase.

For example sample (A+B+C+D)+X is passed through the stationary phase. Initially displacing reagent will be adsorb on stationary phase.

Displacement reagent X replace the mixture by the adsorption on the stationary phase in the mixture. For example X replaces by A,A is replaced by B,B is replaced C,C is replaced by D.

4.Frontal analysis: Frontal analysis consists of passing the sample solution continuously through a adsorb the actives centers of adsorbent are occupied by the more strongly adsorbed components and the least strongly adsorbed components accumulates in the travelling front .pure solvent acts as frontal analysis .frontal analysis leads to resolution only of the least strongly adsorbed solute. Process of this types are used commercially for the removal of relatively small amounts of undesirable components. When these are more strongly adsorbed in the bulk of the material.

****Efficiency of separation (or) Van Deempter Equation:***

Efficiency of separation depends up on the no. of theoretical plates

N=L/H

Where N= No.of plates

L=length of l the column

H=height equivalent theoretical plates

From this

Case(i)N∝L

From this we can say that length of the column increases consequently no. of theoretical plates increases that means efficiency of the separation also increase with increasing of the length of the column.

Case(ii)N∝1/H

From this we can say that the height of the equivalent theoretical plates increases no. of theoretical plates decreases

H=A+B/U+CU

A=nature of the stationary phase

B=nature of the mobile phase

C=nature of the solute

U=velocity of the mobile phase

Now , we can say that efficiency of the separation depends on the S.P,M.P and solutes

H=$2\lambda dp + \frac{2\gamma Dm}{U} + U[f1(k')dp2/Dm$+f2(k')df2/Ds]

$$\lambda = A\ constant\ which\ is\ mseaured\ packing\ irregulairtty$$

dp= particle size of the stationary phase.

U=velocity of the mobile phase

Here f1 and f2 are film thickness of the stationary phase

K'=capacity ratio of the solute between S.P &M.P

Dm=diffusivity of the solute and the M.P

Ds=Diffusivity of the solute and the S.P

$$\gamma = A\ correction\ factor$$

Case(ii):H∝ $2\lambda dp$

That means if the irregular packing of S.P increases then height of the theoretical plates increases then efficiency of separation or resolution decreases.

Qualitative analysis

**Internal standard method:*

In this method a known amount of standard reference compound is directly added to the un known concentration of sample or analyte. In this method peak area or height of the peak of the standard component compare with the peak area of the sample.

"n" components present in the sample and "r" is the one component in the sample then

$$Cr \div Cst = ar \div ast \times ar$$

$$ar = crast \div cstar$$

Where Cr is the concentration of the solute

ar is the area of the peak

Cst is the concentration of the standard

ast is the area of the standard.

If peak height are used instead of peak area.

Cr/Cst=hr/hst× ar

ar=Crhst/Csthr

where hr is the height of the solute

hst is the height of the standard

ar is the response factor

**External standard method:*

In the external standard method a known concentration of standard mixture is passing through one column an unknown concentration of the sample passing through the another stationary phase and development conducted in the same conditions

For example P is present in the both the sample and standard components.

Concentration of solute (P)=CPs

Concentration of solute (P) standard=CPst

Area of peak in the sample=aps

Area of peak in the standard=apst

CPs/C_{Pst}=aps/apst

$Cps=C_{Pst}\times aps/apst$

partition Isotherms:

Partition isotherm depends on the concentration of the sample. If typical partition isotherms giving the stationary phase. concentration of the species as the function of mobile phase concentration

*when M.P concentration are so slow that operation extends over only a short part of the isotherm near the origin

The isotherm can be regarded as linear.

“In this situation concentration of solute in the M.P

A short front tailing area caused by long more type of isotherm. This type of isotherm observed in adsorption chromatography.

The peak shape is caused by the main portion of the solute a more rapidly than the actual retention time of component

*The peak shape is caused by the main portion of solute elute in slowly than the actual retention time of component.

*partition isotherm adsorption isotherm depends on the partition time on the component

*partition isotherm caused the component having more adsorption properties on S.P or least adsorption on S.P than the actual equilibrium properties of the components in the separation

Zones spreading:

The theoretical distribution of a solute during a chromatography assumes that all of it started at the very top of the column. When M.P passes through the zone or spot. The rate of desorption or adsorption takes place between S.P (or) M.P progress down the column by individual solute the assumes random stop and go process.

Each component actually on average value and values about the mean. When dealing the concentration the solutes are not initially comparing to the only the first plate but are over spread in no plates in terms of standard deviation of than gaussian profile of the zone or plate height may be defined as

$H=\sigma^2/H$

Where σ^2 = varience of the zone plate

L=length of the column travels by the center of the zone

Zone spreading consider to be due to a series of molecular diffusion and local non-equilibrium factor. Each factor contributing a certain wavelength to the Gaussian curve.

Zone spreading itself originates in the velocity of flow pattern but the extent of spreading is counted largely diffusion between fast and slow stream parts of the mobile phase

H=$2\lambda dp + \frac{2\gamma Dm}{U} + U[f1(k')dp2/Dm$+f2(k')df2/Ds]

$$\lambda = a = A\ constant\ which\ is\ measure\ for\ packing\ irregularity$$

H=A+B/U+CU

A=$2\lambda dp = Eddy\ diffussion$

B=$2\gamma dm = longitudinal\ diffussion$

F1(k')dp2/Dm=mass transfer of the solute in the mobile phase or diffusion of the solution on M.P

F2(k')df2/Ds=mass transfer of the solute on the stationary phase or diffusion of the solution on S.P

Local non equilibrium effects arise during the mass transfer between mobile phase and stationary phases as the M.P enters and adsorbed the zone it dreams with a solution concentration a smaller than the equilibrium concentration.

*Column length and flow velocity:

After the no. of theoretical plates required for a desired degree of resolution has been a certain by operating conditions.

The time require for separation is simply the time tp is needed to get the one plate multiplied by the no. of plates

T=tp×N

Tp is gives by length of the plate or height of the plate divided by the zone velocity

tp=H/R×v

t=H/RV×N

H/V ratio can be obtain directly by experimental plate and velocity plot.

Long column is potentially preferred for the separation of the components because no.of theoretical plated will be increased in the column consequently resolution of the column.

Volume: The volume of the mobile phase used to wet or wash the stationary phase then in the column.

Retention volume(Rv):

The volume of the mobile phase passed between sample injection point and peak maximum.

UNIT –II

COLUMN CHROMATOGRAPHY

PRINCPLE:-

Mixture of components separated into individual components due to different adsorption properties of the components.

*physical surface attractive force are response for mixture of components separated into individual components. In column chromatography

Stationary phase = solid

Mobile phase=liquid

Mixture of components migrated between two phases; the distribution of two components between 2 phases is response for the separated components. That means the differential rate of components between S.P and M.P responsible for the mixture of components separated into individual components.

Components will be separated due to having different adsorption properties on the stationary phase. It is a Dynamic equilibrium process. The migration of the components are different between S.P and M.P.

Materials used for the preparation of the column:-

Glass columns:

Advantages of glass columns:

It does bit react with organic and inorganic solvents taken as M.P. and also should not react with buffers easily washed.

- We can visualize inside the column
- It is inexpensive
- It should not reacts with M.P and sample

Disadvantages:

Glass columns react with HF to form SiF4.

It cannot with stand high pressure.

Glass columns highly fragile nature

Quarts column:

Advantages:

Quartz columns most use full in the separation of strong acidic and basic components when compared with glass columns.

It does not react with organic and Inorganic solvents and also buffers.

Easily washable

We can visualize the separation in the column.

Disadvantages:

It can react with HF.

Highly expensive.

It cannot stand high pressure

Steel columns:

It can stand in high pressure.

In expensive.

Generally used in the separation of trace elements.

Does not react with acids and buffer.

Disadvantages:

It can not visualize the separation inside the column.

It can reacts with sample at high pressure.

Copper or nickel columns:

Advantages : it can stand in high pressure.

High expensive.

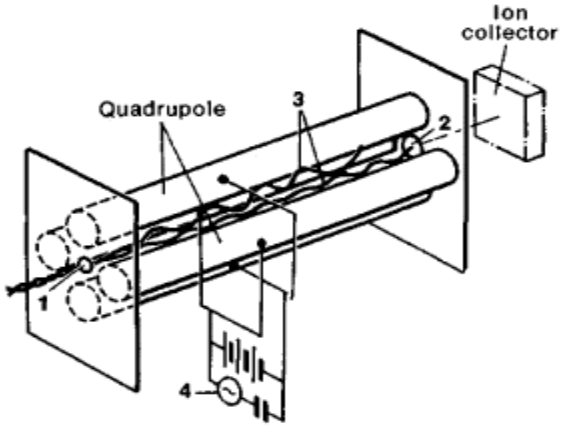
Does not react with acids and buffer.

Disadvantages:

It can not visualize inside the column.

It can react with sample at high pressure.

Quadrapole Mass Analyzer



Quadrupole mass analyzer: (1)and (2) inlet and exit slits of analyzer

(3)trajectory of ions.

(4)high frequency voltage generator

Quadruple mass analyzer is a mass filter by using quadrupole mass analyzer. We can filter non resonate ions. Quadrupole mass analyzer consists 4 poles in this 2 poles are having positively and 2 poles are having negatively charged.

When components are entered into a mass analyzer these ions are oxalate along the z- axis by applying D.C. voltage and radio frequency in these non resonate ions collision with charged poles. And attracted to the poles and filter. Only specific m/z ratio will resonate along the field and have stable path through the detector other will be deflected and colloid with the electrodes and they are filtered out by rapidly varying the voltage ions of one mass after another will take the stable path and be collected by the detector.

Ion trap mass analyzer:

In the simplest form of the instrument an Rf voltage is applied to the ring electrode and the end caps are earthed ions are formed inside in the tap by pulse of electrons form the filament and are the confirmed by opening in the starting region.

An important feature of the device is to achieve mass analyzer selection by the impassion unstable peak conditions to expels ions of a given m/z value to wrote the detector. This may be done by adjusting the D.C –voltage. Rf voltage and the RF frequency. So that trapped ions of consecutive m/z ratio.

Chromatography Media (or) stationary phase:

Organic and inorganic stationary phases taken in column. Chromatography for the separation of components.

Stationary phase

Organic stationary phase	Inorganic stationary phase
Eg: cellulose, starch, sucrose, charcoal	Eg:Na_2CO_3,K_2CO_3,Mgo,CaO,Diatomics,$MgCo_3$
Carbohydrates	Alumina, silica, Mg silicates, salicylic acid , NaOH,
	Mg-Oxalate, Ca-Oxalate,
$Ca_3(PO_4)_2$,Florizil etc	

Ca3(PO4)-taken by hydroxyle Heptile (Hemtile)

Hydroxyle heptile is used for the separation of molecules having high molecular weight preferable for bio chemistry , does not retained low molecular weight substance.

Diatmacious acts as extremely peak these are used for the separation of acidic components.

Charcoal: these are divided into polar and non-polar

Non-polar charcoal prepared at high temperature 1000^oC

Principle:(Dispersion process)

Polar charcoal prepared at low temperature.

Principle: (Hydrogen Bonding) or (Chemi-sorption)

Preparation of Alumina and Silica:

Alumina

Neutral (6.9-7.1)	Basic (10-10.5)	Acidic(3.5-4.5)
Neutal Al_2O_3 preparation for the separation of neutral compounds like nitro compounds, acids, aldehydes , ketones, esters, thers,etc…	Basic Al_2O_3acts as cation exchange resin in Ag solvents. Eg:Amino acids,Amines etc…	Acidic Al_2O_3Acts as Anion exchangeresin Eg: carboxylic weak acids

$NaAlO_2+2Hcl \longrightarrow Al(OH)_3 \xrightarrow{\Delta} Al_2O_3 \xrightarrow{\Delta} Al_2O_3$

900°C 1200°C

(acidic) (neutral)

$Alcl_3+2Hcl \longrightarrow Al(OH)_3 \xrightarrow{\Delta} Al_2O_3 \xrightarrow{\Delta} Al_2O_3$

900°C 1200°C

(basic) (neutral) Silica [Acidic (3.1-4.7)]:

$Na_2SiO_3+2Hcl \longrightarrow H_2SiO_2 \xrightarrow{\Delta} SiO_2 \xrightarrow{\Delta} SiO_2$

900°C 1200°C

(acidic) (neutral)

$Sicl_4+4NH4OH \longrightarrow Si(OH)_4 \xrightarrow{\Delta} SiO_2 \xrightarrow{\Delta} SiO_2$

900°C 1200°C

(basic) (neutral)\

Nature of mobile phase:

It should not react with stationary phase and solutes.

The solute must be soluble to some extent in the mobile phase.

The various factors are influence the mobile phase are

1. Di –electric constant increases solubility also increases.

2. Viscosity
3. Surface tension
4. Boiling point

Examples of mobile phase are : petroleum ether, cyclo hexane, n-hexane, di-ethyl ether, carbon tetra chloride $CHCl_3$ and acetonitrile etc…

Instrumentation of column chromatography:

Initially components are separated by using column. Then the separated compound and mobile phase strikes on continuously circulate platinum chain.

Platinum chain conveys the m.p contains solute in the evaporation chamber.

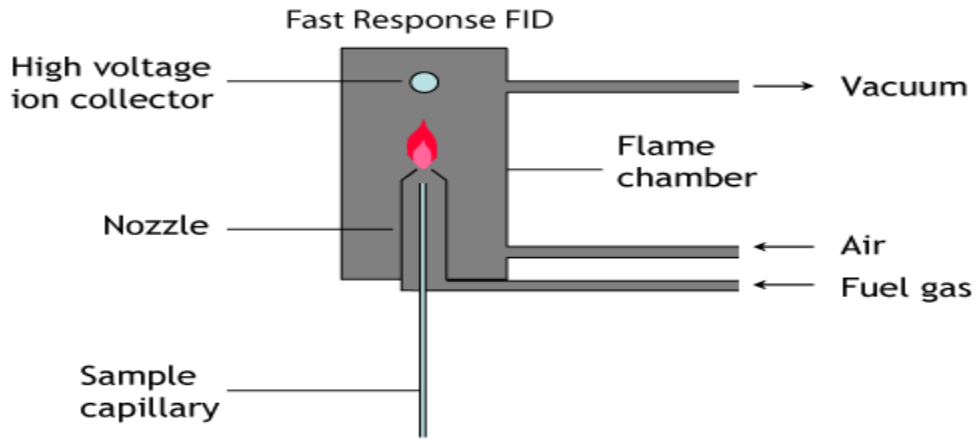
Evaporating chamber contains groob through stainless cartridge heater.

Initially cartridge heater incorporated with nitrogen gas.

When mobile phase+ solute entered into the chamber.

Mobile phase is evaporated from the mixture by the treated with nitrogen gas. When only solutes entered into the detector.

Flame ionization detector:



Principle : combustion flame generate in the chamber by the fuel react with air. The temperature of this flame is greater than 2000°C. All organic and inorganic compounds ionize in this flame. When m.p along with sample entered into the flame. Then the ionization of solute occur due to thermal energy produced by the flame.

The ionization process gives cationic fragments and electrons. These ions are captured by the electrode by applying suitable potential of electrodes by 200-300V.

The strength of the current is directly proportional to the sample

Paraffin, isoparafine, aromatic compounds, naphthalene compounds are detected by FID filter is used to remove the foreign materials.

Applications of column chromatography:

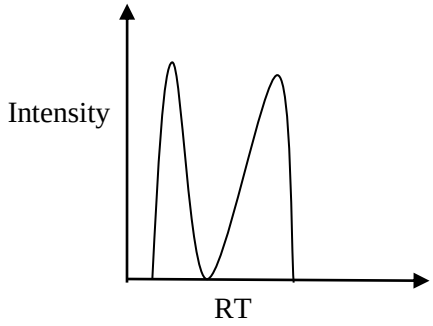
1.separatrion of aromatic compounds from paraffinic and naphthalene compounds.

Stationary phase: Neutral silica,

Mobile phase: n-hexane+chloroform+petroleum+ether

Detector: UV detector

Chromatogram:



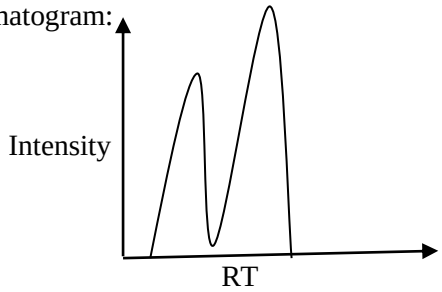
2.separarion of α-cavity from β-cavity

Stationary phase : $CaCO_3$

Mobile phase: Petroleum ether

Detector: UV detector

Chromatogram:



Separation of cis and trans isomers

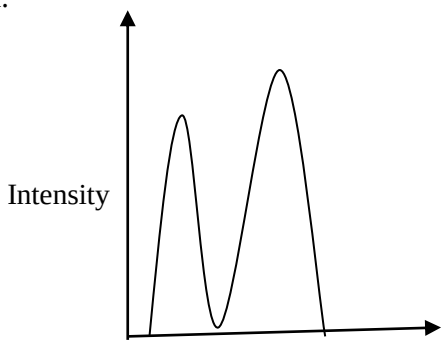
Stationary phase:

Mobile phase: petroleum ether

Here cis isomer forms weak complex with silver and trans isomers forms strong complex with silver

Detector : Eye detector

Chromatogram:



RT

Gel exclusion chromatography (or) Gel filtration Chromatography:

Gel filtration or gel exclusion is also called as size exclusive chromatography. Here in this case the main process is filtration using a gel. Hence its name is gel filtration chromatography. If a solid is separated from mixture of solids it is called sieving. If a solid is separated from the solution is called filtration.

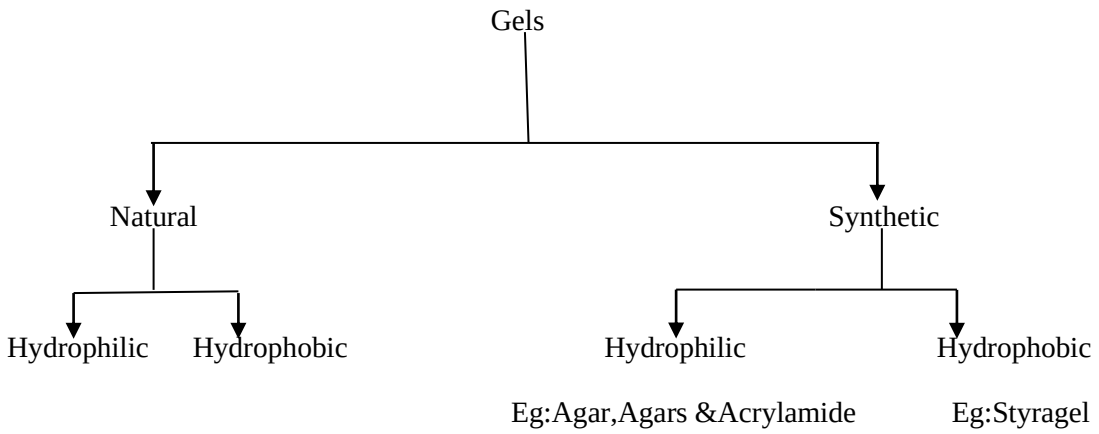
If a soluble component is separated from solution is called gel filtration.

Molecular sieves (may be Inorganic zeolites)

And the organic xero gels can be taken as stationary phase in gel filtration chromatographic technique.

The zeolites are useful for the smaller molecules while organic xero gels are used for the components having high molecular weight. Larger molecules will passes through the column these are excluded from the column.

Prepararion of stationary phase in gel filtration:



Bio –Gels: Bio gels can prepared by the polymerization of acrylamine with N-N Methylene bis acrylamide .

Here which is acts as cross linking agent. In the preparation of pore structure of gels is needed excess addition of cross-linking agent in the preparation of pore structure gels will be decreased.

Sephadex(or)Dextrongels:

Sephadex gels can be prepared by the polymeriasation of epichlorohydroine with straight alkaline chain of dextrons.

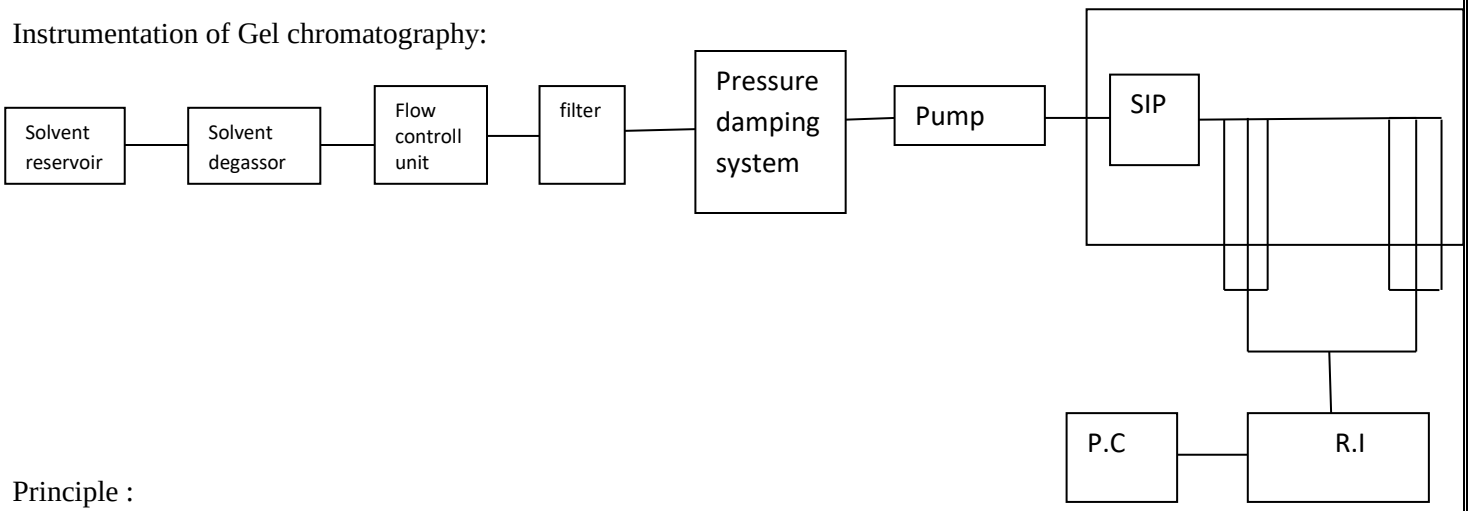
Here Dextrons acts as cross linking agent.

Styrene gels:

Styrene gels can be prepared the polymerization of styrene with dephenyl benzene in presence of toluene at 80oC here divinyl benzene acts as cross linking agent.

Gel type	Fractionating Range
BIO-GEL	
P-2	200-2000
P-6	1000-5000
P-30	20000-50000
P-100	40000-100000
P-300	100000-400000
SEPHADEX	
G-10	UP to 700
G-25	700-5000
G-75	3000-70000
G150	5000-400000
G-200	500000-800000
STYRA GEL	
60-Styra Gels	Up to 800
400-StyraGel	Up to 8000
$5X10^3$-Stya Gels	Up to 100000
$10x10^{15}$-Styta Gels	

Instrumentation of Gel chromatography:



Principle :

Mixture of solid components separated in to individual components with in the forces size of molecule penatate into the gel structure. Beyond the pore size of the molecule excluded from the gel structure.

Solvent reservoir: all organic solvents and buffers acts as mobile phase.

Examples for mobile phase is ethyl alcohol, methanol, methanol +water,Iso-octane etc.

Solvent Degasser:

Solvent degasser is used for the removal of unwanted air particulars present in mobile phase initially M.P treat with nitrogen gas which is captured from a particle present in the M.P

Filter:

Any turbidity (Undesired) present in the mobile phase which is filter by using appropriate filters.

Pressure damping system:

Damping system is used to minimize the pulsed flow of mobile phase (pressure may be increasing or decreasing)

Pump:

Pump is used to deliver the mobile phase to the column by the back and front movement of piston.

Sample injection port:

Liquid samples are introduced by using micro size with out either decomposition or fractionation.

Columns:

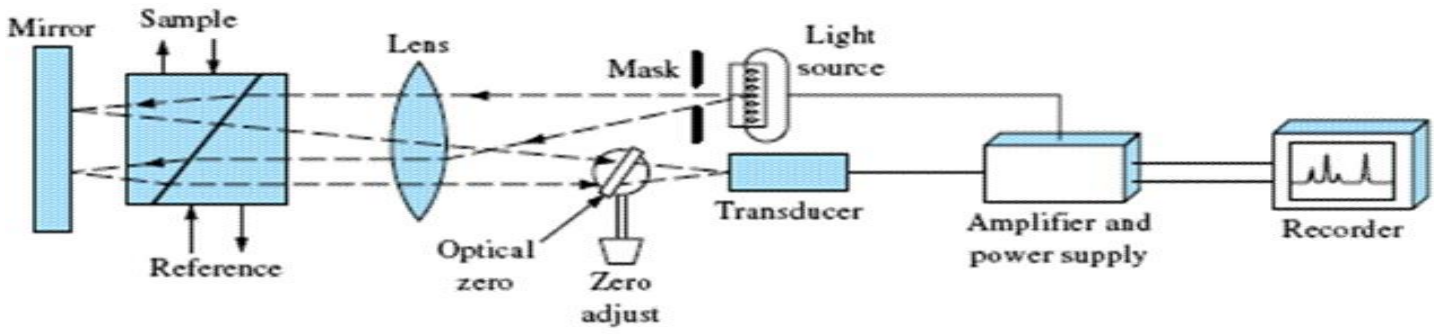
Generally glass and quartz columns are taken in gel filtration technique. Inorganic sieves and organic xero gels acts as stationary phase. Detectors.

Detectors used in gel filtration chromatography is followed.

1.UV detector

2.Refreactive Index detector

Refractive Index detector or RI Detector:



Refractive index detector is bulk detector and also called universal detector.

Response of RI detector does not depend on functional group present in sample the basic principle involved in RI detector is deflection of the beam is caused by the refractive index of the contents of sample and reference cell,

R.I = velocity of light in air

Velocity of light in column

Initially the light is produced from tungsten lamp or mercury lamp then the light focused by optical mass then the mass contains the light region to the cell

And the stricks on lens system, lens collimates the radiation.

The beam of light is passed through sample and reference cell to plane mirror.

Reflex beam of light from plane mirror again passed thorugh reference to photocell.

Here the deflection of beam is rather than its intensity which is determined by the deflection of light is caused by difference in refractive index of the contents between sample and reference cell.

Sensitivity of R.I detector is 1X10-16g/mL

Linear dynamic range 10 to 10^{14} g/mL

Response factor is 0.97 to 1.03

Applications of Gel filtration chromatography:

1.Separation of Poly sacharides :

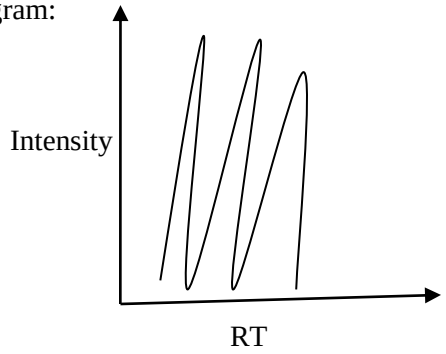
Stationary phase: G150 (sephadex)

Mobile phase: pure water

Flow rate :5min/L

Detector : R.I detector

Chromatogram:



1.Ratinose

2.Maltose

3.Glucose

2.Separation of Poly glycol:

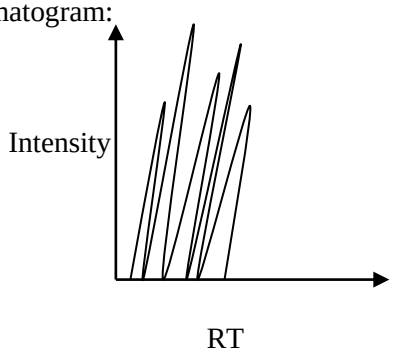
Stationary phase: G10 (sephadex)

Mobile phase: pure water

Flow rate :5min/L

Detector : R.I detector

Chromatogram:



1. Poly ethylene glycol
2. Tetra ethylene glycol
3. Tri ethylene glycol
4. Di ethylene glycol
5. Ethylene glycol

3.Separation of poly ethylene from poly propylene

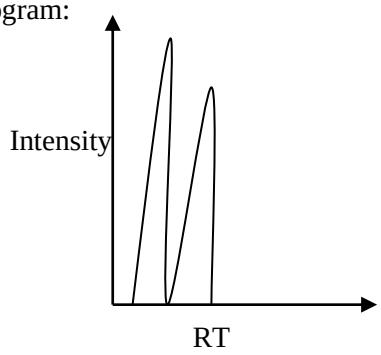
Stationary phase: Styra gel

Mobile phase: toluene or xylene

Flow rate :0.5mL/min

Detector : UV-Fluorescence detector

Chromatogram:



Capillary electrophoresis:

Capillary electrophoresis theory and principle:

A relatively new separation technique i.e capable of separating minute quantities of substance in relatively short time it high resolution is capillary electrophoresis

Mixture of charged particles separated into individual particles basing on there size and charge of the particles in presence of electric field. The mo ssfsf of colloidal or dissolved substance relative of a better solution as a result of an applied field.

This leads to migration of particle or ions towards electrodes fdepending on the effective charge on the particles.

Proteins, peptides, nucleotides, DNA and etc can be separated by using capillary electrophoresis.

The recet application of DNA finger printing forcenic and buld identification is the typical of the value of modern electrophoretic method.

Capillary electrophoresis is also called as “electro zone phoresis” it offers the ability to analse 10-9 nano levels of the sample with over one million theoretical plates with detector semstivity is 10-8 molecular levels.

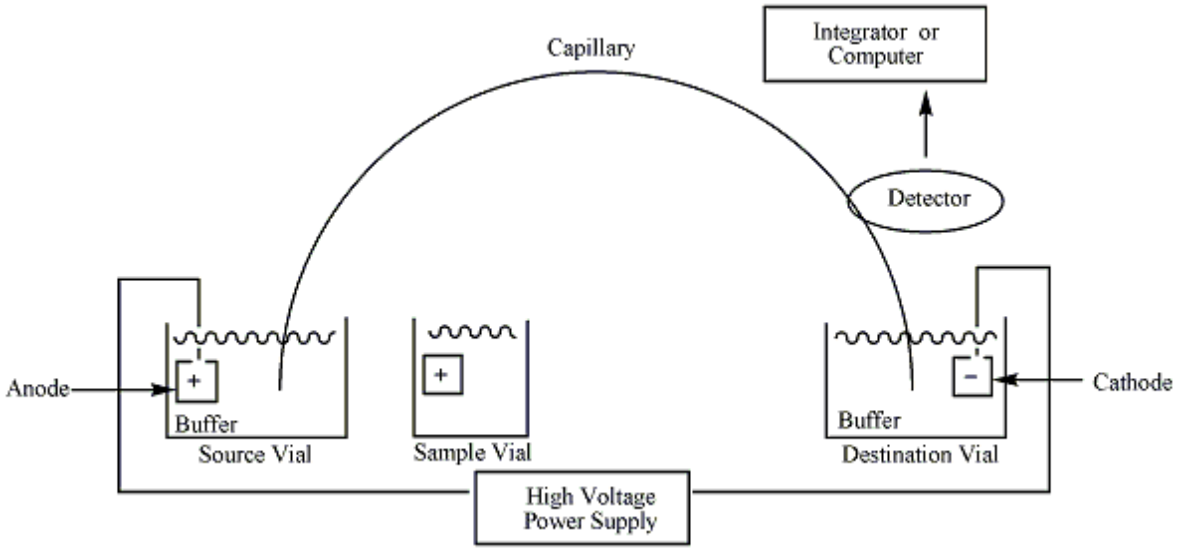
The basic principle involved in electrophoresis is an electro osmosis. Electro osmosis is a bulk flow of solvent all the analyte +ve ions or attracted towards cathode.

Initially capillary tue is filled with ionixable silinoe (sio) groups due to the ionixation of silinones –ve charge produced on the walls of the capillary.

When buffer solultion is passed through this capillary +ve ions present in the buffer is attracted by the anions present on the walls of the capillary.

Instrumentation of capillary electrophoresis:

Diagram



Two beakers are initially filled with buffer solution and one end of the capillary tube is immersed in buffer and second end of capillary tube connected to detector. The detector is taken into close to the cathodes.

When two platinum electrodes also immersed into the buffer solution they connected to DC- voltage above 10000-30000V

A Typical electrophoresis system consists a fused silica capillary and also initially filled with aqueous buffer electrolyte.

Sample introduction:

sample is introduced by inserting anode end of the capillary into the walls of the capillary and then applied electric field to the sample cell is nothing but “electro kinetic sample injection”.

Capillaries:

Fused silica capillaries 30 to 100 meters longer with internal diameter 50-100mμ. Glass and Teflon capillary also used in capillaries and coated with polyamides and also some capillary may be internally bonded with glycerol in order to reduce interaction between the moving molecules and capillary walls.

Applied current field strength:

Capillary electrophoresis required high strength electric field to achieve rapid separation.

High voltage power supply need to provide voltage between 22 to 100 KV. Current strength between 15-200 micro amperes.

Applications of capillary electrophoresis:

Separation of cations or inorganic compounds:

Length of the capillary :64.5cm in the diameter is 75μ

Voltage:462V

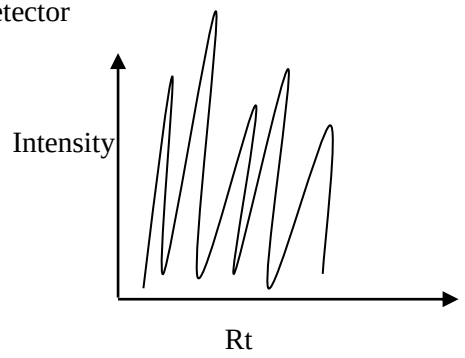
Temperature:35^{o}C

Current strength:14mA

Buffer: Phosphate

Detector: UV-detector

Chromatogram:



1. K^{+}
2. Na^{+}
3. Ba^{+2}
4. Mg^{+2}
5. Li^{+}

2.Separation of Anions:

Length of capillary : 56cm

Voltage:366V

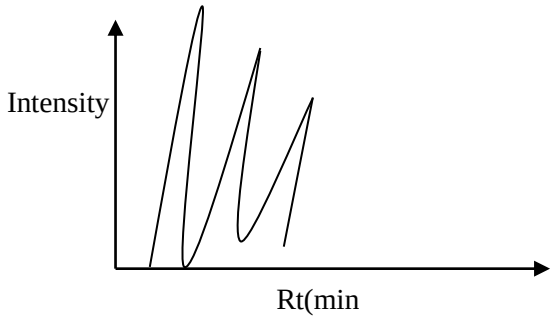
Temperature:25^{o}C

Current strength:9mA

Buffer: Phosphate

Detector: UV-detector

Chromatogram:



1.Cl^{-} 2.$SO4^{-2}$ 3.$NO3^{-}$

3.Separation of acids or organic compounds:

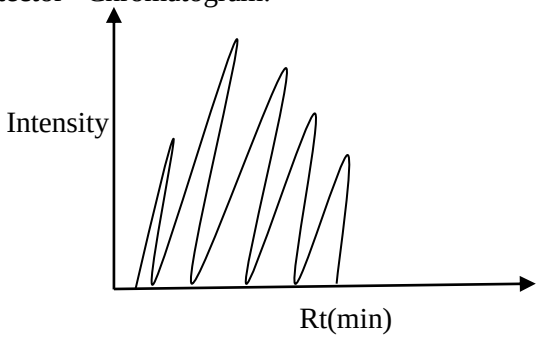
Length of capillary : 805cm

Voltage:310V

Temperature:15^{o}C

Buffer: Phosphate

Detector: UV-detector Chromatogram:



1. Citric acid
2. Formic acid
3. Malic acid
4. Lactic acid
5. Phenyl acetic acid

UNIT –III

GAS CHROMATOGRAPHY

Principle :

Mixture of volatile compounds separated into individual components basing on the Boiling point of the solutes. The separation of the volatile components occurs between stationary phase and Mobile phase (Inert gas)

Gas chromatography technique classified into two types

- Gas liquid chromatography
- Gas solid chromatography

Gas- liquid chromatography:

Stationary phase –liquid coated on the solid

Mobile phase – gas

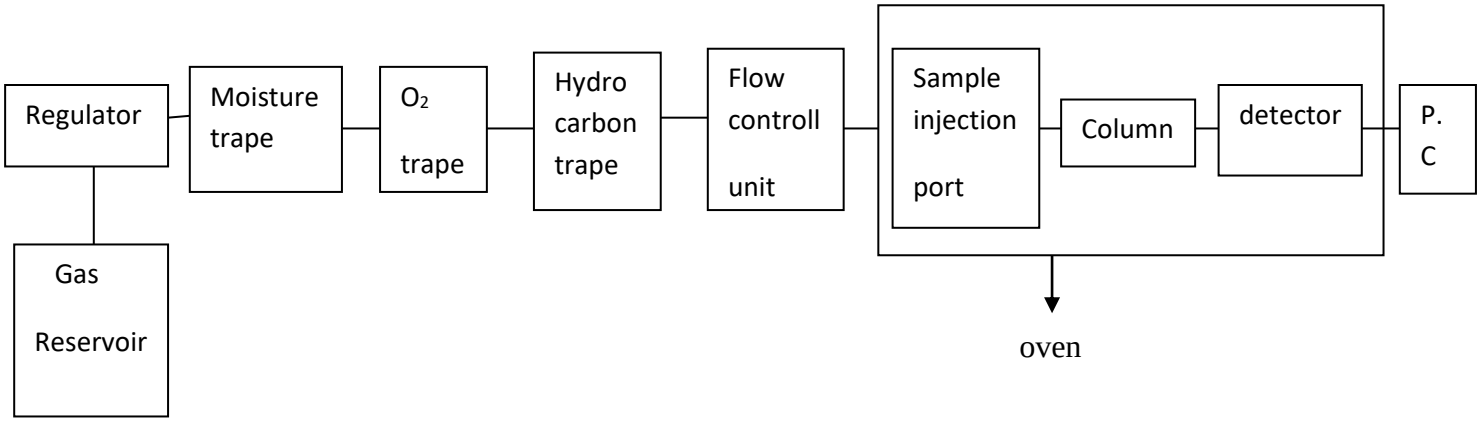
Gas-solid chromatography:

Stationary phase – solid

Mobile phase – gas

Temperature plays a main role in the gas chromatography. Generally pesticides and herbicides Benzene hexa chlorides etc separated by using Gas chromatography.

Instrumentation of Gas Chromatography:



Reservoir of mobile phase:

Generally inert gas used as M.P (Hydrogen, Helium, Neon…etc.). selection of the M.P depends on the availability , purity consumption and reactivity.

Hydrogen has disadvantages like it can react with unsaturated compounds present in the sample.

And it has explosive nature and creates fire. Helium is the second best M.P in gas chromatography because its excellent thermal conductivity and greater flow rate. Nitrogen also used as M.P in gas chromatography is available in pure state.

Trap system:

Presence of contaminates or impurities in M.P may effect on column performance and detector response. If the M.P consists oxygen and hydrocarbon is removed by using trap system.

Purification of M.P is necessary in the separation of components. Generally Oxygen trap, moisture trap hydrogen trap are used in Gas chromatography.

Flow control unit:

It controls the flow rate of the M.P

Sample injection port:

It consists rubber silicon spectrum sample should be introduced into the sample injection port without either decomposition or fractions.

Generally liquid samples are introduced by sing micro syringes having hypo thermic needle though a self silicon rubber spectrum into the chamber. Liquid samples introduced into a nana liters(10^{-9}).

Column:

Packed column and capillary column used in Gas chromatography.

Packed column:

- Length of the packed column is 1-10cm and inner diameter column is 0.2-0.6cm.
- Size of the stationary phase particle should be uniform in size for good packing and have in the range of 60-80 mesh (0.25-0.18)
- Packed columns made of Glass, Stainless Steel, Quartz, Fussed Silicon, Teflon and Copper.
- Generally glass used for the preparation of long columns.
- Quartz and stainless steel is used for the preparation of short columns.
- Copper, Teflon is used for the preparation of inert columns.

Capillary columns:

Capillary columns also made of stainless steel. But stainless steel can react with sample. It is deactivated by treating dimethyl dichloro silane.

Length of the capillary column is 10-100m inner diameter 0.1-0.5µ

Capillary columns are divided into three types.

- Wall coated open tubular column(WCOT)
- Support coated open tubular column(SCOT)
- Porous layer open tubular column(PLOT)

1.Wall coated open tubular column:

The thin liquid film coated on and supported by the walls of capillary. The walls are coated by slowly and a dilute solution of the liquid phase through the column. The solvent is evaporated by passing carrier gas through the column.

Wall coated open tubular column Typically 5000 plates

2. Support coated open tubular column: (SCOT)

Support coated open tubular columns solid micro particles coated with stationary phase are attack to the walls of the capillary. These have highest surface area and greater capacity.

Tubing diameter is 0.5-1.5 m ,sample volume is 0.5µ liters or less.

If a separation require more than 10000 plates.

3. Porous layer open tubular column:

It have solid phase particles attached to the column, wall absorption chromatography.

Particles of Alumina or porous (molecular) polymers are typically used. These column like packed gas solid chromatography column.

These are useful for separation of permanent gases as well as volatile hydrocarbons.

The resolution efficiency of open tubular column is

WCOT>SCOT>PLOT

Advantages of capillary column:

1. They have high sensitivity. These have high resolution and less time of analysis.

Stationary phase:

Polar and non polar particles take as stationary phase in Gas chromatography. Generally used stationary phase particles are poly ethylene glycol are polysiloxanes.

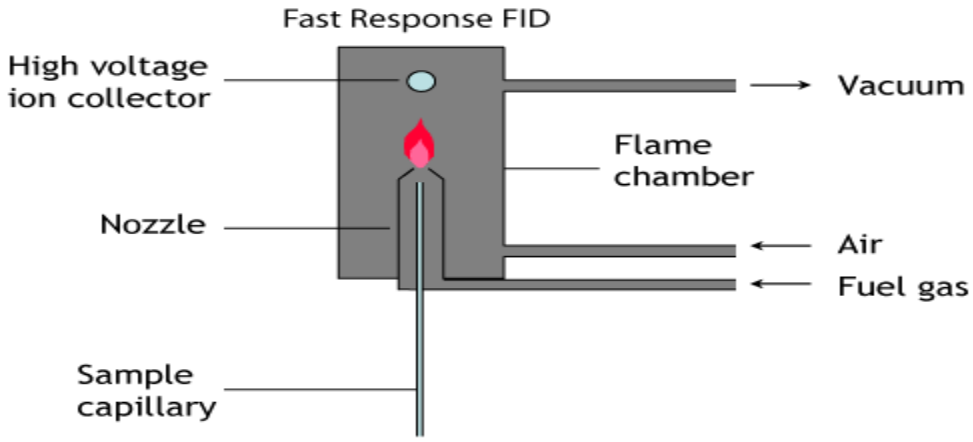
Detectors :

Detectors used in Gas chromatography

1. Flame ionization detector
2. Electron capture detector
3. Nitrogen phosphorous detector
4. Thermal conductivity detector
5. Photo ionization detector

Flame ionization detector:

Flame ionization detector:



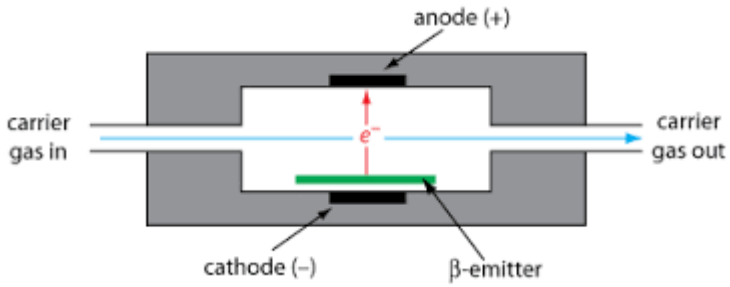
Principle: combustion flame generate in the chamber by the fuel react with air. The temperature of this flame is greater than 2000^{o}C. All organic and inorganic compounds ionize in this flame. When m.p along with sample entered into the flame. Then the ionization of solute occur due to thermal energy produced by the flame.

The ionization process gives cationic fragments and electrons. These ions are captured by the electrode by applying suitable potential of electrodes by 200-300V.

The strength of the current is directly proportional to the sample.

Paraffin, isoparafine, aromatic compounds, naphthalene compounds are detected by FID filter is used to remove the foreign materials.

2.Electron capture detector(ECD):



Principle:

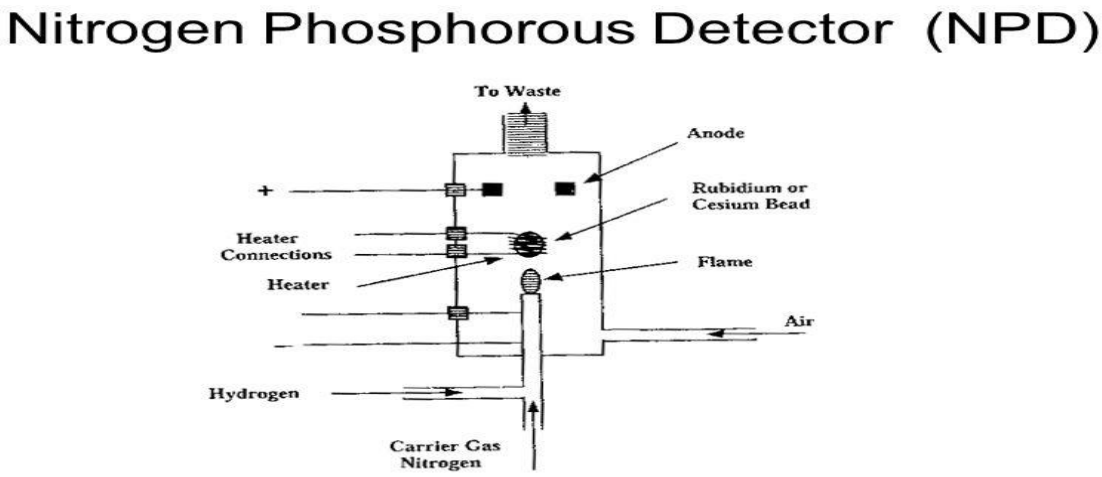
It emits β-particles in the chamber by giving suitable energy to the source.

When only M.P enters into the detector which is produced electron, collision with β-particles present in the chamber. The generated electrons are captured by anode and a constant electron density maintain in the chamber.

When M.P along with solutes having electro negative atom (Cl,Br,O and sullphur) passed through the detector. They will capture the electrons present in the detector. Then we observe decrease in current in the chamber. ECD is a selective detector.

Radioactive source Ni-63 initially washed with 30% KOH in the applying temperature up to 350^{o}C

3. Nitrogen phosphorous Detector:



Nitrogen-containing or Phosphorus-containing species elute into a cool flame (excess H_2). A heated Rb bead interacts with N or P to produce ions that are then collected as a current between electrodes. Like the FID, the NPD is a *Thermionic Detector*.

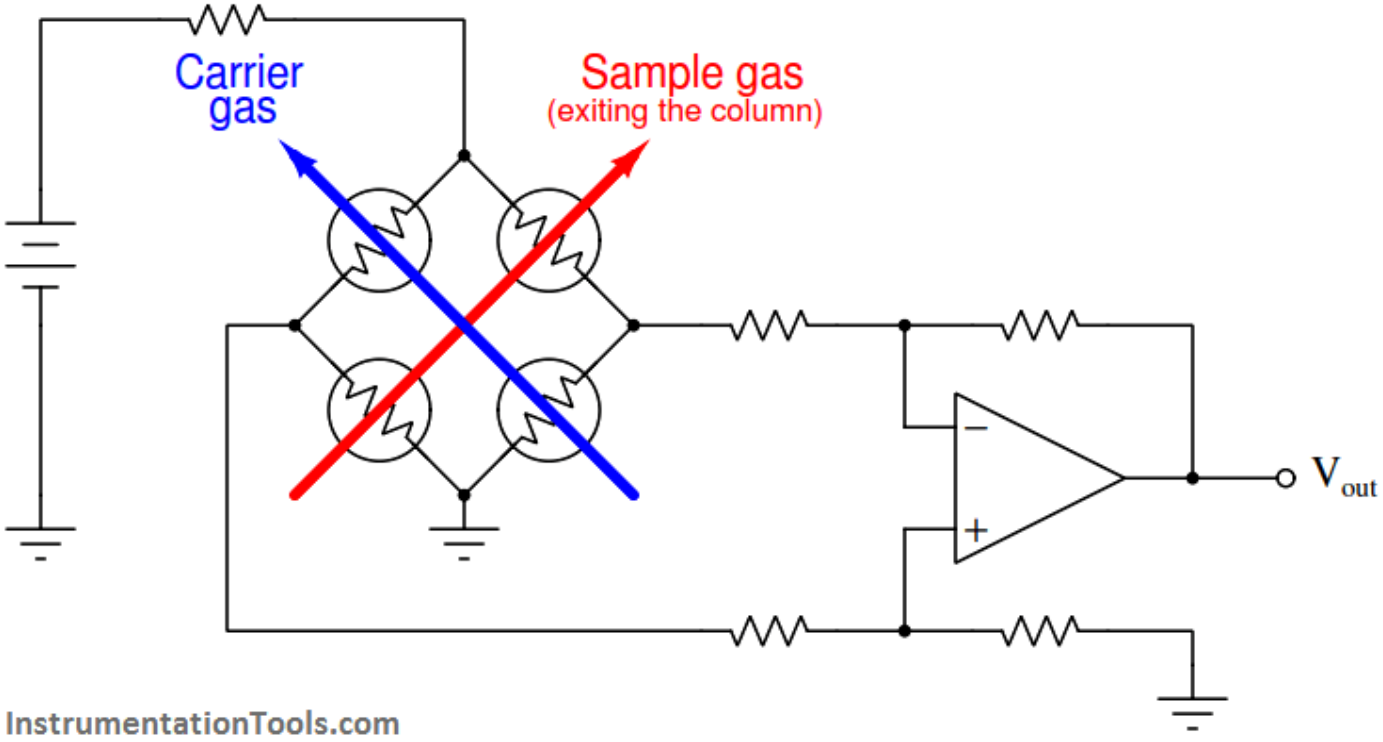
Principle:

Nitrogen phosphorous detector consists Rb-Ce bead. Rb0Ce bead emits electrons by suitable energy to the source. This is known as thermo electronic emission. Combustion flame is produced in the chamber. Fuel react with air.

When M.P along solutes having (N,P) elements entered into the plane they are not completely ionize at the flame. The remaining residue having Nitrogen, Phosphorous absorbed by the Rubidium. Then ejection of electrons Rb-Ce increases in the chamber and work function of the Bead will be decreased." Ejection of electrons directly proportional to the solutes having Nitrogen and Phosphorous."

Thermal conductivity detector(TCD):

Thermal Conductivity Detector (TCD)



Thermal conductivity detector consists two cavities through which the gas (Mobile Phase) flows. Gas is passed through a heated filament wire. The temperature and resistance of wire will carry according to the thermal conductivity of gas.

The pure carrier gas (Mobile Phase) is passed over one filament and the gas containing the sample constituents is passed through another filament.

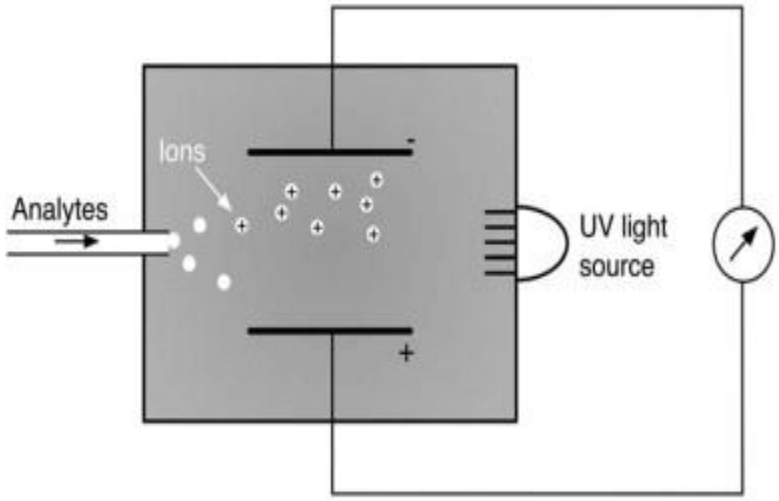
These filaments are connected to two arms of the Wheatstone bridge. Wheatstone bridge circuit that measure the difference in a resistance. As long as, there is no sample gas in the resistance of wire will be the same. But when the same is eluted with a carrier gas a small resistance change will occur in the influent R. the change in resistance is register or recorder. The change in resistance is proportional to the concentration of the sample component in the mobile phase.

Thermal conductivity is particularly used mixture of gases and permanent gases such as CO_2.

Hydrogen and Helium M.P are require in thermal conductivity detector because they have a very high thermal conductivity compare with other gases. So largest change in the resistance occurs in the sample component nitrogen, CO2 and the most of organic vapors have thermal conductivity values of 1/10 of the values of Hydrogen.

Their response is very reproducible they are not the most sensitive detectors.

Photo ionization detector (PID):



Helium lamp, mercury are lamp, deuterium lamp, xenon arc lamp these are the example for UV source.

Principle:

When a carrier gas (M.P) along with solutes enter into chamber. The ionization occur absorption of the UV radiation by the molecules. These ionization process produce ions and electrons. They are captured the ions by the electrodes.

"Current strength is proportional to the ejection of electrons."

Limitations:

Selective ionization of molecules problem arises with a long term use of photo ionization detector.

Especially with regard to contamination of lamp, windows and the life time of the UV lamp.

Temperature programming in Gas chromatography:

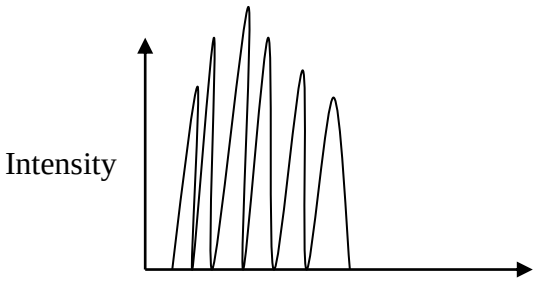
The proper temp. Programming in Gas chromatography is a compare between several factors. The injection temp. Is relatively high consistent in thermal stability of the sample. To give fastest the vaporization to get the sample into the column in a small volume. Decreased spreading and increased resolution spreading. The column temp is compromised between speed, sensitivity and resolution.

Separation can be facilitated by temperature programming and most chromatographs have temperature capability. The temp is automatically increased at a free selected rate during the running of the chromatogram this may be a linear , exponential, step like and soon. In this way the components elutes more can be eluted in a reasonable time without forcing the other from column quickly. Temperature programming from low to higher temperature spreads up separation. The more difficult solutes elute faster at the high temperature the more easily eluted once are better resolved at the lower temperature.

Example : If the consistent is not determined at the non-volatile temperature. It may be converted to a volatile.

Applications of Gas chromatography:

1. Analysis of Aromatic Hydro carbons:
   Column : Hp 55(30mX0.55X0.5µ)
   Mobile Phase:He
   Flow rate:5mL/min
   Temperature programme: 50 $\xrightarrow{5^0C/min}$ 200 $\xrightarrow{10^0C/min}$ 300
   Detector: Flame Ionization detector
   Chromatogram:



1.Napthalene

2. ….Naphthalene

3.Fluorine

4.Anthracene

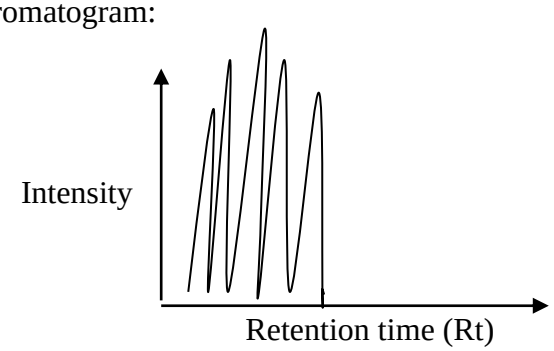
5.Pyrene

6.Benzo Anthracene

2.Analysis of Herbicides:

Column : SPB(30mX0.55mmX0.5µ)
Mobile Phase :Nitrogen
Flow rate:5mL/min
Temperature programme: 60 $\xrightarrow{16^0C/min}$ 260

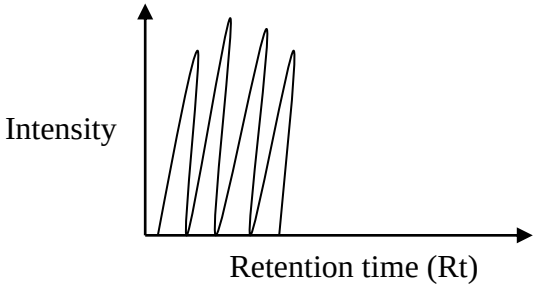
Detector: Nitrogen Phosphorous detector
Chromatogram:



1.Eptane 2. Sutane 3.Varam 4.Tillam 5.Ordam

3.Analysis of Sulphur compounds:

Column : DV-5(30mX0.55X0.5µ)
Mobile Phase: Nitrogen
Flow rate:1mL/min
Temperature programme: 50 5^0C/min 150
Detector: Thermal conductivity detector
Chromatogram:



1.Hydrogen

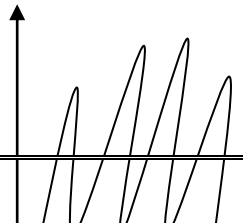
2. Oxygen

3.Methane

4.Ethylene

5. Analysis of Gasolen:

Column: SPB 608 (Fused silica capillary) (30mX0.55X0.5µ)
Mobile Phase: Nitrogen
Flow rate: 2mL/min
Temperature programme: 50 5^0C/min 130
Detector: Flame Ionization detector
Chromatogram:



Intensity

Retention time (Rt)

1.Benzene

2.Toulene

3.Ethyl Benzene

4.Xylene

6.Anallysis of Impurities in Inks:

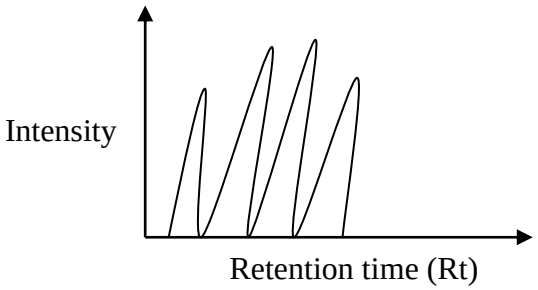
Column: Chromosorb 101

Mobile Phase: Nitrogen

Flow rate :30mL/min

Temperature programme: 50 8^0C/min 280

Chromatogram:



1.Farmaldyhyde

2.Ethyleme Glycol

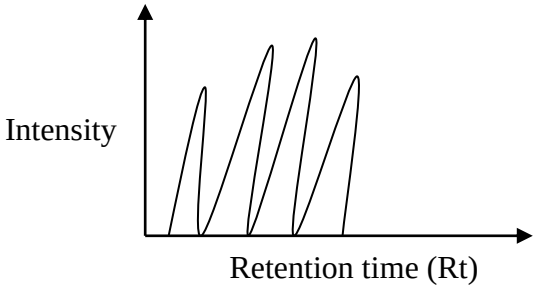
3.Diethyl Glycol Ethyl Ether

4.Glycerine

7.Analysis of Pesticides:

Column :SPB 608 (Fused silica capillary) (30mX0.55X0.5µ)
Mobile Phase: He
Flow rate:5mL/min

Temperature programme: 50 10^0C/min 150 8^0C/min 260^{0}C
Detector: Electron capture detector
Chromatogram:



1.α-BHC

2.β-BHC

3.γ-BHC

4.δ-BHC

SPECIFICATIONS OF DETECTOR:

Detector linearity:

It is a theoretical concept,

An accuracy of the analysis of the separation.

Here α is a numerical term

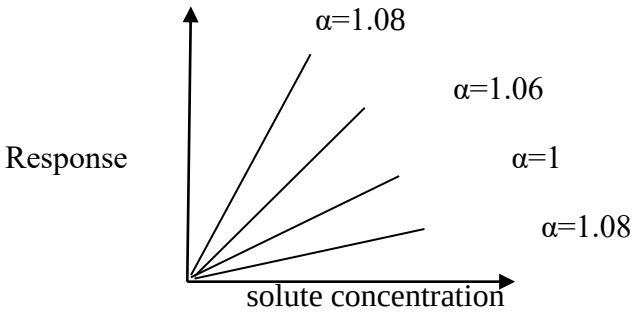
∅=Out pt

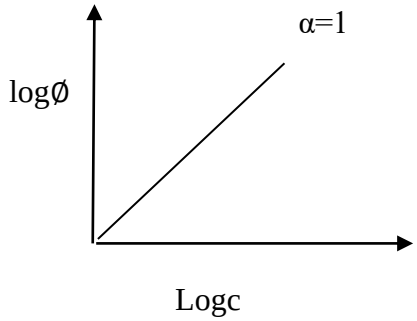
$\varphi = constant$

C=concentration of the substance in detector

α =response factor

The response in their will be unity through true detector (i.e. α =1 acts as true detecto





$\varphi=\psi C\alpha$

Log φ =logψ +αlogC

Detector sensitivity :

Detector sensitivity defined as minimum concentration of the solute peak that can be disernet and ambigiuestly from noise.

Signal by noise = 2

Detector Noise: Detector Noise 3 types

- Long term noise
- Short term noise
- Drift noise

Shorter noise:

Short-term noise consists of base line that has a frequency that is significantly shorter than eluted peak.

It can be easily removed by appropriate noise

Long term noise:

It consists base line petributions that have a frequency similar to that of a eluted peak. This type of noise significant as it is in desirable from very small peaks in the chromatogram

Long term noise cannot be removed by electronic filtering without effecting the profiles of the eluted peak. It is usually arises due to temperature. Pressure and flow rate. Changes in the sensor cells.

Drift noise:

It consists base line petributions that have a frequency greater than a eluted peak.

Pressure sensitivity:

Pressure sensitivity of a detector is the important factor that determines the long term noise. It usually measure as the change in the detector output for a unique change in sensor cell.

Flow sensitivity:

It is another factor that determines the long term noise of the detector. It is usually measured as the change in detector output the unit change in the sensor cell.

Temperature sensitivity:

It is another factor that determines the long term noise of the detector.

It it usually measured as the change in output for a unique change in temperature through the sensor cell.

Linear Dynamic Range: linear Dynamic range of a detector determines of solute concentration over the numerical value of a response index falls within define limits.

The Dynamic range is that range over which detector continuous to response to change in solute concentration in the detector.

Selective Ion Monitoring:

Original fragmentation of molecules gives a large no. of fragments this is difficult to analyze the molecular ion present in the mixture of analyte.

By using selective ion monitoring we can filter unwanted fragrance present in the molecular ions. In selective ion monitoring we can measure desired m/z values containing ions are selectivity monitor. Hence it is called as selective ion monitoring.

In selective ion monitor the spectrum of each molecule detector and stored in systems computer and so the mass spectrum corresponding to given molecular ion peak can be read out. The mass spectrum is generally characteristic for given compound.

Inorganic molecular sieves or zeolites:

Components are separated on basing on the size and structure.

Here stationary phase = Inorganic molecular sieves

Mobile phase = Liquid

Inorganic molecular sieves or zeolites:

Zeolites have honey home structure. Inorganic molecular sieves can be manufactured by the Aluminum hydroxide treated with NaOH and waterglass. It forms internal volume of zeolites. It consists cavities for the purification of permanent gases and smaller organic molecules.

The general formula of zeolite

$Mx/n[(Al_2O_3)_x(SiO_2)_y]_m.H_2O$

Here, x+y tells about nature of the Zeolites.

Classification of zeolites or Inorganic molecular sieves.

Classifaication of molecular sieves depending on pour size of the zeolite and classified into 5 types.

- Type 3A
- Type 4A
- Type 5A
- Type 10X
- Type 13X

1.Type 3A:

Pour size of molecular sieves $3A^0$

Water and ammonia included into the pour structure.

All the hydrogen cab be excluded from the zeolite.

2.Type 4A:

General formula of type 4 series

$NA_{12}[(Al_2O_3)_{12}(SiO_2)_{12}]^2$

Pour size of molecular sieves $4A^o$.

Permanent gases H_2,O_2,CO_2, H_2S are included into the pour structure.

Hydro carbons up to C_{12} can be excluded into the zeolite.

3.Type 5A:

Type 5A can be prepared from type $4A^0$. By the 75% of sodium replaced by calcium and potassium from type 4A.

Type 5A can be used for the purification of straight chains, olefins , Paraffins, chains and the alcohol these are included into pour structure also hydro carbons consists up to remaining aromatic and naphthalene compounds are excluded for the pour structure.

4.Type 10X and Type 13X:

Type 10X pour size molecular sieve is $8A^0$

Type 10X pour size molecular sieve is $10A^0$

General formula is

$NA_8[(Al_2O_3)_8(SiO_2)_{10}]^2$

These are used for the naphthalene and aromatic compound included into the pour structure.

Ion exchange chromatography or ion exclusive chromatography:

Here stationary phase = ion exchange

Mobile phase = liquid

On accomutive domman exclusion principle, opposite charge ions attracted by electrostatic force and same charged ions are repulsive

$R^-–H^+ + Na^+Cl^- \rightleftharpoons R^-\text{-}Na^+ + H^+Cl^-$

R^- = Non labile

H^+ = Labile

Separation of Ethylene Glycol and Nacl:

Mixture Nacl and Ethylene Glycol is passed though the cation exchange resin in the form of Na^+. here Nacl which is excluded from the column and ethylene glycol enter into pour structure or that is included ethylene glycol eluted after Nacl eluted from the column.

Separation of acetic acid+ HCl:

Mixture of acetic acid +HCl is

$HCl \rightarrow H^+ + Cl^-$

CH3COOH contains neutral compound.

Mixture of CH3COOH + HCl is passed through the cation exchange resin in the form of H^+

Here HCl excluded from the Resin.

Separation of mixture acids (Formic acid+Acetic ancid+Butaric acid)

At pH 5-6 initially Formic acid excluded then acetic acid finally butaric acid due to nature of the acids.

*mixture of Formic acid +Acetic Acid+ Butaric acid is passed through the column cation exchange resin is passed at pH 5-6. Here Formic acid and acetic acid is passed through the column and Butaric acid included into the pour structure.

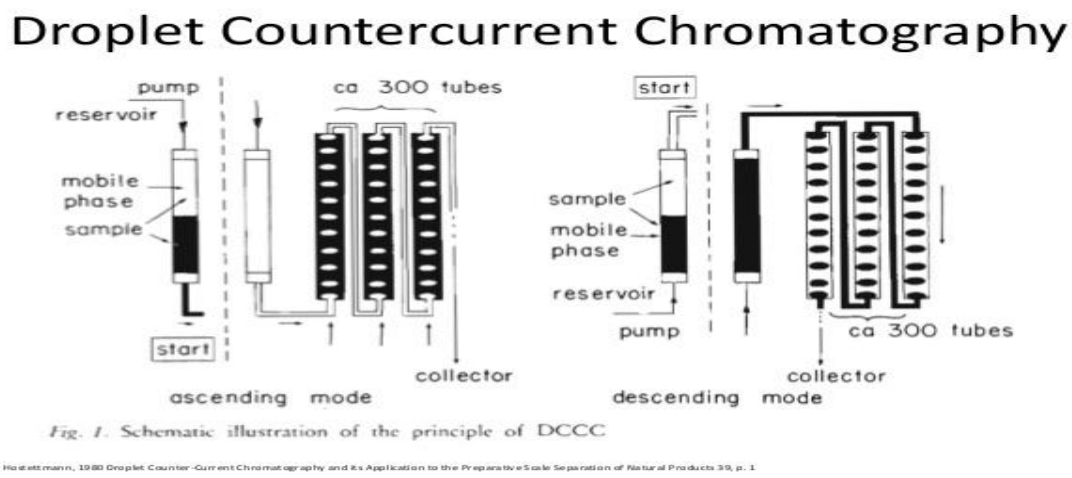


Fig. 1. Schematic illustration of the principle of DCCC

Hostettmann, 1980 Droplet Counter-Current Chromatography and its Application to the Preparative Scale Separation of Natural Products 39, p. 1

Droplet counter current chromatography is a multiple contains extraction method. Is preferred compounds having close partition coefficient. It is an example for liquid chromatography technique.

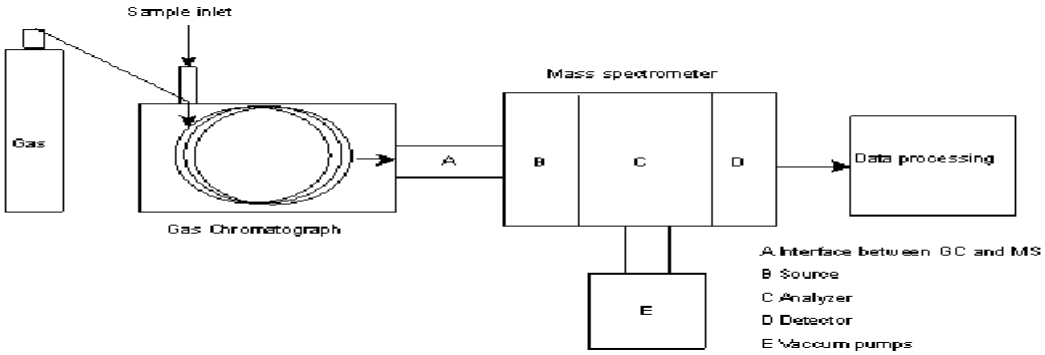
Pyrolysis gas chromatography:

Pyrolysis GC is a specialized sample introduction technique in which a sample is heated in the injection chamber of the gas chromatograph to a temperature at which thermal decomposition of the sample occurs. By chromatographing the pyrolysis products, the structure of the original material can be elucidated, at least in theory. Frequently, no conclusions can be drawn from the pyrolysis products as to the structure of an unknown substance, in which case identification is empirical and based on a fingerprint pattern, which may be characteristic of the particular parent sample.
Many investigations of the optimum conditions for thermal pyrolysis have been reported [148]. Pyrolysis temperatures range from 400–4000°C. The practical problem consists in imparting the desired temperature to the entire sample rapidly and reproducibly. A disadvantage of pyrolysis GC is the relatively poor reproducibility between different types of pyrolysers, as a result of the different temperature behaviour. The duration of the heating also exerts a decisive influence on the pyrogram. There are two types of pyrolysis inlet, pyrolysis chambers or microreactors, and filament systems. Filament systems, in which the sample is deposited as a thin film on the filament and heated using the Curie point principle, may promote catalytic reactions. Another problem is in changes of the filament in the course of time. Using a pyrolysis chamber, the sample is injected directly into the hot furnace. With this type of system, the conditions under which pyrolysis occurs are accurately known. As the temperature is isothermally maintained, the pyrolysis temperature is known and the residence time of the sample in the pyrolysis zone can be controlled.

Gas Chromatography-Mass Spectroscopy

Introduction

Gas chromatography-mass spectroscopy (GC-MS) is one of the so-called hyphenated analytical techniques. As the name implies, it is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. Gas chromatography separates the components of a mixture and mass spectroscopy characterizes each of the components individually. By combining the two techniques, an analytical chemist can both qualitatively and quantitatively evaluate a solution containing a number of chemicals.



Gas Chromatography

In general, chromatography is used to separate mixtures of chemicals into individual components. Once isolated, the components can be evaluated individually.

In all chromatography, separation occurs when the sample mixture is introduced (injected) into a **mobile phase**. In liquid chromatography (LC), the mobile phase is a solvent. In gas chromatography (GC), the mobile phase is an inert gas such as helium.

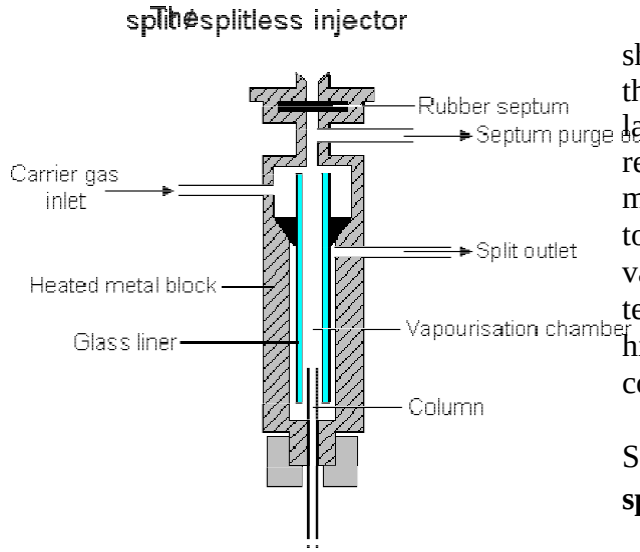
The mobile phase carries the sample mixture through what is referred to as a **stationary phase**. The stationary phase is usually a chemical that can selectively attract components in a sample mixture. The stationary phase is usually contained in a tube of some sort called a **column**. Columns can be glass or stainless steel of various dimensions.

The mixture of compounds in the mobile phase interacts with the stationary phase. Each compound in the mixture interacts at a different rate. Those that interact the fastest will exit (**elute** from) the column first. Those that interact slowest will exit the column last. By changing characteristics of the mobile phase and the stationary phase, different mixtures of chemicals can be separated. Further refinements to this separation process can be made by changing the temperature of the stationary phase or the pressure of the mobile phase.

Our GC has a long, thin fused silica column containing a thin interior coating of a solid stationary phase (5% phenyl-, 95% dimethylsiloxane polymer). This 0.25 mm diameter column is referred to as a capillary column. This particular column is used for semi-volatile, non-polar organic compounds. The compounds must me in an organic solvent.

The **capillary column** is held in an oven that can be programmed to increase the temperature gradually (or ramped), this helps separation. As the temperature increases, those compounds that have low boiling points elute from the column sooner than those that have higher boiling points. Therefore, there are

actually two distinct separating forces, temperature and stationary phase interactions mentioned previously. As the compounds are separated, they elute from the column and enter a detector. The detector is capable of creating an electronic signal whenever the presence of a compound is detected; the greater the concentration in the sample, the bigger the signal. The signal is then processed by a computer. The time from when the injection is made (time zero) to when elution occurs is referred to as the **retention time** (RT).



For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapor - slow injection of large samples causes band broadening and loss of resolution. The most common injection method uses a micro- syringe to inject sample through a rubber **septum** into a flash vaporizer port at the head of the column. The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

Since capillary columns need very little sample, **split/splitless injection** mode is used.

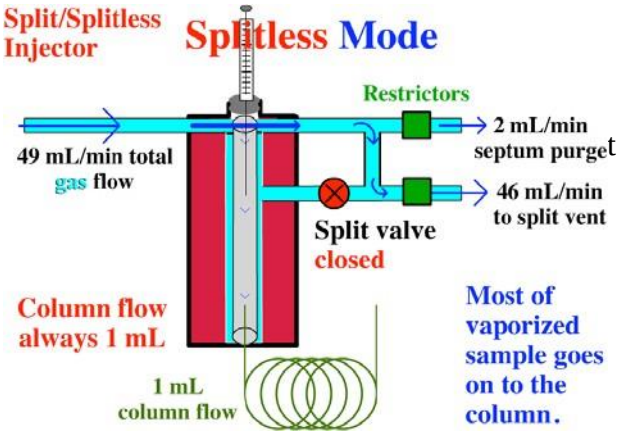
The injector contains a heated chamber containing a **glass liner** into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vaporizes to form a mixture of carrier gas, vaporized solvent and vaporized solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet.

The normal GC injector configuration is split mode as seen left. In this example, a total flow of 49 ml/min comes into the injector from the pressurized carrier gas tank (He is most common; N_2 is cheapest but chromatographically less forgiving).

Note that the total gas flow flows:

1) through the column (green thing at bottom),
2) out the septum purge, and
3) out the split vent and always add up to the incoming flow, 49 ml/min in this example (1 + 2 + 46 = 49 ml/min).

As the above image shows the gas flow passes through the septum purge and the split vent and some of the sample injected into the injector will get vaporized and escape through the split vent. In this example, the split ratio is 1/49 because 49 parts are injected and 1 part goes on column.



race analysis split injection is used. In this mode, the entire analyte sample vaporized in the injector goes onto the column. This is called the splitless mode. All the analyte mass in a 1 µl injection goes onto the column, it is not split.

Finally, the **septum purge** outlet prevents septum bleed components from entering the column. The constant septum purge flow sends clean, carrier gas past the bottom on the septum and out to waste (in this example it is 2 ml/min in whatever mode (splitless or split) the analyst uses). This helps keep the septum clean and prevents sample adsorbed on the septum from getting into the gas in the injector thus creating ghost peaks; that is, analyte peaks that are carried-over from one injection where their concentration is very high to another where they are not supposed to be at all.

While the instrument runs, the computer generates a graph from the signal, called a **chromatogram** (see figure 1). Each of the **peaks** in the chromatogram represents the signal created when a compound elutes from the GC column into the detector. The x-axis shows the RT, and the y-axis shows the intensity (abundance) of the signal. In Figure 1, there are several peaks labeled with their RTs.

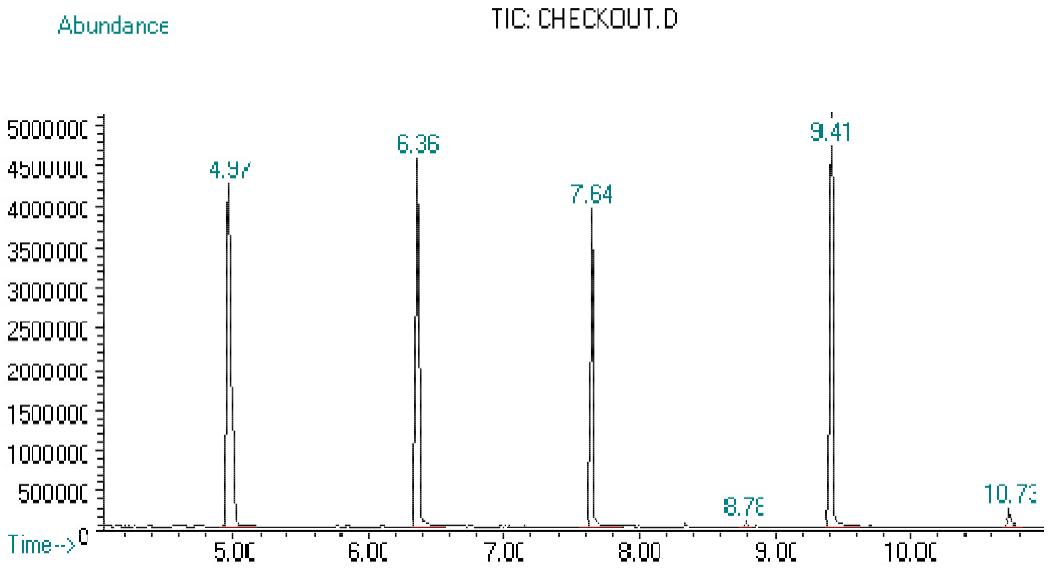


Figure 1: Chromatogram generated by a GC.

If the GC conditions (oven temperature ramp, column type, etc.) are the same, a given compound will always exit (elute) from the column at nearly the same RT. By knowing the RT for a given compound, we can make some assumptions about the identity of the compound. However, compounds that have similar properties often have the same retention times. Therefore, more information is usually required before an analytic al chemist can make an identification of a compound in a sample containing unknown components

Mass Spectroscopy

As the individual compounds elute from the GC column, they enter the **electron ionization** (mass spec) detector. There, they are bombarded with a stream of electrons causing them to break apart into **fragments**. These fragments can be large or small pieces of the original molecules.

The gas molecules exiting the GC are bombarded by a high-energy electron beam (70 eV). An electron which strikes a molecule may impart enough energy to remove another electron from that molecule. Methanol, for example, would undergo the following reaction in the ionizing region:

$$CH_3OH + 1\ e^- \_\_\_\_\_ CH_3OH^{+\bullet} + 2\ e^-$$

(The symbols $^{+\bullet}$ indicate that a radical cation was formed)

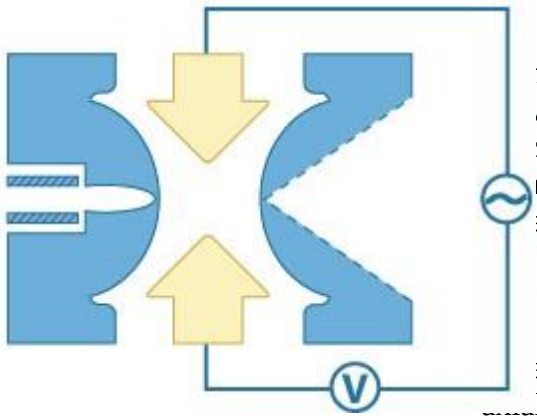
Electron impact Ionization (EI) usually produces singly charged ions containing one unpaired electron. A charged molecule which remains intact is called the **molecular ion**. Instability in a molecular ion, due to the energy imparted by the electron impact, can cause that ion to break into smaller pieces (fragments). The methanol ion may fragment in various ways, with one fragment carrying the charge and one fragment remaining uncharged. For example:

$$CH_3OH^{+\bullet}\ (\text{molecular ion}) \longrightarrow CH_2OH^{+}\ (\text{fragment ion}) + H^{\bullet}$$

(or)

$$CH_3OH\ (\text{molecular ion}) \longrightarrow CH_3^{+}\ (\text{fragment ion}) + {}^{\bullet}OH$$

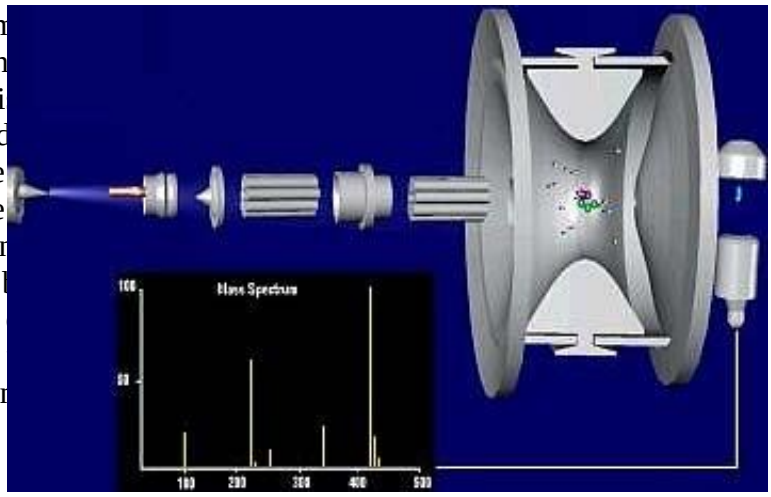
The fragments are charged ions with a certain mass. The mass of the fragment divided by the charge is called the mass to charge ratio **(m/z)**. Since fragments produced by EI have a charge of +1, the m/z represents the molecular weight of the fragment.



Ions created in the EI source are focused into the **ion trap**. The consists of two hyperbolic metal electrodes with their focii ng each other and a hyperbolic ring electrode halfway veen the other two electrodes. The ions are trapped in the e between these three electrodes by AC ~1MHz and DC -oscillating, static) electric fields. The AC radio frequency age oscillates between the two hyperbolic metal electrodes at top' and 'bottom' of the trap ('top' and 'bottom' are in phase) the hyperbolic ring electrode that forms the 'side' of the trap. ions are first pulled up and down axially while being pushed adially. The ions are then pulled out radially and pushed in ally (from the top and bottom). In this way the ions move in a complex motion that generally involves the cloud of ions being long and narrow and then short and wide, back and forth, oscillating between the two states.

The time during which ions are allowed into the trap, termed the "ionization period", is set to maximize signal while minimizing space-charge effects. The ion trap is typically filled with helium to a pressure of about 1 mtorr. **Collisions** with helium dampen the kinetic energy of the ions and serve to quickly focus trajectories toward the center of the ion trap, enabling trapping of injected ions. Ion traps are unique in their ability to perform **multiple stages of mass spectrometry** (**MS^n**), enormously increasing the amount of information obtainable from a molecule. Waveforms are constructed to isolate an ion, induce its fragmentation, then isolate one of the products, induce its fragmentation, etc. Finally, the resultant ions from all of the manipulations are ejected from the trap and detected.

The dynamic range of ion traps is lim
because, when there are too many ions in
trap, space charge effects lead to dimini
performance. Automated scans are used
rapidly count the ions before they go into the
so that the time ions are allowed to enter the
is dependent on the ion flux. This ensures or
certain number of ions get in. This can l
problem when trace elements in particularly
matrices are analyzed because the trap fills
both matrix ions (large number) and trace sar
ions (very small number).



The computer records a graph for each scan. The x-axis represents the m/z ratios and the y-axis represents the signal intensity (abundance) for each of the fragments detected during the scan. This graph is referred to as a **mass spectrum** (see Figure 2).

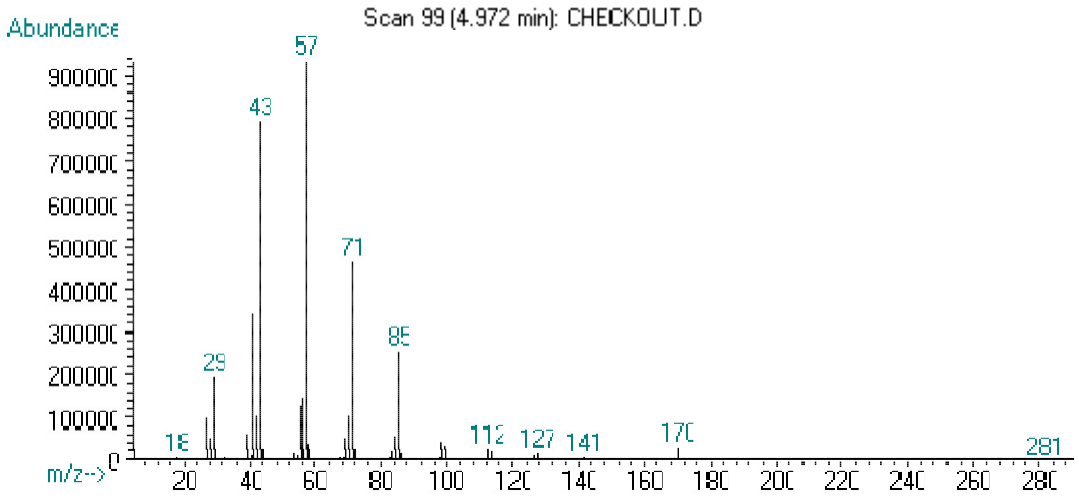


Figure 2: Mass-spectrum generated by an MS.

The mass spectrum produced by a given chemical compound is essentially the same every time. Therefore, the mass spectrum is essentially a fingerprint for the molecule. This fingerprint can be used to identify the compound. The mass spectrum in Figure 2 was produced by dodecane. The computer on our GC-MS has a library of spectra that can be used to identify an unknown chemical in the sample mixture. The library compares the mass spectrum from a sample component and compares it to mass spectra in the library. It reports a list of likely identifications along with the statistical probability of the match.

Following is some general information which will aid EI mass spectra interpretation:

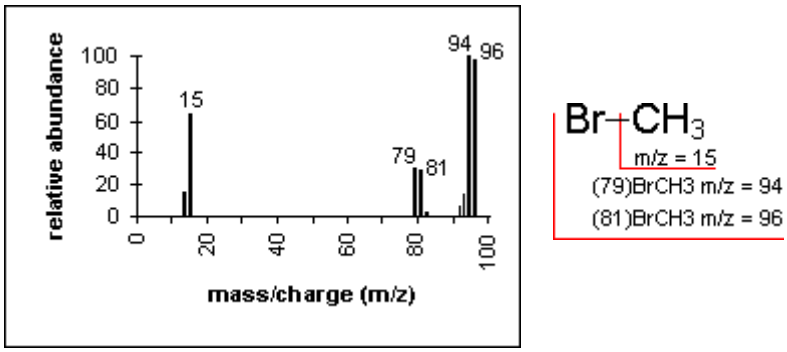
- **Molecular ion** ($M^{\cdot+}$): If the molecular ion appears, it will be the highest mass in an EI spectrum (except for isotope peaks discussed below). This peak will represent the molecular weight of the compound. Its appearance depends on the stability of the compound. Double bonds, cyclic

structures and aromatic rings stabilize the molecular ion and increase the probability of its appearance.

- **Fragmentation**: General rules of fragmentation exist and are helpful to predict or interpret the fragmentation pattern produced by a compound. Functional groups and overall structure determine how some portions of molecules will resist fragmenting, while other portions will fragment easily. A detailed discussion of those rules is beyond the scope of this introduction, and further information may be found in mass spectrometry reference books.
- **Isotopes**: Isotopes occur in compounds analyzed by mass spectrometry in the same abundances that they occur in nature. A few of the isotopes commonly encountered in the analyses of organic compounds are below along with an example of how they can aid in peak identification.

Element	Isotope	Relative Abundance	Isotope	Relative Abundance	Isotope	Relative Abundance
Carbon	^{12}C	100	^{13}C	1.11		
Hydrogen	^{1}H	100	^{2}H	0.016		
Nitrogen	^{14}N	100	^{15}N	0.38		
Oxygen	^{16}O	100	^{17}O	0.04	^{18}O	0.20
Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0

The ratio of peaks containing ^{79}Br and its isotope ^{81}Br (100/98) confirms the presence of bromine in the compound.



Conclusion

When GC is combined with MS, a powerful analytical tool is created. A researcher can take an organic solution, inject it into the instrument, separate the individual components, and identify each of them. Furthermore, the researcher can determine the quantities (concentrations) of each of the components after careful calibration.

UNIT-IV

LIQUID-LIQUID PARTITION CHROMATOGRAPHY

Principle: The basic principle involved in liquid- liquid chromatography is partition mixture of components separated in to individual components different partition between two immiscible phases.

In liquid –liquid chromatography stationary phase is liquid coated on solid. Mobile phase is liquid- liquid chromatography classified into two types.

1. Normal phase liquid chromatography
2. Reverse phase liquid chromatography

Normal phase chromatography : Stationary phase –polar

Mobile phase-non polar

Example for stationary phase, supported particle- Alumina, silica, diatomaceous cellulose and sartch etc., these are acts as supporting particles in liquid chromatography.

Stationary phase in normal phase- Nitro methane, ethylene diamine, water, poly ethyl glycol

Normal polar solvents acts as mobile phase: Butanol, iso –butanol, N-hexane,CCl_4 and chloroform.

Reverse phase chromatography:

Stationary phase: Silicon dimethyl poly slaxanes,

Mobile phase: water, methyl cyanide, water+ acetonitrile, methanol, ethyl alcohol and methanol+ water

Instrumentation of HPLC: (High pressure liquid chromatography or High performance liquid chromatography)

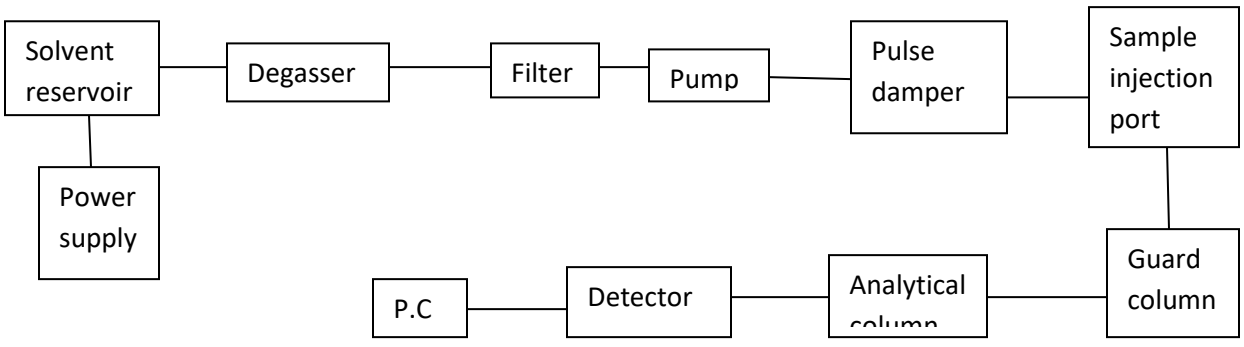
Principle : Mixture of non- volatile components separated into individual components and qualifying them.

Mixture of non – volatile components separated into basing on their different partition between two immiscible lphase by applying the mobile phase.

Stationary phase- liquid coated on solid

Mobile phase-liquid

Block diagram



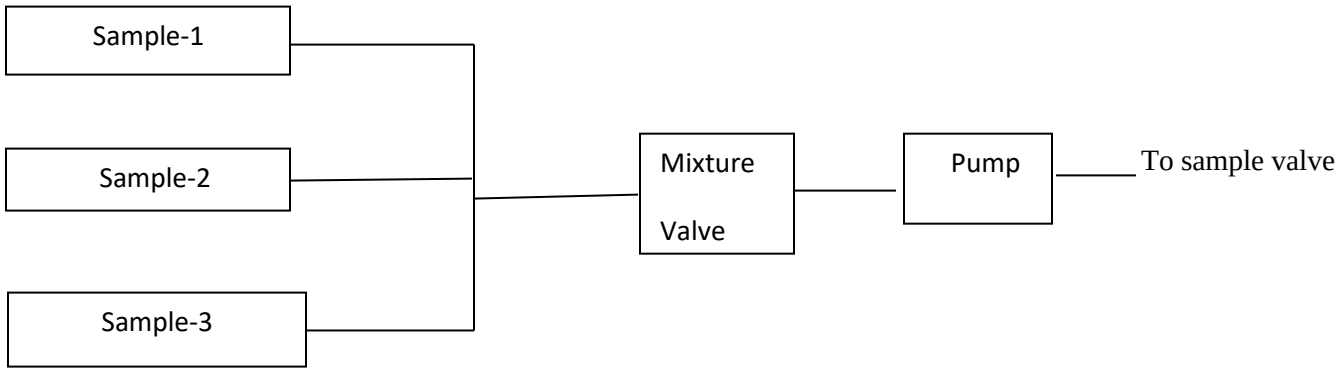
Solvent reservoir system:

Initially solvent reservoir system contains magnetic stirrer. Solvent reservoir is divided into 2 types

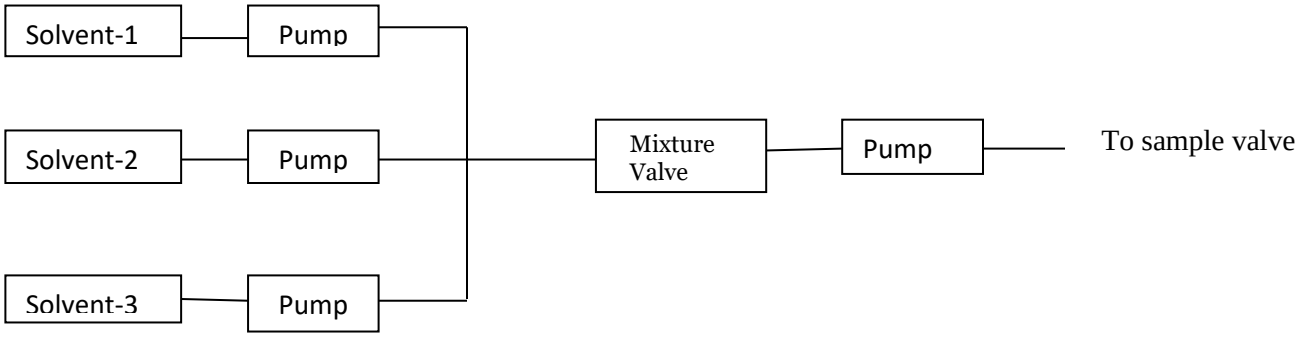
1. Low pressure reservoir system
2. High pressure reservoir system

HPLC grade solvent is 99% pure and mixture of grade solvents taken as mobile phase.

Low pressure reservoir system:



Here in low pressure system mixture of solvent collected to single pump system



Mixture of solvents I,II,III connects individual pump system.

Generally methanol+ water (HPLC grade), acetonitrile+water taken as mobile phase in HPLC . in this methanol+ water has less dispersive nature then acetonitrile+water.

Less dispersive or more polar components separated by using methanol water.

More dispersive or non polar components separated by using acetonitrile+water.

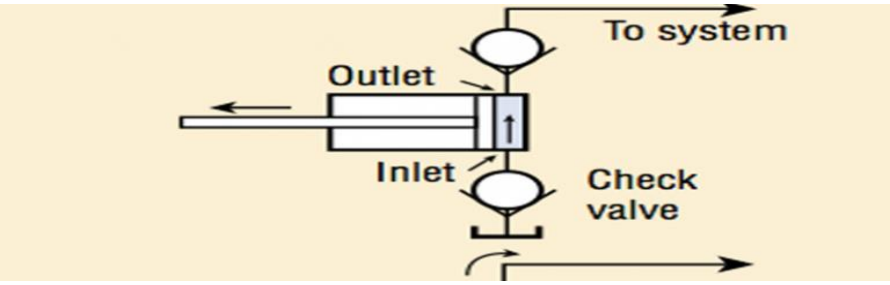
Tetrahydrofuran also acts as mobile phase in HPLC

Solvent degasser system: solvent degasser system is used to remove unwanted air particles present in the mobile phase. That means unwanted particles (impurities) presents in M.P they can be captured by the nitrogen passed through the M.P

Pump system:

Pumps are used to deliver the M.P to the sample injection port

Pneumatic pump system:

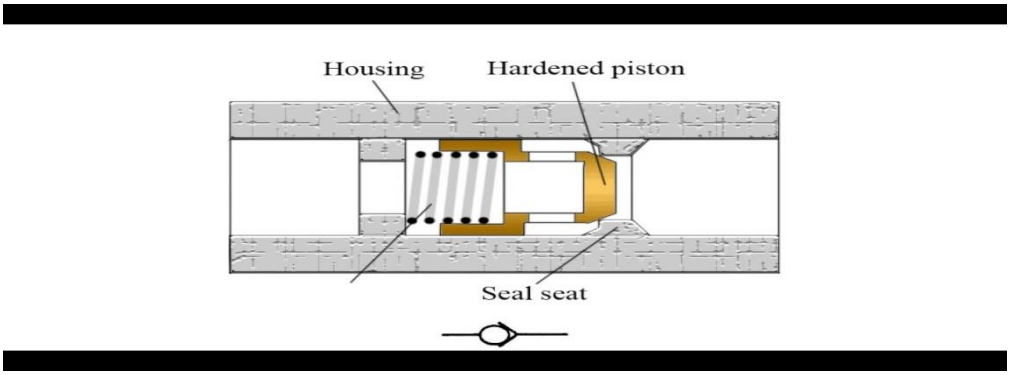


Pumps are delivered the M.P due to back and front movement of piston present in pumps. Pneumatic pumps consists non return valves and air enters port-1 and applies to the upper system which is directly transmitted to the lower piston.

If connected to a liquid chromatogram or column liquid will flow out the left hand side non return valve. This will continue until the maximum movement piston is reached.

Themicroswitch is activated and air pressure transports the port -2.

Non return valve :



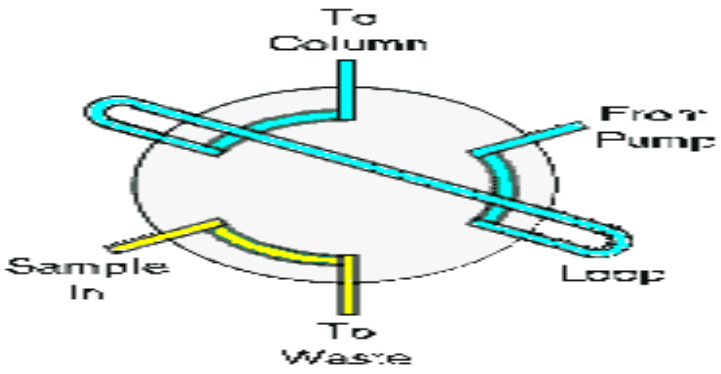
Non return valve consists sapphire seat and sapphire ball. Sapphire ball is rest in a conical seat. The solvent enters through the valve seat and exist above the sapphire ball.

When solvent flow passes though the wall the ball is displacement from seat and the liquid passes it

When the inlet pressure falls the ball is back on the seat.

It can minimize the back flow of M.P

Sample injection port:



Sample injection ort which is consists of stainless steel with six different ports. On to the column.

Teflon with in the ring has three open segments each of which connects a pair of external sample loop of know fixed volume.

In one configuration the cone permits direct flow of mobile phase to the column. And then loop can be filled with sample the cone can be rotated30^0 to make the sample loop connected to column.

Sample can be introduced in sample injection port while using micro syringes initially components are dissolved in a corresponding solvent

Columns:

Generally used HPLC columns are stainless steel quartz, fussed silica and Teflon columns.

Quarts silica columns more inert.

Glass columns with standard high pressures but difficult to know when the glass is broken.

Titanium columns with standard high pressures up to 5000 TSA

Poly ether columns, all organic compounds except methylene chloride separated by using poly ether columns.

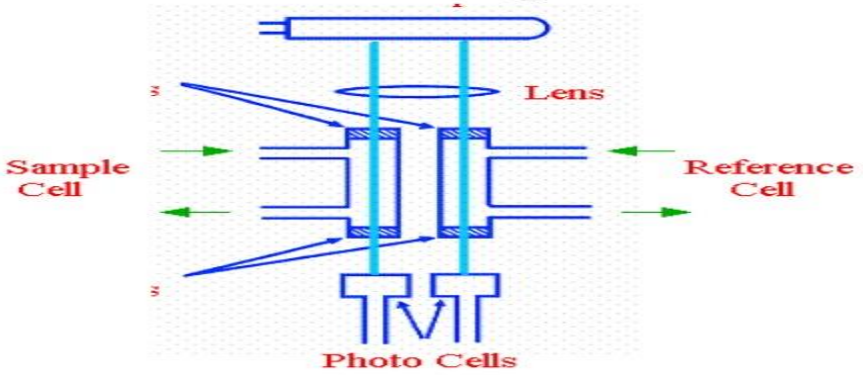
Generally column length 10-15cm, 45mm inner diameter 0.16 cm thickness

Detectors in HPLC:

1. Fixed wave length detector
2. Variable wavelength detector
3. Diode array detector
4. UV fluorescence detector
5. Refractive index detector

Fixed wavelength detector:

Fixed wavelength detectors



Fixed wavelength detector is a specific detector.

The back ground single from the M.P is minimize by using single wavelength detector or fixed wavelength detector

As consequence the detector is also response to both the sample and M.P.

In single or fixed wavelength detector the light emitted at a single wavelength from mercury lamp.

One wavelength with most single wavelength UV lamps other wavelengths also present but other wavelengths has lower intensity when compared with single wavelength.

The single wavelength is produced from mercury lamp. And then passed through quartz lens system into a sample cell and reference cell.

The lens system means it can collimate the radiation to sample cell and reference cell.

Finally the transmitted length focused on photo cell.

The intensity of photo cell can be measured by using Beer's lamberts law.

According to Beer's Lamberts law

$$I_t = I_o \times e^{-KCl}$$

Where Intensity of transmitted light

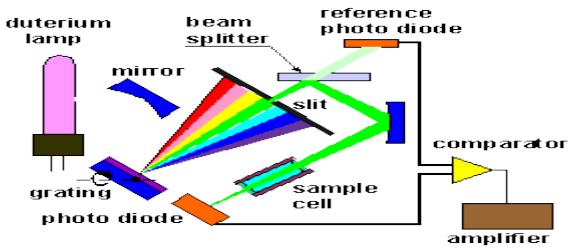
Io= intensity of light when solutes enters into the cell or solutes present in sample cell,

L=path length of cell

C=concentration of the solute present in sample

K= molar adsorption coefficient

Variable wavelength detector:



All the organic and inorganic compounds are separated by using variable wavelength detector.

Variable wavelength detector employs light that emit light over a wide range of wavelength. And by using a monochromator light of particular wavelength can be selected for detection purpose

Light from deuterium lamp is focused means of two curved mirrors on to a diffraction grating and the selected wavelength is reflected back on to the curved mirror. The light then passes through third curved mirror and then focused via plane mirror and a quartz lens through the detector absorption cell.

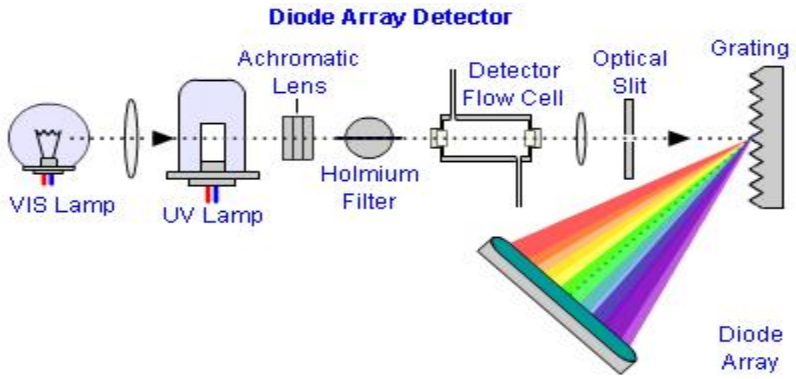
After leaving the absorption cell the transmitted light is caucused by second quartz cell on to the photo cell.

Rotation of the grating allows the wavelength of the light passed through cell to be selected.

Beam splitter is used for it can split the radiation. In which one part of radiation is passed through sample cell other part of radiation cell is passed through reference cell.

Mirrors are used for to adjust the light horizontally or vertically

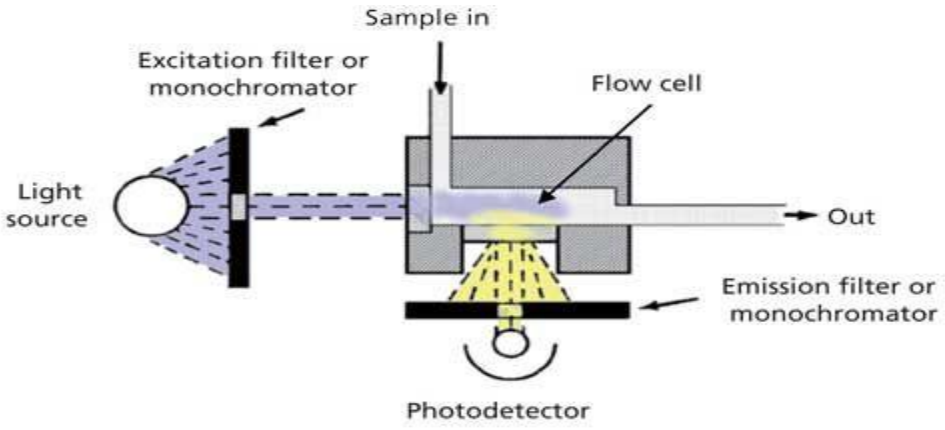
Diode array detector:



A diode array instrument is power ful for study store ancient intermediates I moderately past reaction for kinetic studes and for the qualitative and quantitative determination in the component exciting from a liquid chromatographic colun. Or a capillary electrode persists column. Radiation from a lamp focus on the sample solvent container and then passes into a monochromator with a fixed gradually the dispersive radiation falls on the photo diode array detector

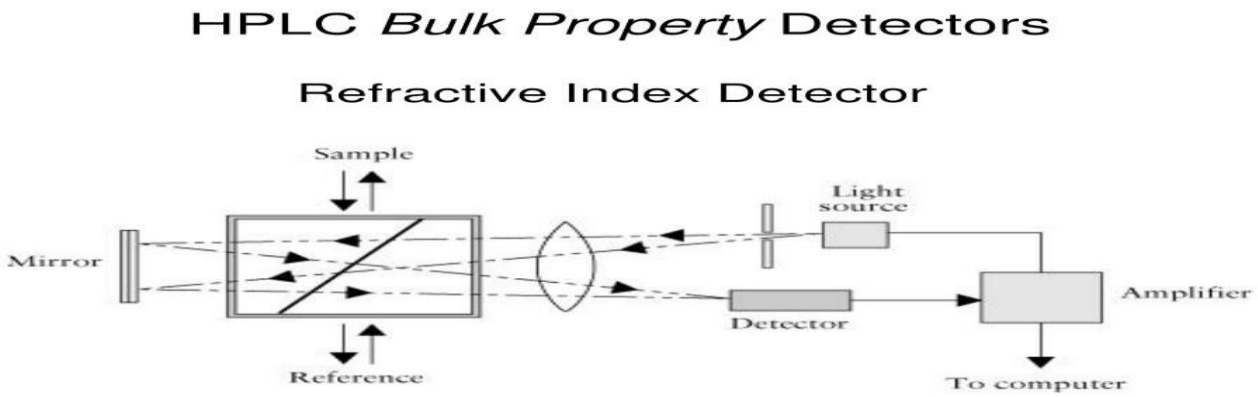
“a single silicon crystal is called chip” it contains several hundred of silicon photo diode connected on series in a single silicon crystal each photo diode has internal capacitor which measure the generator photo current. That means they integrate. The photo current is directly proportional to the concentration of the analyte.

UV-Fluorescence detector:



- UV fluorescence detector is a selective detector for the components having florescence nature.
- The UV radiation produced from the UV lamp is passed through the lens and grating system, wavelength of the radiation can be selected by suing grating.
- Due to rotation of the grating the sufficient excitation is passed through the sample cell.
- The components present in the sample cell these are captured the radiation and then excited to higher excited states. And then finally we can calculate the intensity of emitted solution from solutes by using photo cell.
- Photo cell means the intensity of radiation is converted into electrical signal.

Refractive index detector:



Refractive index detector is bulk detector and it is called as "Universal detector". Response of RI detector does not depend on the functional group present in sample the basic principle involved in RI detector is deflection of the beam is caused by the refractive index of the contents containing sample and reference cell.

R.I=velocity of light in air/velocity of light in column

Initially the light is produced from tungsten lamp or Mercury lamp then the light focused by the optical mass then the mass contains the light region of the cell,

- And the strikes on lens system lens collimates the radiation
- The beam of light is passed through sample and the reference cell is plane mirror
- Reflex beam of light from plane mirror.

Applications:

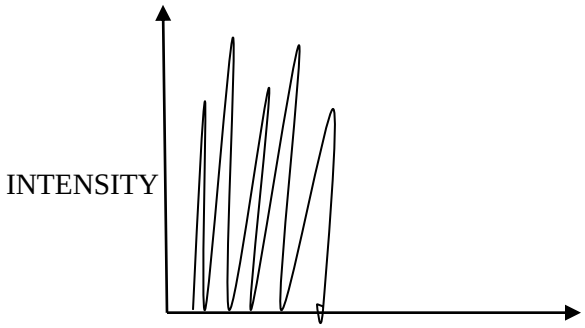
Separation of synthetic cartico steroids (drugs)

Column: Micro pack Si-10

Mobile Phase: 5%ethanol in methylene chloride

Flow rate: 1.55mL/min, pressure: 1000 Si

Detector: Refractive index detector



1. Triamino silanane
2. 6 methyl prednisone
3. Hydro cortisone
4. Dexa metasone
5. Prednisone
6. Separation of drugs of a horse plasma

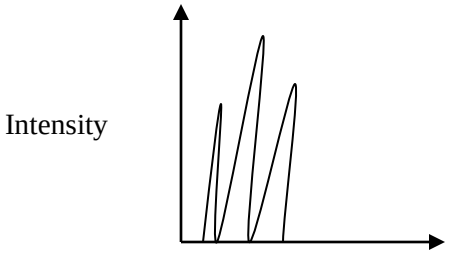
Column: Isri 5 Micron column (1.5cmX4.6)

Mobile Phase: 84% 0.1M KH3PO5 (PH 6.8), 10% Isopropanol, 6% tetra hydro furan

Flow rate: 1mL/min

Detector: UVdetector

CHROMATOGRAM:



1. Oxphen butazone
2. Phenyl nutazone
3. Furosemide

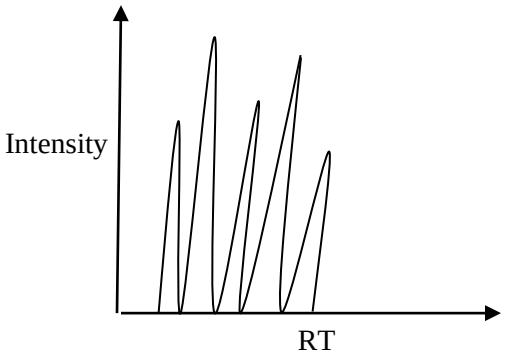
Separation of carbohydrates by using HPLC (organic compounds)

Column: Super cosil NH_2(30cmX4.6ID)

Mobile Phase: Acetonitrile+water(3:1)

Flow rate: 2mL/min,

Detector: Refractive index detector



- Ribose
- Galactose
- Maltoe
- Sucrose
- Rafinose

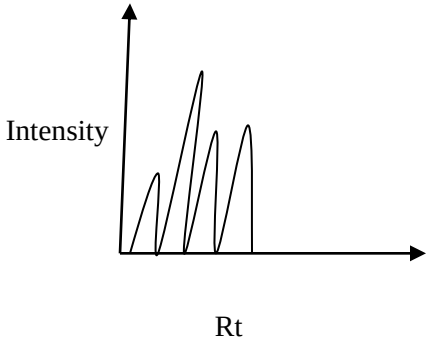
Separation of polycyclic aromatic hydro carbons (Organic compounds)

Column:C18 Reverse phase (30cmX4.6mmID)

Mobile Phase: Acetnitrile+water(9:1)

Flow rate: 5mL/min

Detector: UV florescence detector



1. Naphthalene
2. Anthracene
3. Benzo anthracene
4. Benzo pyrene

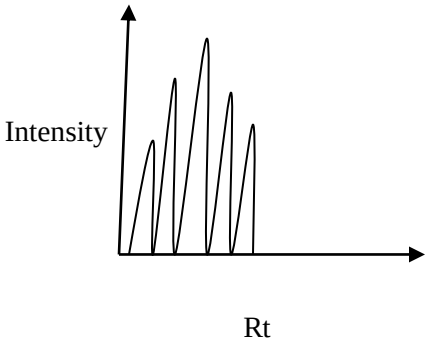
Separation of Di and Tri carboxylic acids

Column: Micropak(15cmX4.6mmID)

Mobile Phase: Acetnitrile

Flow rate: 1mL/min

Detector: UV detector



1. Succinic acid
2. Malic acid
3. α Keto glutaric acid
4. Furaric acid
5. Citric acid

Liquid Chromatography-Mass Spectrometry (LC-MS)

The High Performance Liquid chromatography (HPLC) is one of most common analytical technique used in pharmaceutical industry for determination and quantification of drug substances and its related substances. Due to high reproducibility and accuracy, HPLC is routinely used in pharmaceutical, chemical and pesticide industries.

The Liquid Chromatography-Mass Spectrometry (LC-MS) is hyphenated analytical technique which is combination of Liquid Chromatography (LC) and Mass Spectrometry (MS). HPLC (LC) separates the components of mixtures by passing through chromatographic column. Generally, the separated components cannot be positively identified LC alone. Mass Spectrometry is also used for identification of unknown compounds, known compounds and to elucidate the structure. Mass spectrometry is alone not good for identifying mixtures because mass spectrum mixture is actually complex of overlapping spectra from separated individual components. It is difficult to connect Liquid chromatography (LC) with Mass spectrometry (MS). An interface is used to transfer the liquid eluents from LC to MS.LC-MS is more significantly used in invite dissolution, bioavailability, bioequivalence and pharmaco- dynamics studies . Preparative LC-MS systems can be used for rapid mass-directed purification of specific substances from such mixtures that are important in basic research, pharmaceutical, agrochemical, food and other industries .

Instrumentation

Liquid chromatography-mass spectrometry (LC-MS)

The Liquid Chromatography-Mass Spectrometry (LC-MS) is combination of Liquid Chromatography and Mass Spectrometry which is used with separation power of HPLC with detection power of Mass Spectrometry (MS). The schematic block diagram of LC-MS is shown in below figure 1. The different parts of LC-MS instrument are listed as below.

a. Liquid Chromatography (LC)

b. Mass Spectrometry (MS)

Liquid Chromatography (HPLC): The Liquid Chromatography (LC) is a high performance liquid chromatography in which separation of components of mixture can be carried out by using liquid mobile and solid stationary phase. There are different types of

chromatography like normal phase liquid chromatography, Reversed phase chromatography, Ion-exchange liquid chromatography, Chiral separation and affinity liquid chromatography . By using different packing of columns with high efficiency small amount of complex mixture can be separated. The components of HPLC are listed below

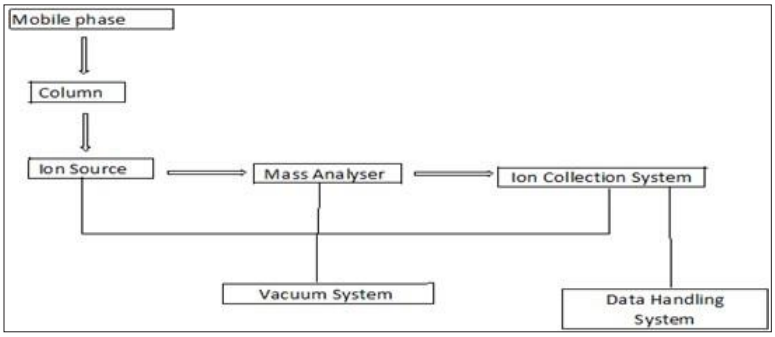


Figure 1: Schematic Block Diagram of LC-MS System.

a. **Pump:** It consists of material which is inert towards solvents or any mixed composition of aqueous buffer and organic solvents. It delivers high volume of mobile phase up to 10mL/min. There are three major types of pumps are used

i.e. reciprocating pump, Syringe pumps and constant pressure pumps.

b.Sample injector: It is used to introduce sample volume into the chromatographic system. Generally sample volume from 1µL to 100µL can be injected. The injection volume can be increase by injector loop up to 2mL volume. There are two major types of injectors used i.e. Automatic injectors and Manual injectors. Automatic injectors are more comfortable and user friendly and are more accurate and precise as compare to manual injectors [3].

c. **Columns:** It is stationary phase which consists of silica material in combination with carbon chain. Generally the column length used is about 50mm to 300mm. The columns used in HPLC are consists of Octadecyl (C18), Octyl (C8), Cyano, Amino, Phenyl packing's. The columns are used on the basis of nature of compounds to be separated [4].

d.Detectors and recorder: The detectors is most important part of HPLC .There are different types of detectors used are UV-Visible detectors, PDA detectors, Refractive index (RI) detectors, Electrochemical detector, Fluorescence detectors and conductivity detectors. The signal received from detector can be recorded as peak and respective data can be stored in a software.

Mass spectrometry: Mass Spectrometry is analytical technique based on the measurement of the mass to charge ratio of ionic species related to the analyte under the investigation.MS can be used to determine the molecular mass and

elemental composition of an analyte as well as in depth structural elucidation of the analyte [5]. In LC-MS there are two key components, ionization source and Interfaces. Below listed are the different components of Mass spectrometers as below.

a. Ionization Sources and Interfaces

b. Mass Analysers

Ionization/Ion Source and Interfaces: The Liquid chromatography separates mixture of components which are in liquid form, usually contains methanol, acetonitrile and water. This liquid containing mixture of components is transferred into the ion source of mass spectrometer. As ion source is under high vacuum. Due to the difference in the pressure it is difficult to mass to vaporize the liquid drops without losing mixture of components. Hence interfaces are used to resolve this problem. The different types of interfaces commonly used in mass spectrometer are described as below.

a. **Direct liquid Introduction (DLI):** The ionization in Direct Liquid Introduction (DLI) is generally accomplished by vaporizing solvent as a chemical ionization and reagent gas. Both the normal and reverse phase solvent system have been used. Reverse phase solvents used are methanol/water, acetonitrile/ water mixture up to 60% water. In general buffer with salts are not allowed as there is chance of capillaries to plug when heated.

The operation of Direct Liquid Introduction (DLI) is combination of thermal energy and liquid flow rate. The liquid enters the interface at limited flow rate only. The analyte ions produced with the help of thermal energy then transferred into ion source through capillary inlet or pinhole diaphragm

Atmospheric-Pressure Ionization (API): In Atmospheric-pressure ionization (API) contains three major steps i.e. Nebulisation, Evaporation and Ionization. There are two main modes of API are Electrospray Ionization (ESI) and Atmospheric-pressure ionization (APCI). In Atmospheric- pressure ionization (API), when stream of liquid (solvent) containing a sample is passed through narrow capillary tube and nebulized at large chamber, mist of small droplets is produced .The ionization process takes place and the proportion of droplets carry an excess of positive or negative electric charge .In large heating chamber the evaporation of solvent takes place. The solvent evaporates from the droplets to form smaller and smaller. The collision takes place between the molecules and ions. The resulting ions then passed through capillary into mass analyser

The Atmospheric-pressure ionization (API) is technique used for wide range of polar and non-polar analytes of moderate molecular weights.

Electrospray Ionization (ESI): The Electrospray Ionization (ESI) is most useful ion source developed by Fenn and his colleague's. In Electrospray Ionization (ESI) the liquid sample passed through a stain steel capillary tube which is maintained at high positive or negative electric potential about 3-5kV [1]. Due to this the charged droplets are formed at the capillary tip which are then undergoes vaporization process. The solvent gets evaporated from droplets, and undergoes reduction in size and surface charge increases. The collision takes place until the highly charged droplets are converted into gas phase ions. These gas-phase ions pass through the capillary sampling orifice into the low pressure region of the ion source

The major advantage of ESI is that the ions are multiply charged, the number of charges increased by 1 to 3 for a molecule 1000Da or above 50000Da. This yields an m/z ratio that is always below 2000. LC-MS with an Electro spray ionization (ESI) is used to measure the molecular weight of peptides, Proteins, Biological samples, Polymers, nucleotides, sugars and organometallics. It is also used frequently in Biological research and medical analysis The Schematic diagram of ESI is shown figure .

b. **Atmospheric Pressure Chemical Ionization (APCI):** The Atmospheric Pressure Chemical Ionization (APCI) include two major steps, evaporation /desolvations of analytes and charged transfer reaction in vapour phase to generate the vapour phase ions.

In Atmospheric Pressure Chemical Ionization (APCI) liquid (solvent) containing sample is nebulised through narrow capillary tube and nebulized into large chamber. In large heating chamber the evaporation of solvent takes place at atmospheric pressure and small droplets are produced. The ionization takes place. Generally ionization takes place at 250 to 400 °C. The ions are then transfer the charges to molecules through chemical reactions. The resulting ions are pass through capillary orifice of mass analyser. It is widely used for less polar and non-polar analytes having moderate molecular weights

c. **Thermo spray and Plasma spray Ionization (TSPI):** The Thermo spray is used as both liquid inlet system as well as ionization source. Plasma spray is modification of thermo spray.

In Thermo spray the liquid sample solution is passed through capillary tube which is heated and which causes the evaporation of solvent. The charged droplets are formed. Due to evaporation of ions are then passed into mass analyser with electrostatic voltage system

The Plasma spray itself does not produce ions but the ions produce in thermo spray, with the help of corona discharge or plasma the number of ions can be increased. The electric discharge induces the more ionization in the neutral molecules. This enhancement increases the ionization of molecule. The plasma spray technique is more sensitive and it is widely used for analysis in clinical and medicine

Mass Analyser: After ionization the ions are transferred into mass Analyser where the separation of ions are done according to their mass to charge (m/z) ratio. Generally mass Analyser used is on its speed, time, rate and its reaction.

Below are the mass Analyser:

a. Quadrupole
b. Time of flight
c. Ion trap
d. FTICR (Fourier transfer ion cyclotron resonance)

a. **Quadrupole Mass Analyser:** It is the most useful and commonly used mass Analyser. It consists of two plain of parallel rods which are located between an ion sources and a detector. The mass Analyser i.e. separation of ion according to their m/z in either time or space [6].

The linear Quadrupole mass Analyser consists of four hyperbolic or cylindrical rods that are placed parallel in a radial array. Opposite rods are charged is a +ve or –ve direct current(DC) potential at which an oscillating radio frequency alternating current (RF) voltage is superimposed.

The combination of DC and RF applied to the rods, trajectories of the ions of one particular m/z are stable these ions are transmitted towards detector. On the other hand ions of unstable m/z are discharged on the rods.

The ions introduced into Quadrupole by mean of low accelerating potential. The ions are oscillating in plane perpendicular to the rod length as they trends through Quadruple filter.

Ions of carrying m/z consequently be travelled towards detector by applying DC and RF voltage at constant ratio. The resolution depends on ratio of DC and RF potentials. Generally the Quadrupole is operated at <4000 m/z and scan speed up to 1000m/z passes. The unit mass resolution means that mass accurately is seldom better than 0.1 m/z .

The RF values are generally in the range 1-2MHZ. The DC Voltage may be 1000V and Maximum RF voltage is 6000v. The schematic diagram of quadruple mass analyser is shown below figure 3.

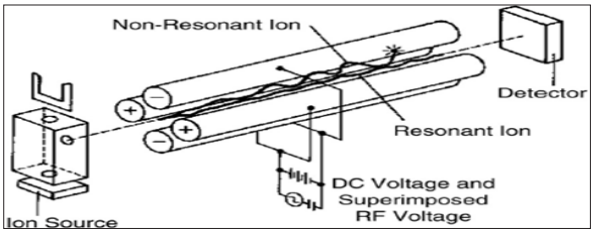


Figure 3: Schematic Diagram of Quadrupole Mass

Detectors

The detector is an important tool of mass spectrometer that produces the current that is proportional to the number of ions strike it. Once the ions are formed passed from analyser they have to be detected and transformed into signal. Below listed are the type of detectors commonly used.

Point Ion Collectors Detector:

In this the ions collectors are placed at fixed point in mass spectrometer. All the ions are focused upon the detector situated at single point. The arrivals of ions can be recorded by the flow of electric current and the data can be recorded. The electric current flow is proportional to the ions arriving at point ion detector.

Array Detector:

An Array detector is collection of point collectors placed in plane. The ions are arrived at a point or across the plane in array detector. The ions with mass to charge (m/z) values are separated and are recorded along plane using point ion collector. Spatially differentiated ions with the mass range are detected simultaneously at the same time in array detector

Applications

Application of LC/ESI-MS in In forensic sciences

LC-MS is used for determination of toxicity, in drug analysis and also in trace analysis. By using small amount of sample the toxins in different material can be determined with LC-MS. Any toxic metabolites in food or beverages can be determined by using LC-MS. E.g. Identification of detergent added into orange juice can be determined by analysing by the juice and detergent sample. The standard surfactant alkyl diphenylether sulphonic acid is used. Both juice and detergent samples are analysed in same chromatographic conditions. The mass chromatograms and mass spectra obtained from the juice and detergent samples are identical with the reference spectra of standard surfactant (alkyl diphenyl ethersulphonic acid)

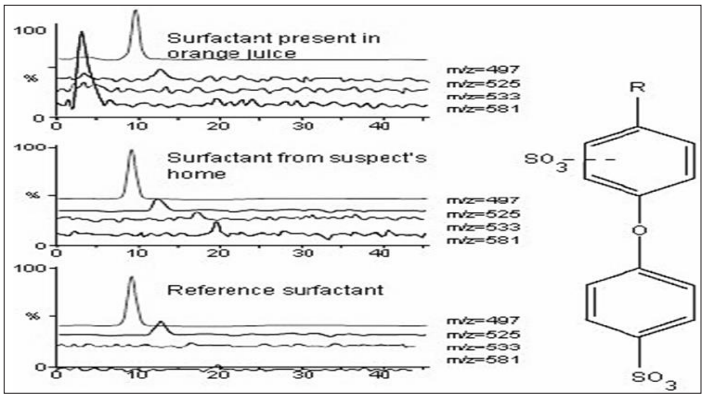


Figure 6: Extracted Ion Chromatogram of Orange Juice using LC-MS using ESI in negative mode.

Application of LC-MS in Doping Test:

The LC/ESI-MS with positive mode can be used for detection in urine of 4-Methyl-2-hexaneamine doping agent. The urine samples are analysed with addition of internal standard of Tuaminoheptane. The suspected primary amine 4-methyl-2-hexaneamine, an analog contained in dietary supplement, to be the unknown compound. The standard used is 4-methyl-2-hexaneamine which exhibits two unresolved peaks at RT 3.43min and 3.78min. which are identical with those of unknown compound . The single reaction monitoring (m/z 116-57) was specific for detection of 4-methyl-2-hexaneamine as shown below figure 7.

Other Applications:

In Pharmacokinetics:

LC-MS is used in the study of absorption, metabolism, and excretion of drugs. Bio analytical methods are used for quantitative and structural elucidation of drugs and its metabolites in the biological samples (plasma, urine, saliva, serum etc.)

In Bioavailability and Bioequivalence study:

Comparative bioequivalence studies in which quantitative determination of drugs or metabolites is measured in biological matrix, pharmacodynamics, clinical trials and In-vitro dissolution tests [27,28].

In determination of molecular weights:

LC-MS is used for determination of molecular weights of known and unknown compounds. It provides the information about molecular weight, structure, identification, quantity of sample components. LC-MS is used for determination of molecular masses of proteins, nucleic acids, polymers and peptides.

In determination of Assay of drug and intermediates:

LC-MS is used in pharmaceutical industry for determination of assay of drug substances, drug products, intermediates and their related compounds [3].

In Agrochemical and pesticides industry:

It is used in determination of different components present in the fertilizers and pesticides .

Environmental Applications:

LC-MS is used for detection of phenyl urea herbicides, detection of low level of carbaryl in food