CHROMATOGRAPHY

Tswett named this new technique chromatography based on the fact that it separated the components of a solution by color.

Chromatography

The word Chromatography is derived fro two Greek words –*Chroma* means – colour and *graphein* to write.

Chromatography is the collective term for a family of laboratory techniques for the separation of mixtures.

Chromatography- Principle

Chromatography is based on the principle of the partition of the solute between two phases/solvents. Chromatography usually consists of a mobile phase and a stationary phase.

1) The mobile phase usually refers to the mixture of the substances to be separated dissolved in a liquid or a gas.

2) The stationary phase is a porous solid matrix through which the sample contained in the mobile phase percolates. The interaction between the mobile and the stationary phases result in the separation of the compounds from the mixture.

3) These interactions include the physico chemical principles such as the adsorption, ion- exchange, molecular sieving and affinity.

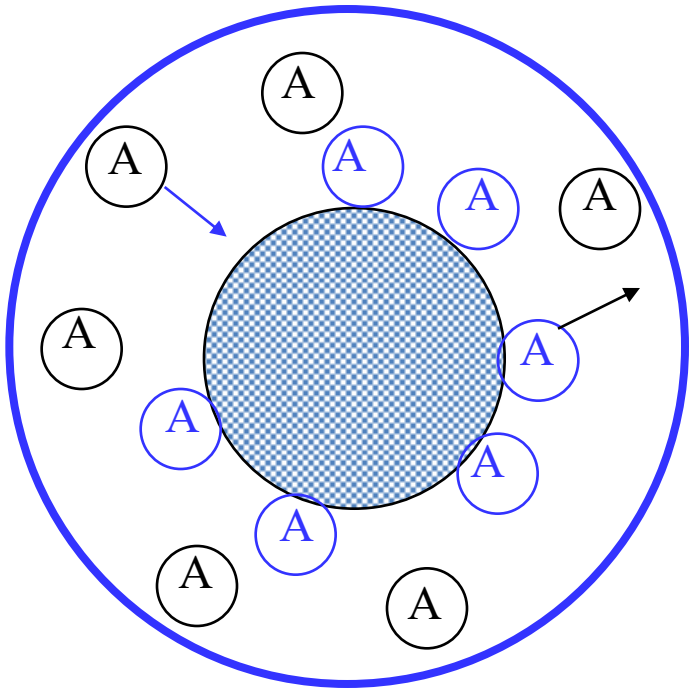
How Does Chromatography Work?

In all chromatographic separations, the sample is transported in a **mobile phase**. The mobile phase can be a gas, a liquid, or a supercritical fluid.

The mobile phase is then forced through a **stationary phase** held in a column or on a solid surface. The stationary phase needs to be something that **does not react** with the mobile phase or the sample.

The **sample** then has the **opportunity to interact** with the stationary phase as it moves past it. Samples that interact greatly, then appear to move more slowly. Samples that interact weakly, then appear to move more quickly. Because of this difference in rates, the samples can then be **separated into their components**.

Chromatography is based on a physical equilibrium that results when a solute is transferred between the mobile and a stationary phase.



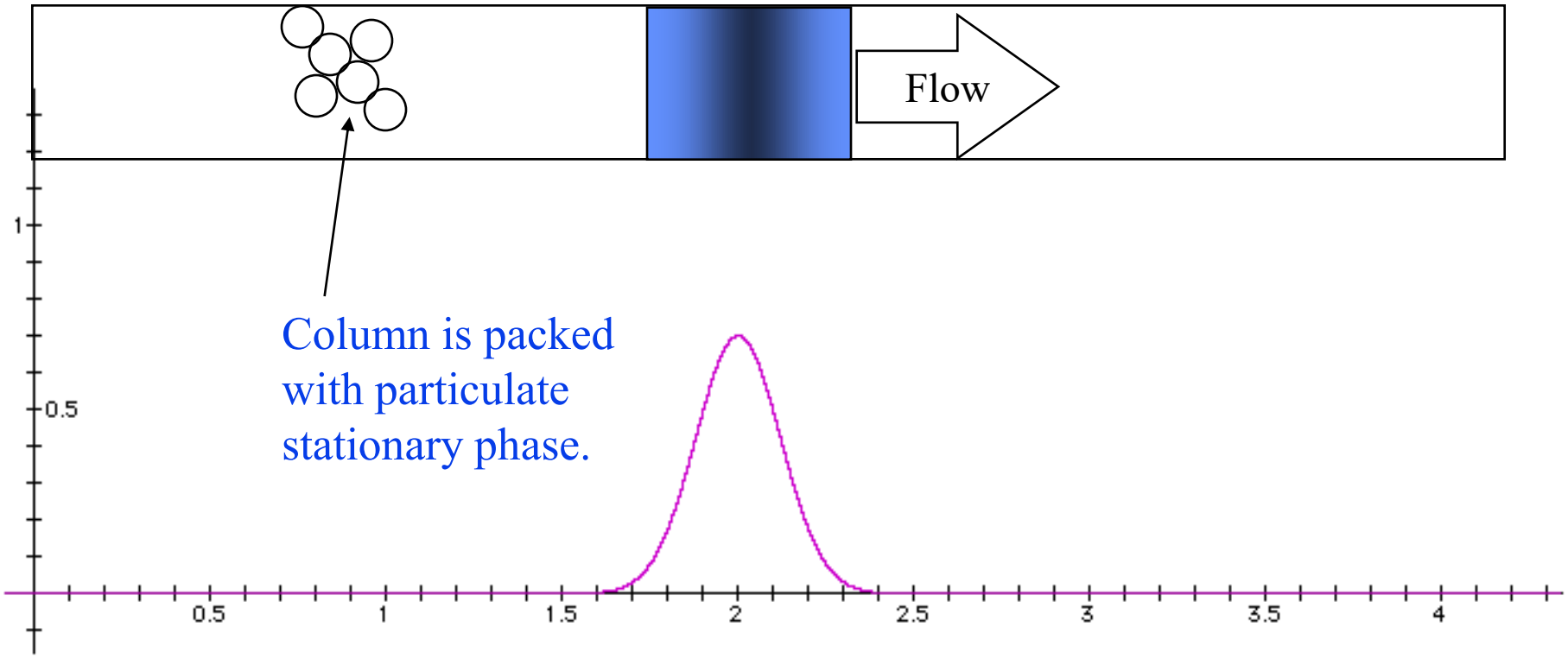
Cross Section of Equilibrium in a column.
"A" are adsorbed to the stationary phase.
"A" are traveling in the mobile phase.

K = distribution coefficient *or* partition ratio

$$K = \frac{C_S}{C_M}$$

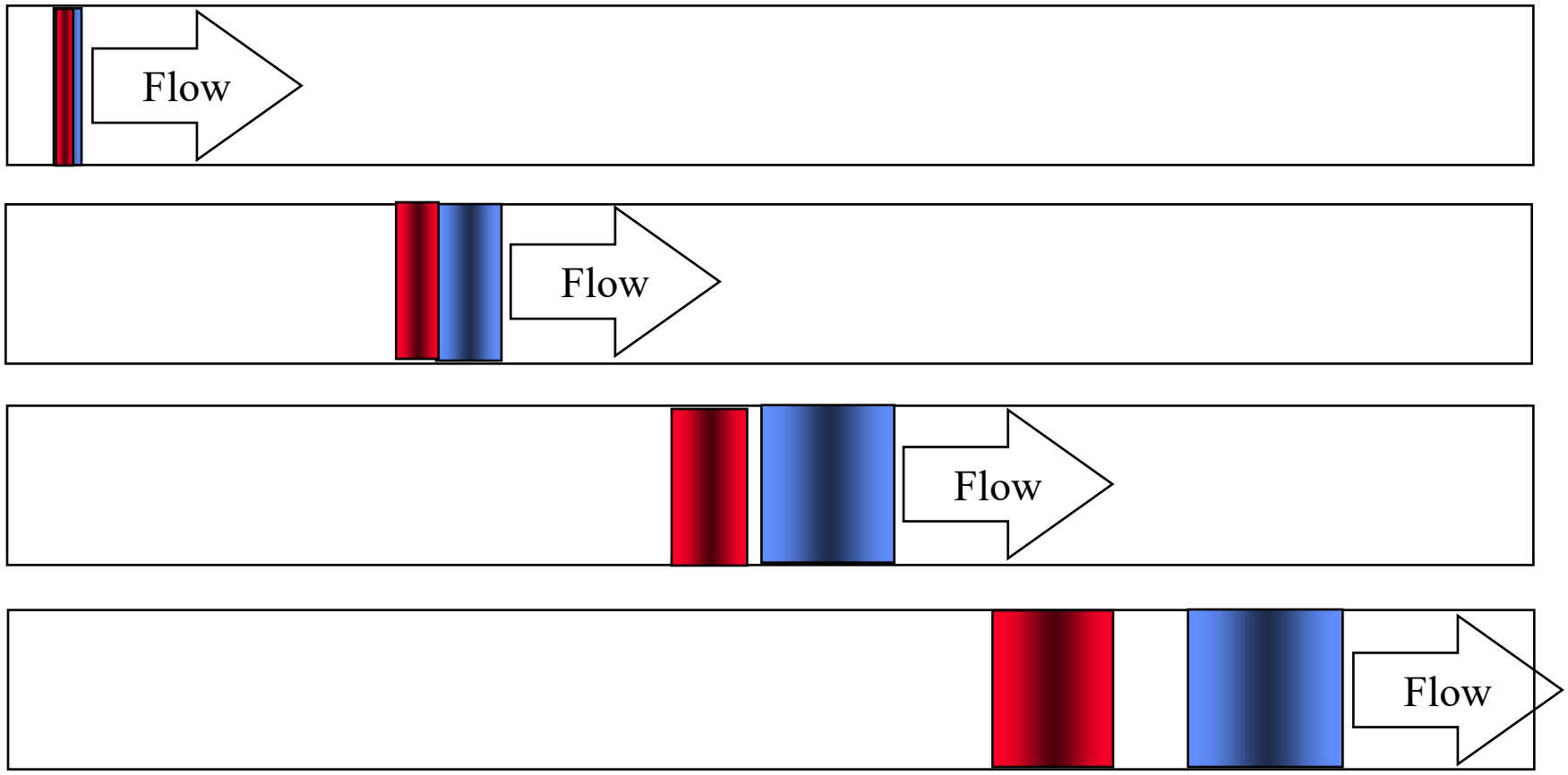
Where C_S is the molar concentration of the solute in the stationary phase and C_M is the molar concentration in the mobile phase.

In a chromatography column, flowing gas or liquid continuously replaces saturated mobile phase and results in movement of A through the column.



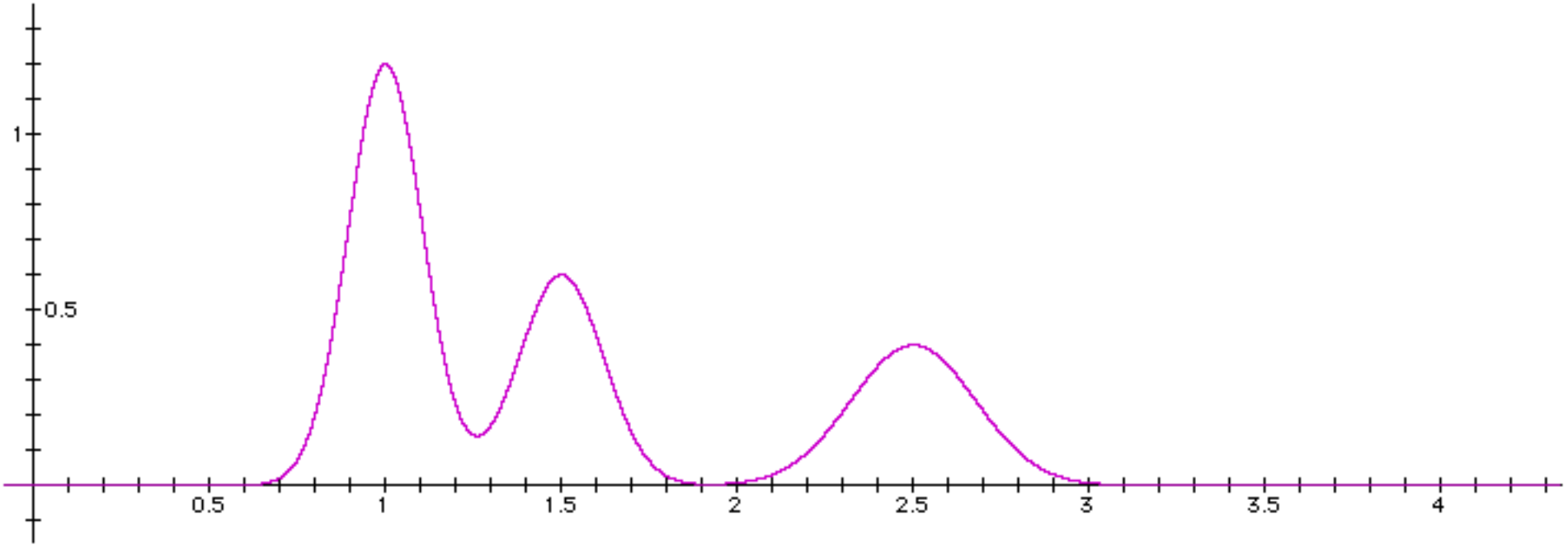
As a material travels through the column, it assumes a Gaussian concentration profile as it distributes between the stationary packing phase and the flowing mobile gas or liquid carrier phase.

In a mixture, each component has a different distribution coefficient, and thus spends a different amount of time absorbed on the solid packing phase vs being carried along with the flowing gas



More volatile materials are carried through the column more rapidly than less volatile materials, which results in a separation.

If a detector is used to determine when the components elute from the column, a series of Gaussian peaks are obtained, one for each component in the mixture that was separated by the column.



Note: The first two components were not completely separated.
Peaks in general tend to become shorter and wider with time.

Chromatography- Classification

Two ways to classify methodology of chromatography:

1)Based on interactions between sample component and stationary phase:

i) Partition

ii) Adsorption

iii) Ion-exchange

iv)Gel-filtration

v) Affinity

vi)High performance liquid chromatography

Partition Chromatography

It is used for the separation of mixture of amino acids and peptides. The molecules of a mixture get partitioned between the stationary and the mobile phase depending on the relative affinity of each one of the phases.

It is undertaken in two ways:

1) Paper chromatography
2) Thin layer chromatography

Paper chromatography

-An analytical technique for the separation and identifying mixtures that are either coloured or can be made coloured

-It is a liquid partition chromatography

-Used for separation of amino acids, sugars, sugar derivatives & peptides

Paper chromatography

1) The stationary phase is water held on a solid support of filter paper (cellulose).

2) The mobile phase is a mixture of immiscible solvents which are mixtures of water, a non polar solvent and an acid or base.e.g.Butanol, acetic acid water or phenol-water-ammonia.

Paper chromatography- Procedure

1) A small spot of sample is applied to a strip of chromatography paper about two centimeters away from the base of the plate. This sample is absorbed onto the paper and may form interactions with it.

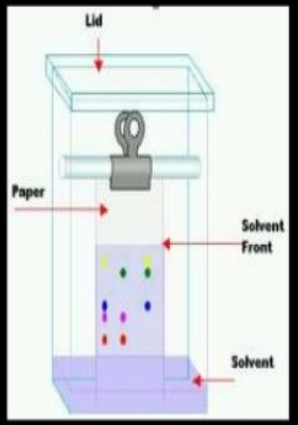
2)The paper is then dipped in to a suitable solvent, such as ethanol or water, taking care that the spot is above the surface of the solvent, and placed in a sealed container.

3) The solvent moves up the paper by capillary action and dissolves the sample mixture, which will then travel up the paper with the solvent solute sample.

Paper chromatography- Procedure

-Different compounds in the sample mixture travel at different rates due to differences in solubility in the solvent, and due to differences in their attraction to the fibers in the paper.

-Paper chromatography takes anywhere from several minutes to several hours



Ascending and Descending Paper Chromatography

Ascending Chromatography

In this method, the solvent is in pool at the bottom of the vessel in which the paper is supported. It rises up the paper by capillary action against the force of gravity.

Descending Chromatography

In this method, the solvent is kept in a trough at the top of the chamber and is allowed to flow down the paper. The liquid moves down by capillary action as well as by the gravitational force.

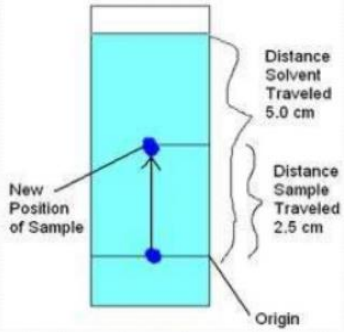
Substances that cannot be separated by ascending method, can be separated by the above descending method.

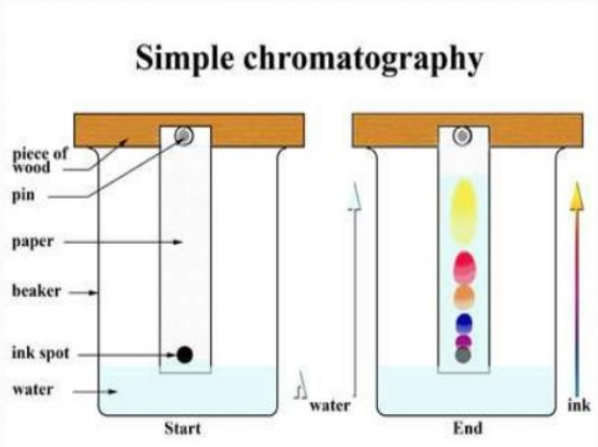
Paper Chromatography -Analysis

After development, the spots corresponding to different compounds may be located by their color, ultraviolet light, ninhydrin or by treatment with iodine vapors.

The paper remaining after the experiment is known as the Chromatogram.

The components which have been separated differ in their retention factor i.e Ratio of distance traveled from the spot or origin by the solute component to that of the distance traveled from the spot or origin by the solvent.





R_f value

R_f, Distance traveled by sample/ distance traveled by solvent.

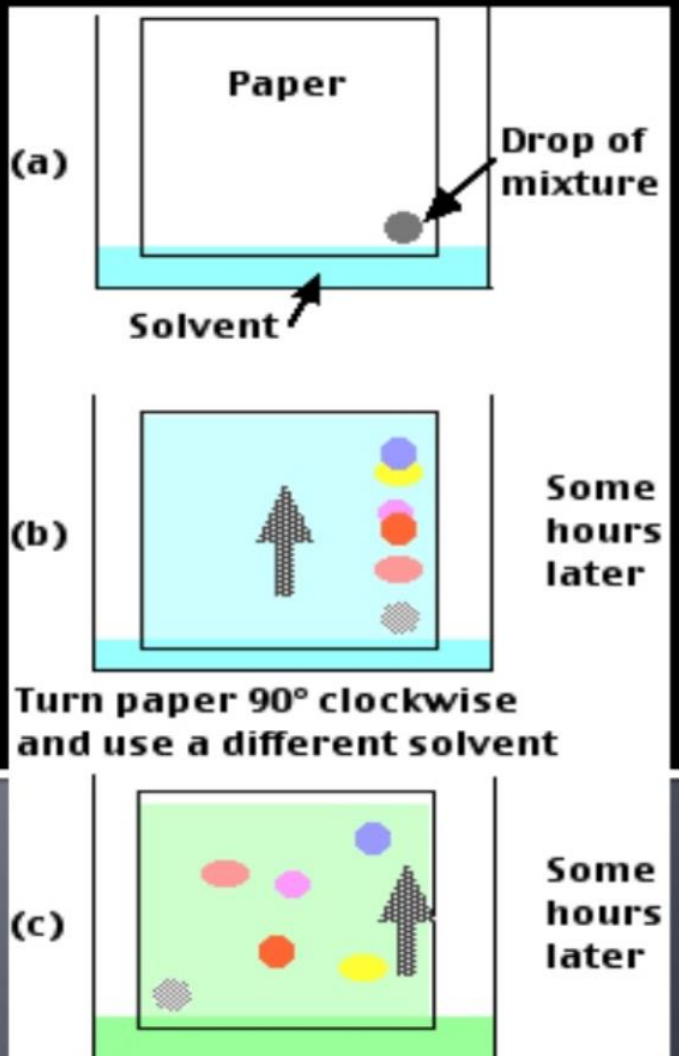
If R_f value of a solution is zero, the solute remains in the stationary phase and thus it is immobile.

If R_f value = 1 then the solute has no affinity for the stationary phase and travels with the solvent front.

Retention Factor can never be greater than one.

Two dimensional chromatography

Sometimes, it is difficult to separate a complex mixture of substances by a single run with one solvent system. In such a case, a second run is carried out by a different solvent system, in a direction perpendicular to the first run. This is referred to as- two dimensional chromatography which enhances the separation of a mixture in to individual components.



Significance of Paper chromatography

1) Paper chromatography is a very easy, simple, rapid and highly efficient method of separation.

2) Can be applied even in microgram quantities of the sample.

3)Can also be used for the separation of a wide variety of materials like amino acids, oligopeptides, sugars, oligosaccharides, glycosides, purines and pyrimidines, steroids, vitamins and some alkaloids like penicillin, tetra cyclin and streptomycin.

4) Not preferred for separating proteins because they are not soluble in many of the solvent systems and are also denatured by them.

5) Paper chromatography is inferior to thin layer chromatography in resolving power.

Thin layer chromatography (TLC)

is a method for identifying substances and testing the purity of compounds.

TLC is a useful technique because it is relatively quick and requires small quantities of material.

Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase.

The stationary phase:

is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.

The mobile phase:

is a developing liquid which travels up the stationary phase, carrying the samples with it.

Components of the samples will separate on the stationary phase according to

how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.

TLC- Procedure

-The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases.

-A small spot of solution containing the sample is applied to a plate, about one centimeter from the base. The plate is then dipped in to a suitable solvent, such as hexane or ethyl acetate, and placed in a sealed container. The solvent moves up the plate by capillary action and meets the sample mixture, which is dissolved and is carried up the plate by the solvent.

- Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent.

Advantages of TLC over paper chromatography

1) In case of paper chromatography , it takes 14-16 hrs for the separation of the components, but in TLC, It takes only 3-4 hrs.

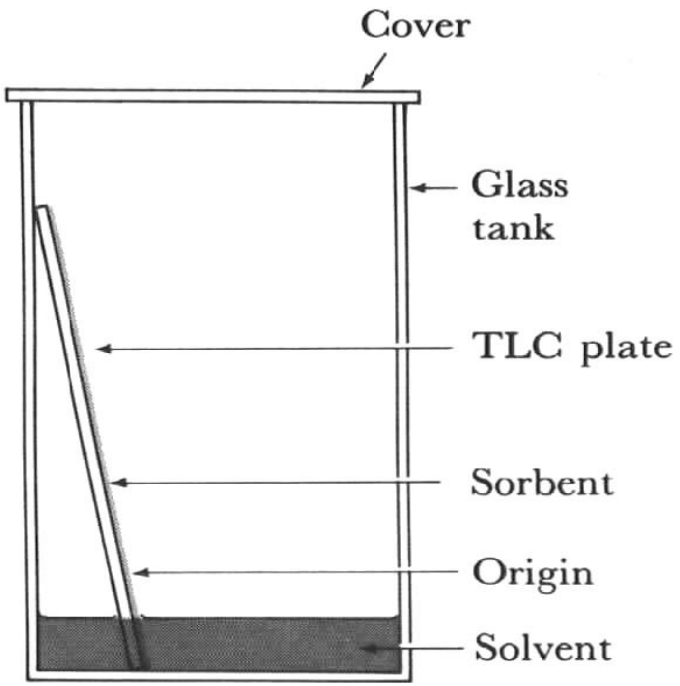
2) TLC has the advantage that the corrosive reagents like sulphuric acid can also be used which pose a limitation for the paper chromatography.

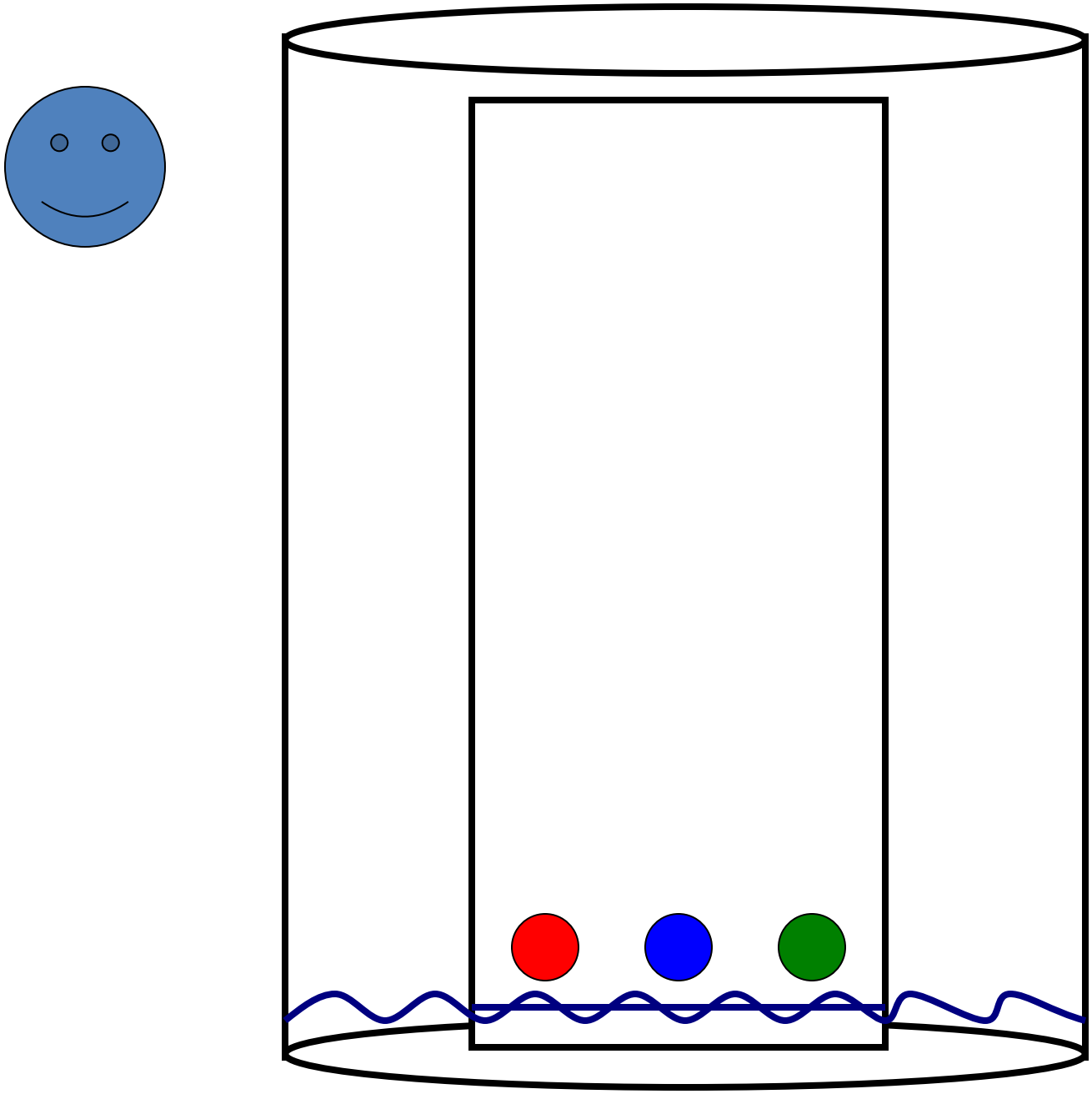
3) It is easier to separate and visualize the components by this method.

4) It has the capacity to analyze multiple samples in a single run.

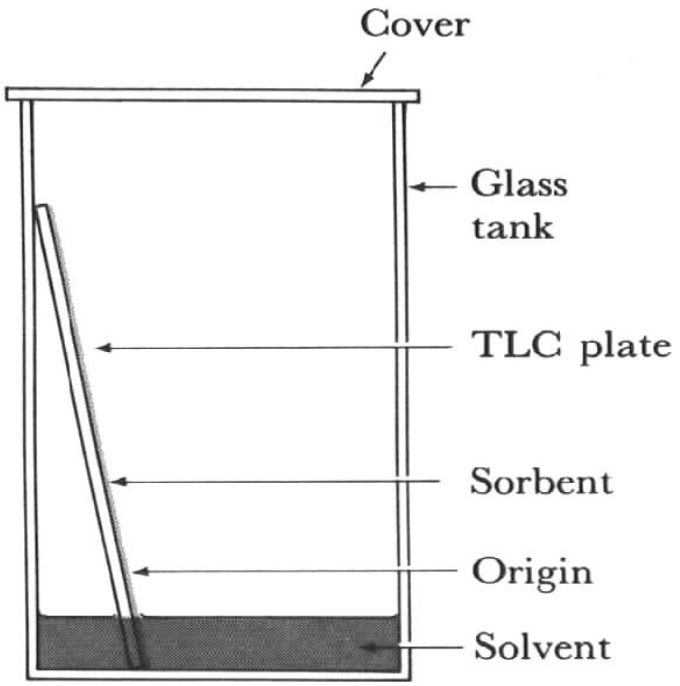
5) It is relatively a low cost.

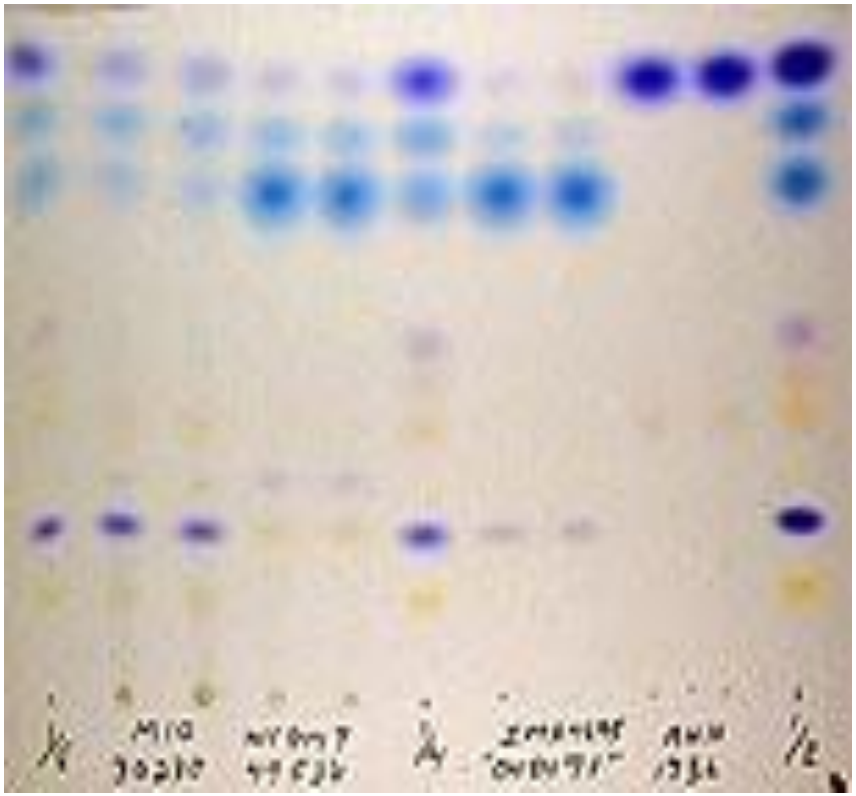
Thin Layer Chromatography (TLC)





TLC





Preparing the Chamber

To a jar with a tight-fitting lid add enough of the appropriate developing liquid so that it is 0.5 to 1 cm deep in the bottom of the jar.

Close the jar tightly, and let it stand for about 30 minutes so that the atmosphere in the jar becomes saturated with solvent.

(Note: R_f values often depend on the temperature and the solvent used in the TLC experiment.

the most effective way to identify a compound is to spot known substances – authentic - next to unknown substances on the same plate.)

In addition, the purity of a sample may be estimated from the chromatogram.

An impure sample will often develop as two or more spots, while a pure sample will show only one spot

Summary

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina).

A small amount of the mixture to be analyzed is spotted near the bottom of this plate.

The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid.

This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

As the solvent moves past the spot that was applied, an equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution.

In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others.

When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized.

If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates.

Gas Chromatography (GC)

- This method depends upon the solubility and boiling points of organic liquids in order to separate them from a mixture. It is both a qualitative (identity) and quantitative (how much of each) tool.

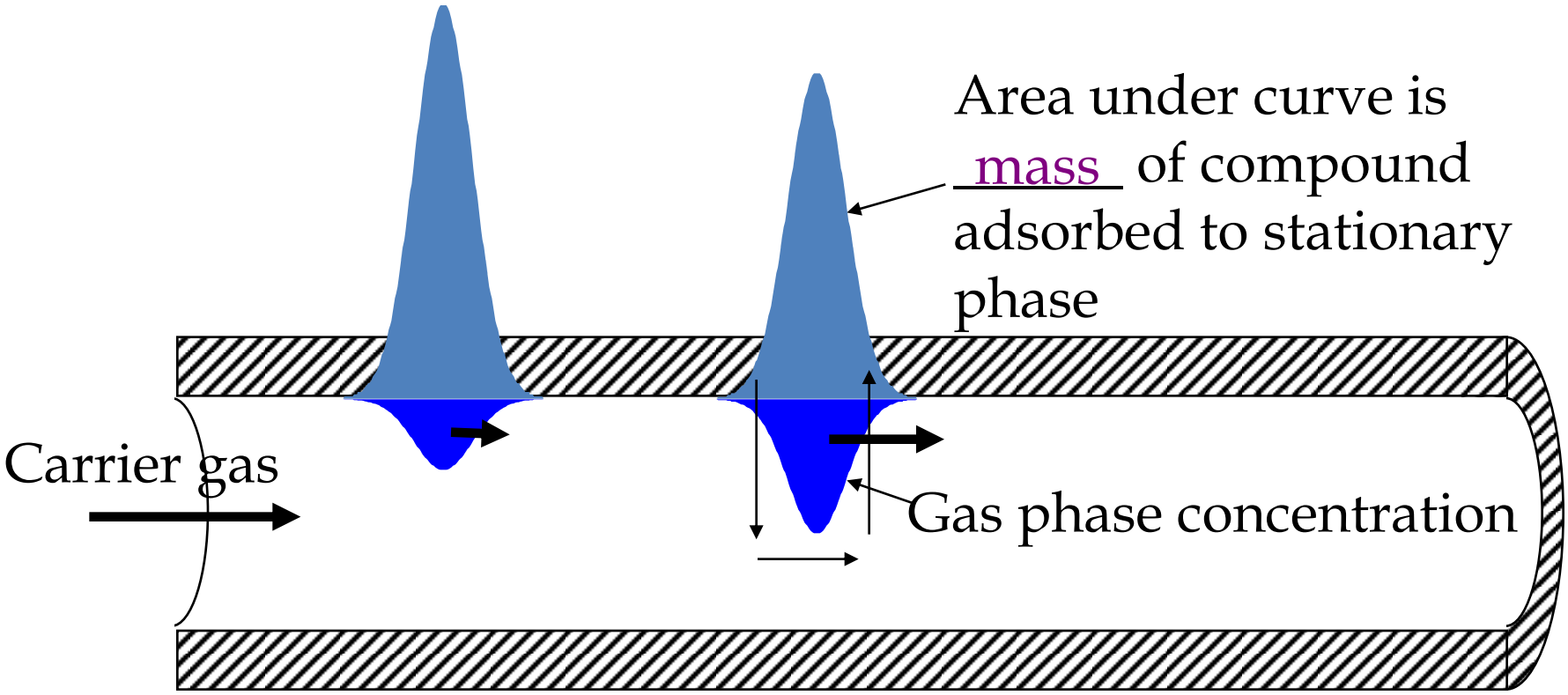
GC Theory

- An inert gas such as helium is passed through the column as a carrier gas and is the moving phase. A sample is injected into a port which is much hotter than the column and is vaporized. The gaseous sample mixes with the helium gas and begins to travel with the carrier gas through the column. As the different compounds in the sample have varying solubility in the column liquid and as these compounds cool a bit, they are deposited on the column support. However, the column is still hot enough to vaporize the compounds and they will do so but at different rates since they have different boiling points. The process is repeated many, many times along the column. Eventually the components of the injected sample are separated and come off of the column at different times (called "retention times").

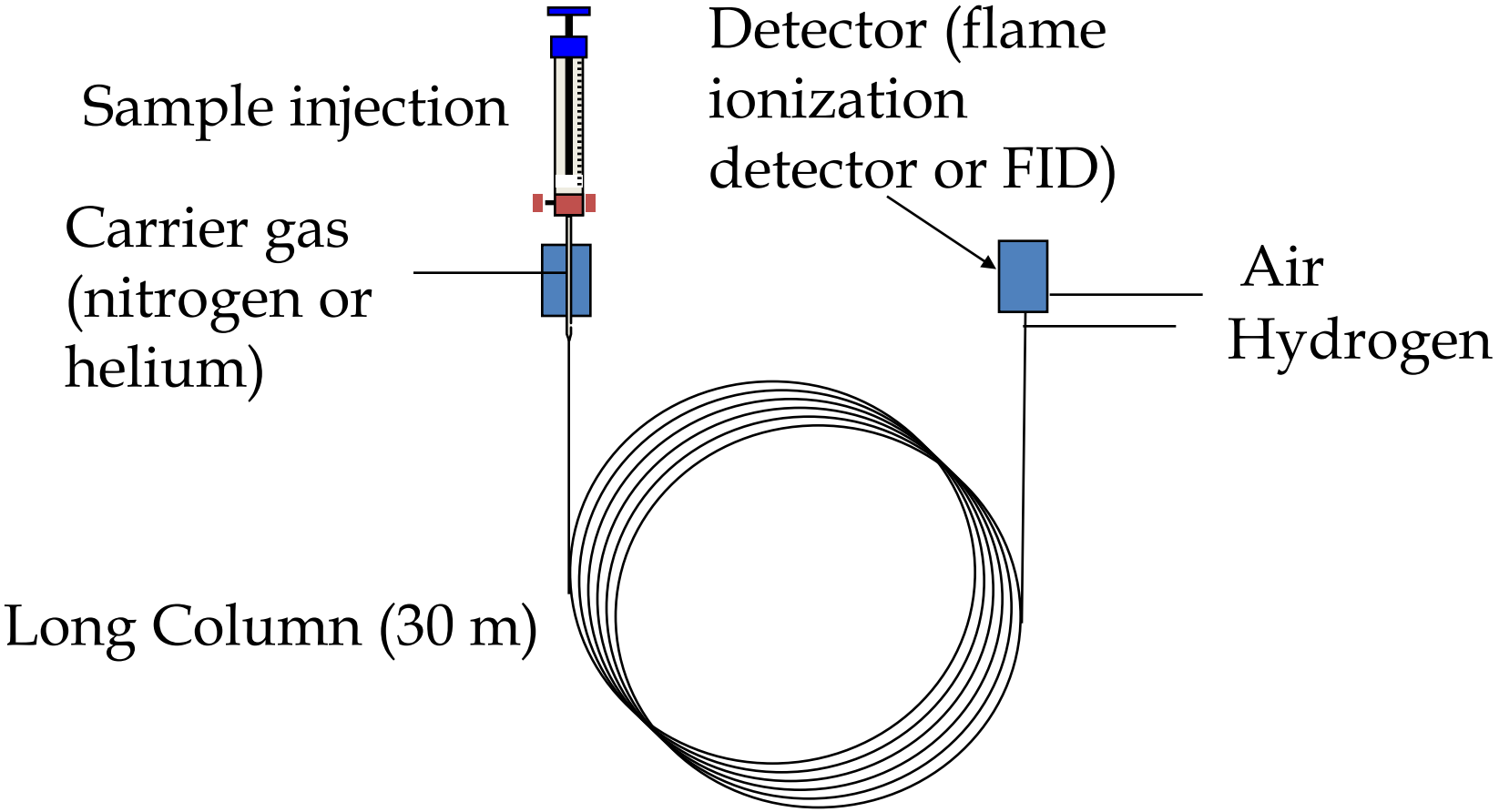
- There is a detector at the end of the column which signals the change in the nature of the gas flowing out of the column. Recall that helium is the carrier gas and will have a specific thermal conductivity, for example. Other compounds have their own thermal conductivities. The elution of a compound other than helium will cause a change in conductivity and that change is converted to an electrical signal. The detector, in turn, sends a signal to a strip chart recorder or to a computer. Detectors come in several varieties, for example, thermal detectors, flame-ionization and electron capture detectors.

Theory of Operation

- Velocity of a compound through the column depends upon affinity for the stationary phase



Process Flow Schematic



Theory GC

- As the gas moves the solute (analyte) through and over the stationary phase, the solute will be in equilibrium with the gas and the solid phase. Since there is a mobile phase, the separation will appear as a chromatogram showing the separation of the analytes.

Theory

- In order to understand GC, need to focus on the general principles of separations which has its roots in solvent extraction. The theory of solvent extraction are used to explain all forms of chromatography i.e. HPLC, LC, GC, EP, and TLC.
- Consider two solvents S1 and S2 and solute X that is in S1. The partition between the two phases.
- $X(S1) \leftrightarrow X(S2)$;

GC Theory

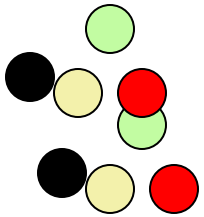
- Partition coefficient K is an equilibrium constant is $K = [X]S_1/[X]S_2$. Suppose that solute X in V_1 (water) is extracted with V_2 (CCl_4). Let m be the moles of X in the system and let q be the fraction of X remaining in phase 1 at equilibrium. The molarity in phase 1 (water) is therefore q_m/V_1.

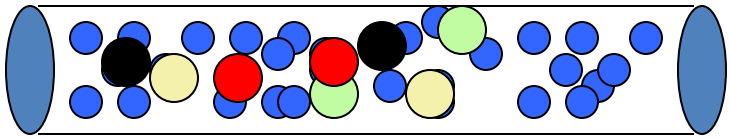
GC Theory

- The fraction of total solute transferred to phase 2 (CCl_4) is (1-q) and the molarity in phase 2 is
- (1-q) m / V_2. Then
- K = ((1-q) m / V_2)/ q m V_1.
- For *GC*:
- K = C_s/C_m where C_s is the concentration in the stationary phase (column) and C_m is the concentration in the mobile phase (gas).

GC Theory

Analyte



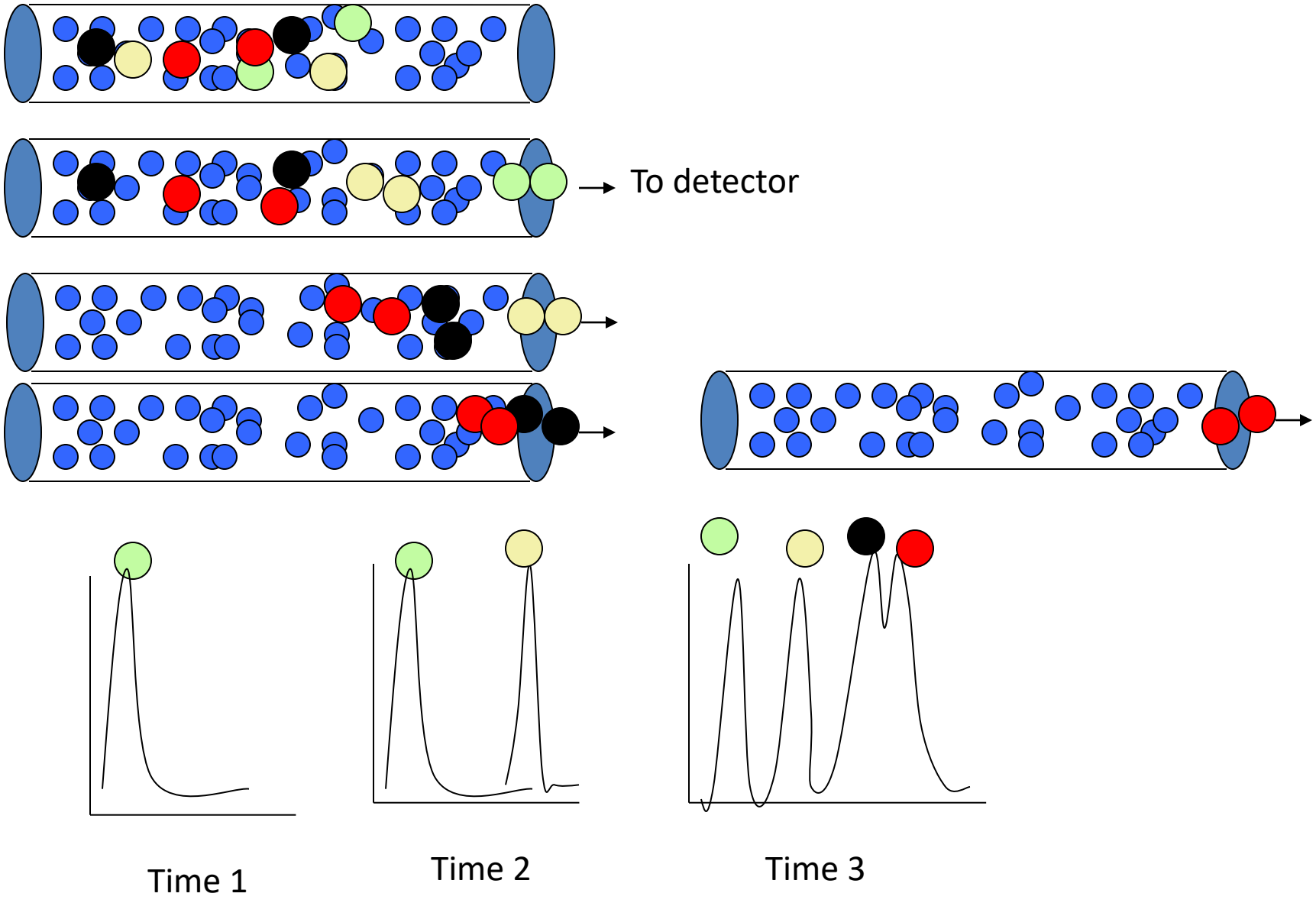


To detector

Column + packing

Time

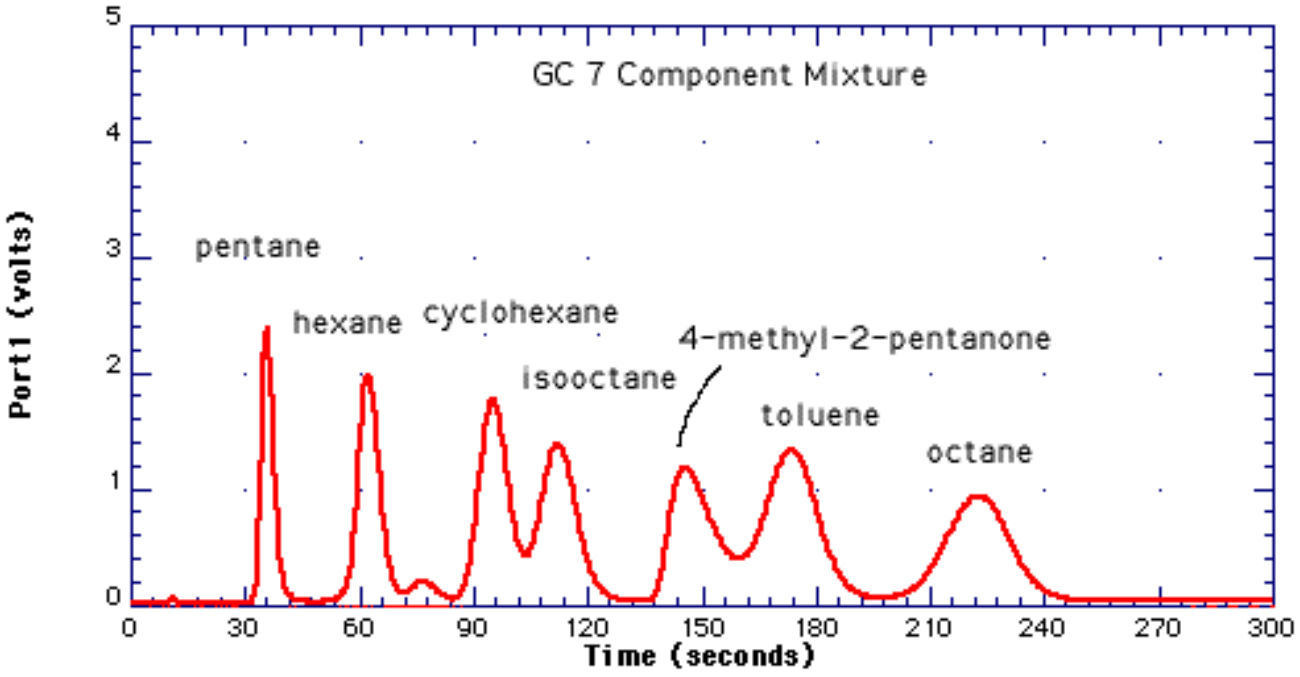
Separations



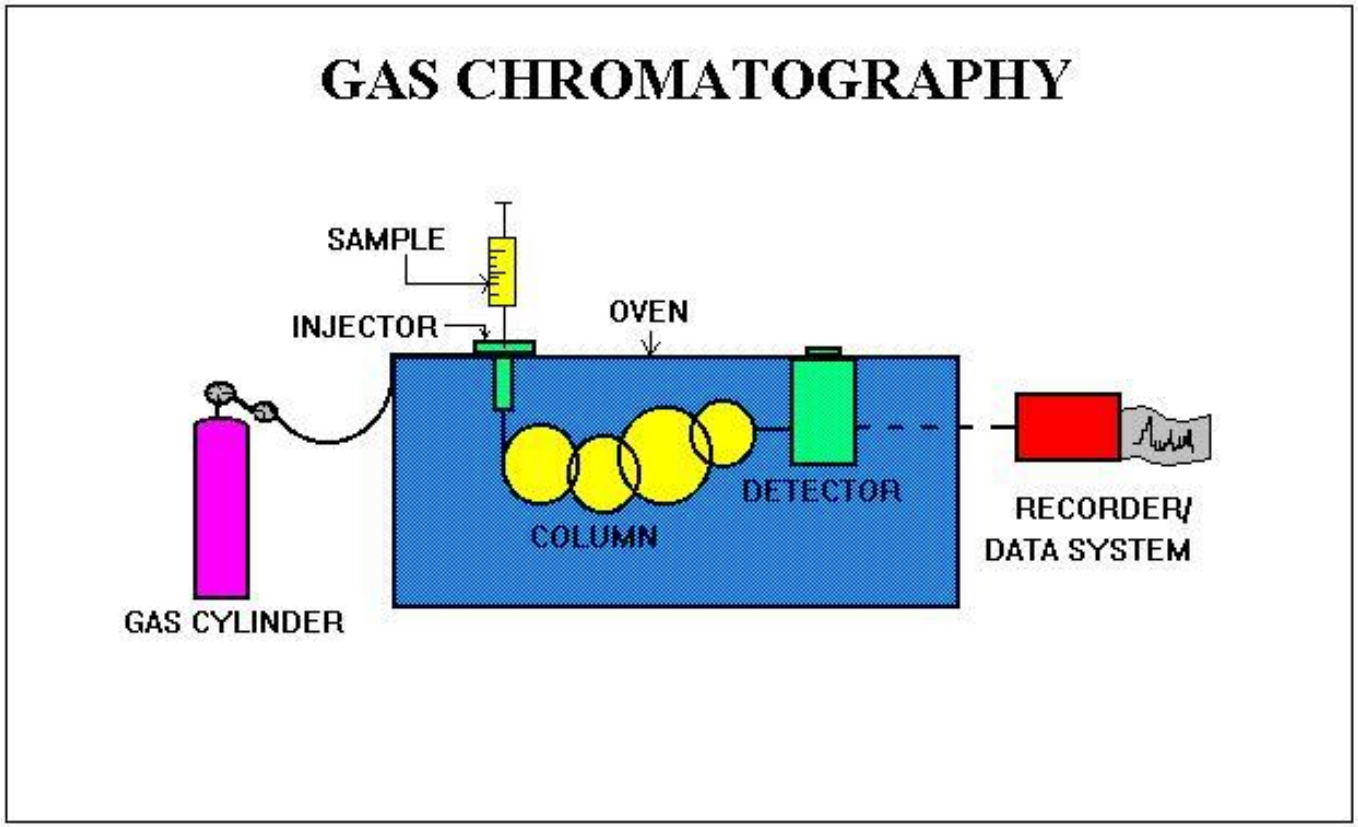
GC General

- The chromatogram shows the order of elution (order of components coming off the column), the time of elution (retention time), and the relative amounts of the components in the mixture. The order of elution is related to the boiling points and polarities of the substances in the mixture. In general, they elute in order of increasing boiling point but occasionally the relative polarity of a compound will cause it to elute "out of order". This is analyzing your sample.

GC Chromatogram



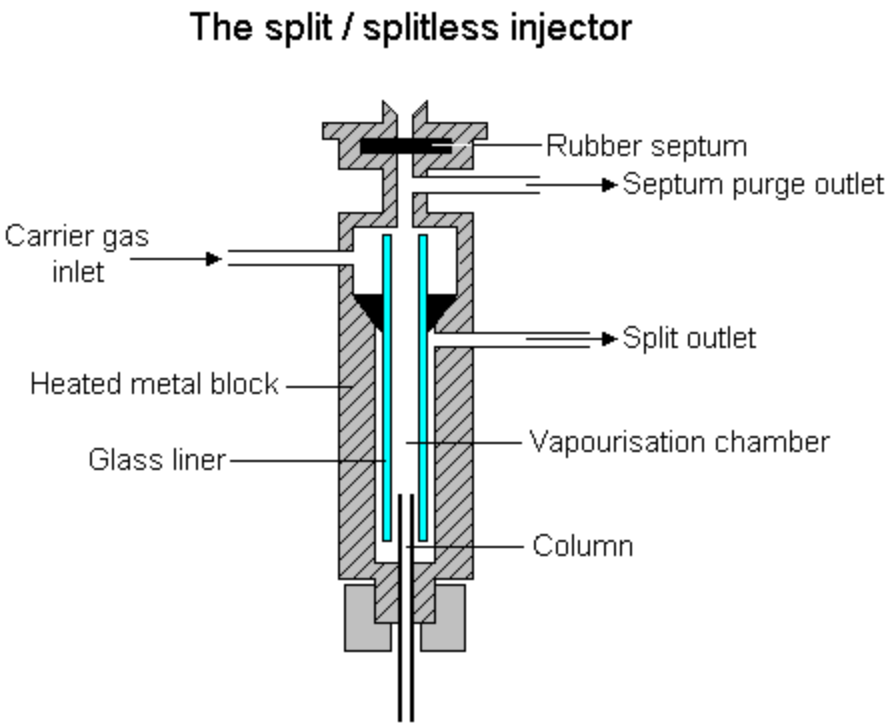
GC Instrumentation



Sample Injections

- The injector should provide consistent and reproducible injections. The micro-syringe is used to load the sample onto the column. The syringe should be clean and accurate and gas tight. The syringe is injected through a rubber septum. The septum should be replaced after many injections to insure gas tightness onto the column. An auto sampler can be used to inject the samples. Typical volumes range from 0.2 to 20 μLs. With capillary columns it is necessary to use a splitter. (See Figure.) A suitable solvent is also necessary for the proper separations and injections.

GC Injector


The split / splitless injector
Rubber septum
Septum purge outlet
Carrier gas inlet
Split outlet
Heated metal block
Vapourisation chamber
Glass liner
Column

Carrier Gas

- This is the mobile phase and should be pure gas so as not to react with the column or analyte. Gas is usually He, Ar, N_2, or H_2. Choice will depend on the type of detector used. He and $_{H2}$ give better resolution (smaller plate height) than N_2. Pressure is also important and as expected the system comes with regulators.

Columns

- The column is the most important component of GC. Here is where the separations take place. All the various equations we discussed above are dependent on properties of the column. There are four types of columns: wall-coated open tubular (WCOT), support coated open tubular (SCOT), micropacked, fused silica open tubular (FSOT), and packed column. The FSOT column is the most flexible. Open tubular is also capillary. Particle size is important because the efficiency of GC column increases rapidly with decreasing particle size of the packing material.

Detectors

- How is the analyte detected? Several detectors are available for GC.
- FID (flame ionization detector) is the most widely used detector. See figure 27-6, Pg 707. Based on the production of ions when compounds are burned then detecting the current produced from the ionization. What compounds can not be detected with this detector?
- TCD (thermal conductivity detector). Operates on the changes in the thermal conductivity of the gas stream brought about by the presence of analyte molecules. See Figure 27-7 on page 708. He is the carrier gas most often used with this detector because it has a high thermal conductivity.

Detection

- The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. FIDs are mass sensitive rather than concentration sensitive; this gives the advantage that changes in mobile phase flow rate do not affect the detector's response. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample.

Advantages of Gas Chromatography

- Requires only very small samples with little preparation
- Good at separating complex mixtures into components
- Results are rapidly obtained (1 to 100 minutes)
- Very high precision
- Only instrument with the sensitivity to detect volatile organic mixtures of low concentrations
- Equipment is not very complex (sophisticated oven)

6) High performance liquid chromatography-

Chromatographic techniques are slow and time consuming. The separation can be greatly improved by applying high pressure in the range of 5000-10,000 pounds per square inch, hence this technique is also referred to as high pressure liquid chromatography.

HPLC requires the use of non compressible resin materials and strong metal columns. The eluents of the columns are detected by methods such as UVabsorption and fluorescence. It can be applied in the form of partition, adsorption, ion exchange or molecular sieve chromatography.

High performance liquid chromatography-

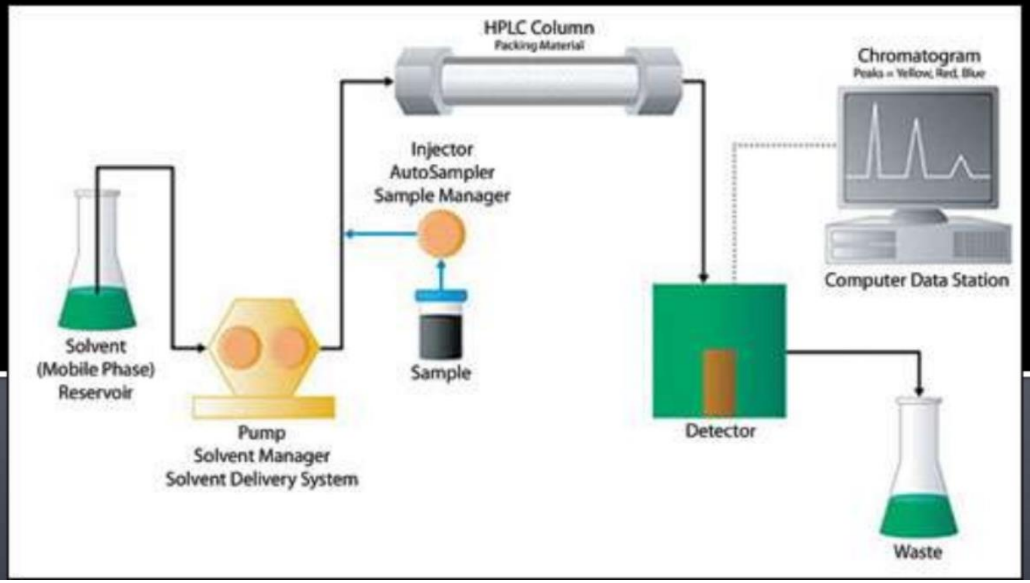
1) The stationary phase- consists of an immobilized thin layer of a liquid on the micro glass or plastic beads, tightly packed in to a narrow column.

2)The mobile phase -consists of a buffered solvent system which is passed under high pressure through the column for eluting the solutes of the sample.

High performance liquid chromatography- Significance

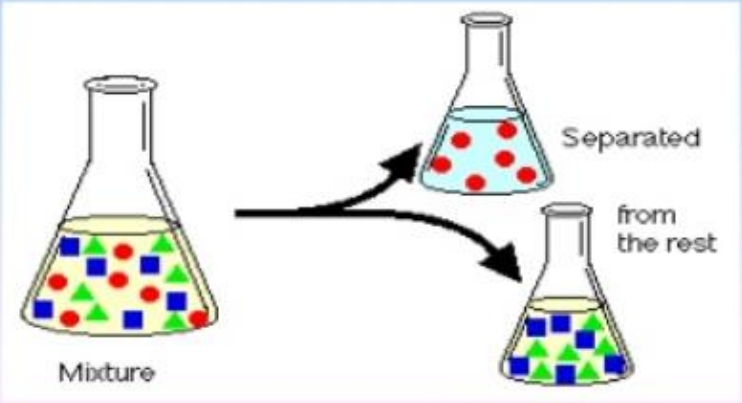
Due to rapidity in action it is used for assaying amino acids, peptides, proteins, carbohydrates, lipids, nucleic acids and related compounds, vitamins, hormones, metabolites and drugs such as
antiarrythmics,antibiotics,antiepileptics,analgesics, bronchial smooth muscle relaxants and anti-depressants.

High performance liquid chromatography- Apparatus



What is Solvent Extraction ?

Solvent Extraction, also known as liquid-liquid extraction, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent.

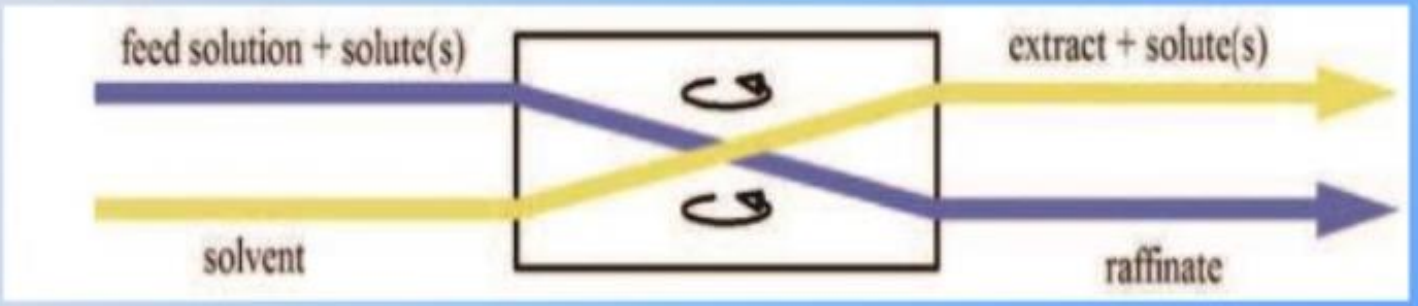


Importance of the process:

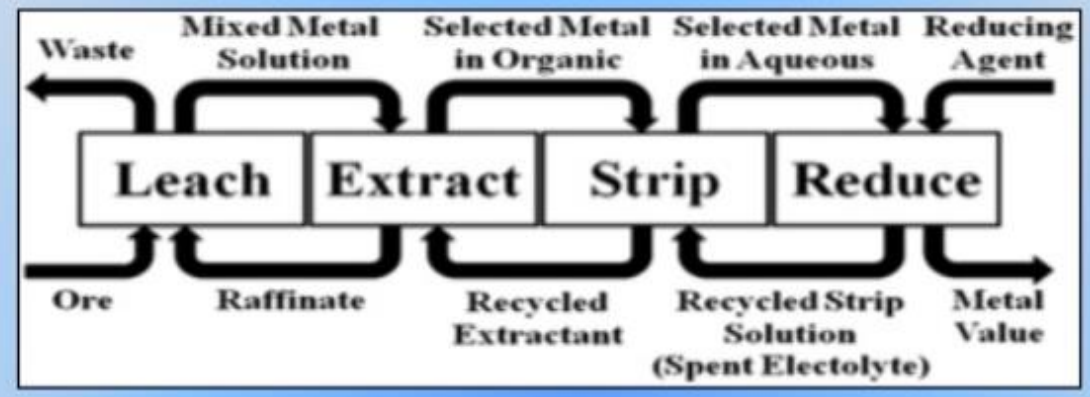
- The Solvent Extraction(**S.E.**) process was first developed as a tool of analytical chemistry. Every metallic element of the periodic table could be virtually separated by this process.
- Back in 1940's , **S.E.** was primarily used to separate nuclear and rare earth elements.
- However, availability of inexpensive and effective reagents led to the establishment of large scale **S.E.** processes for extraction of non-ferrous metals from hydrometallurgical leach liquors.

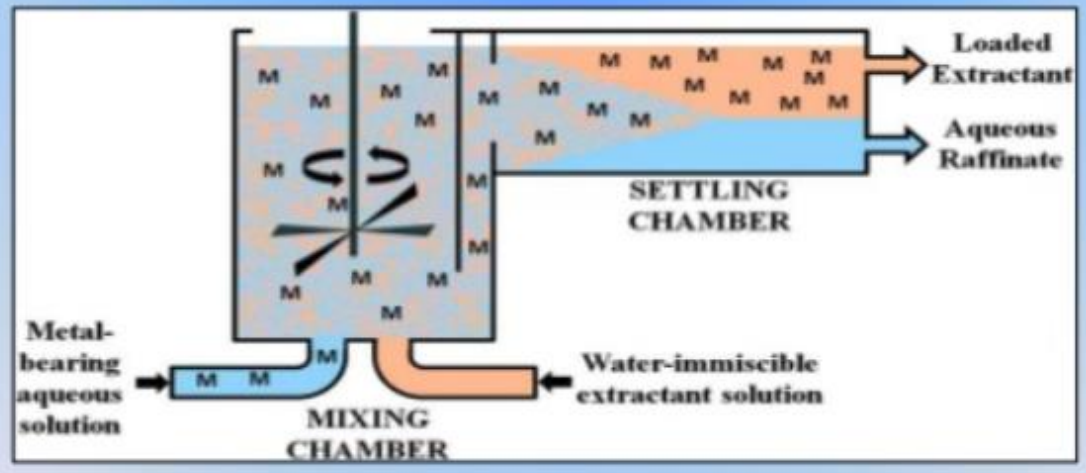
Solvent Extraction, consists of transferring one (or more) **solute**(s) contained in a **feed solution** to another immiscible liquid (**solvent**).

The solvent that is enriched in solute(s) is called **Extract** & the feed solution that is depleted of solute(s) is called **Raffinate**.



S.E. as a part of hydrometallurgy





Types of Solvent Extraction

- SOLVATING EXTRACTION
- CATIONIC EXCHANGE
- ANIONIC EXCHANGE
- CHELATING EXTRACTION

- **SOLVATING EXTRACTION**

Solvent used:

1) Tri Butyl Phosphate (TBP)
2) Tri Octyl Phosphine Oxide(TOPO)
3) Methyl Iso Butyl Ketones(MIBK)

Application:

Extraction/Separation of Lead , Zinc , Uranium, Iron , Cadmium, Hafnium , Zirconium & Plutonium.

- **CATIONIC EXCHANGE**

Solvent used:

1) Di-2-ethylhexy Phosphoric Acid(D2EHPA)
2) Naphthenic Acid
3) Versatic Acid

Application:

Extraction/Separation of Copper, Zinc, Nickel, Cobalt, Silver

- **ANIONIC EXCHANGE**

  Solvent used:

  1) Primary Amines(RNH_2)
  2) Secondary Amines(R_2NH)
  3) Tertiary Amines(R_3N) (R=> C12 – C14)

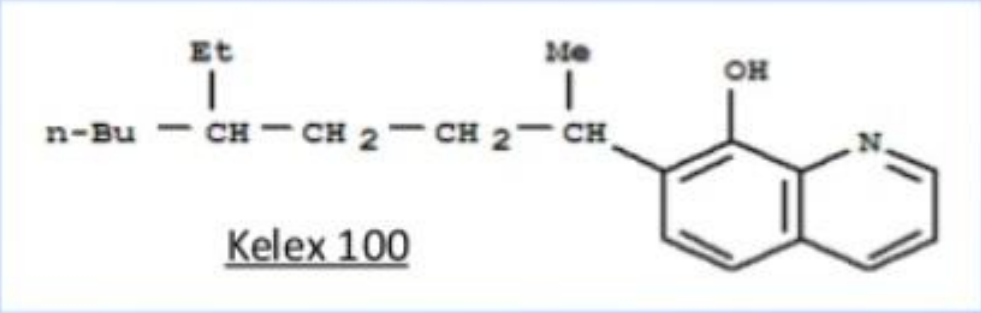
  Application:

  Extraction/Separation of Uranium, Thorium, Vanadium, Cobalt

- **CHELATING EXTRACTION**

Solvent used:

1) Lix63 , Lix65
2) Kelex 100



Kelex 100

Application:

Extraction/Separation of Copper , Nickel, Cobalt

Two basic steps of S.E.

- **Extraction**

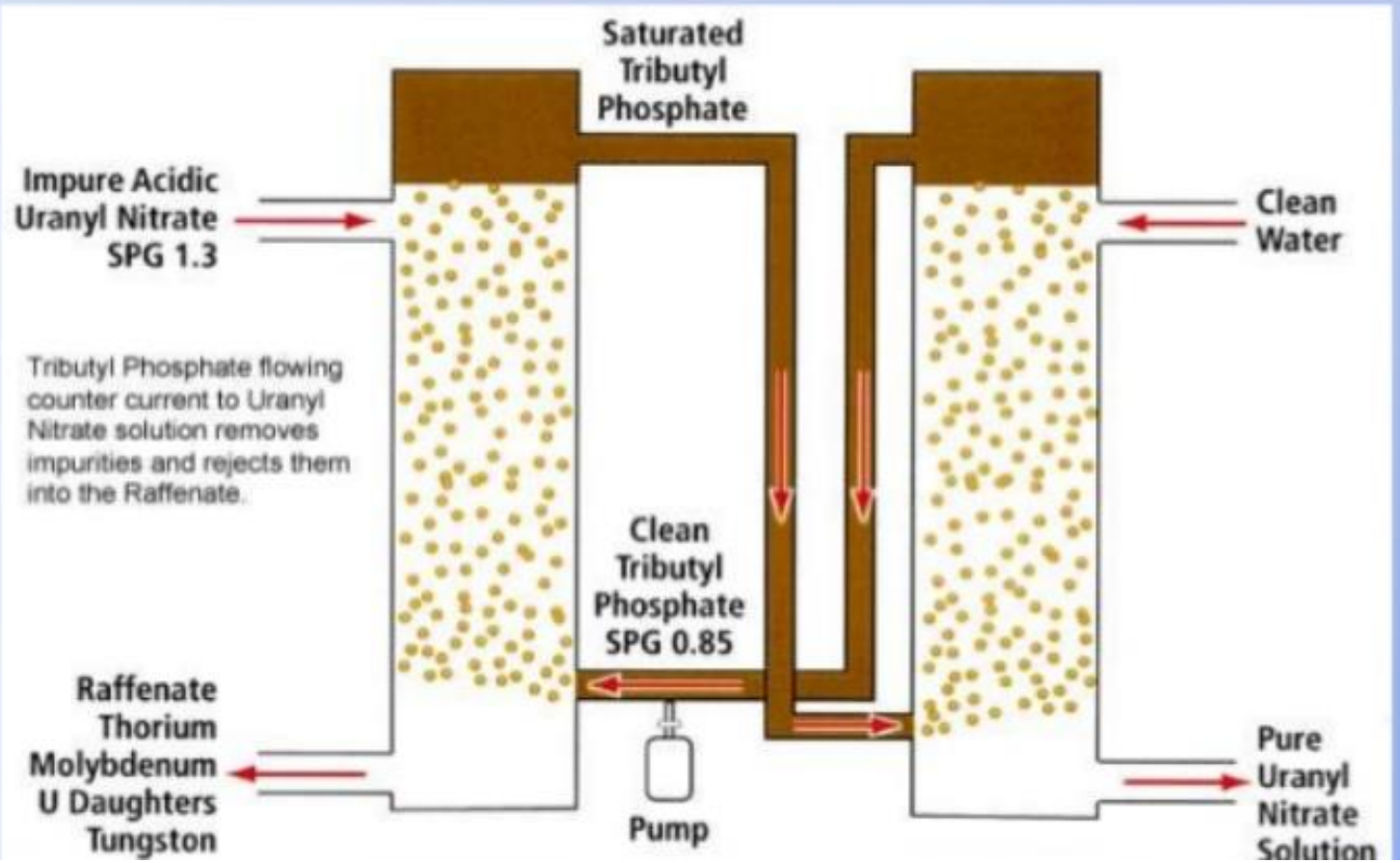
$$UO_2(SO_4)^{4-} + 2(R_3NH)_2SO_4 = (R_3NH)_4UO_2(SO_4)_3 + 2(SO_4^{2-})$$

- **Stripping**

$$(R_3NH)_4UO_2(SO_4)_3 + Na_2CO_3 = R_3N + Na_4UO_2(CO_3)_3^{4-} + H_2O + Na_2SO_4$$

$$2Na_4UO_2(CO_3)_3^{4-} + 6NaOH = Na_2U_2O_7 + 6Na_2CO_3 + 3H_2O$$

Solvent Extraction of Uranium

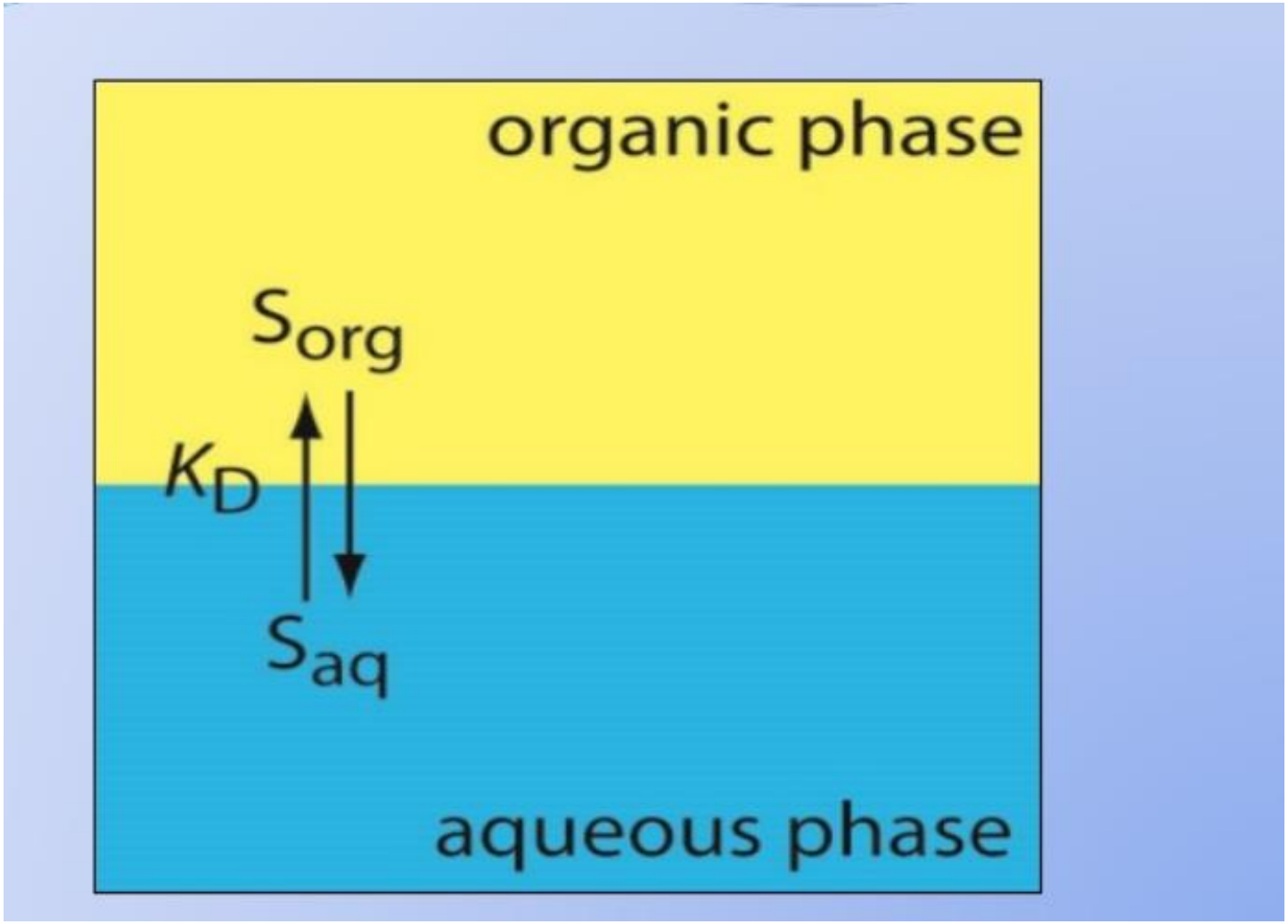


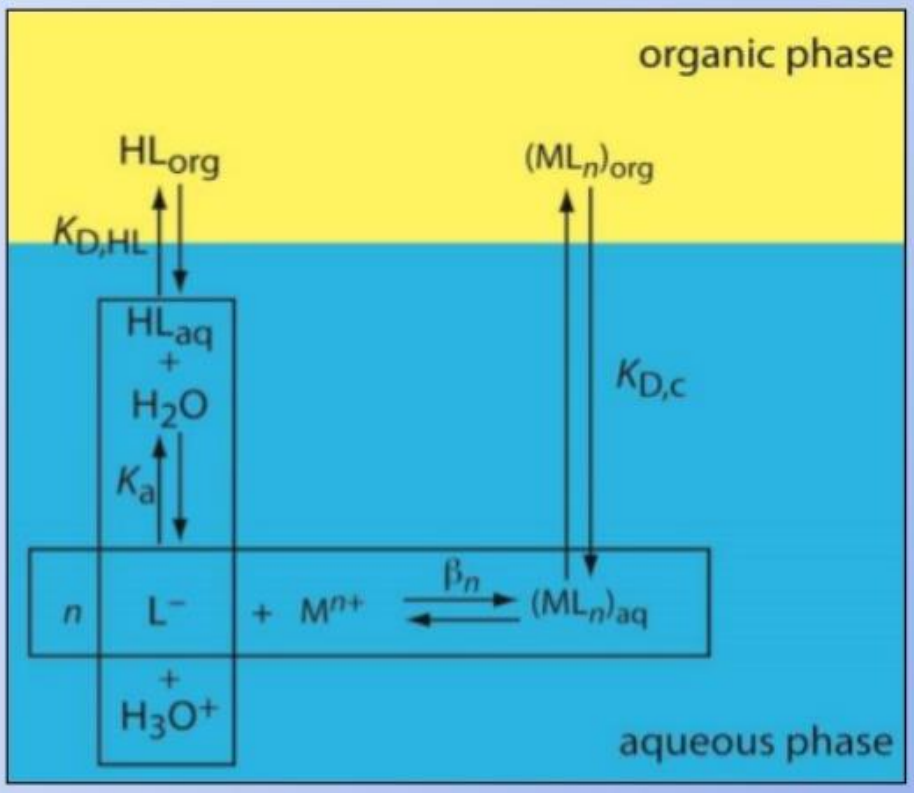
Distribution Coefficient

At a certain temperature, the ratio of concentrations of a solute in each of the solvents is always constant.

This ratio is known as the **Distribution Coefficient(K_D)**

$$K_D = \frac{\text{Conc. of metal ion in Organic Phase}}{\text{Conc. of metal ion in Aqueous Phase}}$$


organic phase
S_{org}
K_D
S_{aq}
aqueous phase


organic phase
HL_{org}
$(ML_n)_{org}$
$K_{D,HL}$
HL_{aq}
+
H_2O
K_a
$K_{D,c}$
β_n
n
L^-
+ M^{n+}
$(ML_n)_{aq}$
+
H_3O^+
aqueous phase

Extraction Equilibria

For a chelating reagent, HR, the extraction reaction for a metal ion M^{n+} can be represented as:

$$M_{aq}^{n+} + n\,HR_{org} \rightleftharpoons MR_{n\,org} + H^{+}_{aq}$$

The Extraction Constant(K_{ex}) is given by:

$$K_{ex} = \frac{[MR_n]_{org}\,[H^+]^n_{aq}}{[M^{n+}]_{aq}\,[HR]^n_{org}}$$

The extraction constant can also be expressed as:

$$K_{ex} = \frac{K_D \cdot [H^+]^n_{aq}}{[HR]^n_{org}}$$

Where,

$$K_D = \frac{[MR_n]_{org}}{[M^{n+}]_{aq}}$$

Extraction Coefficient

- It indicates how much of the metal will move from the aqueous phase to the organic phase in a single contact.

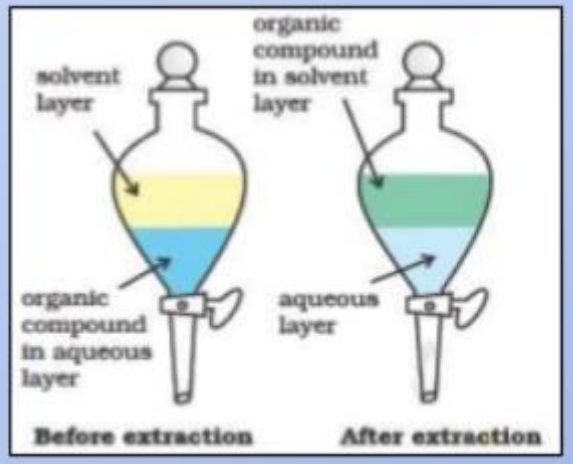
Effective extraction coefficient (E_F) can be expressed as:

$$E_F = \frac{[M^{n+}]_{org}\ (Vol.)_{org}}{[M^{n+}]_{aq}\ \ (Vol.)_{aq}}$$

Single Stage vs. Multistage

❖ **Single Stage S.E.**

- Single stage process is commonly followed in laboratory scale.



- Contacting is carried out by taking two phases together in a separating funnel followed by vigorous agitation so that the phases may disperse in each other as fine droplets. Ceasing the agitation lead to the separation of phases in two distinct layers.

❖ **Single Stage S.E.**

From mass balance it can be shown that:

$$\frac{X_0}{X_1} = 1 + \frac{[M^{n+}]_{org}\ (Vol.)_{org}}{[M^{n+}]_{aq}\ \ (Vol.)_{aq}}$$

where,

X_0 = Conc. of metal ions in the feed solution
i.e. the aqueous phase before any contact.

X_1 = Conc. of metal ions in the aq. raffinate
i.e. the aqueous phase after a single contact.

❖ Single Stage S.E.

From mass balance it can be shown that:

$$\frac{X_0}{X_1} = 1 + E_F$$

where,

X_0 = Conc. of metal ions in the feed solution
i.e. the aqueous phase before any contact.

X_1 = Conc. of metal ions in the aq. raffinate
i.e. the aqueous phase after a single contact.

❖ Multi Stage Counter Current S.E.

- For extraction of metals on Industrial scale, multi stage counter current solvent extraction process is followed.
- The aqueous raffinate from one extraction unit is fed to the next unit as the aqueous feed, while the organic solvent flows in the opposite direction, thereby picking up more metal ions in successive contacts.
- Dividing the organic phase into small parts & making multiple contacts is the most efficient way of extracting.

For a single contact :

$$\frac{X_0}{X_1} = 1 + E_F$$

For multistage contact (assuming **n** contacts):

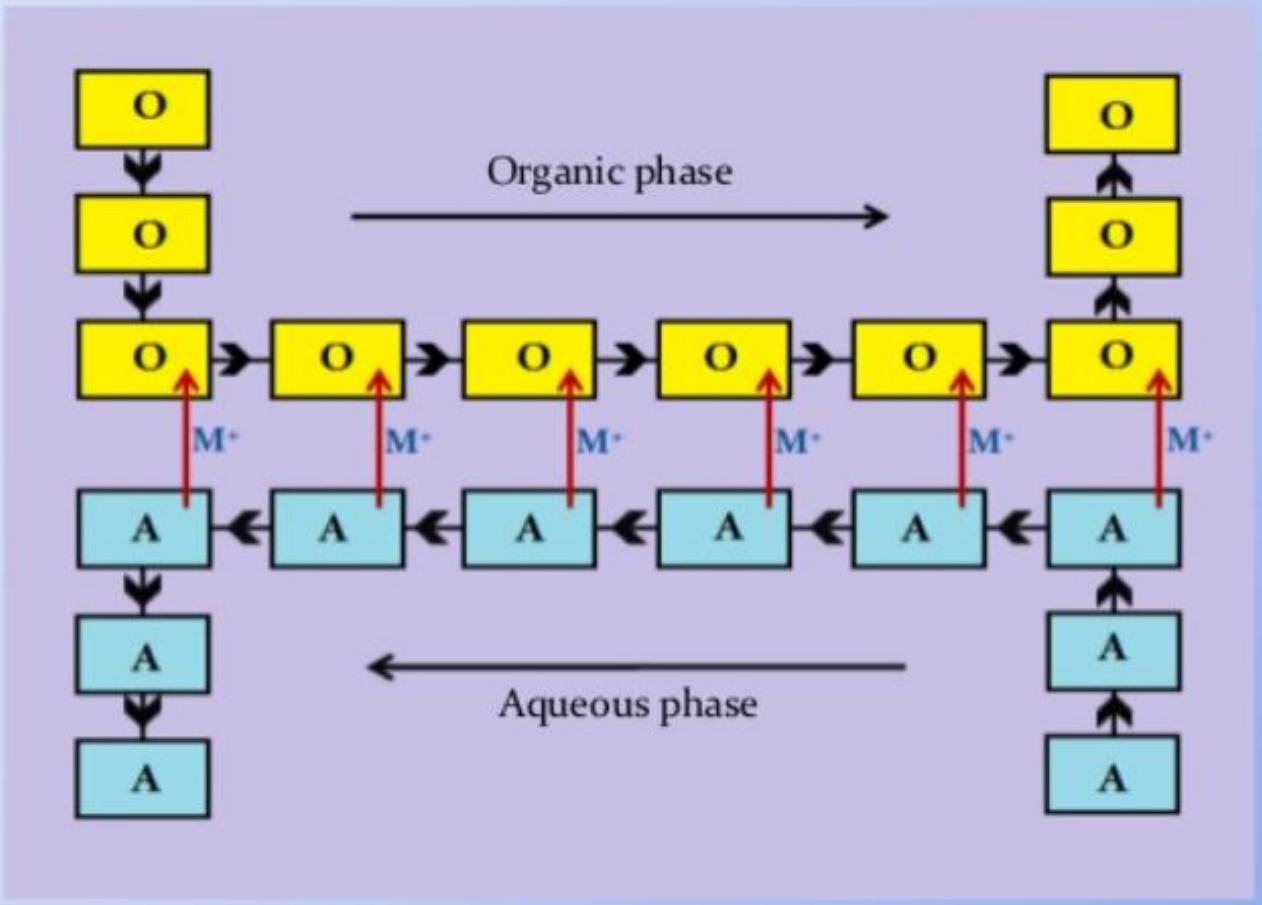
$$\frac{X_0}{X_n} = (1 + E_F/n)^n$$

As $\lim n \to \infty$

$$\frac{X_n}{X_0} = \frac{1}{\exp(E_F)} = \exp(-E_F)$$

So, Multistage contacting is Best

Multistage Counter-current S.E.



Selection of Organic Solvent

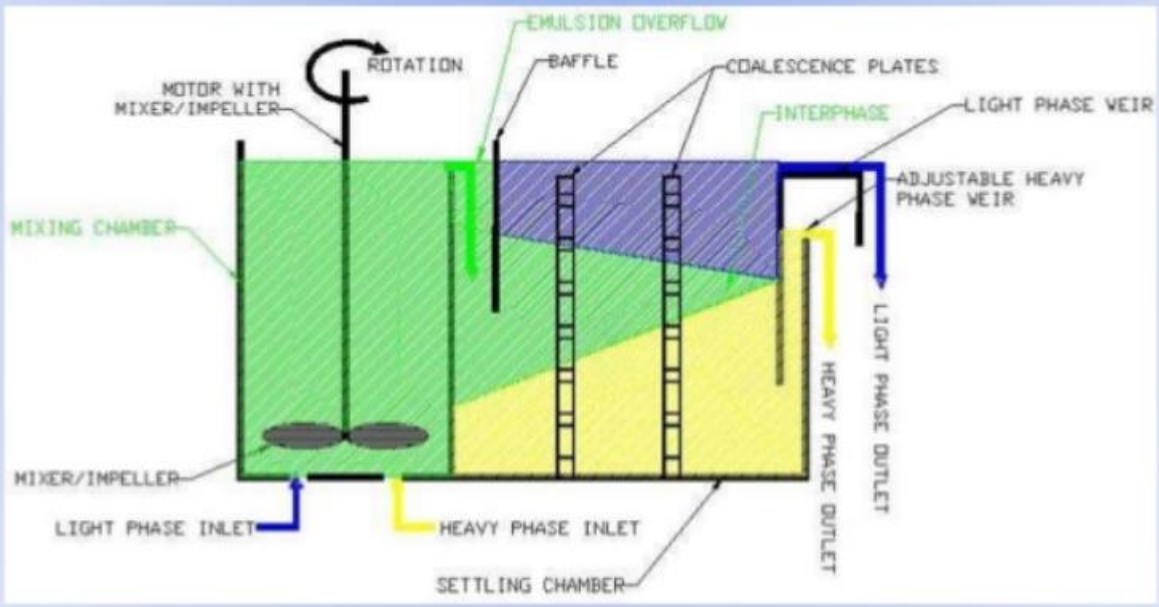
- High extraction capacity
- Selectivity
- Easily Stripped
- Immiscible in aqueous phase
- Sufficient density difference with the aq. phase
- Low Viscosity
- Non toxic
- Non explosive
- Cheap

Diluents

- Diluent is required for dissolving or diluting the organic extractant so that its physical properties like viscosity and density become more favorable for better mixing of the two phases and their separation.
- Aliphatic or Aromatic hydrocarbons or a combination of both, are used as diluent.
- Diluent affects the extraction, scrubbing, stripping and phase separation process quite significantly.
- Examples of some common diluents:
  - Kerosene
  - Benzene
  - Chloroform
  - Xylene
  - Naptha
  - Toluene

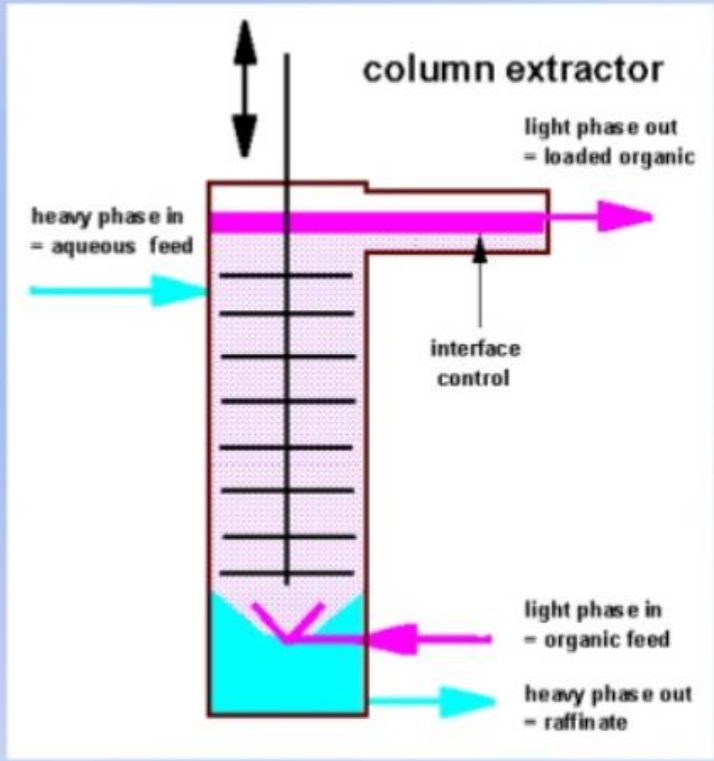
Equipments for Solvent Extraction

- **Mixer-Settlers**



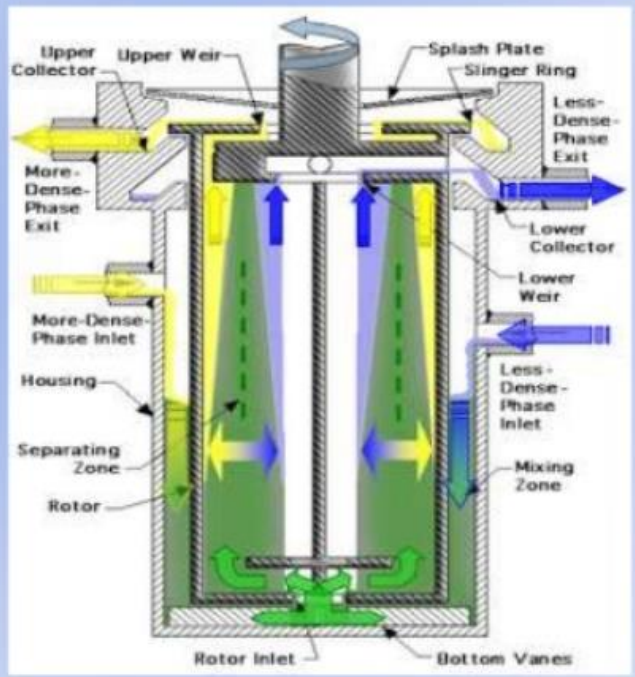
Equipments for Solvent Extraction

- **Column Contactor**



Equipments for Solvent Extraction

- **Centrifugal Contactor**



Application

Today the process of Solvent Extraction is widely applied in miscellaneous fields of Science and Technology

- Analytical Chemistry
- Extraction of Rare-earths & PGM
- Waste water treatment
- Mineral oil treatment & dewaxing
- Food industry(essential oil extraction)
- Perfume industry(fragrance extraction)
- Pharmaceutical industry

Conclusion

In spite of having the advantage of a rapid extraction kinetics with a feasibility of recycling the solvent, there are some drawbacks of this process.

- Use of toxic and flammable organic diluents
- Sometimes the cost of solvent system becomes too expensive
- In several cases there is loss of organic solvent in the process
- Solvent Extraction may not pollute the air as much as other pyrometallurgical processes, but a lot of toxic chemicals find their way into the waterbodies, polluting them.

The extensive application of S.E. has become a field(& industry!) of its own. With proper modeling of the process, invention of new equipments, use of more effective extractants and a better understanding of the science behind it may lead it to be one of the most important extraction processes in metallurgy.

Thin layer chromatography (TLC)

-It is a chromatographic technique used to separate mixtures.

-It involves a *stationary phase* consisting of a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose immobilized onto a flat, inert carrier sheet.

-A *liquid phase* consisting of the solution to be separated which is dissolved in an appropriate solvent and is drawn up the plate via capillary action, separating the solution based on the polarity of the components of the compound in question.

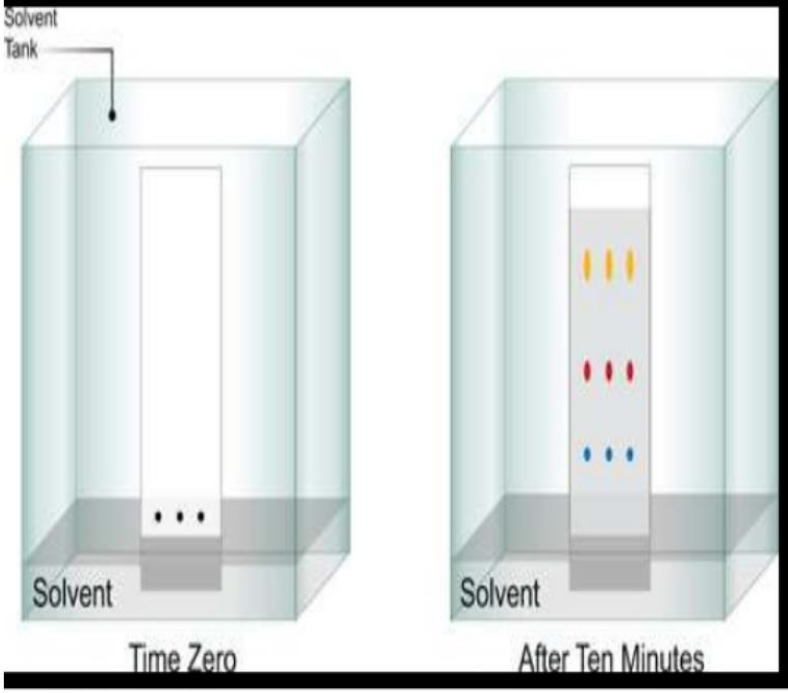
TLC- Procedure

-The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases.

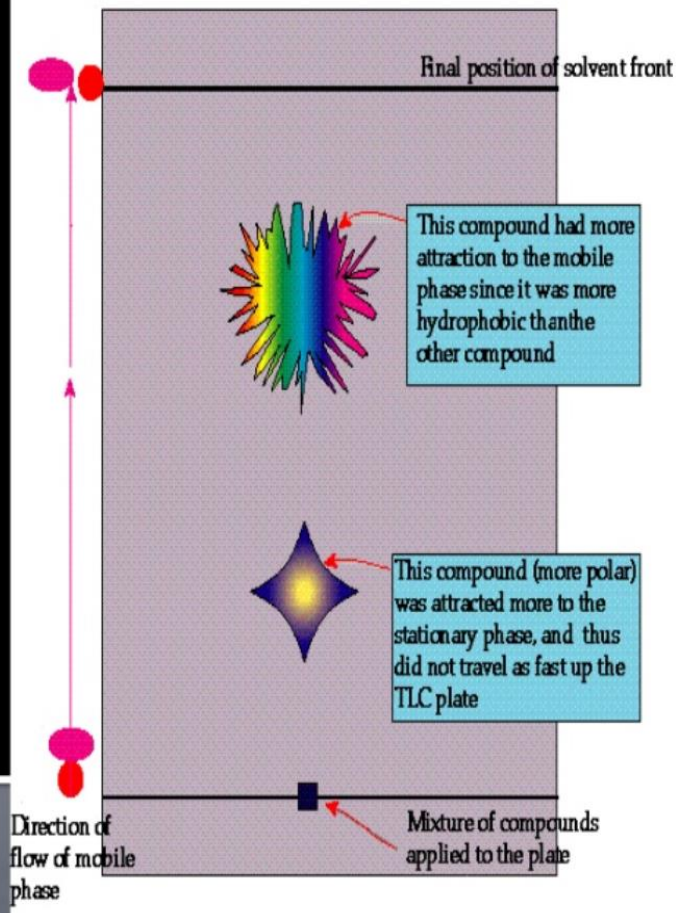
-A small spot of solution containing the sample is applied to a plate, about one centimeter from the base. The plate is then dipped in to a suitable solvent, such as hexane or ethyl acetate, and placed in a sealed container. The solvent moves up the plate by capillary action and meets the sample mixture, which is dissolved and is carried up the plate by the solvent.

- Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent.

TLC -Overview



Thin Layer Chromatogram



The most non-polar compounds will travel further up the plate than the more polar compounds.

Significance of TLC

Its wide range of uses include

-determination of the pigments a plant contains

-detection of pesticides or insecticides in food

-identifying compounds present in a given substance

-monitoring organic reaction

Advantages of TLC over paper chromatography

1) In case of paper chromatography , it takes 14-16 hrs for the separation of the components, but in TLC, It takes only 3-4 hrs.

2) TLC has the advantage that the corrosive reagents like sulphuric acid can also be used which pose a limitation for the paper chromatography.

3) It is easier to separate and visualize the components by this method.

4) It has the capacity to analyze multiple samples in a single run.

5) It is relatively a low cost.

2)Adsorption chromatography

-In this technique the separation is based on differences in adsorption at the surface of the solid stationary medium.

-The adsorbents such as silica gel, charcoal powder and calcium hydroxyapatite are packed in to a column in a glass tube. This serves as the stationary phase.

-The sample mixture in a solvent is loaded on this column.

-The individual components get differentially adsorbed on to the adsorbent.

2)Adsorption chromatography- Elution

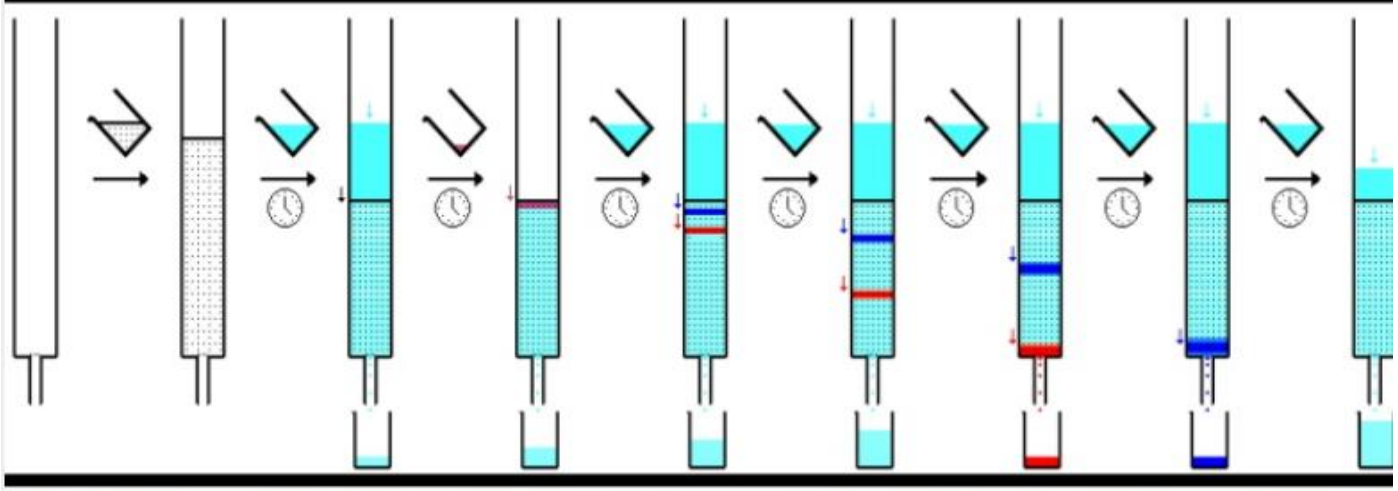
-The elution is carried out by a buffer system (mobile phase). The most weakly held fraction moves fastest, followed by others, according to the order of tightness in adsorption.

-The individual compounds come out of the column at different rates which may be separately collected and identified.

- Amino acids may be identified by Ninhydrin colorimetric method.

-An Automated column chromatography apparatus- fraction collector is frequently used now a days.

Adsorption chromatography



The substance loosely adsorbed to the stationary medium comes in the earlier fraction.

Ion – Exchange chromatography

1) Ion-exchange chromatography (or *ion chromatography*) is a process that allows the separation of ions and polar molecules based on the charge properties of the molecules.

2) It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.

3)The solution to be injected is usually called a *sample*, and the individually separated components are called *analytes*.

4) It is often used in protein purification, water analysis, and quality control.

Ion – Exchange chromatography

-Ion exchange chromatography retains analyte molecules based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups that interact with analyte ions of opposite charge.

- This type of chromatography is further subdivided into cation exchange chromatography and anion exchange chromatography.

i) Cation exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group:

ii) Anion exchange chromatography retains anions using positively charged functional group:

Ion – Exchange chromatography- Procedure

A mixture of amino acids or proteins can be conveniently separated by ion exchange chromatography. The amino acid mixture (at p H around 3.0)is passed through a cation exchange and the individual amino acids can be eluted by using buffers of different p H. Suppose, a mixture of Arginine and Aspartic acid is passed through a cation exchange column. Arginine has extra positive charge and so adheres to the column. But negatively charged Aspartic acid molecules will not adhere and come out first from the column

Ion – Exchange chromatography- Procedure (Contd.)

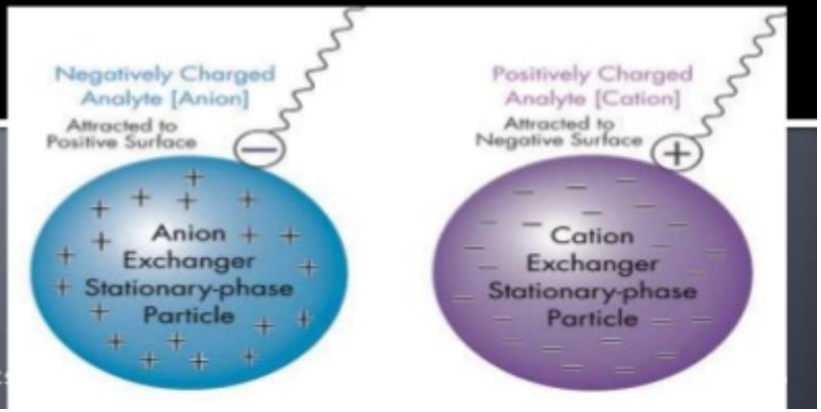
when weak NaOH is passed, the positive charge of Arginine is neutralized. Na+ will replace Arginine in the column, thus Arginine is eluted finally. The various fractions eluted, containing individual amino acids, are allowed to react with Ninhydrin reagent to form colored complexes. This is continuously monitored for qualitative and quantitative identification of amino acids. The amino acid analyzer is based on this method.

Types of ion exchanges resins

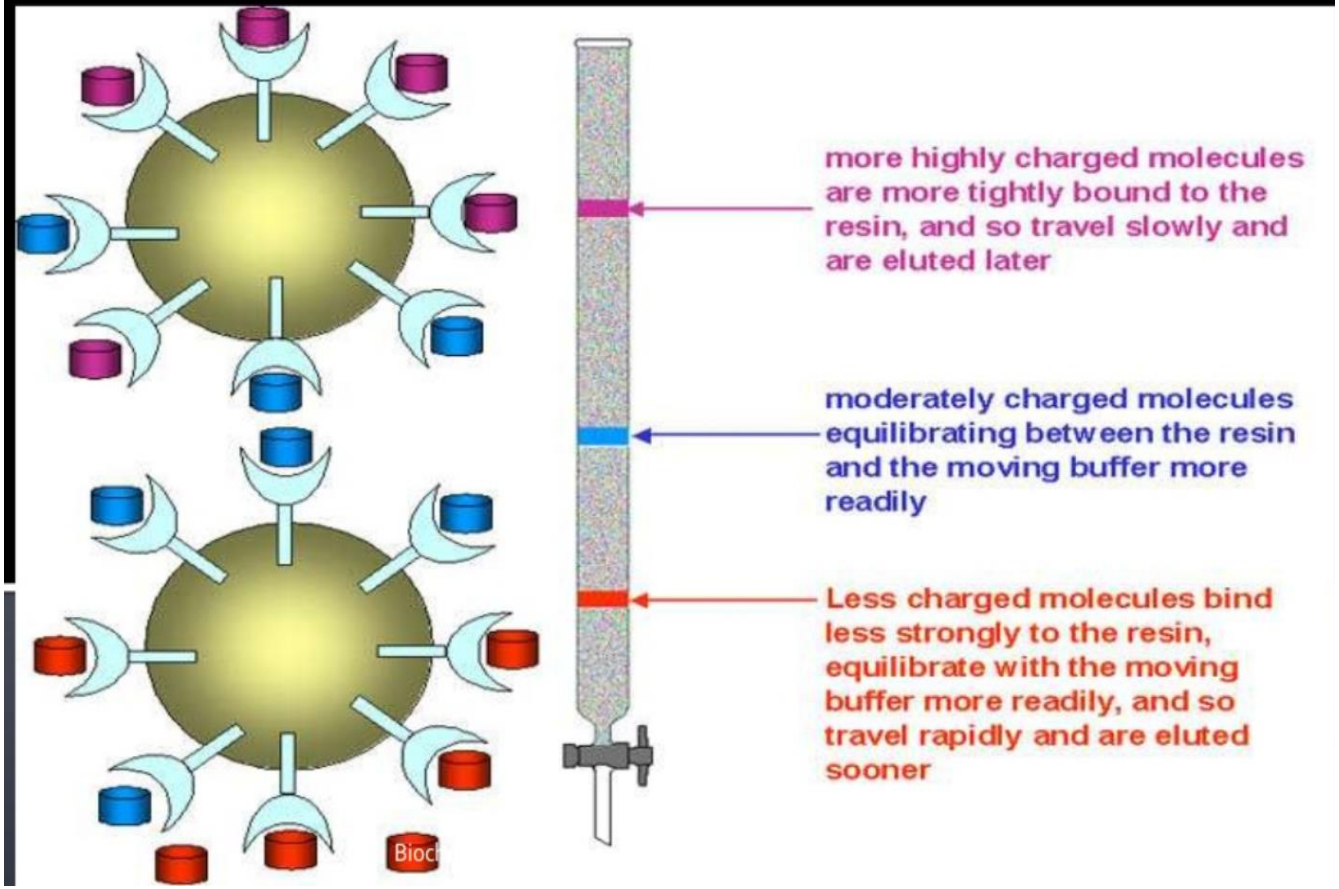
Various types of ion exchanges resins are commercially available-

Cation Exchange resin- Polysterene sulfonate resins, CM-Sephadex gel, CM – cellulose, These bear acidic groups and immobilize cations from adjacent solutions.

Anion exchangers- DEAE cellulose, Trimethyl amino Polysterene, DEAE- Sephadex. All these bear basic groups ionizing into fixed positions and immobilize anions from neighboring solutions.



Ion – Exchange chromatography- An Overview



4) Gel filtration chromatography or Molecular Sieve chromatography

-This is extremely useful in separating ribosomes, viruses, nucleic acids and proteins depending on their particle sizes and shapes.

-In Gel filtration chromatography, the separation of the particles is based on their size, shape and molecular weight.

-This technique is also referred to as molecular exclusion chromatography.

-The apparatus consists of a column packed with sponge like gel beads(usually cross- linked polysaccharides) containing pores.

-The gels serve as molecular sieves for the separation of smaller and bigger molecules.

Gel filtration chromatography or Molecular Sieve chromatography

-The solution mixture containing molecules of different sizes(say protein) is applied to the column and eluted with a buffer.

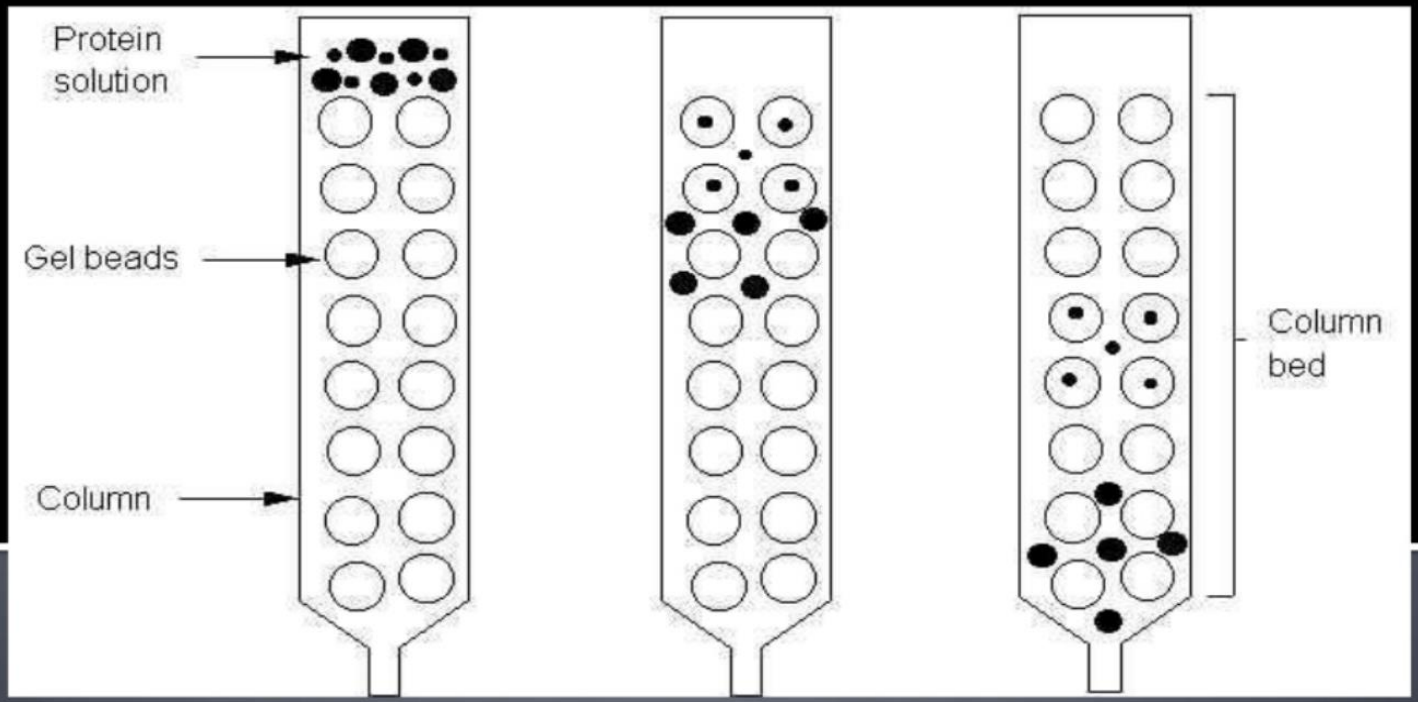
-The larger molecules can not pass through the pores of a gel and therefore move faster.

-The smaller molecules enter the gel beads and are left behind which come out slowly. By selecting the gel beads of different porosity, the molecules can be separated.

-The gel filtration chromatography can be used for an approximate determination of molecular weights.

-This is done by using a calibrated column with substances of known molecular weights.

Gel filtration chromatography-An Overview



Larger particles come out first, while smaller particles come in later fractions

5) Affinity chromatography

-The principle of affinity chromatography is based on the property of specific and non-covalent binding of proteins to other molecules, referred to as substrates or cofactors.

The technique involves the use of ligands covalently attached to an inert and porous matrix in a column. The immobilized ligands act as molecular hooks to selectively pick up the desired protein while the remaining proteins pass through the column. The desired protein captured by the ligands, can be eluted by using free ligand molecules. Alternatively, some reagents that can break protein ligand interactions can also be employed for the separation.

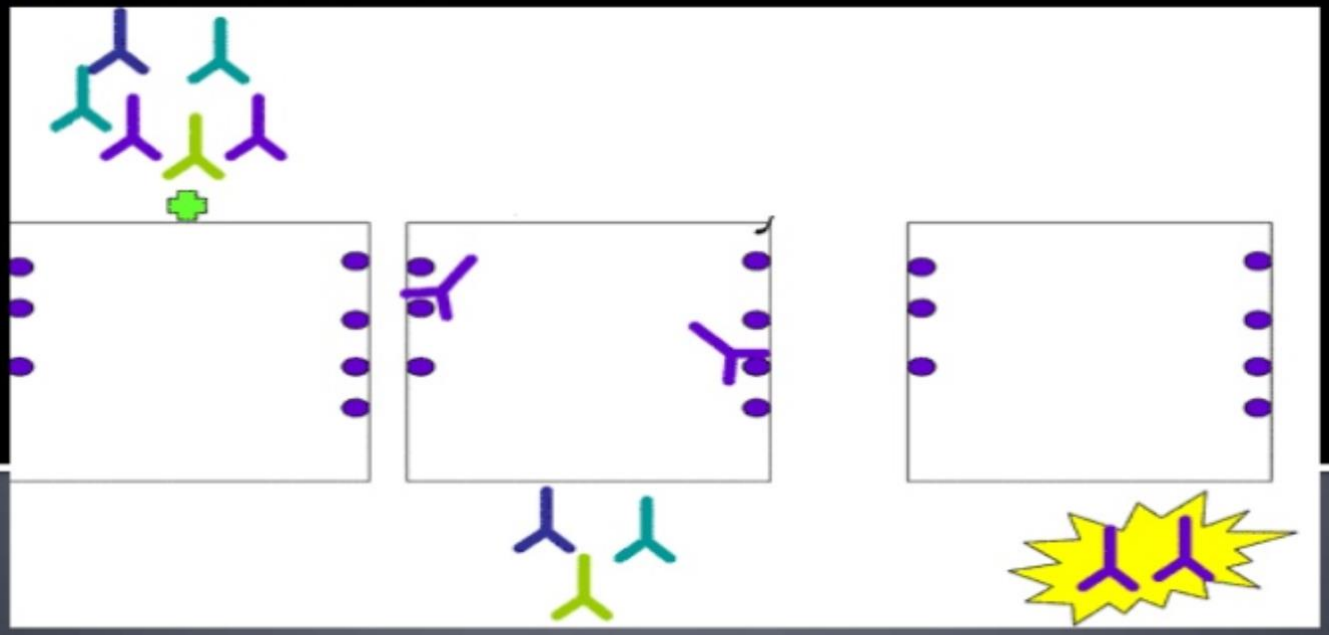
Affinity chromatography- Significance

1) Affinity chromatography is useful for the purification of enzymes, vitamins, nucleic acids, drugs , hormone receptors, antibodies etc.

2)It is also widely used for the estimation of Glycated Hb. Normal Hb does not bind and comes out first, while glycated Hb binds with the Boronic acid used as a ligand. Sorbitol is then added to elute the Glycated Hb which can be quantitated then.

Affinity chromatography- Significance

By using antibodies, antigens can be easily separated. Conversely, antibodies can be purified by passing through a column containing the antigen.



Specific Antibody binds to a specific antigen attached to the stationary medium, the remaining antibodies come out in earlier fractions.

6) Gas liquid chromatography-

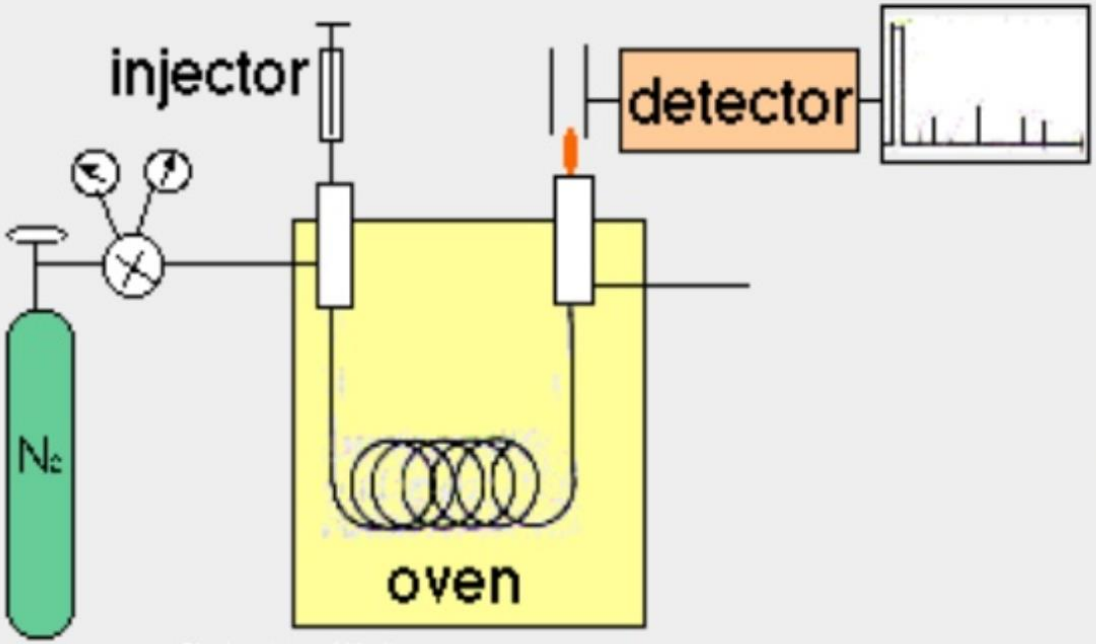
-The method of choice for the separation of volatile substances or volatile derivatives of certain in volatile substances.
-In GLC. the stationary phase is an inert solid material(diatomaceous earth or powdered firebrick), impregnated with a non volatile liquid(silicon or polyethylene glycol).
-This is packed in a narrow column and maintained at high temperature (around 200 degree C).
-A mixture of volatile material is injected in to the column along with the mobile phase, which is an inert gas (argon, helium or nitrogen).

Gas liquid chromatography-

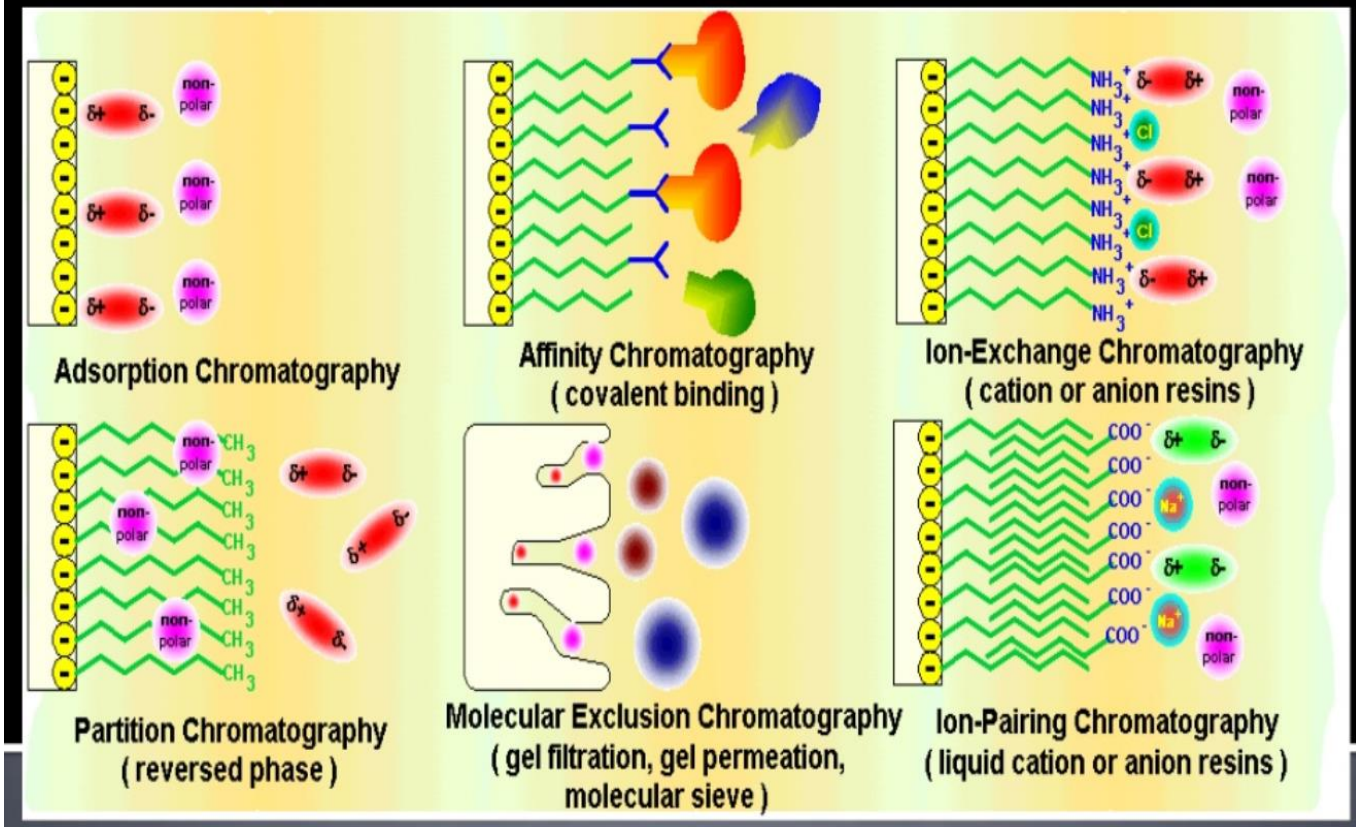
-The separation of the volatile material is based on the partition of the components between the mobile phase(gas) and stationary phase(liquid), hence the name gas liquid chromatography.

-The separated compounds can be identified and quantitated by a detector. Gas liquid chromatography is sensitive, rapid and reliable. It is frequently used for the quantitative estimations of biological materials such as lipids, drugs and vitamins.

Gas liquid chromatography-Apparatus



Summary


Adsorption Chromatography
Affinity Chromatography
(covalent binding)
Ion-Exchange Chromatography
(cation or anion resins)
Partition Chromatography
(reversed phase)
Molecular Exclusion Chromatography
(gel filtration, gel permeation,
molecular sieve)
Ion-Pairing Chromatography
(liquid cation or anion resins)