

# **Introduction to Separations Science**

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# Introduction to Separations Science

- What is separations science?
  - A collection of techniques for separating complex mixtures of analytes
  - Most separations are not an analytical technique in their own right, until combined with an analytical detector (often a type of spectrometer)

# What is a Separation?

- Separations are key aspects of many modern analytical methods. Real world samples contain many analytes, most analytical methods do not offer sufficient selectivity to be able to speciate all the analytes that might be present.
- Most separation methods involve separation of the analytes into distinct chemical species, followed by detection:

( a + b + c + d + ..... )     $\longrightarrow$     (a) + (b) + ( c ) + (d) +.....  
COMPLETE SEPARATION

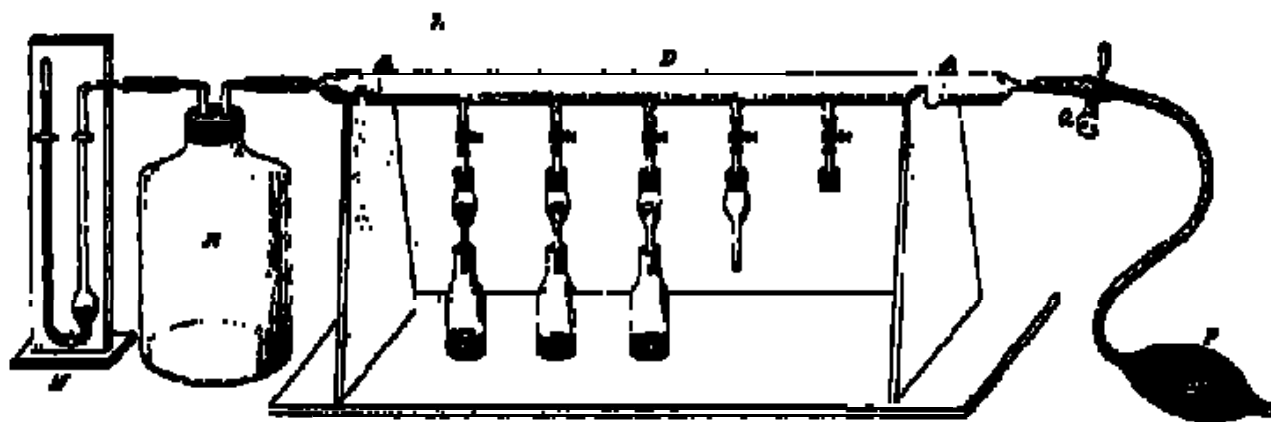
( a + b + c + d + ..... )     $\longrightarrow$     (a) + ( b + c + d+ ..... )     $\longrightarrow$   
PARTIAL SEPARATION

( a + b + c + d + ..... )     $\longrightarrow$     ( a + b ) + ( b + a ) + .....  
ENRICHMENT

DETECTION

# The 100-Year History of Separations

- Russian chemist and botanist Michael Tswett coined the term “chromatography”
- Chromatography was the first major “separation science”
- Tswett worked on the separation of plant pigments, published the first paper about it in 1903, and tested >100 stationary phases
  - Separated chlorophyll pigments by their color using  $\text{CaCO}_3$  (chalk), a polar “stationary phase”, and petroleum ethers/ethanol



Tswett's original adsorption chromatography apparatus

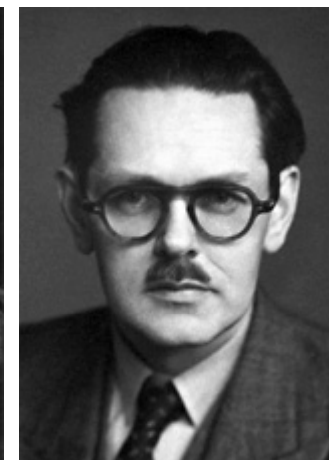


# History of Analytical Chromatography

- Chromatography was “rediscovered” by Kuhn in 1931, when its analytical significance was appreciated
- Chromatography very rapidly gained interest: Kuhn (Nobel prize in Chemistry 1937) separates carotenoids and vitamins
- 1938 and 1939: Karrier and Ruzicka, Nobel prizes in Chemistry
- 1940: established analytical technique
- 1948: A. Tiselius, Nobel prize for electrophoresis and adsorption
- 1952: A. J. P. Martin and R. L. M. Synge, Nobel prize for partition chromatography, develop plate theory
- 1950-1960: Golay and Van Deemter establish theory of GC and LC
- 1965: Instrumental HPLC developed



R. Kuhn



A. J. P. Martin

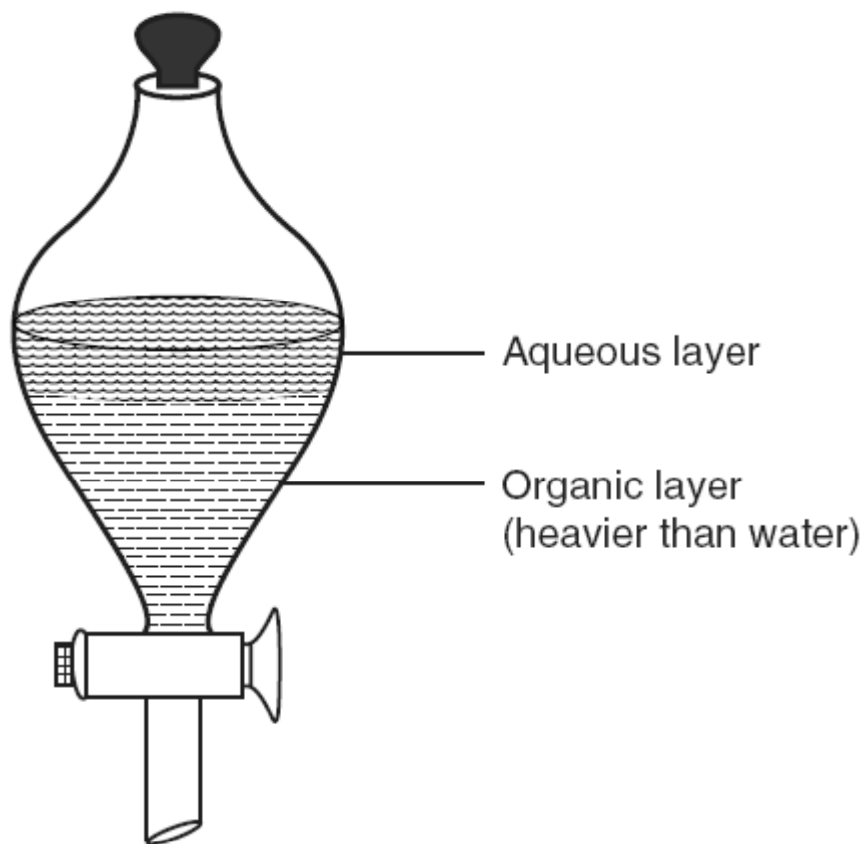


A. Tiselius



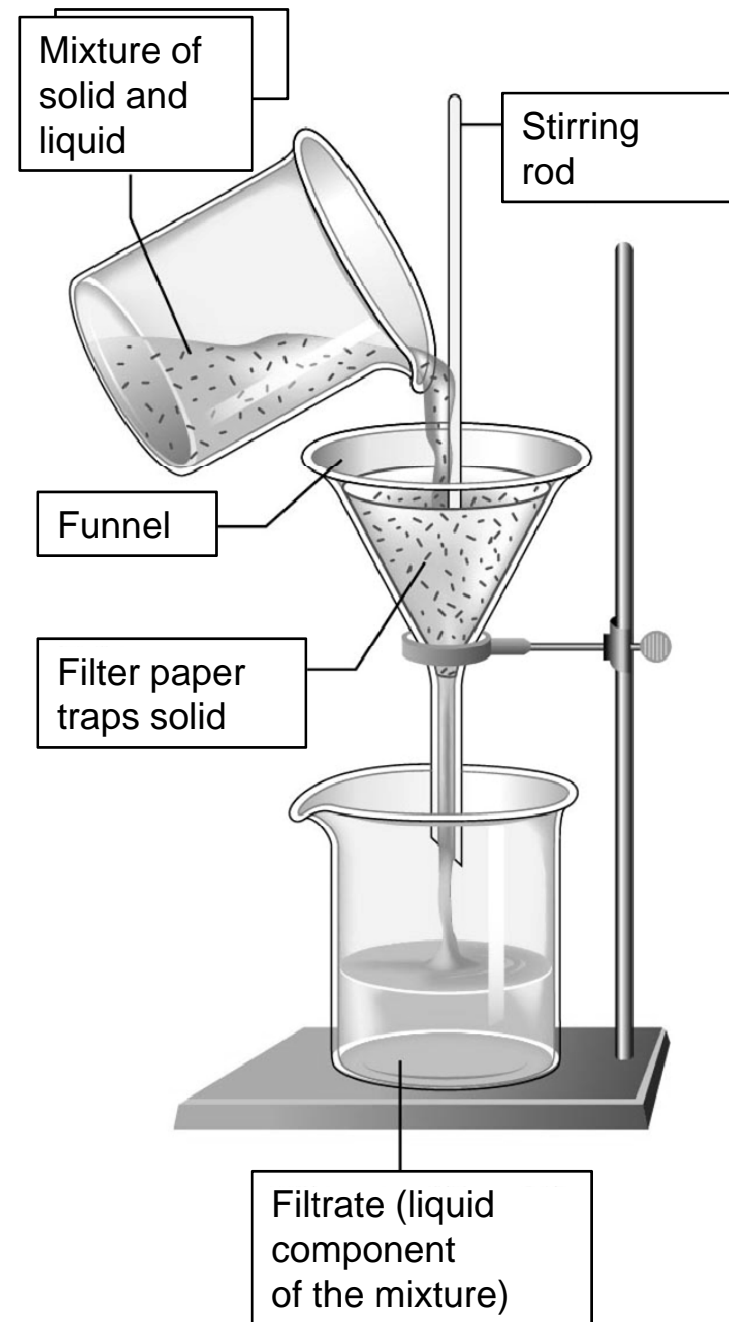
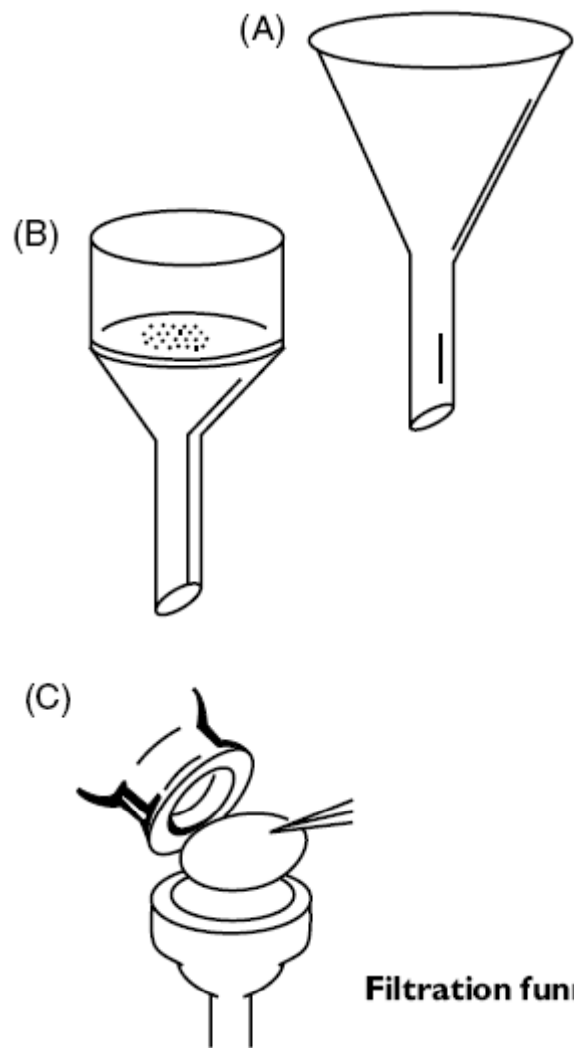
R. L. M. Synge

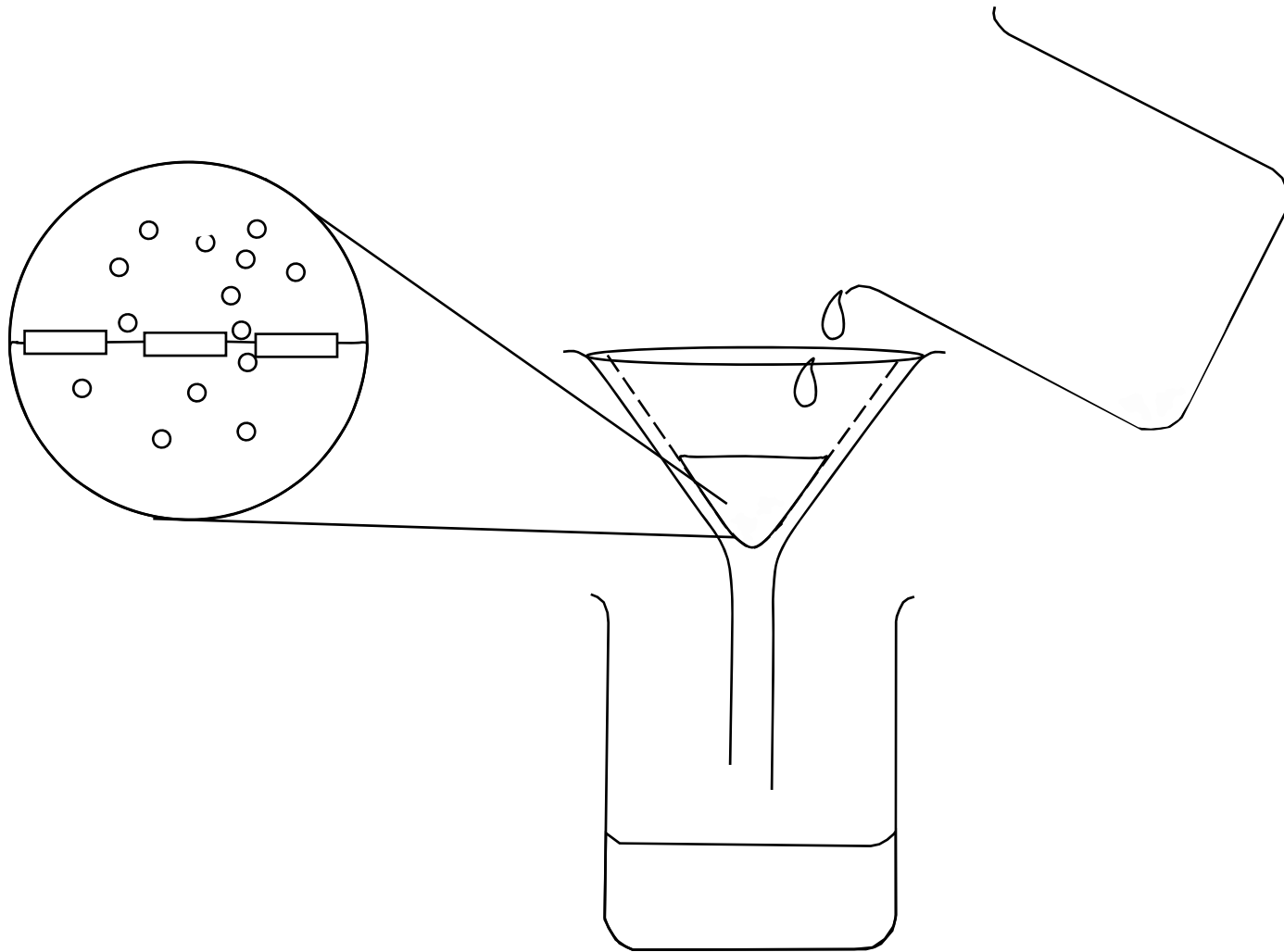
## separatory funnel



$$\text{Distribution coefficient } (K) = \frac{\text{concentration of sample in phase 1}}{\text{concentration of sample in phase 2}}$$

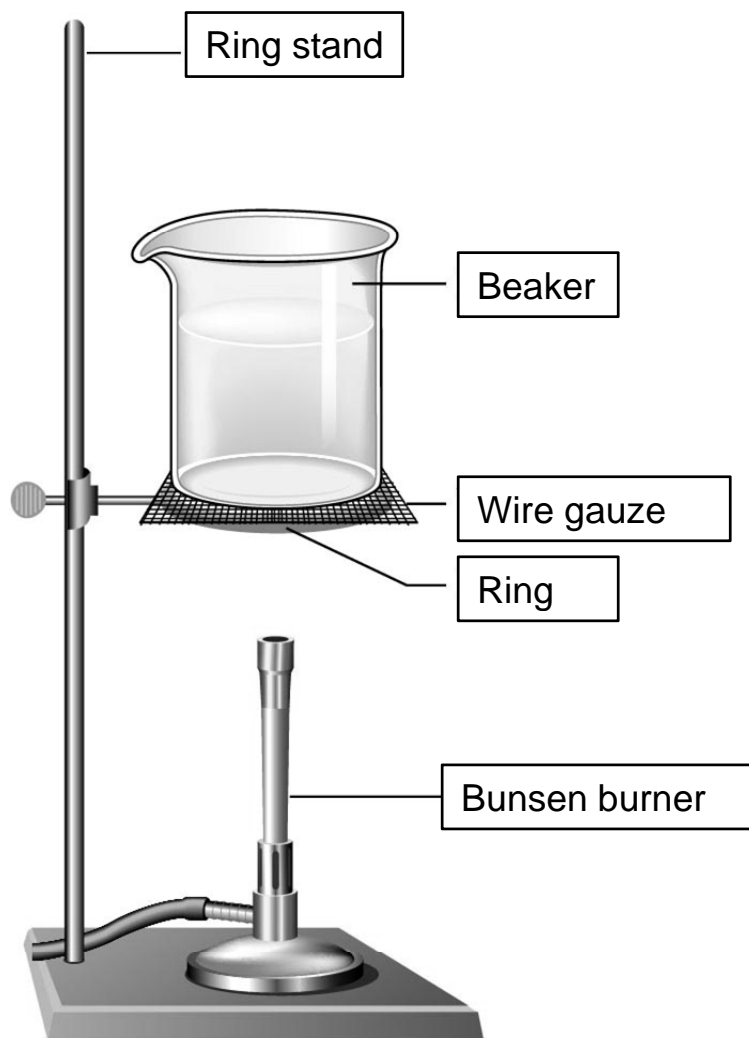
# Filtration



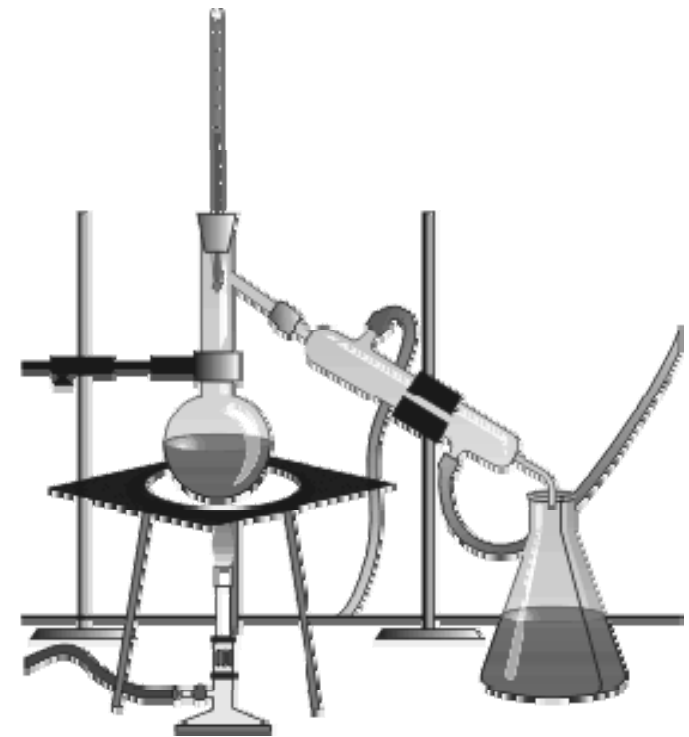




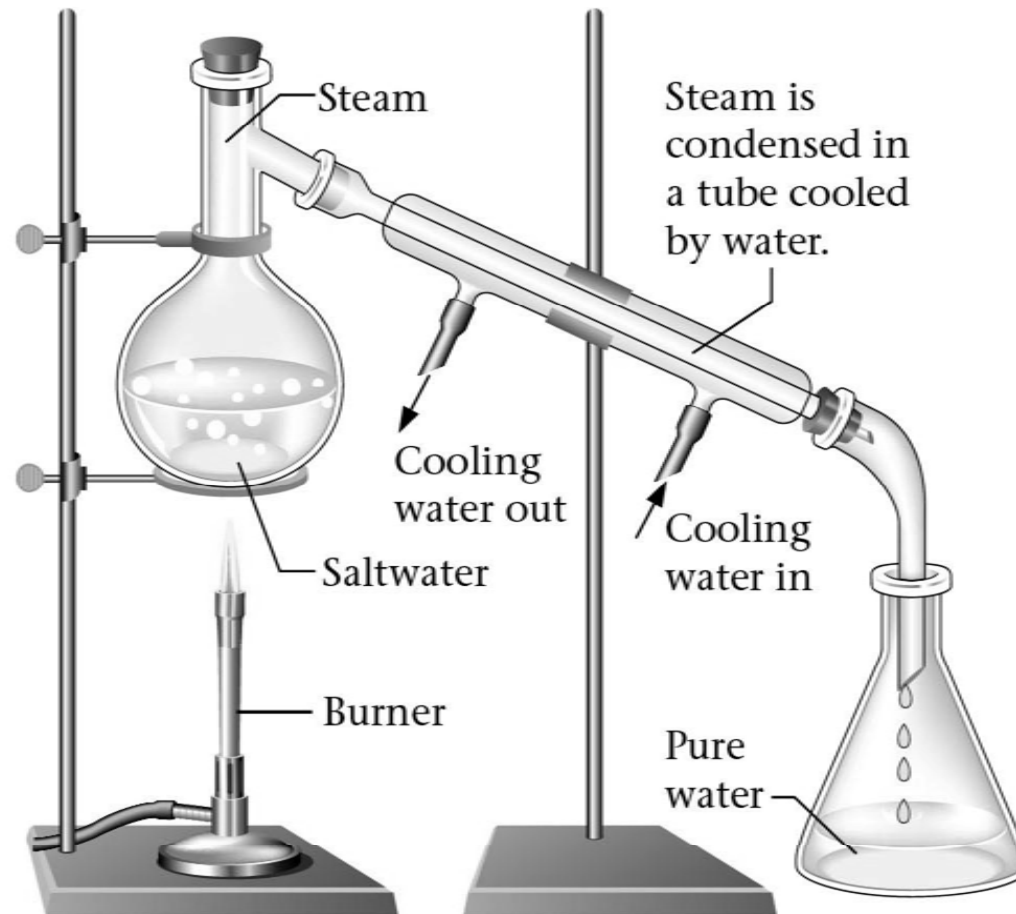
# Setup to heat a solution



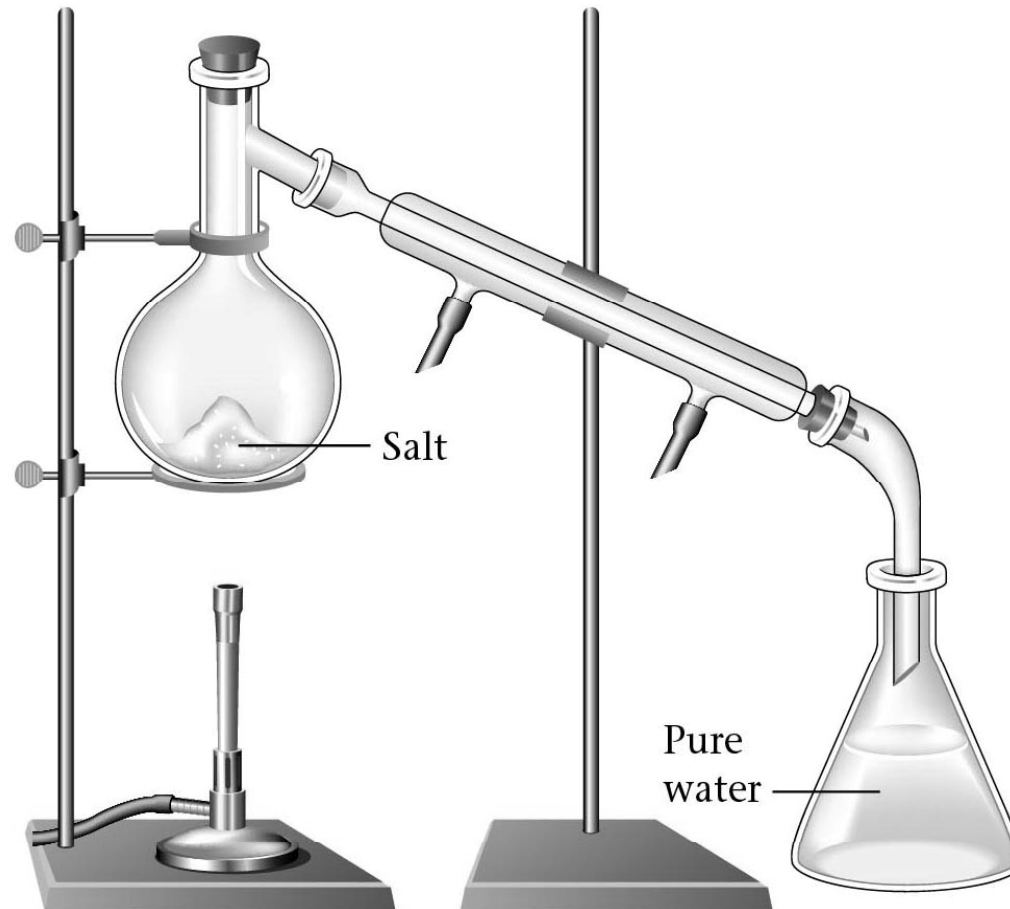
# Distillation



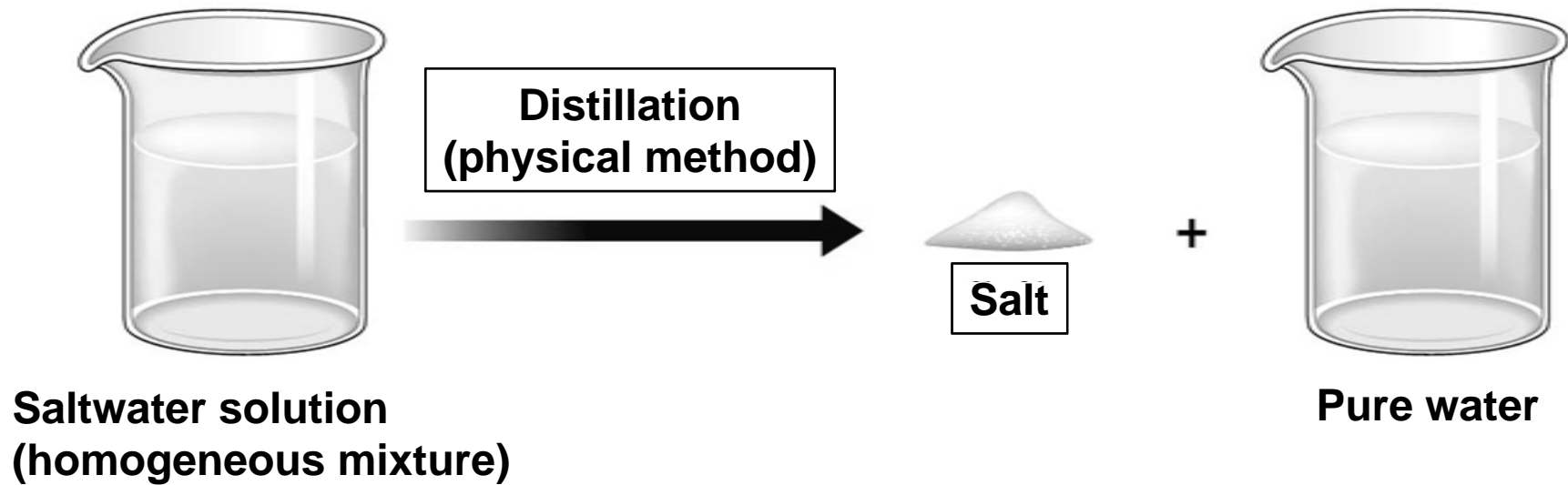
The solution is boiled and steam is driven off.



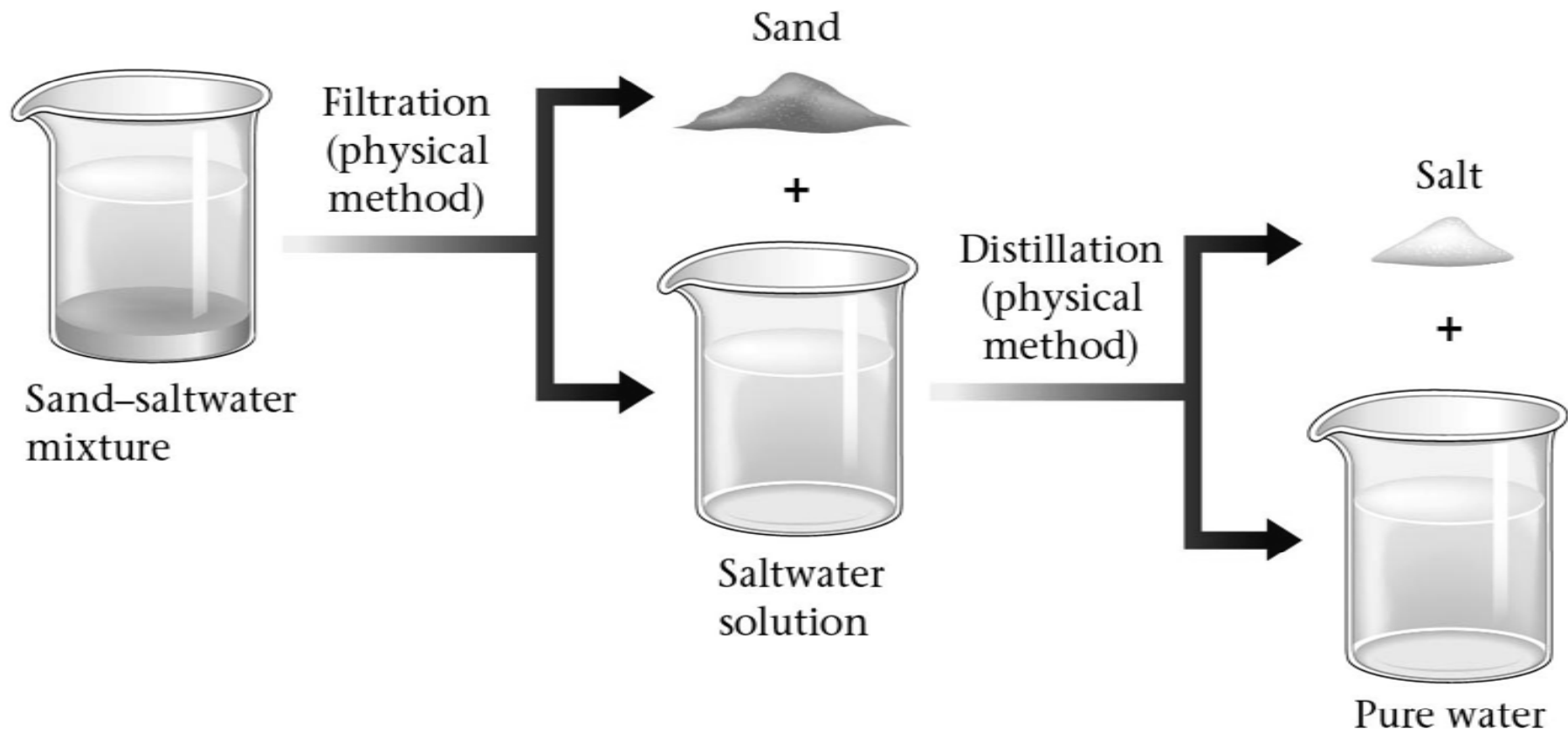
Salt remains after all water is  
boiled off.



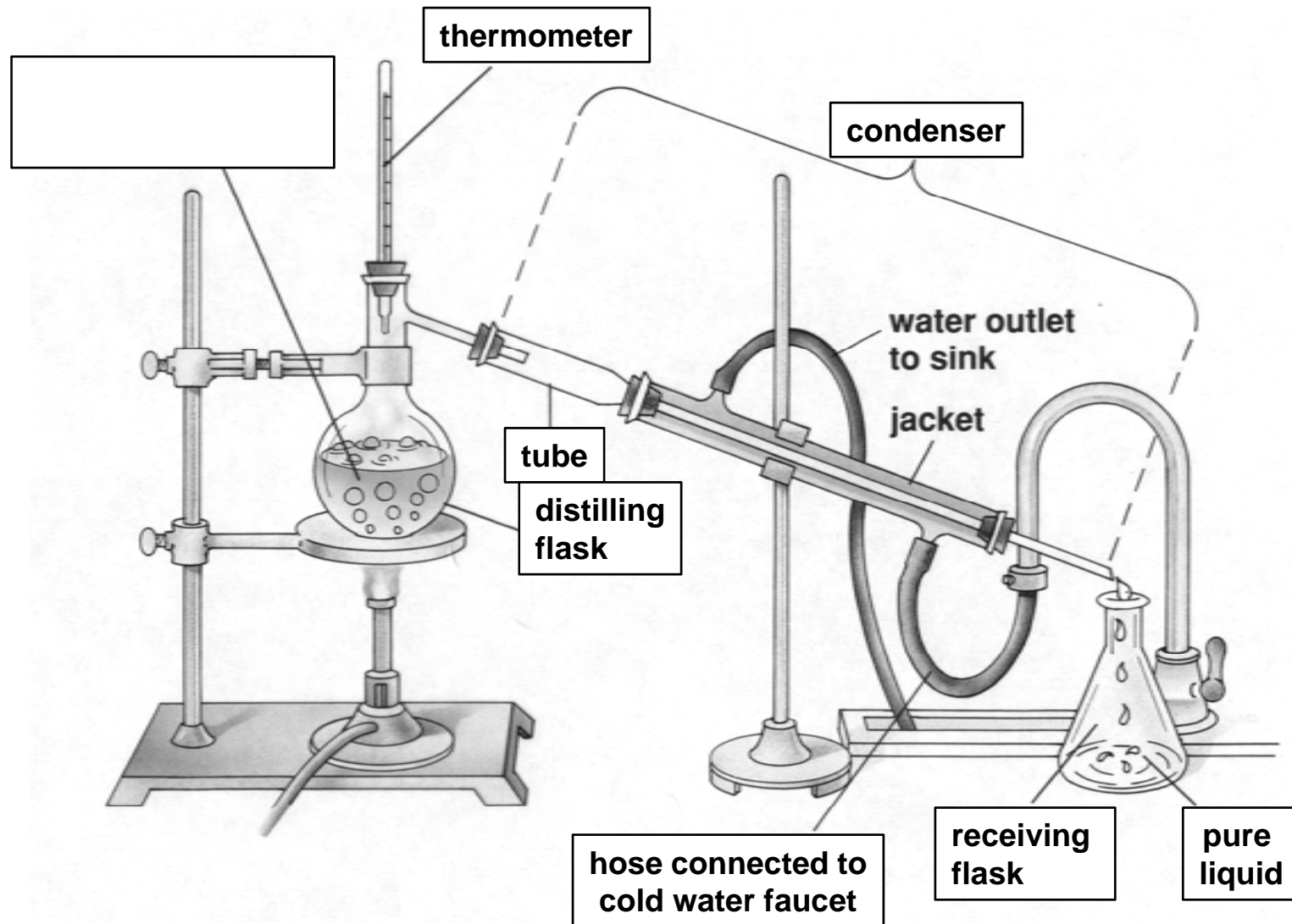
No chemical change occurs when salt water is distilled.



# Separation of a sand-saltwater mixture.



# A Distillation Apparatus



# EXTRACTION

- Is the separation of portions of plant (and animal) tissues using selective solvents through standard procedures (is the process by which a solute is transferred from one phase to a new phase).
- The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or in dry powder form (after removing the solvent).



# THE GENERAL TECHNIQUES OF EXTRACTION

- ✓ Maceration,
- ✓ Infusion,
- ✓ Percolation,
- ✓ Digestion,
- ✓ Decoction,
- ✓ Hot continuous extraction (Soxhlet),
- ✓ Aqueous-alcoholic extraction,
- ✓ Microwave-assisted extraction,
- ✓ Ultrasound extraction (sonication),
- ✓ Supercritical fluid extraction,
- ✓ Phytonic extraction (with hydrofluorocarbon solvents).

# CHOICE OF SOLVENTS

- Successful determination of biologically active compounds depends on the type of solvent used in the extraction procedure.
- The choice of solvent is influenced by what is intended with the extract.



# PROPERTIES OF A GOOD SOLVENT IN PLANT EXTRACTIONS

- ✓ Low toxicity,
- ✓ Ease of evaporation at low heat,
- ✓ Promotion of rapid physiologic absorption of the extract,
- ✓ Low cost
- ✓ Rapid availability,
- ✓ Inability to cause the extract to complex or dissociate.

# SOLVENTS USED FOR ACTIVE COMPONENT EXTRACTION

<b>Water</b>	<b>Ethanol</b>	<b>Methanol</b>	<b>Chloroform</b>	<b>Ether</b>	<b>Acetone</b>
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonol	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxylines			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

# STEPS INVOLVED IN THE EXTRACTION OF MATERIALS

1. Size reduction
2. Extraction
3. Filtration
4. Concentration
5. Drying

# 1. SIZE REDUCTION

## Objective:

- To rupture plant organ, tissue & cell structures so that its ingredients are exposed to the extraction solvent.
- Size reduction maximizes the surface area, which in turn enhances the mass transfer of active principle from plant material to the solvent.

The 30-40 mesh size is optimal.

Hammer mill or a disc pulverizer

# PARAMETERS INFLUENCING THE QUALITY OF AN EXTRACT

- Plant part used as starting material
- Solvent used for extraction
- Extraction procedure

# SELECTION OF PLANT

- Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc.
- Plants are usually air dried to a constant weight before extraction.
- oven drying: every part were cut into pieces  
→dried in an oven at 60°C for 9 hrs. & pulverized.
- Other method for drying the plants is the oven drying at about 40°C for 72 h.

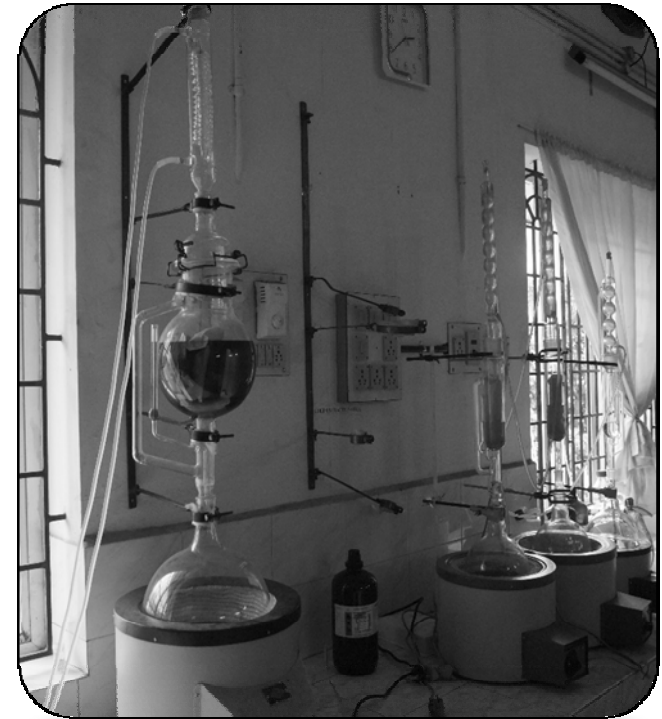


# FILTRATION



# VARIATION IN EXTRACTION METHODS

- Length of the extraction period,
- Solvent used,
- pH of the solvent,
- Temperature,
- Particle size of the plant tissues,
- The solvent-to-sample ratio.



# THE GENERAL TECHNIQUES OF EXTRACTION

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# Maceration

“The process in which material is placed or permitted to soak in a solvent for specific period of time until the cellular structure is softened and penetrated by the solvent and soluble constituents are dissolved and extracted out ”

Example: Tea bags



# Maceration process

- The whole / coarsely powdered material is placed in a stoppered container with the solvent
- Whole of the selected solvent (menstruum) added.
- Allow to stand at room temperature for a period of at least 3 days, shaking occasionally.
- The mixture then is strained, the marc (the damp solid material) is pressed,
- The combined liquids are clarified by filtration or decantation after standing.
- This method is best suitable for use in case of the thermolabile materials.

# INFUSION

- Fresh infusions are prepared by macerating the crude materials for a short period of time with cold or boiling water.
- These are dilute solutions of the readily soluble constituents of crude materials.



# Digestion

- This is a form of maceration in which gentle heat is used during the process of extraction.
- It is used when moderately elevated temperature is not objectionable. (40-50°C)
- It is better to use reflux condenser

Image=microwave  
Digestion system



# Decoction

- In this process, the crude material is boiled in a specified volume of water (1;4) for a defined time,
- Volume is reduced to 1/4<sup>th</sup> the original,
- It is then cooled and strained / filtered.
- This procedure is suitable for extracting → water-soluble, heat-stable constituents (roots,...)





# Percolation

- The solid ingredients are moistened with an appropriate amount of the specified menstruum,
- Allowed to stand for approximately 4 hours in a well closed container, After stand time, the mass is packed



- Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product.
- Sufficient menstruum is added to produce the required volume.
- The mixed liquid is clarified by filtration or by standing followed by decanting.

Percolation extraction system



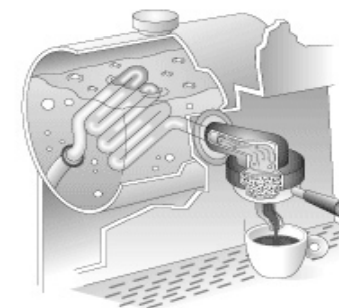
Filter machine



Neapolitan machine



Mocha machine



Espresso machine

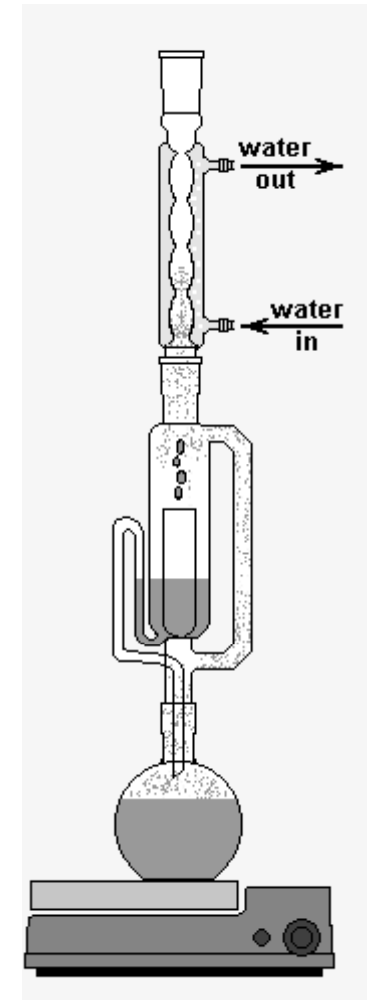
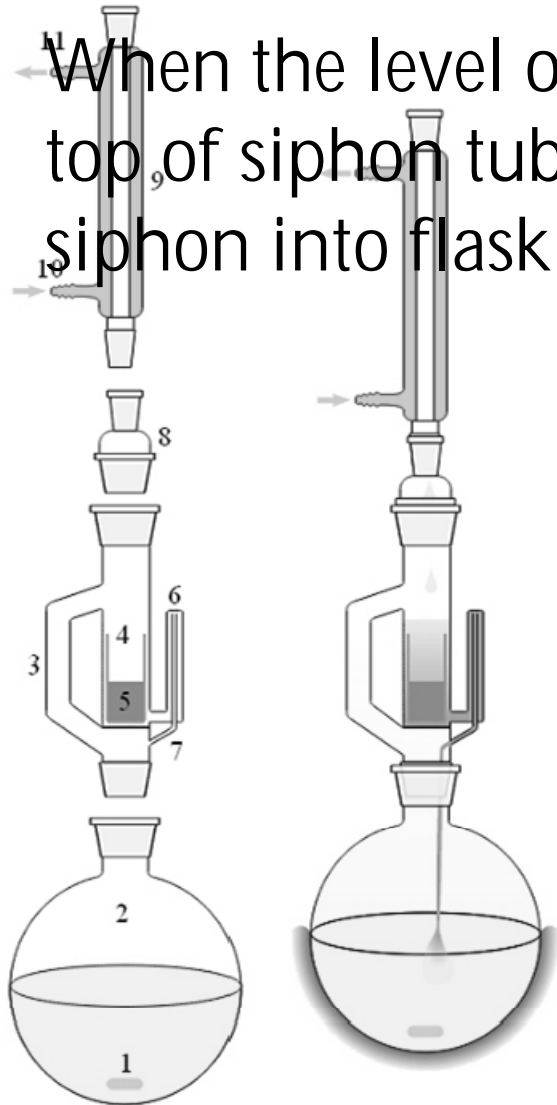
# Hot Continuous Extraction (Soxhlet)

- The finely ground crude material is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber of the Soxhlet apparatus.
- The extracting solvent in flask is heated, and its vapors condense in condenser.
- The condensed extractant drips into the thimble containing the crude material & extracts it by contact.

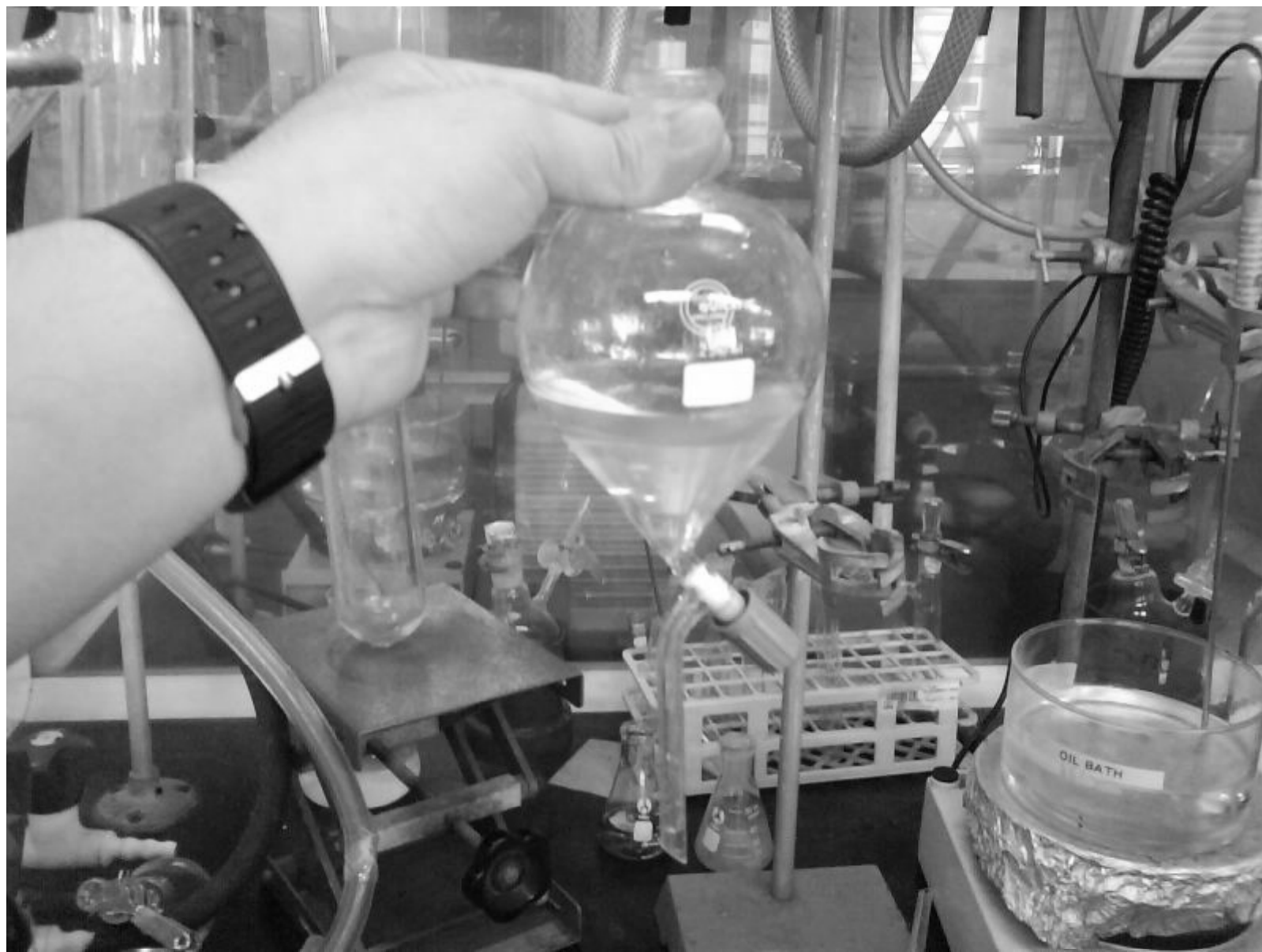


# SOXHLET APPARATUS

- When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber siphon into flask



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



Two phase system, hydrophobic (top) and hydrophilic (bottom) for measuring the partition coefficient of compounds.

# Chromatography

*'A method of separating a mixture of components into individual components through equilibrium distribution between two phases'.*

*OR*

*'A technique by which a mixture is separated into its components on the basis of relative ability of each component to be moved along/through a stationary phase by mobile phase'*

# Introduction

- *Solubility*
- *Adsorption*
- *Volatility*

*2 properties above are essential*

*basic principle: Different speed of components and mobile phase on stationary phase*



# Classification of chromatography

- Analytical ..... to determine the chemical composition of a sample
- Preparative ..... used to purify and collect one or more components of a sample

Types of chromatography:

- Adsorption chromatography
- Partition chromatography

# Types of chromatography:

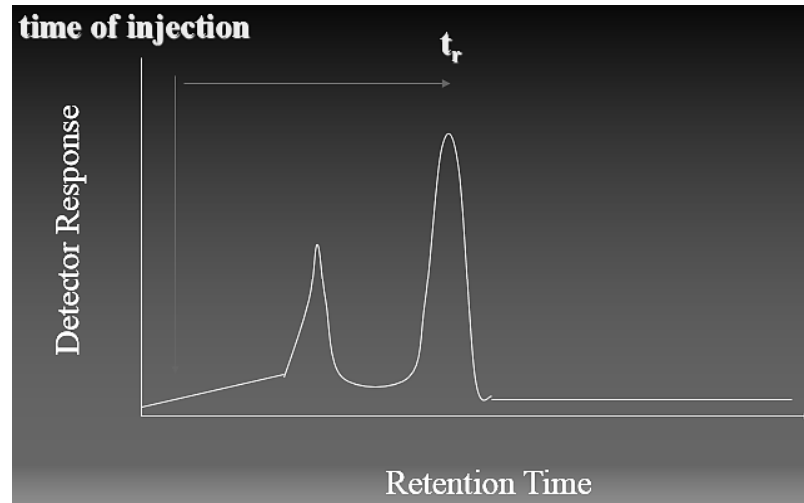
- Gas-Liquid-Partition Chromatography
- Gas-Solid-Adsorption Chromatography
- Ion Exchange Chromatography
- Electron-Exchange Chromatography
- Gel-Filtration Chromatography

## Commonly used terms in chromatography

- The analyte is the substance to be separated during chromatography.
- Analytical chromatography is used to determine the existence and the concentration of analyte(s) in a sample.
- A chromatograph is an equipment that enables the separation

# Commonly used terms in chromatography

- A chromatogram is the visual output of the chromatograph. In the case of an ideal separation, different peaks or patterns on the chromatogram represent different components of the separated mixture.



## Commonly used terms in chromatography

- The eluate is the mobile phase leaving the column.
- The eluent is the solvent that carries the analyte.
- An eluotropic series is a list of solvents ranked according to their eluting power.
- Elution is the process of extracting a substance by washing it with a solvent.

## Commonly used terms in chromatography

- An immobilized phase is a stationary phase that is immobilized on the support particles, or on the inner wall of the column tubing.
- The mobile phase is the phase that moves over the stationary phase. It may be a liquid (LC) or a gas (GC). The mobile phase moves through the stationary phase where the sample interacts with the stationary phase and is separated.

## Commonly used terms in chromatography

- The retention time is the time required for the mobile phase to sweep a component from the stationary phase.
- The retention volume is the volume of the mobile phase required to sweep a component through stationary phase.

## Commonly used terms in chromatography

- The sample is the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated, the phase or the phases containing the analytes of interest is/are referred to as the sample whereas everything else separated from the sample before or during analysis is referred to as waste.
- The solute refers to the sample components in partition chromatography.



## Commonly used terms in chromatography

- The solvent refers to any substance capable of solubilizing another substance, and especially the liquid mobile phase in liquid chromatography.
- The stationary phase is the substance fixed in place for the chromatography procedure. It may be solid, gel or a liquid. e.g ; silica, alumina, cellulose
- The detector refers to the instrument used for qualitative and quantitative detection of analytes after separation.

## Commonly used terms in chromatography

- $R_f$  value or Retention factor ( $R_f$ ) is defined as the ratio of the distance traveled by the center of a spot (solute) to the distance traveled by the solvent front (solvent)

$$\text{Retention factor} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}} = R_f$$

# Choice of a system of Chromatography

- Which system?
- Which mobile phase?
- Which stationary phase?

.....

## Polarity

- What does that mean “polarity” in solvent?
- Polarity of Sample
- Polarity of mobile phase
- Polarity of stationary phase

# Elutropic Series of Solvent

- Light Petroleum (Pet.ether, Hexane, Heptan, etc.)
- Cyclohexan
- Toluene
- Benzene
- Dichloromethane
- Chloroform
- Ethyl ether
- Acetone
- N-propanol
- Ethanol
- Methanol
- Water

Inceasing Polarity



# Stationary Phase

- Cellulose
- Starch
- Sugars
- Magnesium silicate
- Calcium sulfate
- Silicic acid
- Florisil
- Magnesium oxide (Magnesia)
- Aluminum oxide (alumina)
- Activated charcol



Increasing Polarity

# Compounds

- Hydriocarbones
- Olefins
- Ethers
- Halogen compounds
- Aromatics
- Ketones
- Aldehydes
- Alcohols, Amines, Mercaptans
- Acids and strong bases

Increasing Polarity



# Example:

- Silica gel or alumina: stationary phase
- Benzene: mobile phase

-CH<sub>3</sub>

-O-Alkyl

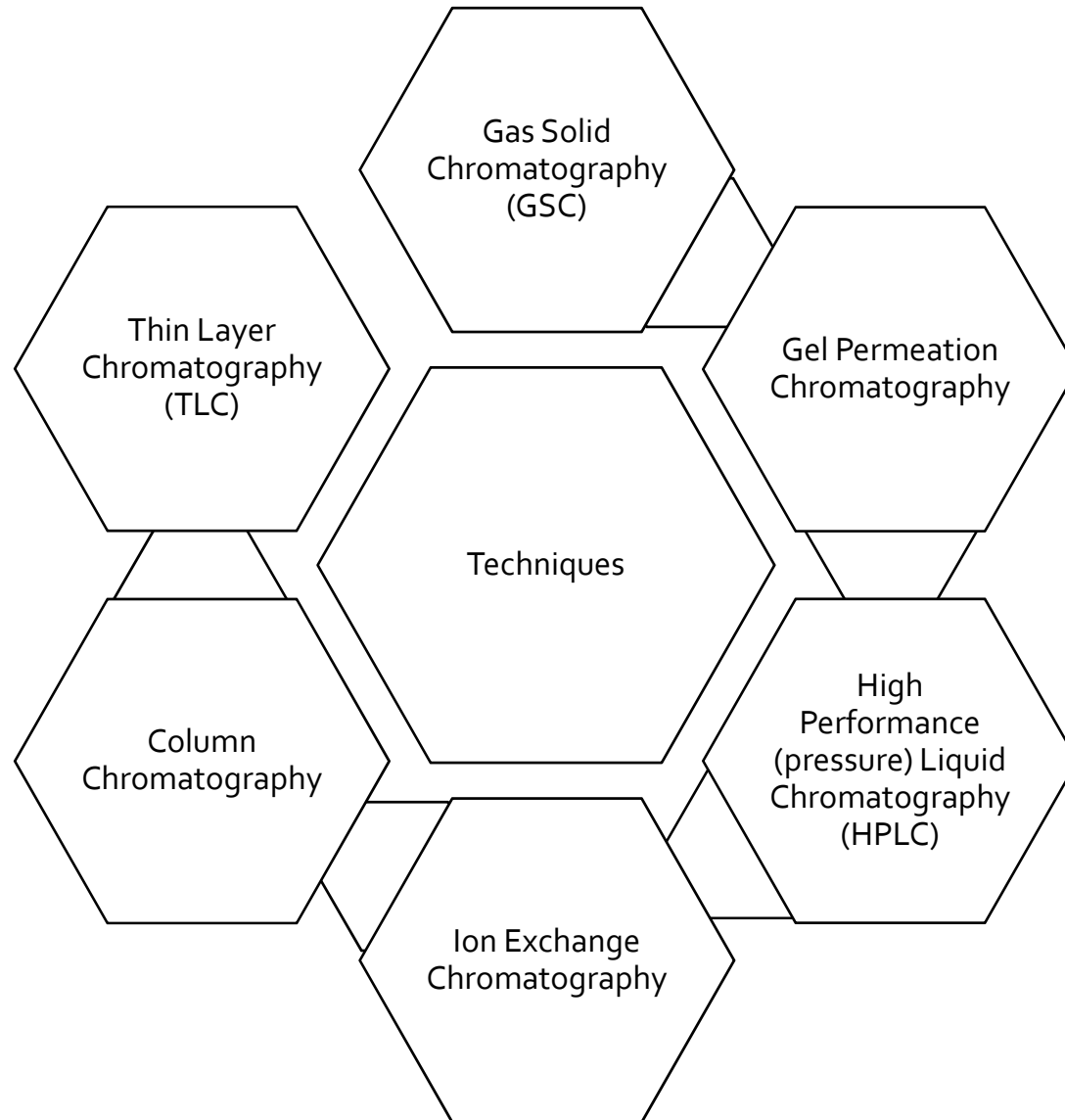
C=O

-OH

-COOH

# Adsorption Chromatography

- It uses a mobile phase or gaseous phase that is adsorbed onto the surface of a stationary solid phase. The equilibration between the mobile and stationary phase accounts for the separation of different solutes. Following are the chromatographic techniques that are included in this category:





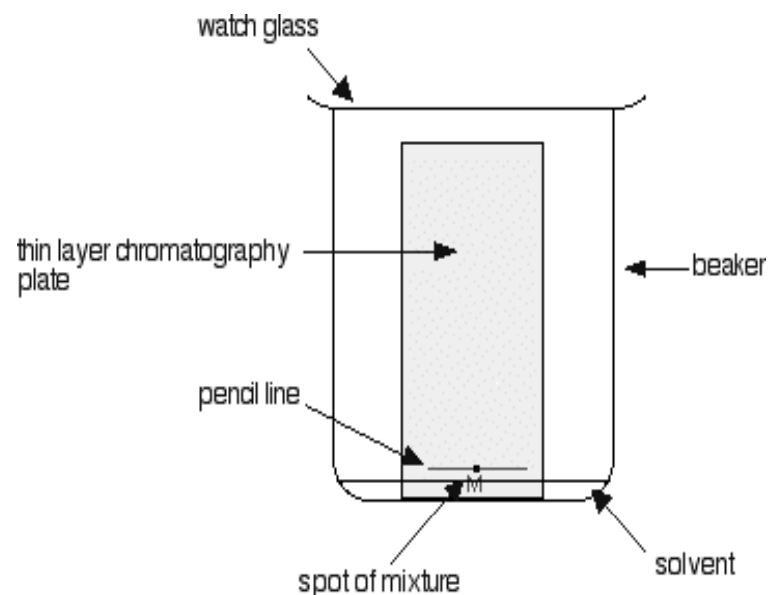
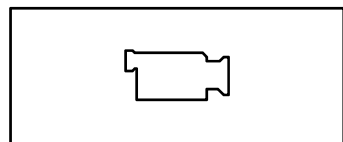
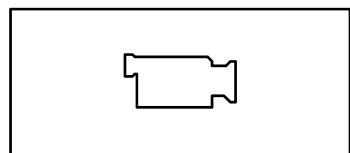
# Thin Layer Chromatography (TLC)

- Thin layer chromatography is similar to paper chromatography, but the Stationary phase is a thin layer of a solid such as silica gel, alumina, or cellulose supported on an inert base such as glass, aluminum foil or insoluble plastic.
- The mixture is 'spotted' at the bottom of the TLC plate and allowed to dry. The plate is placed in a closed vessel containing solvent (the mobile phase) so that the liquid level is below the spot.
- TLC is also called as **drop, strip, spread layer, surface chromatography** and **open column chromatography**.

# Thin Layer Chromatography (TLC)

TLC has advantages over paper chromatography in that its results are more reproducible, and that separations are very efficient because of the much smaller particle size of the stationary phase.

faster runs and the choice between different adsorbents.



# Thin Layer Chromatography (TLC)

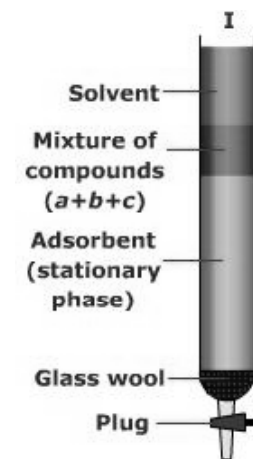
$$\text{Retention factor} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}} = R_f$$

•For substances that are very soluble in the liquid  $R_f$  will be close to ....1

•For substances that are rather insoluble in the liquid  $R_f$  will be close to ....0

# Column Chromatography

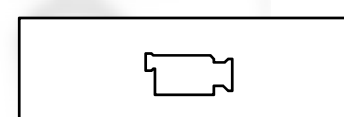
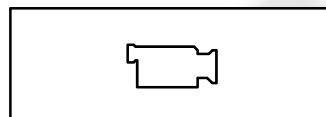
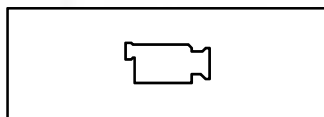
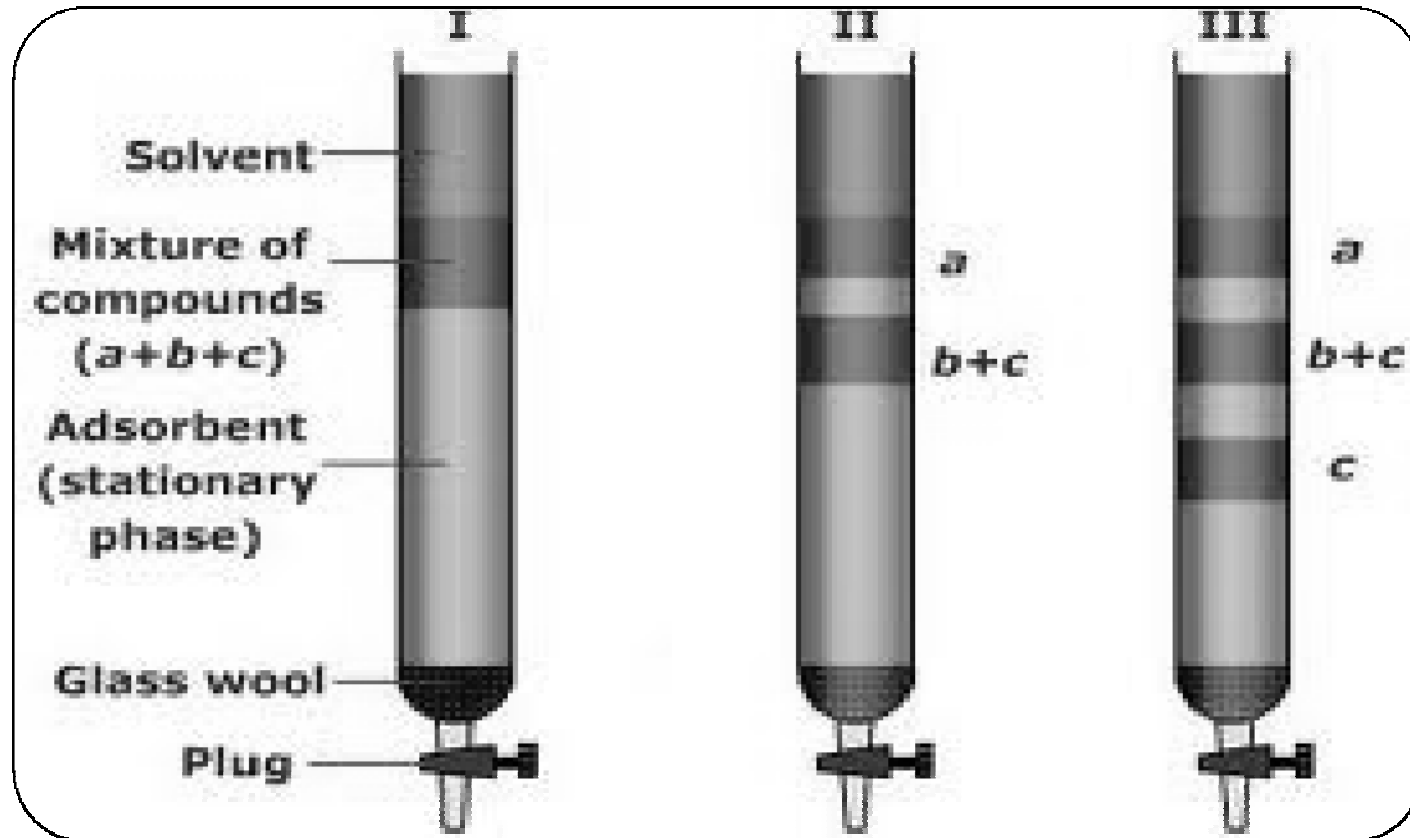
- Column chromatography is a separation technique in which the stationary bed is within a tube. Adsorbents are packed into a column in a glass tube. This serves as the stationary phase.
- Adsorbents such as silica gel, alumina, charcoal powder, calcium hydroxy , cellulose ,..... are used.



# Column Chromatography

- The sample mixture in a solvent is loaded on this column. The individual compounds get differentially adsorbed on to the adsorbent.
- The elution is carried out by the buffer system which is the mobile phase.
- The individual compounds come out of the column at different rates which may be collected separately & identified.

# Column Chromatography

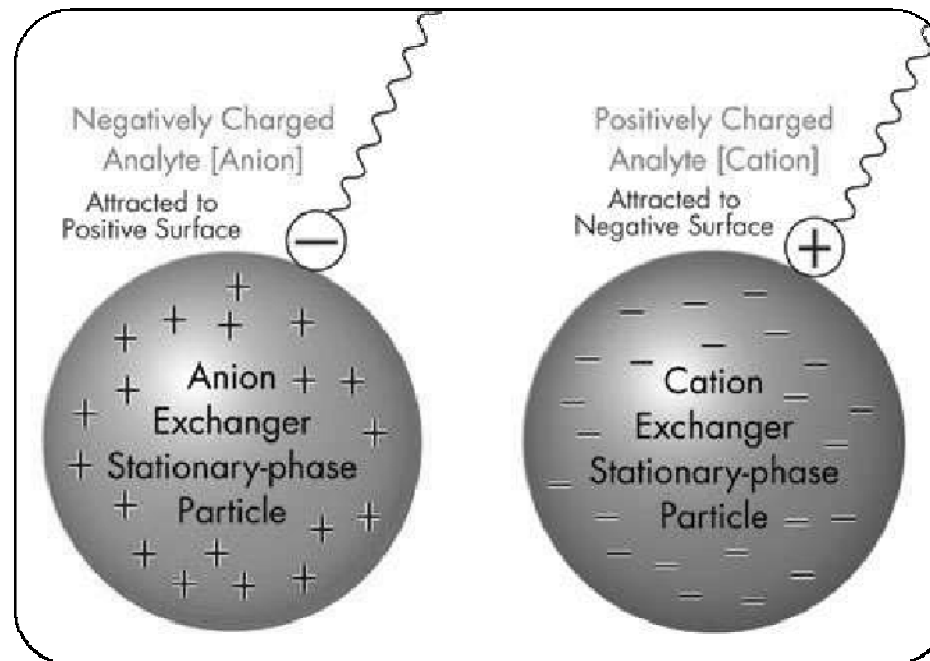


# Ion Exchange Chromatography (IEC)

- Ion exchange chromatography (usually referred to as ion chromatography) uses an ion exchange mechanism to separate molecules on the basis of their electrical charges.
- Ion exchange chromatography uses a charged stationary phase to separate charged compounds including anions, cations, amino acids, peptides, and proteins.

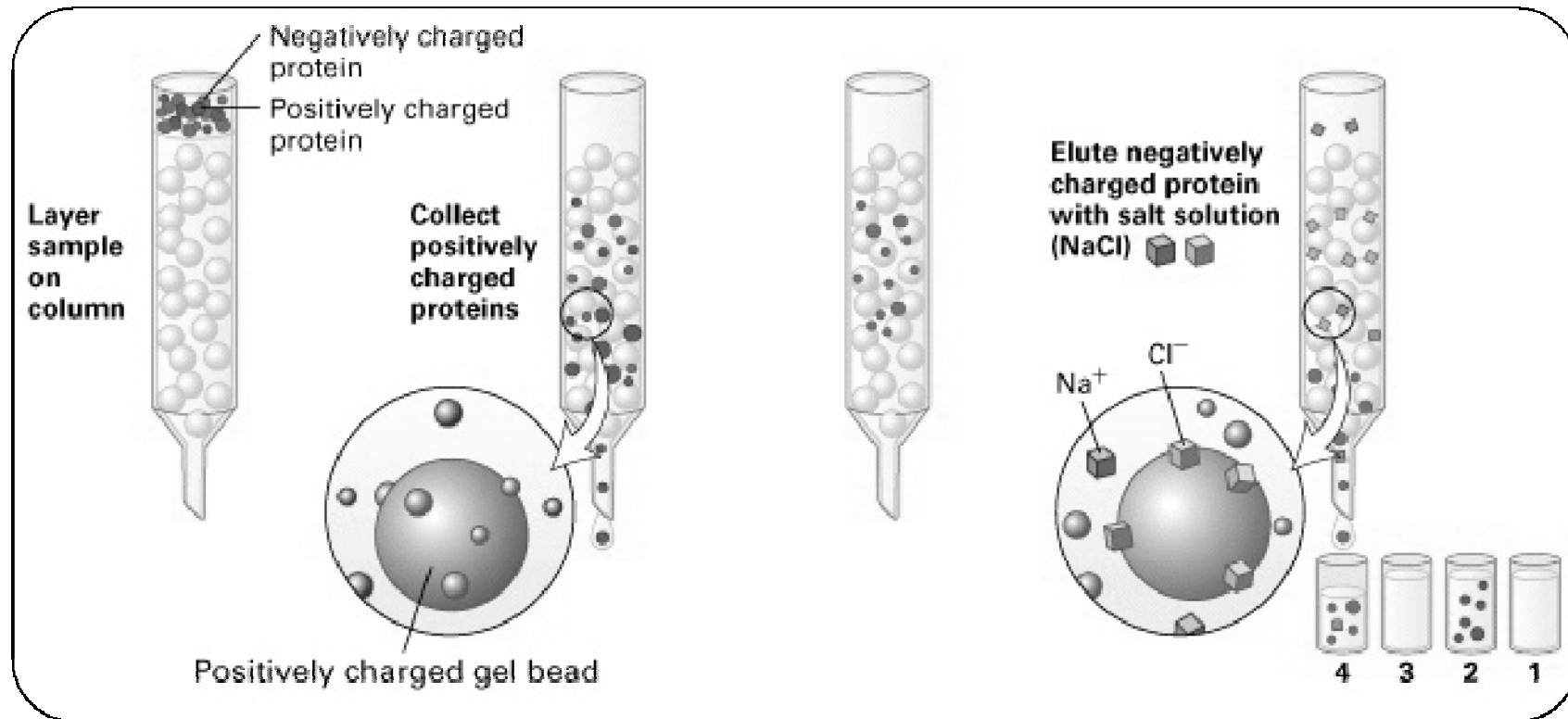
# Ion exchange chromatography

- Cation exchangers & anion exchangers are used as ion exchange resins.
- In conventional methods the stationary phase is an ion exchange resin that carries charged functional groups that interact with oppositely charged groups of the compound.



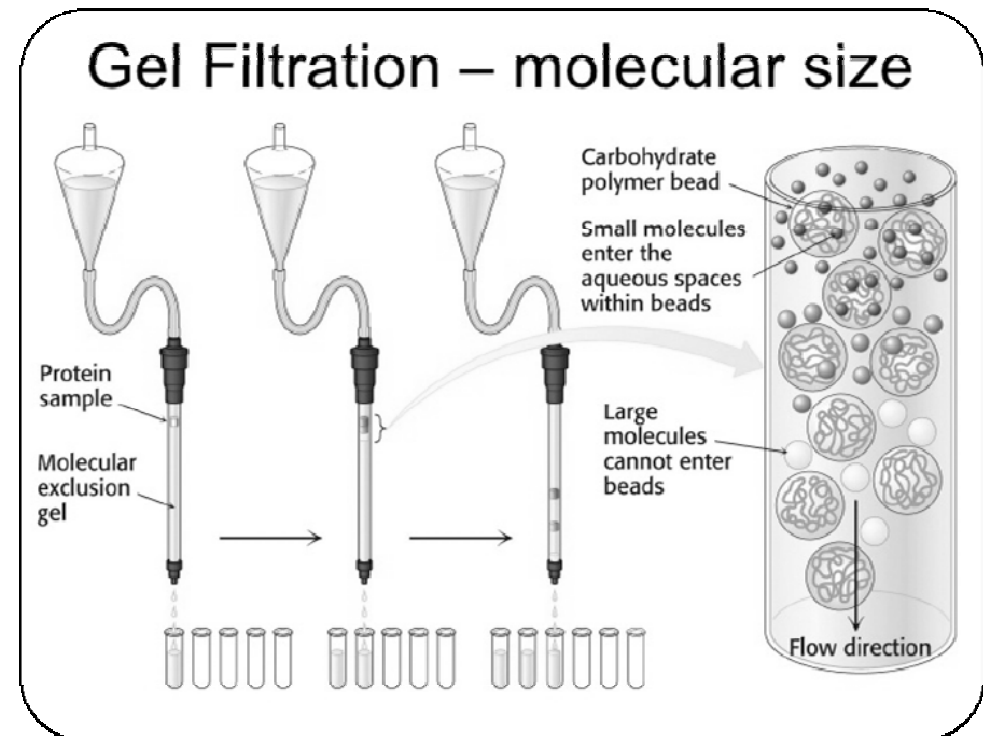


# Ion exchange chromatography



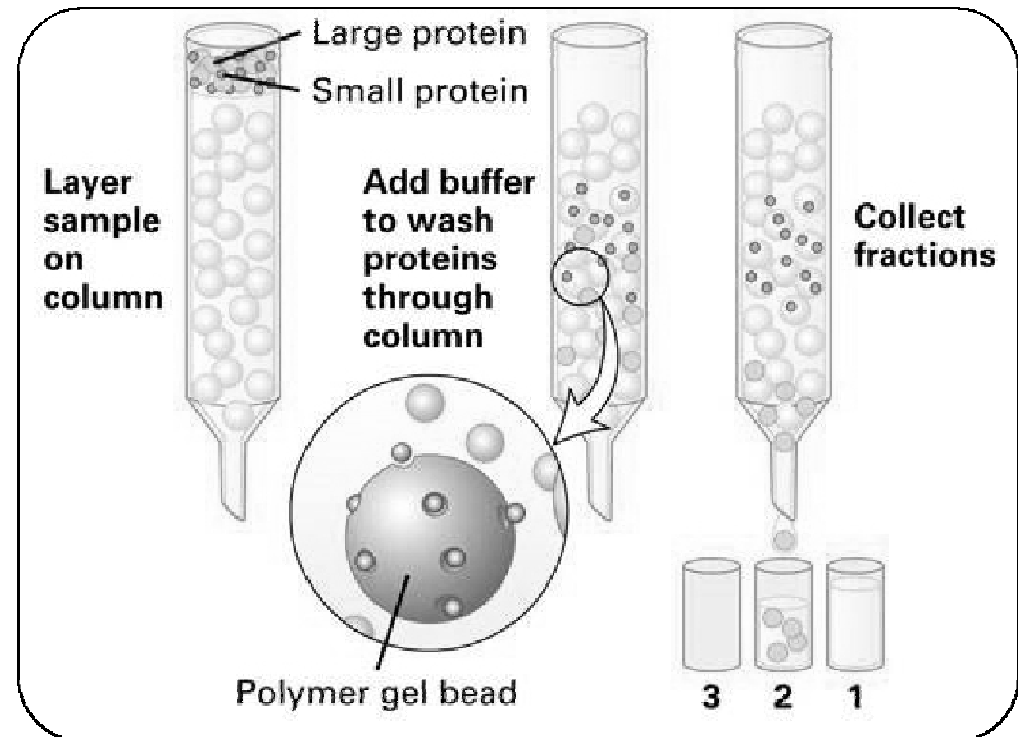
# Gel filtration (Permeation) chromatography

- Size-exclusion chromatography (SEC) is also known as **gel permeation chromatography (GPC)** or **gel filtration chromatography** separates molecules according to their size, shape & molecular weight.
- It is also referred to as molecular sieving or molecular exclusion chromatography.



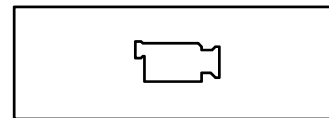
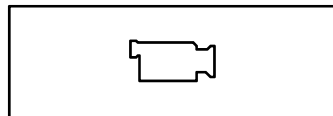
# Gel filtration (Permeation) chromatography

- Smaller molecules are able to enter the pores of the media and, therefore, molecules are trapped and removed from the flow of the mobile phase.
- However, molecules that are larger than the average pore size of the packing are excluded and thus suffer essentially no retention; such species are the first to be eluted. This is how the molecules are separated.



# Gel filtration (Permeation) chromatography

- It is generally a low-resolution chromatography technique and thus it is often reserved for the final, "polishing" step of a purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins.



# Gas chromatography (GC)



- In Gas Chromatography, the components of a vaporized sample are separated as a result of being partitioned between a mobile gaseous phase and a liquid or a solid stationary phase held in the column.
- A sample is being injected at the inlet/injector and vaporized into the chromatographic column.
- The sample is transported through the column by the flow of inert gaseous mobile phase.

# Gas chromatography (GC)

- As the sample passes through the column, they are separated and detected electronically by detector.
- Gas is called “carrier gas”.
- Typical carrier gas: helium or nitrogen.
- Pressure from a compressed gas cylinder containing the carrier gas is sufficient to create the flow through the column.

## *Advantages of Gas Chromatography*

- ★ The technique has strong separation power and even complex mixture can be resolved into constituents
- ★ The sensitivity of the method is quite high
- ★ It gives good precision and accuracy
- ★ The analysis is completed in a short time
- ★ The cost of instrument is relatively low and its life is generally long
- ★ The technique is relatively suitable for routine analysis

# Gas chromatography (GC)

There are two types.

- **Gas-liquid chromatography (GLC)**

  - mobile phase – gas

  - stationary phase - liquid

} Shortened  
to GC

- **Gas-solid chromatography (GSC)**

  - mobile phase – gas

  - stationary phase - solid

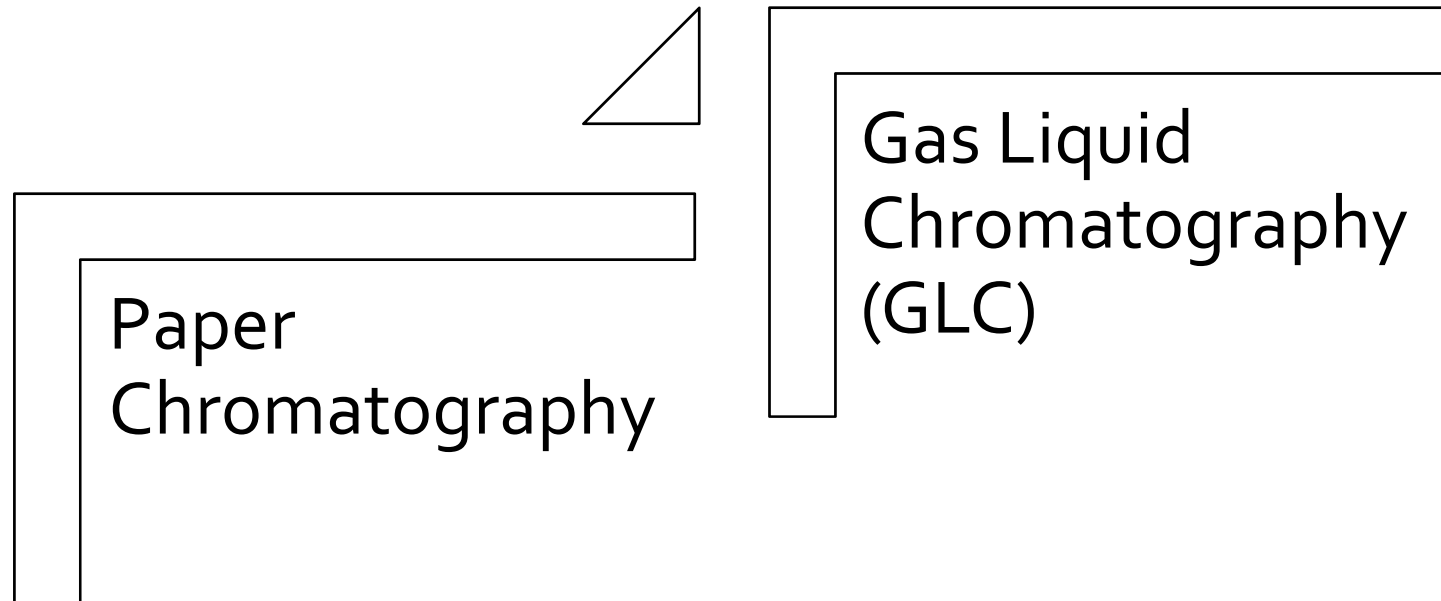


## Gas-solid chromatography (GSC)

- GSC utilizes a solid adsorbent as the stationary phase while gas as a mobile phase and an adsorption process takes place.
- The separation method can be affected by the polarity of stationary phase, temperature, carrier gas flow, length of column, material amount etc.

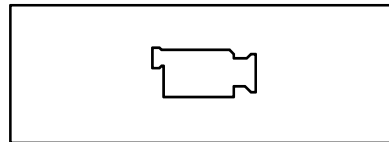
# Partition Chromatography

- This form of chromatography is based on a thin film formed on the surface of a solid support by a liquid stationary phase. Solute equilibrates between the mobile phase and the stationary liquid.



# Gas-Liquid chromatography (GLC)

- In GLC the mobile phase is a gas and stationary phase is a thin layer of a non-volatile liquid bound to a solid support thus a partition process occurs. In such case small inert particles such as diatomaceous earth is coated with the liquid so that a large surface area exists for the solute to equilibrate with.



# INSTRUMENTATION

- A. Carrier gas
- B. Flow regulator
- C. Injector
- D. Column
- E. Detector
- F. Integrator
- G. Display system  
printer/monitor

Thermostated  
oven

