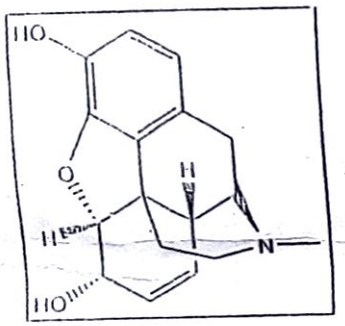
Extraction, Isolation and Purification of Organic Compounds

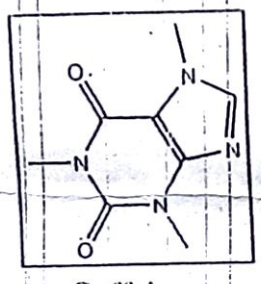
A. Extraction of Organic Compounds

An aspect of **Organic Chemistry** deals with natural products, which refers to organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism. Natural products are obtained from their natural sources or origin by the process called extraction.

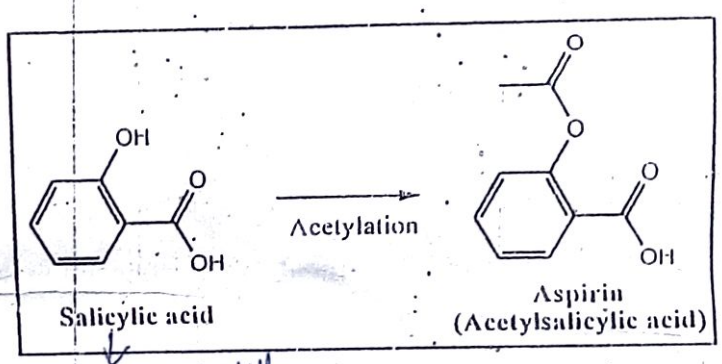
Extraction is a chemical, physical or mechanical means of obtaining something (compounds) from a mixture or crude (plant). Various organic compounds (secondary metabolites) have been extracted and isolated from different medicinal plants. Apparently, medicinal plants play vital roles in management of human health and it has been a source of 'lead' for drug development. For instance, morphine, an analgesic organic compound, was isolated from opium plant by Serturner in 1803. Caffeine, a natural stimulant has been isolated from various plants which include cocoa plant, coffee leaves, tea leaves etc. Also, aspirin (also known as acetylsalicyclic acid) is a synthetic derivative and prodrug of salicylic acid (2-hydroxybenzoic acid) which is usually obtained from the bark of *willow tree.*



Morphine (from opium plant)



Caffeine

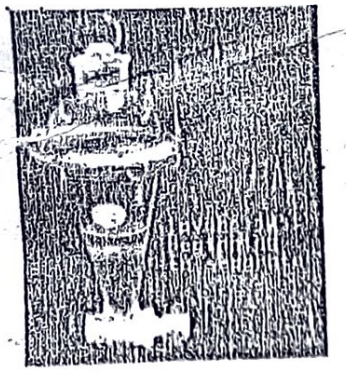


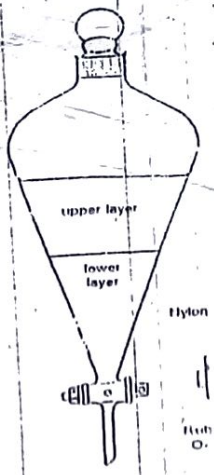
Salicylic acid — **Aspirin (Acetylsalicylic acid)**

There are different kinds of extraction of organic compounds namely;

1. **Solid-Liquid Extraction;** Solid-liquid extraction allows soluble components to be removed from solids using a solvent(s). This can be done by pulverizing/grounding the solid to be extracted in a powder form. Applications of this unit operation include obtaining oil from oil seeds (groundnut), compounds from plant leave or bark, tea (caffeine from tea leave/bag) or leaching of metal salts from ores. The product obtained in this form is called **Extract.** The extract is concentrated either by distillation or evaporation.

2. Solvent extraction; This is also known as **Liquid-liquid extraction** and **partitioning**. It is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. Liquid-liquid extraction is a basic technique in chemical laboratories, where it is performed using a separating funnel.





Separating funnel

The desired component is more soluble in the extracting solvent. This type of process is commonly performed after a chemical reaction as part of the work-up. For instance, Caffeine can be extracted from aqueous medium by dichloromethane. The distribution coefficient K_d is important in this type of extraction.

$$K_d = \frac{\text{concentration of solute in solvent A}}{\text{concentration of solute in solvent B}}$$

- Solvent A has density greater than or less than one
- Solvent B has density equal to one

Partition coefficients are defined as the concentration ration of a chemical between the two media at equilibrium. The media can be gases such as air, liquids such as water or olive oil, or complex mixtures such as blood or other tissues. However, the partition coefficients of an uncharged solute in two different two phase systems of identical ΔΔw2 in general are unequal.

The formula for partition coefficient is,

$$K_d = Cs/Cm.$$

Where,

C_s - Concentration of the solute in stationary phase,

C_m - Concentration of the solute in mobile phase.

Q1. Calculate the partition coefficient K_d between water and hexane where the concentration of solute in the stationary phase is 7.00M and the concentration of solute in the mobile phase is 5.00M.

Given data:

$C_s = 7.00M$

$C_m = 5.00M$

Substitute the values in the corresponding formula.

$K_d = C_s/C_m$ $\quad K_d = 7.00/5.00$ $\quad K_d = 1.4.$

Q2. Consider the aqueous solution of water and chloroform, in which the K_d is given as 6.40 and the concentration of solute in the mobile phase is 0.415M. Identify the concentration of solute in the stationary phase. (A) 2.56 $K_d = \frac{C_s}{C_m}$ $C_s = K_d \times C_m$ $= 6.40 \times 0.415 = 2.65$

5. **Soxhlet-Type Extraction;** Here the pulverized solid is extracted using a hot refluxing solvent in an instrument called soxhlet extractor. The disadvantages of this include (i) poor extraction of polar lipids (ii) long time involved in the refluxing (iii) large volumes of solvents required and (iv) hazards of boiling solvents.

6. **Pressurized Fluid Extraction;** This method is similar to Soxhlet extraction, except that the solvents are used near their supercritical region where they have high extraction properties.

7. **Supercritical fluid extraction (SFE);** This technique resembles Soxhlet extraction except that the solvent used is a supercritical fluid; substance above its critical temperature and pressure. *Supercritical fluids are highly compressed gases which combine properties of gases and liquids in an intriguing manner. Examples include supercritical xenon, ethane and carbon dioxide.* it can diffuse through solids like a gas, and dissolve materials like a liquid. This fluid provides a broad range of useful properties. One main advantage of using SFE is the elimination of organic solvents, thus reducing the problems of their storage and disposal.

8. **Microwave extraction;** This make use of the microwave oven for the extraction process. It a fast and efficient method of extraction.

9. **Ultrasound-Assisted Extraction (UAE);** This method is used for extraction by applying liquid solvents to analytes in solid matrices. This extraction process is fast in comparison with the liquid-liquid methods, because of the contact surface area between solid and liquid phase which is much greater due to particle disruption taking place.

B. **Techniques Used in Isolation and Purification of Organic Compounds**

Common techniques used in isolation and purification include the followings:

1. Chromatography.
2. Filtration.
3. Recrystallization.
4. Distillation.
5. Sublimation.

1) **Chromatography** – This is a method of separation that involves the distribution of the mixture between two phases (*stationary and mobile phases*) based on their polarity, affinity and molecular weight. The concentrated crude extract obtained through the extraction process can be separated into its various components by using chromatography techniques. The sample to be analyzed may be a gas, a liquid, or a solid dissolved in a liquid. The sample to be analyzed is passed over a solid, called a *stationary phase*. The stationary phase is typically powdered silica gel (hydrated SiO_2) or alumina (Al_2O_3). These materials are polar. A *mobile phase* usually a liquid (solvent) or gas is employed to move the sample through the stationary phase thereby separating the sample into its various components.

The common types of chromatography include;

- Thin-Layer Chromatography (TLC)
- Paper chromatography
- Column or Liquid Chromatography or Liquid Solid Chromatography (CC or LC)
- Gas-Liquid Chromatography (GLC)
- High perfomance Liquid Chromatography (HPLC)
- Flash Chromatography
- Ion exchange chromatography (This makes use of ion exchange resins)
- Partition chromatography (Liquid-liquid partitioning)
- Adsorption chromatography (Stationary phase is adsorbent)

The very common one are briefly explained below:

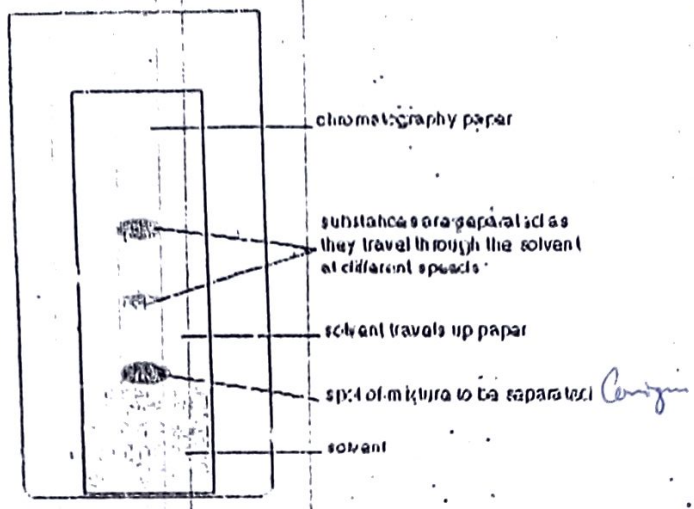
A. **Thin Layer Chromatography (TLC);** A TLC has a stationary phase that is made of aluminium sheet or glass plate coated with adsorbent such as silica gel, alumina or cellulose. The method is Simple, inexpensive, fast, efficient, sensitive, and requires mg quantities of the sample to be analyzed. It is most useful for

(1) Determining the number of components.
(2) Establishing whether two components are the same or not.
(3) Monitoring a reaction's progress.
(4) Determination of purity of samples.

Spotting on TLC plate.

A very small amount of the dissolved sample to be tested is put (spotted) near the bottom of the coated plate/sheet using a small capillary tube. The sheet is then placed in a vertical position in a tank/beaker containing a small amount of liquid. The liquid is called the developing solvent or the mobile phase.

Chromatography



Development/Sample separation

The solvent will wick up (climb up/wet) the stationary phase and dissolves the sample in the spot, move up the phase and partition between the stationary and mobile phase. The sample' components will be deposited at various distance on the plate since they have

(1) different solubility in the solvent and
(2) different affinity for the mobile and stationary phases.

The pattern obtained on the plate after the development is called Chromatogram.

Tutorial Questions- Define the following terms:

A *chromatogram* is the visual output of the chromatograph.

A chromatograph is equipment that enables a sophisticated separation e.g. gas chromatographic or liquid chromatographic separation.

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction.

Effluent is the mobile phase leaving the column.

Partition is the process where a substance divides itself between two immiscible solvents because it is more soluble in one than the other.

Determination of the R_f value

R_f is the retention factor, or how far up a plate the compound travels.

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

Distance travelled by the component is the distance moved by the spot from origin to its present state while distance travelled by solvent is the distance covered by the solvent from the origin to the solvent front. R_f cannot be greater than 1.

Q. Does a polar compound have a larger or a smaller R_f value when the polarity of the stationary phase is increased?

A. The R_f value will decrease as the material will "stick" to the stationary phase more tightly and move more slowly toward the solvent front.

Q. What separates the compounds as a chromatogram develops? Solubility in solvent, polarity of the compound, molecular weight of the compound and affinity to the phases.

Q. If the difference in the distance moved by a compound from the solvent front is 0.3, and the solvent front is 0.9 cm from the origin of the TLC plate. What is the R_f value of the compound?

A. R_f = 0.7/0.9 = 0.78 $A = \frac{0.6}{0.9} = 0.67$

Visualizing the Spots; The developed spot can be viewed:

1. with the naked eye if the compounds are colored.
2. under UV lamp if the compounds are not visible to the eye.
3. by spraying with colour developing reagents such as vanillin reagents or iodine vapour.

to aid visualization

Disadvantage of TLC

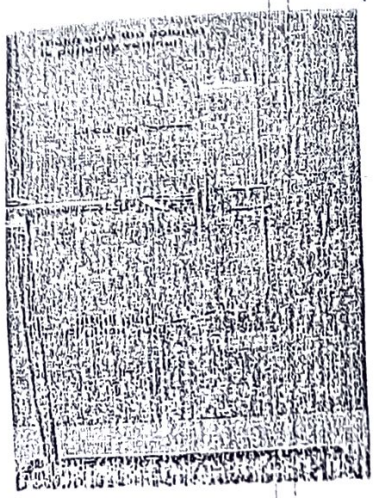
The disadvantages of TLC include:

(1) that the sample is usually not recovered since very small quantities of sample are used.
(2) It cannot be used to separate large quantity of mixtures.
(3) It is not efficient in separating mixture with same polarity and molecular weight.

B. COLUMN OR LIQUID CHROMATOGRAPHY

Larger quantities of sample may be separated by *column chromatography*. The mobile phase is a liquid while the stationary phase is a solid. The equipment consists of a glass (or plastic) column with a control valve (stopcock) on one end. Columns vary in length. Solid support such as silica gel, alumina, reverse phase or cellulose (stationary phase) is put/packed in the column. The sample to be analyzed is added at the top of the column and the desired solvent (eluent) is added at the top thereafter. The solvent exits at the bottom of the column and fresh solvent is continually added at the top of the column. As the solvent moves down the column, the sample also moves down the column. Eluent exiting the column is collected in a series of vials. Each vial is tested for the presence of a component of the sample

used to absorb materials.



Q. Which component in a mixture will exit the column first while eluting using non-polar solvents such as hexane on a silica gel-packed column: the most or the least polar?. Why?

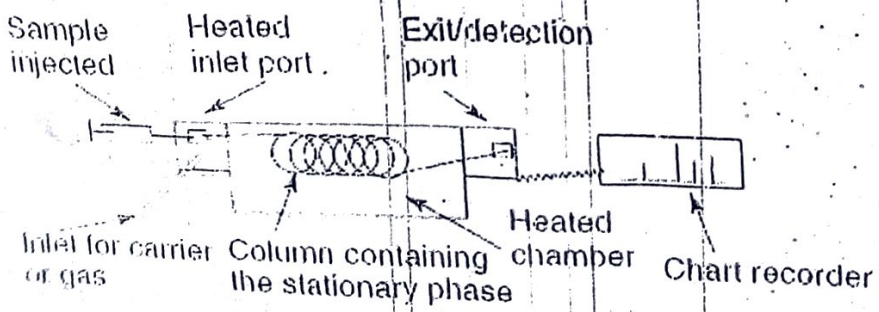
A. The least polar component; the most polar component will "stick" to the polar stationary phase and move down the column at a slower rate.

C Flash Chromatography

This type of gravity elution chromatography is very similar to the column chromatography but it consumes more time. Here, gas pressure is applied to push eluent through column packed with adsorbent such as silica gel of much smaller pore size. However, more efficient separations are obtained as the gas pressure controls eluent flow rate.

D Gas-Liquid Chromatography (GC)

This method is used to separate samples that can be vaporized. The instrument used to carry out these separations is called a Gas Chromatograph. A **carrier gas (mobile phase)**, usually **He**, N, H, Ar and air are used to move the sample through the column on a **stationary phase** *that is a high boiling point liquid absorbed onto a solid*. A sample of a liquid (or gas) is injected into a heated port of a *gas phase. chromatograph (GC)* where the sample is vaporized. An inert carrier gas enters the chromatograph in the heated port and flows through the column packed with a stationary phase. Components in the sample partition between the vapor phase and the stationary phase. Components move in a zigzag pathway through the column and are separated for the same reasons explained for the TLC process. A detection system is used to tell when components exit the chromatograph. The signals are obtained in a readable form on the recorder (Computer system).



GC Chromatography

In a GC, the less polar compound comes out first. The time it takes the compound to travel through the column (**retention time**), the pattern of the chromatogram, peak area and known standard are used to identify (not confirm) the compound.

Features of Gas-Liquid Chromatography

- Analysis of volatile organic liquids
- Quick and easy method
- Can be used for qualitative analysis
- Can be used for quantitative analysis
- Separates very complex mixtures
- Compounds must have high vapor pressure
- Known samples must be available for identification
- Stationary Phase is a non-volative liquid, or packed column coated on solid support or capillary column -- thin film coated on capillary tube
- Mobile phase are inert gas such as He or N_2

Common types of detector for the GLC includes

- Thermal conductivity detector (TCD)
- Flame ionization (FID)
- Mass selective detector (Mass Spectrometry)

Q. A sample containing CCl_4 (boiling point 76°C) and CH_3CH_2OH (boiling point 78°C) is analyzed by GC. Which material is expected to exit the column first?
A. CCl_4, since it is less polar and would "stick" to a lesser extent to the stationary phase.

D High performance Liquid Chromatography (HPLC)

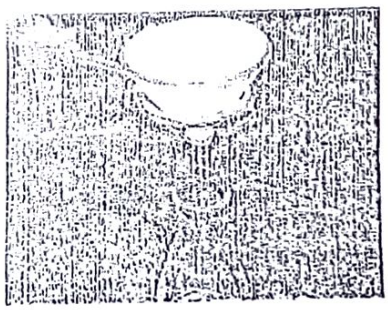
There are about four types of HPLC (partition, adsorption (liquid-solid), ion exchange, size exclusion or gel). This chromatography method gives a more efficient separation than does column chromatography and hence the name *high performance liquid chromatography (HPLC)*. The stationary phase has a greater surface area which gives the components of a mixture more opportunity to interact with the stationary phase surface. The flow rate is increased by using **pressure** to force the solvent (mobile phase) through the column. The sample is injected into the chromatograph with a syringe. The column can be heated to assist separation. A detection system is used to analyze the eluent as it leaves the chromatograph. HPLC can analyse very small samples.

Q. Give reasons why HPLC is better than GC and CC in separating mixtures.
Partition chromatography: a form of separation of solutes utilizing the partition of the solutes between two liquid phases, namely the original solvent and the film of solvent on the adsorption column.
Adsorption chromatography is the type in which the stationary phase is an adsorbent.

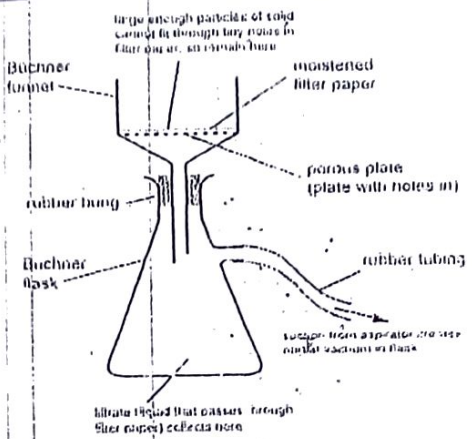
2) **Filtration** - This is a very simple and common method of separation useful in separating solids from liquids. It is used to isolate solid product from a solution or mixture. Depending on the stability of the compound, the filtration could be done cold or hot.

Ordinary or Gravity filtration: makes use filter paper, funnel and conical flask.

Vacuum filtration: When substantial amount of solid is to be recovered from a suspension, **vacuum filtration** (ie filtration under pressure or filtration with suction) could be carried out. This type makes use of the vacuum pump and Buchner funnel to effect proper filtration. The method presents a number of advantages: the filtration may be made in a much shorter time; the liquid may be much more completely separated from the precipitate, in consequence of which the latter will dry more rapidly.



Gravity filtration



Vacuum filtration

3) **Recrystallization** - This is a useful means of removing insoluble solid impurities from a solid dissolved in a suitable solvent. It is based a the principle of **solubility** of different solute in different solvent(s). Further purification of isolated organic solid can be attained by recrystallising the solid from a solvent or mixture of solvents. This is done by

(i) dissolving the impure solid in a appropriate amount of solvent near the boiling point of the solvent.

(ii) filtering the hot solution from particles of insoluble material and dust.

(iii) allowing the filtered solution to cool down thereby causing the dissolved solid to crystallize out.

(iv) separating the new crystals from the supernatant solution.

Examples include the separation of magnessium carbonate from aqueous solution.

Many compounds are so easily soluble in all solvents, even at the ordinary temperature, that they do not separate from their solutions on mere cooling. In this case, in order to obtain crystals, crystal formation is initiated thus:

(i) a portion of the solvent must be allowed to evaporate. (Crystallisation by Evaporation).

(ii) a seed crystal may be added to initiate crystal formation

(iii) the inner wall of the container can be scratched with a glass rod to initiate the formation.

Fractional crystallization is used to separate mixture of solutes by monitoring the solubility or the temperature at which each crystallize out.

A list of commonly used recrystallization solvents is shown in the Table below:

solvent	bp (°C)	solvent	bp (°C)
water	100	ethyl ether	35
methanol	65	dichloromethane	40
ethanol (95%)	78	toluene	111
acetone	56	petroleum ether	35–60
ethyl acetate	77		

Solvents such as DMSO and DMF have too high boiling points and are therefore not used as recrystallising solvents.

Four major criteria for selecting a recrystallizing solvent are:

1. Compound being purified must be insoluble in solvent at room temperature
2. Compound must be soluble at high temperature or in boiling solvent
3. Solvent's boiling point must be lower than the compound's melting point
4. An abundant quantity of crystals must be recoverable from the cool solvent

The **efficiency** of the recrystallization can be determined by calculating the recovery percentage as shown in the equation below.

$$\%\text{recovery} = \left(\frac{\text{mass of recrystallized compound, g}}{\text{mass of crude compound, g}}\right)(100\%)$$

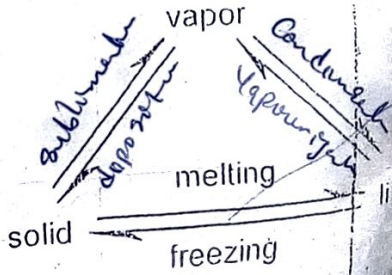
Purity of a recrystallized compound is accessed by observing its color and by measuring its melting point range.

Q. Mention three ways to initiate crystal formation. -adding pure of the solvent to evaporate
-adding seed crystal to the mother
-Scratching the inner part of the vessel

4. **Sublimation**: This technique is used for substances that evaporate from solid state to gaseous state. It is the transition of a substance directly from the solid to the gas phase without passing through the intermediate liquid phase. Sublimation is an endothermic phase transition that occurs at temperatures and pressures below a substance's triple point in its phase diagram.

The **triple point** of a substance is the temperature and pressure at which the three phases (gas, liquid, and solid) of that substance coexist in thermodynamic equilibrium.

The reverse process of sublimation is de-sublimation or deposition, in which a substance passes directly from a gas to a solid phase. Examples of such substances include **iodine, carbon dioxide, Naphthalene** and **snow**. Such substances have high vapor pressures below their melting point.



The features of subliming substances are:

- Vaporizes without melting
- Vaporizes without decomposition
- Vapor condenses to solid
- Impurities present do not sublime
- Generally utilize reduced pressure

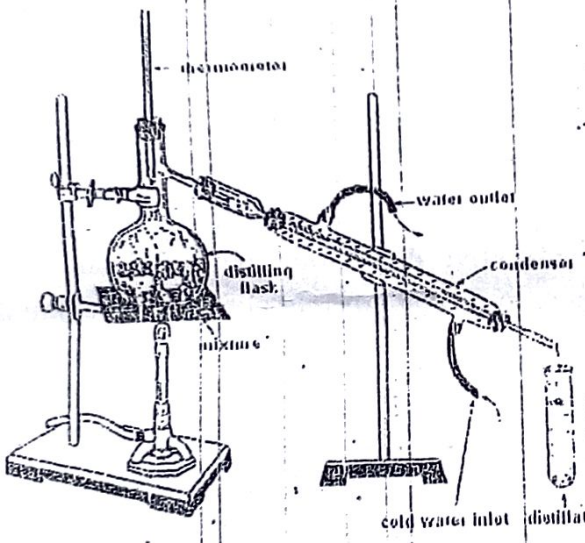
The purity of a solid may be checked by performing a **melting point** determination. A pure compound has a *sharp melting point* whereas impure substances melt over a range of several degrees. A pure liquid has a *sharp and constant* **boiling point** at any particular pressure.

Purification of Organic Liquids

The most common method of purifying organic liquid is by distillation. There are different type of distillation and each method is selected as required.

Distillation: There are different types of distillation techniques

i. **Simple distillation** - This is used for separating a liquid from solid or non-volatile or less volatile liquid impurities. Distillation is the process of heating a liquid until it boils, capturing and cooling the resultant hot vapors in the condenser, and collecting the condensed vapors. It is used to purify water, separate alcohol from water, oil from water, mixture of solvents with wide difference in **boiling points**.



Simple distillation set up.

The disadvantage of simple distillation is that it can only be used to purify mixture with little (10%) impurity.

ii. **Fractional distillation** - It is used for separating mixtures of liquids with close boiling points. This usually requires the use of a special fractionating column. **Fractional distillation** is in order to separate the components well by repeated vaporization-condensation cycles within a packed fractionating column. This separation, by successive distillations, is also referred to as **rectification.** As the solution to be purified is heated, its vapors rise to the fractionating column. As it rises, it cools, condensing on the condenser walls and the surfaces of the packing material. Here, the condensate continues to be heated by the rising hot vapors; it vaporizes once more until it distill out of the column.

Differences between Simple and Fractional Distillation

Simple Distillation	Fractional Distillation
Simple setup	Complicated setup
Fast process	Slow process
Consumes less Energy	Energy intensive
Poorer separation	Better separation
Best for relatively	Best for mixtures pure liquids with close bp

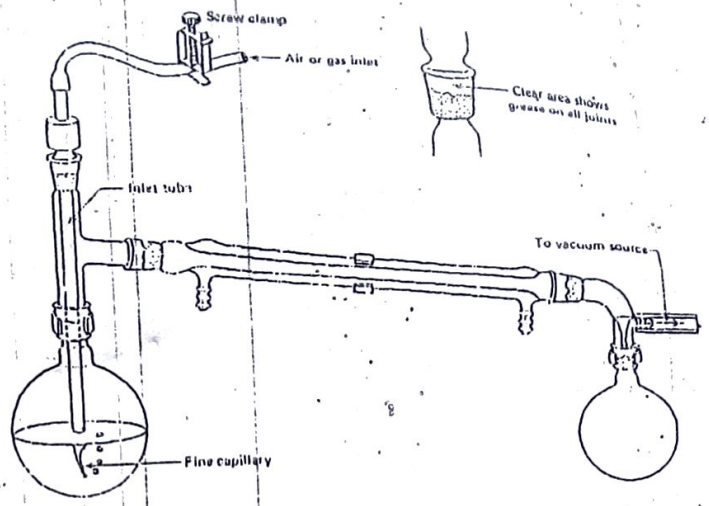
Azeotropes (Azeotropic Mixture)

An azeotrope or a constant boiling mixture is a mixture of two or more substances that boils at a constant temperature, either higher or lower than any of its constituents. Thus an 8.5:1 mole mixture of ethanol and water boils like a pure substance, distilling at 78.2°, which is lower than the boiling point of ethanol (78.5°) or of water (100 °). In contrast, a 1.35:1 mole mixture of methanoic (formic) acid and water boils at 107.1°, which is higher than the boiling points of either methanoic acid (100.7°) or water (100°).

- Constant boiling liquid mixtures
- Cannot be purified further by simple distillation: 95.6% EtOH + 4.4% HOH: bp 78.2°
- Vapor composition is the same as the liquid composition

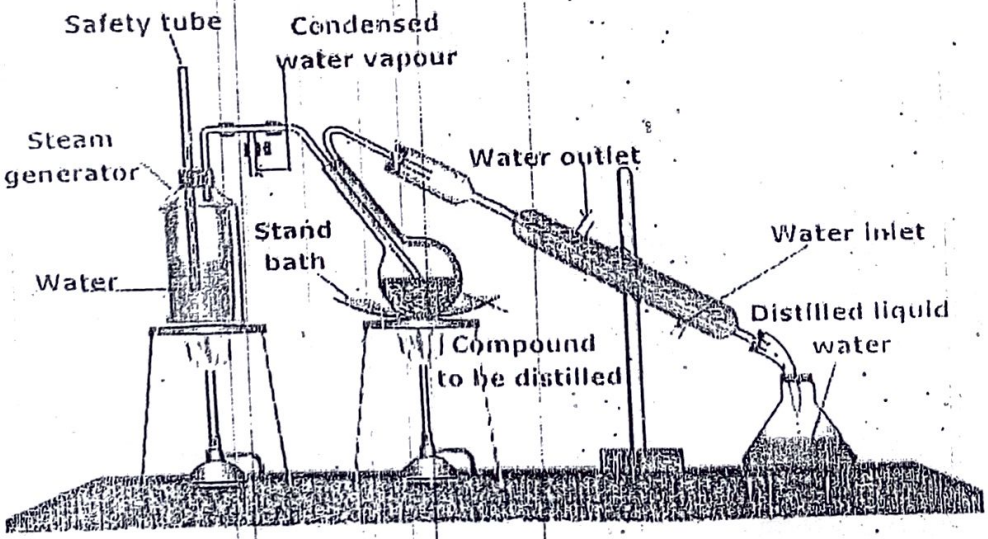
Azeotropic distillation: Also known as **extractive distillation** is carried out by adding **entrainer** (material separation agent) to the mixture to form a separate phase by breaking the azeotrope. For instance, the addition of benzene to the mixture of ethanol and water will neutralize the azeotrope and cause each component to distill at the normal temperature. **Entrainer** is a devise that facilitates the saturation of a gas or steam current by means of liquid. It is applied in the breaking of azeotropes into their respective components. Other common entrainers include pentane, cyclohexane, acetone, diethyl ether, toluene and heptane.

iii. **Vacuum Distillation: Vacuum distillation** is a method of distillation whereby the pressure above the liquid mixture to be distilled is reduced to less than its vapor pressure (usually less than atmospheric pressure) causing evaporation of the most volatile liquid(s) (those with the lowest boiling points) Since boiling point is dependent upon pressure, therefore, as pressure is reduced the bp reduces. A vacuum pump with guage is used to reduce the pressure in the system. High boiling organics can be distilled by reducing the pressure - vacuum distillation



iv. **Steam Distillation (For volatile compounds):** This involves co-distillation with water. It is a special type of distillation (a separation process) for *temperature sensitive* substances like natural aromatic compounds. It once was a popular laboratory method for purification of organic compounds. Two components are immiscible are distilled out together and then separate upon cooling. Each component exerts separate full vapor pressure. This is usually applied in flavor and fragrance industries for the collection volatile oils from natural source. Steam distillation is used to separate

- volatiles from non-volatile impurities
- insoluble compounds in water
- compounds with high molecular weight from low molecular weight
- substances with fairly high vapour pressure near the boiling point of water



Test of Purity: Melting and Boiling Points

The test for the purity of a compound can be carried out by determining the melting point for solids and boiling points for liquids.

Melting Point: The melting point of a compound is the temperature at which the solid and liquid phases exist at equilibrium. A pure organic substance has a sharp melting point.

Mixed Melting Point: In this technique, the purified compound is mixed with a little amount of the known compound. The melting point of the mixture thereafter determined. If the melting point of the mixture is found to be the same with that of the pure compound, then the prepared samples is regarded as a pure compound.

Features of melting point of substances

- Physical characteristic
- Generally reproducible
- Presence of trace impurities depresses mp
- Pure compounds melt over 0.5 to 2 degrees
- Impure compounds have larger melting ranges
- At the melting point – solid and liquid are at equilibrium
- As temperature increases the vapor pressure increases

Boiling point: This is described as the temperature at which the maximum vapour pressure of a liquid is equal to the external pressure.

Features of boiling point of substances

- Vapor pressure of liquid and gas phases are equal
- Boiling point is dependent upon pressure. Therefore, pressure and boiling point are recorded. For instance, water boils at:
    - 100.3° at -285' (1.01 atm)
    - 100.0° at 0' (1.00 atm)
    - 93° at 7520' (0.75 atm)
- Polar compounds have higher boiling point than non-polar compounds
- Increasing molecular weight increases boiling point (constant polarity)
- Boiling point is important for distillation in order to purify organic liquids