SCY1611 – Analytical Chemistry

Unit - I

UNIT - I

SEPARATION AND PURIFICATION TECHNIQUES

Principles involved in the separation of precipitates, solvent extraction and electrophoresis. Purification of solid organic compounds, extraction - use of immiscible solvents, soxhlet extraction, crystallization, use of miscible solvents, fractional crystallization, sublimation. Purification of liquids, experimental techniques of distillation, fractional distillation, vacuum distillation, steam distillation, tests for purity.

1. INTRODUCTION

The separation of mixtures of compounds to give the pure components is of great practical importance in chemistry. Separation techniques constitute an important aspect of experimental chemistry. Almost all compounds of biochemical interest occur naturally as components of very complex mixtures from which they can be separated only with considerable difficulty. Separations can be achieved by differences in physical properties, such as differences in boiling point, or by chemical means, wherein differences in physical properties are enhanced by chemical reactions. In this chapter we will consider some separations of compounds based on differences in physical properties. The chemical and physical aspects of purification of matter are interesting. The purification techniques have undergone vast improvements with the development of modern technology. Though expensive instruments are sometimes used for purifying a compound, the well-known principle of adsorption-desorption is used in chromatography. In spite of the availability of modern gadgets for separation and purification of chemicals, the simple and inexpensive techniques like distillation, filtration, sublimation, etc., continue to remain indispensable in all laboratories.

1.1 Separation Techniques:

Definition:

- It consists of important aspects of experimental chemistry.
- The quest for isolating a chemical in a 100% pure form has resulted in the invention and perfection of innumerable separatory methods.
- The low cost technique is filtration and the computer aided technique are HPLC etc., which are expensive.

Types:

1.2 Precipitation:

It is an important method of separation in various analytical techniques.

In qualitative analysis, the principle of separation of cations is based on by adding suitable reagents.

Eg:

- HCl precipitates Hg, Pb & Ag.
- Thus the separation of these ions from $Cu^{2+,}$ Al^{3+}, Zn^{2+} etc., is possible.
- Mn ions can be separated from Zn ions by adding NaOH.

- In a mixture of Cu & Ni ions, Cu ions are precipitated as sulphide and removed before Ni is estimated gravimetrically using DMG(dimethyl glyoxime).
- A carbonyl compound can be precipitated by adding Borsche's reagent).

1.1.1 Solvent Extraction:

- Extraction with solvents is used as a method for separation of dissolved substances from solutions.
- It can also be used for the separation of one constituent from a solid mixture as well as for the removal of undesired soluble impurities from mixtures.
- Extraction with a second solvent is an application of the Nernst distribution law which states that "at constant temperature a solute distributes itself between two immiscible solvents only in a particular ratio".
- When a substance distributes itself between two solvents without the complications of dissociation or association, it is possible to calculate the weight of the substance that can be removed by a series of extractions.
- If v_1 ml of a solution contains Wg of a substance and if the substance is repeatedly extracted with v_2 ml of another solvent, the weight of the substance W_n remaining in the first solvent after n extractions is given by,

$$W_n = W [K v_1 / K v_1 + v_2]^n$$

Where K = distribution coefficient for the substance between the two solvents.
Larger the value of K, more efficient is the extraction.

1.1.2 Solvent Extraction Using Separating Funnel:

- The extraction with a second solvent can be carried out in the laboratory with a separating funnel.
- It is provided with a ground glass stopper and a stop-cock.
- It is mounted in a ring on a stand.
- When an aqueous solution is shaken with ether in a separating funnel and allowed to settle, two sharply defined layers are formed.
- The two layers can be separated by opening the stop-cock and allowing the lower aqueous layer to drain slowly into a beaker.

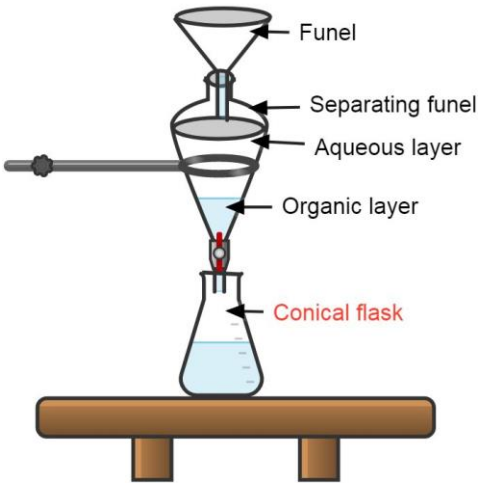


Fig. 1.1. Separating Funnel

1.1.3 Continuous Extraction:

- When one has to separate a component which is slightly soluble in the extracting solvent from a mixture whose other components are essentially insoluble, large quantities of solvent would have to be used.
- The method of continuous extraction can be used in such cases with a smaller quantity of the solvent.
- The apparatus used for continuous extraction when the extracting solvent is lighter than the original solution containing the solute.
- The extracting solvent is kept in a flask which is heated.
- The vapours condense and the liquid solvent drains into the vessel containing the solution to be extracted.
- After extracting a little of the solute, the extracting solvent being lighter rises to the top.
- When the level exceeds a certain limit, it drains once again into the original flask from where it is vaporised and then utilised for extraction once again.

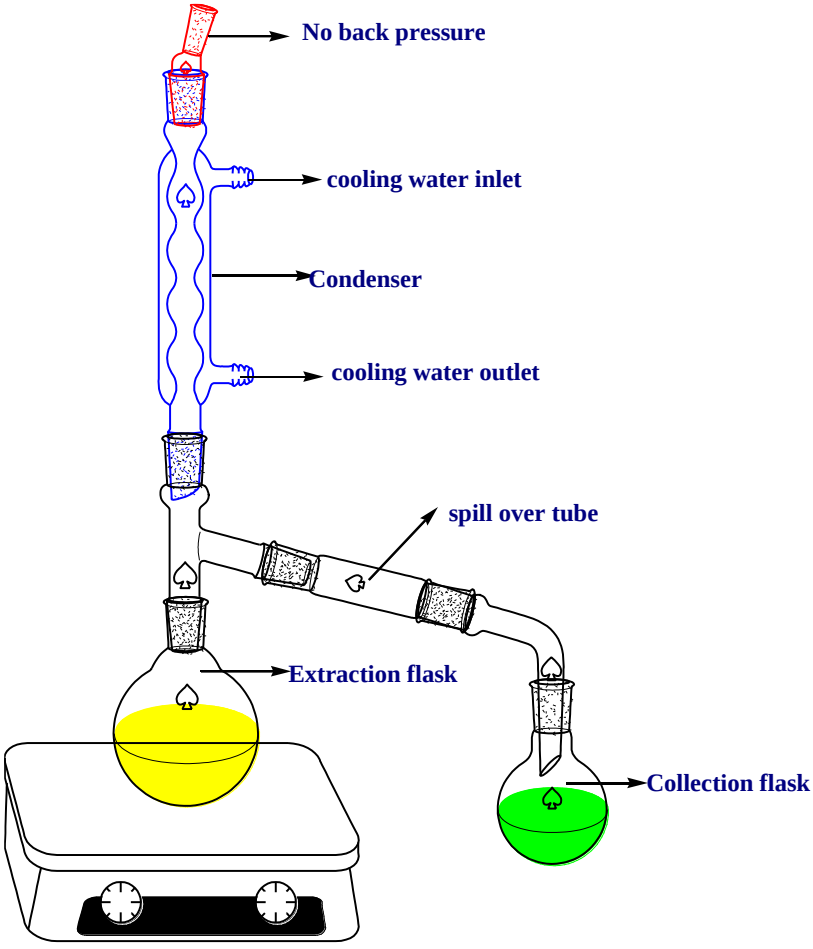


Fig. 1.2 Continuous Extraction

1.1.4 Soxhlet Extraction:

- For separation of the components of a solid mixture by continuous extraction, a Soxhlet apparatus is used.
- The solid is kept in the porous thimble.
- The extracting solvent is taken in the boiling flask.

- When the solvent is heated to reflux, the distillate drains into the porous thimble and extraction occurs.
- As more and more solvent collects in this chamber, the solution drains into the boiling flask by a siphoning action.
- Pure solvent continuously gets vapourized from the boiling flask and the cycle of condensation of the vapour takes place.
- Simultaneously the extraction of the components and draining of the solution into the boiling flask will goes on.

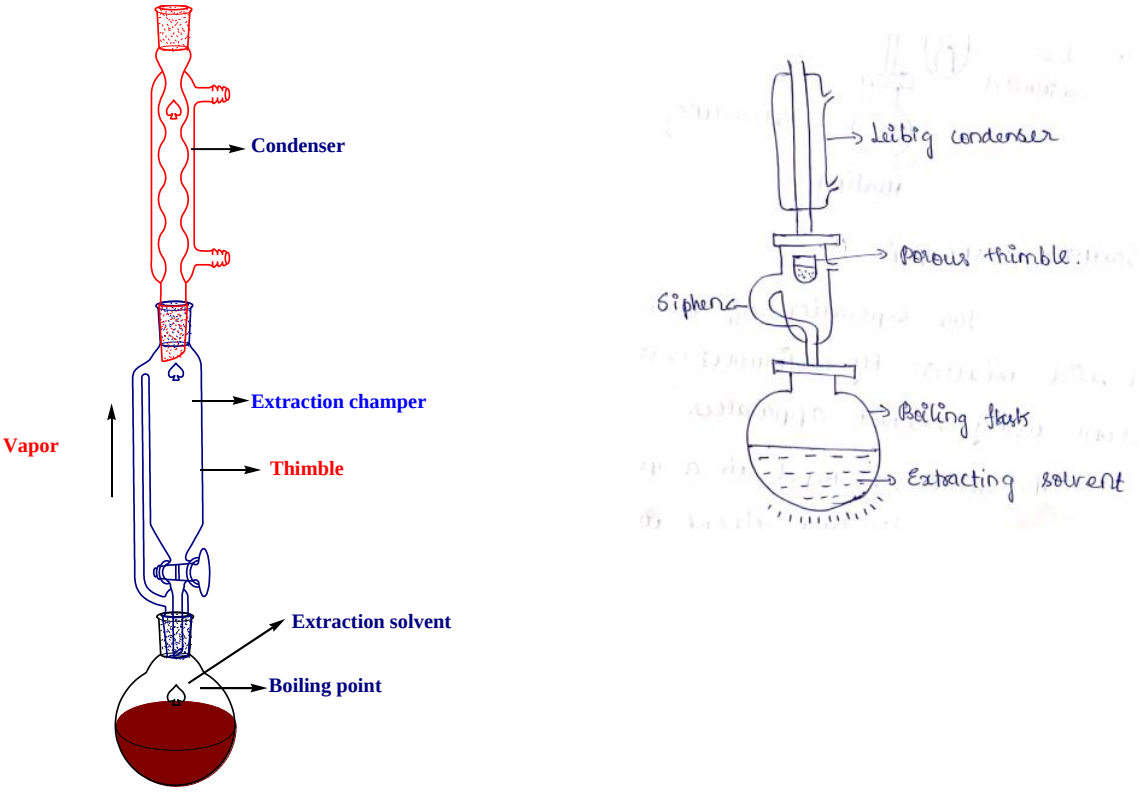


Fig. 1.3. Soxhlet Extraction

The common solvents used are:

- Diethyl ether
- Benzene
- Petroleum ether
- $CHCl_3$
- CCl_4

Conditions for a good Solvent:

- The substance extracted should be highly soluble in water.
- After the extraction the solvent should be easily separable from the solute.

Note: Normally diethyl ether is used as solvent because organic substances generally soluble in ether and ether has low boiling point.

1.1.5 Electrophoresis:

Definition:

It is a separation technique that is based on the migration of charged particles under the influence of an electric field.

Principle:

The principle describes that any charged ion or molecule migrates when placed in an electric field, the rate of migration depend upon its net charge size, shape and the applied electric current. This system consists of two electrodes of opposite charge (anode, cathode), connected by a conducting medium called an electrolyte.

Types:

- Gel electrophoresis.
- Capillary tube electrophoresis.
- Disc electrophoresis.
- Pulsed field electrophoresis.

Process:

- Positively charged ions migrate towards a negative electrode and negatively charged ions migrate towards positive electrode.
- Anions have different migration depending on the total charge, size and shape. For safety reason one electrode is usually at around and the other is based on positive or negative ion.
- This technique is used particularly for macro molecules such as protein, DNA etc.
- A negative charge is added to these molecules so they move towards the positive electrode.
- An gel electrode apparatus consists of a high voltage supplied (DC power), electrodes consist of a buffer and a support for the buffer such as filter paper, cellulose acetate strip, polyacryl amide gel or capillary tube.
- Open capillary tubes are used for many types of samples and other supports like gel are used for biological samples such as protein, DNA etc.
- After separation is completed the support is strained to visualize the separated component.
- Resolution can be improved by isoelectric focussing in this technique and the support gel maintains the pH gradient.
- As the protein migrates down the gel it reaches a pH that is equal to its isoelectric point and at this pH the protein is neutral and no longer migrates which is focussed into a sharp based on the gel.

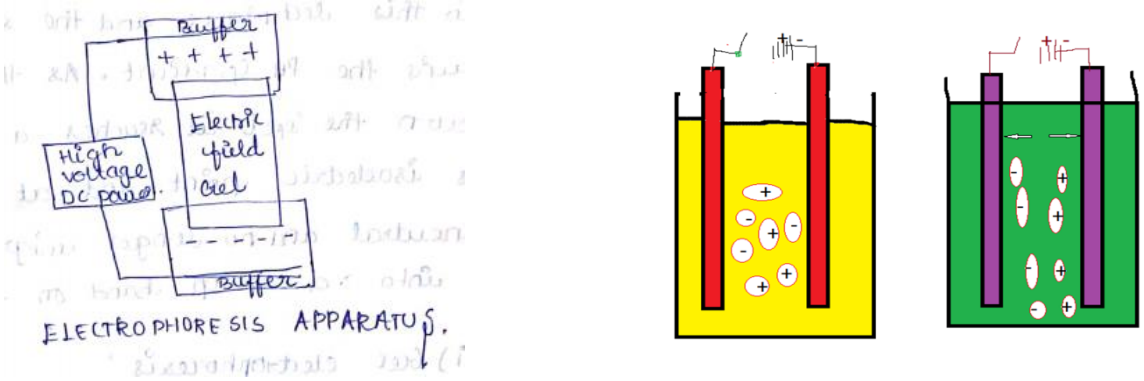


Fig. 1.4. Electrophoresis

Applications:

Electrophoresis plays an important role in the field of,

- Pharmaceutical
- Forensic
- Foods
- Bioscience
- Agricultural field
- Pesticide analysis
- Surfactant analysis
- Transition metal analysis
- Organic compound analysis

1.2 Purification Techniques:

1.2.1 Sublimation:

Definition:

It is a process of converting a solid directly into its vapour on heating without becoming a liquid.

Principle:

It works on the principle that solids have weak intermolecular forces hence a higher vapour pressure which converts it into directly vapour state.

Process:

- The substance to be sublimed is placed in a crucible.
- A narrow ring of pyrex glass is fitted near the rim which support a filter paper with a number of small holes made in upward direction.
- A funnel with a pinch of glass wool or cotton in the stem is kept inverted over the paper.
- The crucible is heated gently.
- The vapours escaped to the holes in the paper and condense on the upper surface of the filter paper and also on the walls of the funnel.
- Heating is stopped when the most of the material in the crucible has vapourized.
- Supply of the heat should be such that the does not become hot and the rate of sublimation is increased by applying suction at the stem of the funnel.
- This will draw the vapour into the condensing chamber.

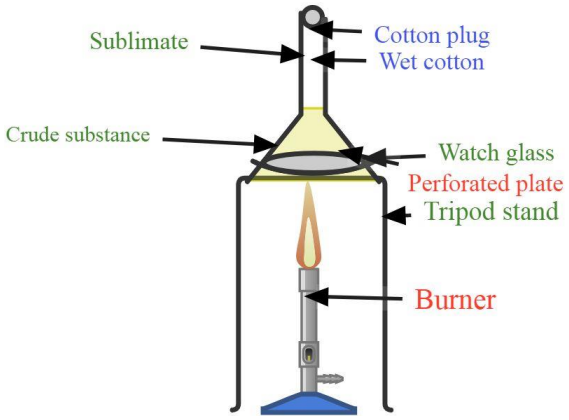


Fig. 1.5. Sublimation

Types of sublimation:

- Simple sublimation
- Sublimation under reduced pressure
- Vacuum sublimation

Advantages:

- Convenient method for purification of solids containing impurities.
- Apparatus is simple and inexpensive.
- Impurities can be easily removed as vapours forming pure crystals of a substance.

1.2.2 Distillation:

Definition:

It is the process of separating the components or substances from a liquid mixture by using selective boiling and condensation.

Principle:

- A liquid present in a closed space will evaporate giving off vapour until the vapour attains a definite pressure.
- This pressure depends on the temperature and the vapour pressure of a liquid in contact with its own liquid is a constant at given temperature.
- It is independent of the absolute amount of liquid and vapour pressure in the system.

The distillation apparatus consists of,

- Distillation flask
- Thermometer
- Round bottom flask
- Liebig condenser
- Funnel
- Condenser tube

Process:

- The liquid is boiled in the distillation flask, condensing the vapour produced by means of a Liebig condenser.
- The condensed pure liquid is collected in a receiver.
- All the joints in the distillation unit must be interchangeable standard joints.
- A small amount of silicone grease may be used at the joints so that dismantling will be easy.
- Sometimes lubrication may contaminate the liquid.
- Bumping of the liquid during boiling may be avoided by adding unglazed porcelain bits.
- These bits should be added before the boiling process and never to be a heated liquid.

Aspects to be followed while distilling a liquid,

- A round bottom flask should be used and not the flat bottom flask.
- Heating must be slow and uniform using a bunsen flame or electric heater.
- The distillate should be collected separately.

- Reject the last portion of the liquid in the flask itself.
- The clamp should hold the coolest part of the neck of the flask.
- The bulb of the thermometer should be below the position where the side tube is fused into the neck of the flask.
- The thermometer tube should not be immersed inside the liquid.
- The glass units must be assembled tightly to avoid loss of vapour or liquid.
- The distillation should be stopped when some liquid is left in the flask.
- Flammable liquids like ethanol, benzene, carbon disulphide etc., should never be heated with a bunsen burner.
- They must be distilled with a steam-bath or controlled electric heating mantle.

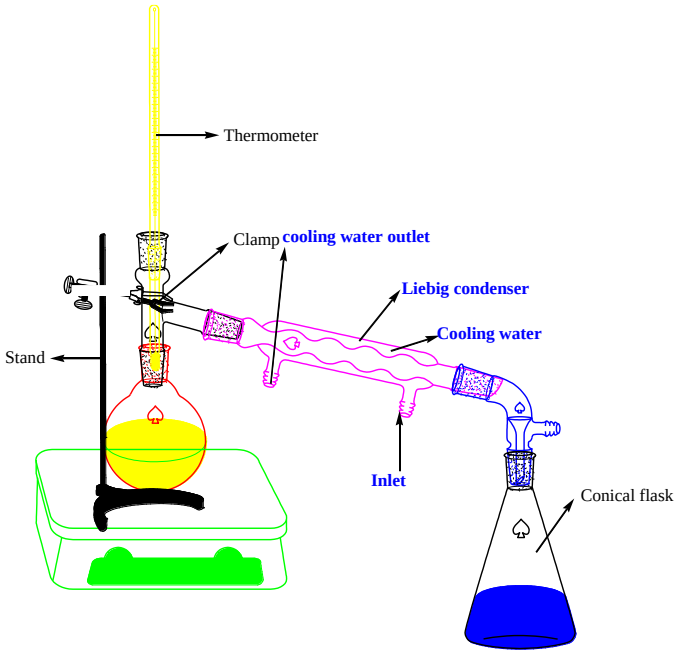
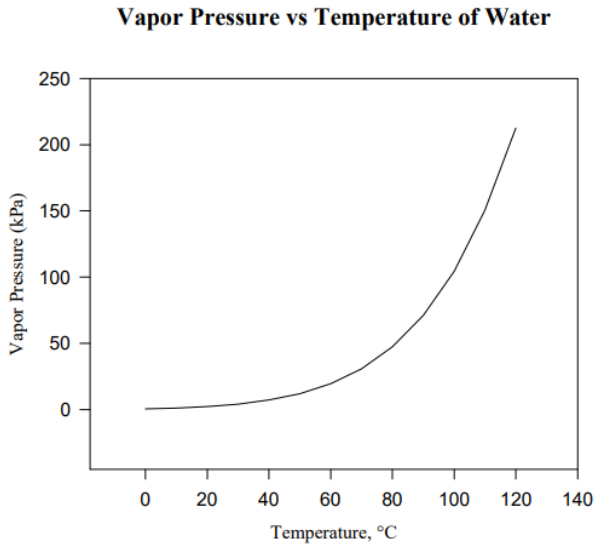


Fig. 1.6. Distillation Apparatus



1.2.3 Fractional Distillation:

Definition:

It is a method of separating two or more liquids having different boiling points.

Principle:

When a mixture of two liquids is boiled, the vapour phase is richer in the more volatile component than the boiling liquid with which it is in equilibrium at any given temperature.
Eg: If the component A is more volatile than B then,
Partial pressure of A/ Partial pressure of B > 1

or

mole fraction of A in the V.P/ mole fraction of B in the V.P >
mole fraction of A in the L.P/ mole fraction of B in the L.P
∴ it is a method used to separate liquids which are miscible with one another
and the boiling point of two liquids should be far from each other.
Eg: A mixture of benzene and toluene have boiling point at 80°C and 110°C can be separated by fractional distillation.

Procedure:

- The mixture of liquids is distilled by heating the flask electrically.
- Care should be taken that the condensed liquid does not drop into the receiver at rapid rate than one drop per second.
- In this distillation a special type of fractionating column is attached to the top of the distillation flask.
- As a mixture of vapour of the two liquids rises in the column the vapours of the higher boiling liquid will condense and fall back into the flask.
- Whereas the vapours of the lower boiling liquid will alone go the top of the column and escape into the condenser.
- The process will proceed slowly and eventually.
- Separate fractions are collected at 3 or 5°C.
- Any distillate passing over between 80.2°C and 81.2°C will be rich in benzene.
- Pure toluene will distills at 110.6°C.
- Each component must be collected at closed temperature ranges as possible.
- Complete separation is not possible at one distillation process.
- The fractionalized liquid should be separately distilled for effective purity of a component.
- Repeated fractionations will give pure component.

Conditions for good fractionation:

- There should be large amount of liquid continuously running through the column.
- Thorough mixing of liquid and vapour should occur.
- A large effective surface area between liquid and vapour should be available.
- There should not be excess cooling in the column.

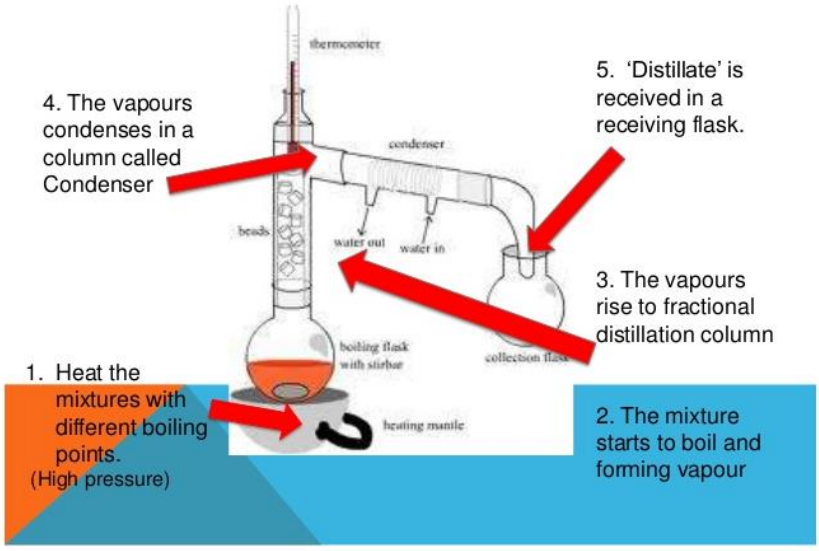


Fig. 1.7. Fractional Distillation Apparatus

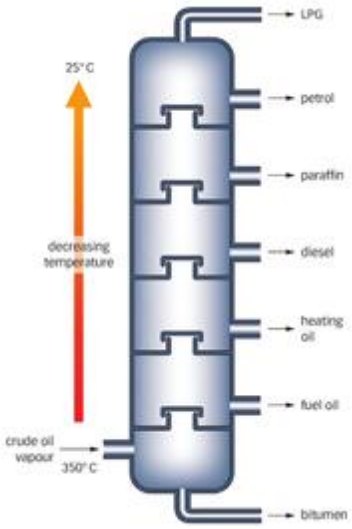


Fig. 1.9. Fractionating Column Setup

1.2.4 Steam Distillation:

Definition:

It is a process in which the separation and purification of volatile organic compounds which are immiscible in water is distilled by steam distillation.

Principle:

In this method a current of steam is blown through the substances to be distilled.
The steam escapes from the substance carrying with it only the volatile component which is being separated from the impurities.

Eg: Mixture of benzene and water.

As the temperature is raised the sum of the two pressures reaches the atmospheric pressure and at this temperature the mixture boils. But this temperature is less than 80°C which is the boiling point of the benzene. Actually the temperature is 69.3°C and partial pressure of benzene is 533mm and for water is 227 mm. ∴ a mixture of benzene and water distills at 69.3°C until one or the other material is exhausted in the distillation flask. Thus passing steam through the benzene will remove as a distillate and this operation is called steam distillation.

Process:

- The organic compound to be distilled is placed in a round bottom flask with a claisen head, which is equipped with a head connected to a water cool condenser?
- The claisen head prevents splattering of the mixture into the condenser during distillation.
- Steam is produced externally in a steam generator and passed through a long tube reaching almost the bottom of the distillation flask.
- Water may condense in the distillation flask filling into the inconvenient levels.
- This problem is avoided by heating the flask gently using bunsen burner or connecting with a bent adapter.
- Instead of passing through the liquid water can be added to the organic compound to be distilled in the distillation flask. This mixture is heated directly to generate steam inside and it is best suitable for steam distillation of small amount of soft liquids.

Precautions:

Steam should be turned off only after opening the pinch clamp on the bent adapter exit tube otherwise due to the development of vacuum in the distillation flask the distilled liquid from the receiver will go back into track.

Uses:

- Used in chemical industries for separating turban oil from crude pine oil.
- Used for separating volatile components of several classes.
- Provides a method of separation of volatile liquids and volatile solids which are insoluble in water from non-volatile substance under mild conditions.

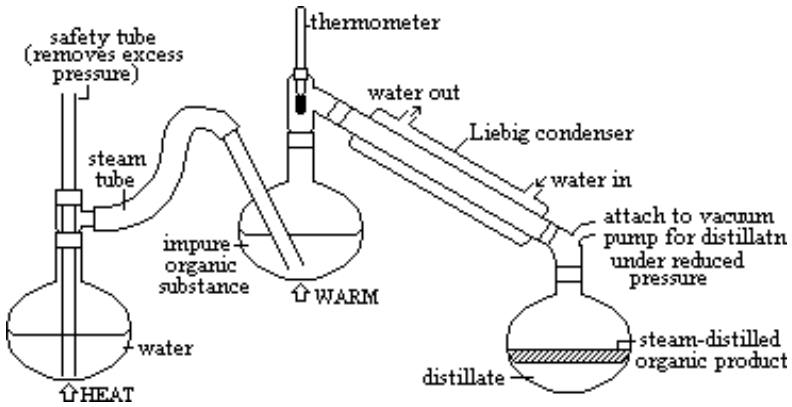


Fig. 1.10. Steam Distillation Apparatus

1.2.5 Vacuum Distillation:

Definition:

It is technique for distilling organic liquids having high boiling temperature.

Principle:

The boiling point of the liquid is that the temperature at which the total vapour pressure is equal to the atmospheric pressure from this it follows that when the external pressure increases the pressure above the liquid is reduced. Hence the boiling point will be decreased. When vacuum is applied (no pressure) the boiling point should be minimum at intermediate pressure. ∴ the boiling will be intermediate and hence normal distillation is effective under diminished pressure.

Need to adopt under diminished pressure:

- A certain liquids have high boiling points ∴ distillation of these under vacuum is easy.
  In many cases boiling temperature at atmospheric pressure ae too high because the compound being distilled may get oxidised or decompose or undergo molecular rearrangements.

- Sometimes impurities present in the liquid may catalyse some reactions at high temperature.

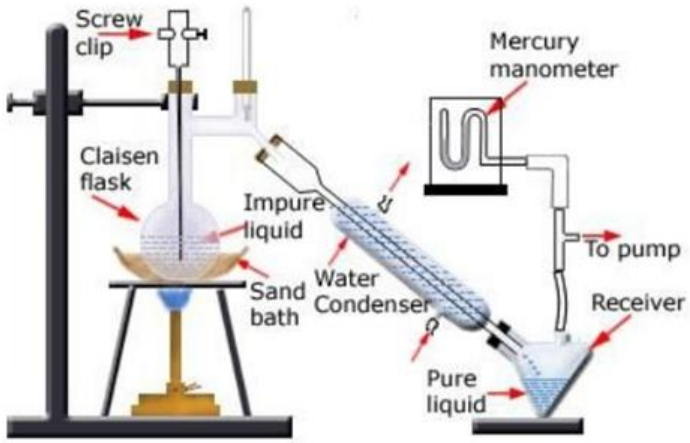


Fig. 1.12. Vacuum Distillation Apparatus

Process:

- For distillation under reduced pressure the distillation apparatus consists of a distillation unit having provision for as aspirator(water pump) or a mechanical oil pump.
- An aspirator commonly reduces the pressure to above 25mm and oil pump to below 1mm.
- The flask should be made up of stout glass otherwise low pressure due to insight it may collapse.
- The distillation apparatus should be tightly assembled with no leakage or loose joints.
- The ground glass joints surfaces of the distillation unit may be lubricated using vaseline or silicon grease.
- Bumping is much more pronounced in distillation under diminished pressure than in ordinary distillation this is avoided by the introduction of a bit of porcelain materials.
- Another way of avoiding bumping is to have a glass tube fitted to the neck of the flask.

- This tube drawn out at its lower end to a capillary which dips into the liquid and closed using a piece of rubber and small screw clamp at its upper end.
- With this arrangement the passage of air into the distilling flask is easily regulated.
- The pressure inside can be read using manometer which is connected to the system between the pump and the distillation unit.

Approximation need to follow for vacuum distillation:

- When the pressure is reduced from 760mm to 25mm, the boiling of a high boiling liquid(250-300°C) is reduced by about 100-125°C.
- Below 25mm,each time the pressure is halved, the boiling point is reduced by about 10°. The exact boiling points at different pressures can also be calculated using the Clausius-Clapeyron equation.

1.2.6 Crystallization:

Definition:

It is a process by which a solid forms, where the atoms or molecules are highly organized into a structure known as a crystal.

Process:

- The solution is heated in an open container
- The solvent molecules start evaporating, leaving behind the solutes.
- When the solution cools, crystals of solute start accumulating on the surface of the solution
- Crystals are collected and dried as per the product requirement
- The undissolved solids in the liquid are separated by the process of filtration.
- The size of crystals formed during this process depends on the cooling rate.
- A large number of tiny crystals are formed if the solution is cooled at a fast rate
- Large crystals are formed at slow cooling rates.

Application of Crystallization:

- Purification of seawater.
- Separation of alum crystals from impure samples.
- In the pharmaceutical industry, crystallization is used as a separation and purification process for the synthesis and isolation of co-crystals, pure active pharmaceutical ingredients (API), controlled release pulmonary drug delivery, and separation of chiral isomers.

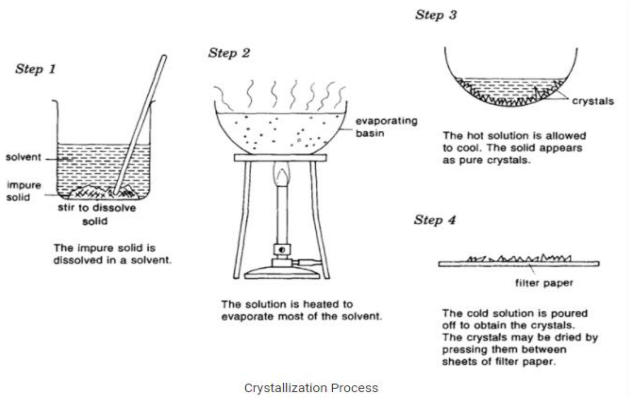


Fig. 1.13. Crystallization Setup

1.2.7 Fractional Crystallization:

Definition:

It is a physical separation process used for purification of organic compounds from a multi-component mixture by crystallization without addition of a solvent.

Principle:

- Fractional crystallization is the method of refining substance based on difference in solubility. The proportion of components in then precipitate will depend in their solubility products.
- $Na_2O + K_2O$ (A), $FeO + Fe_2O_3$ (F), and MgO (M) - Magma

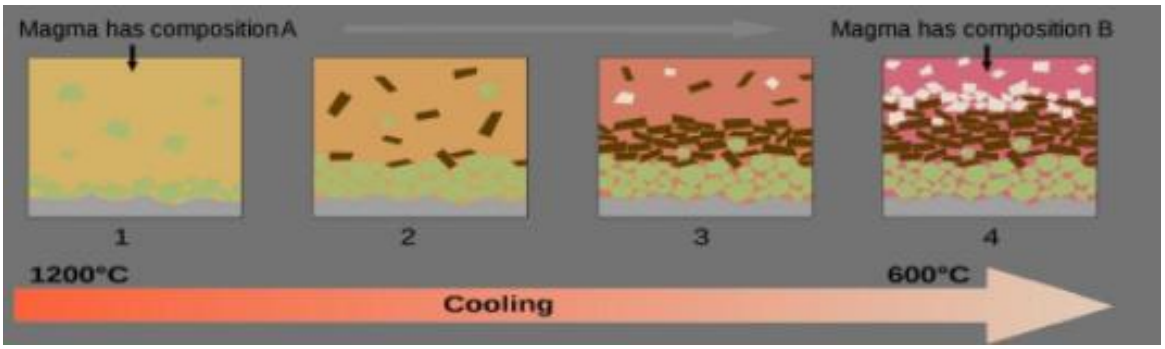


Fig. 1.14. Fractional Crystallization Process

Procedure:

- Add about 25g of the unknown mixtures to a pre-weighed 250ml beaker.
- Weigh the beaker and mixture to determine the mass of the mixture.
- Add 60ml of DI water to the beaker.
- Heat the mixture to 85^0C while stirring.(do not boil)
- Keep the mixture near 85^0C for 5 minutes with stirring.
- While the mixture is heating, setup a vacuum filtration system.
- If a mixture of two or more substances in solution is allowed to crystallize.
- Eg; By allowing the temperature of the solution to decrease, the precipitate will contain more of the least soluble substances.

Application:

- Used in crystal production and purification.
- Used in salt industry.
- Used in silicon wafer production.

1.3 Test for Purity:

The substance purified distillation, sublimation etc., has to be checked for its purity by the following methods:

1. Melting Point
2. Boiling Point
3. Refractive Index
4. Density measurements

1.3.1 Melting Point:

- The melting point of a substance is a temperature at which liquid and solid phase exists in equilibrium with each other.
- The melting point of a pure substance is constant and not affected by experimental conditions.
- It is an intrinsic property (i.e) it is not dependent on the quantity of substance used.
- The pressure of impurity lowers the melting point of a substance.

Determination of Melting Point:

The melting point of the substance can be determined with the help of the following apparatus:

- A beaker containing a liquid and a stirrer
- A thistle melting point apparatus
- A electrically heated metal block

Electrically heated metal block:

- The substance is taken in a capillary tube.
- It is heated by placing in the cavity of a metal block.
- A hole is provided to insert the thermometer which helps to note down the temperature accurately.
- The metal block is heated.
- The change in substance is observed through the magnifying glass.
- The rate of heating can be controlled by rheostat.

Precautions:

- Only a small of the substance is used.
- Thermometer should be calibrated before use.
- The temperature of the bath must be raised slowly by means of a microburner because rapid heating may lead to inaccurate melting points.
- Since the thermometer is not immersed completely, the temperature correction should be carried using the formula,

$$t_{corr} = t + 0.000156\alpha(t-t_0)$$

0.000156 = apparent coefficient of expansion of mercury in glass,
α= length of thread measured in degrees not immersed in the hot liquid,
t= melting point as measured
t_0 = mean temperature of the thread above the liquid
When a short thermometer (50^0C) is used, this correction is not required

Factors affecting melting point:

- Sample Size
- Heating rate
- State of subdivision of the sample

Fig. 1.15. Melting Point Apparatus

1.3.2 Boiling Point:

- When the vapour of a liquid becomes equal to the atmospheric pressure then the liquid starts boiling.
- The boiling point of a pure substance under a particular pressure is constant and therefore this property can be used to check the purity of the liquid.
- The boiling point of a liquid can be determined using the apparatus as shown below.
- The correct boiling point will be recorded by the thermometer only when there is a constant passage of the boiling liquid from distilling flask to the outlet.
- This is achieved by a steady heating, so that a thermometer bulb comes into contact with sufficient boiling vapour.

Precautions:

- The whole of the thermometer bulb should be exposed to the hot vapours during distillation.
- The thermometer is arranged such that the bulb is just below the side tube.
- The bulb should not be immersed in the liquid .
- Bumping should be avoided by adding four to five porcelain bits.

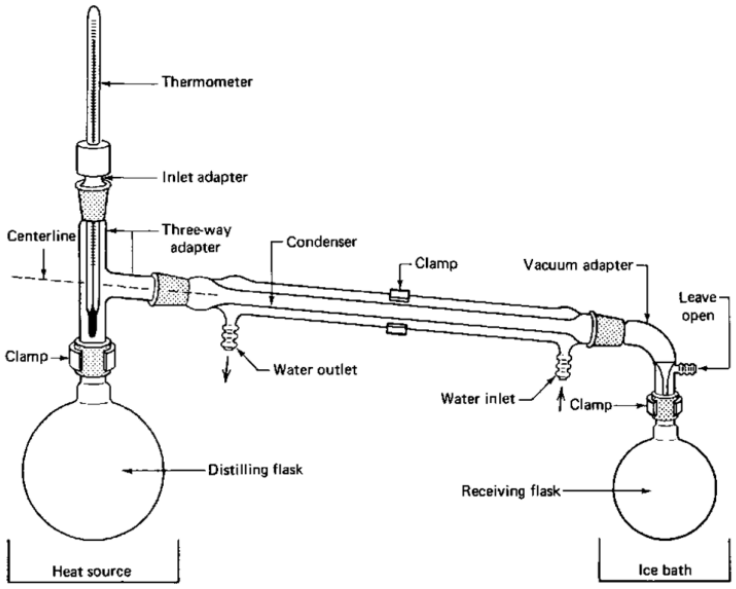


Fig. 1.16. Boiling Point Apparatus

1.3.3 Refractive Index:

- The velocity with which light travels in different media is not a constant.
- This leads to the bending of beam of light as it passes through the interfaces of two different media.
- The angle of refraction is dependent on the density, type of molecules, temperature of medium and also on the wavelength of light.
- Air is chosen as the standard medium with reference to this medium and different media bend light to different angles.
- When all the angles of refraction are measured at the same temperature with same wavelength of light with reference of air, then the measured angle is a property of second medium.
- This property is used as identifying characteristics of the substance constituting the medium.
- Instead of angle of refraction another quantity called refractive index(μ) is actually reported.
- The refractive index is given by the equation,

$$\mu = \text{Sin } i / \text{Sin } p$$

i = angle of incident light in air perpendicular to the interface
p = angle of refracted light perpendicular to the medium.

- Monochromatic light gives more precise value of Θ than white light and
- ∴ Refractive indices for the "D" line of sodium are 589.3 nm.
- This fact is indicated by a subscription and the temperature by a superscription.
- A typical notation of refractive index is "μ^{20}_{D}".
- i.e., the index is measured at 20^0C for Na D line.
- It is measured with an instrument called the refractometer.

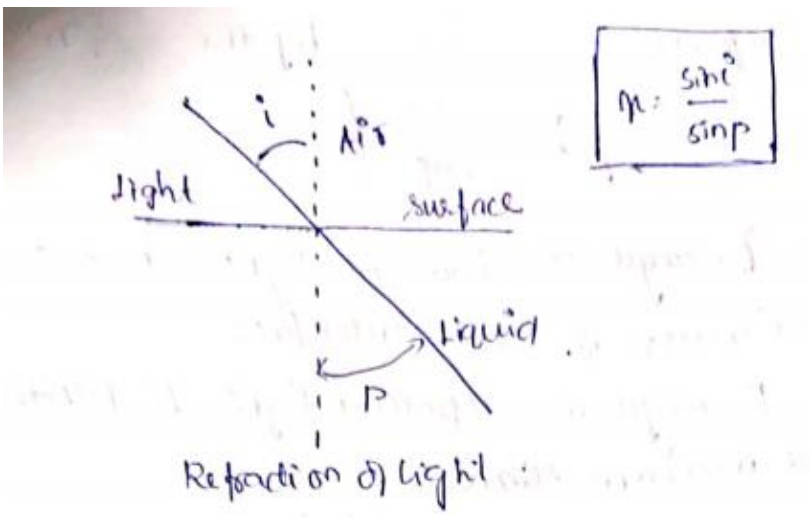


Fig. 1.17. Refractive Index Graph

1.3.4 Density:

- The density of a substance is used to check the purity of the sample it is the measure of concentration of the matter its unit is g/ml at 20^0C.
- The density of a liquid is measured using a small apparatus for picnometer whose volume is known accurately.
- The pycnometer is weighed accurately and then filled with liquid whose density is to be determined.
- The temperature of this is brought to 20^0C and the volume is readjusted.
- If necessary it is also weighed the density may be calculated by dividing the weight in grams by volume in mm.

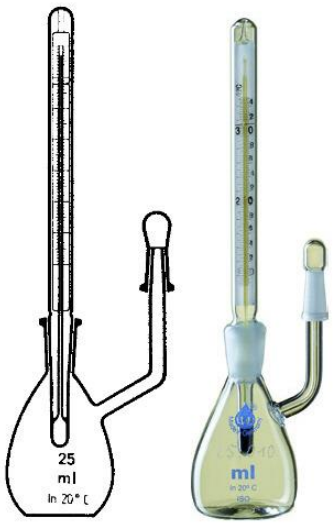


Fig. 1.18. Density Measurement

Reference:

R. Gopalan, P.S. Subramanian, K. Rengarajan, Elements of Analytical Chemistry, Third Edition 2003.

SCY1611 – Analytical Chemistry

Unit - II

UNIT - II

CHROMATOGRAPHY I

Principles adsorption, thin layer, partition and paper. Chromatography column chromatography, adsorbents, preparation of column, adsorption, elution, recovery of substance and applications. TLC - choice of adsorbent and solvent, preparation of chromatogram and applications, Rf value. Paper chromatography, Solvents used and principles, factors affecting Rf value, separation of aminoacid mixtures. Radial paper chromatography.

2. Introduction

Chromatography involves a sample (or sample extract) being dissolved in a *mobile phase* (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through an immobile, immiscible *stationary phase*. The phases are chosen such that components of the sample have differing solubilities in each phase. A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase.

2.1 Chromatography:

Definition:

- Chrome – colour, Graphy – Technique
- It was invented by Russian Botanist Michael Tswet.
- They employed the technique to separate plant pigments like chlorophyll and xanthophyll using a column of calcium carbonate.
- The separated components appeared as coloured bands on the columns and hence the name chromatography.

Principle:

- It is based on the general principle of phase distribution.
- This method involves the removal of components of one phase from that phase when it flows to a secondary stationary phase.
- The stationary phase is always fixed in a column.
- The mobile phase is the solvent flow.
- Separation of two or more components is possible when the equilibrium constant for the distribution of these components vary between the two phases.
- i.e) the substance which interact with the stationary phase strongly will move slowly through the column, whereas the substance which does not interact with the stationary phase will move rapidly.
- This results in the migration of components into separate regions and appearing as bands in the fixed phase.

Types:

- ❖ Adsorption Chromatography
- ❖ Partition Chromatography
- ❖ Ion exchange Chromatography

2.1.1 Adsorption Chromatography:

- A solid which is insoluble in the solvent is chosen as fixed phase.
- The mode of interaction between the components of the mixture and the fixed phase is adsorption.
- Hence this is called as adsorption chromatography.
- Adsorption is caused by electrostatic attraction, hydrogen bonding, vanderwaal's force etc.
  Eg., Column Chromatography, Thin layer chromatography.

2.1.2 Partition Chromatography:

- The fixed phase may be a solid on which liquid is adsorbed to form a stationary phase.
- Distribution of component of a mixture could occur between the adsorbed liquid and the flowing liquid due to difference in their solubility.
- The adsorbed liquid should be immiscible with the flowing solvent.
- This technique is called partition chromatography.
- Eg., Paper chromatography.

2.1.3 Ion Exchange Chromatography:

- It involves an exchange of ions of like sign between a solution.
- The mobile phase could be a liquid or gas which can be forced through the fixed phase by pressure.
- This technique is called Ion exchange chromatography.

2.1.1.1 Column Chromatography:

- It is an example for solid-liquid adsorption chromatography.
- Fixed phase- solid Mobile phase- liquid

Principle:

- A solid which is insoluble in the solvent is choosen as fixed phase.
- The mode of interaction between the components of the mixture and the fixed phase is adsorption.
- Hence this is called as adsorption chromatography.

Process:

- The basis of separation is selective adsorption of the components present in the liquid phase on the solid.
- A long thin column with few millimetres to several centimetres in diameter is used for effective separation.
- The column is made up of fused silica glass.
  The column is packed with an active solid such as silica gel or alumina, charcoal, calcium carbonate, magnesia, starch, sucrose, etc., are also used as solid adsorbents.

- For effective separation the solid adsorbent should be of uniform size particle with high specific area which can contribute to rapid equilibrium of the solute between the two phases.
- The column has to be filled with suitable adsorbent carefully to avoid air gaps.
- The adsorbent is usually made into a paste using solvent like petroleum ether poured into the column and allow to settle.
- During the packing continuous tapping of column with glass rod is required for uniform packing.
- After packing the column with adsorbent a small liquid sample is poured on the top.
- The sample gets adsorbed on the top of the column and eluting solvent is allowed to flow through the column.
- This solvent carries the component of the mixture with it because of the selective adsorption capacity of the solid phase.
- These component moves down the column at different rates.
- The separated components may be recovered from the column by two ways.

1. The solvent can be sent through the column until the bands are eluted from the bottom of the column and collected in different containers.
2. If the solid can be extruded from the column in one piece, the portion of the solid containing different bands may be cut out separately and extracted with appropriate solvents. This method is difficult than the earlier method.

The eluting power of the solvent is given as,

$n\text{-}C_6H_{14} < CCl_4 < C_6H_6 < CHCl_3 < C_2H_5OC_2H_5 < CH_3COCH_3 < C_2H_5OH < H_2O$

Applications:

- Used to remove small amount of impurities whose structure differ widely from that major component.
- Used for separation of structurally similar components .
- Homogeneity of coloured compounds can be tested easily.

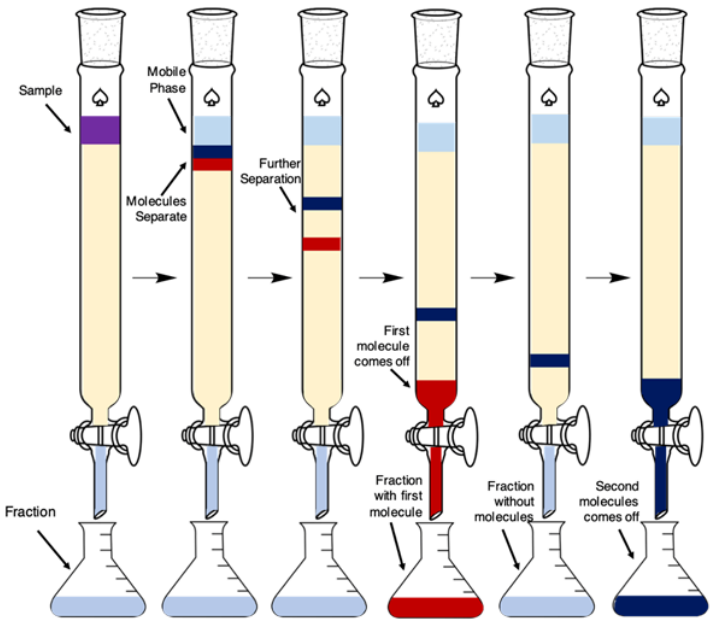


Fig. 2.1. Column Chromatograhy Process

2.1.1.2 Thin Layer Chromatography:

It is an example for solid-liquid adsorption chromatography.

Principle:

- A solid which is insoluble in the solvent is chosen as fixed phase.
- The mode of interaction between the components of the mixture and the fixed phase is adsorption.
- Hence this is called as adsorption chromatography.

Nature of phases:

Stationary Phase- Solid
Mobile phase- Liquid

Process:

Preparation of TLC plates:

- The solid adsorbents like silica gel or alumina are used in TLC.
- A thin layer plate is prepared by spreading an aqueous slurry(paste) of the finely ground solid adsorbent on the clean surface of a glass or plastic plate.
- A small of binder such as plaster of paris, CaSO4, starch etc., is incorporated into the slurry to increase adhesion of the solid property to the glass and to one another.
- The plate is heated in an oven for half an hour and cooled inside the oven itself.
- Care should be taken to avoid the exposure of surface to the atmosphere because the moisture get adsorbed on the plate present in the atmosphere.

Plate Development:

- A drop of the solution mixture to be separated is placed in one edge of the plate and its position is marked with a pencil.
- The plate is then placed in a container and the chamber is closed in along with the glass plate.
- The solvent migrates up in the plate carrying the mixture and the components of the mixture are developed on the plate at different rates.
- Once the solvent has reached the top edge, the plate is removed and dried.
- Coloured material appeared as dots in the chromatogram.
- A number of methods are available for coating the spot of the colourless material.
- There are some detecting agents which sprayed on the chromatogram make the spot coloured.
  Eg., Sulphuric acid and $KMnO_4$ solution. Iodine is also used as another detecting agent.

- Iodine is adsorbed by many organic compounds and consequently brown spots appear on the chromatogram.
- Sometimes the location of the spots can be exhibited by fluorescent spectroscopy.
- Under a given set of conditions the rate of movement of a compound w.r.to the rate of movement of the solvent front is a characteristic property of a compound.
- This property is denoted by a symbol "R_f".
- R_f = Retardation / Retention factor.
- It is obtained by dividing the distance travelled by the solvent front from the original spot. $\therefore R_f = A/B$.

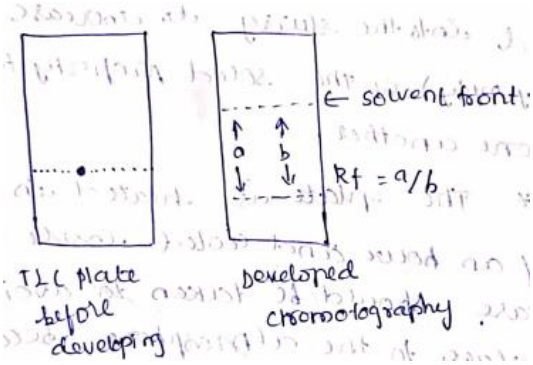


Fig. 2.2. Development of TLC Plates

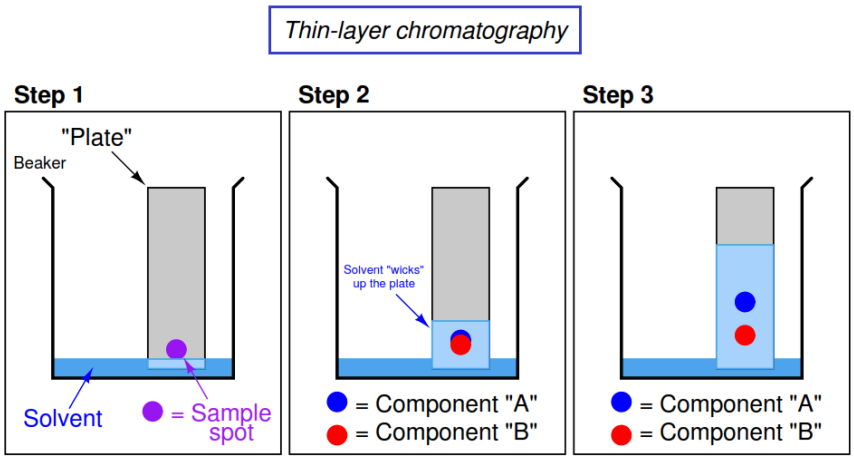


Fig. 2.3. Thin Layer Chromatography

Applications:

- The individual ion present in the mixture can be easily identified by this technique.
- It is used to find out the best eluting agent for column chromatography.
- The technique is easy and can be done rapidly for analysis of mixture of composition.
- Only a small quantity of the sample is required.

Paper Chromatography

It is an example for liquid –liquid partition chromatography.

2.1.2.1 Paper Chromatography:

- The fixed phase may be a solid on which liquid is adsorbed to form a stationary phase.
- Distribution of component of a mixture could occur between the adsorbed liquid and the flowing liquid due to difference in their solubility.
- The adsorbed liquid should be immiscible with the flowing solvent.
- This technique is called partition chromatography.
- Eg., Paper chromatography.

Types:

- **Ascending chromatography**- The technique goes with its name as the solvent moves in an upward direction.

- **Descending chromatography**- The movement of the flow of solvent due to gravitational pull and capillary action is downwards. It is performed in the same way as thin layer chromatography.
  Fixed phase- liquid
  Mobile phase- liquid

Process:

- Specially prepared papers which are reproducible with respect to porosity and thickness are used.
- Such paper contains sufficient porosity for adsorption and hence paper chromatography is classified as liquid-liquid chromatography.
- A milligram mixture of solution is spotted on the paper which is then dipped into the developing solvent.
- The paper is hanged vertically within a wide mouth bottle which contains one centimetre layer of solvent.
- The solvent rises by capillary action and the components are carried along with it at different rates.
- When solvent reaches at most the top edge of the paper, the paper is removed and dried in an oven.
- If the spots are colourless it may be rendered visible by treating with a n appropriate reagent.
- The paper is made up of cellulose acetate, starch, agar-agar etc.

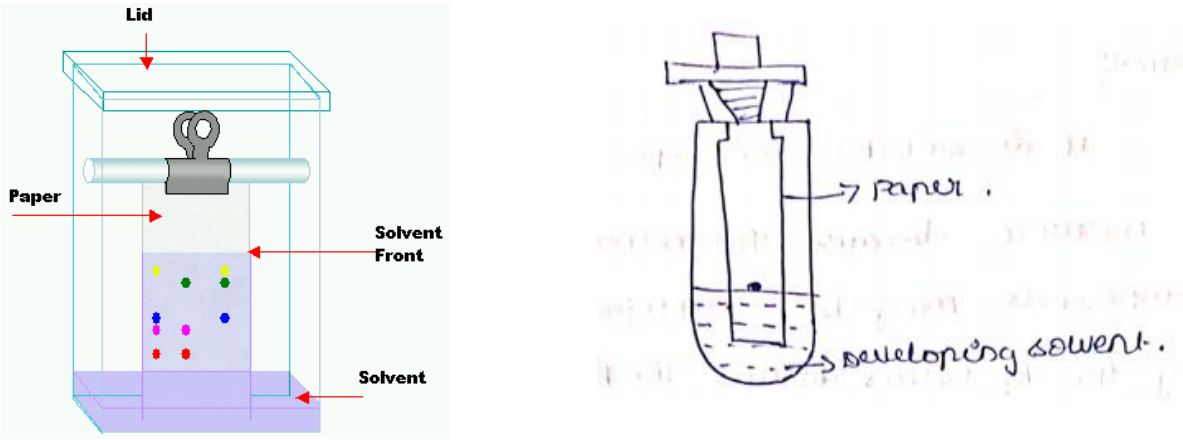


Fig. 2.4. Paper Chromatography

Applications:

- It is suitable for rapid analysis of reaction mixture because the method is very simple.
- Compounds may be identified by comparing the Rf values because the Rf values produced by paper chromatography are more reproducible than Rf values by TLC.

2.1.2.2 Paper Electrophoresis:

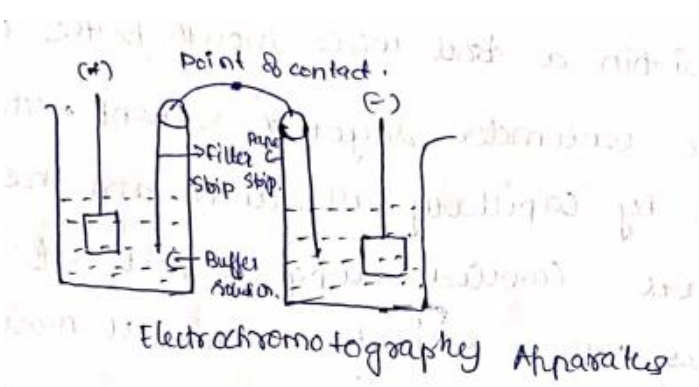
- It is a process in which separation of amino acids can be carried out.
- Ascending chromatography cannot be applied for substances having high molecular weight such as proteins or peptides since the separation are very slow.
- In such cases paper electrophoresis is the alternative method.

Process:

- A strip of paper is held horizontally between two container filled with a buffer solution.
- The paper is soaked with buffer and the sample to be separated is placed at the centre of the strip.
- A DC potential of 100-1000 volts is applied across the electrode which is dipped in the buffer solution.
- The components of the mixture are separated by moving at different speeds in the applied electric field.
- The paper acts as porous supporting material which prevents remixing by diffusion of the separated compounds.
- After passing the current for a suitable for a suitable period paper is removed and dried.
- The separated zones are rendered visible by spraying a suitable detecting agent.
- This method is applicable only to the substances such as acids, bases, amino acids and proteins which carry charge in a buffer of a suitable pH.
- Careful choice of buffer is n essential requirement of electrochromatography.
- In general the pH of the buffer should be roughly in the middle of a Pka range of the components to be separated.
- This technique is applied for water soluble substances if the aqueous buffer can be replaced by organic buffer such as pyridine acetic acid or trimethyl amine formic acid.

Applications:

- Used in clinical diagnosis.
- Used in separation of large molecules such as protein present in the serum such as spinal fluid, gastric juice and other body fluids.
- Inorganic ions can also be separated by this method.
- It is also a valuable tool for the separation of carbohydrates, amino acids, vitamins etc.



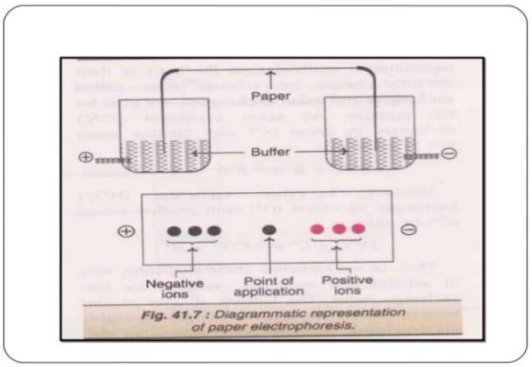


Fig. 2.5. Paper Electrophoresis

2.1.2.3 Radial paper chromatography:

Definition:

It is one of the types of chromatography procedure which owns on a piece of specialized paper. It is a planar chromatography system where a cellulose filter paper acts as a stationary phase on which the separation of compounds occurs.

Principle:

- The principle involved is partition chromatography where the substance are distributed between the liquid phases.
- One of the phases is water which is held in the pores of the filter paper used and other is the mobile phase which moves over the paper.
- The compound in the mixture get separated due to differences in their affinity towards water and the mobile phase during the movement of mobile phase under the capillary action of pores in the paper.

Process:

- It is a type of chromatography which is used for separating chemical mixtures.
- Here the solvent travels from the centre of the circular chromatography through the silica layer plate towards the periphery (boundary).
- This is also known as circular paper chromatography.
- In this method, the solvent travels from the centre towards the periphery of circular chromatography paper.
- The entire system is placed in a petridish for the development of the chromatogram.
- The wick at the centre of the paper dips into mobile phase in a petridish by which the solvent drains onto the paper and moves the sample radially to form the sample spots of different components as concentric rings.

Applications:

- Used for separation of mixture having polar and non-polar compounds.
- Used for the separation of amino acids.
- Used to determine organic compounds, biochemicals present in body fluids.
- Used in pharmacy for the determination of hormones, drugs etc.,
- Used for the evolution of inorganic compounds like sol and complexes.

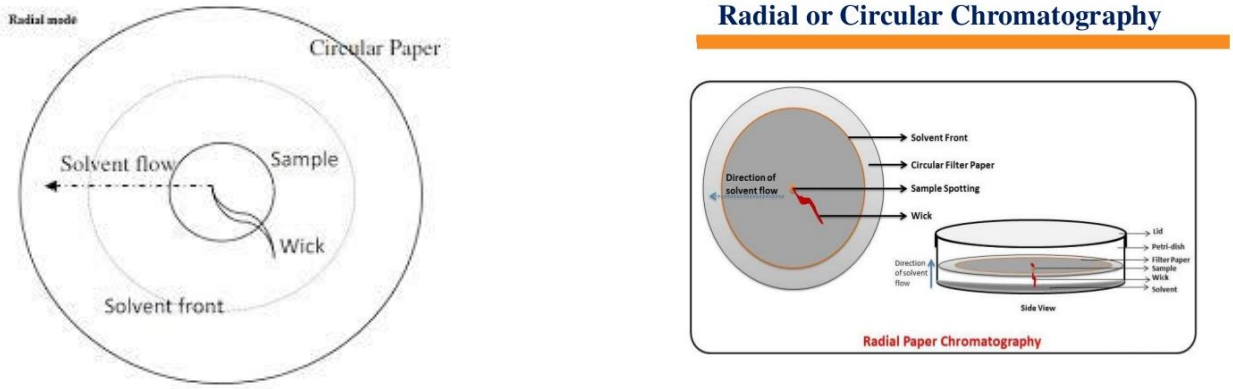


Fig. 2.6. Radial Chromatography

2.2 Factors affecting Retardation/Retention Factor:

Rf values are affected by the following factors:

- Adsorbent
- Solvent
- Chromatography Plate
- Application Technique
- Temperature of the solvent and plate.

Adsorbent:

- TLC plates can be coated with a variety of adsorbents such as silica, alumina etc.,
- Since the Rf value is based on relative affinity of the chemical.
- The change of solvent with the adsorbent will change the Rf value.

Solvent:

- Since the solvent carries in affordable direction, the particular solvent will have substantial impact on the Rf value for the chemical.
- A solvent which has a stronger interaction for a particular chemical will move easily to overcome any affinity of the chemical for the adsorbent layer and move the chemical further in a given period of time.
- Mixture of solvents can also have different effects depending on the proportion of each solvent.

Chromatographic Plate:

- The thickness and uniformity of layer of adsorbent can vary from plate to plate especially if they are handmade.
- This can also change the Rf value of a chemical.

Application Technique:

- The technique of applying too much sample may result large diffuse band of chemical making it difficult for accurate measurement of the distance of the chemical that has been transported.

Temperature of the solvent and plates:

- The solvent can often dissolve the chemicals in a better manner, when it is transporting at higher temperature.

Reference:

R. Gopalan, P.S. Subramanian, K. Rengarajan, Elements of Analytical Chemistry, Third Edition 2003.

SCY1611 – Analytical Chemistry

Unit - III

UNIT - III

CHROMATOGRAPHY- II

Ion exchange chromatography – principle, resins, action of resins, experimental techniques, applications, separation of Zn-Mg, Co-Ni, Cd-Zn, Chloride, bromide. Gas chromatography and high pressure liquid chromatography – principles, Detectors, experimental techniques, instrumentation and applications.

3. Introduction

Chromatography is based on the general principles of phase distribution. The method involves the selective removal of the components of one phase from that phase when it flows past a second stationary phase. The stationary phase is generally a column or strip through which flows the moving phase. The removal of a component by the fixed phase is essentially an equilibrium process. Separation of two or more components is possible when the equilibrium constants for the distribution of these components between the two phase vary. The molecules of a substance which interact strongly with the fixed phase will move only slowly through the column whereas substances which do not interact strongly are carried through more rapidly. These results in the migration of components into separate regions referred to as the bands of the fixed phase.

3.1 Chromatography:

Definition:

- Chrome – colour, Graphy – Technique
- It was invented by Russian Botanist Michael Tswet.
- They employed the technique to separate plant pigments like chlorophyll and xanthophyll using a column of calcium carbonate.
- The separated components appeared as coloured bands on the columns and hence the name chromatography.

Principle:

- It is based on the general principle of phase distribution.
- This method involves the removal of components of one phase from that phase when it flows to a secondary stationary phase.
- The stationary phase is always fixed in a column.
- The mobile phase is the solvent flow.
- Separation of two or more components is possible when the equilibrium constant for the distribution of these components vary between the two phases.
- i.e) the substance which interact with the stationary phase strongly will move slowly through the column, whereas the substance which does not interact with the stationary phase will move rapidly.
- This results in the migration of components into separate regions and appearing as bands in the fixed phase.

Types:

- ❖ Adsorption Chromatography
- ❖ Partition Chromatography
- ❖ Ion exchange Chromatography

3.1.1 Adsorption Chromatography:

- A solid which is insoluble in the solvent is chosen as fixed phase.
- The mode of interaction between the components of the mixture and the fixed phase is adsorption.
- Hence this is called as adsorption chromatography.
- Adsorption is caused by electrostatic attraction, hydrogen bonding, vanderwaal's force etc.
  Eg., Column Chromatography, Thin layer chromatography.

3.1.2 Partition Chromatography:

- The fixed phase may be a solid on which liquid is adsorbed to form a stationary phase.
- Distribution of component of a mixture could occur between the adsorbed liquid and the flowing liquid due to difference in their solubility.
- The adsorbed liquid should be immiscible with the flowing solvent.
- This technique is called partition chromatography.
- Eg., Paper chromatography.

3.1.3 Ion Exchange Chromatography:

- It involves an exchange of ions of like sign between a solution.
- The mobile phase could be a liquid or gas which can be forced through the fixed phase by pressure.
- This technique is called Ion exchange chromatography.

3.1.2.1 Gas Liquid Chromatography:

It is also called as gas liquid partition chromatography.

Definition:

Gas chromatography is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition.

Principle:

The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column." (Helium or nitrogen is used as the so-called carrier gas.)
Fixed phase- liquid
Mobile phase- Gas

Process:

- In GLC the mixture to be separated is vaporized and sent through a column by flowing a inert gas such as He or H_2.

- The inert gas is called carrier gas which carries the gaseous mixture in the mobile phase.
- The column is packed with a solid, on the surface of which is adsorbed a liquid of very low volatility .
- The liquid serves as the stationary phase.
- The components of the mixture moves through the column at different rates due to selective phase distribution between their two phases and get separated.
- In general low boiling and high volatile compounds will move through the column faster than low volatile components because higher the vapour pressure of a gas, the lower will be its solubility in a liquid.
- Apart from volatility polar interactions will also affect the solubility of a solute in a solvent.
- Stationary phase: Poly ethylene glycol, di-ethylene glycol adipate, di-ethylene glycol succinate, and butane diol succinate.
- The basic components of GLC is shown below.

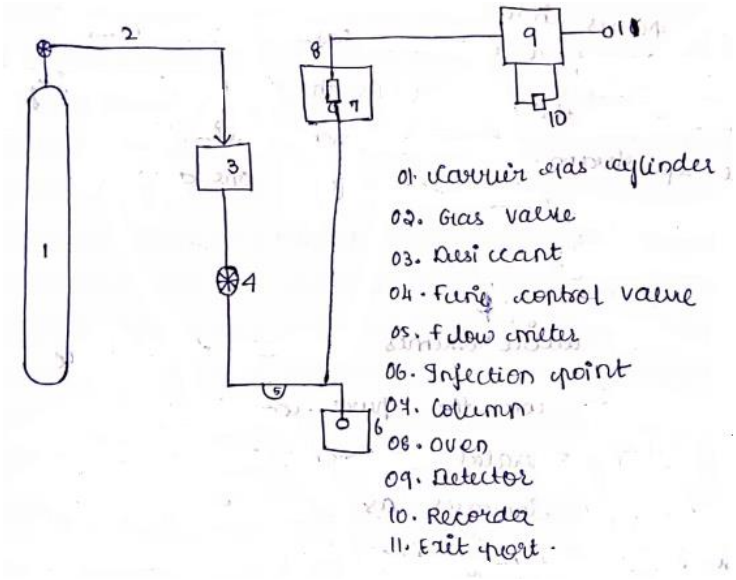


Fig. 3.1. Gas Chromatography

- The column is kept in an oven whose temperature is controlled by thermostat and heating elements.
- The sample is introduced into the flow system at the injection pore.
- This unit is individually heated to facilitate the vaporization of the sample.
- As the sample is vapourised and swept into the column by carrier gas and its component is separated into individual bands in the carrier gas and passes into the detector.
- A simple detector system consists of a instrument called “katherometer” which measures the changes in the thermal conductivity of the gas stream.
- The instrument consists of an electrically heated wire which forms one arm of a wheatstone bridge.
- The wire assumes a steady temperature and resistance when the pure carrier gas flows over it as the separated components reaches the wire and its temperature increases as a result of the decrease in thermal conductivity of its surroundings.
- The consequent change in the system is measured by wheat stone bridge.
- The change in resistance is proportional to the concentration of the component.
- The bridge is coupled to a pen recorder and chromatogram consist of a plot of concentration of the components in the carrier gas Vs time.
- A typical gas liquid chromatogram is shown below.

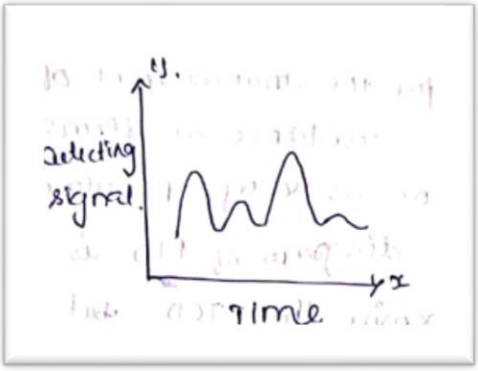


Fig. 3.2. Gas Chromatogram

Applications:

- Used in separating volatile components.
- Used in pharmaceuticals, cosmetics etc.

3.2.1.2 Detectors:

Definition:

A chromatography detector is a device used in gas chromatography (GC) or liquid chromatography (LC) to detect components of the mixture being eluted off the chromatography column.

Ideal properties of a detector:

The detectors used in both GC and HPLC should have the following ideal properties:

1) High sensitivity
2) Good stability and reproducibility.
3) A linear response to solutes.
4) Negligible base line noise.
5) Should be inexpensive.
6) Capable of providing information on the identity of solute.
7) A temperature range from room temperature to at least 400^0c.
8) A short response time independent of flow rate.
9) High reliability and ease of operation.
10) The detector should be non-destructive.
11) Response independent of mobile phase composition.

Types of Detectors

1. Thermal Conductivity Detector- TCD
2. Electron Capture Detector- ECD
3. Flame Ionization Detector- FID
4. Argon Ionization Detector- AID
5. Helium Ionization Detector- HID
6. Flow Dependent Detector- FDD
7. Nitrogen Phosphorus Detector- NPD
8. Flame Photometric Detector- FPD

9. Thermionic Detector- TID
10. Photoionization Detector- PID
11. Atomic Emission Detector- AED
12. Sulfur Chemiluminescence Detector- SFD

The most common type of detectors used are:

1. TCD- Thermal conductivity detector
2. ECD- Electron capture detector
3. FID- Flame ionisation detector.

1. Thermal Conductivity Detector:

Definition:

It is a universal detector and can detect air, hydrogen, CO, N_2, SO_2, inorganic gases etc.
It is a non-specific and non-destructive detector.
For most of the organic molecules the sensitivity of this detector is lower when compared to flame ionization detector.

Principle:

- It is based on the principle of thermal conductivity which depends upon composition of the gas.
- It determines the thermal conductivity difference between the effluent flow and the difference in flow of carrier gas alone which produces potential voltage to this difference.

Process:

- The sample components in the carrier gas passes into the measuring channel.
- The second channel serves as a reference channel where only pure carrier gas flows.
- Electrically heated resistance wires are connected in both the channels.
- The difference in thermal conductivity between column effluent flow(carrier gas + sample component) and the reference flow of carrier gas alone produces a voltage signal proportional to this difference.
- This signal is proportional to the concentration of the sample component.
- Chemically active components like acids and halogenated compounds should be avoided.
- Because they attack the filament wires and change the resistance which permanently reduces the detector sensitivity.
- Oxidising substances such as O_2 can also damage the filaments, therefore gas free environment should be maintained.

Advantages:

- Non-destructive detector.
- Simple in nature.
- Produces large linear dynamic range.

Disadvantages:

- Low sensitivity.

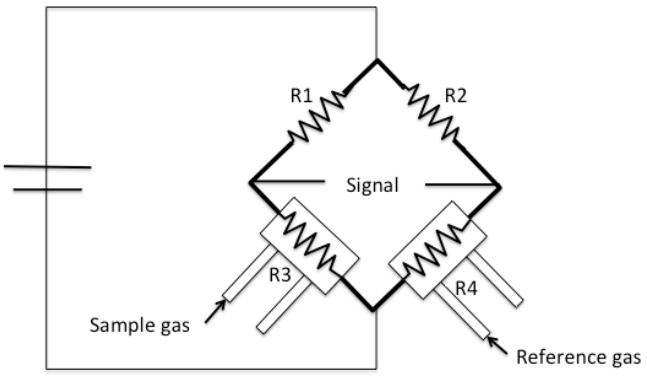


Fig. 3.3. Thermal Conductivity Detector

2. Flame Ionization Detector

Definition:

- It is a scientific instrument that measures analytes in a gas stream.
- It is frequently used as a detector in gas chromatography.
- The measurement of ion per unit time makes this a mass sensitive instrument.

Principle:

- The operation of the FID is based on the detection of ions formed during combustion of organic compounds in a hydrogen flame.
- The generation of these ions is proportional to the concentration of organic species in the sample gas stream.

Process:

- Many organic compounds, when pyrolysed at a temperature of hydrogen or air flame produces ionic species.
- These ions can be collected and the result in ion current is measured as electrometer employed for the measurement of current because of the high resistance or flame.
- The hydrogen flame detector is very popular and sensitive.
- A systematic diagram of FID is shown below.
- It is more sensitive than TCD but it is more sensitive.
- Mixture of solvents can also have different effects depending on the proportion of each solvent.

Advantages:

- Used as an universal detector for organics.
- Carrier gas are not detected.
- Mobile phase impurities are not detected.
- Does not respond to common inorganic compounds.

Disadvantages:

- More expensive.

- Destructive Detector.

Applications:

- It is used to detect traces of hydrocarbons and CO_2 in normal air constituents (O_2, N_2, etc.) and inert gases.

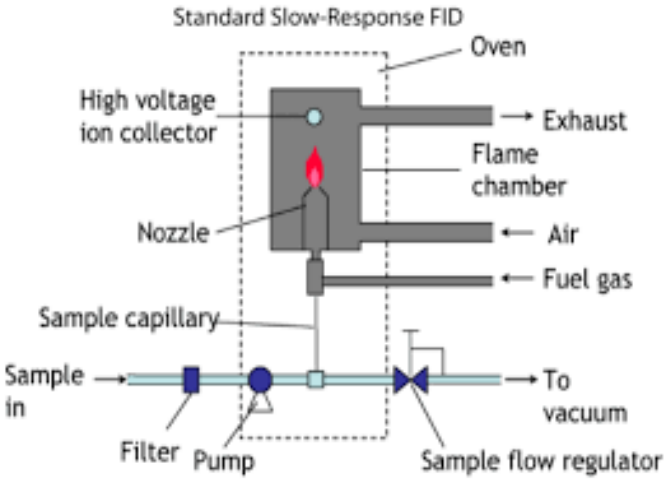


Fig. 3.4. Electron Capture Detector

3. Electron Capture Detector

Definition:

- An electron capture detector (ECD) is a device for detecting atoms and molecules in a gas.
- An ECD has a limited dynamic range and finds its greatest application in analysis of halogenated compounds.

Principle:

- When a gas is passed through the ECD, some electrons of certain molecules which pass through the detector are captured and the current being measured reduces, which results in a positive peak being recorded.
- In order to pass a sample through the ECD, a carrier gas, such as hydrogen or nitrogen must be used.

Process:

- It is a radioactive decay based detector.
- It is selective for electronegative atoms such as halogens, peroxides, quinones and nitro groups.
- The effluent sample from a column is passed over a radioactive β- emitter such as Ni^{63} an electron from the emitter causes ionization of the carrier gas (N_2) and the production of large number of electrons.
- In the absence of organic species a constant standing current between a pair of electrode results from this ionization process.
- The current decreases significantly in the presence of organic molecules containing electronegative functional groups that tends to capture electrons.

Advantages:

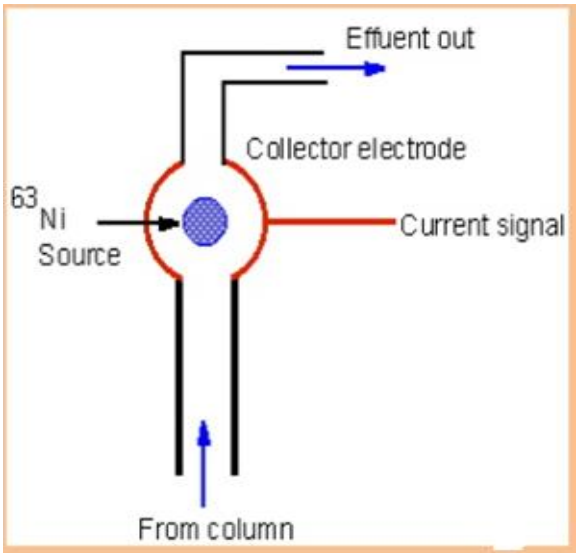
- Non-destructive detector.
- Simple in nature.
- Produces large linear dynamic range.

Disadvantages:

- Affected by fluorides present in the sample.

Applications:

- Used for environmental testing like detection of chlorinated pesticides or herbicides, polynuclear aromatic carcinogens, organometallic compounds.
- Used for selectively halogens, nitro and sulphur containing compounds.
- Used for detecting polynuclear aromatic compounds on hydrides and conjugated carbonium ions.



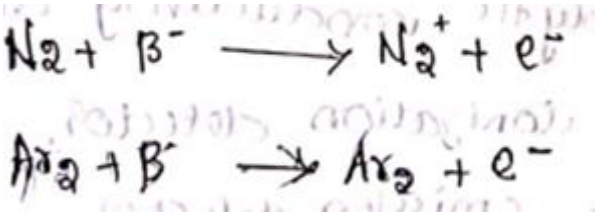


Fig. 3.5. Electron Capture Detector

3.1.2.3 High Performance/Pressure Liquid Chromatography

Definition:

- It is a chromatographic technique that can separate a mixture of compounds and to identify, quantify and purify the individual compounds of the mixture.

Principle:

- The separation is based on the analytes relative solubility between the two liquid phases.
- HPLC utilizes different types of stationary phase and analyte through the column.
- The detector provides a characteristic retention time for the analyte.

Components:

- It consists of a reservoir of mobile phase, a pump, a injector, a separation column and a detector.

Working:

- It is a type of liquid chromatography used to separate compounds that are dissolving in solution.
- A reservoir holds the solvent that is to be injected into the column.
- Compounds are separated by injecting a sample mixture onto the column.
- The different components in the mixture passes through the column at different rates due to their difference in their partition behaviour between the mobile phase and stationary phase.
- The mobile phase must be degassed to eliminate the formation of air bubbles.
- An injector is used to introduce the sample into the HPLC column.
- The column contains the chromatographic packing material needed to affect the separation process.
- This packing of materials is called the stationary phase.
- An injector is needed to collect the separated compound bands as they elute from the column.
- The mobile phase exist the detector and can send the waste or collected as a desired products.
- The detector generate the signals needed to generate the chromatogram on its display and to identify quantitatively the concentration of the sample constituents.
  The flow rate is adjusted of about 0.01 to 10 cm^3 per minute using the pump.

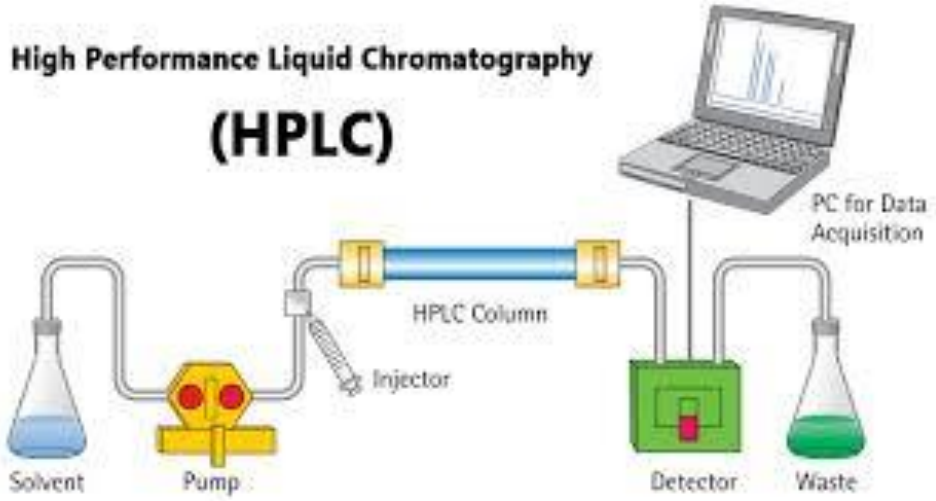


Fig. 3.6. High performance liquid chromatography

Application:

- Used in both quantitative and qualitative applications.
- Used for molecular weight determination.
- Used in environmental chemistry.
- Used in green chemistry.
- Used in polymer chemistry for identification of nature of the polymer.

3.1.3 Ion Exchange Chromatography

Definition:

- It is a chromatography technique in which both cations and anions are identified using ion exchange resins.

Principle:

- It is a process of exchange of ions of like sign between a solution and a solid in contact with the solution.
- IEC separate molecules based on their respective charged groups.
- It retains analyte molecules on the column based on the ionic interaction.
- IEC matrix consists of positive and negatively charged ions.
- Molecules undergo electrostatic interaction with opposite charges and the stationary matrix.
- The stationary phase consists of charged ionisable functional groups or ligands.
- The stationary phase surface displays ionic functional groups such that positively charged molecules bind to cation exchange resin while negatively charged molecules bind to anion exchange resins.

Process:

- They are synthetic high molecular weight organic polymer containing a large number of ionic functional groups per molecule.

Types:

1. **Cation exchange resin:**

It is a styrene divinyl benzene copolymer containing sulphonic acid or carboxylic acid is a functional group. It is denoted as $RSO^{3-}H^{+}.(RH^{+})$.

2. **Anion exchange resin:**

It is a styrene divinyl benzene copolymer containing quarternary ammonium salt as a functional groups. It is denoted as R'N$(CH3)^{+}_{3}$ or $R'OH^{-}$

- A column is packed with an acid resin and treated with HCl to make sure that all exchange points were occupied by H+ ions.
- The first major applications of IEC are the separation of rare earth metals (f-block elements).
- A mixture of rare earth metals as the chlorides is down the column. This results in the displacement of H+ ions by rare earth cations.
- The rare earth ions could be eluted then one after other.
- Since elution with water is very slow therefore a solution of citric acid was used as eluting solvent.
- The cations moved at different rates depending on the solubility of the corresponding complex with citric acid.

$$RSO3^{-}H^{+} + B \rightarrow RSO_3^{-}.BH^{+} \text{ (salt)}$$

$$RSO3^{-}.BH^{+} + OH^{-} \rightarrow H_2O + B^{+}$$

Advantages:

- The capacity of the resins for exchanging is high therefore it is easy to handle as much as 0.1g of the sample at a time.
- The recovery of ions from the column is virtually complete which is an important fact that in dealing with expensive materials as in qualitative analysis.

Disadvantages:

- High in cost.

Application:

- Used for the separation of lanthanides and actinides.
- Used in the separation of inorganic ions in mixture.
- Used in the separation analysis of amino acid mixture from the blood.

3.1.3.1 DEMINERALIZATION PROCESS or ION EXCHANGE PROCESS:

Demineralization process is an ion exchange process in which porous, insoluble, cross linked long chain high molecular weight synthetic resins are used as ion exchangers. The functional groups attached to the polymers are responsible for Ion exchange property. Resins containing acidic groups are capable of exchanging their H+ ions with cations in water where as those containing basic groups are capable of exchanging their OH- ions with anions in water. The organic ion exchange resins are:

1. **Cation exchange resin:**

These are cross linked styrene - divinyl benzene copolymers containing sulphonic acid group (SO_3H) or carboxylic acid group (COOH) which is capable of exchanging reversibly its H^+ ions for cations in water. It is represented as RH^+.

2. **Anion exchange resin:**

These are cross linked styrene - divinyl benzene copolymers containing amino, substituted amino or quaternary ammonium group as integral part of resin. This copolymer on treatment with dilute NaOH becomes capable of exchanging reversibly its OH^- ions for anions in water. It is represented as $R'OH^-$

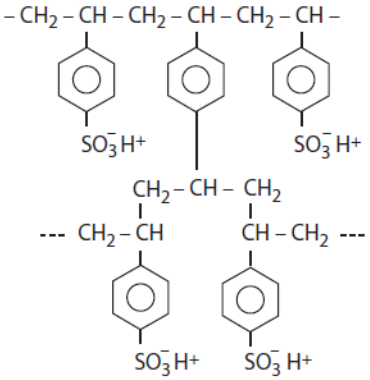


Fig. 3.7. Structure of Cation exchange resin

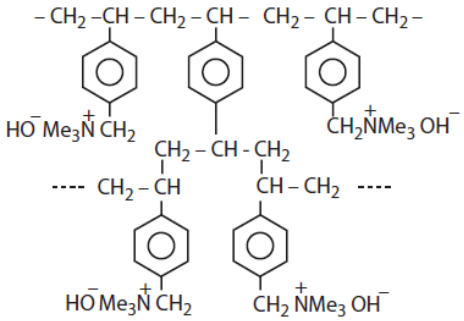


Fig. 3.8. Structure of Anion exchange resin

Softening Process:

The cation and anion exchange resins are kept in two vertical exchangers.

Cation exchange reaction:

Hard water is first percolated through cation exchange resin where the cations are retained in the resin and an equivalent amount of H^+ ions are released in the outgoing water.

$2\ RH^+ + Ca^{2+} \rightarrow R_2Ca^{2+} + 2H^+$

$2\ RH^+ + Mg^{2+} \rightarrow R_2Mg^{2+} + 2H^+$

$RH^+ + Na^+ \rightarrow RNa^+ + H^+$

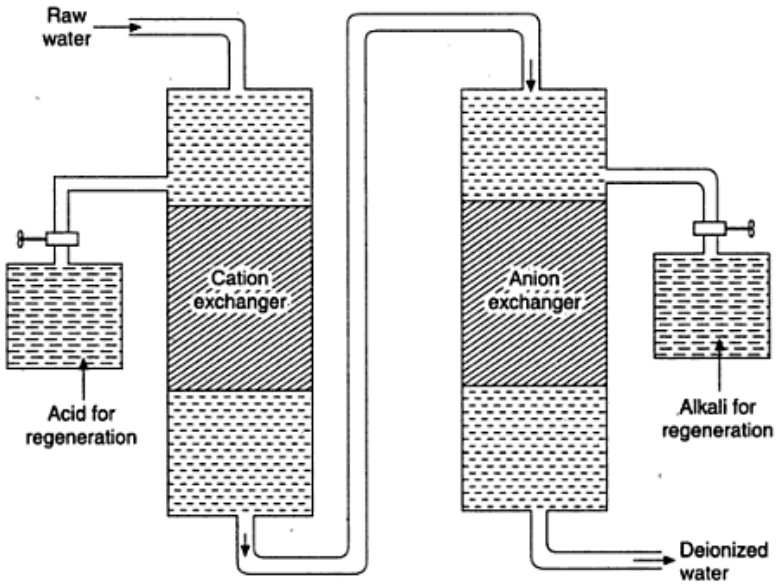


Fig. 3.9. Demineralization cation and anion exchanger

Anion exchange reaction:

The water from cation exchange resin is passed through anion exchange resin which retains the anions and releases an equivalent amount of OH^- ions in the water.

$2\ R'OH^- + CO_3^{2-} \rightarrow R'_2\ CO_3^{2-} + 2\ OH^-$

$2\ R'OH^- + SO_4^{2-} \rightarrow R'_2\ SO_4^{2-} + 2\ OH^-$

$2\ R'OH^- + Cl^- \rightarrow R'Cl^- + OH^-$

H^+ and OH^- ions from cation exchange and anion exchange resins combine to form deionized water which is free from cations and anions.

$H^+ + OH^- \rightarrow H_2O$

The order of exchange process should not be interchanged as water coming out from cation exchange resin is acidic which does not affect the anion exchange resin. Whereas when the order is changed the water coming from the anion exchange resin is alkaline this harms the cation exchange resin in the subsequent step.

Regeneration Process:

When the resin is completely exhausted it loses its ion exchange capacity and are regenerated and reused.

Cation exchange resin:

The exhausted cation exchange resins are regenerated by passing dilute HCl or H_2SO_4.

$R_2Ca^{2+} + 2H^+ \rightarrow 2\ RH^+ + Ca^{2+}$

$R_2Mg^{2+} + 2H^+ \rightarrow 2\ RH^+ + Mg^{2+}$

Anion exchange resin:

The exhausted cation exchange resins are regenerated by passing dilute NaOH.

$R'_2HCO_3^- + 2\ OH^- \rightarrow 2\ R'OH^- + HCO_3^-$

$R'_2\ SO_4^{2-} + 2\ OH^- \rightarrow 2\ R'OH^- + SO_4^{2-}$

Advantages of Demineralisation process

1. Water obtained from demineralisation process contain residual hardness of 2ppm. This can be used in high pressure boilers.
2. Acidic and alkaline waters can be softened by this method.

Disadvantages of Demineralisation process

1. The cost of equipment and resins are expensive.
2. Fe and Mn containing water cannot be treated by this method as they are irreversibly exchanged.
3. Turbid water cannot be treated as they reduce the efficency of the process.

3.1.3.2. Separation of a Mixture by Ion-Exchange:

OBJECTIVE:

The objective of this experiment is to determine the composition of a mixture of metal salts. The mixture components are separated with ion chromatography and quantified by complexometric titration.

INTRODUCTION:

Ion chromatography is a form of liquid chromatography that separates ions by their interaction with ionic sites on the packing (resin). Ion-exchange resins are inert, insoluble materials containing either acidic or basic sites that hold ions by electrostatic attraction. Most commercial ion exchange resins are organic polymers containing sulfonic or carboxylic acid groups (cation exchange resins) or quaternary ammonium groups (anionexchange resins). The ion-exchange process is reversible. Ionic components of the mixture are differentially retained on the column by their differing affinities for ionic sites on the ion-exchange resin. For example, retention of a metal cation on a cation-exchange resin occurs by the following reaction:

$SO^{3-}\ H^+_{(s)} + M^{x+}_{(aq)} \rightarrow SO^{3-}\ M^{x+}_{(s)} + H+_{(aq)}$

Where, M^{x+} = cation of charge x,
(s) = the solid or stationary phase,
(aq) = the aqueous or mobile phase.

The equilibrium constant for this reaction is:

$$K_{eq} = \frac{[\text{-}SO^{3-}\ M^{x+}]_s\ [H^+]_{aq}}{[\text{-}SO^{3-}\ H^+]_s\ [M^{x+}]_{aq}}$$

Different cations have different values of Keq and are therefore retained on the column for different lengths of time. The time at which a given cation elutes from the column can be controlled by adjusting the pH ($[H^+]_{aq}$).

In this experiment, zinc and nickel ions will be separated by anion-exchange rather than by cation-exchange. In 2 M hydrochloric acid, zinc(II) forms negatively charged complexes with chloride,

i.e. $ZnCl^{3-} + R + Cl^- \rightarrow R^+ZnCl^{3-} + Cl^-$

$ZnCl_4^{2-} + 2R^+Cl^- \rightarrow (R^+)_2ZnCl_4^{2-} + 2Cl^-$

Nickel(II) does not form a stable anionic complex with chloride under these conditions. Thus, when a mixture of zinc(II) and nickel(II) in 2 M hydrochloric acid is placed on an anion-exchange resin in the chloride form, only zinc is retained. Once nickel has completely eluted from the column, distilled, deionized water is passed through the column. This lowers the chloride ion concentration in the column to a level where the zinc-chloride complexes are no longer stable and zinc(II) elutes from the column. After separation, the concentration of each metal will be determined volumetrically by titration with ethylene diamine tetraacetic acid (EDTA). Zinc is titrated in a solution buffered at pH = 10 with ammonia using calmagite as the indicator. Nickel is also titrated in a solution buffered at pH = 10 but using murexide as the indicator. Both metals form stable 1:1 complexes with EDTA.

PROCEDURE:

1. Accurately weigh between 3.6 to 3.8 g of pre-dried disodium ethylenediaminetetraacetate dihydrate (EDTA). Dissolve the EDTA in ~200 mL of deionized water. Gentle heating may be required to expedite dissolution. Quantitatively transfer this solution to a 500 mL volumetric flask and dilute to the mark with deionized water.

2. Obtain an unknown sample containing 3 to 6 millimoles each of zinc and nickel from your TA. Record its identification number. Transfer the contents to a 100 mL volumetric flask and dissolve in a mixture of deionized water and HCl. Dilute to the mark bringing the final concentration of HCl to 2 M.

3. Fill two chromatography columns (at least 35 cm long and 1.3 cm in diameter) with Dowex 1X8 strong-base anion exchange resin to the 25 cm level. Take care to remove all air bubbles trapped in the column. Wash the resin by eluting 50 mL of 6 M ammonium hydroxide to remove any metals present as anionic species. Next, elute 100 mL of deionized water followed by 100 mL of 2 M HCl. The flow rate for these washing should be 4 to 5 mL/min. Do not allow the liquid level to drop below the surface of the resin.

4. Pipet a 10.00 mL aliquot of the sample solution onto the top of each column. Drain this aliquot onto the column collecting the eluant into a clean 250 mL Erlenmeyer flask. Wash the inner wall of the column above the resin with several 3-4 mL portions of 2 M HCl, permitting the level of liquid to reach the surface of the resin before adding the next portion.

5. Selectively remove nickel from the column by eluting 50 mL of 2 M HCl solution and collecting the effluent in the flask. Cover the flask and set aside for analysis later in the period.

6. Place a clean 250 mL Erlenmeyer flask under the column outlet and elute the zinc from the column 100 mL of deionized water through the column at a flow rate of 3 to 4 mL/min.

7. Remove the flask and add 20 mL of ammoniacal buffer (pH 10) and 5 drops of Calmagite indicator to the zinc-containing solution. Titrate with the 0.02 M EDTA solution to the point where one or two drops of titrant gives the greatest color change between the wine-red of the zinc-Calmagite complex and the blue of the free indicator. At the end point, record the volume of titrant.

8. Pass another 50 mL portion of deionized water through the column, adding the effluent directly to the flask containing the titration solution from part 7. If the wine- red color of the zinc-Calmagite complex returns, then zinc continues to be eluted from the column and proceed to step.

9. If not, then this is evidence that all of the zinc has been eluted; skip to step 10. 9. If additional zinc has been eluted, collect the column effluent and titrate with EDTA until the end point. Record the volume of EDTA required. Repeat steps 8 and 9 as necessary until all of the zinc has been eluted. Calculate the total volume of EDTA solution required to titrate all of the zinc eluted from the column.

10. Add 20 mL of ammoniacal buffer (pH 10) and 0.5 g of solid 0.2% murexide indicator to the solution containing nickel. Titrate with the 0.02 M EDTA solution until the yellow of the nickel-murexide complex changes to the purple of the free indicator. Record the volume of EDTA solution required.

Reference:

R. Gopalan, P.S. Subramanian, K. Rengarajan, Elements of Analytical Chemistry, Third Edition 2003.

SCY1611 – Analytical Chemistry

Unit - IV

UNIT - IV

Thermal Analysis

Thermal analytical methods - Principle involved in thermogravimetric analysis and differential gravimetric analysis, discussion of various components with block diagram, characteristics of TGA and DTA, factors affecting TGA and DTA curves, thermometric titrations.

4. Introduction

Thermal analysis includes a group of techniques which monitors the change in physical properties such as weight, temperature or enthalpy of a sample material as a function of temperature. The sample is subjected to a programmed heating from an initial lower temperature to a final higher temperature at a specified heating rate during the analysis. The most commonly used techniques include thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC). Thermal analysis has been used to determine the physical and chemical properties of polymers, electronic circuit boards, geological materials, etc. Thermal events that may occur in the sample as it is undergoing a change in temperature include phase transitions, melting, sublimation/volatilization, decomposition, glass transition in polymers, oxidation/reduction, etc. The summary of thermal analysis techniques is given below.

4.1 THERMOGRAVIMETRIC ANALYSIS (TGA)

Definition:

Thermogravimetry is a technique in which a change in weight of the sample is recorded as a function of temperature.

Principle:

The weight of the sample is continuously monitored as a function of temperature when the sample is heated at a controlled heating rate of 10-20^{o}C/minute. When the temperature is increased from ambient to 1200^{o}C, the sample may undergo dehydration, decomposition or volatilization which results in direct weight loss. The online plot of sample weight versus temperature is called a TG thermogram.

Instrumentation:

The major component of TG is the thermobalance or thermogravimetric analyzer for measuring the mass. It includes a thermobalance and a microprocessor controlled tubular furnace. **Fig.1.** shows the instrumentation of TG.

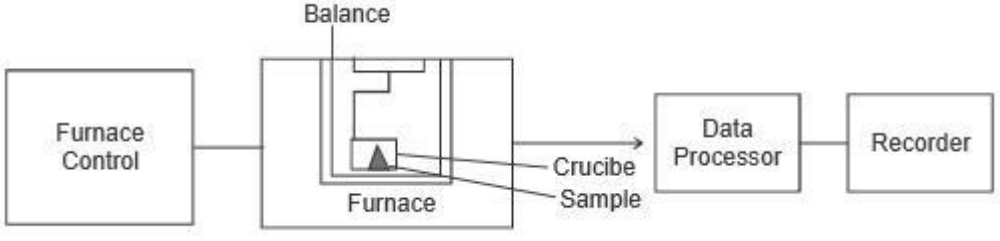


Fig.4 .1. Block diagram of TGA apparatus

Sample:

A solid sample of 5-50 mg is placed in a platinum crucible (sample container) and connected to a sensitive microbalance. The sensitive microbalance can detect a weight change of 1µg of the sample.

Thermobalance:

The balance is placed inside the tubular furnace. A thermocouple, located immediately below the crucible, monitors the furnace temperature. The temperature of the furnace is accurately controlled and programmed for any change by the microprocessor.

The **null point** balance is used in TG. When there is a **change in the weight** of the sample, the balance beam will deviate from its usual position. A sensor detects the deviation and initiates a force that will restore the balance to the null position. The restoring force is proportional to the change in weight. The atmosphere inside the furnace can be controlled by using inert gases such as nitrogen, helium or argon or reactive gases such as oxygen, hydrogen, etc.

Data processor and recorder:

The balance assembly measures the initial weight of the sample and continuously monitors changes in sample weight as heat is applied to the sample inside the furnace. The furnace data and balance data are collected during the experiment and sent to the computer for manipulation. The computer records the TG curve.

The thermogram obtained for calcium oxalate monohydrate is shown in **Fig 2** and the various thermal reactions that occur when calcium oxalate monohydrate is heated from 30 ^{O}C to about 1000 ^{O}C is summarized in Table 2.1. The horizontal portions or plateaus indicate regions where there is no weight loss and the curved portion or downward steps indicate regions of weight loss.

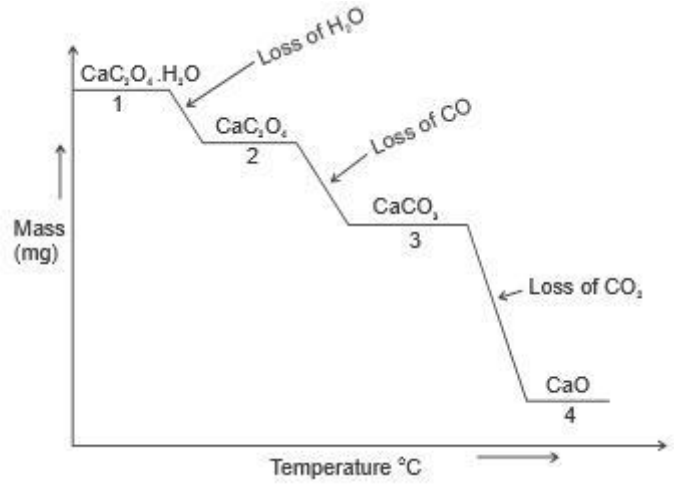


Fig. 4.2. TGA curve for decomposition of $CaC_2O_4.H_2O$

Table 2.1 Summary of thermal reactions in the decomposition of calcium oxalate monohydrate

Temperature range in ^{o}C	Thermal reaction	Change in mass
30 - 130	1st plateau region. $CaC_2O_4.H_2O$ is thermally stable	No change in mass
130 - 190	1st downward step. $CaC_2O_4.H_2O \rightarrow CaC_2O_4 + H_2O$	Loss of water of crystallization and there is decrease in mass .
190 - 400	2nd plateau region. Anhydrous CaC_2O_4.is thermally Stable	No change in mass
400 - 470	2nd downward step. $CaC_2O_4 \rightarrow CaCO_3 + CO$	Decrease in mass due to loss of CO.
470 - 700	3rd plateau $CaCO_3$ is thermally stable.	No change in mass
700 - 840	3rd downward step $CaCO_3 \rightarrow CaO + CO_2$	Decrease in mass due to loss of CO_2
840 - 1000	4th plateau CaO is thermally stable	No change in mass. The residue obtained is CaO.

The themogram obtained for $CuSO_4.5H_2O$ is shown in the **Fig 3** and thermal events are summarized in the Table 2.2.

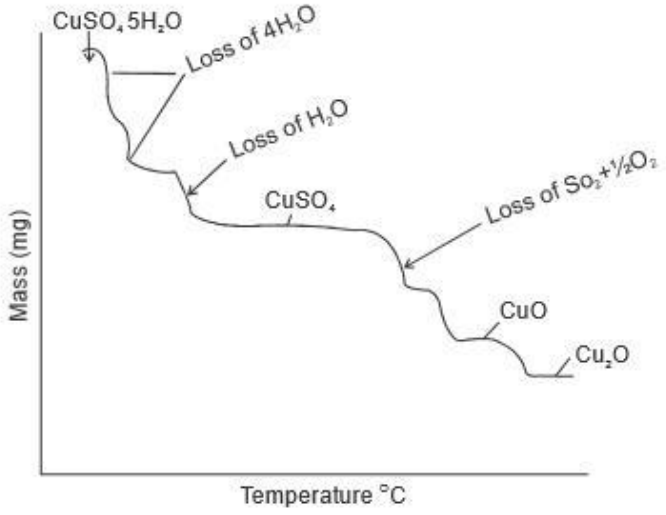


Fig. 4.3. TG curve for $CuSO_4.5H_2O$

Table 2.2 Summary of thermal reactions in the decomposition of $CuSO_4.5H_2O$

Temperature range in °C	Thermal reaction	Change in mass
30 – 90	1st plateau region. $CuSO_4.5H_2O$ is thermally stable	No change in mass
90 – 150	1st downward step. $CuSO_4.5H_2O \rightarrow CuSO_4.H_2O + 3H_2O$	Loss of water of crystallization and there is decrease in mass
150 – 200	2nd plateau region. $CuSO_4.H_2O$ is thermally stable	No change in mass
200 – 275	2nd downward step. $CuSO_4.H_2O \rightarrow CuSO_4 + H_2O$	Decrease in mass due to loss of H_2O
275 – 700	3rd plateau anhydrous $CuSO_4$ is thermally stable.	No change in mass
700 – 900	3rd downward step. $CuSO_4 \rightarrow CuO + SO_2 + 1/2O_2$	Decrease in mass due to decomposition
900 – 1000	4th plateau CuO is thermally stable	No change in mass.
1000 – 1100	4th downward step $2CuO \rightarrow Cu_2O + 1/2O_2$	Reduction of CuO to Cu_2O and there is decrease in mass.

Applications of TGA

1. In the analysis of thermal decomposition of inorganic salts and complexes which are used as catalysts, semiconductors and fine chemicals.

2. The decomposition temperature of commodity plastics and rubber are investigated by TGA. Each kind of polymer has a characteristic thermogram and can be used for identification purposes**. Fig 2.9** shows the thermogram of some common polymers. The thermal stability of polymers deceases in the order PTFE < LDPE < PMMA < PVC. PVC shows two-stage decomposition.

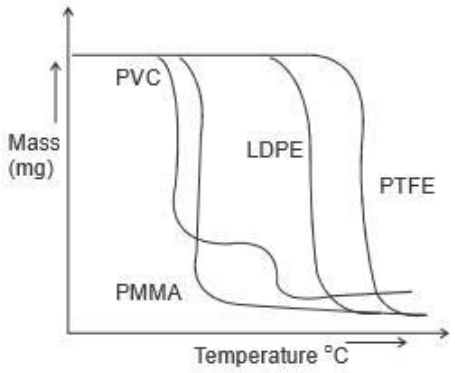


Fig. 4.4. TG curve for the determination of thermal stability of polymers.

3. TGA of pharmaceuticals, coal and minerals is useful in the study of complex thermal reactions.

4. In the determination of composition of alloys and mixtures. E.g. determination of a mixture of calcium and strontium as their carbonates. Both undergo decomposition with evolution of CO_2. But the decomposition of $CaCO_3$ occurs in the temperature range 650-850^oC whereas $SrCO_3$ decomposes in the higher range 950-1150^oC.

5. In qualitative analysis of compounds.

6. In studying the oxidation of alloys.

3.2 DIFFERENTIAL THERMAL ANALYSIS (DTA)

Definition:

Differential thermal analysis is a technique in which the temperature difference (ΔT) between the sample and an inert reference material is measured as a function of sample temperature when both are heated uniformly.

Principle:

When the sample undergoes any transition like melting, dehydration, decomposition etc., there is liberation or absorption of energy by the sample with the corresponding deviation of its temperature from that of the reference. When the sample does not undergo any physical or chemical change, both the sample and the inert reference material are at the same temperature and ΔT is zero. If any endothermic or exothermic reaction occurs in the sample, the temperature of the sample decreases or increases and causes a difference in temperature (ΔT) between the sample and reference. A plot of ΔT vs. T gives the DTA thermogram.

Instrumentation:

The instrument consists of a microprocessor controlled furnace, data processor and recorder and a facility to control the atmosphere. A block diagram of the DTA instrument is shown in **Fig.5.**

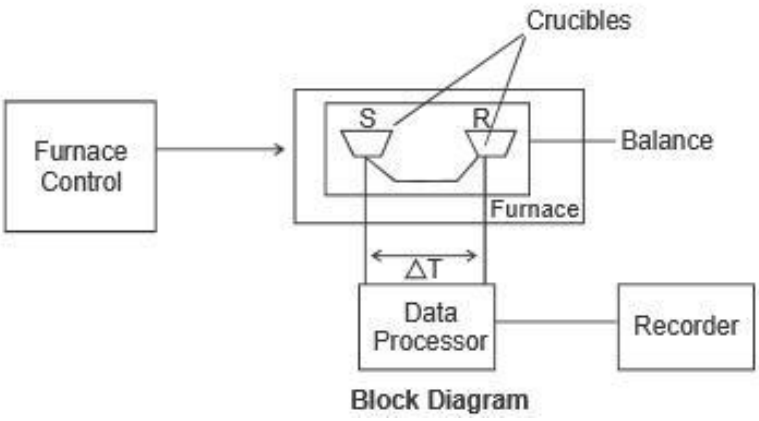


Fig. 4.5. Block diagram of the DTA instrument

Sample holder assembly:

The solid sample and the reference material (usually an inert substance) like alumina of 10 mg is placed in a platinum crucible (sample container) and connected to a sensitive microbalance. The temperature of the sample and reference is measured by an individual thermocouple.

Microprocessor controlled furnace:

The whole sample holder assembly is placed inside the furnace. The sample and the reference are heated at the same heating rate from ambient to 1500^0C. A temperature programmer or furnace control maintains a constant heating rate at 1^0C/min- 100^0C/min.

Facility to control atmosphere:

Sample and reference chamber are designed to permit the circulation of inert gases such as nitrogen or reactive gases such as oxygen or air.

Data processor and recorder:

The difference in temperature (ΔT) between the sample and the reference (S and R) thermocouples is continuously measured. After amplification, the difference in signal is recorded on the y-axis. The temperature of the furnace is measured by an independent thermocouple and recorded on the x axis. The balance and furnace data collected is sent to the PC for manipulation and a DTA plot of (ΔT) vs. T is obtained.

An idealized DTA curve is shown in **Fig.6.** Peak 1 is an exothermic peak and peak 2 is an endothermic peak. Endothermic peaks signify changes in crystallinity or dehydration reactions while exothermic curves results due to chemical reactions such as oxidation.

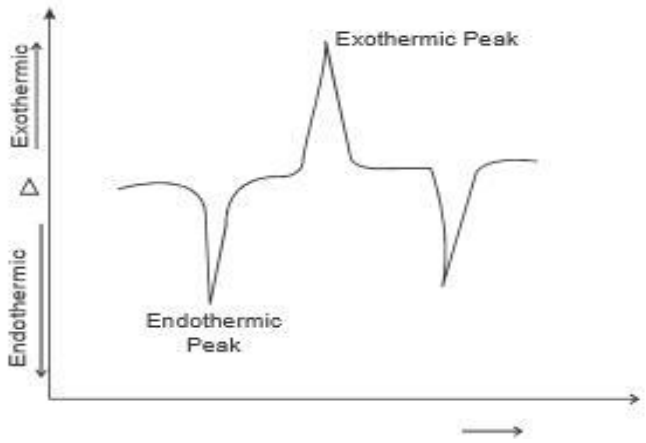


Fig. 4.6. Idealized DTA curve.

Applications of DTA:

Qualitative analysis of materials:

DTA measurements provide a rapid method for the finger printing of minerals, clays and polymeric materials. Eg. **Fig.7.** shows the DTA thermogram of calcium oxalate monohydrate in flowing air (O_2) obtained by increasing the temperature at a rate of 8^0C/min. It contains two endothermic peaks and one exothermic peak. The decomposition and oxidation reactions are shown below.

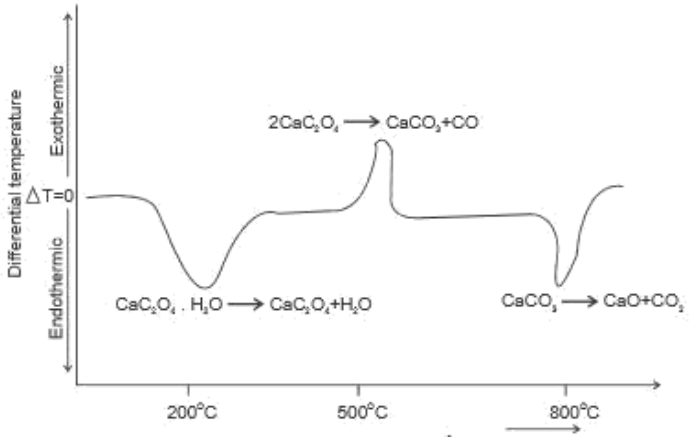


Fig. 4.7. DTA curve of calcium oxalate monohydrate in O_2 atmosphere

DTA provides information regarding processes like fusion, dehydration, oxidation, reduction, adsorption and solid state reactions and in generation of phase diagram and the study of phase transitions.

It provides an accurate way of determining the melting and boiling points for organic compounds.

It has been widely used to study and characterization of polymers and qualitative analysis of polymer mixture. **Fig.8.** is the DTA curve which illustrates the various types of transitions that occurs during heating of a polymer.

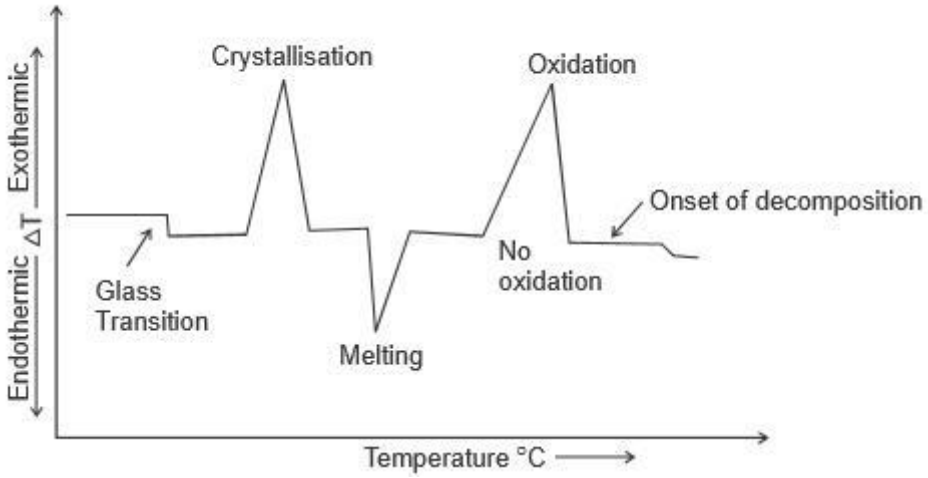


Fig. 4.8. DTA thermogram of polymer

3.3 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Definition:

DSC is a thermal method in which the difference in heat flow into the sample and the reference material is measured as a function of linear increase or decrease of the sample temperature.

Principle:

DSC measures the difference in heating power (heat flow) required to keep the temperature of a sample and a reference material the same**.** The sample and the reference are heated by separate electrical heaters exactly at the same rate. Whenever the sample undergoes an endothermic or exothermic

reaction, there is a change in sample temperature with respect to the reference temperature. The power to the sample heater is modified so that the difference in temperature between the sample and the reference, $\Delta T = 0$. The difference in power supplied to the sample and the reference represents the energy change or enthalpy change (ΔH) in the sample.

Instrumentation:

DSC instrumentation is the same as DTA. DSC is a calorimetric method in which the difference in heat energy (ΔH) is recorded as a function of sample temperature. The block diagram of DSC instrument is shown in **Fig.9.**

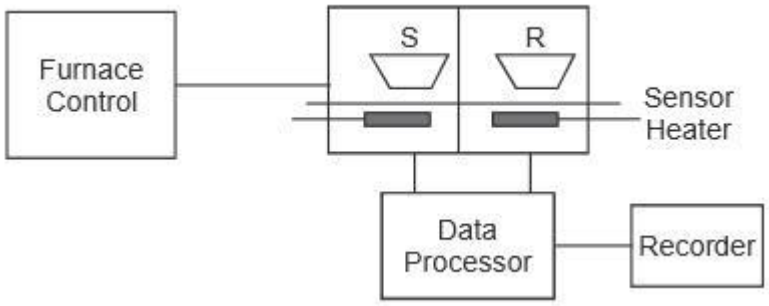


Fig. 4.9. Block diagram of DSC instrument

The main components are a microprocessor controlled furnace provided with individual heater and thermocouples for measuring the temperature of sample and reference, data processor and recorder, and a facility for atmosphere control.

Sample holder assembly:

The solid sample and the reference material (usually an inert substance) like alumina of 10 mg is placed in a separate platinum crucible (sample container) and connected to a sensitive microbalance. The sample and the reference are heated separately and the temperature of the sample and reference is measured by individual thermocouple. The thermocouples measure the difference in heat flow.

Microprocessor controlled furnace:

The whole sample holder assembly is placed inside the furnace. The sample and the reference are heated at the same heating rate from ambient to 700^{o}C. A temperature programmer or furnace control maintains a constant heating rate of 1^{o}C/min -100^{o}C/min.

Facility to control atmosphere:

Sample and reference chamber are designed to permit the circulation of inert gases such as nitrogen or reactive gases such as oxygen or air.

Data processor and recorder:

The difference in heat flow (ΔH) between the sample and the reference (S and R) thermocouples is continuously measured. After amplification, the difference in signal is recorded on the y-axis. The temperature of the furnace is measured by an independent thermocouple and recorded on the x axis. The balance and furnace data collected is sent to the PC for manipulation and the DSC thermogram which is a plot of ΔH vs. T is obtained. A typical DSC thermogram for the decomposition of calcium oxalate monohydrate is shown in **Fig.10.**

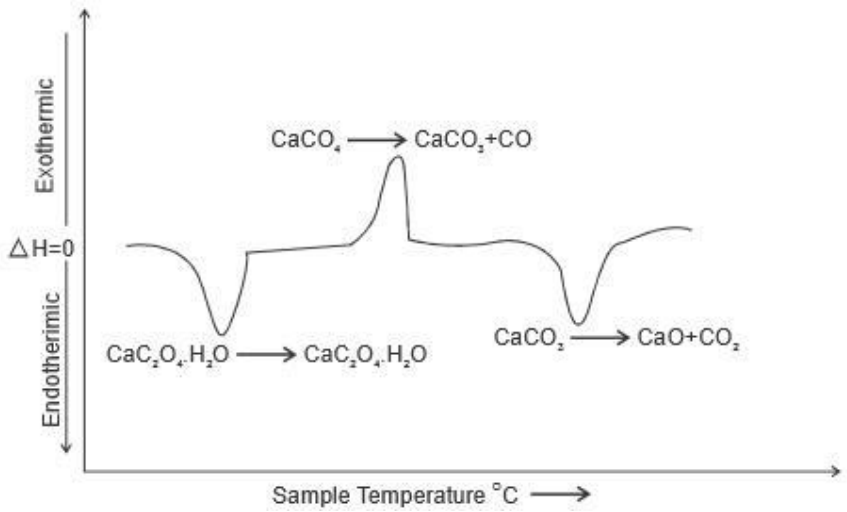


Fig. 4.10. DSC thermogram for the decomposition of calcium oxalate monohydrate

Applications of DSC:

a. DSC is highly useful in the study of phase transitions and temperature induced changes that occur at very low temperatures in the case of polymers, biological samples, pharmaceutical samples, etc.

b. It finds widespread application in calculating the enthalpy of transitions such as enthalpy of melting, enthalpy of crystallization, enthalpy of fusion, etc. of polymeric materials.

c. DSC also provides accurate method of determining melting, boiling and decomposition points for organic compounds.

d. It is used for the determination of purity of drug samples.

3.4 Factors affecting TGA and DTA:

The factors which may affect the TG curves are classified into two main groups.

1) Instrumental Factors:

a) Furnace heating rate:

- The temperature at which the compound or sample decompose depends upon the heating rate.
- When the heating rate is high, the decomposition is also high.
- To observe the various stages of decomposition a slow and constant heating rate should be used.
- A heating rate of 3.5^0C per minute is usually recommended for reliable and reproducible TGA.

b) Furnace atmosphere

- The atmosphere inside the furnace surrounding the sample has a profound effect on the decomposition temperature of the sample.
- One of the following atmospheres can be used inside the furnace as outside.

i) A pure N_2 gas from a cylinder passed through the furnace which provides an inert atmosphere.

ii) Static air is used for maintaining ordinary air inside the furnace as outside.

iii) Dynamic air or compressed air from a cylinder passed through the furnace at a steady rate.

2. Sample Characteristics:

a) Crucible Geometry:

The crucible geometry influences the shape of the thermogravimetric curve. Hence, always use a flat shaped crucible is recommended to obtain reproducible thermogram.

b) Weight of the Sample:

A small weight of the sample is recommended, using a small weight eliminates the existence of temperature gradient through the sample.

c) Particle Size of the sample:

The particle size of the sample should be small and uniform. The use of large particle or crystal may result in apparent, very rapid weight loss during heating.

d) Heat of Reaction:

- This will alter the difference between the sample temperature and furnace temperature and thus will affect the thermogram.
- An exothermic change will cause the sample temperature to lead the furnace temperature.
- An endothermic change will make the sample temperature lag behind the furnace temperature.
- A few simple experiments for measuring the furnace and the sample temperatures will provide the necessary information on the effect of heat of reaction on the thermogram.
- Condensation of volatile products on the cooler sides of the container will introduce serious error in the TGA data and therefore this should be checked and eliminated.

3.5 Thermometric Titrations:

Definition:

It is defined as titration in which end point is determined by measuring the heat generated during chemical reaction.

Principle:

- This involves the measurement of the change of temperature of a solution during titration as a function of the volume of the titrant added.
- The change in temperature is due to heat changes during the reaction.
- The end point in a thermometric titration is indicated by a sharp break in the temperature versus volume curve.

Process:

- Thermometric titrations are carried out in adiabatic conditions to minimize the heat-exchange between the system and the surroundings.
- This can be done in a small Dewar flask.
- The titrant is added from a thermostated automatic burette at a constant rate into the thermally

insulated vessel containing the solution to be estimated.

- The temperature is noted after each addition of titrant.
- The temperature of the system is read accurately with a thermistor-thermometer.

Conditions for thermometric titrations:

- The reaction should be accompanied by sufficient heat change that can be measured.
- The heat of dilution of the product should be negligible.
- The reaction should be a simple, one-step change.
- Transfer of heat between the titration vessel and its surroundings should be avoided.

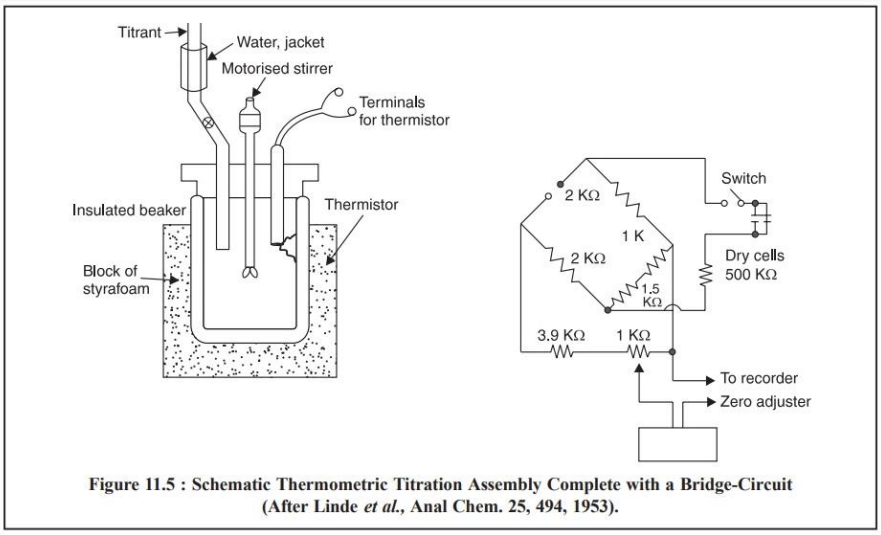


Figure 11.5 : Schematic Thermometric Titration Assembly Complete with a Bridge-Circuit (After Linde *et al.*, Anal Chem. 25, 494, 1953).

Fig. 4.11. Dewar Flask for Thermometric Titration

The **Fig.11.** is the schematic representation of thermometric titration unit. It consists of,

- Motor-driven thermostated burette
- Thermally insulated adiabatic titration vessel (Dewar flask)
- Thermistor bridge set
- Recorder.

Process:

- A known volume of the solution to be estimated is taken in the Dewar flask.
- The tip of the burette is dipped into the solution in the vessel.
- The vessel is covered by a thick, insulating material containing holes for inserting the thermistor, the burette and the stirrer.
- The titrant is added at a constant rate while the solution in the Dewar flask is constantly stirred.
- The thermistor assembly is connected to one arm of a wheatstone bridge and its resistance is recorded directly on a recorder.
- The temperature measuring thermistor unit should be capable of measuring temperature change of 0.01°C.
- The titrant should be 10-100 times more concentrated than the solution in the Dewar flask, so that the titration can be done by the addition of only small quantity of the titrant.

Reference:

R. Gopalan, P.S. Subramanian, K. Rengarajan, Elements of Analytical Chemistry, Third Edition 2003.

SCY1611 – Analytical Chemistry

Unit - V

UNIT - V

Polarography

Principle, concentration polarization, dropping mercury electrode, advantages and disadvantages, convection, migration and diffusion currents, ilkovic equation (derivation not required) and significance, experimental assembly, electrodes, capillary solutions, current voltage curve, oxygen wave, influence of temperature and agitation

5. Introduction:

Polarography was created by Jaroslav Heyrovsky in Feb. 10th 1922. On December 10th 1959 he was awarded the Nobel Prize. The basic idea was to pass the current between two electrodes, one having large surface area and other having very small surface area. Both electrodes can be of mercury metal. • The large electrode can be a pool of mercury at the bottom of the cell. • Small electrode is a drop of mercury coming out of a very fine capillary tube, DME. • Thus, if a steady voltage is applied to such a cell, it is possible to construct a reproducible current voltage curve.

5.1 Polarography:

It is an electrochemical technique of analysing solutions that measures the current flowing between two electrodes in the solution as well as the gradually increasing applied voltage to determine respectively the concentration of a solute and its nature. Hence,

"Polarography is a method of analysis in which the solution to be analyzed is electrolyzed under diffusion controlled condition."

Principle:

The principle in polarography is that a gradually increasing negative potential (voltage) is applied between a polarisable and non-polarisable electrode and the corresponding current is recorded.

Polarisable electrode: Dropping mercury electrode
Non-polarisable electrode: Saturated Calomel electrode

From the current-voltage curve, qualitative and quantitative analysis can be performed. This technique is called polarography, the instrument used is called as polarography and the current-voltage curve recorded is called as polarogram. The point of inflection is called in the current-voltage curve is known as Half-wave potential and is characteristic for every element or functional group (Qualitative aspect). The diffusion current measured is proportional to the concentration of that particular compound (Quantitative aspect). Thus, polarography can be used for both qualitative and quantitative analysis of compounds.

Polarographic measurements are governed by ilkovic equation. Ilkovic equation suggests the linear relationship between the diffusion current(id) and the concentration of electroactive species is shown by the Ilkovic equation.

$id = 607\ n\ C\ D^{1/2}\ m^{2/3}\ t^{1/6}$

id = diffusion current in microamperes,
n = number of electrons required per molecule of electroactive substance
D = diffusion coefficient, in square cm per second
C = concentration in millimoles/L
m = mass of mercury flow from the DME, in mg per second
t = drop time, in seconds.

Working:

Electrolyte is a dilute solution of electro active material to be analyzed in a suitable medium containing excess of supporting electrolyte. Consider a polarographic cell, containing a solution of cadmium chloride, to which an external E.M.F is applied. The positively charged ions present in the solution will be attracted towards the mercury drop of the dropping mercury electrode (DME). The total current flowing through the cell may be regarded as being the "sum of the electrical and diffusive forces." When the applied voltage is increased and the current is recorded a graph will obtained.

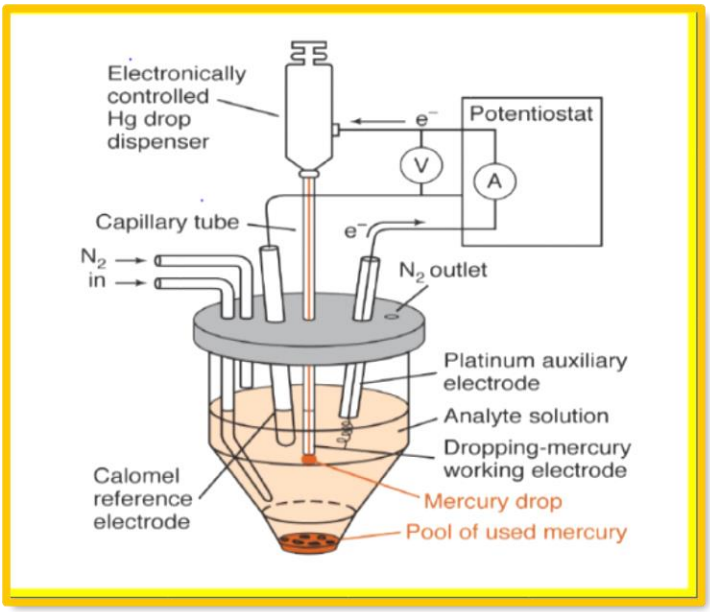


Fig. 5.1. Instrumentation of Polarography

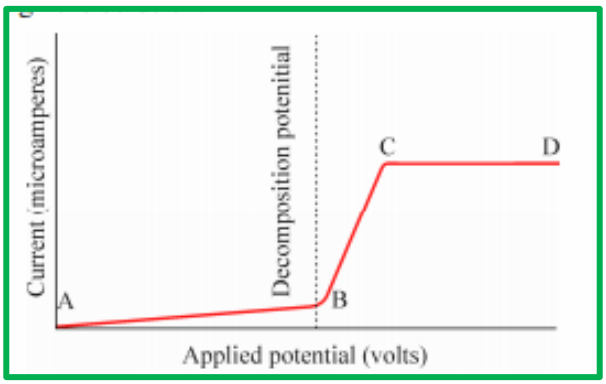


Fig. 5.2. Current voltage curve

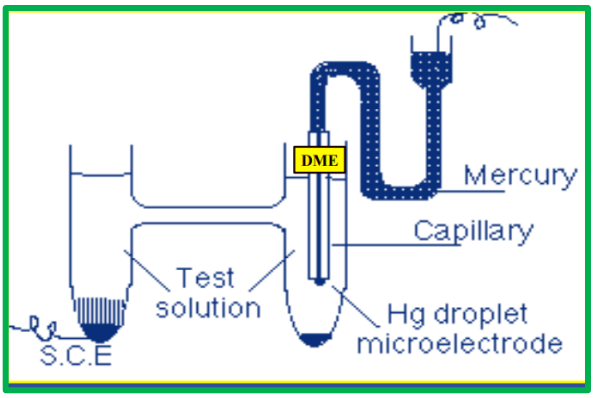


Fig. 5.3. Dropping mercury electrode

ADVANTAGES OF DME:

The dropping mercury electrode has several advantages:

i) Its surface area is reproducible with a given capillary
ii) The constant renewal of the electrode surface eliminates passivity or poisoning effects.
iii) The large hydrogen overpotential on mercury renders possible the deposition of substances difficult to reduce such as aluminium ion and manganese (II) ion (The current-potential curves of these ions are not accessible with platinum micro electrode).
iv) Mercury forms amalgams with many metals.
v) The diffusion current assumes a steady value immediately and is reproducible.
vi) The surface area of the electrode can be calculated from the weight of the drops.

The dropping mercury electrode therefore is useful over the range + 0.3 to − 2.0 V vs. SCE. Above + 0.4 V mercury dissolves and gives an anodic wave due to oxidation to mercury (I) ion. At potentials more negative than − 1.8 V vs SCE visible hydrogen evolution occurs in acid solutions and at − 2.0 V the supporting electrolytes of alkali salts begin to discharge.

DISADVANTAGES OF DME:

- The area of the microelectrode is constantly changing as the size of the drop changes.
- Mercury may be easily oxidized and thus limits the feasible range of electrode.
- The capillary may be easily plugged and thus care must be taken to avoid touching the tip of the capillary with any foreign material.

5.1.1 Types of Currents in polarography:

The Limiting Current:

The limiting current obtained with the dropping mercury electrode is caused by the extreme state of concentration polarization which results from the depletion of the concentration of the electro-reducible or electro-oxidizable substance at the electrode surface by the electrode reaction. The reducible or oxidizable ions are supplied to the depleted region at the electrode surface by two forces:

1. A diffusive force, proportional to the concentration gradient at the electrode surface.
2. An electrical force, proportional to the electrical potential difference between the surface and the solution. So the limiting current is regarded as the sum of 'diffusion current' and a 'migration current'.

The Migration Current:

The migration current is due to the migration of reducible or oxidizable ions which is proportional to the electrical potential gradient at the electrode due to attraction. Unless this potential gradient is removed the limiting current cannot be proportional to the concentration of electroactive ions in the solution. This migration current is usually eliminated by adding 50 or 100 fold excess of an inert supporting electrolyte. Commonly used supporting electrolytes are potassium or sodium salts. The potassium ions cannot be discharged at cathode until the impressed voltage becomes large. The large number of potassium ions therefore remains as a crowd around cathode and restrict the potential gradient to a region so very close to the electrode surface. So there is no longer an electrostatic attraction operative to attract other reducible ions from the bulk of the solution. Under these circumstances the limiting current is solely controlled by the diffusion of the electroactive species through the concentration gradient adjacent to the electrode.

Diffusion Current:

In the presence of excess supporting electrolyte the electrical force on the reducible/oxidizable ions is nullified and therefore, the limiting current is solely diffusion current. Ilkovic (1934) examined the various factors which govern the diffusion current and deduced a theoretical equation as,

$id = 607\ n\ C\ D^{1/2}\ m^{2/3}\ t^{1/6}$

id = diffusion current in microamperes,

n = number of electrons required per molecule of electroactive substance

D = diffusion coefficient, in square cm per second

C = concentration in millimoles/L

m = mass of mercury flow from the DME, in mg per second

t = drop time, in seconds.

The constant 607 is a combination of natural constants, including the Faraday. This is an important equation because it accounts for the linear dependence of id upon c keeping all other factors constant. It is useful in quantitative polarographic analysis.

Residual Current:

Even in the absence of electroactive substance in the solution having only supporting electrolytes a small current is always present which is referred as residual current which consists of two components.

The first is reduction of trace impurities (faradaic current) present in the large concentration of supporting electrolyte used. These include dissolved oxygen, traces of metal ions such as ferric iron, lead and arsenic. Out of these, dissolved oxygen can be removed by passing pure nitrogen for 10 to 15 minutes before taking C-V curve.

The second source is charging current or capacitive current which is non-faradaic and is present whenever the working electrode potential changes. Mercury is unique in remaining electrically uncharged when it is dropping freely into the supporting electrolyte. The small current that is observed from supporting electrolyte solution is because of continuous charging of new mercury drop to the applied potential in order to remain neutral.

The mercury is covered with an electrical double layer of positively and negatively charged ions of the electrolyte. The capacity of the double layer and hence the charging current vary depending on the potential which is imposed upon mercury.

5.1.2 FACTORS AFFECTING LIMITING CURRENT:

- Kinetic current
- Residual current
- Diffusion current
- Migration current

RESIDUAL CURRENT:

RESIDUAL CURRENT = Faradic Current + Condenser current.

MIGRATION CURRENT:

The electro active material reaches the surface of electrode by two processes. The first involves the migration of charge particles in the electrical fields caused by the potential difference existing between the electrode surface and the solution. The second involves the diffusion of particles.

DIFFUSION CURRENT:

Diffusion current is directly proportional to the concentration of the electro active material.

KINETIC CURRENT:

The limited current may be affected by the rate of non-electrode reaction called kinetic current.

APPLICATIONS OF POLAROGRAPHY:

- **INORGANIC COMPOUNDS:** Polarography can be used for estimation of cation and anions.
- **DETERMINATION OF TRACE ELEMENTS:** Polarography can be used for determination of trace elements e.g CO, Al, Cu, Ni, etc.
- **APPLICATION TO ORGANIC COMPOUND:** For quantitative identification of compounds and for quantitative analysis of mixtures.
- **DETERMINATION OF DISSOLVED OXYGEN:** The determination of dissolved oxygen in aqueous solution or organic solvents can be carried out successfully with the help of polarography.
- **DETERMINATION OF PLANT CONTENTS:** A polarographic analysis of content of essential oils.
- **STUDIES OF COMPLEXES:** Polarography is powerful tool for study of composition of complexes if the sample metal ion and complex of that metal ion in the same oxidation state.
- **APPLICATION TO PHARMACEUTICALS:** Oxidation process of medicines like epinephrine and nor-epinephrine.The use of A.C. polarography has proved advantageous in the analysis of tetracycline.
- **ANALYSIS OF BIOLOGICAL SYSTEMS:** The possibility of being able to determine vitamins, alkaloids, hormones, terpenoid substances, and natural colouring substances has made polarography useful in analysis of biological systems

5.2 Polarimetry:

The polarimetric method is a simple and accurate means for determination and investigation of structure in macro, semi-micro and micro analysis of expensive and non-duplicable samples. Polarimetry is employed in quality control, process control and research in the pharmaceutical, chemical, essential oil, flavor and food industries.

Definition:

- Polarimetry is an instrumental analytical method using rotation of polarized light by some substances as a measure of their concentration in a solution.
- The instrument used is called a polarimeter.
- When it is adapted for measuring quality of sugar the name saccharimeter is used.
- In both instruments it is the rotation of polarized light by a substance in a solution which is measured.

Polarimeter:

- Fig. 5.2.1 shows a principle of a polarimeter set up and its main components together with their function. Unpolarized light from the light source is first polarized.
- This polarized light passes through a sample cell. If an optical active substance is in a sample tube, the plane of the polarized light waves is rotated. The rotation is noticed by looking through the analyser as a change in intensity of illumination.
- To reach the same illumination as was without an optical active sample the analyser must be turned around for an angle. Readings are taken in degrees (angle) α or sugar degrees ^{0}Z.

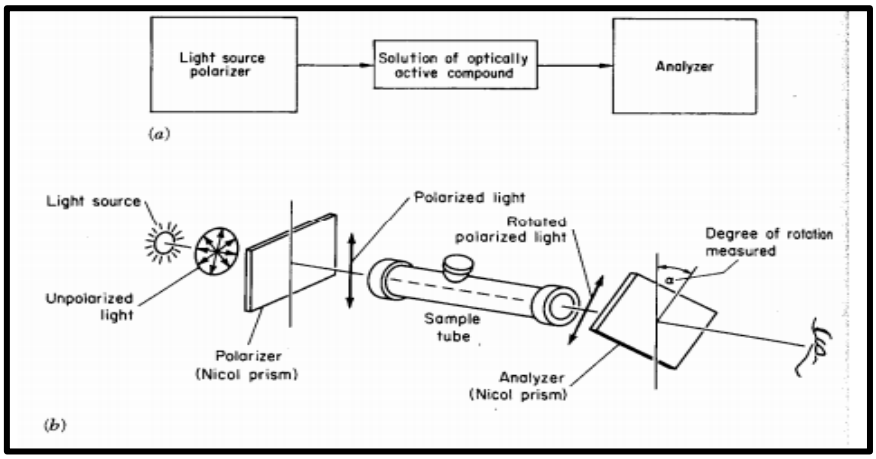


Fig. 5.4. Schematic diagram of Polarimeter

- Figure 5.2.2 shows polarizer (P) and analyzer (A) in a perpendicular position with one another. Both have the structure on a molecular level that polarizes an unpolarized light.
- If they are in a position shown no light passes through analyzer. Polaroid films for example have such a molecular structure that filters (block) all planes of light vibrations except one.
- Nicol prisms are made of calcite ($CaCO_3$) crystals, which show double refraction phenomenon. Both beams are polarized but only one is enough to be used for polarizing unpolarized light.

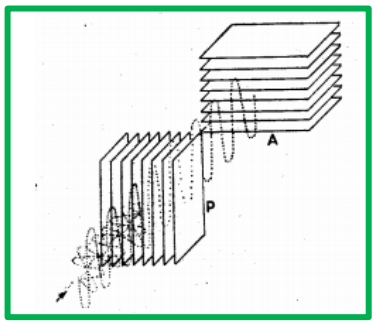


Fig. 5.5. Diagram for polariser and analyser

- Figure 5.2.3 shows the passage of polarized light through a sample tube, which is positioned between polarizer and analyzer. The length of the tube is one of important parameters to be fixed if the measurements were comparable.

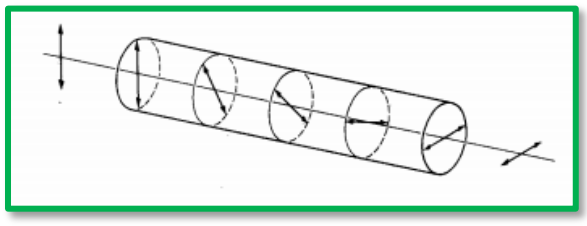


Fig. 5.6. Plane polarisation of light

<u>Optical Rotation:</u>

Certain compounds, mostly organic (notably those containing asymmetric carbon atoms) rotate the plane of polarized light. The phenomenon is called optical rotation and such substances optically

active compounds. Measuring angle of rotation the concentration of a substance in a solution is determined. The measured angle of rotation depends upon many variables:

- The type or nature of sample (example: sugar solution)
- Concentration of the optical active components
- The length of the sample tube
- The wavelength of the light source
- Temperature of the sample

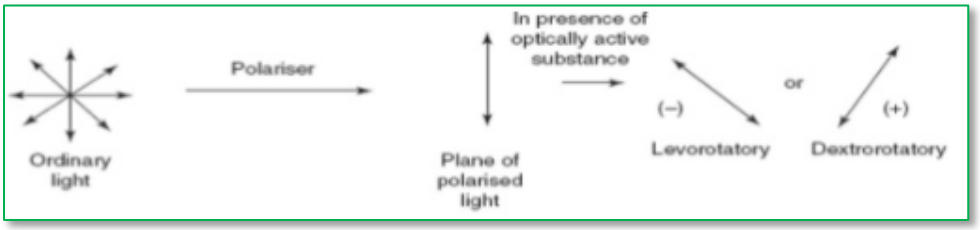


Fig. 5.7. Block diagram of polarimeter

Types of molecules to be analyzed:

- Molecules must be optically active
- Optically active molecules containing asymmetric carbon atom.
-

Instrumentation:

- Optical activity may be detected and measured by an instrument called Polarimeter.
- A polarimeter is used to measure the angle of rotation of an optically active compound.
- There are four principal components in a polarimeter.

1. **The light Source:** This consists of incandescent gaseous sodium metal, which produces monochromatic light of wavelength 589 nm (the sodium D line).
2. **Nicol Prism:** This consists of two optically pure calcite crystal prisms joined together. This is known as the polariser.
3. **A Glass Tube:** In this tube, the sample is taken either as a neat liquid or in the form of a solution.
4. **A Second Nicol Prism:** This is rotatable and is attached with a vernier scale which measures the angle of rotation in degrees. This prism is known as analyser.

The degree of optical rotation depends upon the cell length 'l' and concentration of solution c in grams per millilitre of solution. The specific rotation at 20^0C, using sodium light, is then given by,

$$[\alpha]_D^{20} = \frac{\alpha}{lc}$$

l = length in decimetre

c= concentration in g/ml

α = experimentally observed rotation in degrees.

Problem:

The observed rotation is $+9.3^0$ for a solution which has a concentration of 15 grams in 100 ml solution, in a 20.0 cm long tube. Calculate the specific rotation.

l = 20 cm = 2 dm, c = 15/100 = 0.15 g/ml.

$\therefore [\alpha]_D^{20}$ = 9.3/ (2 x 0.15) = $+31^0$

The molecular rotation is given by,

$[\varphi] = [\alpha]_D^{20}$ M / 100, where [α] is specific rotation and M is the molecular weight of the optically active compound.

Applications:

Polarimetry determines product purity by measuring specific rotation and optical rotation of,

- Amino Acids
- Antibiotics
- Dextrose
- Steroids
- Amino Sugars etc.

5.3 Amperometric titration:

It refers to the measurement of current under a constant applied voltage and under these conditions it is the concentration of analyte which determine the magnitude of current.

In this titration, the potential applied between the indicator electrode (dropping mercury electrode) and the depolarizing reference electrode (SCE) is kept constant and current through the electrolytic cell is then measured on the addition of each increment of titrating solution. It is otherwise called as polarographic or polarimetric titrations.

Principles:

The electrochemical oxidation or reduction of an electro-active species by the application of an appropriate potential of a suitable electrode results in either a steady-state anodic or cathodic current.

However, it is important to note that the current measured amperometrically is distinctly different from measured in the so-called "galvanic cell," which does not require the application of a potential. In amperometry, the current generated from a faradaic reaction is directly proportional to the concentration of the analyte.

According to Ilkovic equation,

$id = 607\ n\ C\ D^{1/2}\ m^{2/3}\ t^{1/6}$
where, id = diffusion current in microamperes,
n = number of electrons required per molecule of electroactive substance
D = diffusion coefficient, in square cm per second
C = concentration in millimoles/L
m = mass of mercury flow from the DME, in mg per second
t = drop time, in seconds.

The diffusion current at an appropriate applied voltage is measured as a function of the volume of the titrating solution. The end point is the intersection of two lines giving the change of current before and after the equivalence point.

Conditions:

- Either the titrate or titrant or both should be electroreducible.
- The potential applied should correspond to the limiting current.

Instrumentation:

Amperometric titration can be carried out using rotating platinum electrode. It consists of a glass tube of length 15 – 20 cm in length and 6 mm in diameter. The platinum wire extends 5-10 mm from the wall of glass tubing. The electrode is mounted on shaft of the motor and rotated at constant speed of 600 RPM. Electrical connection is made to the electrode by copper wire passing through the tubing to the mercury covering the platinum wire seal.

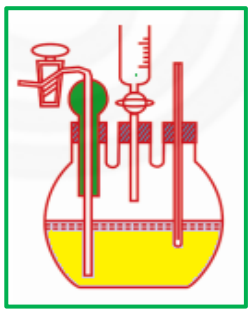


Fig. 5.8. Amperometric titration cell

The general procedure is as follows:

A known volume of the solution for estimation is placed in the titration cell, required amount of supporting electrolyte is added and the cell is connected to the terminals of the instrument. Dissolved oxygen is expelled by passing pure nitrogen gas for 10−15 minutes and initial current is noted. The known volume of the reagent is added from a microburette, N_2 is passed for 2 minutes and then stopped. The current and burette readings are both noted. This procedure is repeated until sufficient readings are obtained and the graph obtained gives end point at the intersection.

Advantages:

1) Diffusion current is 20 times larger than DME which allows measuring the small concentration of ion.
2) The rotating platinum electrode can be used at positive potential up to + 0.9 volt where as DME can be used only +0.4 volt to -2.0 volt.
3) The electrode is simple to construct.
4) Steady diffusion state is reached quickly.
5) Micro concentration of material can be determined because of the larger currents attend at rotating electrodes.

Titration Curves in Amperometry:

A) Titrand is reducible but titrant and product not:

When a solution containing Pb^{+2} ion is titrated against $SO4^{-2}$ ion, a precipitate of $PbSO_4$ is formed. The titration can be performed at fixed potential -0.8 Volt v/s saturated calomel electrode. As titration is proceeds concentration of Pb^{+2} ion decreases and diffusion current also decreases till it becomes minimum at equivalence point. The diffusion current remains constant beyond end point. The values of diffusion current is plotted against the volume of titrant added .The resulting

titration curves is straight line levelling off at end point . The intersection of two extra plotted portions of the curves gives the end point.

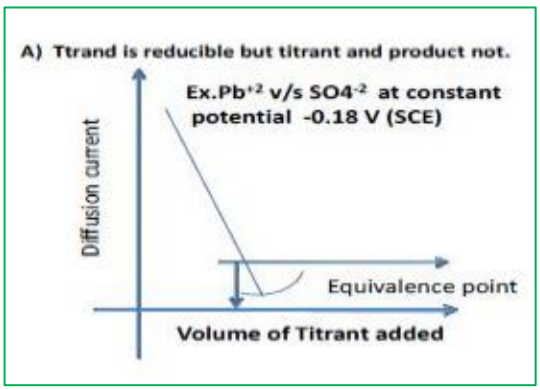


Fig. 5.9. Electroreducible Vs non-electroreducible

B) <u>Titrant is reducible but titrand and product not:</u>

When a solution containing Mg^{+2} ion is titrated against with the reducible species such as 8- hydroxy quinoline because Mg^{+2} ion does not undergoes reduction. Beyond the end point the 8-hydroxyl 4-quinoline undergoes reduction. As its concentration increases diffusion current also increases.

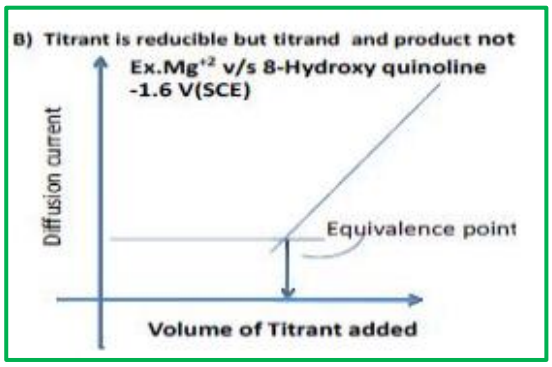


Fig. 5.10. non-electroreducible Vs electroreducible

C) <u>Titrand and titrant both are reducible but product not :</u>

When a solution containing Pb^{+2} ion is titrated against $K_2Cr_2O_7$. The titration is performed at potential of -0.8 Volt v/s SCE. Diffusion current is decreases due to removal of Pb^{+2} ion. The current is minimum at the end point. On further addition of the titrant the current once again increases. V shaped curve is obtained.

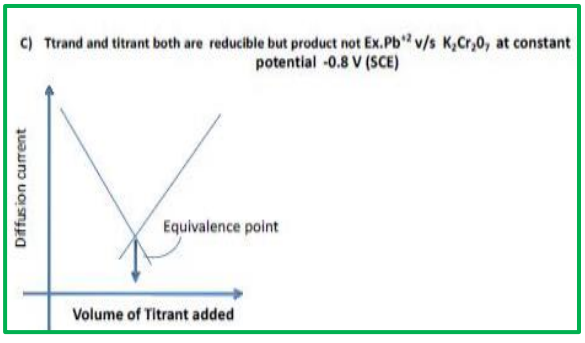


Fig. 5.11. Electroreducible Vs electroreducible

Advantages of Amperometry:

1. The titration can usually be carried out rapidly, since the end point is found graphically; a few current measurements at constant applied voltage before and after the end point are sufficient.

2. Titrations can be carried out in cases in which the solubility relations are such that potentiometric or visual indicator methods are unsatisfactory. For example, when the reaction product is markedly soluble (precipitation titration) or appreciably hydrolysed (acid-base titration). This is because the readings near the equivalence point have no special significance in amperometric titrations. Readings are recorded in regions where there is excess of titrant, or of reagent, at which points the solubility or hydrolysis is suppressed by the Mass Action effect; the point of intersection of these lines gives the equivalence point.

3. A number of amperometric titrations can be carried out at dilutions (ca 10−4 M) at which many visual or potentiometric titrations no longer yield accurate results.

4. 'Foreign' salts may frequently be present without interference and are, indeed, usually added as the supporting electrolyte in order to eliminate the migration current which is not possible in case of conductometric titration.

5. The results of the titration are independent of the characteristics of the capillary.

6. The temperature need not be known provided it is kept constant during the titration.

7. Although a polarograph is convenient as a means of applying the voltage to the cell, its use is not essential in amperometric titrations. The constant applied voltage may be obtained with a simple potentiometric device.

Applications:

1. Widely applicable than Potentiometry and Polarography. This method is widely used for the determination of sulphate which could not be determined accurately by Potentiometric method due to lack of suitable indicator.

2. Successive determination of chloride, bromine, Iodide by using rotating microelectrode.

3. They are used as micro detectors in liquid chromatography.

4. In bioamperometric titration this technique is widely used in karl-fisher moisture titration.

5. Other applications are:
a) Phosphate with uranyl acetate
b) Lead with dichromate ions
c) Sulphate with lead nitrate
d) Iodine with mercuric nitrate

Reference:

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