D.N.R.COLLEGE (AUTONOMOUS): BHIMAVARM
DEPARTMENT OF PG CHEMISTRY

SEPARATION METHODS –II

IV SEMESTER

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UNIT-I

Paper Chromatography

• Paper chromatography definition explains that is an inexpensive and powerful analytical technique, which requires a piece of paper or strips serving as an adsorbent in the stationary phase across which a particular solution is allowed to pass. Or

• Paper chromatography (PC) is a type of planar chromatography whereby chromatography procedures are run on a specialized paper.

• PC is considered to be the simplest and most widely used of the chromatographic techniques because of its applicability to isolation, identification, and quantitative determination of organic and inorganic compounds.

• It was first introduced by German scientist Christian Friedrich Schonbein (1865).

• This analytical tool employs very few quantities of material.

Principle of Paper Chromatography

• Paper chromatography is a form of liquid chromatography where the basic principle involved can be either: – partition chromatography – adsorption chromatography

• Paper Partition chromatography: In paper chromatography separation of component is distributed between phases of liquid.

• Here, one phase of liquid is water that is held amidst the pores of filter paper and the other liquid is the mobile phase that travels along with the filter paper.

• Separation of the mixture is the result that is obtained from the differences in the affinities towards the water and mobile phase when travelling under capillary action between the pores of the filter paper.

• Paper Adsorption chromatography: Paper impregnated with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase

Instrumentation of Paper chromatography

• Stationary phase & papers used • Mobile phase

• Developing Chamber

• Detecting or Visualizing agents

1. STATIONARY PHASE AND PAPERS

• Whatman filter papers of different grades like No.1, No.2, No.3, No.4, No.20, No.40, No.42 etc • In general the paper contains 98-99% of α-cellulose, 0.3 – 1% β -cellulose. Other modified papers

• Acid or base washed filter paper

• Glass fiber type paper.

• Hydrophilic Papers – Papers modified with methanol, formamide, glycol, glycerol etc.

• Hydrophobic papers – acetylation of OH groups leads to hydrophobic nature, hence can be used for reverse phase chromatography.

• Impregnation of silica, alumna, or ion exchange resins can also be made

Instrumentation of Paper chromatography

2. PAPER CHROMATOGRAPHY MOBILE PHASE

• Pure solvents, buffer solutions or mixture of solvents can be used.

3. CHROMATOGRAPHIC CHAMBER

• The chromatographic chambers are made up of many materials like glass, plastic or stainless steel. • Glass tanks are preferred most.

• They are available invarious dimensional size depending upon paper length and development type. • The chamber atmosphere should be saturated with solvent vapour

Steps in Paper Chromatography

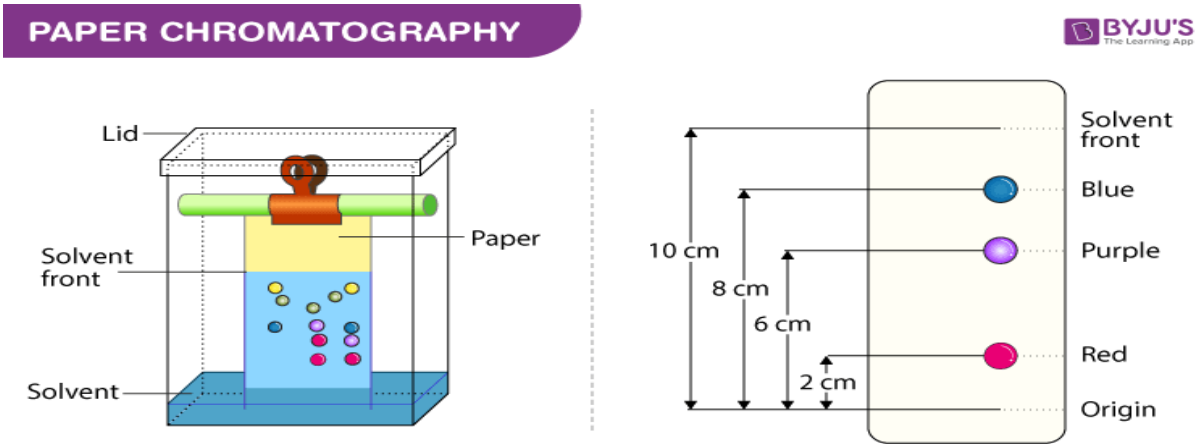
• In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. The basic steps include: Selection of Solid Support

• Fine quality cellulose paper with defined porosity, high resolution, negligible diffusion of the sample, and favoring good rate of movement of solvent. Selection of Mobile Phase
• Different combinations of organic and inorganic solvents may be used depending on the analyte.
• Example. Butanol: Acetic acid: Water (12:3:5) is a suitable solvent for separating amino acids. Saturation of Tank
• The inner wall of the tank is wrapped with filter paper before the solvent is placed in the tank to achieve better resolution. Sample Preparation and Loading
• If the solid sample is used, it is dissolved in a suitable solvent. Sample (2-20ul) is added on the baseline as a spot using a micropipette and air dried to prevent the diffusion.
Development of the Chromatogram
• Sample loaded filter paper is dipped carefully into the solvent not more than a height of 1 cm and waited until the solvent front reaches near the edge of the paper.

Different types of development techniques can be used:

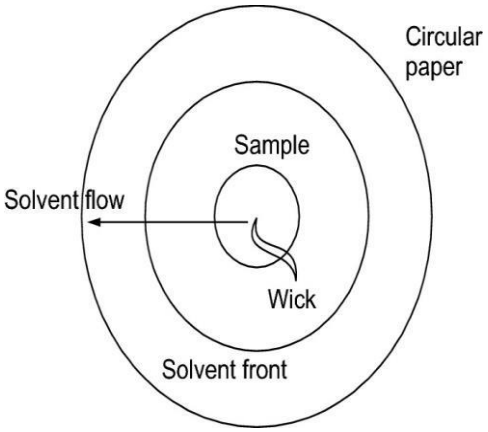
ASCENDING DEVELOPMENT

• Like conventional type, the solvent flows against gravity.
• The spots are kept at the bottom portion of paper and kept in a chamber with mobile phase solvent at the bottom. DESCENDING TYPE
• This is carried out in a special chamber where the solvent holder is at the top.
• The spot is kept at the top and the solvent flows down the paper.
• In this method solvent moves from top to bottom so it is called descending chromatography. **ASCENDING – DESCENDING DEVELOPMENT**
• A hybrid of above two techniques is called ascending-descending chromatography.
• Only length of separation increased, first ascending takes place followed by descending.



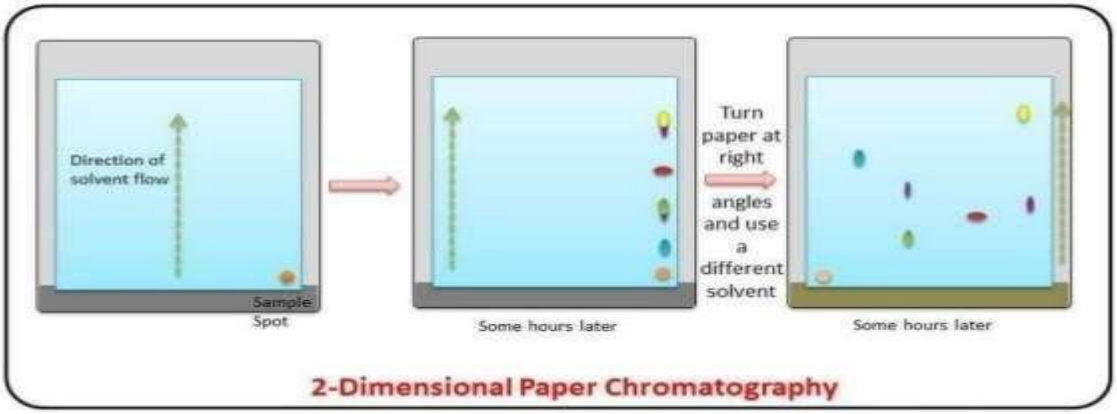
CIRCULAR / RADIAL DEVELOPMENT

• Spot is kept at the centre of a circular paper.
• The solvent flows through a wick at the centre & spreads in all directions uniformly.



TWO-DIMENTIONAL CHROMATOGRAPHY

• This helps in resolving substances that have similar Rf values.



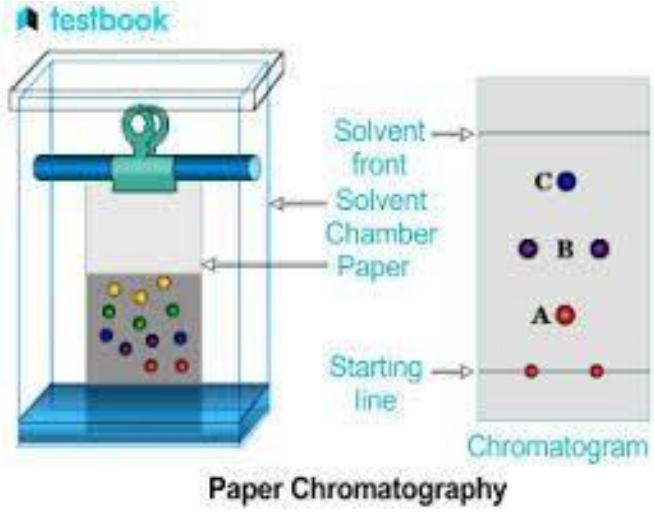
Drying of Chromatogram

• After the development, the solvent front is marked and left to dry in a dry cabinet or oven.

Detection

• Colorless analytes were detected by staining with reagents such as iodine vapor, ninhydrin, etc.

• Radiolabeled and fluorescently labeled analytes were detected by measuring radioactivity and fluorescence respectively



Rf values

• Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the baseline.

• The distance traveled relative to the solvent is a constant for a particular compound as long as other parameters such as the type of paper and the exact composition of the solvent are constant.

• The distance traveled relative to the solvent is called the Rf value.

• Thus, in order to obtain a measure of the extent of movement of a component in a paper chromatography experiment, "Rf value" is calculated for each separated component in the developed chromatogram.

Applications of Paper Chromatography

• To check the control of purity of pharmaceuticals,

• For detection of adulterants,

• Detect the contaminants in foods and drinks,

• In the study of ripening and fermentation,

• For the detection of drugs and dopes in animals & humans

• In analysis of cosmetics

• Analysis of the reaction mixtures in biochemical labs

Advantages of Paper Chromatography

• Simple

• Rapid

• Paper Chromatography requires very less quantitative material.

• Paper Chromatography is cheaper compared to other chromatography methods.

• Both unknown inorganic as well as organic compounds can be identified by paper chromatography method.

• Paper chromatography does not occupy much space compared to other analytical methods or equipments.

• Excellent resolving power

Limitations of Paper Chromatography

• Large quantity of sample cannot be applied on paper chromatography.

• In quantitative analysis paper chromatography is not effective.

• Complex mixture cannot be separated by paper chromatography.

• Less Accurate compared to HPLC or HPTLC

Principle of TLC

- It is based on the principle of adsorption chromatography or partition chromatography or combination of both, depending on adsorbent, its treatment and nature of solvents employed
- The components with more affinity towards stationary phase travels slower.
- Components with less affinity towards stationary phase travels faster

- In TLC, a solid phase, the adsorbent, is coated onto a solid support (thin sheet of glass, plastic, and aluminum) as a thin layer (about 0.25 mm thick). In many cases, a small amount of a binder such as plaster of Paris is mixed with the absorbent to facilitate the coating.
- The mixture (A + B) to be separated is dissolved in a solvent and the resulting solution is spotted onto the thin layer plate near the bottom. A solvent, or mixture of

solvents, called the eluatant, is allowed to flow up the plate by capillary action. At all times, the solid will adsorb a certain fraction of each component of the mixture and the remainder will be in solution. Any one molecule will spend part of the time sitting still on the adsorbent with the remainder moving up the plate with the solvent. A substance that is strongly adsorbed (say, A) will have a greater fraction of its molecules adsorbed at any one time, and thus any one molecule of A will spend more time sitting still and less time moving and vice versa.

- Separation of mixtures in microgram quantities by movement of a solvent across a flat surface; components migrate at different rates due to differences in solubility, adsorption, size or charge; elution is halted when or before the solvent front reaches the opposite side of the surface and the components examined in situ or removed for further analysis.
- Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase

1. The stationary phase:
is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.
2. The mobile phase:
is a developing liquid which travels up the stationary phase, carrying
the samples with it.
Components of the samples will separate on the stationary phase

Selection of adsorbents

- Solubility of compound e.g, hydrophilic or lipophilic
- Nature of substance to be seperated i.e whether it is acidic, basic or amphoteric
- Adsorbent particle size
- Adsorbent should not adhere to glass plate
- Reactivity of compound with the solvent or adsorbent
- Chemical reactivity of compounds with binders

Chromatographic media-coating material Sorbents

That in experiments performed to solve various problems by the adsorption method the use of various sorbents would be necessary. They tested various substances, including aluminum oxides, aluminum silicates, calcium carbonate, kaolin, kieselguhr, magnesium oxide, powdered sugar, silica gels, starch and talc

The separation efficiency obtained in TLC is essentially determined by the mean particle size and the size distribution of the sorption agent used in the preparation of the layer. As can be seen from Fig. Below, the mean particle size of silica gel of a quality suitable for HPTLC is 5 m, that of TLC quality ca. 11 m and that of PSC quality over 20 m.

PREPARATION OF CHROMATOPLATES

Glass plates or flexible plates are commonly used for adsorbent. Size used depends on type of separation to be carried out, the type of chromatographic tank and spreading apparatus available.

The standard sizes are 20 x 5 cm, 20 x 10 cm or 20 x 20 cm.

The surface should be flat without irregularities.

• The standard film thickness is 250um

How to Run Thin Layer Chromatography

Step 1: Prepare the developing container

Step 2: Prepare the TLC plate
Step 3: Spot the TLC plate
Step 4: Develop the plate
Step 5: Visualize the spots

TLC Developing Chambers

- Ascending development
- Descending development,
- Horizontal development.

HPTLC

INTRODUCTION

HPTLC is a sophisticated & automated form of TLC Efficient separation in short time
HPTLC is a form of thin-layer chromatography (TLC) that provides superior separation power using optimized coating material, novel procedures for mobile-phase feeding, layer conditioning, and improved sample application.
The basic difference between conventional TLC and HPTLC is only in particle and pore size of the sorbents.
The principle of separation is similar that of TLC adsorption.
• It is very useful in quantitative and qualitative analysis of pharmaceuticals.

Advantages of HPTLC Over Other Chromatographic Methods

1. In HPTLC, simultaneous processing of sample and standard - better analytical accuracy & precision
2. Lower analysis time & less cost per analysis
3. HPTLC is very simple
4. In HPTLC, the sample preparation is simple
5. Solvent used in HPTLC needs no prior treatment like filtration & degassing
6. In HPTLC, the M.P consumption for sample is extremely low
7. HPTLC allows the use of corrosive & UV absorbing M.P
8. It promotes high separation efficiencies/ resolution of zones due to higher number of theoretical plates.
9. Shorter developing times or analysis time
10. Lower amounts of mobile phase / solvent consumption
11. Enormous flexibility
12. Parallel separation of many samples with minimal time requirement
13. Simplified sample preparation due to single use of the stationary phase.
14. Efficient data acquisition and processing

UNIT-II
ION EXCHNGE CHROMATOGRAPHY

An ion-exchange resin or ion-exchange polymer is a resin or polymer that acts as a medium for ion exchange. It is an insoluble matrix (or support structure) normally in the form of small (0.25–1.43 mm radius) microbeads, usually white or yellowish, fabricated from an organic polymer substrate. The beads are typically porous (with a specific size distribution that will affect its properties), providing a large surface area on and inside them where the trapping of ions occurs along with the accompanying release of other ions, and thus the process is called ion exchange. There are multiple types of ion-exchange resin, that differ in composition if the target is an anion or a cation. Most commercial resins are made of polystyrene sulfonate,[1] followed up by polyacrylate.

Ion-exchange resin beads

Ion-exchange resins are widely used in different separation, purification, and decontamination processes. The most common examples are water softening and water purification. In many cases, ion-exchange resins were introduced in such processes as a more flexible alternative to the use of natural or artificial zeolites. Also, ion-exchange resins are highly effective in the biodiesel filtration process.

Types of resin

Most typical ion-exchange resins are based on cross linked polystyrene. The actual ion-exchanging sites are introduced after polymerisation. Additionally, in the case of polystyrene, crosslinking is introduced by copolymerisation of styrene and a few percent of divinylbenzene. Crosslinking decreases ion-exchange capacity of the resin and prolongs the time needed to accomplish the ion-exchange processes but improves the robustness of the resin. Particle size also influences the resin parameters; smaller particles have larger outer surface, but cause larger head loss in the column processes.

Besides being made as bead-shaped materials, ion-exchange resins are also produced as membranes. These ion-exchange membranes, which are made of highly cross-linked ion-exchange resins that allow passage of ions, but not of water, are used for electrodialysis.

Four main types of ion-exchange resins differ in their functional groups:

strongly acidic cation (SAC), typically featuring sulfonic acid groups, e.g. sodium polystyrene sulfonate or polyAMPS, often used for water softening and demineralization operations.

strongly basic anion (SBA), typically featuring quaternary amino groups, for example, trimethylammonium groups, e.g. polyAPTAC), good for silica, uranium, nitrates removal.

weakly acidic cation (WAC), typically featuring carboxylic acid groups. An ideal choice for dealkalization part and also for softening streams with high salinity levels.

weakly basic anion (WBA), typically featuring primary, secondary, and/or tertiary amino groups, e.g. polyethylene amine. Are effective for demineralization where removal of SiO_2 and CO_2 are not required. Also effective for acid absorption.

Specialised ion-exchange resins are also known such as chelating resins (iminodiacetic acid, thiourea-based resins, and many others).

Anion resins and cation resins are the two most common resins used in the ion-exchange process. While anion resins attract negatively charged ions, cation resins attract positively charged ions.

Anion-exchange resins

Formula: R-OH basic

Anion resins may be either strongly or weakly basic. Strongly basic anion resins maintain their negative charge across a wide pH range, whereas weakly basic anion resins are neutralized at higher pH levels. Weakly basic resins do not maintain their charge at a high pH because they undergo deprotonation. They do, however, offer excellent mechanical and chemical stability. This, combined with a high rate of ion exchange, make weakly base anion resins well suited for the organic salts.
For anion resins, regeneration typically involves treatment of the resin with a strongly basic solution, e.g. aqueous sodium hydroxide. During regeneration, the regenerant chemical is passed through the resin, and trapped negative ions are flushed out, renewing the resin exchange capacity.
Cation-exchange resin
Formula: R−H acidic
The cation exchange method removes the hardness of water but induces acidity in it, which is further removed in the next stage of treatment of water by passing this acidic water through an anion exchange process.
Reaction:
$R{-}H + M^{+} = R{-}M + H^{+}$.
Similar to anion resins, in cation resins the regeneration involves the use of a strongly acidic solution, e.g. aqueous hydrochloric acid. During regeneration, the regenerant chemical passes through the resin and flushes out the trapped positive ions, renewing the resin exchange capacity.
Anion-exchange resin
Formula: $–NR_4^+OH^–$
Often these are styrene–divinylbenzene copolymer resins that have quaternary ammonium cations as an integral part of the resin matrix.[5]
Reaction:
$–NR_4^+OH^– + HCl = –NR_4^+Cl^– + H_2O$.
Anion-exchange chromatography makes use of this principle to extract and purify materials from mixtures or solutions.

Characteristics

Ion exchange resins are often described according to some of the following features.[6]
Capacity: Represents the amount of ions that can be exchanged/stored per unit of mass of the resin. Typically is expressed in miligrams of ion per gram of resin (mg/g).
Swelling: Into contact with solvent, resins can swell (increase in volume). The swelling behavior of a resin is influenced by its chemical composition, polymer structure, and cross-linking. Resins with a higher degree of cross-linking tend to exhibit lower swelling tendencies compared to those with lower cross-linking. Swelling is typically expressed as the percentage increase in volume or weight of the resin when exposed to a specific solvent.
Selectivity: Refers to the resin's preference or ability to selectively adsorb or exchange certain ions over others. It is a fundamental property that determines the resin's effectiveness in separating or removing specific ions from a solution.
Stability: The integrity of the resin can be described in terms of mechanical and chemical resilience of the beads.

Pores

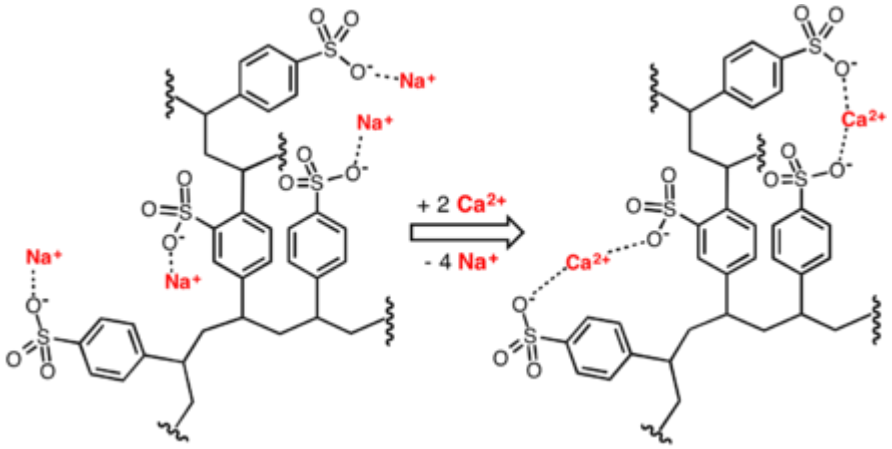
The pore media of the resin particles is one of the most important parameters for the efficiency of the product. These pores make different functions depending on Their sizes and are the main feature responsible for the mass transfer between phases making the whole ion exchange process possible.
There are three main types of pore sizes:
Micropore: With a Slit width less than 2 nm, they are usually found at the end of larger pores and their main characteristic is to have superimposed wall potentials. This means, the particles inside them feel attracted towards their solid walls so they make contact with the active sites.

Mesopore: With a Slit width between 2 and 50 nm these mid-size pores have the main objective to withhold capillary condensation and is usually found before the micropores.
Macropore: With a Slit width bigger than 50 nm, these are the biggest size pores with the main purpose of being the main path for the molecules to enter the particle and later on redistribute through the other smaller channels

Uses

Water softening

In this application, Ion-exchange resins are used to replace the magnesium and calcium ions found in hard water with sodium ions. When the resin is fresh, it contains sodium ions at its active sites. When in contact with a solution containing magnesium and calcium ions (but a low concentration of sodium ions), the magnesium and calcium ions preferentially migrate out of solution to the active sites on the resin, being replaced in solution by sodium ions. This process reaches equilibrium with a much lower concentration of magnesium and calcium ions in solution than was started with.



Idealised image of water-softening process, involving replacement of calcium ions in water with sodium ions donated by a cation-exchange resin

The resin can be recharged by washing it with a solution containing a high concentration of sodium ions (e.g. it has large amounts of common salt (NaCl) dissolved in it). The calcium and magnesium ions migrate from the resin, being replaced by sodium ions from the solution until a new equilibrium is reached. The salt is used to recharge an ion-exchange resin, which itself is used to soften the water.

Water purification

Purified water

In this application, ion-exchange resins are used to remove poisonous (e.g. copper) and hazardous metal (e.g. lead or cadmium) ions from solution, replacing them with more innocuous ions, such as sodium and potassium, in the process cation and anion exchange resins are used to remove dissolved ions from the water.

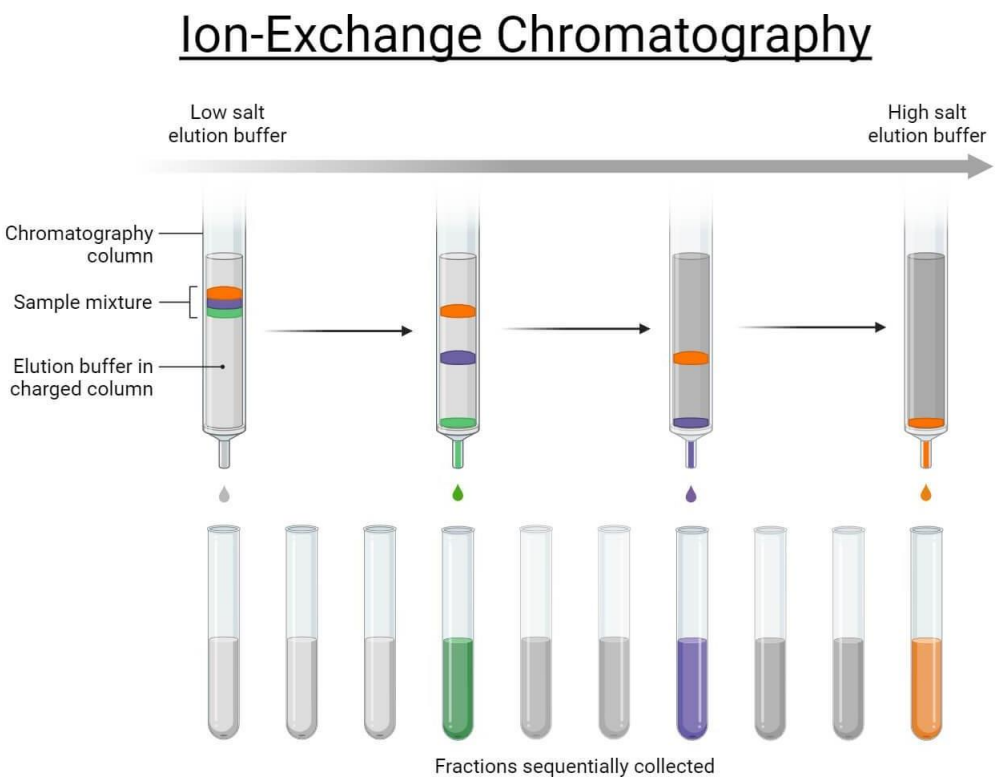
Few ion-exchange resins remove chlorine or organic contaminants from water – this is usually done by using an activated charcoal filter mixed in with the resin. There are some ion-exchange resins that do remove organic ions, such as MIEX (magnetic ion-exchange) resins. Domestic water purification resin is not usually recharged – the resin is discarded when it can no longer be used.

Water of highest purity is required for electronics, scientific experiments, production of superconductors, and nuclear industry, among others. Such water is produced using ion-exchange processes or combinations of membrane and ion-exchange methods.

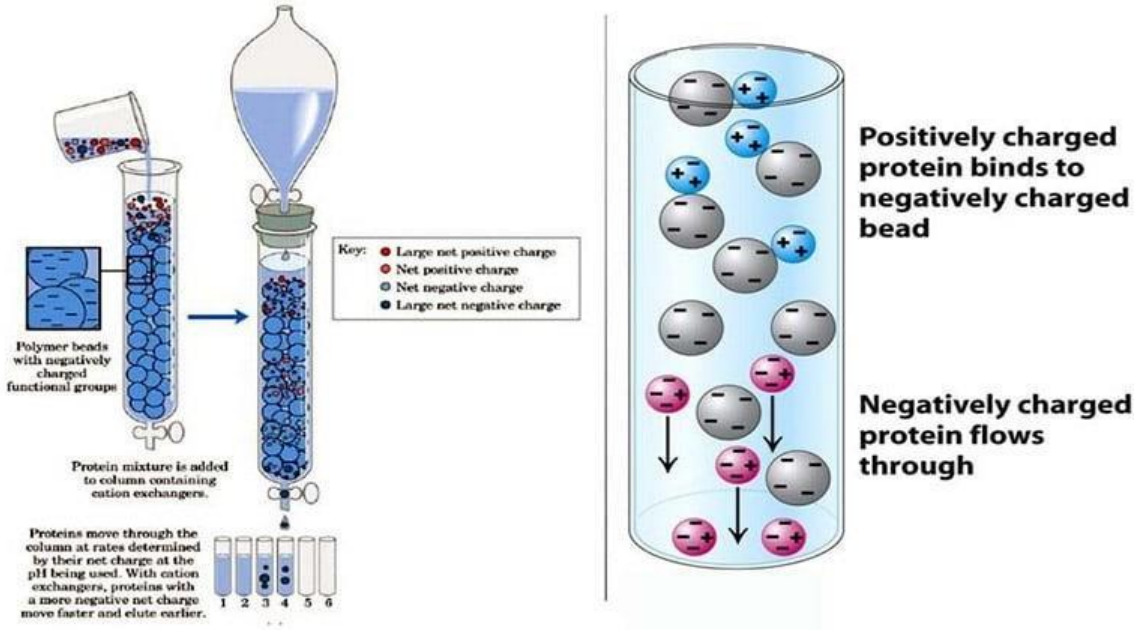
ambient air or direct air capture, since the moisture swing replaces the more energy-intensive temperature swing or pressure swing used with other sorbents. A prototype demonstrating this process has been developed by Klaus Lackner at the Center for Negative Carbon Emissions.

Chromatography is the separation of a mixture of compounds into their individual components based on their relative interactions with an inert matrix.

Ion exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers.
The principle of separation is thus by reversible exchange of ions between the target ions present in the sample solution to the ions present on ion exchangers.

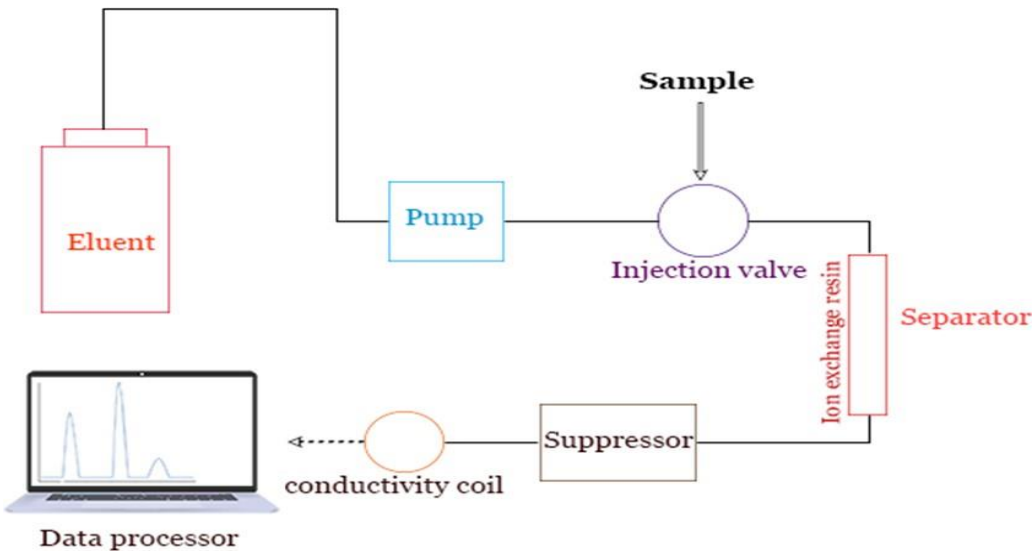


In this process, two types of exchangers i.e., cationic and anionic exchangers can be used.
Cationic exchangers possess negatively charged group, and these will attract positively charged cations. These exchangers are also called “Acidic ion exchange” materials, because their negative charges result from the ionization of acidic group.
Anionic exchangers have positively charged groups that will attract negatively charged anions. These are also called “Basic ion exchange” materials.
Ion exchange chromatography is most often performed in the form of column chromatography. However, there are also thin-layer chromatographic methods that work basically based on the principle of ion exchange.



Working Principle: ion exchange chromatography

This form of chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.
The ion exchangers basically contain charged groups covalently linked to the surface of an insoluble matrix.
The charged groups of the matrix can be positively or negatively charged.
When suspended in an aqueous solution, the charged groups of the matrix will be surrounded by ions of the opposite charge.
In this "ion cloud", ions can be reversibly exchanged without changing the nature and the properties of the matrix.
Instrumentation of ion exchange chromatography



Typical IC instrumentation includes: pump, injector, column, suppressor, detector and recorder or data system.

Pump

The IC pump is considered to be one of the most important components in the system which has to provide a continuous constant flow of the eluent through the IC injector, column, and detector.

Injector

Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. Liquid samples may be injected directly and solid samples need only to be dissolved in an appropriate solvent.

Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 ml of volume with high reproducibility and under high pressure (up to the 4000 psi).

Columns

Depending on its ultimate use and area of application, the column material may be stainless steel, titanium, glass or an inert plastic such as PEEK. The column can vary in diameter from about 2mm to 5 cm and in length from 3 cm to 50 cm depending on whether it is to be used for normal analytical purposes, microanalysis, high speed analyses or preparative work.

Guard column is placed anterior to the separating column. This serves as a protective factor that prolongs the life and usefulness of the separation column. They are dependable columns designed to filter or remove particles that clog the separation column

Suppressor

The suppressor reduces the background conductivity of the chemicals used to elute samples from the ion-exchange column which improves the conductivity measurement of the ions being tested. IC suppressors are membrane-based devices which are designed to convert the ionic eluent to water as a means of enhancing the sensitivity.

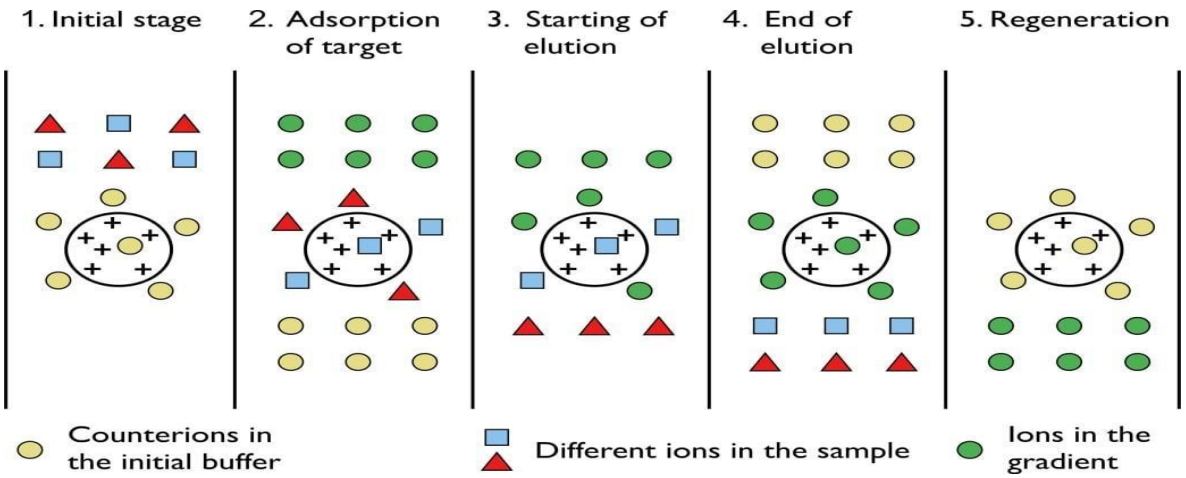
Detectors

Electrical conductivity detector is commonly use.

Data system

In routine analysis, where no automation is needed, a pre-programmed computing integrator may be sufficient. For higher control levels, a more intelligent device is necessary, such as a data station or minicomputer.

Procedure of ion exchange chromatography



Ion exchange separations are carried out mainly in columns packed with an ion-exchanger.
These ionic exchangers are commercially available. They are made up of styrene and divinyl benzene.
Example. DEAE-cellulose is an anionic exchanger, CM-cellulose is a cationic exchanger.
The choice of the exchanger depends upon the charge of particle to be separated. To separate anions "Anionic exchanger" is used, to separate cations "Cationic exchanger" is used.
First the column is filled with ion exchanger then the sample is applied followed by the buffer. The tris-buffer, pyridine buffer, acetate buffer, citrate and phosphate buffers are widely used.
The particles which have high affinity for ion exchanger will come down the column along with buffers.
In next step using corresponding buffer separates the tightly bound particles.
Then these particles are analyzed spectroscopically.
Applications of ion exchange chromatography:
An important use of ion-exchange chromatography is in the routine analysis of amino acid mixtures. The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated and used in clinical diagnosis.
This is most effective method for water purification. Complete deionization of water (or) a non-electrolyte solution is performed by exchanging solute cations for hydrogen ions and solute anions for hydroxyl ions. This is usually achieved by method is used for softening of drinking water.
In the analysis of products of hydrolysis of nucleic acids. In this way, information is gained about the structure of these molecules and how it relates to their biological function as carriers of hereditary information.
Chelating resins are used to collect trace metals from seawater.
To analyze lunar rocks and rare trace elements on Earth.
Advantages of ion exchange chromatography
It is one of the most efficient methods for the separation of charged particles.
It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.
Ion exchange is used for both analytical and preparative purposes in the laboratory, the analytical uses being the more common.

Ion chromatography (or **ion-exchange chromatography**) is a form of chromatography that separates ions and ionizable polar molecules based on their affinity to the ion exchanger. It works on almost any kind of charged molecule—including
small inorganic anions, large proteins,[3] small nucleotides, and amino acids. However, ion chromatography must be done in conditions that are one pH unit away from the isoelectric point of a protein

UNIT-III

SAMPLING OF SOLIDS, LIQUIDS AND GASES

Sampling is the process of obtaining a representative sample (We can not analyze the whole thing!)

A sample is the representative of the whole bulk. It composition should closely reflect the composition of the bulk.

Critical step in analysis as the significance and accuracy depends on sampling

Sample can be solid, liquid, gas and heterogeneous or homogenous

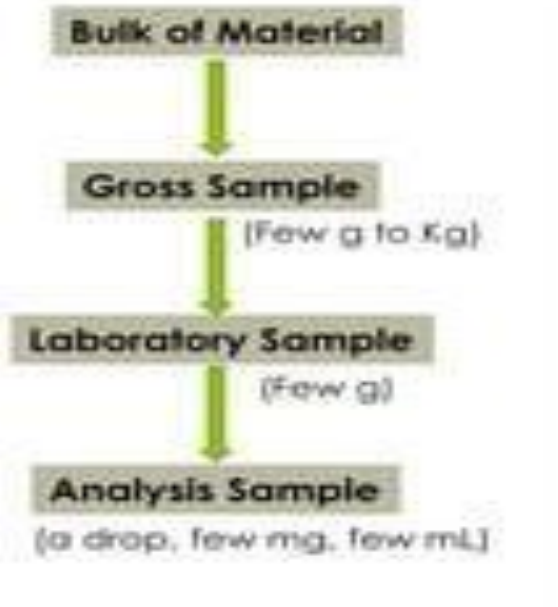
Homogenous Sample: A grab sample is often OK. For instance, in clinical lab, gross sample (blood, urine) can be analyzed directly as it is homogenous.

Heterogeneous Sample: Several individual samples are taken. E.g., analyzing average protein content of shipment of grains, one has to collect little grain from each bag during loading/unloading using a sampling spear (sack sampler) and combine to obtain a gross sample.

Gross Sample consists of several portion of the material to be tested

Laboratory Sample consists of a small portion of gross sample made homo

Analysis Sample is that which is actually analyzed



Theory or Laws of Sampling:-

The theory of sampling is based on certain statistical laws which are not exact but are universally true. The laws governing sampling are of two types.

1. Laws of Statistical regularity, 2) Law of inertia of large numbers.

Laws of Statistical regulating:- According to this law, a moderately large number of samples chosen at random from the universe and mixed uniformly to give an average sample can be termed a representative sample and is expected to represent the composition of the universe with a high probability

Laws of Inertia of Large numbers:- Random sampling from the universe does not mean the selection is done in a haphazard or careless manner but it only means that sampling is done without any bias or prejudice, whereby a portion of the universe has an equal chance of getting included in to sample.

Laws of Inertia of Large numbers:- According to this law, abnormalities will occur in large samples, but abnormalities will occur in large samples, but abnormalities will compensate, leaving the average un alternated.

If follows from this law that, if large numbers of samples are drawn from different parts of the bulk material then the probability that the gross sample will represent the bulk material is quite high. This is in agreement with the observation that the experimental mean (x) approaches the true mean (m) when a hypothetical infinite number of measurements are made. The reliability of the sample is proportional to the square root of the sample size.

Factors Involved in Sampling

- **Nature of bulk material & its homogeneity** :-

If the bulk material is homogenous in nature then sampling is easy and even a small amount of sample will give accuracy in observation & readings. While sampling of heterogeneous bulk will involve a greater number of steps to obtain a sample that will be a true representative of bulk.

- **The expected accuracy of result**

Depending upon the end-use of bulk, the accuracy of bulk sample result is expected, and depending upon it the type or method of sampling is selected.

E.g.: a sampling of drugs will require high accuracy hence will require a costly & elaborate method, while that of raw material used for manufacturing chalk sticks need not be equally elaborate or careful.

- **Cost and time of analysis**:-

The time required for sampling should not be very long and the cost of sampling should not be more than the cost of the product.

Purpose of Sampling:-

- **Judging acceptability** :-

A sampling of bulk many times is done to conclude the material from which sample is done to conclude whether the material from which sample is drawn meets the requirements such as purchase or sales specifications. If it meets requirements it can be accepted otherwise rejected.

- **Detection of contaminations** :-

The second purpose of sampling is to assure that the material under consideration is free from contamination or any unwanted material. E.g.: Urea in drinking milk.

- **Identifying material** :-

The third purpose of sampling is to identify an unknown material. A carefully drawn sample can adequately serve to establish the identity of bulk material under study.

- **Estimation of material** : –

Sampling is sometimes done to make an estimate that a particular element or material is present or absent in a given material.

Types of Sampling

For a sampling technique to be reliable and accurate, the method should confirm to following conditions.

- The sample mean should provide an unbiased estimate of the population mean.
- Sampling procedure should lead us to an accurate estimate of the central tendency and dispersion of bulk material for given time & money.
- Test of significance should be applicable on the sample to estimate populations variance.

The sampling procedure can be broadly classified into two types

A) Random Sampling, B) Non-random or Systematic sampling.

A) <u>Random Sampling</u>:-

Random sampling is the selection of samples without bias and in a way that gives full freedom to the operation of chance factors

- In this method every individual item has an equal chance of being selected; if sample size is large enough then sample produced is most likely represent the bulk.
- This type of sampling requires minimum knowledge of the bulk material in advance of being selected.
- When bulk is of homogenous nature, random sampling is comparatively easy. However, when sample is heterogeneous then different procedure followed.

- Heterogeneous material is first divided into relatively homogenous group i.e. material is first divided into groups possessing similar characteristics. Then from each group samples are drawn at random it is called as stratified sampling.

E.g.: ores exist as lumps of various sizes; hence lumps of different sizes are grouped on basis of their sizes then from each group of sizes samples are drawn at random at mixed.

B) Non-random or Systematic Sampling:-

- This type of sampling appears to be more scientific method than random sampling, though not necessary that it will give better sample.
- In systematic sampling, sample units are drawn in a definite sequence at equal intervals from one another.

E.g.: every tenth tablet is selected from the tableting machine or the twelfth soft drink bottle selected from the bottling plant.

Limitations:

- This method has an element of bias or prejudice in favour of tenth tablet or twelth bottle in above example.
- Due to cyclic fluctuations in tabletting machine or bottling plant exactly the tenth tablet or the twelth bottle may be defective or may be perfect. In either case, sample does not truly represent universe.
- Non- random sampling requires a prior list of the items in population & it gives satisfactory result if items of such a list are arranged in a random manner and samples are drawn at definite intervals.

Problems or Difficulties associated with sampling

a. **Lack of prior information**:- There may be no information on the nature of the distribution of the desired property in the bulk material. The examination of previous data may supply approximate but adequate data required.

b. **Excessive Cost**:- Sampling is immaterial, as long as the sample removed is truly representative of the bulk material and to obtain a representative sample from bulk material, a large number of units may have to be selected, which may involve direct or indirect expenditure.

c. **Physical difficulties**:- The sample has to be removed in such a way as not to disturb the composition of the material at the point at which the sample is removed. But because of the physical nature, condition, or location of material to be sampled, the same cannot be achieved hence required randomness in sampling is not possible. And in the case of heterogeneous material, it is still difficult.

E.g. Steel ingots or cast iron samples are collected by drilling, milling, or sawing the ingots. Drilling causes graphite flakes to become loose thereby increasing the carbon content of the sample.

Sample collected from the interior as well as from the surface of the ingot can only be capable of becoming a true representative. Similarly, the liquid is stored in the drum. Should be sampled from the top, bottom & middle of the drum.

In the case of material forming large pile follows a fixed pattern, not a random pattern, in which larger particles roll down and collect at the outer edge of the pile while smaller particles accumulate at the center of the pile.

Gas Sampling

Gas sampling is a very important concern. A sampling of gases is more difficult than those of liquid or solids. The method used to draw samples of one gas may not be applicable to another gas. Even though gases mix freely by diffusion and become homogenous, there are practical difficulties in drawing a sample of gas. The major difficulty is in the prevention of contamination of gas by atmospheric air. Successive sampling may show the stratifications of the gas samples.

Types of gas sampling

There are two types for sampling for gases and they are,

i) Ambient sampling and ii) Stock sampling

i) Ambient Sampling

Ambient sampling means sampling of air, gases that exist in the free state in air. It is also called atmospheric sampling which is rather difficult than stack sampling.

Factors such as wind (direction, velocity), temperature, height, rain, geographical condition, etc. Should be considered during ambient sampling, because this variable can change the composition of gases from time to time and from place to place.

The method also depends on the chemical and physical properties of the substances which are present in the atmosphere and which are under study.

In collecting air samples, the following points must be satisfied.

a) An accurate airflow device must be used to trap the contamination in the air. Certain

filters or absorbing solutions are used. In general fine filters or specific trapping, solutions are used in and filtration action is controlled by the porosity of the filter and in solutions, certain chemicals are used to trap unwanted components.

b) A widely used technique is to collect air samples in plastic bags of various sizes at definite points at different times. Then all results are computed to give a general pattern.

ii) Stock sampling:-

A sampling of gases from closed systems such as tanks, cylinders, or flowing through pipes is called stack samplings. The industrial gases are generally sampled. Continuously as they flow through pipes by this method. Only care should be taken that the sample collected represents a constant fraction of the total flow and all portions of the stream are sampled.

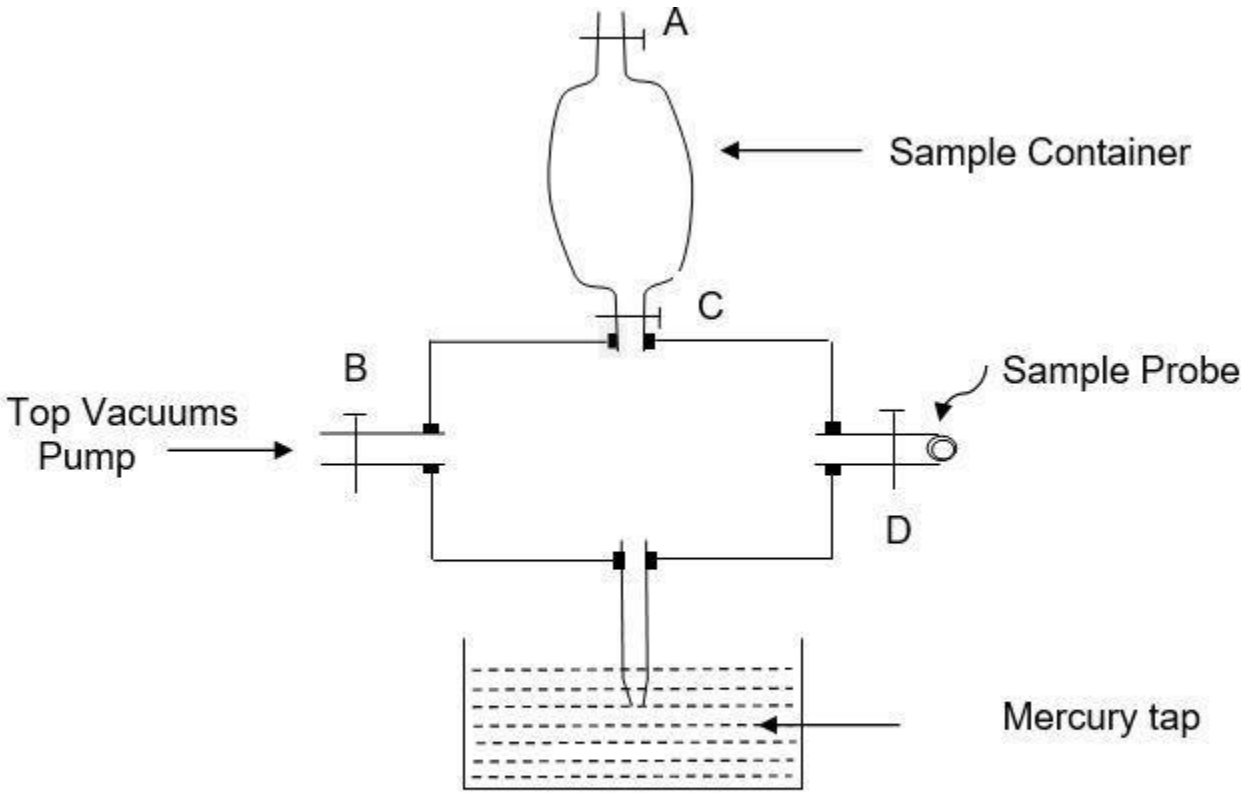
Apparatus used for sampling of gases

Generally, the apparatus used for air sampling consists of

(a) sample probe, (b) Sample container, (c) Delivery line, (d) Mercury trap.

- **Sample Probe** : Sample probe is that part of the apparatus which is attached to or which is extended into vessel or a pipe containing the gas. The probe should extend into vessel to about one-sixth diameter of the gas container.
- **Sample container** : – Sample container is the vessel in which the gas sample is collected and this may vary in size from 250 cm^3 to several cubic centimetres depending upon material to be sampled. The containers are made of glass, steel or iron.
- **Delivery Line** :- Delivery line carries the sample.
- **Mercury trap**: – It is an arrangement connected to the sample container which consists of tube dipped in a vessel containing mercury. It helps in releasing excess of pressure developed.

All the tubes are provided with stopcocks or valves to control gas flow. The stopcocks should be cleaned & well greased with high vacuum grease before a sample is taken; the gas in question is allowed to come in contact with lubricant to establish equilibrium.



Device for sampling gas

Methods used for removal of gas samples

A sampling of relatively pure gas can be done in three following methods.

i) Expansion method, ii) Displacement method, iii) Flushing method

i) **Expansion method**: In this method, the sample container is evacuated by using a vacuum pump attached to the container. The container is also warmed to remove gases absorbed on the container wall.

The sample probe is attached to the gas container (and stopcock D is opened) the gas flows into the sample container by natural expansion.

Since the perfect vacuum is never obtained some residual impurities may still stay on, hence the process of evacuation & filling of the sample gas is repeated several lines. This method is generally used for Ambient sampling.

ii) **Displacement Method**:- In this method, a liquid generally mercury, water, or saturated salt solution is used to displace all the air from the sample container, sample probe & delivery line. This method is generally used to draw samples from cylinders filled with gas at high pressure i.e. in other words for stack sampling.

This method however not suitable for accurate sampling because some gas samples may get dissolved in the liquid used for displacing the air. Another possibility is that sample gas may be saturated with vapors of liquid used for displacing.

iii) **Flushing method**:- In this unwanted gas in the sample container is completely removed or flushed out by the gas being sampled. This may be done by the apparatus by the gas being sampled. This may be done by the apparatus shown in the figure. Initially, the stopcock 'B' is closed. The probe is inserted into the gas container and stopcock 'A', 'C', and 'D' are opened. The gas flows into the sample container and displaces air from pt. When it is determined that all air has been completely flushed out. 'A' is closed and sample gas is allowed to completely fill the sample container, then stopcock 'C' and 'D' are closed.

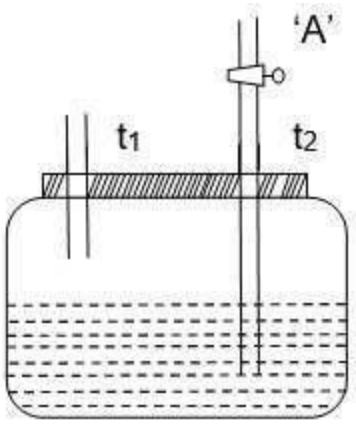
Sampling of Liquids

A sampling of liquids involves three different cases viz. Homogenous liquid, heterogeneous liquids and flowing liquids. In each case, a different technique or procedure has to be employed for sampling.

For a sampling of homogenous & heterogeneous liquid sample thief (figure given below) can be used while multiple tube sampler (fig shown below) can be used for sampling of flowing liquids.

I) <u>Sampling of Homogenous (Static) Liquid:-</u>

A homogenous liquid has the same composition throughout. Hence sample can be withdrawn at random from the top, middle, and bottom layers. For this purpose, sample thief is used.



Sample thief for Liquid Sampling

Sample thief enables the collection of the liquid at different depths without disturbing it. Thus a composite sample can be prepared. Sample thief consists of a bottle shown in the figure. The bottle is fitted with two tubes t1 and t2. The tube t2 is much longer than the height of the liquid in the container, so when the thief is dipped into the container, the tube t2 projects well outside the container, the tube t1 is always open. The thief is lowered to different depths in the container with stopcock 'A' closed. When the thief is at an appropriate depth, 'A' is opened. The liquid enters the bottle through 't_2' by displacing the air through 't_2' and in this way sample is collected.

II) Sampling of Heterogeneous (Static) or Immiscible liquids:-

Heterogeneities of a liquid may arise due to complete immiscibility of components of liquids mixed together or, due to formation of emulsion or suspension or due to volatility of liquid, leaving behind partially crystallized solid. The different procedure has to be used in all different cases.

a) **<u>Heterogeneous liquids</u>** that are immiscible:-

Accurate sampling of immiscible liquids which forms layers is done by determining the volume of each immiscible layer by using the cross-section of the container and the height of the layer samples are then taken from each immiscible layer by using sample thief, care is being taken to see that the volumes of sample collection are in the same ratio as the ratio of the volume of the layers in the container originally.

The individual samples are then mixed to get a gross sample. If the liquid container is non-uniform or odd-shaped, then the liquid has to be transformed into a cylindrical or rectangular container in order to determine the ratio of different liquid layers.

b) **Emulsion or unstable suspensions**:-

The best way to sample an unstable suspension is to separate the two (solid & liquid) phases by filtration. The two phases are weighed and sampled separately. If this is not possible then the entire suspension is rapidly and efficiently stirred to get the uniform mixture and then the resultant liquid is rapidly sampled reproducibility of this sampling technique should be checked.

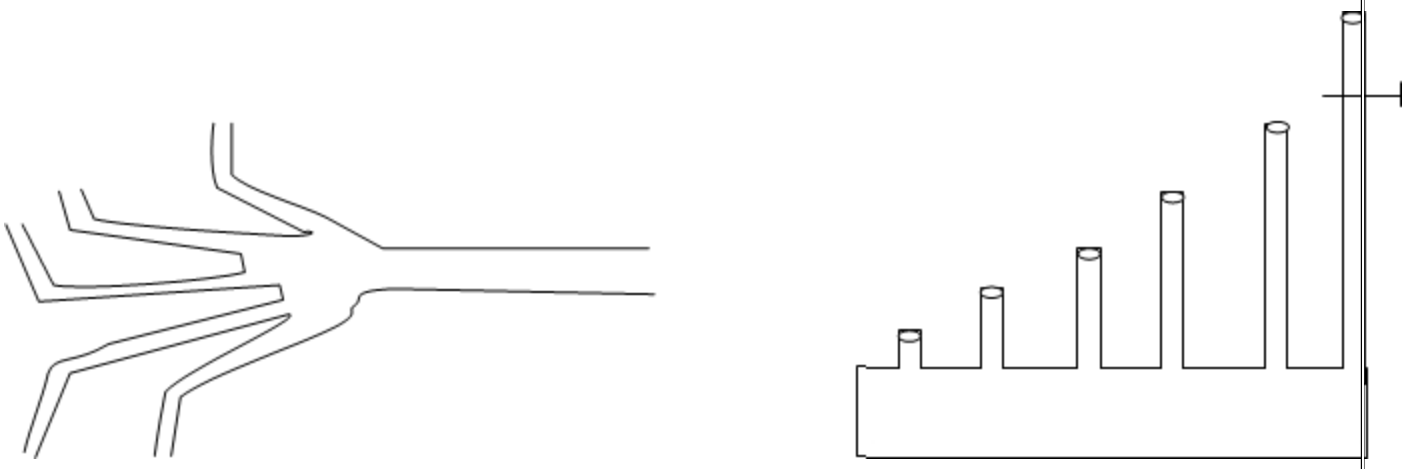
c) **Liquid containing partially crystallized** solids:-

Semi solidified liquids or liquid containing crystallized solids are first heated, before subjecting to sampling. Heating is continued till solid dissolves in the liquid or melts, and then the entire mars is stirred efficiently and then rapidly sampled.

III) Sampling of Flowing (not-static) Liquids :

The sampling technique of flowing liquids has to be modified since the composition of flowing liquid changes with time and position. It is, therefore, necessary to draw samples at different positions and at different time intervals, this leads to the elimination of bias introduced due to variation of composition with time.

Generally, the composition of the liquid at the center of flow is not the same as that at other points of flow. Hence to get a satisfactory sample under this condition a multiple tube sampler is used. This tube consists of tubes of varying lengths ending up into one single long tube. The overall appearance of this device is similar to the palm of a hand, with tubes of varying lengths appearing like fingers.



Multiple Tube Sampler

When such a tube is placed in the path of the flow of a liquid, the tube extends to different points in the liquid stream. Samples collected at random or at regular intervals are mixed to give a gross sample.

General precaution during sampling of liquids.

To obtain a good representative sample of liquid certain requirements are cleanliness of apparatus and containers used preservation of sample composition and the scrupulous care of sampling apparatus. Precaution is also needed during handling, transportation, and storage.

Cleanliness denotes the exclusion of foreign materials from apparatus & containers before and during sampling. Sampling connection, sample container should be rinsed & drained before it is actually used.

The sample composition should be preserved. The volatile sample must be protected against evaporation. Liquid to be sampled if contain solid or immiscible droplets, care should be taken so as to

transfer it quantitatively into the sample. Dissolved gases should not be allowed to escape if they are present.

To allow contraction & expansion, the container should be only 80% filled. Light-sensitive samples should be collected in an opaque or amber-coloured container.

Sampling of Solids

A sampling of solid is more tedious and difficult than a sampling of gases and liquids. This is because of the heterogeneity in which the solids are obtained. A solid exists either in compact form or a particular form. Even if materials appear homogenous they turn out heterogeneous due to localized concentrations of impurities. The sampling technique adopted to obtain a true representative of bulk will depend mainly on the form in which solids exist.

The conversion of a gross sample suitable for analysis in the lab requires reduction of particle size along with reduction of mass, which depends on three factors.

1. Heterogeneity of bulk material, 2. Particle size of bulk material and 3. Degree of accuracy of results.

Sample size in solid sampling:-

The gross sample should have the same particle size distribution as the bulk. The size of the sample depends upon various factors such as the particle size, the bulk size, the degree of precision required, and the actual amount of sample used for testing. In this concept the bulk ratio is important.

$$\text{Bulk ratio} = \frac{\text{Weight of sample}}{\text{Total weight of bulk material}}$$

Another factor used for determining the number and size of increments that are to be brought from bulk to form gross samples is the size to weight ratio.

$$\text{Size: Weight (b)} = \frac{\text{Weight of largest particle}}{\text{Weight of sample}} \times 100$$

For a sample to be a true representative of populations, the bulk ratio should be as large as possible and size: weight be as small as possible. Even due to sampling is minimized when results from a large number of samples are arranged. Sampling error is found to be inversely proportional to the square root of a number of sample average.

If the impurities are present as a particle of large size and cubic shape then it is estimated that the standard deviation 's' of the percentage impurity due to sampling is given by,

$$S = (b.p.)^{1/2}$$

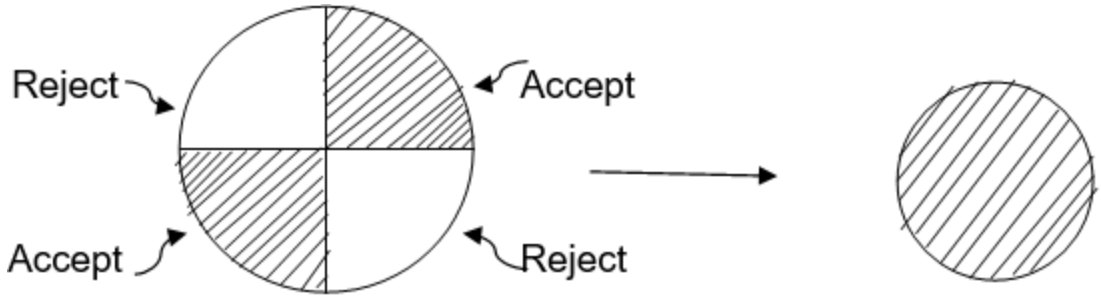
b= Size : weight

p = percentage of impurity

Preparation of Sub-sample: Size Reduction

The Gross sample obtained in a manner is then reduced to a size suitable for analysis. This involves both reduction of particle size as well as bulk. This is done by following different methods, before applying any mentioned methods; the gross sample has to be powdered to coarse size and mixed. This can be done by using a ball mill or grinder. The methods for size reduction are,

a. **Coning & Quartering method**:- This is one of the oldest techniques for reducing the size of the gross sample. The material is first crushed to suitable fineness and is placed into a conical pile. The pile is then flattened, and then divided into quarters, the two opposite quarters are selected, and the other two are rejected. This procedure of coning & quartering is repeated till a sample of the desired size is obtained.



Smaller portion created by accepted pa

b. **The Long pile and alternate shovel method**:- In this method, the material is shoveled in the form of a pile about 10 feet in length and width corresponding to that of the shovel. The alternate shovel is discarded; the sample size thus gets reduced.

c. **Tabelling, rolling,** and quartering:- In this method sample is uniformly spread on a square polythene sheet placed on the table hence the name tabelling. The tabelled material is mixed carefully by lifting one end of the polythene sheet and then repeated from another end this process makes particles roll over one another, hence name rolling. These steps are repeated to make a homogenous mixture. Then this material is uniformly spread on the table and then is divided into four quarters. Two opposite quarters are selected and two are rejected. This is continued till the sample size of the desired amount is obtained.

d. **Method using riffles**:- A riffle consists of a through that is divided into an even number of segments. Alternate segments deliver the sample to the opposite side of the trough the starting material is divided into two approximately equal portions. One part may be paused through the rifle repeatedly until the sample of the desired size is obtained.

Different sampling Equipments and sampling methods.

Sampling equipment used and the method of sampling depends upon the types of solids to be sampled. Solids can be of two types a) Compact solids and b) Particulate solids.

a. **Compact Solids**:- Compact solids consists of various forms of solids such as broken clunk of original solid, resolidified molten material, natural deposits of soil, the material of various hardness. In this case,

the samples are obtained by drilling, sawing, filing material sampling of compact solid involves the following equipment.

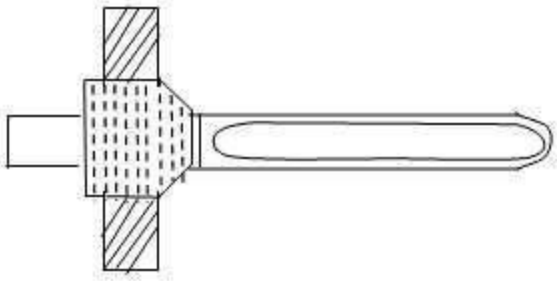
i) Auger Sampler ii) Split – Barrel Sampler iii) Split–tube thief.

i) **Auger Sampler**:- It is a small helical screw of about 4 cm minimum diameter with a 'T' style handle. It is turned into the material and then pulled straight out. The material is then knocked or scraped off with a spatula.

Auger Sampler

ii) **Split-Barrel Sampler**:- The drilling equipment used should provide a clean hole to permit the driving of the sampler to obtain an undisturbed sample. The driving head should be made up of hard steel, is at least 45 cm long. It is detached from the coupler & opened to remove the sample.

iii) **Split-tube thief**:- It is a metallic tube with a slot running the full length of the tube. It has a T-type handle fixed to one end. The end of the tube is sharp and can cut through a container. To remove a sample, the thief is inserted into the container by rotating the handle until the thief reaches the center of the container. The thief is then carefully withdrawn and the material it has scouped out is knocked or scraped out.



Split – tube thief

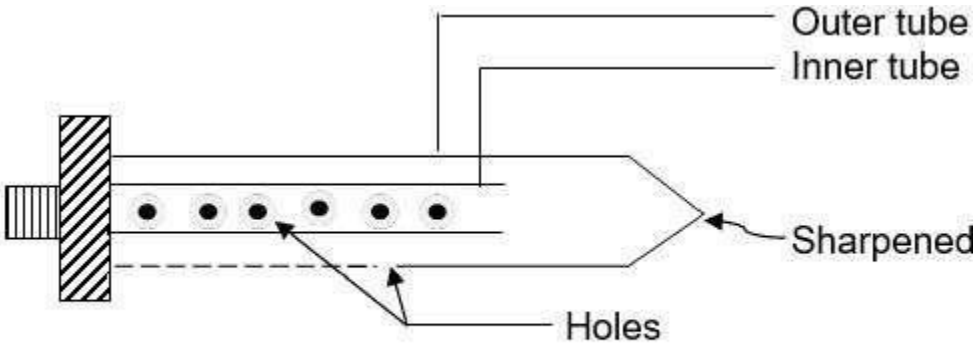
b) **Particulate Solids**:- In particulate form, solids exist as a particle of different sizes. In this case, the particle is important. Sampling is done by making size to weight ratio as the criterion of sample size i.e. 'b' is considered.

$$B = \frac{\text{Weight largest particle}}{\text{Weight of sample}} \times 100$$

Different types of devices used are

Split-tube thief, concentric tube thief & Hand scoups, or shovel.

Concentric tube thief:-



Concentric tube thief

It consists of two concentric tubes which are closely fitted to each other. Both tubes have holes cut into the corresponding positions. Outer tubes have sharp ends & can pierce through containers. During insertion holes are closed, when the thief reaches an appropriate position, the tube is rotated to open holes and material gets deposited into the inner tube. And then holes are closed & the thief is pulled out.

Hand scoups or shovel:- Hand scoup or shovels of suitable size are also used for taking cross-sectional samples of particulate solids while in motion e.g.:- on the conveyer belt. The scoups can also be used to take samples from the surface of drums, bags, barrels & other containers.

UNIT-IV
IMPORTANT OF ANALYTICAL CHEMISTRY AND SOLVENT EXTRACTION

Qualitative and dqunatititative analysis in research and development:

Analytical chemistry is a branch of chemistrywhich dealswith the qualitative and quantitative analysis

Quantitative analysis: which constituents present in the sample is called qualitative analysis

Quantitative analysis: How much amount present in the sample

In analytical research the development technique is done by two methods.

1. methods Development
2.Validation Method.

1. methods Development : For the analysis of unknown drug first we have to check the solubility and we can prepare the sample solution the same is injected to the specified instrument and the mobile phase is passed in specified ratio. That a multiple proportions at certain M.P ratio. The sample is separated very well. So searching of suitable mobile phase with specific ratio's is known as Method development.

2.Validation Method.

- Sensitivity
- selectivity
- specificity
- control chart
- back ground deviation
- LOD
- Youden Plot
- F-test, T-Test

• Solvent extraction, also called liquid-liquid extraction (LLE) and partitioning

• Immiscible liquids are ones that cannot get mixed up together and separate into layers when shaken together. These liquids are usually water and an organic solvent.

Solvent extraction is the process in which a compound transfers from one solvent to another owing to the difference in solubility or distribution coefficient between these two immiscible (or slightly soluble) solvents.

• It is a method of quantitative separation of compounds.

• When extracting solvent is stirred with solution containing solute then solute from original solvent gets transferred into an extracting solvent.

• When stirring is stopped extracting solvent form separate layer and now it contains solute of interest.

Compared with other separation methods, it gives a better separation effect than chemical precipitation, and a higher degree of selectivity and faster mass transfer than the ion exchange method.

• Compared with distillation, solvent extraction has advantages such as low energy consumption, large production capacity, fast action, easy continuous operation and ease of automation.

Commonly used solvents

• ethyl acetate (8.1 %),

• diethyl ether (6.9 %),
• dichloromethane (1.3 %) and
• chloroform (0.8 %) dissolved up to 10 % in water. Water also dissolves in organic solvents:
• ethyl acetate (3 %),
• diethyl ether (1.4 %),
• dichloromethane (0.25 %)
• chloroform (0.056 %).

Uses of solvent extraction process

• Solvent extraction is used in the processing of perfumes, vegetable oil, or biodiesel.
• It is also used to recover plutonium from irradiated nuclear fuel, a process which is usually called nuclear reprocessing.
• The recovered plutonium can then be re-used as nuclear fuel.

The properties of the solvent used for solvent extraction

1. The solvent should be well miscible with the liquid to be extracted.
2. The solvent should not be miscible with the other components of the mixture or react with the solute. 3. The boiling point of the solvent should be low enough (well below the melting point of the solute) such that it can be evaporated easily after collection.
4. It should have a favourable temperature coefficient.

The solvent should be able to dissolve at least one component to a large extent than the rest of the components in the mixture.

• The reaction taking place should be stable and irreversible. Reversible reactions can bring back the dissolved components in their previous form and the extraction will not be completed successfully.
• The compound formed after the reaction should be easily separated from the extracted compound so that it can be reused.
• The density of the compound should be different from the required component to help the separation readily.
• It should be inexpensive and cost-effective.
• The solvent should not be toxic or corrosive as it can harm the extraction instruments.
• Other factors important during solvent selection are viscosity, boiling point, flammability, etc

Distribution coefficient

• When a solution is placed in a separatory funnel and shaken with an immiscible solvent, solutes often dissolve in part into both layers.
• The components are said to "partition" between the two layers, or "distribute themselves" between the two layers.
• When equilibrium has established, the ratio of concentration of solute in each layer is constant for each system, and this can be represented by a value K
• K is called the partition coefficient or distribution coefficient.
• KD=Molarity in organic phase / Molarity in aqueous phase

When a compound is placed in contact with two immiscible liquids then the compound itself get distributed in these two liquids.
• This is an equilibrium process (dissolution equilibrium) governed by temperature of the system.
• Suppose compound A is to be extracted from aqueous solution into organic liquid, then for compound A, dissolution equilibrium can be represented by the equation

$$A\,(aq) \rightarrow A\,(org)$$

Under dissolution equilibrium concentration of compound A in aqueous phase and in organic phase is definite.

• KD=Concentration in organic phase / Concentration in aqueous phase

Distribution ratio

• Many substances undergo dissociation in aqueous phase like weak carboxylic acid such as benzoic acid, phenol, etc.

• Now consider the weak acid HA which will dissociate in aqueous phase as follows

$$HA \rightarrow A^- + H^+$$

• The substances which undergo by accounting dissociation in aqueous layer the distribution coefficient in modified form can be defined as distribution ratio (D)

• $D = [HA]\ org / [A^-]aq + [HA]\ aq$

Solvent extraction techniques

• Batch extraction

• Continuous extraction

• Countercurrent extraction

Batch extraction

• Batch extraction, the simplest and most commonly used method, consists of extracting the solute from one immiscible layer in to other by shaking the two layers until equilibrium is attained, after which the layers are allowed to settle before sampling.

• This is commonly used on the small scale in chemical laboratories.

• The most commonly employed apparatus for performing a batch extraction is a separatory funnel.

• The batch extractions may also be used with advantage when the distribution ratio is large

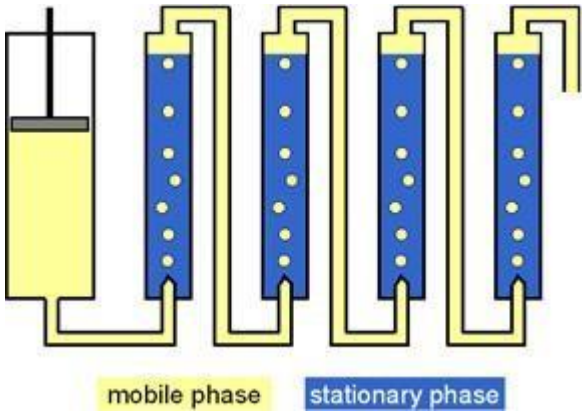
Counter current extraction

• Extraction by continuous counter current distribution is the third general type and is used primarily for fractionation purposes.

• The separation through continuous counter current method is achieved by virtue of the density difference between the fluids in contact.

• In vertical columns, the denser phase enters at the top and flows downwards while the less dense phase enters from the bottom and flows upwards.

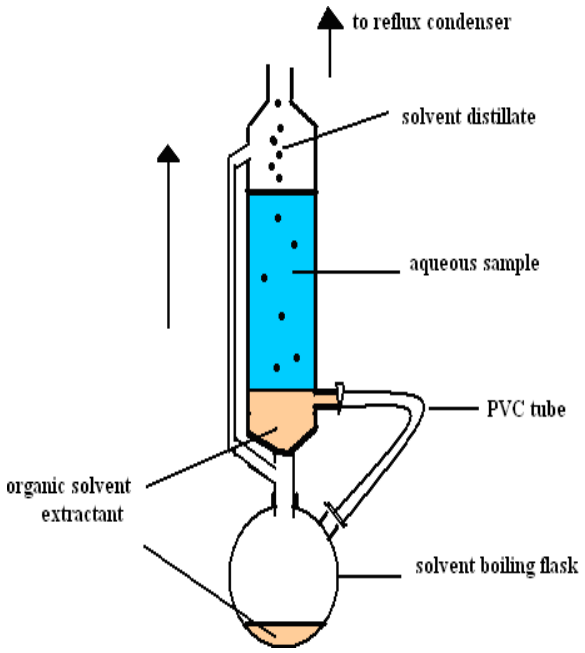
• The choice of method to be employed will depend primarily upon the value of the distribution ratio of the solute of interest, as well as on the separation factors of the interfering materials.



Continuous countercurrent extraction, also known as soxhlet extraction, is extracting chemicals from solid materials. For the determination of crude fat content, soxhlet extraction is used. Fat is found in abundance in many plants' seeds and fruits. Fat content can be used as a criterion for determining the quality of a product. The extraction procedure is widely used across the world. In China, the analysis of oil and grain is done by a traditional method, known as Soxhlet extraction, and it is the chosen standard method. This process requires a lot of time and is usually done with a fat extractor in a lab.

To extract the solid matter, a pure solvent can be used during the usage of the syphon method and solvent reflux. This leads to high extraction efficiency. However, it is also important to note that a solid substance must be discreetly ground before the extraction process. It is important for maximising the area of liquid immersion.

Assemble the device as directed. When the solvent reaches boiling temperature, the vapour rises through the air tube and condenses into a liquid, which drips into the extractor. The phenomenon of syphoning happens when the liquid level surpasses the highest point of the syphon.



Supercritical Fluid Extraction (SFE)

SFE is an instrumental approach not unlike PLE, except a supercritical fluid is used as the extraction solvent rather than a liquid. SFE and PLE employ the same procedures for preparing samples and loading extraction vessels, and the same concepts of static and dynamic extractions are also pertinent. SFE typically requires higher pressure than PLE to maintain supercritical conditions and, for this reason, SFE usually requires a restrictor to better control flow and pressure of the extraction fluid. CO_2 is by far the most common solvent used in SFE due to its relatively low critical point (73 atm and 31°C), extraction properties, availability, gaseous natural state and safety.

A major advantage of SFE over liquid-based methods is that the extraction solvent becomes a gas after extraction and the analytes are conveniently concentrated in the collecting medium (solid-phase trap or liquid). Liquid extraction methods nearly always require a concentration step after extraction. Another key advantage of SFE is that the density of the supercritical fluid and other physicochemical properties can be dramatically altered through control of temperature and pressure. This permits a somewhat higher degree of selectivity and versatility in the extraction process without having to use different solvents. In some cases, SFE can eliminate post-extraction clean-up steps, or at least make clean-up using SPE exceptionally convenient by using the SPE sorbent as a trapping medium in SFE. Due to its many practical advantages, SFE may be considered the first choice for extraction if it is able to meet the needs of the application.

Advantages of super critical fluids

- It is chemically inert and nontoxic.
- It is non-flammable.
- It is non-polar and dissolves oils and fats well.
- Since carbon dioxide is released as a gas at normal temperatures and pressures, solvent removal is easy, allowing more accurate component concentrations to be determined.
- High-purity carbon dioxide can be obtained at a low price, so low running costs can be realized.
- Since carbon dioxide emitted from petrochemical factories is collected, refined and used, it does not increase carbon dioxide emissions.

Applications of solvent extraction

• Determination of iron
• Determination of lead in the blood
• Determination of copper in the alloys such as steel
• Determination of uranium • Separation and Purification of organic compounds by organic chemists