Shree H.N.Shukla Institute of Pharmaceutical Education & Research Rajkot

B.Pharm

Semester V

Subject Name: Pharmacognosy and Phytochemistry II

Subject code: BP503TP

UNIT V:Basics of Phytochemistry

Basics of Phytochemistry

Application of latest techniques like Spectroscopy, chromatography and electrophoresis in the isolation, purification and identification of crude drugs.

Extraction

- It is the method of removing active constituents from a solid or liquid by means of liquid solvent. It can be defined as the process of isolation of soluble material from am insoluble residue by treatment with solvent.
- It is done for separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents.
- In this method the wanted components are dissolved by the use of selective solvents known as menstrum& undissolved part is a marc.
- After the extraction unwanted matter is removed.

Extract: • preparations of crude drugs which contain all the constituents which are soluble in the solvent.

Marc • Solid residue obtain after extraction

Menstrum • Solvent used for extraction

- Extraction is the process of efficiently dissolving and separating the desired constituents from the crude drug with the use of solvent/s

- It is controlled by mass transfer. Plant constituents are usually contained inside the cells. Therefore, The solvent used for extraction must diffuse into the cell to dissolve the desired compounds whereupon the solution must pass the cell wall in the opposite direction and mix with the surrounding liquid.
- An equilibrium is established between the solute inside the cells and the solvent surrounding the fragmented plant tissues the choice of solvent depends upon the charactristics of secondary metabolites like polarity, pH and thermal stability.

Ideal properties of Solvent

- Be highly selective for the compound to be extracted.
- Not react with the extracted compound or with other compounds in the plant material
- Have a low price.
- Be harmless to man and to the environment.
- Be completely volatile.
- Should not mix up with water.
- Should have the big capacity in relation to extractive.
- The density of solvent should be difference from water density.
- Should have the minimum viscosity.

Choice of Extraction method

- Sample size
- Quantity of the exctract required
- Extraction time
- Choice of solvent
- Cost

Drying of Crude drugs

It is necessary to avoid microbial contamination

Done after collection and before drying

Unless specified, drug should be dried below 60o C

Shade dryingLess exposure to heat

• Less chances of chemical alteration

Sundrying :Mosteffiicient,economic

• Use less intense sun light Sun drying

Far infrared drying

Less explored yet

Costly, used for expensive drugs

Vacuum drying

• Low pressure rapid drying method

• For thermolabile compounds

Oven/hot air drying

• Used often

Steps for extraction

1) Size reduction

• Maximizes surface areincrease mass transfer

• 30-40 mesh size is optimum

2)Extraction

• Maceration, percolation, soxhlation, etc

3) Filtration • Throughmusciline cloth, filter paper, filter press

4) Concentration • Evaporation of solvent • Hot air oven, vacuum camber drying, rotary vacuum evaporator

5) Drying • Spray drying, etc

Maceration

- **Maceration means** soaking
- The whole / coarsely powdered crude drug is placed in a stoppered container with the solvent.
- Allow to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter gets dissolved.
- The mixture then is strained, the marc (the damp solid material) is pressed
- The combined liquids are clarified by filtration or decantation after standing.
- This method is best suitable for use in case of the thermolabile drugs.

Modified maceration: is used for unorganized drug like gums and resins

› Pressing is avoided

› Fresh menstruum is used to wash marc and make the volume

Multiple maceration: to obtain concentrated extract, total menstrum is divided into 2 parts (double maceration) or 3 part (triple maceration) and each part is used for maceration separately.

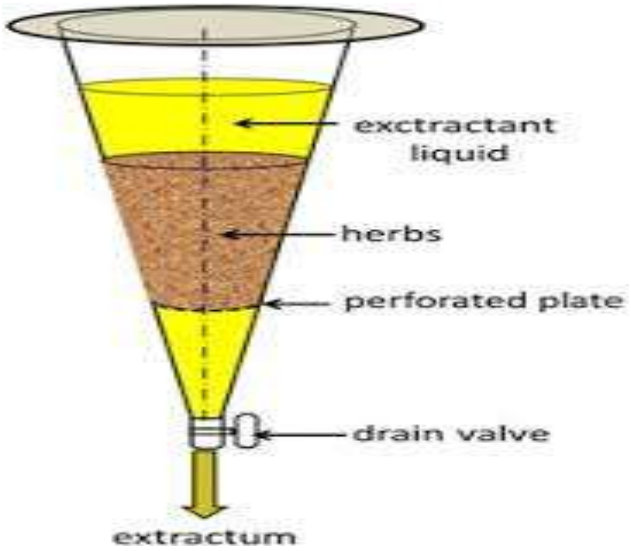
Vacuum maceration: when pressure is reduced to desired extent, menstrum is allowed to enter◊ increases permeability of cell wallsreduced extraction time

Kinetic maceration: the vessel is agitated in constant motion for first 24 hours › 20-50% more yield usually

Re-maceration: first maceration is carried with small amount of menstruum followed by remaining amount of solvent

Circulation maceration: solvent is continuously circulated by pump

Percolation



- Percolate means I pass through
- It is continuous flow of the solvent through the bed of crude drug material to get the extract
- It implies a slow passage of menstrum under the influence of gravity through column of drug powder and during this movement it goes on extracting the drug molecules layerwise.
- In percolation the drug is exhaustively extracted by fresh menstrum.

Percolation Steps

1. Moisten the drug with sufficient quantity if menstrum
2. Allow to stand for 4 hrs in closed vessel
3. Pack it in percolator
4. Add sufficient mensrtum to saturate the drug
5. When liquid starts dropping, close the outlet
6. Add sufficient menstrum to form layer above drug
7. Allow to stand for 24 hrs
8. Allow to percolate until 3/4th of final extract volume
9. Press marc and mix the liquid with extract

10. Clarify further if necessary Adjust final volume

Intermitten percolation: 24 hr maceration and 12 hr maceration are alternated with percolation to effect extraction

Re-percolation: drug is divided into 4/5 lots percolation followed◊ extract is used aasmenstrum for next lot. Same menstrum for every new lot

Hot percolation:percoaltion at elevated temperature increases the efiiciency

Reserved percolation: first part of percolate is reserved and susequent percolate is collected separately. Most of active constituents are in first percolate. Second percoate contains less active constituents

Circulatory percolation:menstrum is continuously circulated

Diffusion percolation:menstrum is made to flow form bottom to top under hydrostatic pressure
Diacolation: same as diffusion just instead of hydrostatic pressure, positive pressure of compressed air is utilized

Strength and limitation:

1. Maceration and its related techniques is the easiest and simple method.
2. Large organic waste is an issue
3. Large volume of solvents is used and proper management of the waste is needed.
4. Alteration in temperature and choice of solvents enhance the extraction process.

Infusion

It is used for extraction of vitamins, volatile ingredients and soft ingredients in which the powdered drug is extracted with hot/cold water.

Extract again with fresh hot/lukewarm water

Press the marc Filter Soak powdered drug in hot/lukewarm water for specified period with/without stirring

DECOCTION

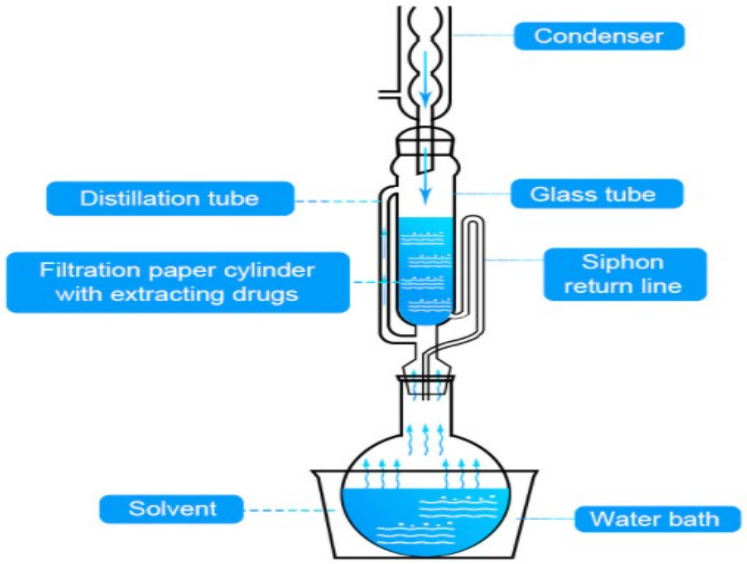
Concetration done by boiling

Drug is boiled with water for for minutes to hours and then filtered

It is suitable for drugs that are hard in nature containing water soluble constituents and are not affected by prolonged heating

HOT CONTINUOUS EXTRACTION

- Also called as Sohxlet extraction



- sample is placed inside a porous bag which is referred as "thimble".
- This thimble is made from a strong filter paper or cellulose, which is place inside a thimble chamber of the Soxhlet apparatus.
- In this extraction process, a suitable solvent is heated in the bottom flask, which is then vaporizes into the sample thimble.
- After reaching the sample thimble, this solvent condenses in the condenser and drip back.
- When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued.

Advantage: Soxhlet extraction method needs a smaller amount of solvent as compared to maceration process

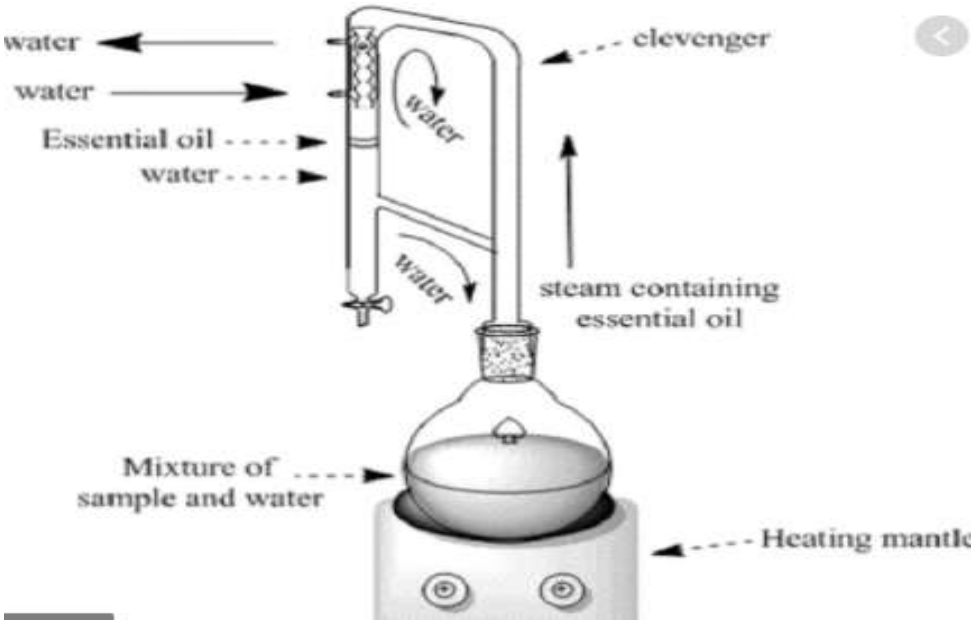
Disadvantage

- Soxhlet extraction method is exposure to hazardous and flammable liquid organic solvents, with potential toxic emissions during extraction
- Use of Solvents is quite costly as they required in more pure form.

- Soxhlet extraction method is associated with pollution problem and therefore it is considered not environmental friendly as compared to advance extraction method such as supercritical fluid extraction (SFE).
- The ideal character required for sample for Soxhlet extraction is also limited that is the sample must be dry and finely divided solid and many factors such as temperature, solvent-sample ratio and agitation speed need to be considered for this method.

Extraction of Volatile Oil

Water Distilation by clavenger Apparatus



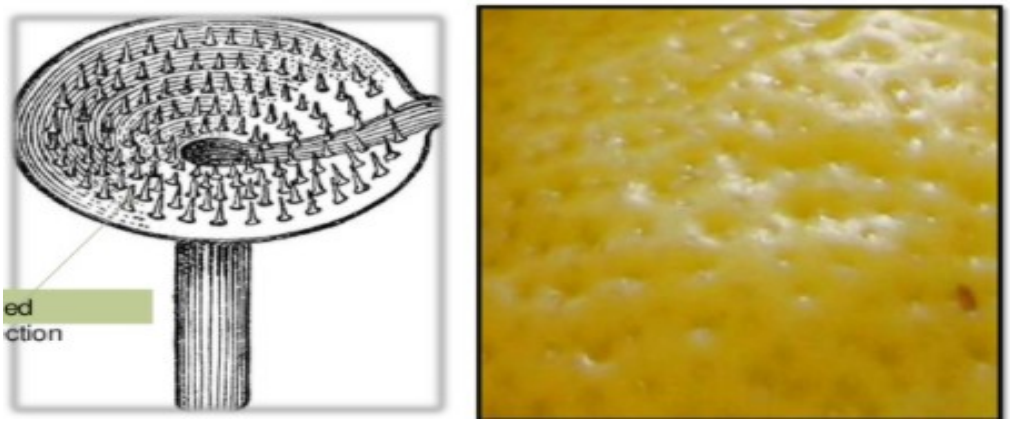
Clavenger apparatus

Steam distillation:

Expression of volatile oils: ›

Sponge: volatile oil is absorbed on the sponge after squeeziing orange peel

› Ecuelle



› Mechanical method:

mechanical/ hydraulic pressure is applied by grater/centrifugation.

Apparatus used for Ecuelle method of extraction

Enflurage:

used for extraction from delicate plant parts

› Fatty material is used

› Kept for 24 hours

› The again fresh petals are loaded

› Continues till fatty material is saturated

coldenflurage

› Fat is heated and petals are stirredstrainput new petals/flowers

› Fatty material is dissolved in suitable lipophilic solvent evaporate ethanol to have pure fragrance › Fat is usually used for soap making

Ultra sound Extraction Method

Also called as sonication extraction

Sound waves of high frequency pulse of 20 to 24 kHz are genrated in ultrasonic bath

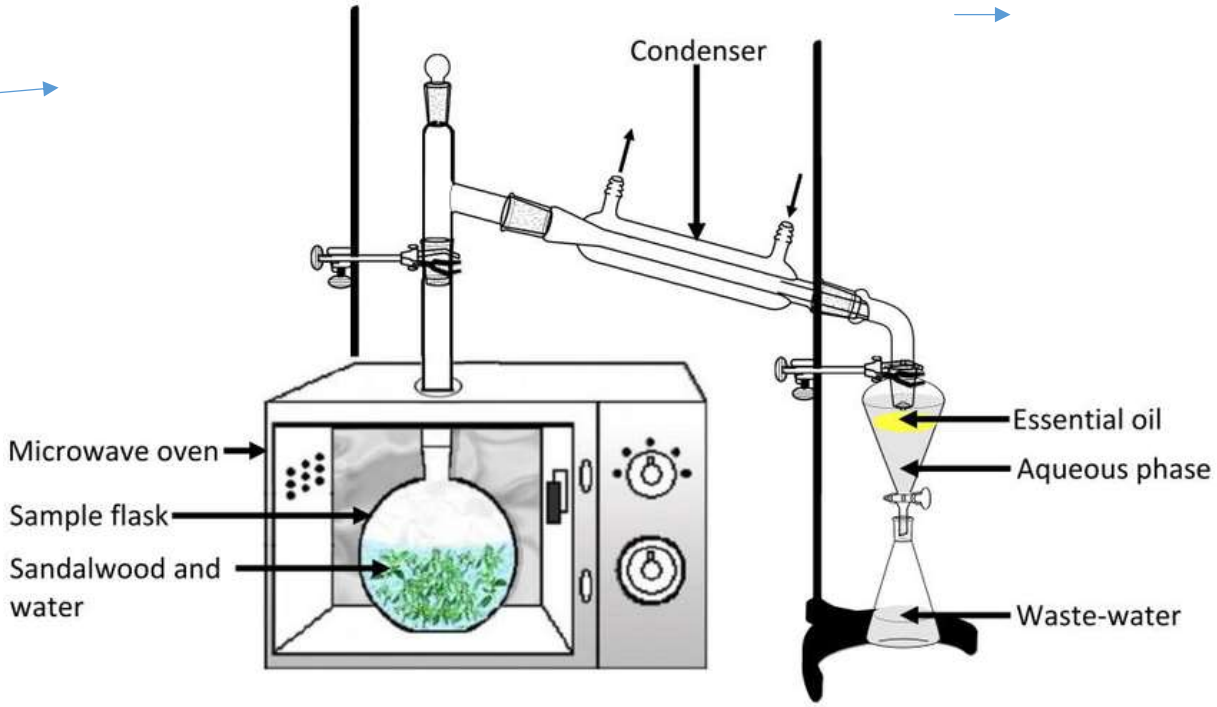
Sample with solvent is kept in suitable solvent.

The ultrasound increases the cell permeabilityand maximum extraction

Microwave Assisted Extraction method

This method is not suitable for large scale

Microwaves are (frequency 300 MHz- 300 GHz) nonionizing electromagnetic waves.



High temperature produced by microwaves evaporate the moisture in cell

dehydrates celluloseruptures cellwallextraction with solvent

Rapid extraction within 5-10 min

Types:

› Solvent extractor

› Solvent free extractor

› Microwave reflux

› Microwave assisted extractor

› Sub-500 W microwave extractor

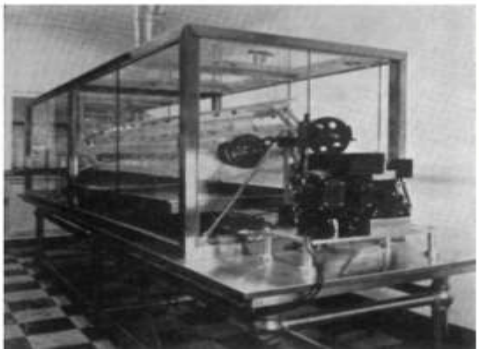
› Drydist model of milestone

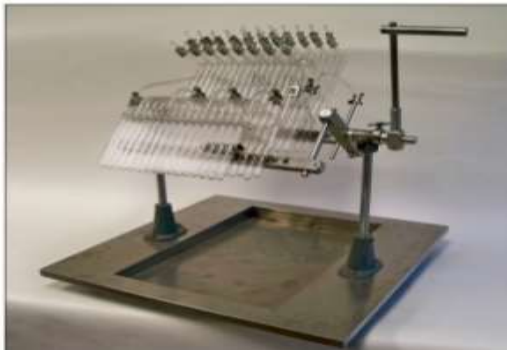
› Monolithic equipment for MAE

› Closed vessel mono model of CEM Co.

Counter current Distribution

Also called as CCD.





It is liquid liquid extraction process based on partition coefficient

The apparatus contains series of tube

First tube contains

› Heavy stationary liquid phase

› Mixture to be separated

› Light mobile liquid phase

The rest of tube contain only stationary phase initiallythe of the tubes contain only stationary phase

Droplet counter current chromatography

Also called as DCC/ DCCC

Is combination of liquid- liquid extraction and column chromatography?

- A liquid stationary phase is held in a collection of vertical glass columns connected in series.
- The mobile phase passes through the columns in the form of droplets.
- The DCCC apparatus may be run with the lower phase stationary and the upper phase being introduced to the bottom of each column.
- Or it may be run with the upper phase stationary and the lower phase being introduced from the top of the column.

- The mobile phase is pumped at a rate that will allow droplets to form that maximize the mass transfer of a compound between the upper and lower phases.
- Compounds that are more soluble in the upper phase will travel quickly through the column, while compounds that are more soluble in the stationary phase will linger.
- Separation occurs depending upon partition coefficient of compounds between the two phases.

Accelerated Solvent extraction (ACE)

- The process uses high temperature and pressure the extraction taking less time and requiring less solvent, and possibly also giving better analyte recovery
- The elevated temperature is employed to increase extraction efficiency of the analyte of interest The elevated pressure (100-200 bars) is used to keep the solvent in a liquid state as the temperature is increased above its boiling point and also increases permeability of solvent.
- The temperatue and pressure is below supercritical point
- The CO2 is not used as solvent

- Requires minutes to complete extraction process

At critical point, substance exists in vapour-liquid equilibrium

Supercritical fluid extraction

- SCF offer liquid like density, gas like viscocity, gas like copressibilty and high diffusivity than liquid
- Carbon dioxide (CO2) is the most used supercritical fluid, sometimes modified by co- solvents such as ethanol or methanol.
- Extraction conditions for supercritical carbon dioxide are above the critical temperature of 31 °C and critical pressure of 74 bar.
- Addition of modifiers may slightly alter this
- The system must contain a pump for the CO2, a pressure cell to contain the sample, a means of maintaining pressure in the system and a collecting vessel.
- The liquid is pumped to a heating zone, where it is heated to supercritical conditions.

- It then passes into the extraction vessel, where it rapidly diffuses into the solid matrix and dissolves the material to be extracted.
- The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out.
- The CO2 can then be cooled, re-compressed and recycled, or discharged to atmosphere.
- SPE is a sample preparation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties.
- It uses the affinity of solutes dissolved or suspended in a liquid (known as the mobile phase) for a solid through which the sample is passed (known as the stationary phase) to separate a mixture into desired and undesired components.
- The syringe barrel/ small glass/ plastic column called acrtidge is packed with stationary phase and sample solution is gently forced with vacuum

Isolation and purification

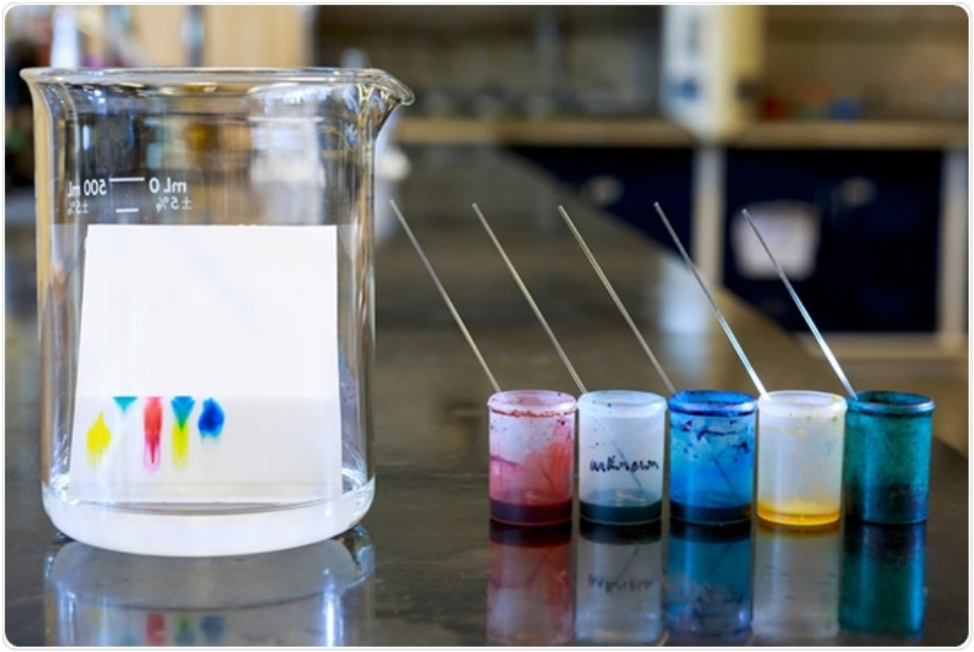
- Depends upon the physical and chemical properties of compounds to be separated and also upon the proportion of the component in the extract
- Methods like fractional crystallization, fractional distillation, fractional liberation, etc
- Chemical derivatization can also be employed based on groups or moieties present in the compound and chemical reactions.

Fractional crystallization

- It is an important method for the purification of compounds from mixture.

- In fractional crystallization the compound is mixed with a solvent, heated, and then gradually cooled so that, as each of its constituent components crystallizes, it can be removed in its pure form from the solution.
- Many natural products are crystalline in nature even in mixture, process such as concentration, slow evaporation, refrigeration are used for crystallization
- Fractional distillation is a process by which components in a chemical mixture are separated into different parts (called fractions) according to their different boiling points.
- This method is used for the separation of the components from volatile mixtures
- Largely using in the separation of hydrocarbons from oxygenated volatile oil egcitral, eucalyptol.
- In this process the groups of compounds having the tendency of precipitation from the solution.
- This process is often used in separation of cinchona alkaloids, morphine etc.
- Some groups of compounds lend themselves to fractional liberation from a mixture , ex : a mixture of alkaloid salts in aqueous solution when treated with aliquots of alkali , will give first the weakest base in the free state followed by base liberation in ascending order of basicity .
- If the mixture is shaken with an organic solvent after each addition , then a fractionated series of bases will be obtained.
- A similar scheme can be used for organic acids soluble in water – immiscible solvents ; in this case, starting with a mixture of the acid salts , it is possible to fractionally liberate the acids by addition of mineral acids .

Chromatography



It is a laboratory technique for the separation of a mixture of phytoconstituents.

Chromatography=from Greek chroma "color and graphein "to write"

- The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase.
- The separation is based on differential partitioning between the mobile and stationary phases.

Principal

Chromatography usually consists of mobile phase and stationary phase.

- The mobile phase = to the mixture of substances to be separated + a liquid or a gas.
- The stationary phase = a porous solid matrix through which the mobile phase along with sample percolates.
- The interaction between the mobile phase and the stationary phase results in the separation of the compounds from the mixture depending upon its partition coefficient between mobile phase and stationary phase.

- Subtle differences in a compound's partition coefficient result in differential retention on the stationary
- Due to this various constituents of the mixture travel at different speeds along with mobile phase, causing them to separate.

Purpose of Chromatography

Chromatography may be preparative or analytical.

- The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification.
- Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture.

Classification of chromatography

1. On the basis of interaction of
a. solute to the stationary phase
b. Adsorption Chromatography
c. Partition Chromatography
d. Ion Exchange Chromatography
e. Size Exclusion Chromatography
2. On the basis of chromatographic bed shape
Two Dimensional Thin Layer Chromatography Paper Chromatography
Three Dimensional Column Chromatography
3. On the basis of physical state of mobile phase
    a. Liquid Chromatography
    b. Gas Chromatography
    c. Super Critical Fluid Chromatography

Chromatographic term

- Chromatograph - equipment that enables a sophisticated separation
- › EX. Gas chromatography or Liquid chromatography

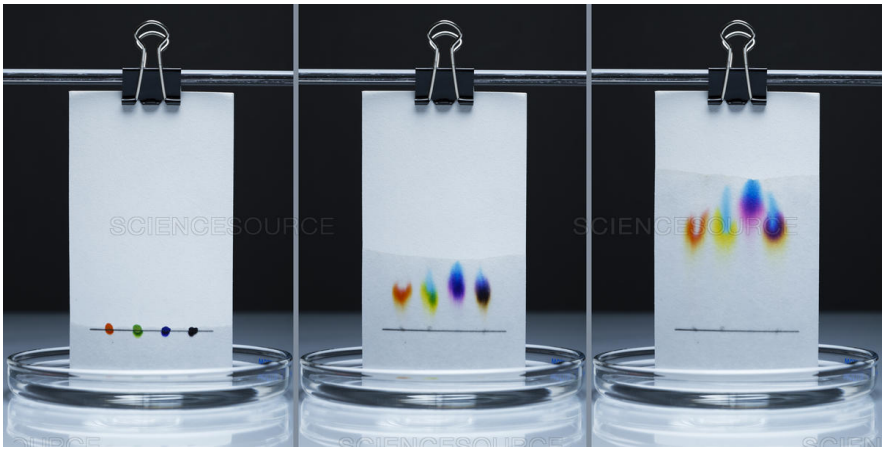
- Eluent - Fluid entering column/ solvent that carries the analyte.
- Eluate - Mobile phase leaving the column.
- Stationary phase - Immobilized phase

- › Immobilized on the support particles or on the inner wall of the column tubing.
- › Examples : Silica layer - Thin Layer Chromatography
- Mobile phase - Moves in a definite direction. Liquid (LC), Gas (GC). The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.
  - Retention time: Time takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.
  - Sample (Analyte) :Substance analyzed in chromatography.
  - Solvent: Any substance capable of solubilizing another substance
  - It is the visual output of the chromatograph

Column chromatography involves the following:

- 1. Adsorption/retention of substance on stationary phase
- 2. Separation of adsorbed substance using mobile phase.
- 3. Recovery of individual components by continuous flow of mobile phase. Stationary phase: silica gel, alumina

Paper chromatography



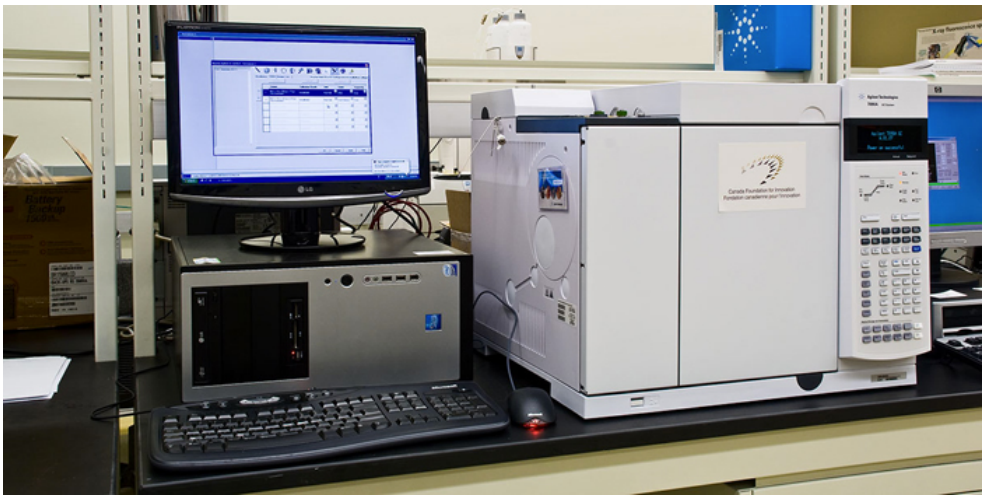
- Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of chromatography paper.
  - The paper is placed in a jar containing a shallow layer of solvent and sealed.
  - As the solvent rises through the paper, it meets the sample mixture, which starts to travel up the paper with the solvent.
  - This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non- polar.
  - More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.
  - Retention factor :R*f* = zero, - Solute remains in the stationary phase and thus it is immobile.
  - R*f* = 1 - Solute has no affinity for the stationary phase and travels with the solvent front.
  - Sometimes,it is rather difficult to separate a complex mixture of substances by a single run with one solvent system. In such a case, a second run is carried out by a different solvent system, in a direction perpendicular to the first run. This is referred to as two dimensional chromatography.

Thin layer chromatography

- Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography.
- However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose .

- Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents.
- The mobile phase runs upward along the plate due to capillary action and the components get separated depending upon their polarity towards stationary phase

Gas chromatography



Gas chromatography (GC), also sometimes known as Gas-Liquid chromatography, (GLC), is a separation technique in which the mobile phase is a gas.

- It is the method of choice for the separation of volatile substances or the volatile derivatives of certain non-volatile substances.
- Stationary phase is an inert solid material (kieselgurh/ firebrick) impregnated with a non-volatile liquid (silicon/PEG)
- This is packed in narrow column and maintained at high temperature around 2000C
- A sample is rapidly heated and vaporized at the injection port. The sample is transported through the column by a mobile phase consisting of an inert gas (Ar/He/N).
- Sample components are separated based partioncoefficeint between mobile phase and staionaryphase .

- The higher a component's affinity for the stationary phase, the slower it comes off the column. The components are then detected and represented as peaks on a chromatogram.
- It is commonly used for quantitative estimation of lipids, drugs and vitamins

High pressure liquid chromatography HPLC

- Also called as high pressure liquid chromatography
- It relies on pumps to pass a pressurized (5000-1000 psi) liquid solvent ie mobile phase containing the sample mixture through a column filled with a solid adsorbent material.
- Stationary phase-immobilized thin layer liquid on micro glass or plastic beads tightly packed on narrow column
- Mobile phase- solvent system passed under high pressure through column

- The eluents can be detected by detectors
- It can be applied in the form of adsorption, ion exchange, partition or molecular sieve chromatography
- Due to rapidity in detection its is used for detection of amino acids, peptides, carbohydrates, proteins, lipids, nucleic acids, vitamins, hormones, drugs, etc

Ion exchange Chromatography

 It retains analyte molecules based on it's ionic interaction

 It can be further divided into:

› Cation exchange chromatography- retains cations on negatively charged stationary phase with negatively charged functional groups and is used when the molecule of interest is positively charged.

› Inion exchange- retains anions on positively charged stationary phase with positively charged functional groups is used when the molecule of interest is negatively charged.

 When amino acid mixture is passed through the cation exchange chromatgraph, individual amino acid can be eluted using buffers of different pH

 A change in pH affects the charge on the particular molecules and, therefore, alters binding.

 Bound proteins are eluted out by utilizing a gradient of linearly increasing salt concentration, usually NaCl.

 With increasing ionic strength of the buffer, the salt ions will compete with the desired proteins in order to bind to charged groups on the surface of the medium.

 This will cause desired proteins to be eluted out of the column.

 Proteins that have a low net charge will be eluted out first as the salt concentration increases causing the ionic strength to increase.

 Proteins with high net charge will need a higher ionic strength for them to be eluted out of the column.

Gel filtration Chromatography

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

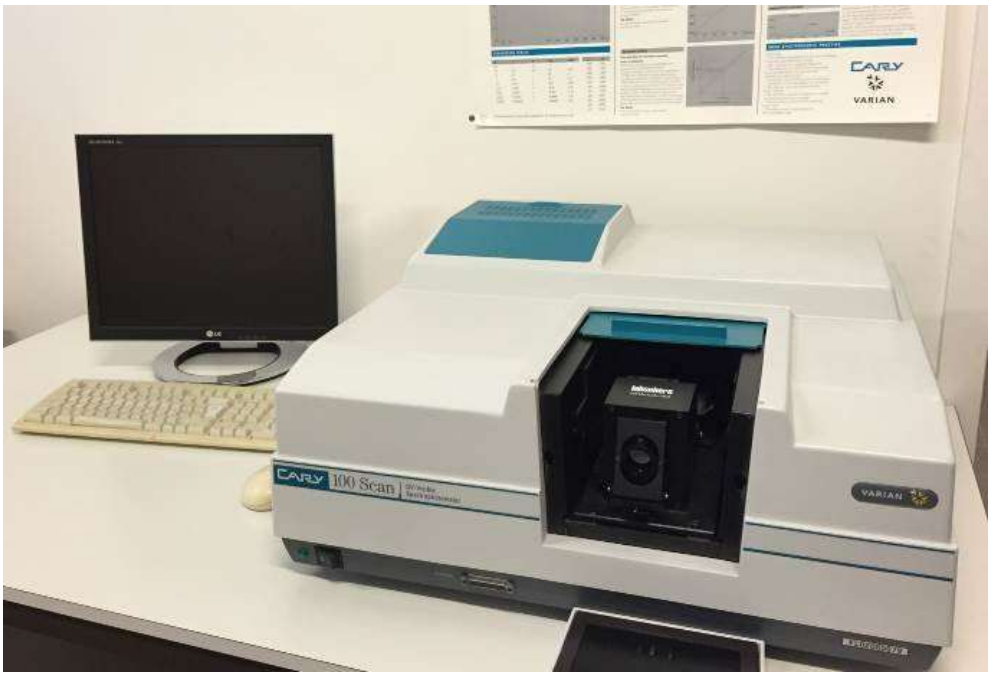
- It is also referred to as molecular sieving or molecular exclusion chromatography.
- The chromatography column is packed with fine, porous beads which are composed of dextran polymers (Sephadex), agarose (Sepharose), or polyacrylamide (Sephacryl or BioGel P). The pore sizes of these beads are used to estimate the dimensions of macromolecules
- Smaller molecules are able to enter the pores of the media and, therefore, molecules are trapped and removed from the flow of the mobile phase.
- Molecules that are larger than the average pore size of the packing are excluded and thus suffer essentially no retention◊ the first edszxcto be eluted. This is how the molecules are separated.
- It is also useful for determining the tertiary structure and quaternary structure of purified proteins, especially since it can be carried out under native solution conditions.

Spectroscopy

- Spectroscopy is the study of the interaction between matter and electromagnetic radiation
- Traditionally, spectroscopy involved the visible spectrum of light, but X-ray, gamma, and UV spectroscopy also are valuable analytical techniques.

- Spectroscopy can involve any interaction between light and matter, including absorption, emission, scattering, etc.
- When a beam of electromagnetic radiation passes through a sample, the photons interact with the sample.
- They may be absorbed, reflected, refracted, etc.
- Absorbed radiation affects the electrons and chemical bonds in a sample.
- In some cases, the absorbed radiation leads to the emission of lower- energy photons.
- Spectroscopy looks at how the incident radiation affects the sample.
- Emitted and absorbed spectra can be used to gain information about the material.

UV –Vis Spectroscopy



UV –Vis Spectroscopyis absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet (10-400nm) and the full, adjacent visible spectral regions (400-800 nm)

Absorption of the ultra-violet radiations results in the excitation of the electrons from the ground state to higher energy state.

Principal

UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

 The expression of Beer-Lambert law is- A = log (I0/I) = Ecl Where, A = absorbance I0 = intensity of light incident upon sample cell I = intensity of light leaving sample cell C = molar concentration of solute L = length of sample cell (cm.) E = molar absorptivity

Chromophore- Chromophore is defined as any isolated covalently bonded group that shows a characteristic absorption in the ultraviolet or visible region (200-800 nm).

Chromophores can be divided into two groups-

› a) Chromophores which contain p electrons and which undergo pie to pie* transitions. Eg: Ethylenes and acetylenes

› b) Chromophores which contain both p and nonbonding electrons. They undergo two types of transitions; pie to pie* and nonbonding to pie*. Eg: Carbonyl, nitriles, azo compounds, nitro compounds

Auxochromes- An auxochrome can be defined as any group which does not itself act as a chromophore but whose presence brings about a shift of the absorption band towards the longer wavelength of the spectrum.

Eg: –OH,-OR,-NH2,-NHR, -SH etc

a) Bathochromic effect- This type of shift is also known as red shift. Bathochromic shift is an effect by virtue of which the absorption maximum is shifted towards the longer wavelength due

to the presence of an auxochrome or change in solvents. The nonbonding to pie* transition of carbonyl compounds observes bathochromic or red shift.

b) Hypsochromic shift- This effect is also known as blue shift. Hypsochromic shift is an effect by virtue of which absorption maximum is shifted towards the shorter wavelength. Generally it is caused due to the removal of conjugation or by changing the polarity of the solvents.

c) Hyperchromic effect- Hyperchromic shift is an effect by virtue of which absorption maximum increases. The introduction of an auxochrome in the compound generally results in the hyperchromic effect.

d) Hypochromic effect- Hyperchromic effect is defined as the effect by virtue of intensity of absorption maximum decreases. Hyperchromic effect occurs due to the distortion of the geometry of the molecule with an introduction of new group.

IR spectroscopy

- IR spectroscopy (which is short for infrared spectroscopy) deals with the infrared region of the electromagnetic spectrum, i.e. light having a longer wavelength and a lower frequency than visible light.

- The IR spectroscopy concept can generally be analyzed in three ways: by measuring reflection, emission, and absorption.
- The major use of infrared spectroscopy is to determine the functional groups of molecules, relevant to both organic and inorganic chemistry
- IR radiation does not have enough energy to induce electronic transitions as seen with UV.
- Absorption of IR is restricted to compounds with small energy differences in the possible vibrational and rotational states.
- For a molecule to absorb IR, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule.
- The alternating electrical field of the radiation (remember that electromagnetic radiation consists of an oscillating electrical field and an oscillating magnetic field, perpendicular to each other) interacts with fluctuations in the dipole moment of the molecule.
- If the frequency of the radiation matches the vibrational frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration.
- What is a vibration in a molecule?

- "Any change in shape of the molecule- stretching of bonds, bending of bonds, or internal rotation around single bonds".
  - Why we study the molecular vibration? › Because whenever the interaction b/w electromagnetic waves & matter occur so change appears in these vibrations.
- FUNDAMENTAL VIBRATIONS •
- Vibrations which appear as band in the spectra.
- NON- FUNDAMENTAL VIBRATIONS
- Vibrations which appears as a result of fundamental vib. Mol. ›
- OVER TONES: These are observed at twice the frequency of strong band. Ex: carbonyl group.
- COMBINATION TONES: Weak bands that appear occasionally at frequencies that are sum/difference of 2 or more fundamental bands.

- FERMI RESONANCE: Interaction b/w fundamental vibration & overtones or combination tones. Ex:CO2
- In IR, the region below 1500 cm-1 is rich in many absorption bands and the region is known as fingerprint region.
  - Here the number of bending vibrations are usually more than the number of stretching vibrations.
  - In this region, small difference in the structure and constitution of a molecule results significant changes in the absorption bands.
  - Many compounds show unique absorption bands in this region and which is very useful for the identification of the compound.
  - Solid run in solution : Dissolve solid sample in non-aqueous solvent (which should be IR inactive) and place a drop of this solution in alkali metal disc and allow to evaporate, leaving a thin film which is then mounted on a sepectrometer. ◦ E.g. of solvents – acetone, cyclohexane, chloroform, carbon tetrachloride etc.
  - Mull technique :Finely powdered sample + mulling agent (Nujol) and make a thick paste (mull). Transfer the mull to the mull plates and the plates are squeezed together to adjust the thickness it is then mounted in spectrometer.
- Pressed pellet technique :Finely powdered sample is mixed with about 100 times its weight of KBr in a vibrating ball mill and the mixture is then pressed under very high pressure in an evacuable die to form a small pellet(1-2mm thick and 1cm in diameter).
  - Solid films:Here amorphous solid is dissolved in volatile solvents and this solution is poured on a rock salt plate (NaCl or KBr), then the solvent is evaporated by gentle heating.
  - Sampling of liquids :Liquid sample can be sandwiched between two alkali halide plates (NaCl , KBr , CaF2).
  - Sampling of gases : Here gases sample is introduced into a glass cell made up of NaCl. › Very few organic compounds can be examined as gases. ◦ E.g.: 1,4-dioxane.
  - Sampling of solutions:Here 1-5% of solution is placed in a solution cell made up of metal halides and a second cell containing the pure solvent act as a reference.
- › Important solvents used are:-chloroform , CCl4, Carbon disulphide etc.

Mass spectrometry

- Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules.
- The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass to charge ratios (m/z) and relative abundances.
- A mass spectrum is the plot of relative abundance of ions against their mass/charge ratio.
- The basic aspect of organic mass spectrometry consist of bombarding the vapour of an organic compound with a beam of energetic electron accelarated from a filament to an energy of 70 eV to form positively charged ions (molecular ions).
- The additional energy of the elecrons is dissipated in breaking the bonds in the molecular ion, which undergoes fragmentation to yield several neutral or positively charged species.
- The ions are then separated according to their mass to charge ration by virtue of magnet and are detected by detector

NMR

- NMR is the most powerful tool available for organic structure determination.
- It is used to study a wide variety of nuclei: › 1H › 13C › 15N › 19F › 31P
- Atomic weight Atomic number Spin number I Odd Odd/even ½,3/2,5/2… Even Even 1 or 0 Even Odd 1, 2, 3… NMR spectra
- A nucleus with an odd atomic number or an odd mass number has a nuclear spin.
    - The spinning charged nucleus generates a magnetic field.
    - When placed in an external field, spinning protons act like bar magnets.
    - The magnetic fields of the spinning nuclei will align either with the external field, or against the field.
    - A photon with the right amount of energy can be absorbed and cause the spinning proton to flip. Energy difference is proportional to the magnetic field strength.
    - This energy is provided through radio waves
    - If all protons absorbed the same amount of energy in a given magnetic field, not much information could be obtained.

- But protons are surrounded by electrons that shield them from the external field.
- Circulating electrons create an induced magnetic field that opposes the external magnetic field. Magnetic field strength must be increased for a shielded proton to flip at the same frequency.
- Depending on their chemical environment, protons in a molecule are shielded by different amounts.
- The number of signals shows how many different kinds of protons are present.
- The location of the signals shows how shielded or deshielded the proton is.
- The intensity of the signal shows the number of protons of that type.
- Signal splitting shows the number of protons on adjacent atoms.

TMS is added to the sample.

- Since silicon is less electronegative than carbon, TMS protons are highly shielded. Signal defined as zero.
- Organic protons absorb downfield (to the left) of the TMS signal.
- Measured in parts per million.
- Ratio of shift downfield from TMS (Hz) to total spectrometer frequency (Hz).

Same value for 60, 100, or 300 MHz machine.

Called the delta scale.

More electronegative atoms deshield more and give larger shift values.

- Effect decreases with distance.
- Additional electronegative atoms cause increase in chemical shift.
- Electrophoresis is the migration of charged particles or molecules in a medium under the influence of an applied electric field.
- Comprehensive term that refers to the migration of charged particle of any size in liquid medium under the influence of an electric field.
- Depending on kind of charge the molecule carry, they move towards either › To cathode › Or toAnode
- An ampholyte become positively charged in acidic condition and migrate to cathode, in alkaline condition they become negatively charge and migrate to anode.
- Commonly used for isolation of amino acids and proteins

- The rate of migration of an ion in electrical field depend on factors,

› Net charge of molecule › Size and shape of particle › Strength of electrical field › Properties of supporting medium › Temperature of operation.