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E-Content for Unit-V of M.Sc. Botany IInd sem on "Solvent Extraction, Paper Chromatography, TLC, HPLC, GC MS"

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SOLVENT EXTRACTION

Introduction: Solvent extraction, also called liquid extraction, is considered to be the most versatile and popular method of separation. The technique is based on principle that a solvate can distribute itself in a certain ratio between two immiscible liquids or solvents such as C_6H_6, $CHCl_3$ or CCl_4., In limiting case, the solute can be more or less, transferred in to the organic phase.

Solvent extraction technique can be used for purpose preparation purification, enrichment, separation and analysis of compound. It has come to the forefront in recent years, as it is elegant, simple, rapid and applicable at tracer and microgram concentration of metal ions.

TECHNIQUES FOR SOLVENT EXTRACTION

(a) Batch Extraction: - Batch extraction is used where a large distribution ratio for the desired separation is readily available. The solute is extracted by distributing it with a second immiscible layer in a separating funnel until partition equilibrium is attained.

(b) Stripping or back Extraction: - Stripping is the removal of extracted solid from organic phase to a more suitable medium for further determination. The metal ion can be back extracted into aqueous phase.

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(C) Continuous Extraction: - It is applicable when the distribution ratio is low. The method makes use of continuous flow of immiscible solvent through the solution to be extracted. Solute is removed continuously with the spent extracting solvent.

(d) Craigs counter current extraction (multiple extraction pioneered by L.C. Craig):- When two or more substances which are simultaneously extracted to different extent to be separated then counter current process is adopted. Here contact between phases occur in large number of discrete steps.

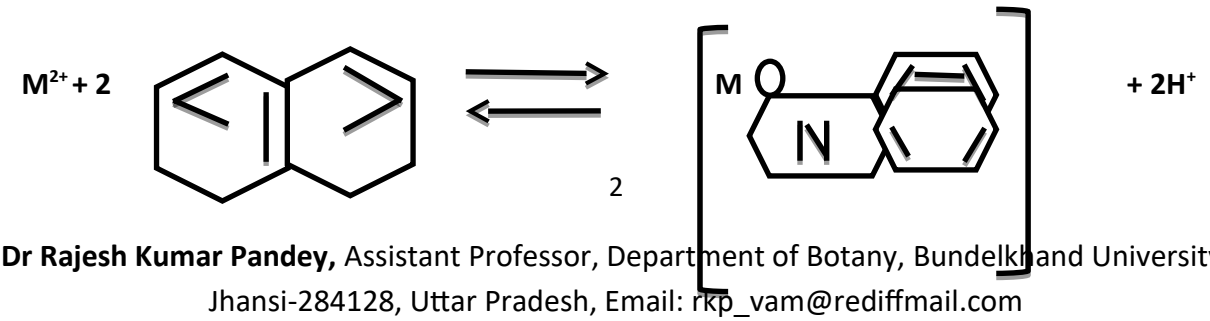
(e) Accelerated solvent Extraction: - It is a technique for the efficient extraction of analyses from a solid sample matrix in to a solvent. The sample and solvent are placed in a closed vessel and heated from 323 to 473k to accelerated the dissolution of analytic and its extraction.

(f) Microware assisted extraction (MAE):- A closed vessel containing sample solvent is placed in a microwave oven and heated by microvan energy. The kinetics of extraction is affected by temperature and it may reach to 423k at 175 psi. This technique reduces time.

APPLICATIONS

(1) Determination of Nikel by synergistic extraction:- Nikel is rapidly and quantitatively extracted using dithizone and 10 –phenanthroline over a broad pH (5.5 to 11.0) range to from a coloured mixture ligand complex having an absorption band at 520 nm. The complex is enough stable to permit the removal of excess dithizone by back extraction with 0.1 M NaOH.

(2) Metal extraction by oxine:- oxine is 8 hydroxy quinolone exist as zwitter ion trivalent metal ion from neutral complexes with three molecule of oxine while only two molecules or reagent are required for divalent metal ion e.g.- Cd, Cu, Ni, Mg, etc.



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The selectivity of extraction is enhanced by using EDTA or cyanides as masking agents**.**

(3) Determination of uranium using oxine:- Take up to 900 mg of uranyl nitrate at pH 8.8 in presence of EDTA. Wave length for maximum absorption is 400 nm.

(4) The solvent extraction has also been successfully employed to the following determination.

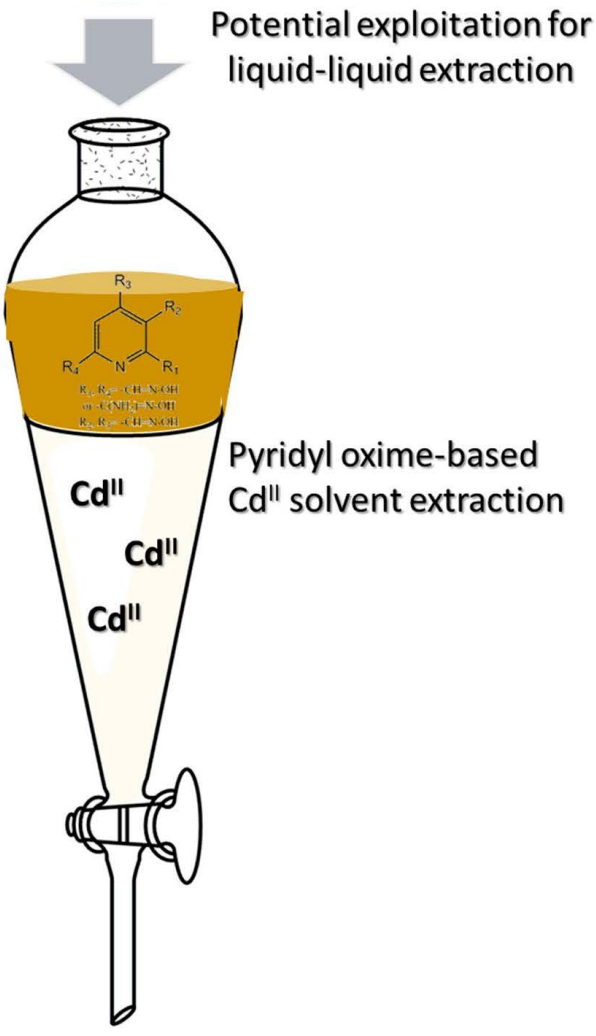
(i) Copper as diethyldithiocarbonate

(ii) Beryllium as acetylacetone complex

(iii) Molybdenum as thiocyanate complex

(iv) Lead as dithizone complex

(v) Copper as neo cuproin complex



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CHROMATOGRAPHY

General Principle & Techniques

1. Definition of Chromatography: -

Chromatography many be define as a method of separating a mixture of component in to individual components through equilibrium distribution between two phases. **Chromatography =chroma (Greek)** mean colour **+ graphy (Greek) mean** writing

Essentially chromatography technique is based on the difference in the rate at which the components of mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called moving phase)

The chromatographic method of separation in general involves following steps.

1. Adoration and retention of substance or substances on stationary phase.
2. Separation of adsorbed substance by mobile phase.
3. Recovery of separated substances by a continuous flow of mobile phase the method is called elution.
4. Qualitative and quantitative analysis of eluted substance.

2. Basic Principle of Chromatography: - The method of chromatography has revolutionized experimental organic chemistry over the part 30 – 35 years. These methods are by for more powerful of the techniques for separating mixtures and isolating pure substances.

“Chromatography can be defend as resolution of multicomponent mixture by distribution between two phases one stationary another mobile.

The various methods of chromatography are categorized by the phase involved calcium, this layer, and paper (solid –liquid) partition (liquid –liquid) and vapour phase (gas –liquid). The

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principal mechanism on which separating depends is different solubility or adsobitnity of the mixture component with respect to two phase involved.

PAPER CHROMATOGRAPHY

1. **Introduction: -** The credit for the present full pledged status of paper chromatography in the realm of separation techniques goes to Cambridge School of Works, A.J.P Martin and Coworkers R. Consden and , A.H. Gorden and R.L.M Single.

2. **Principle: -** The Technique is a type of partition chromatography in which the substances ore distributed between two liquids i.e. one is stationary phase (usually water and other is moving liquid or developing solvent and called moving phase the components of the mixture to be separated migrate at different rate and appear as spots at different points at the paper. In this technique a drop of the test solution is applied as a small spot on a filter paper and the spot is dried. The paper is kept in a close chamber and the edge of the filter paper is dipped in to a solvent called developing solvent as soon as the filter paper gets the liquid through its capillary axis when it reaches the spot of the test solution; the various substances and moved by solvent with various speeds. When the solvent has moved these cations to suitable heights (15–18cm) the paper is dried and various spots are visualized by suitable reagents called visualizing reagents. The movement of substances relate to solvent is extracted as in terms of Rf or (RF) valves.

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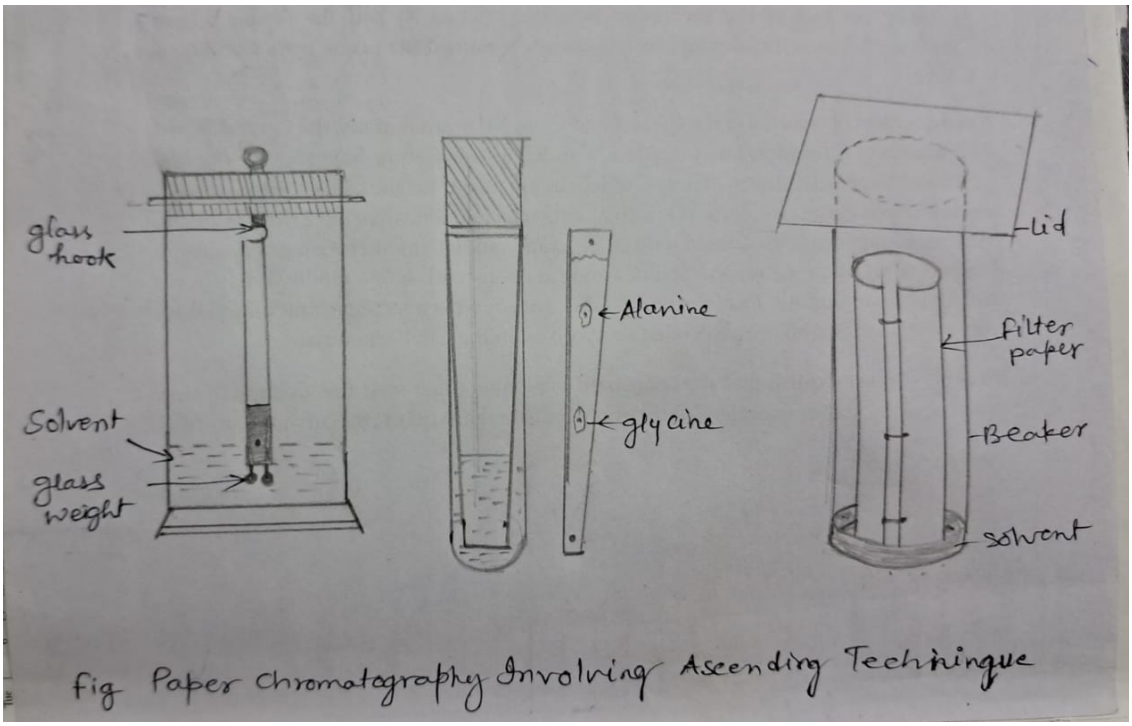
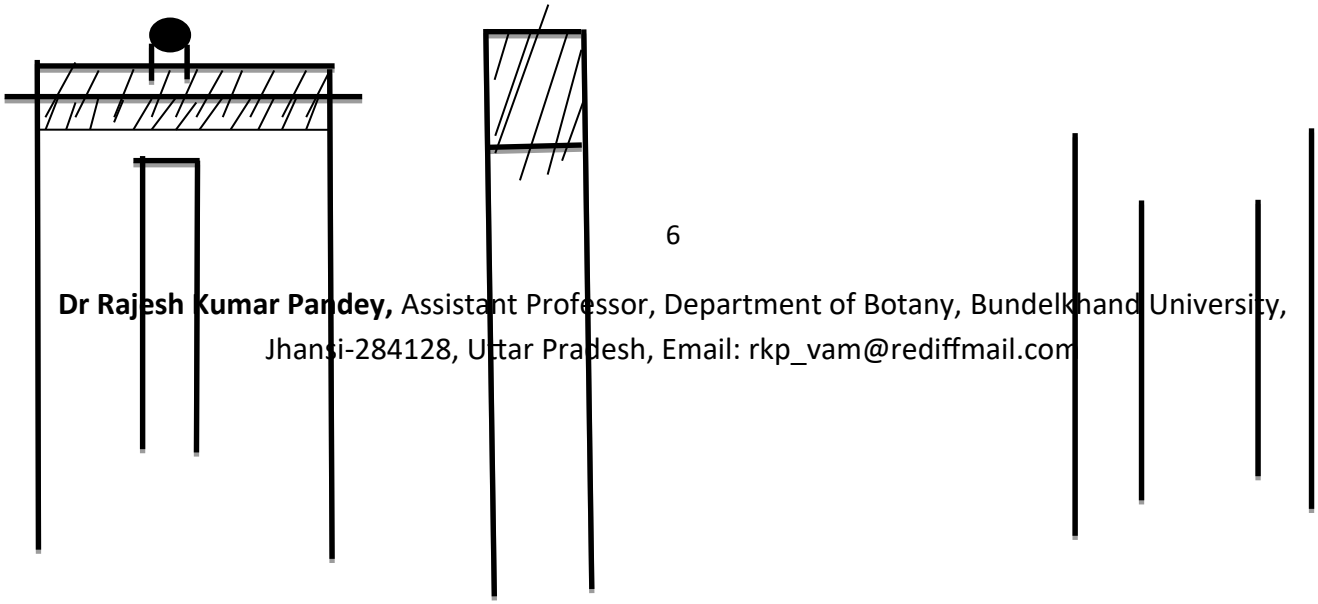


fig Paper Chromatography Involving Ascending Technique



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3. Types of paper chromatography

(a) Descending chromatography

(b) Ascending chromatography

(c) Ascending chromatography

(d) Radial paper chromatography or Circular chromatography

4. Application of paper chromatography

The technique of paper chromatography has revolutionized bio chemistry where analyses with vanishingly. Small sample volumes are region.

. Paper chromatography can be used for identification, separation of compound

. Paper chromatography is also used in purification process.

. In examination of reaction.

. High speed of separation.

. Easy process.

. Useful for most of chemical compounds.

Glass plate solvent form

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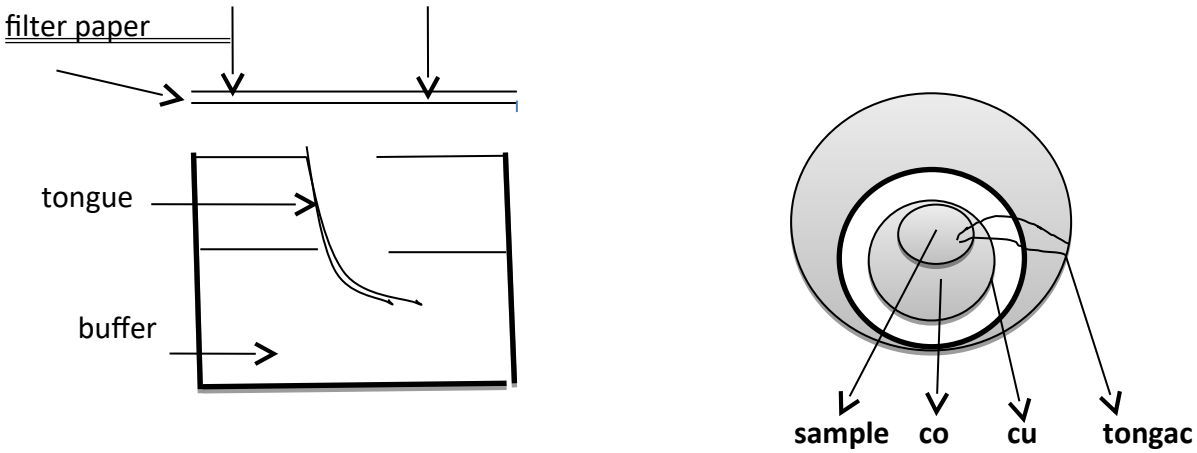

Radial paper chromatography

THIN LAYER CHROMETOGRAPHY

Thin layer chromatography is a technique where the components of mixture are separated by differential migration through a planner bed of a stationary phase, the mobile phase flowing by virtue of capillary forces. The solutes are detected in site on the surface of the thin layer plate by visulising reagents after the chromatography has been completed. T.L. C is similar to paper chromatography, but stationary phase is finely divided sorbent spread as thin layer on supporting flat plastic.

A typical T.L.C. Procedure

A typical TLC procedure consist of the following steps.

I. The sufficient mobile phase to provide about a 0.5 am depth of liquid is poured in to a development tank, or chamber which is then covered and allowed to stand for several minutes to allow the atmosphere in the tank to become saturated with solvent vapor.

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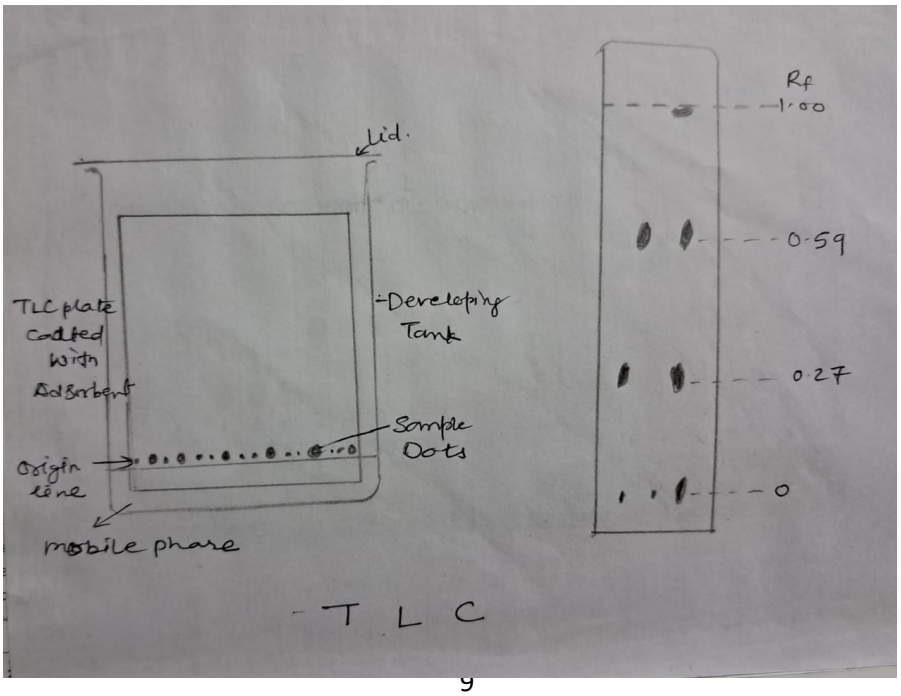
II. Small volumes of liquid samples and standards, or solution are spotted on to the sorbent surface of a TLC plat along a line close to and parallel with one edge. This plate is the positioned in the tank with this edge in contact with the mobile phase and the cover is placed.

III. The mobile phase in drawn through the bed of sorbent from the edge of plate principally by capillary action and this development process is halted shortly before the solvent front reaches the opposite site of the plate sample component and standard migrate in parallel path in the direction of flow of the mobile phase. Separating in to discrete zones or spots.

IV. The plate is removed from development tank dried in the current of warm water solute spots are located by a approximate methods.

V. Each solute is characterized by the distance migrated relative to the solvent front i.e. its Rf value which is lie between 0 and 1 and unknowns are identified by companion with standards run simultaneously.



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APPLICATION OF T L C

The various application of TLC are as follows.

(a) As a check on process: - TLC has been used checking the other separation producer and purification process. It has also been checking of distillation process and checking for progress of purification of molecular distillation.

(b) In organic chemistry: - The main use of TLC lies in the isolation and separation of individual component of mixture the main reason for popularity of TLC as an analytical and preparation are as follows.

- **i.** It can be used for many compounds.
- **ii.** High separation speed.
- **iii.** High selectivity.

(c) Application in inorganic chemistry: - For checking the purity of sample.

- **(i) For checking the purity of sample**
- **(ii)** As a purification process**.**
- **(iii)** Examination of reaction.
- **(iv)** For identification of organic compound.
- **(v)** TLC can also be used for charactering and isolating a wind range of oraganic compounds such as alcohols, acid, glycols, alkaloids, amines, amino acid, and proteins.

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COLUMN CHROMATOGRAPHY:

Introduction: - it was developed by American petroleum chemist D.T. Day 1900, M.S Tswett the polish botanist, in 1906 used adsorption column in his investigation of plant pigments. It was not unit about 1930 that the method was used extensively by chemists, column chromatography is known as adsorption chromatography.

Principal: - It is known that rate of adsorption varies with given absorbent, for different materials. This principle of different materials. This principle of selective adsorption is used in column chromatography.

In this method the mixture to be separated is dissolved in a suitable solvent and allowed to pass through a tube containing absorbent the component which has greater adsorbing power is adsorbed in the upper part of the column. The next component is adsorbed in the lower portion. of the column which has lower adsorption power.

Then the first component, this process continued as a result, material is partially separated and adsorbed in the various parts of the column. Separation of component can be improved. By

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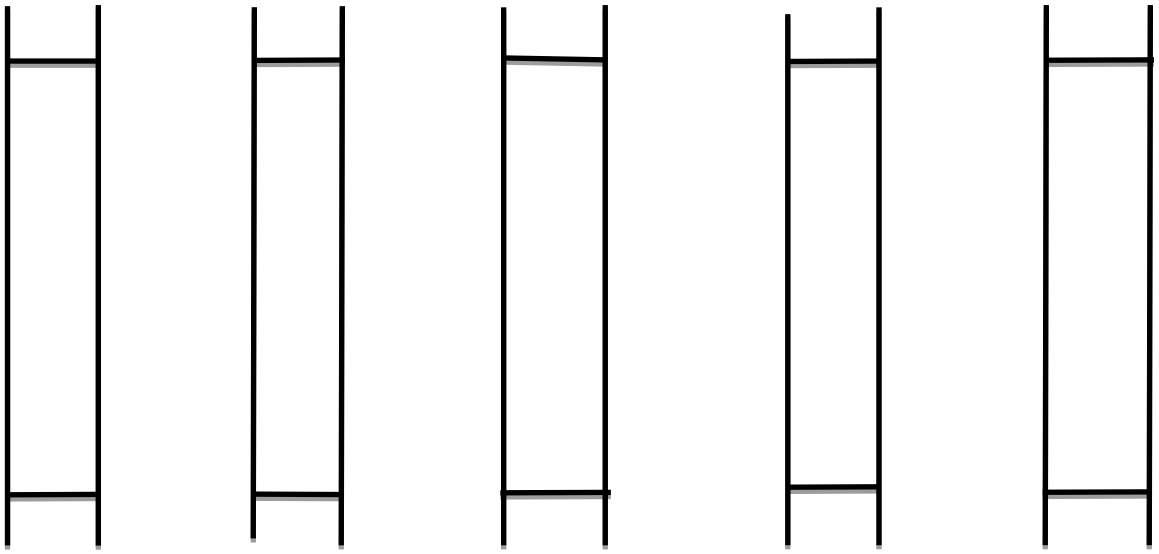
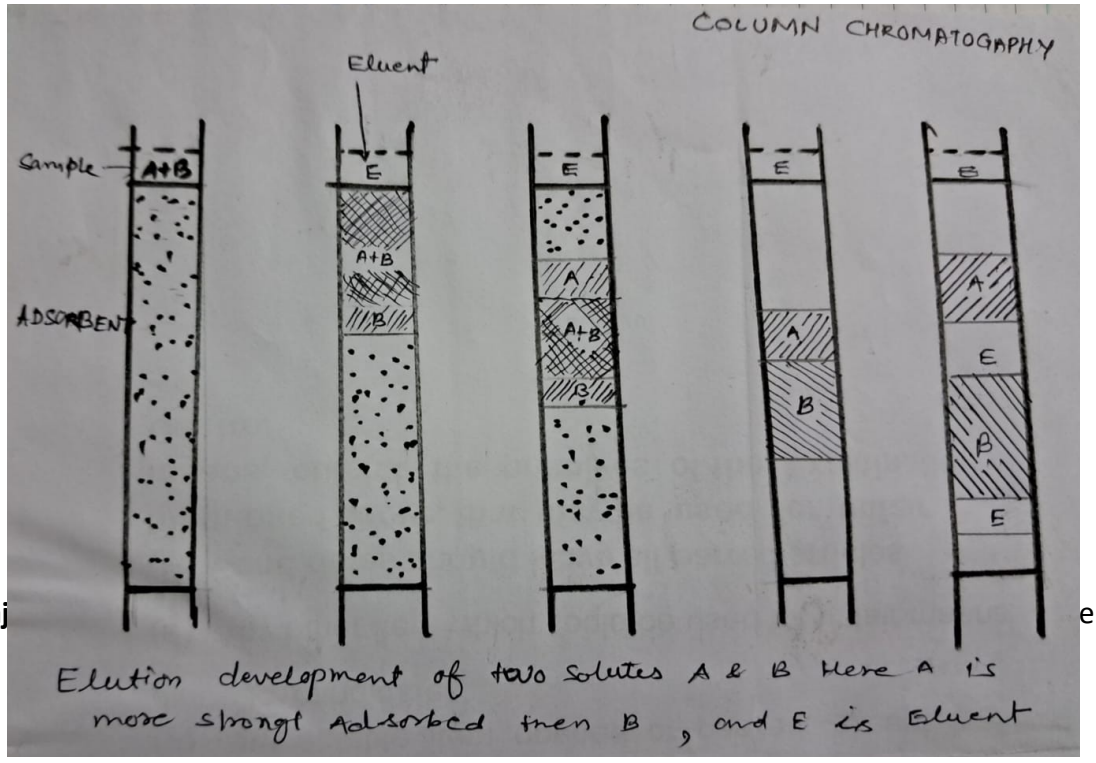
passing either the original or some other suitable solvent, which leads. the formation of more defined bands. A banded column is component can be separated these are known as eluent.





3. Experimental details

(a) Apparatus: - A simple straight glass tube tapered at bottom and often with a topic is still commonly used the ratio of length to diameter should be greater than 20:1 a ratio of 40:1 is taken as standard this tube is 20 -30 cm long and 2 -3 cm in diameter. This may hold 50 - 100 of adsorbent and may retain several grams of adsorbate.

(b) Adsorbents

General requirement: - In order to obtain filtration properties, the adsorbent should have following characteristics.

1. Their particles should be spherical in shape and uniform in size.
2. Their mechanical stability must be great enough. To prevent formation fine dust might be deposited in the chemical of the packing.

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3. They should not recent chemically either with the eluting solvent or with sample solvents.
4. They should contain as small amount of soluble component as possible.
5. They should be catalytically inactive and as a rule have natural surface. How ever exception ore ion exchangers.

Factor affecting column Efficiency

Various factors are described as follows.

I. **Nature of solvents: -** Solvent of low viscosities, are generally used for high efficiency separations the reason for this is that of rate of flow. in inversely proportional to viscosity and hence it becomes necessary to select a solvent of lowest viscosity and proper elution strength.

II. **Dimensions of columns: -** It is possible to improves the column efficiency by increasing the length width ratio of the column for common preparative separation sample column packing rations have found to range from 1:20 to 1:100.

III. **Particle diameter of column:** -Generally polar, adsorbent possess a pore diameter of ≤ 20 Å according to kpen decrease in average in a pore diameter from 170 Å -20 Å does not affect efficiency.

IV. Temperature of the column: Difficult soluble sample are generally separated at higher temperature while other sample are separated at room temperature.

Application of Column Chromatography

(a) Analytical use: - Glass or copper made capillaries are used for analyzing sample.

(b) Separation of geometrical isomer: - Winterstein reported the first chromatography separation of cis/ trans isomer of bixin and crocetin dimethyl ester.

(c) Separation diastereamers :- In some case a dcriating having an optically active partner cannot be separated from latter. However, such separation has been realised by column chromatography on various adsorbents.

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(d) Separation of tautomeric mixture: - Component of tautomeric mixture requires high temperature for separation which is not possible in gas chromatography so in such cases column chromatography is widely used.

(e) Separation of racemates: - Racemic mixture can also be separated by column chromatography.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Introduction: - HPLC has its origin in column chromatography. Much higher effectiveness and hence better reductions are achieved in HPLC through the use of smaller particle of stationary phase and pumping of the mobile phase through the column under pressure for achieving high flow rates.

Principle: - Microgram to gram quantities of mixture can be separated by passage sample by means of a pressurized flow of a liquid mobile phase through a column. Containing a stationary solid phase components mixture migrate through the column at different rate due to different relative affinities for the mobile and stationary phase based on charge, size or adsorption.

Apparatus and Instrumentation: - Solvent delivery system, injector, stainless steel column pump, detector and recorder, most of them are manufactured from inertness as they come in contact with mobile phase during operation.

Application: Complementary to gas chromatography it is used largely for separation of non-volatile substance including ionic and polymeric sample it is used in pharmaceutical bio chemical, food, products forensic chemistry and pesticide industries, it is also extremely helpful in clinical environmental studies.

Disadvantages of HPLC: -

I. Universal detection system not available.
II. Column performance very sensitive.
III. Setting of the packed bad.
IV. The accumulation of particulate matter or strongly adsorbed material at the top.

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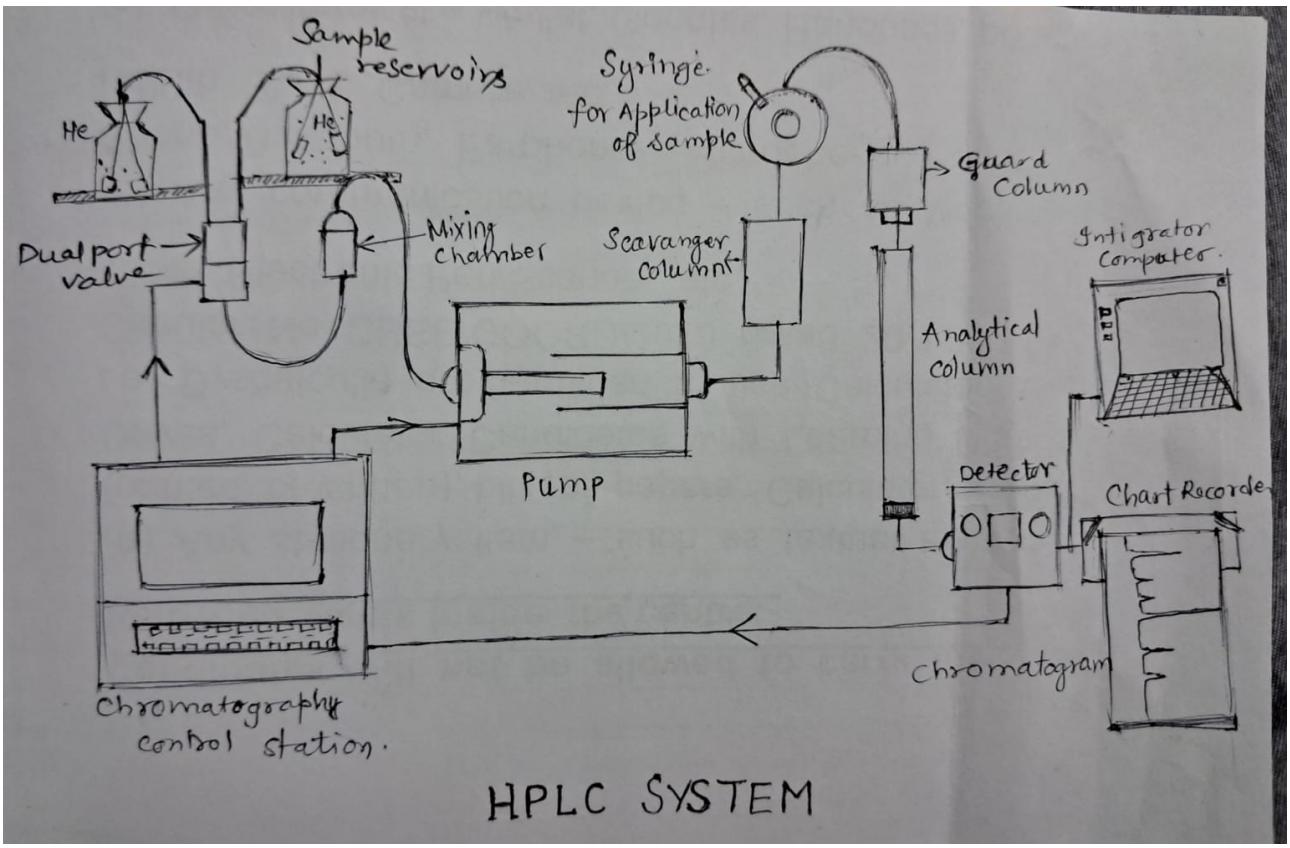

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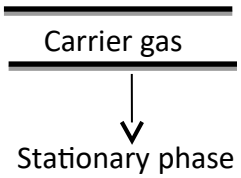
GAS – CHROMATOGRAPHAY (GC)

Introduction: - GAS chromatography is so called because mobile phase is a gas in of two types viz gas liquid chromatography (G.L.C) and gas solid chromatography (G.S.C) depending upon whether the stationary phase is a liquid or solid respectively. Thus, for G.L.C stationary phase is liquid the sorption process is predominantly one of partition. For G. S. C the stationary phase is solid as adsorption phase the major role.



Principle: -

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When the vapour of sample in gas stream is allowed to pass through a column containing stationary phase as liquid or solid component of mixture nitrate with different rate due to difference in B.P, solubility or adsorption microgram quantity.

Apparatus & instrumentation: - A gas chromatography consists of following important component like, regulator, injection part, glass or fused quartz column, thermostat detector, and recorder.

These components are joined together to.

I. Provide constant flow of mobile phase.
II. Permit to the introduction of sample phase in to flowing mobile phase.
III. Contain the sufficient length of stationary phase.
IV. Maintain column at appropriate temperature.
V. Detect the sample component when they elute out from the column.
VI. Provide a readable signed proportional in magnitude to the amount of each component.

Application of Gas Chromatography

I. Gas chromatography is suitable is separation of thermally stable volatile organic and inorganic components.
II. It is used for analysis of gaseous sample like , solution and volatile solution.
III. It can be used for preparation of pure substance or narrow fraction as standard for further investigation.
IV. On an industrial scale, it can be utilized for elemental analysis of organic compounds.
V. It can be used to determine specific surface area in adsorption study.
VI. Recently, it has been utilized for element analysis of organic compounds.

A few examples of application of gas chromatography are given as follow.

(a) **In food industry**: - G.C can be use in analysis of fruit juice, wines, bears, beverages oil, dairy product etc. The determination of antioxidants food preservations, decomposition, products contamination of adulterant are rationally used.

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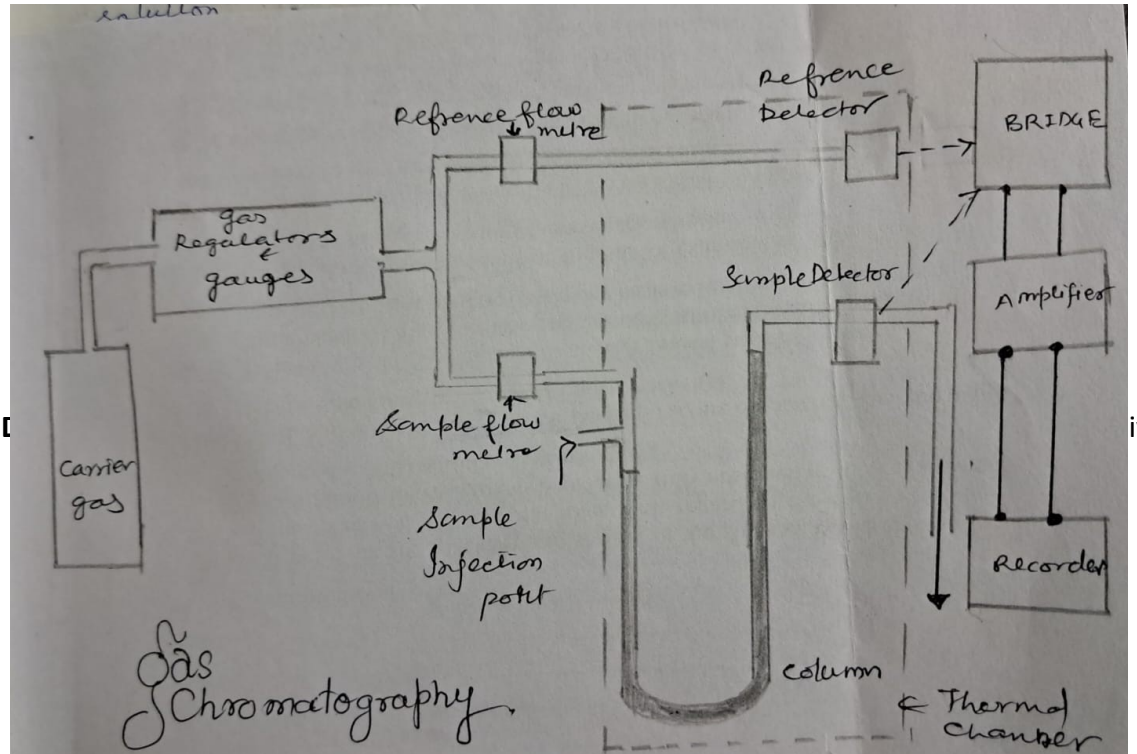
(b) **Drug and pharmaceuticals**: - For quality control, analysis of drugs and medicine, for monitoring of metabolites in biological system.

(c) **Petroleum industry: -** Gas chromatography is successfully used for separation determination of many compounds in petroleum product.

(d) **Clinical chemistry:** - Blood, urine, biological fluids can be analyzed for protein, carbohydrate, amino acids, steroids, vitamins, barbiturates either directly or after preparation of appropriate volatile derivatives.



D iversity,

TECHNIQUES AND PRINCIPLE

THERMAL ANALYSIS

These methods are based upon the measurement of the dynamic relationship between temp. such as mass (m), change in mass (Δm) heat of reaction etc.

The most important technique is given below. T.G.A, D.T.A. and D.S.C.

Thermogravimetry analysis. (T.G.A.): - It is a technique where by mass of a substance is recorded continuously as the temp. is linearly increased from room temp. to a temp as high as 1200^0C.

Instrumentation: - The main component of T.G.C are shown in the form of black diagram in figure and are .

I. Analytical thermobalance for the measurement of mass with reproducibility of order of ±10 microgram.
II. Furnace linked with microprocessor-controlled power source, generally programmed to increase the temp. linearly at a predetermined rate of 0.5 -25^0C/min from room temp to 1200^0C.
III. Environmental control equipment for providing inert atmosphere for sample where ever desired.
IV. A recorder that given a graph of “m” as a function of “T”

Application: -

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In quantitative analysis

In qualitative analysis.

Differential thermal analysis D.T.A

D.T.A employs a similar type of furnace heating programmer and recording device as employed in T.G.A. but the furnace of D.T.A. contains two chambers which are identical and symmetrically located and are connected with temperature sensor. The block diagram of data D.T.A. is shown in Figure.

Instrumentation & function: - The sample is placed in one chamber and material such as α-Al_2O_3 is placed in the other chamber temp. of furnace and two chamber are then gone linearly increased at the rate of 5^0C to 120^0C/minute. The difference in temperature between sample (S) and furnace (R) ($\Delta T=T_S-T_R$) is continuously measured a function of T, usually reference temp. T_R for the temp., measurement temp. Sensor is directly placed in the sample or attached to the chamber, so we get the highest thermometric accuracy in DTA of the thermal method.

Because the sample undergoes transition with liberation or absorption of energy hence the deviation of sample. Temp. from that of reference tells the analyst whether the transition was exothermic or endothermic and the temp of transmitter.

Application:-

- **I.** In practical chemistry.
- **II.** Generalization of phase diagram & phase transition.
- **III.** For determination of specific heat and heat of reaction.
- **IV.** For the determination thermal diffusity.
- **V.** In analytical chemistry.
- **VI.** In organic chemistry.

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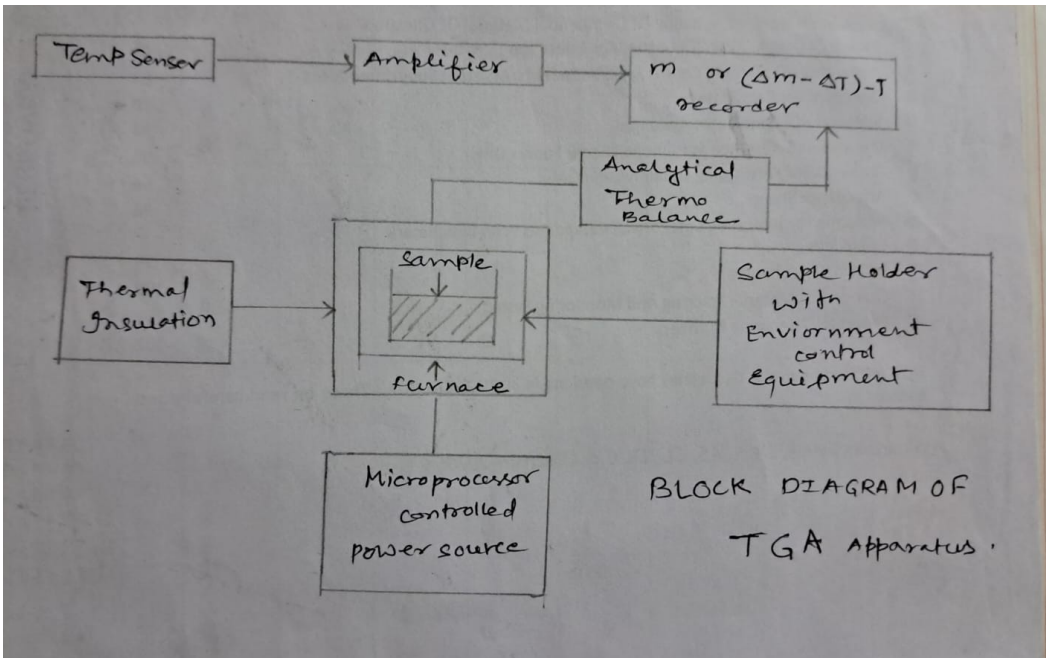
VII. In inorganic chemistry.



DIFFERENTIAT SCANNING COLORIMETRY DSC

The DSC, the heat energy is supplied at varying rate of the sample or reference as to keep their temp. equal and this heat energy is recorded as a function of temperature. Or time which both substance and reference material are heated or cooled at a predetermined rate.

Instrumentation: - Most of the components identical of DTA apparatus. Except that it contains individual heater for sample and reference material whenever there is a difference b/w sample and reference material due to exothermic and endo thermic process (transition) in a sample heat is supplied from secondary power unit is to the cooler of the two so that temp equally is restored.

Block diagram of DSC apparatus it shown in figure.

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It is to be noted that DSC thermogram is similar in appearance to DTA curve with only difference that Y axis has dh/ dt instead of st.

The area wider a DSC peak can directly be related to the enthalpy change occurring.

Application: -

I. Enthalpy of transition such as melting, crystallization and fusion by polymeric materials can be measured DSC.

II. Percentage of crystallization of polymeric material can be measured by DSC.

III. % crystallization = $\frac{\Delta H_f \text{ sample}}{\Delta H_f \text{ (100\% crystalline polymer)}} \times 100$

IV. Purity of drug sample can be determined within 1%,

V. The melting, boiling & decomposition points of organic compound can be conveniently and accurately determined by DSC.

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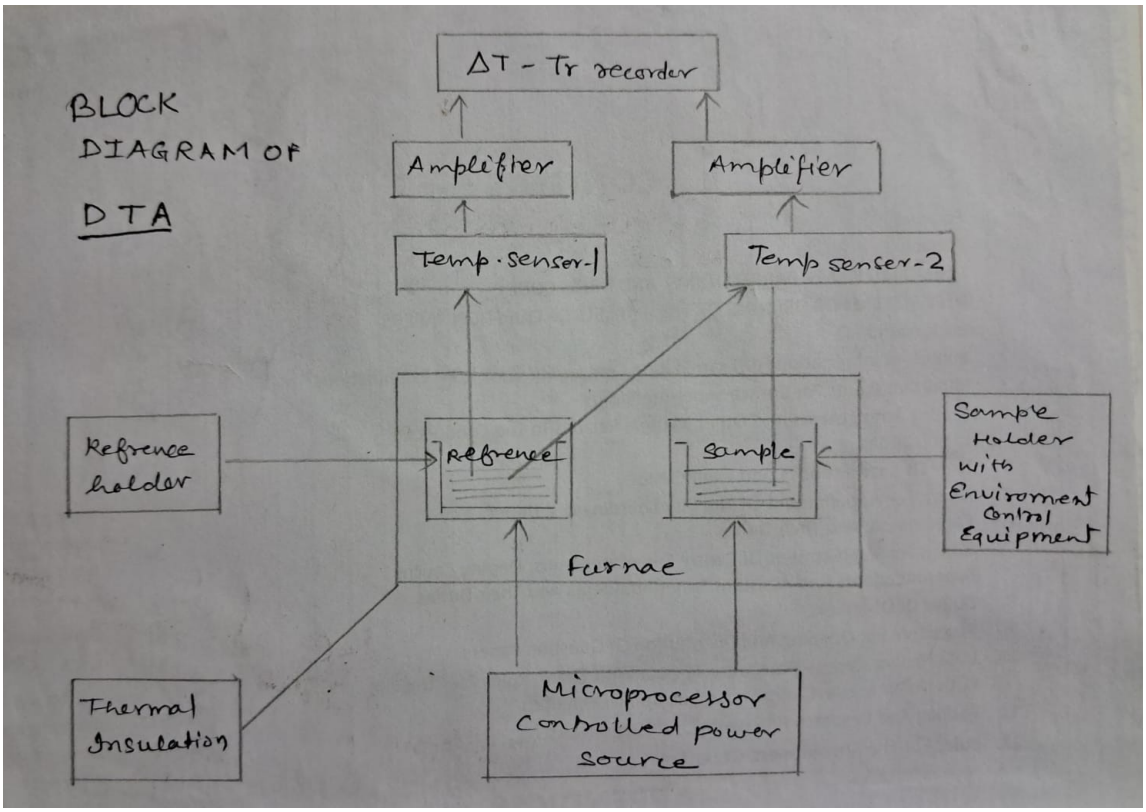

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