2.3 Chromatography

Chromatography is an analytical technique for separating compounds on the basis of the differences in their affinity for a stationary phase and a mobile phase.

Principles

Adsorption chromatography

In adsorption, the binding of a compound to the surface of the solid phase takes place. Adsorption chromatorgraphy is a technique in which small differences in the adsorption behaviour of substances between a moving solvent (liquid or gas) and a stationary solid phase are used to separate them. When the moving phase is a liquid it is called liquid - solid chromatography or adsorption column chromatography. When the moving phase is a gas it is called gas - solid chromatrogarphy (GSC).

Partition chromatography

In partititon, the relative soubility of a compound in two phases result in the separation of the compound. Partition chromatography is a technique in which mixture of substnaces are separated by means of partition between the moving solvent and a stationary liquid, which is held on a suitable solid support. When the solvent (moving phase) is a liquid it is called liquid - liquid chromatography. When the solvent (moving phase) is a gas, the technique is called vapour chromatography or gas - liquid chromatography.

In the liquid - liquid chromatography, the solid support for the stationary liquid is provided by either cellulose or most silica gel. This solid support may be in the form of this sheet. Such a technique is called paper chromatography (PC). The solid support may be thin layers, then it is called thin layer chromatography (TLC). The solid support may be a packed column, then it is called partition column chromatography (PCC). The stationary liquid phase in all the above techniques is water.

Ion exchange chromatography

Ion exchange is a process in which an interchange of ions of like sign takes place between a solution and an insoluble solid (ion exchange) in contact with the solution. This process is utilized to separate a mixture of

ions. Here a reversible exchange of ions takes place between ions in a liquid phase (mobile phase) and an ion exchange resin (an insoluble substance containing ionic sites) which is the stationary phase.

1. Column Chromatography

Column chromatography is defined as a separation process involving the uniform percolation of a liquid solute through column packed with finely divided material.

Principle :

The separation in the column is effected either by direct interaction between the solute components and the surface of the stationary phase or by adsorption of solute by the stationary phase. Column chromatography involves adsorption, partition or ion exchange phenomena. In adsorption column chromatography, the substances are preferentially adsorption by the adsorbent packed in the column. In partition column chromatography of components of a mixture distribute themselves in different ratios between two different solvents and thus get separated. In this method the column is packed with silica gel or cellulose which contains significant amounts of water. In ion exchange column chromatography, ions are exchanged between the mobile phase and stationary phase.

1. Adsorbents :

Characteristics :

A good adsorbent should posses the following characteristics.

i. The adsorbent must not react with materials under investigation.

ii. The adsorbent's colour should not interfere with the colour of the chromatogram. Preferably it should be colourless.

iii. The adsorbent should be insoluble in the solvent used.

iv. The physical and chemical properties should not change under the experimental conditions.

v. ...hould be fine enough to told the substances and give sharp bands. It ...ld not be so fine that passage through the column is greatly retarded.

...na and magnesia give satisfactory separation with most of the mat... ...lumina has higher selective power. It gives sharper and narrower band...

Classification of absorbents :

Adsorbents may be cl... chromatography in which they...

1. *Adsorbents used in ads...*
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2. *Adsorbents used in pa...*
Adsorbents used in thi... silica gel, calcite, kieselguh... the stationary liquid phase.

3. *Adsorbents used in io...*
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i. Sulphonated pol...

ii. Quarternary amo...

Requirements of goo...

i. It should not react ... used. It should be i...

ii. It should not catalys... or the solvent.

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iv. It should be colour... of the components...

2. Solvent and the...

The separation in ... phenomena. In a chron... for...

Classification of absorbents :

Adsorbents may be classified according to the type of column chromatography in which they are used. They are as follows :

1. *Adsorbents used in adsorption column chromatography* should be activated. For example, the widely used adsorbent is aluminium oxide or alumina. It is activated by heating it to about 200°C in current of air or CO_2 and is used in adsorption coloumn chromatography. Other adsorbents used are magnesium oxide or magnesia, magnesium carbonate, calcium carbonate, calcium sulphate, barium carbonate, charcoal, sucrose, talc, starch, cellulose and Fuller's earth. The Inorganic adsorbents are activated by heating them to 200-220°C.

2. *Adsorbents used in partition column charomatography :*
Adsorbents used in this technique should be inert, e.g. Cellulose, starch, silica gel, calcite, kieselguhr etc. The role of adsorbent here is just to support the stationary liquid phase.

3. *Adsorbents used in ion exchange column chromatography :*
The adsorbents used in this technique are ion exchange resins. They are either cation exchange resins or anion exchange resins. E.g.,

i. Sulphonated polystyrene
ii. Quarternary amonium compounds.

Requirements of good adsorbent in column chromatography :

i. It should not react with the substances to be separated and the solvent used. It should be inert.

ii. It should not catalyse the decomposition of the substance to be separated or the solvent.

iii. It should not dissolve in the solvent to be used.

iv. It should be colourless so that its own colour does not mask the colours of the components of the mixture to be separated.

2. Solvent and their role :

The separation in a column involves adsorption, partition and exchange phenomena. In a chromatographic separation, different solvents may be used for

i. Placing the solute on the column
ii. Developing the chromatogram and
iii. eluting the adsorbed materials.

But in practice a single solvent is used for all the above said purposed. Some times two or more solvents are used. The solvent must be pure. Non polar solvents effect better separation. Therefore they are used to prepare the solutions of mixtures. The development should be carried out with a some what polar solvent. Even more polar solvent should be used for elution. Some common solvents are given below in the increasing order of their polarity : petroleum ether < carbon tetrachloride < cyclohexane < carbon di sulphide < ether < acetone < chloroform < alcohols < water < pryidine < organic acids. In ion exchange column chromatography, the solvent is water. The selective desorption of ions is carried out by altering the pH or concentration of ions in the eluting solvent.

3. Preparation of column :

The adsorption column can be or almost any size, shape and length. Usually, the column is made of thin walled pyrex glass tube 5 - 20 cm long and with a diameter of 1 cm. One end of the tube is either drawn out or it is closed with a rubber stopper. Thus powdered adsorbent is retained. The liquid passes through.

The column is packed with dry powder. Wet packing is more common. Wet packing has certain adventages everyday packing. For wet packing, the column is clamped in a vertical position. A thick slurry of the absorbent in a suitable medium is poured through the open gradually and allowed to settle under gravity until a column of desired height is obtained. The tap at the lower end is then opened to allow the liquid to run out until it just covers the top of the medium. In the dry packing, the dry powdered adsorbent is introduced through the open end. Suction is applied from the bottom. The column is tapped with light object like a pencil until no more settling takes place. The top should be solid and unbroken.

The efficiency of column depends on

i. The particle size of the solid adsorbent. It should be uniform. It should be uniform. It should not be too fine to affect the rate of separation or two coarse so that separation becomes irregular.

ii. It should have high specific area. This helps attainment of equilibrium of solute between stationary and mobile phase.

iii. There should be no air gaps. Air gaps lead to mixing of separated zones and allow the soultion of the mixture to pass through without effecting separation of the mixture.

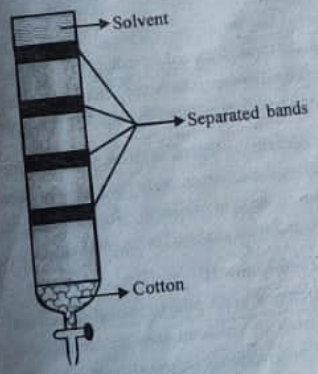
Adsroption :

We know that the separation of a mixture by column chromatography involves adsorption, partition or ion - exchange. The method of separation is the same for all the three types. The difference is that the column must be packed suitabley.

A solution of the mixture to be separated is prepared in a relatively non-polar solvent. It is introduced into the column in a stepwise manner with the help of a pipette. The stop clock at the bottom is opened slightly to allow the solvent to run out until a small amount of the solution remains in the column covering the top of the packed material. First the components of the mixture are adsorbed at the top of the column.

4. Development of Chromatogram

When all the solution has been poured, the developing solvent is then introduced. It is allowed to flow steadily through the column.



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... etc.,

3. ***Hydrophobic solvents :***
A hydrophobic solvents also can be used E.g., Kerosene, hydrocarbons, dimethyl formamide etc.

Mobile phase :
Several combinations are possible for a mobile phase. Mixtures of two, three or more solvents, solution of salts, buffers etc., are generally used E.g.,

1. Isopropyl alcohol, water and ammonia mixture in the ratio 9:2:1.
2. n - Butyl alcohol, water and acetic acid mixture in the ratio 4:5:1.

The choice of solvents depends on the nature of the substance to be separated. The choice is usually made by refering to the literature or by trail and error method.

Mechanism :
In this method, the dissolved substances are applied as a small spot on cellulose bound filter paper. The paper is then dipped into a vessel containing the mobile phase. The mixture are partitioned between the solvent held on the paper (stationary phase) and organic solvent (mobile phase). The separation is effected by the differential migration of the mixture of substances.

Two types of forces operate when a drop of solution is applied on the filter paper and treated with a solvent.

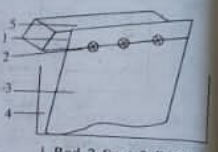
i. The propelling force drags the substance in the direction of the flow of the solvent. The propelling force depends on the solvent flow and the solubility of substances in the solvent. The compound with higher solubility will move rapidly along the paper than the less soluble one. This leads to a separation.

ii. The retarding force drags the substance behind towards its point of application. This retardation depends on the adsorption and partition. Thus, when a drop of solute is treated with the solvent on the strip of a paper the more strongly adsorbed component adsorbed will move along the paper with the slovent. The process of partition is also operative on the paper. The cellulose of the filter paper always contains a small amount of water. Partition of the substance takes place between water in the cellulose and the mobile organic solvent. This also causes separation of substances.

The paper is made into a cylinder. The two ends are clipped together. It is then placed into the solvent tank (figure). The tank is always closed with a lid. The solvent moves up the paper. The solute is partitioned between water in the filter paper and the solvent. An equilibrium is established between the two phases.

ii. The descending technique :

Here, the solvent is placed at the top of the tank. The paper is hanged such that solvent flows down the paper. A small amount of the solvent is placed at the bottom of the tank to replace air inside the tank with the vapour of the solvent. In this method, the solvent moves down the paper. Since the solvent can be allowed on run off the paper, the separation can be improved by increasing the length of the paper (figure).



1. Rod, 2. Spot, 3. Paper, 4. Trough, 5. Solvent

iii. Radial or disc development :

This technique is used in special cases. In this method a circular paper is marked with a pencil as shown in figure. A wick is cut parallel to the radius from the edge to the wick in the centre. The sample solution is applied at the upper end of the wick in the centre. The paper is dried. It is placed over a petridish containing the developing solvent with the wick dipping in the liquid.

The solvent flows through the wick to the sample spot and carries the solute with it. Thus the different components of a mixture are separated. This technique is called circular chromatography.

f. Drying the chromatogram :

After the solvent has moved a certain distance for a certain time, the chromatogram is taken out from the tank and the position of the solvent front is marked with a pencil. The chromatogram is now dried by blowing hot air from a hair dryer or by any other suitable method.

g. Location of amino acids :

A 300 mg of ninhydrin dissolved
spots. Different amino acids appear
and compared with the literature for

Thus the mixture of amino aci

Radial paper chromatography

In this technique the equi
Chromatographic techniques excep
the circular disc (see the previous p

Electrochromatography (Pap

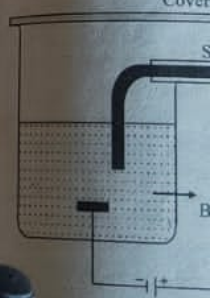
This is a technique to separa
placed on a strip of paper, by passi

Principle :

Under the influence of applied
different speeds and thus get sepa

Procedure :

The apparatus is set up as sho



SAP = Sample applicatio
BS = B

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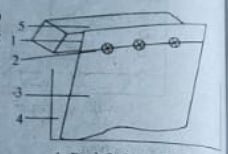

1. Rod, 2. Spot, 3. Paper, 4. Trough, 5. Solvent

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r suitable method.

6. ***Location of amino acids :***
300 mg of ninhydrin dissolved in 100 ml acetone is used as the locating agent. Different amino acids appear as blue spots. The R_f values are recorded and compared with the literature for identification.

Thus the mixture of amino acids is separated and identified.

Radial paper chromatography (Circular chromatography)

In this technique the equipment consists of two glass plates Chromatographic techniques except that the development is done by using the circular disc (see the previous page).

Electrochromatography (Paper electrophoresis) :

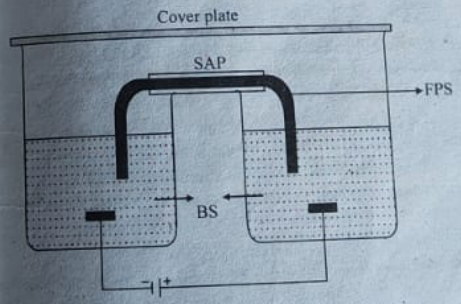
This is a technique to separate the components of a mixture from its placed on a strip of paper, by passing current.

Principle :

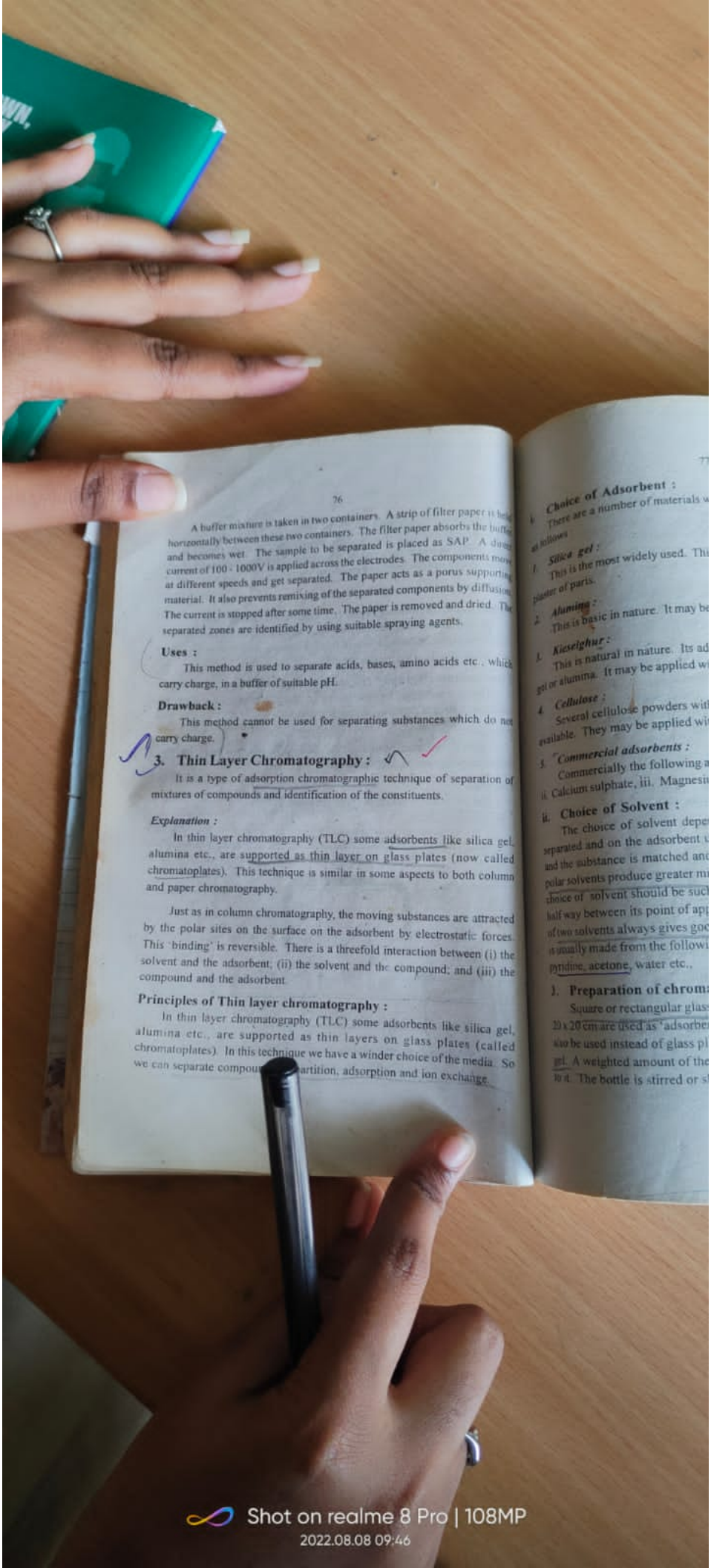
Under the influence of applied field, the components in a mixture move at different speeds and thus get separated.

Procedure :

The apparatus is set up as shown in figure.



SAP = Sample application poin, FPS = Filter paper strip
BS = Buffer solution



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A buffer mixture is taken in two containers. A strip of filter paper is held horizontally between these two containers. The filter paper absorbs the buffer and becomes wet. The sample to be separated is placed as SAP. A direct current of 100 - 1000V is applied across the electrodes. The components move at different speeds and get separated. The paper acts as a porus supporting material. It also prevents remixing of the separated components by diffusion. The current is stopped after some time. The paper is removed and dried. The separated zones are identified by using suitable spraying agents.

Uses :

This method is used to separate acids, bases, amino acids etc., which carry charge, in a buffer of suitable pH.

Drawback :

This method cannot be used for separating substances which do not carry charge.

3. Thin Layer Chromatography :

It is a type of adsorption chromatographic technique of separation of mixtures of compounds and identification of the constituents.

Explanation :

In thin layer chromatography (TLC) some adsorbents like silica gel, alumina etc., are supported as thin layer on glass plates (now called chromatoplates). This technique is similar in some aspects to both column and paper chromatography.

Just as in column chromatography, the moving substances are attracted by the polar sites on the surface on the adsorbent by electrostatic forces. This 'binding' is reversible. There is a threefold interaction between (i) the solvent and the adsorbent; (ii) the solvent and the compound; and (iii) the compound and the adsorbent.

Principles of Thin layer chromatography :

In thin layer chromatography (TLC) some adsorbents like silica gel, alumina etc., are supported as thin layers on glass plates (called chromatoplates). In this technique we have a winder choice of the media. So we can separate compou... partition, adsorption and ion exchange.

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i. Choice of Adsorbent :

There are a number of materials w... as follows

1. ***Silica gel :***
This is the most widely used. Thi... plaster of paris.
2. ***Aluming :***
This is basic in nature. It may be...
3. ***Kieselghur :***
This is natural in nature. Its ad... gel or alumina. It may be applied w...
4. ***Cellulose :***
Several cellulose powders wit... available. They may be applied wi...
5. ***Commercial adsorbents :***
Commercially the following a... ii. Calcium sulphate, iii. Magnesi...

ii. Choice of Solvent :

The choice of solvent depe... separated and on the adsorbent ... and the substance is matched and... polar solvents produce greater m... choice of solvent should be such... half way between its point of app... of two solvents always gives goo... is usually made from the followi... pyridine, acetone, water etc.,

1. Preparation of chroma...

Square or rectangular glass... 20 x 20 cm are used as 'adsorbe... also be used instead of glass pl... gel. A weighted amount of the... to it. The bottle is stirred or s...

...cen in two containers. A strip of filter paper ...
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...partition, adsorption and ion exchange.

i. Choice of Adsorbent :

There are a number of materials which are used as adsorbents. They are as follows :

1. ***Silica gel :***
This is the most widely used. This is used along with a binding agent like plaster of paris.

2. ***Alumina :***
This is basic in nature. It may be applied with or without plaster of paris.

3. ***Kieselghur :***
This is natural in nature. Its adsorbing capacity is less than either silica gel or alumina. It may be applied with or without plaster of pairs.

4. ***Cellulose :***
Several cellulose powders with a variety of ion exchange properties are available. They may be applied with or without plaster of paris.

5. ***Commercial adsorbents :***
Commercially the following adsorbents are used. i. Polyamide powder, ii. Calcium sulphate, iii. Magnesium silicate, iv. Powdered glass.

ii. Choice of Solvent :

The choice of solvent depends on the nature of the substances to be separated and on the adsorbent used. Generally the polarity of the solvent and the substance is matched and then the choice of solvent is made. More polar solvents produce greater migration and thus give better separation. The choice of solvent should be such that the position of the compound must be half way between its point of application and the solvent front. Combination of two solvents always gives good separation than single solvent. The choice is usually made from the following solvents : petroleum ether, CCl_4, benzene, pyridine, acetone, water etc.,

3. Preparation of chromatogram :

Square or rectangular glass plates with sizes ranging from 2.5 x 20 cm, to 20 x 20 cm are used as 'adsorbent supports' in TLC. Plastic and metal foils can also be used instead of glass plates. The most widely used adsorbent is silica gel. A weighted amount of the adsorbent is taken in a bottle. Water is added to it. The bottle is stirred or shaken vigorously until we get a homogeneous,

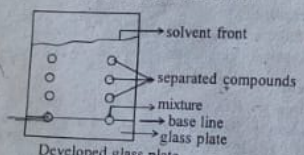
thick, mobile slurry. Two plates are put together. They are held by the thumb and forefinger at one end. Now they are dipped in the slurry, taken out and are held vertically. The solvent dries up. The dry plates are separated. Now, we get two chromatoplates. There are also other apparatus for the preparation of chromato plates.

Activation :

TLC involves adsorption. So, water or other polar solvents affect the development. So, they must be removed from the chromatoplates. This is known as activation. This is done by drying the plates in an over at 100 – 150°C for about 2 hours.

4. Sample application :

A small amount of the sample is dissolved in a small volume of a volatile solvent such as benzene, ether or ethanol. The choice of solvent depends on i) the nature of the substance to be separated and ii) the nature of the adsorbent. For polar substances polar solvents are used.



Developed glass plate

Less polar substances should be dissolved in a suitable non - aqueous solvent. Activated silica gel or alumina should be used as adsorbent. A base line is drawn, about 2.5 cm from one edge of the plate. The samples are applied in small sptos, 1 cm apart, on the base line. The solvent is evaporated. Solutions of standard substances are also applied by the side of the test samples.

5. Development of the chromatogram :

The chromatogram is usually developed by ascending method in a developing chamber called tank. The atmosphere in the tank is saturated with the solvent vapour by placing a paper impregnated with the solvent around the side of the tank. The chromatoplate is placed between the glass plates and the through is filled with the solvent. The tank is closed firmly with the lid. After a certain time when the solvent has moved to about 10 – 20 cm above the origin, the plate is removed from the tank and the solvent front is carefully marked. The solvent is evaporated.

6. Location of compound o

Coloured compounds can be i
colourless compounds, physical or

a. Water is sprayed on the chron
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b. The developed chromatogran
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c. Crystals of iodine are placed
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Applications of Thin Layer

1. TLC may be considered
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2. The technique is extremely
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Separation of a mixture of dy

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6. Location of compound on the chromatogram :

Coloured compounds can be identified by visual inspection. To identify colourless compounds, physical or chemical methods are adopted.

a. Water is sprayed on the chromatogram. Hydrophobic compounds show their presence as optically dense waxy areas.

b. The developed chromatogram is inspected under UV light in a darkened box. Many compounds appear as dark sports in a light background.

c. Crystals of iodine are placed in the tank during development. It imparts a dark brown colour to the spots.

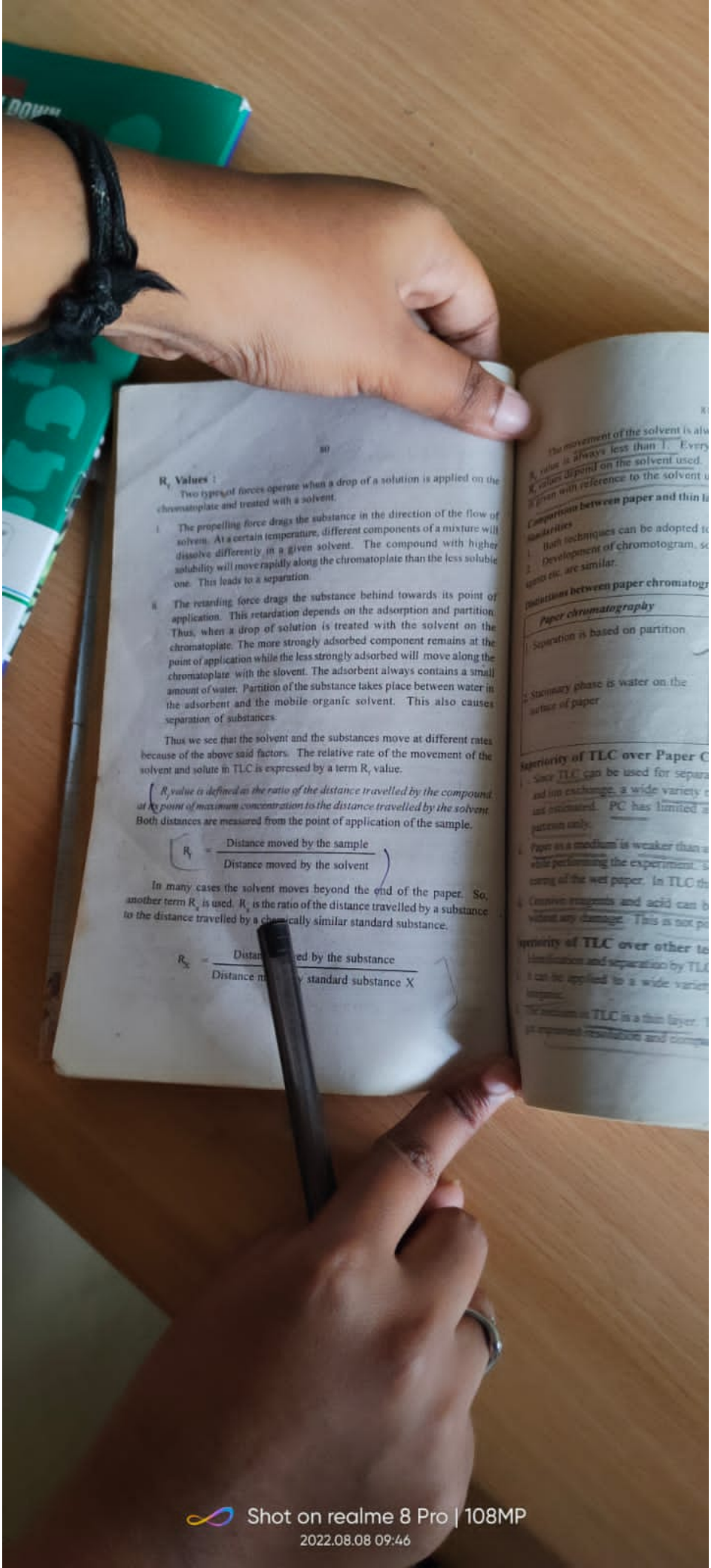
d. The sample is mixed with a very small amount of a radioactive isotope and applied on the plate. The radioactivity is measured by a Geiger Muller counter after development. They may be detected by autoradiography also.

Applications of Thin Layer Chromatography :

1. TLC may be considered basically for qualitative identification, quantitative separation in the preparation of organic and inorganic materials.
2. The technique is extremely suited for analysis of components which are available in traces only.
3. A large number of inorganic compounds have been separated, identified and quantitatively analysed.
4. The applications of TLC include the detection of by - products in synthetic processes, determination of the presence of impurity, removal of impurities and isolation of pure compounds and analysis of inorganic cations and anions. For example Ni, Mn, Co and Zn may be separated using TLC A mixture of acetone and hydrochloric acid is used as the solvent for development. A suitable spraying agent may be used.

Separation of a mixture of dyes :

The chromatoplate is prepared with kieselghur or silica as adsorbent. It is activated in an oven at 100 – 150°C for about half an hour. The solvent used for development of the chromatogram is a mixture of methyl alcohol, acetic acid and isoprophyl alcohol in the ratio 2:2:1. The solvent is taken in rimless beaker to a depth of about 1 cm. A drop of the mixture of dyes is placed 2 cm above the bottom of the chromatoplate. It is placed in the solvent for sufficient time till the solvent reaches the top of the Chromatoplate. The plate is removed and dried. Various sports would be noticed showing that the dyes have been separated.



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R_f Values :

Two types of forces operate when a drop of a solution is applied on the chromatoplate and treated with a solvent.

i. The propelling force drags the substance in the direction of the flow of solvent. At a certain temperature, different components of a mixture will dissolve differently in a given solvent. The compound with higher solubility will move rapidly along the chromatoplate than the less soluble one. This leads to a separation.

ii. The retarding force drags the substance behind towards its point of application. This retardation depends on the adsorption and partition. Thus, when a drop of solution is treated with the solvent on the chromatoplate. The more strongly adsorbed component remains at the point of application while the less strongly adsorbed will move along the chromatoplate with the slovent. The adsorbent always contains a small amount of water. Partition of the substance takes place between water in the adsorbent and the mobile organic solvent. This also causes separation of substances.

Thus we see that the solvent and the substances move at different rates because of the above said factors. The relative rate of the movement of the solvent and solute in TLC is expressed by a term R_f value.

R_f value is defined as the ratio of the distance travelled by the compound at its point of maximum concentration to the distance travelled by the solvent.

Both distances are measured from the point of application of the sample.

$$R_f = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the solvent}}$$

In many cases the solvent moves beyond the end of the paper. So, another term R_x is used. R_x is the ratio of the distance travelled by a substance to the distance travelled by a chemically similar standard substance.

$$R_x = \frac{\text{Distance moved by the substance}}{\text{Distance moved by standard substance X}}$$

The movement of the solvent is alw
R_f value is always less than 1. Every
R_f values depend on the solvent used.
is given with reference to the solvent u

Comparison between paper and thin l

Similarities

1. Both techniques can be adopted t
2. Development of chromotogram, s
spots etc. are similar.

Distinctions between paper chromatogr

Paper chromatography
1. Separation is based on partition.
2. Stationary phase is water on the surface of paper

Superiority of TLC over Paper C

1. Since TLC can be used for separa
and ion exchange, a wide variety
and pulverised. PC has limited a
pattern only.
2. Paper as a medium is weaker than a
while performing the experiment. S
curing of the wet paper. In TLC th
3. Corrosive reagents and acid can b
without any damage. This is not po

Superiority of TLC over other te

identification and separation by TL
2. It can be applied to a wide variet
reagents.
3. The medium in TLC is a thin layer. T
in separation, resolution and compa

The movement of the solvent is always greater than the solute. Therefore, R_f value is always less than 1. Every compound has a different R_f value. R_f values depend on the solvent used. So R_f value of a particular compound is given with reference to the solvent used.

Comparision between paper and thin layer chromatography :

Similarities :

1. Both techniques can be adopted to separate a same set of mixture.
2. Development of chromotogram, solvent used for development, spraying agents etc. are similar.

Distinctions between paper chromatography and thin layer chromatography

Paper chromatography	*Thin layer chromatography*
1. Separation is based on partition.	Separation is based on partition, adsorption and ion exchange.
2. Stationary phase is water on the surface of paper	Stationary phase is some adsorbent like silica gel, alumina which are supported as thin layers on glass plates

Superiority of TLC over Paper Chromatography :

i. Since TLC can be used for separations based on partition, adsorption and ion exchange, a wide variety mixtures can be separated, identified and estimated. PC has limited applications only, which depend on partition only.

ii. Paper as a medium is weaker than a glass plate. So, damages may occur while performing the experiment. so extreme care is to be taken to avoid tearing of the wet paper. In TLC this risk is not there.

iii. Corosive reagents and acid can be sprayed on TLC chromatoplates without any damage. This is not possible in PC.

Superiority of TLC over other techniques :

i. Identification and separation by TLC can be done within 20 - 40 minutes.

ii. It can be applied to a wide variety of compounds, both organic and inorganic.

iii. The medium in TLC is a thin layer. The particle size is very small. So we get improved resolution and compact spot.

iv. It is applicable to the compounds which are decomposed by heat.

v. TLC is sensitive and gives sharper zones.

Here also, as in column chromatography, the moving substance is attracted by the polar sites on the surface of the adsorbent by electrostatic forces. This binding is reversible. There is a three fold interaction between the

i. solvent and the adsorbent

ii. the solvent and the compound and

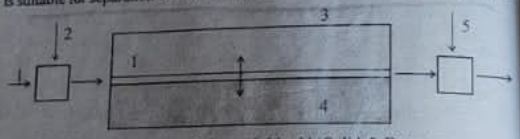
iii. the compound and the adsorbent

This produces good separation of components.

4. Gas - Liquid chromatorgraphy

Theory / Principle :

We know that chromatography refers to the physical method of separation based on the distribution of components between a mobile phase and a stationary phase. If the mobile phase is a gas it is called gas chromatography. If the stationary phase is a liquid, then it is called gas - liquid chromatography. Gas - liquid chromatography is an important technique which has a liquid as a stationary phase distributed over the surface of a solid support. The techniques is suitable for separation of materials which are volatile without decomposition.



1. Gas, 2. Sample, 3. Column, 4. Liquid / Solid, 5. Detector

The sample is introduced into the moving carrier gas stream (figure). It is carried by the gas through the column. The column contains a liquid of low vapour pressure held upon an inert support or only a liquid in a capillary column. The non - volatile liquid forms the stationary phase. The carrier gas forms the mobile phase. the components of the mixture sample distribute themselves between the two phases. The solubility of different components in the liquid phase will be different. Therefore these components are carried

along the column at different rates. Th
column in distinct zones (peaks) separa
the vapours of the components are de
recorded.

Procedure :

There are six essential parts of a l

i. A gas cylinder containing a c
ii. A sample injection systems.
iii. The column
iv. The thermal compartment
v. The detection system
vi. The recorder.

i. *Carrier gas supply :*
The most commonly used carri
carrier gas should be pure. The flow

ii. *Sample injection system :*
A small amount of the sampl
syringe. Solid sample can be diss
by a syringe.

iii. *The column :*
The columns are betwen 12
diameter. They are made from st
may be coiled, or bent in V or W
The partition columns are packe
celite, ground fire brick or glas
volatile liquid phase. The choic
the substance to be separated.

Capillary columns are oper
steel. They are 0.1 to 1 mm in d
of the capillary tube is coated

iv. *Thermal compartmen*
The temperature of
vapour jacked contai

hich are decomposed by heat.

ones.

ography, the moving substance is
e of the adsorbent by electrostatic
is a three fold interaction between

mponents.

the physical method of separation
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e it is called gas chromatography.
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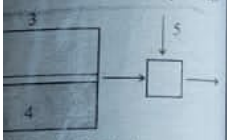
technique which has a liquid as a
f a solid support. The technique
e volatile without decomposition.



id / Solid, 5. Detector

carrier gas stream (figure). It is
olumn contains a liquid of low
r only a liquid in a capillary
tionary phase. The carrier gas
he mixture sample distribute
bility of different components
these components are carried

along the column at different rates. They finally emerge at the end of the column in distinct zones (peaks) separated by the carrier gas. On emerging the vapours of the components are detected by suitable methods and are recorded.

Procedure :

There are six essential parts of a laboratory gas chromatograph (GC).

i. A gas cylinder containing a carrier gas.
ii. A sample injection systems.
iii. The column
iv. The thermal compartment
v. The detection system
vi. The recorder.

i. *Carrier gas supply :*

The most commonly used carrier gases an He, H_2, N_2, Ar and CO_2. The carrier gas should be pure. The flow rate of the carrier gas should be constant.

ii. *Sample injection system :*

A small amount of the sample is introduced into the carrier gas with a syrings. Solid sample can be dissolved in a suitable solvent and introduced by a syrings.

iii. *The column :*

The columns are betwen 120 cm to 500 cm in length and 2 - 10 mm in diameter. They are made from stainless steel, copper glass or plastic. They may be coiled, or bent in V or W shape. Partition columns are used in GLC. The partition columns are packed with an inert support like finely divided celite, ground fire brick or glass beeds. These inert supports carry a non - volatile liquid phase. The choice of liquid phase depends up on the nature of the substance to be separated. Some liquids used are silicon oil, greases etc.,

Capillary columns are open tubes made of nylon, glass, copper or stainless steel. They are 0.1 to 1 mm in diameter and 30 to 300 mm in length. The inside of the capillary tube is coated with a liquid partitioner.

iv. *Thermal compartments :*

The temperature of the column can be maintained uniformly by the use of vapour jacked containing benzene, toluene, etc.

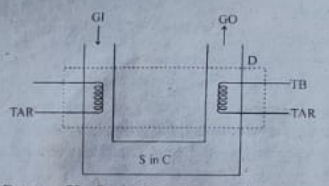
v. *Detection system :*

Detectors measure either the concentration of the solute in mole fraction in the carrier gas or the flow rate of the solute, (in moles per unit time). Some of the detectors which are commonly used are (a) thermal conductivity detector (b) gas density detector (c) flame ionisation detector etc.

1. Thermal conductivity detectors :

Principle :

Thermal conductivities of gases are different. So a change in composition of the gas changes thermal conductivity. Thermal conductivity detectors are based on the change in the thermal conductivity of the gas stream. For this purpose, an instrument called Katharometer is used. A schematic diagram is shown below.



D = Detector, GI = Gas in, GO = Gas out, S in C = Sample in column, TB = To battery, TAR = To amplifier and recorder

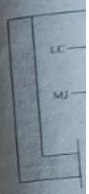
When the current is passed through the instrument, the thermal conductivity of the surrounding gas changes with a change in the temperature of the wire. The resistance of the wire is measured. From that, the thermal conductivity of the gas can be calculated and the change in the gas mixture in the effluent stream can be monitored.

2. Flame ionization detector :

Principle :

Organic compounds are pyrolysed when introduced into ...ogen oxygen flame. As a result of pyrolysis, ions are produced. These ...ected and the resulting current is measured.

A hydrogen flame ionisat...



CE = Colum...
To DC = To Source of dir...
MJ = M...

The column effluent ... which acts as cathode. A...

As the composition ... and electrons will also ch... change in composition of...

This is the most pop...

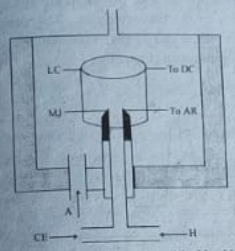
vi. *Recorder :*

All the detectors giv... passed through an ampli... drive the recording char...

Working Technique

The carrier gas ob... flow regulator. This er... sample is introduced int... of the sample injector ... highest boiling compo... injector. Its sweeps off... The components of th...

A hydrogen flame ionisation detector is shown below :



CE = Column Effluent, A = Air, H = Hydrogen,
To DC = To Source of direct current, To AR = To Amplifier and recorder,
MJ = Metal Jet, LC = Loop of Cylinder

The column effluent gas is mixer with hydrogen and burnt at a metal jet which acts as cathode. A loop of cylinder acts as the anode.

As the composition of the gas in the flame changes, the number of ions and electrons will also change. Thus the current flow will change with the change in composition of the gas eluted from gas chromatographic column.

This is the most popular detector.

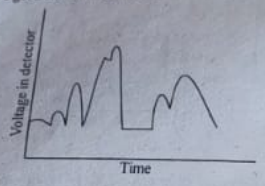
vi. Recorder :

All the detectors give rise to a small and weak electrical signal. These are passed through an amplifier and fed into the recorder. The amplified signals drive the recording chart strip. We get a series of peaks on the paper.

Working Technique :

The carrier gas obtained from a steel gas cylinder is passed through a flow regulator. This enters into the sample injector. A small amount of the sample is introduced into the sample injector using a syringe. The temperature of the sample injector is maintained slightly above the boiling point of the highest boiling component of the sample. The carrier gas enters the sample injector. Its sweeps off the vapourised sample and passes through the column. The components of the sample are distributed between the stationary and the

mobile phases. They pass down the column at different rates. This results in the separation of the components of the sample. The carrier gas with the separated components enters the detectors. The detector measures the change in concentration of the carrier gas. This change is amplified fed into a recorder. We get a chromatogram (figure).



Interpretation of Gas - liquid Chromatography :

The peak is generaly bell shaped. Usually the time required for components to travel through the column is compared with that of known compounds. The area of the peak is a direct measure of the concentration of each compound present in the sample.

Applications / Importance :

1. We can quantitatively determine the amount of a component in a mixture with GLC. The peak area is measured. This is a quantitative measure of a particular component.

   E.g., β - Diketo ligands like acetylacetone, trifluoro acetyl acetone (TFA) and hexafluoro acetylacetone (HFA) form stable volatile chelates with Al, Be, Cr (III) and a number of other metal ions. These can be identified, separated and estimated by GLC.

   It is the newest and most versatile technique for analysing complex mixtures of substances in industry and research. The speciality of this technique is that, it gives one peak per substance in the mixture.

86

...e column at different rates. This ...
...of the sample. The carrier gas with ...
...detectors. The detector measures ...
...ier gas. This change is amplified fed ...
...Figure).

...ime

Chromatography :

...haped. Usually the time required ...
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...e the amount of a component in a mixture ...
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...ylacetone, trifluoro acetyl acetone (TFA) ...
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...atile technique for analysing complex ...
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87

2. Fatty acids have been separated by this technique. The other mixtures separated by this technique are
   i. Benzene and toluene,
   ii. Chloro methanes
   iii. Species with similar boiling points but with different polar characteristics.

   It can be used for quantitative analysis of mixtures also. Peak heights, peack areas are compared with those of known substances and their quantity determined.

3. It has been used in elemental carbon, hydrogen and nitrogen analysis of organic compounds.

   They are converted to CO_2, H_2O and N_2 by burning them in the presence of oxidising catalysts like MnO_2. The gases are detected.

4. In petrolium industry it has been used in the analysis of crude petroleum products, fractions, gasolines, waxes, LPG, sulphur and nitrogen compounds etc.

5. In food industry it hasbeen used to account for the colour and flavour of food. It has been used for the determination of residual solvents in spices, oleoresins and for pesticides in food.

6. In cosmetics and perfume industry it is used to determine the composition of the various cosmetics, the quality of ingredients and components of subtle fragrances.

7. In plastic industry it is used to identity plastics, determination of ethers in acrylic copolymers etc.

8. In many research fields in the above industries and in many other fields it finds use.

5. Ion - Exchange Chromatography :

It is a special category of column chromatography in which the stationary phase is an ion exchange resin.

Principle :

It involves exchange of ions with like signs between a solution and a solid in contact with the solution.

Types of resins :

Ion exchange resins are granular insoluble organic compounds with giant molecules with exchangeable ions. The ion exchange resin are two types. They are

i. Cation exchange resin and ii. Anion exchange resin.

A cation exchange resin can be represented as $(Res\ A^-)B^+$ where Res is the basic polymer of the resin to which is attached the anion A^- and mobile cation B^+. The cation exchanger exchanges its mobile cation with the cation of the solution.

Example :

$$2(Res\ SO_3^-)Na^+Ca^{2+} \rightleftharpoons (Res\ SO_3^-)Ca^{2+} + 2Na^+$$

An anion exchange resin can be represented as $(Res\ B^+)A^-$. Anion exchange exchanges its mobile anion with the anion of the solution.

Example :

$$[Res\ N(CH_3)_3]^+OH^- + H^+Cl^- \rightleftharpoons [Res\ N(NH_3)_3^+]Cl^- + H_2O$$

Each of these subdivided as follows

i. *Strongly acidic cation exchange resins :*

Eg., Sulphonated polystyerene resins. They are useful in the pH range of 1 - 14. These are usd to separate cations, inorganic compounds, lanthanides, vitamins, peptides and aminoacids.

ii. *Weakly acidic cation exchange resins :*

Eg., Carboxylic polymerthacrylate resins. They are useful in the pH range of 5 - 14. These are used to separate cations, biochemical compounds, transition elements, aminoacids, antibiotics and organic bases.

iii. *Strongly basic anion exchange resin*

Eg., Quarternary ammonium polystyr

pH range 0 - 12. These are used to sep

vitamin B complex, fattyacids etc.

iv. *Weakly basic anion exchange resins*

Eg., Phenol formaldehyde and polya

useful in the pH range of 0 - 9. These are

of metals, amines of different valencies, v

Requirements of a good resin :

1. It must be sufficiently cross linked
2. It must be sufficiently hydrophilic so
the structure at a constand and fini
3. The swollen resin must be denser
4. It should be chemically stable.
5. It most contain a sufficient number

Action of ion exchange resins :

Principle :

Ion exchange resins behave as a
electric charge, which is distributed ove
The surplus charge is compensated b
exchange resins comprise of static ions
ions. These mobile ions are exchanged
process. In this ion exchange process
available heat of exchange is low. Th
place by diffusion occuring in two dif

1. *Film diffusion :*

In this method diffusion of ions
is adjacent to the resin particle. Thi
and with small ions.

2. *Particle diffusion :*

In this method diffusion of ions
particles. This method dominates i
ions.

...tography :

...n chromatography in which the station...

...with like signs between a solution an...

...insoluble organic compounds with ...
The ion exchange resin are two typ...

...Anion exchange resin.

...represented as $(Res\ A^-)B^+$ where Res...
...ch is attached the anion A^- and mob...
...nges its mobile cation with the cation...

$...SO_3^-)Ca^{++} + 2Na^+$

...e represented as $(Res\ B^+)A^-$. Ani...
...with the anion of the solution.

$... \rightleftharpoons [Res\ N(NH_3)_3^+]Cl^- + H_2O$

...*esins* :
...ins. They are useful in the pH ran...
...s, inorganic compounds, lanthanid...

...*ins* :
...esins. They are useful in the pH ran...
...e cations, biochemical compound...
...ics and ...c bases.

iii. *Strongly basic anion exchange resins*

Eg., Quarternary ammonium polystyrene resins. These are useful in the pH range 0 - 12. These are used to separate anions, halogens, alkaloids, vitamin B complex, fattyacids etc.

iv. *Weakly basic anion exchange resins* :

Eg., Phenol formaldehyde and polyamine polystyrene resins. These are useful in the pH range of 0 - 9. These are used to separate anionic complexes of metals, anions of different valencies, vitamins and aminoacids.

Requirements of a good resin :

1. It must be sufficiently cross linked to have only a negligible solubility.
2. It must be sufficiently hydrophilic so as to permit diffusion of ions through the structure at a constand and finite rate.
3. The swollen resin must be denser than water.
4. It should be chemically stable.
5. It most contain a sufficient number of accessible ionic exchange groups.

Action of ion exchange resins :

Principle :

Ion exchange resins behave as a porous network, carrying a surplus electric charge, which is distributed over the surface and throughout the pores. The surplus charge is compensated by ions of opposite charge. Thus ion exchange resins comprise of static ions attached to the resin - part and mobile ions. These mobile ions are exchanged with similar ions during ions exchange - process. In this ion exchange process, no chemical bonds are formed as the available heat of exchange is low. The actual ion exchange process is taking place by diffusion occuring in two different ways.

1. *Film diffusion* :

In this method diffusion of ions takes place across the liquid film which is adjacent to the resin particle. This method dominates in dilute solutions and with small ions.

2. *Particle diffusion* :

In this method diffusion of ions takes place within the pores of the resin particles. This method dominates in concentrated solutions and with large ions.

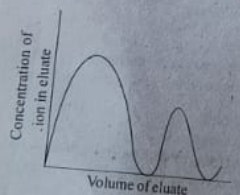
Experimental Techniques :

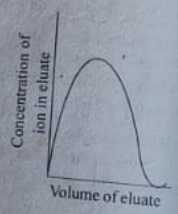
The ion - exchange chromatorgraphy is carried out in a chromatographic column. The column consists of a burette provided with a sintered glass disc or glass wool plug at the lower end. The column is packed with wet ion - exchange resin uniformly. The top of the resin bed is covered with a glass wool pad. The column should never be allowed to drain out.

Let us suppose that we which to replace the Cl^- ions by OH ions. The resin column is washed with a concentrated solutionof NaOH to ensure the column contains only OH^- ions and no other anion. The soltion containing Cl^- ions is run through the column Cl^- is exchanged by OH^-. The effluent will contain a quantitative yield of the hydroxide compound. The same process occurs when a cation exchanger is used.

If a mixture of small quantities of two or more different cations X, Y, etc., is passed through an ion - exchange column, they get separated. If cation X is held more firmly by the exchange resin than cation Y, Y will flow out of the bottom of the column before X. This separation techniques is called ion - exchange chromatography. The liquid entering the column is called influent. The liquid leaving the column is called the effluent. The process by which the absorbed ions are removed from the column is known as elution. The solution used for elution is eluent and the solution obtained as a result of elution is called the eluate.

If a solution of suitable eluent is passed through a column containing an ion X, the effluent is continuously analysed, and the concentration of X is plotted against the volume of eluate, an elution curve is obtained. (Figure).





If the column contains sev
curves are obtained for each io
curves are sufficiently far apart.
If the elution curves overlap on

Application of Ion - Exch

Ion - exchange chromatog
actinides, tansition metals, pho
serves as a standard method in
actinides. Industrually, it is use
metals from ores and separatio

Some specific examples are giv

Separation of rare earths

A column is packed with a
first to ascertain that all exchan
mixture of rare eraths (lanthani
The rare earth ions get exchan
ions are eluted with a solution
citric acid. Depending upon t
eluted at different rates.

Separation of Zn and Mg

Principle :

Zinc is absorbed from 2M
while megnesium is not, zinc in

Procedure :

A column of the anion e
chloride form is prepared. Th
allowed to flow thorugh the col
is washed with 50 cc of 2M H
liquid to fall below the top of t
in a receiver (conical flask). T
is changed. Now Zinc is el
approximately 0.25 M HNO_3

graphy is carried out in a chromatograph
burette provided with a sintered glass d
d. The column is packed with wet ion
of the resin bed is covered with a gla
be allowed to drain out.

to replace the Cl^- ions by OH^- ions. Th
entrated solutionof NaOH to ensure th
no other anion. The soltion containi
is exchanged by OH^-. The effluent wi
droxide compound. The same proces
ued.

two or more different cations X, Y, etc.
column, they get separated. If cation X
sin than cation Y, Y will flow out of the
s separation techniques is called ion -
entering the column is called influen
the effluent. The process by which the
lumn is known as elution. The solution
ution obtained as a result of elution is

passed through a column containing an
nalysed, and the concentration of X is
an elution curve is obtained. (Figure).

If the column contains several ions of similar charge, Y, Z, etc , elution curves are obtained for each ion by the use of suitable eluants. If the elution curves are sufficiently far apart, (figure) a quantitative separation is possible. If the elution curves overlap only incomplete is possible.

Application of Ion - Exchange Chromatography :

Ion - exchange chromatography is used in the separation of rare - earths, actinides, tansition metals, phosphates, complex ions and even isotopes. It serves as a standard method in the discovery and isolation of trace amount of actinides. Industrually, it is used in the metal finishing industry, extraction of metals from ores and separation of rare earths.

Some specific examples are given below :

Separation of rare earths :

A column is packed with an acid resin and treated with hydrochloric acid first to ascertain that all exchange centres were occupied by hydrogen ions. A mixture of rare eraths (lanthanides) as their chlorides is sent down the column. The rare earth ions get exchanged with hydrogen ions. Later, the rare earth ions are eluted with a solution of citric acid. The cations form a complex with citric acid. Depending upon the stability of these complexes, these ions are eluted at different rates.

Separation of Zn and Mg :

Principle :

Zinc is absorbed from 2M HCl acid by a column of anion exchange resin while magnesium is not, zinc in 2M HCl forms negativly charged chloro complex.

Procedure :

A column of the anion exchange resin using 15g of zerolit FF in the chloride form is prepared. The column is made up in 2M HCl, the mixture is allowed to flow thorugh the column at a rate of about 5cc/ minute. The column is washed with 50 cc of 2M HCl. Care is taken not to permit the level of the liquid to fall below the top of the column of the resin. The effluent is collected in a receiver (conical flask). This will contain all the magnesium. The receiver is changed. Now Zinc is eluted with 30 cc water followed by 80 cc of approximately 0.25 M HNO_3. Thus zinc and magnesium are separated.

Separation of chloride and bromide ion :

Principle :

An anion exchange resin originally in the chloride form is converted into the nitrate form by washing it with sodium nitrate solution. A concentrated solution of the chloride and bromide mixture is introduced at the top of the column. The halide ions exchange rapidly with nitrate ions in the resin forming a band at the top of the column. Now this band eluted with sodium nitrate solution. Chloride ions are more rapidly eluted than bromide ions. Thus they are separated.

Procedure :

A column of the anion exchange resin using 40g of Zerolit FF in the chloride form is prepared. The column is washed with 0.6M sodium nitrate until the effluent contains no chloride ions. Now the column as washed with 50cc of 0.3M sodium nitrate solution. The mixture of chloride and bromide is mixed with 0.3M Sodium nitrate and mixture is placed a the top of the column sodium nitrate is passed through the column at the rate of 1cc/minute. Chloride ions are eluted first. Bromide ions are eluted next. Thus they are separated.

Separation of Co and Ni :

Principle :

Cobalt forms a monovalent complex anion probably $[CoCl_3]^-$ in 9M HCl while nickel does not. This anion is rapidly extracted from its solution by a strongly basic anion exchanger such as zerolit FF. The anionic chloro complex of nickel is not retained by the resin, as it is not stable. It can be washed out of the column with 9M HCl. Later, the column is eluted with water. The cobalt complex is decomposed and passes out in the effluent as cobalt (III) chloride.

Procedure :

A column of the anion exchange resin using 30g of zerolit FF inthe chloride form is prepared. 50cc and 9M HCl is passed through the column. 10 cc of the mixture of Co and Ni is placed at the top of the column. 100cc of 9M HCl is passed through the column. Nickel is eluted out, it is collected. Then 50cc water is passed through the column at the rate of 5cc / miunte. The anionic cobalt chloro complex is decomposed and comes out of the column as cobalt chloride is collected. Thus cobalt and nickel are separated.

paration of Cd and Zn :

Principle :

Cadmium and Zinc form neg
sorbed by a strongly basic anio
luted with 2M NaOH containing
e resin. It is later eluted with 1

Procedure :

A column of the anion ex
chloride form is prepared. The
100g / dm^3 AR NaCl (reagent 1.
of the column. It is brought int
of 2M NaOH containing 20g l
the column at the rate of 4cc
resin is washed to remove Na
the column at the rate of 4cc
Zn and Cd are separated.

Advantages of ion - ex

i. The exchange capacity
effected fairly quickly.

ii. The recovery of ions
expensive materials
would be no loss. A
separation is nearly

Factors determini
an ion - exchange re

i. *Nature of exchang*
A low aqueous co
of exchange increases

$Na^+ < Ca^{2+} < Al^{3+}$

bromide ion :

ginally in the chloride form is converted in
ith sodium nitrate solution. A concentrate
ide mixture is introduced at the top of t
e rapidly with nitrate ions in the resin forming
Now this band eluted with sodium nitrate
rapidly eluted than bromide ions. Thus the

hange resin using 40g of Zerolit FF in the
olumn is washed with 0.6M sodium nitrate
ride ions. Now the column as washed with
ion. The mixture of chloride and bromide is
nd mixture is placed a the top of the column
e column at the rate of 1cc/minute. Chloride
s are eluted next. Thus they are separated

mplex anion probably $[CoCl_4]^-$ in 9M HCl
is rapidly extracted from its solution by a
as zerolit FF. The anionic chloro complex
n, as it is not stable. It can be washed out
he column is eluted with water. The cobalt
out in the effluent as cobalt (III) chloride

resin using 30g of zerolit FF inthe chloride
is passed through the column. 10 cc of the
top of the column. 100cc of 9M HCl is
s eluted out, it is collected. Then 50cc
t the rate of 5cc / miunte. The anionic
d and comes out of the column as cobalt
alt and nickel are separated.

Separation of Cd and Zn :

Principle :

Cadmium and Zinc form negatively charged chloro complexes which are adsorbed by a strongly basic anion exchange resin such as zerolit FF. zinc is eluted with 2M NaOH containing 20g of NaCl / dm^3. Cadmium is retained on the resin. It is later eluted with 1M HNO_3.

Procedure :

A column of the anion exchange resin using 30g of Zerolit FF in the chloride form is prepared. The column is washed with 0.12M HCl containing 100g / dm^3 AR NaCl (reagent I. The mixture of Cd and Zn is placeld on the top of the column. It is brought into the column with the help of reagent I. 150 cc of 2M NaOH containing 20g lf AR NaCl / dm^3 (reagent II), is passed through the column at the rate of 4cc / minute. Zn is eluted out. It is collected. The resin is washed to remove NaOH. Now 150 cc of 1M HNO_3 is passed through the column at the rate of 4cc / minute. Cd is eluted out. It is collected. Thus Zn and Cd are separated.

Advantages of ion - exchange chromatography :

i. The exchange capacity of the resins is very high. So separation can be effected fairly quickly.

ii. The recovery of ions from the column is nearly 100% complete. So expensive materials can be separated by this technique so that there would be no loss. Aslo, quantitative works could be carried out since separation is nearly 100% complete.

Factors determining the distribution of ions between an ion - exchange resin and a solution :

i. *Nature of exchanging ion :*

A low aqueous concentrations and at ordinary temperatures, the extent of exchange increases with increasing valency of the exchanging ion, i.e.,

$$Na^+ < Cu^{2+} < Al^{3+} < Th^{4+}$$

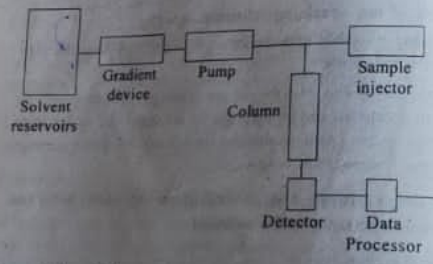
ii. Under similar conditions and constant valency, for univalent ions, the extent of exchange increases with decrease in size of the hydrated cations. $Li^+ < H^+ < Na^+ < NH_4^+ < K^+ < Rb^+ < Cs^+$, while for divalent ions, the ionic size is an important factor but the incomplete dissociation of salts on bivalent metals also plays a part.

The order is $Cd^{2+} < Be^{2+} < Mn^{2+} < Mg^{2+} = Zn^{2+} < Cu^{2+} = Ni^{2+} < Co^{2+} < Ca^{2+} < Sr^{2+} < Pb^{2+} < Ba^{2+}$.

6. High pressure liquid chromatography *(not in syllabus)*

The modoner liquid chromatography comprises the following basic components.

i. A solvent delivery system including pump
ii. Sample injection system
iii. A chromatographic column
iv. A detector and recording system.



1. *Solvent delivery system :*

The mobile phase is pumped under pressure from one or several reservoirs and flows through the column at a constant rate. The pump is the most important component of HPLC, because its performance directly affects the retention time, reproducibility and detector sensitivity. Most of the work in analytical HPLC is done using pressures between about 400 – 1500 psi.

Pounds per square inch
cubic centimeter