SEPARATION METHODS OF ANALYSIS (CHM 703)

If it were possible to identify or quantitatively determine any element or compound by simple measurement, no matter what it's concentration or the complexity of the matrix, separation technique would be of no value to the analytical chemist.

But most techniques fall short of this ideal, because of the interference with the required measurement by other constituents of the sample. Many techniques for separating and concentrating the species of interest have just been devised. Such techniques are in at exploiting differences in physicochemical properties between the various components of mixture volatility; solubility, charge, molecular size, shape, and the polarity are the most useful. In this respect, a change of phase has occurred during distillation or formation of new phase as in precipitation, can provide a simple means of isolating a desired component.

Usually, more complex separation procedures are required for multi component sample most depend on the selective transfer of materials between two immiscible phases. The most widely used techniques and the phase system associated with them are summarized as follows:

Classification of separation techniques

S/NO	Technique	Phase system
1.	Solvent extraction	Liquid - liquid
2.	Gas chromatography	Gas - liquid
3.	Liquid chromatography	Liquid - liquid Liquid - solid
4.	Thin - layer chromatography	Liquid - solid Liquid - liquid
5.	Ion – exchange and gel – formation chromatography	Liquid - solid Liquid - liquid
6.	Supercritical fluid chromatography and	Supercritical fluid - liquid or solid – liquid.

	electrophoresis	

All separation techniques one or more achieve chemical equilibrium. Consequently, the degree of separation achieved can vary greatly according to experimental condition to a large extent; attainment of optimal condition to a large extent, attainment of optimal condition has to be approached empirically rather than by the application of a rigid theory in the following section which deals with solvent extraction, chromatography and electrophoresis. The minimum theory necessary for an understanding of the basic principle is presented.

SOLVENT EXTRACTION

Solvent extraction is a selective transfer of ug to g in quantity between 2 immiscible phases (liquid). Separation is based on solubility differences and selectivity is achieved by pH control and complexation.

Separating funnel is used for batch extraction and special glass apparatus, soxhlet (for cont. extraction). Batch methods are rapid, simple and versatile.

Disadvantages of batch method.

(1) It sometimes requires large quantities of organic solvents.
(2) Poor resolution of mixture of organic components.

THEORY

Solvent extraction is sometimes called liquid extraction. It involves the selective transfer of a substance from one liquid phase to another e.g. aqueous solution of iodine and sodium chloride is shaking with ccl4 (carbon tetra-chloride) and the liquid allowed to separate. Most of the iodine will be transferred to ccl4 layer and will sodium chloride will remain in aqueous layer. The extraction of soxhlet is governed by Nernst partition or distribution law which states that at equilibrium, a given solute will always be distributed between two essentially immiscible liquid in the same proportion.

$$\frac{[A]\ \text{organic}}{[A]\ \text{aqueous}} = K_D \qquad \text{(same temperature and pressure)}$$

Where [ ] represents the concentration and K_D is "partition coefficient" and it is independent of the concentration of the solute.

'A' must exist in the same form in both phases. Equilibrium is established when the chemical potential (free energy) of the solute in the two phases are equal and is usually achieved within a few minutes of shaking. The value of K_D is a reflection of the relative solubilities of the solute in the two phases.

The value of K_D breaks down if 'A':

1. dissociates
2. polymerizes
3. form complexes with other components e.g. solvents.

Analytically, the total amount of solute present in each case is better described in distribution 'D',

Where D = $\frac{[\mathrm{CA}]o/ml}{[\mathrm{CA}]\mathrm{aq/ml}}$ CA = amount of solute

If there is no interaction, $D = K_D$.

Efficiency of Extraction.

It depends on the magnitude of D or K_D and on the relative volumes of the liquid phases

The % of extraction is given by:

$$\Sigma = \frac{100D}{[\mathrm{D} + \frac{\mathrm{Vaq}}{\mathrm{Vo}}]}$$

For equal volumes of aqueous and organic solvent,

$$\Sigma = \frac{100D}{[D + 1]}$$

If D is large i.e. tending towards 100, a single extraction may affect virtually quantitative transfer of the solute, whereas with small values of D, several extractions would be required. The amount of solute remaining in aqueous solution is readily calculated for any number of extractions with equal volumes of organic solvents from the following equation.

$$[Caq]_n = Caq \left[\frac{Vaq}{(DVo+Vaq)}\right]^n$$

Where $[Caq]_n$ is the amount of solute remaining in aqueous phase.

Vaq = the volume of aqueous solvent after n extraction

Vo = the volume of organic solvent after n extraction.

If the value of D is known, the equation above is useful in determining the outmost condition for quantitative transfer of material.

For example, the complete removal of 0.1g of iodine from $50cm^3$ of an aqueous solution of iodine and sodium chloride is carried out using $CC1_4/H_2O$; the value of D was given as 85.

Calculate the efficiency using:

1. $25cm^3$ of CCl_4 once
2. Batch extraction three times.

Which method out of the two would be most efficient?

1. $\{Caq\}1 = 0.1 \left\{ \frac{50}{(85 \times 25) + 50} \right\}^n$

$= 0.0023$

% efficiency $= \frac{0.1 - 0.0023}{0.1} \times 100$

$= 97.7\%$

2. $\{Caq\}3 = 0.1 \left\{ \frac{50}{\left(85 \times \frac{25}{3}\right) + 50} \right\} 3$

$= 2.86 \times 10^{-5}$

% efficiency $= \frac{0.1 - 2.86 \times 10^{-5}}{0.1} \times 100$

$= 99.9\%$

It is therefore clear that extracting several times with small volumes of organic solvent is more efficient than one extraction with large volume.

Assignment

A solution of 6g of substance X in 50cm^3 of aqueous solution is in equilibrium at room temp with ether solution of aqueous liquid containing 108g of X in 100 cm^3 with:

a. 100cm^3 of ether once

b. 50cm^3 of ether twice at room temperature.

Which process is more efficient in both methods a & b? Bear in mind that 100cm^3 of ether is used in both a & b.

CHROMATOGRAPHY

The word chromatography is used to describe separation into components. All the techniques depend upon the same basic principle i.e. variation in the rate in which different components of a mixture migrates through a stationary phase under the influence of a mobile phase.

Rates of migration vary because of differences in distribution ratios. The process whereby a solute is transferred from a mobile phase to a stationary phase is called SORPTION.

Chromatographic techniques are based on four sorption mechanisms namely:

- Surface Adsorption
- Partition
- Ion – exchange
- Exclusion.

Both adsorption and partition may occur simultaneously. For example, a stationary phase of Al_2O_3 is highly polar and normally exhibit strong adsorptive properties. However, this may be modified by the presence of adsorbed water which introduces a degree of partition into the overall sorption process by acting as a liquid stationary phase.

Paper (cellulose) is relatively non-polar and retains a large amount of water which functions as partition medium. Nevertheless, residual polar group in the structure of paper can lead to adsorptive effect.

The third sorption is that of ion exchange. Here, the stationary phase is a permeable polymeric solid containing fixed charge groups and mobile counter-ions which can exchange with the ions of the solute as the mobile phase carries it through the structure.

There are two types:

- Cation Exchanger, $R^- X^+$
- Anion Exchanger, $R^+ X^-$

The fourth type of mechanism is Exclusion. Strictly speaking, it is not a true sorption process as the separating solute remains in the mobile phase throughout. Separations occur because of variation in the extent to which the solute molecule can diffuse through the inert, porous stationary phase. This is normally a gel structure which has a small pore size and into which small molecules up to a certain critical size can diffuse. Molecules larger than the critical size are excluded from the gel and move unhindered through the column or layer while the smaller ones are retarded to an extent dependent on molecular size.

ADSORPTION SYSTEM

Stationary Phase

Almost any polar solid can be used, the most common choices being silica gel or alumina (Al_2O_3). The adsorbent used in column chromatography are ranged in the order of decreasing adsorptive power.

Alumina → Charcoal → Silica gel → $MgCO_3$ → $CaCO_3$ → Starch → Sucrose → Cellulose.

Silica gel and alumina are highly polar materials that adsorb molecules strongly. Activity is determined by the overall polarity and the number of adsorption site. In silica gel, the adsorption sites are the Oxygen atom and silanol groups (-Si - OH) which readily form H – bonds with polar molecules.

Adsorption site of different types are present on the surface of alumina but unlike silica gel, a proportion of them are hydroxyl (OH^-) groups. The amount of water present on the surface has a profound effect on activity by blocking adsorption site. If the water is progressively removed by oven – drying, the material becomes correspondingly more active. The choice of stationary phase and its degree of activity is determined by the nature of the sample. If sample component are adsorbed too strongly, they may be difficult to elute, or chemical changes may occur. Weakly polar solute should be separated on highly active adsorbent, otherwise, they may elute rapidly with little or no resolution.

Strongly polar solute is better separated on adsorbent of low activity. Silica gel can be prepared with a wider range of activities than alumina and is less likely to induce chemical changes.

Mobile Phase

The eluting power of a solvent is determined by the overall polarity, the polarity of the stationary phase and the nature of sample component.

The list below shows some widely used solvent in order of their eluting power, this being known as **ELUOTROPIC SERIES.**

SOLVENT	SOLVENT POLARITY PARTITION BASED	SOLVENT POLARITY ADSORPTION BASED
n–hexane	0.1	0.01
Cyclohexane	-0.2	0.04
CCl_4	1.6	0.18
Toluene	2.4	0.29
benzene	2.7	0.32
methylene dichloride	3.1	0.42
n–propanol	4.0	0.82
Tetrahydrofuran	4.0	0.57
ethyl acetate	4.4	0.56
Isopropanol	3.9	0.82
Chloroform	4.1	0.40
Acetone	5.1	0.56
Ethanol	4.3	0.88
Acetonitrile	5.8	0.65
Methanol	5.1	0.95
Water	10.2	_

It is important that a given solvent should not contain impurities of a more polar nature e.g. water or acids, alcohol in chloroform, aromatics in saturated hydrocarbons, as resolution may be impaired.

PARTITION SYSTEM.

In a partition system, the stationary phase is a liquid coated onto a solid support (silica gel, kieselguhr or cellulose). There is a very wide choice of pairs of liquids to act as stationary - mobile phases. It is not necessary for them to be totally immiscible, but a low mutual solubility is desirable.

A hydrophilic (water loving) liquid may be used as stationary phase with a hydrophobic (water hating) mobile phase or vice versa.

CHARACTERIZATION OF SOLUTE

1. The rate of movement of a solute is determined by its distribution ratio, D as:

$$D = \frac{Conc.of\ stationary\ phase}{Conc.of\ mobile\ phase}$$

The larger the value of D, the slower will be the progress of the solute through the system, and the components of the mixture will therefore reach the end of the column in order of increasing value of D.

In paper and thin layer chromatography, the separation process is altered at a stage which leaves separation components *in situ* on the surface in the form of spot. The rate at which a solute has moved is then determined by its Retardation factor, (R_f), which is defined as follows:

$$R_f = \frac{distance\ trav\ elled\ by\ the\ centre\ of\ solute\ spot}{distance\ trav\ elled\ by\ the\ front\ of\ mobile\ phase}$$

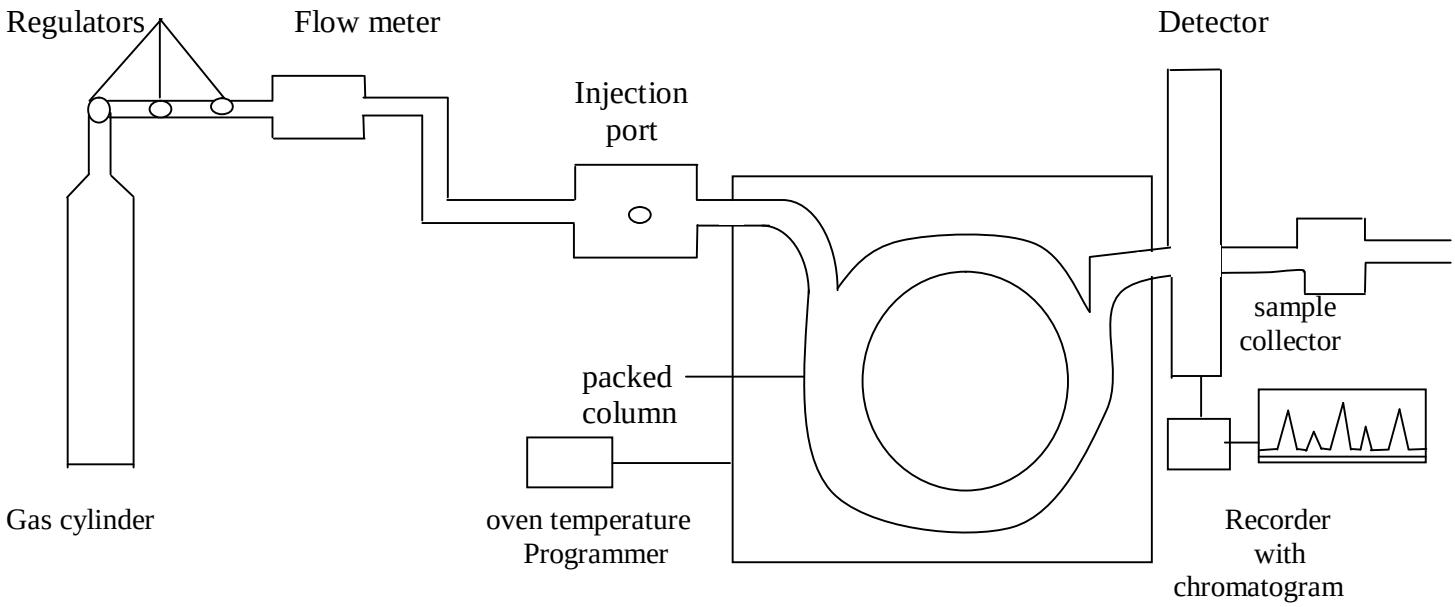
For good separation, the components should have different R_f.

GAS CHROMATOGRAPHY

PRINCIPLE

It involves the separation of mixtures in µg quantities by passage of the vaporized sample in a gas stream through a column containing a stationary liquid or solid phase. Components migrate at different rates due to difference in boiling point, solubility, or adsorption.

Apparatus and Instrumentation.



Schematic Diagram of Gas Chromatography

The apparatus is made up of injection port, heated metal or fused quartz glass column, detector and recorder.

Mode of Operation

The mobile phase or the carrier gas is supplied from a cylinder via a pressure - reducing head at pressure of 10-40psi (1atm = 14.6psi) with a flow rate of 2 – 50cm^3 min^{-1}. Typical carrier gases used are N_2, H_2, Ar, He, CO_2 etc. (i.e. gases that do not react). Fine control of carrier gases is achieved by

flow controller. For optimum result, it is advisable to dry the gas before use by passing it through molecular sieve to remove water vapour.

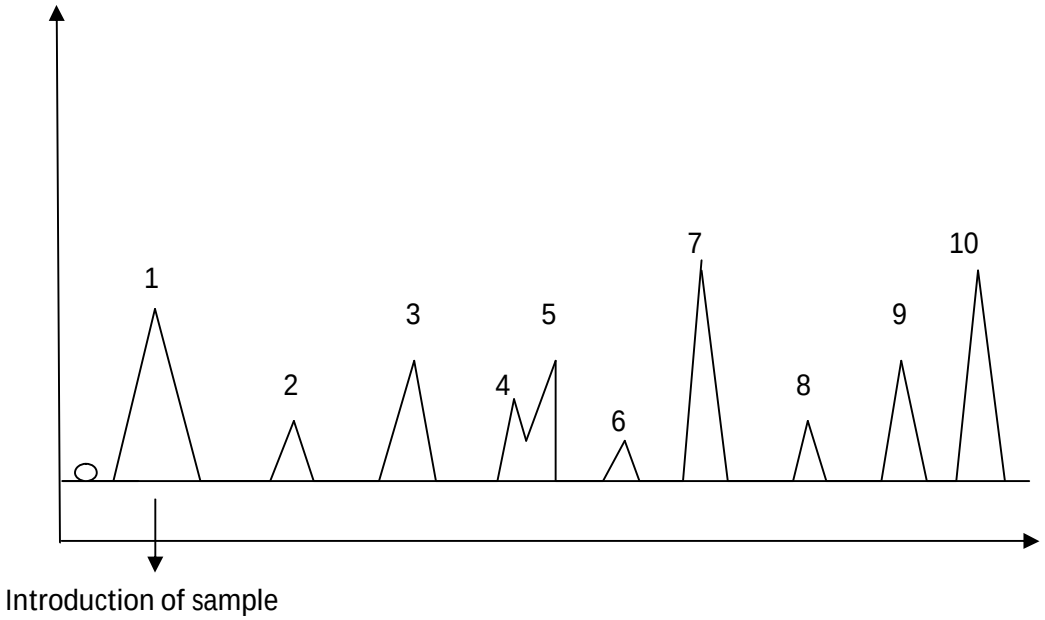
Gas chromatography, so called because mobile phase is a gas, comprises gas – liquid chromatography (GLC) and could be gas – solid chromatography (GSC). For GLC, the stationary phase is a high boiling liquid and the sorption process is predominantly one of partition. For GSC, the stationary phase is a solid and adsorption plays the major role.

Samples which must be volatile and thermally stable at the operative temp are introduced into the gas flow via an injection port located at the top of the column.

Gas samples require a large volume gas-tight syringe or gas sampling valve as they are much less dense than liquid. For packed column, 0.1 – 10 μL of a liquid sample or solution may be injected into a heated zone or flash vapourizer positioned just ahead of the column and constantly swept through with carrier gas.

A continuous flow of gas elutes the component from the column in order of increasing distribution ratio from where they pass through a detector connected to a chart recording system. The chart recorder gives different histogram of the components at different retention time in the sample.

Typical example is as shown below:



Typical elution profile: The separation of aliphatic esters by Gas-liquid Chromatograohy.

1. Methyl formate
2. Methyl acetate
3. Ethyl formate
4. Ethyl acetate
5. n-propyl formate
6. Isopropyl acetate
7. n-butyl formate
8. 2^o butyl acetate
9. Isobutyl acetate
10. n-butyl acetate.

Different detectors are used in GC depending on the type of sample to be analyzed.

Typical examples are:

1) Flame Ionization Detector (FID)

2) Electron Capture Detector (ECD)

2) Flame Photometric Detector (FPD)

QUALITATIVE ANALYSIS IN GAS CHROMATOGRAPHY

Identification of the component peaks of a chromatogram which may be numerous can be achieved in two ways:

- Comparism of retention time, t_R.
- Trapping the eluted component for further analysis by other analytical techniques such as IR and mass spectrometry.

Valid comparism can be made using relative retention data which are dependent only on column temperature and type of stationary phase.

QUANTITATIVE ANALYSIS IN GC

The integrated area of a peak is directly proportional to the amount of solute eluted. Peak height can also be used but less reliable. Accurate measurement can be carried out by the following:

i. **Geometrical method**: The area of the isosceles triangle (Δ) formed in the chromatogram can be calculated by multiplying the height by the width at half height, i.e. $A = b \times h/2$.
ii. **Cutting out and weighing**: This method is fairly precise and very useful for unsymmetrical peak but subject to errors arising from variation in thickness and moisture content of paper.

iii. **Automatic integration**: Electronic integrators are the most rapid and precise means of determining peak areas. They have a digital output derived by feeding the detector signal into a voltage-to-frequency converter which produces a pulse rate proportional to the input signal. The total number of pulse is a measure of the peak, and this can be printed out directly or stored until required.

EFFICIENCY AND RESOLUTION

The ideal chromatographic process is one in which the component of a mixture form narrow bands which are completely resolved from one another. The narrowness of a band or peak is a measure of the efficiency of the process while resolution is assessed to resolve peak of components with similar retention time (t_R) or retardation factor (R_f) values.

Efficiency, N for column separation is related to t_R as follows:

$$N = \frac{(tR}{\sigma} \qquad \sigma = standard\ deviation$$

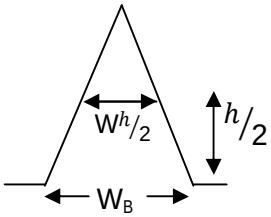
It is also easier to measure in terms of base line width

$$N = 16\ (\frac{tR}{WB})^2$$

$$N = 5.5\ (\frac{tR}{Wh/2})^2$$

WB = baseline width.

$W^{h}/_{2}$= peak width measured at half of peak height.



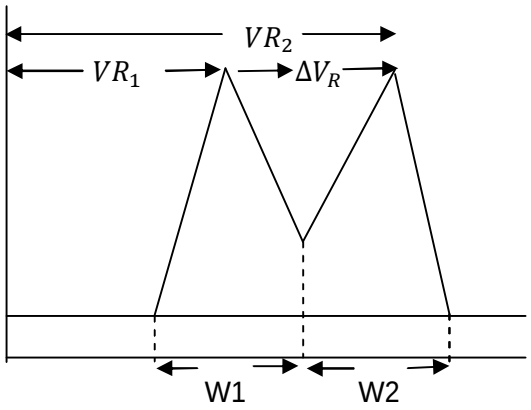
The parameter N is usually referred to as the "Plate Number" but an alternative mean of quoting efficiency is in terms of plate height, H or HETP (Height Equivalent to Theoretical Plate). N is related to H as:

$N = {}^{L}/_{H}$ L = total length of the column

In practice, Rs (resolution) is measured from a chromatogram by relating the peak-to-peak separation to the average peak width. This is expressed by the equation:

$$Rs = \frac{2\Delta\ VR}{(W1+W2)}$$

where V_R = retention volume.



ΔV_R is the separation of peak maxima, W1 and W2 are the respective peak widths. R_f value of 1.5 or more indicates cross contamination of 0.1% or less.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

It has its origin in classical column chromatography, although in both theory and practice, it is similar to gas chromatography. In column chromatography, the sample is introduced into a liquid mobile phase which flows through a column usually containing silica or alumina under the influence of gravity. Flow rate are in the order of $0.1cm^3\ min^{-1}$, which result in extremely lengthy separation time, and quite inadequate efficiency for separation of multi-component mixture. The poor performance is largely due to very slow mass transfer between stationary and mobile phases and poor packing characteristics leading to a large multiple path effect.

It was later recognized that the problems could be overcome through the use of smaller particles of stationary phase, and that rapid separations would require higher flow rate, necessitating the pumping of mobile phase through the column under pressure.

The means of meeting this basic requirement were developed in HPLC. The mobile phase is typically pumped at pressure up to about 3000psi, and flow rate of $1\text{-}5cm^3min^{-1}$ can be achieved through 10-25cm stainless steel column packed with particles as small as 3µm in diameter.

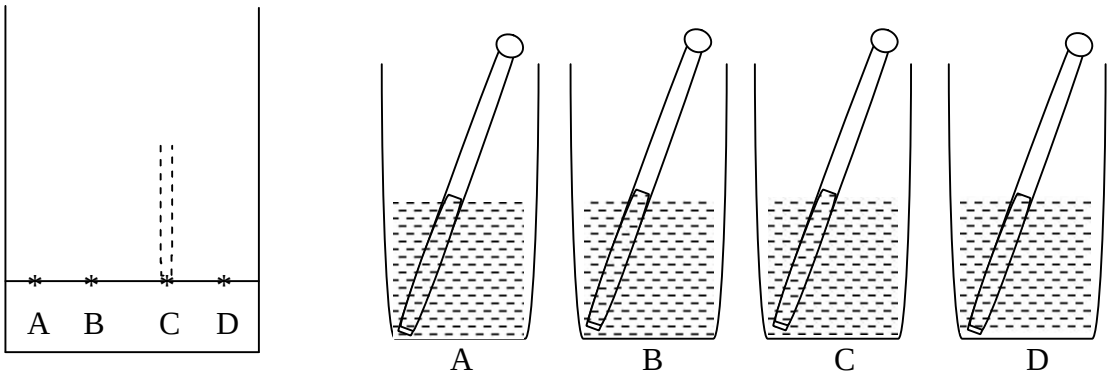
HPLC can be used largely for the separation of non-volatile substances including ionic and polymeric sample. This is complementary to gas chromatography. The use of HPLC in all its form is growing steadily and may eventually exceed that of GLC. This is because all four sorption mechanisms can be exploited and the technique is well suited to a very wide range of compound types including ionic, polymeric and labile (volatile) materials.

PAPER CHROMATOGRAPHY

Partition chromatography on sheet or strips of filter paper is one of the simplest and the most widely used of chromatographic technique. "Whatman 3mm" is used for preparative work.

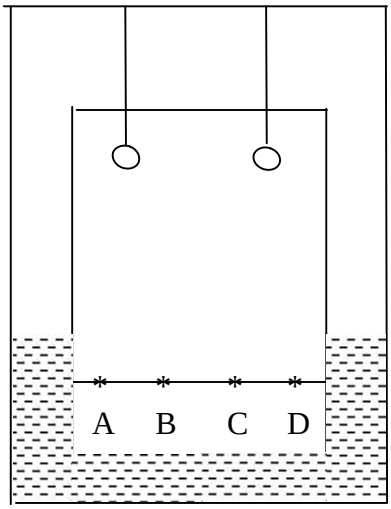
METHOD

Samples are applied on the paper from the solution. Extracts from biological tissue often require preliminary purification before they can be applied. The reason for this is that large amount of protein or salt can interfere with the partition process by extracting water from the solvent. Proteins can be precipitated with alcohol and salts removed by electrolytic method (ion-exchange). Having selected the size and grade of paper to be used, a pencil line is drawn parallel to one edge and at a suitable distance from it. A number of small crosses are mount on the line corresponding to the number of samples to be applied and the nature of each sample written on in pencil. A drop of each sample is spotted on the appropriate position with a short length of capillary tubing or a platinum loop.



The spot should be about 5mm in diameter. Large spots lead to poorer separation. If more test substance is required than is present in 1 drop (especially with dil. solution), the spot is allowed to dry by hair dryer and a similar application made. When the spots are dry, the paper is ready for development, in which solvent flows through the paper to produce separation. The solvent for development depends upon the nature of substances to be separated. It is essential to use one – phase solvent which corresponds in composition to the organic layer. Development can be carried out either by allowing the solvent to travel up the paper (the ascending technique) or down the paper (the descending technique).

Some of the solvent is poured into the bottom of the tank so as to saturate the atmosphere with its vapour.



Both ascending & descending techniques are employed. The ascending method usually gives better result with very volatile solvent. The disadvantage of this technique is that compounds with low R_f values are often incompletely separated. With the descending technique, the developing solvent can be allowed to run off the edge of the paper under the influence of gravity so that one is able to increase considerably the effective length of the run and this improves the separation. When the solvent has travelled the required distance, the position of the solvent formed is noted by tearing the paper slightly (or cut it) at the edges. Drying is carried out in the fume cupboard by electric hair dryer. The next is to locate the separated compounds. If they are coloured, there is no problem, but many compounds, especially of biological interest are colourless and hence invisible. Several methods are used, both physical and chemical methods to spot the compounds present.

The physical methods utilize particular properties of the compound such as fluorescence or radioactivity, while chemical methods involve reacting the substance with reagents to get coloured products.

Physical Methods

1. ***Fluorescence***: A number of unsaturated organic compounds fluoresce i.e. they have the property of absorbing UV light of short (invisible) wavelength and emitting light of longer (visible) wavelength. These compounds, although invisible on chromatogram in ordinary light, can readily be detected under UV lamp. The wavelength of the light emitted and hence the colour

when seen is a characteristic of the compound and is therefore useful for the purpose of identification.

2. ***Radioactivity***: The wide spread use of nuclear power labeled radioactive compounds for research. This can be detected on chromatograms by means of special counter (Geiger Muller counter and Scintillation counter).

Physical methods of location have the advantage over chemical methods in that the substances on the chromatogram are not converted into other compounds and so they can be removed for further studies.

Chemical Methods

Colourless compounds are converted to coloured compounds by treatment with "locating reagents". The locating reagent can be a gas (H_2S), for location of metallic ions which form metallic sulphide. Solutions of reagents can be applied by dipping the paper into a solution of the reagent or by spraying the solution on the paper e.g. the most widely used reagent for detecting amino acid is "ninhydrin".

Ninhydrin is a white solid which when applied to the paper at a 0.1 – 0.25% solution is a suitable solvent.

The reaction can be represented as:

Amino acid	+	ninhydrin	⟶	coloured product
Colourless		colourless (white)		blue - lilac

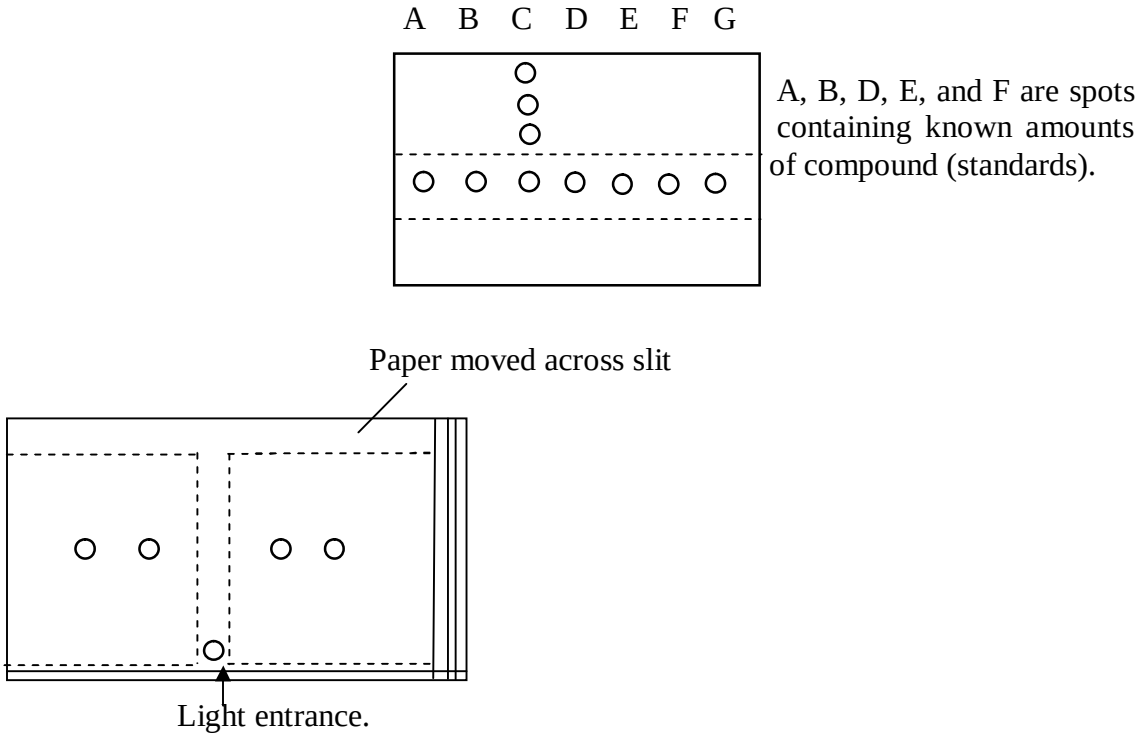
Also, sensitive methods of detecting reducing substances e.g. glucose and maltose (malt sugar) is by paper chromatography. This is dipped into appropriate volume of $AgNO_3$ and NaOH in alcohol. A grey spot is an indication of reducing sugar.

Both physical & chemical methods described are for qualitative analysis.

Quantitative Measurement

Sometime, it is required to make a quantitative measurement of a particular measurement in a mixture in addition to the usual qualitative ones.

The paper is developed dried and the marginal strips cut out and treated with suitable reagents. The compound so eluted can then be determined by one of the multitude of methods available for substances in solution (colour reactions, titration, micrograviметric procedures etc.) If a reagent is available, which react with the component to produce a colour, the intensity of which is proportional to the amount of that component present then quantitative measurement can be made directly on the paper. Such methods are always comparative in nature. For more accurate work, the intensity of each spot is measured with a photoelectric device known as **"photo densitometer"**. A thin strip is cut from the chromatogram (area enclosed by the dotted line), placed between two sheets of glass, and into the holder of the densitometer. The machine is set at zero on the section of the strip free from any compound and the strip is then moved in small stages passed the beam and there will be deflection. The deflection is proportional to the concentration of the material. Such deflection or dryer is recorded.



The graph of that deflection is plotted against concentration (standards). It obeys Beer-Lambert's law.

Advantages of Paper Chromatography

One of the major advantages of is the sensitivity with which compound can be located after separation. Amount as little as 0.1μg can be detected with routine reagent. The lower limit for the detection of most compounds is between 1-50 μg (ppb).

Paper chromatography has played major role in elucidating the chemistry of compounds.

THIN – LAYER CHROMATOGRAPHY

Comparatively, paper chromatography is a versatile technique but its use is limited by the fact that separation can be conducted only with fibrous materials such as cellulose, since other valuable media such as silica gel, alumina and gel filtration beads cannot be made into sheets. This problem can be overcome by supporting thin – layer of these substances on an appropriate base. The usual method is to support them on glass base: in which case the product is referred to as **"chromatoplate"**.

A thin layer supported on solvent – resistant plastic sheet is called **"chromatosheet"**. Both are available commercially. Two types of layers are useful; solid layers which adhere to the support by the virtue of the adherent qualities of the material itself or because of a binding agent incorporated with it and also the loose layers.

Thin – layer separation resemble those of paper in some ways, but much wider choice of media means that separation by partition adsorption, gel exclusion filtration and ion – exchange can be performed by this technique.

The particular properties of thin layer also allow shorter development times to be achieved.

PREPARATION OF CHROMATOPLATE

Solid layers are prepared by applying 'slurry' of the chosen medium in a suitable liquid unto a clean glass plate. It is essential for optimum results to be achieved by making the layers to be uniform.

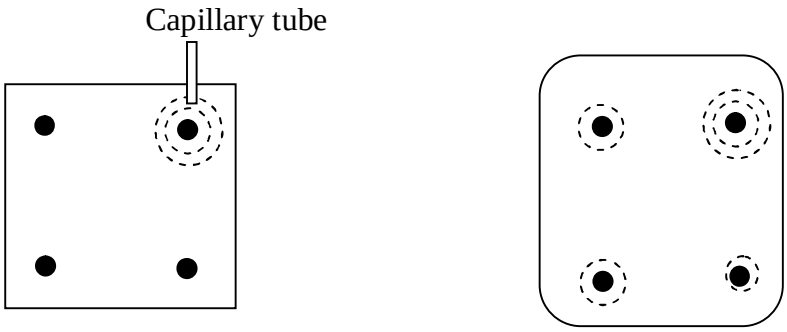
The particle size, surface structure and adhesiveness of medium must be carefully controlled. If reproducible results are to be obtained must be carefully controlled, if reproducible results are to be obtained. If the material adheres badly, a binding agent such as calcium sulphate ($CaSO_4$) is often

incorporated in small quantity. Other additives to thin layer media include “special fluorescing agent”, which allows spots on the developed chromatogram to be seen under UV light.

The thickness of layers for thin layers for thin layer chromatography is 0.25mm. Thinner layer usually gives a more rapid but less effectives separation.

Choice of medium and solvent

Almost any material can be used for thin layer, and the factors involved in the choice have been discussed earlier. The choice of solvent will depend on the nature of substances being separated and on the material on which it is being separated. A general rule is to match the polarity of the solvent to that of the substances being separated. Sometimes, trial solvents are applied by capillary tube to the center of each spot as shown below.



APPLICATION OF SAMPLE

The method is similar to that of paper chromatography except that the delicacy of many of the layers makes it necessary to take much more care.

Application of Thin – Layer Chromatography

Thin layer does not provide quantitative information of the highest precision and accuracy. The same procedure for quantitative evaluation in PC can also be followed. TLC is very widely used for qualitative purposes. Almost any mixture can be at least partially resolved. Inorganic applications such as separation of metals in alloys, soil and geological samples, and polar organic system, such as mixture of amino acids or sugars in urine are particularly suited for this exercise. TLC is ideally suited for a lot of complex reactions, quality control, purity checks, clinical diagnosis and forensic tests.

ION EXCHANGE CHROMATOGRAPHY

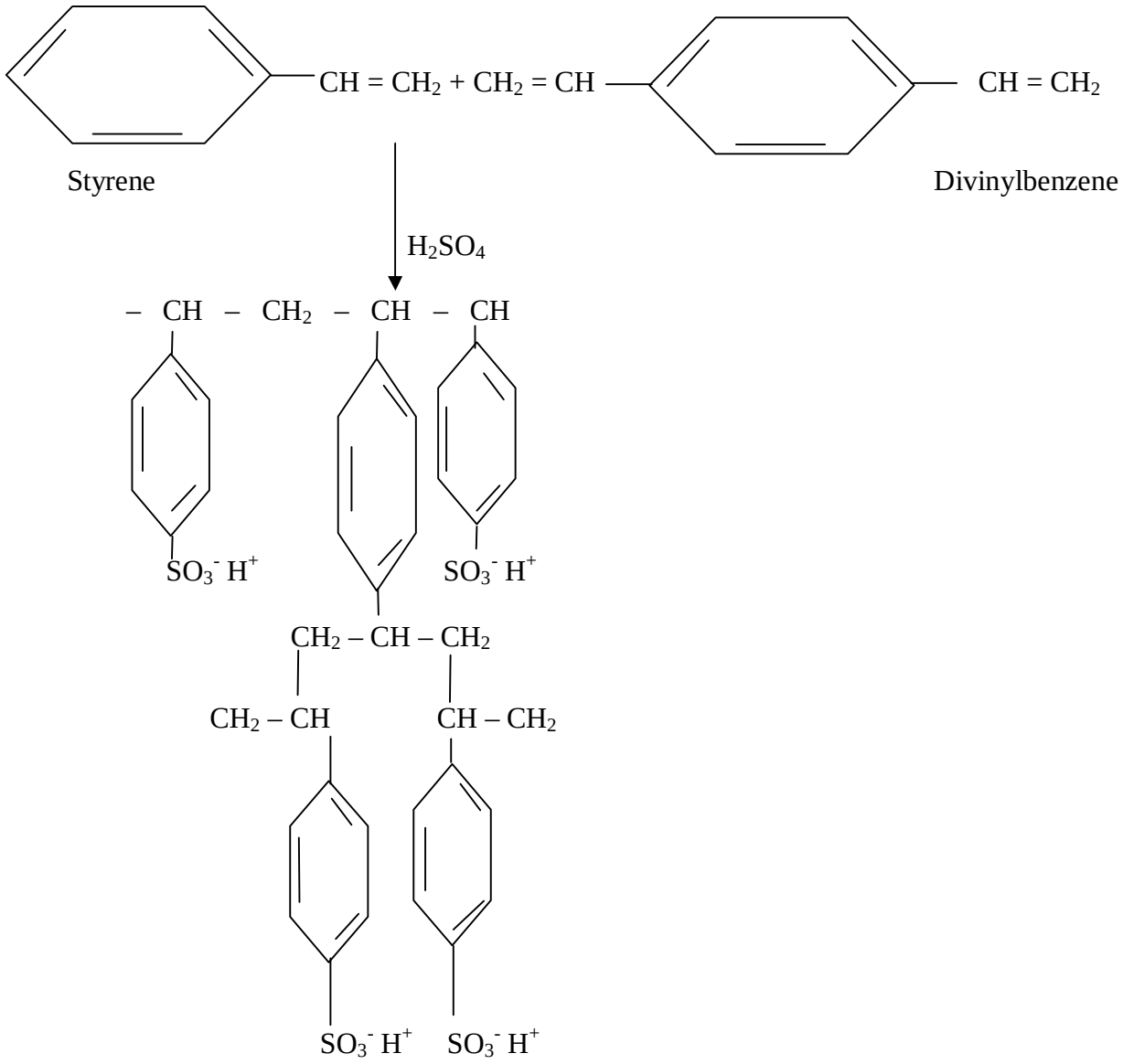
Ion-exchange is generally understood to mean the exchange of ions of like signs between a solution and a solid highly insoluble in contact with it. The solid (ion-exchanger) contains ions of its own and for the exchange to proceed sufficiently; the solid must have an open, permeable molecular structure, so that ions and solvent molecules can move freely in and out. All ion-exchanger sub value in analysis must have the following characteristics:

1. They are almost insoluble in water and in organic solvent.
2. They contain active or counter ions that will exchange reversibly with other ions in surrounding solution without any appreciable physical change occurring in the materials.
3. The ion-exchanger is of complex nature and is in fact polymeric.

The polymer carries an electric charge that is exactly neutralized by the charges on the counter ion. These active ions are cations in CATION EXCHANGER and anions in ANION EXCHANGER. Thus, a cation exchanger consists of a polymeric anion (R^- X^+) with active cation, while an anion exchanger is a polymeric cation (R^+ $X^{-)}$ with active anions.

CATION EXCHANGER

We may define a cation exchange resin as a high molecular weight cross linked polymer containing sulphonic, carboxylic, phenolic etc group, as an integral part of the resin and an equivalent amount of cations e.g. the copolymerization of styrene and divinylbenzene, followed by sulphonation.

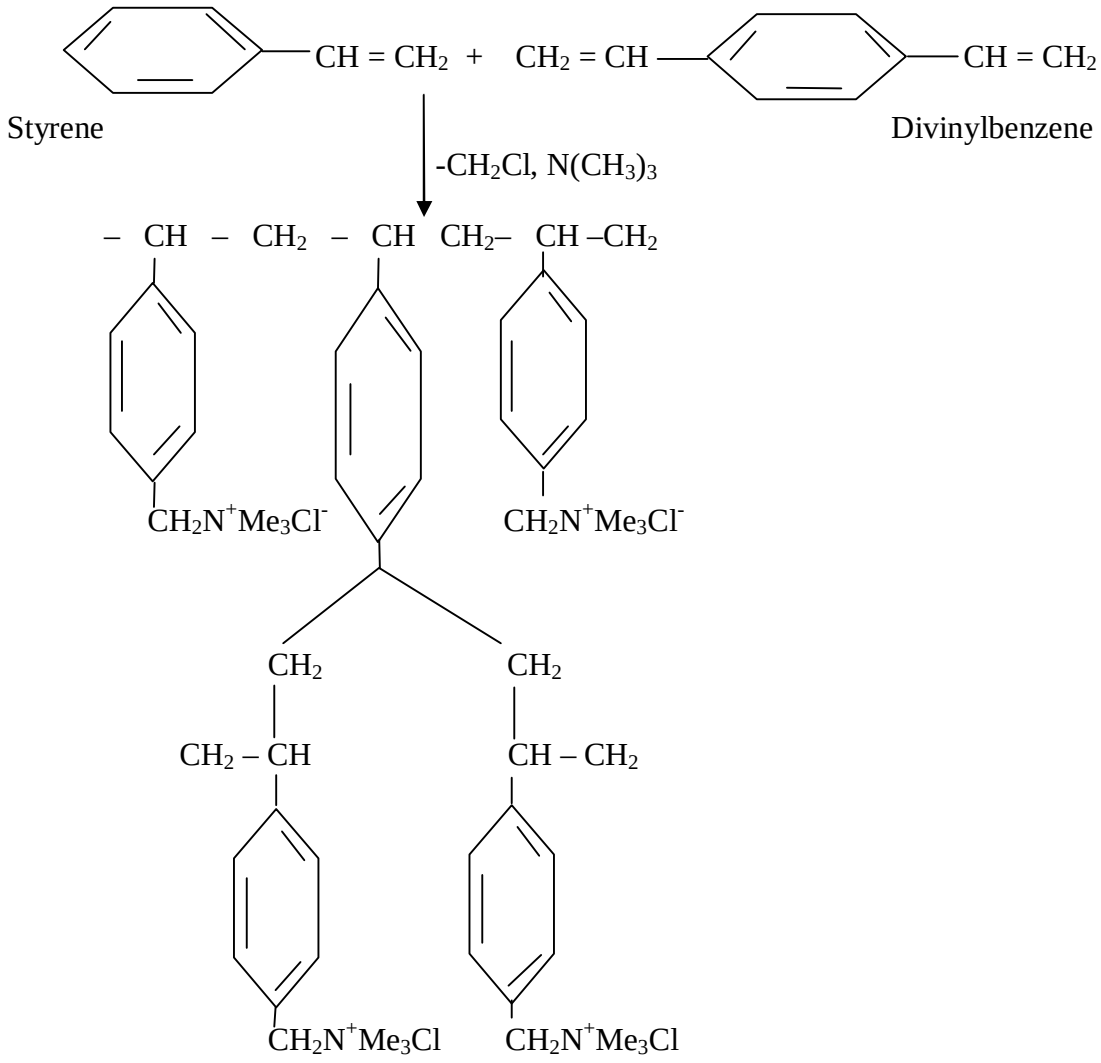


Cation Exchanger

ANION EXCHANGER

Anion exchangers are likewise cross linked high molecular weight polymer. Their basic character is due to the presence of amino, (NH_2), substituted amino or quaternary ammonium group. The resin is a polymer containing the amino, or quaternary ammonium group as integral part of polymer lattice and an equivalent amount of anions such as chlorides, hydroxyl or sulphate ions e.g. copolymerization of

styrene and a little of divinylbenzene followed by chloromethylation (addition of CH_2Cl) and interaction with a base such as trimethylamine.



Anion Exchanger

ACTION OF ION EXCHANGE RESIN

Cation exchange resin can be exchanged for cations in solution.

$(\text{Resin } A^-)B^+ + C^+ \text{ solution} \leftrightarrow (\text{Resin } A^-)C^+ + B^+ \text{ solution}$

If the solution contain several ions (C^+, D^+, E^+), the exchanger may show different affinities for them thus making separation possible.

FACTORS AFFECTING ION EXCHANGE BETWEEN RESIN AND SOLUTION.

Nature of exchanging ions

At low aqueous concentration and at ordinary temperature, the extent of exchange increases with increasing valency of the exchanging ions, i.e. $Na^+ < Ca^{2+} < Al^{3+} < Th^{4+}$.

Under similar condition and constant valency for univalent ions, the extent of exchange increases with decrease in size of the hydrated cation i.e. $Li^+ < H^+ < Na^+ < NH4^+ < K^+ < Rb^+ < Cs^+$, while for divalent ions, the ionic size is an important factor, but incomplete dissociation of salts of bivalent metals also plays a part ($Cd^{2+} < Be^{2+} < Mn^{2+} < Mg^{2+} = Zn^{2+} < Cu^{2+} = Ni^{2+} < Co^{2+} < Ca^{2+} < Sr^{2+} < Pb^{2+} < Ba^{2+}$

With strongly basic anion exchange resins, univalent anions appear to behave similar to univalent cation.

$$F^- < OH^- < HCO_3^- < Cl^- < HSO_3^- < CN^- < Br^- < NO_3^- < I^-$$

In dilute solution, polyvalent anions are generally absorbed preferentially.

When a cation in solution is being exchanged for anion of different valency, the relative affinity of the high valent ion increases in direct proportion to the dilution. Thus, to exchange a higher valent ion on the exchanger for one of lower valency in solution, exchange will be favoured by increasing the concentration, while if the lower valent ion is in the exchanger, and the higher valent ion is in solution, exchange will be favoured by high dilution.

Nature of Ion-Exchange Resin

The absorption of ion will depend upon the nature of the functional group in the resin. It will also depend upon the degree of cross linking. As the degree of cross linking is increased, resin becomes more selective towards ions of different sizes.

ELECTROPHORESIS

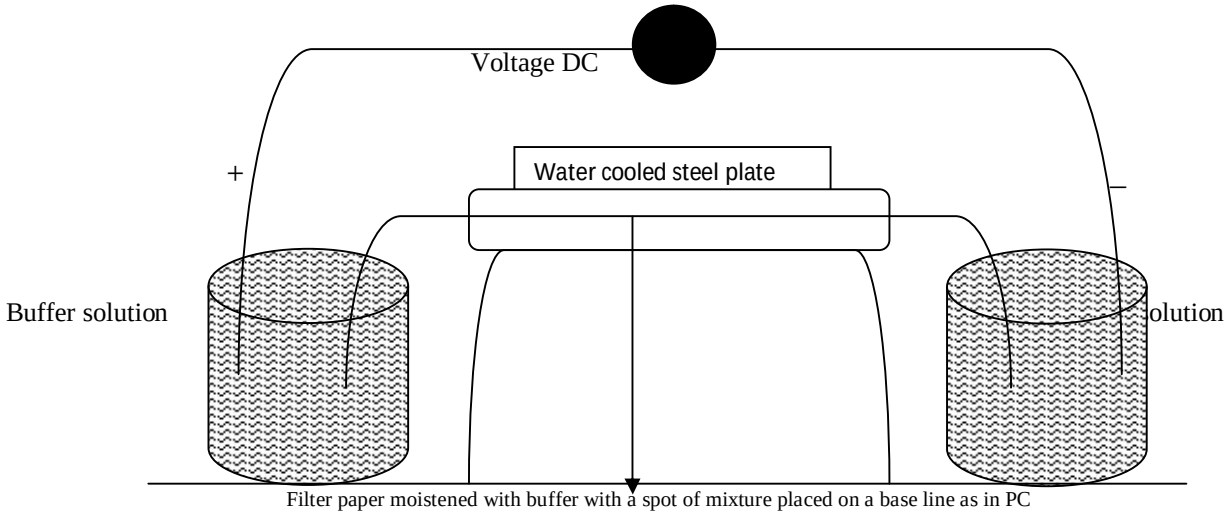
Electrophoresis is a technique which is closely associated with chromatography, and is often used in conjunction with it.

Separations depend upon the difference in the electrical properties of the components in the mixture, so that often substances which would be difficult to separate by chromatographic methods are readily separated by this technique. Electrophoresis is an incomplete form of electrolysis in which the charged particles are stopped somewhere along their path to the electrode.

There are two types:

a. ***Free Electrophoresis,*** in which the separated substances are in solution and are therefore free to diffuse the moment the current, is switched off.
b. ***Zone Electrophoresis,*** in which the separation is carried out on a supporting medium such as starch gel or strips of filter paper.

A pencil line is drawn perpendicular to the length of the filter paper and spots of samples applied to positions mount on the line. The paper is carefully moistened with a buffer solution suitable to effect separation, and the two ends dipped into pots of the same buffer solution as shown below.



Filter paper moistened with buffer with a spot of mixture placed on a base line as in PC

When the current is passed, the supporting medium act as a 'bridge' between the two pots of buffer solution and any substance in the mixture which bears an electrical charge will migrate. In

many cases, molecules which bear no electrical charge can have complex ions with ions present in the buffer solution.

Many sugar molecules can be separated in this way. After a suitable time, the current is switched off and the paper is removed from the apparatus. The rate of migration of substance during electrophoresis depends on several factors, e.g. the voltage applied, the structure of the ion e.t.c.

After allowing the paper to dry, the separated components can be located with a ‘locating reagent’ if they are not naturally coloured. In the same way described for paper chromatography, it is necessary to put on the filter paper a non-moving marker, i.e. a compound which will have no electrophoretic mobility since there is a tendency for substances to move in a direction opposite to that of their electrophoretic movement by electro osmosis.