EMT 516: SEPARATION TECHNIQUES IN ENVIRONMENTAL ANALYSIS (2 UNITS)

Lecturers: Professor O. Bamgbose & Dr. O.S. Olatunji

SYNOPSIS

Solvent extraction methods in analysis. Types of separation, distribution coefficient, single batch extraction, continuous bath extraction, Bath processes, application of solvent extraction. Principles of chromatography theoretical plates, resolution and band spreading, paper, thin layer, ion-exchanger, column, gel-permeation, GLC, electrophoresis.

LECTURE CONTENT

SEPARATION METHODS

Objective:

1. Separation of Interferences
2. Concentration
3. Increasing defection limit
4. Separation of material of interest. Analytes from mixture of closely related substances.

e.g: in the sensitive but unselective colorimetric analysis of In (III) using 4-(-2 pyridylazo) resorcinol, which forms a pink 2:1 complex with In^{3+}. E = 4.3 x 10^{-4} m-1cm-^{1} at 510nm) Elements such as Zn, Pb,Cr, Al, Sn, Cd, Cu, Mn, Fe, Co, Ni, V, Zr and Bi all interferes with the determination.

To analyze a mineral containing just 0.1% in requires the separation of the above elements.

One procedure involves
(i) Dissolution in Hu plus HNO_3
(ii) Removal of Sn^{4+} as volatile $SnBr_4$
(iii) Precipitation of Pb^{2+} with SO_4^{2-}
(iv) 1n (OH)3 and the OH^- of other several metals are Precipitated with NH_3 and redissolved in HCl.
(v) Addition of KI give InI_3, which can be extracted into Diethylether.
(vi) Finally, the In can be extracted back into aqueous Solution and analysed colorimetrically.

Isolation of one component of a mixture for analytical or preparative purposes is an important and challenging aspect of the practice of chemistry.

SOLVENT EXTRACTION

Definition:

Extraction: A method of separation in which a solid or solution is contacted with a liquid solvent (the two being essential mutually insoluble) to transfer one or more components into the solvent.

Extract: Material separated from liquid or liquid or solid mixture by a slvent

Extractant: The liquid used to remove a solute from another liquid

Solvent extraction:

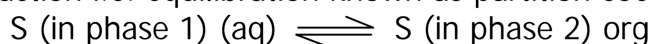
In its simplest form is the transfer of a solute from one solvent to another which is for all purpose and intent immiscible with the primary solvent.

The most common case is the extraction of an aqueous solution with an organic solvent.
Diethylether, Benzene and other organic solvents are common solvents used, that are less dense than water, hence they sit on top. CH_2Cl_2, $CHCl_3$ and CCl_4 are immiscible with water but denser than water.

In both phases (i.e. organic and aqueous) some of each solvent is found in both phases, but one phase is predominantly water and the other phase predominantly organic. Thus the volume of each phase after mixing/equilibration is not exactly equal to the original volume mixed. For the purpose of study, we will assume that volume changes do not occur.

Solvent extraction is essentially a partition technique which depends on the distribution of a solute substance between the two solvent (i.e. differential solubility of a solute in 2 solvent)

Supposing a solute S is partitioned between phase 1 and 2, the equilibrium constant for the reaction i.e. equilibration known as partition coefficient is given as:



S (in phase 1) (aq) $\rightleftharpoons$ S (in phase 2) org

$$K = [S]_2/[S]_1 \qquad \text{-----------------(1)}$$

i.e. the rate of the solubility of solute in organic solvents to the solubility of solute in aqueous solvent.

$$K = \frac{\text{Solubility of solute in organic solvent}}{\text{Solubility of solute in aqueous solvent}}$$

K = Partition coefficient applies only, if neither of the solvent is saturated by the solute; but yet the ratio will be in the proportion of their solubilities.

K is thus a thermodynamic parameter (i.e. state of function) given the above conditions, such that k is constant. If k is large, the extraction process is quantitative and good transfer is effected in a single stage. If K is low, then the extraction requires multi stages transfer.

Note: The substance of interest i.e. solute must exist in the same form in both solvents i.e. either as monomer, molecule, polymer etc.

Thus, if during a separation process, an amount of solute is transferred from the aqueous phase 1 to the organic phase 2, and the amount of solute left in aqueous is q, then molarity or concentration of solute in phase one is given as;

$$[S]_{aq} = q_m/V_1 \qquad \text{----------} \ (2)$$

Thus the amount of solute transferred to the organic phase 2 is given as 1 – q = p, and the molarity or concentration of solute in phase 2 is given as;

$$[S]_{org} = (1 - q)_m/V_2 \ \text{-----------} \ (3)$$

Thus the partition coefficient K is given as:

$$K = [S]_{2corg}/ [S]_{1aq}$$
$$= (1 - q)_m/V_2/ q_m/V_1$$
$$= (1 - q)_m/ V_2 \div q_m/V_1$$
$$= (1 - q)_{\not{m}}/ V_2 \times V_1/q_{\not{m}}$$
$$K = (1-q) V_1/V_2q$$
$$KV_2q = V_1 - qV_1$$
$$KV_2q + qV_1 = V_1$$
$$q (KV_2 + V_{1)} = V_1$$
$$q = V_1/ KV_2 + V_1 \longleftrightarrow V_1/ V_1 + KV_2$$

If k is substituted for by D

$$q = V_1/ V_1 + DV_2$$

it should be noted that q is constant for any extraction process since K is a constant; and the efficiency of separation depends on D, Distribution ratio.
Thus for n numbers of extraction, the amount of solute left or transferred in the 2 solvent is given thus:

$$q = V_1/ V_1 + DV_2$$

N = number of times extraction is carried out.

Thus, the fraction of solute left in the aqueous phase depends on K and the two volumes. For instance if the 2 phases are separated and fresh solvent 2 is mixed with phase 1 (aq), the fraction of solute remaining in the aq phase 1 is given as:

$$q.q = (V_1/V_1 + DV_2)^2 = q^2$$

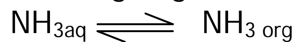
DISTRIBUTION RATIO

Distributive ratio denoted by the symbol D is the ratio of the solubility of the solutes in all its form in the organic solvent to the solubility of the solutes in all its form in the aqueous solvent.

$$D = \frac{\sum \text{solubilities of solutes in phase 2}}{\sum \text{solubilities of solutes in phase 1}}$$

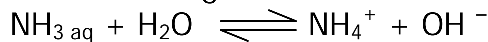
Note that D is not a thermodynamic parameter; thus varies with experimental and environmental conditions. If K is known and the experimenttal conditions are defined, then D can be calculated.

Consider NH_3 going into a non ionizing solvent



$$NH_{3aq} \rightleftharpoons NH_{3\ org}$$

But NH_3 in an ionizing solvent, results into some degree of dissociation



$$NH_{3\ aq} + H_2O \rightleftharpoons NH_4^+ + OH^-$$

In non-ionizing solvent

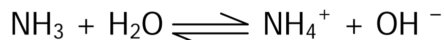
$$K = [NH_3]_{org}/[NH_3]_{aq} \qquad \text{----------(1)}$$

In Ionizing solvent, D is given by

$$D = [NH_3]_{org}/ /[NH_3]_{aq} + /[NH_4^+]_{aq} \qquad \text{---------(2)}$$

Thus in ionizing solvent defined by pH, D changes with changes in pH

Consider ammonia in water



$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

$$k_b = [NH_4^+]\ [OH^-]_{aq}/[NH_3]_{aq} \qquad \text{--------------a}$$

$$k_a = [NH_3]_{aq}/[NH_4^+]\ [OH^-]_{aq} \qquad \text{--------------b}$$

But we know that

$$k_w = [H^+]\ [OH^-]$$

$$[OH^-] = k_w/[H^+] \qquad \text{-------------c}$$

Substituting equation c into a

$$K_b = [NH_4^+]k_w/[NH_3]\ [H^+]$$

: - $[NH_4^+] = k_b\ [NH_3]\ [H^+]/k_w$ ---------------d

Substituting equation a into equation 2

$D = [NH_3]_{org}/[NH_3]_{aq} + k_b [NH_3]_{aq} [H^+]_{aq}/K_w$--------(3)

But

$K_w = k_a . k_b$

$Kw/kb = ka$

$:1/k_a = k_b/k_w$ -----------------e

Substituting equation e into 3

$D = [NH_3]_{org}/[NH_3]_{aq} + [NH_3]_{aq} [H^+]_{aq}/k_a$

$D = [NH_3]_{org}/[NH_3]_{aq} [1 + [H^+]_{aq}/k_a]$

$= k/1 + [H^+]_{aq}/k_a = \quad k/k_a + [H^+]_{aq}/k_a$

$D = k\, k_a/k_a + [H^+]_{aq}$

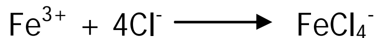
Note: Optimum separation occurs when the amount of solute transfered from the aqueous phase (solvent) to organic phase (solvent) is about 99.9%.
Efficiency of separation is high when D i.e. distribution ratio is high. Thus the denominator must be as small as possible and this occur at low pH (i.e. when a base is to be extracted into water (NH_4^+) at the same time. Use high pH to extract an acid into water (A^-).
When the solute is in molecular form, the efficiency of solute transfer from aqueous solvent to organic phase is high.

TECHNIQUES EMPLOYED IN SOLVENT EXTRACTION

Some inorganic species are basically not extracted by organic solvent, hence can be made extractible by organic solvent, by making the inorganic species non-polar. This can be achieved by: (1) Ion pair formation
(2) Use of metal Chelators (chelates)

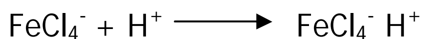
(1) Ion Pair Formation

Ion pair could sometimes be extracted into an organic phase from an aqueous phase. For example, Fe^{3+}, which is soluble in water, could be extracted as $FeCl_4^-$ from 6M HCl into diethylether, as tightly associated unit or ion pair written as $FeCl_4^-H^+$, with no net charge.
The mechanism is thus:



$Fe^{3+} + 4Cl^- \longrightarrow FeCl_4^-$

This attracts H^+ to form a neutral molecule



$FeCl_4^- + H^+ \longrightarrow FeCl_4^- H^+$

The distribution coefficient is a delicate function of conditions.

Thus, too much or too little HCl may lead to incomplete extraction of $FeCl_4^-H^+$. The optimum condition for the process is found by trial and error.

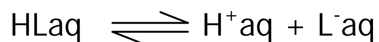
(2) Extraction with a Metal Chelator

One of the processes of separating metal ions from each other, is to selectively complex one ion, using an organic ligand (chelator) and extract it into an organic solvent.

Ligands commonly used for this purpose are:
(i) Dithizone
(ii) 8 - Hydroxylquinoline
(iii) Cupferron

Each ligand can be represented as a weak acid, HL which loses one proton when it binds to the metal ion.

Consider HL in an ionizing solvent.



$HLaq \rightleftharpoons H^{+}aq + L^{-}aq$

for which

$Ka = [H^{+}]aq\,[L^{-}]aq/\,[HL]aq$ ----------1

On chelation of the metal ion M^{n+} by ligand ion nL^{-}

$nL^{-}aq + M^{n+}\,aq \rightleftharpoons MLn\ aq$

for which the formation constant is

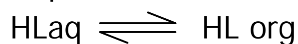
$\beta = [ML_{2}]aq/M^{n+}\,aq[L^{-}]^{n}aq$ ----------2

Each of the ligands can react with different metal ions, but some selectivity is achieved by controlling pH. Most complexes extracted into organic solvent are neutral because charged complexes are not very soluble in organic solvents e.g. Fe $(EDTA)^{-}$ or Fe$(1,10\text{-phenanthroline})_{3}^{2+}$

Deriving an equation for the distribution co-efficient of a metal between the two phases (aqueous & organic) using metal chelates:

Assumptions:
(i) Metal ions in aqueous phase (in form of M^{n+})
(ii) All metals in organic phase are in the form MLn

Thus the partition coefficient for ligand and complex are as follows:

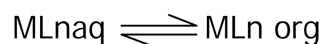


$HLaq \rightleftharpoons HL\ org$

for which

$K_{L} = [HL]org/\,[HL]aq$ ----------3

and



$MLnaq \rightleftharpoons MLn\ org$

for which

$Km = [MLn]org\ /\ [MLn]aq$ ----------4

Therefore, Distribution coefficient is given as

$$D = \frac{[\text{Total metal}]org}{[\text{Total metal}]aq} \sim \frac{[MLn]org}{[M^{n+}]aq} \quad 5$$

thus from equation 2

β = [MLn]aq / [Mnt]aq $[L^-]^n$ aq

β [Mnt]aq $[L^-]^n$ aq = [MLn]aq

[MLn]aq = β $[M^{nt}]$aq $[L^-]^n$ aq ----------6

from equation 4

Km = [MLn]org / [MLn]aq

Km [MLn]aq = [MLn]org

[MLn]aq = [MLn]org/km ----------7

Substituting equation 7 into 6

[MLn]org/km = β $[M^{n+}]$aq $[L^-]^n$ aq

[MLn]org = km β $[M^{n+}]$aq $[L^-]^n$ aq----------8

But from equation 1

Ka = $[H^+]$aq $[L^-]$aq / [HL]aq

Ka^n = $[H^+]^n$aq $[L-]^n$aq / $[HL]^n$aq

$[L^-]^n$ aq = Ka^n $[HL]^n$aq / $[H^+]^n$aq ----------9

Substituting equation 9 into 8

[MLn]org = km β $[M^{n+}]$aq $Ka^n[HL]^n$aq / $[H^+]^n$aq

[MLn]org / $[M^{n+}]$aq = km β ka^n $[HL]^n$aq / $[H^+]^n$aq 10

Since: [MLn]org / $[M^{n+}]$aq = D

equation (10) becomes

D = km β ka^n $[HL]^n$aq / $[H^+]^n$aq ----------11

Thus, since most of the ligand i.e. HL is in the organic phase,

D = km β kan $[HL]^n$aq / K^n_L $[H^+]^n$aq -----12

Thus equation 12 implies that distribution coefficient for metal ions extraction depends on pH and the ligand concentration. Since equilibrium constants are different for each metal, it is possible to select pH for which D is large for one metal and small for the other, e.g Cu^{2+} can be separated from Pb^{2+} and Zn^{2+} by extraction at pH = 5 using Dithizone.

Note that ions that form an extractable complex with a non selective chelator might be isolated by masking other ions in the sample, e.g. citrate & tartrate forms polar complexes with many ions, and they remain in aqueous phase while the desired ion might be extracted with another chelator.

Relative stability constants are important in this scheme. Also, sometimes a relatively polar ion can be extracted into organic phase in the presence of hydrophobic counterion. Tetrabutylammonium cation $[(C_4H_9)_4N^+]$ is commonly used for this purpose.

Extraction of Metal ions with non-polar molecule

Another way to extract metal ion, is to use a non-polar molecule with one good ligand atom that may occupy one coordination site of a metal ion, thus pulling it into the organic phase.

Example of hydrophobic ligand that can interact through their oxygen atoms with cations are

Trioctylphosphine oxide $[CH_3(CH_2)_7]_3\ P+ - 0$

$[CH_3(CH_2)_7]_3\ P = 0$

and

Dioctylsulphoxide $[CH_3(CH_2)_7]_3\ S = 0$

Crown ethers and ionophores are designed to extract metal ions into non-polar solvent.

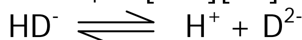
For example: consider the extraction of Pb^{2+} using Dithizone

Dithizone demoted by H_2D

H_2D in an ionizing solvent yields

$H_2D \rightleftharpoons HD^- + H^+$

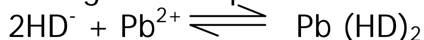
for which $Ka_1 = [HD^-][H^+]aq / [H_2D]aq$ ----------1



$HD^- \rightleftharpoons H^+ + D^{2-}$

For which $Ka_2 = [H^+][D^{2-}]aq / [HD^-]aq$ ----------2

Any of the ligand ion species HD^- or D^{2-} can react with the solute i.e. metal ion Pb^{2+}

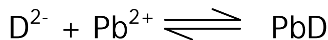


$2HD^- + Pb^{2+} \rightleftharpoons Pb\ (HD)_2$

for which the formation constant is

$\beta = [Pb\ (HD)_2)]aq / [Pb^{2+}]\ [HD^-]^2aq$ ----------3

and



$D^{2-} + Pb^{2+} \rightleftharpoons PbD$

for which the formation constant is

$\beta = [PbD)]aq / [Pb^{2+}]\ [D^{2-}]aq$ ----------4

Condition for extraction:

1. H_2D (ligand or chelator) is made in organic phase
2. Pb^{2+} is in aqueous phase
3. Right pH to consume H^+ so as to have HD^- and D^{2-} to consume Pb^{2+}.

Note that the amount of Pb^{2+} that forms complex depends on the stability of the complex depends on the stability of the complex and this depends on the pH.

Procedure

i. Complexing agent is prepared in organic solvent.
ii. The separating funnel is then mixed (equilibrated)

The Dithizone H_2D partitions itself between the aqueous and organic phases for which partition coefficient is given as

$$KD = [H_2D]org / [H_2D]aq \qquad ----------5$$

Also the distributive coefficient for chelation in ionizing solvent is given as:

$$[Pb(HD)_2]org / [Pb(HD)_2]aq + [Pb^{2+}] \qquad ----------6$$

But the partition of the metal chelate is given by partition coefficient as

$$K = [Pb (HD)_2]org / [Pb (HD)_2]aq ----------7$$

from equation 2

$$[Pb (HD)_2]aq = \beta [Pb^{2+}]aq [HD^-]^2aq$$
$$[Pb (HD)_2]aq =[Pb(HD)_2]aq/ \beta [HD^-]^2aq \quad 8$$

Substituting equation 8 into 6

$$D = [Pb(HD)_2]org / [Pb(HD)_2]aq + [Pb(HD)_2]aq / \beta [HD^-]^2aq \quad 9$$

For separation with high efficiency, the concentration of the metal chelate $[Pb(HD)_2]aq$ tends to zero, and the amount of Pb^{2+} ions in the aqueous phase tends to zero too hence

$$D = [Pb(HD)_2]org /0 + [Pb(HD)_2]aq/\beta[HD^-]^2aq$$
$$= [Pb (HD)_2]org/[Pb (HD)_2]aq/\beta[HD^-]^2aq \qquad ----------10$$

we know from equation 7 that

$$[Pb (HD)_2]org / [Pb (HD)_2]aq = K$$

equation 10 becomes

$$D = K_{[Pb(HD)2]} \Big/ 1/\beta[HD^-]^2aq \qquad = \qquad K_{[Pb(HD)2]} \,.\, \beta[HD^-]^2aq----------11$$

where HD^- is the amount of ligand dissolved in the aqueous phase.

Thus from equation 1 & 5

$$D = K_{Pb(HD)2}.\ \beta.\ \frac{Ka_2\ [H_2D^-]^2aq}{[H^+]^2aq}$$

$$D = \frac{K_{Pb(HD)2}\ .\ \beta.\ Ka_2\ [H_2D^-]^2org}{K^2D\ [H^+]^2aq} \qquad ----------12$$

COUNTER CURRENT DISTRIBUTION

This is a serial extraction process derived by L.C. Crag in 1942. The process is a powerful improvement of liquid extraction. Although this process is almost totally supplanted by chromatographic methods of separation, its theory is worth studying

because it provides a basis for understanding chromatography. However automated countercurrent separations apparatus using a centrifugal field to help separate phase is still the method of choice for certain large-scale separations of delicate drugs and biological molecules.

The objective and importance of countercurrent distribution is to separate two or more solutes from each other by a series of partitions between two liquid phases. (That is separation of 2 or more solutes with close distribution coefficient ratios).

CHROMATOGRAPHY

This depends on the combination of the two phases i.e. the stationary phase and the mobile phase.

MOBILE	STATIONERY	TYPES
Liquid	liquid	Partition chrom (paper)
Liquid	solid	Adsorption chrom (TLC, Column chrom, ion exchange
Gas	Liquid	Gas liquid chrom
Gas	solid	Gas solid chrom

COLUMN CHROMATOGRAPY (ADSORPTION CHROM)

Column chromatography is a form of adsorption chromatography. The adsorbent in solid form is usually packed into a glass column. Glass column are glass-tubes and are presented in various sizes between 20-60cm long and 1-4cm wide (internal diameter)

The sand and glass wool prevents the adsorbent from passing through the tap while the sand at the top of the column (acid-washed sand prevents air from entering the column because air bubbles in the column leads to inefficiency of the column.)

ADSORBENT

The commonly used adsorbents are alumina, silica, gel, Ca $(OH)_2$, Mg-silicate, $CaCO_3$, fuller's earth and natural clays which are mainly magnesium aluminum silicate. The type of adsorbent used depends on the property of the material to be separated.

SOLVENT

Solvents serves two purposes:

1. As developers – to effect the fullest separation of all the zones of the chromatogram
2. As eluents – to remove different component of the mixture as they are separated from the column.
   The eluent properties functions by being adsorbed on the column (adsorption, then displacement of the solute molecule), the more strongly adsorbed a solvent the greater, its eluding power.

CHOICE OF SOLVENT

The following parameters determine which solvent is best suited for carrying out the separation.

a) Must be a good solvent for the component to be eluted
b) The eluent must be well adsorbed so that the solvent action is assisted by its displacing action at intervals.
c) The solvent used must be easily removable from the adsorbed component (i.e solvent with low boiling point).
d) The solvent should not react with any of the component of the mixture.

TECHNIQUE FOR COLUMN CHROMATOGRAPHY

The column is packed with the adsorbent either by dry or wet packing. For the dry packing method, this is done by adding the adsorbent to the column already containing the solvent. While for the wet packing, this is done by pouring a slurry of the adsorbent into the column and allowing it to settle. The adsorbent is held in place by a plug of glass wool. The sample to be separated in suitable solvent (0.1 - lml) is them placed on top of a column.

A suitable solvent (eluent) is added and allowed to flow slowly (about 2mls per minute) through the column. At no time must the eluenting solvent fall below the column material level (if this is allowed air gets into the column and the separation efficiency is affected). Fractions of equal volume are collected at intervals and the amount of solute in each fraction determined by a suitable analytical method.

Alternatively, (i.e. an alternative way is by using suitable analytical method) after developing the chromatograph, in case of coloured bands. The adsorbent is extruded and allowed to dry in air after which it is cut into portions according to the colour bands. The different portions are then separately extracted and analysed i.e. by cutting each section placing in a conical flask then boiling with a suitable solvent e.g. chloroform to extract the coloured bands from the column thereafter filter to separate the coloured material from the extract.

For compounds that possesses no colour, assess the position of solute by using UV light, if material fluorescence or by the preparation of coloured derivatives.

It is also possible to identify the position of solute by using streaking or brush method, the developed colour is extruded from the tube and streaked length wise with a brush dipped in test reagent. The colours appearing on the surface of the column indicate the position of the solute. The zones are and then analysed.

APPLICATION

1. Separation of simple or complex mixtures e.g. amino acids, vitamins, pesticides and carboxylic acids.
2. Purification of substances e.g. nitrobenzene is a colourless substance but after its preparation a yellow product is usually obtained. These can be purified by passing though a column as in column chromatography.
3. Separation of biopolymers.

PAPER CHROMATOGRAPHY (PARTITION)

Special type of partition chromatography. The filter paper acts as a solid support in partition chromatography. The stationary phrase is an aqueous solvent while the mobile phase is an organic solvent which may be water immiscible (hydrophobia).

There are two types of techniques, descending and ascending of which the ascending is more often used.
The separation of the components of the mixture has been effected primarily because of partition between the solvents flowing along the filter paper and the water held in the filter paper.

Ascending Techniques: Operation of:

On an appropriate paper about 30cm. sq draw one (1) pencil line about 3cm towards one end of the paper. On this second line make some pencil mark about 2-3cm apart giving sufficient allowance for the ends. The other end of the paper is then serated with a scissors, so as to aid uniform flow. Using a micropipette or capillary tube a few drops of sample solution is applied at each marked spot. The paper is then allowed to dry and then introduced into a chamber previously saturated with the stationary phase (solvent) and containing a trough / beaker of the solvent e.g. phenol saturated with water.

The spotted end of the paper is dipped into the solvent trough and the solvent rises up the length of the paper until it gets to the end of the paper or pre-determined spot or time. The paper is then allowed to dry outside the chamber or by use of an air-gun. The position of the components are then located by spraying or dipping the paper in a specific reagent e.g. I_2 or using UV light or other photoelectric means. If the compound contains radioactive materials, then radioactive tracer techniques can be used.

RF of various spots are then determined by measurement, RF is usually in %. The components of the mixture are thus distributed along the length of the paper. There by leading to separation. The position of the different components can be determined in a number of ways e.g. by spraying the paper with suitable reagent. The retardation factor (RF) is then determined which represent a ratio of

$$\frac{\text{The distance travelled by compound from origin}}{\text{Distance travelled by solvent from origin}}$$

RFA = A= AX/XY   RFB = B= BX/XY

PAPER

Usual paper is whatman paper Nos. 1, 3 and 4. Other papers are Schleicher and schull (SS) 589 and 595. Solvent travels faster on thick paper than on thin paper.

SOLVENTS

Include isopropyl alcohol, phenols, methanol and pyridine, it is not un-usual to use miscible solvents. The solvent for paper chromatography are prepared from commercial products. Generally prepared by shaking the organic solvent with water, then separating the two layers using the organic layer as mobile phase and the aqueous layer as stationary phase or simply by mixing the correct volume of water and organic solvent.

QUANTITIVE PAPER CHROMATOGRAPHY

Two chromatograms are run, one is sprayed with the location reagent to identify the components of the mixture, the other one is then place alongside, the second one and the area occupied by the substance is cut out and further cut into smaller pieces. Each set is extracted a number of times with the appropriate solvent, then analysed in the usual way.

USES: for amino acids analysis

Thin Layer Chromatography

The technique of TLC is similar to that of ascending paper chrom. In TLC a chromatoplate is used as the column. (A glass plate coated with the adsorbent). TLC is usually preferred to paper chrom. Because the process is simple, takes much shorter time (minutes instead of hours) and better separation is obtained.

Adsorbents

Mostly widely used adsorbent is silica gel which is available in various forms with or without binders (binders help to increase the adhesive capacity; usually plaster of

Paris). Other Adsorbent in use include aluminum oxide which is available in acid form (pH4) and basic form (pH9) to neutral (pH7). Cellulose powder (which is best for amino acid and stays much firmer on plate than other adsorbent). $CaSO_4$, $MgSiO_4$ sephadex, polyamides.

Glass plate
About 3mn thickness is used. Sizes are standard usually 20cm by 5cm (small plates), 20cm by 20cm (standard plate) or the use of microscopic slides 2.5cm by 7.5cm.

Technique of Operation
A slurry of adsorbent is made by mixing and shaking the adsorbent with suitable diluents solvent, usually water in specified proportion.
The slurry is then poured into the container of the spreading apparatus called the spreader which is placed on top of one of the plate to be coated, which itself is resting on the base of the aluminium base of the spreading apparatus. Some spreaders can take 5 plates of 20 by 20 or 20 plates of 20 by5cm. The slurry is then spread uniform in one direction of the plate with the thickness of the coating is preset at 0.25 – 0.30mm. The wet plates are then allowed to dry in the air. The colour changes from shinning wet colour to white.
The plates are then dried in an oven at 80^0C for ½ an hour and are later activated at a temperature of 110 – 120^0C for 1-2hrs in an oven.

Activation removes all the remaining water, including the chemically bonded ones. In this state, the plates are now ready for use. If the plates had been stored for a long period then they have to be reactivated.

The material to be separated is dissolved in a minimum amount of solvent possible (conc solution) then spotted on the plate as in paper chrom.

The plate is then placed inside a TLC tank containing a certain amount of developing solvent and which has been pre-saturated with the solvent.

There are many types of tanks e.s. Shandon – the tanks are cylindrical in shape.
When the solvent raches the top of the plate or predetermined spot, it is then allowed to dry. (Components are then located as in paper chrom).

Quantitative TLC
For analytical work, pre TLC is used, thickness of plate coating is usually 0.5 – 2mm: plates of 20 by 20 or more usually 20 by 100cm are used. The conc solvent of the mixture is applied along an imaginary line about 2-3cm from one end with the end of a capillary tube or pipette or the spotting instrument.
Put in tank and develop, then dry. Transfer plate to dark room and with help of uv light different bands can be located (best type of silica gel to use is the pf series e.s pf 254).

Use a tube to mark the bands when in the UV room, then scrape the silica gel in the bands out separately and extract each with chloroform (CHCl) and boil.

Filter off the silica gel and the resulting filtrate would contain the separated component.

Application

Identification of drugs, forensic science and to detect contaminant in biochemical preparation

ION-Exchange Chromatography

Ion exchange is a process in which an interchange of ion of like signs occur between a solution and an essentially insoluble solid in contact with solution. The insoluble solid act as a stationary phase and it's referred to as ion exchange.

Ion Exchanger

Synthetic ion-exchange resins are high molecule weight polymeric materials containing a large number of ionic functional grps per molecule. They are obtained by co-polymerisation of styrene and diviny, /benzene. (DVB)

The above structure is repeated severally and the number of cross-linkages determine by ratio of DVB to styrene. Increasing the cross –linkage increase rigidity, reduce swallow and reduces porousity and solubility of the polymeric structure.

Types of ion Exchanger

1. Cationic exchanger for which we have strongly acidic and weakly acidic
2. Anionic exchanger for which we have strongly basic and weakly basic.

Cationic Exchangers

Strongly acidic cationic exchanger: after the production of the polymeric structure i.e the resin, it can be further treated to form the type of ion-exchanger so desire. Acidic functional groups can therefore be introduced. For a strongly acidic cationic exchanger this is prepared by sulphonation in which a sulphonic acid group is attached to nearly every aromatic nucleus, sulphonic acid are strong acid and therefore strongly dissociate.

The resins contain free H^+ that can be exchange easily.

Weakly Acid Cationic Exchanger: It is prepared by introducing $COOH^+$ acid functional group into the structure – x $ReOOH^+$

The exchange capacity of this type of resin is pH dependent (exchange capacity is the total number of equivalence of replaceable protons per unit volume of resin).

TYPE	NAME	DRY CAPACITY (meg/ml)	TRADE NAMES
Strong cationic	Sulfonated polystrene	1.9	Dowex 50, Amberlite IR20
Weak cationic	Condensed acyclic acid	4.2	Amberlite IRC 50
Strong anionic	Polystyrene^{+} CH_2NMe_3+cl	1.2	Dowex, Amberlite IRA 400
Weak anionic	Polystyrene^{+} ^{2O}amine	2.0	Dowex 3, Amberlite IR45

Strongly Anionic Exchanger

Are prepared with a tertiary amine yielding a strongly basic quaternary ammonium group e.g

$2(ResNMe_3^{+})$ C + $SO^{2-}{}_4$............... $(ResNMe_3^{+})_2SO_4^{2-}$

Weak Anionic Exchangers

These are prepared from 2 amine, yielding a weakly basic tertiary amine e.g.
$(ResNMe_3^{+})OH$

Principle of ion-Exchange Chromatography

This depends on the exchange of ions between resin and solution $ResSO_3H^{+}$+ $A^{+}{}_{aq}$ - $ResSO_3A^{+}$ + H^{+}

The cation A+ formerly in solution diffuse into the structure of the resin while H+ formerly in resin diffuse back into the solution therefore, there is an exchange of ions between the resin and the solution

Determination of Resin Exchange Capacity

This is also known as ion-exchange capacity and is defined as the amount of exchangeable ion per gram of the resin. This depends on the number of active ion per unity weight of the resin and is generally expressed as mill equivalent per gram. The higher the resin capacity the greater the cation or anion exchanged of the resin. The exchange capacity is determined by the following steps.

1. Take a known weight of the resin in H+ form, let the weight =W
2. Pack the column as in column x matography

3. Convert the resin into the H+ form by washing with strong acid
4. Wash off the free acid until effluent is at pH4
5. Elute the column with about 10M NaCL (the H+ is now replaced by the Na^+ and free H+ comes out of the column)
6. Collect the effluent
7. Titrate the effluent against standard alkaline
8. Calculate the total equivalent of the H^+ in the solution e.g. if

a = molarity of base, V = liter volume

mmoles of base aV = to mmoles of H^+
:Exchange capacity = aV/W

Application

1. Purification and demineralization e.g household water softening in which is all the multiple charged acation are replaced with Na, deionized water is prep by passing raw water through cation ion-exchanger which replaces all cations with H^+ and then through an anion-exchanger which replaces all anions with OH ion.
2. Determination of concentration of salt: this is done by conversion of all cation to H^+ or anion to OH^-, followed by a simple acid base titration.
3. Concentration of a trace constituent: when an ion is present at low concentration, it can be concentrated by collecting it on an ion exchange column and eluting with a small volume of elute.

Gas Chromatography

Gas chromatography is a typical chromatographic technique in which the eluting substance is a gas. The method is suitable for separating compounds that can be vaporized without decomposition or association. The mixture to be separated is volatilized at an elevated temperature and introduced into a stream of mobile phase which carries it through the column. The column either contains a surface active solid or a liquid of low volatility held onto a porous solid support. Each component of the mixture is distributed between the mobile and stationary phases either by partition or adsorption. The components of the mixture are separated based on the differences in their affinities for the stationary and mobile phase . The components which are most stable in the gaseous mobile phase will be eluted first and those which are more adsorbed or more soluble in the stationary phase comes out later. The components on leaving the column enter the detector which gives an electrical response for each of the components. This electrical output is amplified and fed to a strip chart recorder which gives a plot of the detector response versus time. When all the operating conditions of a gas chromatography are kept constant, the time from the point of injection of a sample of the appearance of component peak is constant and is the retention time used

as a mean of identifying components. The height or the area under the peak is proportional to the amount of eluted component. By comparison of the peak area or height, of the component in a sample with that of the standard of known concentration, the concentration of the component can be estimated.

Instrumentation.

1 & 2
This is supplied from a cylinder via a pressure gauge. For a gas to be useful as a carrier gas, it must be inert so that there is no interaction between the carrier gas and the liquid phase, solid support or sample. It must be available in the pure form and shouldn't be expensive. Most common gases used are Hydrogen, Helium and Argon. Fine control of gas pressure is achieved either by a needle valve or by a mass flow controller. The MFC allows a constant flow rate to be maintained when the temperature is measured during a separation (with a sample needle valve, the flow rate will decrease with a rise in temperature due to an increase in the viscosity of the carrier gas).

Injection Port

Liquid samples are introduced into the carrier gas stream by injecting a small quantity through a self sealing silicon rubber septum with a hypodermic syringe. The injection chamber is usually a metallic block with a hypodermic syringe. The injection chamber is usually a metallic block with a heating device. The injection block is maintained at a higher temperature than that in the column oven otherwise the compound will condense and may not reach the column. Sample may be injected directly into the column (on column injection) or into a chamber enclosed in a heated metal block situated just ahead of the column.

Column

The column is made up of three parts namely:

1. Column material
2. Solid support
3. Stationary phase

The column is the heart of the gas chromatograph in which separation process occur.
Column material: it consists of a coil of tubing usually stainless steel, glass or copper between 1-2m in length with an internal diameter of 3-6mm. Columns have mostly a U-shape or W-shape or in the coiled formed ( ) or just a straight column. Shape of the column depends on the shape of the column oven.

Stationary Phase

A good liquid stationary phase should have the following characteristics.

- Must not enter into irreversible reaction with the solid support, column material, carrier gas or sample components

- At the column operating temperature it must have low vapour pressure and low viscosity.
- It should be able to spread uniformly on the support phase i.e. form a thin stable film over the support material.
- Must possess selective solubility for the solute so as to allow for separation.

The Liquid phases could be group into two namely:

i. Non polar and
ii. Polar

Generally the choice of stationary phase depends on the type of compound being separated. Usually for efficient separation, the liquid stationary phase should be similar in chemical structure to the components of the mixture e.g. Hydrocarbon compounds are separated with a hydrocarbon liquid phase (squalene).

Stationary Phase	**Polarity**	**Maximum Operation Temperature**	**Applications**
Liquid Paraffin	Non polar	100^0C	Saturated Hydrocarbons
Squalene	Non Polar	140^0C	Halogenated Hydrocarbons
Apiezon L	Non Polar	200^0C	High boiling points Hydrocarbons
Silicone Oil	Non polar	250^0C	Aromatics
Dinonylphthalate	Polar	150^0C	Esters, Alcohols
Polyethylene glycol (PEG)	High polar	120^0C	Hydrogen bonded compounds
PEGA (Adipate)	High Polar	240^0C	Amines, esters
PEGS (succcinate)	High Polar	220^0C	Fatty acids, esters

Solid support – the solid support serves to provide large uniform inert surface area for distribution of the liquid stationary phase. It is desirable that the solid support should have the following characteristics to have good properties.

a) It should be inert.
b) It should have large surface area.
c) The particles of the solid support must be of regular shape and uniform size to ensure uniform coating. The average particles radius is given by 6.35/Mesh size.

Most of the materials used for solid support are got from fire bricks which are diatomite and are micro amorphous in nature, occur as deposits usually formed from skeletal part of single cell algae and have been marketed under various trade names such as Celite, chromosorb, keselghur. The support material must be totally inert and as such pre-treatment of the materials are necessary. This consist of acid or alkali washing to remove trace metals and silanizing to convert Si –OH groups to silyl ethers. (Si-O-Si$(CH_3)_3$. The silanization process is necessary because the Si-OH group may interact with the stationary phase and the solute. This is carried out by treatment with dimethyldicholorosilane (HMDS).

Some Solid supports are marketed already acid washed and silanised.

Packing the column

This is done by mixing the solid support material with the right amount of stationary phase dissolved in a suitable low boiling solvent like acetone, benzene, chloroform, toluene or pentane. The solvent is then evaporated with stirring to ensure uniform coating. Filling of the column is done by placing the column on a vibrator and pouring the solid support thought a funnel (easy with straight or U-shaped column). Hand vibrators can be used for coiled columns. A suction pump at very low pressure is normally used for coiled columns.

Conditioning of Column

This is done by connecting one end of the column to the gas inlet and leaving free the detector end. It is done to expel all volatile substances that may be impurities to the column (the other end is not connected so that the impurities may not deposit on the detector. The gas passed out must have a temperature slightly higher than that of the required working temperature. (Aging is done for 24hrs)

Oven

The oven provides the working temperature of the column which must be even throughout its entire volume. To ensure the uniformity, a fan is operated continuously in the oven. If the components do not differ much in boiling points, the analysis can be done under isothermal conditions which means the working temperature is not changed during the analysis. If the components cover a wide range of boiling points, then a temperature programming is used.

Detectors

Ideally should have a rapid response to presence of solute, a wide range of linear response, high sensitivity and stability. There are two main types

i. Differential detectors
ii. Integral detectors

Integral detectors accumulate the instantaneous signal and give the total amount which has been measured up to a given instant function of cumulative concentration while differential detectors measures instantaneous rate of emergence of the component.

Most detectors are of the differential type. They measure changes in the physical property of the gas stream e.g. thermal conductivity, density, flame ionization, electrolytic conductivity, flame ionization and electron capture are widely used.

Thermal conductivity Detector.

Based on the principle that a hot body loses heat at rate which depends on the thermal conductivity and therefore the composition of the surrounding gas. Also referred to as a Katharometer. Consist of two heated filaments of a metal which has a high coefficient of resistance e.g. platinium. The metal forms two arms of a Wheatstone bridge circuit. Pure carrier gas flows through one channel and the effluent from the column through the other. The rate of heat loss from each filament determines its temperature and therefore its resistance. When pure carrier gas flows through two wires there is no reading on the galvanometer, but on the passage of a vaporized component, the resistance changes and produces an out of balance signal in the bridge circuit. This imbalance is registered as a deflection of the recorder pen.

H_2 and He can be used as carrier gas because of their sensitivity to heat change. The katharometer has moderate sensitivity: the response is non-linear and is sensitive to changes in temperature and flow rate so that it is not particularly suitable for quantitative work.

Flame ionization detector (FID)

Ionization detectors depend on the principle that the electrical conductivity of a gas is directly proportional to the concentration of charged particles within it. Effluent gas from the column is mixed with hydrogen and burned at a small metal jet.

The jet forms one electrode of an electrolytic cell, the other a loop of wire being place just above the flame i.e. the effluent gas from the column passes between two electrodes across which a direct current potential of about 200V is applied. An ionizing source (the flame) partially ionizes the carrier gas allowing a steady current to flow between the electrode and through a resistor where a corresponding voltage drop is amplified. When a sample component is eluted from the column, it is also ionized in the electrode gap, thereby increasing the conductivity and producing a response in the recorder circuit.

Effluent gas from the column is mixed with hydrogen and burned at a small metal jet. The jet forms one electrode of an electrolytic cell, the other a loop of wire being placed just above the flame. FID responds to virtually all organic compounds with exception of formic acid and air and other inorganic compounds. Also its response to water is very low. FID has a very high sensitivity, it is stable to variations in temperature and flow rate and has the widest linear range of any detector in common use.

Electron capture Detector (ECD)
Unlike the FID, the electro capture detector depends on the recombination of ions with free electrons and therefore measures a reduction in signal (negative detector).

As the nitrogen carrier gas flows through the detector, a tritium or ^{63}Ni sources ionizes the gas forming slow electrons which migrate towards the wire anode under an applied potential of 20-50V. This drift of slow electrons constitutes a steady current when only the carrier gas is present. If a solute with a high electron affinity is eluted from the column, some of the electrons are captured thereby reducing the current in proportion to its concentration. ECD is very sensitive to compounds containing halogens and sulphur, anhydrides, peroxides, conjugated carbonyls, nitrites, nitrated and organometallics. However it is insensitive to hydrocarbons, alcohols. Ketones and amines. It had been found particularly useful in the analysis of halogen containing pesticides (10^{-13}) though ECD is the most sensitive; its linear range is poor and is also sensitive to temperature changes.

Comparison of Kathorometer ECD & FCD

Detector	Minimum Detectable Quantity	Linear Range	Temperature ^0C	Remarks
Thermal Conductivity	10^{-9}	10^4	450	Non destructive. Temp & Flow Rate sensitive
FID	10^{-11}	10^7	400	Destructive Excellent Stability
ECD	10^{-13}	10^2	350	Non destructive. Contaminated Temp sensitive.

Quantitative Analysis
Identification of the component peaks of a chromatogram can be achieve by comparison of retention volumes or by trapping the eluted components for further anlaysis by other analytical techniques such as I.R., UV or MS.

Comparison of retention volumes
Provided that operating conditions remain constant and are reproducible, the retention volumes of the components of a sample can be compared directly with those of

standards or synthetic mixtures. An unfamiliar peak can sometimes be identified by spiking a sample with a pure substance whose presence is suspected.
Most chromatograms consist of peaks.

V_R – is the distance between point of injection and peak maximum. (This is the volume of carrier gas needed to elute the solute from the column).
V_g – dead volume – distance between point of injection and the maximum of the air peak – this is the volume equal to the volume of gas inside the column.

$$V_R - V_g = V^1_R$$

In an operating condition of constant pressure and constant carrier gas flow rate, then both T_R and V_R are characteristics of liquid phase and sample e.g. if gas flow rate = F_c

The $V_R = F_c T_R$

Values of $V^I R$ are obtained for the sample components to be identified and also for a standard chromatograph under identical conditions preferably by adding to it the sample. The relative adjusted retention volume x for component is given by.

$$x = \frac{V^1_{R\,(x)}}{V^1_R \text{ standard}}$$

Note that, uncorrected V_R is not used for identification.

Quantitative Analysis

After analyzing the sample, then it is important to be able to obtain quantitative information. The integrated area of a peak is directly proportional to the amount of solute eluted. Peak height can be used as a measure of peak area, though less accurate. The following methods have been used for estimation of peak area.

1. Peak height: Measuring of the height of the peaks. This is applicable to well formed peaks the heights are then compared to those of standards.
2. Peak area: estimation of peak area has been carried out by the following methods.
   a) Peak height X width at ½ height
   b) Triangulation
   c) Cut & weight
   d) Plannimeter
   e) Disc integrator
   f) Electronic digital integrator

Determination of peak areas

1 Peak height: Generally dependent on sample size and sample feed volume. Peak height is usually measured from the baseline to the peak of the peak maximum.

Peak Areas

a. There are several ways in which the area of a peak can be determined.

   Peak height x width at ½ height

b. Since a normal peak is approximately a triangle (½ x b x h) technique is defined and simple reproducibility is dependent on the operation.
   Triangulation

   Area = ½ x BC x AD

c. Tangents are drawn to the two sides of the peak. Then use the area of the triangle to represent the area of the peak. The method is very precise for well shaped peaks

Cut and weigh

This involves cutting out the peaks and weighing on an analytical balance. It is good for dissymmetric peaks; the major disadvantage is that of destroying the chromatogram. (The moisture content of the paper most also be taken into consideration).

Plannimeter

This is a mechanical device in which one of the arms is used to trace the peak and the area is read off the counter. It is time consuming and not very precise, reproducibility varies from person to person.

Disc Integrator

Disc is rotating at a rate proportional to the movement of paper. Then the integrator pen will draw traces along the sides which is proportional to the area of the displacement. The disc integrated the area under each peak simultaneously as it is been draw.

Electronic Digital Integrator

Input signal is fed into the integrator. The integrator converts voltage into frequency. An output pulse is produced which is proportional to the area of the peak.

Applications

Fuels gases, auto exhaust gases, identification of natural products, pesticides, water, phenol, hydrocarbons.