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 electrophoresis	Supercritical fluid - liquid or solid – liquid.

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

$= 0.0023$

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

$= 97.7\%$

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

$= 2.86 \text{ x } 10^{-5}$

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

$= 99.9\%$

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:

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

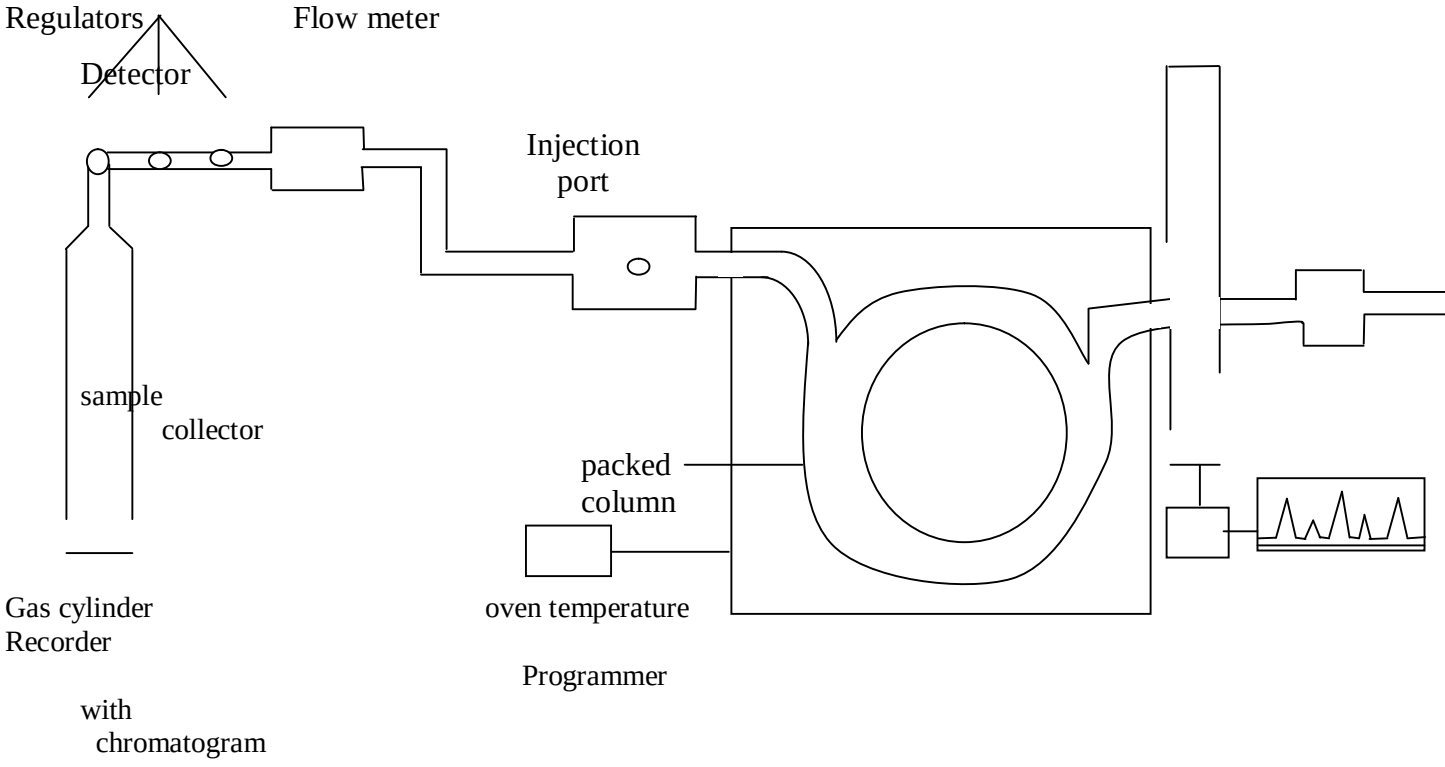
For good separation, the components should have different R_f.

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.

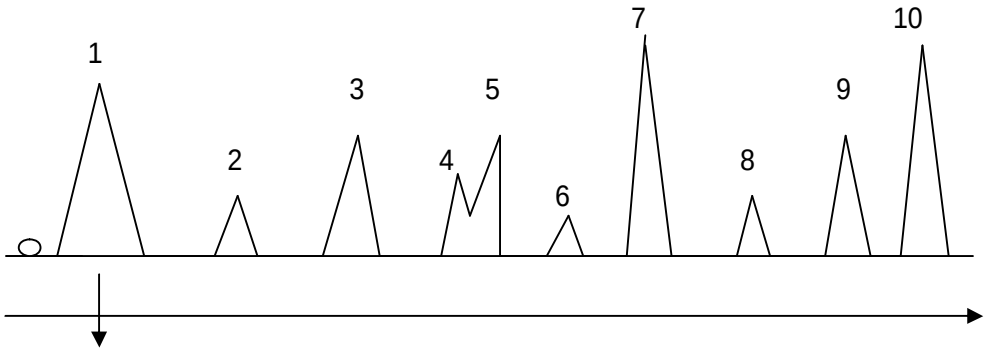
Gas chromatography, so called because mobile phase is a gas, comprises gas – liquid chromatography (GLC) and could be gas – solid chromatography (GSC). For GLC, the stationary phase is a high boiling liquid and the sorption process is predominantly one of partition. For GSC, the stationary phase is a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