Department of Chemistry /College of Sciences/ University of Baghdad

Subject: Analytical Chemistry 4

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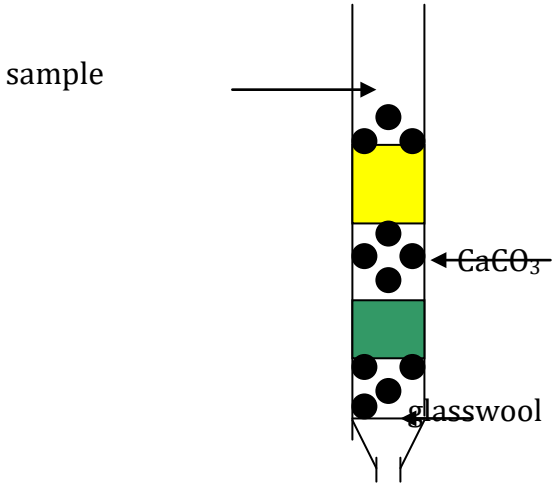
Introduction to chromatography

Chromatography is a variety of physicochemical techniques for the separation of components within mixtures; all methods are based on the distribution of a component between two immiscible phases, the stationary phase and mobile phase. The chromatographic methods are divided according to the physical state of these phases. The invention of chromatography is drawback to Russian Botanist "Mikhail **Tswett"** who has introduced column chromatography in 1906. He employed the technique to separate various plant pigments, such as chlorophylls and xanthophylls, by passing solutions of these species through glass columns packed with finely divided calcium carbonate. The separated species appeared as colored bands on the column, which accounts for the name he chose for the method. The term of *chromatography* is derived from Greek ***chroma*** meaning **"color"** and ***graphein*** meaning to **"writing".**



What is meant by Chromatography?

Chromatography is a physical method which has revealed to separate the sample components distributed between two immiscible phases –first, the stationary phase which can be solid or liquid supported on solid and second, mobile phase which can be a liquid , a gas , or supercritical phase, and moved continuously across the stationary phase. The distributions of the component to be separated between phases are Either

- by solubility difference of solute in both phases – this called a p*artition chromatography.*

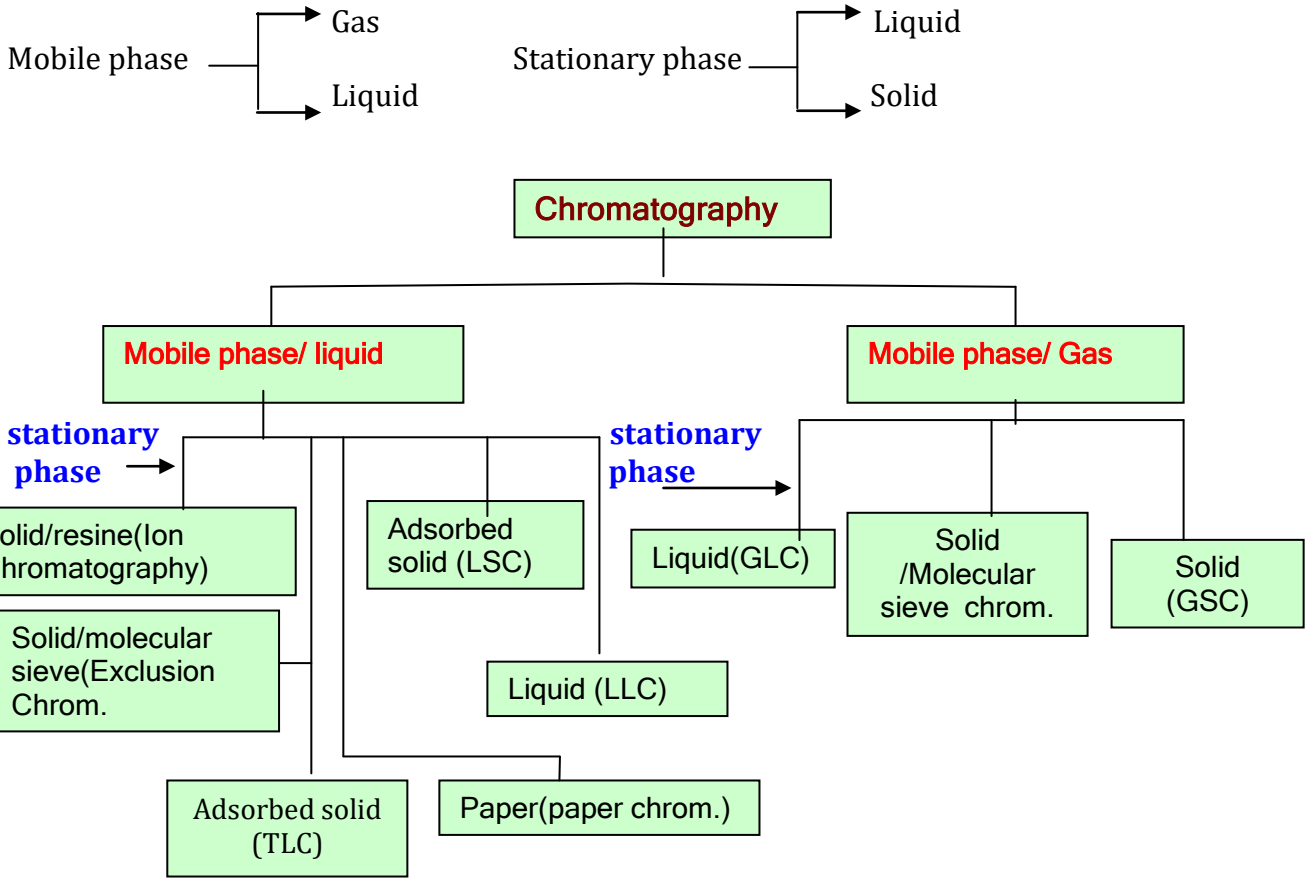
or

- by adsorption difference of solute in the stationary phase–this called *adsorption chromatography.*

Classification of Chromatographic Methods:

There are several types of classifications for the chromatographic methods; we can mention part of them as flows:

1- depending on the physical nature of two phases(stationary and mobile) and consequently they classified by first referring to name of mobile phase;



2- Chromatography is subdivided into categories on the basis of mechanism of interaction of the solute with the stationary phase, as follows

(a) ***Adsorption Chromatography****:* a solid stationary phase and a liquid or gas mobile phase are used. Solute is adsorbed on the surface of the solid particles .the more strongly a solute is adsorbed, the slower it travels through the column. A typical example of this type is the Thin-layer Chromatography (TLC) in which the stationary phase is solid supported on a glass plate (Planer Chrom.)

(b) ***Partition Chromatography:*** a high-boiling liquid stationary phase is bonded to a solid surface, which is typically the inside of the silica (SiO2) chromatography column in gas chromatography, solute equilibrate between the stationary liquid and the mobile phase, which is the flowing gas in GC.

(c) ***Ion-exchange Chromatography:*** Anion such as $-SO_3^-$ or cation such as $-N(CH_3)_3^+$ are covalently attached to the stationary solid phase, usually a *resin*, in this type of chromatography. Solute ions of the opposite charge are attracted to the stationary phase by electrostatic force. The mobile phase is a liquid.

(d) ***Molecular Exclusion Chromatography:*** also called *gel filtration* or *gel permeation chromatography.* This technique separates molecules by size , with the larger solutes passing through most quickly.

The Chromatographic Process

A chromatographic separation is illustrated in **Figure 1.1.** A sample is introduced at the beginning of the chromatographic column. The solute molecules are distributed between the stationary and mobile phases according to an appropriate distribution constant. As the mobile phase moves, the compounds become separated.

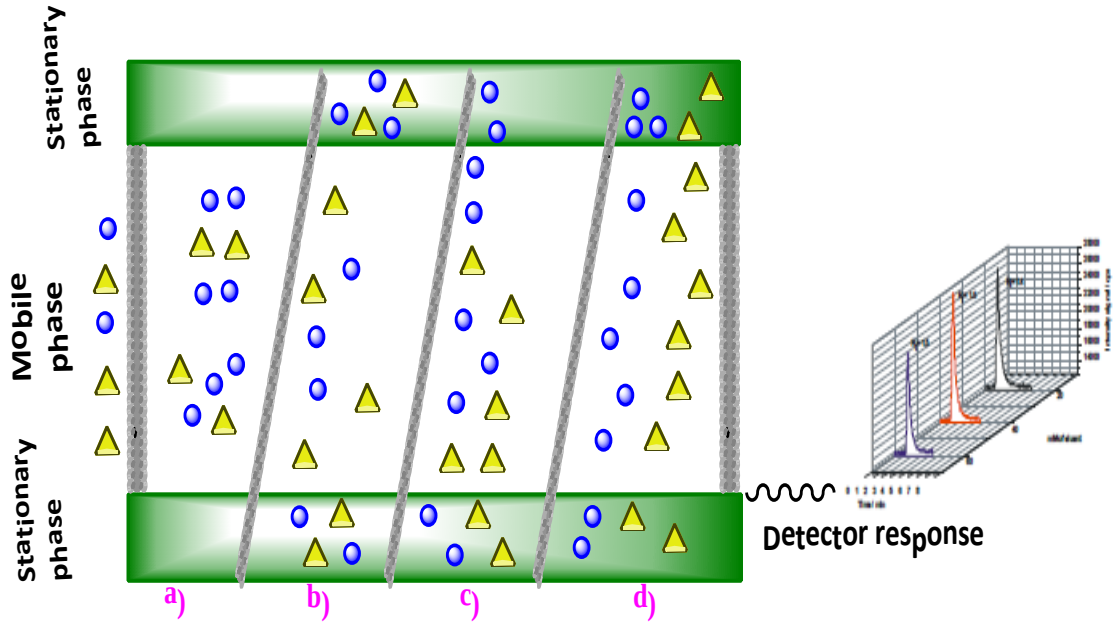


Figure 1.1: The chromatographic process

Figure 1.1 is illustrated the chromatographic process whereas a two-component mixture is injected at the head of an open tubular column **(Figure 1.1-a)**. The two compounds are distributed between the mobile and stationary phases (**Figure 1.1-b). Figure 1.1-c** shows the compounds begin to separate as they progress down the column. The compounds are completely separated (**Figure 1.1-d)**.

Physical principles of chromatographic separation
Retention parameters

In a chromatographic method, we observe a dynamic equilibrium for analytes between the phases involved. This balance is the **equilibrium distribution**. A successful separation is given only if the **distribution coefficient** D_A of the substances to be separated is sufficiently different. $\boldsymbol{D_A}$ is defined as the ratio of the concentration of a substance (A) between mobile ($_M$) and stationary phase ($_S$). Substances with a high distribution coefficient $\boldsymbol{D_A}$ are more strongly retained by the stationary phase than those with small distribution coefficients.

$$D_A = \frac{c_{AS}}{c_{AM}} \tag{1.1}$$

In a chromatographic column, two different analytes are separated if they spend different times in the stationary phase as in **Figure 1.2 (a)**. The time necessary for the non-retained analytes to move is called the **hold-up time** $\boldsymbol{t_M}$, also sometimes referred to as dead time or void time. The analyte **retention time** $\boldsymbol{t_S}$ is defined as the time for solutes not to move along the column. As in Equation 1.2, the gross retention time or residence time $\boldsymbol{t_R}$ of analytes on the stationary phase is obtained from the analyte retention time and column hold-up time:

$$t_R = t_S + t_M \tag{1.2}$$

When injection is made at time t = zero. The non-retained signal shows at $\boldsymbol{t_R = t_M}$ and is called the **hold-up time**; two retained signals (analyte affinity for the stationary phase) show at time t_{R1} and t_{R2} (retention times of signals A and B respectively). This is illustrated in **Figure 1.2 (a)** (idealized chromatogram).

The **retention volume** $\boldsymbol{V_R}$ is calculated from the solute retention time and a constant of flow rate of mobile phase F:

$$V_R = t_R F \tag{1.3}$$

The asymmetry factor A_S is defined as the ratio of the distances (tail portion-b and front portion-a) between the central verticals and the slopes of the distribution at 10% of their height as shown in **Figure 1.2 (b)**.
As in Equation 1.4, the asymmetry factor A_S is calculated of peak distortion:

$$A_S = \frac{b}{a} \tag{1.4}$$

Both a and b are measured at 10% of the peak height as shown in **Figure 1.2 (b)**. The individual analytes behave independently of one another through the chromatographic process. Consequently, after repeated sorption and desorption of solutes on the stationary phase, they produce a randomized aggregation of retention times. Sometimes some unwanted interaction occurs through the chromatographic process.

This is usually indicated by non-symmetrical signals. In the actual sample, asymmetric signals can be categorized as tailing or fronting depending on the style of the asymmetry. For asymmetry factors > 1, the asymmetry is called tailing. Tailing effects occur via a fast increase of the chromatographic signal followed by a relatively slow decrease; primarily responsible for this effect are adsorption processes.

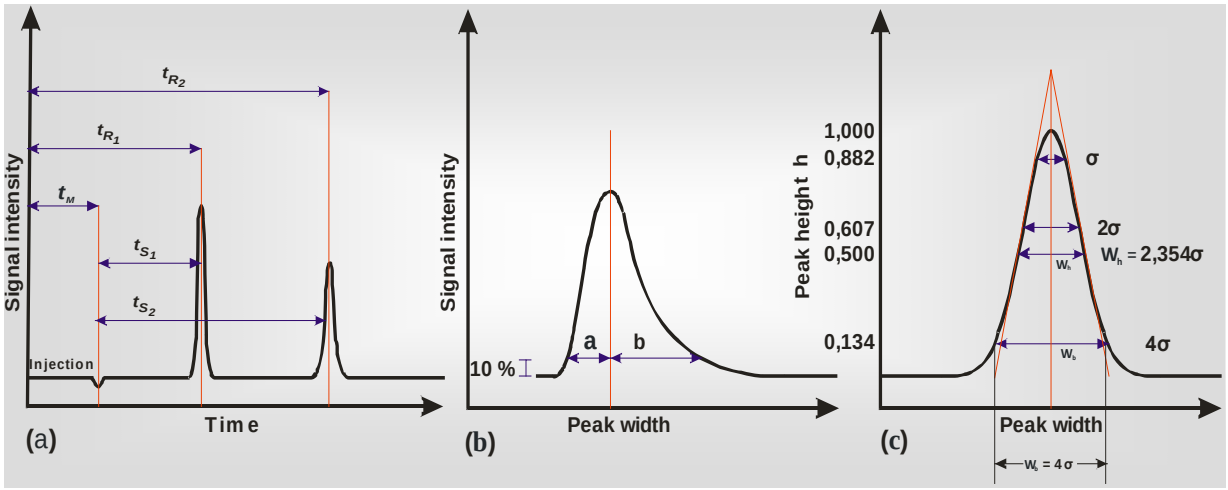


Figure 1.2: (a) Idealized chromatogram. (b) Definition of the asymmetry factor. (c) Gaussian distribution with characteristic parameters.

For asymmetry factors < 1, the asymmetry is called fronting. The image of the peak shape of this effect is the opposite of the tailing, fronting effect which happens if the stationary phase does not have a sufficient number of suitable adsorption sites.

Retention factor, selectivity, and resolution

The **retention factor *k'*** indicates the factor by which the analyte is staying longer on the stationary than in the mobile phase. Mathematically, it is defined as a product of the **distribution coefficient D_A** and the ratio of the volume of the stationary phase V_s to the mobile phase as shown in Equation 1.5:

$$k' = D_A \cdot \frac{V_s}{V_m} = \frac{C_s}{C_m} \cdot \frac{V_s}{V_m} = \frac{t_{ms} - t_m}{t_m} = \frac{t_s}{t_m} \tag{1.5}$$

Small values of the retention factor k' mean that the analyte is eluted near the hold-up time; therefore, the separation will be poor. Large values of the retention factor k' mean that the longer the analysis time, the wider the peak and the lower the sensitivity.

For multi-component systems, it is not only sufficient that the retention factor is in an acceptable range but also that they have to differ sufficiently from each other. To determine this parameter, the selectivity α is introduced, which refers to the relative retention of separation of two components. The selectivity is defined as the ratio of the analyte retention times of two different peaks as follows:

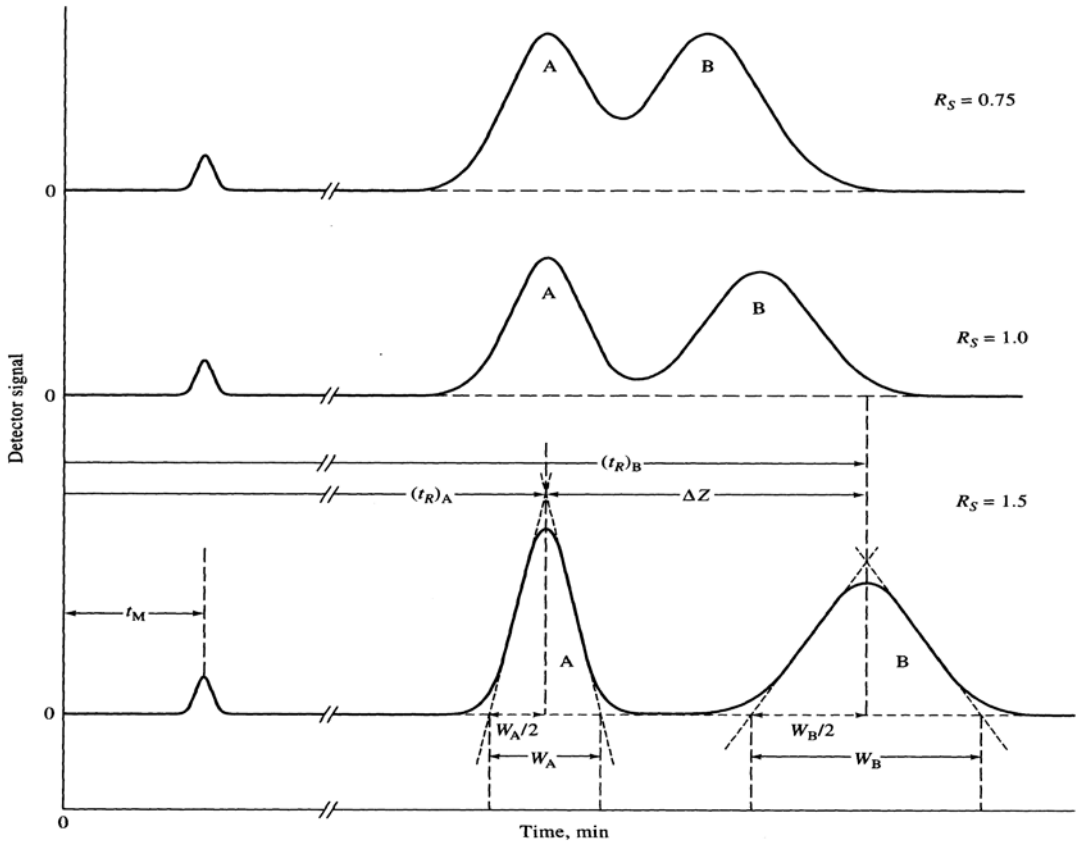
$$\alpha = \frac{t_{s_2}}{t_{s_1}} = \frac{t_{ms_2} - t_m}{t_{ms_1} - t_m} \tag{1.6}$$

If there are no thermodynamic differences between the two solutes under certain chromatographic circumstances, α = 1 and coelution occurs, no separation is possible. The larger value of α means a better separation of analytes. With increasing selectivity, the time required for the separation of solutes also increases.

The ultimate goal of any chromatographic separation is to separate the solutes of a mixture into separate bands. A better measure to describe the quality of separation is the resolution *R*. The resolution *R* of two signals is defined as the difference between the distances of two peaks maxima divided by the arithmetic mean of peak width *w* at base:

$$R = \frac{t_{R_2} - t_{R_1}}{(w_1 + w_2)/2} = \frac{2\Delta t_R}{w_1 + w_2} \tag{1.7}$$

Where (t_{R1}, t_{R2}) are retention times for peaks 1 and 2, respectively, (w_1, w_2) are the widths of the peaks at baseline, as shown in **Figure 1.2**. If the difference in retention times of two peaks with the base width is large, we obtain a high resolution. A resolution of R = 2.0 (8σ-separation) is enough for quantitative analysis but is not desirable because the items related to cost are too large for analysis. At a resolution of R = 0.5, it is still possible to be recognized as separate peaks of two analytes. A qualitative separation requires a resolution of R = 1; for quantification, a resolutions in the range of R = 1.2-1.5 is required.



The selectivity factor, α, can also be manipulated to improve separations. When α is close to unity, optimizing k' and increasing N is not sufficient to give good separation in a reasonable time. In these cases, k' is optimized first, and then α is increased by one of the following procedures:

1. Changing mobile phase composition
2. Changing column temperature
3. Changing composition of stationary phase
4. Using special chemical effects (such as incorporating a species which complexes with one of the solutes into the stationary phase)

Theoretical concepts of the chromatography

The process to explain the mechanism of migration and separation of compounds on the column has been the source of considerable controversy. Therefore, there are two theories to explain the chromatography process. First, the plate theory (developed by *Martin and Synge*) and second, the dynamic theory (proposed by *Van Deemter*).

1- The plate theory

In 1940, *Martin and Synge* introduced the plate theory to describe chromatography by analogy to distillation and extraction. It is sometimes beneficial to deal with the equilibrium concept in chromatography; e.g. the theoretical plate value is a fanciful part of the column. The plate model supposes that the chromatographic column is contains a large number of separate layers, called ***theoretical plates*** (Figure 1.3). Separate equilibrations of the sample between the stationary and mobile phase occur in these "plates". The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.

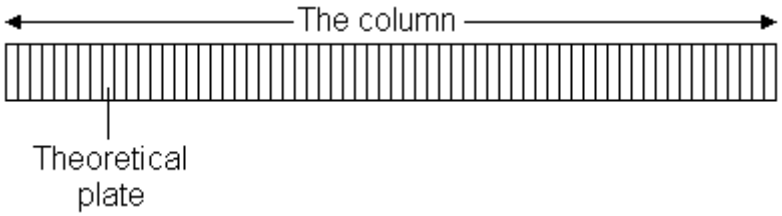


Figure 1.3: the virtual column showing theoretical plates

The number of the theoretical plate N of a column can be calculated using the half-widths and the total retention times from the chromatogram:

$$N = \left(\frac{t_R}{\sigma}\right)^2 = 16\left(\frac{t_R}{w_b}\right)^2 = 5.54\left(\frac{t_R}{w_h}\right)^2 \qquad (1.8)$$

Here, t_R is the retention time, σ is the peak standard deviation, w_b is the peak width at the baseline and w_h is the peak width at half height. The parameters σ, w_b, *and* w_h can be obtained from the **Figure 1.2 (c)**.
The height equivalent of a theoretical plate H or *HETP* can also be used to describe the separation performance and is given by:

$$HETP = \frac{L}{N} = \frac{\sigma^2}{L} \quad (1.9)$$

Here, L is the column length. The concept of theoretical plates explains the appearance of Gaussian peaks. Namely, it is assumed that the compound passes down the column by transfer of the mobile phase from one plate to the other, due to irregularities in the equilibrium of the compound between the mobile and stationary phases caused by diffusion and continuous flow of the mobile phase, the compounds move through the column more slowly (interact strongly from plate to plate) or more rapidly (interact weakly from plate to plate). As a result, the narrow peaks with an increasing retention time of compounds on the stationary phase become broader.

We always observe some band broadening even for a non-retained signal. In some cases, it is advisable to calculate the effective plate number N_{eff} by using the corrected retention time t_R-t_m instead of the retention time t_R in Equation 1.8:

$$N_{eff} = \left(\frac{t_R - t_m}{\sigma}\right)^2 \quad (1.10)$$

The value of the number of theoretical plate N is usually used as an expression of the efficiency of a column. Smaller values of height equivalent of a theoretical plate H mean large values of N. Large values of N mean that the system is closer to equilibrium and therefore more efficient.

2- The dynamic theory (van Deemter equation)

In all of the above discussions in the theoretical plate model, the solute diffusion and the velocity of the mobile phase in the column were not taken into consideration. Consequently, the velocity must have an impact on the progress of the solutes in the column outlet. This dispersion affects the outcome of the quality of the analysis carried out. There is no real equilibrium created between the analytes in the mobile and stationary phases, due to the always-flowing mobile phase in a chromatographic column. The peak broadening happens because of several effects occurring in the chromatographic column. The first approach that deals with band broadening in chromatography was proposed by *Van Deemter* in 1956.

The Van Deemter equation describes the factors affecting band broadening in a chromatographic separation. The van Deemter equation is:

$$H = A + \frac{B}{u} + Cu \qquad (1.11)$$

Here *A*, *B*, and *C* are constant factors of multi-path effects, eddy diffusion, longitudinal diffusion, and mass transfer, respectively, and *u* is the average linear velocity of the mobile phase in the column.

Term A describes *eddy diffusion*, also known as the packing factor. Non-retained analytes will not leave directly from the column inlet to the column outlet. The solute also faced particles of the stationary phase and it must move around them. Consequently, non-retained solutes may follow to a multi-pathway in their travel via the column.

The term A (eddy diffusion) in the van Deemter equation is:

$$A = 2\,\lambda\,d_p \qquad (1.12)$$

In Equation 1.12, d_p is the average particle diameter and **λ** is an experimental packing factor (Coefficient describing the quality of the packing). The more homogenous particles size in the column (uniform particles), the closer the λ to one, therefore, it is an indication for the packing quality of the column.

Term B describes *longitudinal diffusion*. As a band of solute molecules travels in the mobile phase, it will tend to diffuse in all directions, attributed to the con-

centration gradient in the column. Thus, analyte diffusion along the travel direction of the mobile phase in the chromatographic column will lead to peak broadening. To reduce the longitudinal diffusion the mobile phase velocity will set to a reasonable value. The term B (longitudinal diffusion) in the van Deemter equation is described by:

$$B = 2\,\gamma\,D_m \tag{1.13}$$

In Equation 1.13, D_m is the diffusion coefficient of the analyte in the mobile phase and δ is an obstruction factor, which describes the obstruction of the free longitudinal diffusion due to collisions with particles of the stationary phase.

Term C is related to the *resistance to mass transfer*. The analyte molecules should be able to partition between the stationary and mobile phases in order for an analyte to be retained. Accordingly, this implies two processes. First, ***resistance to mass transfer in the mobile phase C_m***: The analyte molecules are diffusing continuously from the mobile phase to the stationary phase and back again during their travel through the column. This transfer process is not immediate; a limited time is required for solutes to diffuse through the mobile phase in order to access the interface and enter the stationary phase. This term is given by:

$$C_m u = \frac{f(k')\,r^2}{D_m} u \tag{1.14}$$

In Equation 2.14, $f(k')$ is a constant which represents a function of the retention factor, and r is the column radius. Term C_m emanates from mass transfer in the mobile phase, which is the first part of the C term in the van Deemter equation.

Second, ***resistance to mass transfer in the stationary phase C_S***: Once more, the analytes are in contact with the stationary phase and may leave and reenter the mobile phase by diffusion. Before reentering the mobile phase, the analytes have a more or less dispersed way through the stationary phase and, therefore, varying distances for back diffusion to the surface on the stationary phase. The term C_s is given by:

$$C_s u = \frac{f(k')\,d_f^2}{D_s} u \tag{1.15}$$

Here, d_f is the thickness of the film of stationary coated on the support and D_s is the diffusion coefficient of the analyte in the stationary phase. Term C_s arises

from mass transfer in the stationary phase, which is the second part of the *C* term in the van Deemter equation. The total resistance to mass transfer *C* is:

$$Cu = C_m u + C_s u \qquad (1.16)$$

This phenomenon of eddy diffusion, longitudinal diffusion, and resistance to mass transfer are pictured in Figure 1.3. A typical graphic of the plate high *H* versus the average linear velocity of the mobile phase *u* in the column is shown in Figure 1.3.

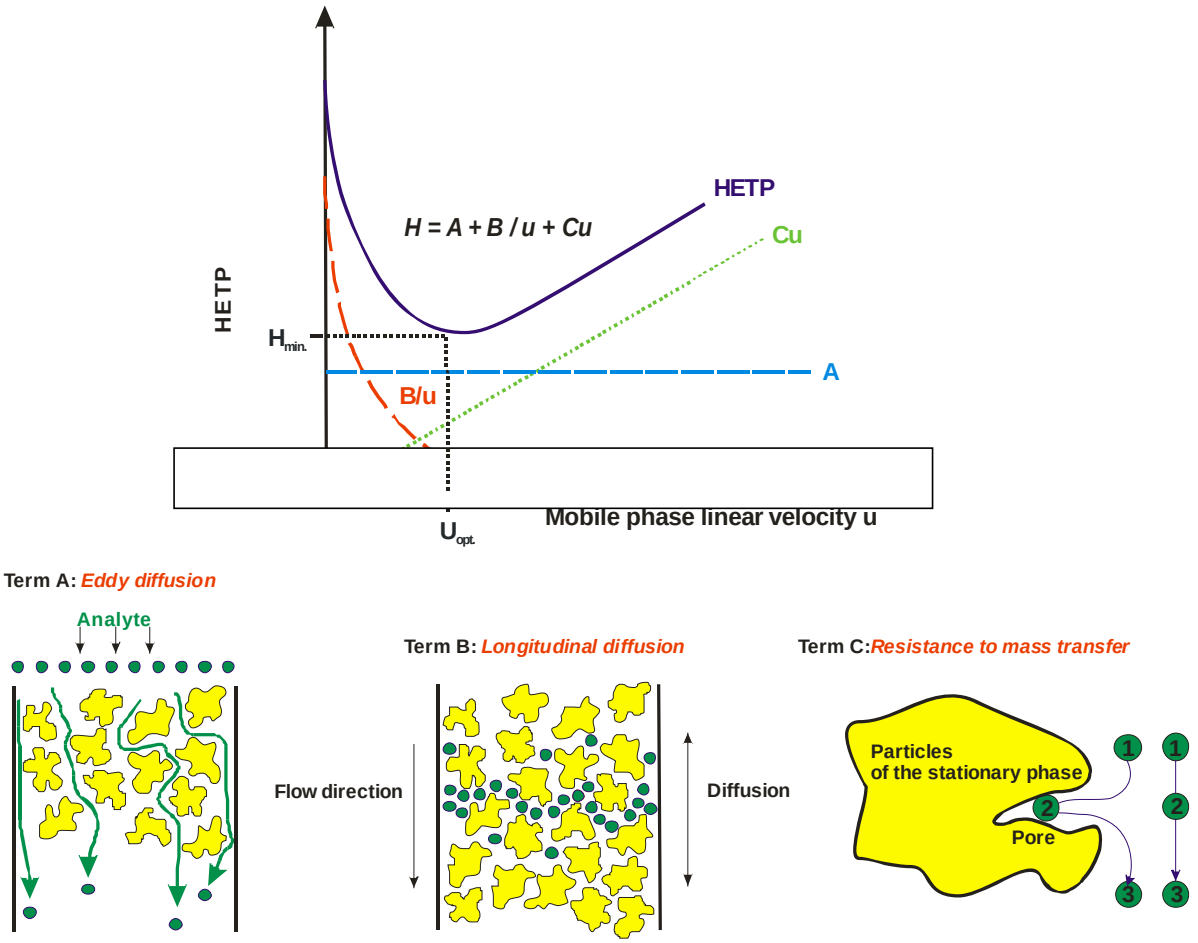
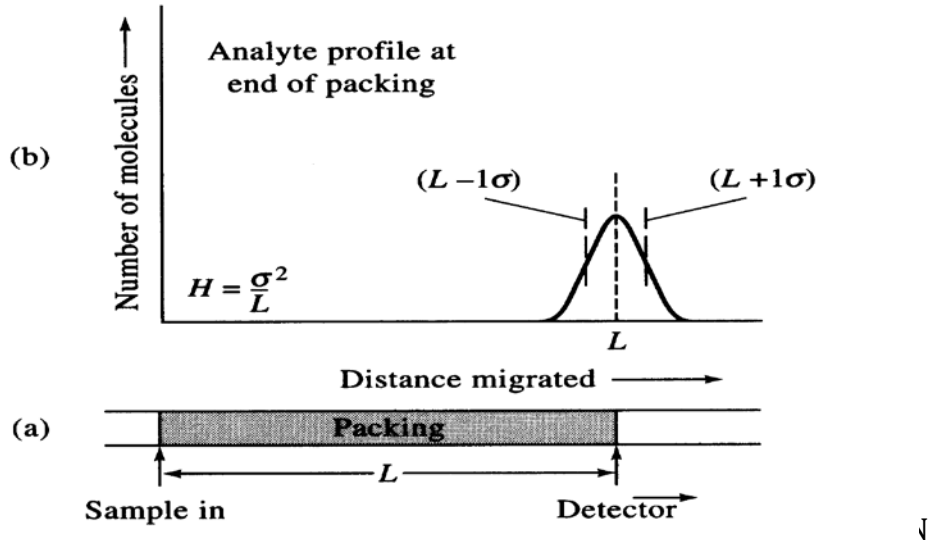


Figure 1.3: General illustration of a Van Deemter curve with representation of the individual terms A, B and C.

How to calculate H and N from a chromatogram:



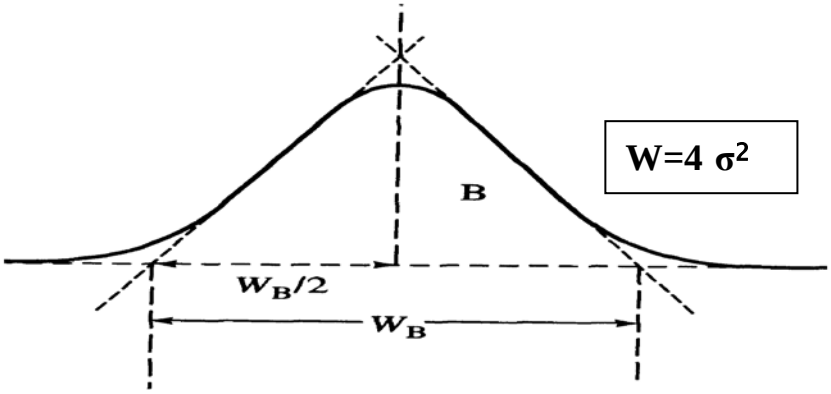
The relationship between the peak variance σ^2 and distance migration (column length) or retention time t_R understood as the ***Height Equivalent to a Theoretical Plate*** H (or HETP)

$$H = \frac{\sigma^2_L}{L} = \frac{[cm^2]}{[cm]} = \text{unit H= cm} \quad \ldots\ldots\ldots\ldots\ldots\ldots (15)$$

or

$$H = \frac{\sigma^2_L \cdot L}{t_R^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (16)$$

Practically, we can use the chromatogram directly to determine the numbers of theoretical plates (N) and for this purpose we approximate the peak width at mid-peak height (b½) by using baseline W



If we substitute σ^2 in Eq.16, we get

$$H = \frac{W^2 L}{16\ t_R^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (17)$$

We back to Eq. 13 , we get ;

$$L$$

$$N = \frac{}{W^2 L/16\, t_R^2}$$

And by rearrangement,

$$N = 16\left(\frac{t_R}{W}\right)^2 \quad \text{.....(18)}$$

So, we can calculate N from any chromatogram using Eq. (18) or the modified Eq. (14).
We can conclude from the plate theory that this theory

(a) Give us an idea about the number of equilibrations or number of theoretical plates, each equilibration expresses one theoretical plate. This theory is useful from theoretical point of view because it gives an idea for the separation efficiency.

(b) But this theory does not give ideas about the factors that increase the theoretical plate. This question is answered by the so called the rate theory.

Solved Problems

Example 1:

A mixture of benzene, toluene, and methane was injected into a gas chromatograph. Methane gave a sharp peak spike in 42 s, whereas benzene required 251 s and toluene was eluted in 333 s. find the adjusted retention time and capacity factor for each solute and relative retention?

Solution:

The adjusted retention times are:
benzene: $t'_R = t_R - t_M = 251-42=209$ s
Toluene : $t'_R = t_R - t_M = 333-42=291$ s

The capacity factors are;

$$\text{Benzene: } k' = \frac{t_R - t_M}{t_M} = \frac{251-42}{42} = 5.0$$

$$\text{Toluene: } k' = \frac{t_R -}{t_M} = \frac{333-42}{42} = 6.9$$

$$\alpha = \frac{t'_R \text{ (toluene)}}{t'_R \text{ (benzene)}} = \frac{333-42}{251-42} = 139$$

Example 2:

A peak with resolution time of 407 s has a width at the base of 13 s . A neighboring peak is eluted at 424 s with a width of 16 s .find the resolution for these two components.

Solution:

$$\text{Resolution} = \frac{\Delta t_R}{W_{av}} = \frac{424-407}{\frac{1}{2}(13+16)} = 1.1$$

Example 3:

A solute with a retention time of 407 s has a width at the base of 13 s on a column 12.2 m long. Find the number of plates and plate height.

Solution:

$$N = 16 \left(\frac{t_R}{W}\right)^2$$

$$N = 16 \left(\frac{407}{13}\right)^2 = 1.57 \times 10^4$$

$$H = \frac{L}{N} = \frac{12.2}{1.57 \times 10^4} = 7.77 \times 10^{-4}\ \text{cm}$$

Example 4:

Two solutes have a relative retention of α =1.08 and capacity factors k'_1 =0.5 and k'_2=5.4. The number of theoretical plates is nearly the same for both compounds. How many plates are required to give a resolution of 1.5? of 3.0? if the plate height is 0.20 mm,how long must the column be for a resolution of 1.5?

Solution:

$$\mathbf{R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k'_B}{1+k'_B}\right)}$$

$$R_s = 1.5 = \frac{\sqrt{N}}{4}\left(\frac{1.08-1}{1.08}\right)\left(\frac{5.4}{1+5.4}\right)$$

$$N = 8.65 \times 10^3 \text{ plates}$$

To double the resolution to 3.0 would require four times as many plates = 3.46×10^4 plates. For a resolution of 1.5, the length column required is (0.20 mm/plate) 8.65×10^3 plates) =1.73 .

Example 5:

Substances A and B have retention times of 16.40 and 17.63 min, respectively, on a 30.0 cm column. An unretented species passes through the column in 1.30 min. the peak widths (at base) for A and B is 1.11 and 1.21 min, respectively. Calculate (a) the column resolution, (b) the average number of plates in the column,(c) the plate height, (d) the length of column required to achieve a resolution of 1.5, and (e) the time required to elute substance B in the column that gives R=1.5

Solution:

$$R = \frac{2(17.63-16.40)}{1.11+1.21} = 1.06$$

$$N_1 = 16 \left(\frac{16.40}{1.11}\right)^2 = 3493 \text{ plates}$$

and

$$N_2 = 16 \left(\frac{17.63}{1.21}\right)^2 = 3397 \text{ plates}$$

(c) $H = L/N$ $30.0/3445 = 8.7 \times 10^{-3}$ cm

(d) k and α do not change greatly with increasing N and L. Thus, substituting N_1 and N_2 into Eq. 24 and dividing one of the resulting equations by the other yield

$$\frac{(R)_1}{} = \frac{\sqrt{N_1}}{}$$

$$(R)_2 \qquad \sqrt{N_2}$$

Where the subscripts 1 and 2 refer to the original and longer columns, respectively. Substituting the appropriate values for N_1 , $(R)_1$, and N_2, $(R)_2$ gives

$$\frac{1.06}{1.5} = \frac{\sqrt{3445}}{\sqrt{N_2}}$$

$$N_2 = 3445 \left(\frac{1.5}{1.06}\right)^2 = 6.9 \text{ x } 103 \text{ plates}$$

But

$$L = NxH = 6.9x10^3 \text{ x } 8.7x10^{-3} = 60 \text{ cm}$$

(e)Substituting $(R)_1$ and $(R)_2$ into Eq. 26 and dividing yield

$$\frac{(t_R)_1}{(t_R)_2} = \frac{(R)_1^2}{(R)_2^2} = \frac{17.63}{(t_R)_2} = \frac{(1.06)^2}{(1.5)^2}$$

$$(t_R)_2 = 35 \text{ min}$$

Thus, to obtain the improved resolution, the column length and, consequently, the separation time must be doubled.

Problems and exercises

1- Define the followings: (a) elution (b) mobile phase (c) stationary phase (d) retention time (e) selectivity factor (f) plate height (g) resolution (h) partition ratio (i) retention factor(j) plate height

2- Describe a method of determining the number of plates in a column.

3- Which quantities can improve the resolution of a chromatographic separation and how can be determined from a chromatogram?

4- Name three methods based on mechanical phase separation.

5- If the width of a chromatogram is 15s , retention time 33 s and the length of column is 25 cm . Calculate n and H?

6- Calculate the resolution between the peaks of two components A and B in liquid chromatography, if the retention time of A and B are 7.42s and 8.92s with peak width of 0.87s and 0.91s respectively. Is the resulting resolution is suitable for quantitative analysis.

7-The following data are obtained from liquid chromatographic column with length of 24.7 cm , flow rate= 0.313 mL/min, Vm= 1.37 mL , Vs = 0.164 mL. A chromatogram of a mixture of species A, B, C and D provided the following data:

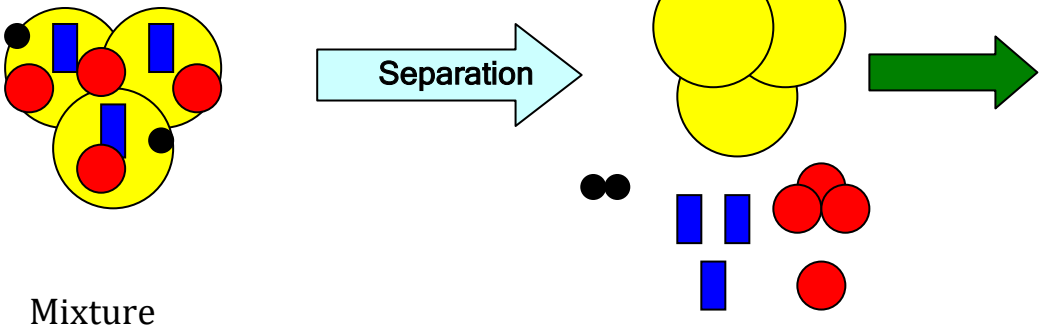
component	Retention time , min	Width of peak base(w) , min
Non-retained	3.1	-
A	5.4	0.41
B	13.3	1.07
C	14.1	1.16
D	21.6	1.72

Calculate:

(a) the number of plates from each peak
(b) The mean and standard deviation for (N)
(c) The plate height (H) for the column.
(d) Capacity factor and distribution coefficient for A, B, C andD
(e) The resolution (R) for B and C and selectivity factor and the column length required to separate these species with a resolution of 1.5.

Paper and Thin-layer Chromatography

Chromatography is a technique for separating mixtures into their components in order to analyze, identify, purify, and/or quantify the mixture or components.



***Analyze**

***Identify**

***Purify**

***Quantify**

Mixture

Components

Chromatography is used by scientists to:

- Analyze–examine a mixture, its components, and their relations to one another.
- Identify–determine the identity of a mixture or components based on known components.
- Purify – separate components in order to isolate one of interest for further study.
- Quantify – determine the amount of the mixture and/or the components present in the sample.

Paper Chromatography (PC):

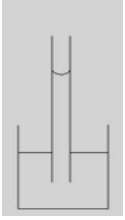
Historically important technique used to separate amino acids, nucleotides and metabolites. As we learn from previous chapters, chromatographic separation involves TWO PHASES;

MOBILE PHASE (gas or liquid)
STATIONARY PHASE (liquid or solid)

In paper chromatography, the mobile phases pass through the sample by **CAPILLARY ACTION** in the paper.

MOBILE PHASES = Solvents polar solvent mixture (eg. water:ethanol)

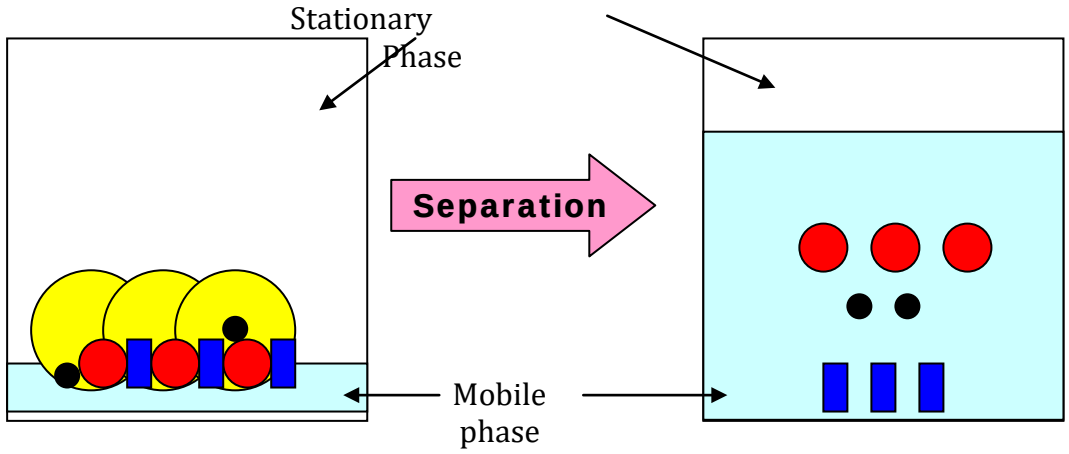
STATIONARY PHASE = Chromatography paper (cellulose-based paper)



Or, separates dried liquid samples with a liquid solvent (mobile phase) and a paper strip (stationary phase)

Hence, paper chromatography is based on the separation of components mixture in the sample solution by the movement of a liquid (mobile phase) through the spaces of a porous material (filter paper) or a small diameter tube against the forces of gravity (i.e. **by capillary action**) and the degree to which a material (solute) dissolves into a liquid (solvent)- i.e. **absorption.**

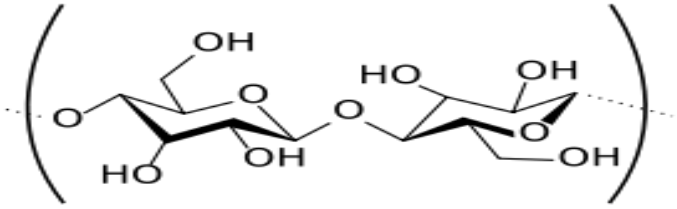
Illustration of Chromatography



Components	Affinity to Stationary Phase	Affinity to Mobile Phase
Blue	----------	Insoluble in Mobile Phase
Black	✓ ✓ ✓ ✓ ✓ ✓	✓ ✓
Red	✓ ✓	✓ ✓ ✓ ✓ ✓
Yellow	✓	✓✓✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓

In principle, paper chromatography, like thin layer chromatography, substances are distributed between a stationary phase and a mobile phase. The stationary phase is usually a piece of high quality filter paper. The mobile phase is a developing solution that travels up the stationary phase, carrying the samples with it. Components of the sample will separate on the stationary phase according to how strongly they adsorb to the stationary phase versus how much they dissolve in the mobile phase.

Paper consists primarily of **CELLULOSE**(see structure below) ,a high molecular weight polymer of the carbohydrate **GLUCOSE** $(C_6H_{10}O_5)n$ .Three water-like **hydroxyl units** can **HYDROGEN BOND** to water or to **IONIC** dye molecules



Other types of paper can be used, so the majority of PC has been carried out on standard filter paper material, however, there are still commercially available a range of chromatographic papers:

- pure cellulose;
- silica gel loaded;
- ion exchange cellulose; or
- resin loaded

These papers are manufactured to a high specification with controlled porosity, thickness, and matting characteristics and are low in metal content.

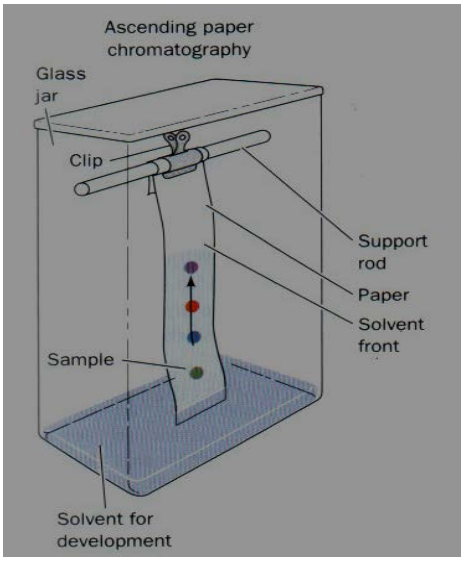
Solvent systems for PC applications:

Compound class	Solvent	Proportions	Analyte
Hydrophilic compounds	Phenol/water	Sat. soln.	Amino acids
	Phenol/water/ammonia	200 : 1	
	Butanol/water/acetic	4 : 1 : 5	
	Butanol/water/pyridine	1 : 1 : 1	
	Isopropanol/water/ammonia	9 : 1 : 2	
	Butanol/ammonia	Sat. soln.	Fatty acids
Moderately hydrophilic substances	Pyridine/EtOAc/water	2 : 1 : 2–12 : 5 : 4	Sugars
	Formamide/chloroform	1 : 9–9 : 1	
	Formamide/$CHCl_3$/benzene[a]	1 : 9–9 : 1	
Inorganic	Acetone/water/conc. HCl	87 : 8 : 5	Co, Mn, Ni, Cu, Fe (chlorides)
	Pyridine/water	9 : 1	F, Cl, Br, I (Na salts
	n-Butanol/HCl (3 mol litre^{-1})	Sat. soln.	Hg, Pb, Cd, Cu, Bi (chlorides)
	Pentan-2,4-dione (sat. soln. in water)/acetone/conc. HCl	149 : 1 : 50	As, Sb, Sc (chlorides)

[a] Paper impregnated with 60% ethanolic formamide prior to use.

The filter paper holds the components until the solvent dissolves them and carries them up the filter paper.

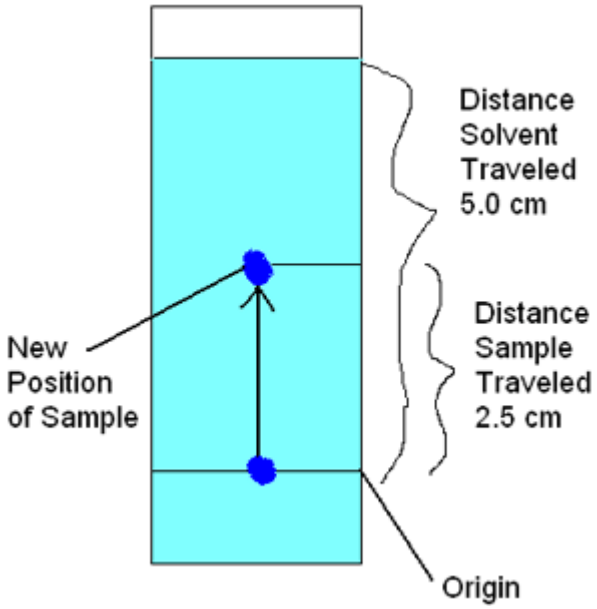
The solvent travels up the filter paper by capillary action.



The solvent's attraction to itself pulling it up is greater than the force of gravity pulling it down. The separation of components depends on their solubility with the solvent and their affinity to the solvent and filter paper. The solvent can only move the components if they are soluble in it and the more soluble a component is the more there is available to move up the filter paper. Solutes will dissolve into solvents that have similar properties. Polar solvents dissolve polar solutes and non-polar solvents dissolve non-polar solutes. (Like dissolves like.)

A component will travel up the filter paper at a rate that is determined by its affinity to the filter paper and solvent. Since each component has its own solubility with the solvent and its own affinity to the solvent and filter paper, they can be separated in multiple ways by using mixtures of different solvents and different filter papers.

To measure how far each component travels, we calculate the retention factor (R_f value) of the sample. The **R_f value is the ratio between how far the component travels and the distance the solvent travels from a common starting point (the origin).** If one of the sample components moves 2.5 cm up the paper and the solvent moves 5.0 cm, then the R_f value is 0.5. You can use R_f values to identify different components as long as the solvent, temperature, pH, and type of paper remain the same. In the image below, the light blue shading represents the solvent and the dark blue spot is the chemical sample.



When measuring the distance the sample traveled, you should measure from the origin (where the middle of the spot originally was) and then to the center of the spot in its new location.

To calculate the R_f value, we use the equation:

$$R_f = \frac{\text{distance traveled by the sample component}}{\text{distance traveled by the solvent}}$$

In our example, this would be:

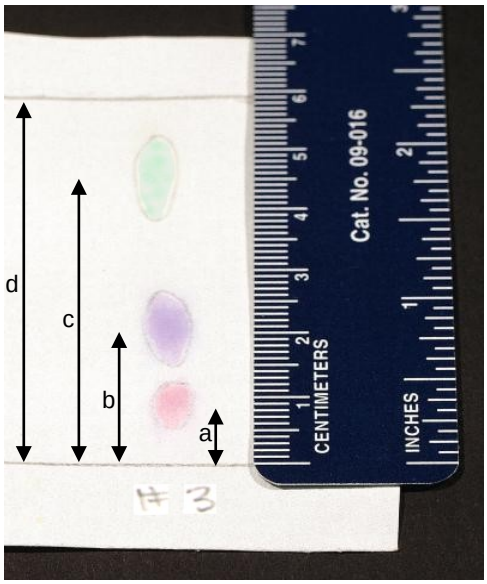
$$R_f = \frac{2.5\text{ cm}}{5.0\text{ cm}} = 0.5$$

Note that an R_f value has no units because the units of distance cancel. In general, if different components are separated as shown below, we can determine the R_f value of each compound in the sample.

- Determine the R_F values for each colored spot in the knowns and the unknown.

$$R_{F(a)} = \frac{a}{d}$$

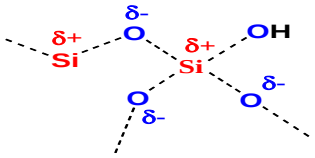
- Use your computed R_F values to identify the components of your unknown.



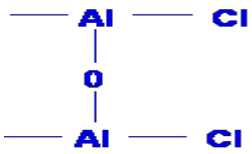
Thin-Layer Chromatography (TLC):

Thin Layer chromatography (planar chromatography) is a classical liquid chromatographic method of separating mixtures. In TLC the ***stationary phase*** (S.P) is solid which is spread over a glass sheet (support material) and in general, it is a porous solid powder of small particles (5-40 mm) in diameter coated on open bed.

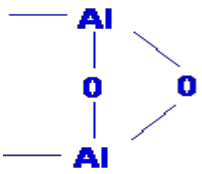
Commonly used S.P s is *silica gel*, **alumina**, *polyamides* and *cellulose*



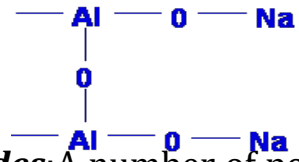
Silica gel



Acidic Alumina pH 4.0



Neutral Alumina at pH 7.5



Basic Alumina PH 9.5

Polyamides:A number of polyamides have been used as S.P in TLC. Some examples include:

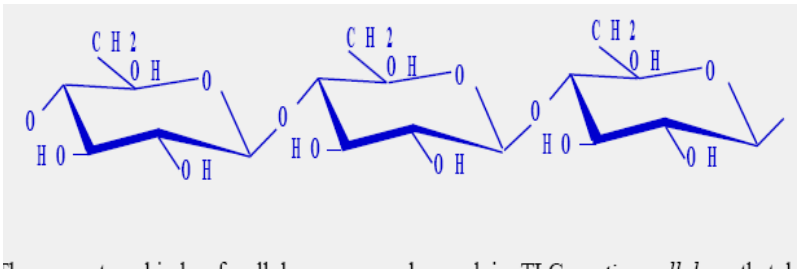
(a) Polyamide 6,6 (Nylon 6,6); (b) Polyhexamethylenediamine, polyamide 6 (Nylon 6).

In general, polyamides can be obtained by polycondensation of a dicarboxylic acid with diamine as shown below:

$$-[NH-(CH_2)_6NH-CO-(CH_2)_X-C]_n$$

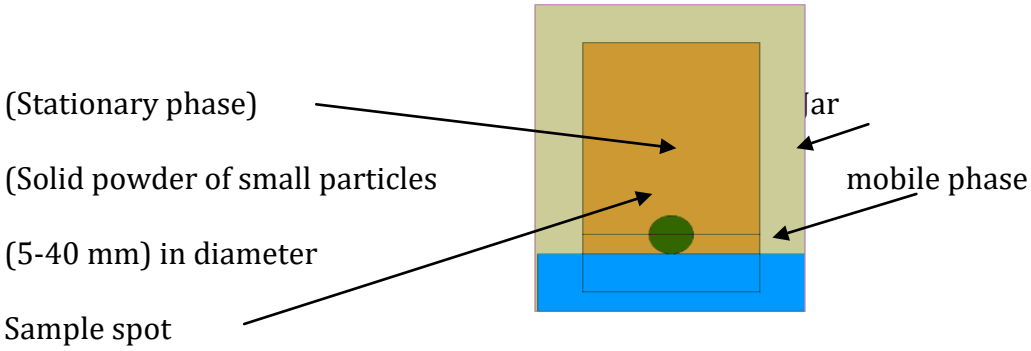
If X=4 then the product is Nylon6.6; X=8 then the product is Nylon 6.10 etc..

Cellulose: Two types (native and microcrystalline), native has b/w 400-500 units/chain and microcrystalline (obtained from partial hydrolysis has 40-200 units/chain).



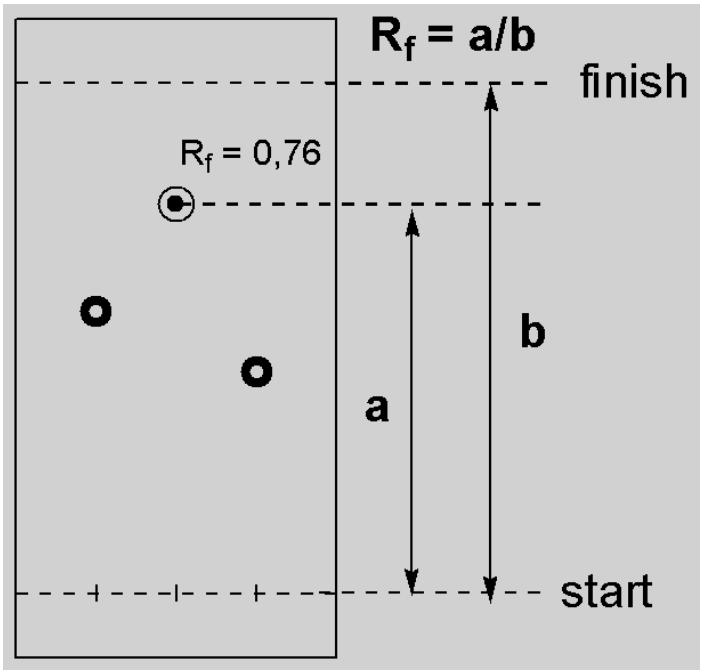
The *mobile phase* (M.P) is a volatile liquid, which is drawn up the plate by capillary action. The Commonly used M.P includes:

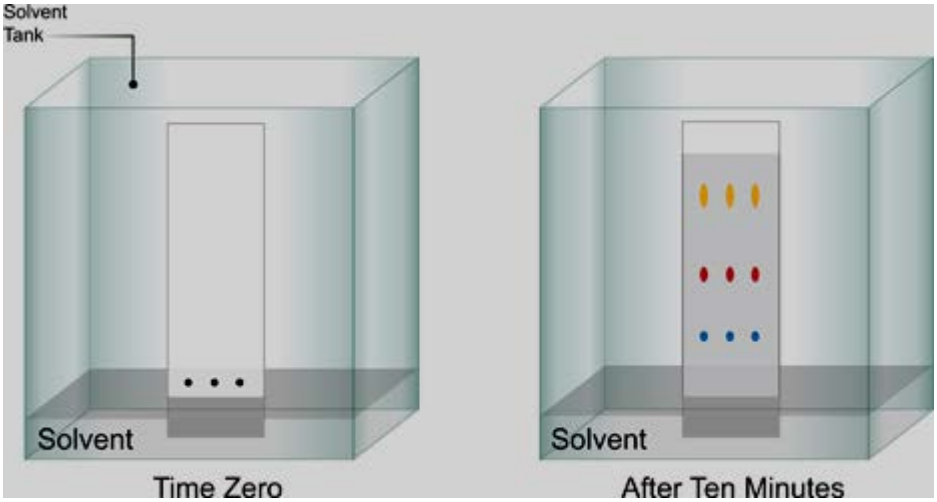
-Chloroform, Toluene, acetic acid, CH_3OH and small amount of H_2O.



- TLC involves spotting a dilute solution (1%) of sample on one end of a small sheet that has been coated with silica gel (SiO_2) or alumina (Al_2O_3), known as the stationary adsorbent phase.

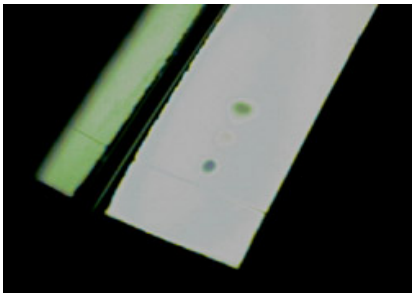
- The sheet is placed upright inside a jar in a small pool of solvent. As the solvent rises up the sheet by capillary action, the components travel at different rates based on competing interactions with the mobile (solvent) and adsorbent phases.
- SiO_2 is used for separation of more polar compounds while Al_2O_3 is used in the separation of non-polar compounds.
- A polar solvent will carry a polar compound farther while a non-polar solvent will carry a non-polar compound farther.
- Rf value is the ratio of the distance the spot travels from the origin to the distance the solvent travels.



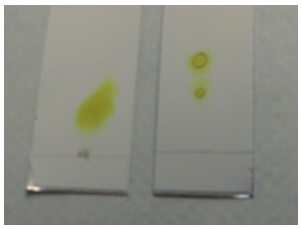


If your samples are colored, mark them before they fade by circling them lightly with pencil. Most samples are not colored and need to be visualized with UV lamp. Hold a UV lamp over the plate and mark any spots which you see lightly with pencil.

UV lamp



Here are two proper sized Spots, viewed under a UV lamp



Here's what overloaded plates look like compared to well-spotted plates. The plate on the left has a large yellow smear; this smear contains the same two compounds which are nicely resolved on the plate next to it.

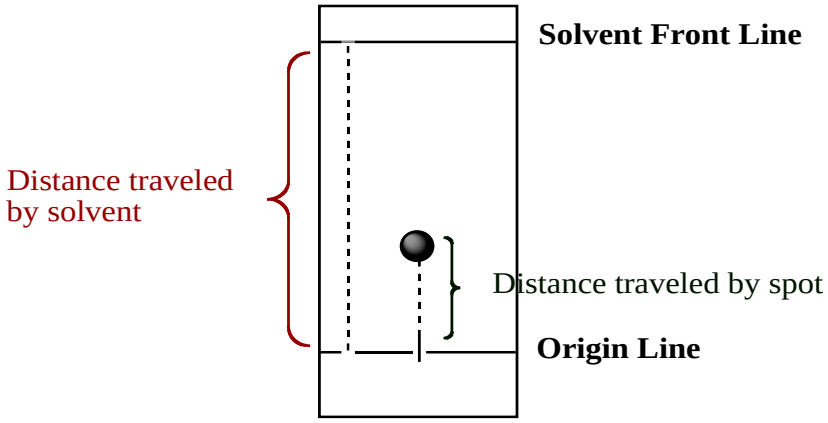
Qualitative TLC; for qualitative analysis, spots on TLC plates are characterized by Retardation Factor R_f:

R_f value importance
R_f =0 -→ means solute interact strongly with the S.P (spot does not move)
R_f =1 -→ means solute is not retention and no separation from the solvent front

R_f between 0-0.99 Indicate solute is separated from the solvent front and move

Calculating R_f values

$$R_f = \frac{\text{distance traveled by spot (compound)}}{\text{distance traveled by solvent (M.P)}}$$



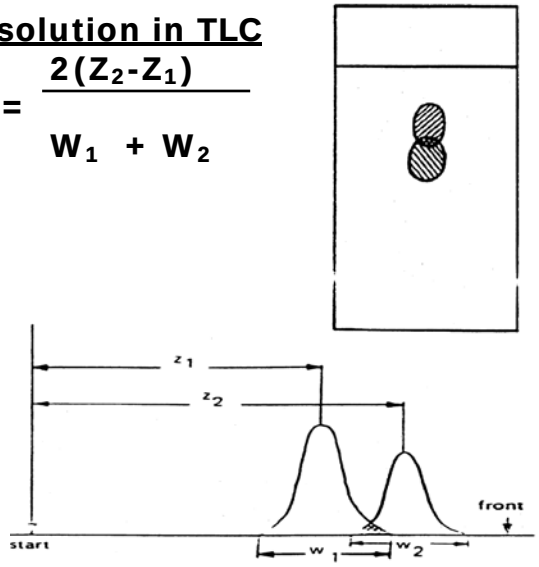
Normal phase TLC

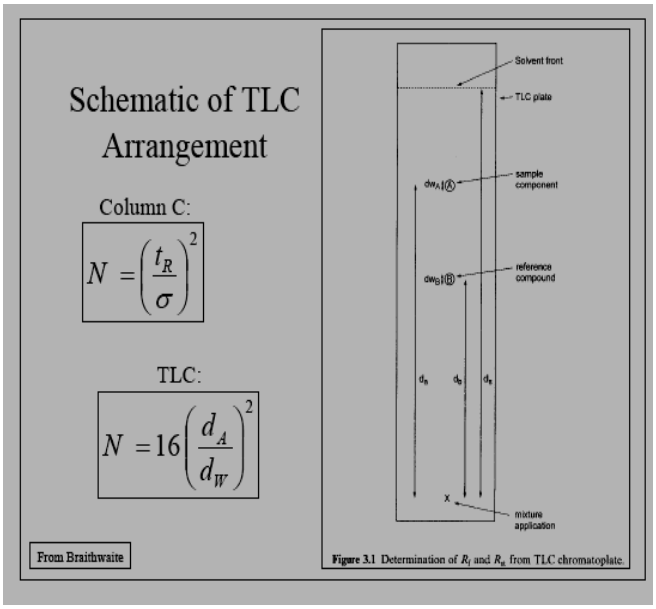
More polar compounds = smaller R_f values
Less polar compounds = larger R_f values

Efficiency and Resolution in Thin Layer Chromatography:

Resolution in TLC

$$R_s = \frac{2(Z_2 - Z_1)}{W_1 + W_2}$$





If R_s = 1-→ Two spots are reasonably separated
If R_s >1 ---→ Much better separation
If R_s <1 ---→ Poor separation

Factors that influence separation and rate of elution:

1- Polarity of mobile phase(solvent)

- more polar mobile solvents displace substrate from stationary phase, more easily than less polar solvents(all substrates)
- more polar the mobile phase , faster the substrate travels
- Can increase polarity to point where get no separation at all.

	Solvent
Least polar	Hexane
	Carbon tetrachloride
	Toluene
	Chloroform
	Diethyl ether
	Ethyl acetate
	Acetone
	Methanol
	Acetic acid
Most polar	Water

2- Substrate interactions with stationary phase

- stronger interaction, more slowly the substance moves
- Polar substrates more move slowly than non-polar one

(Polarity= ability of substance to bind to stationary phase)

	Compound type
Least polar	Alkanes
	Alkenes
	Ethers
	Alkyl halides
	Aromatics
	Aldeydes and Ketones
	Alcohols
	Amines
	Organic acids
Most polar	Salts

Advantages of TLC

1- Simultaneous Analysis: number of samples/standards can be analyzed simultaneously. However, with column chromatography samples are analyzed sequentially.
2- Simultaneous separation of unresolved components samples which are difficult to resolve in one dimension can be developed in two different solvent systems, which runs in perpendicular direction for e.g., in 2D-TLC.
3- Harsh separation conditions can be used for separation since TLC plates are used only once, harsh separation conditions that would degrade the S.P can be used. In contrast, in HPLC harsh conditions or dirty samples can destroy the column.
4- Solutes movement is always observable If no components are lost to vapor surrounding the plate, all components must be somewhere on the plate .In contrast to HPLC or GC in which sample components may never elute and are lost.

Liquid-Liquid Extraction (Solvent Extraction)

The solvent extraction processes depend on the distribution of solute between two immiscible liquid phases. This distribution is subjected to the equilibrium process and this equilibrium state occur when the free energy of solute between two phases are equal.

Principle: Selective transfer of material in microgram to gram quantities between two immiscible liquid phases; separations based on solubility differences; selectivity achieved by pH control and complexation.

When a phase containing a solute, *S,* is brought into contact with a second phase, the solute partitions itself between the two phases.

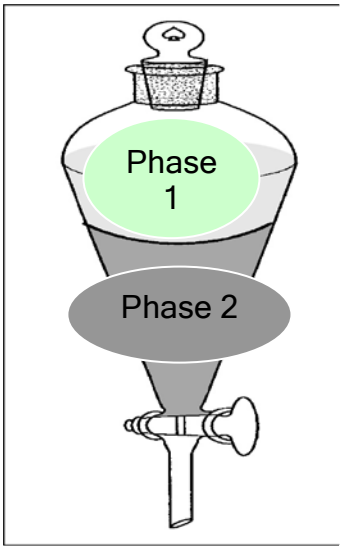
$$S_{\text{phase 1}} \rightleftharpoons S_{\text{phase 2}}$$

The equilibrium constant for this reaction

$$K = \frac{[S_{Phase}]_2}{[S_{Phase}]_1}$$

is called the distribution constant, or **partition coefficient.** If K_D is sufficiently large, then the solute will move from phase 1 to phase 2. The solute will remain in phase 1, however, if the partition coefficient is sufficiently small.

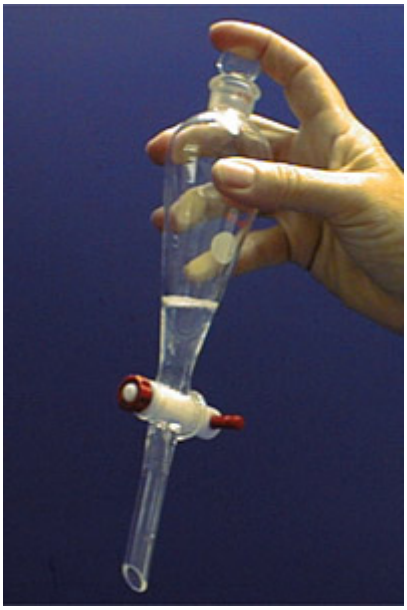
Liquid–liquid extractions are usually accomplished with a separatory funnel (see Figure). The two liquids are placed in the separatory funnel and shaken to increase the surface area between the phases. When the extraction is completed, the liquids are allowed to separate, with the denser phase settling to the bottom of the separatory funnel. Liquid–liquid extractions also may be carried out in the sample container by adding the extracting solvent when the sample is collected. Pesticides in water, for example, may be preserved for longer periods by extracting into a small volume of hexane added to the sample in the field.



(1) Pour in liquid to be extracted.

(2) Pour in the solvent

(3) Add a stopper



(4) Pick up the separatory funnel with the stopper in place and the stopcock closed, and rock it once gently.

(5) Point the stem up and slowly open the stop-cock to release excess pressure. Close the stop-cock. Repeat this procedure until small amount of pressure is released when it is vented.

Distribution Coefficient:

The extraction of a solute in this manner is governed by the *Nernst partition* or *distribution law* which states that " at equilibrium, a given solute will always be distributed between two essentially immiscible liquids in the same proportions at constant temperature on condition that the solute molecular state (A) in both phases is constant" . Thus, for solute A distributing between an aqueous and an organic solvent,

$$K_D = \frac{[A]_o}{[A]_{aq}}$$

Distribution (partition) coefficient
An equilibrium constant describing the distribution of a solute between two
Phases; only one form of the solute is used in defining the partition coefficient (K_D).

where [ ] denote concentrations (strictly activities) of A in both organic and aqueous respectively, and K_D is known as the *equilibrium distribution* or *partition coefficient* which is independent of total solute concentration.

It should be noted that constant temperature and pressure are assumed, and that (A) must exist in exactly the same form in both phases. Equilibrium is established when the chemical potentials (free energies) of the solute in the two phases are equal and is usually achieved within a few minutes by vigorous shaking. The value of K_D is a reflection of the relative solubility's of the solute in the two phases.

The above law does not take the activities of various species of solute into consideration (where the solute state in both phases is the same), therefore,

- It is imprecise thermodynamically and can be applied only for the dilute solutions where the activities proportions close to one.
- It can not be applied when the species encounter dissociation, association or polymerization etc ... in each phase.

Example:
This law is used to extract simple nonpolar molecules like I_2 It is known that I_2 have low solubility in polar solvent like water, but it is good soluble in nonpolar solvent like CCl_4. Thus, to extract I_2 and separated from aqueous solution, an auxiliary phase should be added to aqueous phase and the later should be chosen such that it does not miscible with water but the solubility of I_2 in auxiliary phase is good.

When CCl_4 add to an aqueous phase containing I_2 and mix together in the separating funnel by shaking, then I_2 will distribute between two phases where an equilibrium state occurs

$$I_{2\,(aq)} \rightleftharpoons I_{2\,(CCl4)}$$

$$K_D = \frac{[I_2]_o}{[I_2]_{aq}} = \text{constant quantity}$$

Due to the high solubility of I_2 in CCl_4, it is expected that large proportion of I_2 molecules from aqueous to organic phase.

In many practical situations solute A may dissociate, polymerize or form complexes with some other components of the sample or interact with one of the solvents. In these circumstances the value of K_D does not reflect the overall distribution of the solute between the two phases as it refers only to the distributing species. Analytically, the total amount of solute present in each phase at equilibrium is of prime importance, and the extraction process is therefore better discussed in terms of the **distribution ratio *D***.

Distribution Ratio (D)

A ratio expressing the total concentration of solute in one phase relative to a second phase; all forms of the solute are considered in defining the distribution ratio (*D*).

$$D \equiv \frac{\text{total conc. of solute in org. phase}}{\text{total of conc. of solute in aqueous phase}}$$

or

$$\mathbf{D = \frac{[S_{org.}]_{Total}}{[S_{aq}]_{total}}}$$

When the solute exists in only one form in each phase, then the partition coefficient and the distribution ratio are identical. If, however, the solute exists in more than one form in either phase, then K_D and *D* usually have different values. For example, if the solute exists in two forms in the aqueous phase, A and B, only one of which, A, partitions itself between the two phases, then

$$D = \frac{[S_{org}]_A}{[S_{aq}]_A + [S_{aq}]_B} \leq K_D = \frac{[S_{org}]_A}{[S_{aq}]_A}$$

This distinction between K_D and D is important. The partition coefficient is equilibrium constant and has a fixed value for the solute's partitioning between the two phases. The value of the distribution ratio, however, changes with solution conditions if the relative amounts of forms A and B change. If we know the equilibrium reactions taking place within each phase and between the phases, we can derive an algebraic relationship between K_D and D.

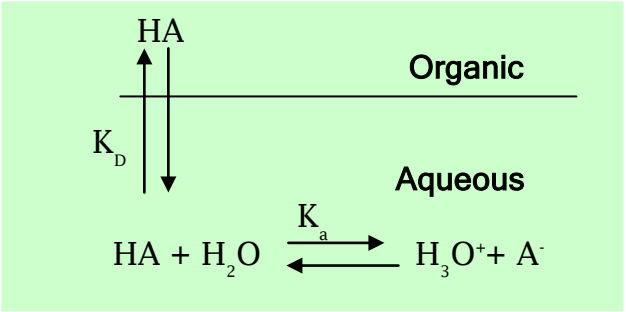
For the purpose of deriving the relationship between D and K_D from the involved equilibrium processes, we take the following example,

Weak acid (such as, benzoic acid HBz):

When benzoic acid is distributed between ether and water, there are different equilibrium states between two phases;

1- A weak acid is ionized and the equilibrium processes take place between two phases.

$$HA \rightleftharpoons H^+ + A^-$$



So, the equilibrium constant is,

$$K_a = \frac{[H^+]_w[A^-]_w}{[HA]_w} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

2- The acid is distributed between the aqueous and organic phases:

$$(HA)_w \rightleftharpoons (HA)_o$$

$$K_D = \frac{[HA]_o}{[HA]_w} \quad \ldots\ldots\ldots\ldots\ldots (2)$$

Then, the distribution ratio D is,

$$D \equiv \frac{[HA_o]_{tot}}{[HA_w]_{tot}} = \frac{[HA]_o}{[HA]_w + [A^-]_w} \quad \ldots\ldots\ldots (3)$$

Since the position of acid–base equilibrium depends on the pH, the distribution ratio must also be pH-dependent. To derive an equation for *D* showing this dependency, we begin with the acid dissociation constant for HA and solving Eq.1for $[A^-]_w$

$$[A^-]_w = \frac{K_a [HA]_w}{[H^+]_w} \quad \ldots\ldots\ldots\ldots\ldots (4)$$

And substituting into Eq. 3 gives

$$D \equiv \frac{[HA]_o}{[HA]_w + \{Ka\ [HA]_w / [H^+]_w\}} \ldots\ldots\ldots (5)$$

Factoring $[HA]_w$from the denominator, we get,

$$D \equiv \frac{[HA]_o}{[HA]_w \{1+K_a / [H^+]_w\}} \ldots\ldots\ldots (6)$$

and substituting Eq. 2

$$D \equiv \frac{K_D}{1 + \{K_a / [H^+]_w\}} \quad \ldots\ldots\ldots (7)$$

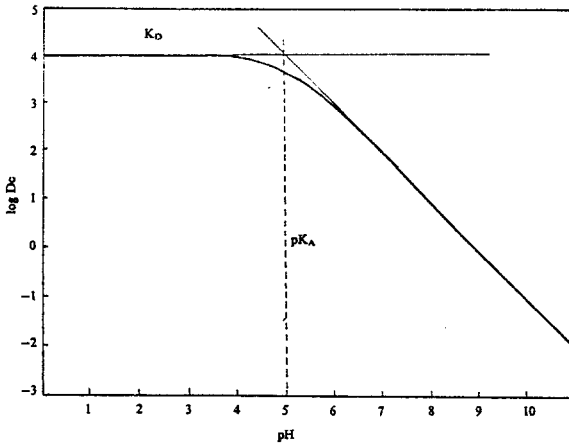
Eq.7 can be simplified to yield:

$$D \equiv \frac{K_D[H^+]_w}{[H^+]_w + K_a} \quad \ldots\ldots\ldots (8)$$

In this case of a weak acid, D is dependent on the solution pH. If we plot D versus pH, we obtain the diagram as shown below, revealing two straight line regions:

- when $[H^+]_w > K_a$, then $D=K_D$
- and when $K_a > [H^+]_w$ then:

$$D \equiv \frac{K_D[H^+]_w}{K_a}$$

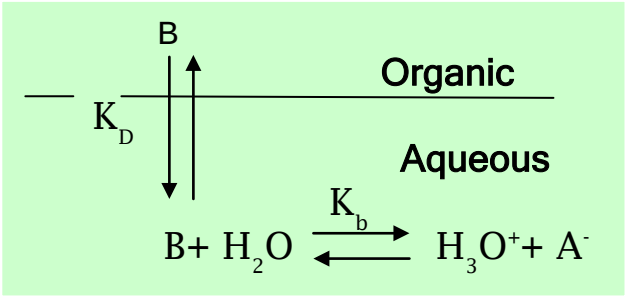


i.e. at low pH, where the acid is undissociated $D \approx K_D$, and the acid is extracted with greatest efficiency.

At high pH, where dissociation of the acid is virtually complete, *D* approaches zero and extraction of the acid is negligible. Graphical representation of equation (8) for benzoic acid shows the optimum range for extraction, Curves of this type are useful in assessing the separability of acids of differing *K*a values. A similar set of equations and extraction curve can be derived for bases, e.g. amines.

The same approach can be used to derive an equation for the distribution ratio when the solute is a molecular weak base, B.

$$D = \frac{K_D\, K_a}{K_a + [H^+]_{aq}}$$



The resulting distribution ratio is where *K*a is the acid dissociation constant for the weak base's conjugate weak acid.

Percentage Extraction (%E)

Stated as the number of millimoles of solute in organic phase divided by the total number of millimoles of solute in both phases and multiply by 100.
Suppose $[A]_o$ the conc. of solute in organic phase of volume V_o and the conc. of solute in aqueous phase $[A]_a$ of volume $V_{a,}$ then

$$\%E = \frac{[A]_o V_o}{[A]_o V_o + [A]_a V_{a,}} X\ 100 \quad \ldots\ldots (9)$$

From Eq.9, we can see that the percentage extracted of solute is connected with distribution ratio D. if we divide the nominator and dominator by $[A]_a$, we get,

$$\%E = \frac{[A]_o / [A]_a V_o}{[A]_o/ [A]_a V_o + [A]_a/ [A]_a V_{a,}} X\ 100$$

Then we get ,

$$\%E = \frac{D V_o}{DV_o + V_{a,}} X\ 100 \quad \ldots\ldots\ldots\ldots (10)$$

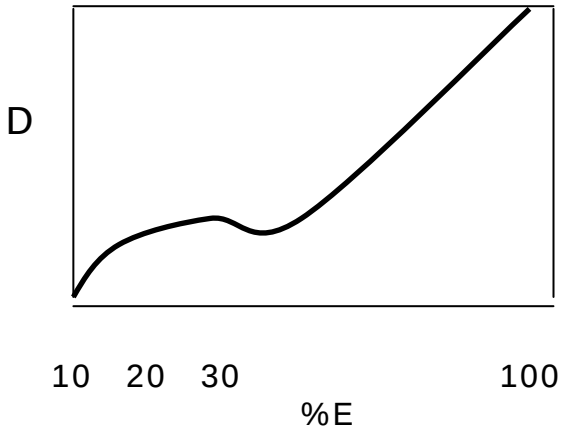
Rearrangement of Eq. 10 , we obtain,

$$\%E = \frac{100D}{D + (V_{a,} / V_o)} \quad \ldots\ldots\ldots\ldots (11)$$

If $V_{a,} = V_o$, we get,

$$\%E = \frac{100D}{D + 1} \quad \ldots\ldots\ldots\ldots\ldots (12)$$

We can see from the Figure, when the %E approach to 100% , the distribution ratio (D) approach to infinity. In this case the solute hold quantitatively if the value of D is less than 0.001.



We note from Eq. 3, the extracted fraction increases by decreasing the ratio(V_a / V_o) i.e. by increasing the organic layer. Thus, the most efficient method is to increase the amount extracted by using the same volume of solvent for batch extractions with individual small volume of this solvent .For example, if the value of D=10 and V_a / V_o =1, the %E = 91%, and if we decrease V_a,/ V_o to 0.5 (by doubling V_o) , this will increase %E to 95% , but by carrying out two consecutive extractions (twice extraction) with V_a,/ V_o =1 the extraction efficiency will increase to 99% .

The factors affecting the separation efficiency:

The probability of transferring an amount of solute that present in phase 1 into phase 2 is a function of different factors , that is:

$$\frac{m_1}{m_1+m_2} \int (W_1, W_2, V_1, V_2, n) = S \quad \text{.....(13)}$$

where, m_1 the weight of solute A in phase 1
m_2 the weight of solute A in phase 2

1- W_1, W_2 the forces affecting between the particles in phase1 and phase 2 and these forces depend on many factors such the solubility product and distribution. in general , we can summing up these forces as follows:
   (a) the solute or solvent or both have a constant dipole moment.
   (b) The possibility of creating a dipole moment via induction between the particles of a species because of the other species has a constant dipole moment.
   (c) The forces other than polarization forces like dispersion force.
   (d) Chemical forces such as the ability of creating ionic lattice, formation of hydrogen bonds, complex formation or occurring oxidation-reduction reactions.

2- V_1 and V_2 the volume of phase 1 and 2, respectively.

3- the time of extraction (n)

It was found experimentally from the extraction methods that the partition (dividing) of organic solvent used for extraction and re-extraction for many times is most efficient from the extraction of one time with large volume of organic phase.

Also, it was shown that the distribution coefficients are useful because it allows us to calculate the concentration of remaining component (analyte) in aqueous solution for certain number of extractions.Suppose the following simple extraction system:

$$\mathbf{A}_{aq} \rightleftharpoons \mathbf{A}_{org}$$

$$\mathbf{K_D} = \frac{[\mathbf{A}]_o}{[\mathbf{A}]_{aq}}$$

We indicated here that the amount or concentration of analyte A remaining in aqueous phase after n extractions with organic phase is m_n, where,

$$m_n = m_a \left(\frac{V_1}{DV_2 + V_1}\right) \qquad \ldots\ldots\ldots\ldots\ldots\ldots (14)$$

m_a represents the amount of analyte (original concentration) before doing the extraction process and dissolved in light solvent (aqueous).If the analyte dissolved in the heavy phase , i.e.

$$m_n = m_a \left(\frac{V_2}{DV_1 + V_2}\right) \qquad \ldots\ldots\ldots\ldots\ldots\ldots (15)$$

Many of literatures reveal that the analyte of component is dissolved in aqueous phase and in this case we can use Eq. 15 , i.e. the equation of heavy phase become,

$$m_n = m_a \left(\frac{V_a}{DV_o + V_a}\right)^n \qquad \ldots\ldots\ldots\ldots\ldots\ldots (16)$$

m_n = amount of analyte that remains in aqueous phase V_a after n times of extractions with volume V_o of organic phase.

m_a = the amount of analyte originally present in the aqueous phase..

n = extraction times.

D = distribution ratio.

V_a= volume of aqueous phase.

V_o= volume of organic phase.

It is possible to calculate the fraction remaining un-extracted in aqueous phase, the fraction extracted in organic phase and the percent extracted by using the following formulas:

$$\phi_S = \frac{m_n}{m_a} = \left(\frac{V_a}{DV_o + V_a}\right)^n \quad \text{....(17)}$$

Where ϕ_S = the remaining fraction un-extracted in aqueous phase

Then ,

$$\Phi_m = 1 - \phi_S$$

or,

$$\Phi_m = 1 - \left(\frac{V_a}{DV_o + V_a}\right)^n \quad \text{.....(18)}$$

Where Φ_m = the extracted fraction in organic phase.

The percent extracted = Φ_m X 100

If the value of *D* is known, equation (4) is useful for determining the optimum conditions for quantitative transfer.

Suppose, for example, that the complete removal of 0.1 g of iodine from 50 mL of an aqueous solution of iodine and sodium chloride is required. Assuming the value of D for carbon tetrachloride/water is 85, then for a single extraction with 25 mL of CCl_4,

$$m_n = m_a \left(\frac{V_a}{\quad}\right)^n$$

$$DV_o + V_a$$

$$m_n = 0.1\ \left(\frac{50}{85x25 + 50}\right) = 0.0023g \text{ in } 50 \text{ mL}$$

i.e. 97% of the I_2 is extracted
For three extractions with 8.33 mL of CCl_4,

$$m_n = 0.1\ \left(\frac{50}{85x8.33 + 50}\right)^3 = 0.000029g \text{ in } 50 \text{ mL}$$

i.e. 99.79% of the I_2 is extracted which for most purposes can be considered quantitative.

It is clear therefore that extracting several times with small volumes of organic solvent is more efficient than one extraction with a large volume. This is of particular significance when the value of *D* is less than 102.

Homework: A 1 g of solute is dissolved in 100 mL water. Calculate the amount of solute remaining in aqueous phase after (a) single extraction with 90 mL of organic phase (b) single extraction with 30 mL of organic phase (c) three extractions with 30 mL of organic extraction. D= 10, and comment on the results.

Selectivity of Extraction

Often, it is not possible to extract one solute quantitatively without partial extraction of another. The ability to separate two solutes depends on the relative magnitudes of their distribution ratios. For solutes A and B, whose distribution ratios are *D*A and *D*B, the separation factor b is defined as the ratio *D*A/*D*B where *D*A>*D*B. Table 4.2 shows the degrees of separation achievable with one extraction, assuming that *D*A = 102, for different values of *D*B and b. For an essentially quantitative separation b should be at least 105.

A separation can be made more efficient by adjustment of the proportions of organic and aqueous phases. The optimum ratio for the best separation is given by the *Bush-Densen equation* as follows.

$$\mathbf{V_o / V_a = (1/D_A D_B)^{1/2}}$$

Separation of two solutes with one extraction, assuming equal volumes of each phase;

D_A	D_B	β	%A Extracted	%B Extracted
	10	10	99.0	90.9
	1	10^2	99.0	50.0
10^2	10^{-1}	10^3	99.0	9.1
	10^{-2}	10^4	99.0	1.0
	10^{-3}	10^5	99.0	0.1

Successive extractions, whilst increasing the efficiency of extraction (B) of both solutes, may lead to a poorer separation. For example, if $D_A = 10^2$ and $D_B = 10^{-1}$, one extraction will remove 99.0% of A and 9.1% of B whereas two extractions will remove 99.99% of A but 17% of B. In practice, a compromise must frequently be sought between completeness of extraction and efficiency of separation. It is often possible to enhance or suppress the extraction of a particular solute by adjustment of pH or by complexation. This introduces the added complication of several interrelated chemical equilibria which makes a complete theoretical treatment more difficult.

Applications of Solvent Extraction

The technique is used predominantly for the isolation of a single chemical species prior to a determination and to a lesser extent as a method of concentrating trace quantities. The most widespread application is in the determination of metals as minor and trace constituents in a variety of inorganic and organic materials, e.g. the selective extraction and spectrometric determination of metals as colored complexes in the analysis of metallurgical and geological samples as well as for petroleum products, foodstuffs, plant and animal tissue and body fluids.

Separation procedures for purely organic species do not possess the same degree of selectivity as systems involving metals because of a general lack of suitable complexing and masking reactions.
Nevertheless, classes of compounds such as hydrocarbons, acids, fats, waxes, etc., can often be isolated prior to analysis by other techniques.

Problems and exercises

1- The distribution ratio of solute (A) between ether and water is 10. (a) Calculate the percent extracted of (A) if 100 mL of water contains 1 g of (A) extracted in 100 mL of ether (b) What is the percent extracted of (A) if the aqueous layer is extracted twice with 50 mL of ether.
2- It is possible to separate Fe^{3+} from the other element like chromium from aqueous acidic solution .if 50 mL of this solution contains 0.25 g of Fe^{3+} is treated with 150 mL of ether in one extraction . How many milligram of iron will remain in aqueous solution (use D=50).
3- In twice extraction with 20 mL of organic solvent, 89% of solute was extracted from 100 mL of aqueous solution. Calculated D (org/aq).
4- Weak acid (HX) has a distribution ratio between water and organic solvent equal to 10 and at pH=5 a half of acid is extracted in organic layer. Calculate the dissociation constant of HX.
5- A solute with a partition coefficient of 4.0 is extracted from 10 mL of phase 1 into phase 2 (a) what volume of phase 2 is needed to extract 99% of the solute in one extraction.(b) what is the total volume of phase 2 needed to remove 99% of the solute in three equal extraction instead?.
6- A solute, S, has a distribution ratio between water and ether of 7.5. Calculate the extraction efficiency if a 50.0-mL aqueous sample of S is extracted using 50.0 mL of ether as (a) a single portion of 50.0 mL; (b) two portions, each of 25.0 mL; (c) four portions, each of 12.5 mL; and (d) five portions, each of 10.0 mL. Assume that the solute is not involved in any secondary equilibria.
7- What volume of ether is needed to extract 99.9% of the solute in problem 6 when using (a) one extraction; (b) two extractions; (c) four extractions; and (d) five extractions?

Ion-Exchange Chromatography

In this technique, separation is based on the exchange of ions (anions or cations) between the mobile phase and ionic sites on the stationary phase. The charged species are covalently bound to the surface of the stationary phase. Sample ion retention is based on the affinity of different ions on the support and other solution parameters including counter ion type, ionic strength and pH.

What is the Ion Exchange?

It means that the exchange of the ions having a similar charges between a solution (mobile phase) and a solid highly insoluble body (stationary phase) in contact with it. The later is called *ion exchanger* and characterized to:

- Contains ions of its own,
- Must have an open, permeable molecular structure so that ions and solvent molecules can move freely in and out.

Or it is based on exchange equilibria between ions in solution and
ions of like charge on surface of essentially insoluble, high-molecular weight solid.

What are Ion-Exchangers?

These are organic or inorganic polymers used to exchange cations or anions from a solution phase. These are used as the stationary phases in ion-exchange chromatography and organic type composed of cross-linked polymers and active ionizable functional groups.

General Properties of Exchange Media

An ideal ion exchanger is one that has the following criteria:

1. A regular and reproducible composition and structure;
2. High exchange capacity;
3. A rapid rate of exchange (i.e. an open porous structure);
4. Chemical and thermal stability and resistance to 'poisoning' as well as radiation stability when used in the nuclear industry;
5. Mechanical strength stability and attrition resistance;
6. Consistency in particle size, and compatibility with the demands of the use of large columns in industry.

In addition some applications demand the ability to exchange a specific ion(s) selectively from high concentrations of other ions.

What main types of Ion Exchangers are?

1- ***Natural*** such as, certain clay minerals, also many natural substances such as proteins, cellulose, living cells and soil particles exhibit ion exchange properties which play an important role in the way the function in nature.

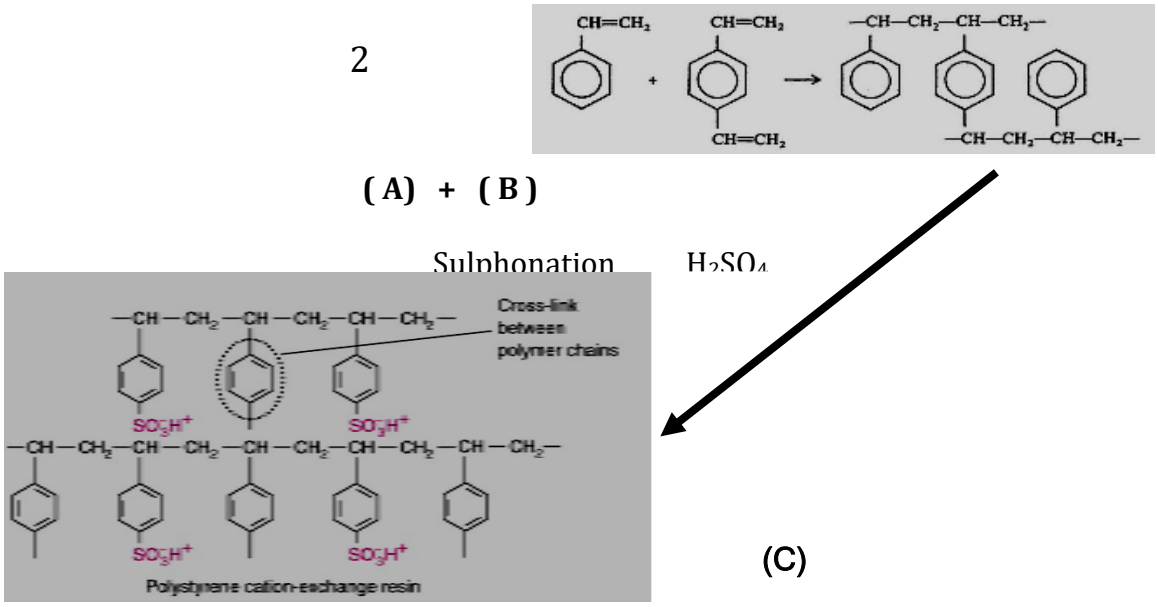
2- ***Synthetic***, whether inorganic or organic resins. For analytical work synthetic *organic ion exchangers* are chiefly of interest,although some inorganic materials, e.g. zirconyl phosphate and ammonium 12-molybdophosphate, also possess useful ion exchange capabilities and have specialized application.

Organic ion exchangers are subdivided into two main classes:

1- Cation Exchange Resins :

These are polymers having high-molecular weights, likewise cross-linked consists of a fixed anion which form an integral part on resin body such as, sulfonic acid group (HSO_3^-) or carboxylic acid group (-COOH) and active cations (like H^+ or Na^+) which are capable to be exchange with other cations in solution.

A widely used cation exchange resin is that obtained by the copolymerization of styrene (A) and a small proportion of divinylbenzene (B), followed by sulphonation; it may be represented as (C)



Cation exchangers are of two types:

- ***Strongly acidic cation exchanger* (SCX):** Aryl–$SO_3^-H^+$ (work with pH: 5-14) and generally used to separate the strong ionic compounds or multi-groups materials such as proteins and peptides.

- ***Weakly acidic cation exchanger* (WCX):** $R–CO^{2-}H^{+}$(work with pH: 1-14) and used to separate the complex mixtures.
  - exclude neutrals and negative ions
  - Suitable for both inorganic analytes and organic analytes (retains charged and elutes neutrals)

The proton on the sulphonic group exchanges with other cation in solution: in general;

$$RX^{-}C^{+} + M^{+} \Leftrightarrow RX^{-}M^{+} + C^{+}$$

$$nRSO_3^{-}H^{+} + M^{n+} \Leftrightarrow (RSO_3)_nM + nH^{+}$$

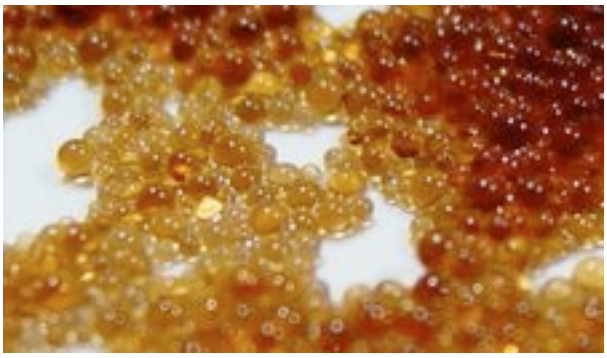
and

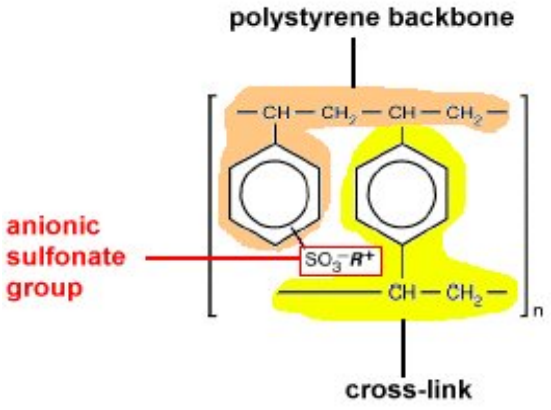
$$nRCO_2^{-}H^{+} + M^{n+} \Leftrightarrow (RCO_2)_nM + nH^{+}$$

– contain bound negative groups

When a cation exchanger containing mobile ions C^{+} is brought into contact with a solution containing cations M^{+} the latter diffuse into the resin structure and cations C^{+} diffuse out until equilibrium is attained. The solid and the solution then contain both cations C^{+} and M^{+} in numbers depending upon the position of equilibrium. The same mechanism operates for the exchange of anions in an anion exchanger.

The divinyl benzene (DVB) units of the ion exchanger specifically determine the swelling of polystyrene chains. The solid granules of resin swell when placed in water to give a gel structure, but the swelling is limited by the cross-linking.





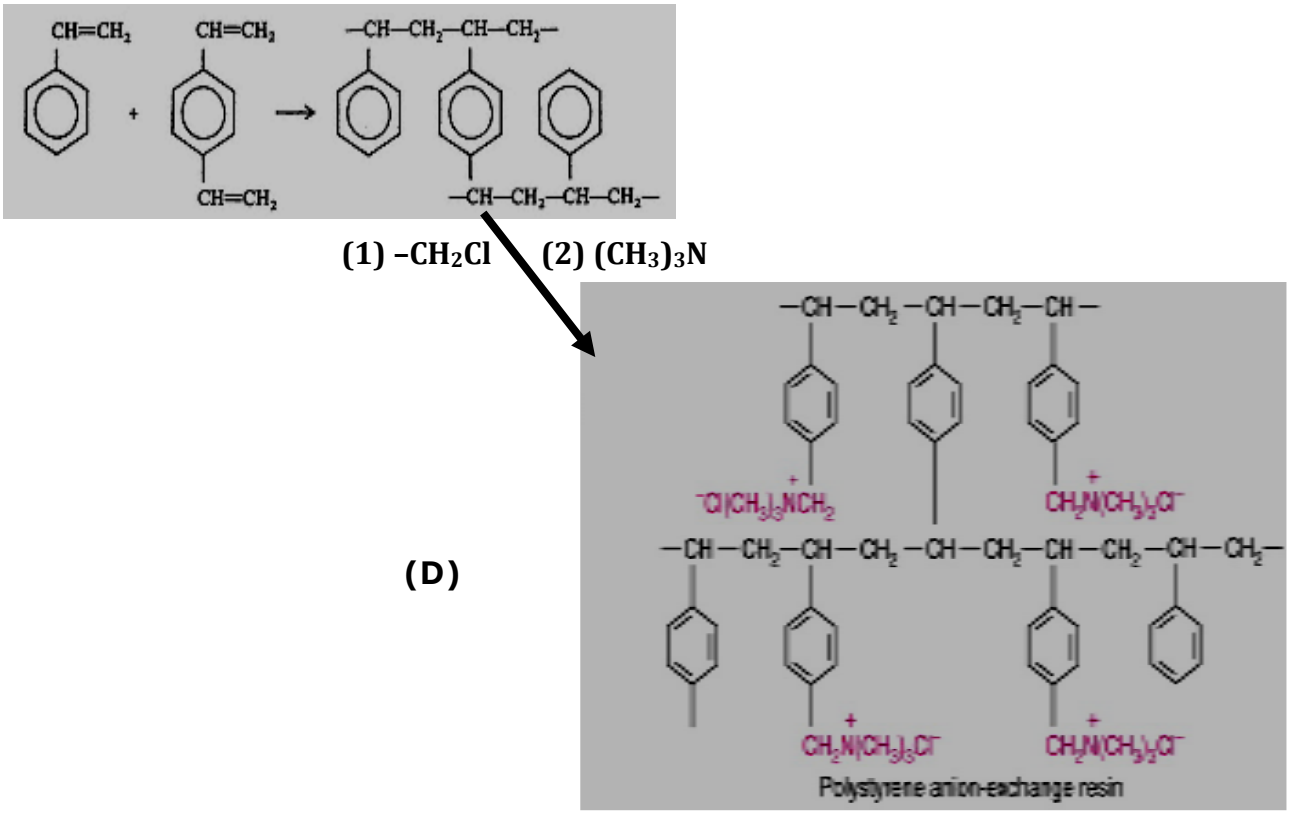
In the above example the divinyl benzene units 'weld' the polystyrene chains together and prevent it from swelling indefinitely and dispersing into solution. The resulting structure is a vast sponge-like network with negatively charged sulphonate ions attached firmly to the framework. These fixed negative charges are balanced by an equivalent number of cations: hydrogen ions in the hydrogen form of the resin and sodium ions in the sodium form of the resin, etc. These

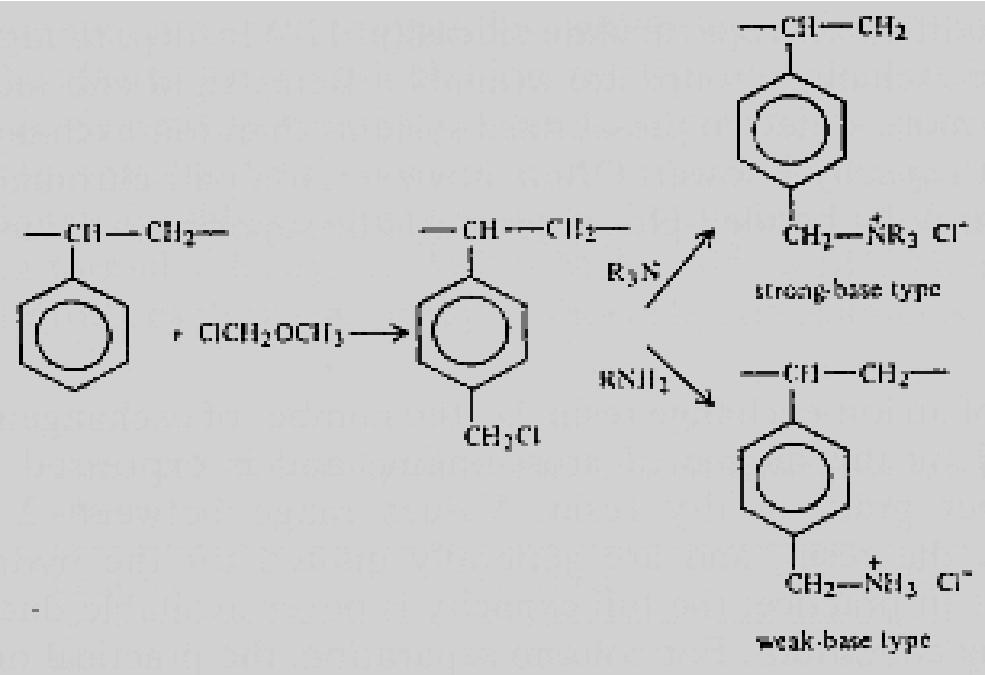
ions move freely within the water-filled pores and are sometimes called mobile ions; they are the ions which exchange with other ions.

2- *Anion Exchange Resins*:

Anion exchange resins are organic polymer containing polystyrene chains linked between themselves by divinyl benzene bridges (DVB). On the polymer chains, amino (RNH_3^+), substituted amino (R_2NH^+), or quaternary ammonium groups R_4N^+ can be added, and active anions (like OH^- or Cl^-) which are capable to be exchange with other anions in solution. They are likewise cross-linked, high-molecular-weight polymers.

A widely used anion exchange resin is prepared by co-polymerization of styrene and a little divinyl benzene, followed by chloromethylation (introduction of the $-CH_2Cl$ grouping, Say, in the free para position) and interaction with a base such as trimethylamine. A hypothetical formulation of such a polystyrene anion exchange resin is given as (D).





Anion exchangers are two types:

- **Strongly basic** (SAX): Aryl–$N(CH_3)_3^+Cl^-$, works with pH(0-12)
- **Weakly basic** (WAX): R–$NH(R)_2^+Cl^-$, works with pH(0-12)

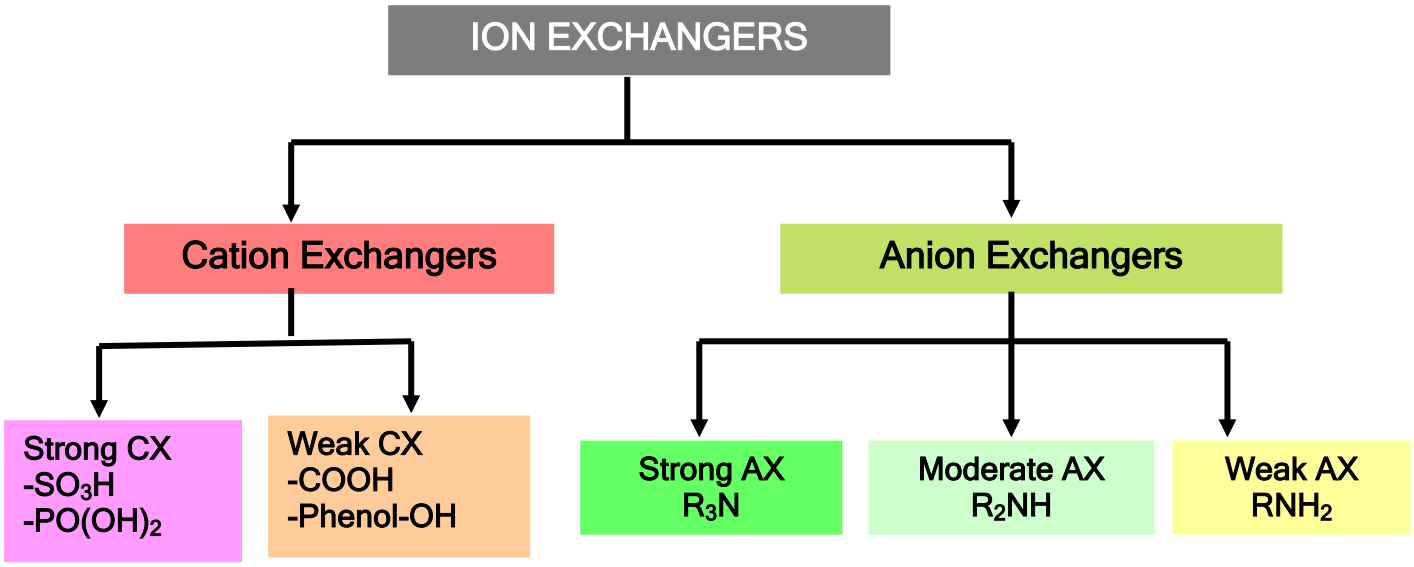
contain bound positive groups

$$\mathbf{RX^+A^- + M^+B^- \Leftrightarrow RX^+B^- + M^+ + A^-}$$

$$\mathbf{RNR_3^+Cl^-_{(solid)} + NO_3^-{}_{(sol)} \Leftrightarrow RNR_3^+ NO_3^-{}_{(solid)} + Cl^-{}_{(sol)}}$$

The sample ion can exchange with the counter ion to pair with the covalently attached charge on the support. When the sample ion is paired with the charged group on the support, it does not move through the column. Again, sample ion retention is based on the affinity of different ions on the support and other solution parameters including counter ion type, ionic strength and pH.

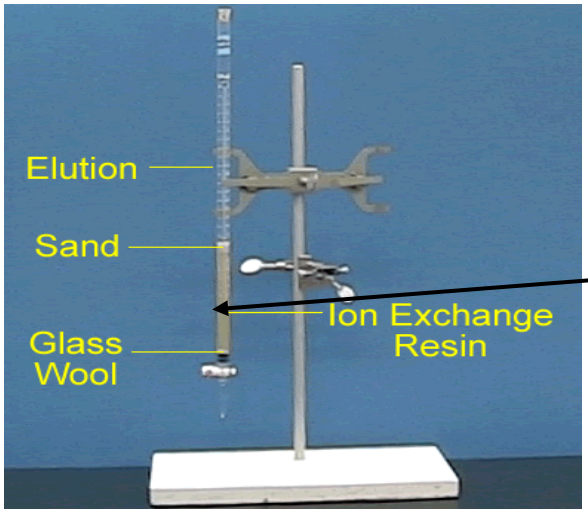
Classification of Organic Ion Exchange Resins

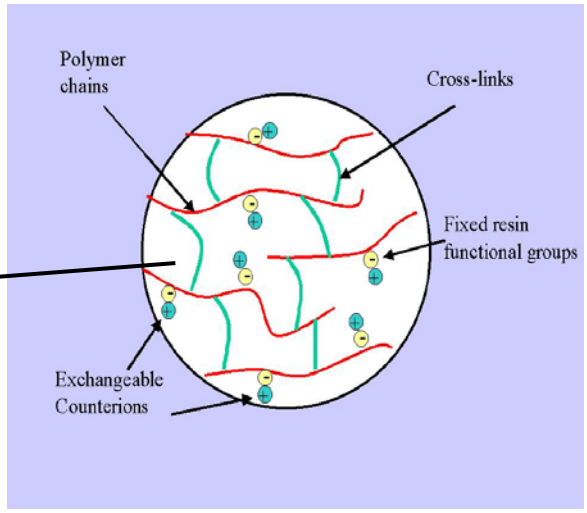


- Ion exchange resins are organic polymer containing polystyrene chains linked between themselves by divinyl benzene bridges (DVB).
-On the polymer chains, sulfonic groups SO_3H or quaternary ammonium groups R_4N^+ can be added.

- In the case of sulfonic resins, the proton can be replaced by metallic actions; the resin is **cationic exchanger.**
- In the case of quaternary ammonium resins, the positive charge must be neutralized by an anion X^- (R_4N^+, X^-), this anion can be constituted of an anionic metallic complex $MX_n^{(n-m)-}$ with n: number of ligands linked to the metal
m: metallic ion charge,

These resins are **anionic exchangers**





How ion exchange resins work

Cation exchange resins (for example) contain free cations which can be exchanged for cations in solution (soln).

$$(Res.A^-)B^+ + C^+ (soln) \leftrightarrow (Res.A^-)\,C^+ + B^+ (soln)$$

If the experimental conditions are such that the equilibrium is completely displaced from left to right the cation C^+ is completely fixed on the cation exchanger. If the solution contains several cations (C^+, D^+, and E^+) the exchanger may show different affinities for them, thus making separations possible.

A typical example is the displacement of sodium ions in a sulphonate resin by calcium ions:

$$2(Res.SO_3^-)Na^+ + Ca^{2+} (soln) \leftrightarrow (Res.SO_3^-)2\,Ca^{2+} + 2Na^+ (soln)$$

Similarly, strongly basic anion exchange resins, e.g. a cross-linked polystyrene containing quaternary ammonium groups, are largely ionized in both the hydroxide and the salt forms. Some of their typical reactions may be represented as:

$$2(Res.NMe_3^+)Cl^- + SO_4^{2-}{}_{(soln)} \leftrightarrow (Res.NMe_3^+)_2SO_4^{2-} + 2C1^-{}_{(soln)}$$

$$(Res.Nme_3^+)Cl^- + OH^-{}_{(soln)} \leftrightarrow (Res.Nme_3^+)OH^- + Cl^-{}_{(soln)}$$

How can the separation be accomplished?

We can resort the first approximation *coulomb's law* to calculate the attraction force between the ion exchanger site having a charge **q(resin)** and the counter ion charge **q(ion)** separated by distance of **(r):**

$$F \alpha \frac{q(ion)\ X\ q(resin)}{r^2} = \frac{q(ion)\ X\ q(resin)}{r\ (hydrated\ ion)^2}$$

From the above equation, the attraction force between the exchanger site and ion increases as the charge of ion increases, but decreases with increasing the hydrated ion radius (size). Consequently, the ion exchanger has a large attraction force toward ions of largest charge and smallest radius. Inasmuch as the charge increases or/and the radius of ion decreases the retention time (t_R) for ion on the exchanger increases.

(i.e. whenever the attraction force between the exchanger site and ion of interest increases, the relative retention time or retention time increases in stationary phase).

The effect of charge on retention in an ion-exchange column can be expressed by relative retentions. For example:

1. Nature of exchanging ions.

(a) At low aqueous concentrations and at ordinary temperatures the extent of exchange increases with increasing charge of the exchanging ion, i.e.

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

(b) Under similar conditions and constant charge, for singly charged ions the extent of exchange increases with decrease in size of the hydrated cation:

$$Li^{+} < H^{+} < Na^{+} < K^{+} \approx NH_4^{+} < Rb^{+} < Cs^{+}$$

While for doubly charged ions the ionic size is an important factor but the incomplete dissociation of salts of such cations also plays a part;

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

(c) With strongly basic anion exchange resins, the extent of exchange for singly charged anions varies with the size of the hydrated ion in a similar anions are generally obsorved preferentially.

$$F^{-} < HCO_3^{-} < Cl^{-} < HSO_3^{-} < CN^{-} < Br^{-} < NO_3^{-} < I^{-} \ll SO_4^{2-}$$

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

2. Nature of ion exchange resin.

The absorption of ions will depend upon the nature of the functional groups in the resin. It will also depend upon the degree of cross-linking: as the degree of cross-linking is increased, resins become more selective towards ions of different sizes (the volume of the ion is assumed to include the water of hydration); the ion with the smaller hydrated volume will usually be absorbed preferentially

Selectivity

The affinity between a resin and an exchangeable ion is a function both of the resin and the ion. Ion exchange is an equilibrium process which for a cationic resin can be represented by the equation

$$n\mathrm{R^-H^+} + \mathrm{M}^{n+} = (\mathrm{R^-})_n\mathrm{M}^{n+} + n\mathrm{H^+}$$

where R represents the resin matrix.

The equilibrium constant, also known as the *selectivity coefficient*, is given by

$$K = \frac{[\mathrm{M}^{n+}]_\mathrm{R}[\mathrm{H^+}]^n}{[\mathrm{M}^{n+}][\mathrm{H^+}]^n_\mathrm{R}}$$

where $[M^{n+}]_R$ and $[H^+]^n_R$ are the concentrations (strictly activities) of the exchanging ion and the hydrogen ion within the resin structure. Thus, the greater the affinity for a particular ion, relative to hydrogen, the greater the value of *K*. Selectivity coefficients are functions of the proportions of the exchanging ions, the total concentration of the solution and the degree of crosslinking.

In dilute solutions (<0.1 M) values of *K* increase with increasing formal valency, i.e. $M^{4+} > M^{3+} > M^{2+} > M^{+}$.

Partition coefficient K_D

The partition of a metallic ions M between an aqueous phase and the ion exchange resin is characterized by the partition coefficient K_D

$$\mathbf{K_D = C_{MR} / CM^{a-1}}$$

With C_{MR} = concentration of M in the resin (Mole for a gram of resin)
CM^{a-1} = concentration of M in the aqueous phase in mole/L. The dimension of K_D is L/g

Capacity of Ion exchanger

- Capacity is defined as the number of counter-ion equivalents in a specified amount of material. Capacity and related data are primarily used for two reasons:- for characterizing ion-exchange materials, and for use in the numerical calculation of ion-exchange operations. Capacity can be defined in numerous ways:

1. Capacity (*Maximum capacity*, *ion-exchange capacity*) Definition: Number of inorganic groups per specified amount of ion-exchanger.
2. Scientific Weight Capacity Units: Meq/g dry H^+ or Cl^- formTechnical Volume Capacity Units: eq/liter packed bed in H^+ or Cl^- form and fully water-swollen.
3. *Apparent Capacity* (Effective Capacity) Definition: Number of exchangeable counters ions per specified amount of ion exchanger. Units: meq/g dry H^+ or Cl^- form (apparent weight capacity). Apparent capacity is lower than maximum capacity when inorganic groups are incompletely ionized; depends on experimental conditions (pH, conc., etc).

Applications of Ion Exchange Resins:

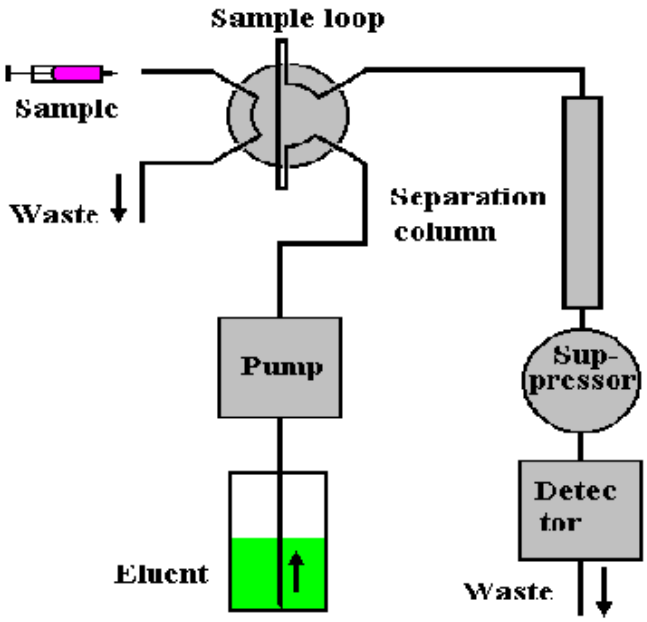
Ion exchange and exchange resins have numerous applications. for example:

1- In scientific studies, exchange resins are used to isolate and collect various ionic **species**, cations on a cation resin, and anions on an anion resin.
2- In industry resins are used to purify water by removing all ions from it. Upon passage of a water sample through both a cation resin (H^+ form) and an anion resin (OH^- form) the cations and anions in the water are retained. The H^+ and OH^- ions released from the resin then combine to form additional water. Deionized water also is a source of pure water containing no ionic chemical compounds.
3- Ion exchange also is used to remove ionic compounds from boiler water used in the steam generation of electric power.
4- Ion exchange resins also are used in the separation and purification of various chemicals. Rare **earth** elements are separated from their ores and purified in this manner.
5- Separation of amino acids

Ion Chromatography:

Ion chromatography is used for water chemistry analysis. Ion chromatographs are able to measure concentrations of major *anions,* such as fluoride, chloride, nitrate, nitrite, and sulfate, as well as major *cations* such as lithium, sodium, ammonium, potassium, calcium, and magnesium in the parts-per-billion (ppb) range. Concentrations of organic acids can also be measured through ion chromatography.

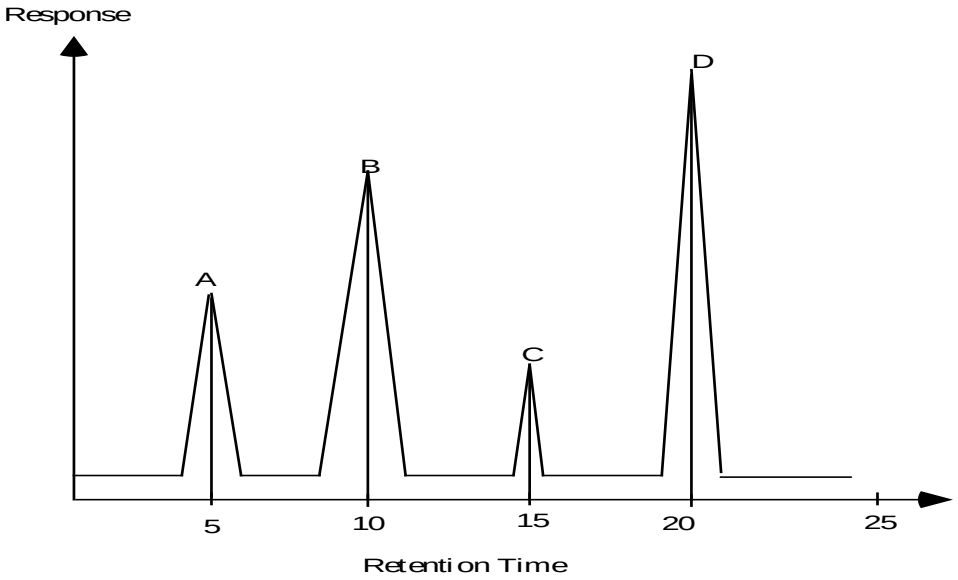
Ion chromatography includes all rapid liquid chromatography separations of ions in columns coupled online with detection and quantification in a flow-through detector". The following diagram shows the arrangement of ion chromatographic system.



- The solvent in reservoir (mobile phase or the eluent) passes from the selector/programmer to a high pressure pump. The mobile phase then passes from the pump to the sampling device, usually a simple rotating

valve that, on rotation, places the sample, in line with the mobile phase flow which, then, passes onto the column in which ions of interest are separated.

- The exit flow from the column passes either to an ion suppressor (this column uses selectively to remove the mobile phase ions coming from the separating column) or directly to the detector. The detector is usually an electrical conductivity detector but the UV detector and other types of detector can also be used under certain circumstances.
- The output from the detector sensor is modified by the detector electronics and the electronically modified output, which is now linearly related to ion concentration, is either passed to a potentiometric recorder (now largely obsolete) or to an A/D converter and thence to a computer. The computer output is either observed on a monitor or presented in printed form by the computer printer as shown in following chromatogram.



- If the electrolyte solution used as mobile phase is HCl (usually used in cations separation) , anion exchanger type OH^- is used in the suppressor column, thus the Cl^- ion in dilute HCl exchanges with OH^- and in turn with H^+ form H_2O.
- If we want to separate anion in the sample, and the mobile phase is dilute NaOH, the separating column is cation exchanger and the suppressor column is anion exchanger. So H^+ ions in suppressor column react with OH^- and form H_2O.