Chromatographic Techniques

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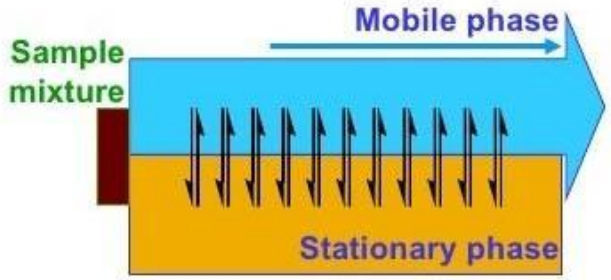
Definition and application

Chromatography is a physical method of separating a mixture of compounds by continuous distribution of components between two phases. One phase moves over the other in a continuous manner.

Chromatography helps to

1. Know the number of compounds in a sample mixture.
2. Identify the compounds
3. Know the relative quantities of the compounds in a sample
4. Separate the compounds quantitatively

Fundamental mechanism



Equilibrium establishes at each point (ideally)

$$A(mobile) \rightleftharpoons A(stationary)$$

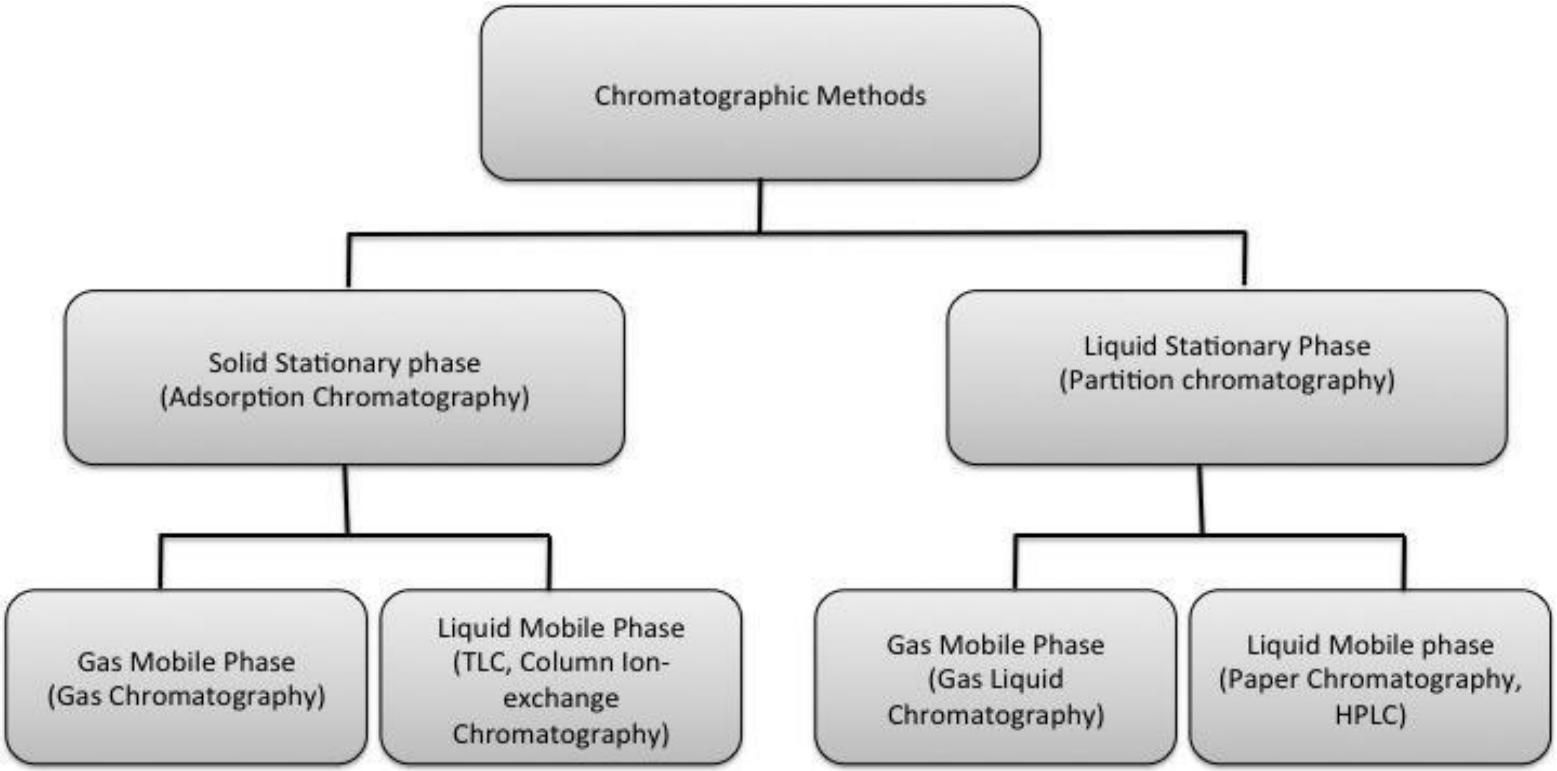
$$K_c = \frac{(a_A)_S}{(a_A)_M}$$

The molecules of the mixture interact with the molecules of the Mobile and Stationary Phase → Retardation of rate of movement of molecules

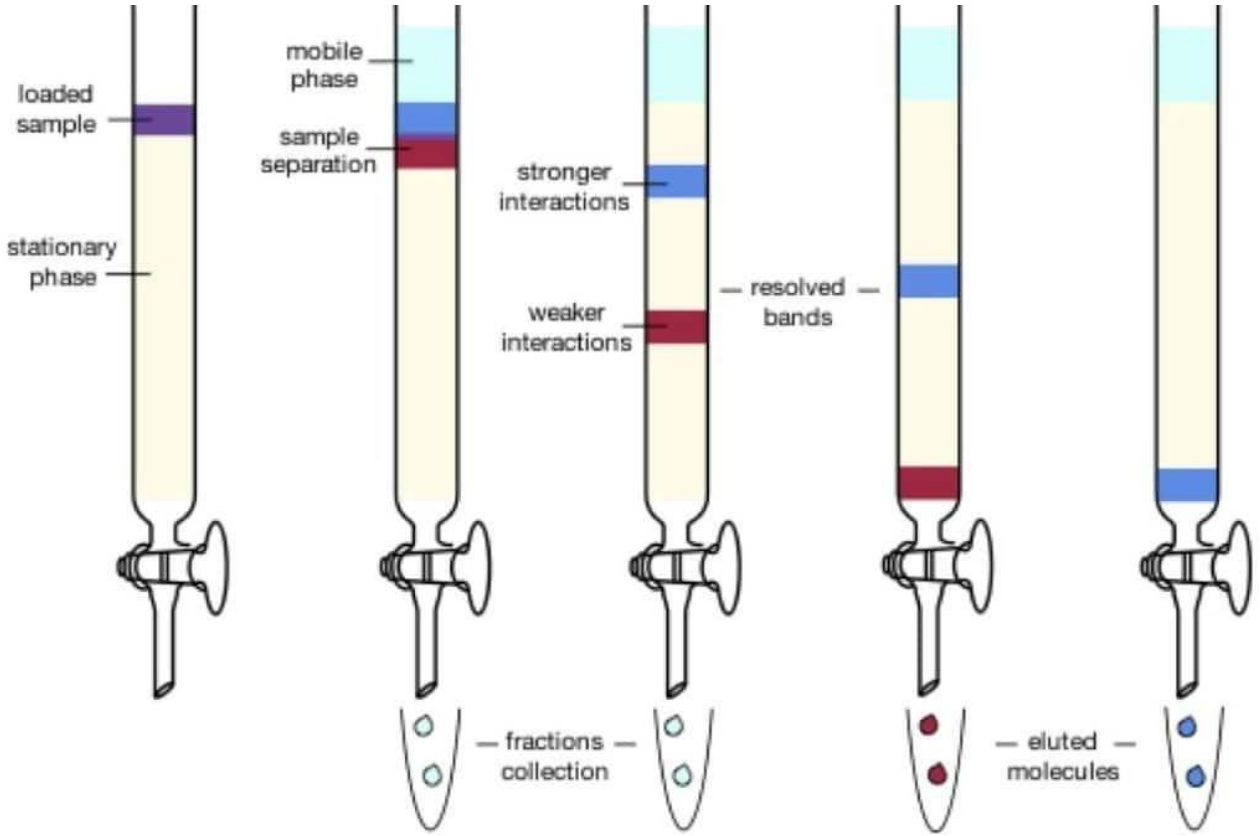
$$K_c = \frac{c_S}{c_M}$$

Each molecule interacts differently with MP and SP → Different distribution coefficients and different net rates of migration

Classification of Chromatography



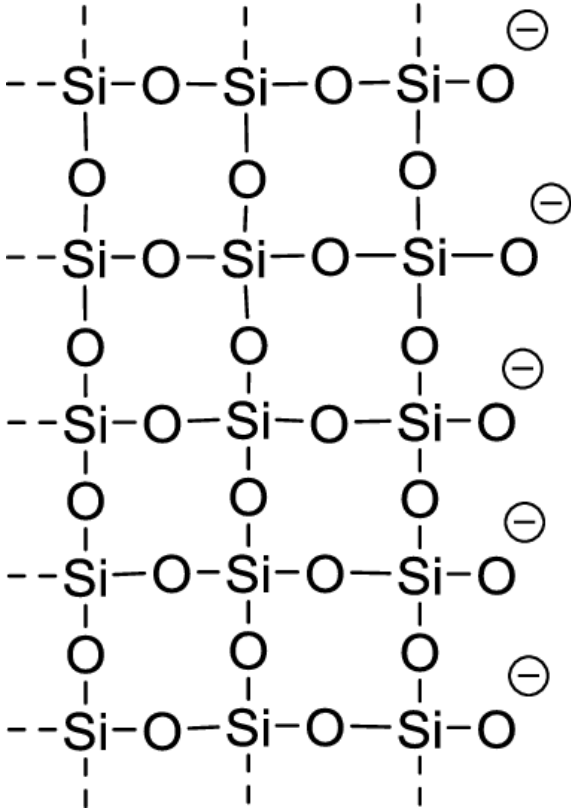
Technical side column chromatography



Technical side column chromatography

Technical side column chromatography

Silica in the laboratory

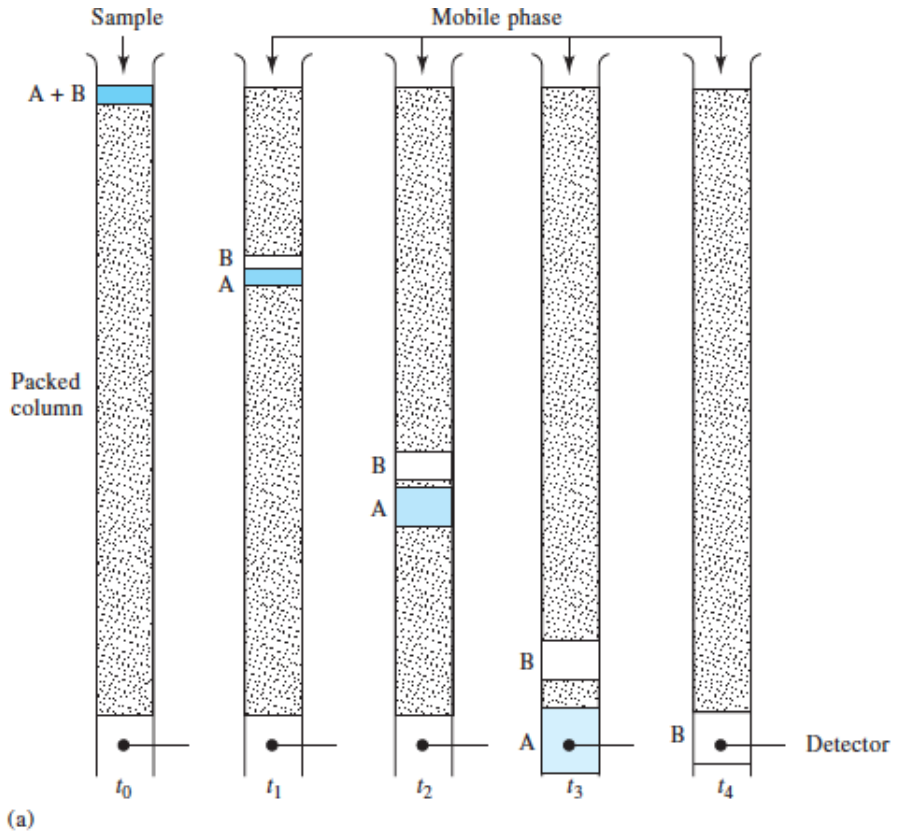


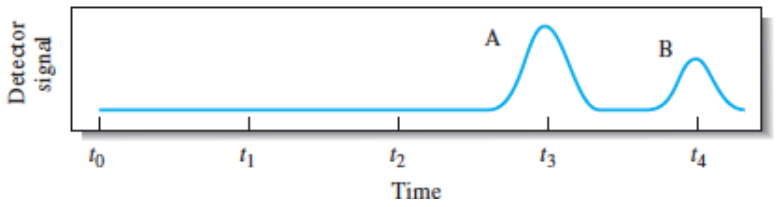
Molecular structure of silica

Technical side column chromatography

Automated column chromatography set up

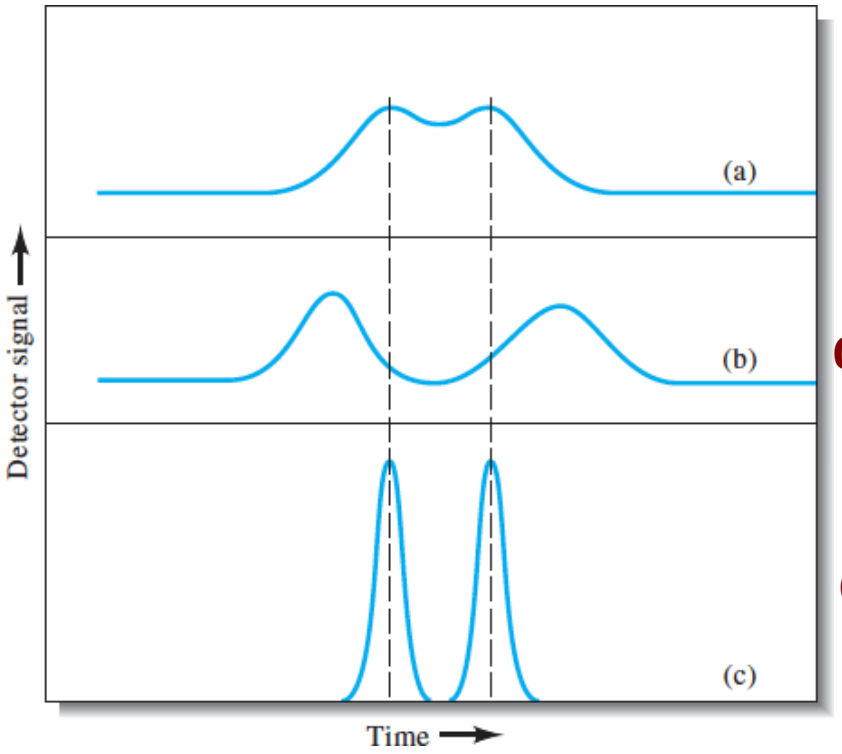
The detector signal at the various stages of elution





Chromatogram

Column performance

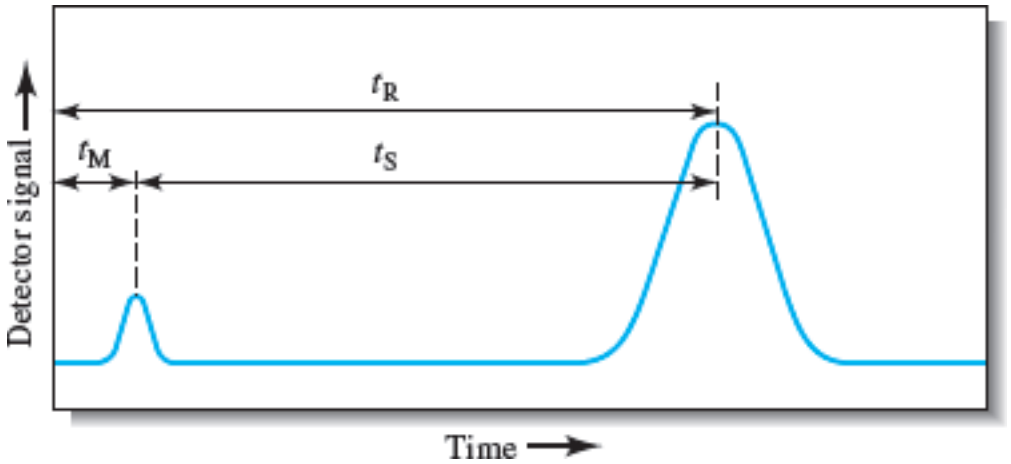


Chromatogram with overlapping peaks

Chromatogram with increased band separation

Chromatogram with decreased band width

Column performance



$t_R = t_S + t_M$

t_R = Retention time

t_M = Void time

t_S = Time the analyte was retained by the stationary phase

$$\bar{v} = \frac{L}{t_R} \qquad u = \frac{L}{t_M}$$

$\bar{v}$ = Average velocity of solute migration

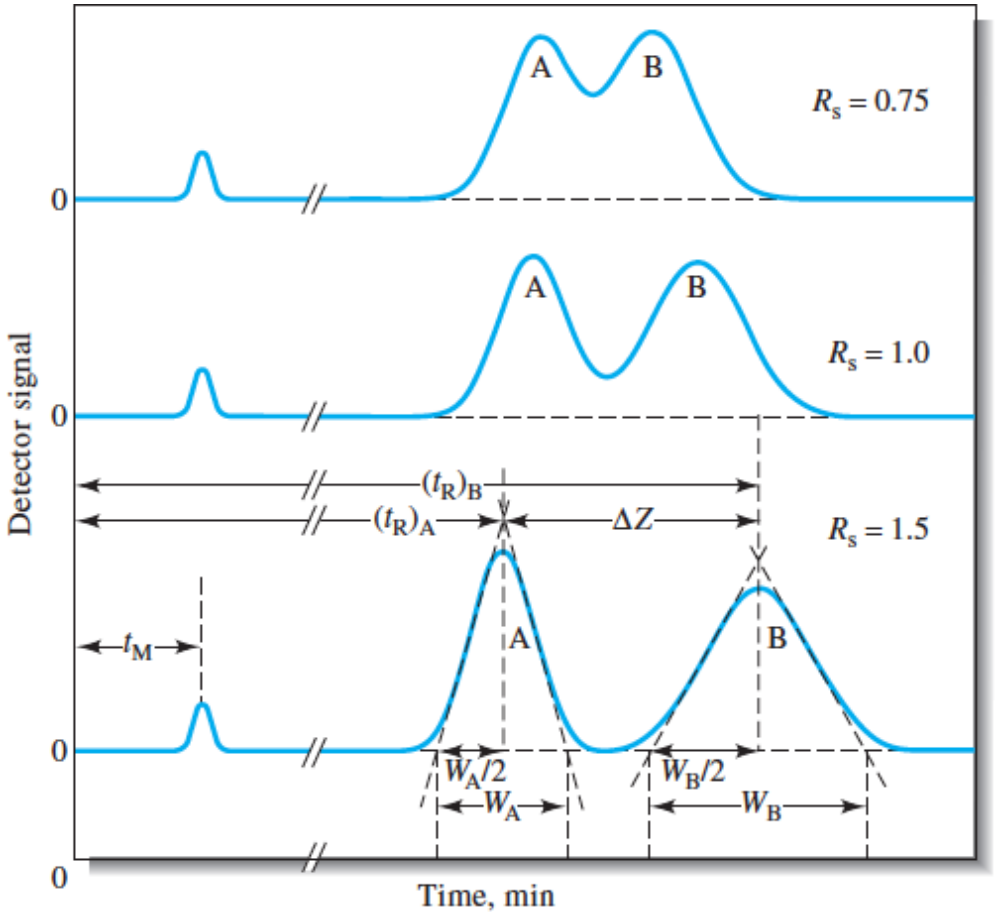
u = Average velocity of mobile phase migration

L = Length of the column packing

Column resolution

The resolution, R_s, of a column tells us how far apart two bands are relative to their widths. The resolution provides a quantitative measure of the ability of the column to separate two analytes.

$$R_s = \frac{\Delta Z}{\frac{W_A}{2} + \frac{W_B}{2}} = \frac{2\Delta Z}{W_A + W_B} = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$



Retention factor

At any given time during the migration through the system, there is a distribution of molecules of each component between the two phases.

If n_s and n_m are the number of molecules in the stationary and mobile phases, respectively, at a given time, then

$k = n_s/n_m$ is called the retention factor

When n_s is much larger than n_m, the migration is very slow and the analyte elutes with high retention.

$$\overline{v} = u \times \frac{\text{no. of moles of solute in mobile phase}}{\text{total no. of moles of solute}}$$

Retention factor

$k = n_s/n_m$

$$\bar{v} = u \times \frac{\text{no. of moles of solute in mobile phase}}{\text{total no. of moles of solute}}$$

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k_A}$$

$$k_A = \frac{t_R - t_M}{t_M} = \frac{t_S}{t_M}$$

- Ideally k should be 1-5.
- k>20 elution time is too long
- k<1 means the solute emerges from the column near the void time

- In gas chromatography, retention factor can be varied by changing the temperature
- In liquid chromatography retention factor can be manipulated to give better separation by changing the composition of the mobile phase.

Selectivity factor (α)

$$\alpha = \frac{K_B}{K_A} = \frac{k_B}{k_A}$$

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

K_B is the distribution constant for more strongly retained species B, and K_A is the The same constant for less strongly held or more rapidly eluted species.

k_B and k_A are the retention factor of solute B and A, respectively

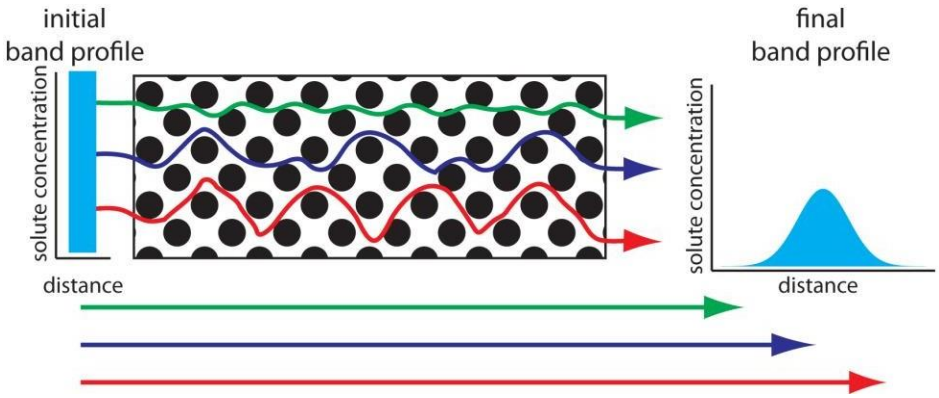
$(t_R)_B$ and $(t_R)_A$ are the retention time of the solute B and A, respectively

t_M is the void time

Band broadening

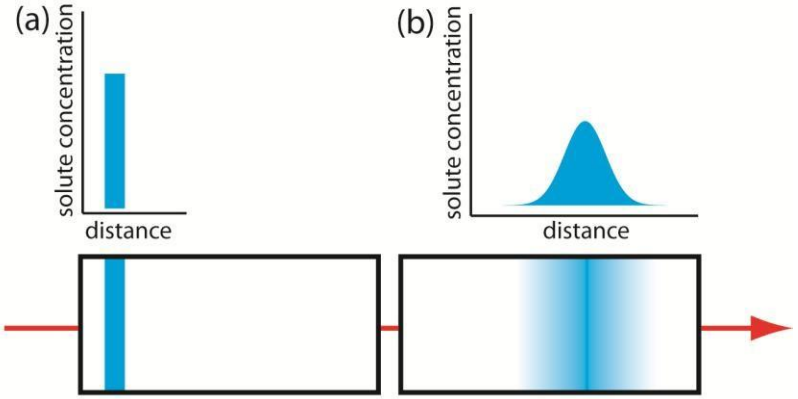
Reasons for band broadening:

- Eddy diffusion
- Longitudinal diffusion
- Resistance to mass transfer



Eddy diffusion

Multiple paths: variation in path length



Longitudinal diffusion

Natural tendency of a compound to diffuse from more to less concentrated region

Resistance to mass transfer meaning:

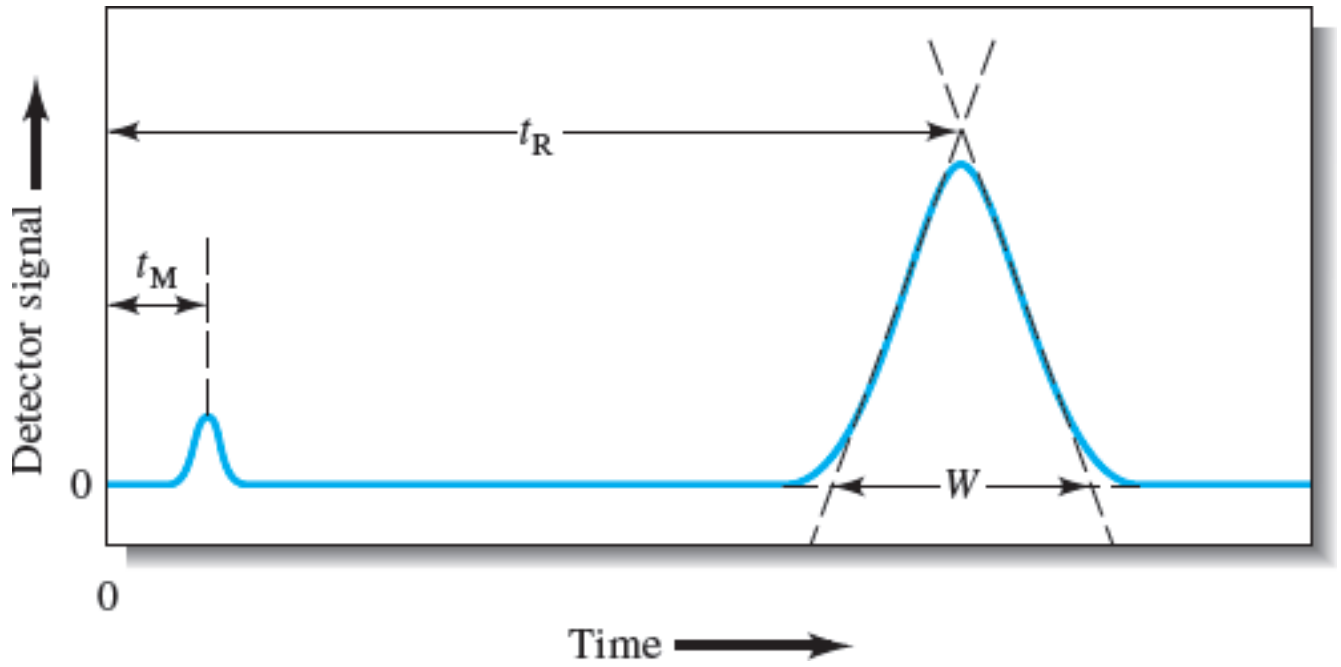
Solute's movement within the mobile phase or within the stationary phase is not fast enough to maintain an equilibrium partitioning of solute between the two phases

Quantification of Band broadening

$$N = \frac{L}{H} \qquad \sigma = \frac{LW}{4t_R}$$

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

N: Number of theoretical plate
H: Plate height
L: Length of the column
σ : Standard deviation
W: Width of the peak at its base



Factors that affect resolution

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B}{1 + k_B}\right)$$

Retention factor: Increase retention factor of a solute -> choose a mobile phase that is Weaker solvent. Solute take more time to elute as it spends more time in the stationary phase.
Also gradient elution can be used.

Selectivity: Composition of the liquid can be changed. For example change in polarity and pH

Column efficiency: Increase the length of the column, decrease H by adjusting the velocity of the mobile phase. Diffusion can be also be reduced by decreasing particle size.

Migration rates and distribution constant

$$\bar{v} = u \times \text{fraction of time solute spends in mobile phase}$$

$$\bar{v} = u \times \frac{\text{no. of moles of solute in mobile phase}}{\text{total no. of moles of solute}}$$

$$\bar{v} = u \times \frac{c_M V_M}{c_M V_M + c_S V_S} = u \times \frac{1}{1 + c_S V_S / c_M V_M}$$

$$\bar{v} = u \times \frac{1}{1 + K_c V_S / V_M}$$

The two volumes can be estimated from the method by which the column is prepared.

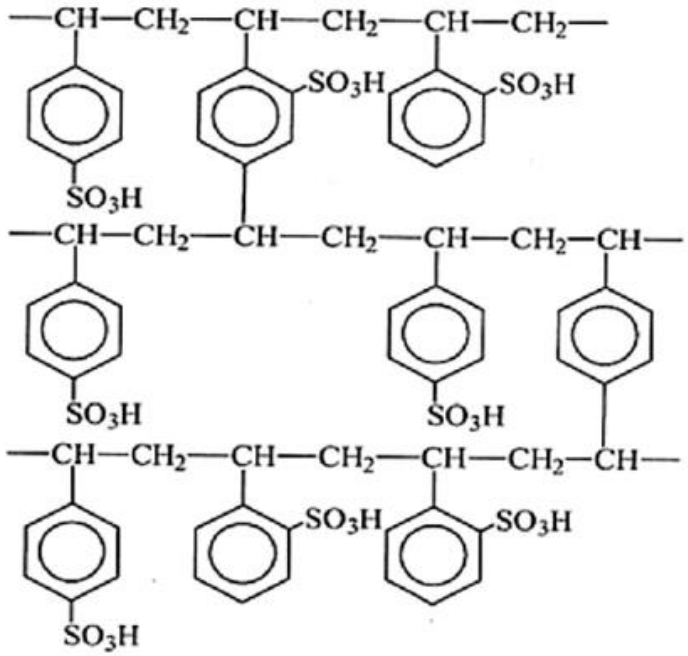
Ion exchange chromatography

It is a technique for separating mixtures of charged compounds such as cations (K^+, Na^+, Ca^{2+}, Cu^{2+}) and anions (Cl^-, Br^-, I^-) or amino acids, proteins that can develop a charge in acidic or basic media.

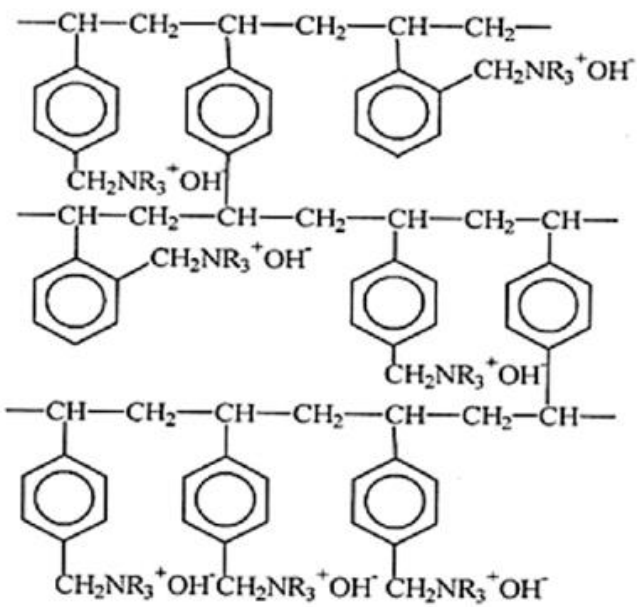
Ion-exchange chromatographic separations are done by using porous resin beads to which are bonded acidic groups such as $–SO_3H$ or –COOH or basic groups such as $–CH_2N^+R_3X^-$ or $–CH_2NR_2$.

Depending on the type of functional group, ion exchange chromatography can be classified as, a) cation-exchange chromatography, b) anion-exchange chromatography

Molecular structure of ion-exchange resin



Sulphonated ST-DVB copolymer (strong acid cation exchange resin)



Quaternary ammoniated ST-DVB copolymer (srong base anion exchange resin). R= CH_3 is known as Type-1 anion resin

Resin use and regeneration

$$x\mathrm{RSO_3^-H^+} + \mathrm{M^{x+}} \rightleftharpoons (\mathrm{RSO_3^-})_x\mathrm{M^{x+}} + x\mathrm{H^+}$$

solid soln solid soln

$$x\mathrm{RN(CH_3)_3^+OH^-} + \mathrm{A^{x-}} \rightleftharpoons [\mathrm{RN(CH_3)_3^+}]_x\mathrm{A^{x-}} + x\mathrm{OH^-}$$

solid soln solid soln

$\mathrm{Resin\text{-}SO_3^-H^+ + Na^+Cl^- = Resin\text{-}SO_3^-Na^+ + H^+Cl^-}$

$\mathrm{Resin\text{-}SO_3^-H^+ + Ca^{2+}\,2Cl^- = (Resin\text{-}SO_3^-)_2Ca^{2+} + 2H^+Cl^-}$

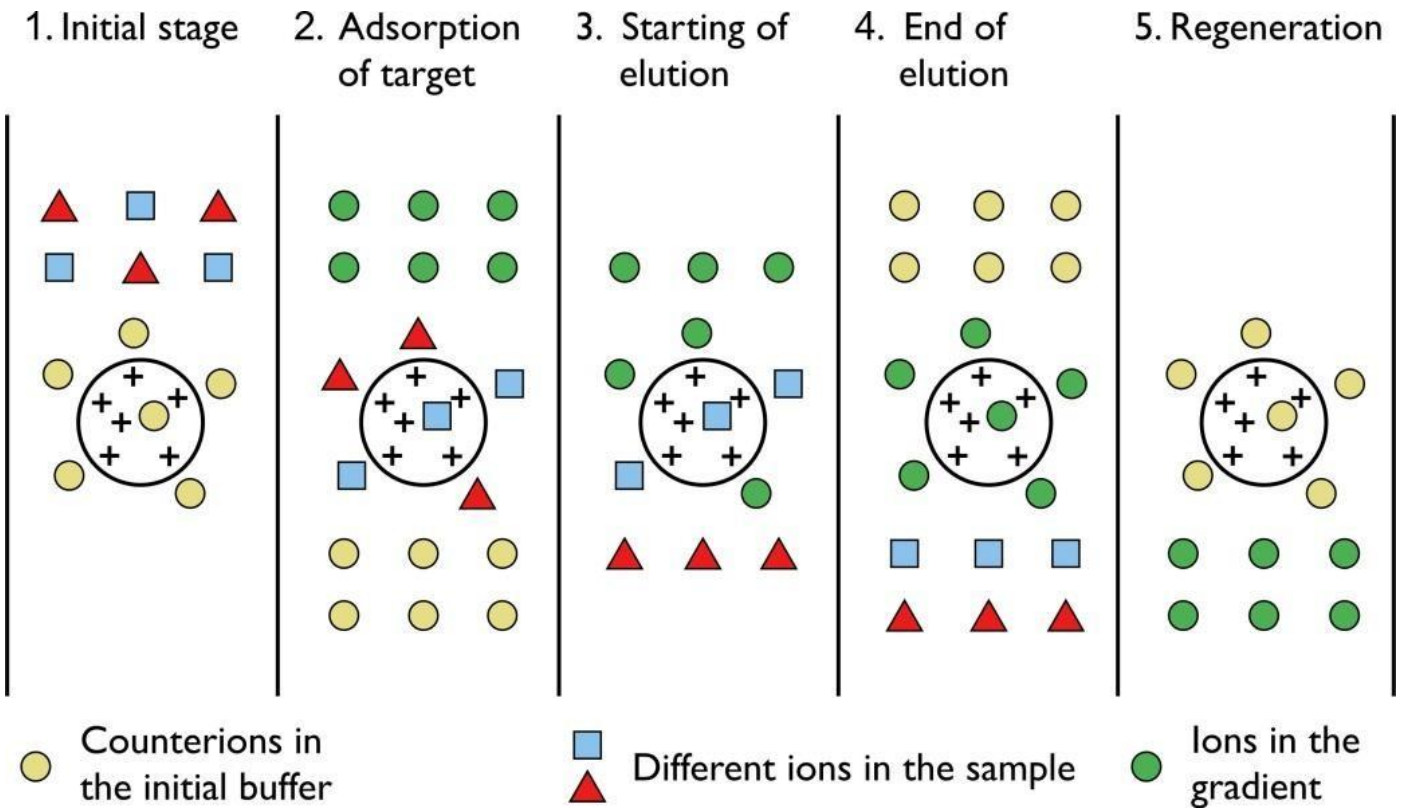
$\mathrm{Resin\text{-}CH_2\text{-}N^+Me_3Cl^- + Na^+OH^- = Resin\text{-}CH_2\text{-}N^+Me_3OH^- + Na^+Cl^-}$

$\mathrm{2\ Resin\text{-}CH_2\text{-}N^+Me_3Cl^- + SO_4^{2-} = (Resin\text{-}CH_2\text{-}N^+Me_3)_2SO_4^{2-} + 2Cl^-}$

The cation and anion exchange resins are reversible and can be regenerated.

$\mathrm{(Resin\text{-}SO_3^-)_2Ca^{2+} +\ strong\ H^+Cl^- = Resin\text{-}SO_3^-H^+ + Ca^{2+}\,2Cl^-}$

Steps of an ion exchange chromatography



Ions with smaller hydrated ionic radius are held more strongly by the resin than the larger hydrated ionic radius. $Ag^{+} > Cs^{+} > Rb^{+} > NH_4^{+} > K^{+} > Na^{+}$

The binding strength, $I^{-} > Br^{-} > Cl^{-} > F^{-}$