CHROMATOGRAPHIC TECHNIQUES

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History: The history of modern chromatography can be traced to the turn of the century when the Russian botanist Mikhail Tswett (1872–1919) used a column packed with a stationary phase of calcium carbonate to separate colored pigments such as from plant extracts. The sample was placed at the top of the column and carried through the stationary phase using a mobile phase of petroleum ether. As the sample moved through the column, the pigments in the plant extract separated into individual colored bands, which accounts for the name he chose for the method (from the Greek *chroma* meaning "color" and *graphein* meaning "to write"). Once the pigments were adequately separated, the calcium carbonate was removed from the column, sectioned, and the pigments recovered by extraction.

►**Stationary phase:** The stationary phase in chromatography is a phase that is fixed in place either in a column or on a planar surface. Generally, the stationary phase is a finely divided solid or liquid which is supported over a solid.

►**Mobile phase:** The mobile phase in chromatography is a phase that moves over or through the stationary phase carrying the analyte with it.

►**General definition of chromatography:** Chromatography is a technique in which the components of a mixture are separated based upon the rates at which they are carried through a stationary phase by a gaseous or liquid mobile phase.

►**Classification of chromatographic techniques:** Chromatographic processes can be classified according to the type of equilibration process involved, which is governed by the type of stationary phase. Various bases of equilibration are: (a) adsorption, (b) partition, (c) ion-exchange, and (d) pore penetration.

(a) Adsorption chromatography: The stationary phase is a solid on which the sample components are adsorbed. The mobile phase may be a liquid (liquid-solid chromatography) or a gas (gas-solid chromatography); the components distribute between the two phases through a combination of sorption and desorption processes. Thin-layer chromatography (TLC) is a special example of sorption chromatography in which the stationary phase is a planar surface, in the form of a solid supported on an inert plate.

(b) Partition chromatography: The stationary phase in partition chromatography is a liquid supported on an inert solid. Again, the mobile phase may be a liquid (liquid-liquid partition chromatography) or a gas (gas-liquid chromatography, GLC). Paper chromatography is a type of partition chromatography in which the stationary phase is a layer of water adsorbed on a sheet of paper.

(c) *Ion exchange and size exclusion chromatography*: Ion exchange chromatography uses an ion exchange resin as the stationary phase. The mechanism of separation is based on ion exchange equilibria. In size exclusion chromatography, solvated molecules are separated according to their size by their ability to penetrate a sieve-like structure (stationary phase).

►**Principle of chromatography:** While the mechanism of retention for various types of chromatography differs, they are all based on the establishment of an equilibrium between a stationary phase and a mobile phase. A small volume of sample is placed at the top of the column, which is filled with the chromatographic particles (stationary phase) and solvent. Mobile phase solvent is added to the column and is allowed to emerge slowly from the bottom of the column. The individual components interact with the stationary phase to different degrees:

$$A_m \leftrightarrows A_s$$

The distribution equilibrium is described by the distribution constant:

$D = [A_s]/[A_m]$where $[A_s]$ is the concentration of component A on or in the stationary phase at equilibrium and $[A_m]$ its concentration in the mobile phase. The equilibrium constant is governed by the temperature, the type of compound, and the stationary and mobile phases.

It is also called the distribution coefficient or distribution ratio or partition coefficient in partition chromatography. Solutes with a large D value will be retained more strongly by the stationary phase than those with a small value. The result is that the latter will move along the column (be eluted) more rapidly.

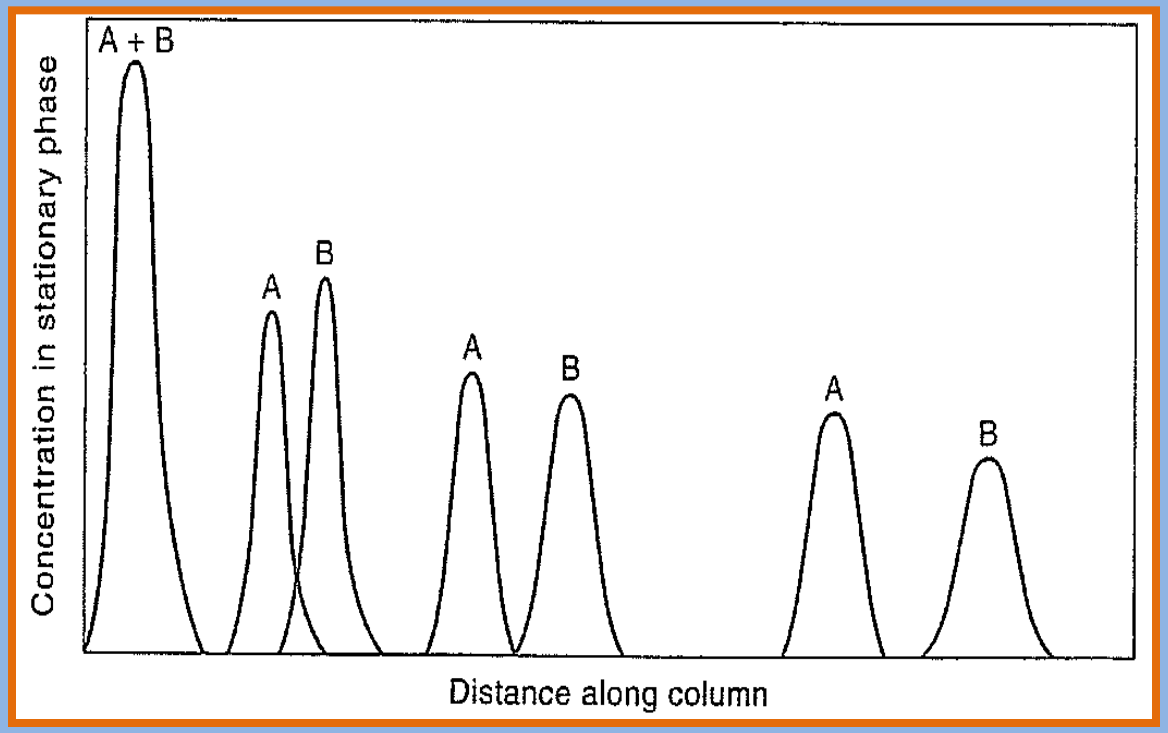

Figure: Distribution of two substances A and B, along a chromatographic column.

Steps for a chromatographic separation:

(i) Adsorption or retention of a substance or substances on the stationary phase.

(ii) Separation of the adsorbed substances by the mobile phase.

(iii) Recovery of the separated substances by a continuous flow of the mobile phase.

(iv) Qualitative and quantitave analysis of the eluted substances.

Elution chromatography:

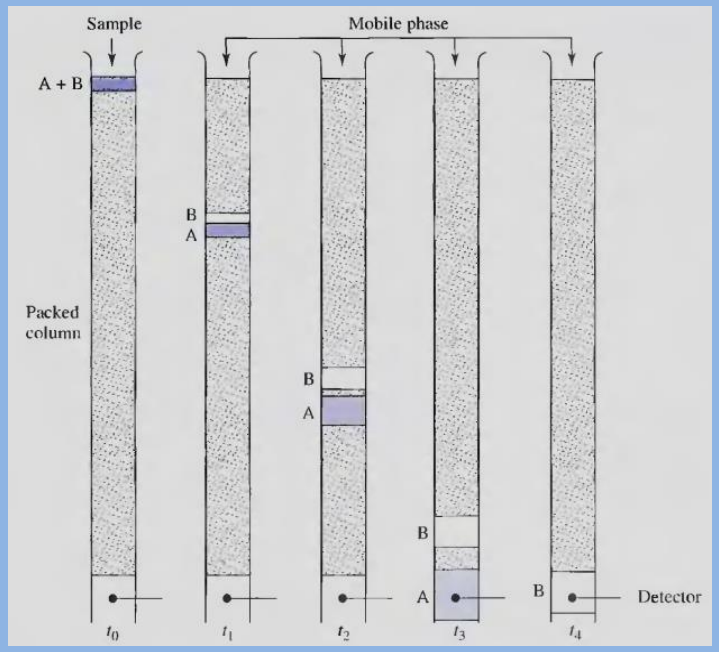

Figure: Separation of mixture of components A and B by elution chromatography

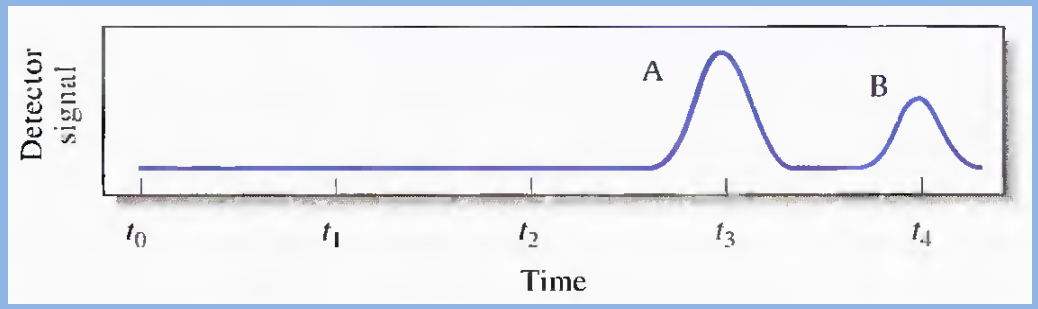

Figure: Detector signal at various stages of elution

►**Dead time and Retention time:**

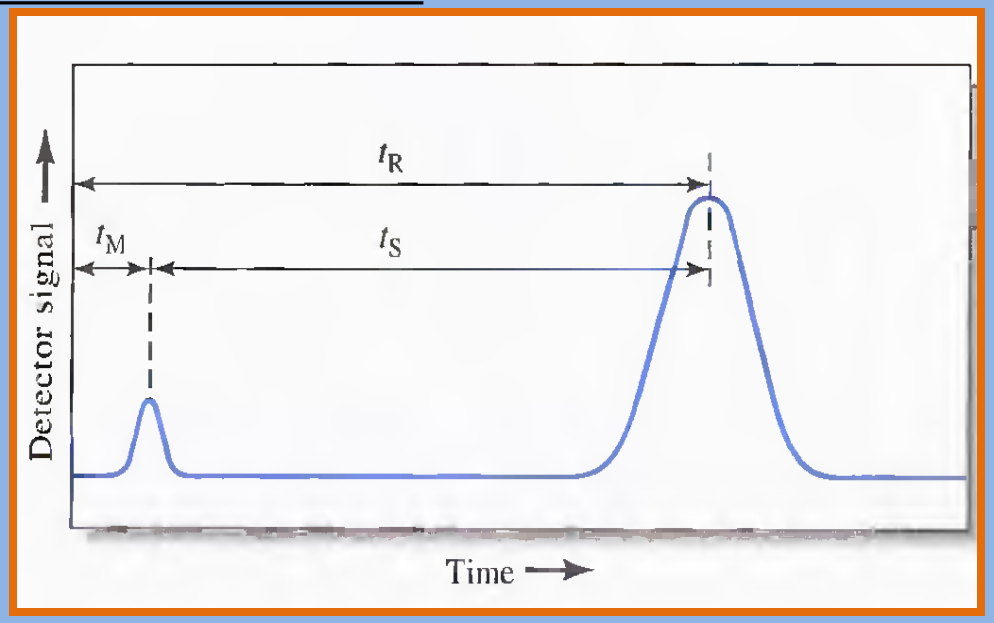

Figure: Dead time and retention time

►**Column selectivity: Relative migration rates:** The relative selectivity of a chromatographic column for a pair of solutes A and B is given by the *selectivity factor* α, which is defined as:

$$\alpha = K_B/K_A$$

where K_B is the partition ratio for the more strongly retained species B and K_A is the constant for the less strongly held or more rapidly eluted species A. According to this definition, α is always greater than unity. The following equation gives an expression that permits the determination of α from an experimental chromatogram:

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

►Peak profiles and band broadening:

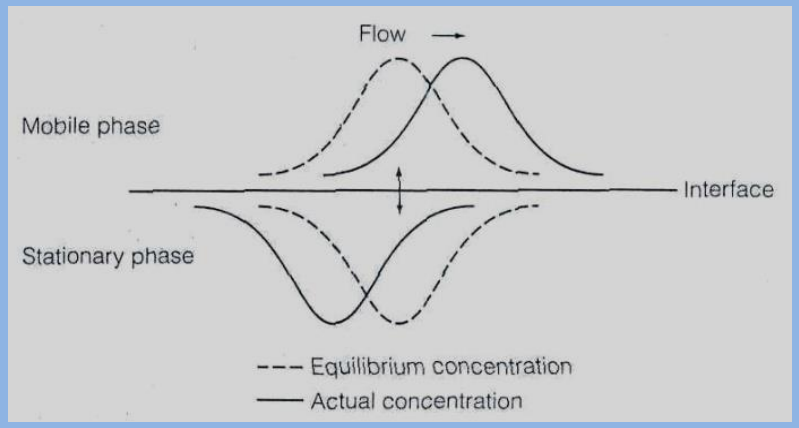

Figure: The symmetrical nature and broadening of a chromatographic peak

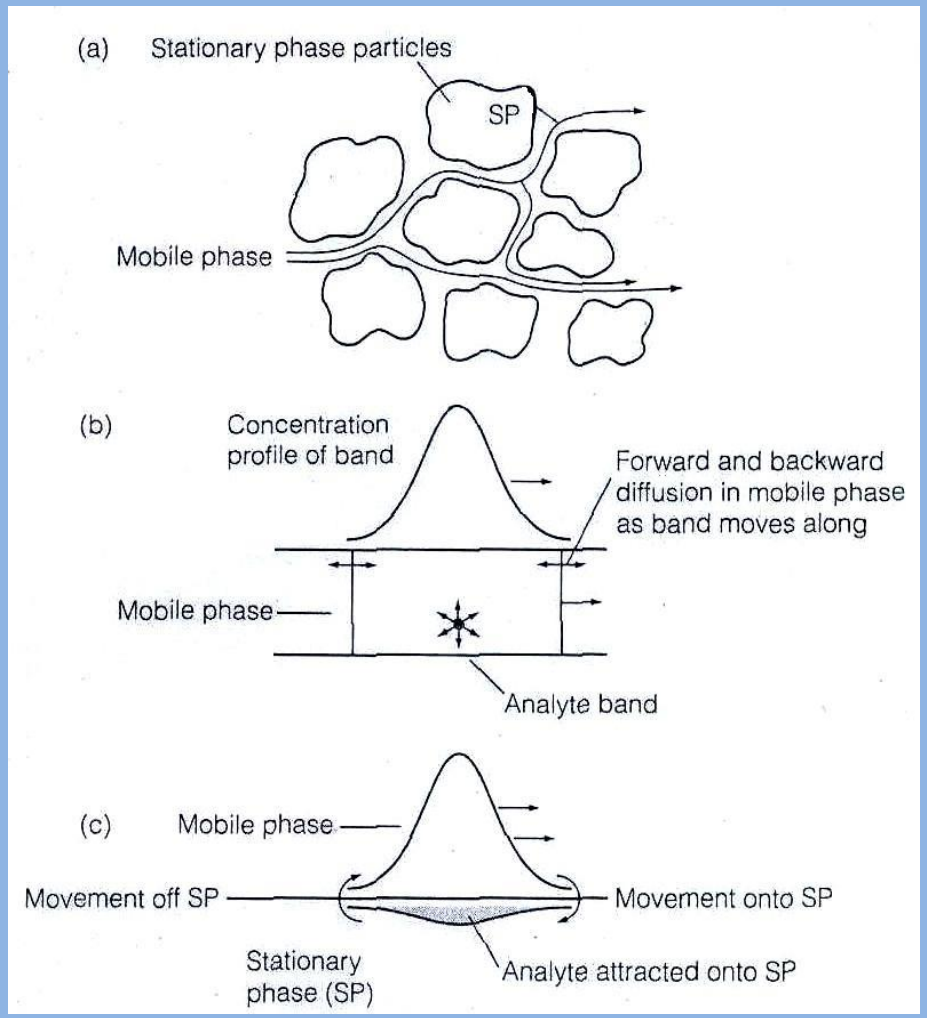

Figure: Illustration of the three principal causes of band broadening: (a) multiple-path effect; (b) longitudinal diffusion effect; (c) mass-transfer (non-equilibrium effect)

►**<u>Efficiency and resolution</u>:** Two means of assessing the quality of a chromatographic separation are to measure the extent of band broadening of individual solute peaks (efficiency) and the degree of separation of adjacent peaks (resolution).

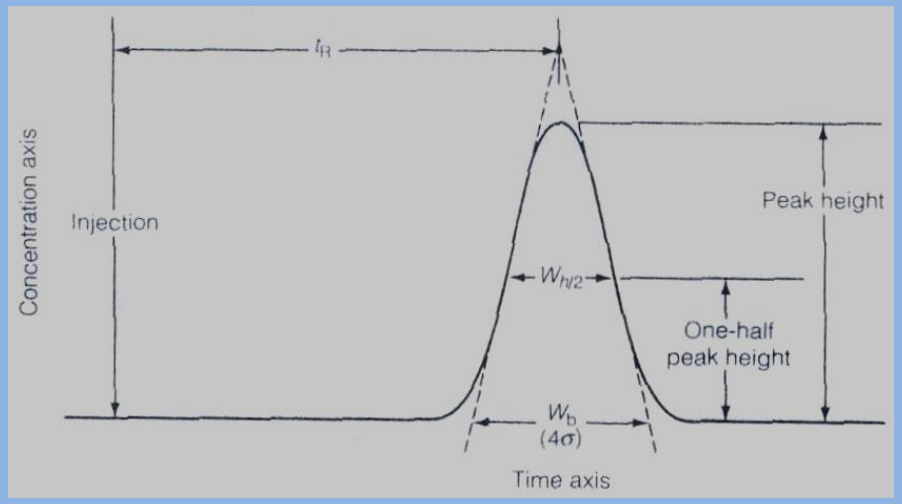

Figure: Measurement of chromatographic efficiency from a Gaussian peak

$N = 16(t_R/W_b)^2$
and $N = 5.54(t_R/W_{h/2})^2$
N = Plate number

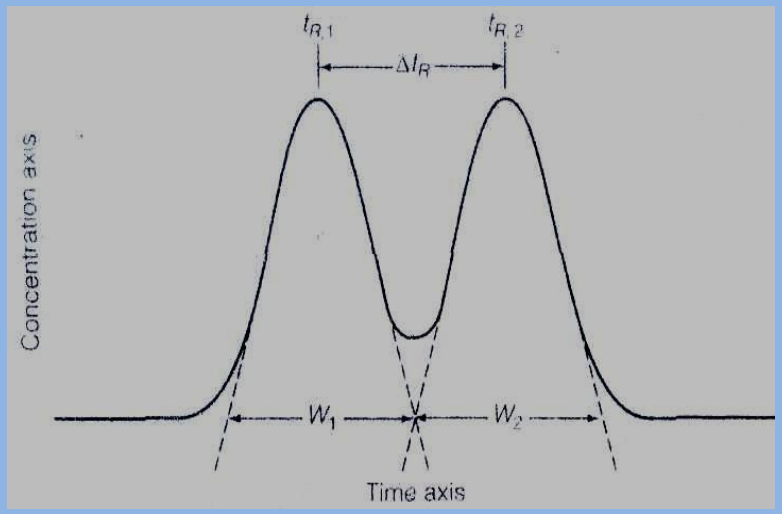

Figure: Measurement of the resolution of two adjacent Gaussian peaks

►**Thin-layer chromatography:**

♥*Definition*: Thin layer chromatography is a technique where the components of mixtures separated by differential migration through a planar bed of a stationary phase, the mobile phase flowing by virtue of capillary forces. The solutes are detected *in situ* on the surface of the thin-layer plate by visualizing reagents after the chromatography has been completed.

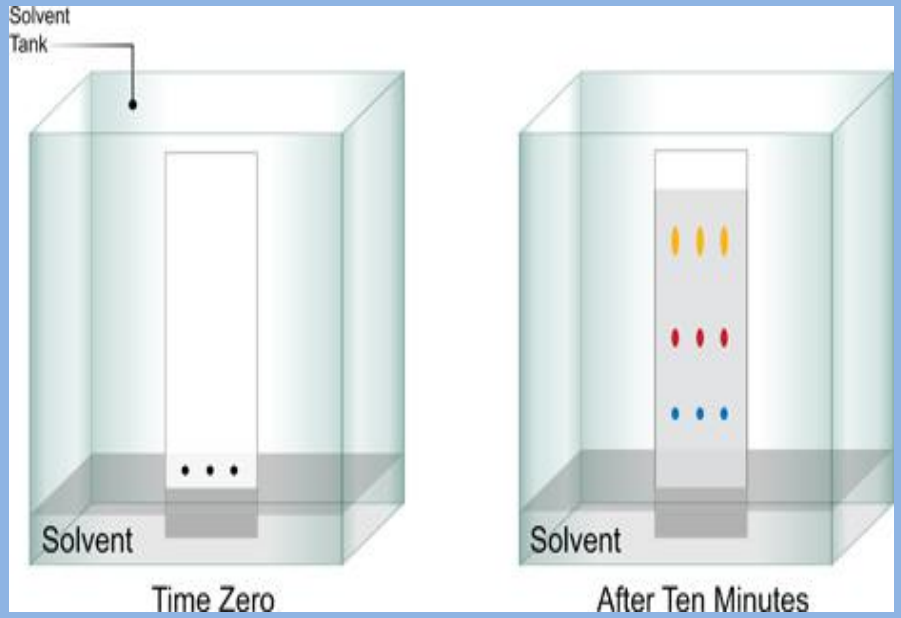

Figure: TLC plates during and after development and visualization

Each solute is characterized by the distance migrated relative to the solvent front, i.e. its R_f value, which will lie between 0 and 1, and unknowns are identified by comparisons with standards run simultaneously. Practical values of R_f are evaluated from the ratio:
R_f = (Distance moved by solute / Distance moved by the solvent front)

►Paper chromatography:

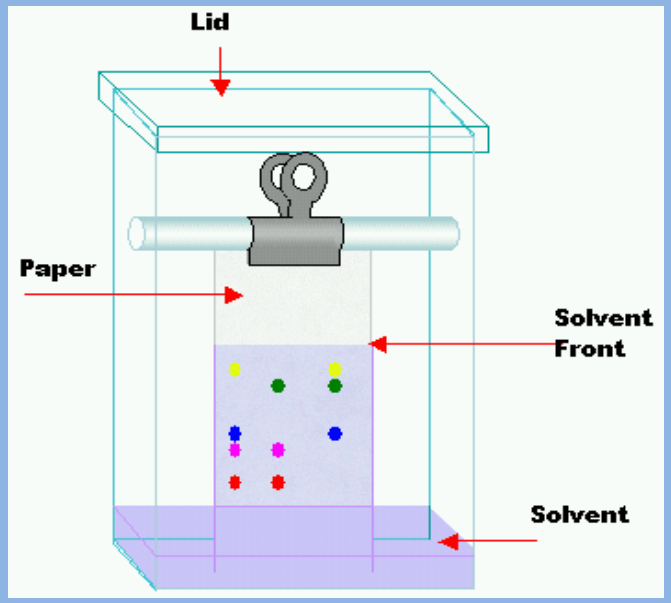

Figure: Paper chromatography setup

►**Ion exchange chromatography:**

While most of other types of chromatography are used principally for separations of complex organic substances, ion exchange chromatography is particularly well suited for the separation of inorganic ions, both cations and anions, because the separation is based on the exchange of ions in the stationary phase. It has also proved to be extremely useful for the separation of amino acids.

►**Cation exchange resins**

$$nRzSO_3^-H^+ + M^{n+} \leftrightarrows (RzSO_3)_nM + nH^+$$
$$nRzCO_2^-H^+ + M^{n+} \leftrightarrows (RzCO_2)_nM + nH^+$$
(Res) (sol^n) (Res) (sol^n)

where Rz represents the resin.

►**Anion exchange resins**

$$nRzNR_3^+OH^- + A^{n-} \leftrightarrows (RzNR_3)_nA + nOH^-$$
$$nRzNH_3^+OH^- + A^{n-} \leftrightarrows (RzNH_3)_nA + nOH^-$$
(Res) (sol^n) (Res) (sol^n)

where R represents organic groups, usually methyl.

►**Commonly used ion exchangers:**
Strong-acid cation exchanger: Dowex-50
Weak-acid cation exchanger: Amberlite IRC-50
Strong-base anion exchanger: Dowex-1
Weak-base anion exchanger: Dowex-3

►**Distribution coefficient:** The rate at which two constituents separate in the column is determined by the ratio of the two corresponding distribution coefficients, where the distribution coefficient is given by the equation:

$$K_d = \frac{\text{amount of solute on resin}}{\text{weight of resin (gm)}} \div \frac{\text{amount of solute in solution}}{\text{volume of solution (ml)}}$$

► **Determination of the capacity of an ion exchange resin:**

Cation exchange resin: An aqueous solution of Na_2SO_4 is allowed at a specific rate to pass through the column which is originally in the H^+ ion form and the effluent is collected in a conical flask. The effluent is titrated with standard NaOH solution using phenolphthalein as indicator. The reaction which occurs may be written as:

$$R^-H^+ + Na^+ \leftrightarrows R^-Na^+ + H^+$$

The capacity of the resin in milliequivalent per gram is given by $a.v/W$, where a is the normality of the NaOH solution, v is the volume in ml, and W is the weight (gm) of the resin.

Anion exchange resin: An aqueous solution of $NaNO_3$ is allowed at a specific rate to pass through the column which is originally in the Cl^- ion form and the effluent is collected in a conical flask. The effluent is titrated with standard $AgNO_3$ solution using K_2CrO_4 as indicator. The reaction may be represented as:

$$R^+Cl^- + NO_3^- \leftrightarrows R^+NO_3^- + Cl^-$$

The capacity of the resin expressed as milliequivalent per gram is given by $b.v/W$, where v ml of b normal $AgNO_3$ are required by W gm of the resin.