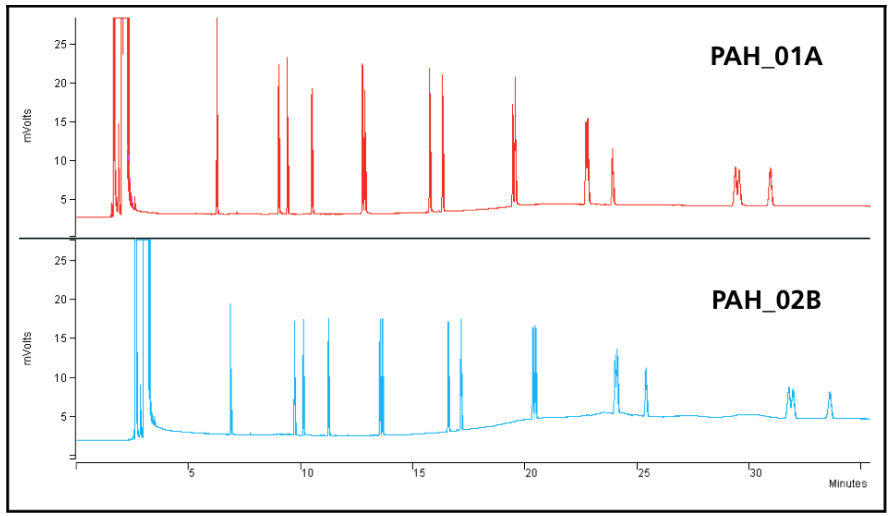
Introduction to Analytical Separation

Chromatography

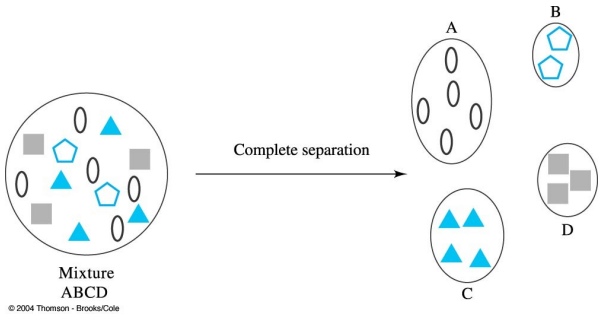


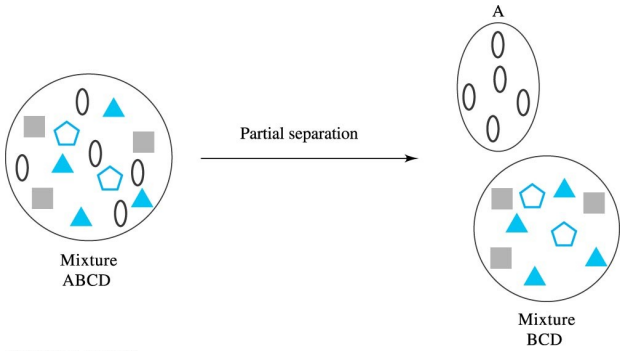
Chromatography is a broad range of physical methods used to separate and or to analyze complex mixtures. The components to be separated are distributed between two phases: a stationary phase bed and a mobile phase which percolates through the stationary bed.

Separation Techniques

In any analytical technique, it is important to remove foreign species, or interferent, that can attenuate the signal from the analyte or produce a signal that is indistinguishable from that of the analyte.

Separation principles can involve (a) mixture that is separated to individual components or (b) mixture in which the species of interest is separated from the other components.





Experimental Techniques

Separation Methods

Method	Basis of Method
Mechanical phase separation	
Precipitation and filtration	Difference in solubility of compounds formed
Distillation	Difference in volatility of compounds
Extraction	Difference in solubility in two immiscible liquids
Ion exchange	Difference in interaction of reactants with ion-exchange resin
Chromatography	Difference in rate of movement of a solute through a stationary phase
Electrophoresis	Difference in migration rate of charged species in an electric field
Field-flow fractionation	Difference in interaction with a field or gradient applied perpendicular to transport direction

Separation by Selective Precipitation

Analyte can be separated by selective separation using a number of precipitating agents such as (1) acidity, (2) sulfides, (3) inorganic, (4) organics, (5) electrolytic, (6) salt-induced.

- Acidity -Controlled acidity can be used to separate certain species via precipitation Table 30.2
- Sulfides - Most cations form sparingly soluble sulfides (except G1,G2) that will precipitate. T 30.3

Chemical Techniques

Separations Based on Control of Acidity

Reagent	Species Forming Precipitates	Species Not Precipitated
Hot concd HNO_3	Oxides of W(VI), Ta(V), Nb(V), Si(IV), Sn(IV), Sb(V)	Most other metal ions
NH_3/NH_4Cl buffer	Fe(III), Cr(III), Al(III)	Alkali and alkaline earths, Mn(II), Cu(II), Zn(II), Ni(II), Co(II)
$HOAc/NH_4OAc$ buffer	Fe(III), Cr(III), Al(III)	Cd(II), Co(II), Cu(II), Fe(II) Mg(II), Sn(II), Zn(II)
$NaOH/Na_2O_2$	Fe(III), most +2 ions, rare earths	Zn(II), Al(III), Cr(VI), V(V), U(VI)

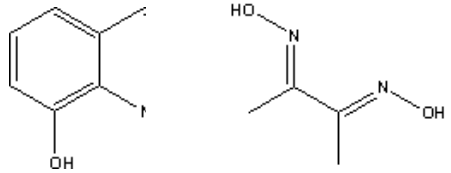
Chemical Techniques

Precipitation of Sulfides

Elements	Conditions of Precipitation*	Conditions for No Precipitation*
Hg(II), Cu(II), Ag(I)	1, 2, 3, 4	
As(V), As(III), Sb(V), Sb(III)	1, 2, 3	4
Bi(III), Cd(II), Pb(II), Sn(II)	2, 3, 4	1
Sn(IV)	2, 3	1, 4
Zn(II), Co(II), Ni(II)	3, 4	1, 2
Fe(II), Mn(II)	4	1, 2, 3

*1 = 3 M HCl; 2 = 0.3 M HCl; 3 = buffered to pH 6 with acetate; 4 = buffered to pH 9 with $NH_3/(NH_4)_2S$.

- Inorganics - Although sulfides and hydroxides salts are ideal because certain cations can be selective precipitation, phosphates, carbonates, oxalates can also be used but are less selective.
- Organics -Organics such as dimethyglyoxime, 8-hydroxyquinoline can be used as precipitation reagents because range of solubility of the reaction products.

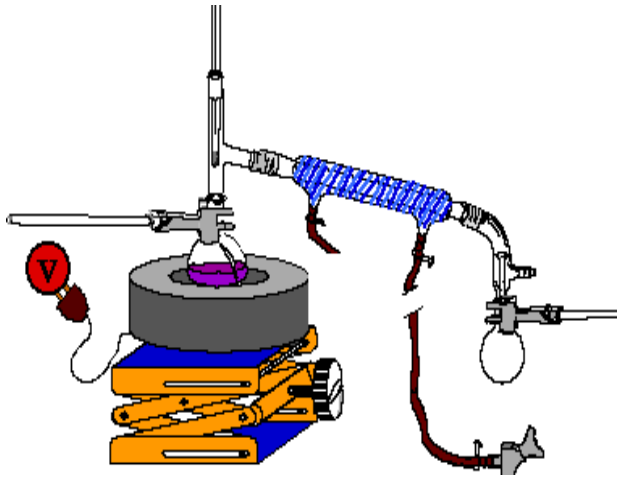


- Electrolytic - Electrochem potential can be used to change oxidation state of species to cause precipitation.
- Salt-Induced- Proteins can be separated by adding high concentrations of salts. Salting out the protein results from Debye-Huckel theory which suggest that a high salt concentration the electrostatic attraction decreases causing the protein to precipitate.

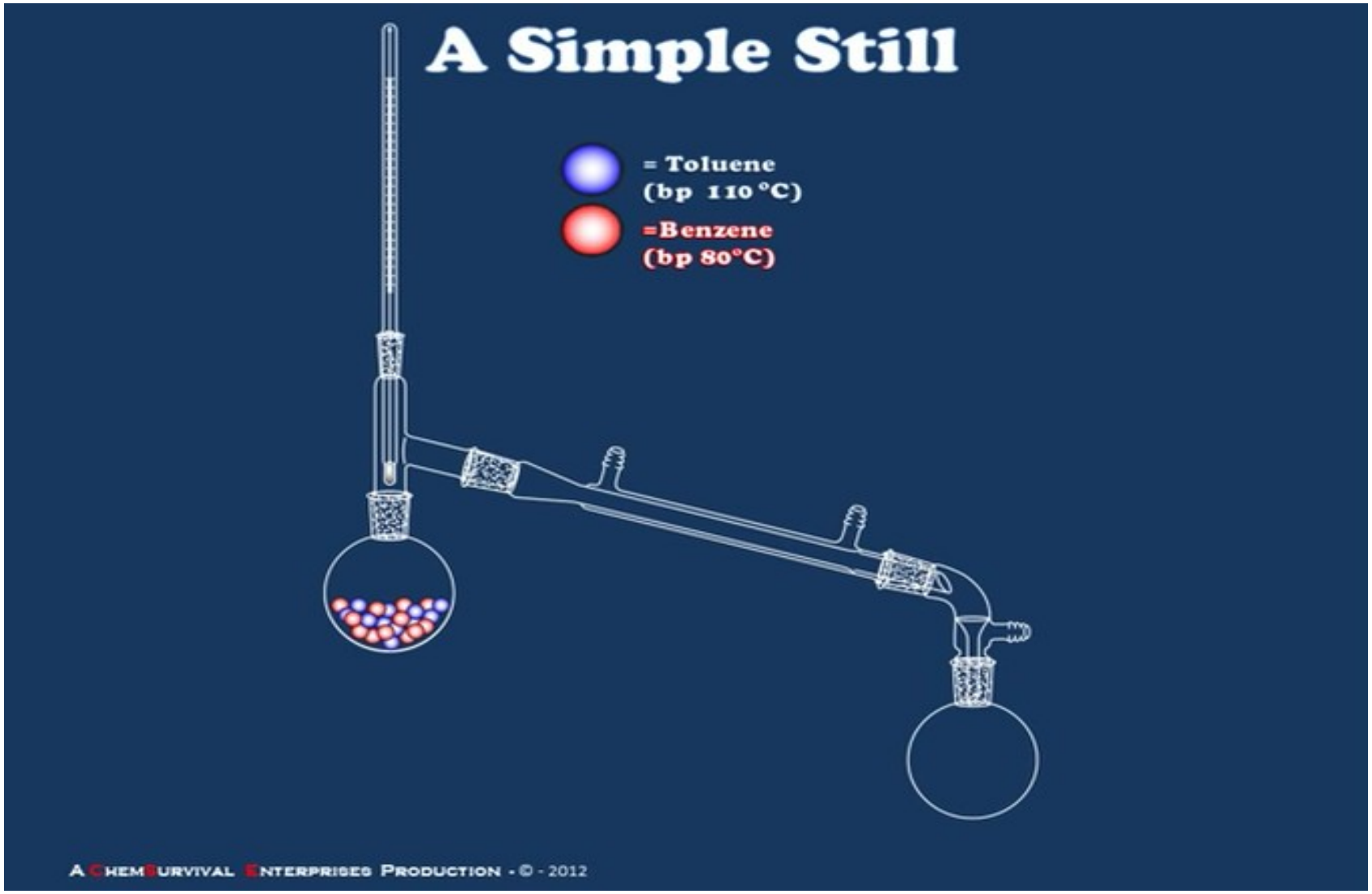
Separation by Distillation

Distillation is commonly used in organic chemistry to separate volatile analytes. Although the term is most commonly applied to liquids, the reverse process can be used to separate gases by liquefying components using changes in temperature and/or pressure. Distillation is based on the fact that the vapor of a boiling mixture will be richer in the components that have lower boiling points. As the solution to be purified is heated, its vapors rise to the fractionating column. As it rises, it cools, condensing on the condenser walls and the surfaces of the packing material.

Here, the condensate continues to be heated by the rising hot vapors; it vaporizes once more. However, the composition of the fresh vapors are determined once again by Raoult's law. Each vaporization-condensation cycle (called a theoretical plate) will yield a purer solution of the more volatile component. More theoretical plates lead to better separations. The fractionating column improves separation by providing a larger surface area for the vapor and condensate to come into contact. This helps it remain at equilibrium for as long as possible.



Separation by Distillation



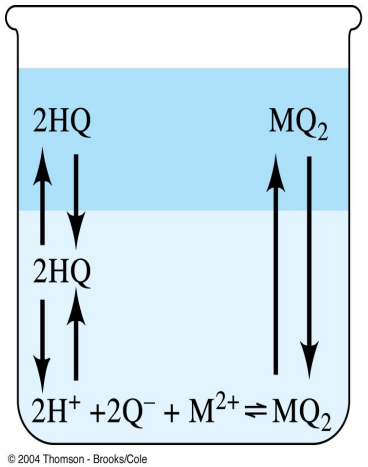
Separation by Extraction

Extraction is used to separate immiscible liquids.

The principle of extraction is based on the distribution law. $A_{(aq)} \leftrightarrow A_{(org)}$

This equilibrium can be written as $K = [A]_{org}/[A]_{aq}$ or by using activities, $K = [\alpha_A]_{org}/[\alpha_A]_{aq}$ in which K is called the **distribution constant**. The distribution constant can be used to calculate the concentration of analyte remaining in a solution after a certain number of extractions. The concentration of A remaining in an aqueous solution after i number of extractions with organic solvent ($[A]_i$) is given by $([A]_i = (V_{aq} / V_{org}K + V_{aq})^i [A]_o$

The graph shows that efficiency of multiple extractions falls off rapidly as a total fixed volume is subdivided into smaller and smaller portions.



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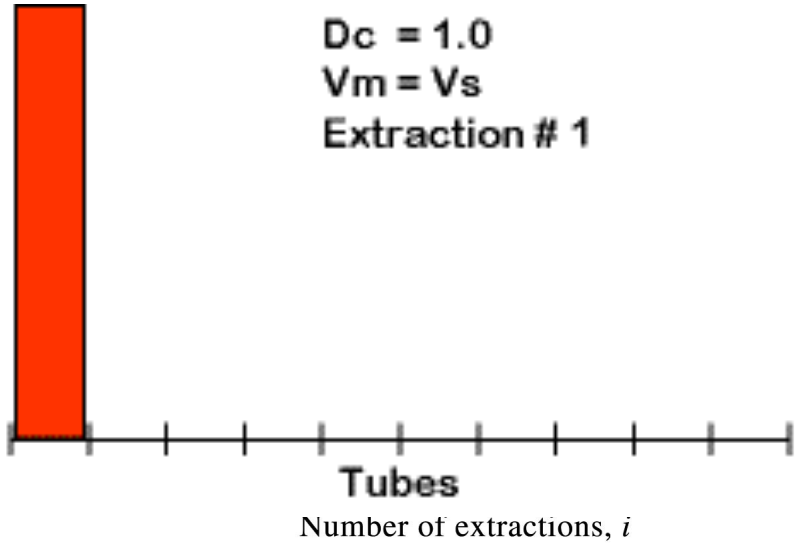
$S_{(in\ phase\ 1)}$! $S_{(in\ phase\ 2)}$

$$K = \frac{A_{S_2}}{A_{S_1}} = \frac{[S]_2}{[S]_1}$$



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Shown is equilibria in the extraction of an aqueous cation M^{2+} into an immiscible organic solvent containing 8-hydroxyquinoline.

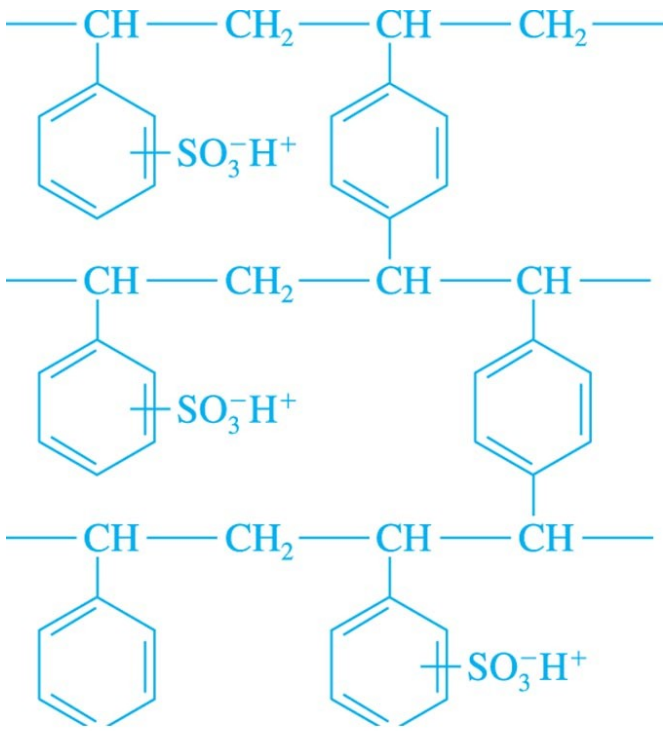
Separation by Ion Exchange

Ion exchange is a process whereby solute ions in the mobile phase can exchange with counter-ions carrying the same charge and associated with oppositely charged groups chemically bound to the stationary phase. The stationary phase is a permeable polymeric solid, such as an insoluble organic resin or a chemically modified silica, containing fixed charge groups and mobile counter ions. Both cationic and anionic ion-exchangers are available, the exchange process being represented by the following equations-

Cation-exchange: $nR^-H^+ + X^{n+} = (R^-)_nX^{n+} + nH^+$

Anion-exchange: $nR^+Cl^- + Y^{n-} = (R^+)_nY^{n-} + nCl^-$

where R represents the polymeric resin or silica, and X^{n+} and Y^{n-} are solute cations and anions respectively or valency n.

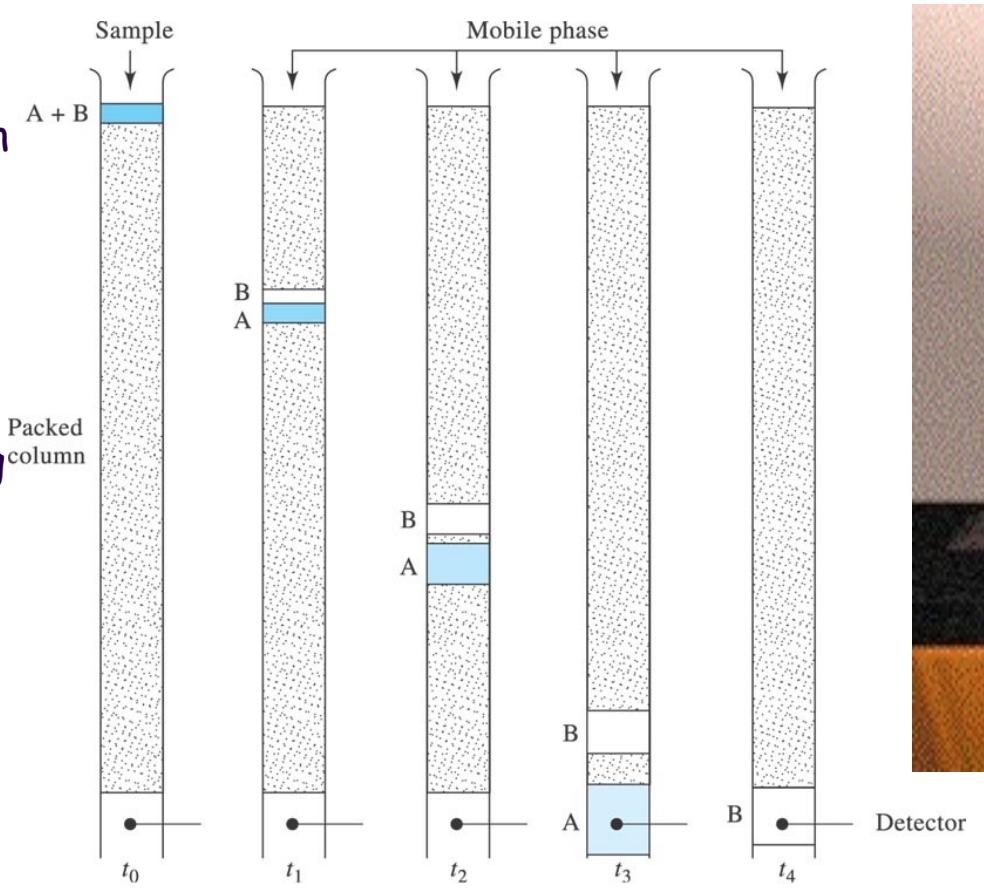


Structure of a cross-linked polystyrene ion-exchange resin. Similar resins are used in which the $-SO_3^-H^+$ group is replaced by $--COO^-H^+$, $-NH_3^+OH^-$, and $-N(CH_3)_3^+OH^-$ groups.

Chromatographic Separations

Chromatography is a process in which compounds are separated from one another by passing a mixture through a column that retains some compounds longer than others.

Separation by chromatography. Solute B has a greater affinity than solute A for the stationary phase, so B remains on the column longer than A. The term chromatography is derived from experiments in 1903 by M. Tswett, who separated plant pigments with a column containing solid $CaCO_3$ particles (the stationary phase) washed by hydrocarbon solvent (the mobile phase). The separation of colored bands led to the name chromatography, from the Greek word chromatos, meaning color.



Chromatographic Parts

The mobile phase (solvent moving through the column) in chromatography is either a liquid or a gas. The stationary phase (the substance that stays, fixed inside the column) is either a solid or a liquid that is usually covalently bonded to solid particles or to the inside wall of a hollow capillary column. Partitioning of solutes between the mobile and stationary phases gives rise to separation. In gas chromatography, the mobile phase is a gas; and in liquid chromatography, the mobile phase is a liquid. Fluids entering the column is called the eluent. Fluid exiting the column is called eluate. The process of passing liquid or gas through a chromatography column is called elution.

The Elution process

Eluent in

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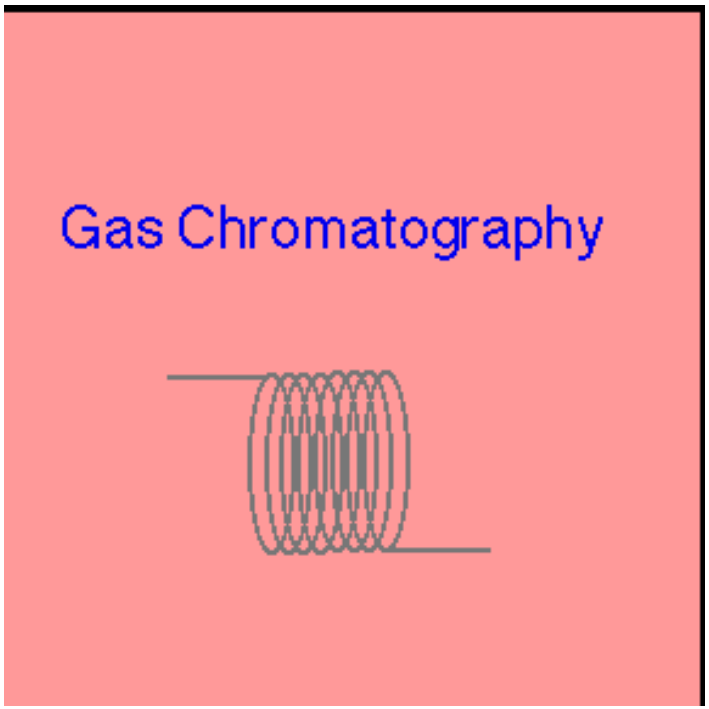
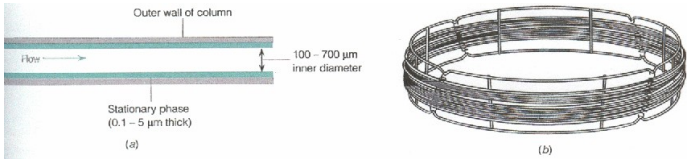
Eluate out





Chromatography Types

Chromatography classification-

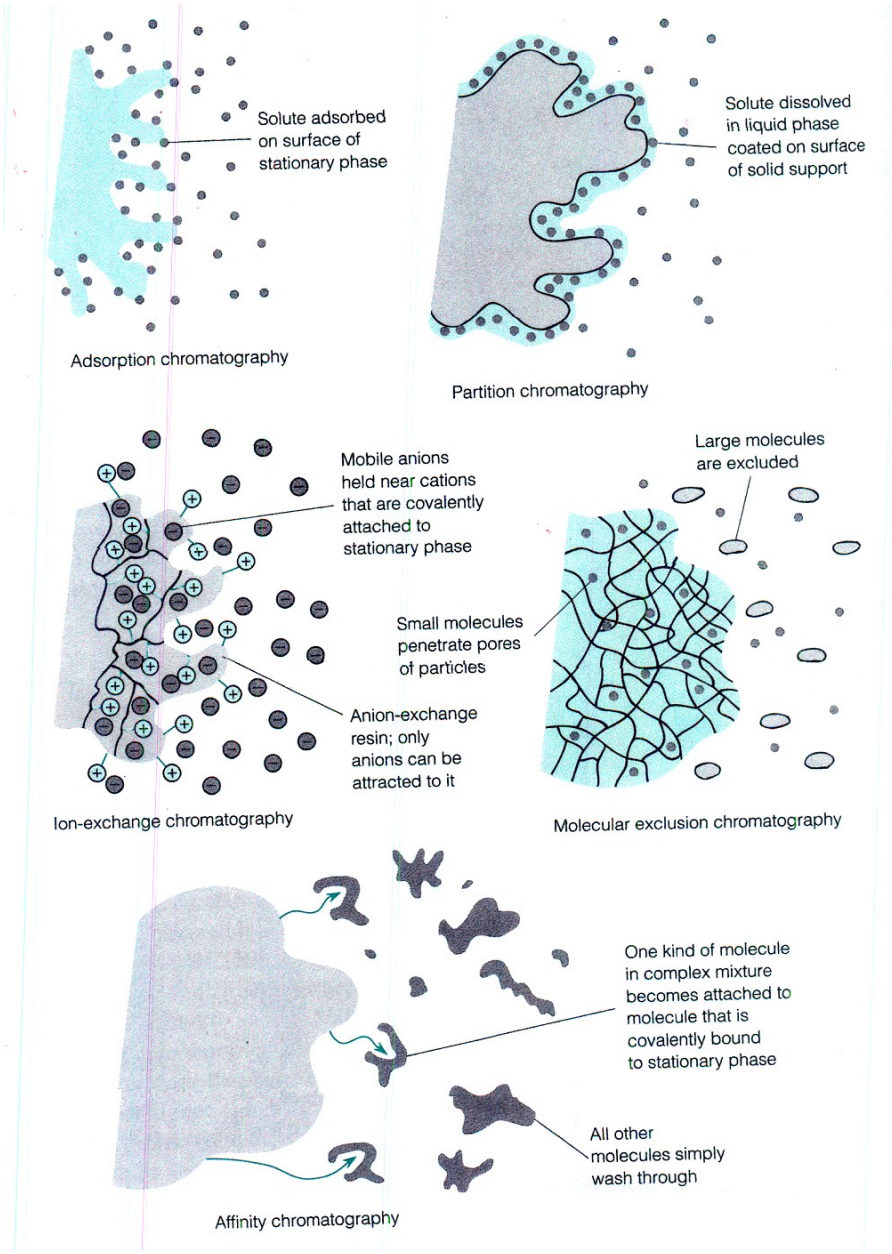
·**Adsorption** - solid stationary phase and a liquid or gaseous mobile phase with solute adsorbed on the surface of the solid particles.

·**Partition** - thin liquid stationary phase coated on the surface of a solid support. Solute equilibrates between the stationary liquid and mobile phase.

· **Ion-exchange** - ionic groups such as $-SO_3^-$ or $-N(CH_3)_3^+$ covalently attached to the stationary solid phase, which is usually a resin. Solute ions are attracted to the stationary phase by electrostatic forces. The mobile phase is a liquid.

·**Molecular exclusion** - (gel filtration, gel permeation) separates molecules by size with large molecules passing through most quickly. There are no attraction between stationary phase and solute. Small molecules are trap in the pores of the stationary phase and large molecules are able to pass through faster.

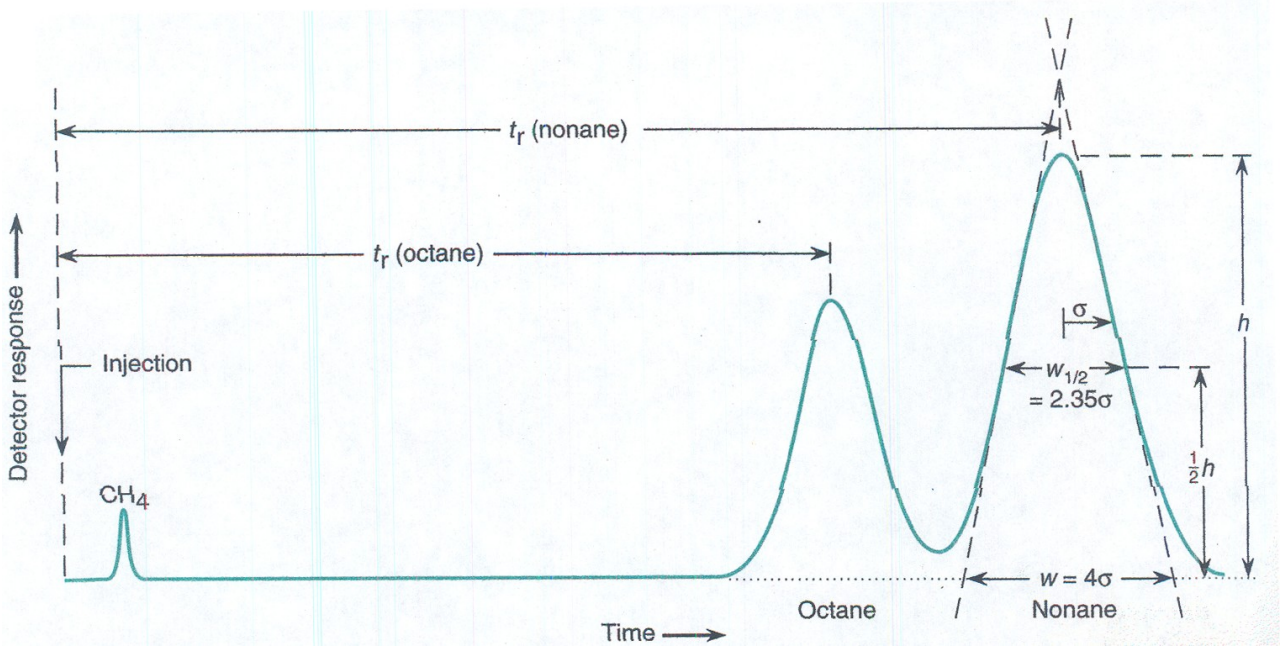
·**Affinity** - is the most selective by employing specific interaction between one kind of solute molecule and a second molecule that is covalently attached (immobilized) to the stationary phase.



Chromatograms

A chromatogram shows the detector response as a function of time (or elution volume) in a chromatography run. Each peak corresponds to a different substance eluted from the column. The retention time, t_r, is the time needed after injection for an individual solute to reach the detector.

Schematic gas chromatograms, showing measurement of retention time (t) with width at half-height ($w_{1/2}$). The width at the base (w) is found by drawing tangents to the deepest parts of the Gaussian curve and extrapolating down to the baseline. The standard deviation of the Gaussian curve is σ. In gas chromatography, a small volume of CH_4 injected with the 0.1 to .2L sample is usually the first component to be eluted.



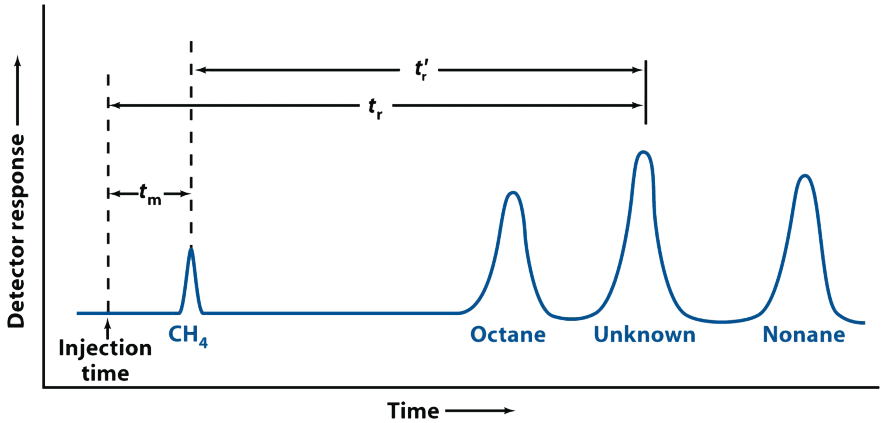
Chromatograms Retention times

Retention time, t_r, is the time needed after injection for component to reach the detector. The retention volume, V_r, is the volume of this component.

The **adjusted retention time**, t'_r, is additional time the solute component travels to detector after the un retained solvent.

$$t'_r = t_r - t_m$$

where t_m is the retention time for the un-retained solvent.



Analyzing two components, 1 & 2, the **relative retention, α (or separation factor)** is the ratios of the adjusted retention times. $\alpha = t'_{r2} / t'_{r1}$

The **unadjusted relative retention, γ** is the ratios of the unadjusted retention times. $\gamma = t_{r2} / t_{r1}$

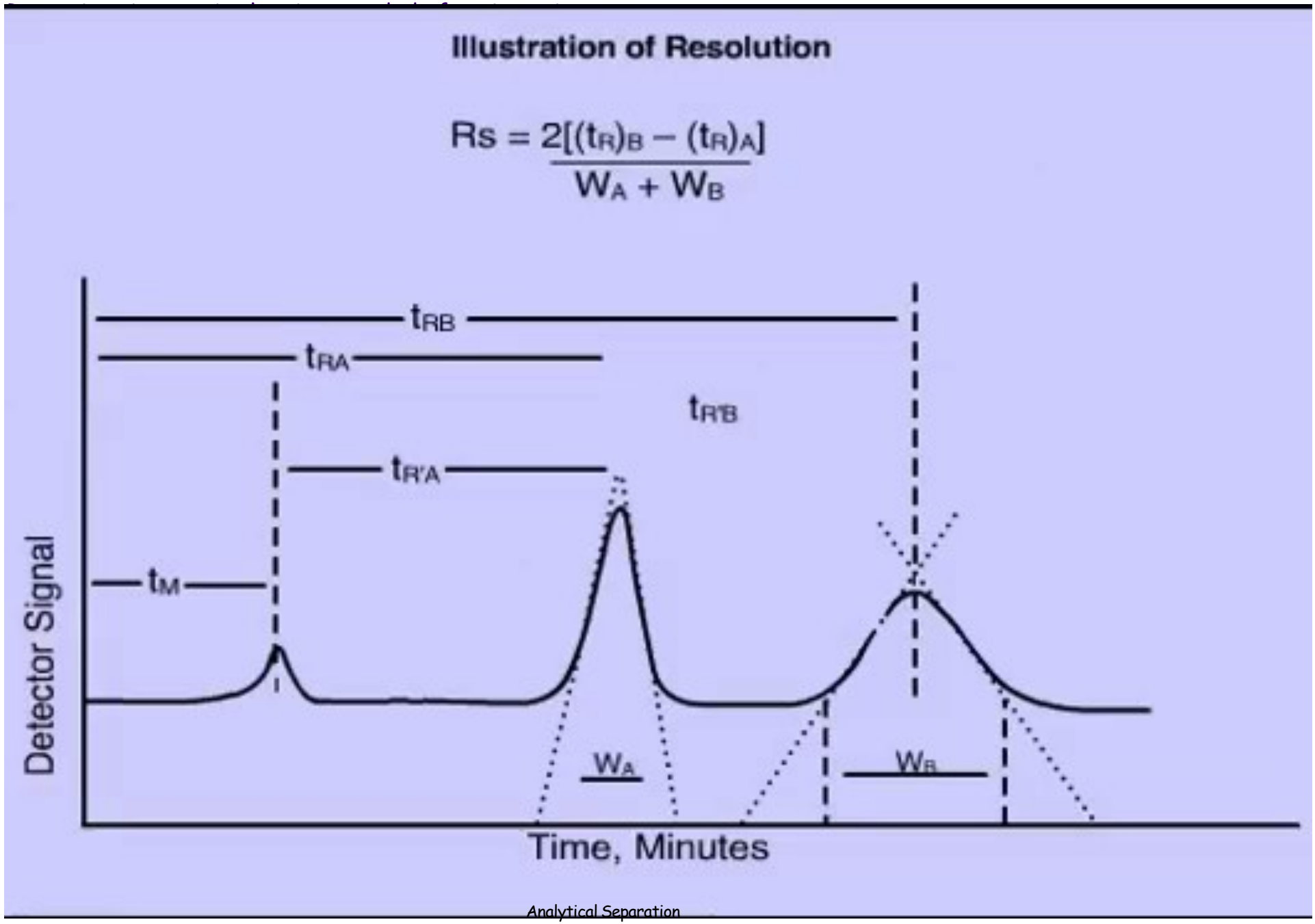
The **retention factor, k** is defined for each peak as: $k = t_r - t_m / t_m$

The longer the component is retained, the greater the retention factor.

Which can also be defined as:

$$k' = \frac{\text{time solute spreads in stationary phase}}{\text{Time solute spreads in mobile phase}}$$

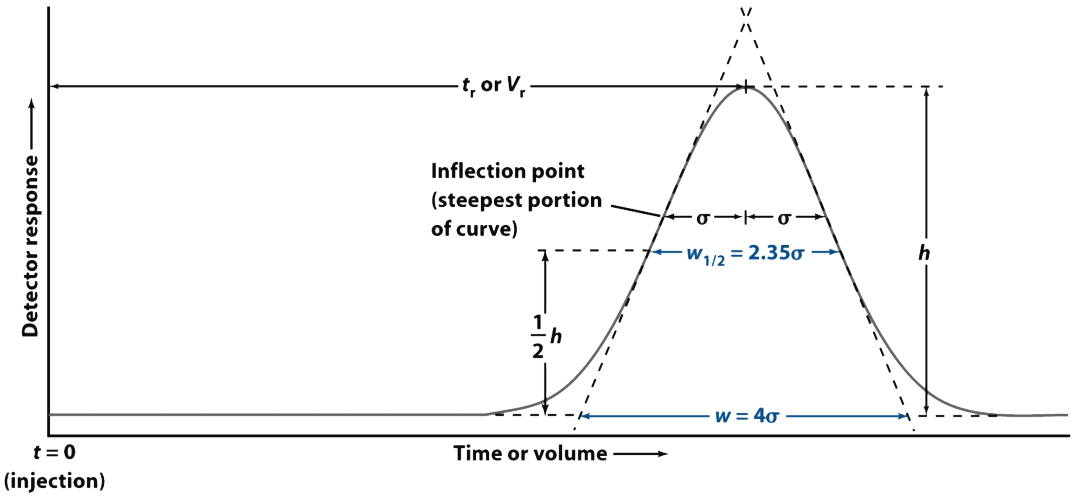
Chromatograms Retention times

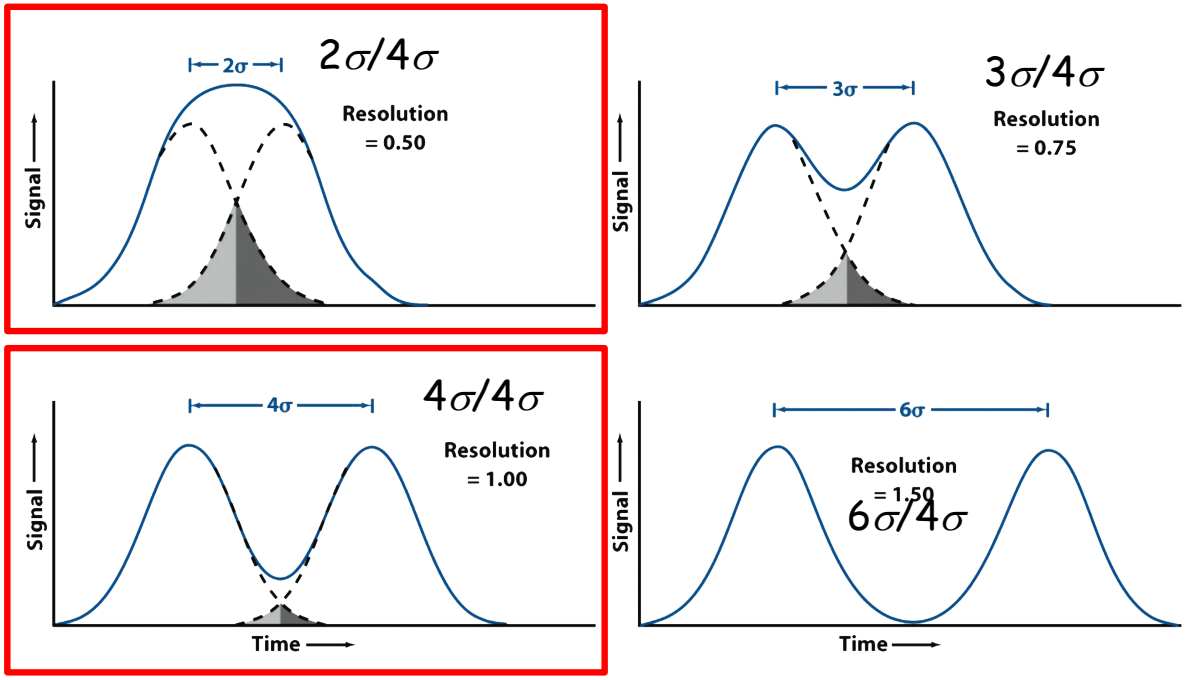


Resolution

Two factors are responsible for resolution. The first is the difference in elution time between peaks and the second is peak broadening. Longer in column, larger the broadening of the signal.

Chromatogram with common measurements: w, $w_{1/2}$, s





Resolution of two peaks is define as:

$$\text{Resolution} = \frac{\Delta t_r}{w_{av}} = \frac{\Delta V_r}{w_{av}} \text{ or}$$

If $w_{\frac{1}{2}}$ is used,

$\Delta t_r = 0.589 \Delta t_r$

$$\text{Resolution} = \frac{0.589 \Delta t_r}{w_{\frac{1}{2}av}}$$

Factors affection Resolution

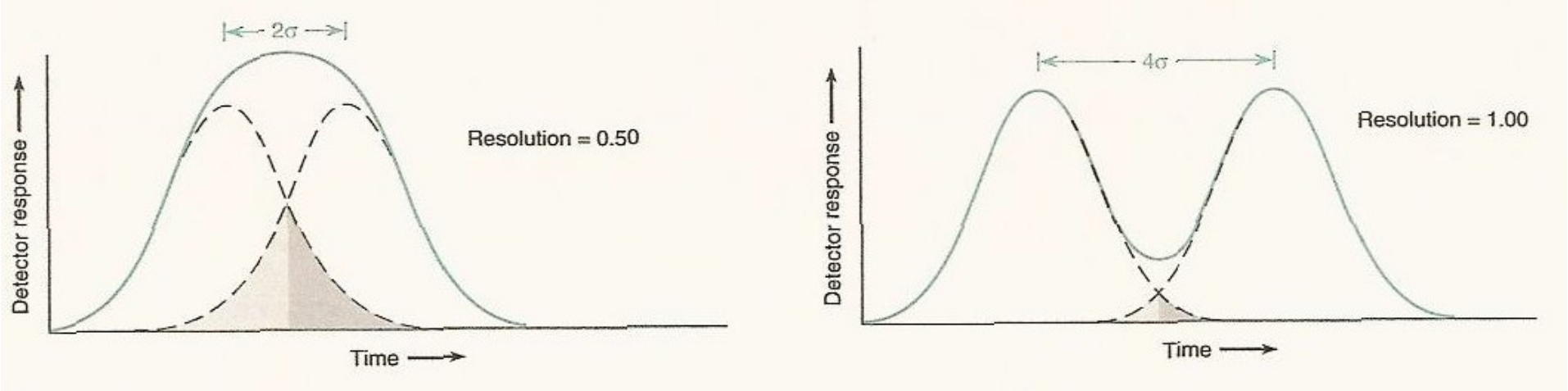
The resolution between neighboring peak is equal to the peak separation (Δt_r) divided by the average peak width (w_{av}) measured at the base.

Resolution = Δt_r / w_{av} = 0.589 Δt_r / $w_{1/2av}$

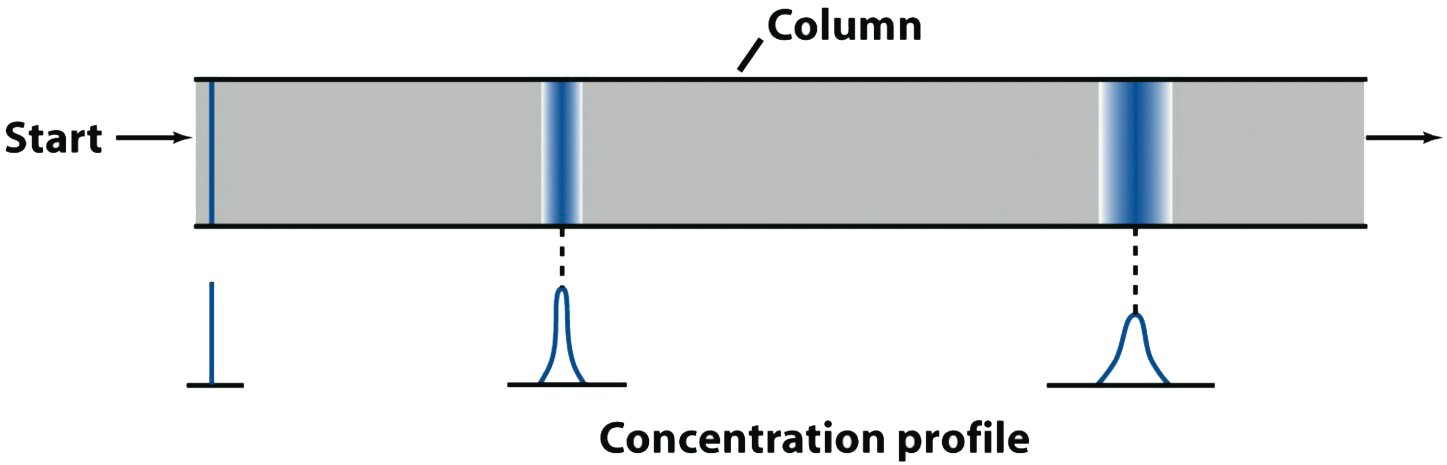
Later we see-

Resolution = ($N^{1/2}$/ 4) (γ-1) where γ is the separation factor t_B/t_A

The resolution of Gaussian peaks of equal area and amplitude. Dashed lines show individual peaks; solid lines are the sum of two peaks.



Poor Resolution Due to Diffusion



One main cause of spreading is diffusion. A solute component placed in a column broadens as it progress through the column, this is due to diffusion of the solute as it travels along the column. The diffusion coefficient measures the rate by which a substance spreads over time.

Diffusion Coeffiecient:

$$\text{Flux}\ \left[\frac{\text{mol}}{\text{m}^2 \bullet \text{s}}\right] \quad J = -D\frac{d_c}{d_x}$$

Broadening of Chromatography band by diffusion:

$$c = \frac{m}{\sqrt{4\pi Dt}} e^{-x^2/(4Dt)}$$

m = moles per unit cross section

c = mol/m^3,D = diffusion coeff.

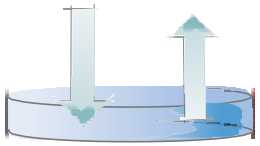
t = time, x = distance along column,

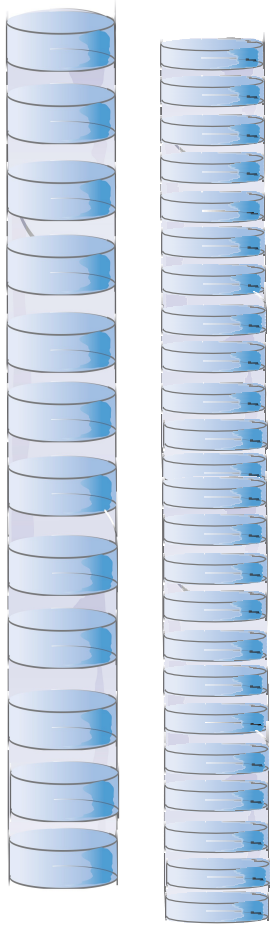
Standard deviation of band:

$$\sigma = \sqrt{2Dt}$$

Plates heights a measure of column efficiency

A column plate height (height equivalent to theoretical plate) is a constant proportionality that takes into account the variance, σ^2, of the band and the distance it travels in the column.





From the diffusion coefficient

$$\sigma^2 = 2Dt = 2D\frac{x}{U_x} = \left(\frac{2D}{U_x}\right)x = Hx$$

$$H = \frac{\sigma^2}{x}$$

The plate height is an estimate of the length of a column that would allow one equilibration between solute in the mobile and stationary phase. The smaller the plate height the better the ability to separate desired component in a column. If a column is L, distance long then the number of theoretical plates can be calculated

Number of theoretical plates: $N = \frac{L}{H}$

for x=L and $\sigma = \frac{w}{4}$,

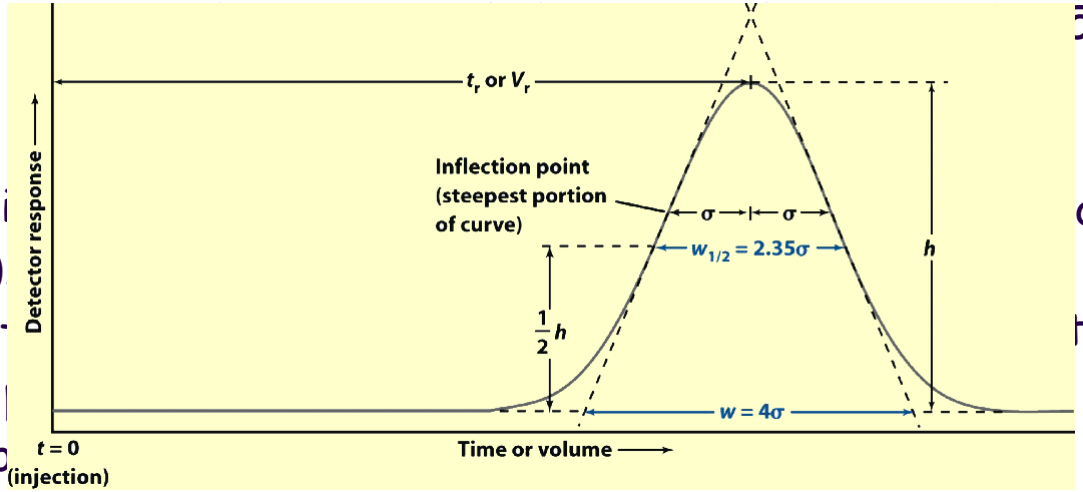
$$N = \frac{t_r^2}{\sigma^2} = \frac{16t_r^2}{w^2} = \frac{5.55\,t_r^2}{w_{\frac{1}{2}}^2}$$

for $H = \frac{\sigma^2}{x}$, $N = \frac{L\,x}{\sigma^2} = \frac{L^2}{\sigma^2} = \frac{16L^2}{w^2}$

Note, $L = t_r$ and $w_{1/2} = 2.35\sigma$

Theoretical Plates

An ideal chromatographic peak has a Gaussian shape. If the height of the peak is h, the width at half-height, $w_{1/2}$ is equal to 2.35σ, where σ is the standard deviation of the peak. Theoretical plates describes the number of levels the equilibrium steps between injection and elution. The more equilibration steps (the more theoretical plates), the narrower the bandwidth when the compound emerges.



$5\ t_r^2\ ) / w^2_{1/2}$

Retenti olume (such as milliliters of eluate) units. Ifa column is divided into N te height, H, is the length of one column (L) by the number of theo

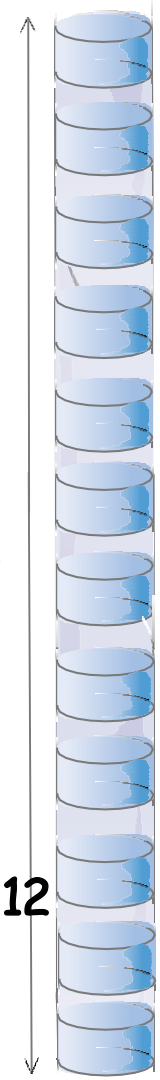
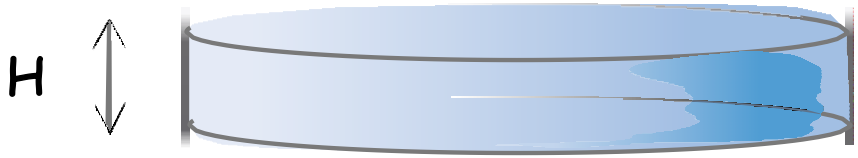


Plate height: H = L/N

The smaller the plate height, the narrower the peaks. The ability of a column to separate components of a mixture is improved by decreasing plane height.



Summary of Chromatography Equations

The important equation use for chromatography techniques are summarize as-

Summary of chromatography equations

Quantity	Equation	Parameters
Partition coefficient	$K = C_s/C_m$	C_s = concentration of solute in stationary phase C_m = concentration of solute in mobile phase
Adjusted retention time	$t'_r = t_r - t_m$	t_r = retention time of solute of interest t_m = retention time of unretained solute
Retention volume	$V_r = t_r \cdot u_v$	u_v = volume flow rate = volume/unit time
Capacity factor	$k' = t'_r/t_m = KV_s/V_m$	V_s = volume of stationary phase V_m = volume of mobile phase
	$k' = \frac{t_s}{t_m}$	
t_s = time solute spends in stationary phase		t_m = time solute spends in mobile phase
Relative retention	$\alpha = \frac{t'_{r2}}{t'_{r1}} = \frac{k'_2}{k'_1} = \frac{K_2}{K_1}$	Subscripts 1 and 2 refer to two solutes
Separation factor	$\gamma = t_2/t_1$ $(\gamma > 1)$	t_2 = retention time of solute 2 t_1 = retention time of solute 1
Number of plates	$N = \frac{16t_r^2}{w^2} = \frac{5.55t_r^2}{w_{1/2}^2}$	w = width at base $w_{1/2}$ = width at half-height
Plate height	$H = \frac{\sigma^2}{x} = \frac{L}{N}$	σ = standard deviation of band x = distance traveled by center of band L = length of column N = number of plates on column
Resolution	Resolution $= \frac{\Delta t_r}{w_{av}} = \frac{\Delta V_r}{w_{av}}$	Δt_r = difference in retention times ΔV_r = difference in retention volumes w_{av} = average width measured at baseline in same units as numerator (time or volume)
	Resolution $= \frac{1}{4}\sqrt{N}(\gamma - 1)$	N = number of plates γ = separation factor $(\gamma > 1)$

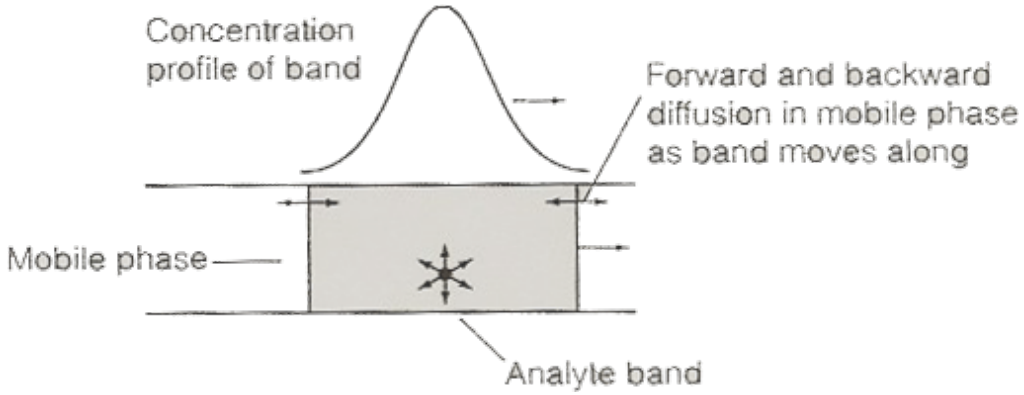
Band Broadening Mechanisms

Longitudinal Diffusion-

A solute band that is introduce into the chromatography column inescapably separate due to longitudinal diffusion. The diffusion occurs because solute continuously separates from the concentrated zone. The farther a band travels, the more time it has had to diffuse and the broader the signal. Similarly, the slower the flow rate, the longer time the solute spends in the column and longitudinal diffusion is maximized.

Longitudinal broadening is related to the flow rate, u.

Broadening μ 1/u

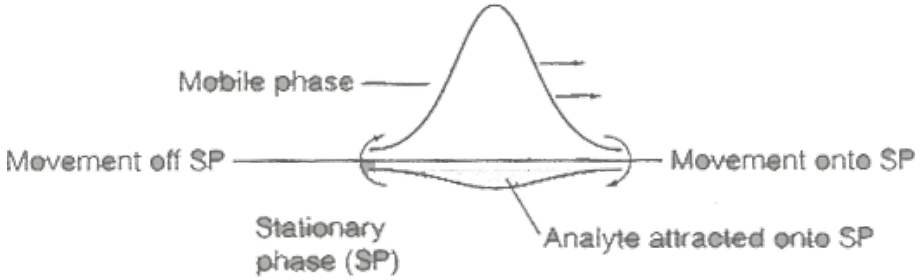


Resistance to Mass Transfer-

For analytes to separate in chromatography, the solute must have time to equilibrate between mobile and stationary phase, If the solute cannot equilibrate rapidly between phase then the solute in the stationary phase will lag behind the solute in the mobile phase resulting in broadening The broadening due to the finite rate of mass transfer between the phases worsens as the flow rate increases.

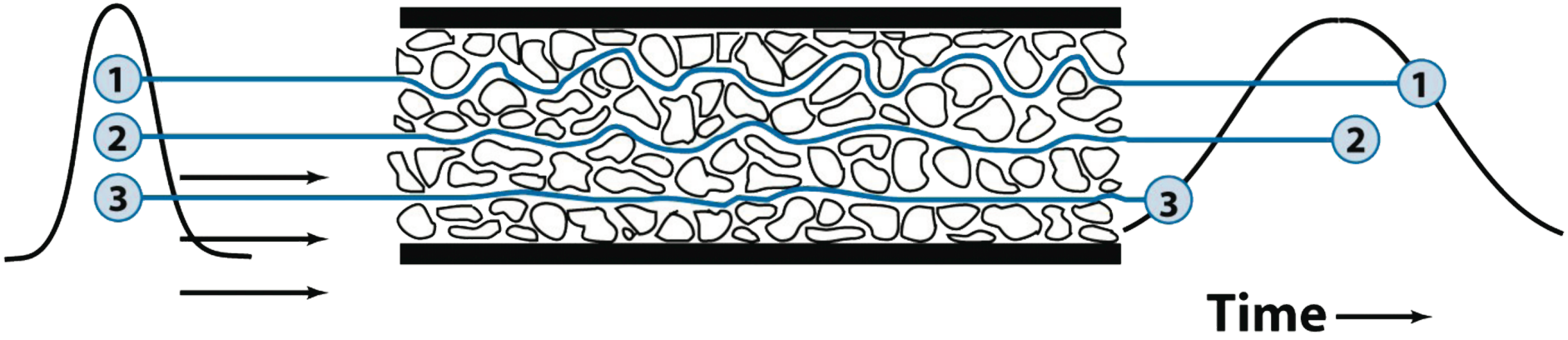
Broadening by finite rate of mass transfer.

Broadening μ u



Multiple Flow Path Broadening (Eddy Diffusion)

Broadening can occur because of the path the solute takes in the column. For packed column, solute travel slightly different total distances through a particular stationary phase causing concentration profiles to broaden symmetrically. This phenomena is known as the multiple-path effects.



Typical pathways of three molecules during elution. Note the distance traveled by molecule 1 is greater than that traveled by molecule 2 which is greater than that of molecule 3. This results in the broadening of the chromatogram peak as it arrives at the detector.

Van Deemter Equation

$$H = A + B/u + Cu$$

The three processes that contribute to peak broadening described in the van Deemter equation are:

A-term: eddy diffusion: The column packing consists of particles with flow channels in between. Due to the difference in packing and particle shape, the speed of the mobile phase in the various flow channels differs and analyte molecules travel along different flow paths through the channels. [Constant function of column]

B-term: longitudinal diffusion: Molecules traverse the column under influence of the flowing mobile phase. Due to molecular diffusion, slight dispersions of the mean flow rate will be the result. [Faster flow rate, less diffusion, narrower peaks]

C-term: resistance against mass transfer. A chromatographic system is in dynamic equilibrium. As the mobile phase is moving continuously, the system has to restore this equilibrium continuously. Since it takes some time to restore equilibrium (resistance to mass transfer), the concentration profiles of sample components between mobile and stationary phase are always slightly shifted. This results in additional peak broadening. [Slower flow rate, better separation]

Van Deemter H (Height)-u (flowrate) Curve

$$H = A + B/u + Cu$$

The van Deemter equation is graphically expressed in the H-u curve, which is a plot of the plate height as a function of the mobile phase velocity.
The H-u curve shows that:

The A-term is independent of u and does not contribute to the shape of the H-u curve..

The contribution of the B-term is negligible at normal operating conditions (Liquid solute). This is due to the fact that the molecular diffusion coefficient in a liquid medium is very small.

The C- term increases linearly with mobile phase velocity and its contribution to the H-u curve is therefore considerable. A small C-term leads to a fairly flat ascending portion of the H-u curve at higher mobile phase velocities. This means that the separation can be carried out at higher mobile phase velocities without sacrificing separation quality.

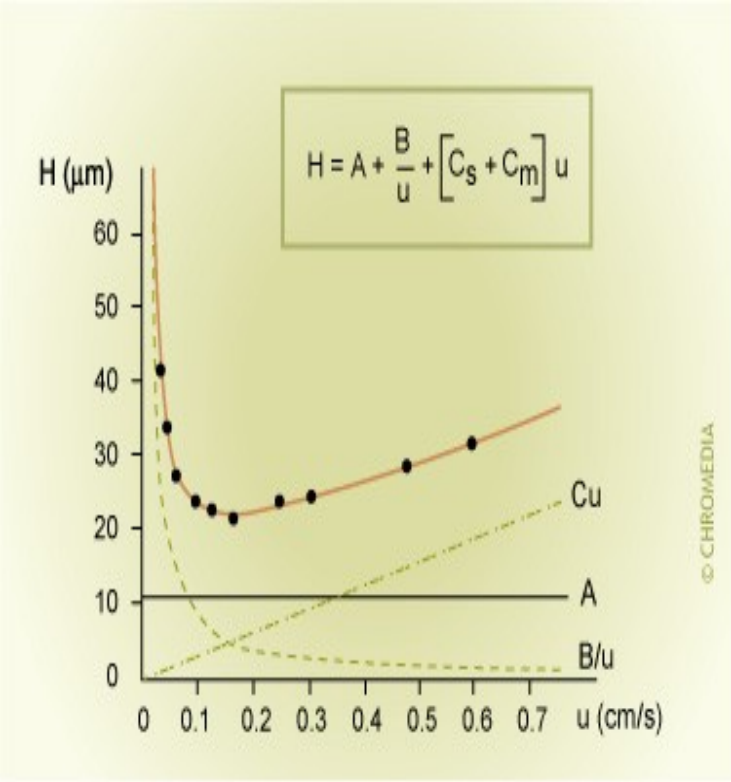
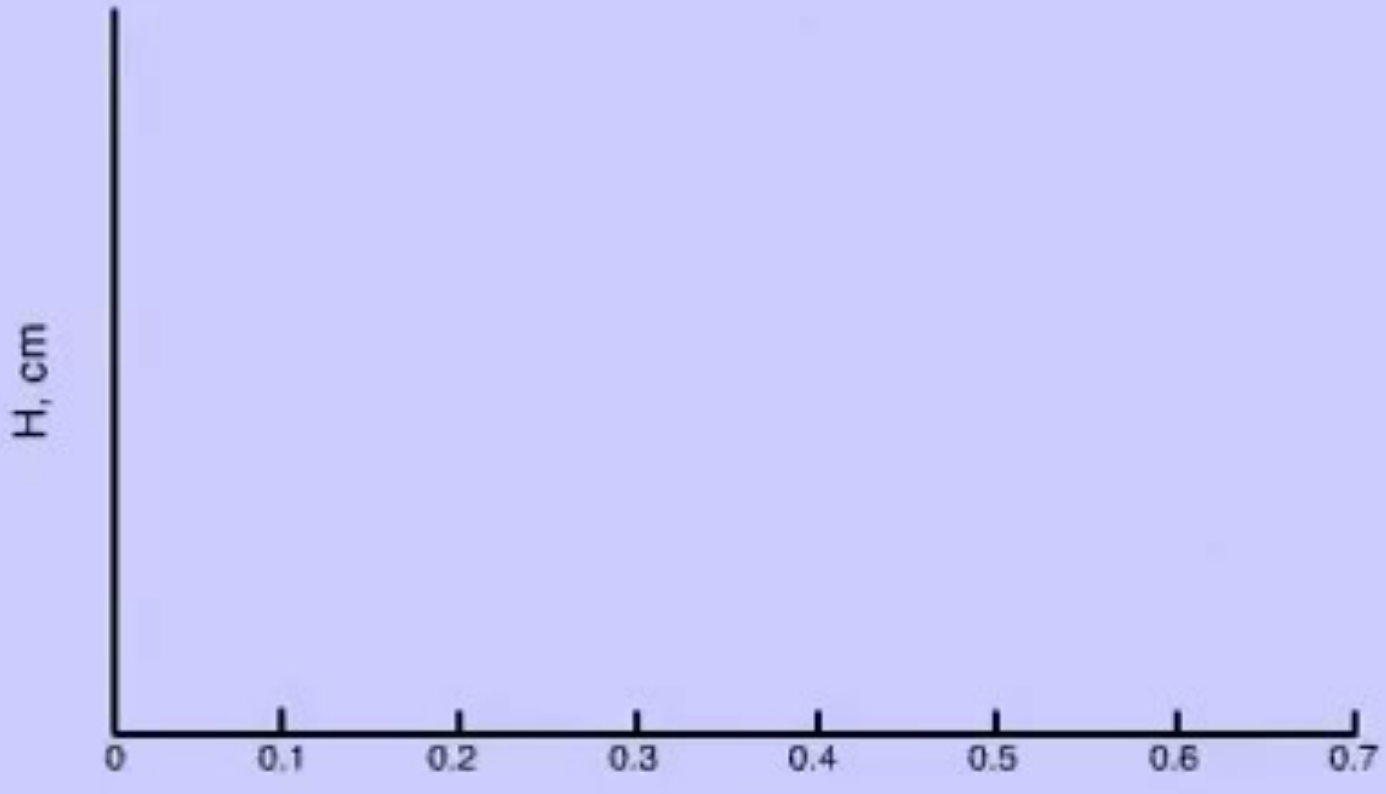


Plate Height Equation



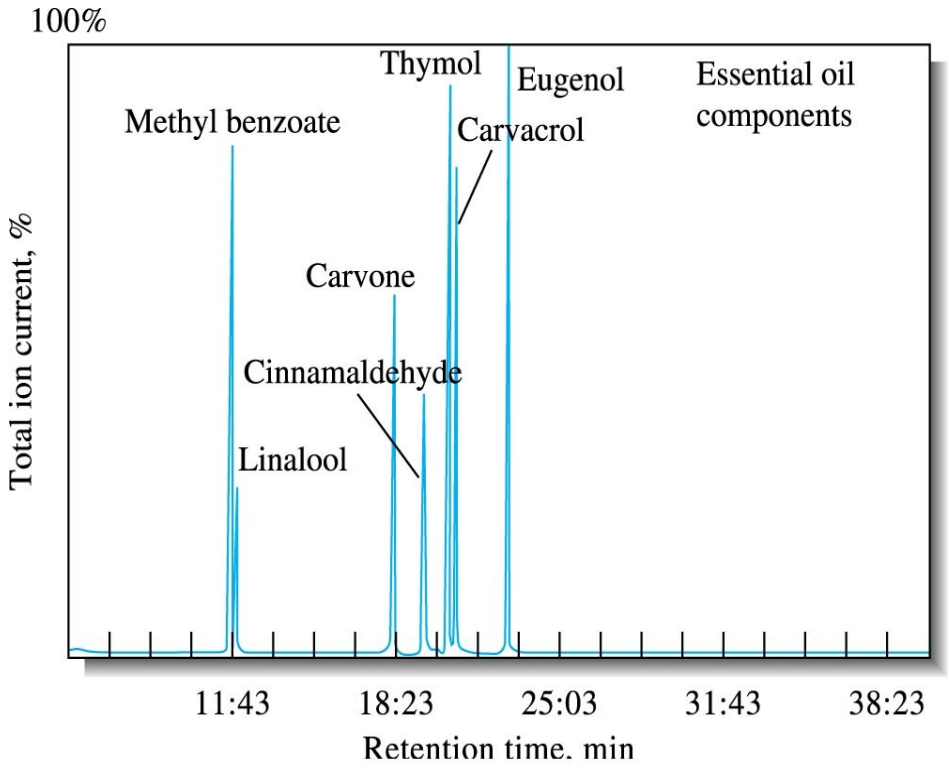
Theoretical Van Deemter Curve

Quantitative and Qualitative Analysis

The For qualitative analysis, the simplest way to identify a chromatographic peak is to compare its retention time with that of an authentic sample of the suspected compound. This can be done by Co-chromatography, in which authentic sample is added to the unknown, then the relative size of one peak will increase.

For qualitative analysis, each chromatographic peak can be directed into a mass spectrometer or infrared spectrometer to record a spectrum as the substance eluted from the column. The area of a chromatographic peak is also proportional to the quantity of analyte. Internal standards are frequently used.

These days computer-controlled chromatographs will find peak areas automatically and integrate the area under the peak.

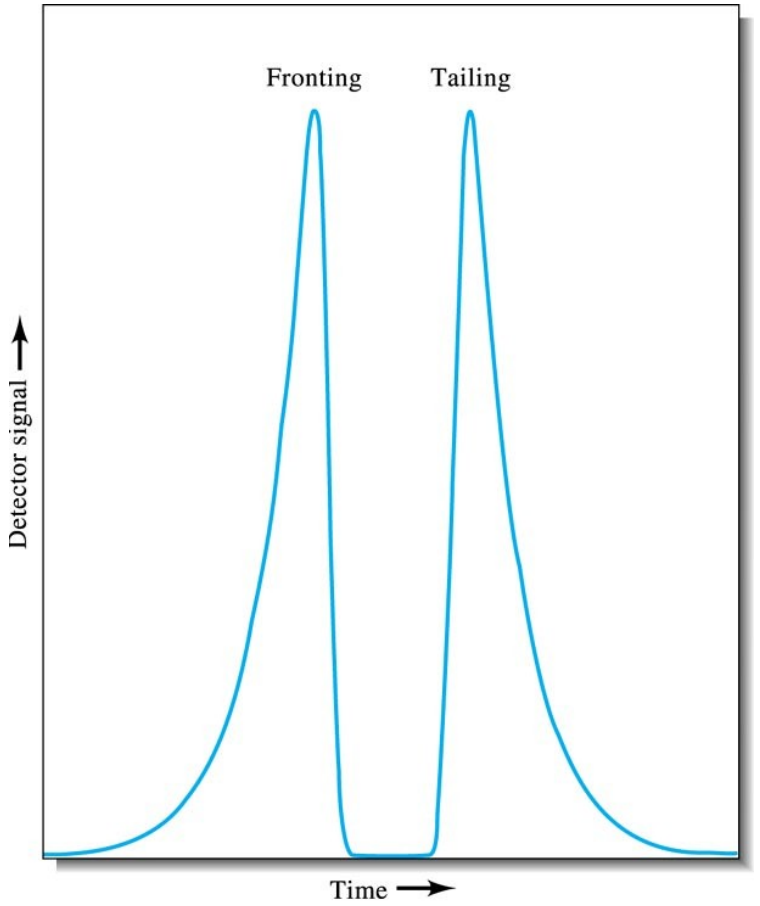


Band anomalies from Column inefficiencies

Peak shape anomalies due to column inefficiencies lead to peak distortions.

Overloading gives chromatographic bands that have ordinary front edge but abrupt cut off rears.

Tailing chromatograms show grossly elongated tails.



Peak asymmetry

The concentration profile of a migrating solute is fundamentally symmetrical (Gaussian), only if the solute distribution ratio is constant. Deviation from Gaussian occurs from changes in the solute distribution ratio at higher concentration levels, lead to two type of peak asymmetry, or skew, describe as tailing and fronting. Both of these can be prevented by reducing the amount of solute chromatographed, although slow de-sorption may still cause some tailing.