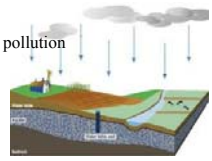
Analytical Separations

An Introduction to Chromatography

Lead Paint Prompts Mattel to Recall 967,000 Toys

Ground water pollution



Hydraulic fracturing

In general matter do not exist in a pure form but in a 'mixed' state.

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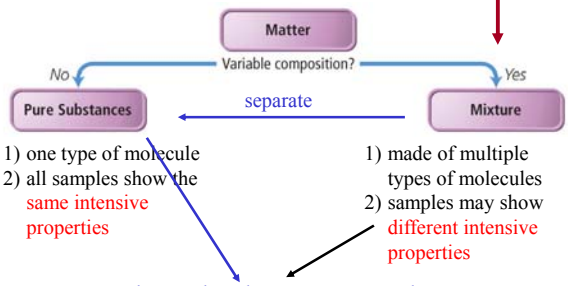
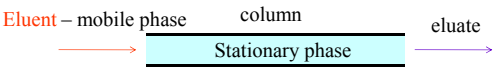


CHROMATOGRAPHY

Chromatography refers to a group of instrumental techniques used for the separation of a mixture of analytes into their individual 'components'.

Chromatography allows the identification and quantification, of the components separated, as a function of time.

The objective of chromatographic techniques is to separate mixtures into its components in the smallest of space and in the shortest of time.



The 'mixture' introduced into a tube containing a material - stationary phase - s.p. - (column); s.p. 'holds' the analytes tentatively.

Another phase is moved over the s.p.; mobile phase – m.p. (eluent) that elutes the 'held' analytes off the s.p.

The 'analytes' enters the column at the same time and leaves the column at different times (separation).

A device 'looks' at the eluate to detect the eluted components (detector). The detector response is plotted – chromatogram.

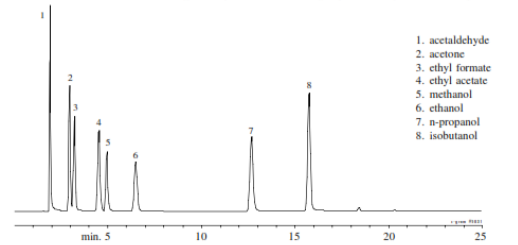
Two types of columns: packed and open tubular.

In chromatography a mixture is separated by distributing its components between two phases.

The *stationary phase* remains fixed in a tube the *mobile phase moves over the stationary phase* carrying the components of the mixture. The stationary phase alters the movement of the components in a mixture; components move slower than the mobile phase.

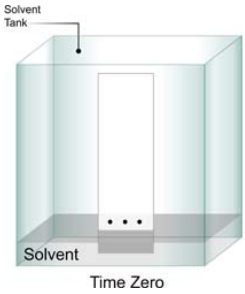
The components interact with the mobile and/or stationary phases differently due to the differences of their solubility of in the mobile phase and the stationary phase. Components will move at different speeds through the column, enabling the separation of the components in the mixture from one another.

The Rtx®-Wax maintains column efficiency at 20°C allowing separation of volatile components.



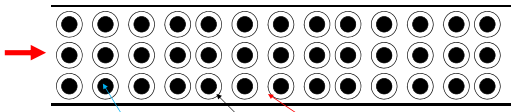
30m, 0.53mm ID, 1.0µm Rtx-Wax (cat.# 12455). 0.1µl split injection of volatile solvents.
Oven temp.: 20°C (hold 10 min.) to 100°C @ 5°C/min.; **Inj./det. temp.:** 200°C;
Carrier gas: helium; **Linear velocity:** 40cm/sec. set @ 20°C; **FID sensitivity:** 8 x 10^{-11} AFS; **Split ratio:** 10:1

Thin Layer Chromatography



http://www.waters.com/waters/en_US/HPLC---High-Performance-Liquid-Chromatography/nav.htm?cid=10048919&locale=en_US

Column



Stationary phase
nonvolatile *liquid*

Inert support
diatomaceous earth;
silica

Mobile phase
gas (or liquid)

Gas-liquid chromatography

In gas chromatography (GC), the mobile phase is an inert gas such as helium and the m.p. does not take an active role in the separation process.

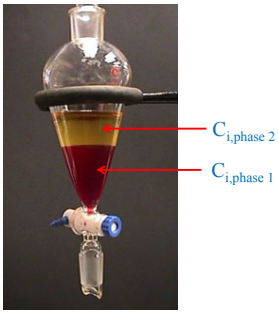
The stationary phase is generally **coated or chemically bonded** to an inert support (diatomaceous earth/silica), both in GC and LC.

In chromatographic systems the separation of analytes is initiated with the sample mixture being introduced (injected) into the **mobile phase**.

There are numerous modes of chromatography.
A partial classification based on the ***state of phases*** and ***mechanism of separation.***

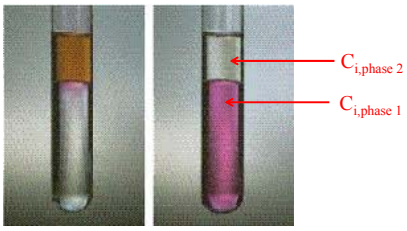
mobile phase	**stationary phase**	**acronym**	**mechanism**
gas	solid	*GSC*	*adsorption chromatography*
	liquid	***GC****(GLC)*	***partition chromatography***
liquid	solid	*LSC*	*adsorption chromatography*
	liquid	***LC****(LLC)*	***partition chromatography***
liquid – single phase		***CE***	*electromigration*

Partitioning of an analyte between two phases – key to chromatography!



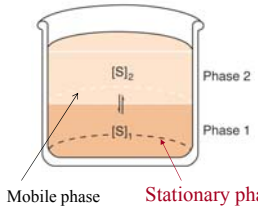
http://w.harpercollege.edu/tm-ps/chm/100/dgodambe/thedisk/labtech/sepfun2.htm

http://www.flickr.com/photos/14833125@N02/7932966792/



http://faculty.sdmiramar.edu/fgarces/zCourse/All_Year/Ch100_MM/aMy_FileLec/04MM_LecNotes_Ch100/05_CompoundBonding/505_IMF_Water/505_SiMatterIMF.htm

Partitioning of an analyte between two phases is a thermodynamic process and is characterized by an equilibrium constant, K (function of temperature).



Partition Coefficient, K_i for analyte i,

$$K_i = \frac{C_{i,phase1}}{C_{i,phase2}}$$

$$D_i = \frac{C_{i,phase2}}{C_{i,phase1}}$$

The 'temporary holding' of analyte occurs in the stationary phase.

In liquid chromatography (LC), the mobile phase is a *solvent, that takes an active* role in the partitioning/separation of analytes.

In gas chromatography (GC), the mobile phase is an inert gas such as helium and the m.p. does not take an active role in the separation process.

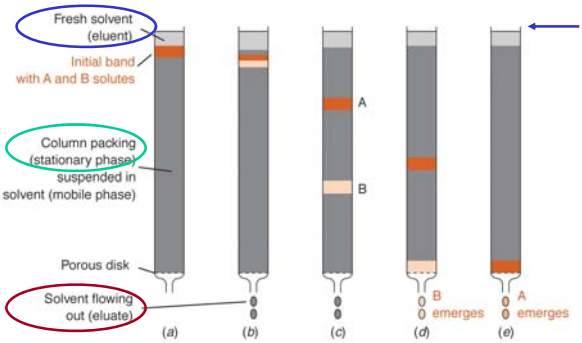
Each analyte in the mixture partitions to a different degree between the phases, therefore retains differently, depending on the K value of the analyte.

Chromatography, in general involves the *partitioning and re-partitioning of analytes between two phases* (extraction) *many, many times*; i.e. multiple 'extractions' done during the separation process.

These multiple '*partitionings*' are achieved by moving one phase (mobile phase) relative to the other (stationary phase); thereby constantly refreshing the system with the mobile phase.

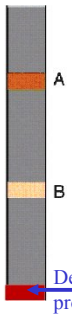
This process enables their separation from each other.

LC (Macro-scale column chromatography)



Mobile phase moves faster (m.p. velocity = u) than analytes.

Under proper stationary/mobile phase combination, *each analyte* migrates with a *unique* velocity, which is less than the mobile phase velocity u.



(B retained less than A on the stationary phase)

Detector: generates a signal proportional to amount of analyte passing through.

Components in a mixture (analytes) separate from each other as distinct bands because each analyte *interact* (retain) differently with the stationary phase and the mobile phase.

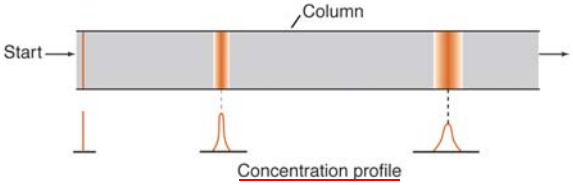
There are different mechanisms involved depending on the type of interaction implemented e.g. partitioning, adsorption, size exclusion etc.

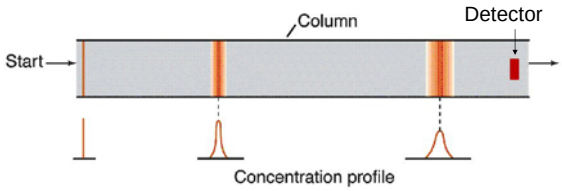
In GC and LC differential partitioning of the components is the predominant mechanism of separation. Sufficient time is required to establish the partitioning of each component between the mobile and stationary phases.

Moving an analyte through the column *results in the broadening of the analyte band – concentration profile*.

Low broadening (preferred) leads to superior chromatograms.

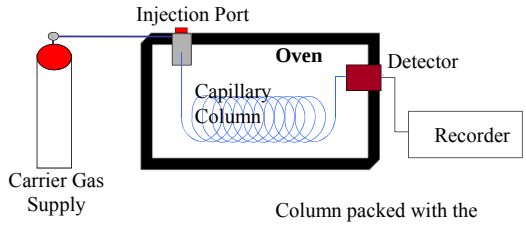
A very thin segment of test material at the starting point of the column.





Detector: generates a signal proportional to amount of analyte passing through and it's response factor.

Gas Chromatograph



Column packed with the stationary phase through which the mobile phase is moved.

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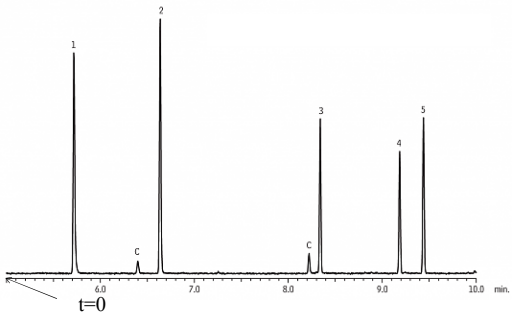
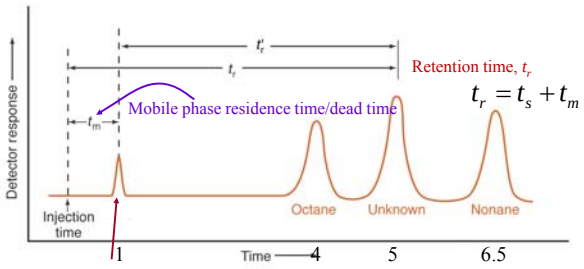
Typical Chromatogram

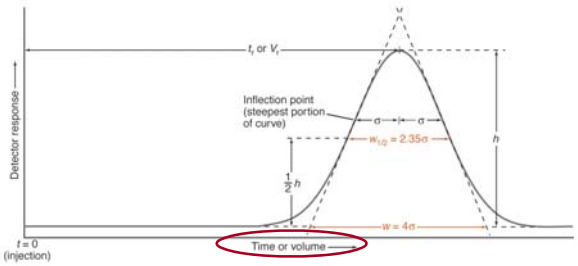


Chromatogram: A graphical plot of the ***detector signal*** as a function of time.



Any un-retained substances, move at the same velocity as the mobile phase, u.

Adjusted retention time, t'_r $t'_r = t_r - t_m$



Chromatographic peaks (from good experimental parameters) takes a Gaussian shape.

x- axis: time of run or volume of the mobile phase.

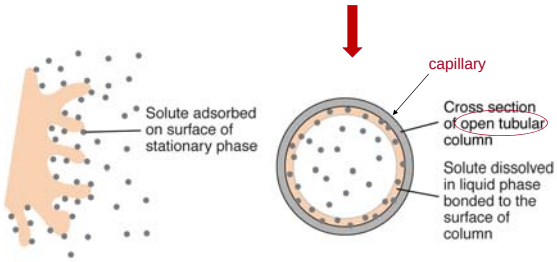
Types of Chromatography
(A classification by mechanism of retention)

1. **Partition Chromatography** GC(GLC), LC(LLC)
2. Adsorption Chromatography
3. Ion-exchange Chromatography
4. Molecular Exclusion Chromatography
5. Affinity Chromatography

Partitioning into the phases is controlled by the polarities of the analyte and the phases involved (Like dissolves like) and the volume ratio of the phases.

mp
sp

$$K = \frac{m_s V_m}{m_m V_s} \quad \& \quad \frac{m_s}{m_s + m_m} = \frac{1}{1 + V_m / KV_s}$$

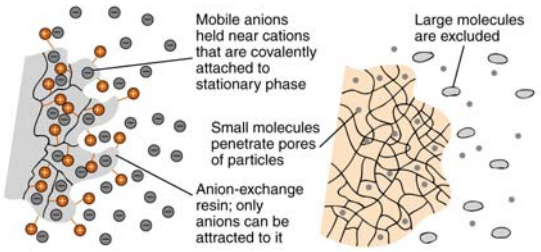


Adsorption chromatography

Solute adsorbs on the surface of the stationary phase

Partition chromatography

Solute dissolves in the liquid bonded or coated to the surface of a column



Ion-exchange chromatography

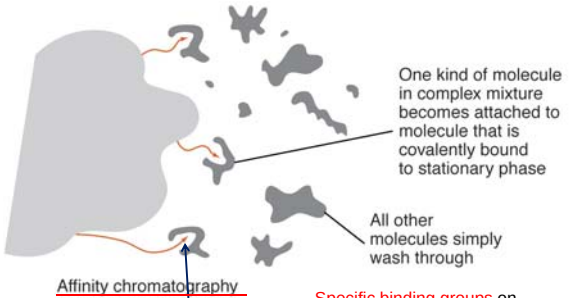
Ions interact with oppositely charged groups on stationary phase

LC

Molecular exclusion chromatography

Solute avoids the small pores in a porous support

LC



Affinity chromatography

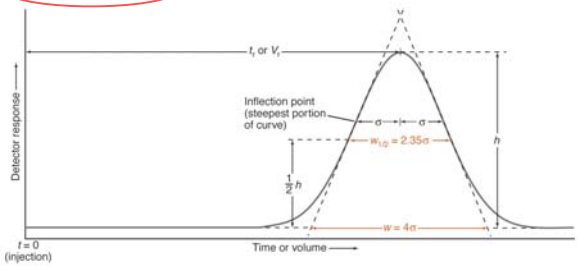
Non-covalent interactions

Specific binding groups on stationary phase attach molecule

LC

σ = standard deviation

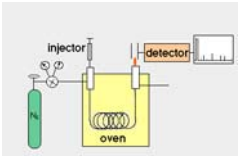
σ^2 = variance



σ; a measure of the band broadening.

The value of σ is a measure of the *broadening* of the peak.

It arises from *in-column* effects and *extra column* effects.



http://www.sfu.ca/bisc/bisc-429/GLC.html

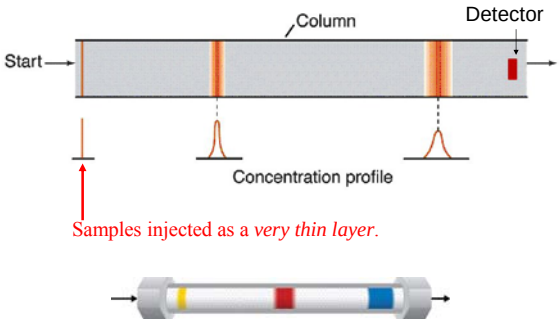
$$\sigma^2 = \sigma^2_{obs} = \sigma^2_{col} + \sigma^2_{inj} + \sigma^2_{det}$$

Note: only variances are additive.

Band Broadening (In-column effects)

- Multiple paths taken by analyte molecules; Eddy diffusion
- Longitudinal Diffusion
- Mass Transfer Effects

Band Broadening (In-column)



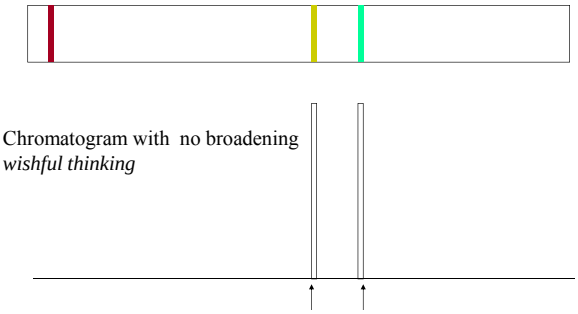
http://www.waters.com/waters/en_US/HPLC---High-Performance-Liquid-Chromatography/nav.htm?cid=10048919&locale=en_US

Band *broadening imposes a limitation* on the ability to ***resolve*** consecutive peaks *because of the overlap* of consecutive analyte bands (i.e. chromatographic peaks).

If there is no broadening the separated regions would come out of the column with region thickness same as at the beginning of the separation process.

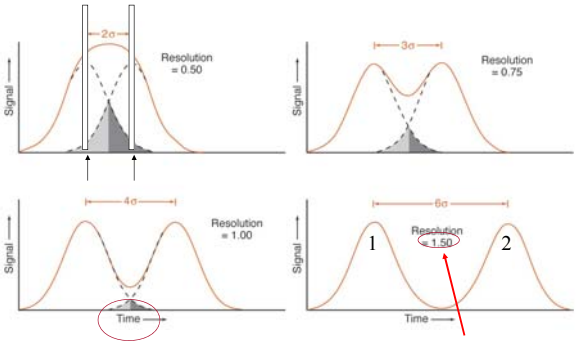
Resolution of peaks –

the ability to distinguish two consecutive analyte segments from each other.



Chromatogram with no broadening
wishful thinking

For two peaks with *equal detector signals*,



Optimum resolution

Resolution: Defined for two consecutive peaks

$$R = \frac{2(t_{r,2} - t_{r,1})}{w_2 + w_1} = \frac{\Delta t_r}{w_{average}} = \frac{0.589\Delta t_r}{w_{1/2,avg}}$$

$$w_{average} = \frac{w_2 + w_1}{2}$$

For two similar peaks, #1 eluting before #2.

Note: x-axis is in time units, $t_{r,i}$ is the elution time.

(x axis may be expressed as elution volume)

The objective is to separate mixtures into individual components and subsequently identify them.

For quantification R values preferred ≥ 1.5.

$$R = \frac{2(t_{r,2} - t_{r,1})}{w_2 + w_1}$$

Enhance retention time difference (Better to optimize) — SELECTIVITY OF THE COLUMN

Decrease band widths — EFFICIENCY OF THE COLUMN

SELECTIVITY: Ability of a chromatographic system to impart **differential migration velocities** to different analytes.

Better selectivity of the system allows separation and isolation of each component.

EFFICIENCY: Ability of the chromatographic system to produce **compact** bands.

More compact bands arise from more efficient chromatographic systems.

SELECTIVITY

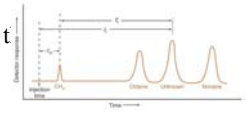
For separation to occur each analyte in the mixture must leave the column at different (elution) times, t_r.

Since all analytes travel the same length of the column, L, separable analytes would have different migration velocities,

$v_r < u$ and $u = L/t_m$.

What makes different v_r values for different analytes?

……. the mechanism involved in t process.

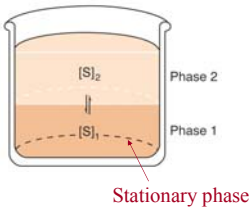


1. **Partition**
2. Adsorption
3. Ion-exchange
4. Molecular Exclusion
5. Affinity

Partition mechanism in a *packed column* is the most complicated system, other columns have less complications.

Packed column:
A tube filled uniformly with an inert material (diatomaceous earth) covered uniformly with the stationary phase. Mobile phase is moved over the stationary phase.

Partitioning of an analyte between two phases is a thermodynamic process and is characterized by an equilibrium constant, K (function of temperature).



Partition Coefficient, K_i for analyte i

$$K_i = \frac{C_{i,phase1}}{C_{i,phase2}}$$

$$D_i = \frac{C_{i,phase2}}{C_{i,phase1}}$$

$$K_i = \frac{C_{s,i}}{C_{m,i}}$$

For $K_i \neq 0$, analyte disperses in both phases.
For $K_i = 0$, analyte flushes out, no retainment in s.p.

Large K_i, analyte prefers s.p. at equilibrium, and therefore retained in s.p. stronger, vice versa.

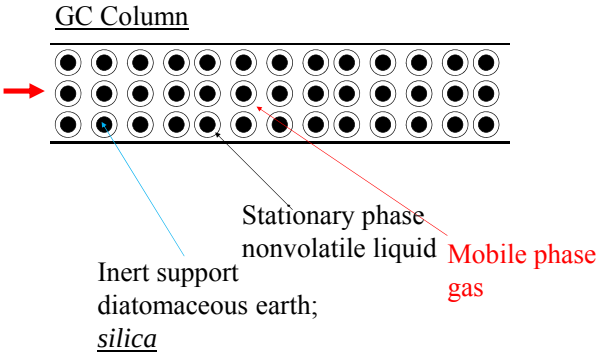
Different K_i *values* of different analytes makes different analytes to be *retained differently* on s.p. Thus they moves through the column at *different velocities*, resulting in *different* $t_{r,i}$; separation.

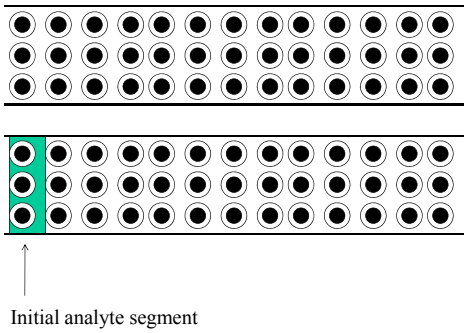
Plate Theory of Band Broadening (In-column effects; Packed column)

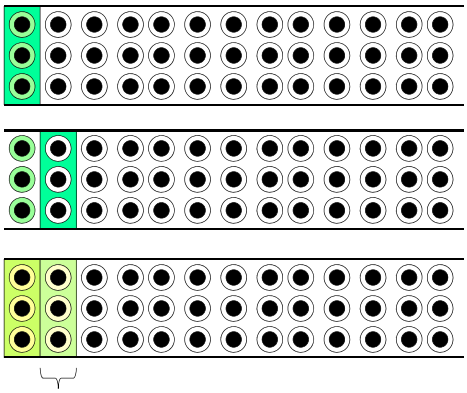
Partitioning of an analyte *i* between the two phases is characterized by its partition coefficient, K_i.

In a column many 'partitions' of the analyte occurs as the analyte moves thro' the column.

Each partitioning/equilibrium 'region' is referred to as a *plate*. *Equilibrium thermodynamics invoked.*







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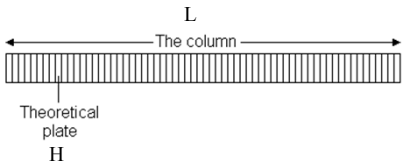


Plate theory: Each equilibration 'region' is a theoretical plate.

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

HETP: Height equivalent of a theoretical plate. The '*length*' of an equilibrium '*region*' in the column; plate height.

The smaller the HETP, more compact the peak be. And also the higher would be the *number of equilibrations* in the column (N).

Higher number of N (*theoretical plates*) ensures smaller HETPs, compact peaks thus better separations.

A measure of the "effective length of an equilibrium region" is termed the ***height equivalent of the theoretical plate***, HETP, H.

Decreasing the peak width is an integral part of method development in chromatography.

Smaller H values (i.e. larger N values) generate smaller peak widths.

For a column of length L, $N = \frac{L}{H}$

Number of theoretical plates (N) is a measure of the compactness of eluting peaks.

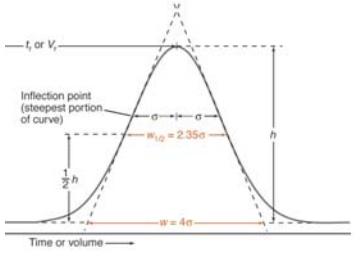
N measures the EFFICIENCY of a column.

Major objective: increase efficiency of columns.

To increase N is to decrease H. $H = \frac{L}{N}$

Ideal peaks are Gaussian in shape.

Characterized by σ.



Narrow peaks are generated by more EFFICIENCT columns;
with larger number of theoretical plates.

Efficiency of column can be calculated using the data of a peak.

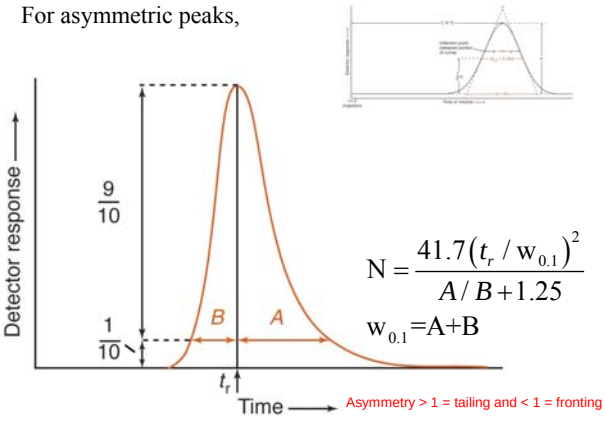
$$N = \left(\frac{t_r}{\sigma}\right)^2$$

$$N = 5.55\left(\frac{t_r}{w_{1/2}}\right)^2$$

$$N = 16\left(\frac{t_r}{w_b}\right)^2$$

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

For asymmetric peaks,

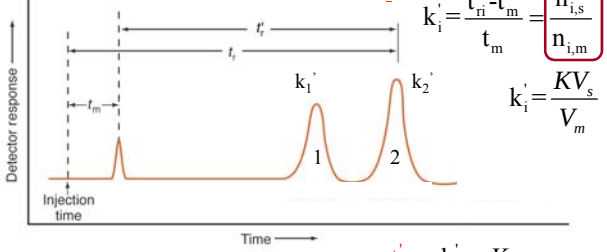


$$N = \frac{41.7\left(t_r / w_{0.1}\right)^2}{A/B + 1.25}$$

$w_{0.1}$=A+B

Asymmetry > 1 = tailing and < 1 = fronting

Capacity factor of an analyte, k_i':



$$k_i' = \frac{t_{ri} - t_m}{t_m} = \frac{n_{i,s}}{n_{i,m}}$$

$$k_i' = \frac{KV_s}{V_m}$$

Relative Retention, $\alpha_{2,1}$: $\alpha_{2,1} = \frac{t_{r2}'}{t_{r1}'} = \frac{k_2'}{k_1'} = \frac{K_2}{K_1}$

Separation factor, γ $\gamma = \frac{t_{r2}}{t_{r1}}$

The **capacity factor** *(retention factor);

There are two primary column-related factors that affect capacity factor: *volume of the stationary phase and the volume of the mobile phase*.

The other important factor is the *partition coefficient of the analyte*.

$$k_i' = \frac{n_{i,s}}{n_{i,m}} = \frac{C_{i,s}v_{bs}}{C_{i,m}v_{bm}} = \frac{C_{i,s}V_s}{C_{i,m}V_m} = \left(\frac{K_iV_s}{V_m}\right) = \frac{K_i}{\beta}$$

column

$$k_i' = \frac{n_{i,s}}{n_{i,m}} = \frac{C_{i,s}v_{bs}}{C_{i,m}v_{bm}} = \frac{C_{i,s}V_s}{C_{i,m}V_m} = \left(\frac{K_iV_s}{V_m}\right) = \frac{K_i}{\beta}$$

band

Volume ratio is the same for the smaller *band* and the entire *column* because of the uniformity of the column.

Capacity factor :

Expression that measures the degree of retention of an analyte relative to an un-retained peak, where t_R is the retention time for the sample peak and t_m is the retention time for an un-retained peak. Thus the higher the capacity factor, the longer the retention time

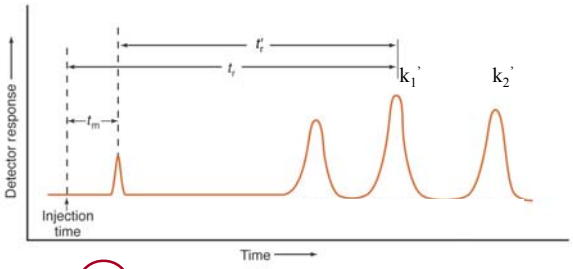
Any retention shifts (capacity factor) are due to the changing column phase ratio or the changing the chemistry of partitioning.

The **relative selectivity** is directly related to how different analyte molecules *interacts* with the phases involved in the column.

If the analyte strongly interacts with the stationary phase, it is because of strong **"intermolecular forces"** of the analyte with the stationary phase. It is a function of the structure of **both the analyte molecule and the phases**, especially the stationary phase.

If the *analyte – s.p. structures are similar*, then these *attractive forces for one another are strong*. If attractive forces are weak, then analyte to stationary phase attraction is weak, and retention is less.

Resolution between consecutive peaks



$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha_{2,1}-1}{\alpha_{2,1}}\right)\left(\frac{k'_2}{1+k'_{avg}}\right) = \frac{\sqrt{N}}{4}\left(\frac{t_b}{t_a}-1\right)$$

Efficiency Relative retention Capacity factor

Velocity of Migration of Analyte (i) Band :

$$v_i = \frac{1}{\left(\frac{K_i V_s}{V_m}\right)+1}u$$

$$k_i' = \left(\frac{K_i V_s}{V_m}\right) = \frac{K_i}{\beta}$$

$$v_i = f(u, K_i, \beta)$$

u = linear velocity m.p.
V_s = volume of s.p. column
V_m = volume of m.p. column
K_i = partition coefficient of analyte i in the sp/mp system.
k_i'=capacity factor
β= phase ratio; V_m/V_s

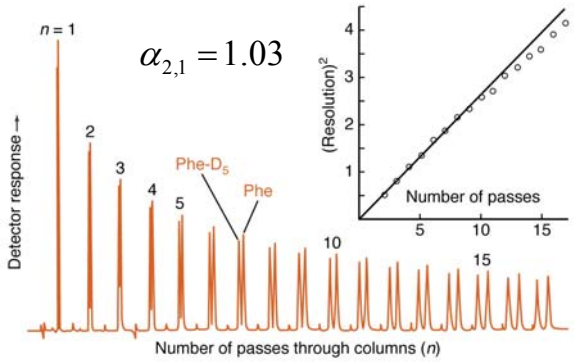
Resolution between two (consecutive) peaks is related as;

$$R = \frac{\Delta t_r}{w_{avg}} = \frac{\Delta t_r}{4\sigma_{avg}} = \frac{0.589\Delta t_r}{w_{1/2}}$$

$$R \propto \sqrt{N} \qquad R \propto \sqrt{\frac{L}{H}}$$

Because HETP, H, is an intensive property, by changing the physical length of the column alone, resolution can be increased.

In general, capillary columns (open-tubular) provide higher resolutions than packed columns (GC).



Alternatively think of the above plot as coming from columns of length L, 2L, 3L,…

Plate theory assumes instantaneous attainment of partitioning; that is the instantaneous equilibration of analytes in a 'plate'.

Rate Theory of Band Broadening (In-column effects; Packed column)

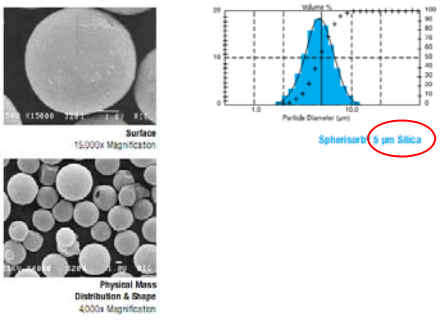
- Multiple paths taken by analyte molecules, Eddy diffusion
- Longitudinal Diffusion
- Mass Transfer Effects

HETP: $H = \frac{\sigma^2}{L}$ Extra-column broadening ~constant

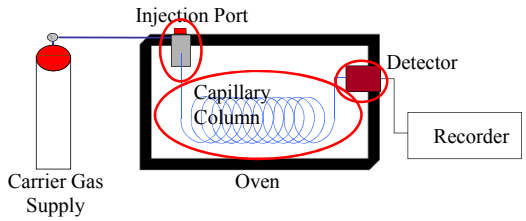
$$H = \frac{\Sigma\sigma_i^2}{L} = \frac{\sigma_{column}^2 + \sigma_{injector}^2 + \sigma_{detector}^2}{L}$$

$$H_{inj} = \frac{(\Delta t_{injector})^2}{12 t_r}; H_{det} = \frac{(\Delta t_{detector})^2}{12 t_r}$$

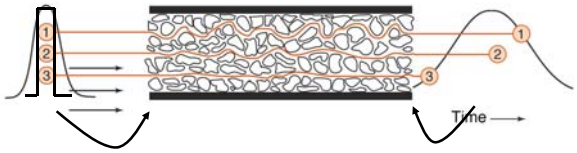
The band broadening arises due to several reasons, and any model explaining in-column band broadening must consider the realistic features in the column; *kinetic and thermodynamic* factors. Each band broadening factor would contribute to σ_{column}.



Gas Chromatograph



1. Eddy Diffusion (multiple path):



Packed particles are uneven, 'pseudo-spheres'; of average size d_p. Larger particles generate a larger range of multiple paths (packed columns); $H_{Eddy} = \lambda d_p = A$

H_{eddy} is a constant for a column because d_p is a constant.

Practically, add *extra-column* contribution to A.

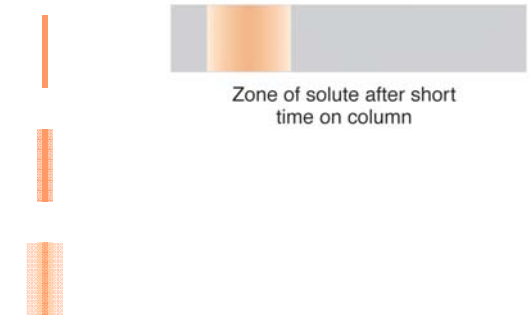
2. Longitudinal Diffusion:

Diffusion: Natural tendency for matter to move from high concentration regions to lower concentration regions.

Occurs in both m.p. and s.p. (more in m.p. in GC)

Longitudinal broadening relates – *inversely* to the mobile phase velocity (i.e. u); *directly* to the diffusion coefficient (time of residence of analyte in the m.p. column (t=L/u) (diffusion broadening in s.p.–small in GC, so ignored.);

$$H_D = \frac{2\gamma D_m}{u} = \frac{B}{u}$$ u = linear velocity (u_x)



Diffusion

A plug of analyte diffuses *out* to produce a Gaussian curve of distribution of concentration. The σ of it, is given by the Einstein's Law;

$$\sigma^2 = 2Dt$$

$$t = \frac{L}{u}\ ;\ \textit{longitudinal diffusion in m.p.}$$

$$\sigma^2 = 2D\frac{L}{u}\ ;\ \textit{by substitution}$$

In GC D = D_m
γ = 0.6 packed
γ = 1 capillary

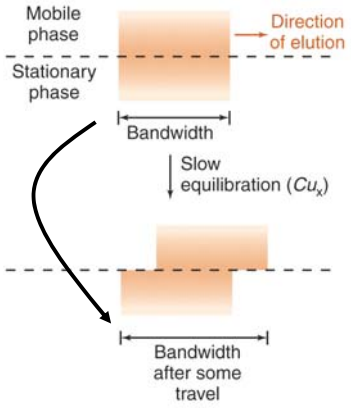
$$\textit{HETP from diffusion;}\ H_D = \frac{\sigma^2}{L} = \frac{2D}{u} = \frac{2\gamma D}{u} = \frac{B}{u}$$

3. Mass Transfer Effects:

Ideal equilibration must allow enough time to reach that state, in reality *it is not the case* because the m.p. is in constant motion; and *equilibration is not instantaneous.*

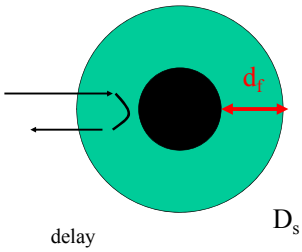
That is to say *complete equilibration does not occur* fast enough; partial (non) - equilibrium system.

a. Stationary phase, H_{stat}
b. Mobile phase, H_{mob}



3 a. Stationary phase

"An incomplete equilibrium" due to a time lag between molecules in the s.p. relative to those in m.p.



$$H_{stat} \propto d_f^2; \qquad H_{stat} \propto \frac{1}{D_s}; \qquad H_{stat} \propto u;$$

$$H_{stat} = \frac{qk'}{(k'+1)^2}\frac{d_f^2}{D_s}u = \boxed{C_{stat}u}$$

For open tubular open tubular column q=2/3
packed column q=1/6.

Table 23-1 Representative diffusion coefficients at 298 K

Solute	Solvent	Diffusion coefficient (m^2/s)
H_2O	H_2O	2.3×10^{-9}
Sucrose	H_2O	0.52×10^{-9}
Glycine	H_2O	1.1×10^{-9}
CH_3OH	H_2O	1.6×10^{-9}
Ribonuclease (FM 13 700)	H_2O (293 K)	0.12×10^{-9}
Serum albumin (FM 65 000)	H_2O (293 K)	0.059×10^{-9}
I_2	Hexane	4.0×10^{-9}
CCl_4	Heptane	3.2×10^{-9}
N_2	CCl_4	3.4×10^{-9}
$CS_2(g)$	Air (293 K)	1.0×10^{-5}
$O_2(g)$	Air (273 K)	1.8×10^{-5}
H^+	H_2O	9.3×10^{-9}
OH^-	H_2O	5.3×10^{-9}
Li^+	H_2O	1.0×10^{-9}
Na^+	H_2O	1.3×10^{-9}
K^+	H_2O	2.0×10^{-9}
Cl^-	H_2O	2.0×10^{-9}
I^-	H_2O	2.0×10^{-9}

$$D_{gas} = \frac{kT}{6\pi\eta r};\ \text{gas phase diffusion coefficient}$$

$$D_{gas} \approx 10000 D_{liquid}$$

3 b. Mobile phase

Larger number of streams, *smaller* diffusion coefficients and faster carrier gas velocities decrease broadening.

$$H_{mob} = \frac{k'^2 d_p^2}{96(1+k')^2 D_m} u = C_{mob} u$$

For packed column

$$C_{mob} = \frac{1+6k'+11k'^2}{24(k'+1)^2}\frac{r^2}{D_m}$$

For open-tubular column

Diffusion coefficient D_m is very sensitive to temperature changes; D_m increases with temperature.

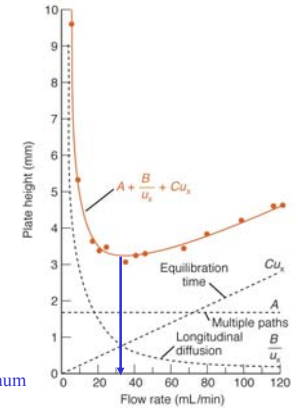
Overall: **Van Deemter Equation**:

$$H_{column} = A + \frac{B}{u} + (C_{stat} + C_{mob})u$$

$$H_{column} = A + \frac{B}{u} + Cu$$

Expressions for capillary (WCOT, SCOT,PLOT) differs from the packed column, however the trends are the same. See text.

$$H_{column} = \lambda d_p + \frac{2\gamma D_m}{u} + \left(\frac{qk'}{(k'+1)^2}\frac{d_f^2}{D_s} + \frac{1+6k'+11k'^2}{24(k'+1)^2}\frac{r^2}{D_m}\right)u$$



$u_{optimum}$

Smaller d_p → lesser # differing multiple paths lengths → H smaller: accomplished in capillary GC (open tubular) – $d_p \approx d_s$.

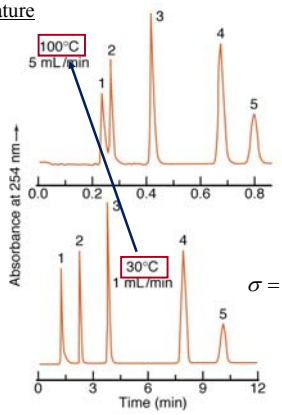
$$H_{column} = \lambda d_p + \frac{2\gamma D_m}{u} + \left(\frac{qk'}{(k'+1)^2}\frac{d_f^2}{D_s} + \frac{k'^2 d_p^2}{96(k'+1)^2 D_m}\right)u$$

(packed column)

Diffusion coefficient D_m is very high in GC; equilibration fast; capillary column - s.p. coated on surface, m.p. small allows - faster equilibration possible!

Effect of Temperature

Retention time reduces; faster equilibration. *Flow rate needs raising.*



Later eluting peaks widen; always.

$$\sigma = \sqrt{2Dt} = \sqrt{\frac{2DL}{u}}$$

Table 23-2 Summary of chromatography equations

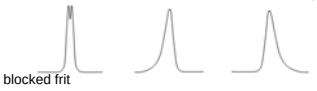
Quantity	Equation	Parameters
Partition coefficient	$K = C_s/C_m$	C_s = concentration of solute in stationary phase C_m = concentration of solute in mobile phase
Adjusted retention time	$t_r' = t_r - t_m$	t_r = retention time of solute of interest t_m = retention time of unretained solute
Retention volume	$V_r = t_r \cdot u_v$	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
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	$\text{Resolution} = \frac{\Delta t_r}{w_{av}} = \frac{\Delta V_r}{w_{av}}$	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)
	$\text{Resolution} = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k_2'}{1+k_{av}'}\right)$	N = number of plates α = relative retention k_2' = capacity factor for second peak k_{av}' = average capacity factor

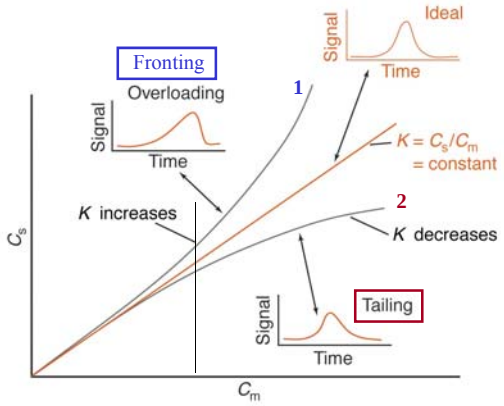
Asymmetric bands:

The *partition coefficient is a constant* for a given 'column', temperature, analyte combination; but,

1. If the injections overloads the column, the s.p. over saturates making the *above assumption* fail, C_s larger than expected.

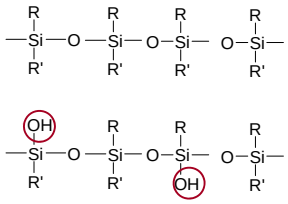
2. If the 'inert' *support* is not actually inert and interacts attractively with the analytes (defective regions in the column) the *'assumption'* fails too, C_s smaller than expected.





Fronting due to overloading, it is alleviated by using diluted samples. The number of 'sites' available for analyte to partition in the s.p. is less than needed.

Tailing can be (mostly) is because of ***adsorption*** on the s.p.

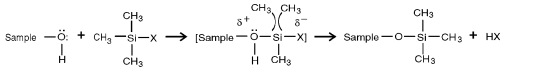


To avoid tailing the active sites on the silica particles must be 'deactivated' - ***silanization***.

Surface hydroxyl groups often are the active sites forms H-bonds with polar solutes.

HMDS = $(CH_3)_3SiNHSi(CH_3)_3$

X = $NHSi(CH_3)_3$



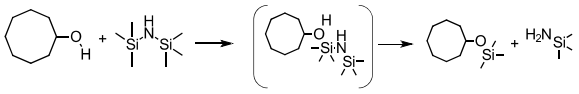


Table 23-3 **Comparison of packed and wall-coated open tubular column performance**[a]

Property	Packed	Open tubular
Column length, L	2.4 m	100 m
Linear gas velocity	8 cm/s	16 cm/s
Plate height for methyl oleate	0.73 mm	0.34 mm
Capacity factor, k', for methyl oleate	58.6	2.7
Theoretical plates, N	3 290	294 000
Resolution of methyl stearate and methyl oleate	1.5	10.6
Retention time of methyl oleate	29.8 min	38.5 min

a. Methyl stearate ($CH_3(CH_2)_{16}CO_2CH_3$) and methyl oleate (*cis*-$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2CH_3$) were separated on columns with poly(diethylene glycol succinate) stationary phase at 180°C.

SOURCE: L. S. Ettre, *Introduction to Open Tubular Columns* (Norwalk, CT: Perkin-Elmer Corp., 1979),