ADVANCES IN FOOD ANALYSIS

INTRODUCTION TO CHROMATOGRAPHY

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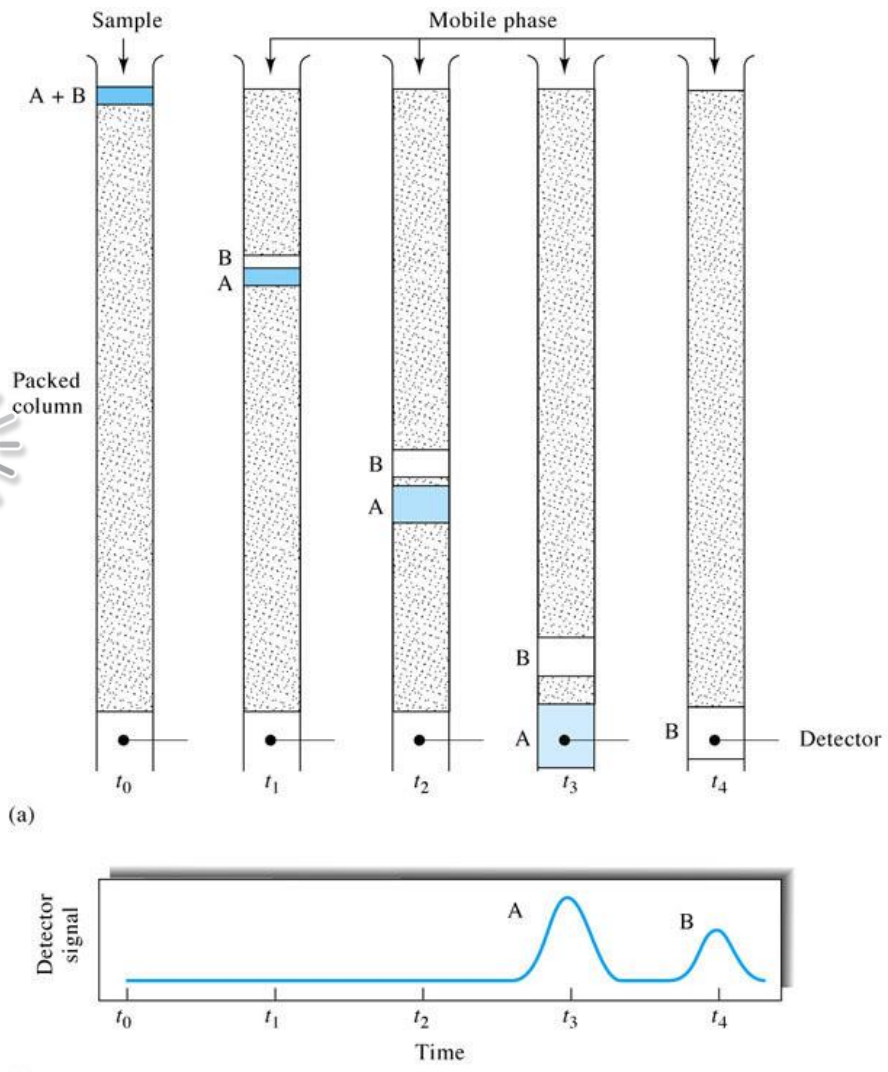
Chromatography

1.) Definition

- A separation technique based on the different rates of travel of solutes through a system composed of two phases
  - **A stationary phase**
  - **A mobile phase**

- Detect compounds emerging in column by changes in absorbance, voltage, current, etc

Chromatogram (not spectrum)



Chromatography

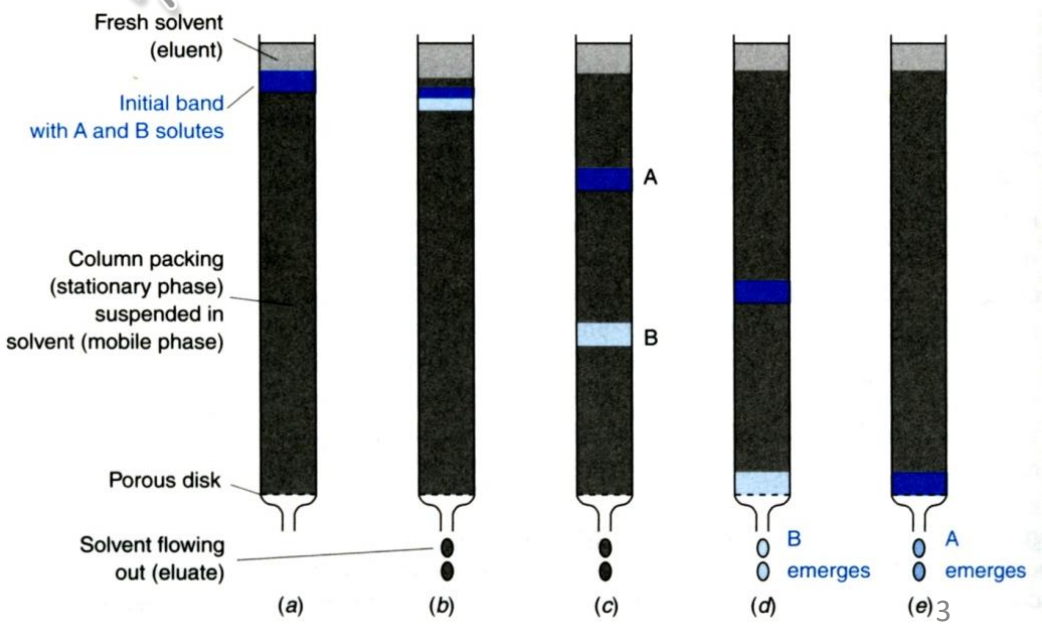
2.) System Components and Process

- Stationary Phase: the chemical phase which remains in the column (chromatographic system)
- Mobile Phase (eluent): the chemical phase which travels through the column

eluent in → COLUMN → eluate out

- Support: a solid onto which the stationary phase is chemically attached or coated

Solute are separated in chromatography by their different interactions with the stationary phase and mobile phase

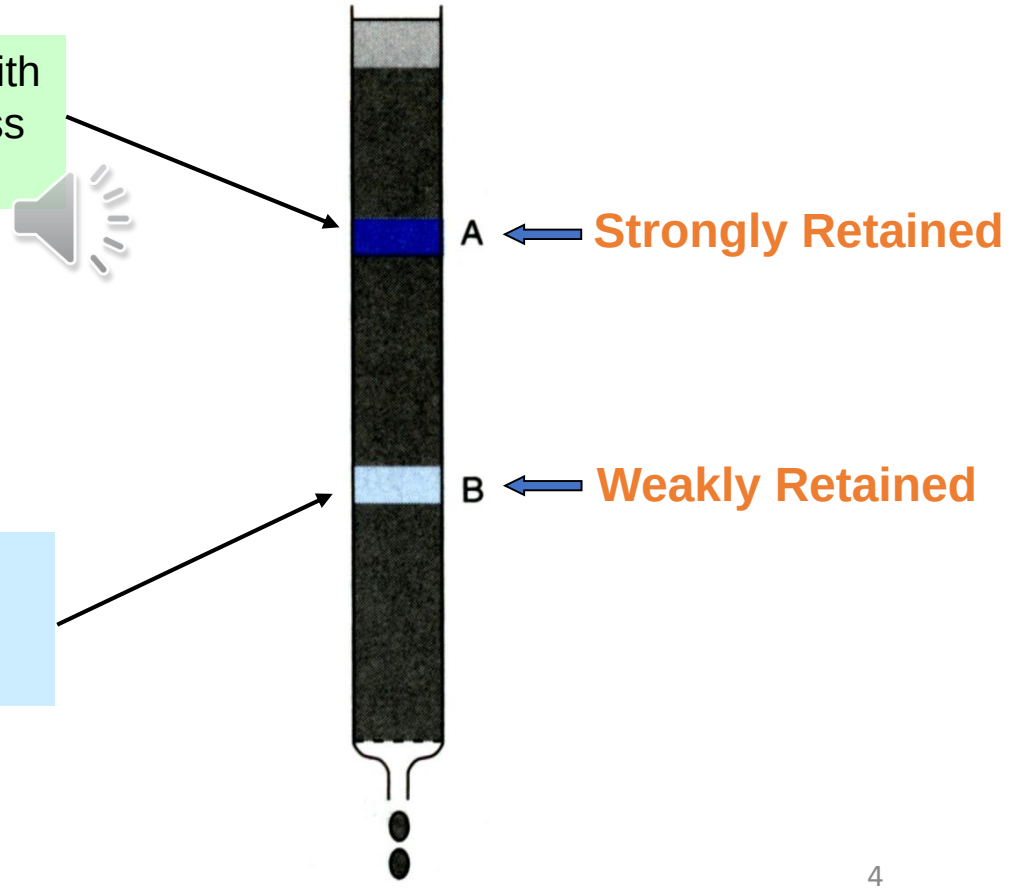


Chromatography

2.) System Components and Process

Solutes which interact more strongly with the stationary phase take longer to pass through the column

Solutes which only weakly interact with the stationary phase or have no interactions with it elute very quickly



Types of Chromatography

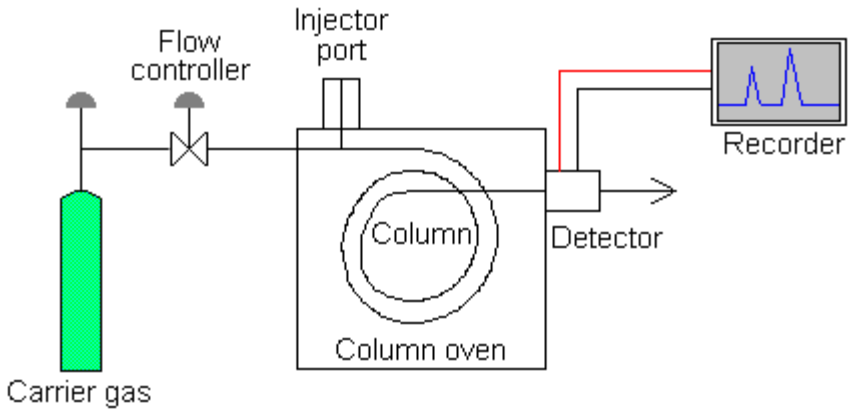
1.) The primary division of chromatographic techniques is based on the type of mobile phase used in the system:

Type of Chromatography	Type of Mobile Phase
Gas chromatography (GC)	gas
Liquid chromatograph (LC)	liquid

2.) Further divisions can be made based on the type of stationary phase used in the system:

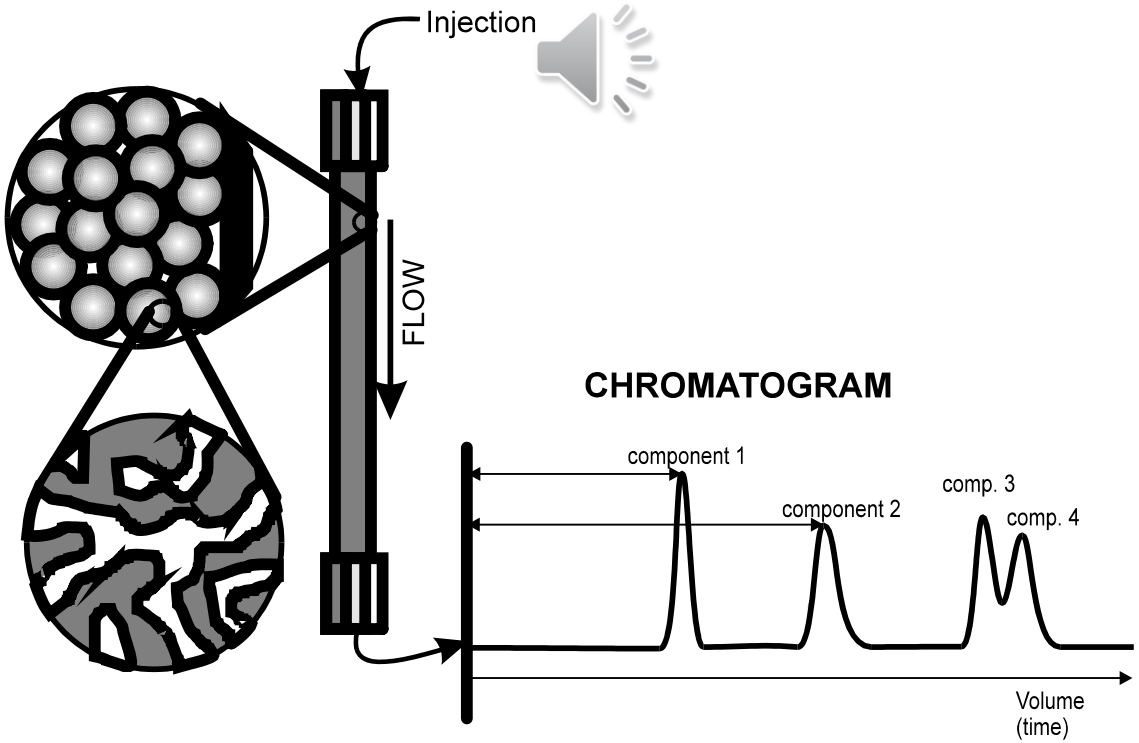
Gas Chromatography

Name of GC Method	Type of Stationary Phase
Gas-solid chromatography	solid, underivatized support
Gas-liquid chromatography	liquid-coated support
Bonded-phase gas chromatography	chemically-derivatized support



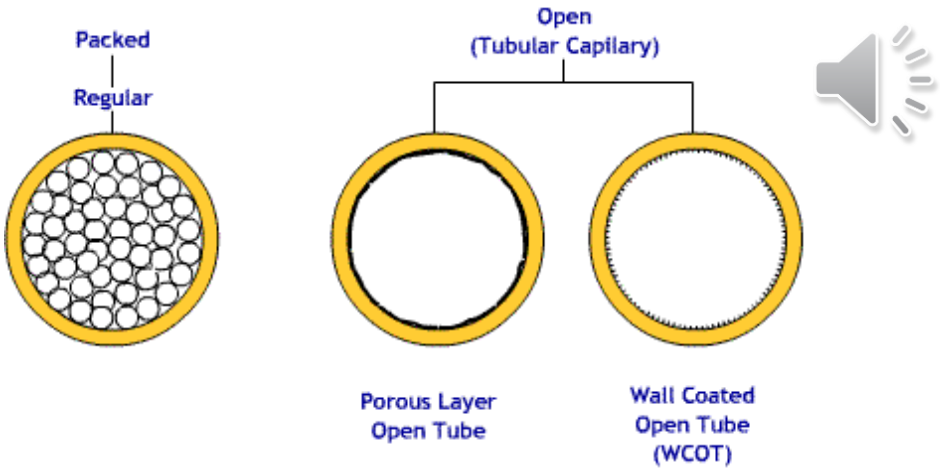
Liquid Chromatography

Name of LC Method	Type of Stationary Phase
Adsorption chromatography	solid, underivatized support
Partition chromatography	liquid-coated or derivatized support
Ion-exchange chromatography	support containing fixed charges
Size exclusion chromatography	porous support
Affinity chromatography	support with immobilized ligand



3.) Chromatographic techniques may also be classified based on the type of support material used in the system:

Packed bed (column) chromatography
Open tubular (capillary) chromatography
Open bed (planar) chromatography



Packed column versus Capillary in GC

One of the main effort of analysts has been focused on increase of separation power

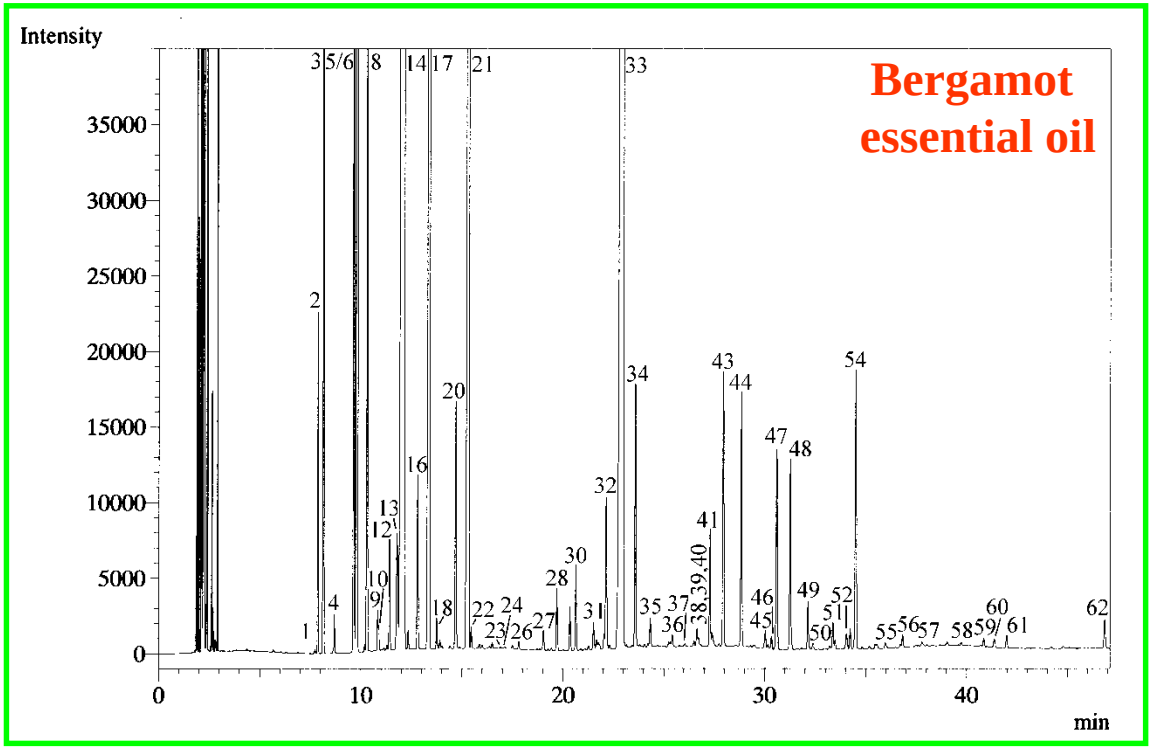
1D GC

Golay M.J.E., **1958**, Gas chromatography. New York:Accademic press

Packed column → capillary column

peak capacities in the 20-50 range

peak capacities in the 400-600 range

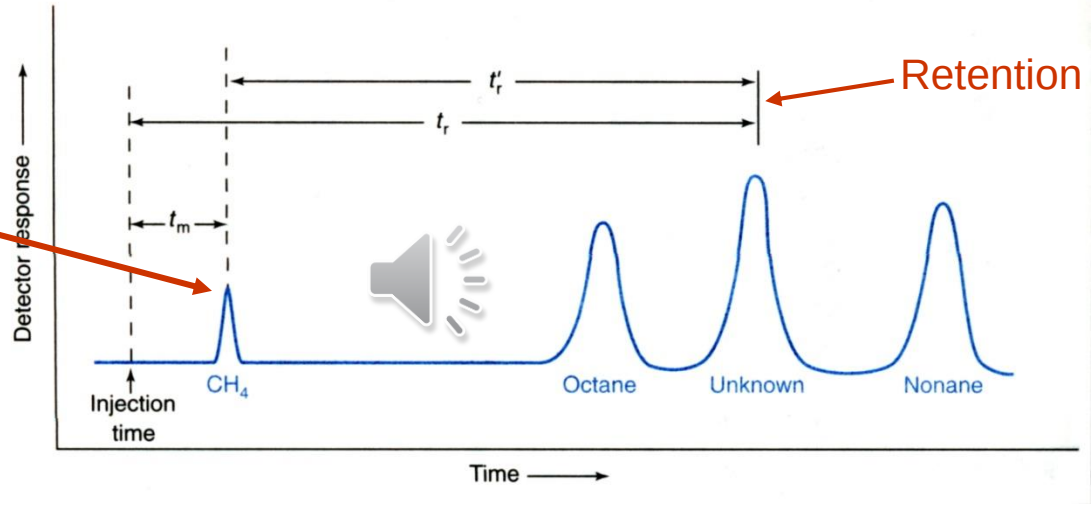


Chromatography

3.) Chromatogram

- Chromatogram: graph showing the detector response as a function of elution time.

Non-retained solute (void volume)



- Retention time (t_r): the time it takes a compound to pass through a column
- Retention volume (V_r): volume of mobile phase needed to push solute through the column

The strength or degree with which a molecule is retained on the column can be measured using retention time or retention volume.

Chromatography

4.) Fundamental Measures of Solute Retention

- Adjusted retention time (t_r'): the *additional* time required for a solute to travel through a column beyond the time required for non-retained solute

$$t_r' = t_r - t_m$$

where: t_m **= minimum possible time for a non-retained solute to pass through the column**

- Relative Retention or Separation factor (α): ratio of adjusted retention time between two solutes

$$\alpha = \frac{t_{r2}'}{t_{r1}'}$$

where: $t_{r2}' > t_{r1}'$ **, so** $\alpha > 1$

- **Greater the relative retention the greater the separation between two components**

Measures of Solute Separation:

Separation factor (α) – parameter used to describe how well two solutes are separated by a chromatographic system:

$$\alpha = k'_2/k'_1 \qquad k' = (t_R - t_M)/t_M$$

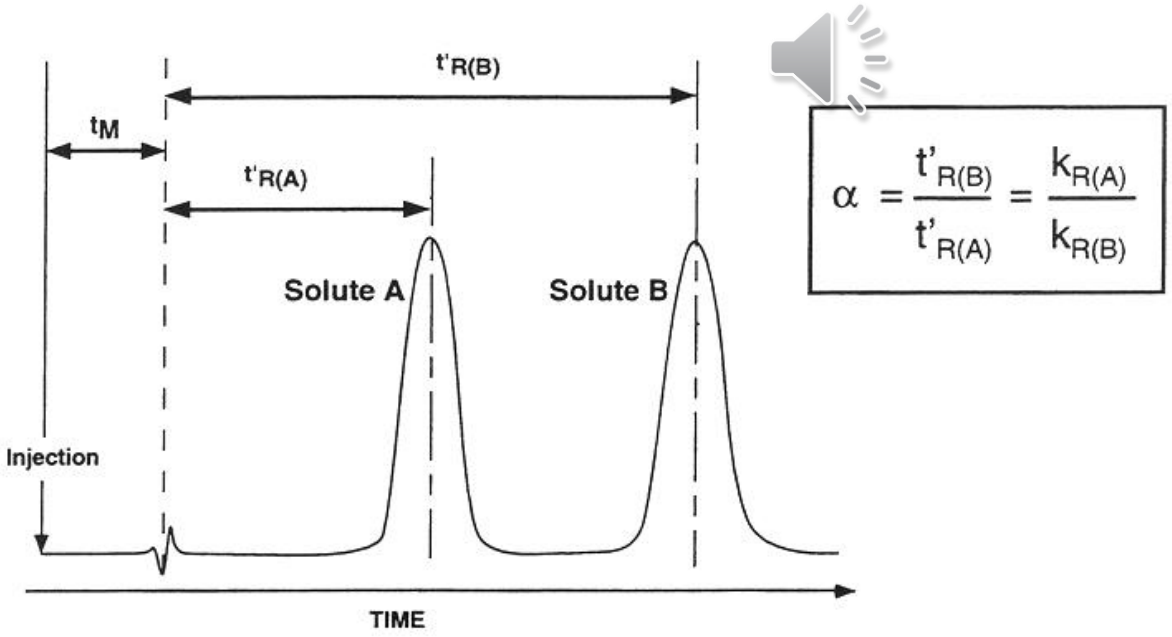
where:

k'_1 = the capacity factor of the first solute

k'_2 = the capacity factor of the second solute,

with $k'_2 \geq k'_1$

A value of α >1.1 is usually indicative of a good separation



$$\alpha = \frac{t'_{R(B)}}{t'_{R(A)}} = \frac{k_{R(A)}}{k_{R(B)}}$$

It is a measure of selectivity

Does <u>not</u> consider the effect of column efficiency or peak widths, only retention.

Chromatography

4.) Fundamental Measures of Solute Retention

- Retention factor or Capacity factor (*k*):

$$k' = \frac{t_r - t_m}{t_m}$$

- The longer a component is retained by the column, the greater the capacity factor
  - **Capacity factor of a standard can be used to monitor performance of a column**

- Capacity factor is equivalent to:

$$k' = \frac{\text{time solute spends in stationary phase}}{\text{time solute spends in mobile phase}} \rightarrow k' = K\frac{V_s}{V_m}$$

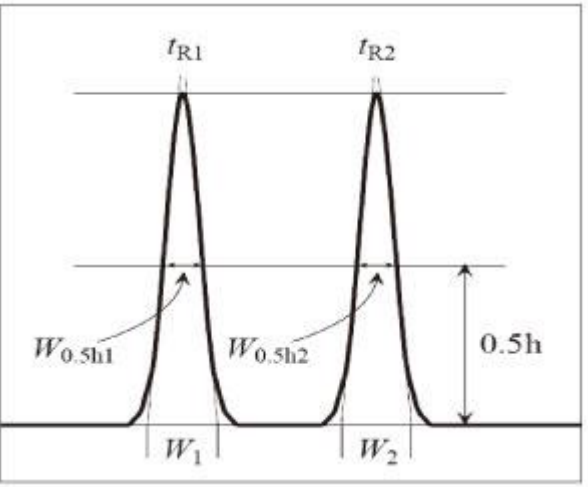
where: V_s = volume of the stationary phase
V_m = volume of the mobile phase
K = partition coefficient

Capacity factor is directly proportional to partition coefficient

Chromatography

5.) Efficiency of Separation

- Resolution (R_s) is defined as:



$$R_s = \frac{(t_{r_2} - t_{r_1})}{(w_{b_2} + w_{b_1})/2}$$

where: t_{r2}, t_{r1} = retention times of solutes 1 and 2 ($t_{r2} > t_{r1}$)
w_{b2}, w_{b1} = baseline widths of solutes 1 and 2

Or

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

where: N = number of theoretical plates

α relative retention time or separation factor

$$\alpha = \frac{k_2}{k_1} = \frac{t_{R2} - t_0}{t_{R1} - t_0}$$

k retention factor or capacity factor

$$k = \frac{t'_R}{t_M} \qquad t'_R = tR - t_0$$

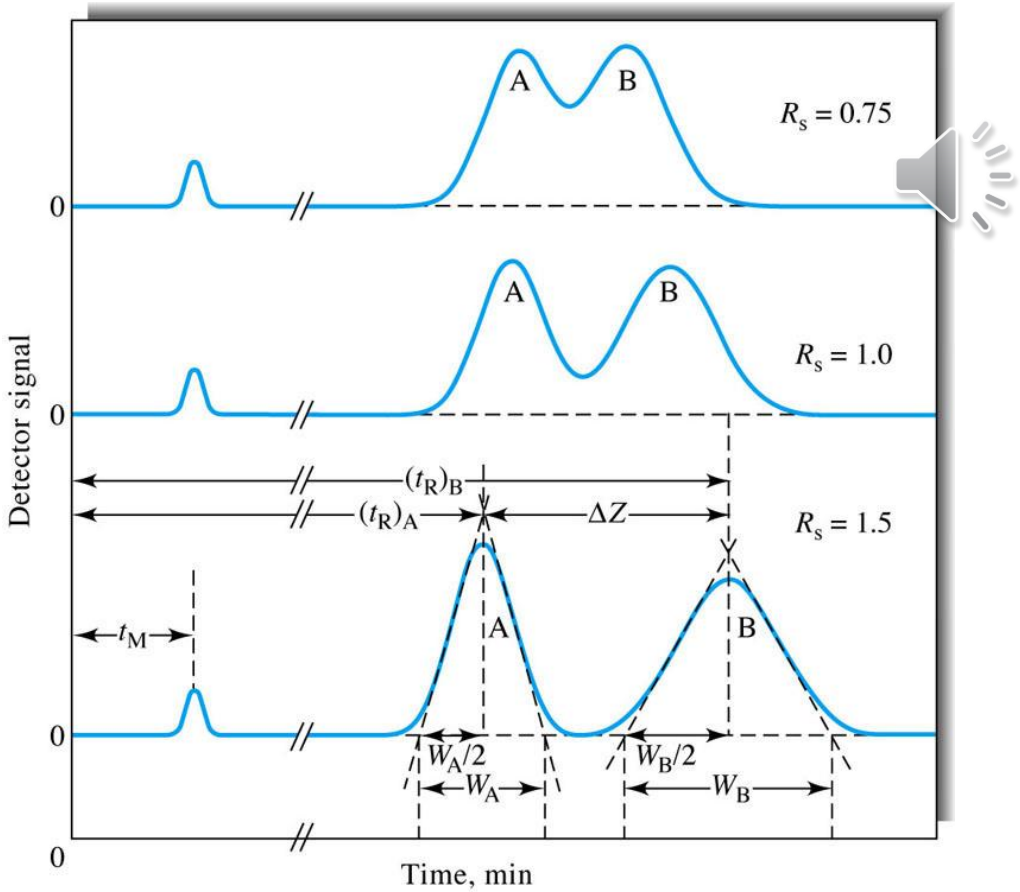
Resolution (R_S) – resolution between two peaks is a second measure of how well two peaks are separated:

$$R_S = \frac{t_{r2} - t_{r1}}{(W_{b2} + W_{b1})/2}$$

where:

t_{r1}, W_{b1} = retention time and baseline width for the first eluting peak

t_{r2}, W_{b2} = retention time and baseline width for the second eluting peak



R_s is preferred over α since both retention (t_r) and column efficiency (W_b) are considered in defining peak separation.

R_s = 1.5 represents *baseline resolution*, or complete separation of two neighboring solutes → ideal case.

R_s = 1.0 considered adequate for most separations.

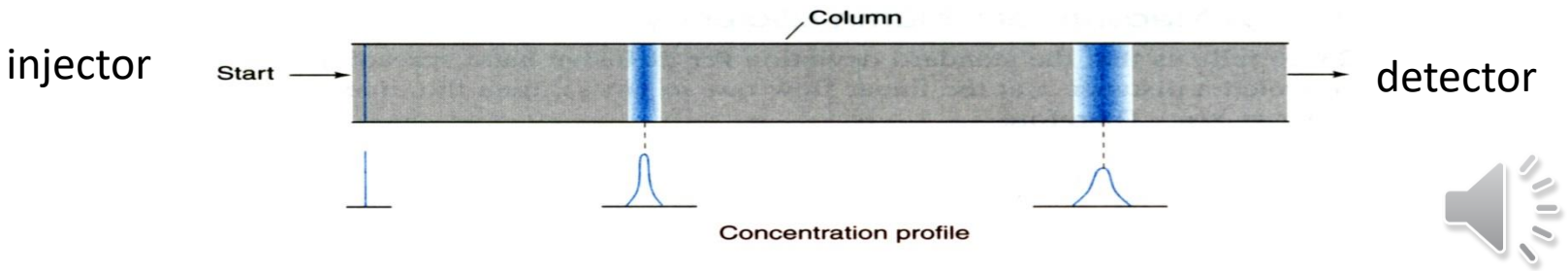
Chromatography

6.) Why Bands Spreading?

- *Remember*: Efficiency is dependent on peak width
- A band of solute spreads as it travels through the column
  - **described by a standard deviation (σ)**

$$\sigma^2 \text{ variances} = \sigma^2 \text{ extra column} + \sigma^2 \text{ column}$$

[total system band spread] [instrument] [intra-column]



- Factors include:

a. Eddy diffusion
b. Mobile phase mass transfer
c. Stationary phase mass transfer
d. Longitudinal diffusion

Van Deemter equation

- Sample injection
- others

Description of Band Spread

- Plate height (H) is proportional to band width
  - **The smaller the plate height, the narrower the band**

Van Deemter equation

$$H = A + \frac{B}{u} + Cu$$

Eddy diffusion — **Longitudinal Diffusion** — **equilibration time**

where: **μ = linear flow rate**
A,B,C = constants for a given column and stationary phase

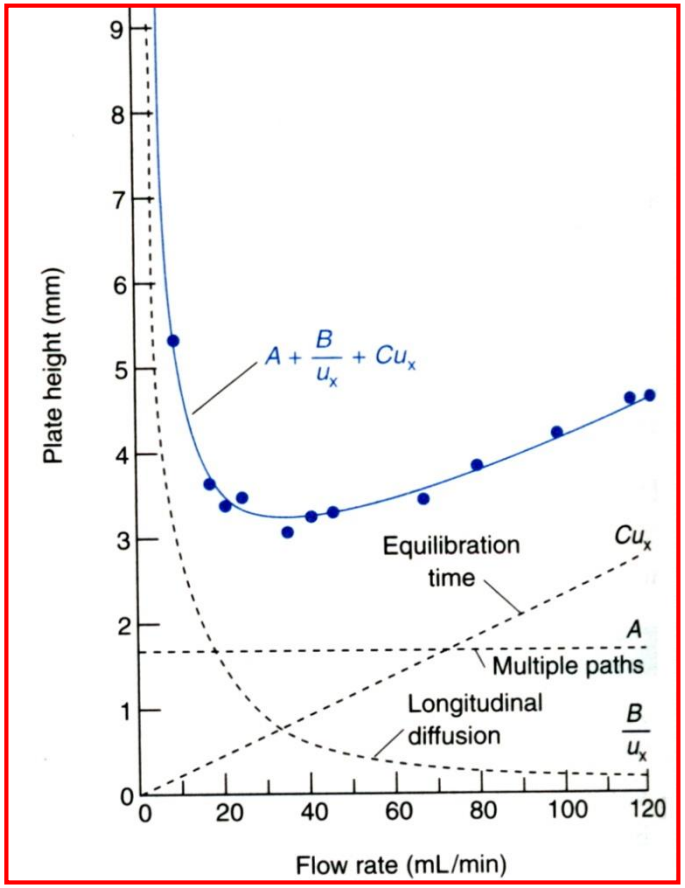
μ (reported also as u or v) = linear velocity (flow-rate x V_m/L)

H = total plate height of the column

A = constant representing **eddy diffusion** (multiplicity of eluent path)

B = constant representing **longitudinal diffusion** in the mobile phase

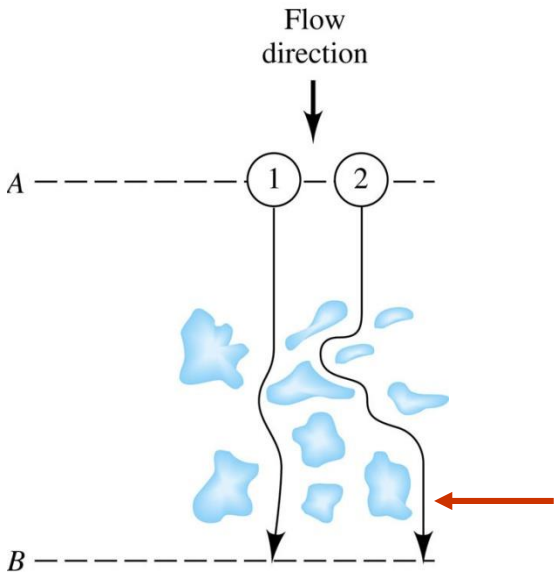
C = constant representing **resistance to mass transfer** in the column related to the diffusion process in the mobile (**C_m**) and stationary phase (**C_s**) [$C=C_m+C_s$]



$$H = A + \frac{B}{u} + Cu$$

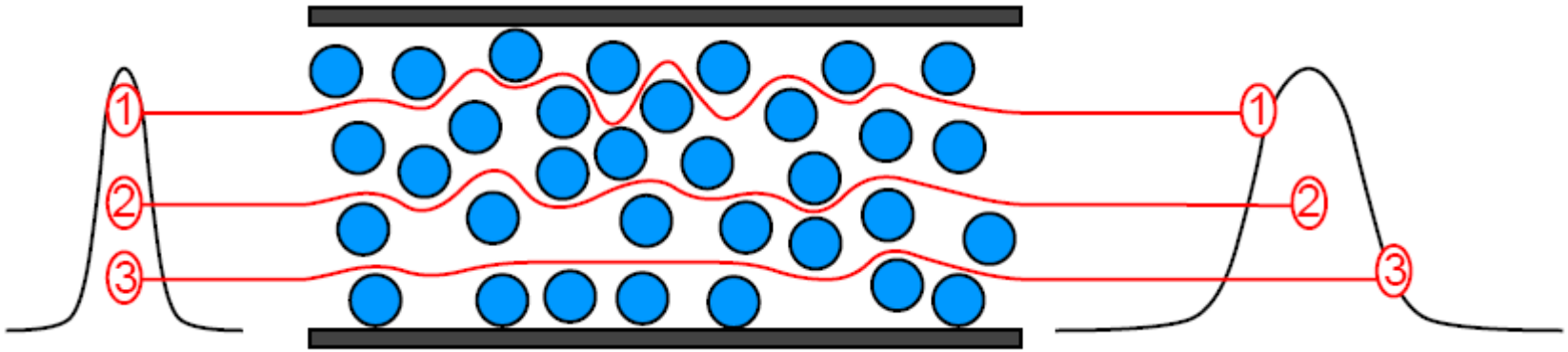
Eddy diffusion

a process that leads to peak (band) broadening due to the presence of multiple flow paths through a packed column.



As solute molecules travel through the column, some arrive at the end sooner then others simply due to the different path traveled around the support particles in the column that result in different travel distances.

Longer path arrives at end of column after (1).

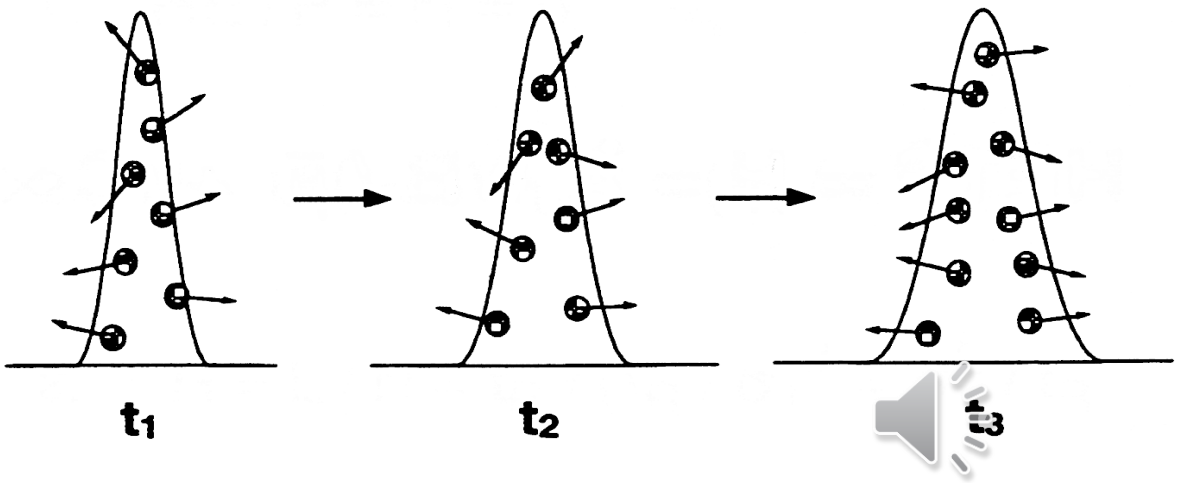


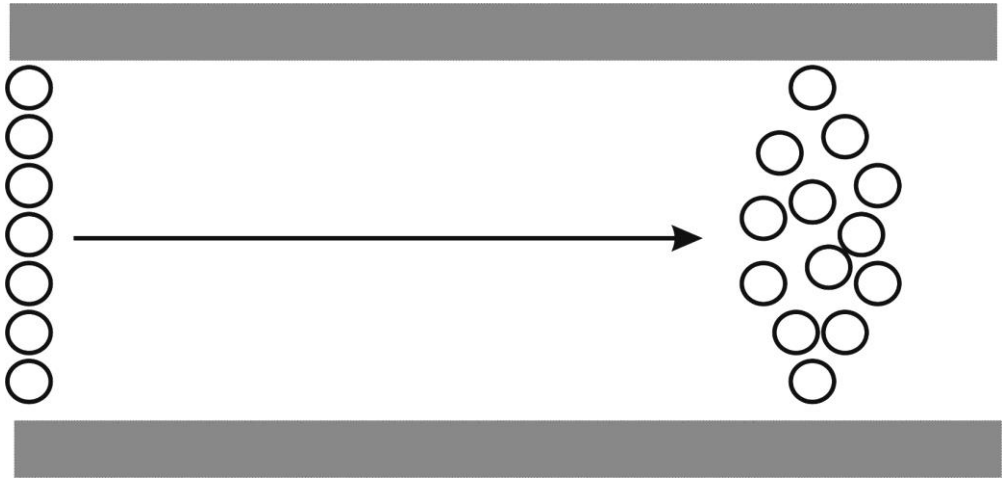
Smaller particle diameter size reduce the effect of band broadening due to Eddy diffusion

$$H = A + \frac{B}{u} + Cu$$

Longitudinal diffusion

due to the diffusion of the solute along the length of the column in the flowing mobile phase.





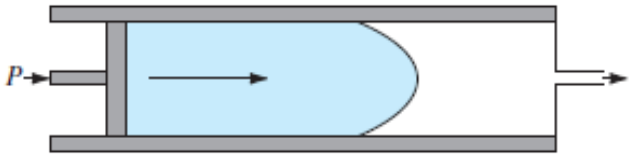
The degree of band-broadening due to longitudinal diffusion depends on:

1) the diffusion of the solute

2) the flow-rate of the solute through the column

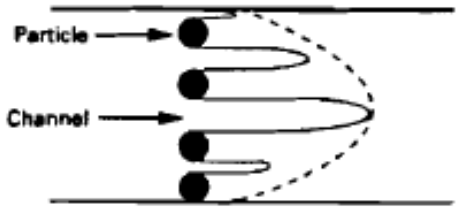
Mass transfer

$$H = A + \frac{B}{u} + Cu$$

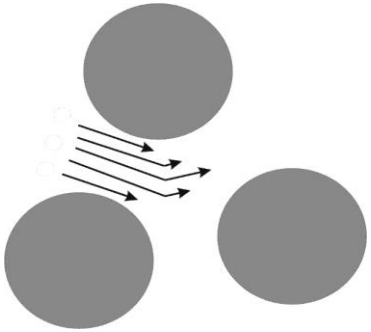


Mobile phase mass transfer (C_m)

Due to the presence of different flow profile within channels or between particles of the support in the column.



A solute in the center of the channel moves more quickly than solute at the edges. it will tend to reach the end of the channel first leading to band-broadening



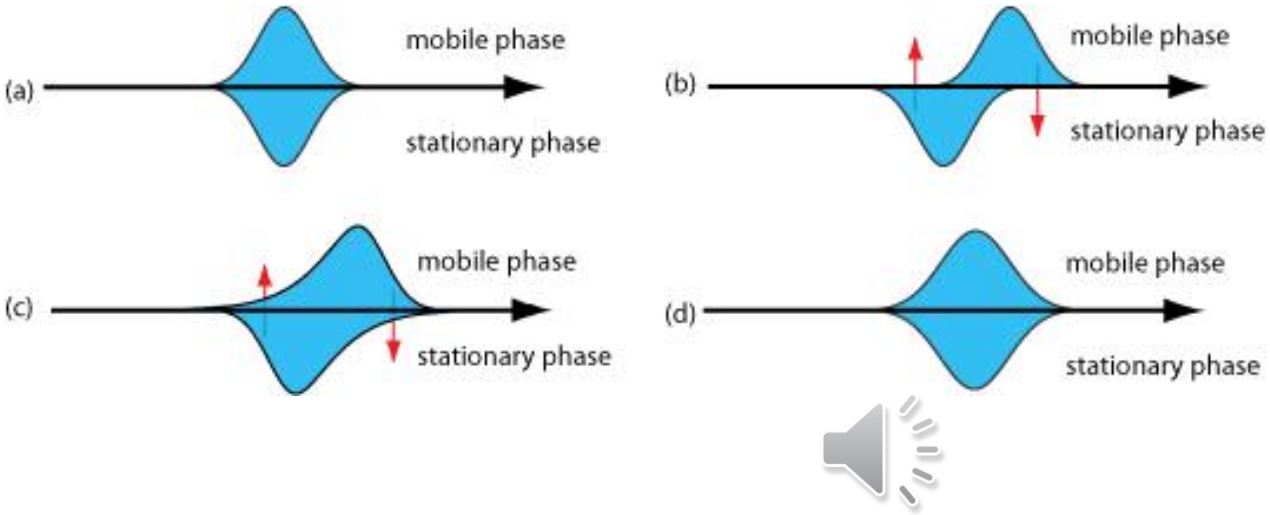
The degree of band-broadening due to eddy diffusion and mobile phase mass transfer depends mainly on:

1) the size of the packing material
2) the diffusion rate of the solute

$$H = A + \frac{B}{u} + Cu$$

Stationary phase mass transfer (C_s)

Due to the diffusion into the stationary phase.



Since different solute molecules spend different lengths of time in the stationary phase, they also spend different amounts of time on the column, giving rise to band-broadening.

The degree of band-broadening due to stationary phase mass transfer depends on:

1) the retention and diffusion of the solute
2) the flow-rate of the solute through the column
3) the kinetics of interaction between the solute and the stationary phase

Description of Band Spread

- Plate height (H) is proportional to band width
  - **The smaller the plate height, the narrower the band**

Van Deemter equation

$$H = A + \frac{B}{u} + Cu$$

Eddy diffusion — **Longitudinal Diffusion** — **equilibration time**

where: **μ = linear flow rate**
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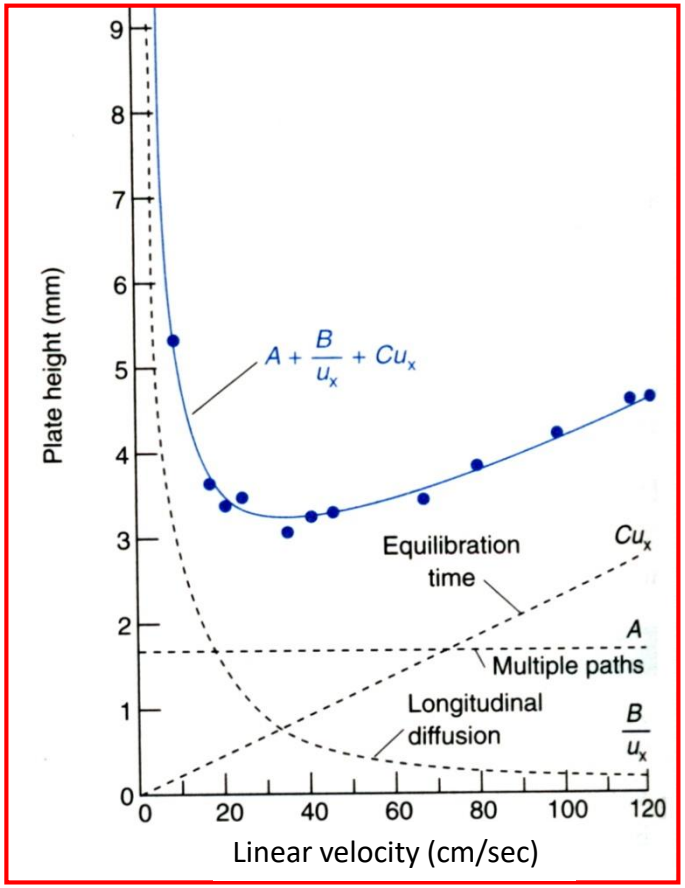
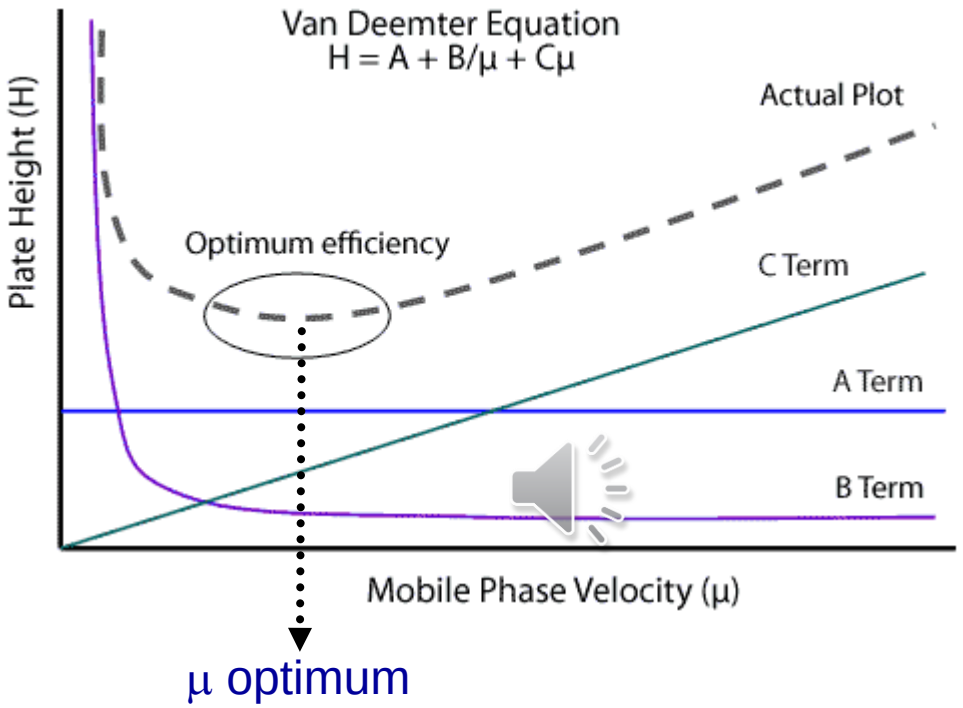


H-u curve

Plot of van Deemter equation shows how H changes with the linear velocity (flow-rate) of the mobile phase

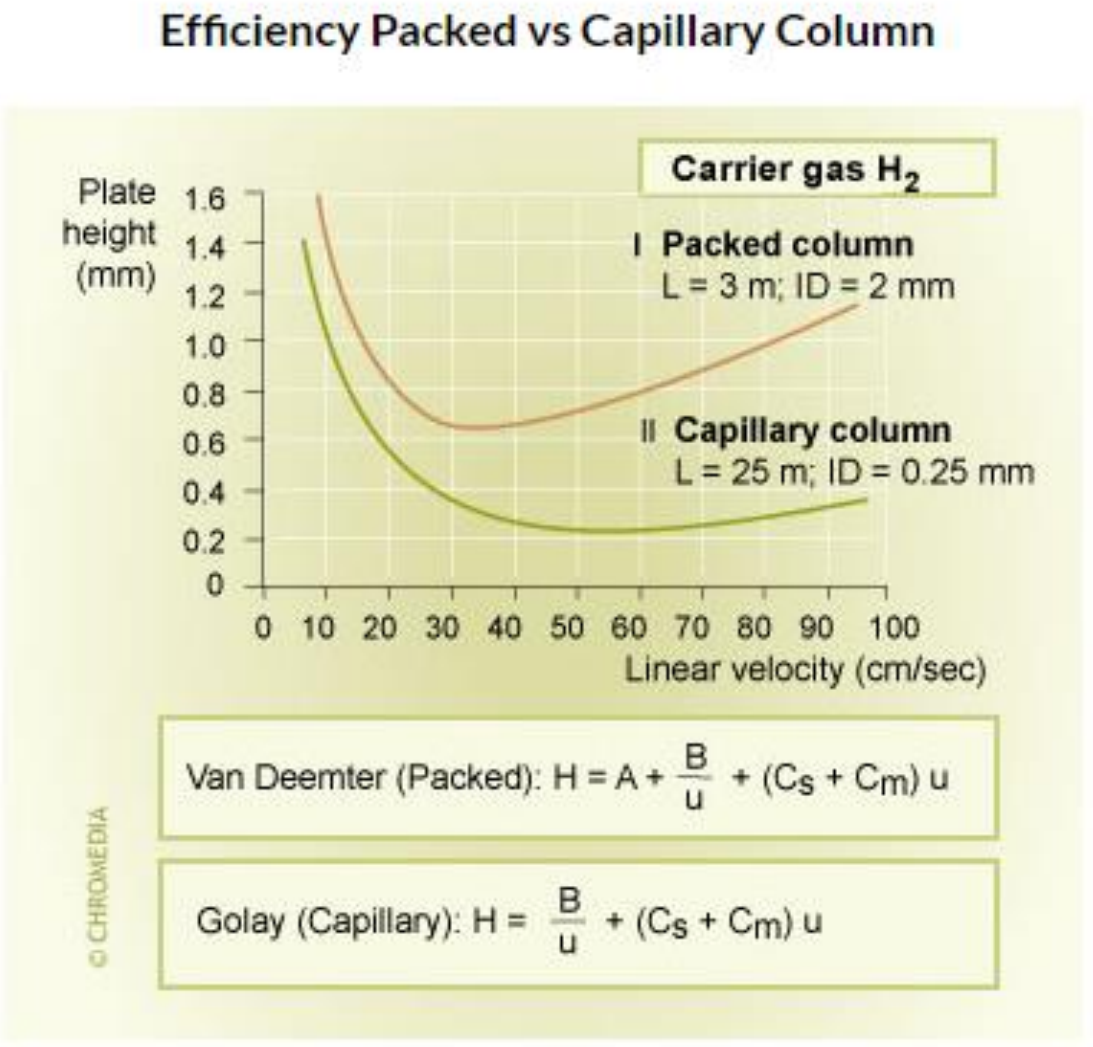


Optimum linear velocity (μ_{opt}) - where H has a minimum value and the point of maximum column efficiency:

$$\mu_{opt} = \sqrt{B/C}$$

μ_{opt} is easy to achieve for gas chromatography, but is usually too small for liquid chromatography requiring flow-rates higher than optimal to separate compounds

Packed column versus Capillary



Lower value of the Height of the Theoretical Plate (H) = better efficiency (*N*)

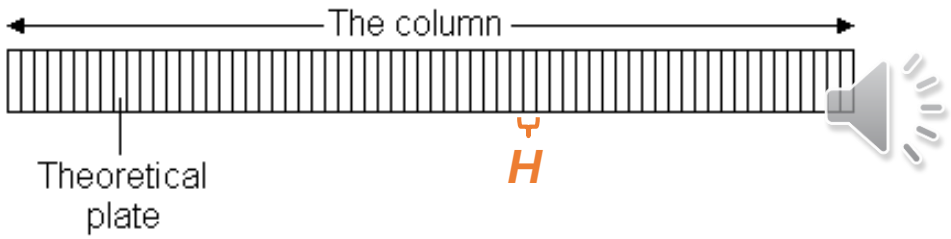
Chromatography

Measure of **Column Efficiency (N)**

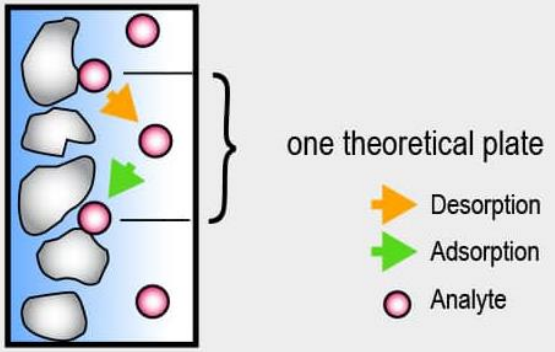
- Height Equivalent of a Theoretical Plate (H or HETP)
  - **The distance along the column that corresponds to one "theoretical" separation step or plate (N)**

$$N = L / H$$

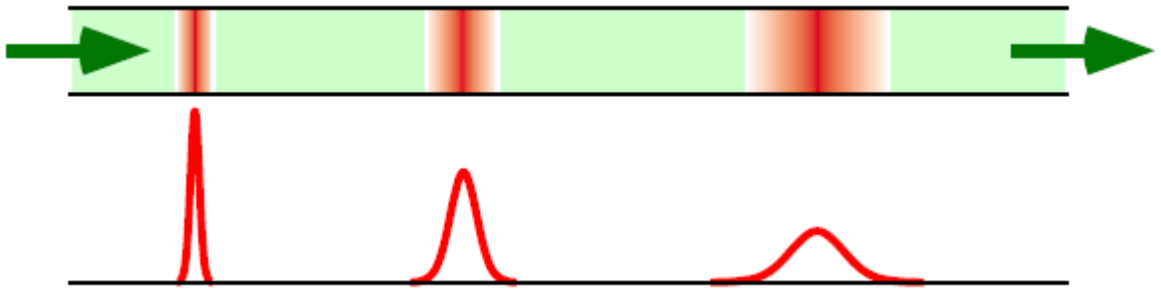
where: **L = length of column**
N = number of theoretical plates



The more theoretical plates available within a column, the more equilibrations between the stationary and mobile phases are possible and the better the quality of the separation.



- As *H* decreases, more separation steps per column length are possible
  - **Results in a narrower peak width and better separation between two neighboring solutes**



Chromatography

Measure of **Column Efficiency**

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

- Number of Theoretical Plates (N)
  - As N increase (number of separating steps) → greater the separation between two compounds

Efficiency is related *experimentally* to a solute's peak width.

- an efficient system will produce narrow peaks
- narrow peaks → smaller difference in interactions in order to separate two solutes

Efficiency is related *theoretically* to the various kinetic processes that are involved in solute retention and transport in the column

- determine the width or standard deviation (σ) of peaks

u_{min} HPLC << u_{min} GC

H_{min} HPLC << H_{min} GC

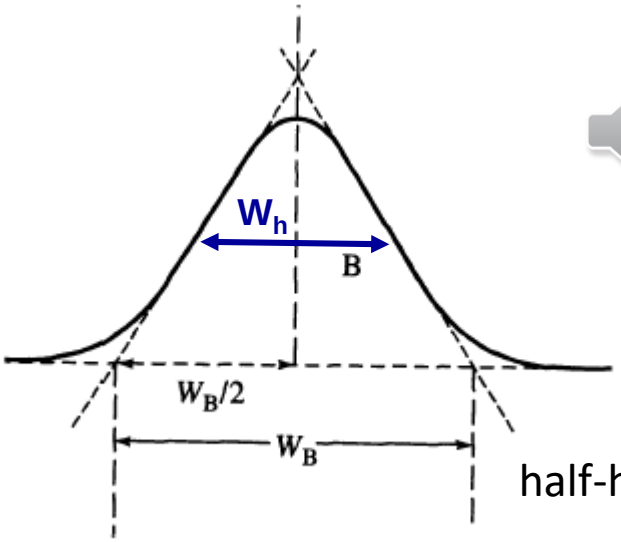
But L in HPLC ~ 5-25 cm et

L in GC ~ 50 m → $N_{GC} > N_{HPLC}$

https://www.youtube.com/watch?v=_ZPgkLQPuKI

Chromatography

Measure of **Column Efficiency**



half-height method

Estimate σ from peak widths, assuming Gaussian shaped peak:

$W_b = 4\sigma$

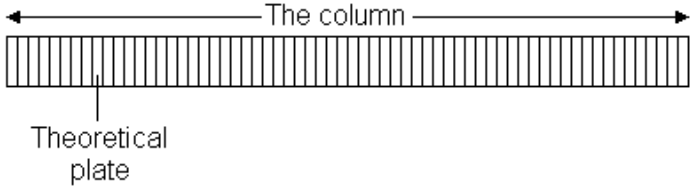
$W_h = 2.354\sigma$

Dependent on the amount of time that a solute spends in the column (k' or t_R)

$N = (t_R/\sigma)^2$

For a Gaussian peak:

$$N = 16\left(\frac{t_r}{w_b}\right)^2 = 5.55\left(\frac{t_r}{w_{1/2}}\right)^2$$



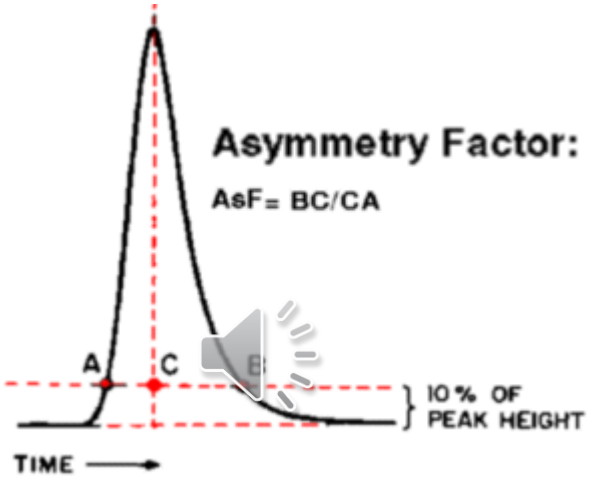
where: **w_b = baseline width of peak (in time units)**
$w_{1/2}$=half-height peak width

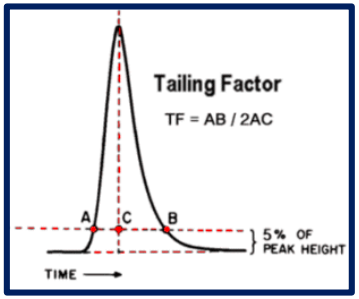
half-height method

Peak Asymetry

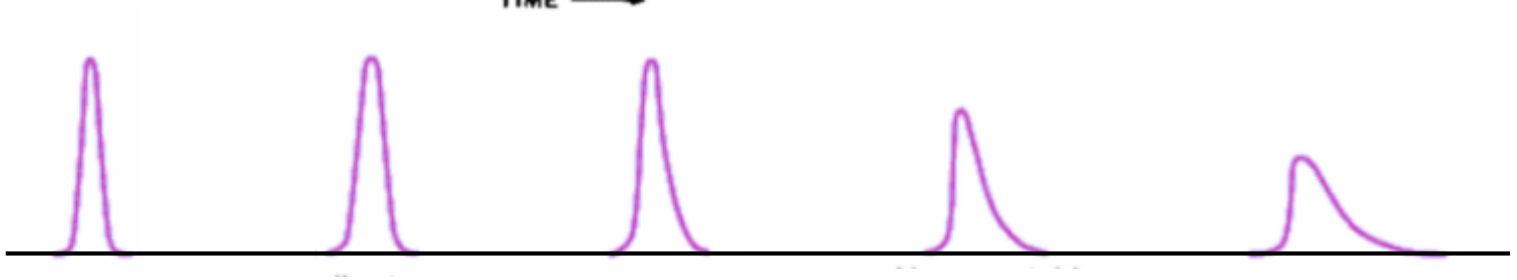
Asymetry measure

$$0.8 < A_s < 1.2$$





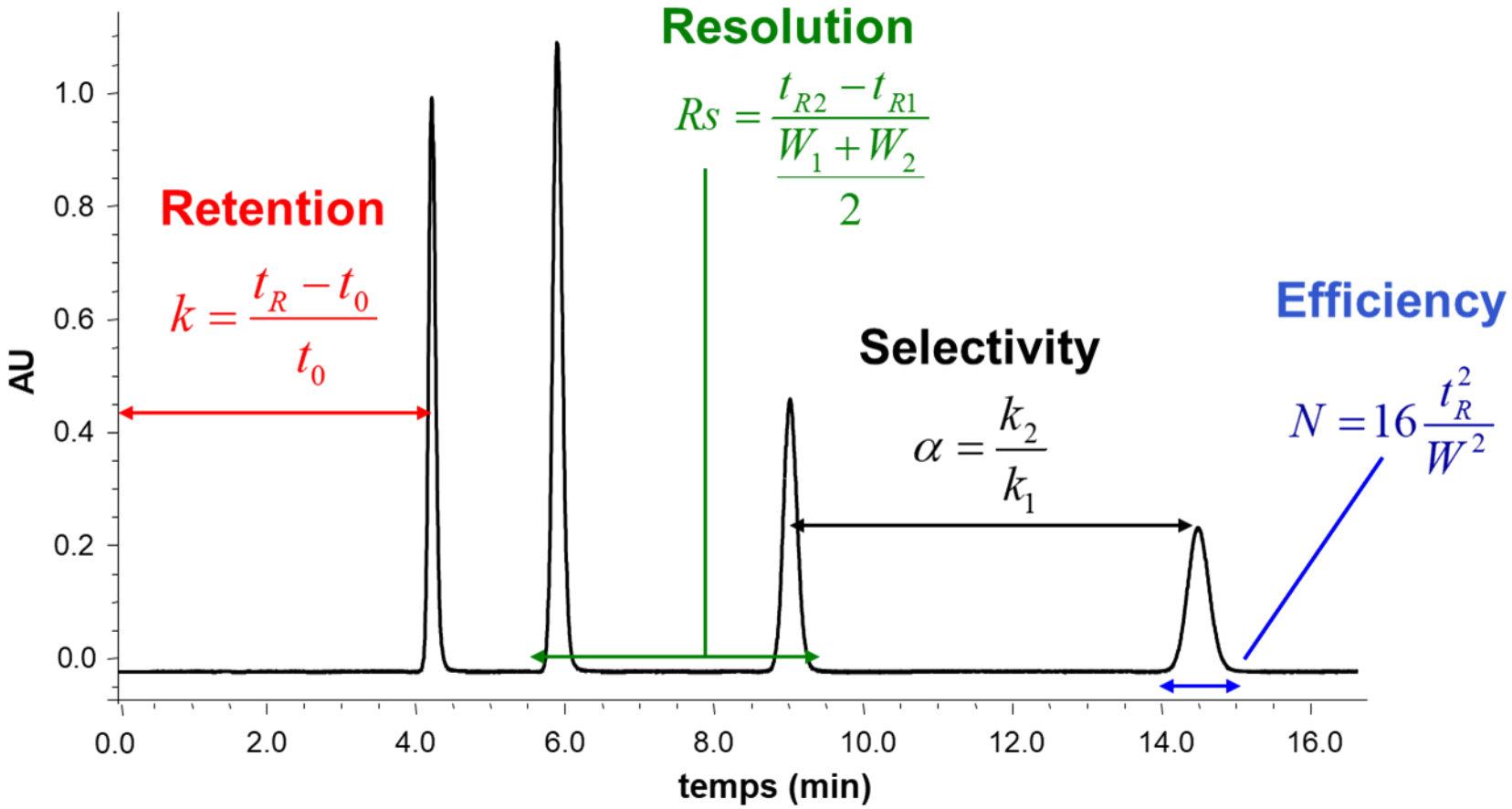
Example of asymetry



A_s=1.0 A_s=1.05 A_s=1.2 A_s=2 A_s=4

Excellent Good Unacceptable Very bad

Resolution, Selectivity, Retention and Efficiency



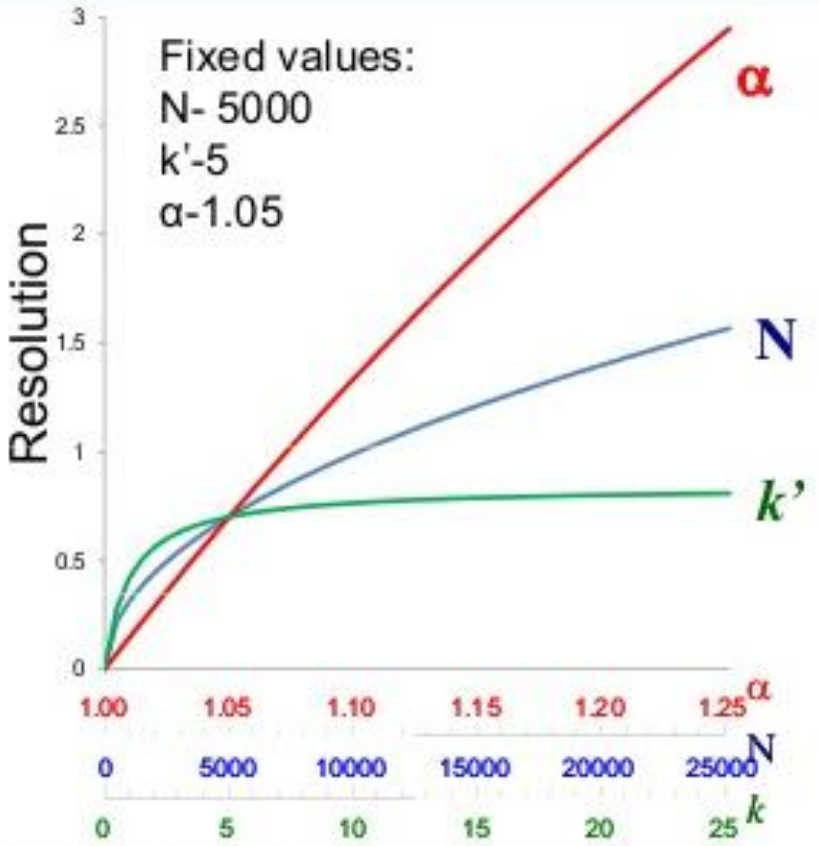
Resolution, Selectivity, Retention and Efficiency

Efficiency Retention Selectivity

$$R = \frac{\sqrt{N_2}}{4} \cdot \frac{k_2'}{k_2' + 1} \cdot \frac{\alpha - 1}{\alpha}$$

$$\alpha = \frac{k_2'}{k_1'}$$

Selectivity (α) has the greatest impact on improving resolution



Stationary phase, gradient delay volume, mobile phase, pressure / flow rate, temperature affect selectivity