Principles of Chromatography

Excellent resource: Quantitative Chemical Analysis
By Daniel C. Harris
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What is Chromatography?

Chromatography is the ability to **separate** molecules using **various partitioning** characteristics of molecule to remain in a stationary phase versus a mobile phase. Once a molecule is separated from the mixture, it can be isolated and quantified.

Can chromatography identify components?

Not without the detector – chromatography is **the process of separation**!

Why is chromatography called chromatography?

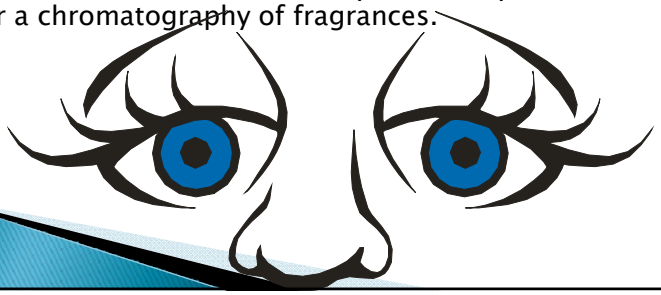
First application by M. S. Tswett 1903

For the separation of plant pigments. Since the components had different colors the Greek *chromatos*, for *color*, was used to describe the process.

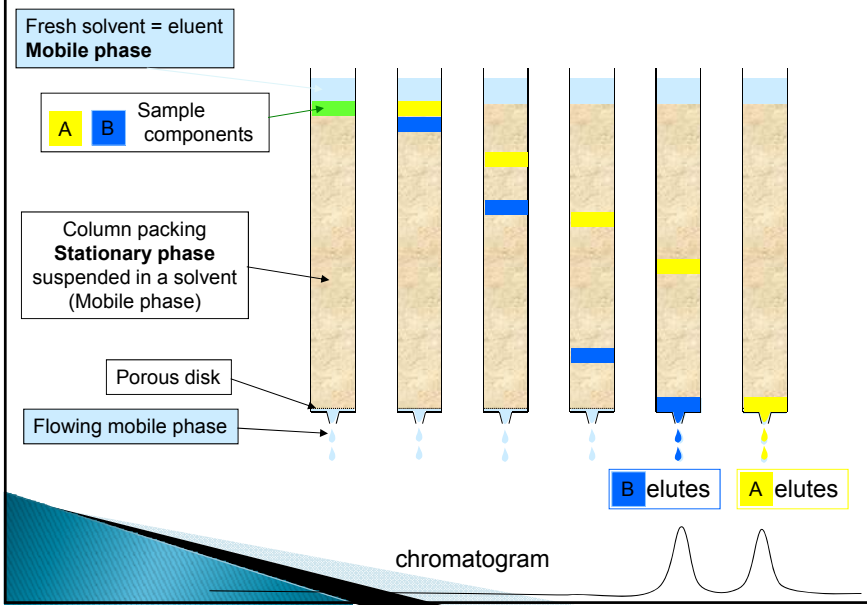
So, the detector was not needed?

IT WAS!!! YOU ALWAYS NEED A DETECTOR TO IDENTIFY chromatographically separated COMPONENTS.

In this case, the detector is an eye, Similarly, a nose can be used for a chromatography of fragrances.



Chromatographic Separation



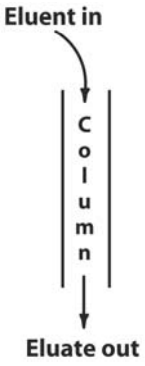
Chromatographic separation

Major components:

- Mobile phase flows through column, carries analyte.
  - Gas = Gas Chromatography (GC)
  - Liquid = Liquid Chromatography (LC), Thin Layer Chromatography (TLC)
  - Supercritical fluid = Supercritical Fluid Chromatography (SFC)
- Stationary phase stays in a place, does not move.
  - GC, LC placed inside of the column
  - TLC – layer of a sorbent on the plate
- The SEPARATION is based on the partitioning between the mobile and stationary phase.

Basic Chromatographic terminology

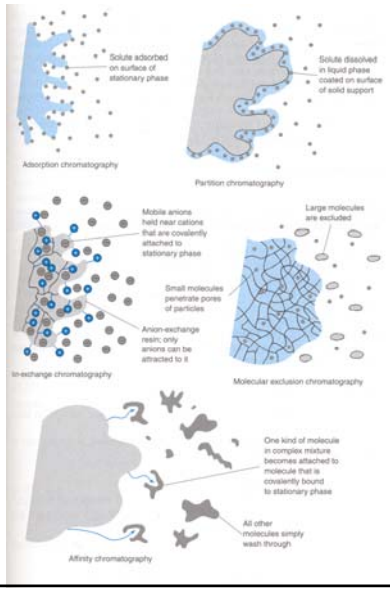
- **Chromatograph:** Instrument employed for a chromatography.
- **Stationary phase:** Phase that stays in place inside the column. Can be a particular solid or gel-based packing (LC) or a highly viscous liquid coated on the inside of the column (GC).
- **Mobile phase:** Solvent moving through the column, either a liquid in LC or gas in GC.
  Eluent: Fluid entering a column.
  Eluate: Fluid exiting the column.
- **Elution:** The process of passing the mobile phase through the column.
- **Chromatogram**: Graph showing detector response as a function of a time.
- **Flow rate:** How much mobile phase passed / minute (ml/min).
- **Linear velocity:** Distance passed by mobile phase per 1 min in the column (cm/min).

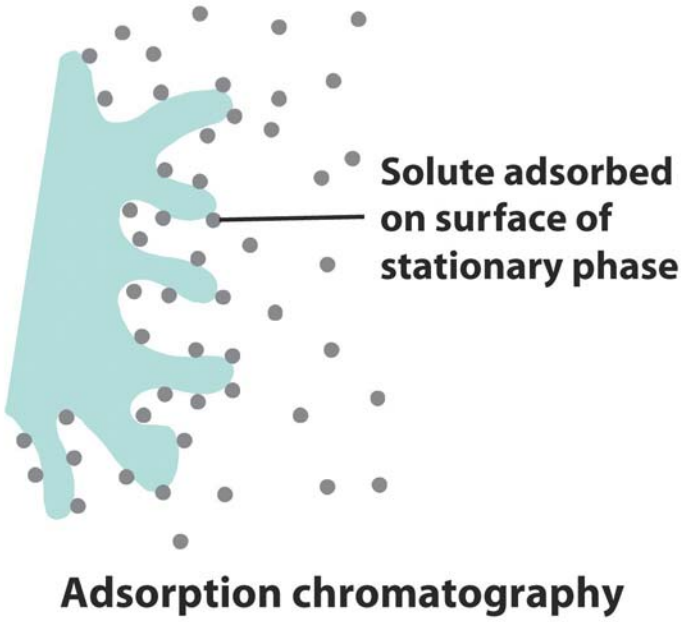


Types of chromatography on the basis of interaction of the analyte with stationary phase

- the interaction determines retention times of analytes

- **Adsorption** – of solute on surface of stationary phase; for polar non-ionic compounds
- **Ion Exchange** – attraction of ions of opposite charges; for ionic compounds anions or cations
- **Partition** - based on the relative solubility of analyte in mobile and stationary phases
- **Size Exclusion (gel filtration, gel permeation)** – separates molecules by size; sieving - not real interaction, small molecules travel longer
- **Affinity** – specific interactions like a particular antibody to protein

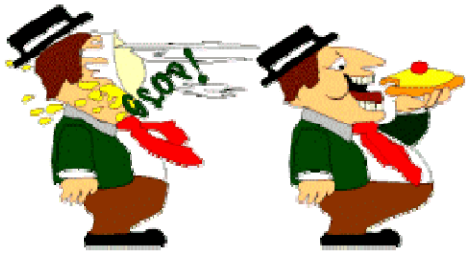




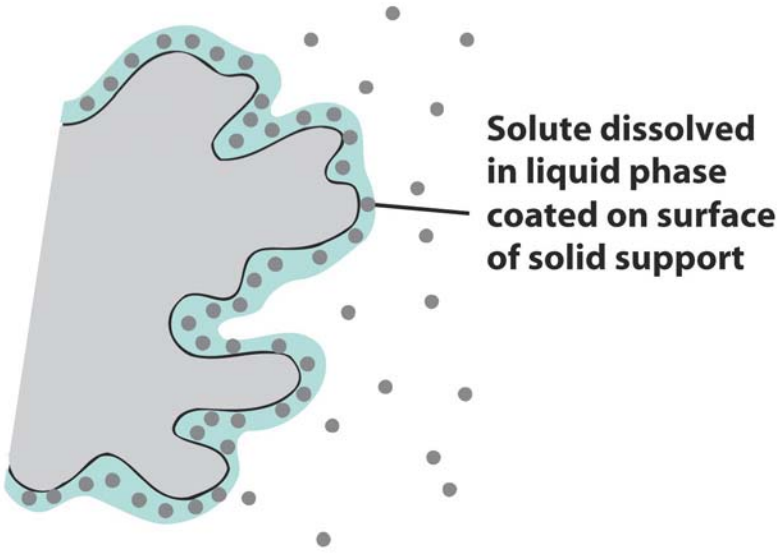
Difference between ad / ab - sorption

Adsorption / Absorption

Adsorption Absorption



Chemie für Mediziner Prof. J. Gasteiger et al.



Partition chromatography

Partition coefficient K

- Based on thermodynamic equilibrium

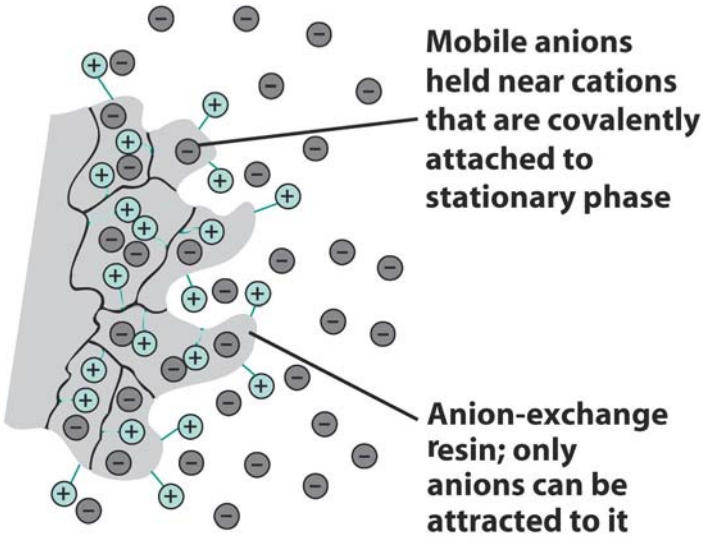
Ratio of Analyte

$$K = \frac{C_s}{C_m}$$

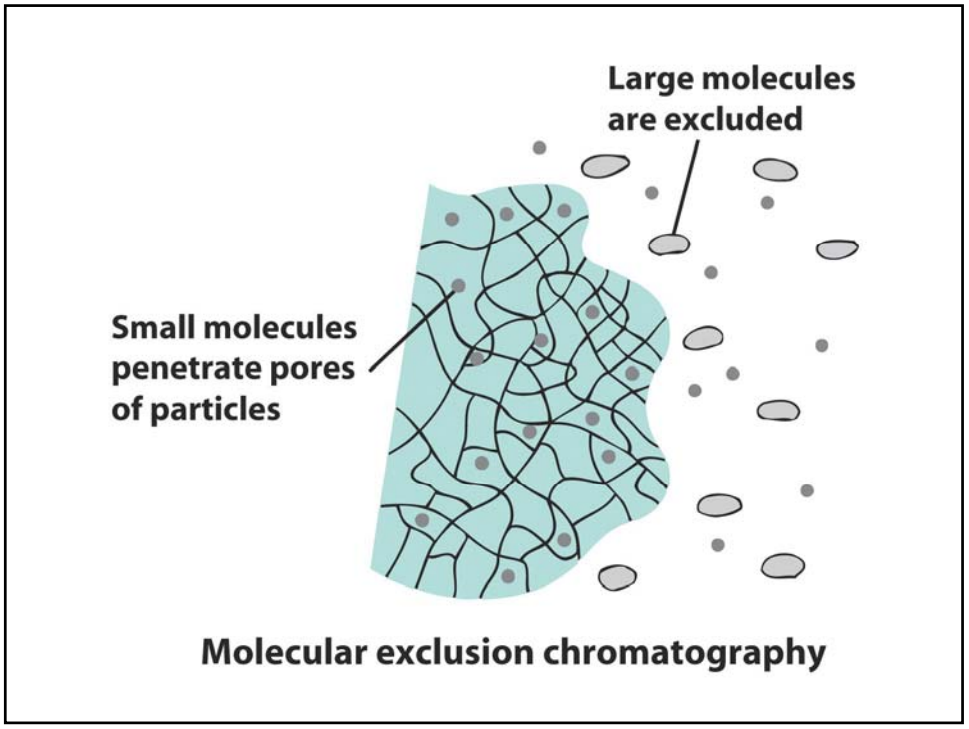
C_s: concentration in stationary phase

C_m: concentration in mobile phase

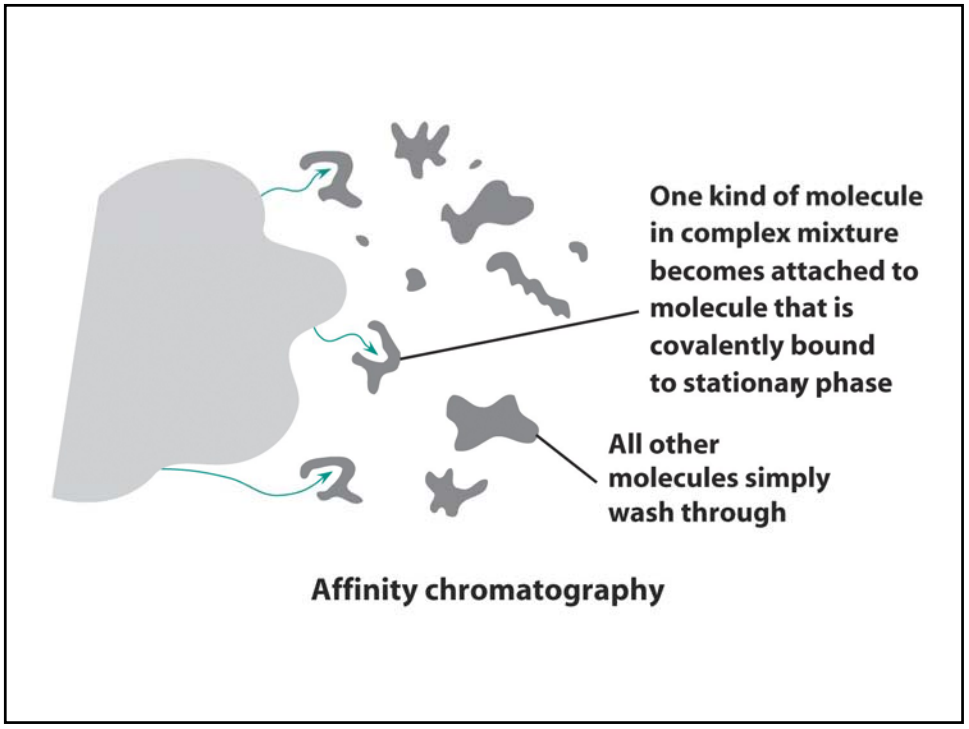
The same principle as Liquid Liquid Extraction



Ion-exchange chromatography



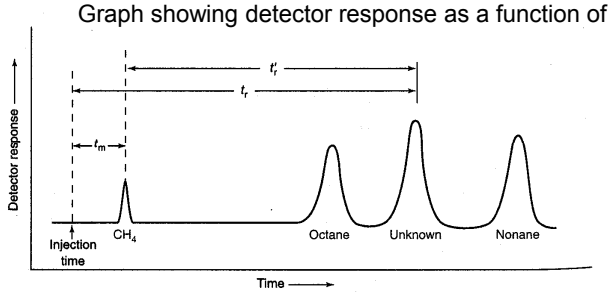
Molecular exclusion chromatography



Affinity chromatography

Chromatogram

Graph showing detector response as a function of elution time.



t_r retention time = time between injection and detection of the analyte.

t_m = time at which an unretained analyte or mobile phase travels through the column.

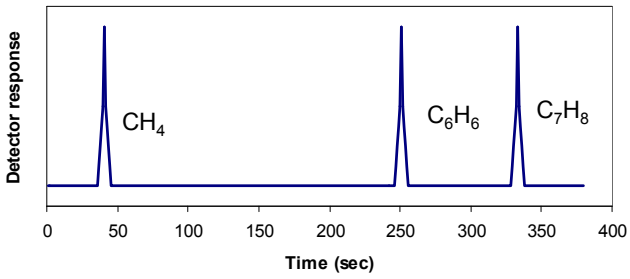
Adjusted retention time $t'_r = t_r - t_m$

Unadjusted relative retention $\gamma = t_{r2}/t_{r1}$

Relative retention (separation factor) $\alpha = t'_{r2}/t'_1$ a ratio of relative retention times $\alpha > 1$, indicates quality of the separation; $\uparrow\alpha$ = greater separation $\alpha = k_2/k_1$

Retention factor $k = (t_r - t_m)/t_m$ $\uparrow k$ = greater retention

FIND THE ADJUSTED RETENTION TIME AND THE RETENTION (CAPACITY) FACTOR FOR BENZENE AND TOLUENE ASSUMING THAT METHANE IS UNRETAINED.



Methane t_r = 42 s
Benzene t_r = 251 s
Toluene t_r = 333 s

t_m = time at which unretained analyte travels through the column Also $\bar{u} = L/tm$

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

Relative retention (Separation factor) $\alpha = t'_{r2}/t'_{r1} = k_2/k_1$

Retention factor $k = (t_r - t_m)/t_m$

Relationship between retention factor and partitioning coefficient

$$k = \frac{t_R - t_m}{t_m} \qquad K = \frac{C_S}{C_m}$$

$$k = \frac{\text{time analyte spends in stationary phase}}{\text{time analyte spends in mobile phase}} = \frac{\text{moles of analyte in stationary phase}}{\text{moles of analyte in mobile phase}}$$

$$k = \frac{C_S V_S}{C_m V_m} \qquad k = K\frac{V_S}{V_m}$$

$$\alpha = \frac{t'_{R2}}{t'_{R1}} = \frac{k_2}{k_1} = \frac{K_2}{K_1}$$

The greater ratio of partition coefficients the greater separation of two analytes

Scaling up from 2 to 20 mg

$$k = K\frac{V_S}{V_m}$$

Volume of the column = $\pi \cdot r^2 \cdot L = \frac{\pi \cdot d^2 \cdot L}{4}$

If column length is maintained

$$\frac{m_2}{m_1} = \frac{r_2^2}{r_1^2} \qquad \frac{F_2}{F_1} = \frac{r_2^2}{r_1^2}$$

From 2 to 20 mg, the initial separation d=1 cm
F = 0.3 mL/min

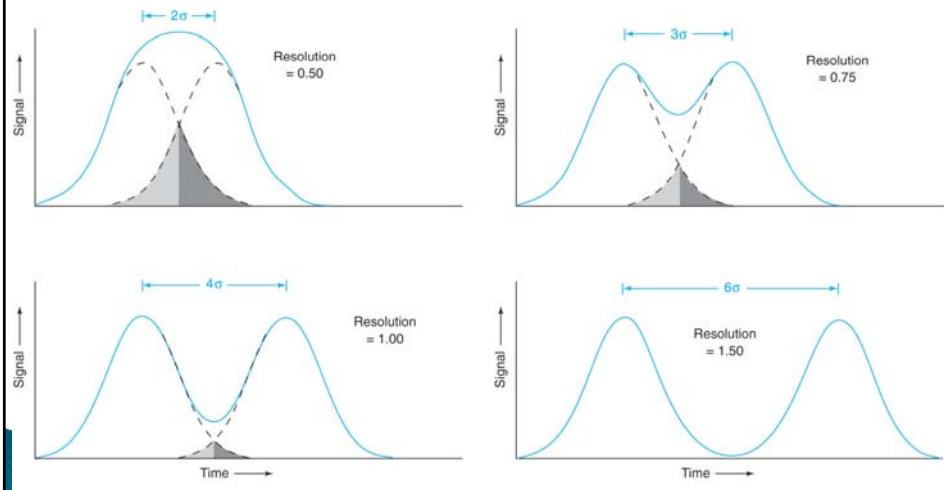
$$\frac{20}{2} = \frac{r_2^2}{0.5^2} \qquad r_2 \approx 3cm$$

F = 3 mL/min

Resolution of separation

Resolution of two peaks from one another = $\Delta tr/w_{av} = 0.589\Delta tr/w_{1/2av}$

We Want Resolution > 1.5



The separation is worse with the increasing peak width

Resolution of separation

A solute with a retention time of 5 min has a width of 12 s at the base.

A neighboring peak is eluted at 5.4 min with a width of 16 s.

What is the resolution for those two components?

Resolution of two peaks from one another = $\Delta tr/w_{av}$ We Want Resolution > 1.5

t_{r1} = 5*60=300 s t_{r2} =5.4*60=324 s w_{av}=(12+16)/2=14

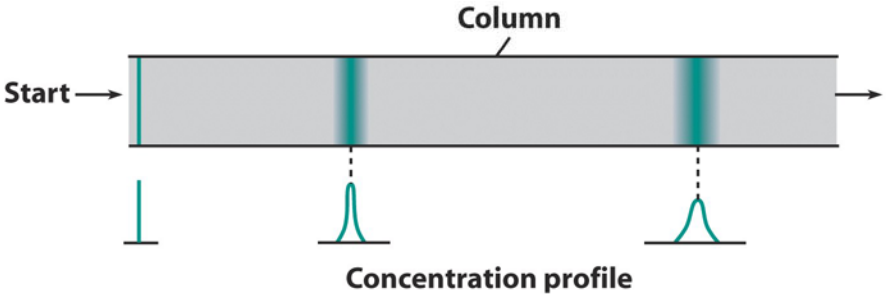
R = (324-300)/14=1.7

What happens if the peaks elute at 10 and 10.4 min with widths 16 and 20 s, respectively?

R = 24/18 = 1.33

Note the role of peak width in separation

So, why do peaks broaden?



Band width is proportional to the diffusion coefficient (D) of the molecule in the solvent and its elution time (tr).

HOW GOOD IS THIS COLUMN FOR SEPARATION?

Separation efficiency for certain compound is expressed by a number of theoretical plates (N).

$N = 5.55\ (tr/\ w_{1/2})^2$ sometimes also $N = 16\ (tr/\ w_b)^2$

$w_{1/2}$ is the width of the peak at half height
w_b is the width of the peak at the base (less precise)

Related parameter is the plate height H = Height equivalent to one theoretical plate (HETP)

$H = L/\ N$

where L is the column length

It allows to compare stationary phase of different columns.

The small height plate => narrow peaks => better separation

Compare column efficiencies

On a gas chromatographic column L= 30 m compounds elute in 5 min with $w_{1/2}$=5 s. What's a number of theoretical plates and what's the plate height?

$N = 5.55\ (tr/\ w_{1/2})^2$ **H = Length of column/ N**

NOTE they are inversely proportional!

$N=5.55 \times (300/5)^2=19980$ $H=30 \times 10^3/19980=1.5$ mm

- On a liquid chromatographic column L=25 cm, compounds elute in 5 min with $w_{1/2}$=5 s. What's a number of theoretical plates and what's the plate height?

$N=5.55 \times (300/5)^2=19980$ $H=250/19980=1.2 \times 10^{-2}$ mm

The smaller height plate, the narrower chromatographic band, better separation !!!!

Factors affecting resolution

$$R = \frac{\sqrt{N}}{4}(\gamma - 1) = \frac{\sqrt{N}}{4}(\frac{t_{r2}}{t_{r1}} - 1)$$

$$H = \frac{L}{N}$$

Increasing the column length 2x will improve resolution √2

Van Deemter Equation

tells us how the column and flow rate affect the plate height (i.e., peak broadening).

$$H \sim A + B/\bar{u} + C\bar{u}$$

We want H to be low = so all the parameters A,B, and C should be as low as possible

ū is average linear velocity (cm/s)

A multiple pathways, diffusion through packed column (is eliminated in GC)

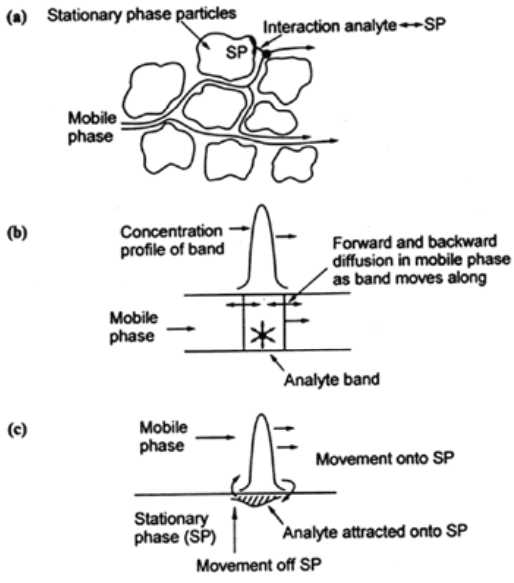
B longitudinal diffusion (molecular diffusion)
GC bigger molecule of gas used as a mobile phase, the bigger B
LC more viscous mobile phase => bigger B

C mass transfer – transfer of the analyte in and out of stationary phase,
faster is the interaction between analyte and stationary phase means smaller C

The smaller height plate, the narrower chromatographic band, better separation

$$H \sim A + B/\bar{u} + C\bar{u}$$

H ~ Multiple paths (A) + Longitudinal Diffusion (B/u_x(linear flow rate)) + Mass transfer (C^*u_x)



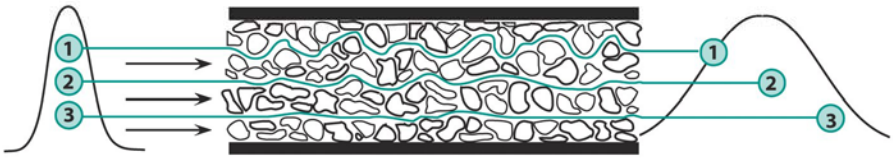
Van Deemter Equation

For packed columns, A is a problem with **non-homogenous** particles as a **packing**. A reduces with a smaller homogenous packing and a smaller particle size
This is not a problem for GC.

Diffusion along axis ↓ by ↑ flow rate is balanced by a back pressure of a column for LC. B is reduced with smaller diameter packings.

Related to **transfer of solute between phases**. ↑ N with ↑ temp. It is represented by practical problems such as sample and column degradation.

Band spreading on packed columns



van Deemter equation for plate height:

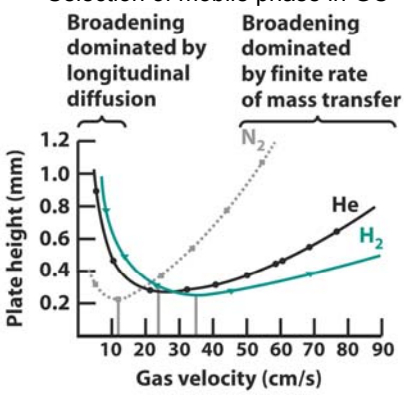
$$H \approx A + \frac{B}{u} + Cu$$

Multiple paths | Longitudinal diffusion | Equilibration time

van Deemter Equation in Respect to Chromatographic Conditions

We always want the plate height low, H↓

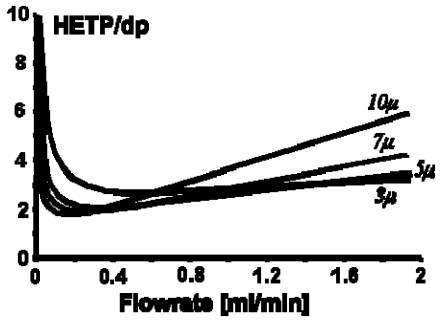
Selection of mobile phase in GC



Hydrogen can operate at most flow rates.
Hydrogen is explosive, therefore helium is preferred.

Van Deemter equation

Selection of stationary phase in HPLC
size of particles to fill columns



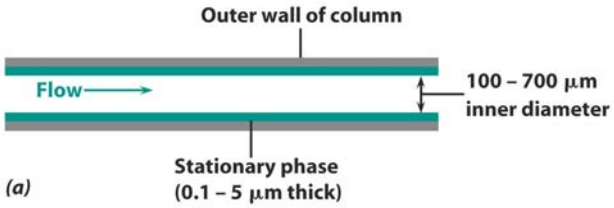
Smaller particle size is better.

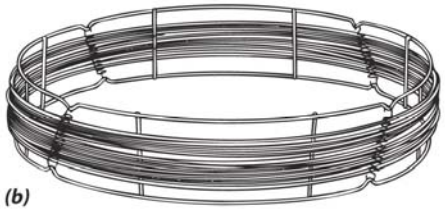
van Deemter equation for plate height:

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

Multiple paths — Longitudinal diffusion — Equilibration time

Open tubular columns





van Deemter equation for plate height:

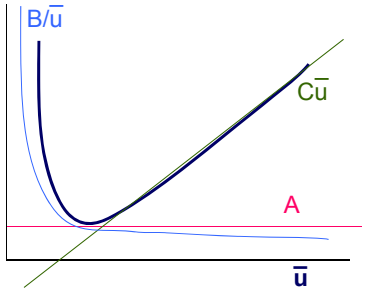
$$H \approx \cancel{A} + \frac{B}{u} + Cu$$

Multiple paths | Longitudinal diffusion | Equilibration time

Draw van Deemter curve

$H \sim A + B/\bar{u} + C\bar{u}$

- **Label axes**
- **Explain H and $\bar{u}$**
- **Explain what are the parameters A, B, and C and how they affect separation efficiency of the column.**
- **Can you show which part of curve is affected by which parameter**



H=L/N
length/per number of plates

Linear velocity of mobile phase