

Chapter 23 Introduction to Analytical Separations

continued

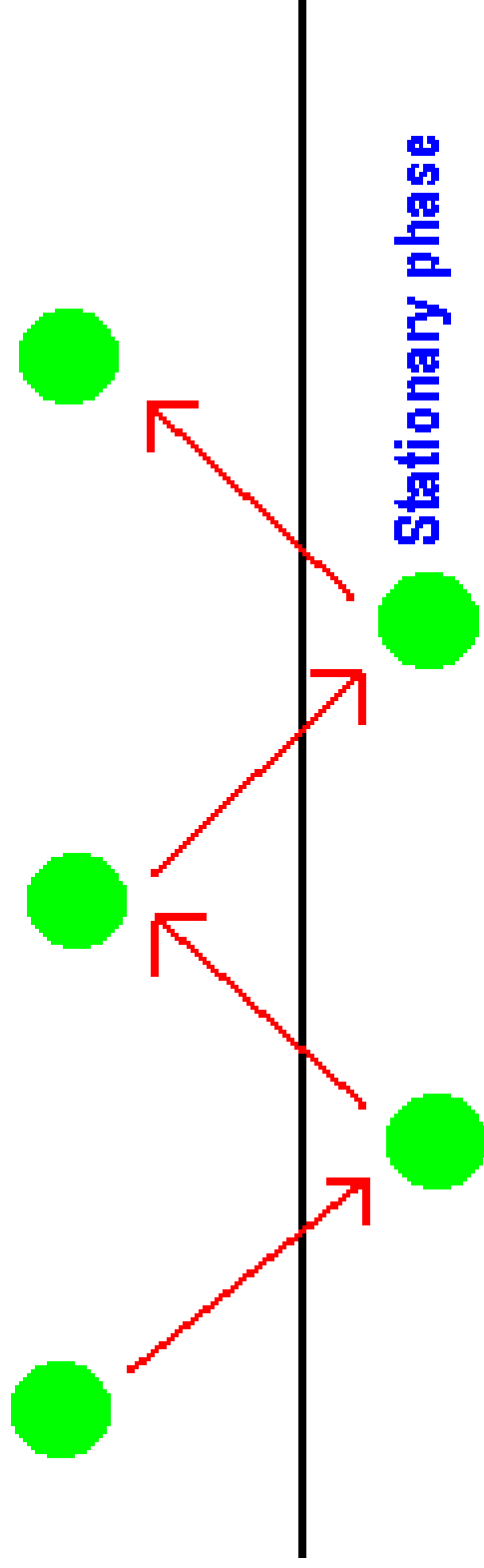
What is chromatography?

The separation of a mixture by distribution of its components between a mobile and stationary phase over time

- Mobile phase - phase that moves through chromatograph
 - gas (GC)
 - water (LC)
 - organic solvent (LC)
 - supercritical fluid (SCFC)
- Stationary phase - column; phase that is stationary in chromatograph that has a bonded phase with reactive groups imparted to stationary phase in order to achieve selectivity

A simple conceptual model of dynamic interactions in chromatographic separations

Mobile phase =====>



***Degree of retention is determined by
the relative amounts of time the
analyte spends in each phase***

Review of stationary phase – attractive forces

- Adsorption - for polar non-ionic compounds
- Ion Exchange - for ionic compounds
 - Anion - analyte is anion; bonded phase has positive charge
 - Cation – analyte is cation; bonded phase has negative charge
- Partition - based on the relative solubility of analyte in mobile and stationary phases
 - Normal – analyte is nonpolar organic; stationary phase MORE polar than the mobile phase
 - Reverse – analyte is polar organic; stationary phase LESS polar than the mobile phase
- Size Exclusion - stationary phase is a porous matrix; sieving

Mobile phase - phase that moves analyte along the solid phase and through the chromatograph

Mobile phases:

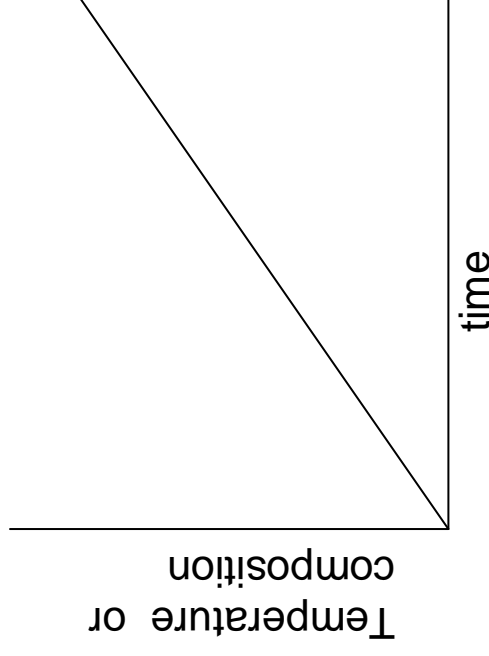
- gas (GC)
- water (LC)
- organic solvent (LC)
- supercritical fluid (SCFC)

How to affect the partitioning (interactions) in the mobile phase:

- temperature (GC)
- gradient (mix) of solvents (LC)

Gas mobile phase:

- Temperature is the most conveniently manipulated variable that controls/affects retention

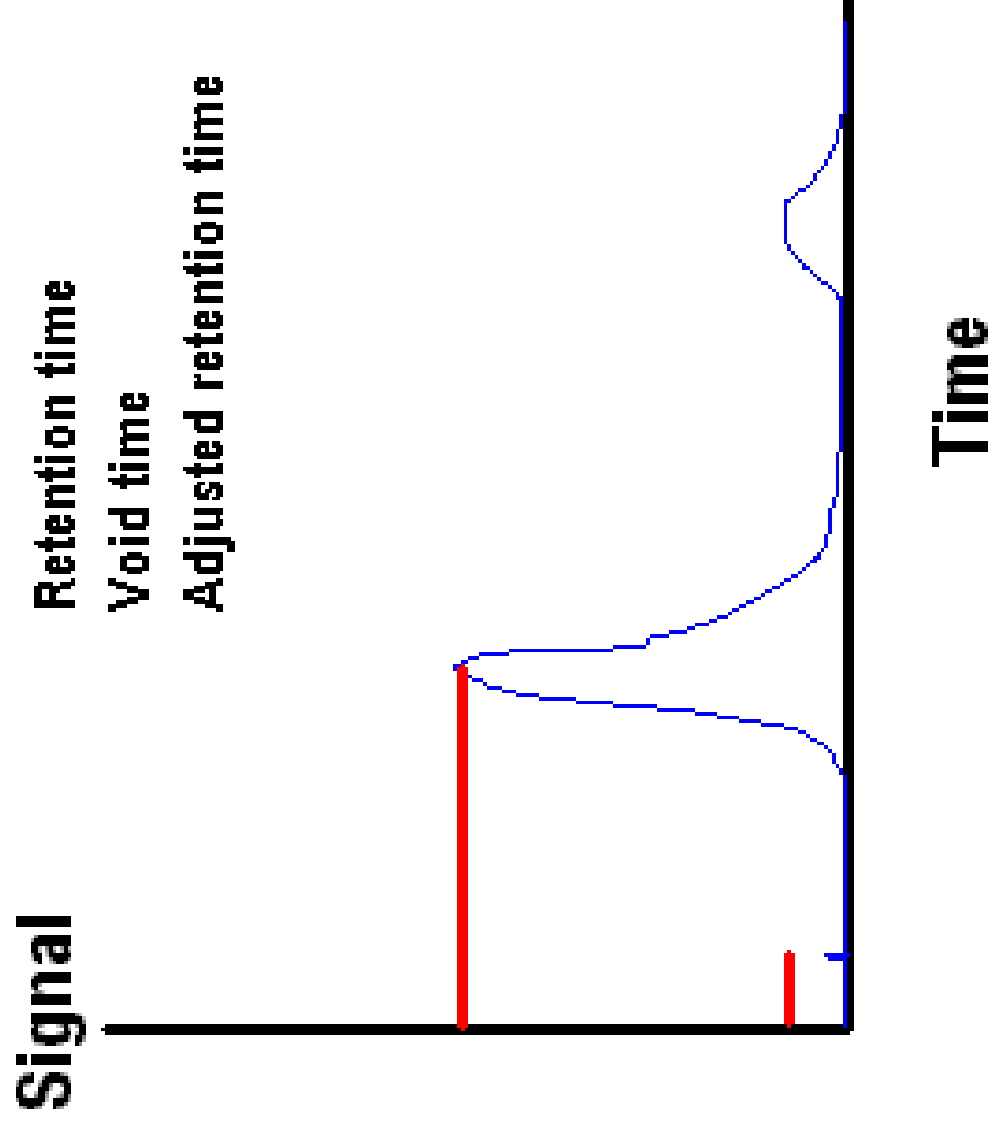


- Use temperature programming to optimize chromatography for solutes of varying boiling points

Liquid mobile phase:

- Single solvent used as mobile phase (isocratic elution)
- Two or more solvents used as the mobile phase by continuously changing the solvent composition to improve the interaction with the mobile phase (gradient elution)

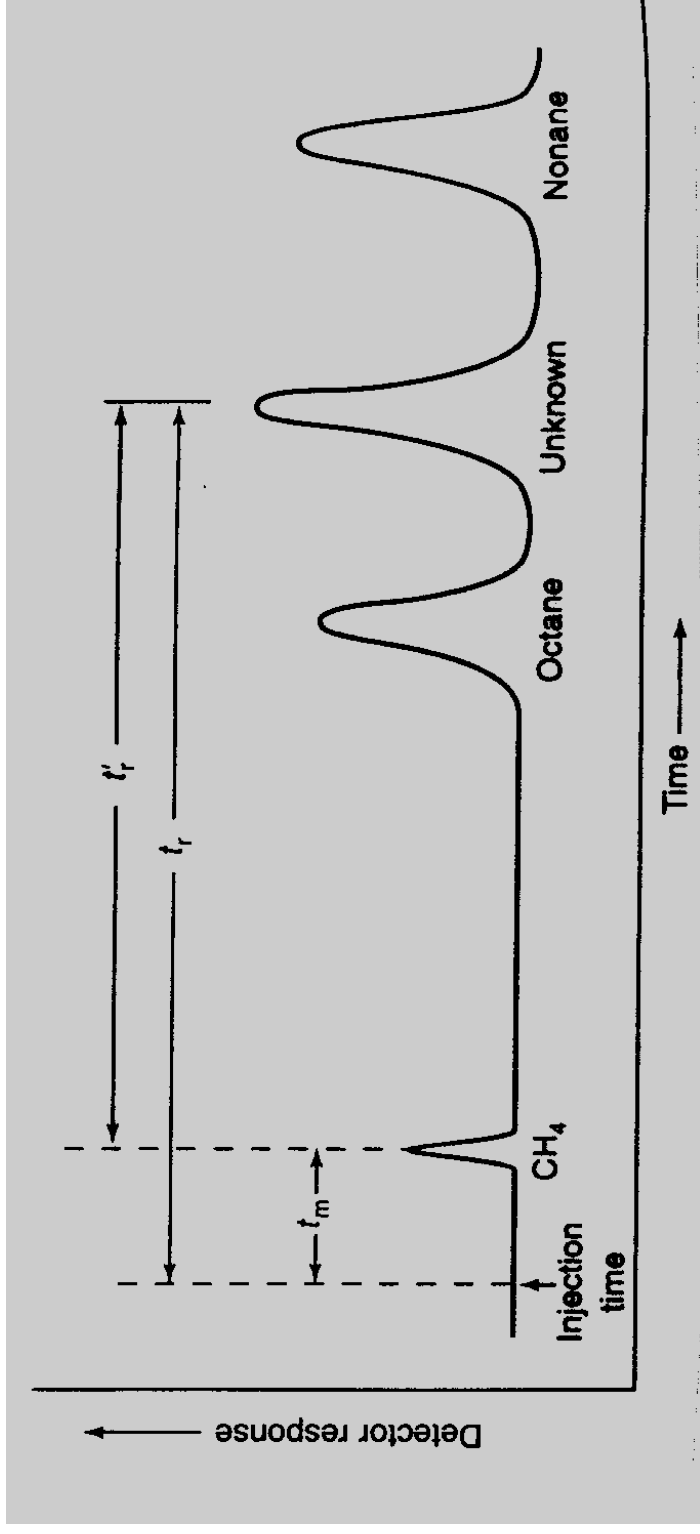
A chromatogram



Retention and the capacity factor, k'

$$k' = K (V_{\text{stationary}} / V_{\text{mobile}}) = (t_R - t_m) / t_m$$

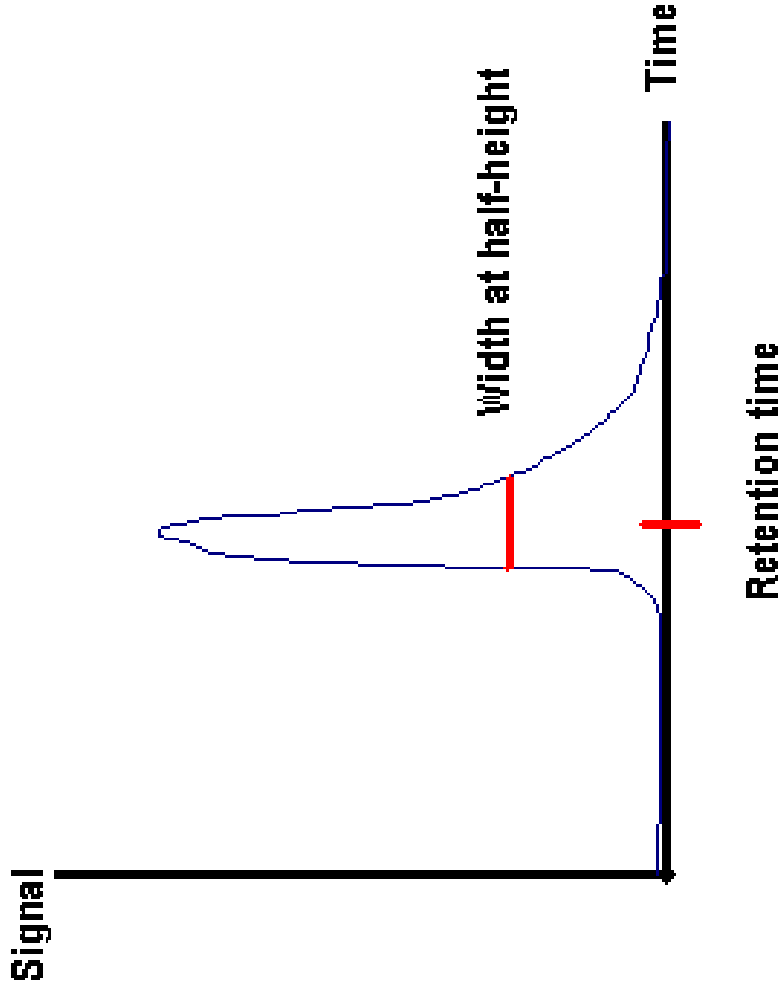
where K is the partition coefficient, V is volume, and t is retention time



Peak quality: what do we want?

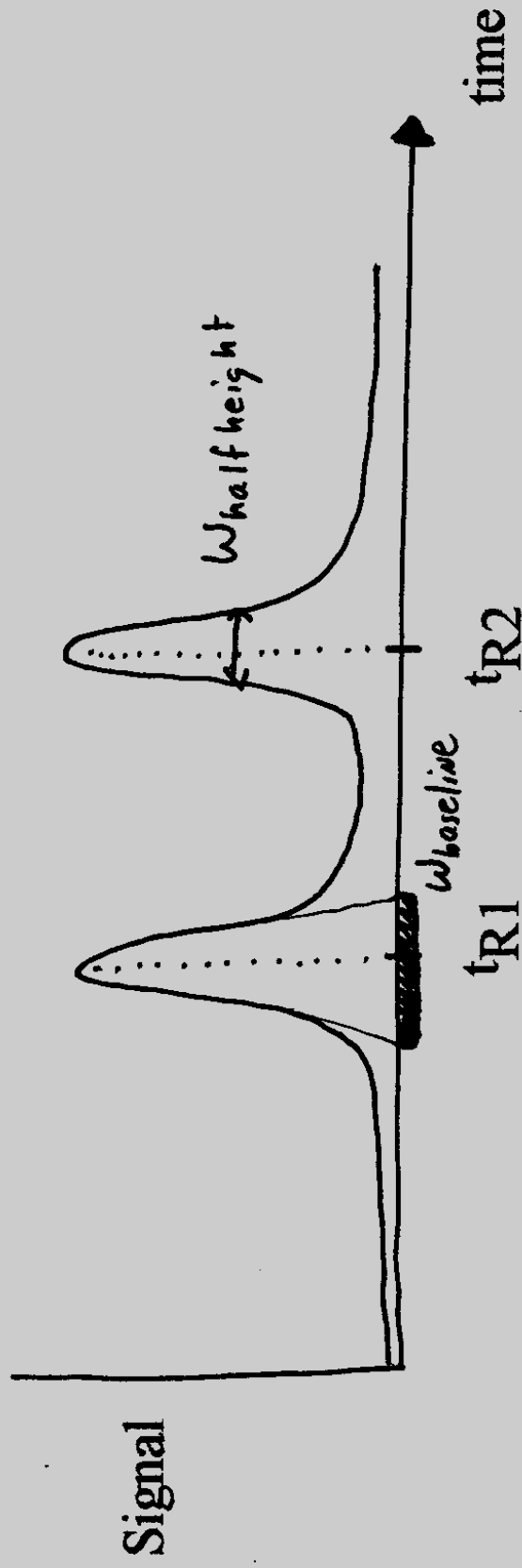
The quality of a chromatographic peak is determined by

- Kinetics
- Heterogeneous retention sites
- Mass transport
- Bed packing



Definition of resolution of two bands

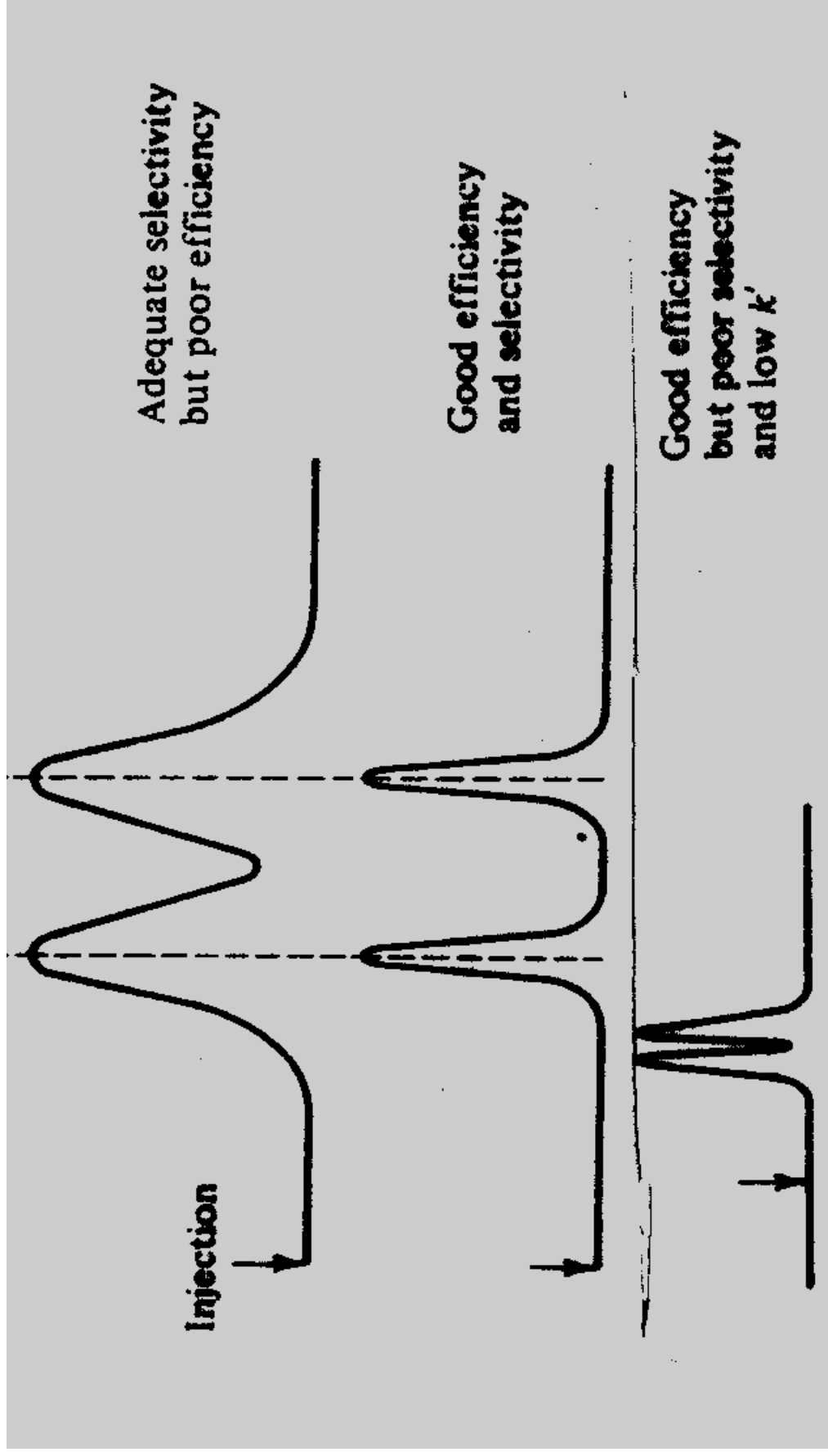
$$\text{Resolution} = (t_{R2} - t_{R1}) / 0.5(w_1 + w_2)$$



$$\text{Resolution} = \Delta t / w_{\text{ave}} = (t_{r2} - t_{r1}) / 0.5 (w_1 + w_2)$$

where: t = retention time w = peak width

Good vs. bad chromatography



Describe quality of chromatographic separation by the *theoretical plates*

- *One theoretical plate corresponds to the length of stationary phase required for one “equilibration” or “extraction step” of the solute between the stationary and mobile phase*

One theoretical plate is equivalent to one extraction

- *There is no “plate” but relates the width of a band of solute to the distance it travels in the column.*

- *The smaller the plate height, the narrower the band width of the peak, the better the separation.*

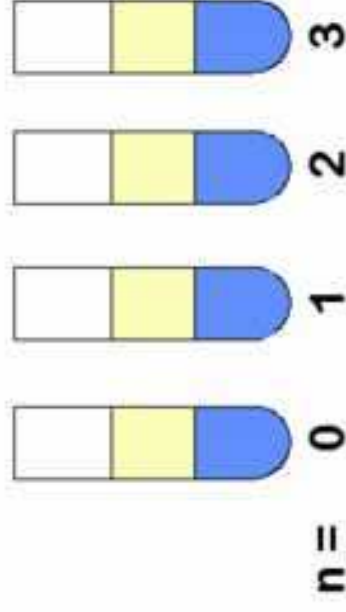


Plate Height:

width of band / length of column

Number of plates on column:

$$(\text{retention time})^2 / (\text{peak width})^2$$

where *retention time* is related to the length of the column

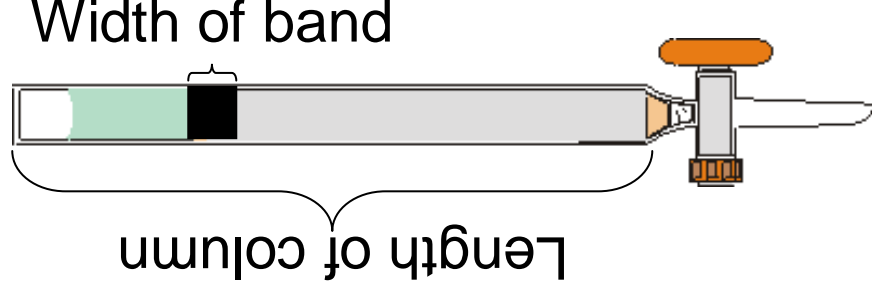
So...

For the same length column

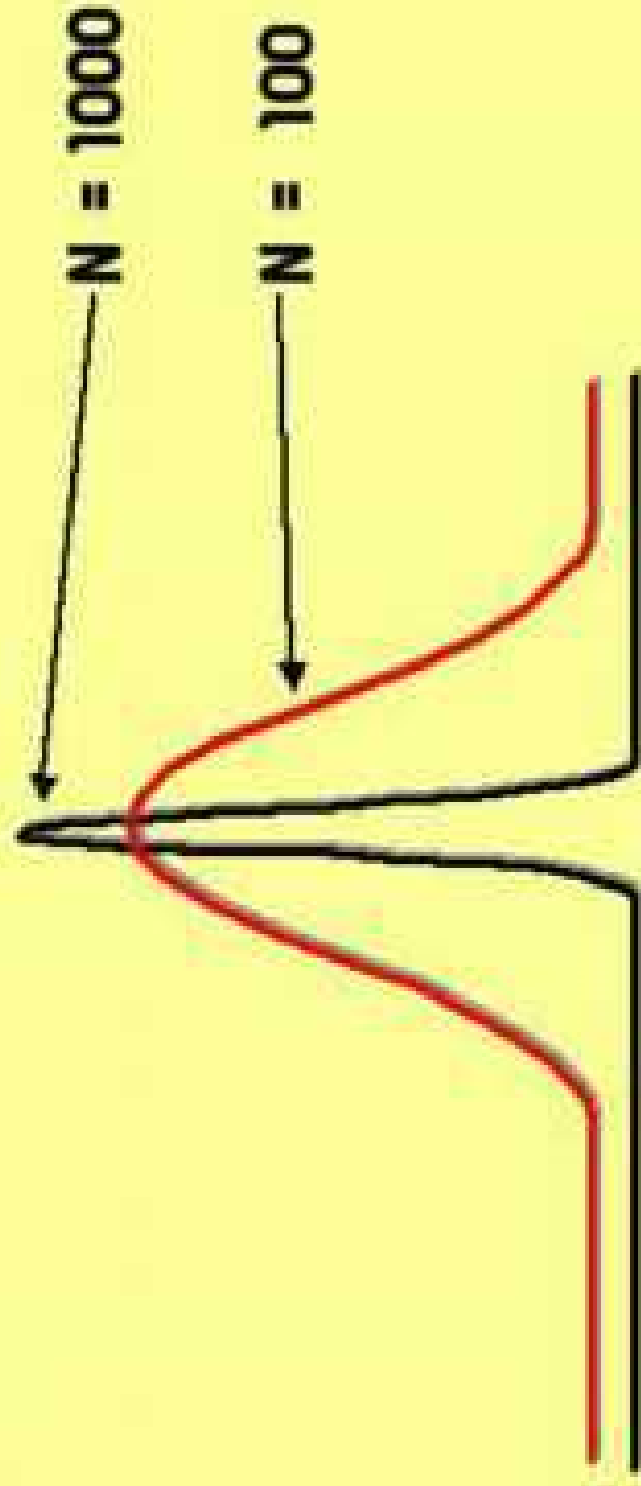
the smaller the plate height

the more theoretical plates present in the column,
the more extraction steps

THE BETTER THE SEPARATION!!!



In this example, we have materials with the same elution time but different numbers of plates.



<http://ull.chemistry.uakron.edu/analytical/Chromatography/>

Van Deemter Equation –

Calculation of theoretical plate height

$$H = A + B/u_x + Cu_x$$

u_x = linear flow of mobile phase

A = Multiple paths or eddy diffusion

B = Longitudinal diffusion

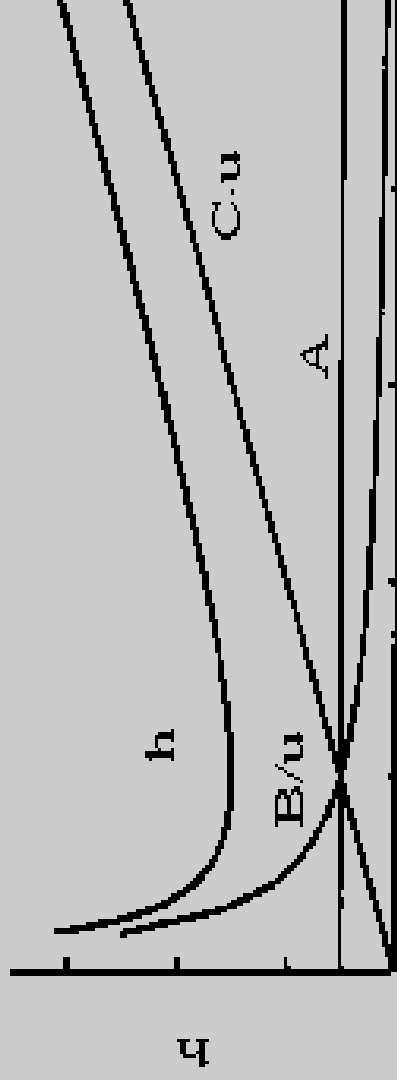
C = Equilibration time (for partitioning)

In order to optimize separation, you don't have to calculate H, but understand how different physical phenomenon contribute to H so you can optimize flowrate, type of column, etc.

Van Deemter Equation :

(R: Van Deemter, et. al. *Chem. Eng. Su.*, 1956, 5, 271)

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

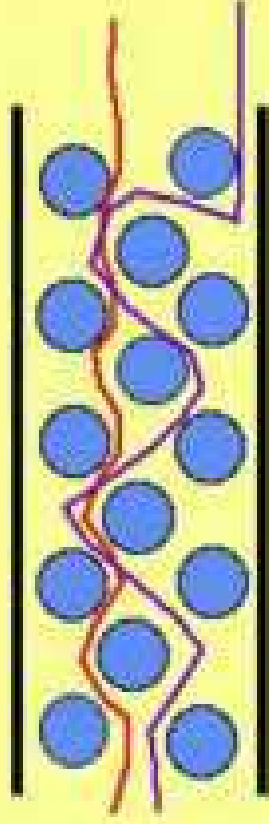


Graph of the van Deemter equation

$$H = \frac{A}{u} + B/ux + Cux$$

A term – multipath or eddy diffusion

This term accounts for the effects of packing size and geometry.



The range of possible solute paths results in a minimum peak width.

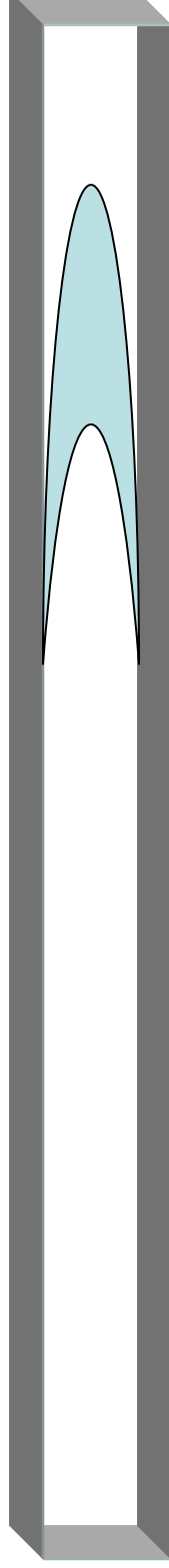
Can affect A term by better packing of column (no deadspace or loose packing) and packing with small diameter material.

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

B term – longitudinal diffusion

Broadening due to diffusion in the mobile phase

- The analyte diffuses from the concentrated region in the center to less concentrated regions (LeChatelier's principle).
- The greater the flow rate, the less time is spent in the column and the less time the analyte has to diffuse to less concentrated regions.

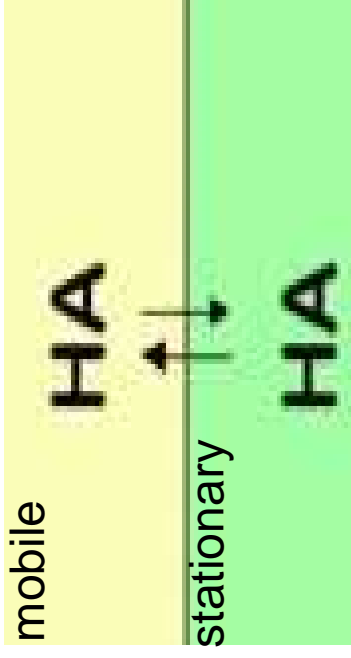


$$H = A + B/u_x + C u_x$$

C term – equilibration time (partitioning)

Finite time required for the analyte to equilibrate between the mobile and stationary phases

- Thick or viscous stationary phases require longer equilibration times for the analyte.
- Minimize the C term by having thin layer and/or less viscous stationary phases and slowing down the flow rate to allow the analyte time to equilibrate.



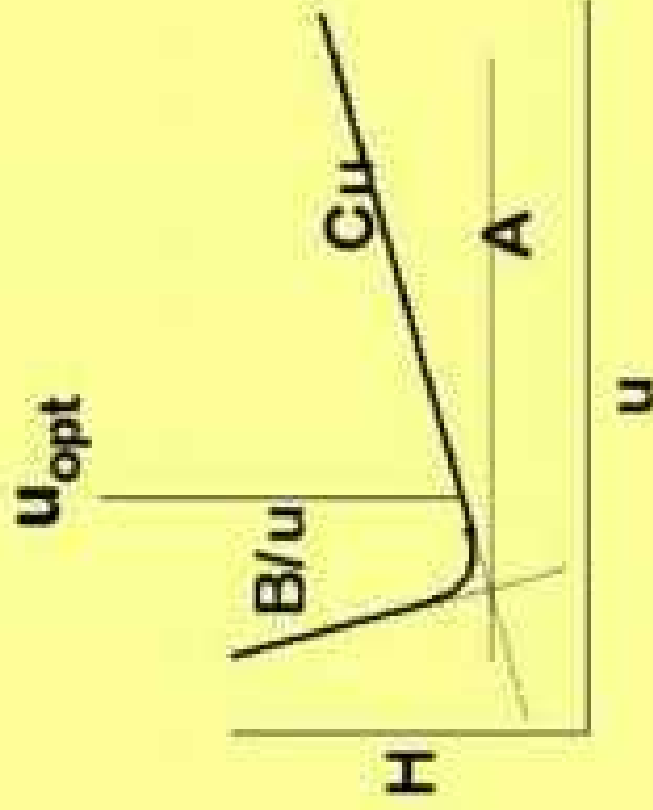
Consequences of Van Deemter Equation

- **Flow too high:** separation is encumbered by the C term - mass transport rate limits establishment of equilibrium
- **Flow too low:** separation is encumbered by the B term - diffusion in longitudinal and axial directions causes excessive “spreading” (dispersion) of solute band
- **Minimize the A term:** need good packing or open tubular column

OPTIMIZE FLOW RATE

The best velocity is a function of the Van deemter equation and practical conditions. You need to have a useable analysis time.

Also, since the effects of B are greater than C , it is best to set the flow a little on the high side in case it changes slightly during the analysis.



Detection and identification in chromatography

Chromatography is a separation process only!

“Matching retention times” does not constitute identification!

Three basic types of detectors

- **Positive identification:** IR, MS
- **Selective detectors**
 - Electron capture, photoionization (GC)
 - Fluorescence, amperometric (HPLC)
- **Peak-indicating** (non-selective...universal)
 - Thermal conductivity, flame ionization (GC)
 - Refractive index, UV absorption, evaporative light scattering, conductivity (HPLC)