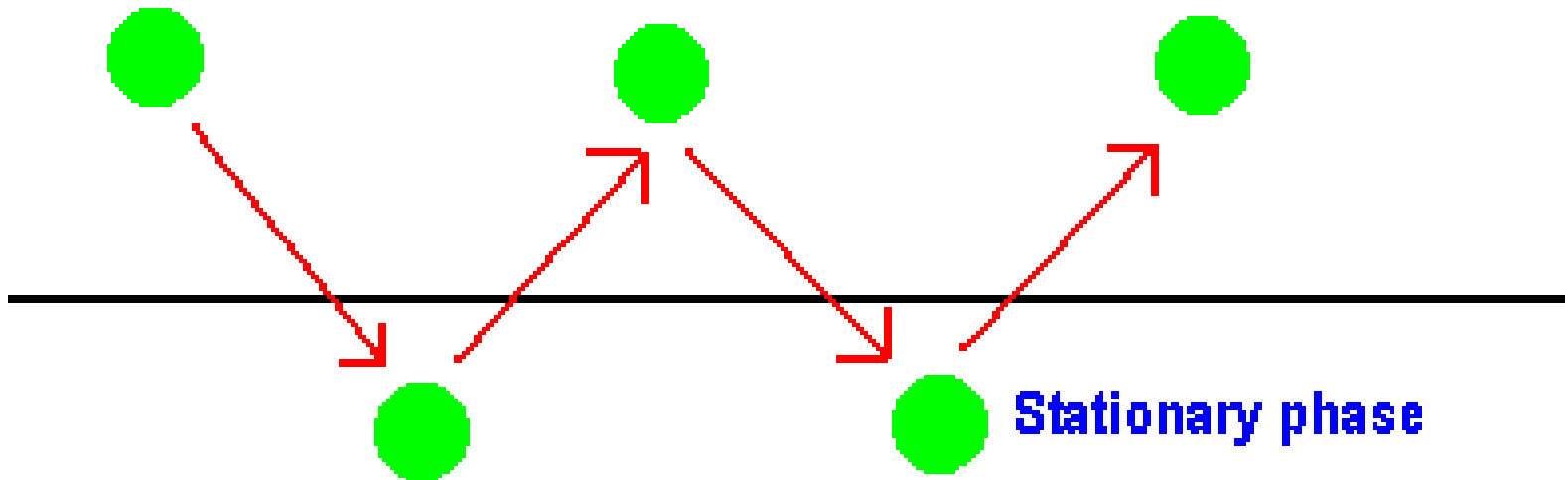
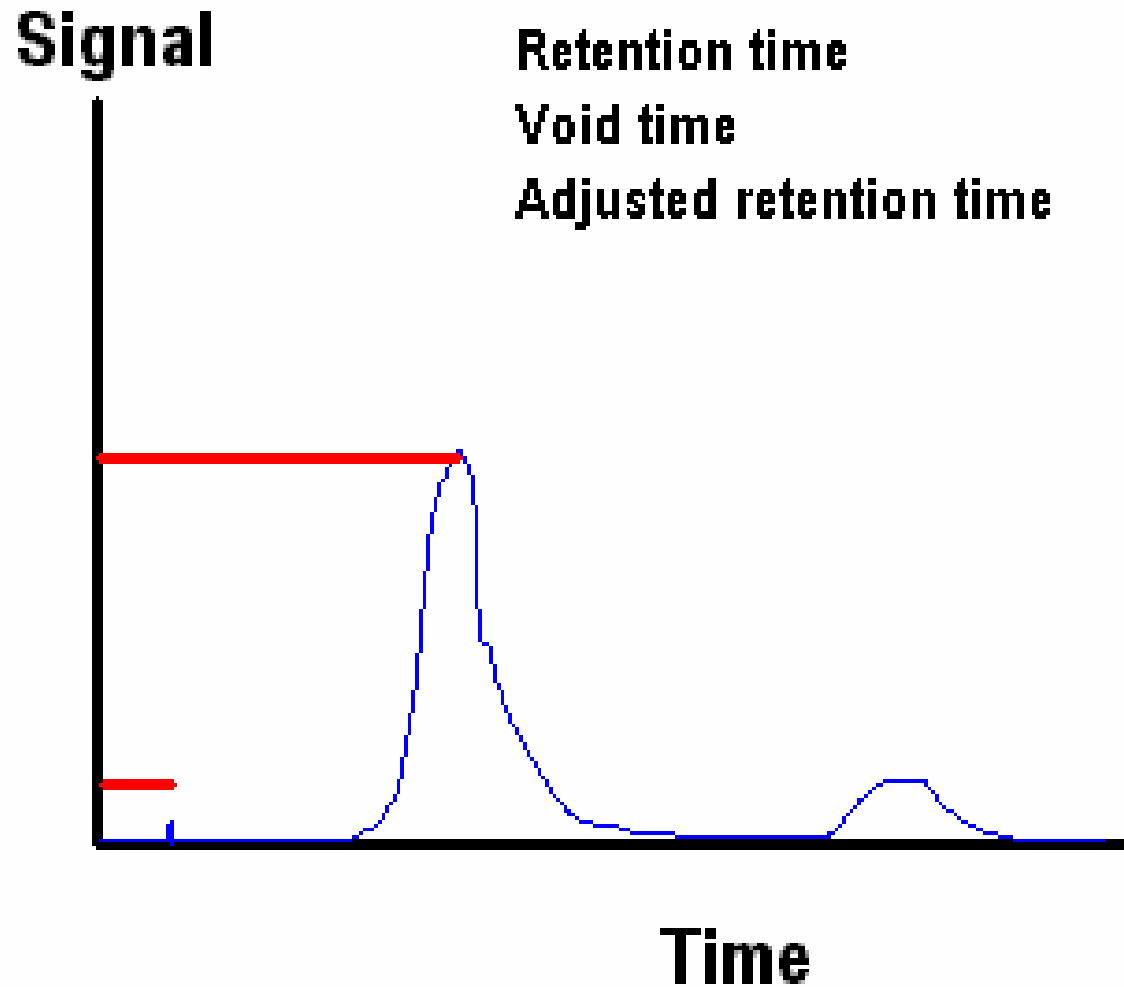


**Chromatography:** Separation of components of a mixture by exploiting differences in partitioning between a stationary phase and a mobile phase.

**Mobile phase** =====>



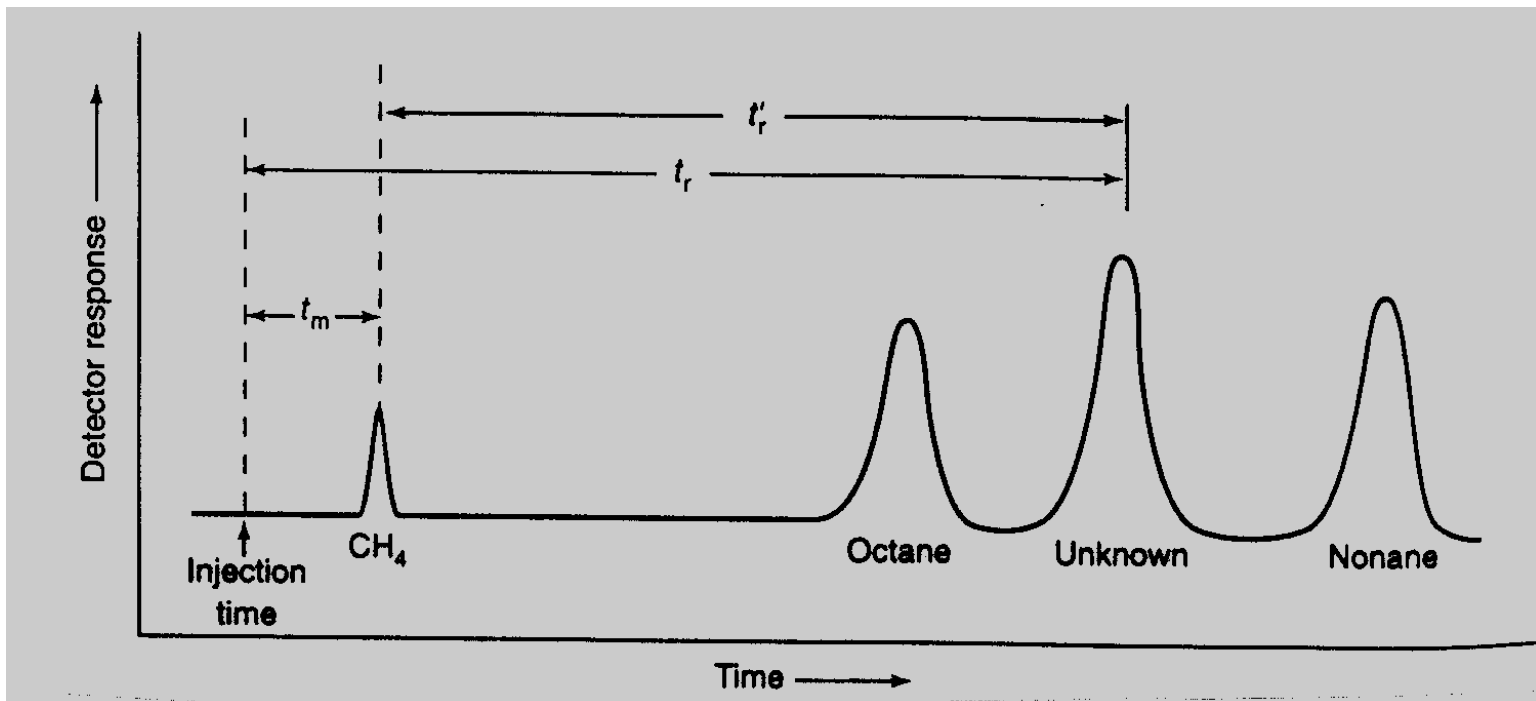
# A chromatogram



# Retention and the capacity factor, $k'$

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

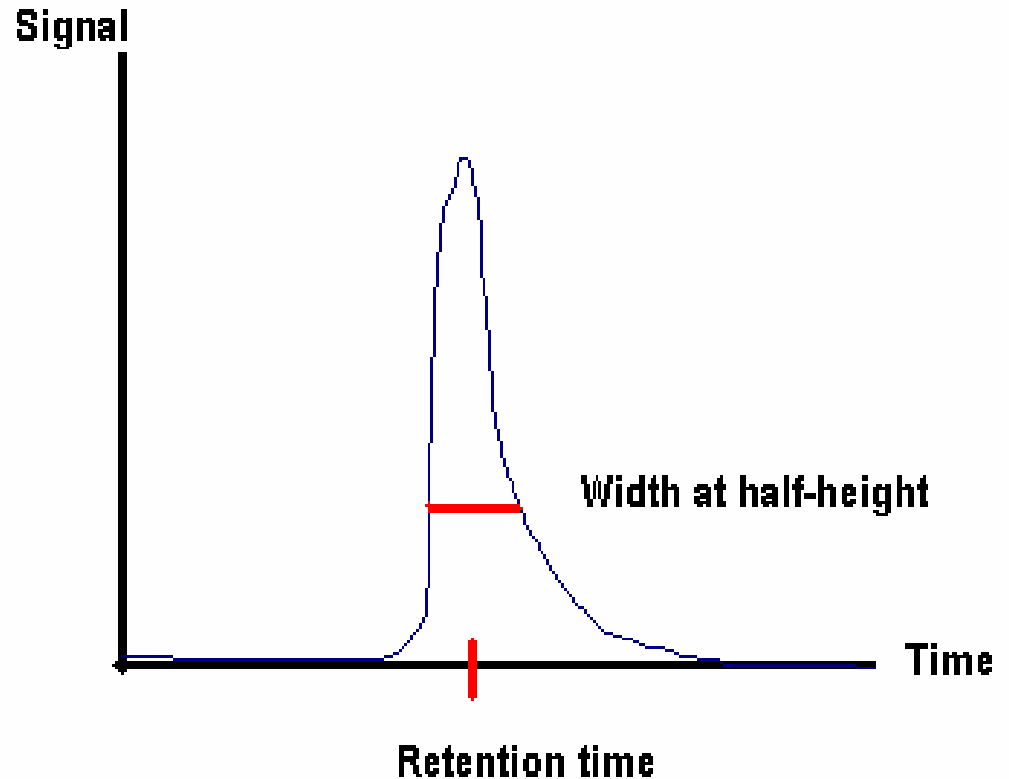
where  $K$  is the partition coefficient,  $V$  is volume, and  $t$  is retention time



# Peak quality: what do we want?

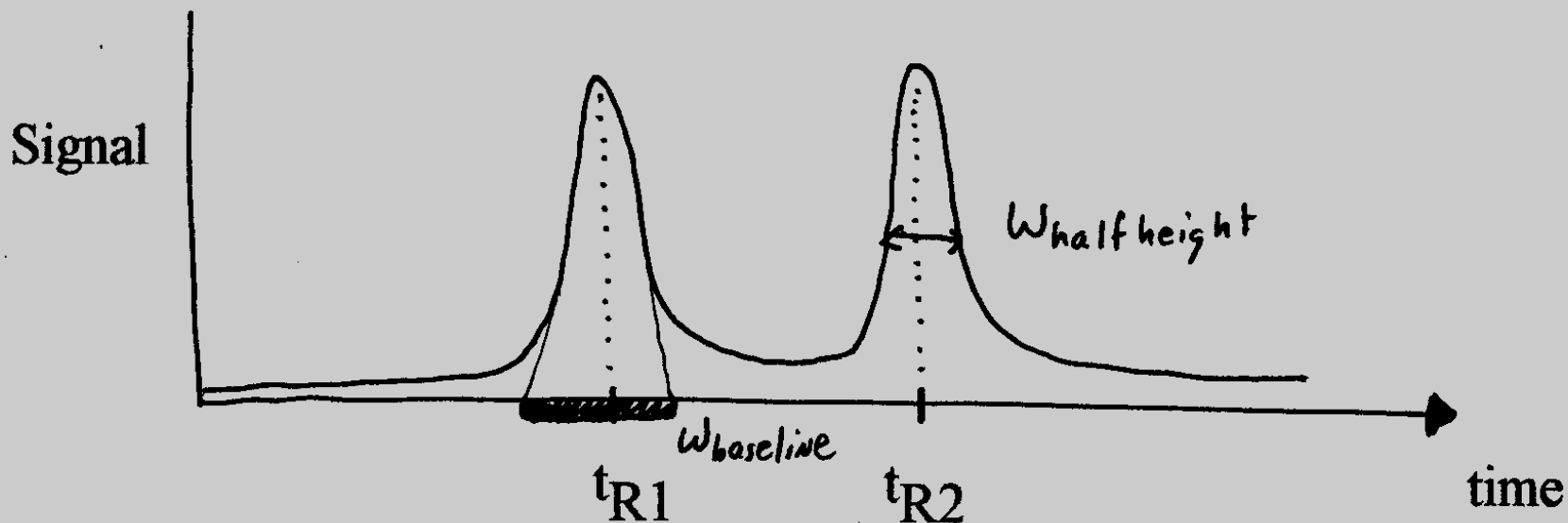
The quality of a chromatographic peak is determined by

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



# Definition of resolution of two bands

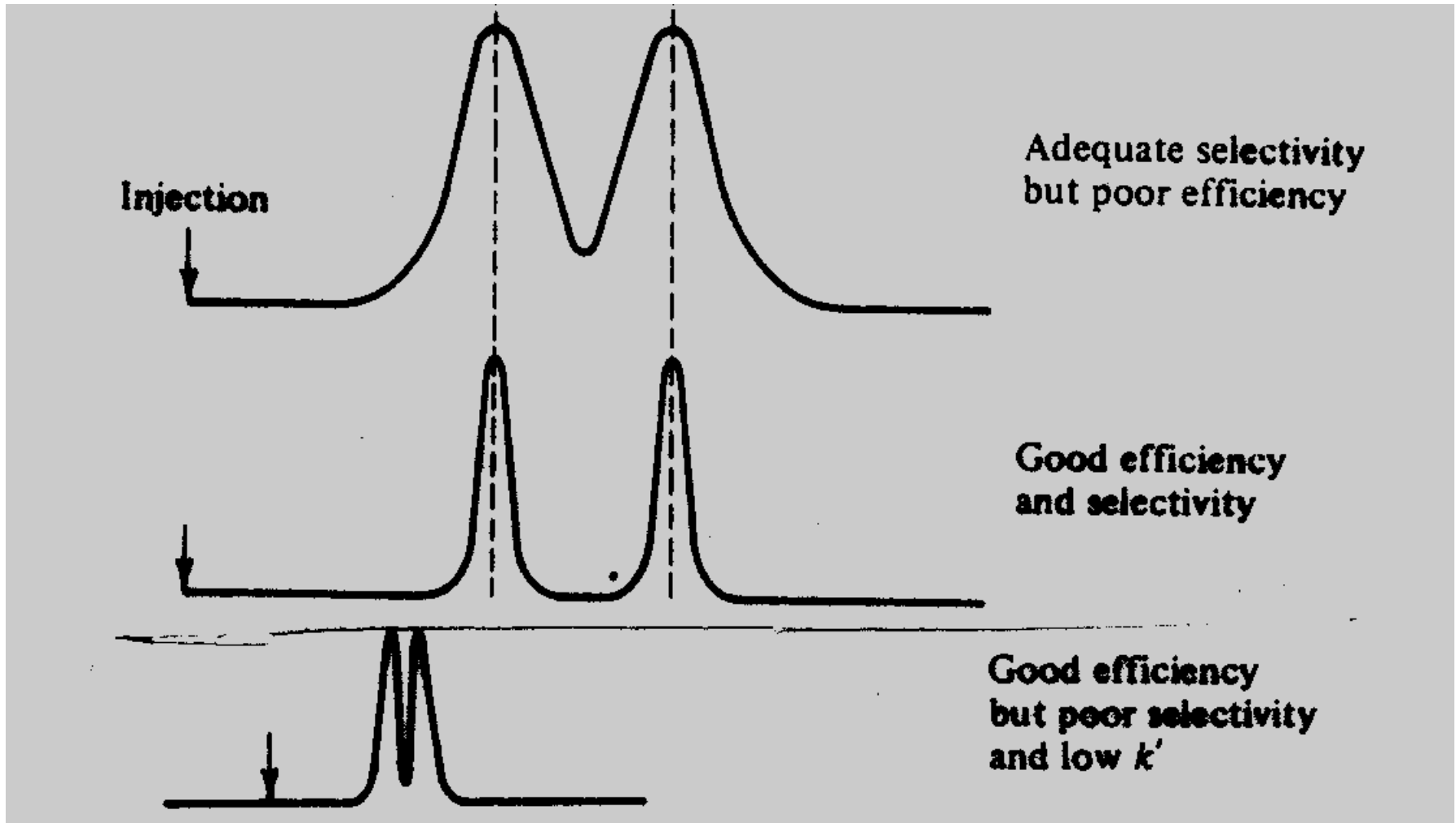
$$\text{Resolution} = (t_{R2} - t_{R1}) / 0.5(w_1 + w_2)$$



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

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*
- *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.*

*One theoretical plate is equivalent to one extraction*

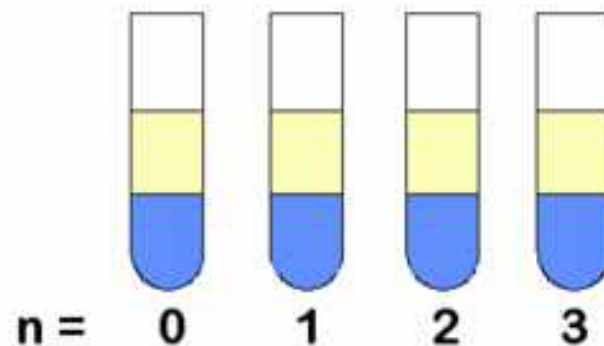


Plate Height:

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...

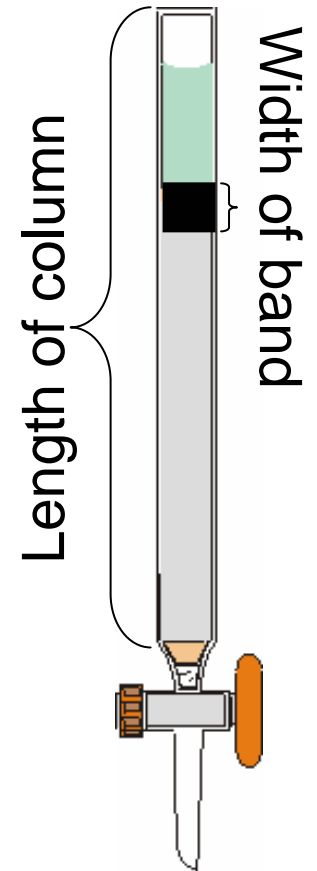
For the same length column

the smaller the plate height

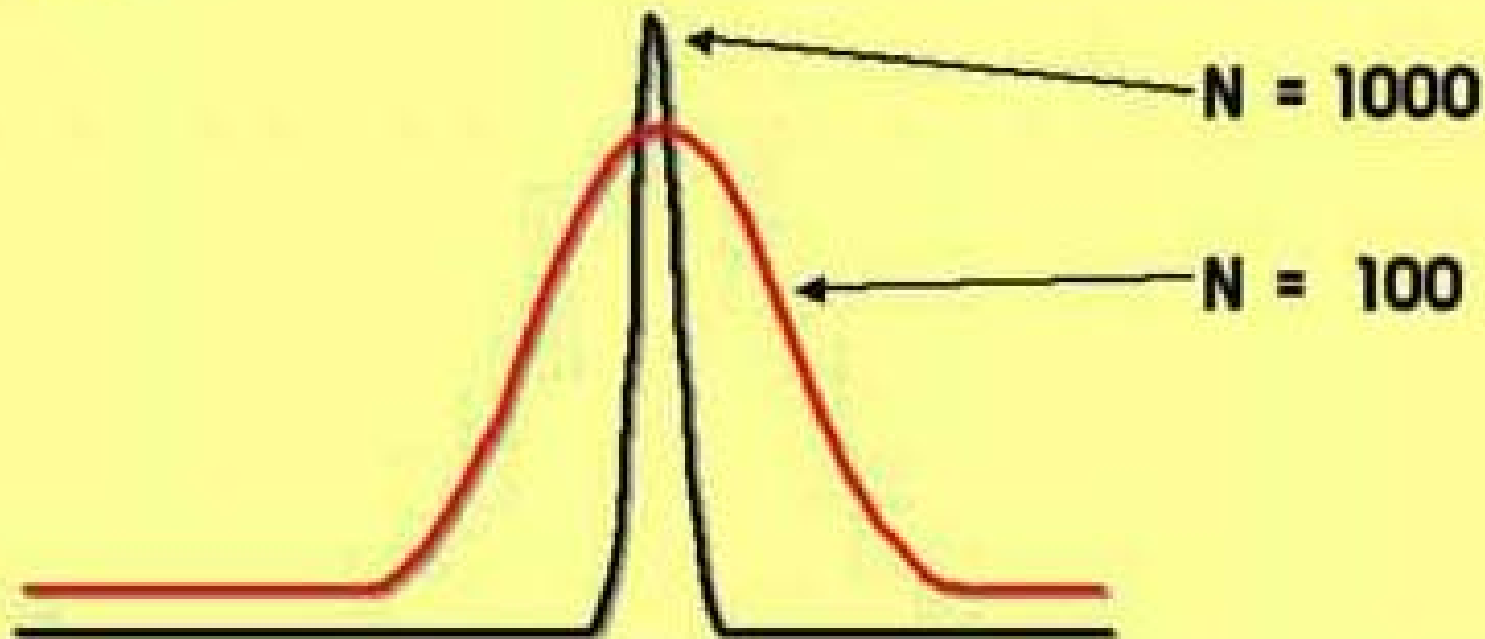
the more theoretical plates present in the column,

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

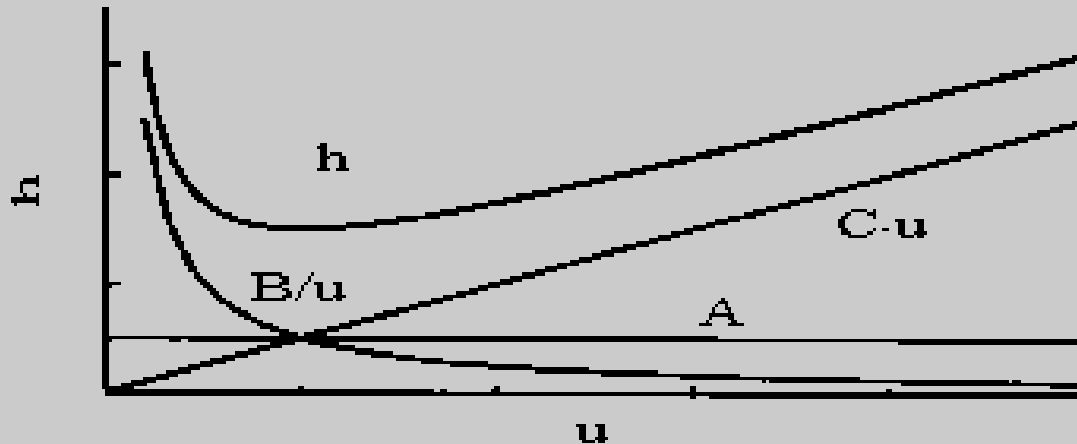
C = Equilibration time (for partitioning)

*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)

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

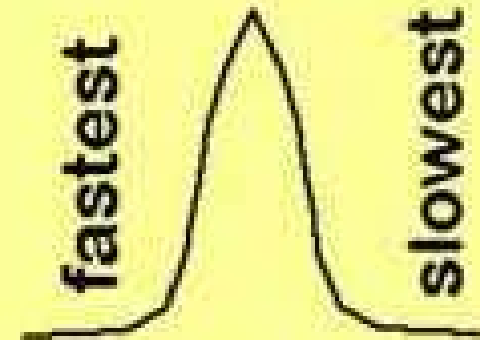
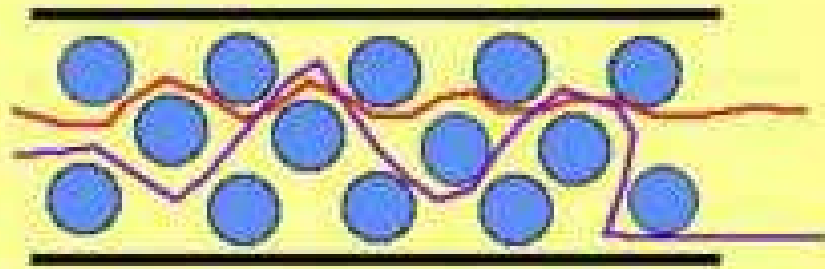


Graph of the van Deemter equation

$$H = \underline{A} + B/u_x + C u_x$$

## 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.

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

## 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.

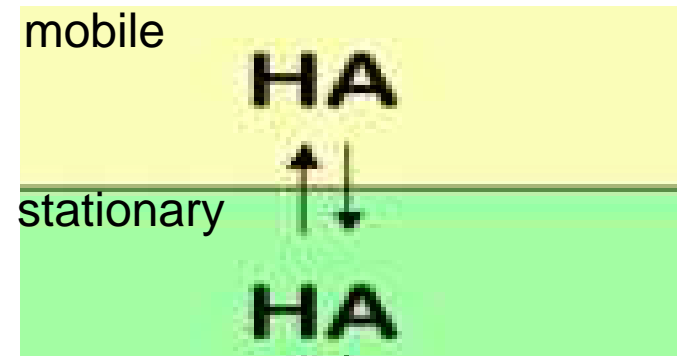


$$H = A + B/u_x + \underline{C}u_x$$

## 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.



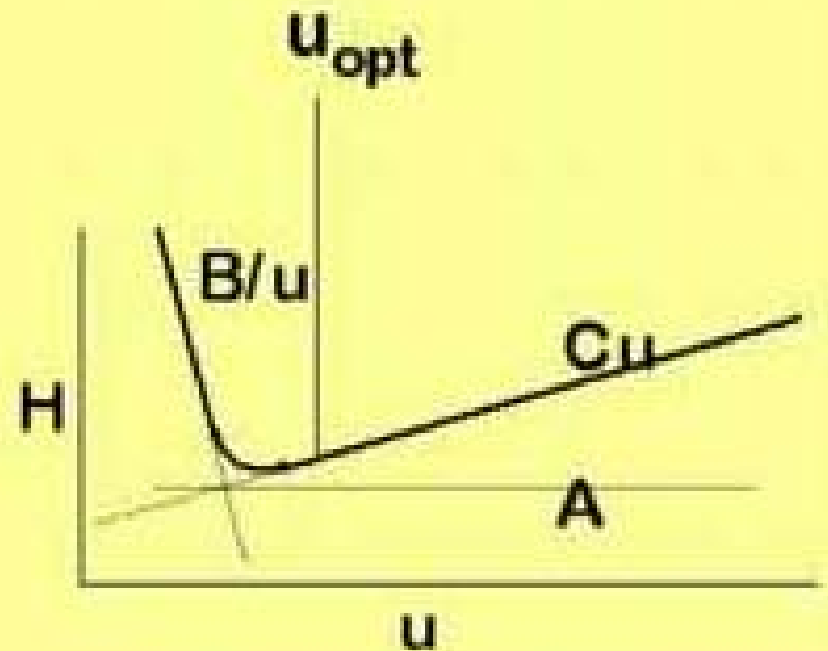
# Consequences of Van Deemter Equation

- ***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)

# Types of Chromatographic Techniques

- Gas chromatography (GC)
  - Mobile phase = gas (typically He)
  - Stationary phase = coated column or packing material (typically based on partitioning)
- Liquid chromatography (LC or HPLC)
  - Mobile phase = solvent (water or organic)
  - Stationary phase = coated packing material (based on partitioning or ion exchange or size exclusion)
- Capillary electrophoresis (CE)
  - Mobile phase = electrolyte-containing solution
  - Stationary phase = glass capillary tube

(separation accomplished by applying a voltage and allowing the charged analytes to migrate in electric field)
- Supercritical Fluid Chromatography (SCFC)
  - Mobile phase = supercritical fluid (typically CO<sub>2</sub>)
  - Stationary phase = coated packing material

(SCFC has similarities to GC and LC)

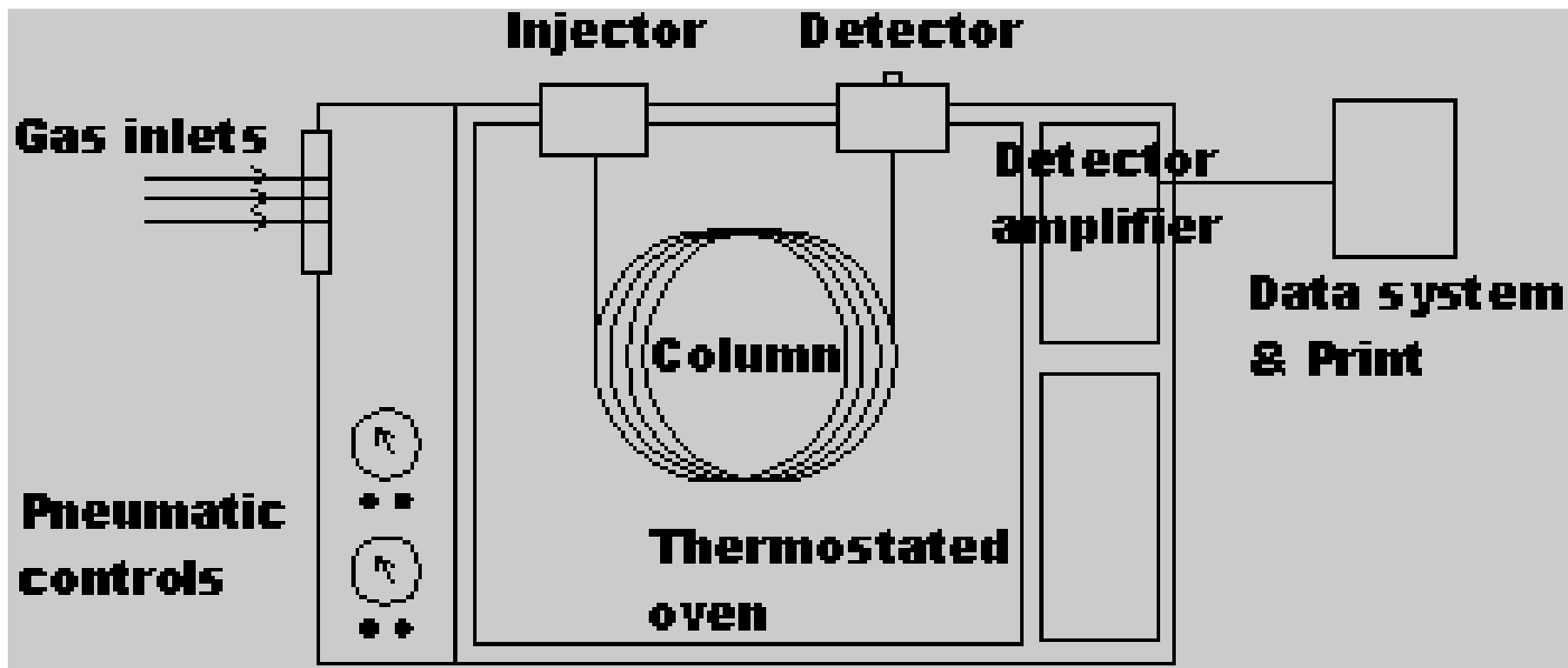
# Gas Chromatography (GC) - requirements

- Thermal stability
- Volatile (a few millitorr at column temp.)
- Ability to detect the substance

## Applications of GC

- Most non-electrolytes with molecular mass < 300, unless thermally labile
- Heavier substances: PAH's, volatile metal chelates, organometallics
- Volatile inorganic halides (e.g.  $WF_6$ )
- Pyrolysis products
- Improve behavior of some substances via derivatization; make more volatile, less polar
  - alcohols  $\implies$  esters
  - amines  $\implies$  amides

# A generic gas chromatograph



# High Performance Liquid Chromatography

*HPLC – used to stand for high pressure liquid chromatography but is now generally understood to mean high performance liquid chromatography*

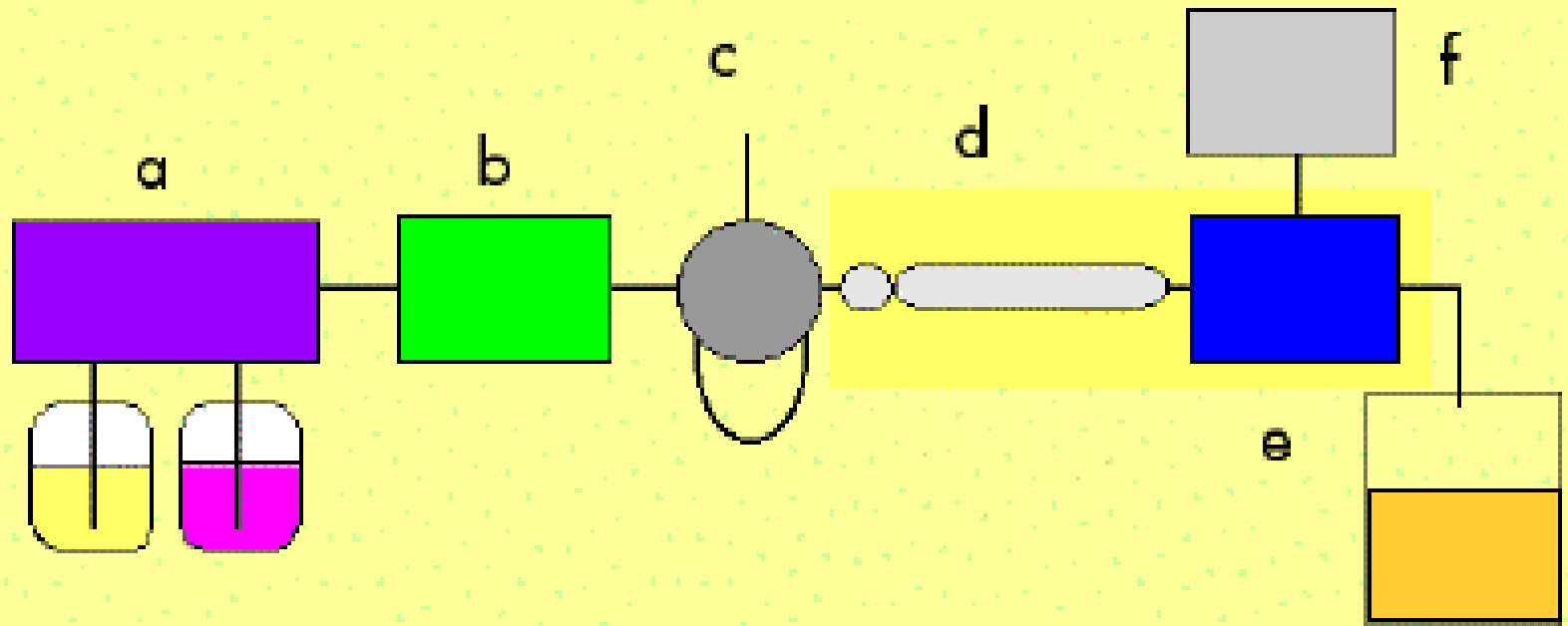
## Purposes and applicability of HPLC

- Separate mixtures where GC won't work
- Application to small, large molecules
- Ionic, highly polar, labile compounds
- Polymers

## Requirements for HPLC

- Solute is soluble in mobile phase, does not react adversely with stationary phase
- Elution occurs in a timely fashion (retention times are generally longer in HPLC compared to GC)
- Detection must be compatible with “liquid”

# A generic liquid chromatograph



a - gradient controller  
b - pump/dampning system  
c - sample introduction

d - column/precolumn  
e - detector  
f - data output

# Why is GC usually preferred to LC?

- Faster
- More theoretical plates ==> better resolution available
- Lower operating costs
- Better detection schemes available

## Limitations of HPLC

1. Low number of theoretical plates
2. No “universal” separation mode
3. Operating costs and waste disposal - use of solvents

# Detection and identification in chromatography

***Chromatography is a separation process only!***  
***“Matching retention times” does not constitute identification!***

Recall: Chromatography is a race of the analytes from the start to the end of the column. The retention time provides how long it took to get through the column, but does not directly identify the chemistry of the analyte.



**Non-Selective Detector (universal)** – useful in that this type of detector “sees” all analytes, but disadvantage is little chemical information gained which is needed for identification

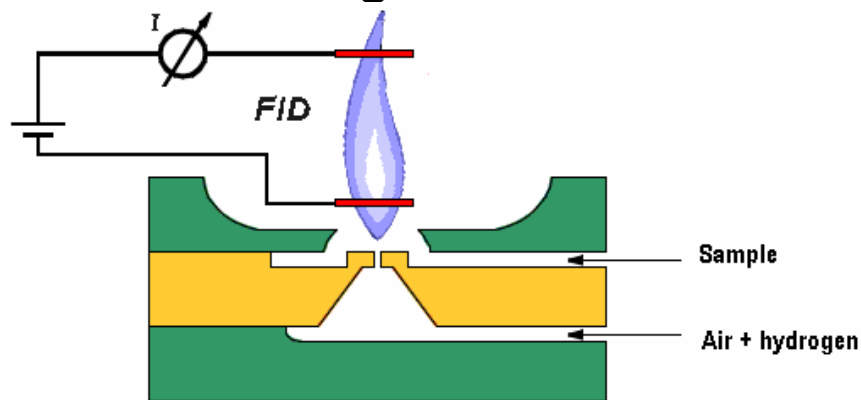
### Examples: GC

- Flame ionization  
(see below)
- Thermal conductivity

**Flame ionization** - combustion in a  $H_2$ -air flame yields gas-phase carbocations when reduced carbon atoms are present in the carrier gas stream. Widely used as a general, sensitive non-selective detector

### Examples: HPLC

- UV-vis absorption
- Refractive index
- Conductivity
- Evaporative light scattering



**Selective Detector** – useful in that this type of detector can identify specific analytes based on their chemistry, but disadvantage is a smaller group of analytes can be detected and identified (i.e.. not all analytes are “seen” by these detectors)

### Examples of GC

- Mass Spectrometry
- IR
- Photoionization
- Electron Capture
- Flame emission

### Examples of HPLC

- Fluorescence
- IR
- Chemiluminescence
- Amperometric
- Mass Spectrometry
- ICPMS

Most powerful detector:

**Mass Spectrometers** – MS offers highly specific chemical information for identification and is the most universal of the selective detectors (i.e. you can get a mass spectrum of most analytes) as well as very sensitive.

# GC/MS of extract from pine needles treated with permethrin (insecticide)

- Top chromatogram is MS detection of mass 10 to 500 amu
- Bottom chromatogram is Selective Ion Mode only detecting 183, 163, 91, 77 amu

