Introduction and Theory of Chromatography

CU- Boulder

CHEM 5181

Mass Spectrometry & Chromatography

Prof. Jose L. Jimenez

Reading: Braithwaite & Smith Chapters 1 & 2

Last Updated: Nov. 2013

Business Items

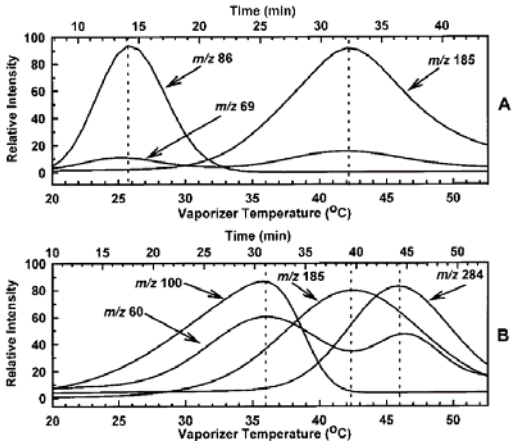
24	C1	Thu	14-Nov-13	JLJ	Chromatography I						
25	C2	Tue	19-Nov-13	JLJ	Chromatography II - GCxGC-MS invited lecture -- Lucas Smith, LECO			13			
26	L1	Thu	21-Nov-13	JLJ	Labview I						w/ David & Rainer
		Tue	26-Nov-13		NO CLASS -- FALL BREAK						
		Thu	28-Nov-13		NO CLASS -- THANKSGIVING						
25	SNR1	Tue	3-Dec-13	JLJ	MS Detectors & Signal-to-noise						
26	L2	Thu	5-Dec-13	JLJ	Labview II						w/ Don & Ken
27	L3	Tue	10-Dec-13	DT	Labview III						David & Don (Jose @ AGU)
28	L4	Thu	12-Dec-13	JLJ	Labview IV						w/ David & Rainer
		Tue	17-Dec-13	JLJ	4:30-7 pm: FINAL EXAM						

- Next Tue: invited lecture from Lucas Smith, LECO
- HW due Tue (could extend some of it, but overlap with 1st LV HW)
- FCQs online + our own feedback form, response rate counts towards class participation grade for everyone

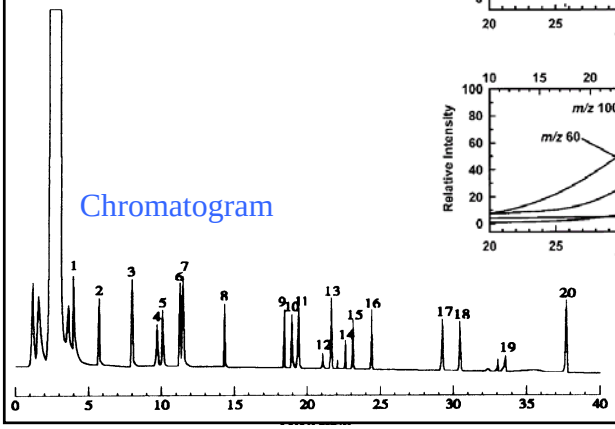
Why is Chromatography So Successful?

Single-Step Thermogram

Ziemann et al.
UC-Riverside
(now CU-Boulder)

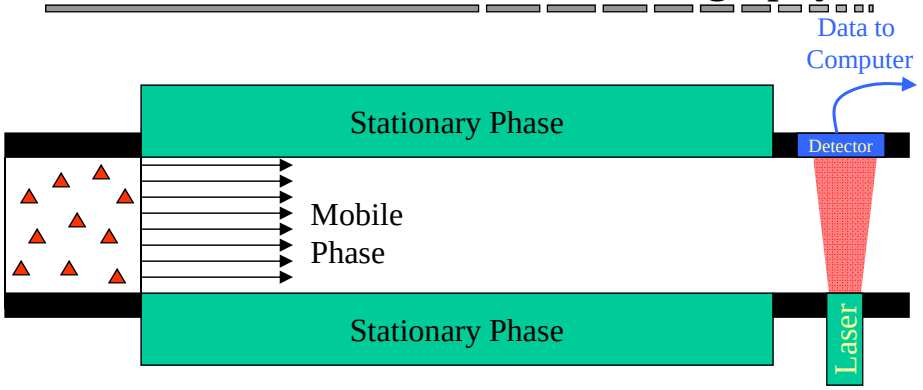


Chromatogram



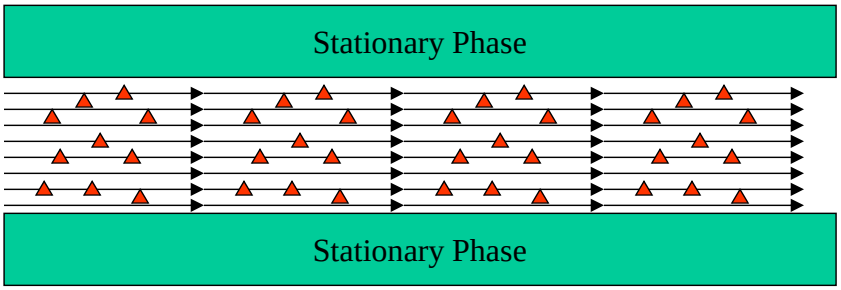
Much higher selectivity, by doing single step over and over

Schematic of Column Chromatography



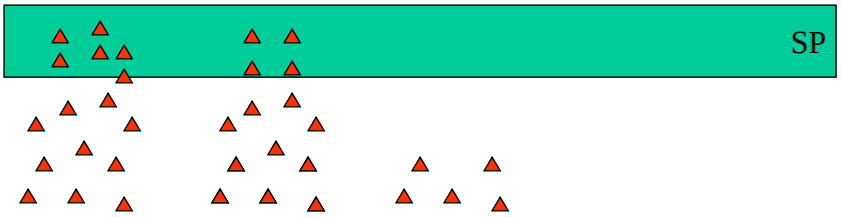
- Sequence of events
  - At t=0 we will open the gate and let the analyte into the column
  - Analyte will be carried by mobile phase
  - Analyte may partition to stationary phase
  - Analyte will be detected by its absorption of light at the detector

Schematic of Column Chromatography II



- For simplicity, we will assume that the mobile phase moves in steps rather than continuously
- If analyte had no affinity to the stationary phase, it would just follow the mobile phase
  - Emerge at the detector after t_M *(mobile phase time)*

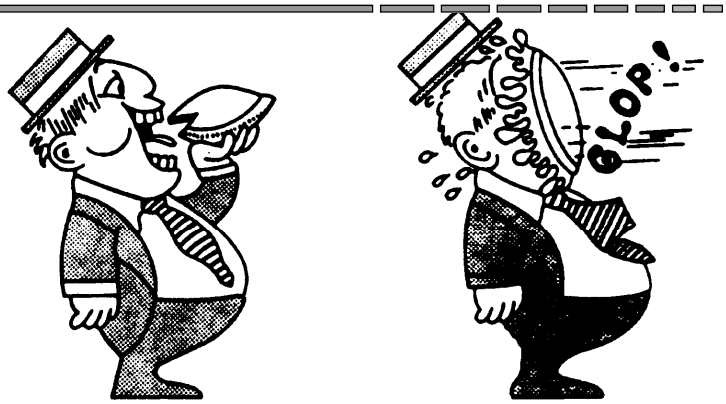
Schematic of Column Chromatography III



- If analyte has some affinity to the stationary phase, it will be retarded
  - Equilibrium
  - Kinetics
    - Molecular mass transfer: diffusion
  - Emerge at the detector after "*retention time*" t_R

$$K = \frac{C_S}{C_M}$$ *Distribution Ratio*

Mechanisms of Partitioning to the Stationary Phase



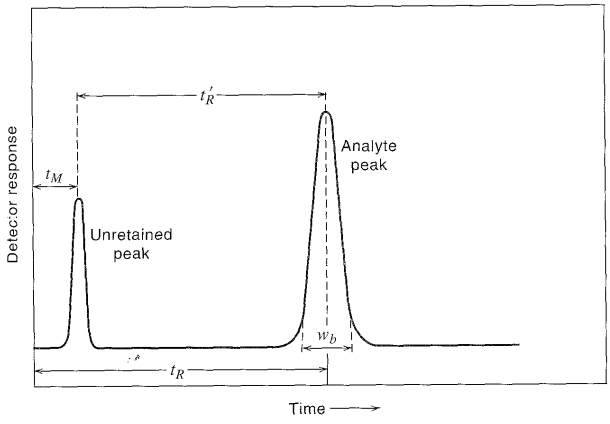
Dissolve into the bulk

Stick to the surface

Q: (A) Left is Absorption, Right is Adsorption
(B) Left is Adsorption, Right is Absorption
(C) I don't know

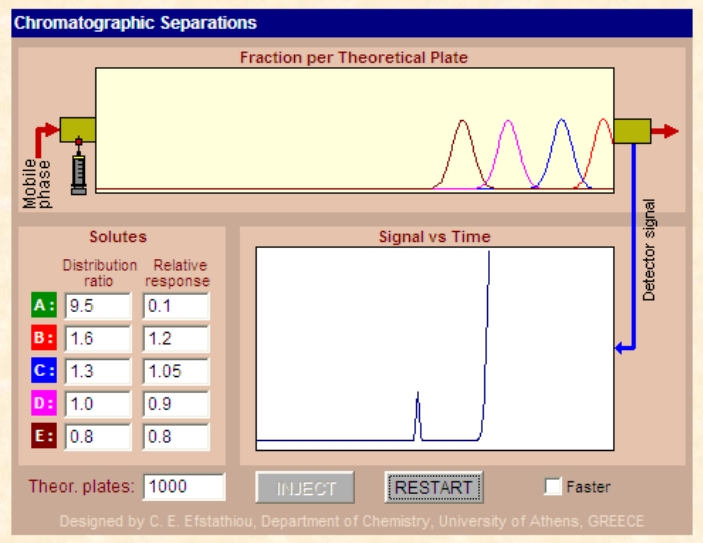
Fig. from McNair & Miller, Basic Gas Chromatography, Wiley, 1998

Retention and Mobile Phase Time



- Simplest chromatogram w/ 2 components
  - Unretained peak (t_M)
  - Analyte peak (t_R)
  - *Corrected retention time* (t'_R)

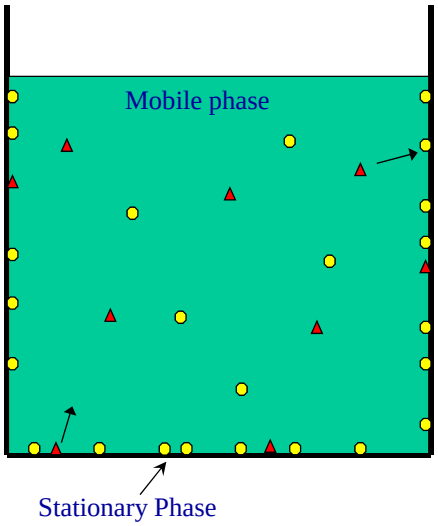
Chromatography Simulator



Investigate
- Effect of K
- Effect of N
- Effect of RR

- http://www.chem.uoa.gr/Applets/AppletChrom/Appl_Chrom2.html

Using the Distribution Ratio: Single Step ("Plate")

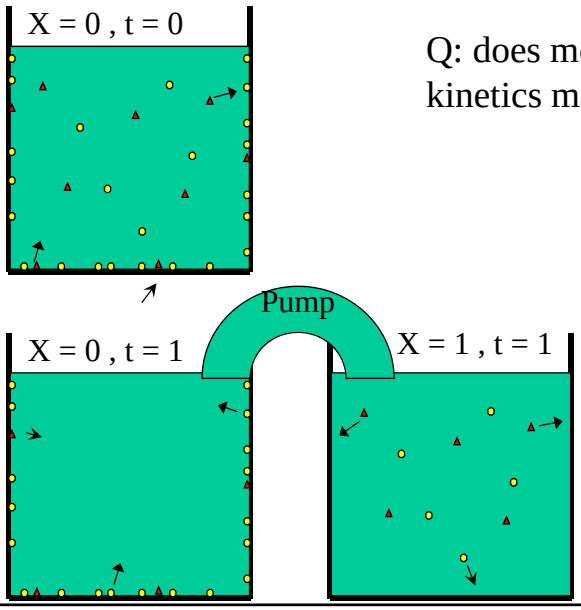


▲ Analyte A

● Analyte B

- Q: If each symbol represents a μmol, given 10 cm^2 of surface to which analytes adsorb, and 100 cm^3 of liquid in which analytes are dissolved, what are K_A & K_B?
- Large K has more affinity for stationary phase
- Small K has more affinitiy for the mobile phase

The first step in a separation



Q: does molecular-level kinetics matter?

Key concepts:

- Enriched in component which prefers mobile phase
- Not very good separation in 1-step (like thermogram). It is repetition that makes it great.

Partitioning in Real Chromatography

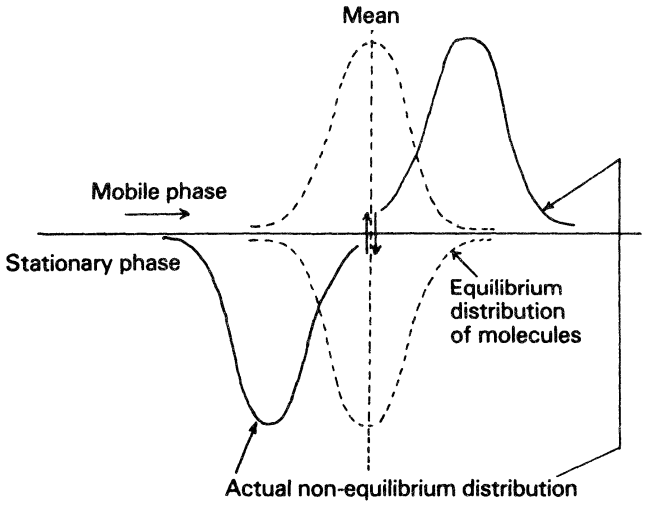


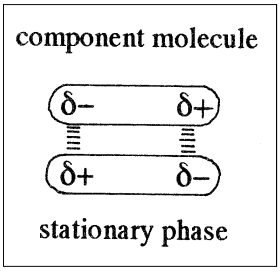
Figure 2.5 Equilibrium process during separation.

Factors Influencing Retention…

- are those that influence distribution (equil.)
  - Stationary phase: type & properties
  - Mobile phase: composition & properties
  - Intermolecular forces between
    - Analyte & mobile phase
    - Analyte & stationary phase
  - Temperature

Intermolecular Forces I

- Based on electrostatic forces
  - "Like-attracts like" or "oil and water" (similar electrostatic properties)
    - Polar/polar & non-polar/non-polar
  - Molecules with dissimilar properties are not attracted
- Polar retention forces
  - Ionic interactions (IC)
  - Hydrogen bonding (permanent dipoles)
  - Dipole-Induced dipole



Intermolecular Forces II (Dipole)

- Polar forces (cont.):
  - Energy of dipole-dipole interaction

$$\varepsilon_D = -\left(\frac{2\mu_A^2\mu_S^2}{r^6 kT}\right)$$

μ: dipole moment, A: analyte, S: stationary phase

Table 2.1 Dipole moments of some organic groups, debye units

		μ_A
R–CH=CH$_2$	Alkene	0.4
R–O–Me	Methyl ether	1.3
R–NH	Amine	1.4
R–OH	Alkanol	1.7
R–COOH	Carboxylic acid	1.7
R–Cl	Chloride	1.8
R–COOMe	Methyl ester	1.9
R–CHO	Aldehyde	2.5
R–CO–R	Ketone	2.7
R–CN	Nitrile	3.6

  - Factor of 10 variation on permanent dipole moment
    - Factor of 10^4 variation on interaction energies
  - As r^6 => mainly at the surfaces

Intermolecular Forces III (London)

- London's Dispersion Forces
  - Most universal interaction between molecules
    - Only one for non-polar species
  - Due to induced dipoles
    - Relatively weak
  - Energy of interaction:

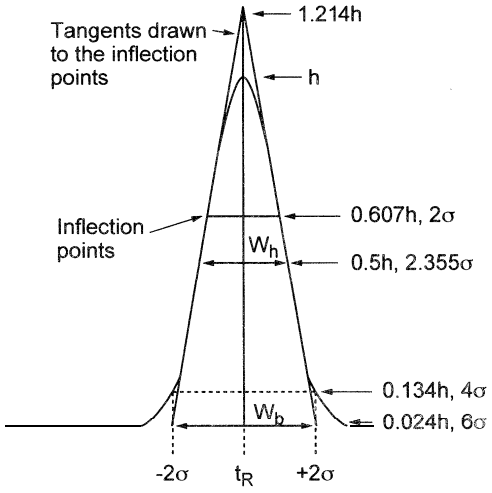
$$\varepsilon_L = \frac{3\alpha_A\alpha_P I_A I_S}{2r^6(I_A + I_S)}$$

  - α is the polarizability, I: ionization potential, A: analyte, S: stationary phase

Clicker Question

A. The chemical nature of the mobile phase affects distribution in gas-chromatography (GC) & liquid chromatography (LC)
B. The mobile phase affects distribution in GC but not LC
C. The mobile phase affects distribution in LC but not GC
D. The mobile phase affects doesn't affect distribution in either GC or LC
E. I don't know

Separating Efficiency – Peak Width



Assume Gaussian Peaks
FWHM = $w_h = 2.36\sigma$
HWHM = 1.18σ

***Base Width* of Peak (w_b)**
- Width at intersection of tangents at inflexion points (+/- σ) and the baseline
- $w_b = 4\ \sigma$
- 95.5% of molecules are within w_b
  - Assuming that the Gaussian model holds!

When in doubt, use NORMDIST in Excel or GAUSS in Igor

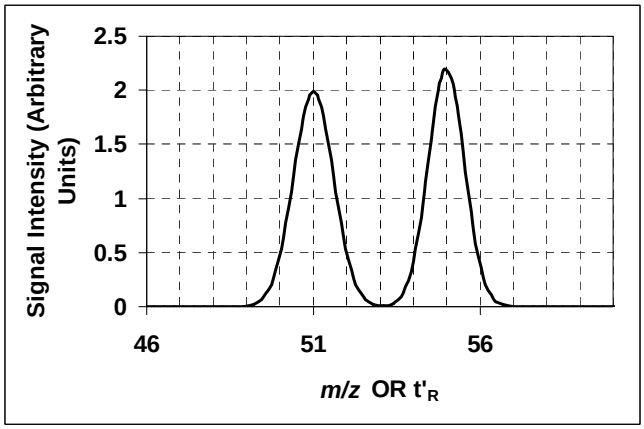
Peak heights of a Gaussian peak and width as a function of standard deviation.

Figure 2.3, Braithwaite & Smith

Resolution in Chromatography

- Objective: accurate measurement of individual peak areas (spectra)

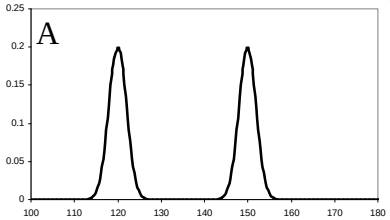
$$R_s = \frac{\Delta t}{2 * FWHM}$$

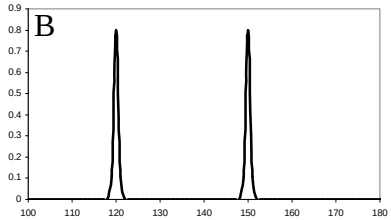


- *What is the resolution if this is a mass spectrum?*
- *If it is a chromatogram?*

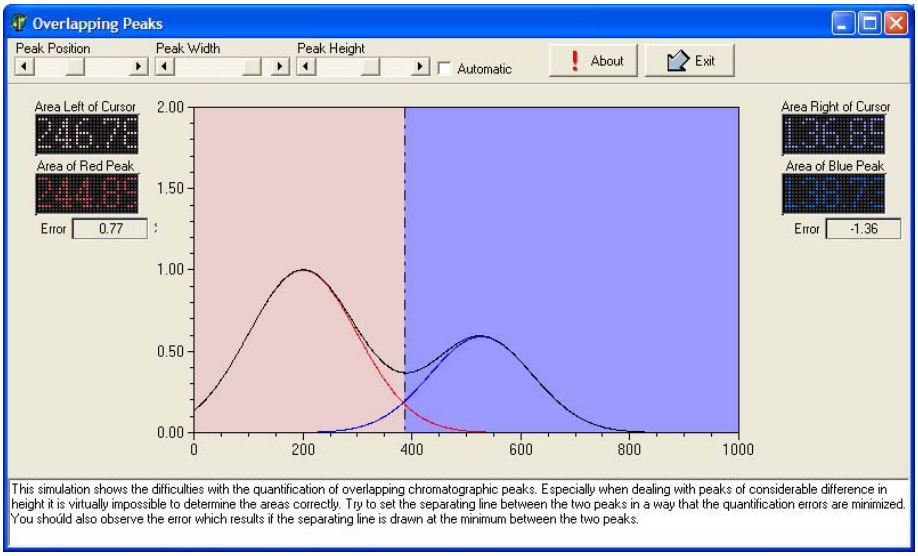
Clicker Question

- Analysis B is more desirable than A
  - A. In MS & Chrom.
  - B. In MS but not Chrom.
  - C. In Chrom but not MS
  - D. In neither MS nor Chrom
  - E. I need a coffee





Resolution vs Peak Integration



- http://www.vias.org/simulations/simusoft_peakoverlap.html

Can One Have Too Much Resolution?

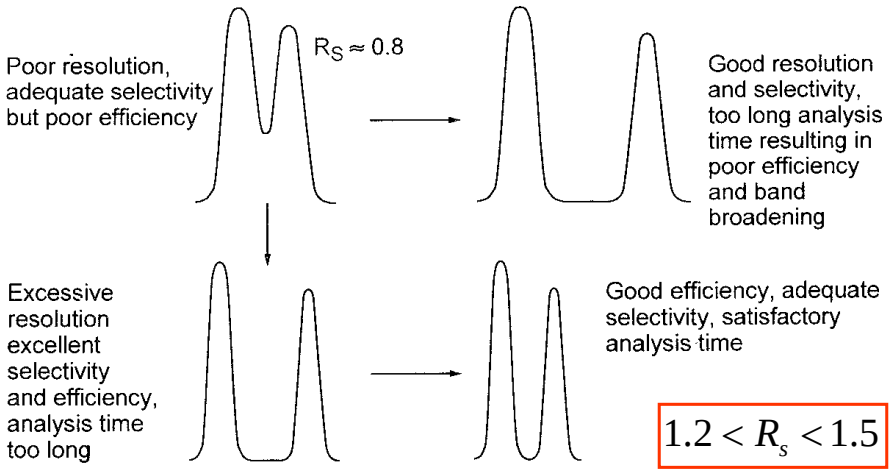
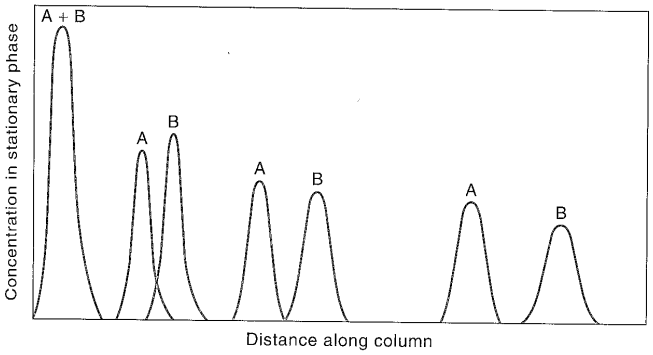


Figure 2.8 Resolution, selectivity and column efficiency.

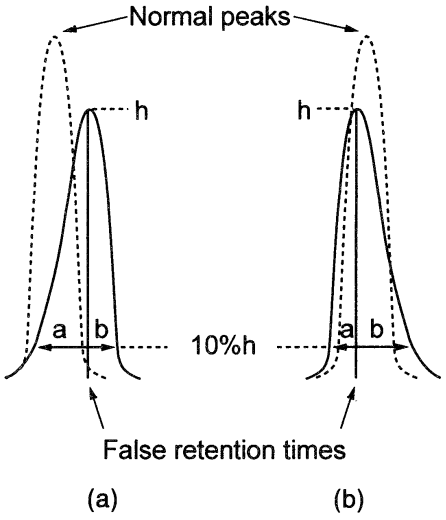
What to do if you have too much resolution?

- If you have too much resolution, you can
  - Shorten the column
  - Increase temperature (GC), flow rate

  => Shorten the analysis



Separating Efficiency – Peak Asymmetry



Q: what can cause peak asymmetry?

Figure 2.4 Peak asymmetry. (a) Fronting and (b) tailing.

Separating Efficiency – Peak Asymmetry

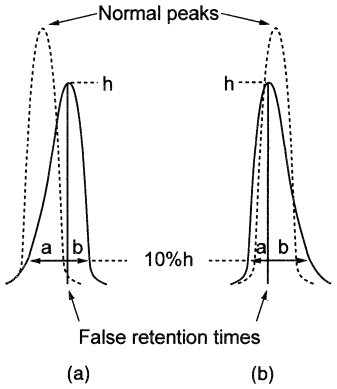


Figure 2.4 Peak asymmetry. (a) Fronting and (b) tailing.

- *Tailing:* some part of the stationary phase binds analyte molecules more strongly
- *Fronting:* some molecules move ahead (inject too much sample => saturate Stat. Phase)
- Peak Asymmetry

$$A_s = \frac{b}{a} \text{ at } 10\% \text{ h}$$

- $0.9 < A_s < 1.2$ for acceptable chromatography

Equations for Calculation of Chromatographic Figures of Merit for Ideal and Skewed Peaks, J.P. Foley and J.G. Dorsey, Anal. Chem., 55: 730-737, 1983

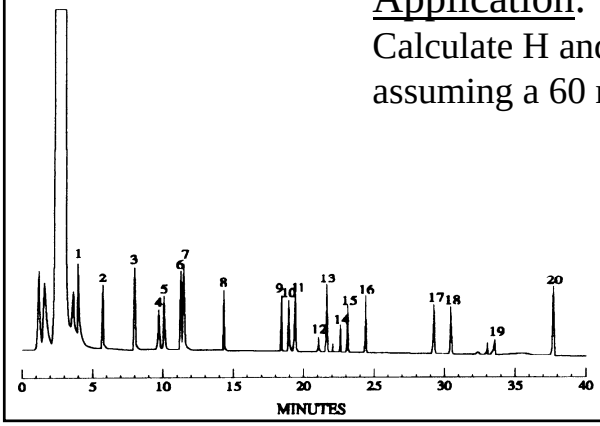
Separating Efficiency – Peak Width II

- Described by variance σ^2 (units: s^2)
  - σ determined from Gaussian fit to peak
- Classical chromatography theory
  - Separation in N discrete steps ("plates")
  - $\sigma^2 \alpha \frac{1}{N}$ or $\sigma \alpha \frac{1}{\sqrt{N}}$
  - Also broadening increases as t_R increases:
  - $\sigma \alpha t_R \frac{1}{\sqrt{N}}$ and in practice: $N = \left(\frac{t_R}{\sigma}\right)^2$

Height Equivalent of a "Plate"

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

- *Height Equivalent to One Theoretical Plate (HETP)*



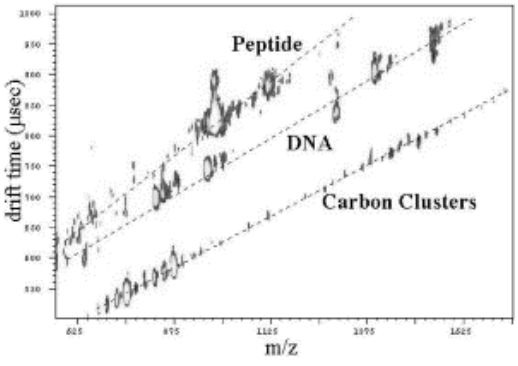
Application:
Calculate H and N for peak 18, assuming a 60 m column

A. H ~ 5 μm
B. H ~ 50 μm
C. H ~ 500 μm
D. H ~ 5 mm
E. I don't know

Concept of Peak Capacity (ϕ)

$$\phi = \frac{\text{Time or Area Available for Separation}}{\text{Time or Area of an Individual Peak}}$$

Fig. 4. Mobility–mass plot of a complex mixture containing multiple classes of ions. In this case peptide, DNA, and carbon cluster ions are observed. Lines are superimposed onto the plot to indicate the mobility–mass trends for each class of molecule.



- ϕ of IMS-MS for peptides ~ 2600
- ϕ for LC-FTICR ~ 6 x 10^7

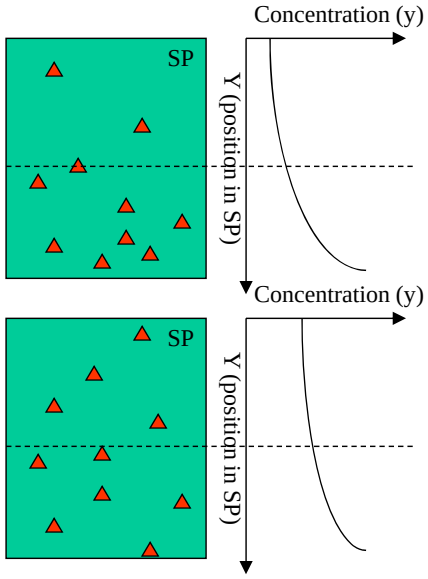
Peak capacity of ion mobility mass spectrometry: Separation of peptides in helium buffer gas. Brandon T. Ruotolo, Kent J. Gillig, Earle G. Stone and David H. Russell. Journal of Chromatography B 782, 1-2, 25, 2002, Pages 385-392. http://dx.doi.org/10.1016/S1570-0232(02)00566-4

Diffusion: Fick's 1st Law

- When there is a gradient in concentration of a species that can diffuse in medium

$$j_A = -D_A \frac{dC_A}{dy}$$

- j_A: molecular flux of A (moles cm^{-2} s^{-1})
- C_A: concentration of A (moles cm^{-3})
- D_{AB}: diffusivity of A in B (cm^2 s^{-1})
  - ~ 0.1-0.01 cm^2 s^{-1} in gases
  - ~10^{-5} cm^2 s^{-1} in liquids



Clicker Question

- When an analyte is diffusing in the stationary phase, equilibrium will be reached faster

A. When D_A is small
B. When D_A is large
C. When SP thickness is large
D. A and C
E. I don't know

From Bird, Stewart & Lightfoot, Transport Phenomena, 2nd Ed. 2002

Mass Transfer Kinetics: Fick's Law

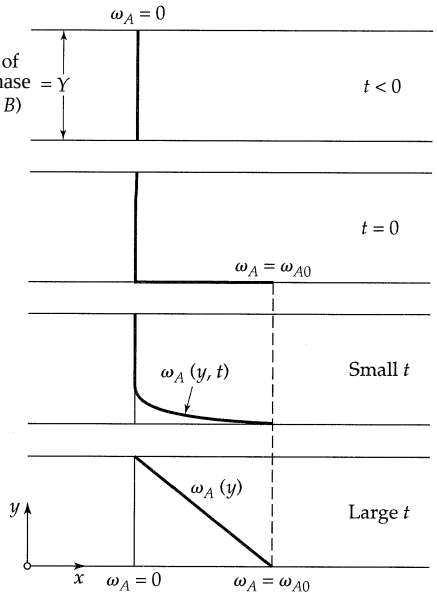
- Initially no substance A
- Pulse at the surface
- Transient concentration
- Steady state profile at long times

$$\frac{W_{Ay}}{S} = \rho D_{AB} \frac{\omega_{A0} - 0}{Y}$$

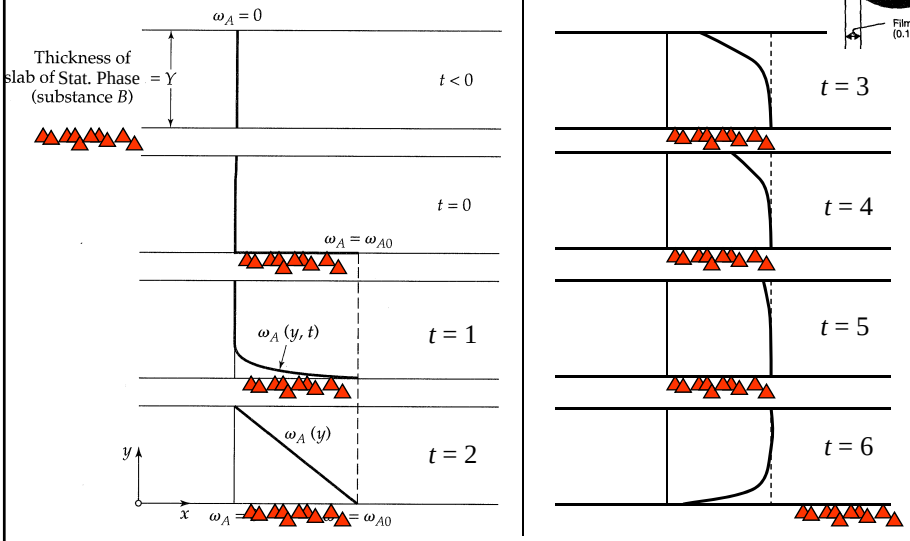
  - W_{Ay}: mass flux of A
  - ω_A: mass fraction of A
  - D_{AB}: diffusivity of A in B
  - S: surface area; ρ : density

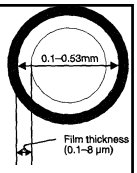
$$j_{Ay} = -\rho D_{AB} \frac{d\omega_A}{dy}$$

  - j_{Ay}: molecular mass flux of A



Situation in Column Chromatography

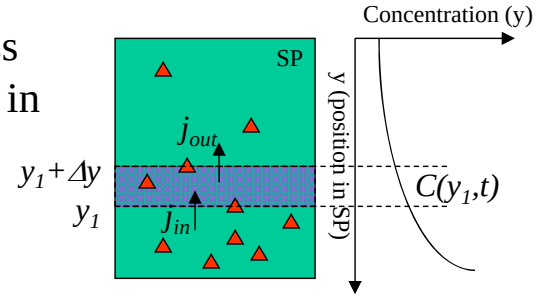




Mass transfer takes time => separation + limit on resolution

Diffusion: Fick's 2nd Law

- Conservation of mass for diffusing species in control volume
  - Per unit area perpendicular to diffusion



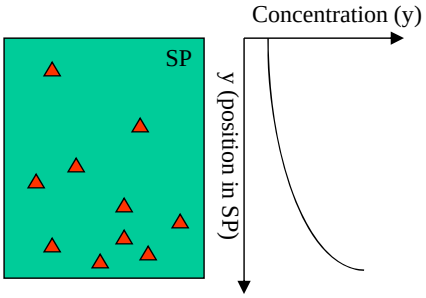
$$\frac{\partial C_A(y_1,t)}{\partial t} = j_{in} - j_{out}$$

$$\frac{\partial C_A(y_1,t)}{\partial t}\Delta y = -D\frac{\partial C_A(y_1,t)}{\partial y} + D\frac{\partial C_A(y_1+\Delta y,t)}{\partial y}$$

Diffusion: Fick's 2nd Law II

- When things are changing in time:

$$\boxed{\frac{\partial C_A(y,t)}{\partial t} = D_A\frac{\partial C_A(y,t)}{\partial y^2}}$$



- Once C_A is the same everywhere, we have reached equilibrium in the SP

Q: can we estimate order-of-magnitude of time needed?

Time Scale of Mass Transfer

- For transfer across SP thickness Y
- *Start with:*

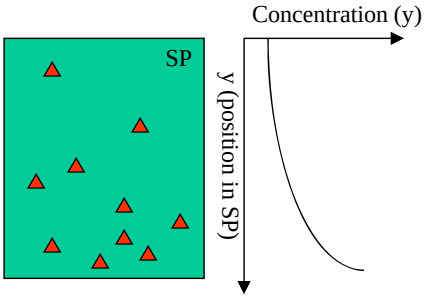
$$\frac{\partial C_A(y,t)}{\partial t} = D_A \frac{\partial C_A(y,t)}{\partial y^2}$$

- *"Order-of-magnitude analysis"*

$$\frac{\Delta C_A}{\tau_D} = D_A \frac{\Delta C_A}{Y^2}$$

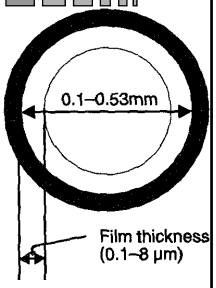
- Simplifying:

$$\boxed{\tau_D = \frac{Y^2}{D_A}}$$

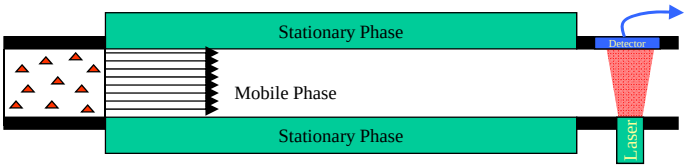


Numerical Example of Mass Transfer

- Assume
  - Column diameter = 100 μm
  - Film thickness: Y = 1 μm
  - Diffusivity of analyte in stationary phase $D_{AB} = 10^{-6}$ cm^2 s^{-1}
  - Solubility of A in B is 1% of volume
  - MW_A = 100 g mol^{-1}
- Questions
  - What is the time scale of mass transfer?
  - What should be the time scale of flow along 1 mm of the column?
  - What is the max. amount of analyte that can be in the stationary phase per 1 mm of column length?

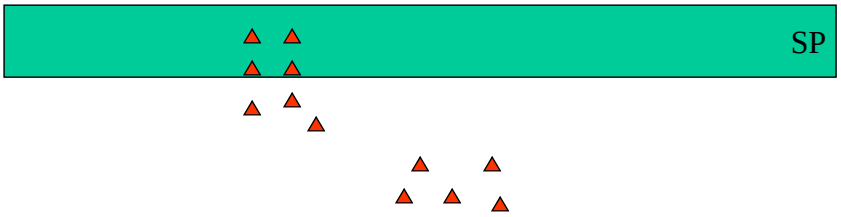


Band Broadening Processes



- Non-column broadening
  - Dispersion of analyte in
    - Dead volume of injector
    - Connection between injector & column
    - Connection between column & detector
  - Emphasis on minimizing dead volume (injectors, fittings…)
- Column broadening: Van Demteer model

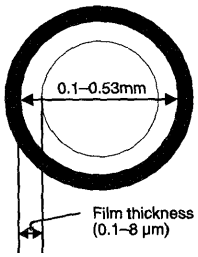
Schematic of Column Chromatography III



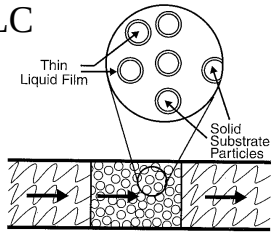
- If analyte has some affinity to the stationary phase, it will be retarded
  - Equilibrium $K = \frac{C_S}{C_M}$
  - Kinetics
    - Molecular mass transfer: diffusion
  - Emerge at the detector after "*retention time*" t_R

GC vs. HPLC Columns

GC



HPLC



- Most GC columns do NOT have particles
- Most HPLC columns do have particles
- Why?
  - A. Particles are needed to prevent liquid flow for being too fast
  - B. Particles are needed in HPLC to shorten diffusion distance in MP
  - C. Particles are not needed in GC because diffusion is very fast
  - D. B & C
  - E. I don't know

Effect of Mobile Phase velocity on H

- Experiment
  - Repeat the same separation, same column and mobile phase
  - Determine H vs. flow rate
  - Observe an optimum, H increases to both sides
  - Goethe: "there is nothing more practical than a good theory"

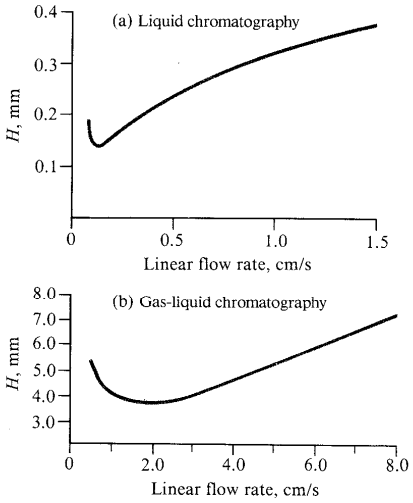
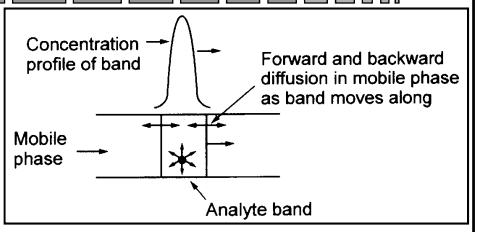


FIGURE 24–7 Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.

Skoog & Leary, 4th Ed

Van Deemter Model: "*B*" Term

- **Longitudinal Diffusion**
- Basically molecular diff., as if mobile phase was not moving



Clicker 1
A. B term ↑ as u ↓
B. B term ↓ as u ↓
C. B term $\neq f(\bar{u})$
D. Don't know

Clicker 2. B term is
A. more imp in GC
B. More imp in HPLC
C. Similar importance
D. I don't know

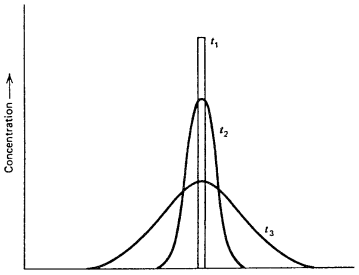
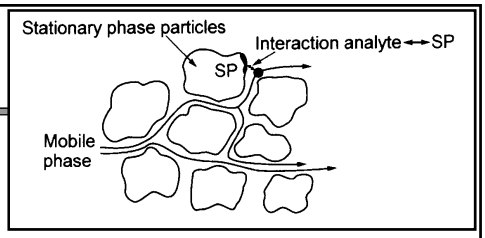


Fig. 3.8. Band broadening due to molecular diffusion. Three times are shown: $t_3 > t_2 > t_1$. From Miller, J. M., *Chromatography: Concepts and Contrasts*, John Wiley & Sons, Inc., New York, 1987, p. 31. Reproduced courtesy of John Wiley & Sons, Inc.

Van Deemter Model: "*A* Term" Broadening



- **'Eddy diffusion' & unequal pathways**
- Molecules may travel unequal distances
- Particles (if present) cause eddies & turbulence
- A depends on size of stationary particles (want *small)* and their packing (want *uniform)* (or coating in TLC plate)
  - GC: 150 μm, HPLC: 5-10 μm

$$A = \lambda d_p$$

Clicker
A. A term ↑ as u ↓
B. A term ↓ as u ↓
C. A term $\neq f(\bar{u})$
D. Don't know

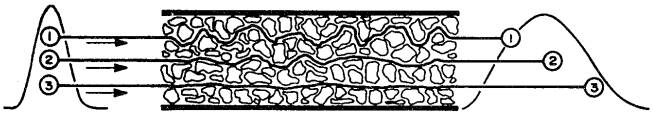
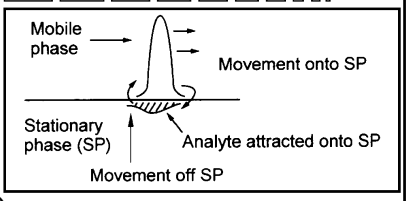


Fig. 3.11. Illustration of eddy diffusion.

Van Deemter Model: "*C*" Terms I



- Accounts for finite time for mass transfer (equil. btw analyte in stationary and mobile phase not instantaneous)
- Most important effect in GC & HPLC
- C_S accounts for stationary phase mass transfer
  - d_f: stationary phase film thickness
  - D_S: diffusion coeff. of analyte in SP
  - Thinner films reduce mass transfer time & broadening
    - But also reduce capacity of the column

$$C_s = \frac{d_f^2}{D_s}$$

Van Deemter Model: "*C*" Terms II

- C_M accounts for mass transfer on the mobile phase interface with the SP
- In packed columns:
  - d_p is particle diameter

$$C_M = \frac{d_P^2}{D_M}$$

- In open columns
  - d_c is column diameter

$$C_M = \frac{d_C^2}{D_M}$$

Clicker 1
A. C term ↑ as u ↓
B. C term ↓ as u ↓
C. C term $\neq f(\bar{u})$
D. Don't know

Clicker 2. C_M term is
A. More important in GC
B. More important in HPLC
C. Similar importance
D. I don't know

Van Deemter Model of Band Broadening

- Tries to explain previous experiment
- H: plate height
- $\bar{u}$: average linear velocity

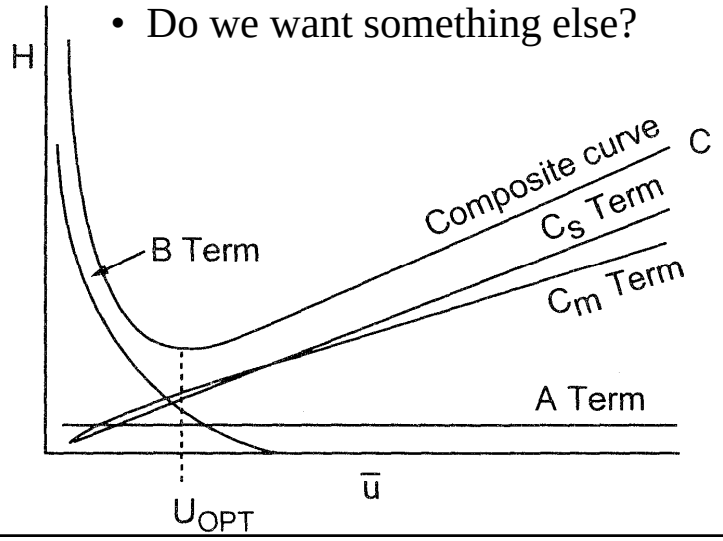
$$\bar{u} = \frac{L}{t_M}$$

$$H = A + \frac{B}{\bar{u}} + C_S\bar{u} + C_M\bar{u}$$

- H: as small as possible (calculate H_{min}?)
- Some terms decrease, other increase with $\bar{u}$
  - There should be optimum $\bar{u}$
- There are alternative models (see reading)

Optimum Mobile Phase Velocity

- We want N highest, H lowest
- Do we want something else?



Optimum Mobile Phase Velocity: GC & HPLC

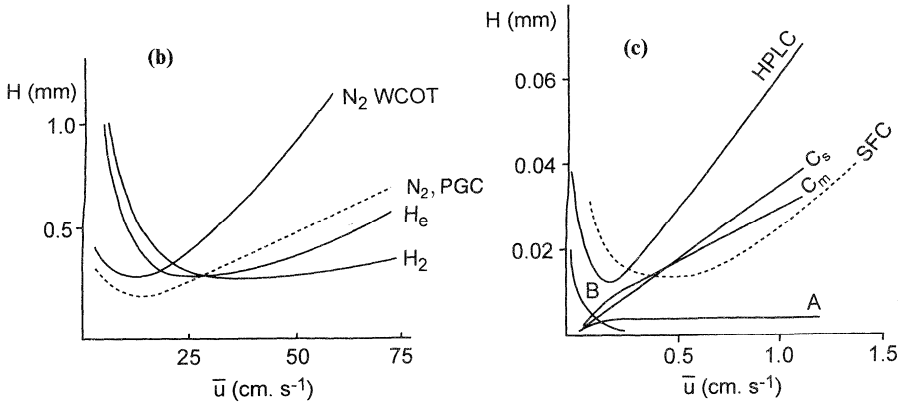


Figure 2.7 Van Deemter plots of plate height, H, against average linear velocity, $\bar{u}$, of the mobile phase: (a) the contribution of each term to the composite curve; (b) plots for W_{COT} GC columns using N_2, He, H_2 carrier gas and N_2 for packed column GC, PGC; (c) plots for HPLC and SFC composite curve.

Q: differences in A, B, C between Supercritical Fluid Chrom. & HPLC?