Separations Methods

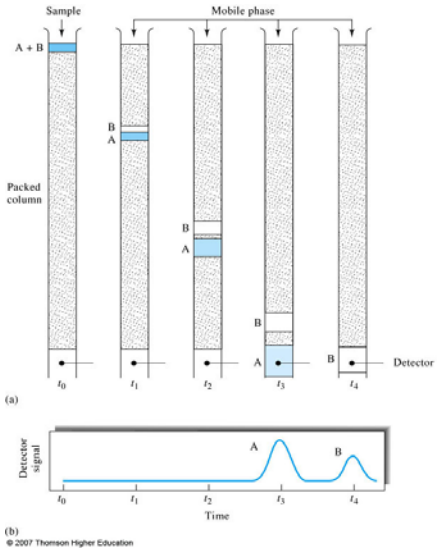
- Rarely is a sample collected in a form ready for analysis
  - need sample preparation
  - must deal with sample matrix and possible interferents
- Possible solutions
  - do wet-chemical methods (precipitation, complexation, etc.) to "purify" sample
  - Design analysis that discriminates against interferents
    - find the "magic" wavelength for AA, etc.
  - Design a method that allows you to look at the sample one component at a time
    - Separations, "Chromatography"
- Chromatographic separations rely on varying equilibria for the distribution of analyte between a *mobile phase* and a *stationary phase*.

$$A_{m.p.} = A_{s.p.} \qquad K = \frac{[A_{s.p.}]}{[A_{m.p.}]}$$

  - K =*partition coefficient*
  - Different "types" of chromatography result from different mobile phase:stationary phase combinations

Separations Basics

- Mixture introduced as narrow "band"
- Differing partition coeff. result varying migration times
- On elution, bands are broader than initial band
  - dilution



Options for Separations

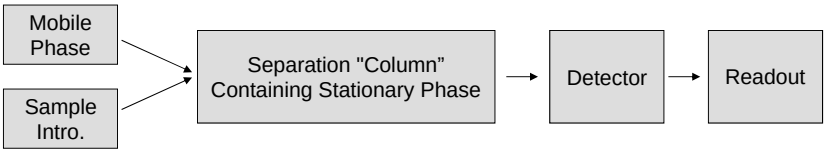
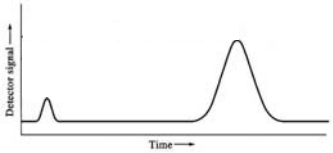


TABLE 26-1 Classification of Column Chromatographic Methods

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
1. Gas chromatography (GC)	a. Gas-liquid chromatography (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	b. Gas-solid	Solid	Adsorption
2. Liquid chromatography (LC)	a. Liquid-liquid, or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	b. Liquid-solid, or adsorption	Solid	Adsorption
	c. Ion exchange	Ion-exchange resin	Ion exchange
	d. Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	e. Affinity	Group specific liquid bonded to a solid surface	Partition between surface liquid and mobile liquid
3. Supercritical fluid chromatography (SFC; mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

Describing the Results of a Separation and Quantifying Separation Quality



- Retention Time:
- Dead Time:
- Capacity Factor (retention factor): Tendency of compound to remain on the column.

$$k'_A = K_A \frac{V_S}{V_M} = \frac{t_R - t_M}{t_M}$$

- Selectivity Factor: Tendency of compound to remain on the column (compared to other compounds).

$$\alpha = \frac{K_A}{K_B} = \frac{k'_A}{k'_B} =$$

Describing the Results of a Separation and Quantifying Separation Quality

- "Ideal" peak shape in chromatography is Gaussian
  - due to random distribution of migration rates for a given molecule
  - often, Gaussian shape *isn't* observed
    - result of column inefficiency
    - due to several possible factors
- Quantifying Column Efficiency: "Theoretical Plates"
- Separation is a result of several distinct events
  - each is a theoretical plate
  - the more theoretical plates (events) that occur, the better the separation is likely to be

$$N = L/H$$

  - L:
  - H:

$$H = \frac{\sigma^2}{L} = L\left(\frac{W}{4t_R}\right)^2$$

  - N:

$$N = \left(\frac{4t_R}{W}\right)^2 = \left(\frac{2.35t_R}{W_{1/2}}\right)^2$$

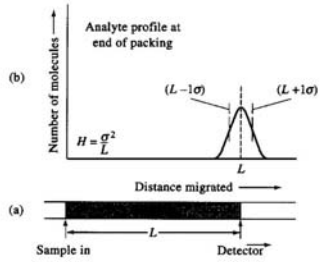


Figure 26-5 Definition of plate height $H = \sigma^2/L$.

Causes for Peak Nonideality

- Most of nonideality comes as a result of combination of mass transfer processes
  - Effects plate height
  - Described by *van Deemter* equation

$$H = A + \frac{B}{u} + Cu = A + \frac{B}{u} + (C_s + C_m)u$$

(Remember, small H is better!)

  - A = Multipath Term
    - A depends on:
  - B/u = Longitudinal Diffusion Term
    - B/u depends on:
  - C = Mass Transfer Term
    - C depends on:

TABLE 26-2 Variables That Influence Column Efficiency

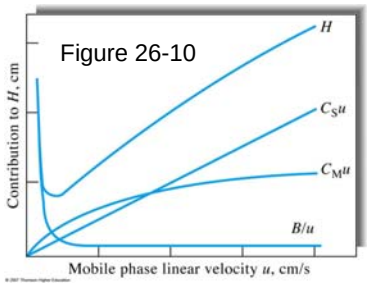
Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	cm s^{-1}
Diffusion coefficient in mobile phase*	D_M**	$cm^2\ s^{-1}$
Diffusion coefficient in stationary phase*	D_S	$cm^2\ s^{-1}$
Retention factor (Equation 26-12)	k	unitless
Diameter of packing particles	d_p	cm
Thickness of liquid coating on stationary phase	d_f	cm

TABLE 26-3 Processes That Contribute to Band Broadening

Process	Term in Equation 26-23	Relationship to Column* and Analyte Properties
Multiple flow paths	A	$A = 2\lambda d_p$
Longitudinal diffusion	B/u	$\frac{B}{u} = \frac{2\gamma D_M}{u}$
Mass transfer to and from stationary phase	$C_S u$	$C_S u = \frac{f(k)d_f^2}{D_S}u$
Mass transfer in mobile phase	$C_M u$	$C_M u = \frac{f'(k)d_p^2}{D_M}u$

More Inherent Column Characteristics

- Plate height depends on flow rate



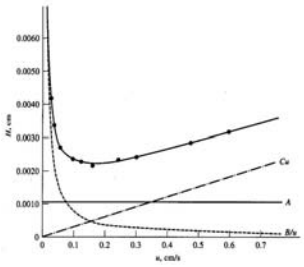
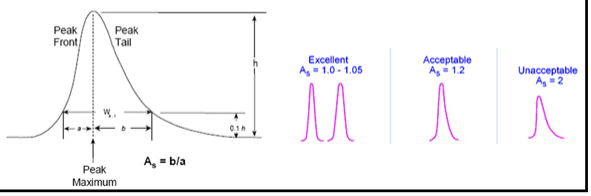


Figure 26-9 A van Deemter plot for a packed liquid chromatographic column. The points on the upper curve are experimental. The contributions of the various rate terms are shown by the lower curves: *A*, multipath effect; *B*/*u*, longitudinal diffusion; *Cu*, mass transfer for both phases. *(From E. Katz, R. L. Open, and R. P. W. Scott, J. Chromatogr., 1983, 270, 51. With permission.)*

- Other issues van Deemter doesn't address…
  - Peak Fronting
  - Peak Tailing

Asymmetry



More Inherent Column Characteristics

- Final separation is typically a compromise between quality and time
  - "Easily" controllable experimental conditions
    - Mobile Phase composition
    - Flow rate
  - Other controllable factors
    - stationary phase identity
    - column size
    - particle size

TABLE 26-4 Important Chromatographic Quantities and Relationships

Name	Symbol of Experimental Quantity	Determined From
Migration time, unretained species	t_M	Chromatogram (Figure 26-7)
Retention time, species A and B	$(t_R)_A$, $(t_R)_B$	Chromatogram (Figures 26-7 and 26-12)
Adjusted retention time for A	$(t'_R)_A$	$(t'_R)_A = (t_R)_A - t_M$
Peak widths for A and B	W_A, W_B	Chromatogram (Figures 26-7 and 26-12)
Length of column packing	L	Direct measurement
Volumetric flow rate	F	Direct measurement
Linear flow velocity	u	F and column dimensions (Equations 26-6 and 26-7)
Stationary-phase volume	V_S	Packing preparation data
Concentration of analyte in mobile and stationary phases	c_M, c_S	Analysis and preparation data

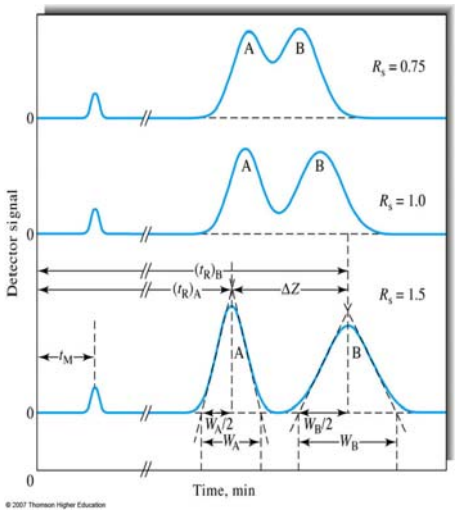
What makes a "good" separation?

- Resolution: ability to distinguish multiple species

$$R_S = \frac{\Delta Z}{W_A/2 + W_B/2} = \frac{2\Delta Z}{W_A + W_B}$$

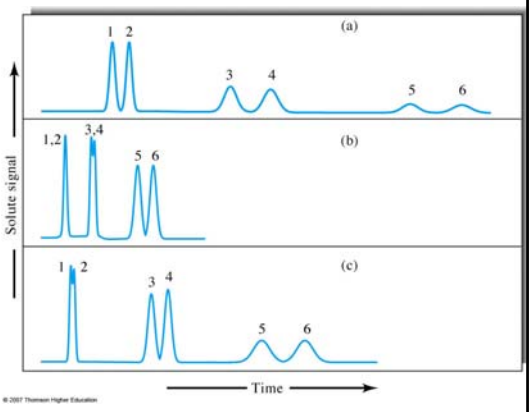
- Resolution is strongly dependent on the capacity factors for the two compounds that you want to separate

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



Optimizing Separations…Still

- In a complex mixture, the conditions need for optimal separation of some components won't be optimal for others
  - General Elution Problem



- Decrease separation time and improve the separation of multiple components by adjusting conditions during the run
  - LC: Gradient Elution
  - GC: Temperature Programming
- http://www.chem.uoa.gr/Applets/AppletChrom/Appl_Chrom2.html

Last Words

- Qualitative and Quantitative Analysis with Separations
- Peak Height vs. Peak Area for Quantitative Analysis
  - Peak area is more reliable
  - Peak height is easier to measure!
- The smaller plug you inject, the narrower the peaks will be.
- Dead Volume
- Important things to consider:
  - You are comparing chromatograms of two samples run under the same set of conditions., Both show peaks at the same retention time, does this mean that both samples contain the same compound?
  - In one chromatogram, peaks corresponding to two components have the same area. Does this mean that the two components are present at the same concentration?