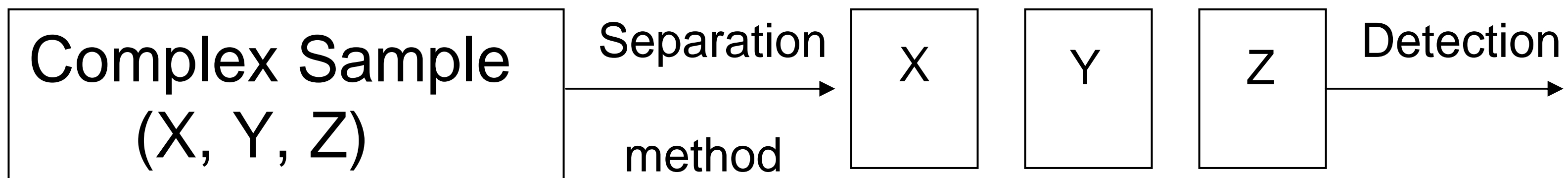
Chapter 26 – Introduction to Separation Science (Chromatography)

Read: pp. 762-783 Problems: 26-2,5,6,7,10

Separation science is a key aspect of most every analytical methodology! Most often, a real world sample contains many analytes that are responsive to the particular perturbation (e.g., light), and most analytical methods do not offer sufficient selectivity for all the analytes that might be present.

Therefore, most methods involve analyte separation first, followed by detection.



Separation Principal and The Chromatograph

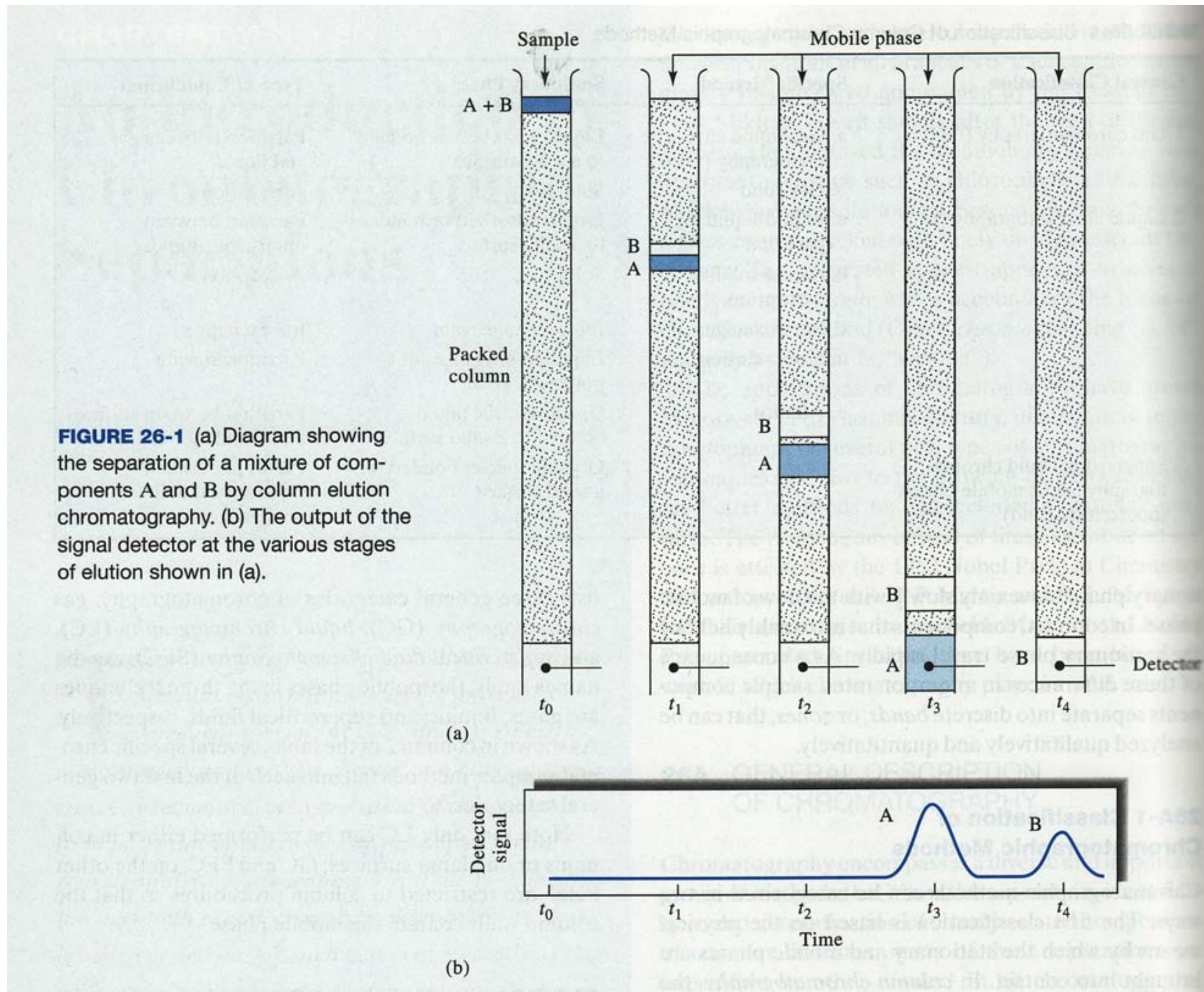
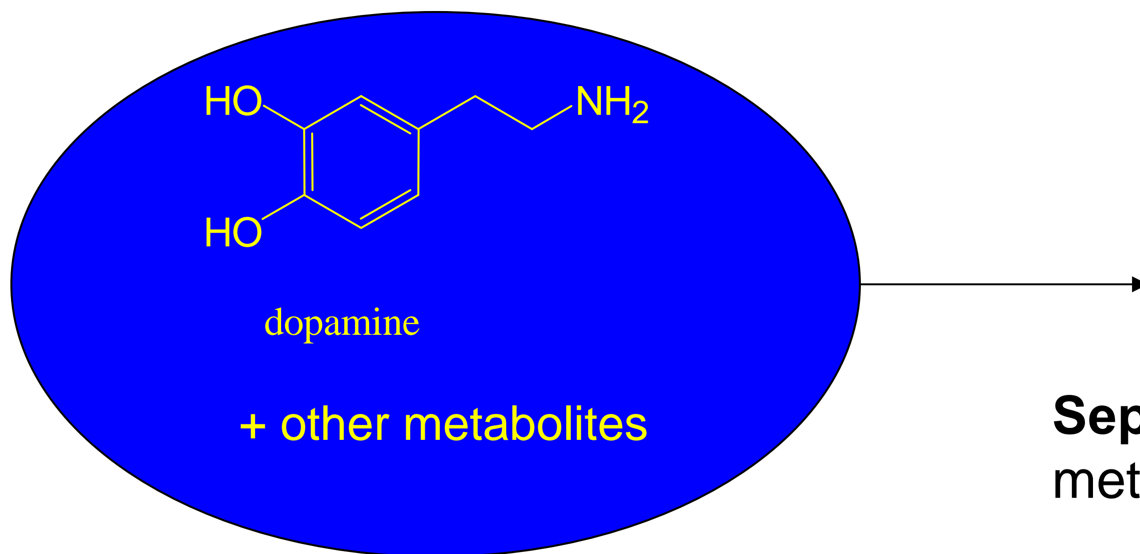


FIGURE 26-1 (a) Diagram showing the separation of a mixture of components A and B by column elution chromatography. (b) The output of the signal detector at the various stages of elution shown in (a).

A Chemical Problem

Imagine that you are tasked with investigating how electrical impulses are transferred from nerve cell to cell in the brain of Parkinson's patients and how these signals are affected by changing concentrations of one primary neurotransmitter, dopamine.



Need to separate the aromatic amines so that each can be individually detected.

Separation and **Detection** method must be selected!

Polar, water soluble, aromatic molecules

Types of Separation Methods

Mobile Phase + Stationary Phase

TABLE 26-1 Classification of Column Chromatographic Methods

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Liquid chromatography (LC) (mobile phase: liquid)	Liquid-liquid, or partition	Liquid adsorbed on a solid	Partition between immiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Liquid-solid, or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
Gas chromatography (GC) (mobile phase: gas)	Gas-liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Gas-solid	Solid	Adsorption
Supercritical-fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

Basics of the Separation

Mobile phase and Stationary phase

Migration time ⟶

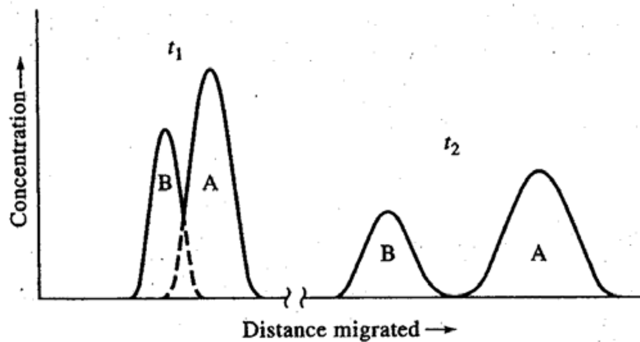


Figure 26-2 Concentration profiles of analyte bands A and B at two different times in their migration down the column in Figure 26-1. The times t_1 and t_2 are indicated in Figure 26-1.

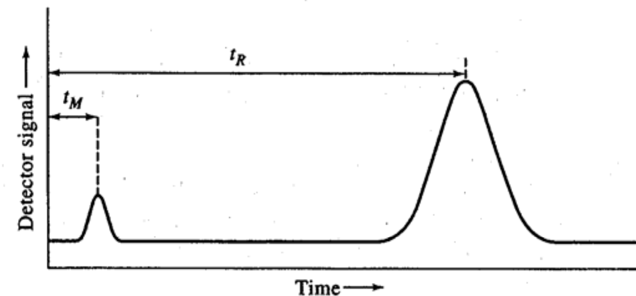


Figure 26-4 A typical chromatogram for a two-component mixture. The small peak on the left represents a species that is not retained on the column and so reaches the detector almost immediately after elution is started. Thus its retention time t_M is approximately equal to the time required for a molecule of the mobile phase to pass through the column.

The longer the analytes (solutes) spend interacting with the stationary phase, the better the separation efficiency is but the broader (in time) the peaks get.

Differential Interaction of Solute with Stationary and Mobile Phases

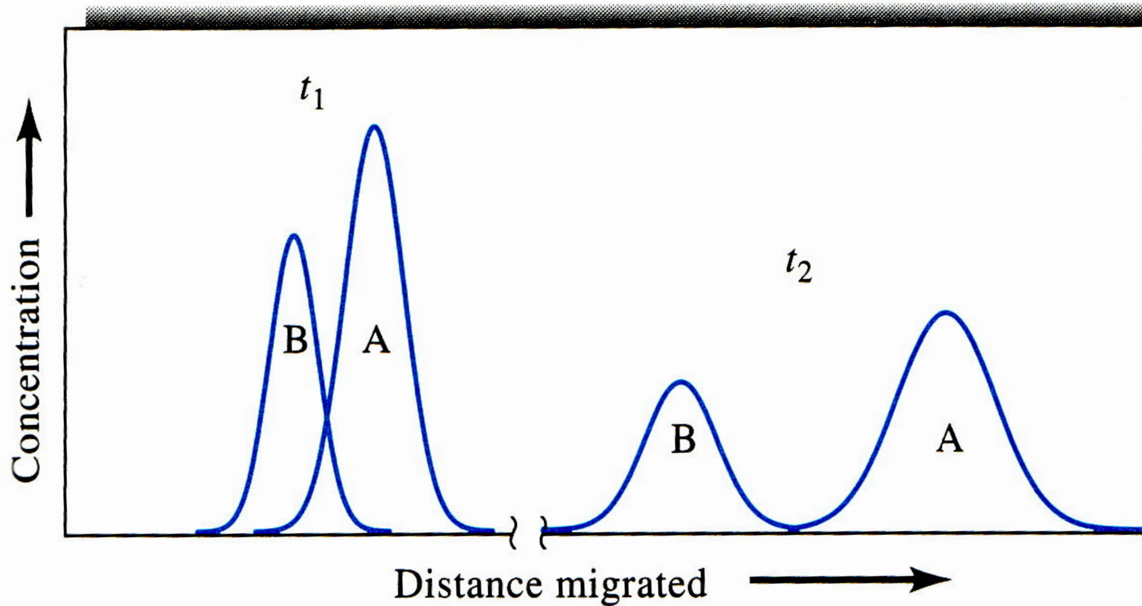


FIGURE 26-2 Concentration profiles of solute bands A and B at two different times in their migration down the column in Figure 26-1. The times t_1 and t_2 are indicated in Figure 26-1.

Asymmetric Peak Shapes

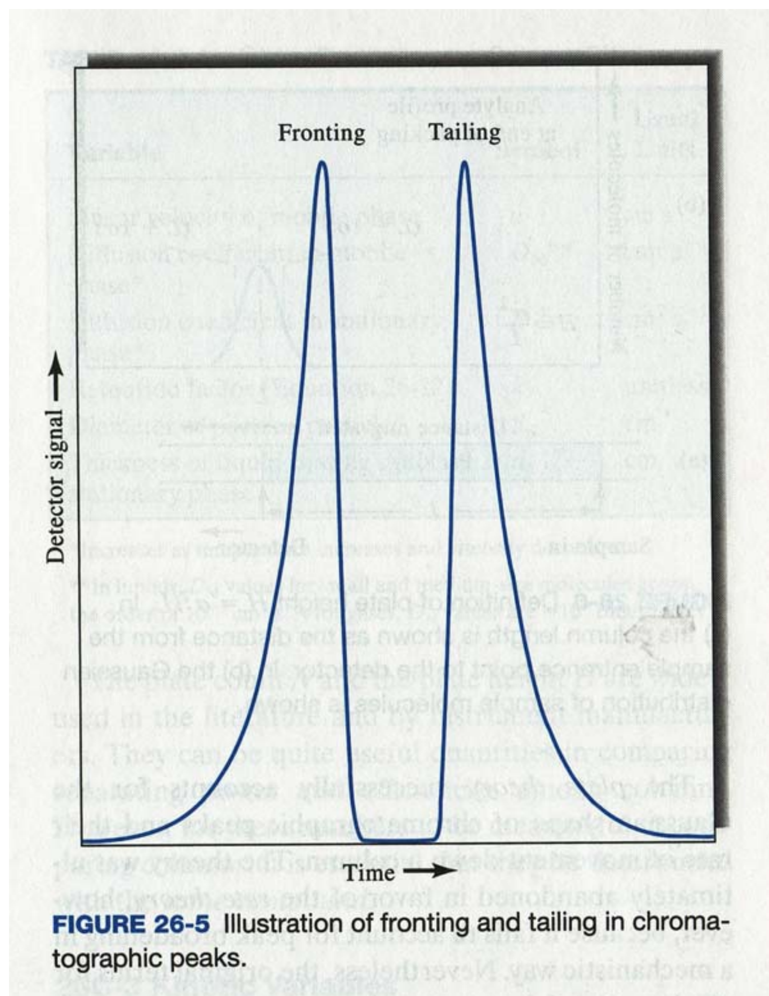


FIGURE 26-5 Illustration of fronting and tailing in chromatographic peaks.

Basics of the Separation

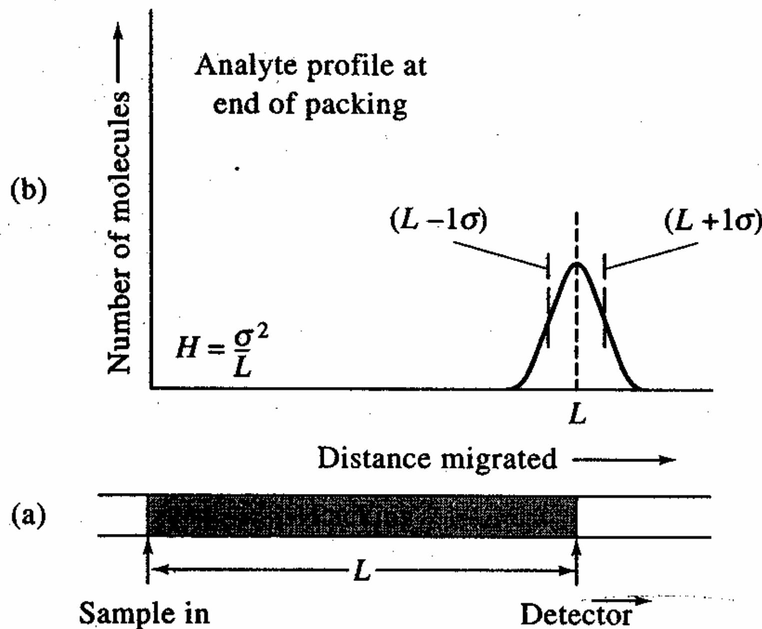


Figure 26-5 Definition of plate height $H = \sigma^2/L$.

N = L/H (efficiency)

N = number of plates
L = column length (cm)
H = plate height (cm/plate)

A pseudo-equilibrium is established on the column as the analyte (solute) develops a distribution within the mobile phase and the stationary phase.

K_{eq} (partition coefficient) = C_{stat}/C_{mobile}

Basics of the Separation Method

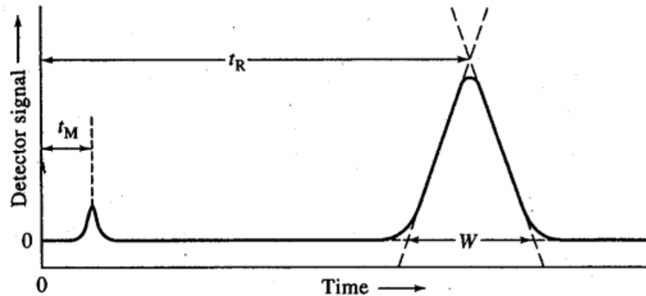


Figure 26-6 Determination of the standard deviation τ from a chromatographic peak: $W = 4\tau$.

k´ (capacity factor) = $(t_r - t_m)/t_m$

α (selectivity factor) = k'_b/k'_a

u (mobile phase flow vel., cm/s) = L/t_m

K_{eq} (distribution coeff.) = $(k'V_m)/V_s$

N (plate number) = L/H = $16\,(t_r/W)^2$

R_s (resolution) = $2[(t_r)_b - (t_r)_a]/(W_b + W_a)$

TABLE 26-2 Variables That Affect Column Efficiency

Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	$cm \cdot s^{-1}$
Diffusion coefficient in mobile phase*	D_M	$cm^2 \cdot s^{-1}$
Diffusion coefficient in stationary phase*	D_S	$cm^2 \cdot s^{-1}$
Retention factor (Equation 26-8)	k'	unitless
Diameter of packing particle	d_p	cm
Thickness of liquid coating on stationary phase	d_f	cm

*Increases as temperature increases and viscosity decreases.

Chromatographic Experimental Quantities

TABLE 26-4 Important Chromatographic Experimental Quantities and Relationships

Name	Symbol of Experimental Quantity	Determined From
Migration time, nonretained species	t_M	Chromatogram (Figure 26-6)
Retention times, species A and B	$(t_R)_A$, $(t_R)_B$	Chromatogram (Figure 26-6)
Adjusted retention time, species A	$(t'_R)_A$	$(t'_R)_A = (t'_R)_A - t_M$
Peak widths, species A and B	W_A, W_B	Chromatogram (Figure 26-6)
Length of column packing	L	Direct measurement
Flow rate	F	Direct measurement
Volume of stationary phase	V_S	Packing preparation data
Concentration of analyte in mobile and stationary phases	c_M, c_S	Analysis and preparation data

Separation Figures of Merit

TABLE 26-5 Important Derived Quantities and Relationships

Name	Calculation of Derived Quantities	Relationship to Other Quantities
Linear mobile-phase velocity	$u = L/t_M$	
Volume of mobile phase	$V_M = t_M F$	
Retention factor	$k' = (t_R - t_M)/t_M$	$k' = \frac{KV_S}{V_M}$
Distribution constant	$K = \frac{k' V_M}{V_S}$	$K = \frac{c_S}{c_M}$
Selectivity factor	$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$	$\alpha = \frac{k'_B}{k'_A} = \frac{K_B}{K_A}$
Resolution	$R_s = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$	$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_B}{1 + k'_B}\right)$
Number of plates	$N = 16\left(\frac{t_R}{W}\right)^2$	$N = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{1 + k'_B}{k'_B}\right)^2$
Plate height	$H = L/N$	
Retention time	$(t_R)_B = \frac{16R_s^2 H}{u}\left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1 + k'_B)^3}{(k'_B)^2}$	

Factors Influencing Peak Widths

Retention time ⟷ Peak width ⟷ Resolution ⟷ Efficiency

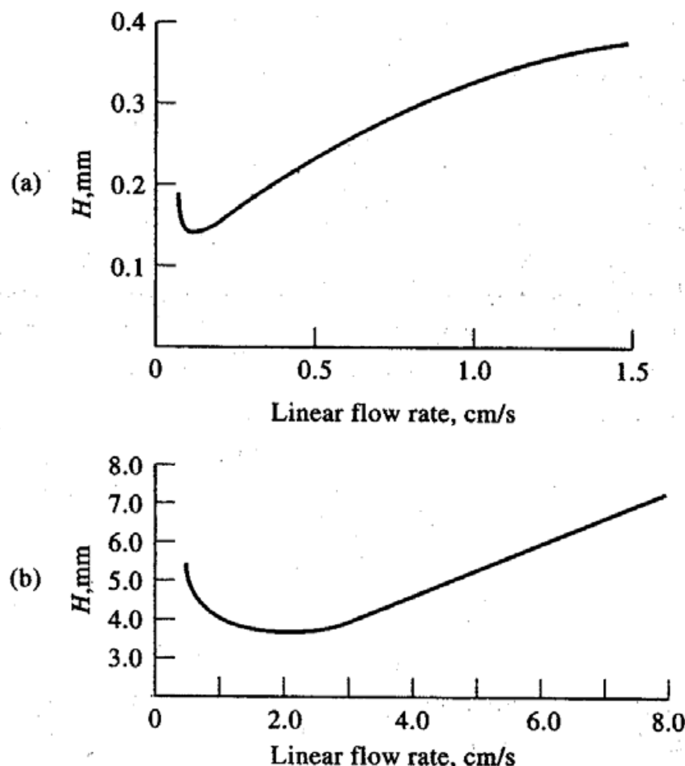


Figure 26-7 Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.

What one wants is the shortest retention time that gives adequate resolution of all closely eluting components!

$$N = L/H$$

One wants a small value for H!

Factors Influencing Peak Widths

What factors make H large?

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

A

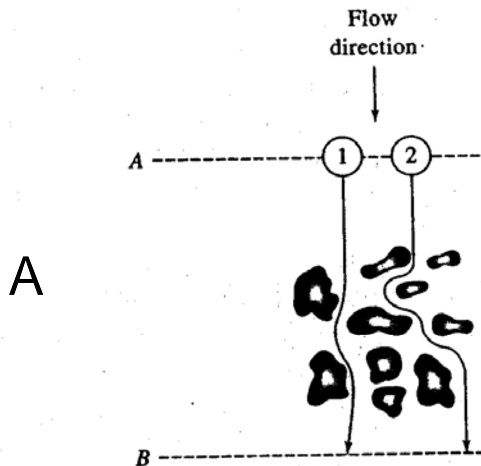


Figure 26-8 Typical pathways of two molecules during elution. Note that distance traveled by molecule 2 is greater than that traveled by molecule 1. Thus, molecule 2 would arrive at *B* later than molecule 1.

TABLE 26-3 Kinetic Processes That Contribute to Peak Broadening

Process	Term in Equation 26-19	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 liquid stationary phase	$C_S u$	$C_S u = \frac{f_S(k')d_f^2}{D_S} u$
Mass transfer in mobile phase	$C_M u$	$C_M u = \frac{f_M(k')d_p^2}{D_M} u$

*u, D_S, D_M, d_f, d_p, k' are as defined in Table 26-2.
$f(x)$ = function of x.
λ, γ: constants that depend on the quality of the packing.
B: coefficient of longitudinal diffusion.
C_S, C_M: coefficients of mass transfer in stationary and mobile phases, respectively.

B

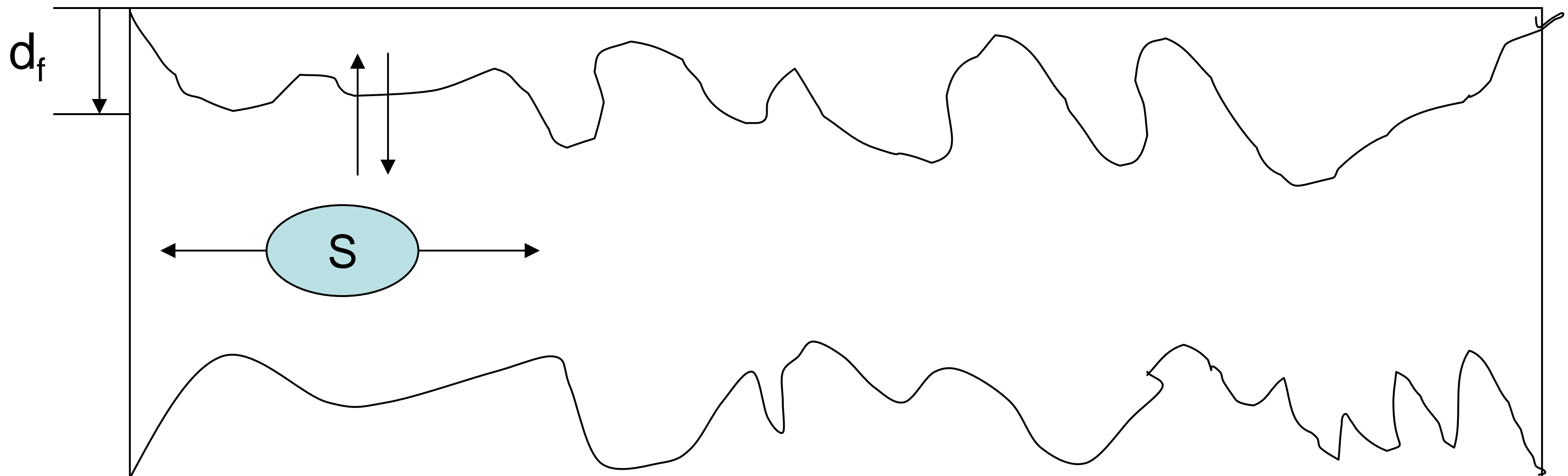
Factors Influencing Peak Widths

C_s mass transport to and from stationary phase
C_m mass transport in mobile phase

$C_s \propto d_f/D_s$

$C_m \propto d^2_p/D_m$

Migration time →



Remember: the stationary phase is pretty uniform in terms of its structure and chemistry. Therefore, differential rates of migration can be established

Factors Influencing Peak Widths

$H = A + B/u + Cu$

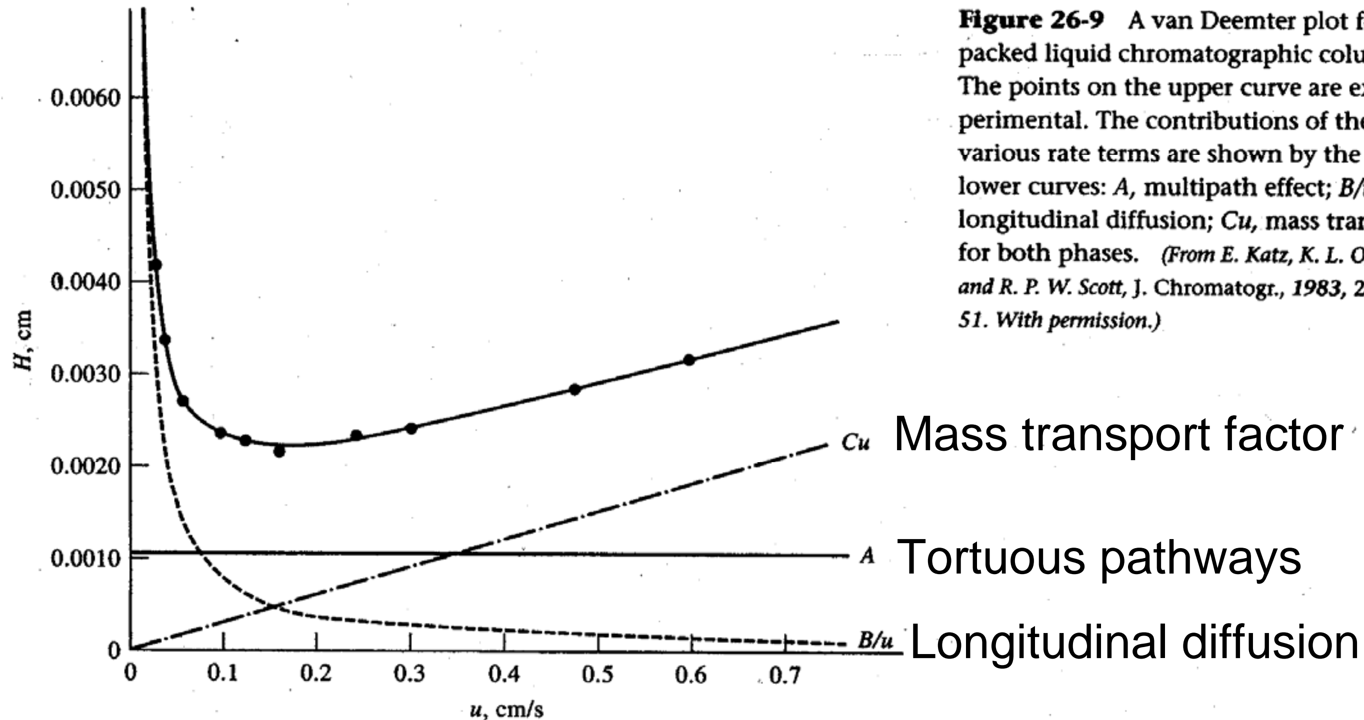


Figure 26-9 A van Deemter plot for a packed liquid chromatographic column. The points on the upper curve are experimental. The contributions of the various rate terms are shown by the lower curves: *A*, multipath effect; *B/u*, longitudinal diffusion; *Cu*, mass transfer for both phases. *(From E. Katz,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

Remember $N = L/H$!

Factors Influencing Peak Widths

The plate height, H, depends on the particle size!!
A and C terms of Van deemter equation increase with particle size.

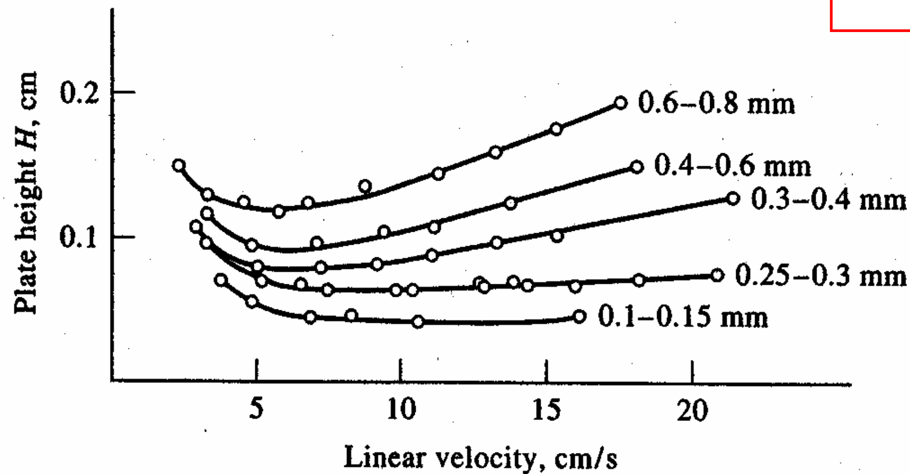


Figure 26-10 Effect of particle size on plate height. The numbers to the right are particle diameters. *(From J. Boheman and J. H. Purnell, in* Gas Chromatography, 1958, *D. H. Desty, Ed. New York: Academic Press, 1958. With permission of Butterworths, Stoneham, MA.)*

Resolution (Time) in Chromatograms

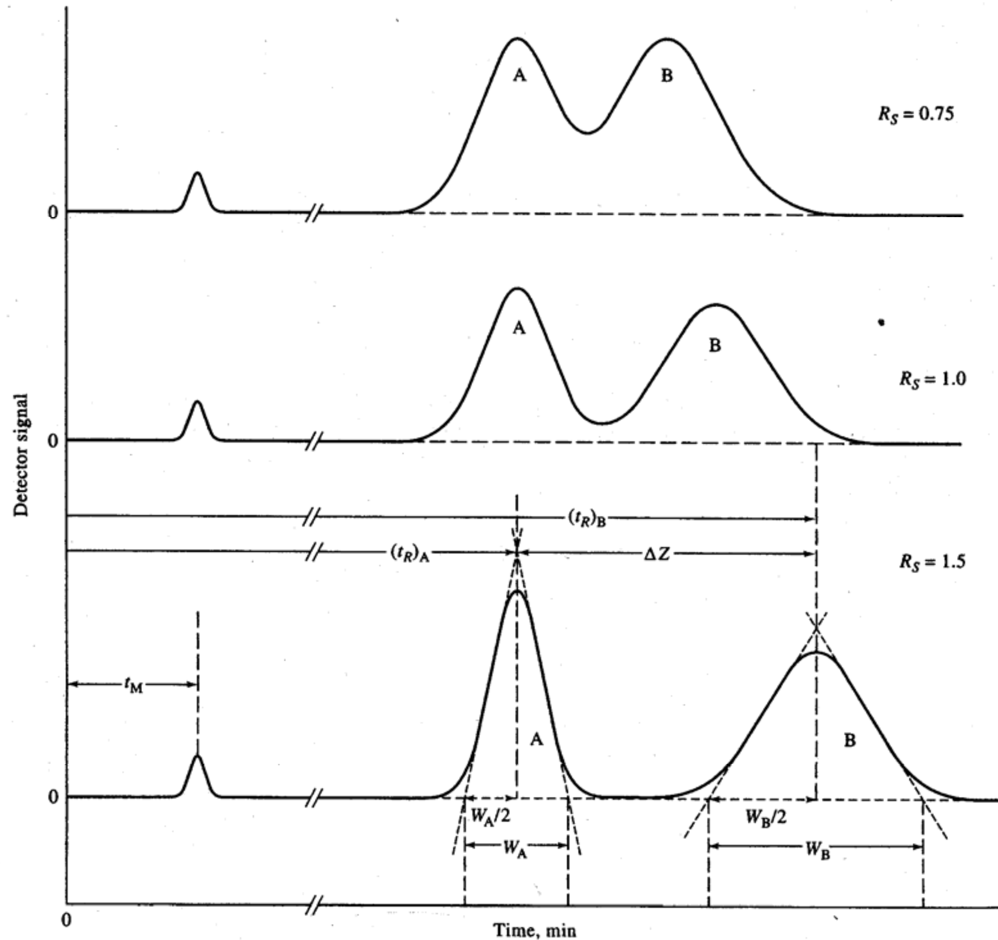


Figure 26-11 Separations at three resolutions. Here, $R_s = 2\Delta Z/(W_A + W_B)$.

Adjustments of the stationary phase material and properties, the linear flow velocity of the mobile phase, and the mobile phase composition are all varied to optimize the separation.

Desired large N, high resolution and short analysis time!

The General Elution Problem

Gas or Liquid Chromatography

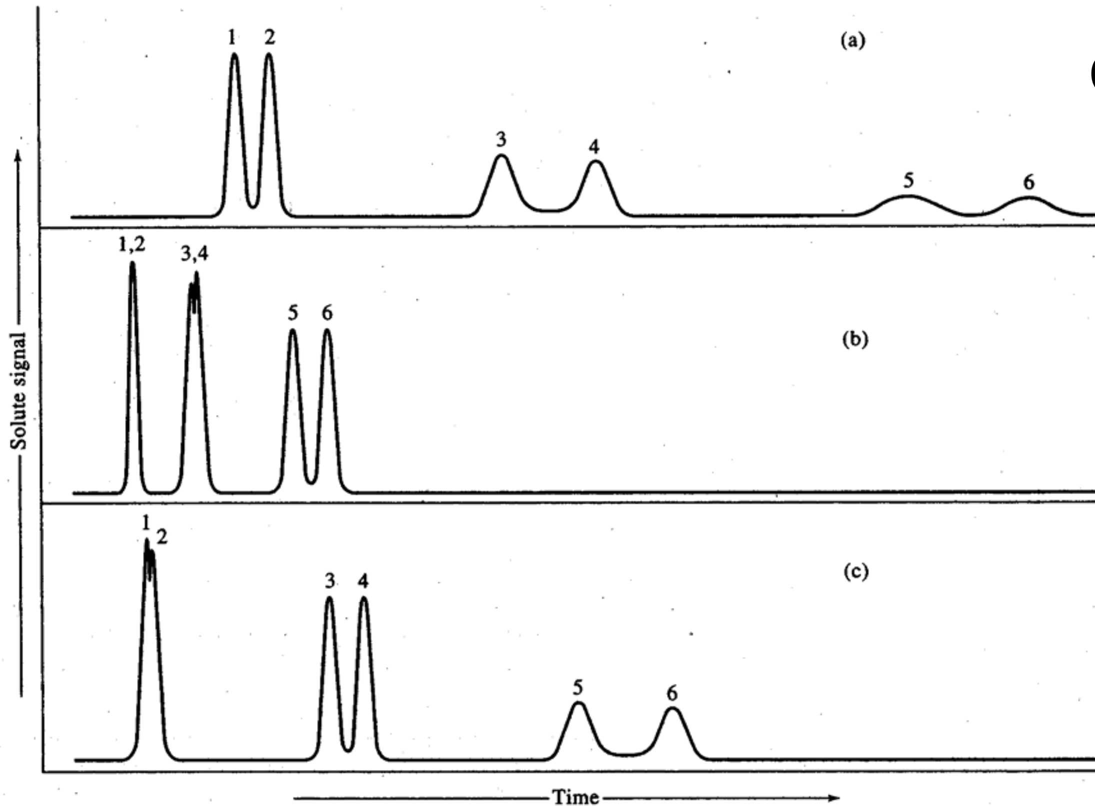


Figure 26-14 Illustration of the general elution problem in chromatography.

To solve this problem, the separation conditions are often not kept constant during the entire run, but rather are altered at some point during the run. Typical separations often begin with one set of conditions and end with a another set.