Chromatographic separations

Chapter 26

The "stuff" you do before you analyze a "complex" sample

It is all about "Reducing Interferences"

Table 24-1

Methods for Eliminating Interferences in a Chemical Analysis

Method	Basis of Method
1. Masking	Immobilization of interferent as a nonreactive complex
2. Mechanical phase separation	
a. Precipitation and filtration	Difference in solubility of compounds formed
b. Distillation	Difference in volatility of compounds
c. Extraction	Difference in solubility in two immiscible liquids
d. Ion exchange	Difference in stability of reactants with an ion-exchange resin
3. Chromatography	Difference in rate of movement of a solute through a stationary phase
4. Electrophoresis	Difference in migration rate in an electrical field gradient

Chromatography basics

- *Mobile* and *Stationary* phase
- *Retention - Migration*
- *Bands or zones*
- *Equilibrium!*

- *Column vs. planar*
- *Liquid vs. gas vs. SF*
- *High vs. low resolution*
- *Partition*
- *Adsorption*
- *Ion exchange*
- *Size exclusion*

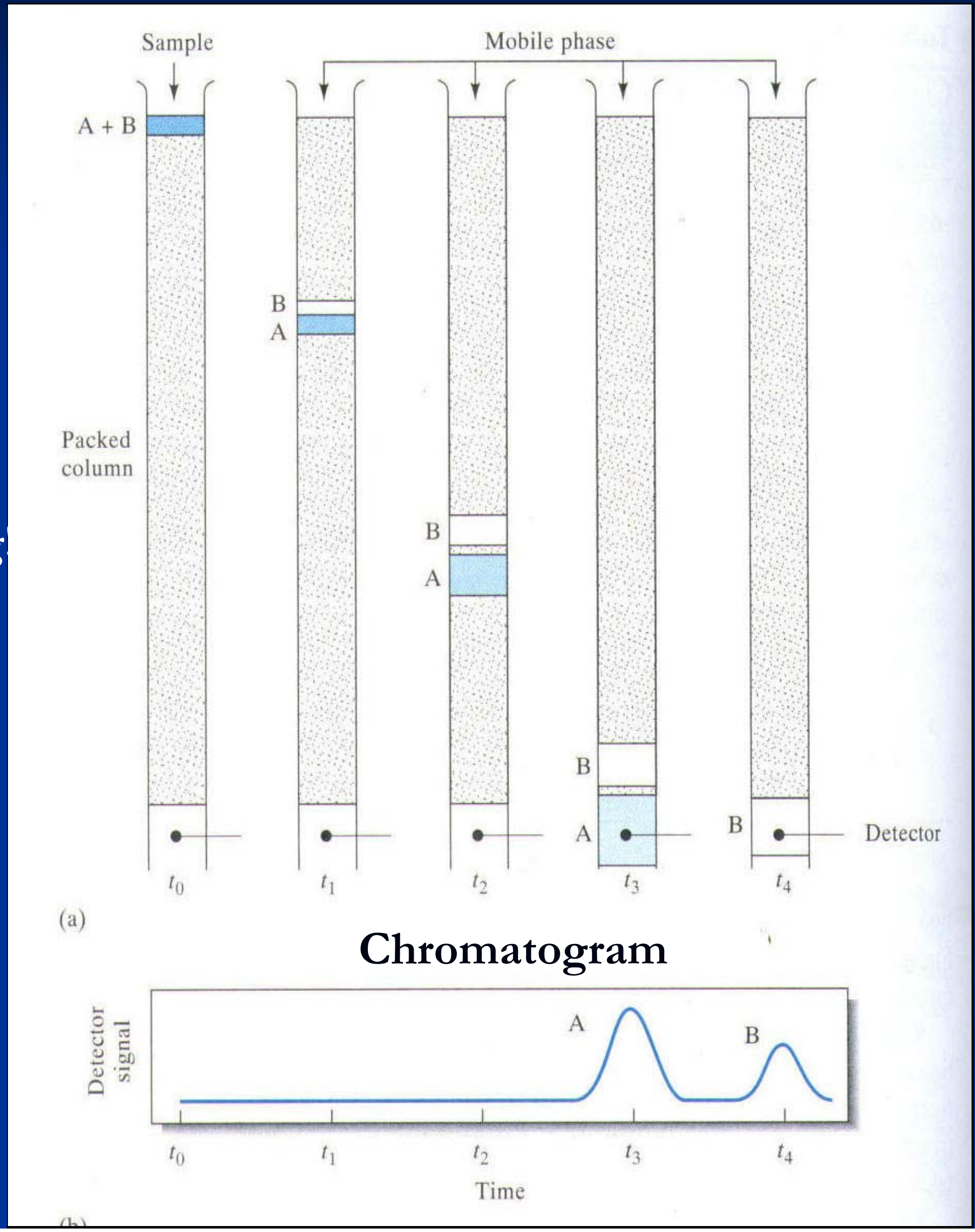
Chromatography

A. Column Chromatograpgy, B. Planar Chromatograpgy

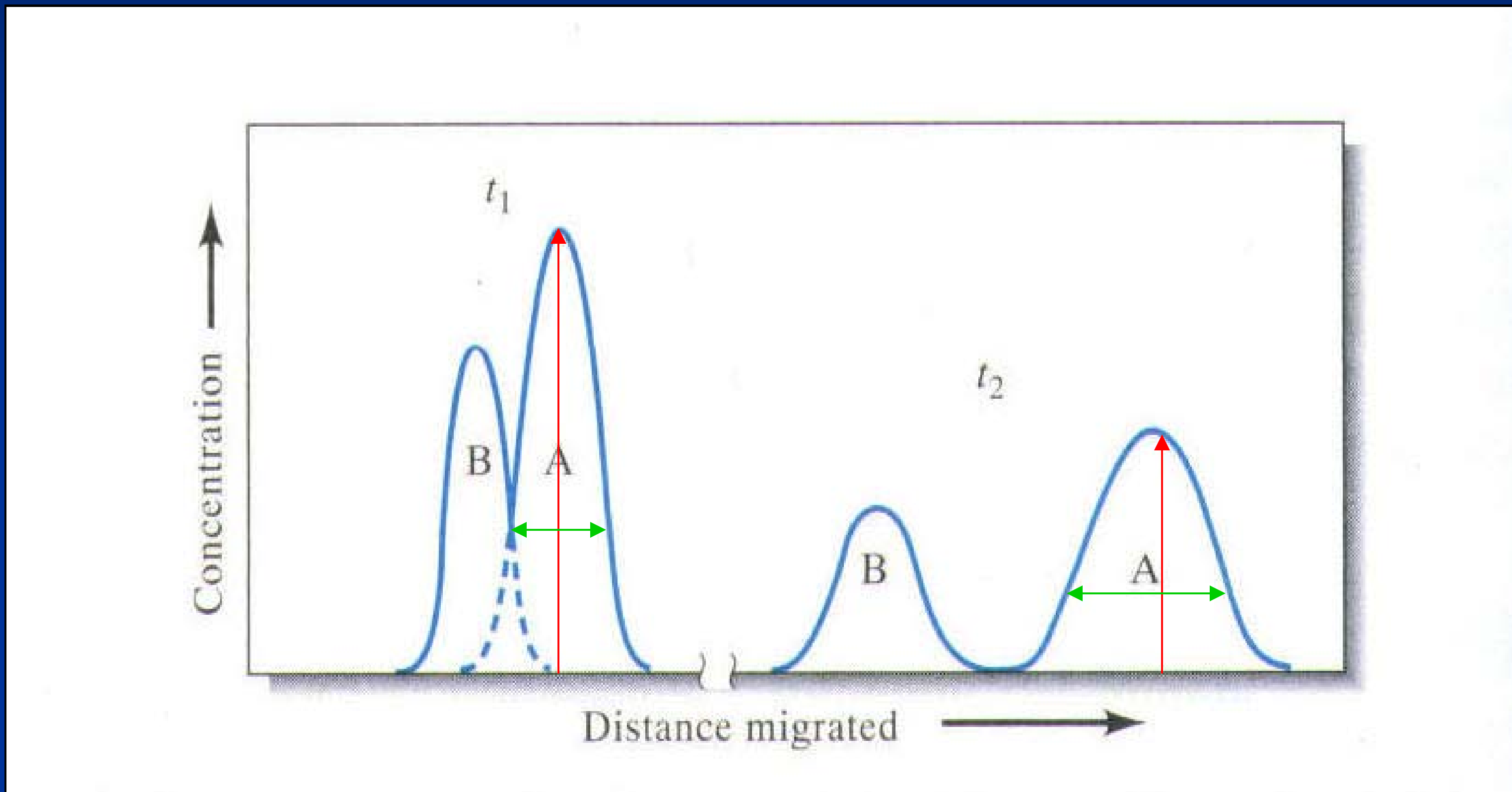
General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Liquid chromatography (LC) (mobile phase: liquid)	Liquid-liquid, or partition	Liquid adsorbed on a solid	Partition between immiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid 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

Column Chromatography

Dilution &
Peak broadening!



Chromatography: Peak separations

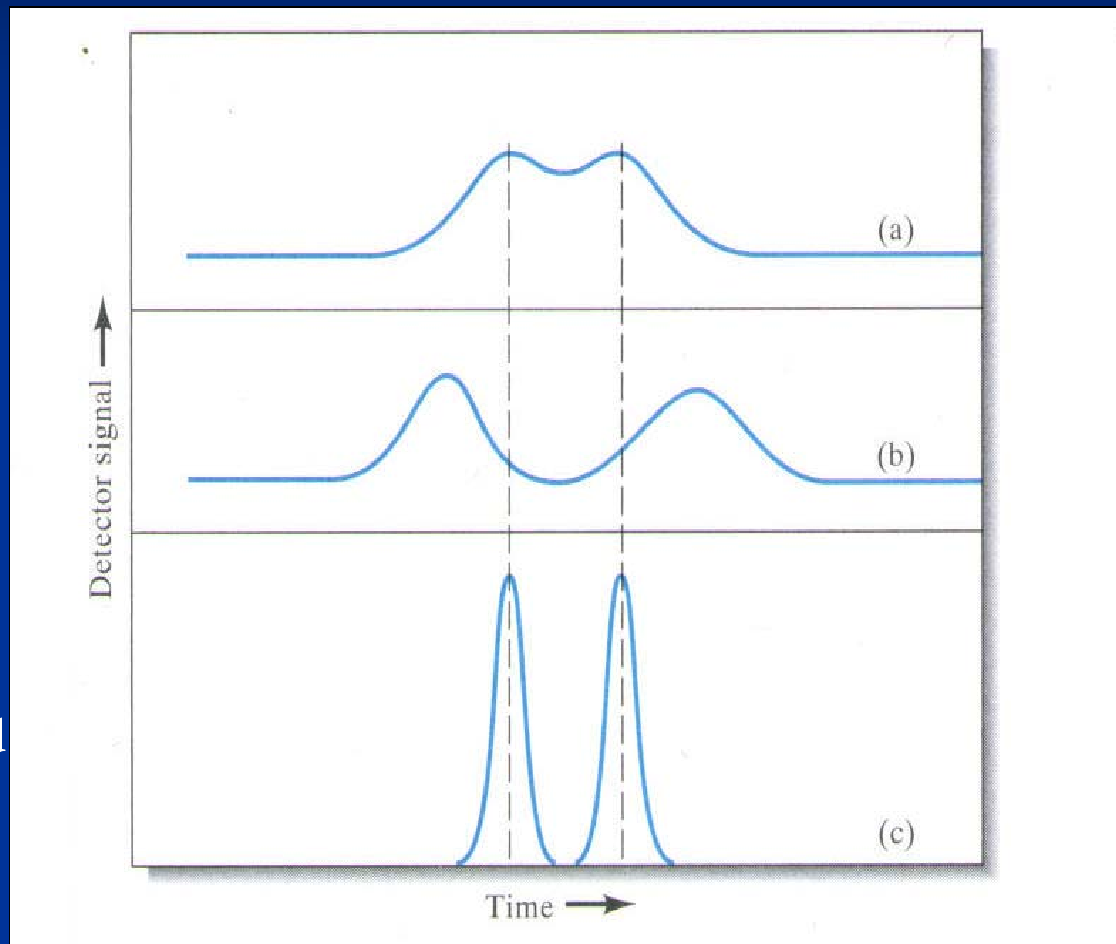


Chromatography: Peak Resolution

Poor resolution

More separation

Less band spread



Chromatography:

Distribution Constant (recommended by IUPAC)
(old term: partition coefficient)

A mobile ↔ A stationary

$$K_c = \frac{c_S}{c_M}$$

stationary
mobile

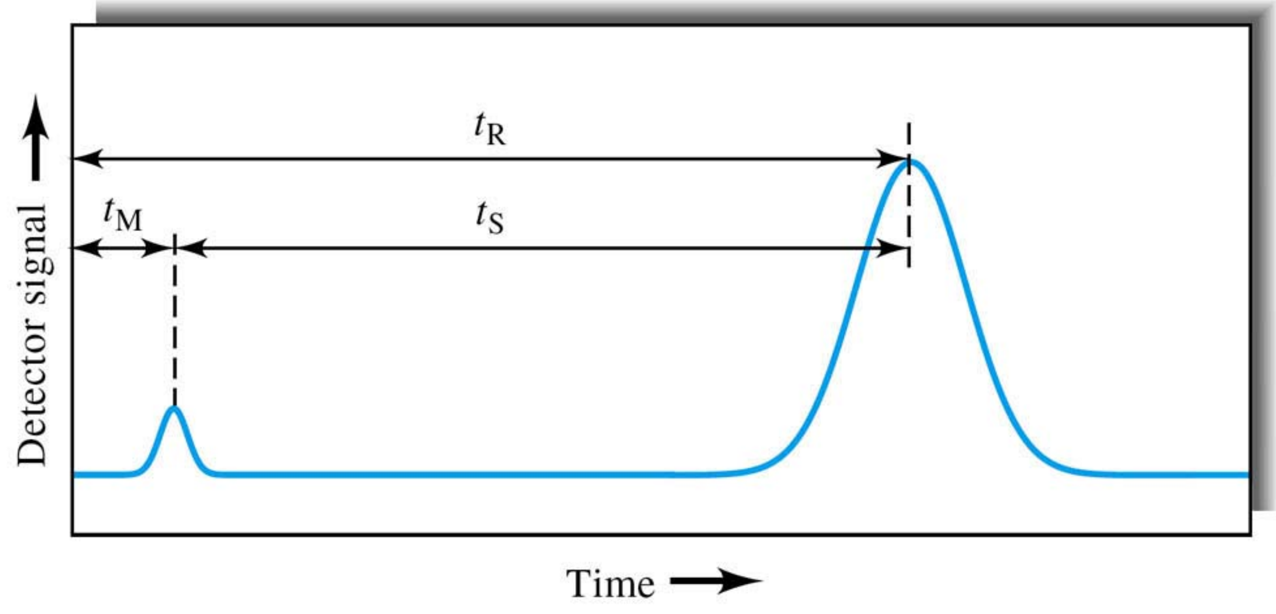
$C_S = n_S/V_S$, $C_M = n_M/V_M$

K ~ constant ➔ linear chromatography

>>>K >>> Retention in the stationary phase → Retention times

How to manipulate K?

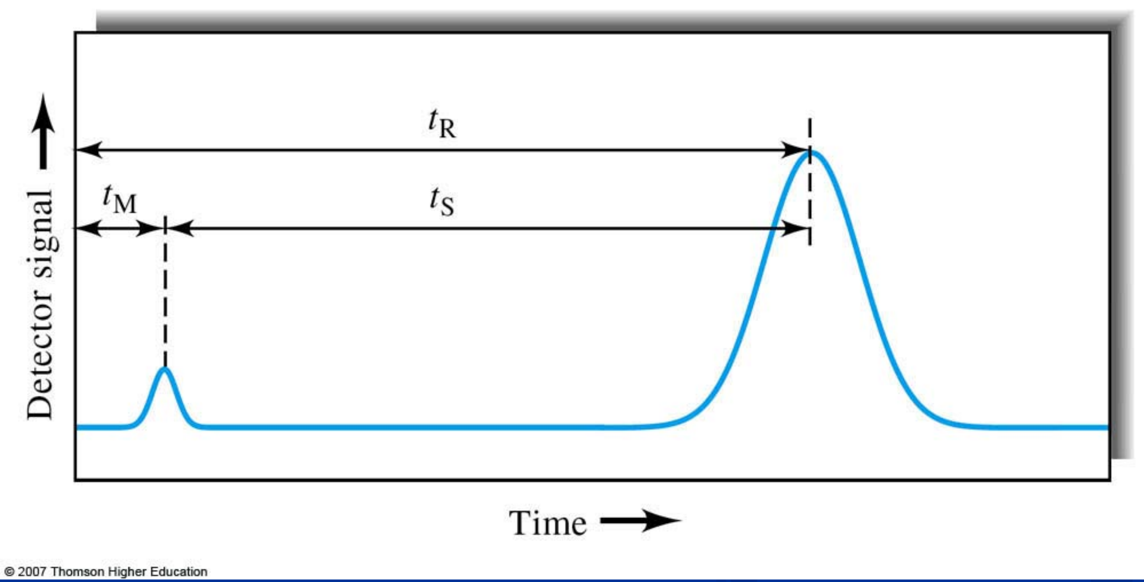
Chromatography Retention Times



t_M = retention time of mobile phase (dead time)
t_R = retention time of analyte (solute)
t_S = time spent in stationary phase (adjusted retention time)
L = length of the column

Chromatography: Velocities

Linear rate of solute migration!



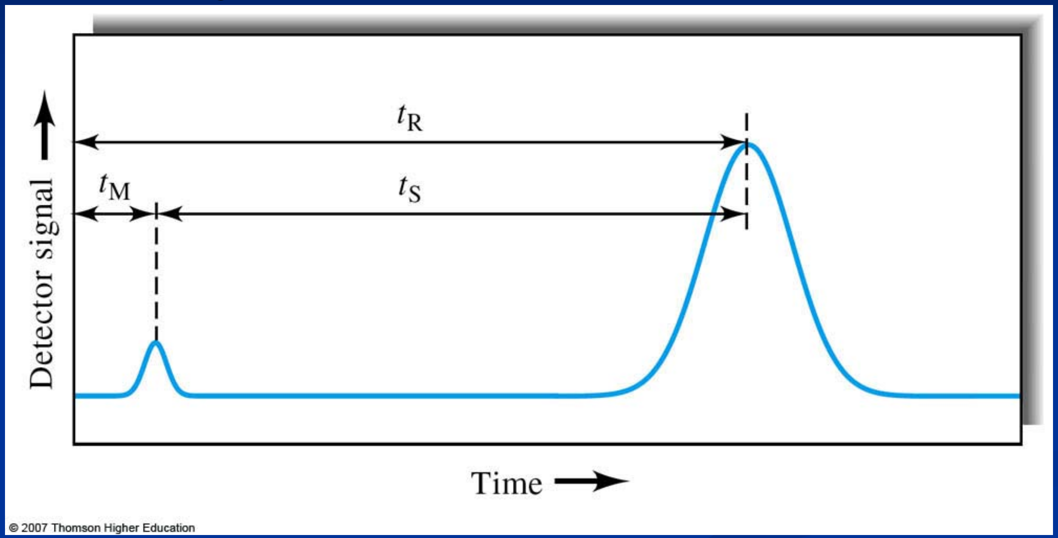
Velocity = distance/time → length of column/ retention times

Velocity of solute: $$\bar{v} = \frac{L}{t_R}$$

Velocity of mobile phase: $$\mu = \frac{L}{t_M}$$

Chromatography

Velocity/Retention time and Kc



$$\bar{v} = \mu \times \text{fraction of time in mobile phase}$$

$$\bar{v} = \mu \times \frac{\text{moles of solute in mobile phase}}{\text{total moles of solute}}$$

$$\bar{v} = \mu \times \frac{c_M V_M}{c_M V_M + c_S V_S}$$

Chromatography

Velocity Relationships

$$\bar{v} = \mu \times \frac{c_M V_M}{c_M V_M + c_S V_S}$$

$$\bar{v} = \mu \times \frac{1}{1 + c_S V_S / c_M V_M}$$

$$K = \frac{c_S}{c_M} \quad \text{Distribution Constant}$$

$$\bar{v} = \mu \times \frac{1}{1 + K\, V_S / V_M}$$

Chromatography

Retention Factor : are we there yet?

$$\bar{v} = \mu \times \frac{1}{1 + K\,V_S / V_M}$$

$$k_A = K_A V_S / V_M \quad \text{(Retention Factor)}$$

$$\bar{v} = \mu \times \frac{1}{1 + k_A}$$

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k_A}$$

$$k_A = \frac{t_R - t_M}{t_M}$$

← **Adjusted retention time**

Relative retention time:

RRT = t_R / t_{Rs}

t_{Rs} = retention time of internal standard

Chromatography

Selectivity Factor: can you separate from your neighbor

B retained more than A → $\alpha > 1$

$$\alpha = \frac{K_B}{K_A}$$

Distribution Constant

$$\alpha = \frac{k_B}{k_A}$$

Retention factor

$$k_A = \frac{(t_R)_A - t_M}{t_M} \quad and \quad k_B = \frac{(t_R)_B - t_M}{t_M}$$

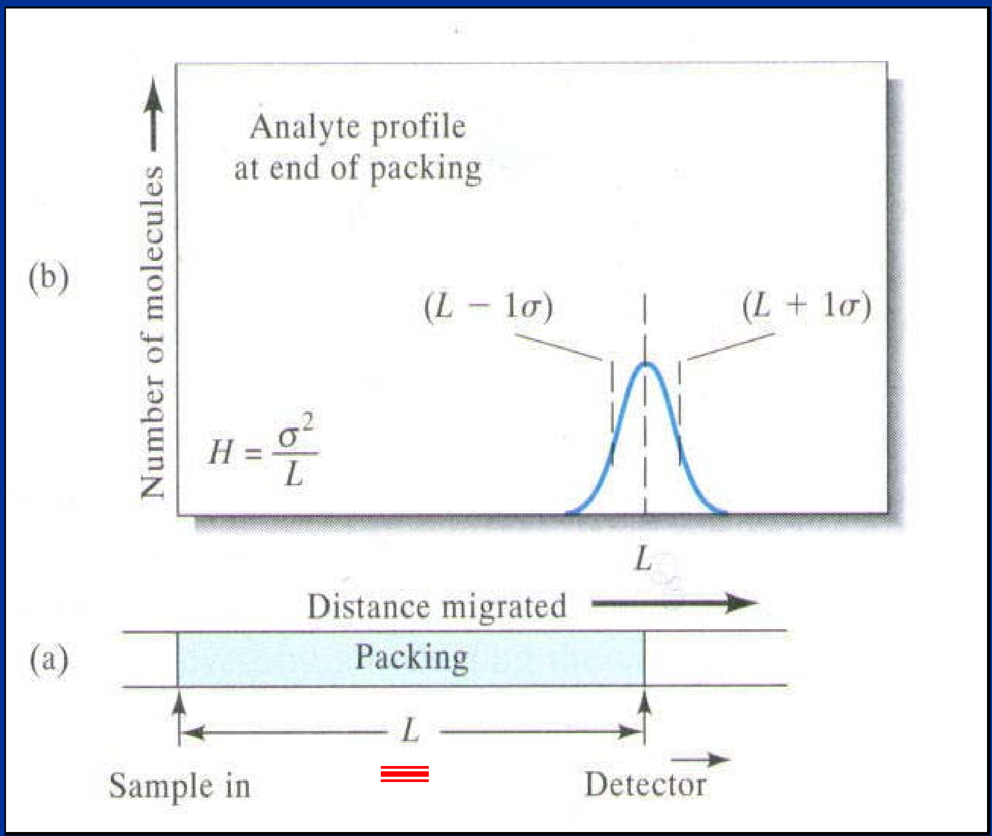
$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

Retention time

Chromatography

Column Efficiency - Theoretical Plates

Plate and Rate Theories



$$H = \text{plate height}$$

$$N = \text{number of plates}$$

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

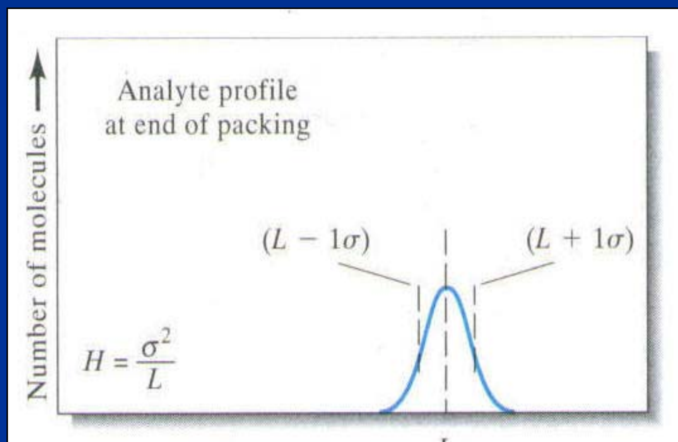
$$H = \frac{\sigma^2}{L}$$

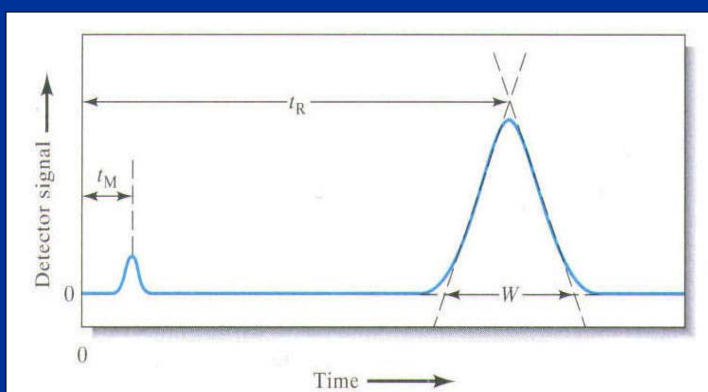
L = length of column packing

$\sigma \rightarrow$ standard deviation $\sigma^2/L \rightarrow$ variance per unit length.

Chromatography

Relation between column distance and retention times





$L =$ column length (distance)

$\sigma =$ standard deviation in distance

$t_R =$ retention time

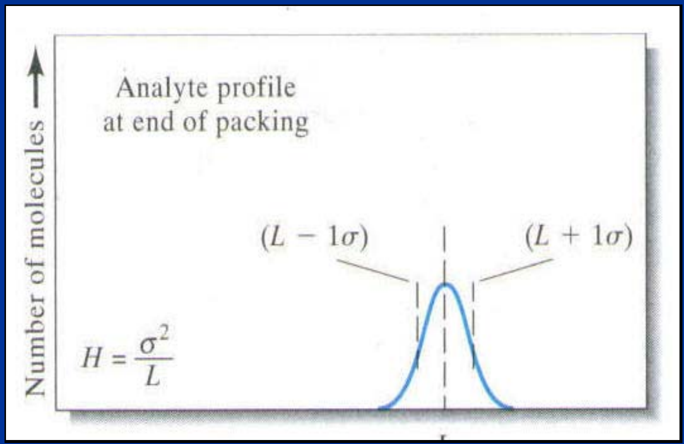
$\tau =$ standard deviation in time

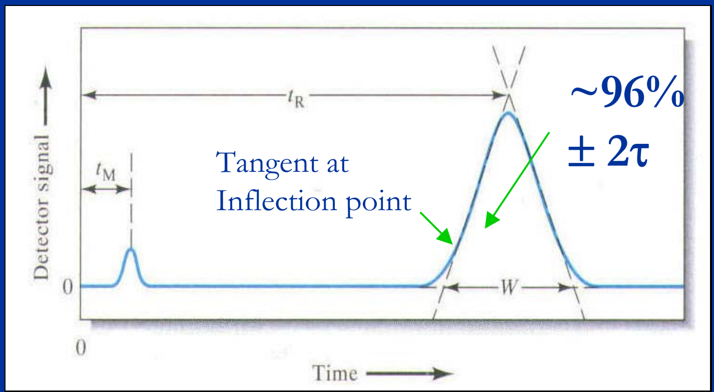
$$\frac{\sigma}{L} = \frac{\tau}{t_R}$$

$$\tau = \frac{\sigma}{L/t_R}$$

Chromatography

Relation between column distance and retention times





$$\frac{\sigma}{L} = \frac{\tau}{t_R}$$

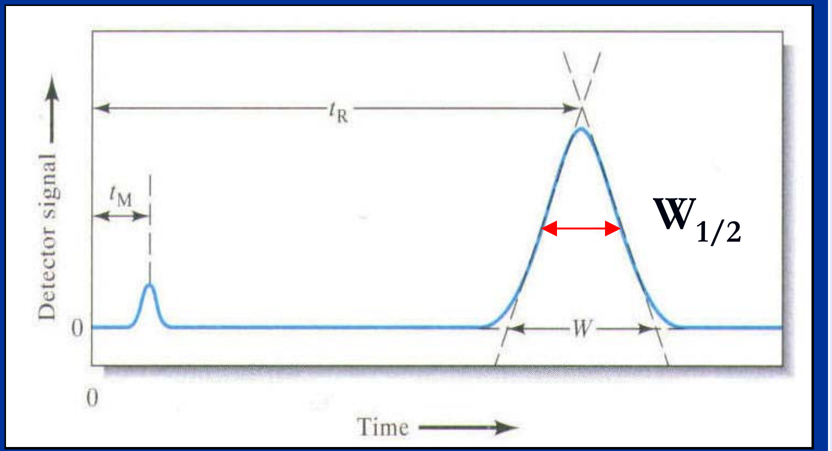
$$\sigma = \frac{\tau L}{t_R}$$

$$W = 4\tau$$

$$\sigma = \frac{W L}{4 t_R}$$

$$\boxed{H = \frac{\sigma^2}{L} = \frac{W^2 L}{16 t_R^2}}$$

Chromatography
Determining the Number of Theoretical Plates



$$N = \text{numberof pates}$$

$$N = 16\left(\frac{t_R}{W}\right)^2$$

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$

Summary of Plate Theory

- Successfully accounts for the peak shapes and rate of movement
- Does not account for the “mechanism” causing peak broadening
- No indication of other parameters’ effects
- No indication for adjusting experimental parameters

Rate Theory

- Zone broadening is related to Mass Transfer processes

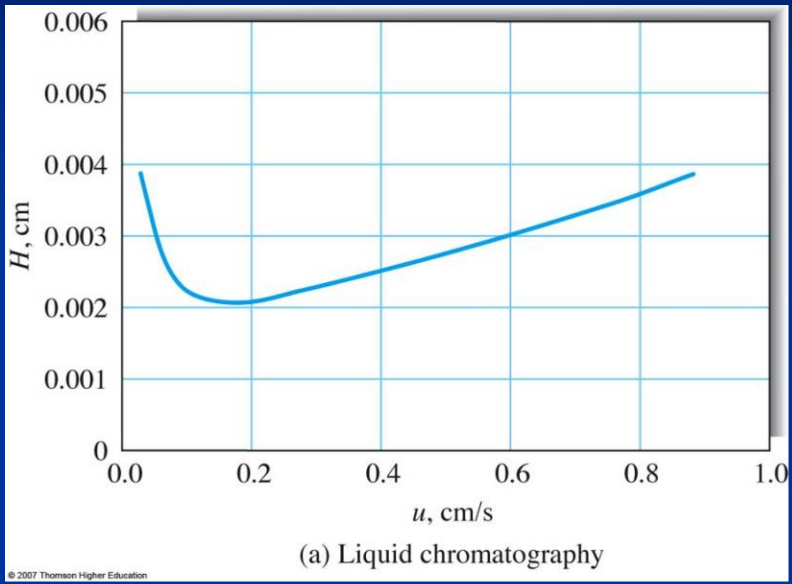
Column Efficiency
Kinetic variables

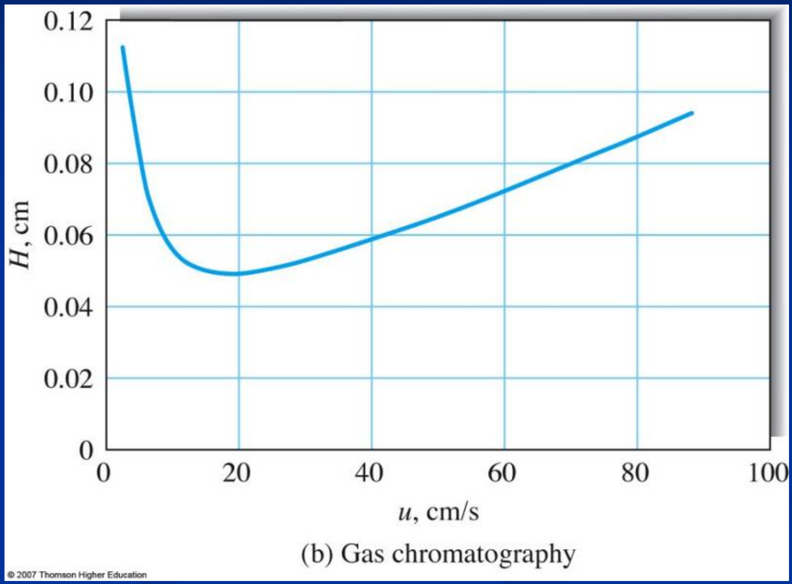
TABLE 26-2 Variables That Influence Column Efficiency

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

Zone Broadening

Flow Rate of Mobile Phase





Liquid chromatography ***Gas chromatography***

Note the differences in flowrate and plates height scales

Why GC normalluy has high H, but also high overall efficiency?

Zone Broadening

Kinetic Processes

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	$C_M u$	$C_M u = \frac{f'(k) d_p^2}{D_M} u$

Van - Deemter Equation

λ and γ are constants that depend on quality of the packing.

B is coefficient of longitudinal diffusion.

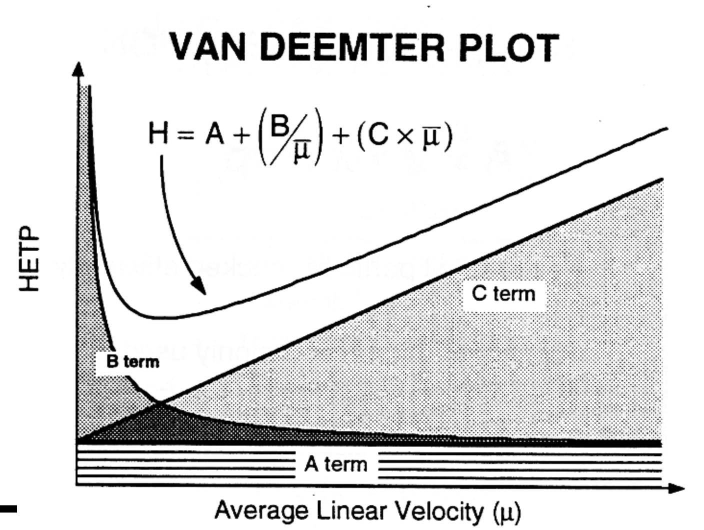
Cs and Cm are coefficients of mass transfer in stationary and mobile phase, respectively.

$$H = A + B/\mu + (C_S + C_M)\mu$$

Zone Broadening

Kinetic Processes

Process	Term in Equation 26-19	Relationship to Column* and Analyte Properties
Multiple flow paths	A	
Longitudinal diffusion	B/u	
Mass transfer to and from liquid stationary phase	$C_S u$	
Mass transfer in mobile phase	$C_M u$	

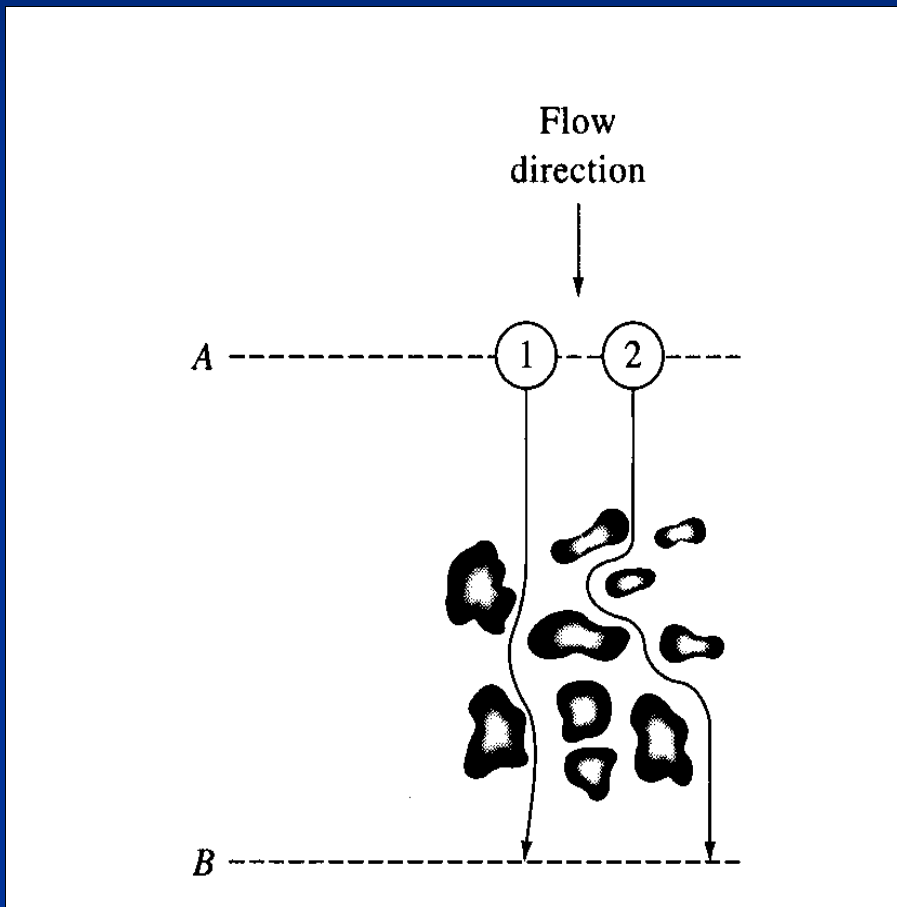


Van - Deemter Equation

$$H = A + B/\mu + (C_S + C_M)\mu$$

Zone Broadening

Multiple Pathways



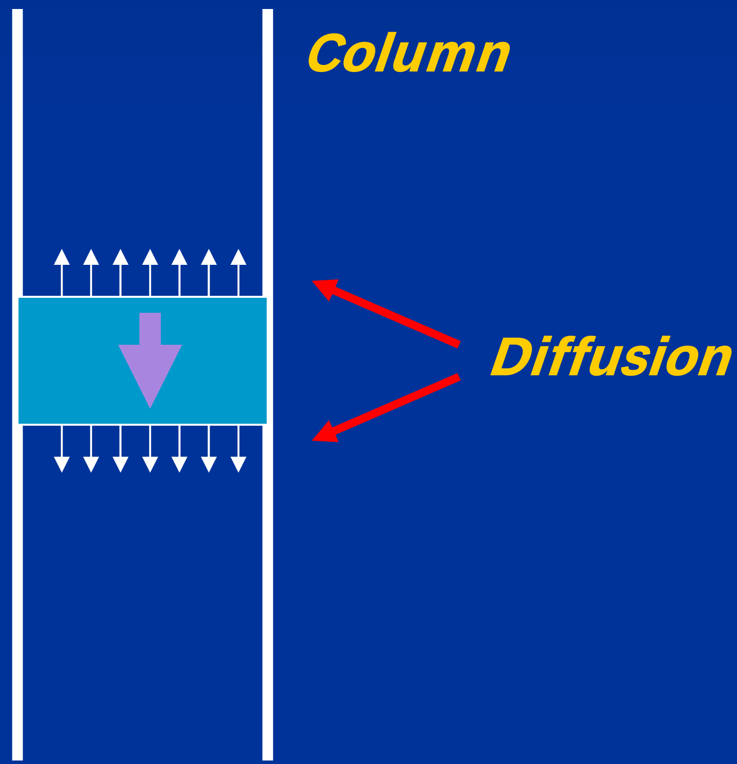
Eddy Diffusion: band broadening process results from different path lengths passed by solutes.

1. Directly proportional to the diameters of packing
2. Offset by ordinary diffusion
3. Lower mobile-phase velocity, smaller eddy diffusion

Stagnant pools of mobile phase retained in stationary phase.

Zone Broadening

Longitudinal Diffusion



- The higher the μ, the smaller the H
- Much smaller in LC than in GC

Zone Broadening

Mass Transfer between Phases

- Slow equilibrium of solute between mobile and stationary phases
- Time is required for solute molecules to diffuse from the interior of these phase to there interface where transfer occur

Zone Broadening
Longitude vs. Mass Transfer

- Longitude
  - Parallel to the flow
  - Inversely proportional to the flow of the mobile phase
- Mass Transfer
  - Diffusion tends to be right angles to the flow
  - The faster the mobile phase moves, the larger the band broadening

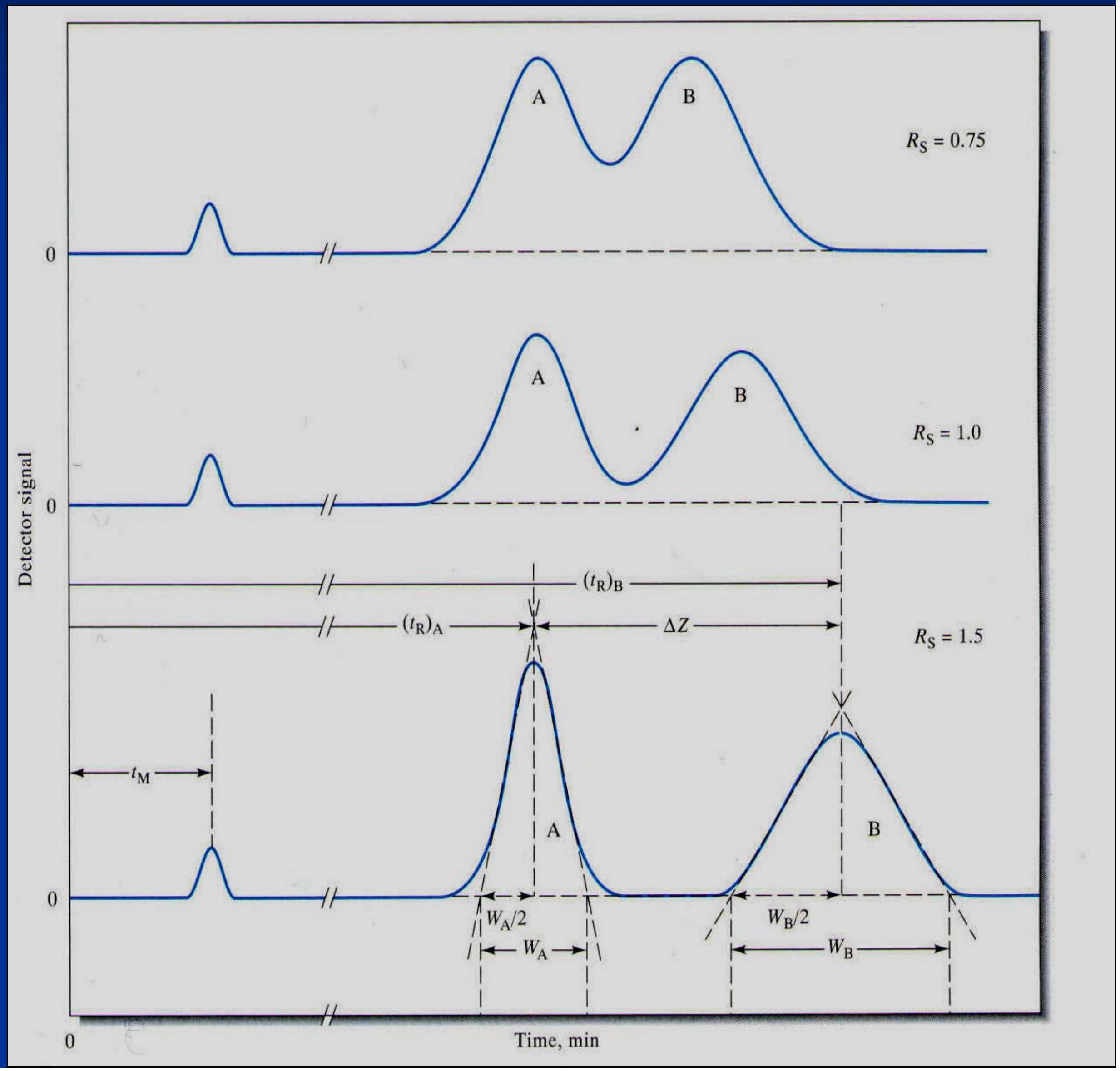
Chromatography

Resolution

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

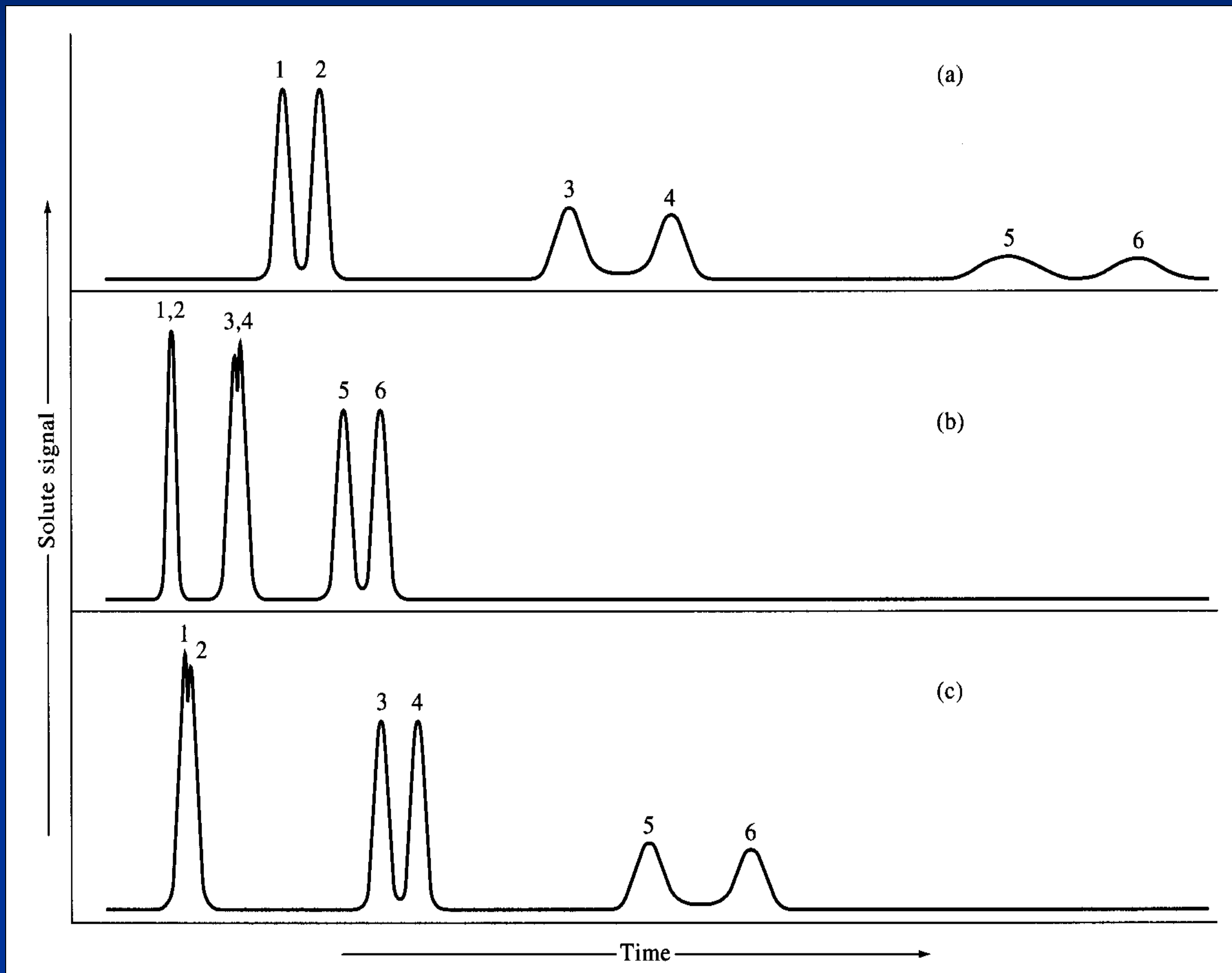
$$R_s = \frac{2\Delta Z}{W_A + W_B}$$

$$R_s = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$



Chromatographic

Separations with a twist



Chromatographic Definitions

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

Chromatographic 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	$(t_R)_B = \frac{16R_s^2 H}{u}\left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1 + k'_B)^3}{(k'_B)^2}$	

Quantitative Analysis

- Peak areas
- Peak height
- Calibration and standards
- Internal Standard method

Summary

- Relate to column chromatography
- Retention times
- Velocities of mobile and component
- Height equivalent of theoretical plates
- Peak or zone broadening
- Resolution