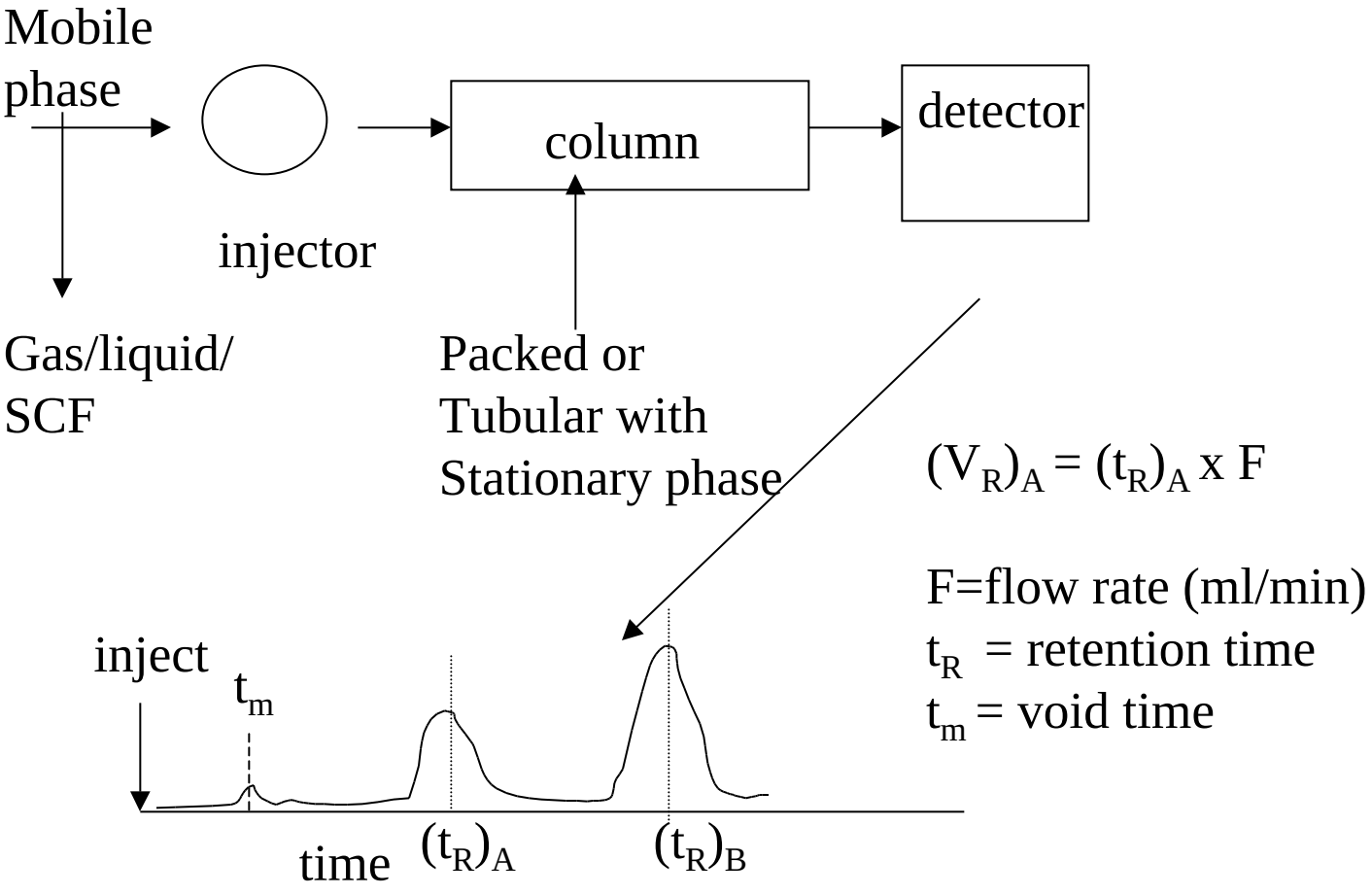
Separations---Chromatography and Electrophoresis

Chromatography--one of most diverse and important analytical methods--

- Used initially primarily to purify species
- With advent of sensitive detectors---now used often as method of choice for qualitative and quantitation of given analyte, or several analytes simultaneously!

Basic system:



$(V_R)_A = (t_R)_A \times F$

F=flow rate (ml/min)

t_R = retention time

t_m = void time

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 Size exclusion	Ion-exchange resin Liquid in interstices of a polymeric solid	Ion exchange 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

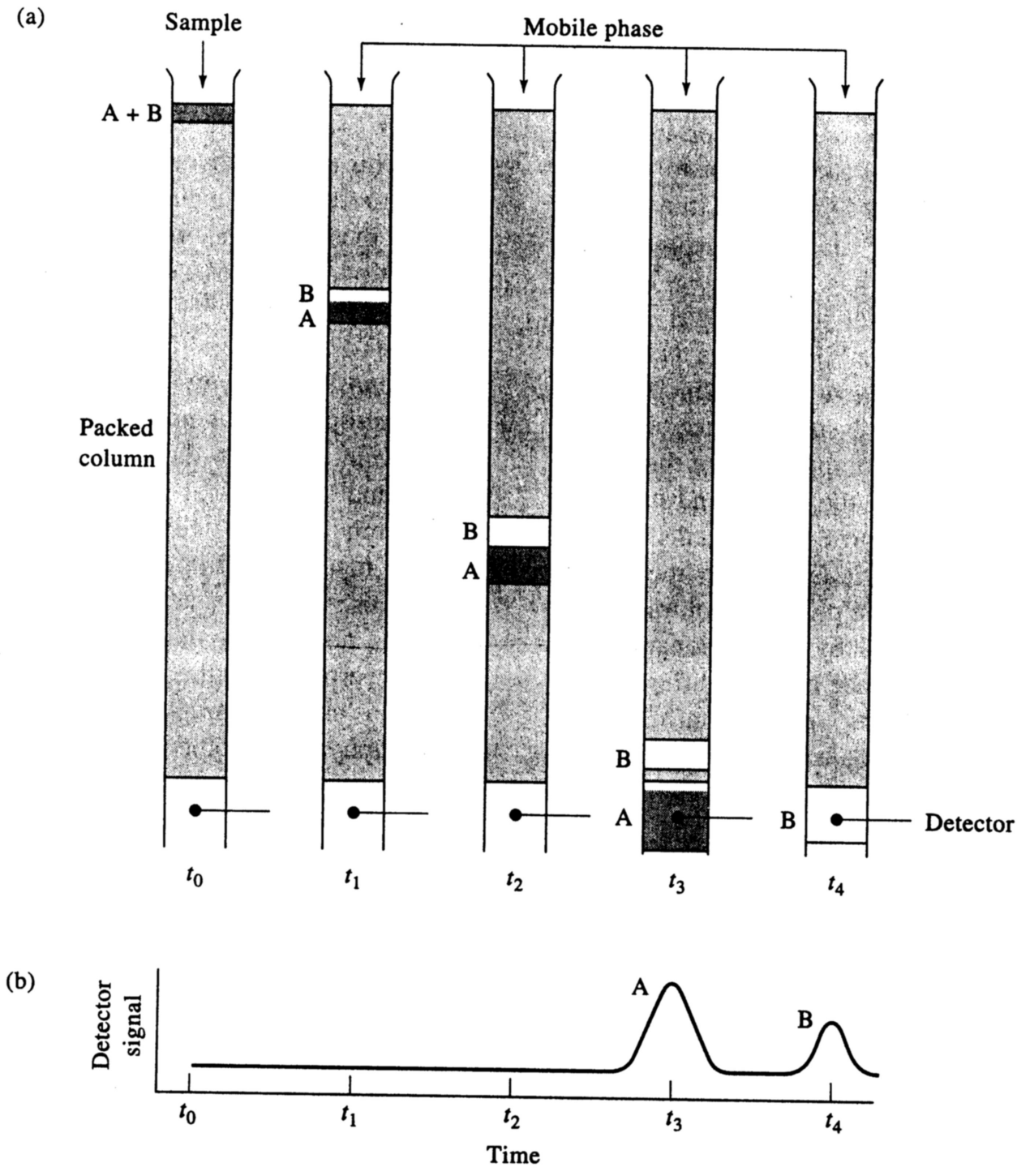


Figure 26-1 (a) Diagram showing the separation of a mixture of components A and B by column elution chromatography. (b) The output of the signal detector at the various stages of elution shown in (a).

Types of Stationary Phases:

- Bulk liquid--defined volume (partitioning phase)
- Monolayer of bonded species (adsorption/partitioning)

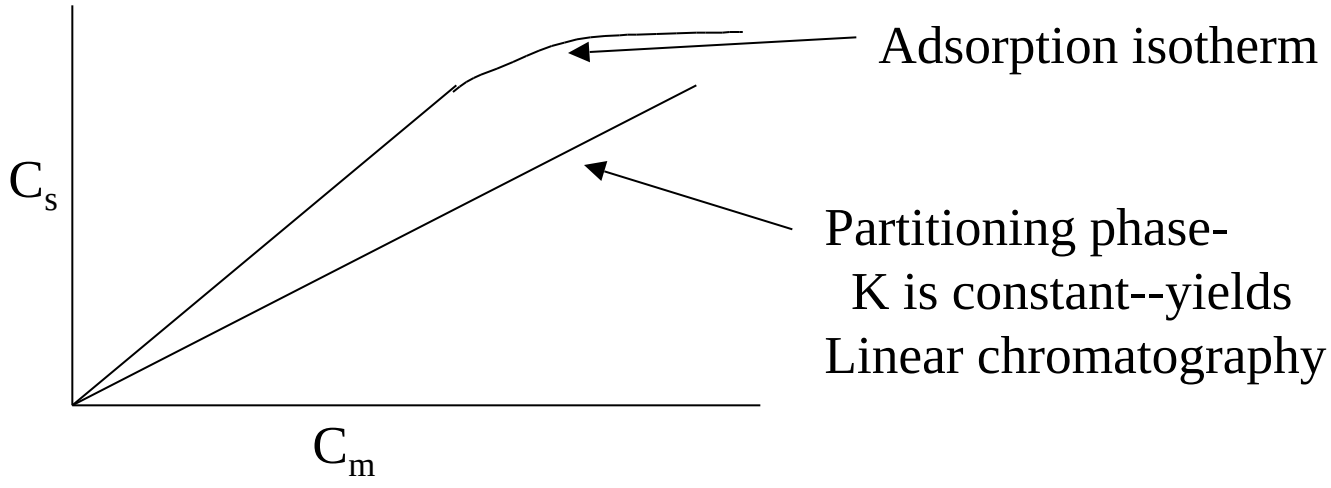
Solid Support (adsorption)

Separation on stationary phase based on different rates of movement of solutes down column due to partitionary/adsorption between mobile phase and stationary phases---Distribution of solute between the two phases determines the migration rate!

$$A_{mobile} \longleftrightarrow A_{stationary}$$

(Could be dissolved or adsorbed)

$$K = \text{partition coeff} = \frac{C_S^A}{C_M^A}$$



For solute partitioning between mobile and Stationary phases---

-Average linear migration rate = $\nu_A = L/(t_R)_A$

Where L is the column length!

For solute not able to interact with stationary phase- then migration rate is:

$\mu = L/t_m$ = linear velocity in mobile phase only

Want to relate retention time or migration rate to K, the partition coeff.

$\nu = \mu$ x fract. of time solute spends in mobile phase

If Fract. is 1, then $\nu = \mu$

The fraction of time is related to the average moles of solute in mobile phase at any given instant, divided by the total # of moles of solute

$$\nu = \mu \frac{C_m V_m}{C_m V_m + C_s V_s} = \frac{\mu}{1 + \frac{C_s V_s}{C_m V_m}}$$

$$\nu = \frac{\mu}{1 + K\frac{V_s}{V_m}}$$

But: $\nu = L/t_R$

$\mu = L/t_m$

$t_R = V_R/F$

$t_m = V_m/F$

Then:
$$\frac{L}{V_R/F} = \frac{L}{V_m/F} \; x \; \frac{1}{1 + K\frac{V_s}{V_m}}$$

Divide both Sides by L/(1/F)
$$\frac{1}{V_R} = \frac{1}{V_m} \; x \; \frac{1}{1 + K\frac{V_s}{V_m}}$$

$V_R = V_m + KV_s$ Fundamental eqn of Chrom.

Define $K(V_s/V_m)$ as capacity factor = k'

$$k'_A = \frac{K_A V_s}{V_m} \qquad k'_B = \frac{K_B V_s}{V_m}$$

$$therefore: --$$

$$\nu = \mu \frac{1}{1+k'}$$

$$\frac{L}{t_R} = \frac{L}{t_m}\frac{1}{1+k'}$$

$$\frac{1}{t_R} = \frac{1}{t_m}\frac{1}{1+k'} = \frac{1}{t_m + t_m k'}$$

$$t_R = t_m + t_m k'$$

$$k' = \frac{t_R - t_m}{t_m} \leftarrow$$

Equation to calculate Capacity factor from Chrom. Data

Selectivity--ability to separate two similar species-

$$\text{Selectivity Factor} = \alpha = \frac{k'_B}{k'_A} = \frac{(t_R)_B - t_m}{(t_R)_A - t_m}$$

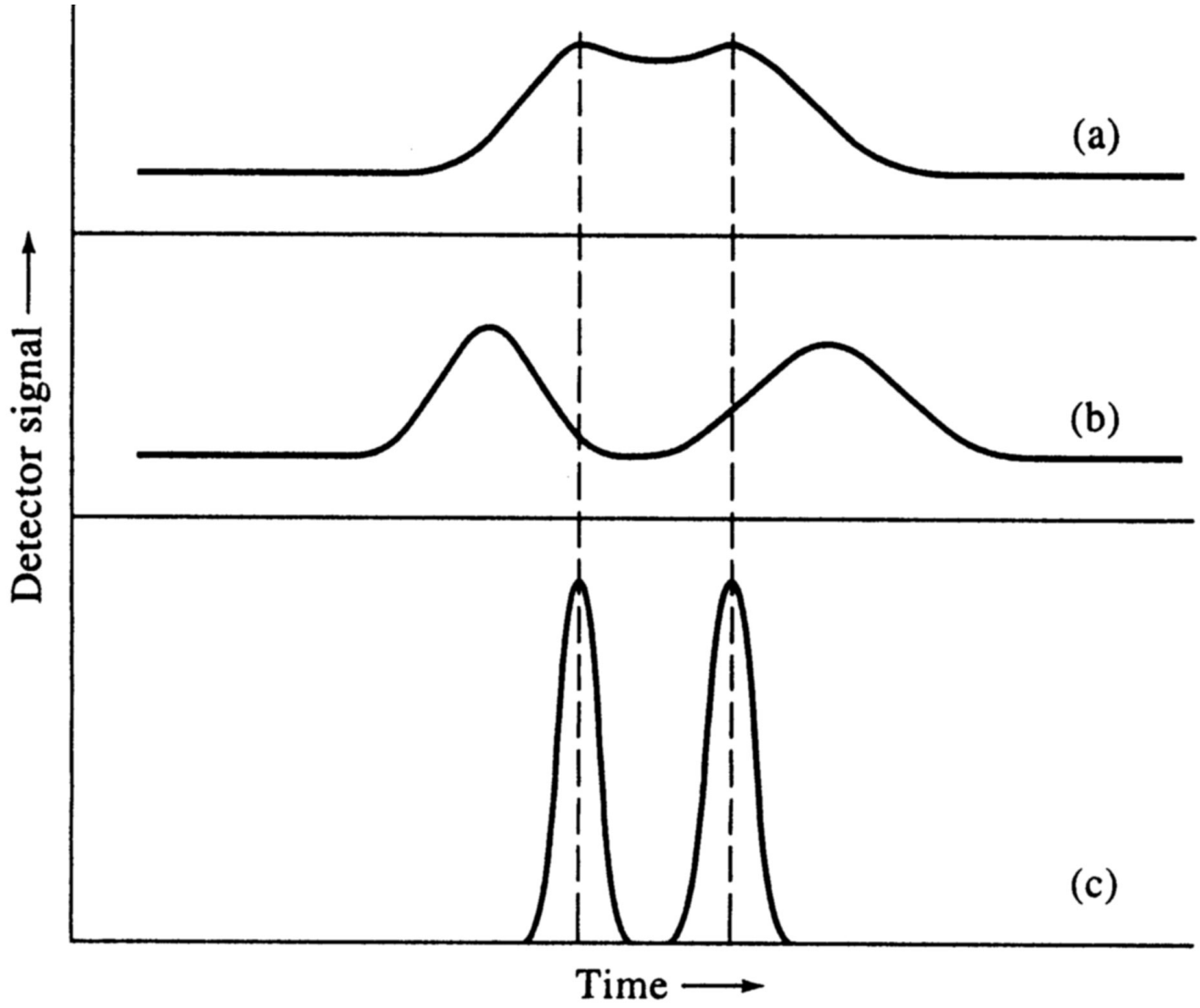


Figure 26-3 Two-component chromatograms illustrating two methods of improving separators: (a) original chromatogram with overlapping peaks; improvements brought about by (b) an increase in band separation, and (c) a decrease in band spread.

Band Broadening: Efficiency of Chromatographic Columns/Systems

Plate theory---(1941) ---treats chromatographic column as if it were divided into discrete units---each would correspond to an individual extraction tube/flask in which an equilibrium step between solute in mobile phase and stationary phase takes place.

N = # theoretical plates = L / H

H = H.E.T.P. = height equivalent to a theoretical plate

decrease H, increase N

H is related to variance ((s.d.)^2) of solute band for given length of column

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

σ^2 = variance of band at end of column length L in distance

Need to convert to time measurable units--since chromatogram is recorded in time--not distance

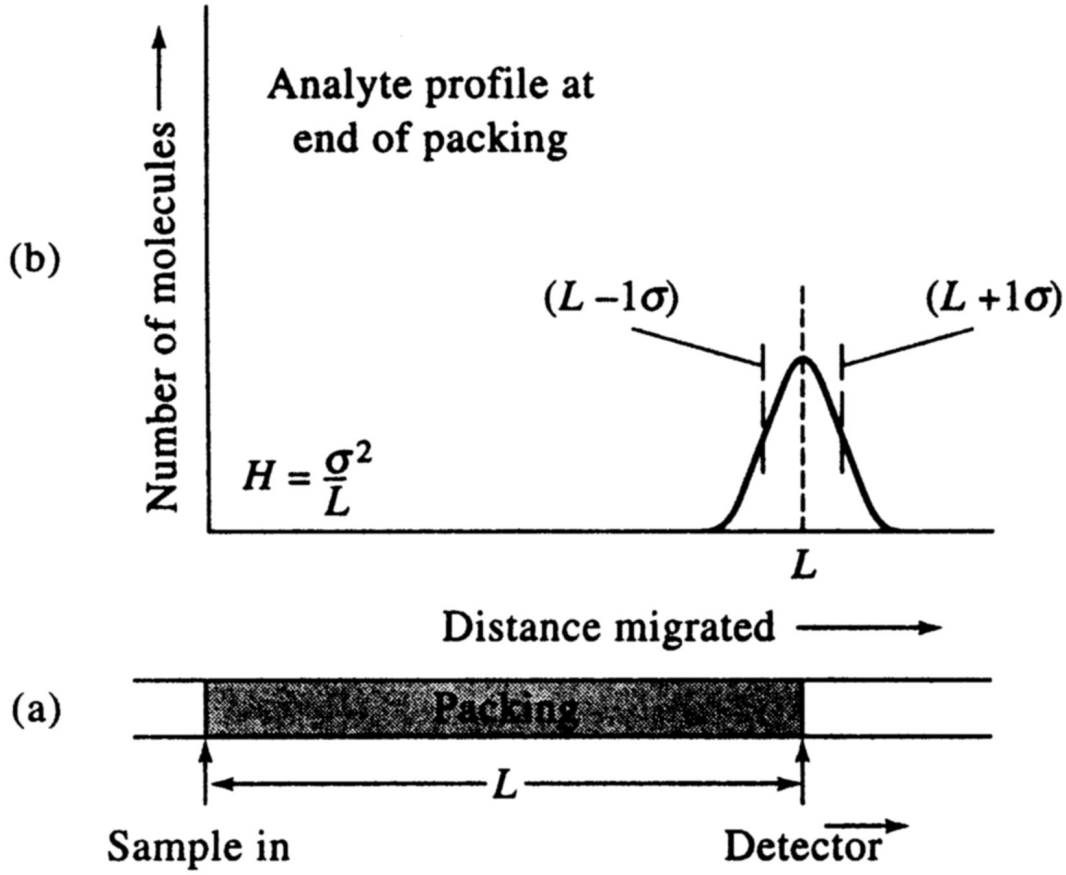
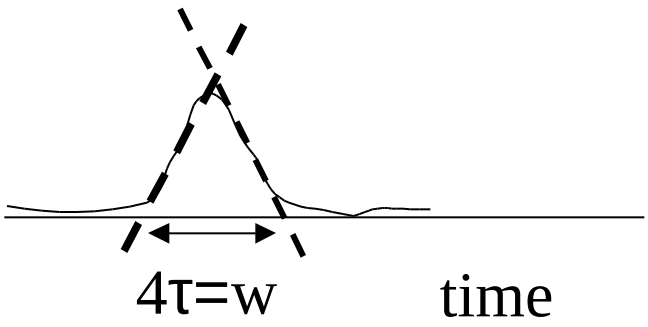


Figure 26-5 Definition of plate height $H = \sigma^2/L$.



Recall--for Gaussian distribution 96% of data points fall within +/- 2σ of mean

τ is s.d. of band in time units (sec)!

$$\tau = \frac{w}{4} = \frac{\sigma}{L/t_R} \longrightarrow w = \frac{4\sigma}{L/t_R}$$

$$\sigma = \frac{wL}{4t_R} \longleftarrow \frac{wL}{t_R} = 4\sigma$$

Now recall that $H = \sigma^2 / L$

$$H = \frac{(wL/4t_r)^2}{L} \longrightarrow H = \frac{w^2 L}{16 t_R}$$

But N = L / H

$$\textit{therefore};\ \ N = 16\left(\frac{t_R}{w}\right)^2$$

This is one equation that you can use to calculate N for a given column/system from experimental data

Another equation often used employs the peak Width at 1/2 peak height ($w_{1/2}$)--often easier to determine--

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

While plate theory provides means to convert observed band broadening into a number of plates and thus H--this value is not a constant---it varies due to the fact that the column is not really divided into finite segments where true equilibrium is achieved--
Indeed, the mobile and stationary phases within a column never come into complete equilibrium--in terms of solute interactions---since mobile phase is always moving!! (flowing)

Rate theory of chromatography considers the non-Equilibrium of solute due to flowing mobile phase-

The rate theory showed that column efficiency and plate number was dependent on many variables including the linear velocity of the mobile phase- μ-- (see Fig. 26-7)

Rate theory culminated in van Deemter equation:

$$H = A + B/\mu + C\mu$$

A=multiple flow path or eddy term
B= longitudinal diffusion term
C=mass transfer term --can be broken into two separate terms--C_s = mass transfer related to stationary phase; C_m = mass transfer related to mobile phase

Thus--: $H = A + B/\mu + (C_s + C_m)\,\mu$

Various constants A, B, C highly dependent on nature of packing material

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.

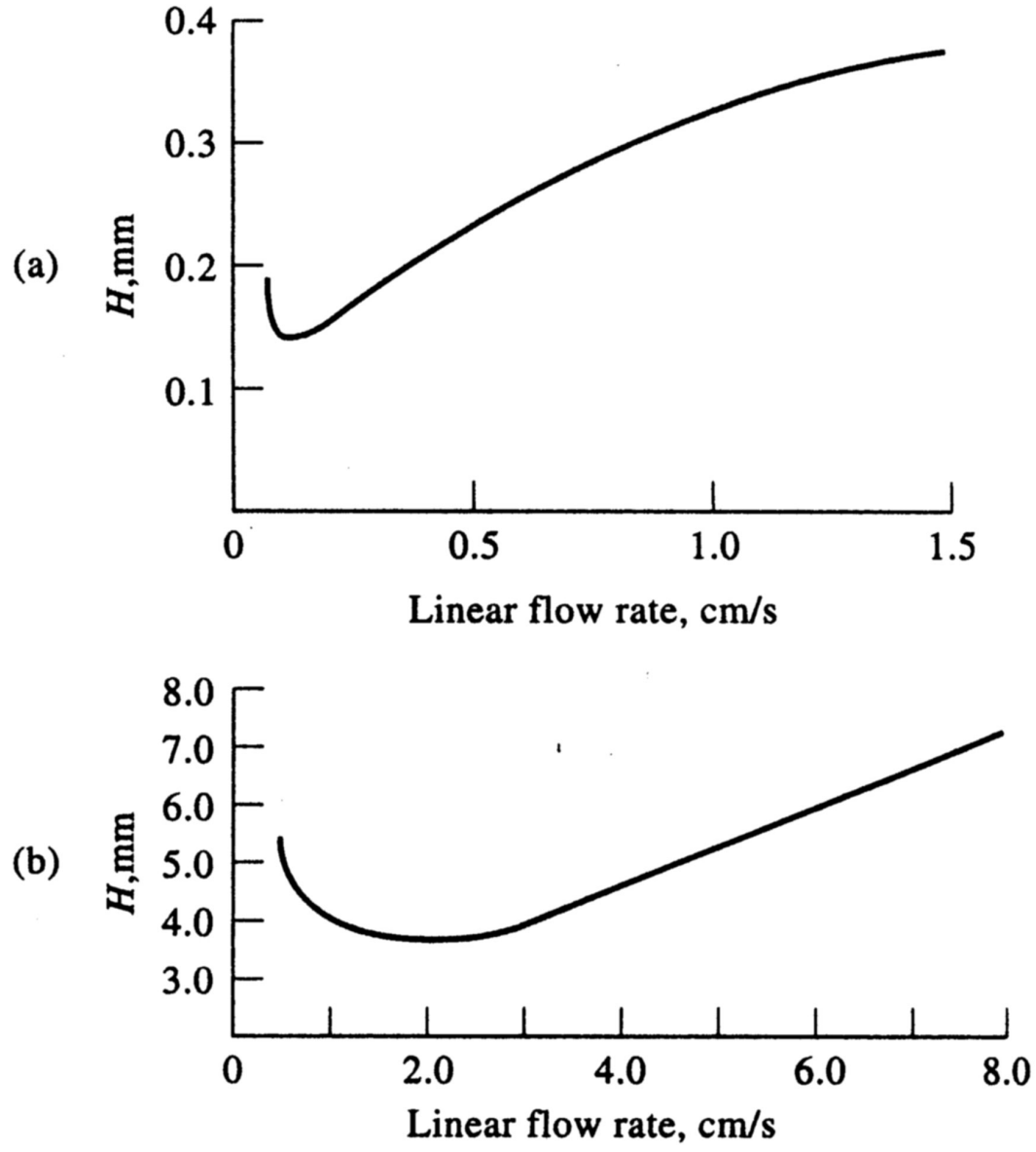


Figure 26-7 Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.

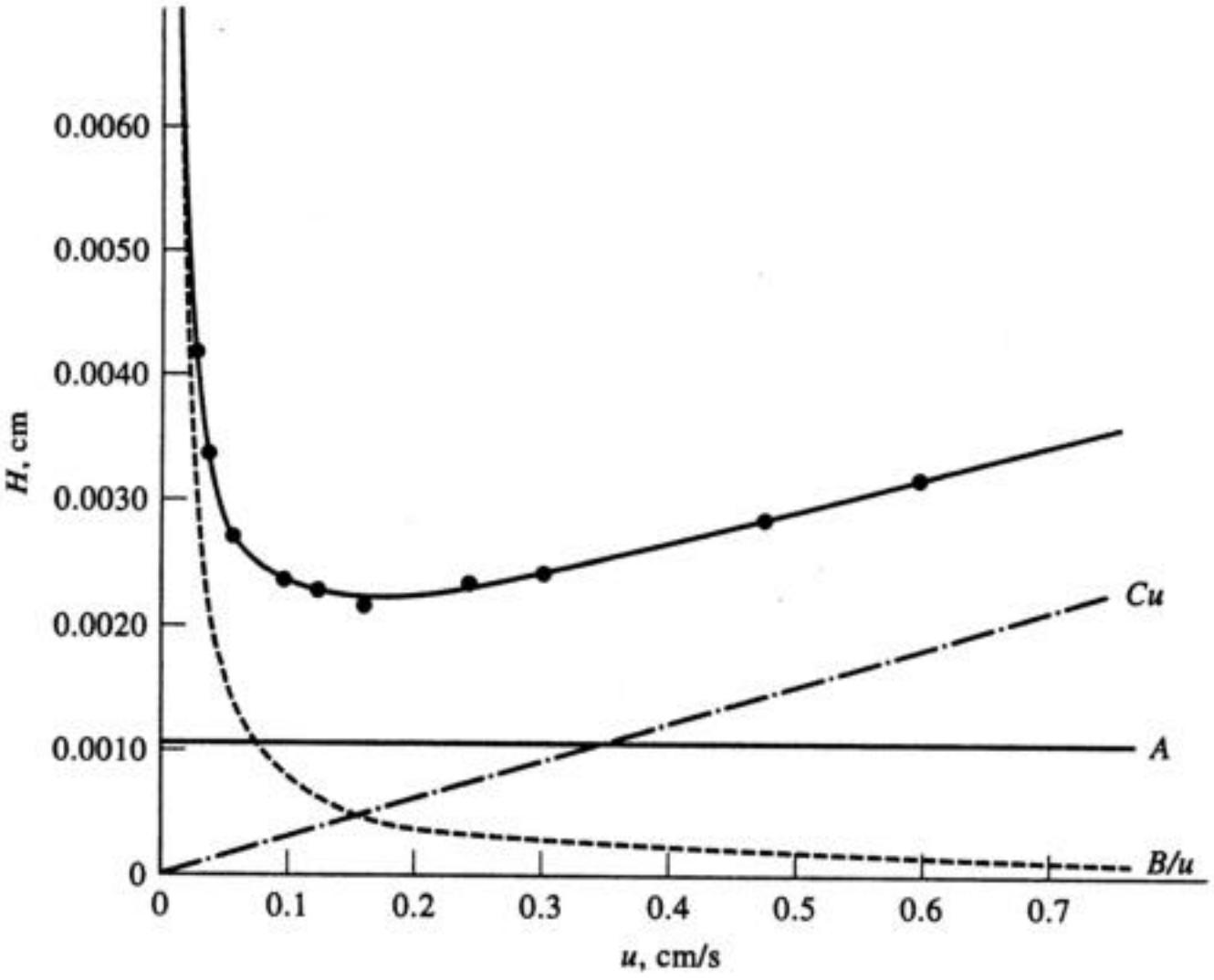


Figure 26-9 A van Deemter plot for a packed liquid chromatographic column. The points on the upper curve are experimental. The contributions of the various rate terms are shown by the lower curves: *A*, multipath effect; *B/u*, longitudinal diffusion; *Cu*, mass transfer for both phases. *(From E. Katz,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

See Fig. 26-9 for influence of each term of van Deemter equation on H-----

A-term---$A = 2kd_p$

Solute species don't all have the same path to take To get to the end of the column---some take shorter Paths than others (see Fig. 26-8); --less difference if packing particle size is small! ---less band broadening due to multipath effect-- at very low flow rates, the multipath effect or A term is greatly reduced---because diffusion will dominate and control band broadening!

B-Term--due to diffusion of solutes in mobile phase- very important in GC--since D_m is much larger compared to liquid phase--10^{-2} cm^2/sec vs. 10^{-6} cm^2/sec

$B = 2k D_m$ ----k = 0.6 or so for packed column
k = 1.0 for unpacked columns--tubular
Inverse relation to μ ---if μ is fast, bandspreading due to longitudinal diffusion is much less, --less time for diffusion to occur

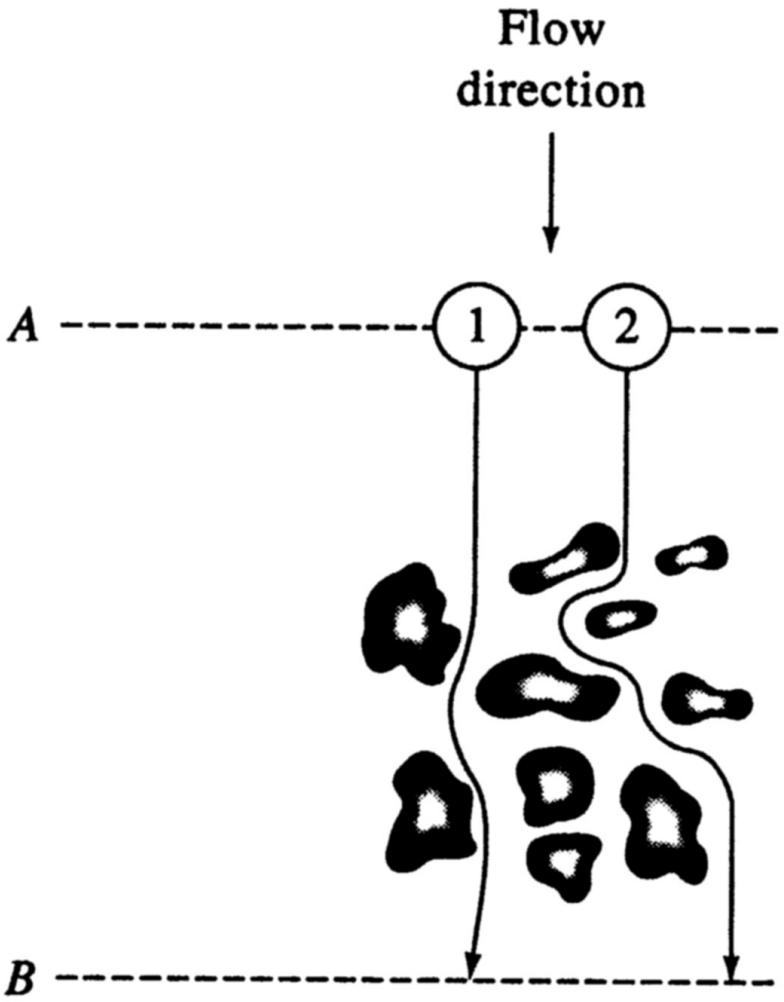
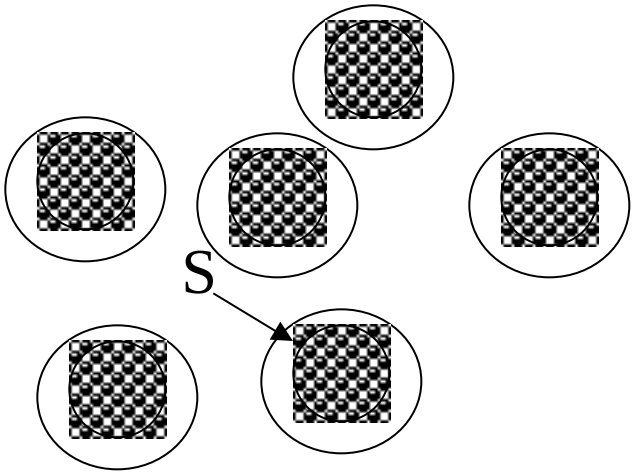


Figure 26-8 Typical pathways of two molecules during elution. Note that distance traveled by molecule 2 is greater than that traveled by molecule 1. Thus, molecule 2 would arrive at *B* later than molecule 1.

C- term; mass transfer terms--related to kinetics of interaction between solute in mobile phase with stationary phase---

if μ is slow, then contribution due to non-equilibrium mass transfer becomes less---more time for Solute to attain equilibrium with stationary phase in given segment of column.



$C_s = f(k')d_f^2 / D_s$; d_f = thickness of stationary phase film

want thin films of stationary phases!

For adsorption process--

$C_s \alpha\ 2\ t_d k' / (1+k')^2$

t_d =avg desorption time of solute; $t_d = 1/k_d$

k_d first order rate constant for desorption

Fast kinetics---C_s decreases; better efficiency

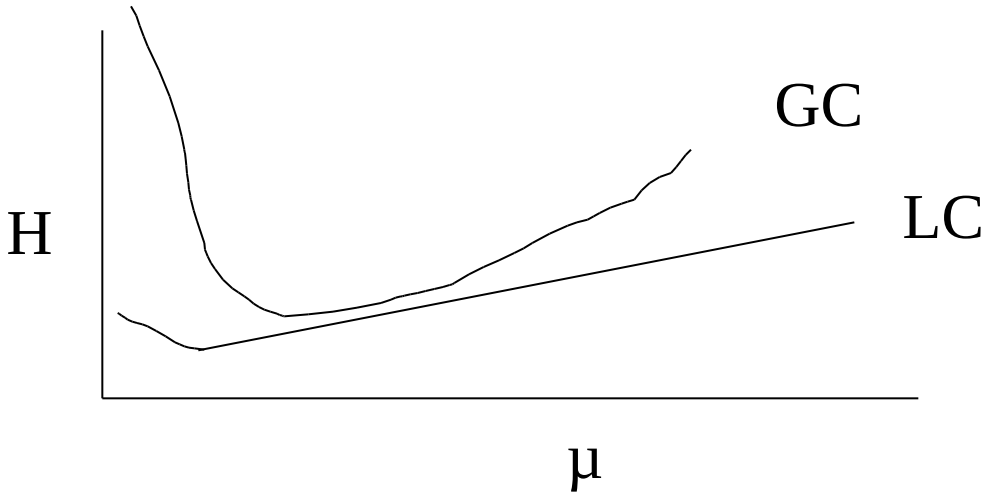
$C_m \alpha f(k')d_p^2 / D_m$

Larger particle size--means that gaps between Particles larger---not packed as tight;--longer distance to diffuse to stationary phase!

For tubular columns---$C_m \alpha r^2/D_m$ --r is radius of column

Can decrease H and hence increase N by the following---

- smaller particles---in LC this leads to high pressure
- Narrower tubular columns
- Thinner films of stationary phases



Resolution--ability to separate two components--
depends on K values (α), and N

Definition of Resolution:

$$R_s = \frac{\Delta t_R}{w_A/2 + w_B/2} = \frac{2\Delta t_R}{w_A + w_B}$$

If R_s = 1.5 --gives essentially complete resolution
of two solutes---only 0.3% overlap

If R_s = 1.0 ---4% overlap of peaks

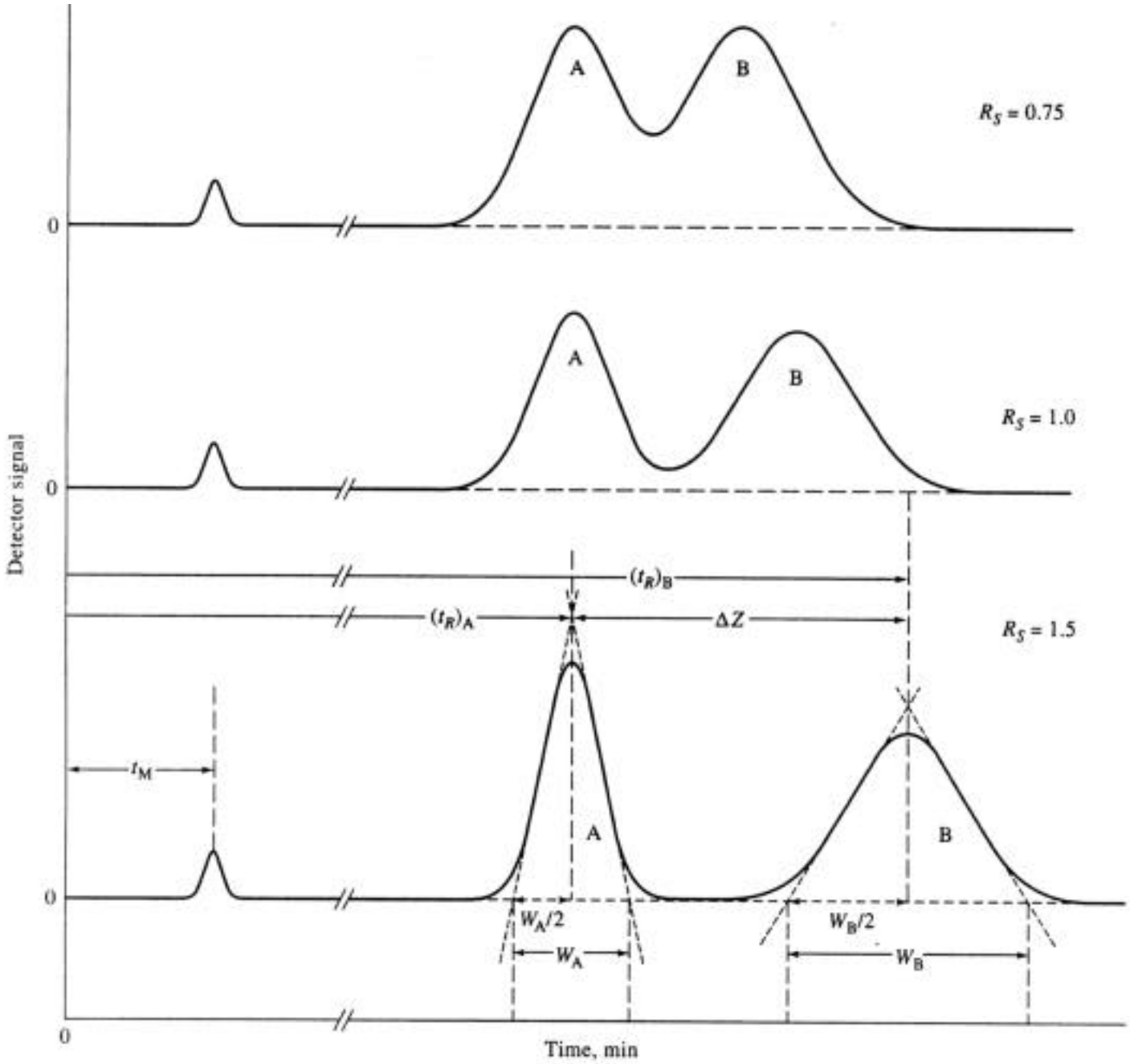
Resolution eqn:

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

k'_B = longer retained solute capacity factor
Increase R_s by increasing α and increase N
Can increase N by making column longer---
or reducing H---better to reduce H since longer L
means longer analysis time!

$$N = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{1 + k'_B}{k'_B}\right)^2$$

Equation to determ/.
N to achieve given R_s


A
B
$R_S = 0.75$
0
A
B
$R_S = 1.0$
0
Detector signal
$(t_R)_B$
$(t_R)_A$
ΔZ
$R_S = 1.5$
t_M
A
B
0
$W_A/2$
W_A
$W_B/2$
W_B
0
Time, min