Introduction to Chromatographic Separations (Chapter 26)

Many determinations involve separation followed by analysis

- chromatography
- electrophoresis

Chromatography:

sample transported by mobile phase

electrostatic or van der Waals'

some components in sample interact more strongly with stationary phase and are more strongly retained

sample separated into zones or bands

Elution Chromatography:

flushing of sample through column by continual mobile phase (eluent) addition

migration rate   fraction time spent in mobile phase

Planar chromatography - flat stationary phase, mobile phase moves through capillary action or gravity

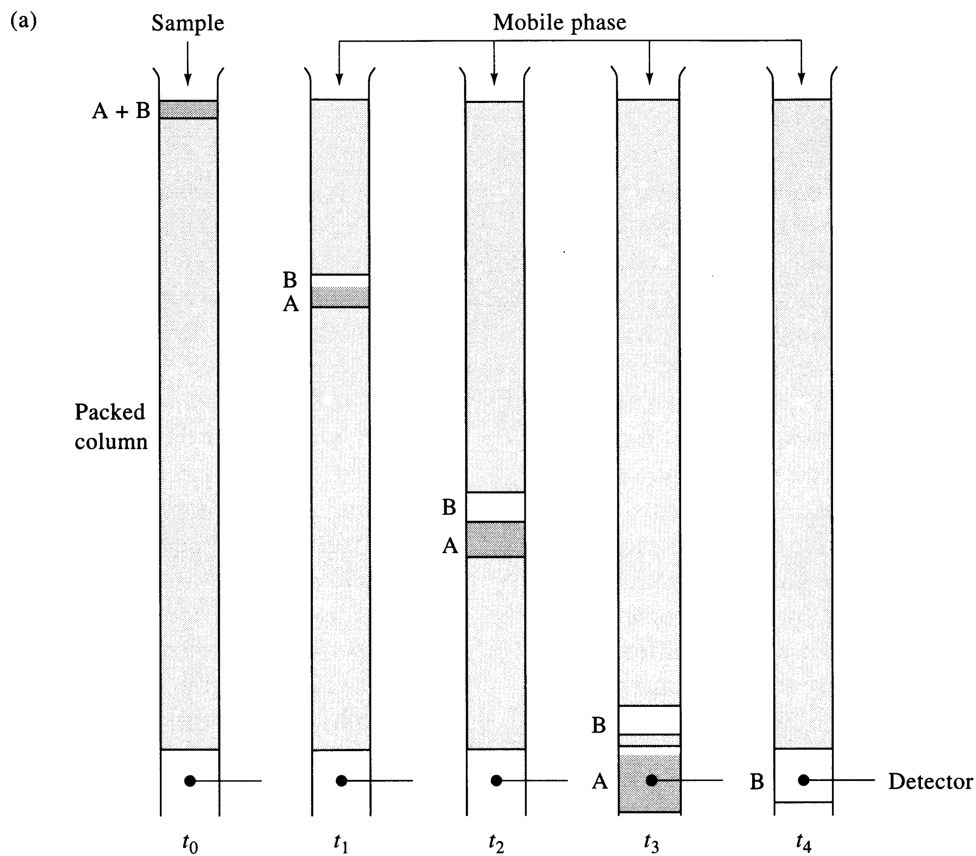
Column chromatography - tube of stationary phase, mobile phase moves by pressure or gravity

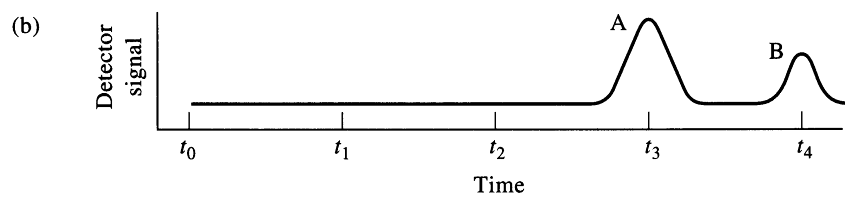
Table 26-1:

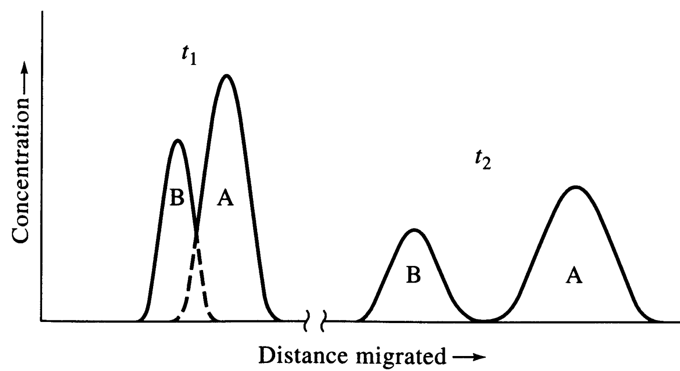
TABLE 26-1 Classification of Column Chromatographic Methods

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

Fig 26-1, 26-2







Important:

- chromatogram (concentration versus elution time)
- more strongly retained species elutes last (elution order)
- analyte is "diluted" during elution (dispersion)
- zone broadening proportional to elution time

By changing experimental conditions, non-separated bands can be separated

(A) adjust migration rates for A and B (increase band separation)

(B) adjust zone broadening (decrease band spread)

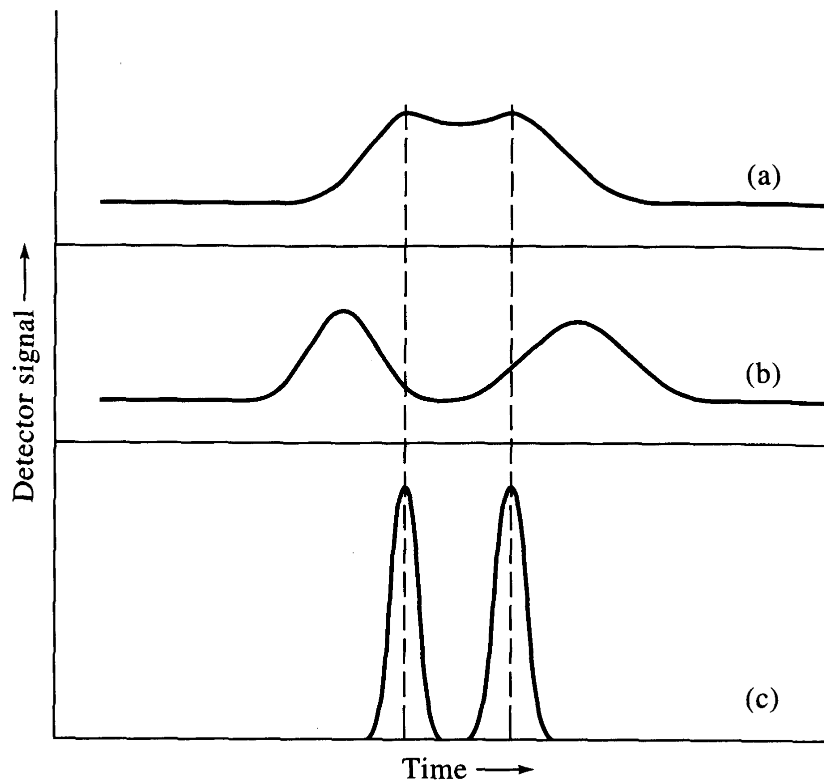


Fig 26-3

(A) Adjusting Migration Rates:

Analyte A in equilibrium with two phases

A_{mobile} $A_{stationary}$

$$K = \frac{c_{stationary}}{c_{mobile}}$$ partition ratio

We know elution time is related to amount of time in mobile phase - can we quantify this?

<u>Retention Time t_R</u>:

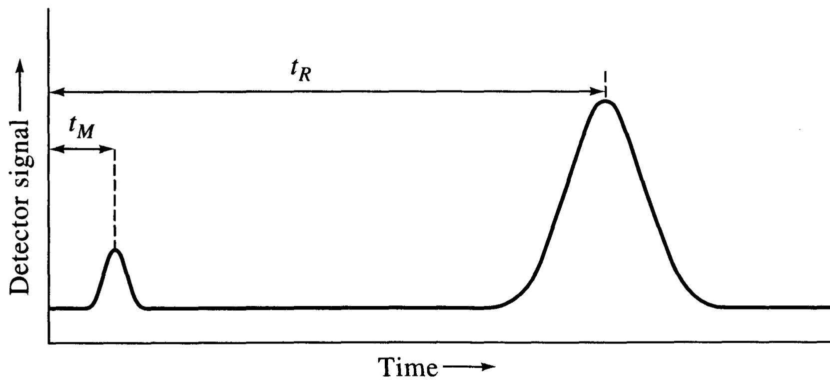


Fig 26-4

t_R retention time for retained species

average migration rate $\bar{v} = \frac{L}{t_R}$ column length

t_M time for unretained species (dead time)

same rate as mobile phase molecules

average rate migration $u = \frac{L}{t_M}$ dead time

Ideally

- t_R independent of volume injected
- produces Gaussian peaks

Retention Time (t_R) and Partition Ratio (K):

Migration rate of analyte is

$$
\begin{aligned}
\bar{v}_A &= u \times \text{fraction time A spends in mobile phase} \\
&= u \times \frac{\text{\# mols A in mobile phase}}{\text{total \# mols A}} \\
&= u \times \frac{c_M V_M}{c_M V_M + c_S V_S} \\
&= u \times \frac{1}{1 + c_S V_S / c_M V_M} \\
&= u \times \frac{1}{1 + K_A (V_S / V_M)}
\end{aligned}
$$

estimate from column packing

Capacity Factor k'_A:

$$k'_A = K_A (V_S / V_M) \qquad \text{[unitless] for analyte A}$$

$$\bar{v} = u \times \frac{1}{1 + k'_A}$$

How is k'_A related to t_R and t_M?

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k'_A}$$

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

When k'_A is 1.0, separation is poor

When k'_A is >30, separation is slow

When k'_A is 2-10, separation is optimum

Relative Migration Rates - Selectivity Factor ( ):

How do we compare elution of two components A and B?

$$\text{selectivity factor} \quad = \frac{K_B}{K_A} \quad \text{partition ratios}$$

$$= \frac{k'_B}{k'_A} \quad \text{capacity factors}$$

$$= \frac{t_{R(B)} - t_M}{t_{R(A)} - t_M} \quad \text{retention times}$$

larger = better separation

(B) Adjusting Zone Broadening:

- Individual molecule undergoes "random walk"
- Many thousands of adsorption/desorption processes
- Average time for each step with some +ve or -ve differences
- Add up to give Gaussian peak (like random errors)
- Breadth of band increases down column because more time

- Zone broadening is affected by separation efficiency - more efficient, less broadening

Column Efficiency:

number of plates | length of column

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

height of 1 theoretical plate

Plates are only theoretical - column efficiency increases with N

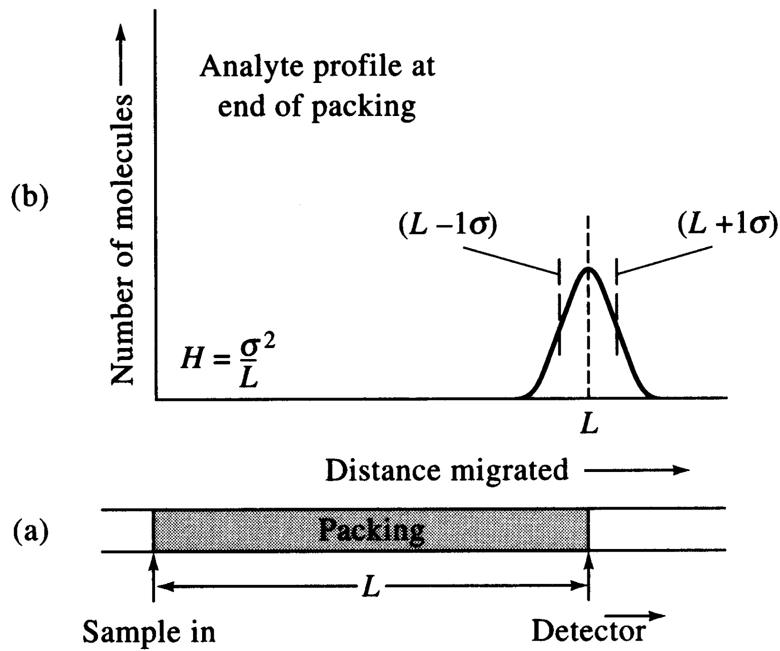
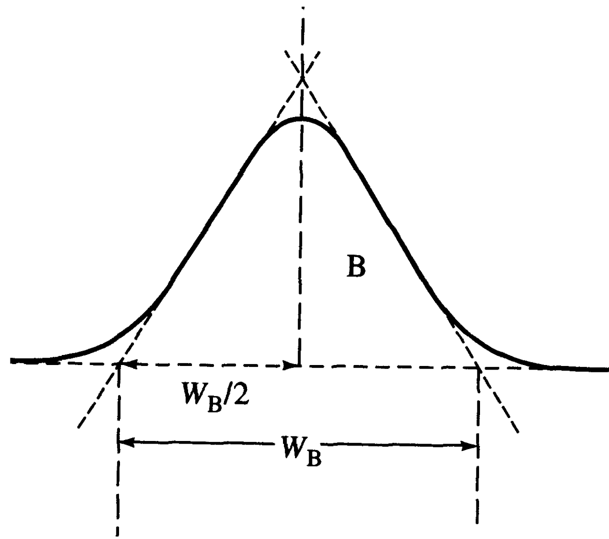


Fig 26-5

Efficient column has small plate height - less zone broadening

$$H = \frac{^{2}}{L} \qquad \frac{[cm^2]}{[cm]} \quad \text{Units } H = cm$$

Experimentally, H and N can be approximated from the width of the base of the chromatographic peak

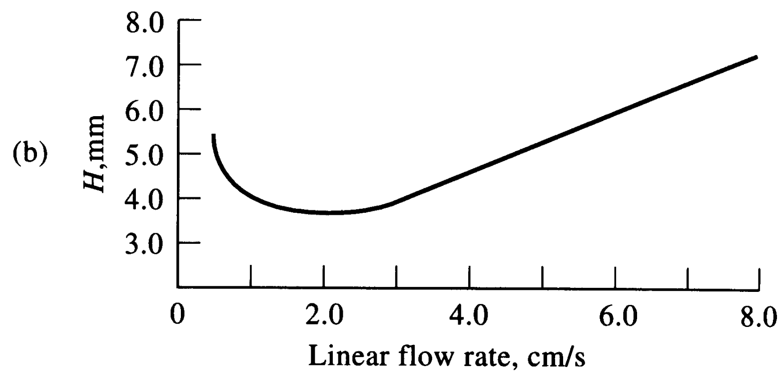


$$N \quad 16 \quad \frac{t_R}{W_B}^{\;2}$$

Other Variables Affecting Peak Width (Zone Broadening):

Mobile Phase Velocity:

> Higher mobile phase velocity, less time on column, less zone broadening
>
> However, plate height H also changes with flow rate - plot of H versus u called van Deemter plot (Fig 26-7)



Van Deemter equation:

$$H = A + B/u + Cu$$
$$= A + B/u + (C_S + C_M)u$$

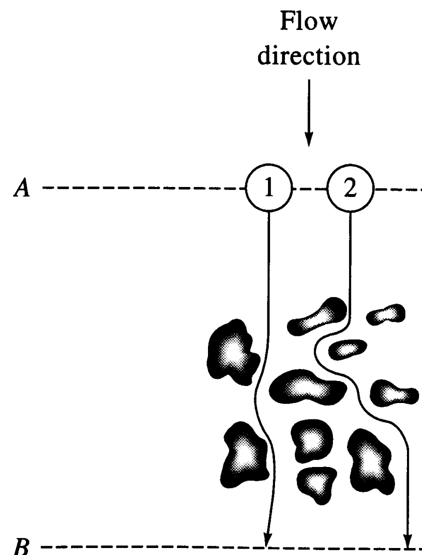
linear velocity mobile phase cm/s

A - multipath term

B - longitudinal diffusion term

C - mass transfer term for mobile and stationary phases

A - Multipath term: (Fig 26-8)



- Molecules move through different paths
- Larger difference in pathlengths for larger particles
- At low flow rates, diffusion allows particles to switch between paths quickly and reduces variation in transit time

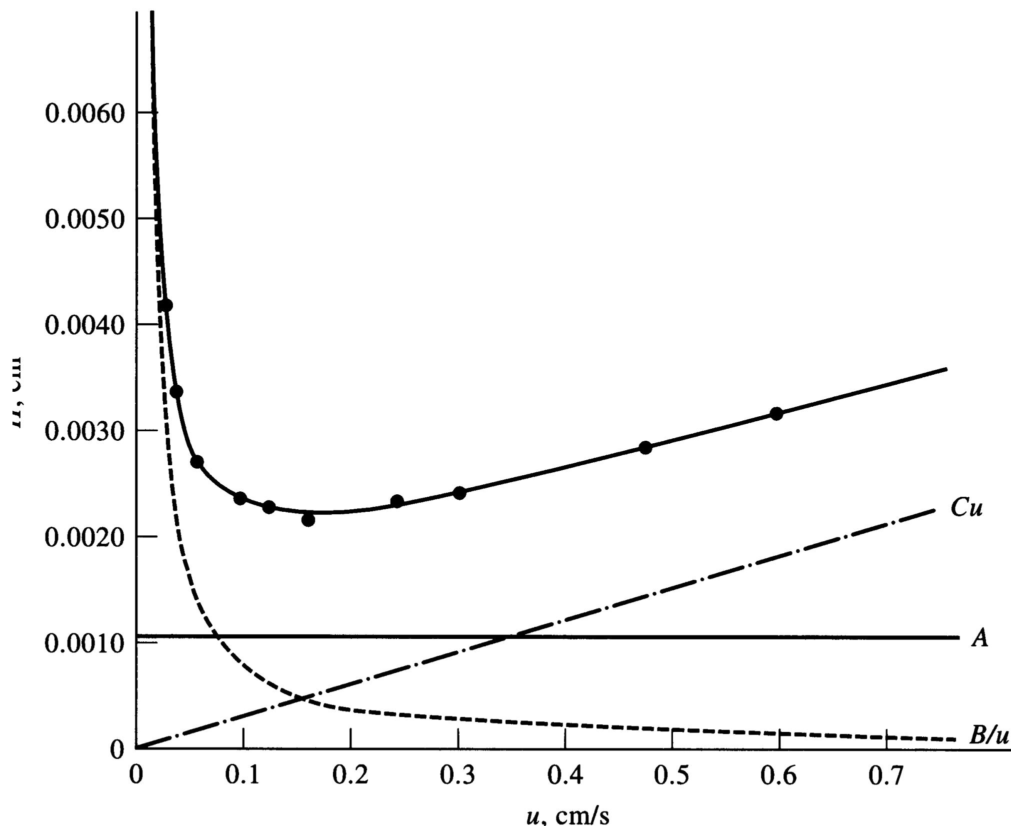
B - Longitudinal Diffusion term:

- Diffusion from zone (front and tail)
- Proportional to mobile phase diffusion coefficient
- Inversely proportional to flow rate - high flow, less time for diffusion

C - Mass Transfer Coefficients (C_S and C_M):

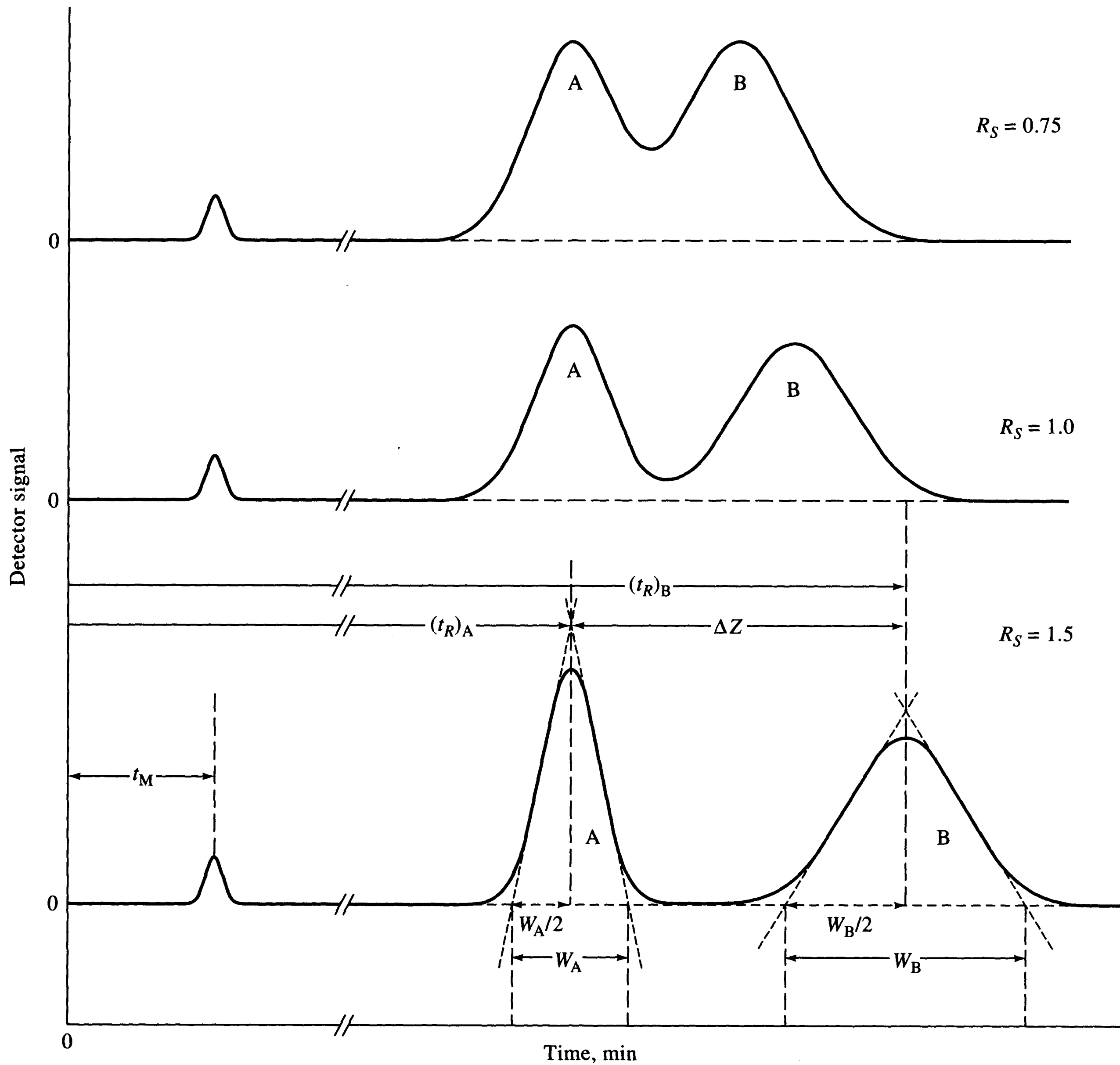
- C_S is rate for adsorption onto stationary phase
- C_M is rate for analyte to desorb from stationary phase
- Effect proportional to flow rate - at high flow rates less time to approach equilibrium

van Deemter plot (Fig 26-9)



Optimization of Column Efficiency:

Column Resolution R_S: (Fig 26-11)



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

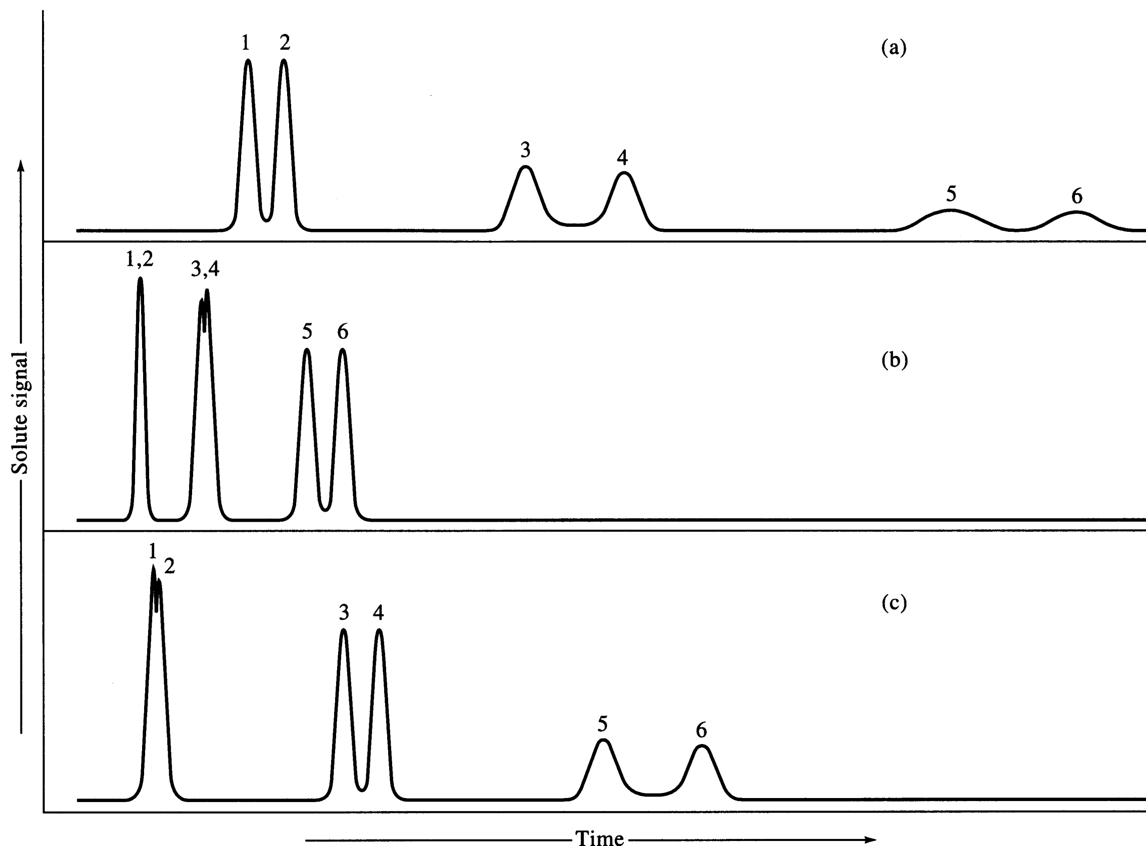
$$= \frac{2\left[t_{R(B)} - t_{R(A)}\right]}{W_A + W_B}$$

Resolution (and zone broadening) depends on

- u (linear flow rate) - low flow favors increased resolution (van Deemter plot)
- H (plate height) (or N number of plates) - use smaller particles, lengthen column, viscosity of mobile phase (diffusion)
- (selectivity factor) - vary temperature, composition of column/mobile phase
- k_A' (capacity factor) - vary temperature, composition of column/mobile phase

General Elution Problem:

For multiple components, conditions rarely optimum for all components (Fig 26-14 effect of k'_B)



Resolution of first eluting peaks results in long t_R's. Acceptable t_R's for last eluting peaks results in poor resolution of first eluting peaks.

Solution? Change column conditions *during* elution

- change in liquid mobile phase composition - gradient elution or solvent programming
- change in temperature for gas chromatography - temperature programming