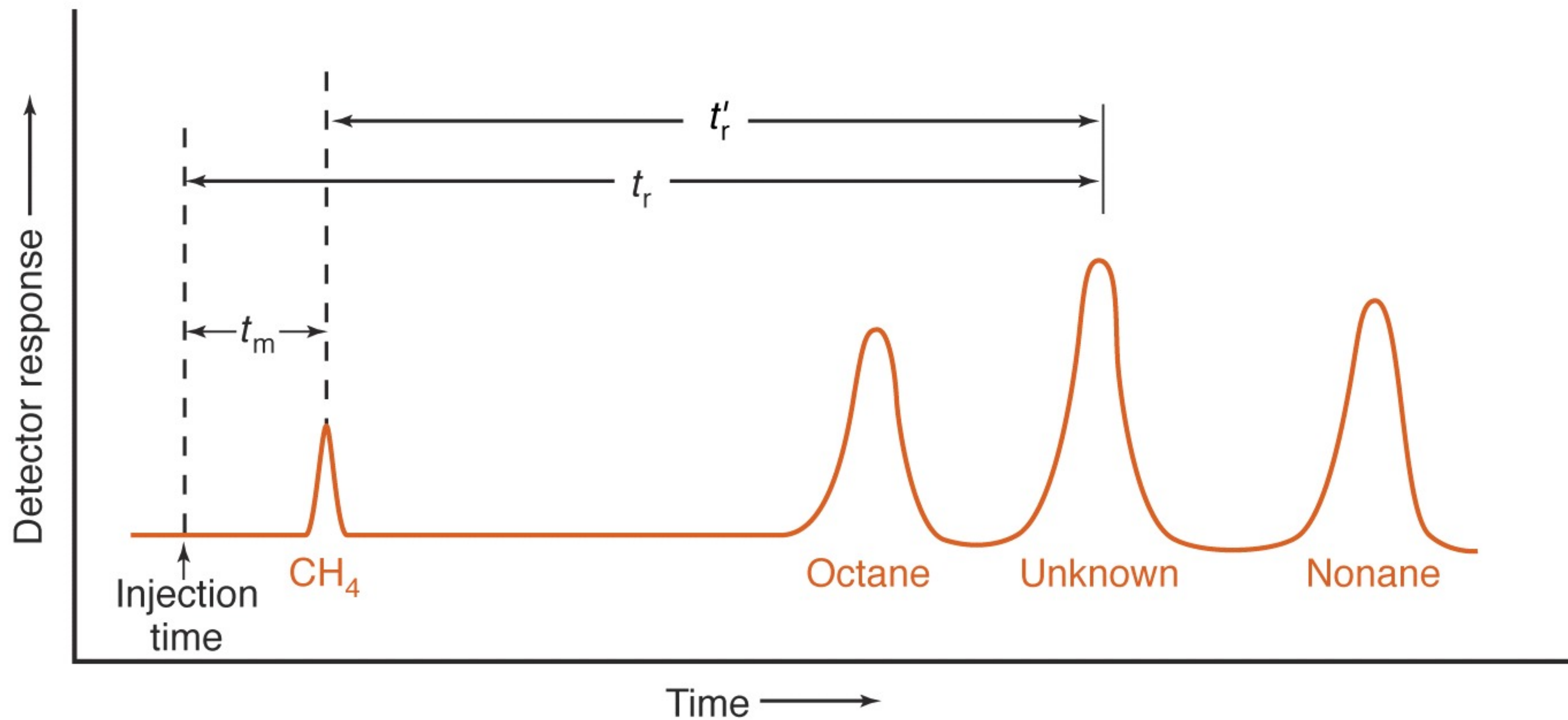
Theory of Chromatography

The Chromatogram

- A ***chromatogram*** is a graph showing the detector response as a function of elution time.
- The ***retention time***, t_R, for each component is the time needed after injection of the mixture until that component reaches the detector.



t'_r
t_r
t_m
Detector response
Injection time
CH_4
Octane
Unknown
Nonane
Time

The Chromatogram (cont.)

- ***Retention volume***, V_R, is the volume of mobile phase required to elute a particular solute from the column:

  $$V_R = t_R \times F$$

  where F is the mobile phase flow rate
- The ***dead time***, t_m, is the time of travel of unretained mobile phase through the column.

The Chromatogram (cont.)

- The ***adjusted retention time***, t_R', for a solute is the additional time required for solute to travel the length of the column beyond the time required by unretained solvent:

  $$t_R' = t_R - t_m$$

  In GC, t_m is usually taken as the time needed for CH_4 to travel through the column.

The Chromatogram (cont.)

- For any two components 1 and 2, the ***relative retention***, α, is the ratio of their adjusted retention times:

$$\alpha = \frac{t'_{R2}}{t'_{R1}}$$

where $t'_{R2} > t'_{R1}$, so $\alpha > 1$.

The Capacity Factor

- For each peak in the chromatogram, the ***capacity factor***, *k'*, is defined as:

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

$$k' = \frac{\text{time solute spends in stationary phase}}{\text{time solute spends in mobile phase}}$$

The Capacity Factor (cont.)

$$k' = \frac{\text{time solute spends in stationary phase}}{\text{time solute spends in mobile phase}} = \frac{\text{moles of solute in stationary phase}}{\text{moles of solute in mobile phase}}$$

$$k' = \frac{C_s V_s}{C_m V_m}$$

$$\frac{C_s}{C_m} = K$$

$$k' = K \frac{V_s}{V_m} = \frac{t_R - t_m}{t_m} = \frac{t'_R}{t_m}$$

Relative Retention – Alternative Expressions

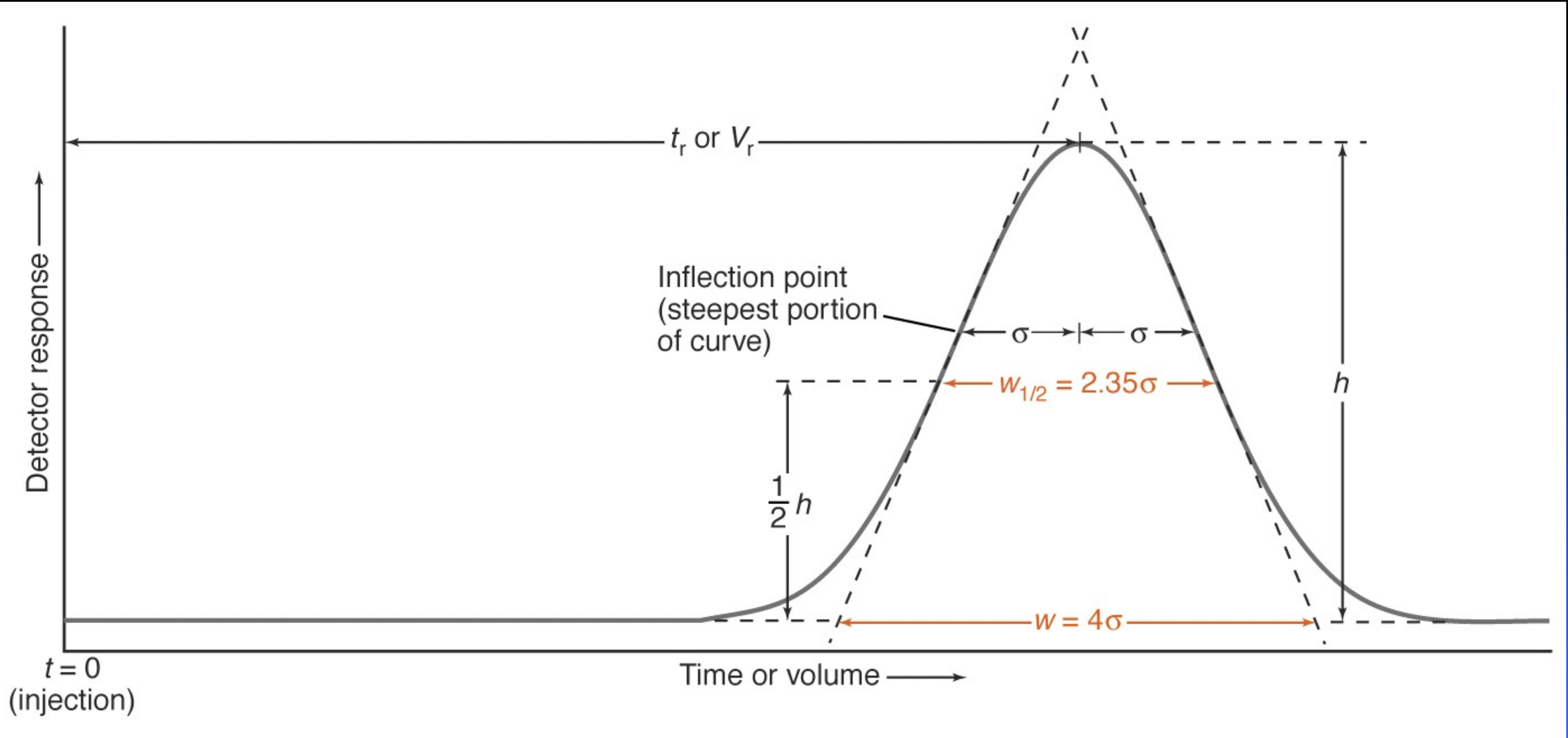
$$\alpha = \frac{t'_{R2}}{t'_{R1}} = \frac{k'_2}{k'_1} = \frac{K_2}{K_1}$$

Efficiency of Separation

Resolution

Solute moving through a column spreads into a Gaussian shape with standard deviation σ. Common measures of breadth are:

- The width $w_{1/2}$ measured at half-height
- The width w at the baseline between tangents drawn to the steepest parts of the peak (inflection points).



t_r or V_r
Detector response
Inflection point
(steepest portion
of curve)
σ
σ
$w_{1/2} = 2.35\sigma$
h
$\frac{1}{2}h$
$w = 4\sigma$
t = 0
(injection)
Time or volume

Resolution (cont.)

It can be shown that:

$$w_{1/2} = 2.35\sigma$$

and

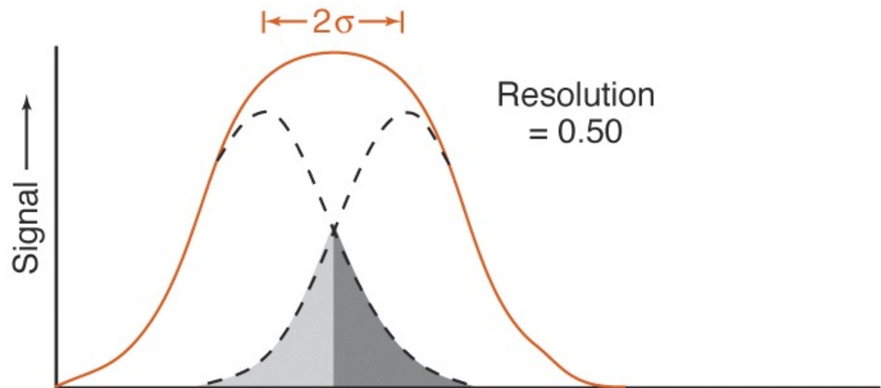
$$w = 4\sigma$$

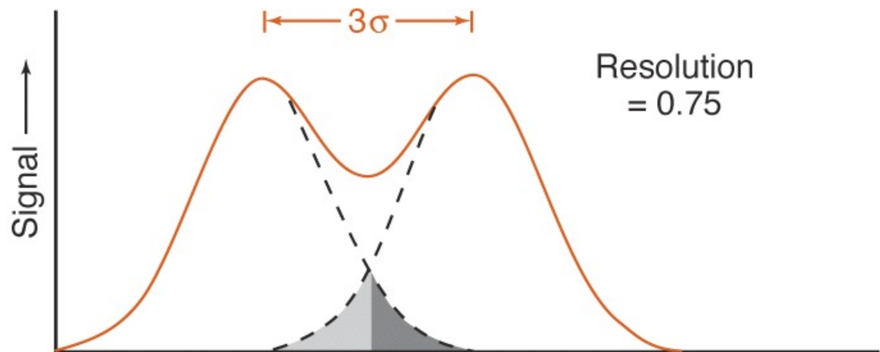
Resolution (cont.)

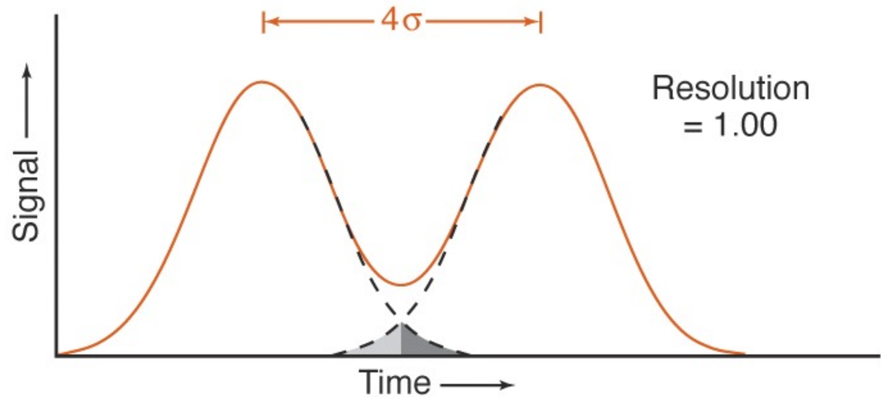
In chromatography, the ***resolution*** of two peaks from each other is defined as

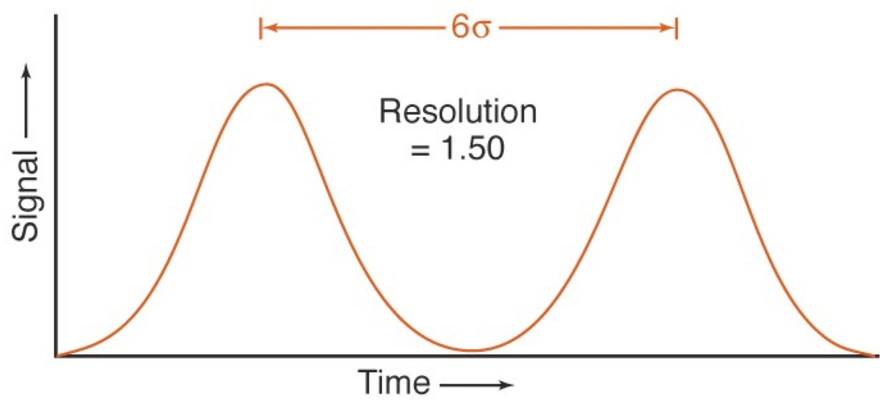
$$R_s = \frac{\Delta t_R}{w_{av}} = \frac{\Delta V_R}{w_{av}} = \frac{0.589 \Delta t_R}{w_{1/2 av}}$$

where Δt_R or ΔV_R is the separation between peaks and w_{av} is the average width of the two peaks.


2σ
Resolution
= 0.50
Signal


3σ
Resolution
= 0.75
Signal


4σ
Resolution
= 1.00
Signal
Time


6σ
Resolution
= 1.50
Signal
Time

- So, separation of mixtures depends on:
  - width of solute peaks (want narrow)
    efficiency
  - spacing between peaks (want large spacing)
    selectivity

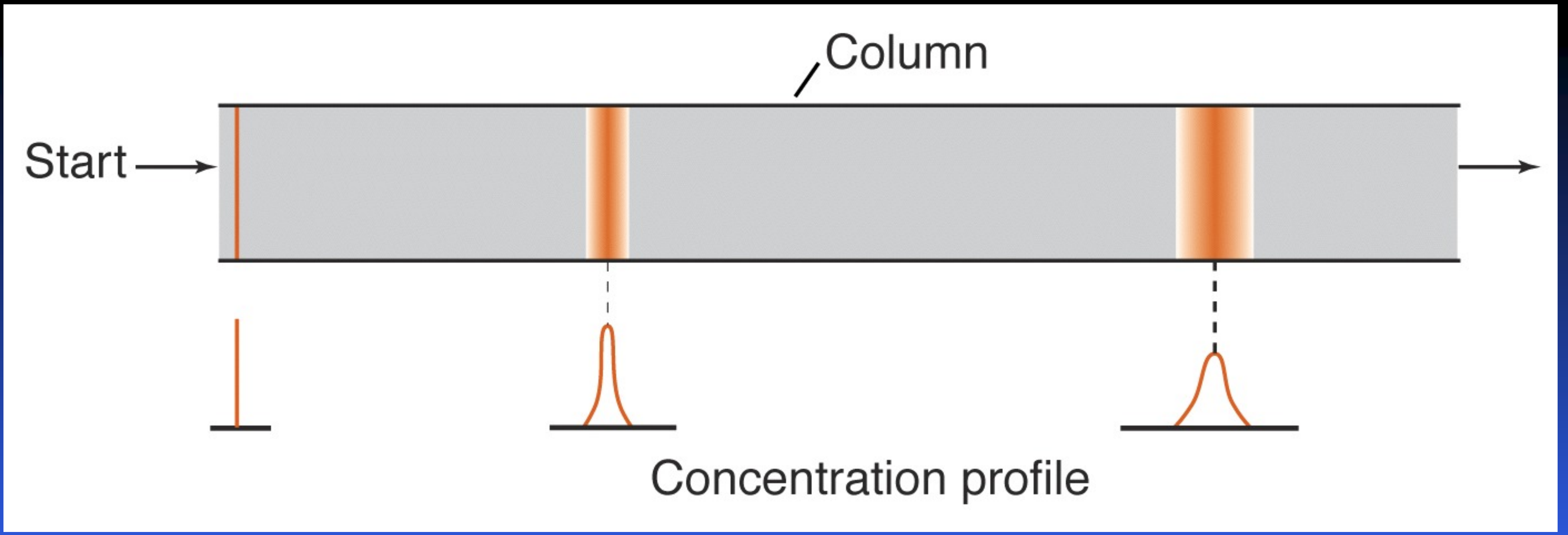
Example

- What is the resolution of two Gaussian peaks of identical width (3.27 s) and height eluting at 67.3 s and 74.9 s, respectively?

- ANS: Resolution = 2.32

Diffusion

A band of solute broadens as it moves through a column. Ideally, an infinitely narrow band applied to the inlet of the column emerges with a Gaussian shape at the outlet.


Column
Start
Concentration profile

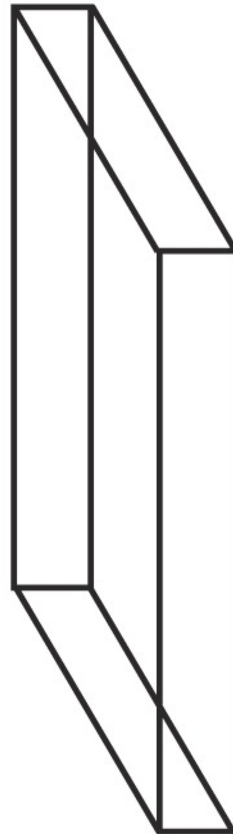
Diffusion (cont.)

One main cause of band spreading is *diffusion*. The ***diffusion coefficient*** measures the rate at which a substance moves randomly from a region of high concentration to a region of lower concentration.

Diffusion (cont.)

The number of moles crossing each square meter per second, called the ***flux***, is proportional to the concentration gradient:

$$\text{flux}\quad\left(\frac{\text{mol}}{\text{m}^2\cdot s}\right)\equiv J=-D\frac{dc}{dx}$$


High concentration
Low concentration
Flux = J
mol per m²
per second
Position = x
Concentration = c
x + dx
c − dc

Broadening of Chromatographic Band by Diffusion

If solute begins to move through a column in an infinitely sharp layer with *m* moles per unit cross-sectional area of the column and spreads by diffusion alone, then the Gaussian profile of the band is described by

$$c = \frac{m}{\sqrt{4\pi Dt}} e^{-x^2 / (4Dt)}$$

$$\sigma = \sqrt{2Dt}$$

Table 23-1 Representative diffusion coefficients at 298 K

Solute	Solvent	Diffusion coefficient (m²/s)
H_2O	H_2O	2.3×10^{-9}
Sucrose	H_2O	0.52×10^{-9}
Glycine	H_2O	1.1×10^{-9}
CH_3OH	H_2O	1.6×10^{-9}
Ribonuclease (FM 13 700)	H_2O (293 K)	0.12×10^{-9}
Serum albumin (FM 65 000)	H_2O (293 K)	0.059×10^{-9}
I_2	Hexane	4.0×10^{-9}
CCl_4	Heptane	3.2×10^{-9}
N_2	CCl_4	3.4×10^{-9}
$CS_2(g)$	Air (293 K)	1.0×10^{-5}
$O_2(g)$	Air (273 K)	1.8×10^{-5}
H^+	H_2O	9.3×10^{-9}
OH^-	H_2O	5.3×10^{-9}
Li^+	H_2O	1.0×10^{-9}
Na^+	H_2O	1.3×10^{-9}
K^+	H_2O	2.0×10^{-9}
Cl^-	H_2O	2.0×10^{-9}
I^-	H_2O	2.0×10^{-9}

The Theory of Chromatography: Column Efficiency

- Plate theory - older; developed by Martin & Synge
- Rate theory - currently in use

Plate Theory - Martin & Synge

- View column as divided into a number (N) of adjacent *imaginary* segments called theoretical plates
- within each theoretical plate complete equilibration of analytes between stationary and mobile phase occurs

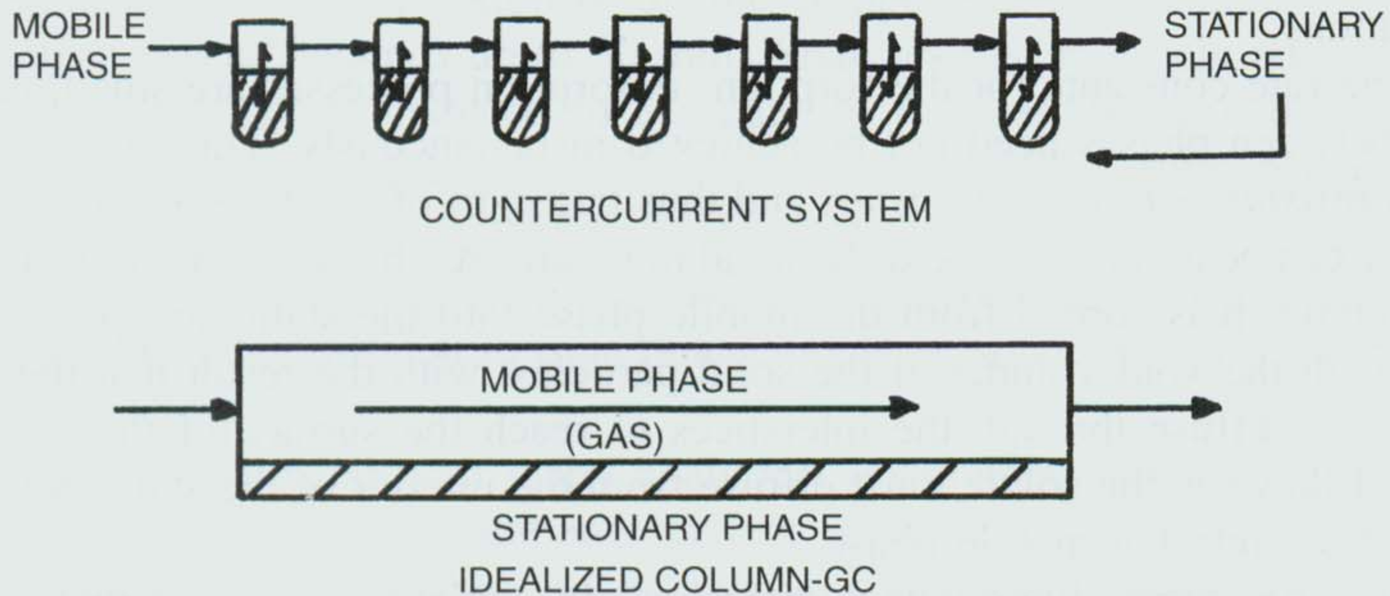


FIGURE 2.19 Comparison of countercurrent extraction and the chromatographic process.

Plate Theory - Martin & Synge

- Significance?
  Greater separation occurs with:
  - greater number of theoretical plates (N)
  - as plate height (H or HETP) becomes smaller
- $L = N \times H$ or $H = L / N$
  where L is the length of column, N is the number of plates, and H is the plate height

First Important Prediction of Plate Theory

Band spreading - the width of bands increases as their retention time (volume) increases:

Plate height is the constant of proportionality between the variance of the band and the distance it has traveled:

$$\sigma^2 = 2Dt = 2D\frac{x}{u_x} = \left(\frac{2D}{u_x}\right)x = Hx$$

Second Significant Prediction of Plate Theory

The smaller HETP, the narrower the eluted peak

Plate Theory - Practical Considerations

- Not unusual for a chromatography column to have millions of theoretical plates
- Columns often behave as if they have different numbers of plates for different solutes present in same mixture

Number of plates on column:

$$N = 16\left(\frac{V_R}{w_b}\right)^2$$

w_b – base width of the peak

This equation is a measure of the ***efficiency*** of a column.

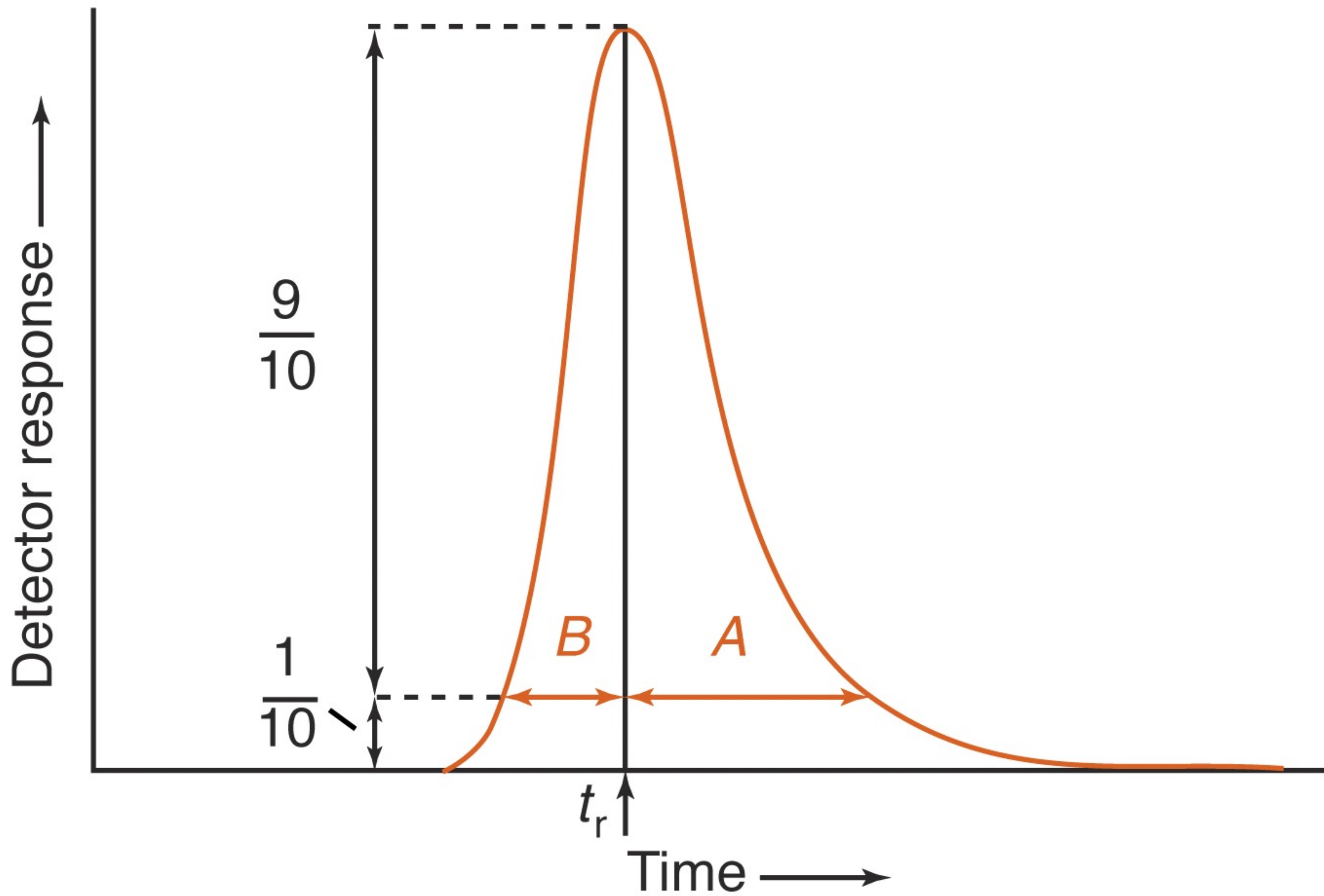
Sometimes the number of plates is measured at the bandwidth at half-height $w_{1/2}$:

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

Estimating the Plate Number for Asymmetric Peaks

The Dorsey-Foley equation:

$$N = \frac{41.7\left(t_R / w_{0.1}\right)^2}{A/B + 1.25}$$



Detector response
9/10
1/10
B
A
t_r
Time

N can be Estimated Experimentally from a Chromatogram

Knowing the number of theoretical plates and the length of the column, we can determine the HETP, ***height equivalent to a theoretical plate***:

$$H = \text{HETP} = \frac{L}{N} = \frac{L}{16}\left(\frac{w_b}{V_R}\right)^2 = \frac{L}{16}\left(\frac{w_b}{t_R}\right)^2$$

Effective Number of Theoretical Plates

Introduced to characterize open tubular columns – uses adjusted retention volume V_R' in lieu of total retention volume V_R:

$$N_{\mathrm{eff}} = 16\left(\frac{V_R'}{w_\mathrm{b}}\right)^2 = 16\left(\frac{t_R'}{w_\mathrm{b}}\right)^2$$

Effective Number of Theoretical Plates (cont.)

The N_{eff} value is useful for comparing a packed and an open tubular column when both are used for the same separation.

N and N_{eff} are related by the expression

$$N_{eff} = N\left(\frac{k'}{k'+1}\right)^2$$

Rate Theory

- Based on a random walk mechanism for the migration of molecules through a column
- Takes into account:
  - mechanism of band broadening
  - effect of rate of elution on band shape
  - availability of different paths for different solute molecules to follow
  - diffusion of solute along length

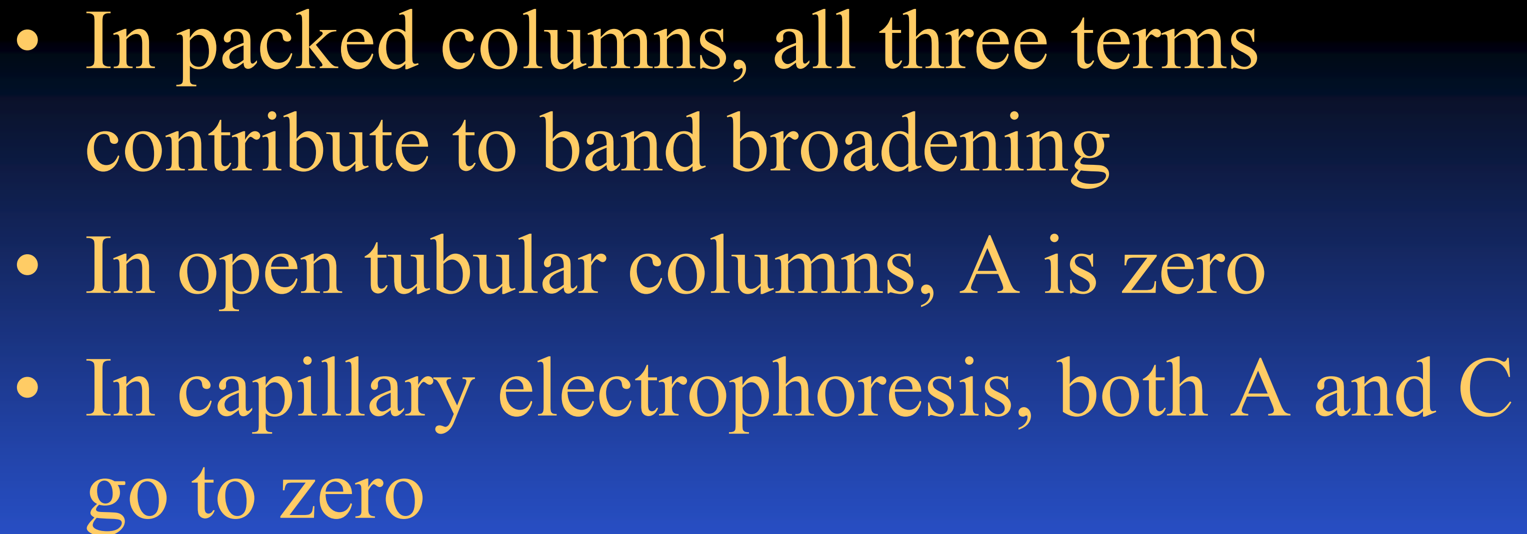
Van Deemter Equation for Plate Height

$$H = A + \frac{B}{u_x} + C u_x$$

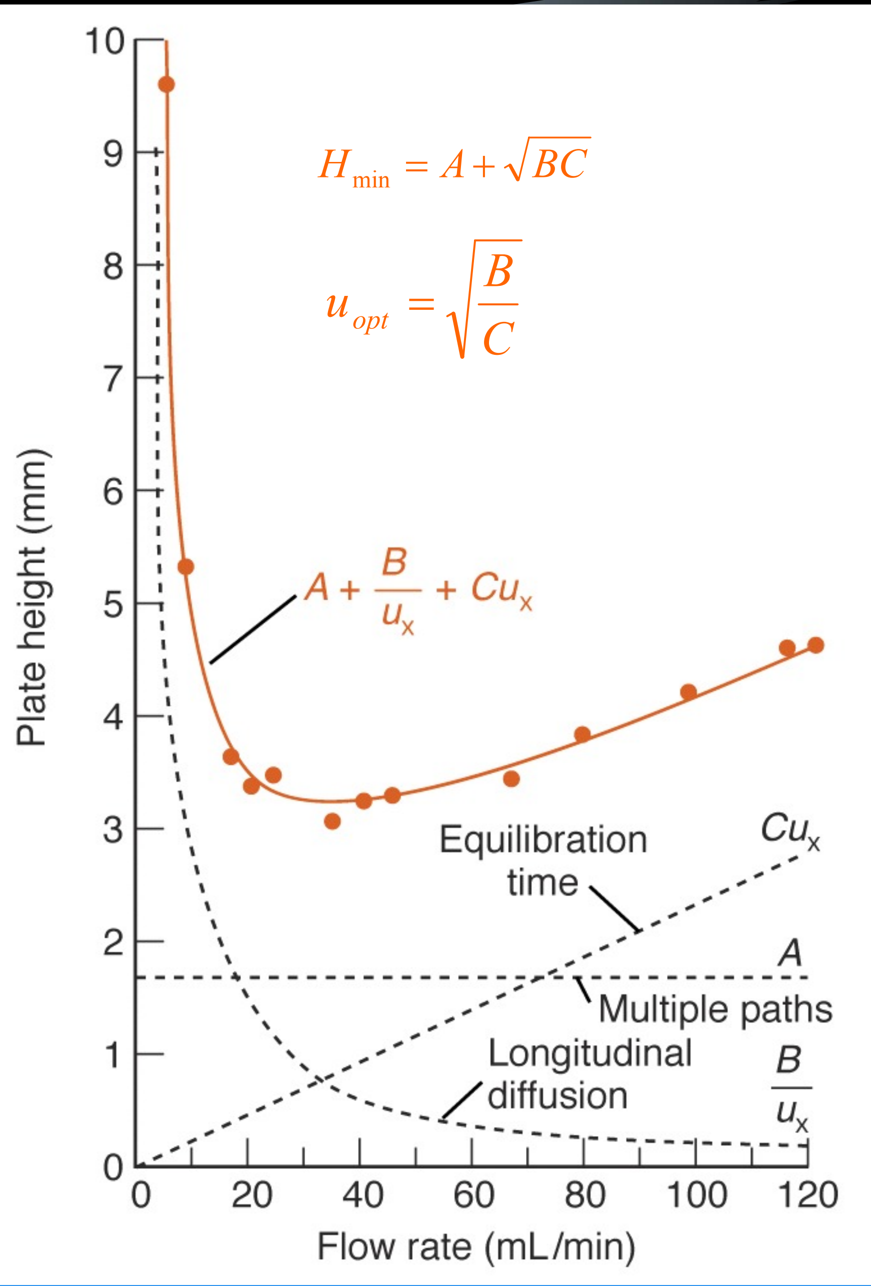
Multiple paths

Longitudinal diffusion

Equilibration time



- In packed columns, all three terms contribute to band broadening
- In open tubular columns, A is zero
- In capillary electrophoresis, both A and C go to zero



$H_{min} = A + \sqrt{BC}$
$u_{opt} = \sqrt{\frac{B}{C}}$
$A + \frac{B}{u_x} + Cu_x$
Plate height (mm)
Equilibration time
Cu_x
A
Multiple paths
Longitudinal diffusion
$\frac{B}{u_x}$
Flow rate (mL/min)

Longitudinal Diffusion

- Gives rise to B/u_x term
- Solute continuously diffuses away from the concentrated center of its zone
- The greater the flow rate, the less time is spent in the column and the less longitudinal diffusion occurs

Longitudinal Diffusion (cont.)

The variance resulting from diffusion is

$$\sigma^2 = 2D_m t = \frac{2D_m L}{u_x}$$

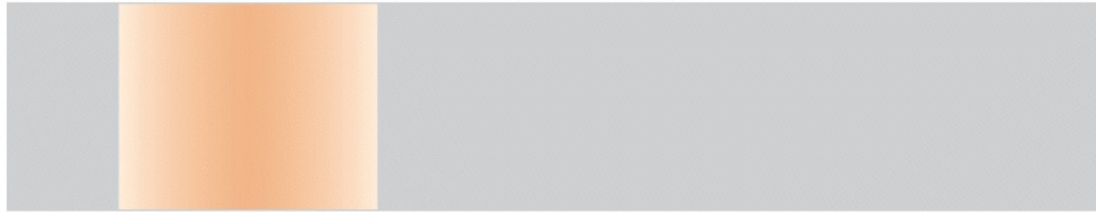
Plate height due to longitudinal diffusion:

$$H_D = \frac{\sigma^2}{L} = \frac{2D_m}{u_x} \equiv \frac{B}{u_x}$$

Longitudinal Diffusion (cont.)

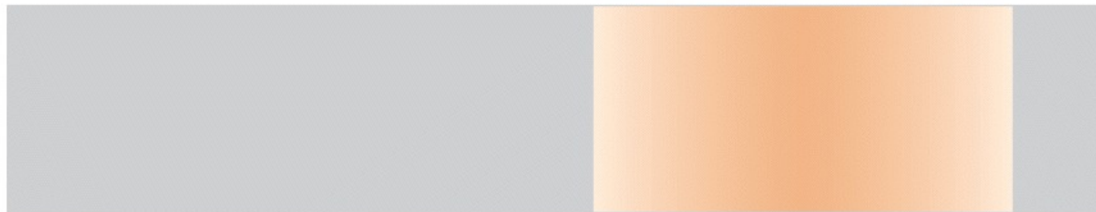
In packed columns, the tortuosity coefficient γ is used to account for irregular diffusion patterns and is usually less than unity ($\gamma \sim 0.6$), because molecular diffusivity is smaller in packed columns than in open tubes ($\gamma = 1$):

$$H_D = \frac{2\gamma D_m}{u_x}$$



Zone of solute after short time on column

Longitudinal diffusion (B/u_x)



Zone of solute after longer time on column

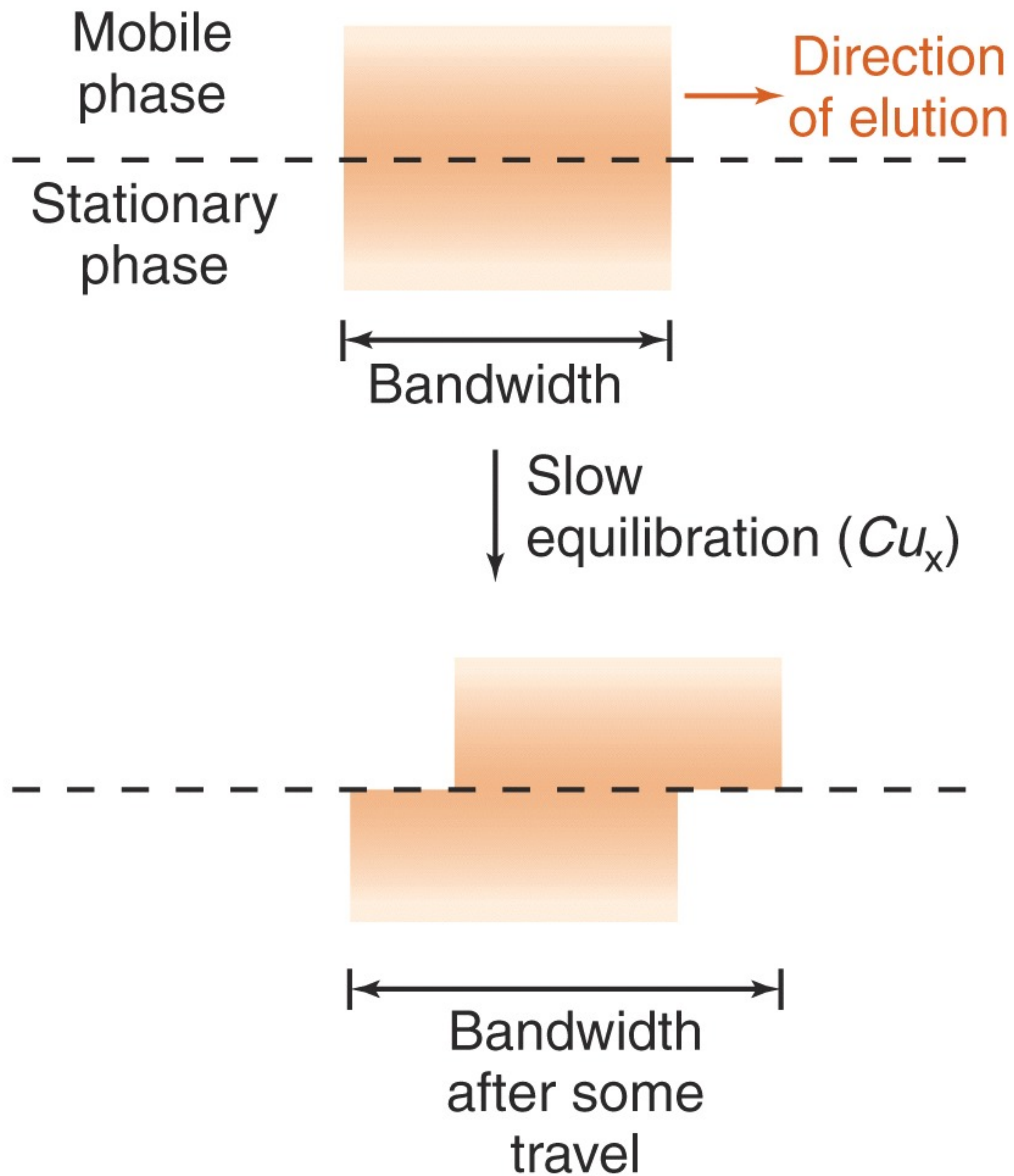
Direction of travel

Longitudinal Diffusion (cont.)

Because longitudinal diffusion in a gas is much faster than in a liquid, the optimum flow rate in gas chromatography is higher than in liquid chromatography

Nonequilibrium (Resistance to Mass Transfer Term)

- This term comes from the finite time required for the solute to equilibrate between the mobile and stationary phases
- Some solute is stuck in the stationary phase, but the remainder in the mobile phase moves forward resulting in spreading of the zone
- The slower the flow rate, the more complete equilibration is and the less band broadening occurs


Mobile phase
Stationary phase
Direction of elution
Bandwidth
Slow equilibration (Cu_x)
Bandwidth after some travel

Resistance to Mass Transfer (cont.)

Plate height due to finite equilibration time:

$$H_{\text{mass transfer}} = Cu_x = (C_s + C_m)u_x$$

where C_s describes the rate of mass transfer through the stationary phase and C_m describes the rate of mass transfer through the mobile phase. Specific equations for C_s and C_m depend on the type of chromatography.

Resistance to Mass Transfer (cont.)

For gas chromatography in an open tubular column, the terms are:

Mass transfer in stationary phase:

$$C_s = \frac{2k'}{3(k'+1)^2} \frac{d_f^2}{D_s}$$

Mass transfer in mobile phase:

$$C_m = \frac{1 + 6k' + 11k'^2}{24(k'+1)^2} \frac{r^2}{D_m}$$

Resistance to Mass Transfer (cont.)

k' – the capacity factor

d_f – the thickness of stationary phase film

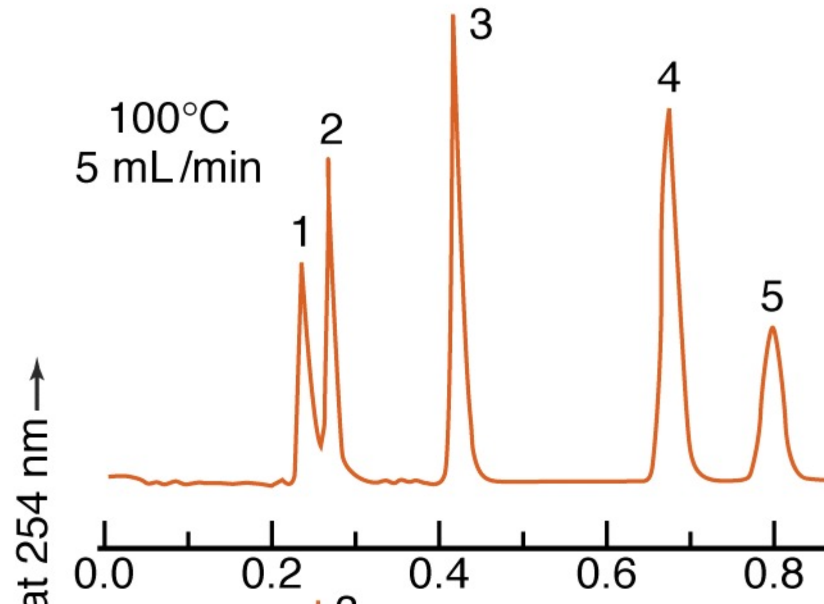
D_s – the diffusion coefficient of solute in the stationary phase

r – the column radius

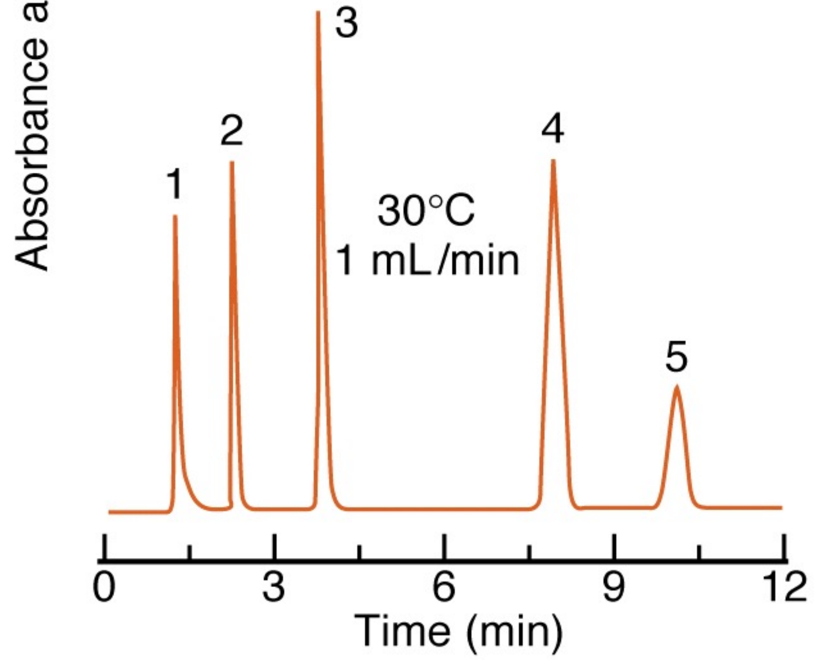
D_m – the diffusion coefficient of solute in the mobile phase

Resistance to Mass Transfer (cont.)

- Efficiency is increased by:
  - Decreasing stationary phase thickness
  - Reducing column radius
  - Increasing temperature



100°C
5 mL/min
1
2
3
4
5
0.0
0.2
0.4
0.6
0.8
Absorbance at 254 nm



1
2
3
4
5
30°C
1 mL/min
0
3
6
9
12
Time (min)

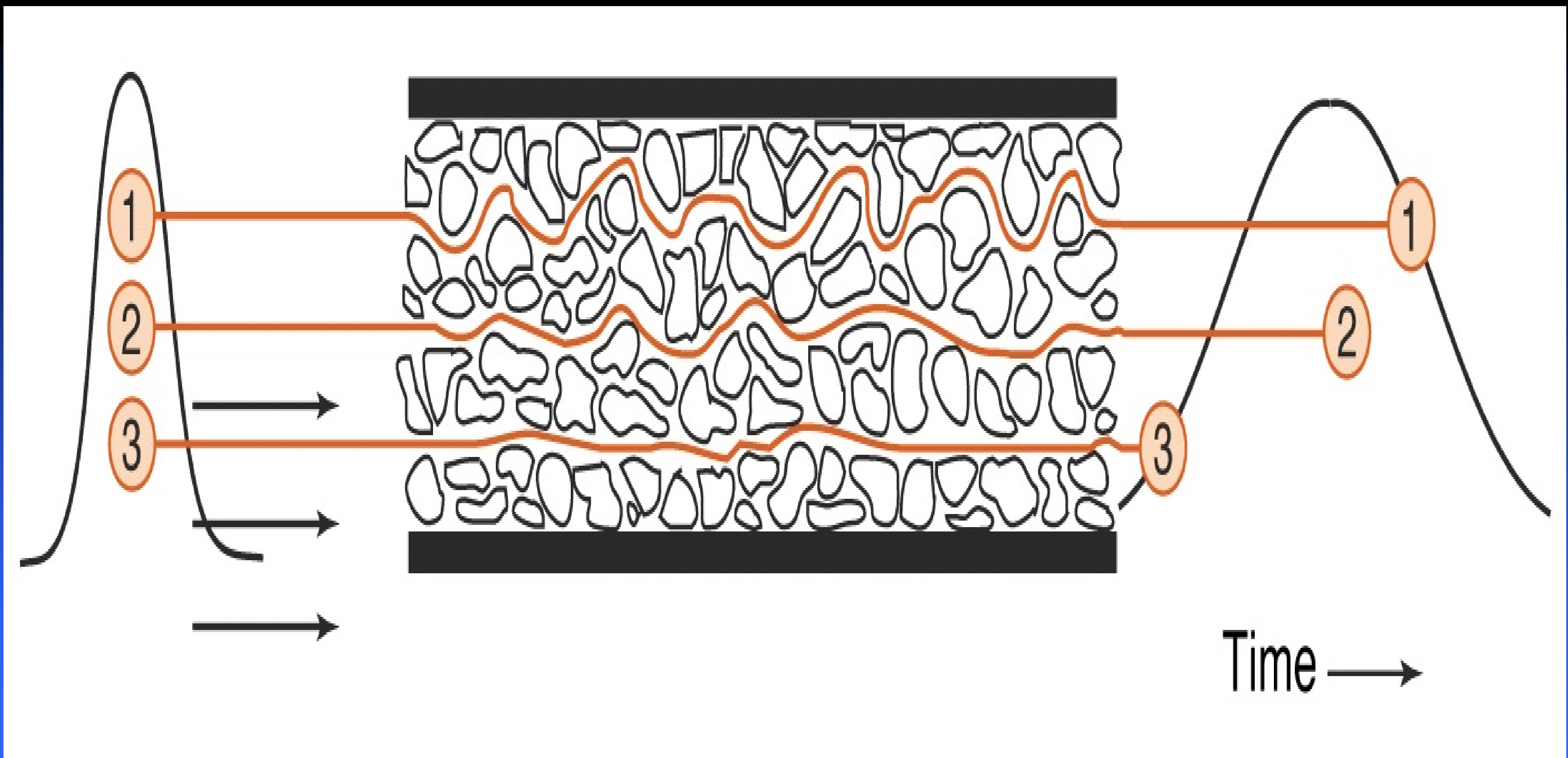
Multiple Flow Paths (Eddy Diffusion)

The term A accounts for the multitude of pathways that could be followed through the column by one molecule:

$$A = 2\lambda d_p$$

λ – dimensionless constant characteristic of packing

d_p – the particle diameter


1
2
3
1
2
3
Time

Multiple Flow Paths (Eddy Diffusion) (cont.)

The *A* term can be minimized by:

- Eliminating column packing (open tubular columns)
- Reducing the particle size of the packing
- Packing the column more uniformly (spherical particles of similar size)

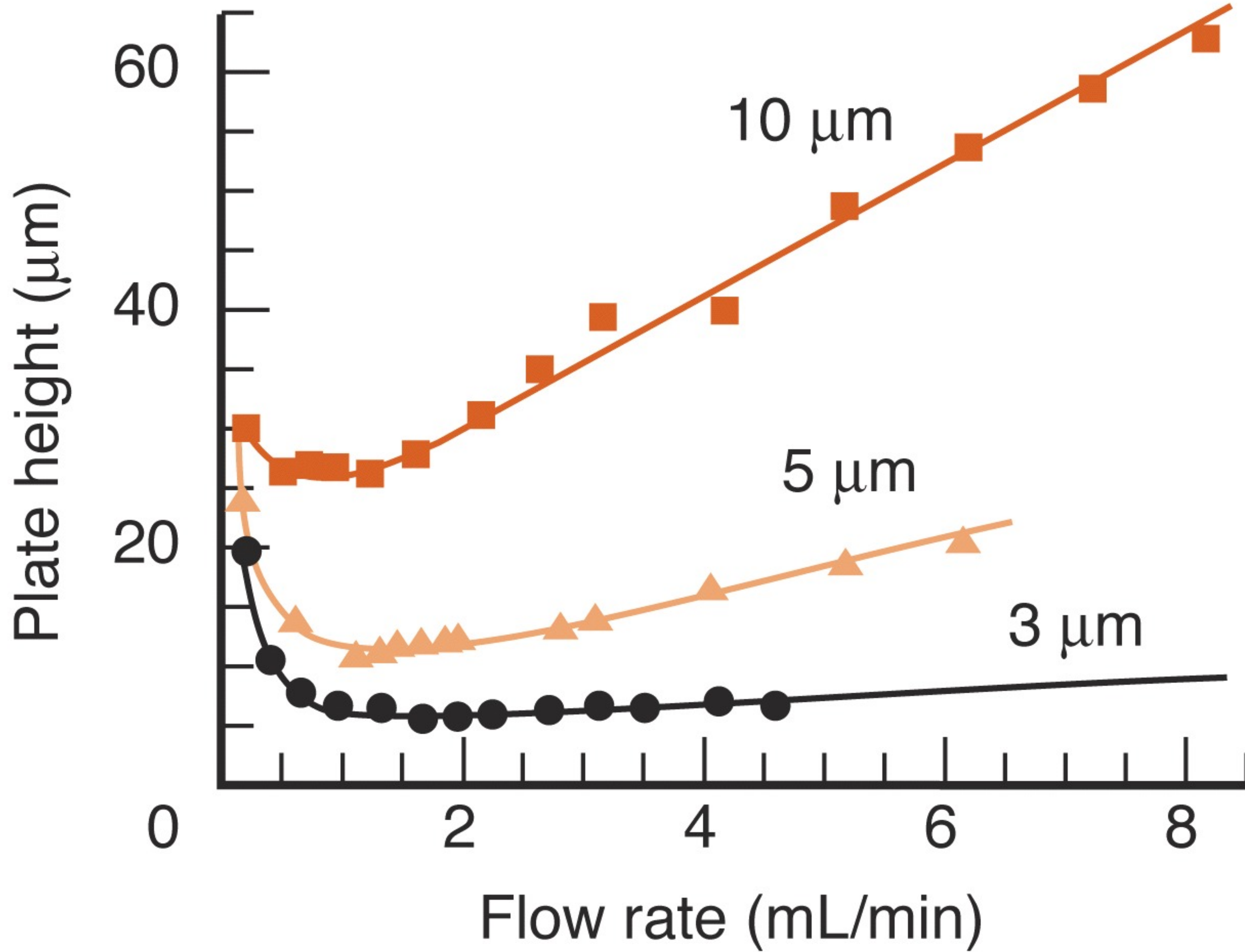


Plate height (μm)
60
40
20
0
2
4
6
8
10 μm
5 μm
3 μm
Flow rate (mL/min)

Table 25-1 Performance as a function of particle diameter

Particle size d_p (μm)	Retention time (min)	Plate number (*N*)	Required pressure (bar)
5.0	30	25 000	19
3.0	18	42 000	87
1.5	9	83 000	700
1.0	6	125 000	2 300

Theoretical performance of 33-μm-diameter × 25-cm-long capillary for minimum plate height for solute with capacity factor $k' = 2$ and diffusion coefficient $= 6.7 \times 10^{-10}$ m^2/s in water-acetonitrile eluent.

SOURCE: J. E. MacNair, K. D. Patel, and J. W. Jorgenson, "Ultrahigh-Pressure Reversed-Phase Capillary Liquid Chromatography with 1.0-μm Particles," *Anal. Chem.* **1999**, *71*, 700.

Alternative Plate Height Equation: The Knox Equation

- Used to compare column efficiencies
- Makes use of so-called *reduced parameters* (dimensionless quantities):
  - Reduced plate height: $h = H/d_p$
  - Reduced velocity: $\nu = ud_p/D_m$

$$h = a\nu^{1/3} + \frac{b}{\nu} + c\nu$$

The Knox Equation (cont.)

For well-packed columns of varying particle size and differing conditions, the coefficients a, b and c will be roughly constant: e.g. $a = 1$, $b = 2$, and $c = 0.05$ for porous particles.

Modification of the van Deemter Equation: the Giddings Equation

Giddings realized that the eddy diffusion and resistance to mass transfer in the mobile phase must be treated dependently:

$$H = \sum_{i=1}^{5} \frac{1}{{}^{1}\!/_{A} + {}^{1}\!/_{C_1 u}} + \frac{B}{u} + C_s u + C_m u + H_e$$

The Giddings Equation

H_e – extracolumn band broadening

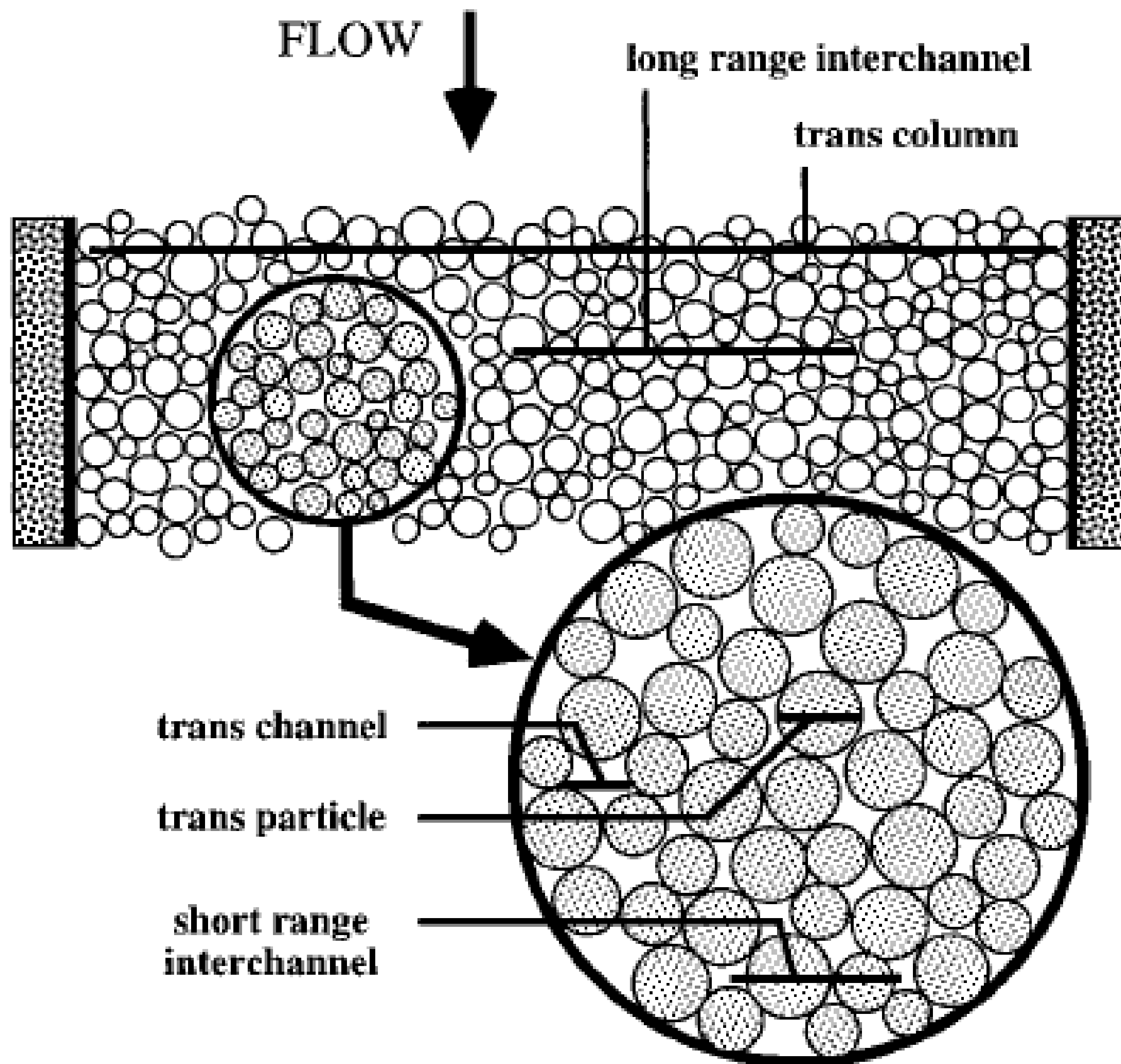
$$C_1 = \left(\frac{c_b d_p^2}{D_m} \right)$$

c_b – a proportionality constant; $c_b \approx 1$

The Giddings Equation (cont.)

The five possible mechanisms of band broadening are:

- Through channels between particles
- Through particles
- Resulting from uneven flow channels
- Between inhomogeneous regions
- Throughout the entire column length


FLOW
long range interchannel
trans column
trans channel
trans particle
short range
interchannel

Factors Affecting Resolution

A more usable expression for resolution is

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

efficiency selectivity retention

Required Plate Number

If k_2' and α are known, the required number of plates can be calculated:

$$N_{\mathrm{req}} = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{k_2' + 1}{k_2'}\right)^2$$

The R_s value is set at the 6σ level or 1.5

Required Column Length

The N_{req} parameter can be used to determine the length of column necessary for a separation. We know that $N = L/H$; thus

$$L_{\mathrm{req}} = 16 R_s^2 H \left(\frac{\alpha}{\alpha - 1} \right)^2 \left(\frac{k_2^{'} + 1}{k_2^{'}} \right)^2$$

Minimum Analysis Time

The minimum analysis time $t_{\min}$ is

$$t_{\min} = 16 R_s^2 \frac{H}{\bar{u}} \left(\frac{\alpha}{\alpha - 1} \right)^2 \frac{\left(k_2' + 1 \right)^3}{\left(k_2' \right)^2}$$

The Major Objective in Chromatography

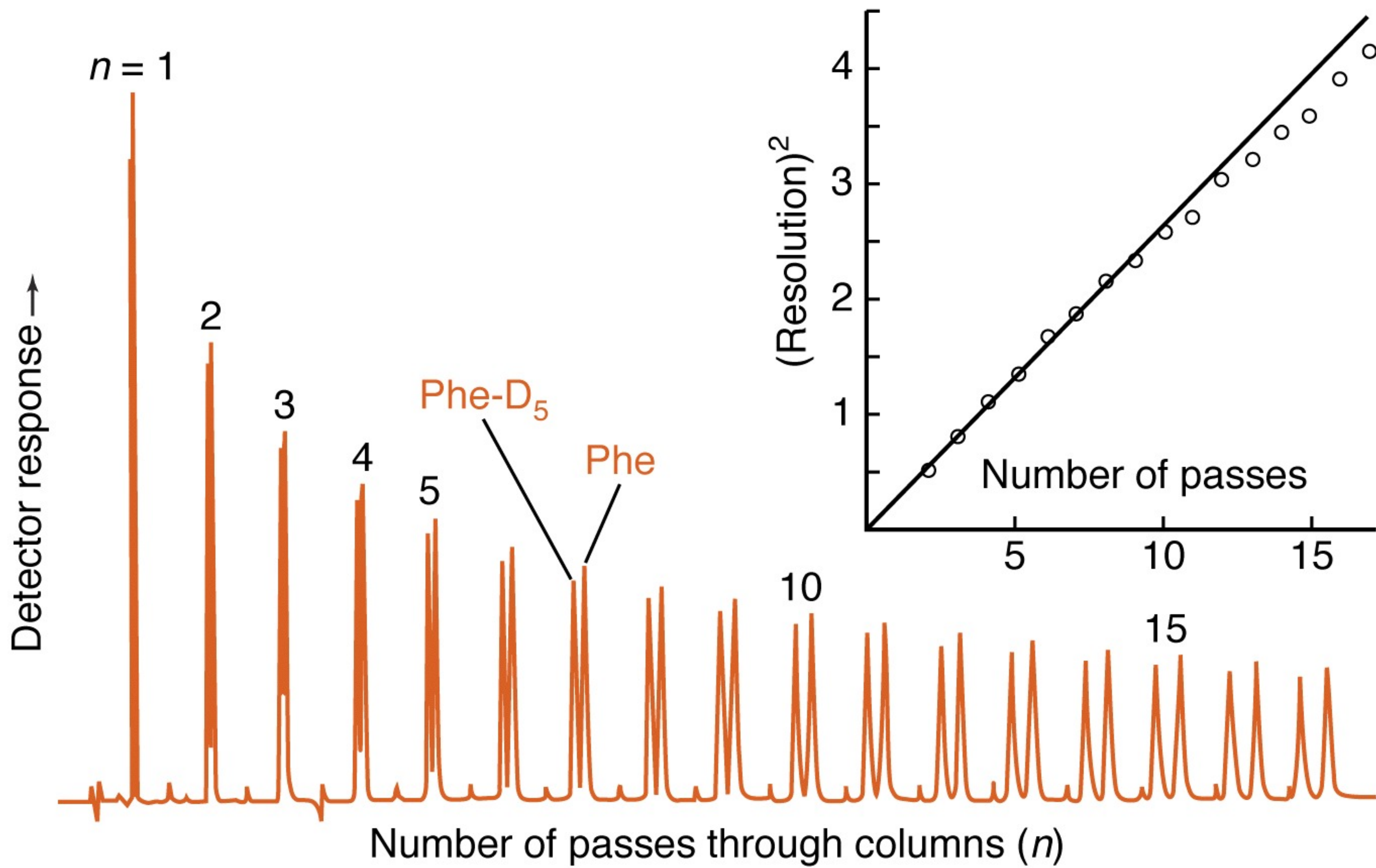
The goal in chromatography is the highest possible resolution in the ***shortest possible time***. Optimization techniques aim at choosing conditions that lead to a desired degree of resolution with a minimum expenditure of time

Optimization Techniques

- Minimizing plate height:
  - Reducing d_p
  - Reducing column diameter
  - Changing column temperature
  - Reducing the thickness of the liquid film
  - Optimizing the flow rate of the mobile phase

Optimization Techniques (cont.)

- Resolution also improves with L, but it expensive in terms of time of analysis
- Variation in the selectivity factor:
  - Changing the composition of the mobile phase (in HPLC)
  - Changing the column temperature
  - Changing the stationary phase
  - Using special chemical effects



n = 1
2
3
4
5
Phe-D5
Phe
10
15
Detector response
Number of passes through columns (n)
(Resolution)2
Number of passes
4
3
2
1
5
10
15

Effect of Capacity Factor on Resolution and Elution Time

- Increases in k' enhance resolution but at the expense of elution time
- The optimum k' values are from 1 to 5
- The easiest way to improve R_s is by optimizing k'
- In GC, k' can be improved by temperature programming
- In LC, k' is improved by gradient elution

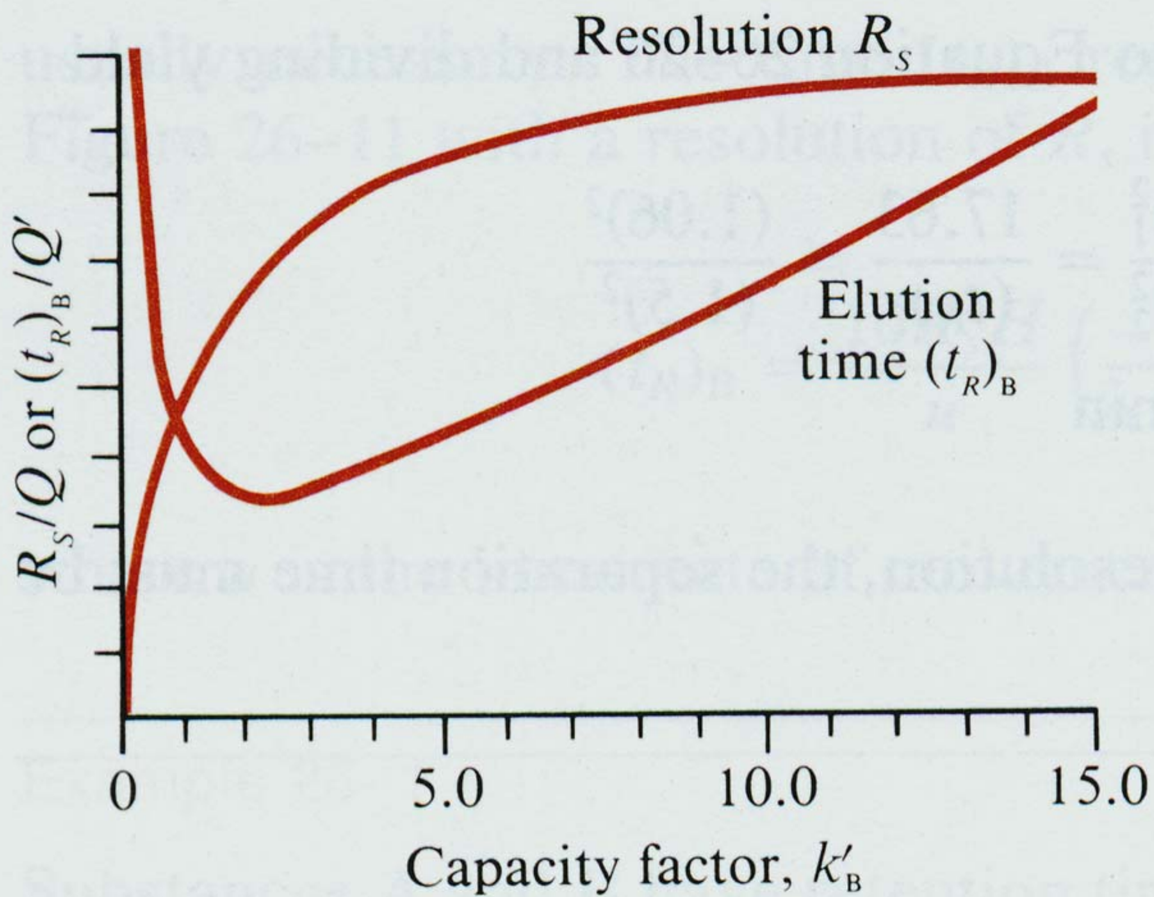

Resolution R_S
Elution time $(t_R)_B$
R_S/Q or $(t_R)_B/Q'$
0
5.0
10.0
15.0
Capacity factor, k'_B

Table 23-2 Summary of chromatography equations

Quantity	Equation	Parameters
Partition coefficient	$K = C_s/C_m$	C_s = concentration of solute in stationary phase C_m = concentration of solute in mobile phase
Adjusted retention time	$t'_r = t_r - t_m$	t_r = retention time of solute of interest t_m = retention time of unretained solute
Retention volume	$V_r = t_r \cdot u_v$	u_v = volume flow rate = volume/unit time
Capacity factor	$k' = t'_r/t_m = KV_s/V_m$ $k' = \frac{t_s}{t_m}$	V_s = volume of stationary phase V_m = volume of mobile phase t_s = time solute spends in stationary phase t_m = time solute spends in mobile phase
Relative retention	$\alpha = \frac{t'_{r2}}{t'_{r1}} = \frac{k'_2}{k'_1} = \frac{K_2}{K_1}$	Subscripts 1 and 2 refer to two solutes
Number of plates	$N = \frac{16t_r^2}{w^2} = \frac{5.55t_r^2}{w_{1/2}^2}$	w = width at base $w_{1/2}$ = width at half-height
Plate height	$H = \frac{\sigma^2}{x} = \frac{L}{N}$	σ = standard deviation of band x = distance traveled by center of band L = length of column N = number of plates on column
Resolution	$\text{Resolution} = \frac{\Delta t_r}{w_{av}} = \frac{\Delta V_r}{w_{av}}$	Δt_r = difference in retention times ΔV_r = difference in retention volumes w_{av} = average width measured at baseline in same units as numerator (time or volume)
	$\text{Resolution} = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_2}{1 + k'_{av}}\right)$	N = number of plates α = relative retention k'_2 = capacity factor for second peak k'_{av} = average capacity factor

Advantages of Open Tubular Columns

Compared with packed columns, open tubular columns can provide:

- Higher resolution
- Shorter analysis time
- Increased sensitivity
- Lower sample capacity

Advantages of Open Tubular Columns (cont.)

Compared with packed columns, open tubular columns allow

- Increased linear flow rate or a longer column or both
- Decreased plate height, which means higher resolution

Table 23-3 **Comparison of packed and wall-coated open tubular column performance**[a]

Property	Packed	Open tubular
Column length, L	2.4 m	100 m
Linear gas velocity	8 cm/s	16 cm/s
Plate height for methyl oleate	0.73 mm	0.34 mm
Capacity factor, k', for methyl oleate	58.6	2.7
Theoretical plates, N	3 290	294 000
Resolution of methyl stearate and methyl oleate	1.5	10.6
Retention time of methyl oleate	29.8 min	38.5 min

a. Methyl stearate ($CH_3(CH_2)_{16}CO_2CH_3$) and methyl oleate (*cis*-$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2CH_3$) were separated on columns with poly(diethylene glycol succinate) stationary phase at 180°C.

SOURCE: L. S. Ettre, *Introduction to Open Tubular Columns* (Norwalk, CT: Perkin-Elmer Corp., 1979),

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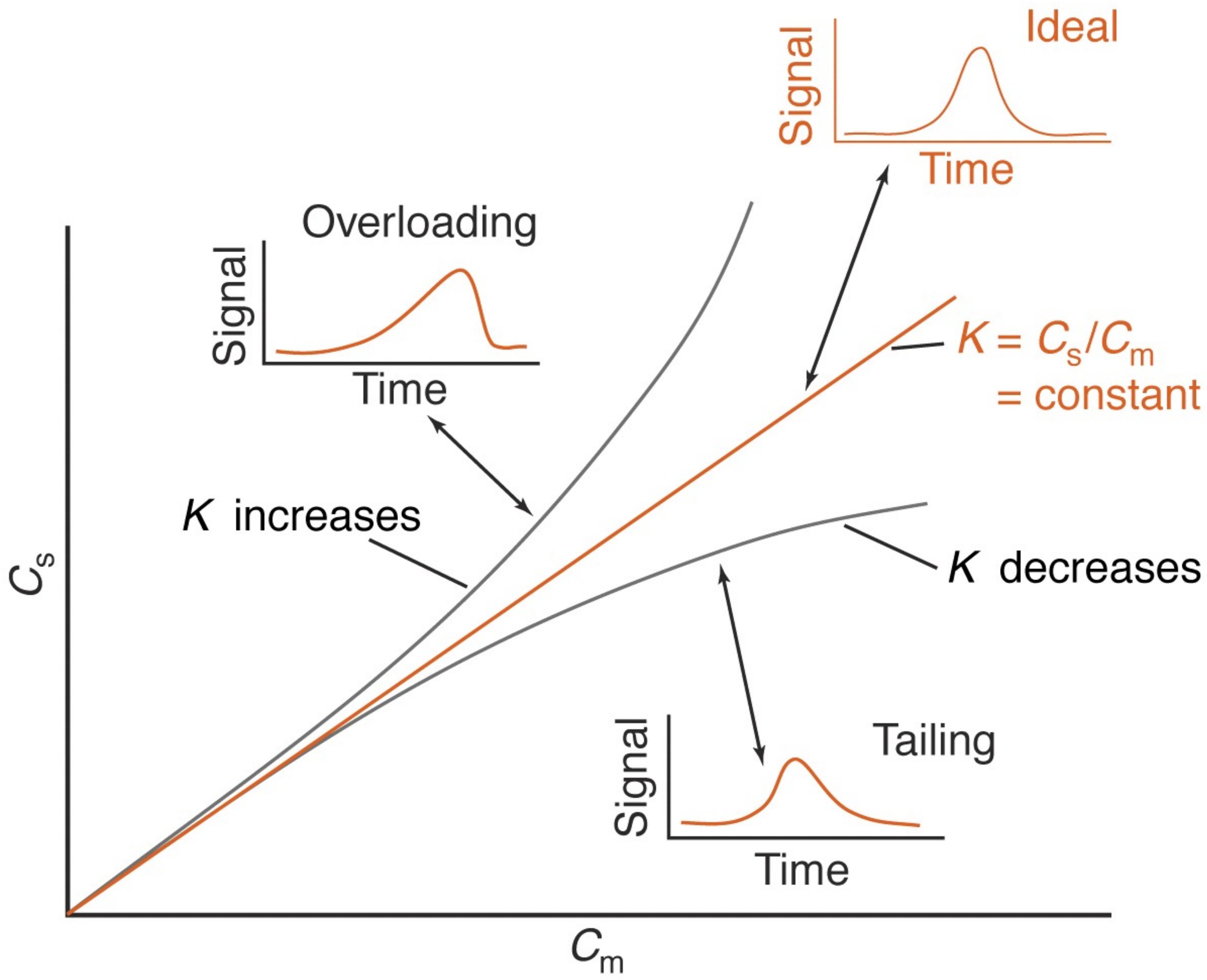
Ideal
Signal
Time
Overloading
Signal
Time
K = Cs/Cm
= constant
K increases
K decreases
Tailing
Signal
Time
Cs
Cm