Chapter 26: An Introduction to Chromatographic Separations

- Column Chromatography
- Migration Rates
  - Distribution Contstants
  - Retention Times
  - Selectivity Factor
- Zone Broadening & Column Efficiency
- Optimizing Performance
- Resolution

Intro to Chromatography

- Chromatography is a separation technique
- HPLC & GC are our primary focus
- Also discuss low pressure column chromatography & TLC (thin layer)
- All chromatographic techniques have
  - Stationary phase – solid or viscous liquid phase typically in a column
  - Mobile phase – moves sample in contact with 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 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

Partitioning = type of equilibrium where the analyte divides itself between two phases

For liquid-liquid extraction – two liquids

For chromatography – mobile vs. stationary phases

Define a partition ratio K (or distribution constant)

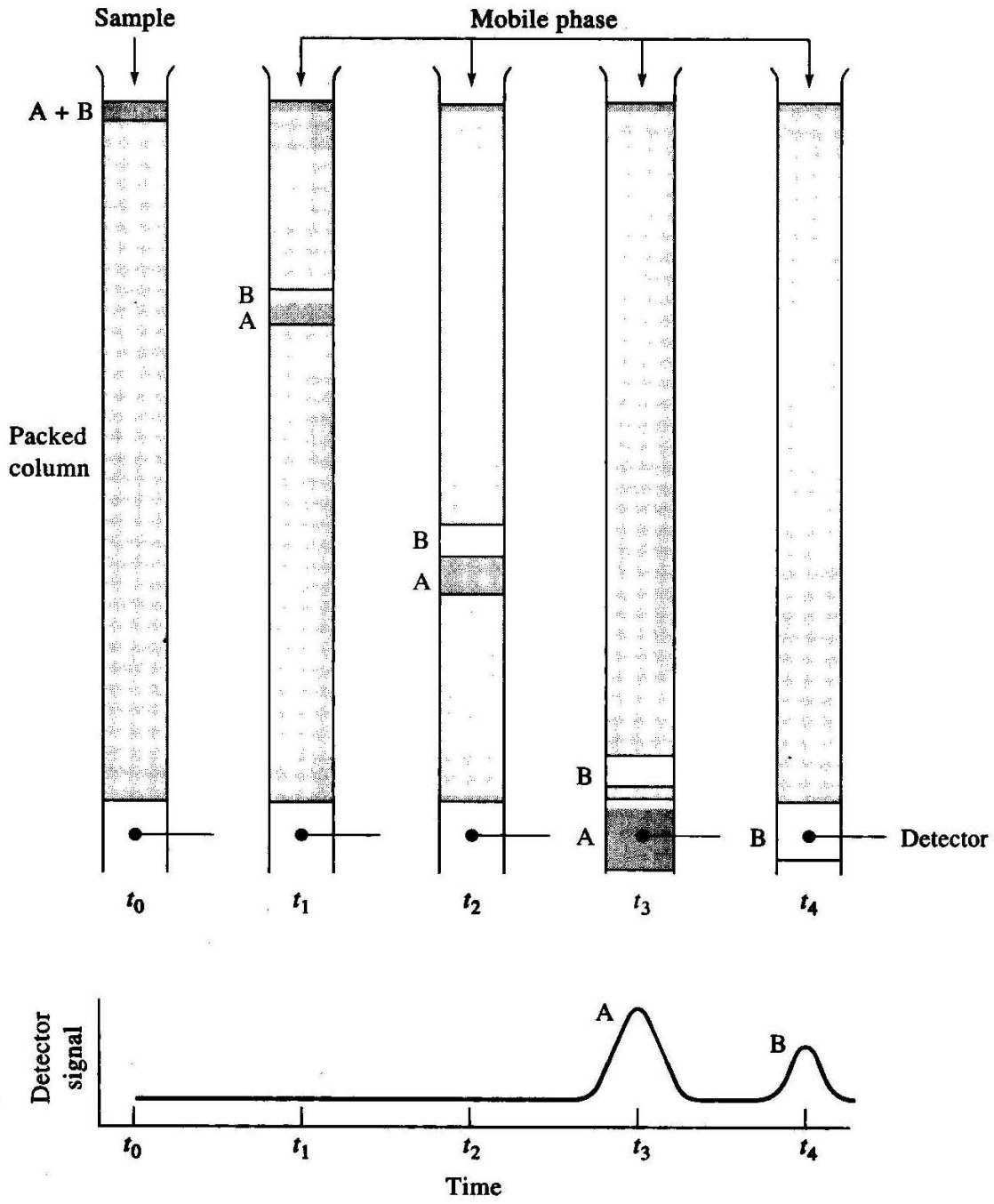
$$K = \frac{C_s}{C_M}$$

where C_s & C_M are concentrations of analyte in stationary & mobile phases

- Prefer if K is constant over conc. range
- If not constant we can work in a narrow range where it is constant
- This is linear chromatography

- Example of non-linear chromatography
  - Pour aqueous solution of A & B on column
  - Allow water to drain out, both A & B stick
  - Wash column with 50% MeOH, A removed
  - Wash column with 100% MeOH, B removed
- Used extensively for sample cleanup in GC

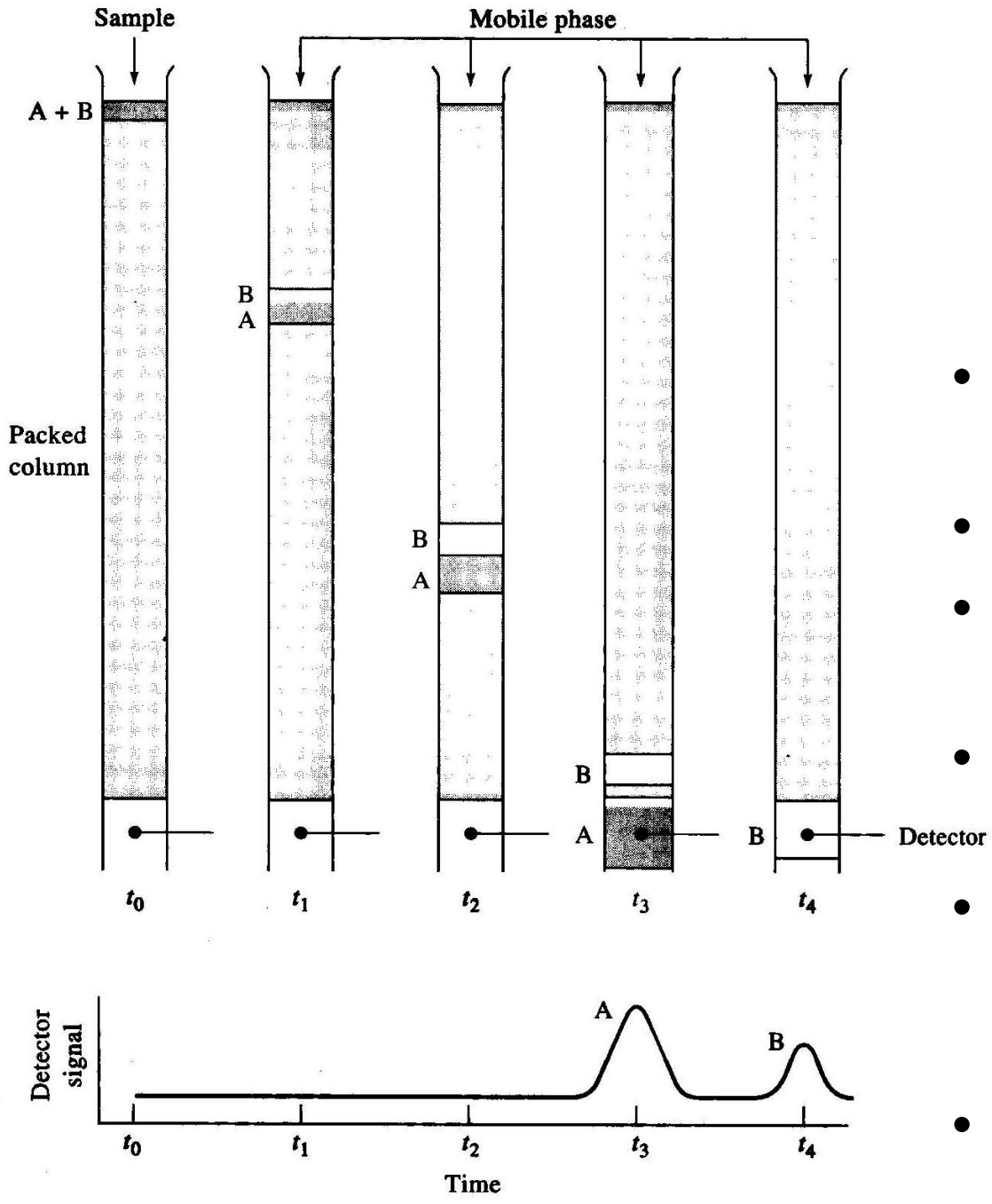
- From now on everything is linear chromatography
- In linear chromatography a constant flow rate of mobile phase moves through column
- K is typically constant or nearly constant
- Elution = process by which analyte is flushed through the column by mobile phase (which could be a liquid or a gas)



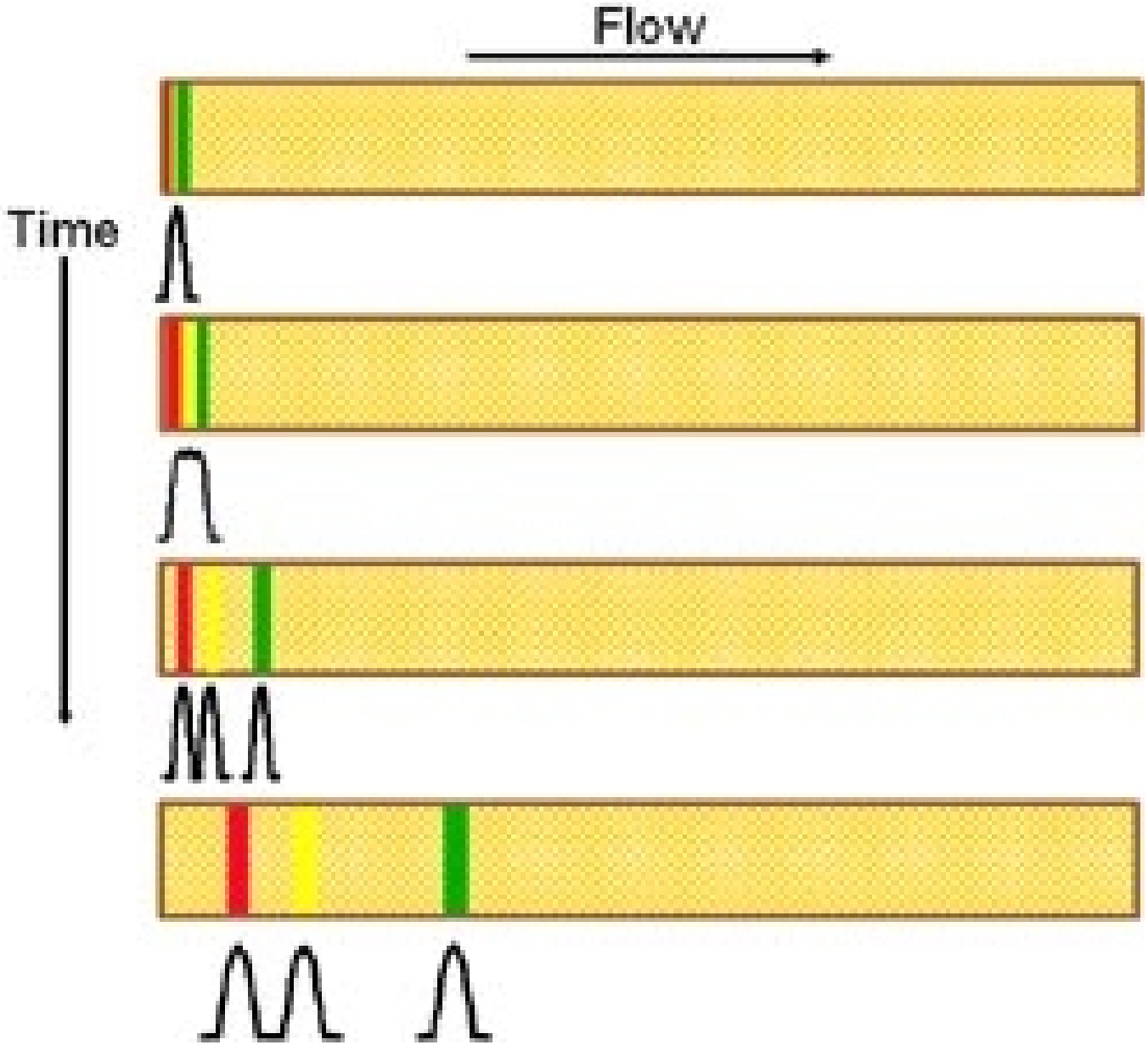
Overview of chromatographic process – packed column

- Inject at t_o
- Separate t_1 to t_3
- Detect at t_4

← Resulting chromatogram



- A & B retained by column differently
- B has higher K
- B takes longer to elute from column
- Detector sees A first then B
- Peak heights & peak areas are proportional to conc.
- Band broadening


Flow
Time

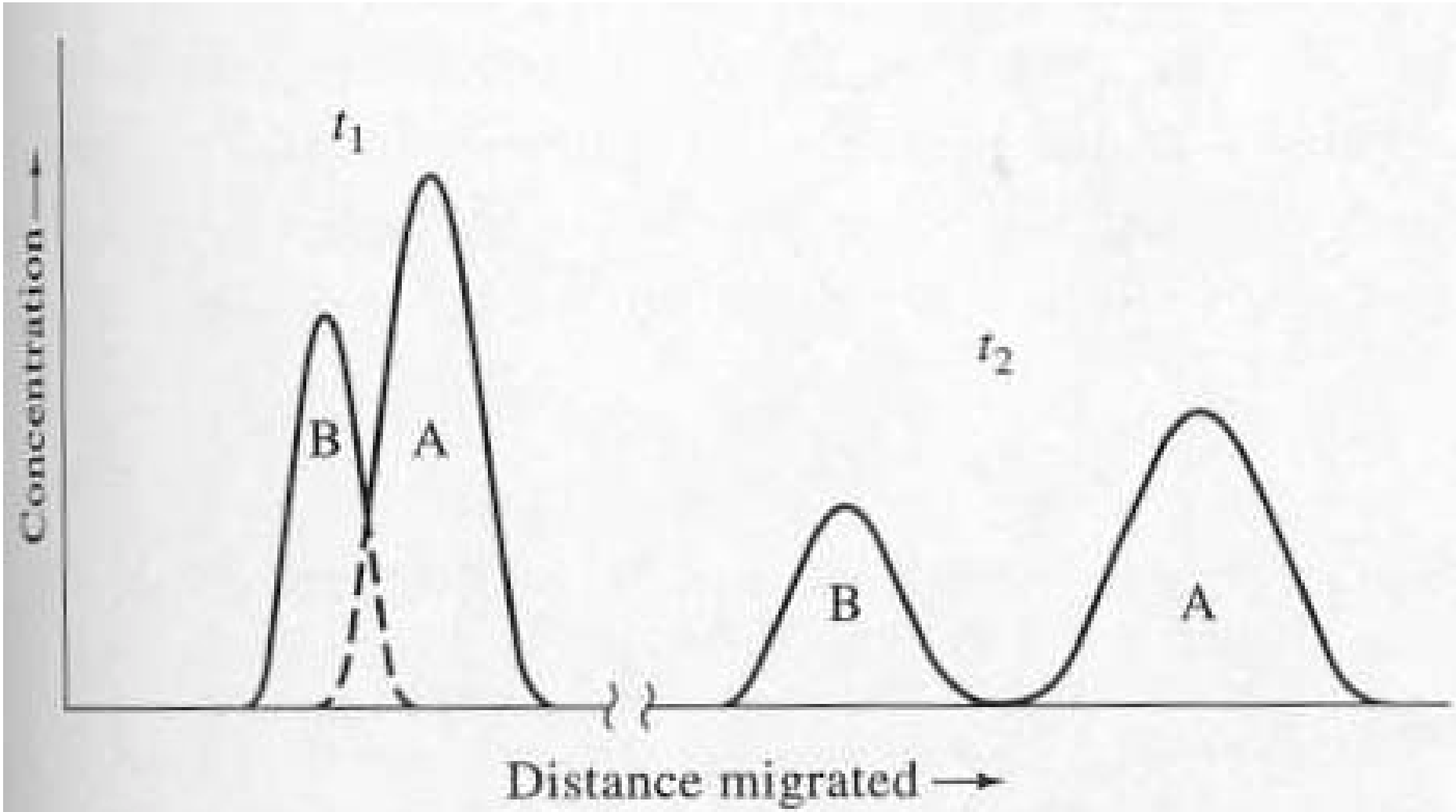


Figure 26-2 Concentration profiles of analyte bands A and B at two different times in their migration down the column

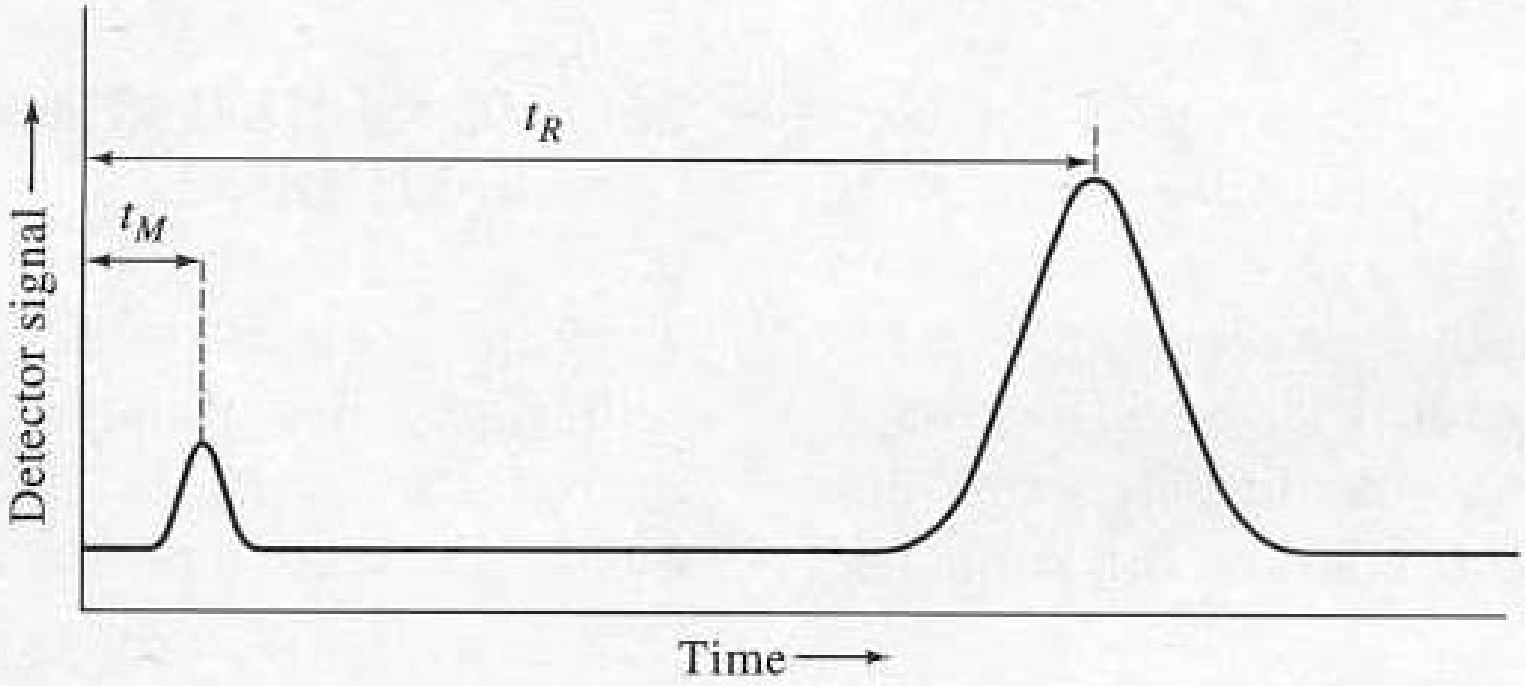


Figure 26-4 A typical chromatogram for a two-component mixture. The small peak on the left represents a species that is not retained on the column and so reaches the detector almost immediately after elution is started. Thus its retention time t_M is approximately equal to the time required for a molecule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

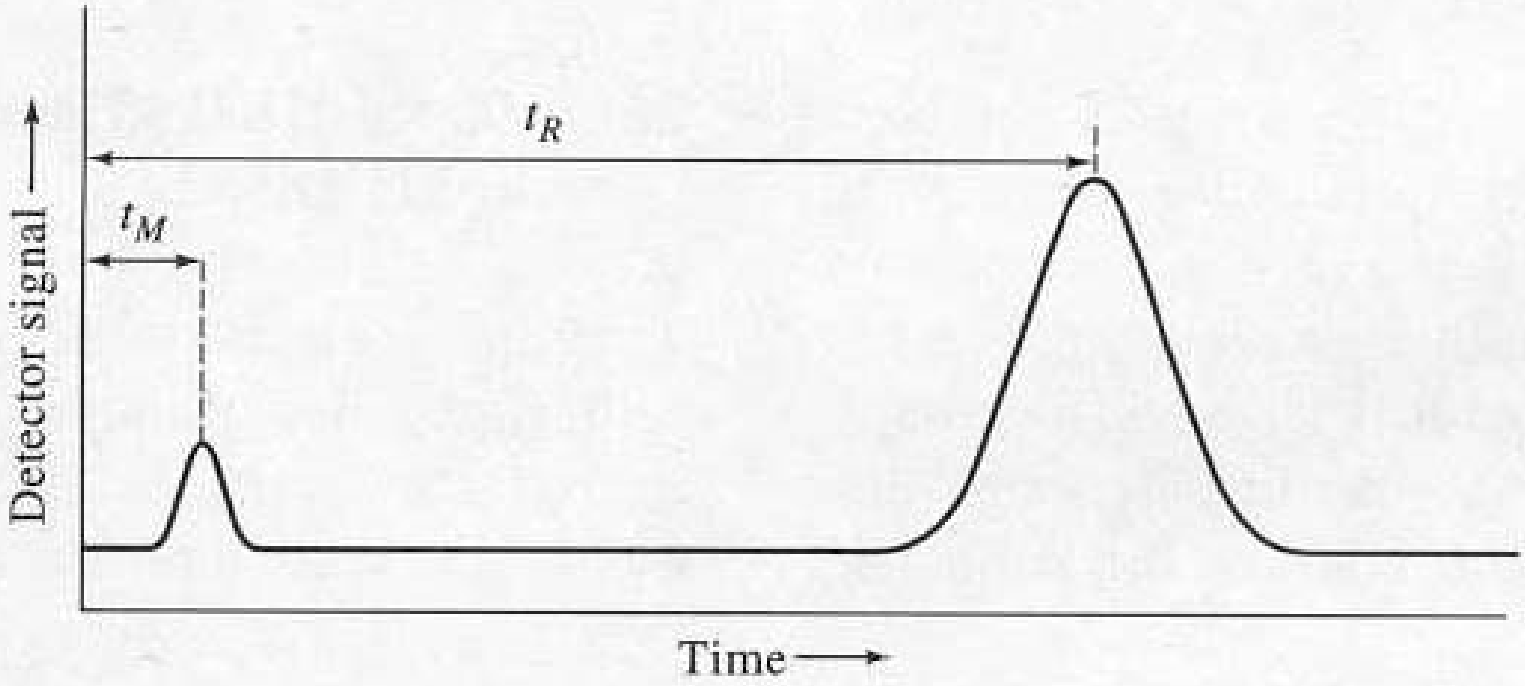
t_M = time for unretained molecule to reach detector or dead time
t_R = retention time, time for retained species to reach detector

Define $\bar{v}$ as average linear rate of <u>solute</u> migration & L as column length, then

$$\bar{v} = \frac{L}{t_R} \qquad \frac{\text{distance}}{\text{time}} = \text{velocity}$$

Similarly if define μ as average linear rate of movement of molecules of <u>mobile phase</u>

$$\mu = \frac{L}{t_M}$$



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t_M = time for unretained molecule to reach detector or dead time
t_R = retention time, time for retained species to reach detector

Relating retention time t_R to K (= C_s/C_M)

$\bar{\nu}$ = μ x fraction of time analyte is in mobile phase

$$\bar{\nu} = \mu \times \frac{\text{moles of analyte in mobile phase}}{\text{number of moles of analyte}}$$

$$\bar{\nu} = \mu \times \frac{C_M V_M}{C_M V_M + C_s V_s} = \mu \times \frac{1}{1 + C_s V_s / C_M V_M}$$

Substituting $K = C_s/C_M$

Gives

$$\boxed{\bar{\nu} = \mu \times \frac{1}{1 + K V_s / V_M}}$$

More useful relationships - <u>capacity factor **k'**</u>

(comes from K) K in concentration, **k'** in moles

$$\mathbf{k'} = \frac{\text{amount of analyte in stationary phase}}{\text{amount of analyte in mobile phase}}$$

So for A → $k_A' = \frac{K_A V_s}{V_M} = \frac{n_s}{n_M}$ n = # of moles

From previous slide

$$\bar{\nu} = \mu \times \frac{1}{1 + K\, V_s/V_M}$$

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

From previous equation →

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

Can plug in $\bar{\nu} = L/t_R$ & $\mu = L/t_M$

Rearrange and get

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

Now have k_A' in terms of something easily measured in chromatogram

Compares how long it takes a species to move through system compared to unretained species

Relative because ratio, Numerator = Net Retention

One step further → Selectivity factor (α) describes differential migration

For two components

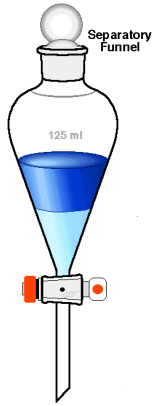
$$\alpha = \frac{K_B}{K_A} = \frac{k_B'}{k_A'}$$

And from chromatogram

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

Allows calculation of the resolving power of a chromatographic system (i.e. column with A & B)

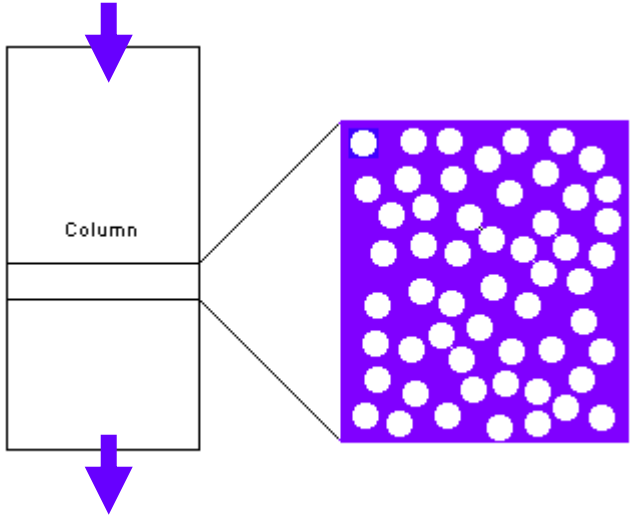
Chromatographic Plate Theory vs. Rate Theory

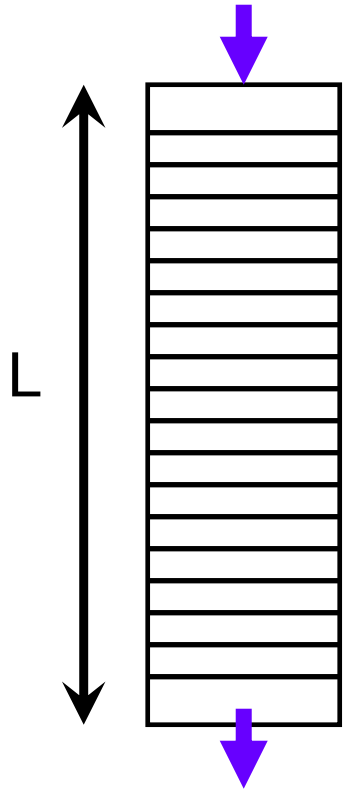


- Plate theory based in liquid-liquid extraction (successive extractions)
- $K = C_{org}/C_{water}$
- Chromatographic column can be thought of in the same way (only continuous process)
- $K = C_s/C_M$

Stationary phase bead

Mobile phase (liquid)



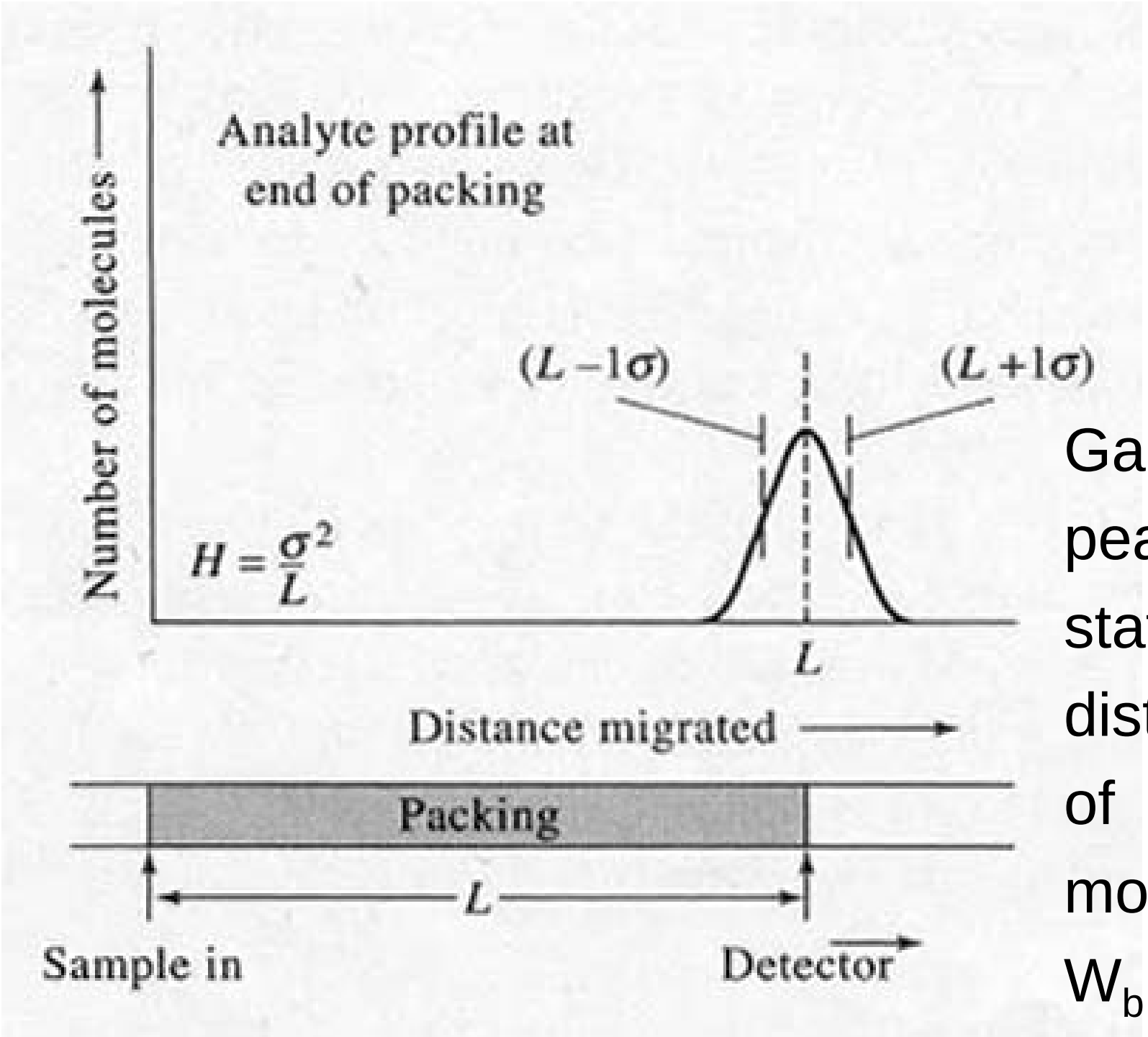


- Divide chromatographic column up into steps or segments called theoretical plates
- The theoretical concept is that these theoretical plates are equilibrium units for $K = C_s/C_M$
- The more theoretical plates a column has, the more efficient it is
- If column length = L & N = number of plates, then H = height equivalent to theoretical plate

$L = NH$

or

$N = L/H$

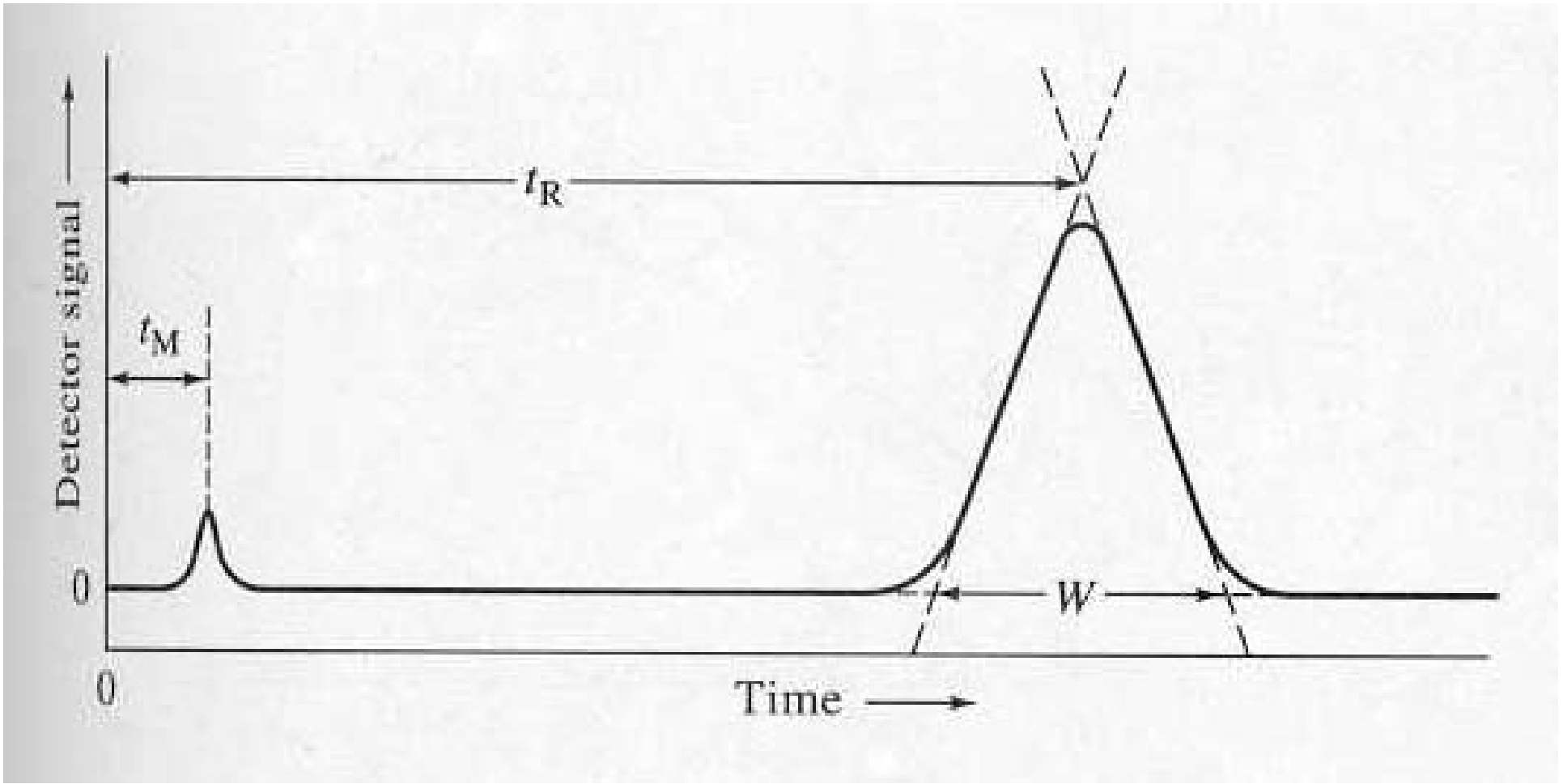


Gausian peaks – statistical distribution of molecules

$W_b = 4\sigma$

Gausian distribution (bell curve)

$W = 4\sigma$



Can derive N = number of plates

$N = 16\ (t_R/W_b)^2$ W_b = base width

$N = 16\ (t_R/4\sigma)^2 = (t_R/\sigma)^2$

$N = 5.54\ (t_R/W_{½})^2$ $W_{½}$ = width at half height

Column manufacturers use N

to characterize column – N varies widely

Shortcomings of Plate Theory

- Assumes K is independent of concentration
- Assumes equilibration is rapid relative to velocity of mobile phase – not true, in reality solute may pass a plate without entering
- Assumes no longitudinal diffusion (= non ideal effect that causes band broadening)
- Does not address several factors caused by mobile phase velocity (fast or slow) Rate Theory
- Assumes discrete units or plates for equilibrium rather than a semi continuous process through the column

Rate Theory of Chromatography

$$H = H_L + H_S + H_M + H_{SM}$$

H = height equivalent to theoretical plate (as in Plate Theory)

H_L = contribution due to longitudinal diffusion

H_S = stationary phase mass transfer contribution

H_M = diffusion associated with mobile phase effects

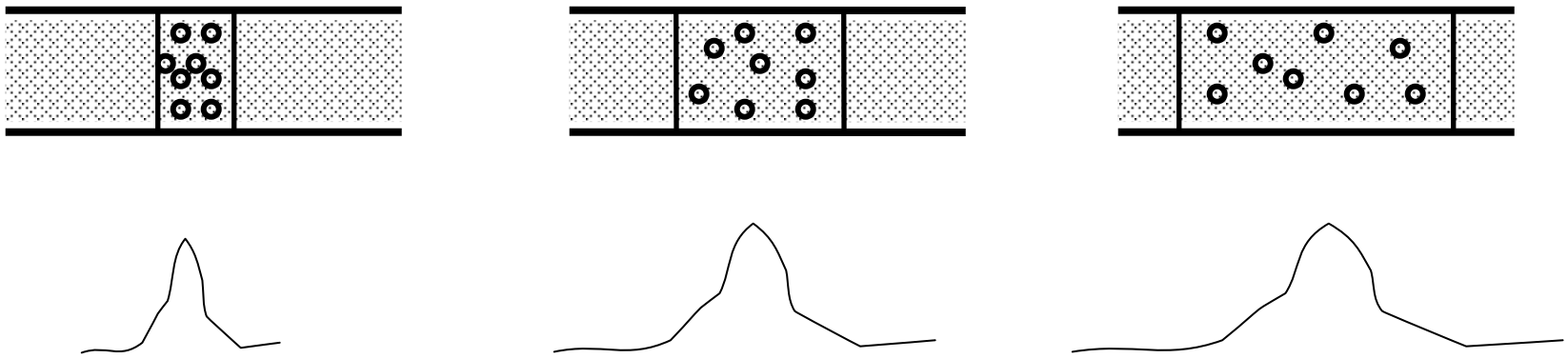
H_{SM} = diffusion into or mass transfer across a stagnant layer of mobile phase (neglect)

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

van Deemter Equation A, B & C are coefficients, μ = velocity

1) Longitudinal Diffusion $H_L = (B/\mu)$

$t = 0$ $0 < t < t_R$ t_R

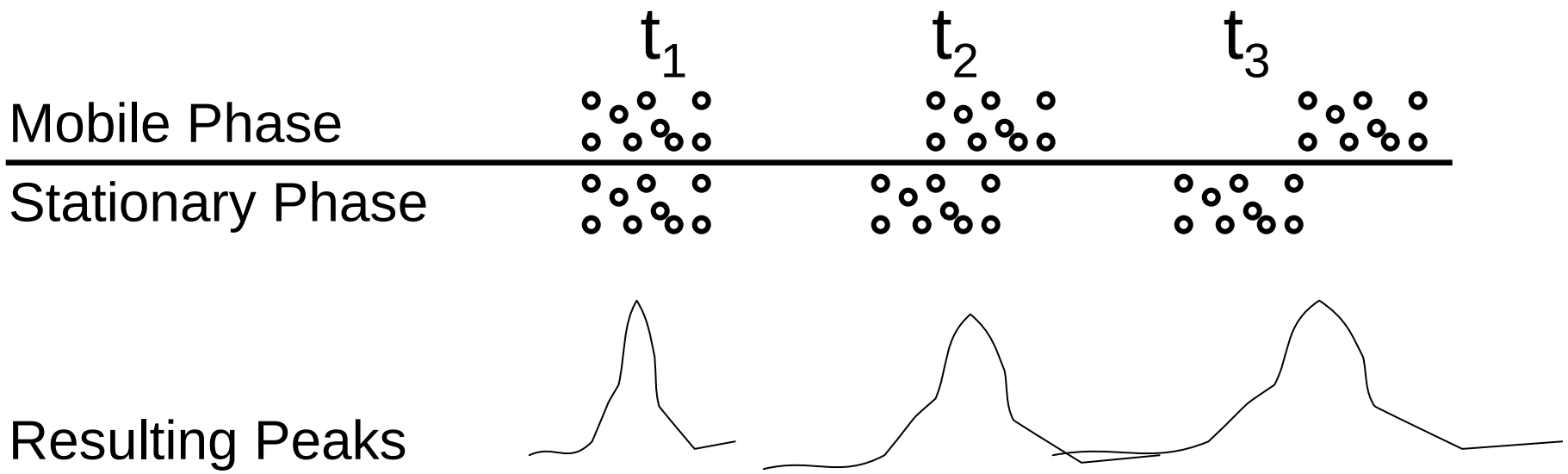


$\sigma_L^2 = 0$ $\sigma_L^2 = 2 D_M t_M$

Variance due to longitudinal diffusion = 0 at start

Variance increases with time & diffusion coefficient D

2) Mass transfer in & out of stationary phase

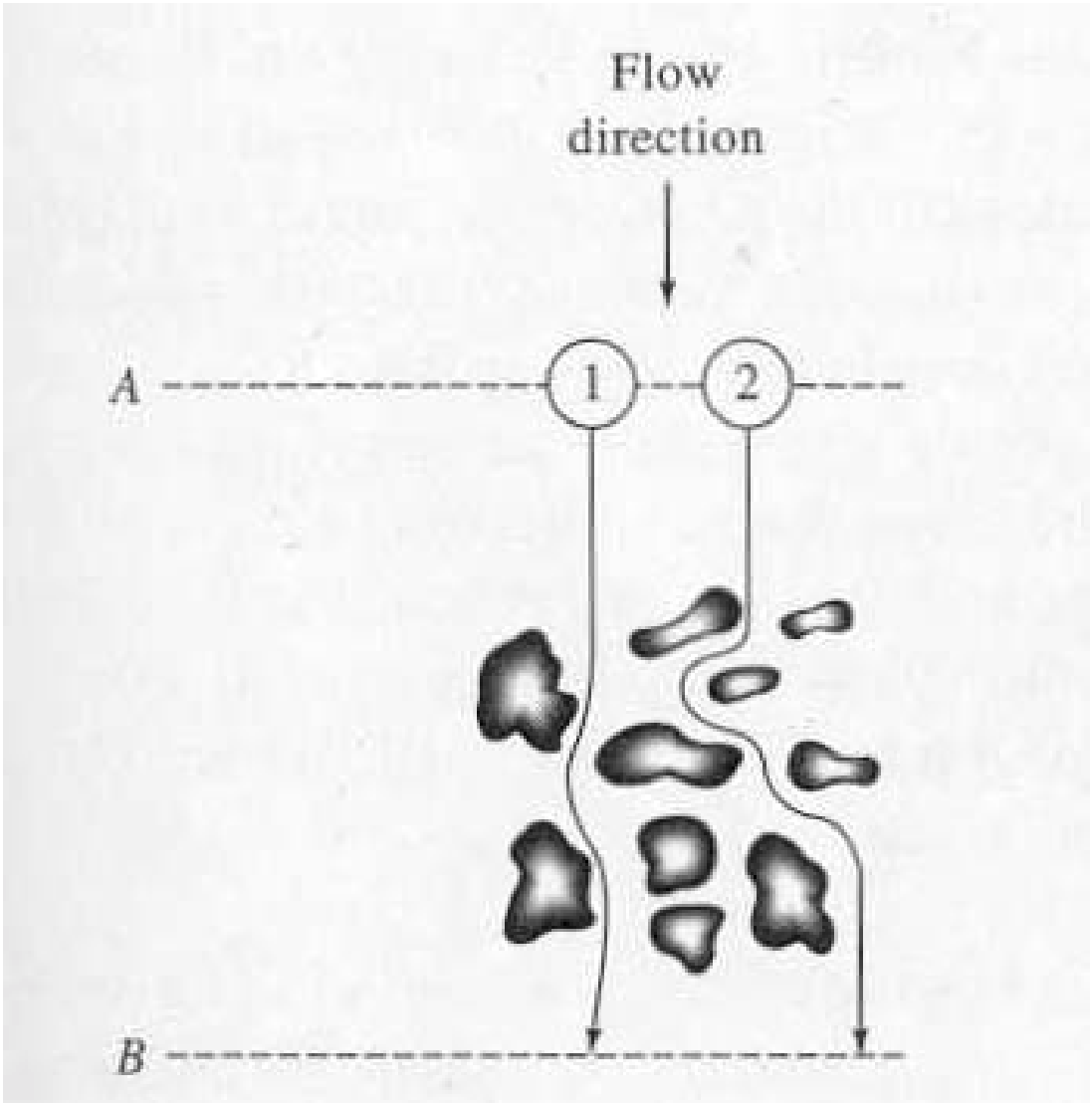


Broadening of peaks is a function of mobile phase velocity (moving molecules faster than those in stationary phase)

Not the same as longitudinal diffusion $H_S = C\mu$

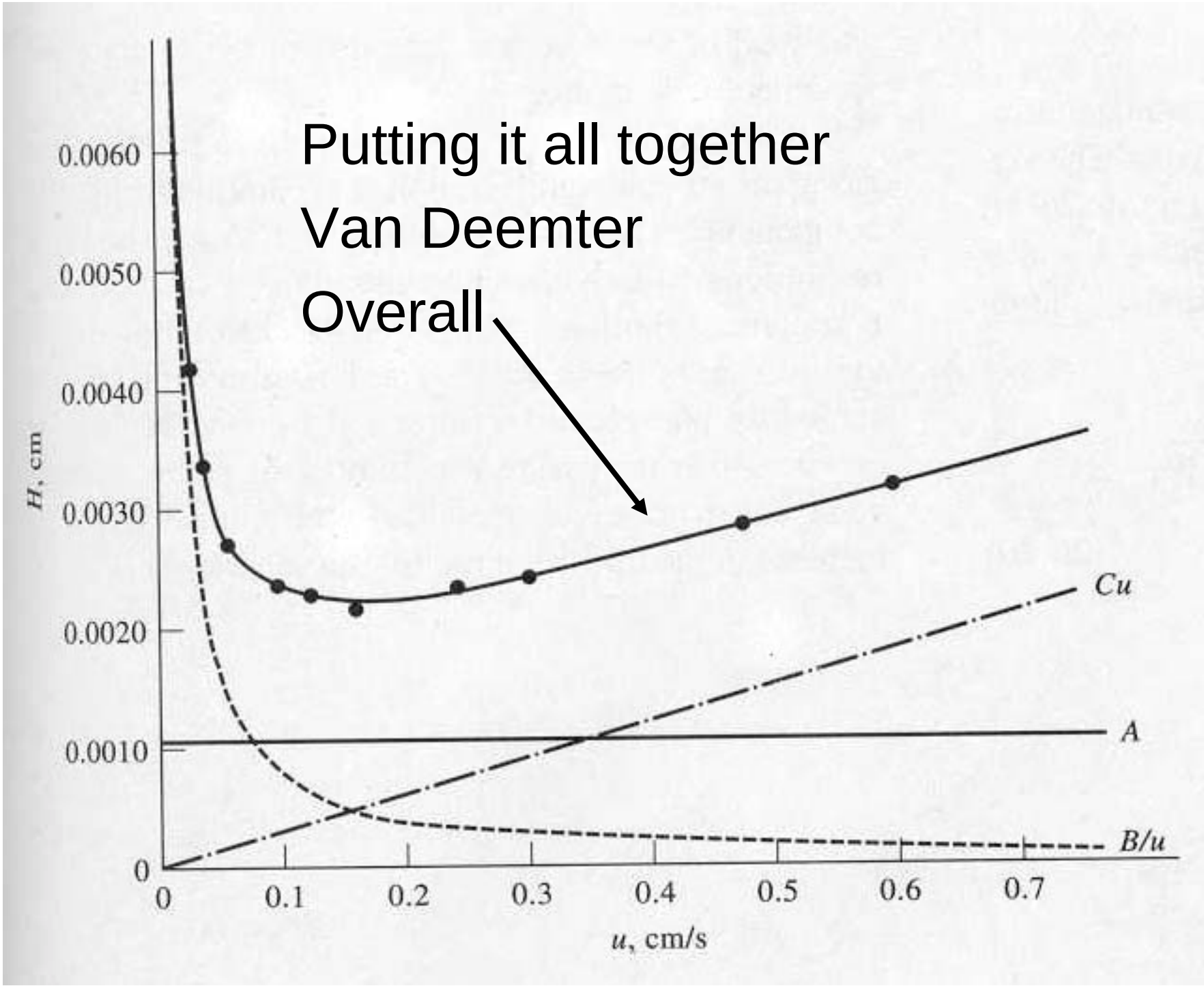
In Plate Theory condition at t_1 assumed to hold throughout

3) Uneven Flow or Eddy Diffusion



Path 1 is shorter than path 2

$H_M = A$


Putting it all together
Van Deemter
Overall
0.0060
0.0050
0.0040
0.0030
0.0020
0.0010
0
H, cm
Cu
A
B/u
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
u, cm/s

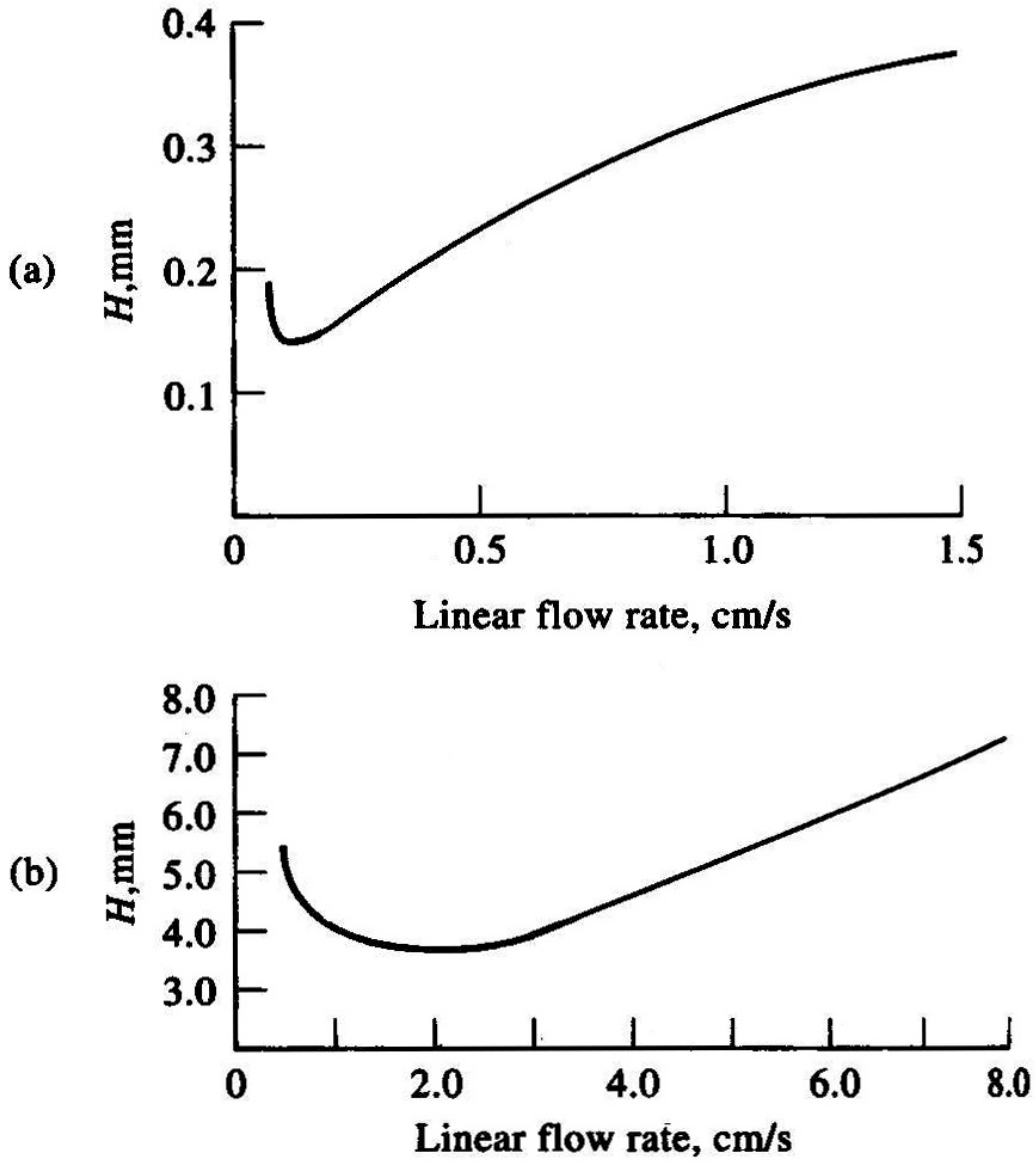


Figure 26-7 Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.

Finding optimum

Homework due 4/7/05

Chapter 26

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