Chapter26 An Introduction to Chromatographic Separations

26A General Description of Chromatography

26A-1 Classification of Chromatographic Methods

Two ways:

Column Chromatography

Planar Chromatography

a) Liquid chromatography

--- Liquid - liquid (LLC)

--- Liquid - solid (LSC)

--- Liquid - bonded phase (LSB)

--- Ion - exchange (IEC)

--- Gel - permeation (GPC)

b) Gas chromatography

--- Gas - liquid (GLC)

--- Gas - solid (GSC)

--- Gas - bonded phase

c) Supercritical-fluid chromatography

d) Electrochromatography

TABLE 26-1 Classification of Column Chromatographic Methods

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
1. Gas chromatography (GC)	a. Gas-liquid chromatography (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	b. Gas-solid	Solid	Adsorption
2. Liquid chromatography (LC)	a. Liquid-liquid, or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	b. Liquid-solid, or adsorption	Solid	Adsorption
	c. Ion exchange	Ion-exchange resin	Ion exchange
	d. Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	e. Affinity	Group specific liquid bonded to a solid surface	Partition between surface liquid and mobile liquid
3. Supercritical fluid chromatography (SFC; mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

26A-2 Elution in Column Chromatography

- **Analyte Dilution**

Fig 26-1 illustrates an important general characteristic of the chromatographic process-namely, dilution of analytes always accompanies chromatographic separation.

- **Chromatograms**

If a detector that responds to solute concentration is placed at the end of the column and its signal is plotted as function of time as shown in the lower part of Fig 26-1.

- **Improving Column Performance**

Fig 26-2 shows concentration profiles for the bands containing solutes A and B in Fig 26-1 at time t_1 and a later time t_2.

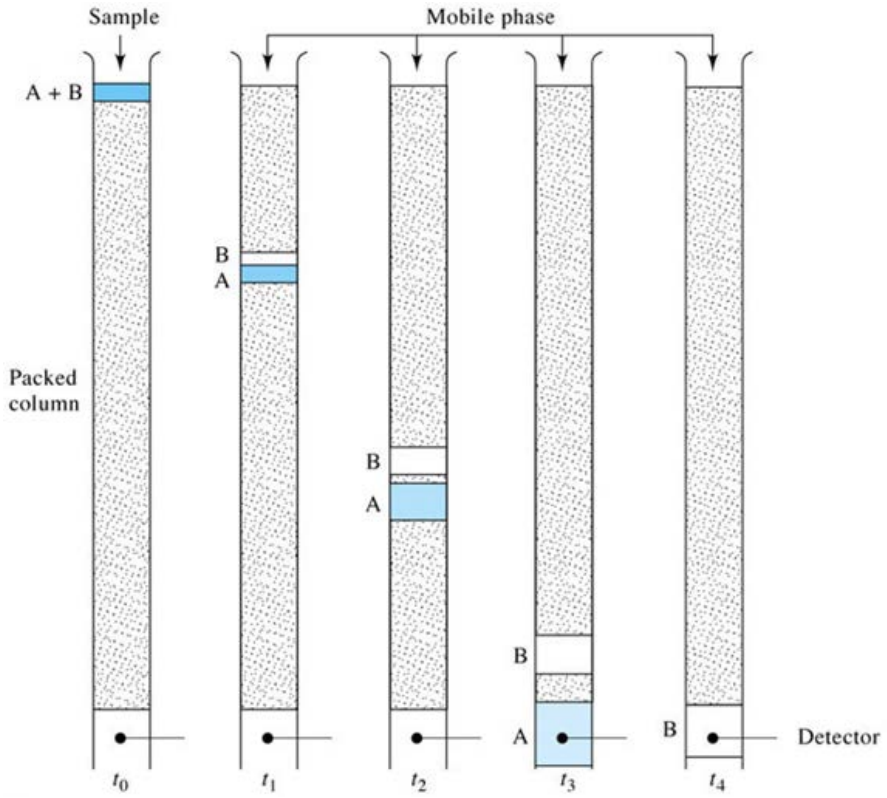


Fig. 26-1 Elution Chromatography

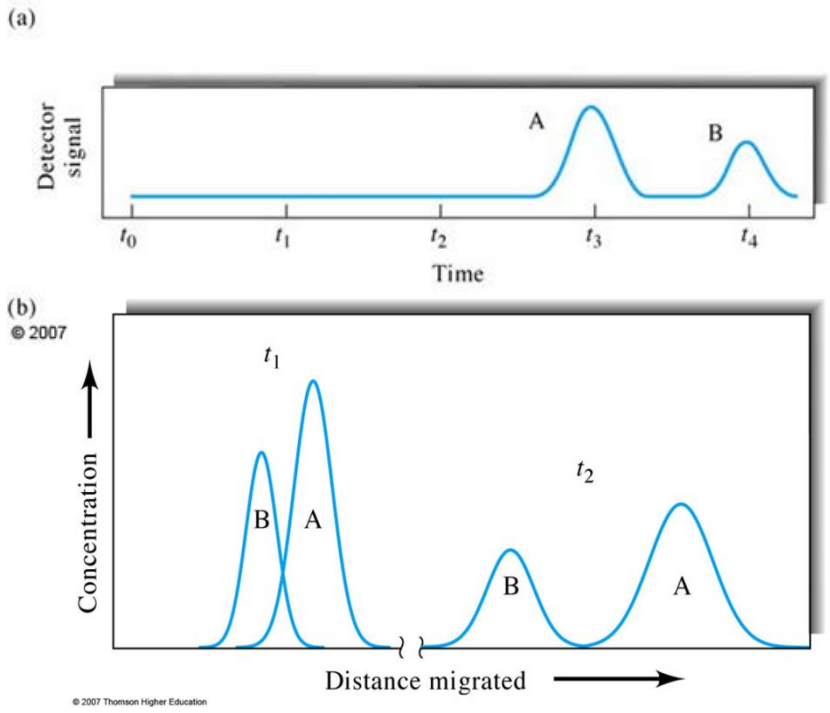


Fig. 26-2 Concentration profiles of analyte band A and B

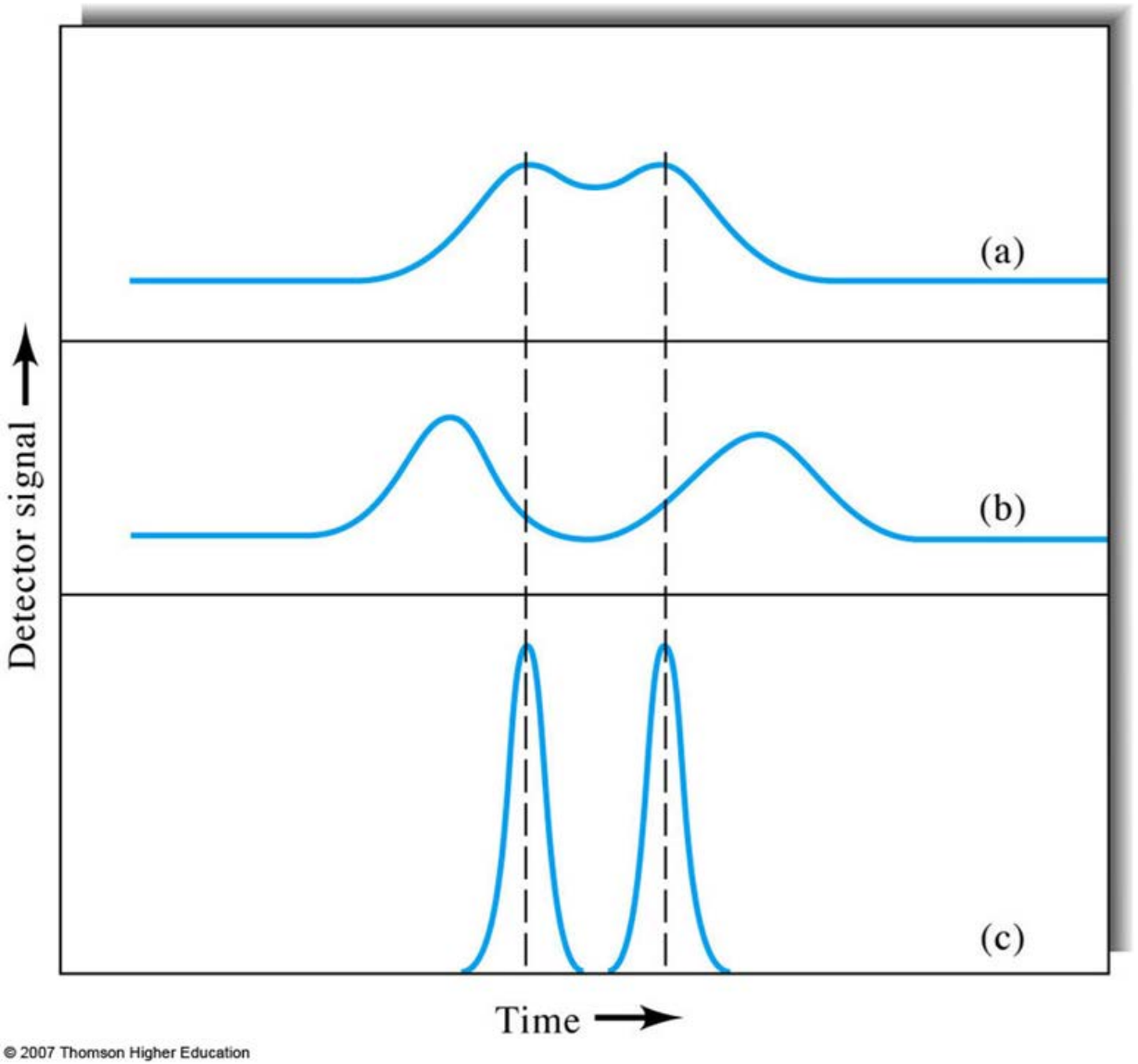


Fig. 26-3. Two component chromatogram illustrating two methods for Improving separation

26B Migration Rates of Solutes

26B-1 Distribution Constants

Often, the distribution equilibria involved in chromatography are described by relatively straightforward equations that involve the transfer of an analyte between the mobile and stationary phases.

$$\mathbf{A}_{\text{mobile}} \leftrightarrow \mathbf{A}_{\text{stationary}}$$

$$K_c = \frac{(a_A)_S}{(a_A)_M}$$

$$K_c = \frac{c_S}{c_M} = \frac{n_S / V_S}{n_M / V_M}$$

26B-2 Retention time

The average linear rate of solute migration:

$$t_R = t_S + t_M$$

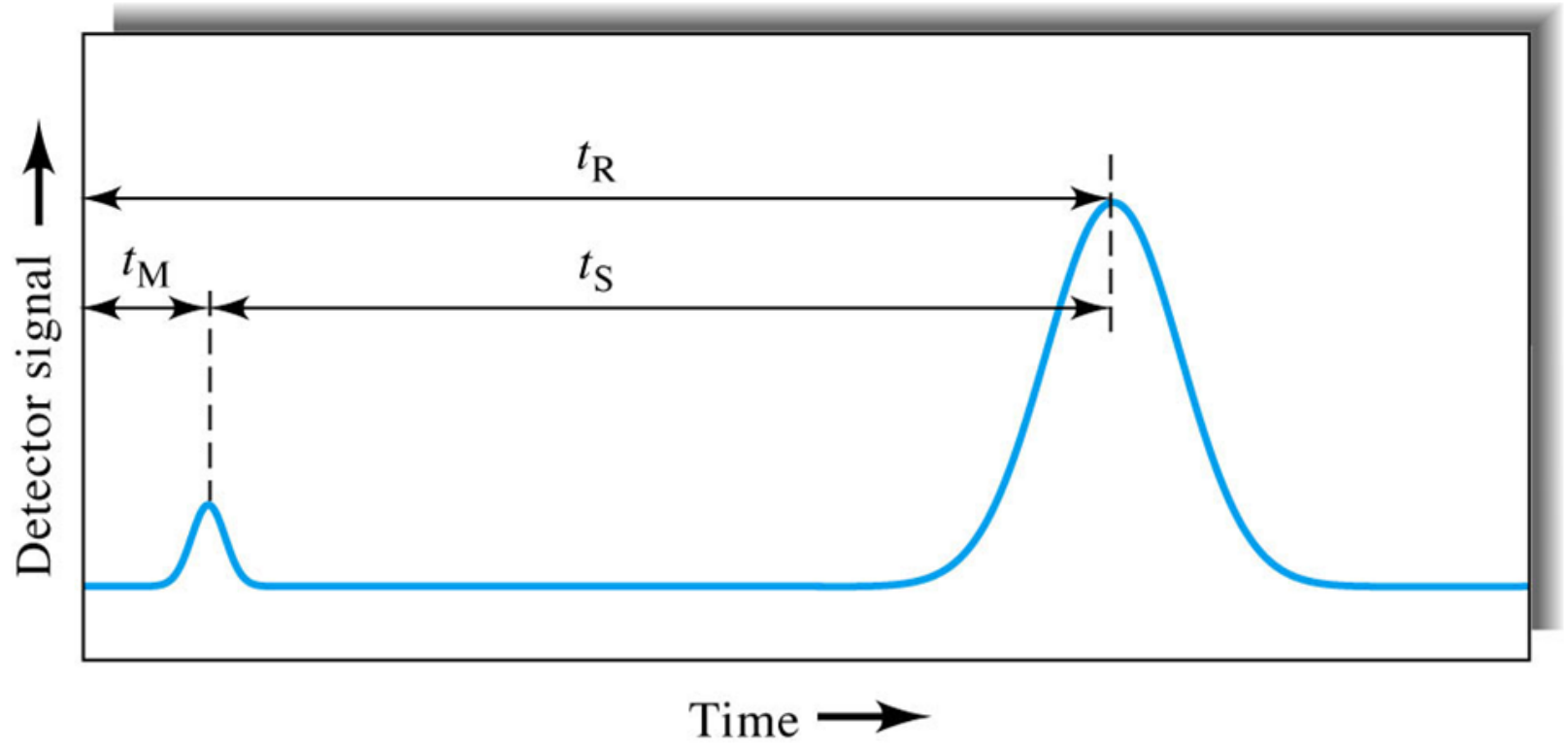
$$\bar{v} = \frac{L}{t_R}$$

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

26B-3 The Relationship between Volumetric Flow Rate and Linear Flow Velocity

The average linear rate of molecules of the mobile phase:

Fig. 26-4. A typical chromatogram for a two-component mixture.



$$F = u_o A = u_o \times \pi r^2$$

$$F = \pi r^2 u_o \varepsilon$$

26B-4 The Relationship between Retention Time and Distribution Constant

$$\bar{v} = \frac{L}{t_R}$$

= u x fraction of time solute spends in mobile phase

= u x moles of solute in mobile phase / total moles of solute

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

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

26B-5 The Rate of Solute Migration: The Retention Factor

The retention factor k is an important experimental quantity widely used to compare the migration rates of solutes in columns.

$$k'_{\mathrm{A}} = \frac{K_{\mathrm{A}} V_S}{V_M}$$

$$\bar{v} = u \times \frac{1}{1 + k'_{\mathrm{A}}}$$

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k'_{\mathrm{A}}}$$

$$k'_{\mathrm{A}} = \frac{t_R - t_M}{t_M}$$

26B-6 Relative Migration Rates: The Selectivity Factor.

The *selectivity factor* α of a column for the two solutes A and B is defined as

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

from

$$k'_A = \frac{K_A V_S}{V_M} \qquad k'_A = \frac{t_R - t_M}{t_M}$$

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

26C Band Broadening and Column Efficiency

26C-1 The Rate Theory of Chromatography

The *rate theory* of chromatography describes the shapes and breadths of elution bands in quantitative terms based on a random-walk mechanism for the migration of molecules through a column.

26C-2 A Quantitative Description of Column Efficiency

Two related terms are widely used as quantitative measures of chromatographic column efficiency:

1) Plate height, 2) Plate count plate,

$N = L/H$ (26-16)

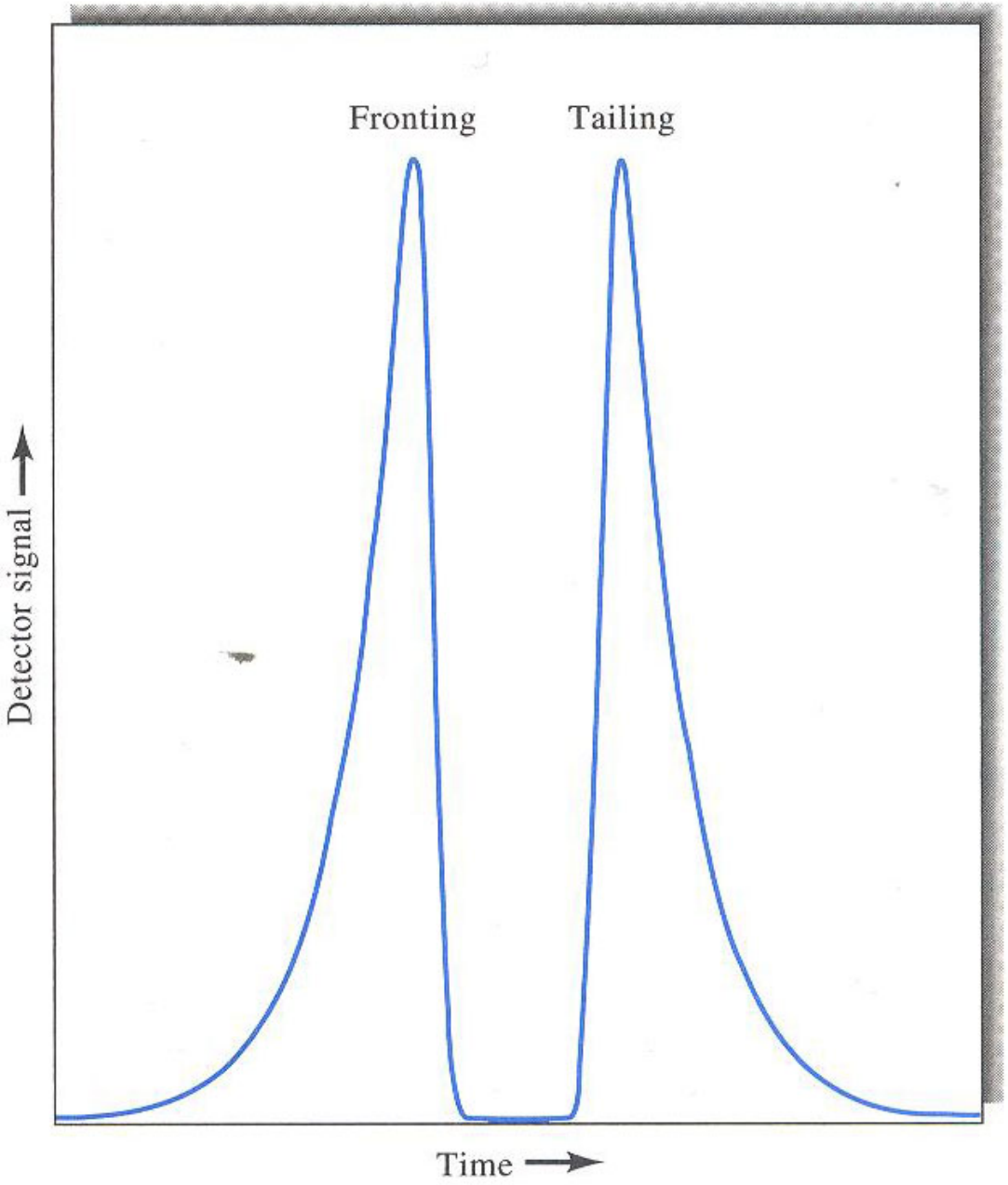


FIGURE 26-5 Illustration of fronting and tailing in chromatographic peaks.

- **The Definition of Plate Height**

As shown in Section a1B-1 of Appendix 1, the breadth of a Gaussian curve is described by its standard deviation σ or its variance σ^2.

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

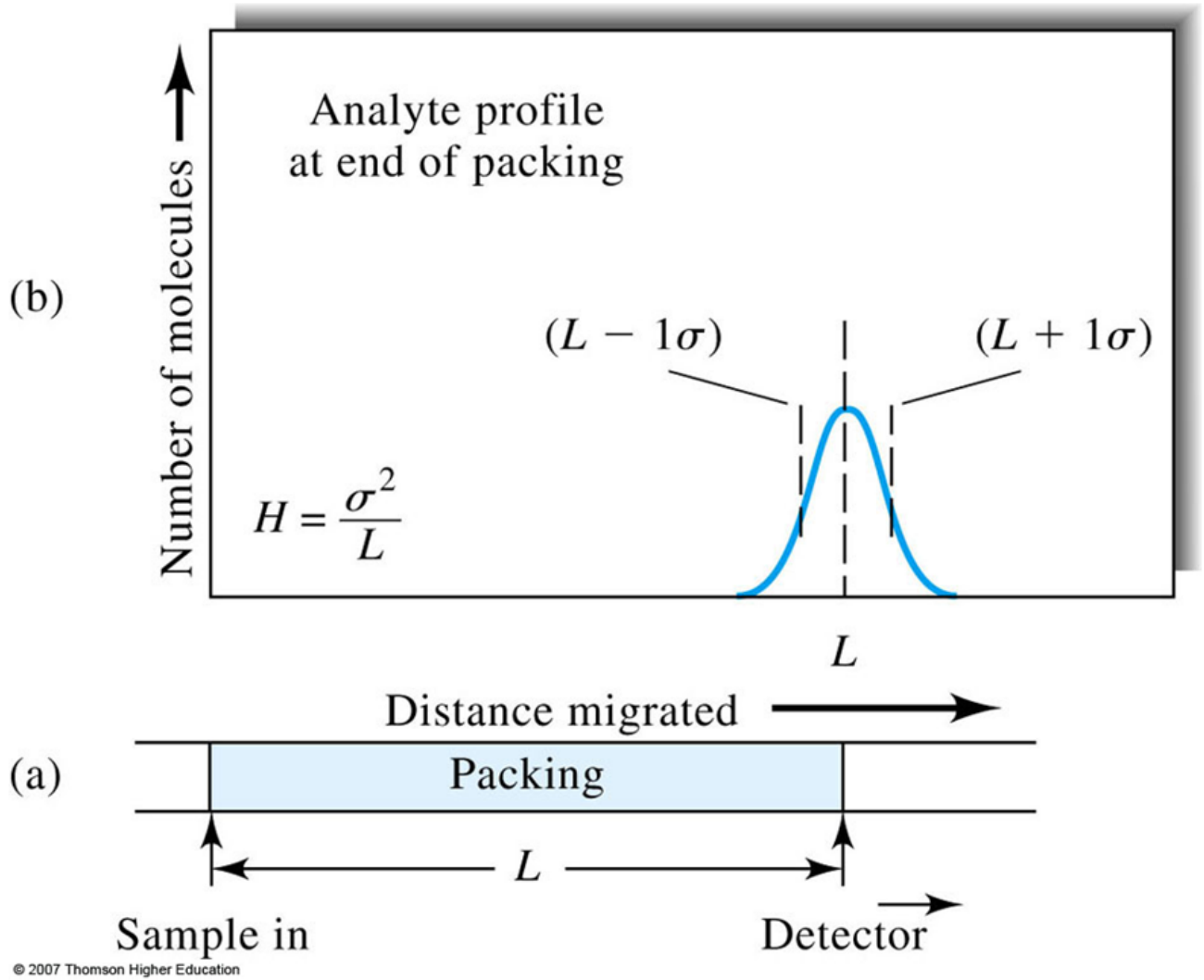


Fig. 26-6. Definition of plate height $H=\sigma^2/L$.

- **The Experimental Evaluation of H and N**

Fig 26-7 is a typical chromatogram with time as the abscissa. Fig 26-7 illustrates a method for approximating τ and σ from an experimental chromatogram.

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

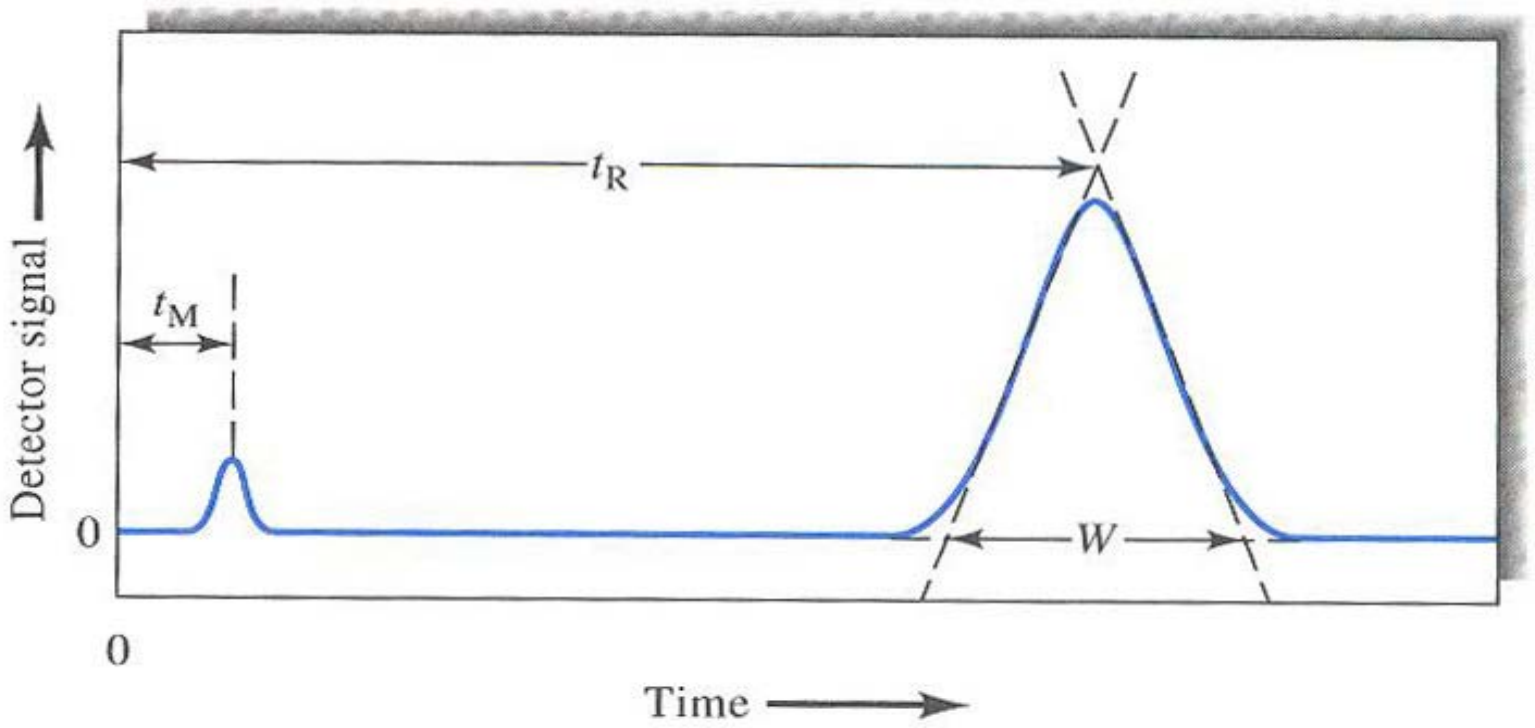
$$\sigma = \frac{LW}{4t_R}$$

$$H = \frac{LW^2}{16t_R^2}$$

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

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

FIGURE 26-7 Determination of the number of plates $N = 16\left(\frac{t_R}{W}\right)^2$.



26C-3 Kinetic Variables Affecting Column Efficiency

- **The Effect of Mobile-Phase Flow Rate**

The extent of band broadening depends on the length of time the mobile phase is in contact with the stationary phase, which in turn depends on the flow rate of the mobile phase.

- **Theory of Band Broadening**

The efficiency of chromatographic columns can be approximated by the expression

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

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

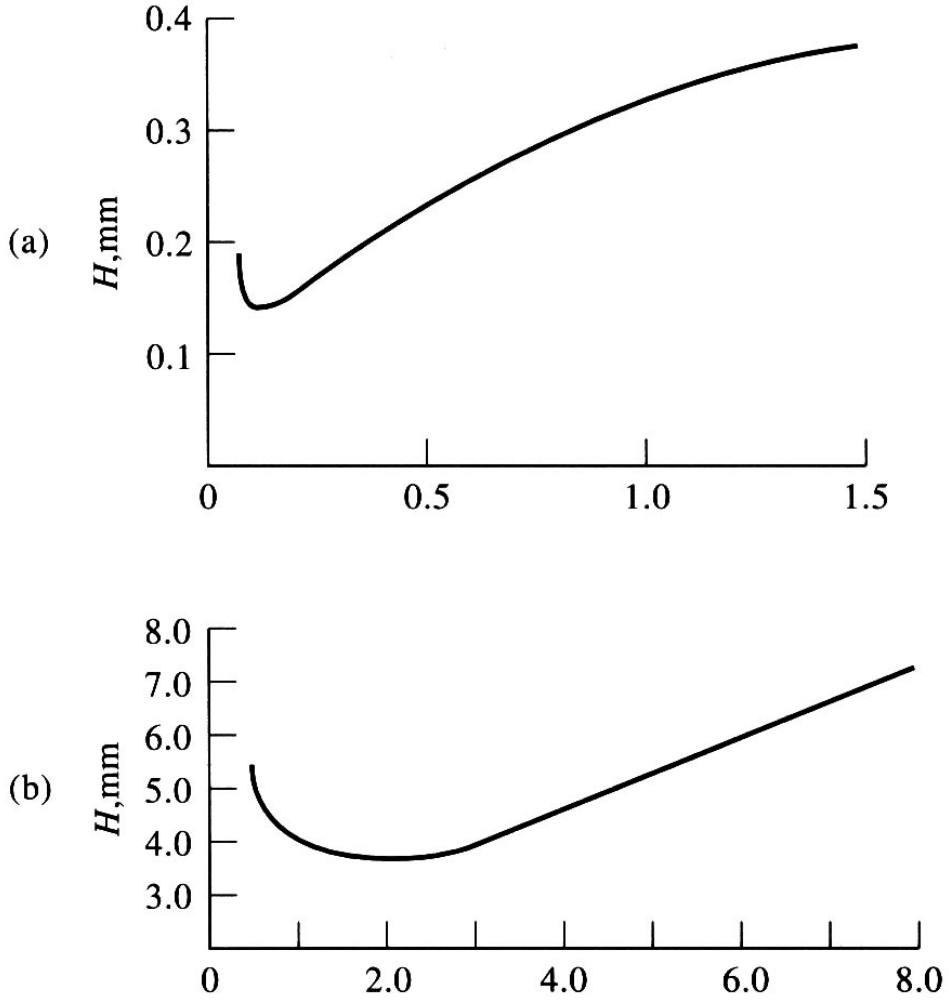


Fig. 26-8 Effect of mobile-phase flow rate on plate height for LC and GC

- **The Multipath Term A.**

  As shown in Fig 26-9, the length of these pathways many differ significantly.

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

· The Longitudinal Diffusion Term B/u.

Diffusion is a process in which species migrate from a more concentrated part of a medium to a more dilute region.

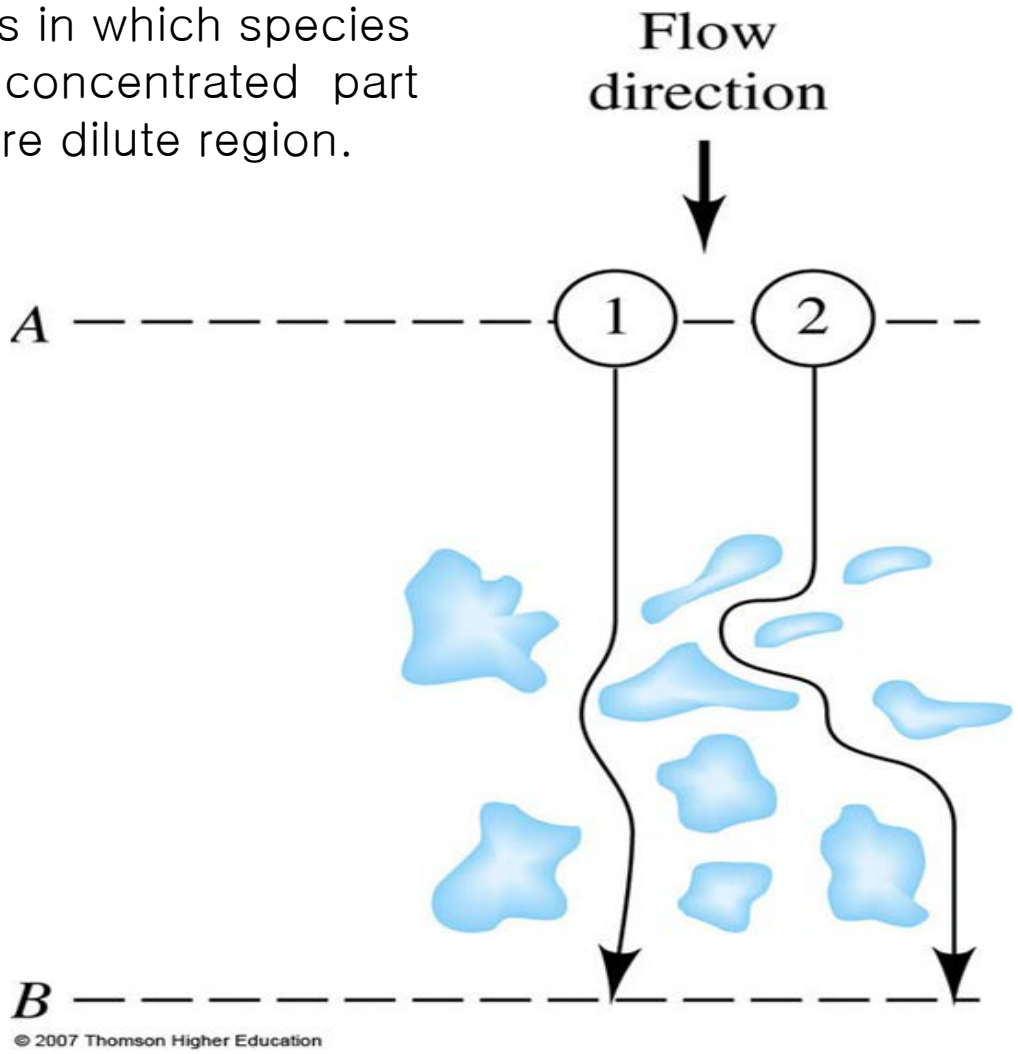


Fig 26-9. Typical pathways of two molecules during elution.

- **The Stationary-Phase Mass-Transfer Term $C_s u$.**

When the stationary phase is an immobilized liquid, the mass-transfer coefficient is directly proportional to the square of the thickness of the film on the support particles d_f^2 and inversely proportional to the diffusion coefficient D_s of the solute in the film.

- **The Mobile Phase Mass-Transfer Term $C_M u$.**

The mass-transfer processes that occur in the mobile phase are sufficiently complex that we do not yet have a complete quantitative description.

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

A Van Deemter plot (Fig. 26-10)

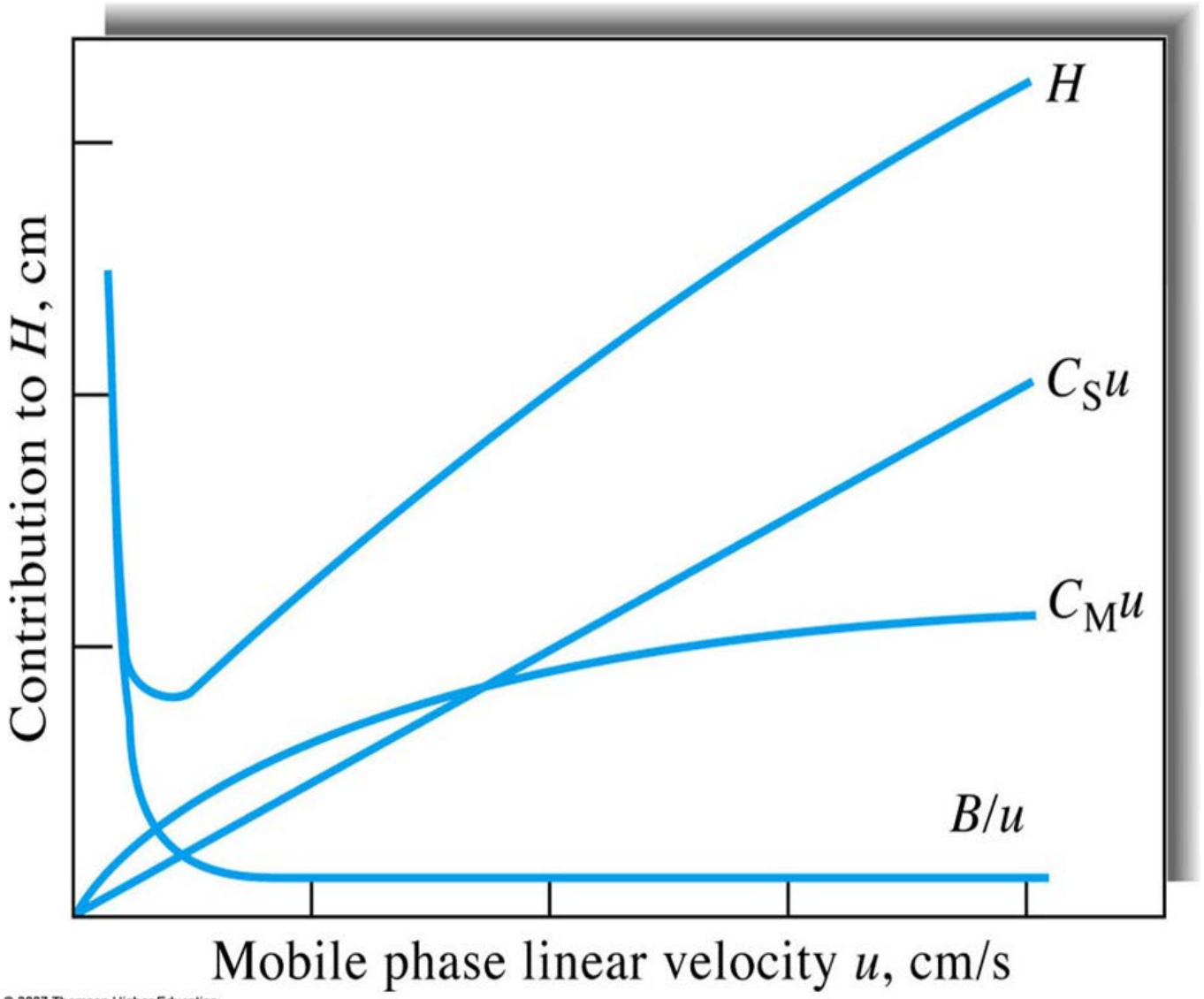


Fig 26-10. Contribution of various mass-transfer terms to plate height.

- **Effect of Mobile-Phase Velocity on Terms in Equation 26-23.**

Fig 26-10 shows the variation of the three terms in Equation 26-23 as a function of mobile-phase velocity.

- **Summary of Methods for Reducing Band Broadening.**

For packed columns, the most important variable that affects column efficiency is the diameter of the particles making up the packing.

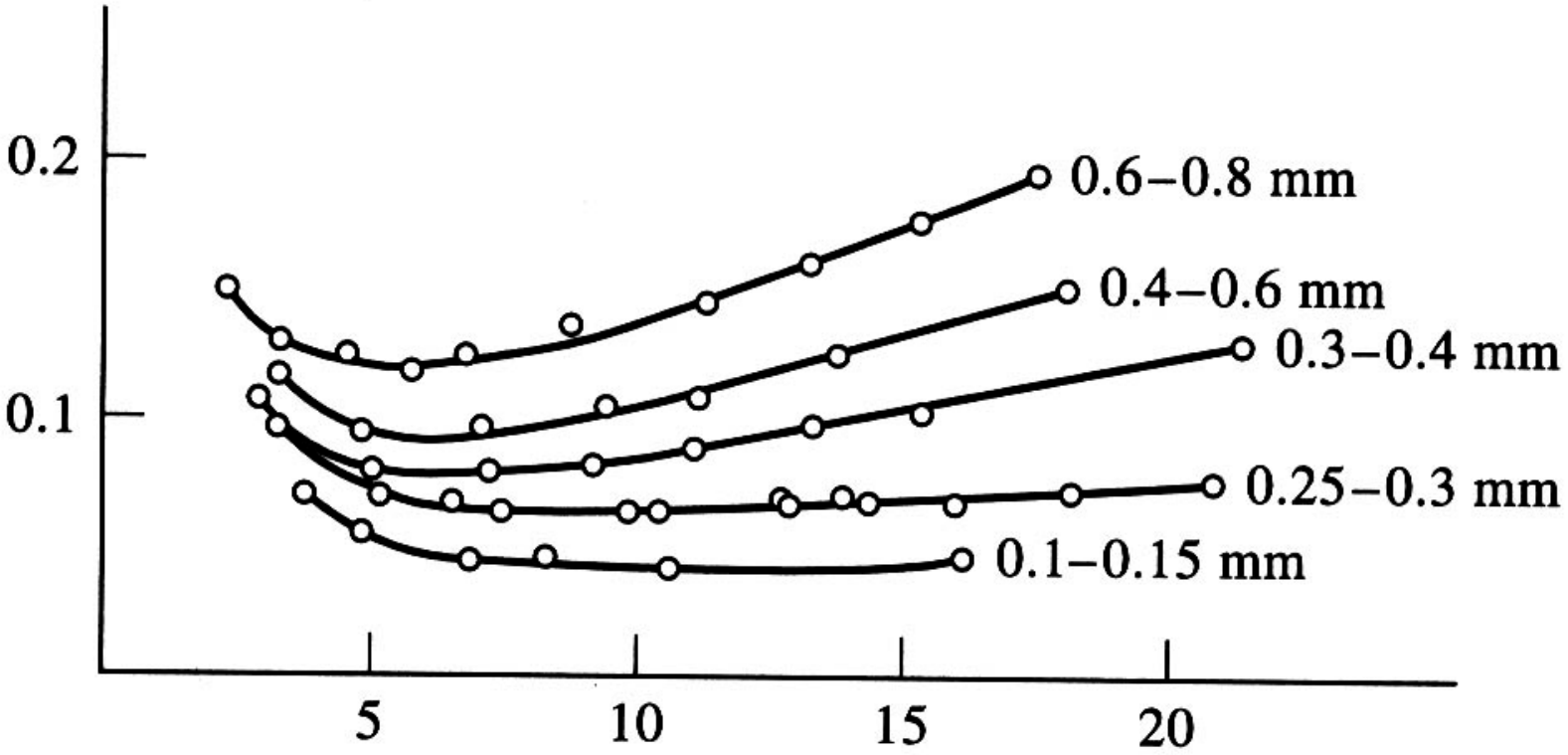


Fig 26-11. Effect of particle size on plate height for a packed GC column.

26D Optimization of Column Performance

26D-1 Column Resolution (R_s)

The resolution R_s of a column tells us how far apart two bands are relative to their widths.

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

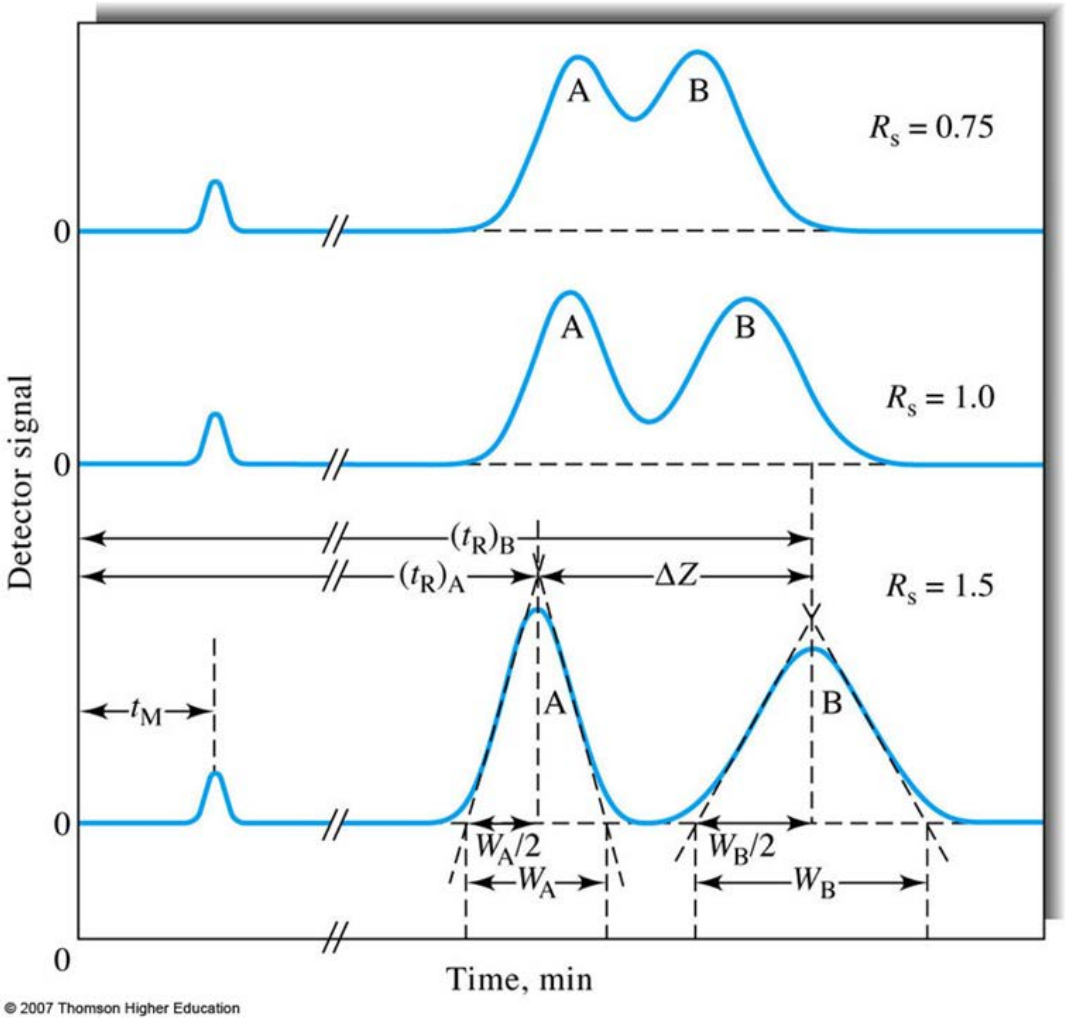


Fig 26-12.
Separation at three resolution values: R = $2\Delta Z/(W_A + W_B)$.

26D-2 The Effect of Retention and Selectivity Factors on Resolution

It is useful to develop a mathematical relationship between the resolution of a column and the retention factors k_A and k_B for two solute, the selectivity factor α , and the number of plates *N* making up the column.

$$W_A = W_B \approx W$$

$$R_s = \frac{(t_R)_B - (t_R)_A}{W}$$

$$R_s = \frac{k'_B - k'_A}{1 + k'_B} \times \frac{\sqrt{N}}{4}$$

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

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

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

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

26D-3 The Effect of Resolution on Retention Time

The completion time of a separation is determined by the velocity v of the slower-moving solute, as given in Equation 26-4.

$$\bar{v}_B = \frac{L}{(t_R)_B}$$

$$(t_R)_B = \frac{NH(1 + k'_B)}{u}$$

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

EXAMPLE 26-1

Substances A and B have retention times of 16.40 and 17.63 min, respectively, on a 30.0-cm column. An unretained species passes through the column in 1.30 min. The peak widths (at base) for A and B are 1.11 and 1.21 min, respectively. Calculate (a) the column resolution, (b) the average number of plates in the column, (c) the plate height, (d) the length of column required to achieve a resolution of 1.5, (e) the time required to elute substance B on the column that gives an R_s value of 1.5, and (f) the plate height required for a resolution of 1.5 on the original 30-cm column and in the original time.

Example 26-1

a) $$R_S = \frac{2(17.63\ \text{min} - 16.40\ \text{min})}{(1.11\ \text{min} + 1.21\ \text{min})}$$

$$= 1.06$$

b) $$N = 16\left(\frac{16.40\ \text{min}}{1.11\ \text{min}}\right)^2 = 3.49 \times 10^3$$

$$N = 16\left(\frac{17.63\ \text{min}}{1.21\ \text{min}}\right)^2 = 3.40 \times 10^3$$

$$N_{\text{av}} = \left(\frac{3.49 \times 10^3 + 3.40 \times 10^3}{2}\right)$$

$$= 3.44 \times 10^3 = 3.4 \times 10^3$$

c) $$H = L/N = \frac{30.0\ \text{cm}}{3.44 \times 10^3}$$

$$= 8.7 \times 10^{-3}\ \text{cm}$$

d) $$\frac{(R_s)_1}{(R_s)_2} = \frac{\sqrt{N_1}}{\sqrt{N_2}} \qquad \frac{1.06}{1.5} = \frac{\sqrt{3.44 \times 10^3}}{\sqrt{N_2}}$$

$$N_2 = 3.44 \times 10^3 \left(\frac{1.5}{1.06}\right)^2 = 6.9 \times 10^3$$

$$But,\ L = NH = 6.9 \times 10^3 \times 8.7 \times 10^{-3} = 60cm$$

e) $$\frac{(t_R)_1}{(t_R)_2} = \frac{(R_s)_1^2}{(R_s)_2^2} = \frac{17.63}{(t_R)_2} = \frac{(1.06)^2}{(1.5)^2} \qquad (t_R)_2 = 35\text{min}$$

f) $$\frac{(t_R)_B}{(t_R)_B} = \frac{(R_s)_1^2}{(R_s)_2^2} \times \frac{H_1}{H_2}$$

$$H_2 = H_1 \frac{(R_s)_1^2}{(R_s)_2^2} = 8.7 \times 10^{-3}\ \text{cm}\ \frac{(1.06)^2}{(1.5)^2}$$

$$= 4.3 \times 10^{-3}\ \text{cm}$$

26D-4 Variables That Affect Column Performance

- **Variation in N**

An obvious way to improve resolution is to increase the number of plates in the column(Eq. 26-25).

- **Variation in H**

In Ex. 26-1f , we showed that resolution can be significantly improved at no cost in time if the plate height can be reduced.

- **Variation in Retention Factor**

Often, a separation can be improved significantly by manipulating the retention factor k_B .

$$R_s = \frac{Qk'_B}{1 + k'_B}$$

$$(t_R)_B = Q' \frac{(1 + k'_B)^3}{(k'_B)^2}$$

- **Variation in Selectivity Factor**

Optimizing *k* and increasing *N* are not sufficient to give a satisfactory separation of two solutes in a reasonable time when α approaches unity.

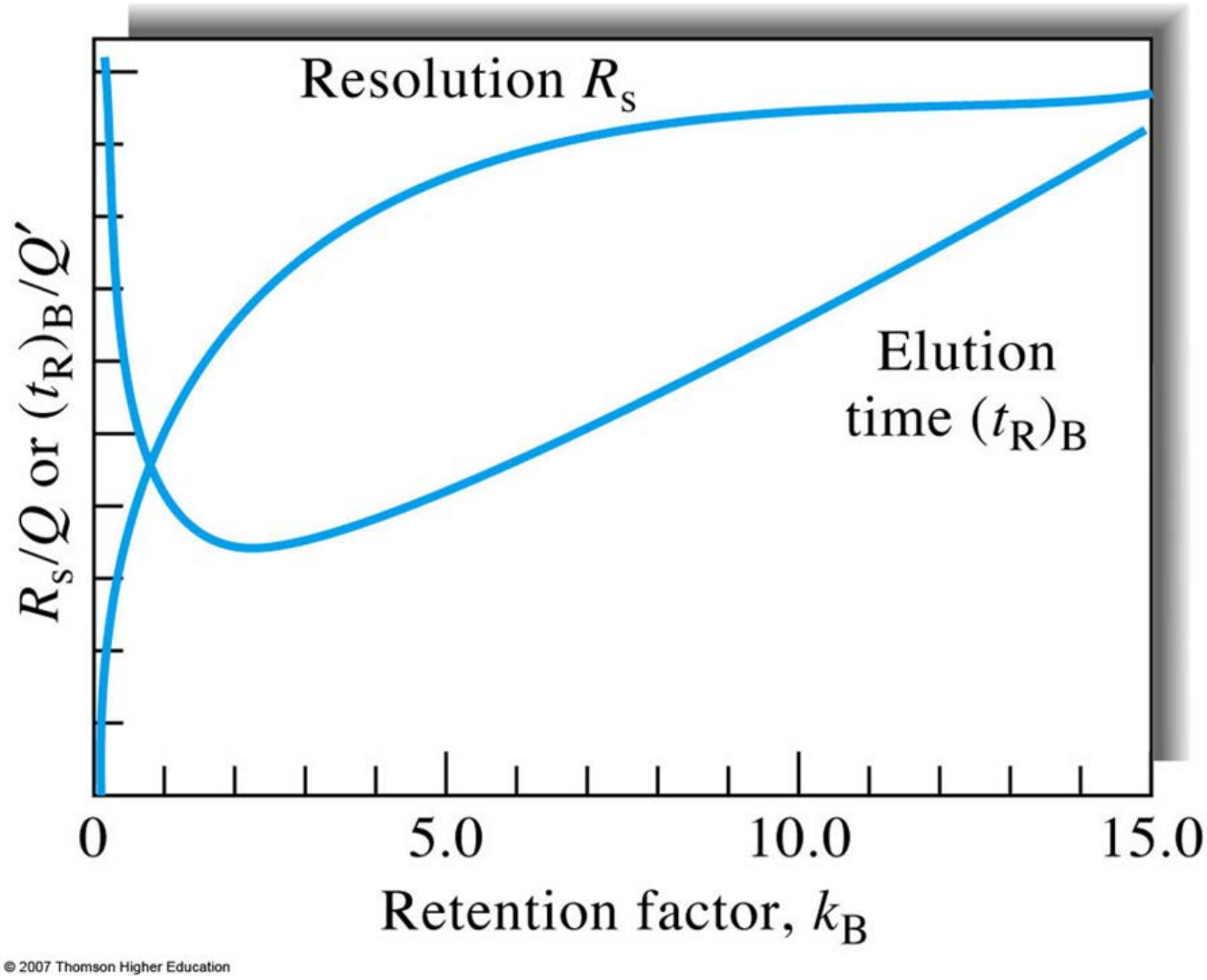


Fig26-13. Effect of retention factor k_B on resolution and elution time ($t_R)_B$

26D-5 The General Elution Problem

Fig 26-15 shows hypothetical chromatograms for a six-component mixture made up of three pairs of components with widely different distribution constants and thus widely different retention factors.

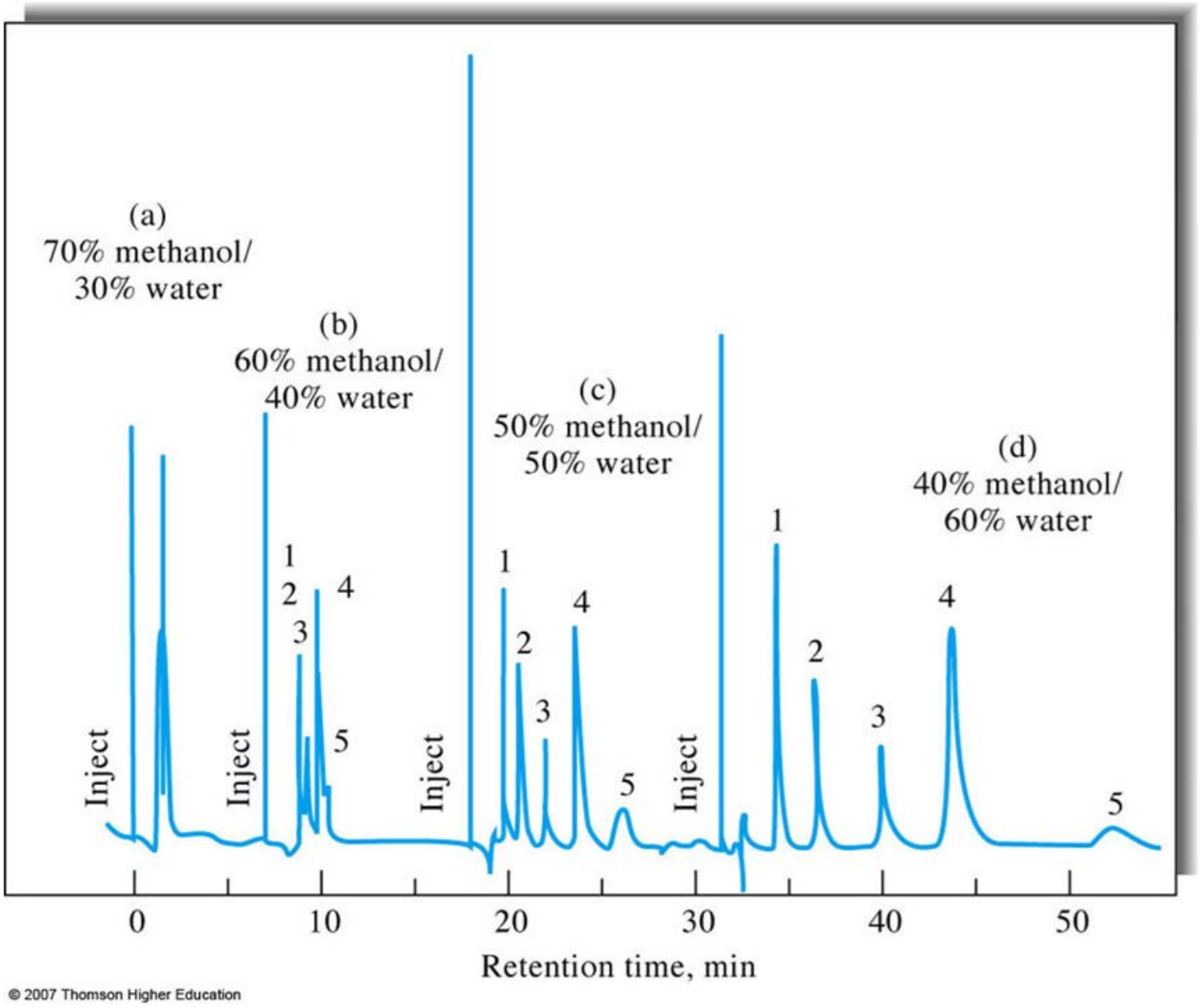


Fig. 26-14 Effect of solvent variation on chromatograms.

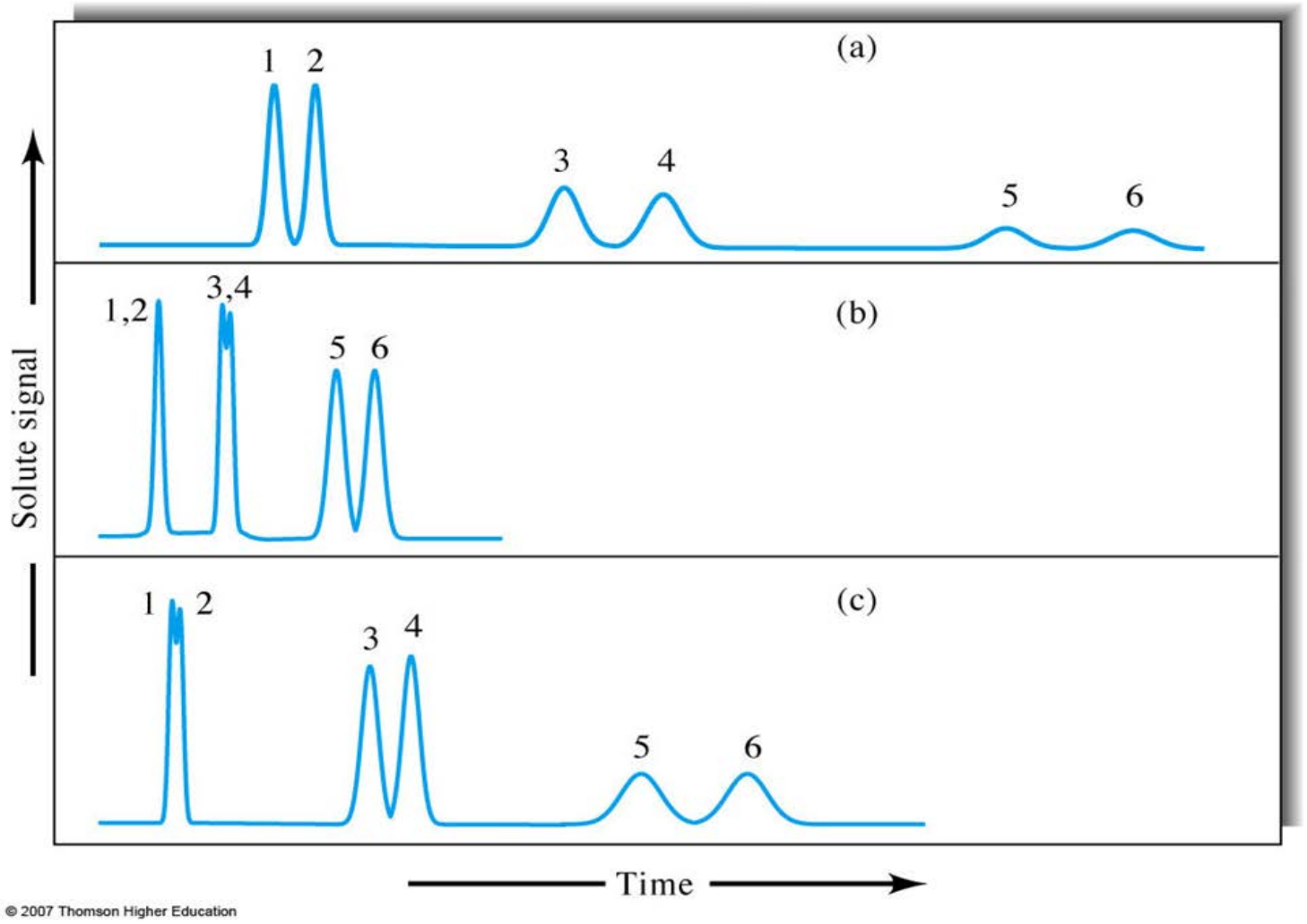


Fig. 26-15. The general elution problem in chromatography.

26E Summary of chromatographic relationships

The number of quantities, terms, and relationships employed in chromatography is large and often confusing. Tables 26-4 and 26-5 summarize the most important definitions and equations that will be used in the next three chapters.

26F Applications of chromatography

26F-1 Qualitative Analysis

A chromatogram provides only a single piece of qualitative information about each species in a sample, its retention time or its position on the stationary phase after a certain elution period.

TABLE 26-4 Important Chromatographic Quantities and Relationships

Name	Symbol of Experimental Quantity	Determined From
Migration time, unretained species	t_M	Chromatogram (Figure 26-7)
Retention time, species A and B	$(t_R)_A$, $(t_R)_B$	Chromatogram (Figures 26-7 and 26-12)
Adjusted retention time for A	$(t'_R)_A$	$(t'_R)_A = (t_R)_A - t_M$
Peak widths for A and B	W_A, W_B	Chromatogram (Figures 26-7 and 26-12)
Length of column packing	L	Direct measurement
Volumetric flow rate	F	Direct measurement
Linear flow velocity	u	F and column dimensions (Equations 26-6 and 26-7)
Stationary-phase volume	V_S	Packing preparation data
Concentration of analyte in mobile and stationary phases	c_M, c_S	Analysis and preparation data

TABLE 26-5 Important Derived Quantities and Relationships

Name	Calculation of Derived Quantities	Relationship to Other Quantities
Linear mobile-phase velocity	$u = \frac{L}{t_M}$	
Volume of mobile phase	$V_M = t_M F$	
Retention factor	$k = \frac{t_R - t_M}{t_M}$	$k = \frac{K V_S}{V_M}$
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	$H = \frac{L}{N}$	
Retention time	$(t_R)_B = \frac{16R_s^2 H}{u}\left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1 + k_B)^3}{(k_B)^2}$	

26F-2 Quantitative Analysis

- **Analyses Based on Peak Height**

The height of a chromatographic peak is obtained by connecting the baselines on either side of the peak by a straight line and measuring the perpendicular distance from this line to the peak.

- **Analyses Based on Peak Areas**

Peak areas are independent of broadening effects due to the variables mentioned in the previous paragraph.

- **Calibration and Standards**

The most straightforward method for quantitative chromatographic analyses involves the preparation of a series of external-standard solutions that approximate the composition of the unknown.

- **The internal-Standard Method**

The highest precision for quantitative chromatography is obtained using internal standards because the uncertainties introduced by sample injection are avoided.

- **The Area-Normalization Method**

Another approach that avoids the uncertainties associated with sample injection is the area-normalization method.

EXAMPLE 26-2

The area normalization method was applied to the determination of normal-, secondary-, iso-, and tertiary-butyl alcohol. To determine the relative response factor for the alcohols, a standard solution of the alcohols was prepared and its gas chromatogram observed. The results were as follows:

Alcohol	Weight Taken, g	Weight % Alcohol	Peak Area A, cm^2	Weight % Area	Relative Response Factor F
n-Butyl	0.1731	24.61	3.023	8.141	1.000
i-Butyl	0.1964	27.92	3.074	9.083	1.116
s-Butyl	0.1514	21.52	3.112	6.915	0.849
t-Butyl	0.1826	25.96	3.004	8.642	1.062
	$\sum$ wt = 0.7035	$\sum$ % = 100.00	$\sum A$ = 12.213		

The relative response factors were obtained by dividing the data in column 5 by 8.141, the first entry in column 5.

A sample containing only the four alcohols yielded the area data in the second column below. Calculate the weight percent of each alcohol present.

▸ *Solution*

The results are shown in column 4 below.

Alcohol	Peak Area, cm^2	Area × *F*	Weight % Alcohol
n-Butyl	1.731	1.731	18.18
i-Butyl	3.753	4.188	43.99
s-Butyl	2.845	2.415	25.36
t-Butyl	1.117	1.186	12.46
		$\Sigma = 9.521$	99.99

Unfortunately, it is often not practical to arrange conditions so that all of the components of a mixture are eluted from a column in a reasonable period. As a result, the area-normalization method has limited applications.