

Lecture 9

Principles of Chromatography
(See also Chapter 26 in Skoog,
Holler, and Newman and hand-
written notes Lecture 7 and 8.)

What affects column efficiency?

TABLE 26-2 Variables That Affect Column Efficiency

Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	$\text{cm}\cdot\text{s}^{-1}$
Diffusion coefficient in mobile phase*	D_M	$\text{cm}^2\cdot\text{s}^{-1}$
Diffusion coefficient in stationary phase*	D_S	$\text{cm}^2\cdot\text{s}^{-1}$
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.

Mobile Phase Flow rate and Plate Height (H)

variations

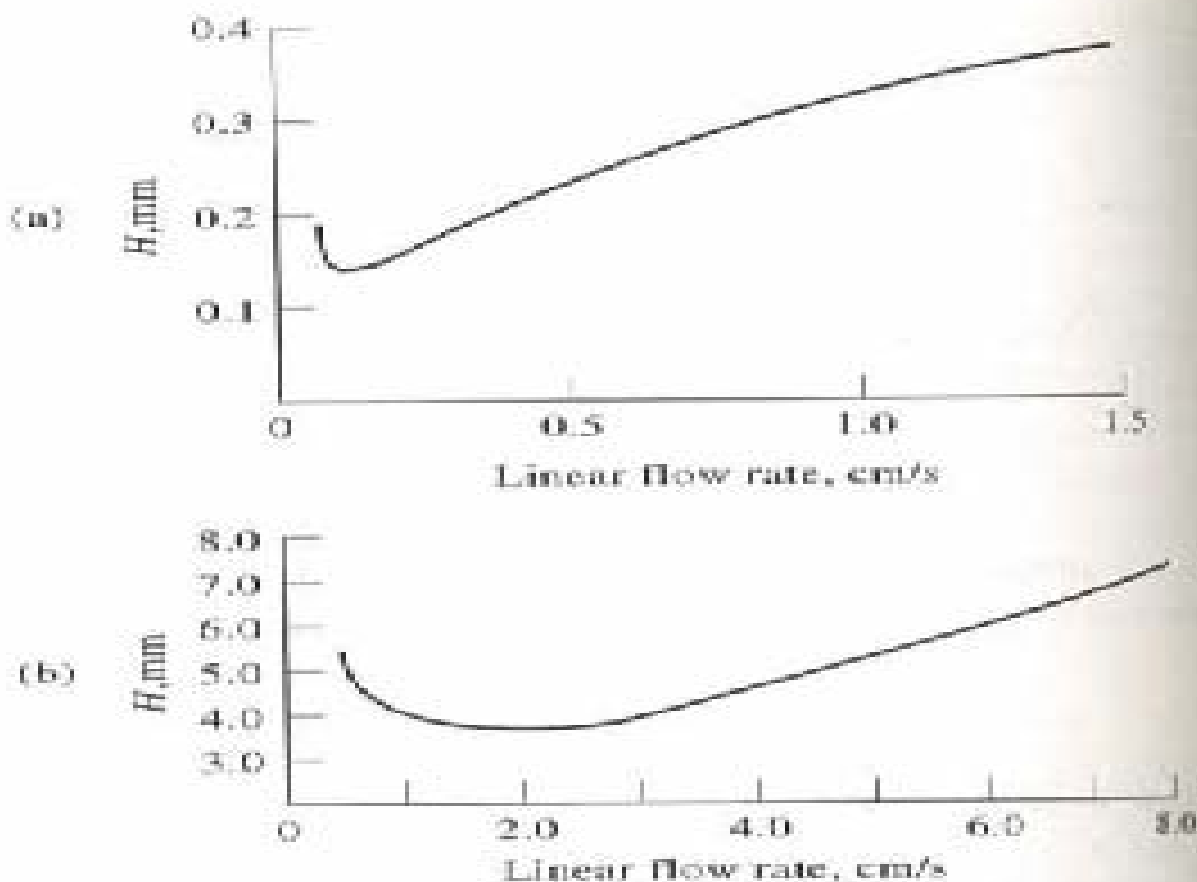


Figure 26-7 Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.

Factors that Effect Peak Broadening

TABLE 26-3 Kinetic Processes That Contribute to Peak Broadening

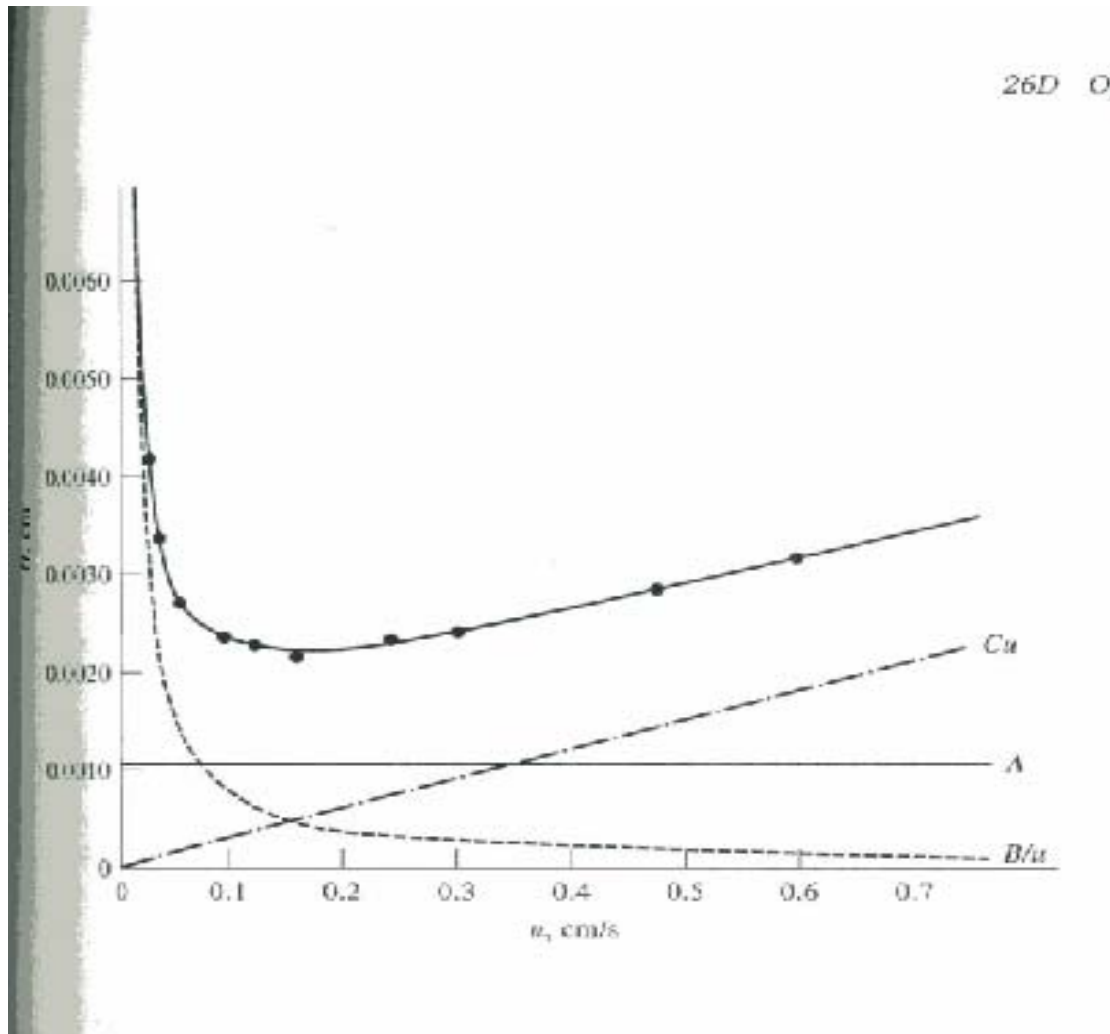
Process	Term in Equation 26-19	Relationship to Column* and Analyte Properties
Multiple flow paths	A	$A = 2\lambda d_p$
Longitudinal diffusion	B/u	$\frac{B}{u} = \frac{2\gamma D_M}{u}$
Mass transfer to and from liquid stationary phase	$C_S u$	$C_S u = \frac{f_S(k')d_f^2}{D_S} u$
Mass transfer in mobile phase	$C_M u$	$C_M u = \frac{f_M(k')d_p^2}{D_M} u$

TABLE 26-2 Variables That Affect Column Efficiency

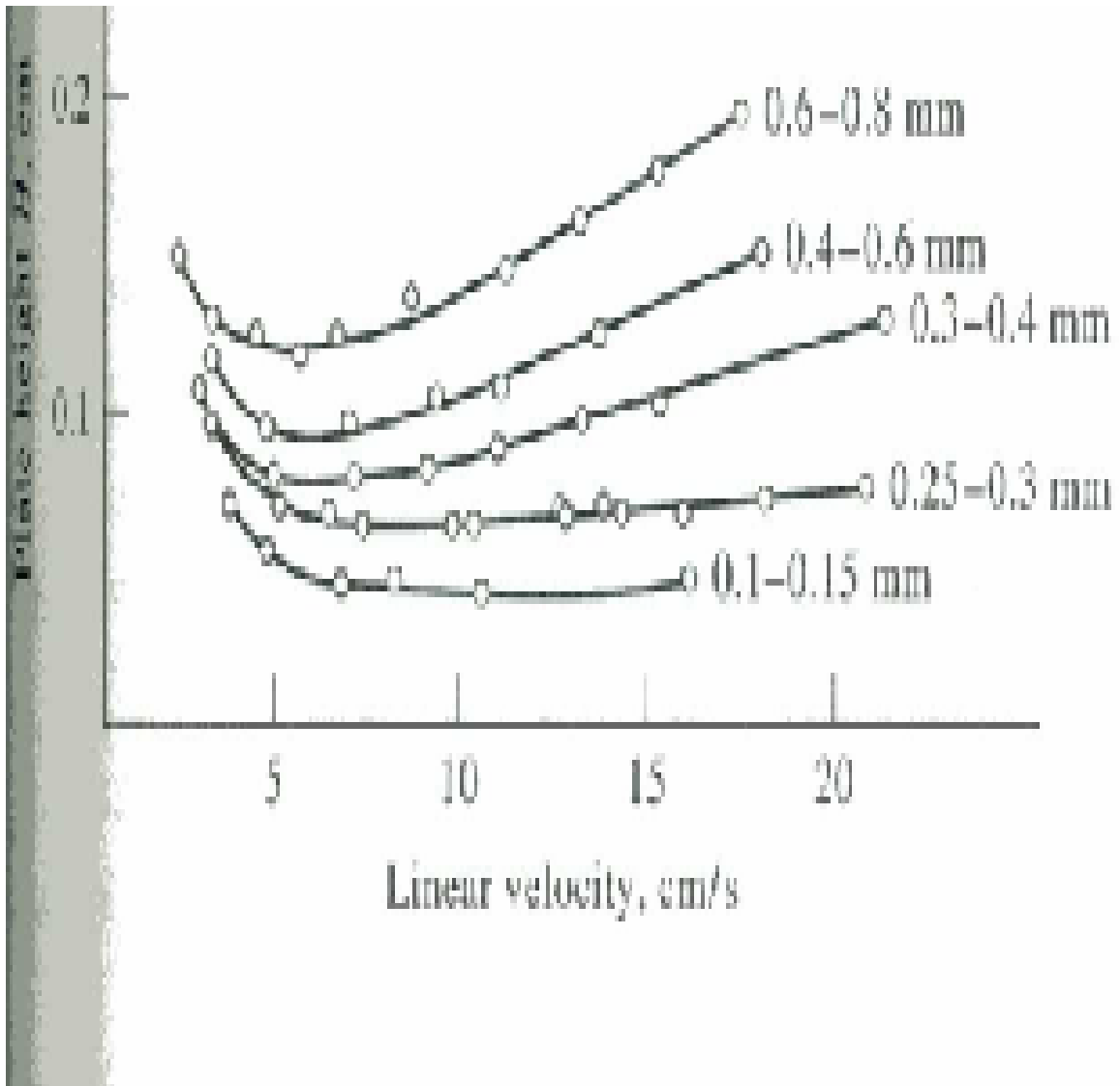
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*Increases as temperature increases and viscosity decreases.

Van Deemter Plot



Effect of particle size (stationary phase) on plate height



Column Resolution

$$R_s = \frac{\Delta Z}{W_A/2 + W_B/2} = \frac{2\Delta Z}{W_A + W_B} = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B} \quad (26-20)$$

the overlap is about 0.5%. The resolution for a given stationary phase can be improved by lengthening the column, thus increasing the number of plates. An adverse consequence of the added plates, however, is an increase in the time required for the separation.

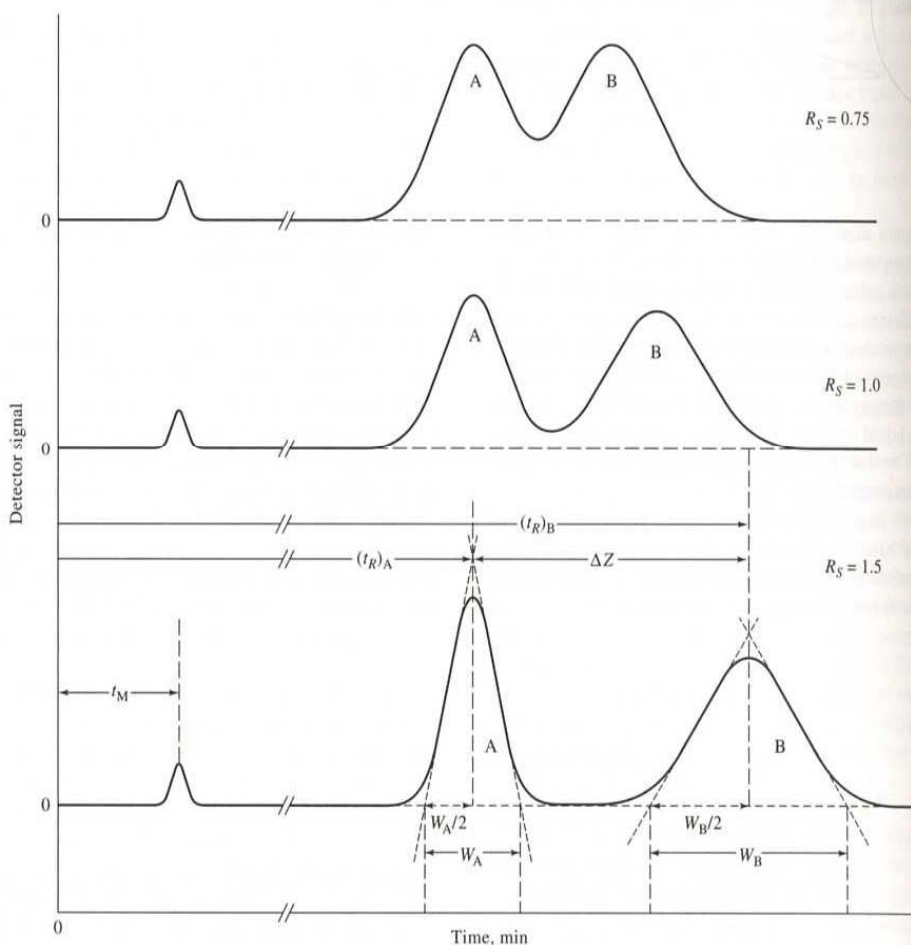


Figure 26-11 Separations at three resolutions. Here, $R_s = 2\Delta Z/(W_A + W_B)$.

Summary – The General Elution Problem

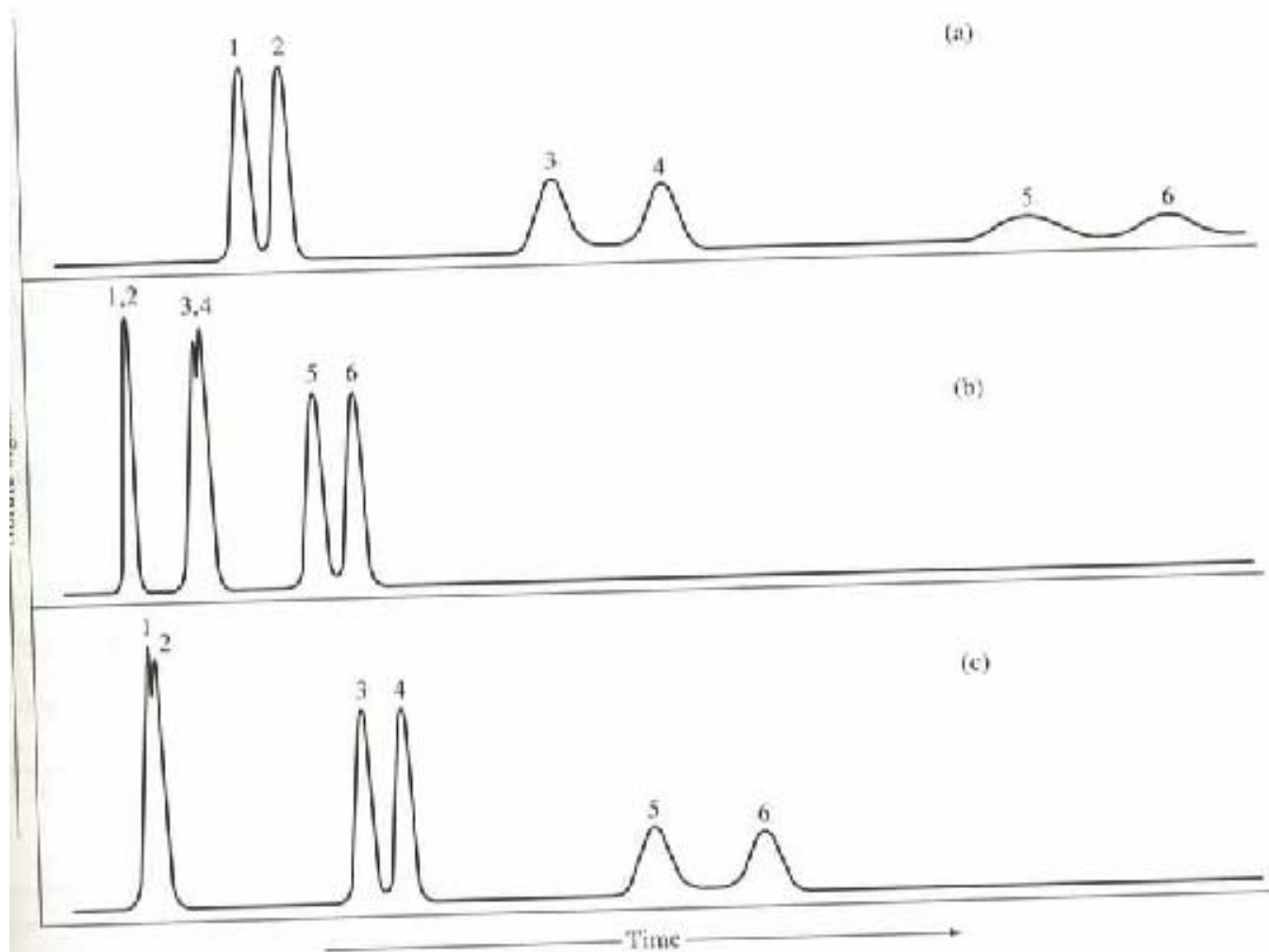


Figure 26-14 Illustration of the general elution problem in chromatography.

TABLE 26-4 Important Chromatographic Experimental Quantities and Relationships

Name	Symbol of Experimental Quantity	Determined From
Migration time, nonretained species	t_M	Chromatogram (Figure 26-6)
Retention times, species A and B	$(t_R)_A, (t_R)_B$	Chromatogram (Figure 26-6)
Adjusted retention time, species A	$(t'_R)_A$	$(t'_R)_A = (t_R)_A - t_M$
Peak widths, species A and B	W_A, W_B	Chromatogram (Figure 26-6)
Length of column packing	L	Direct measurement
Flow rate	F	Direct measurement
Volume of stationary phase	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	$u = L/t_M$	
Volume of mobile phase	$V_M = t_M F$	
Retention factor	$k' = (t_R - t_M)/t_M$	$k' = \frac{KV_S}{V_M}$
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	$H = L/N$	
Retention time	$(t_R)_B = \frac{16R_s^2 H}{u} \left(\frac{\alpha}{\alpha - 1} \right)^2 \frac{(1 + k'_B)^2}{(k'_B)^2}$	