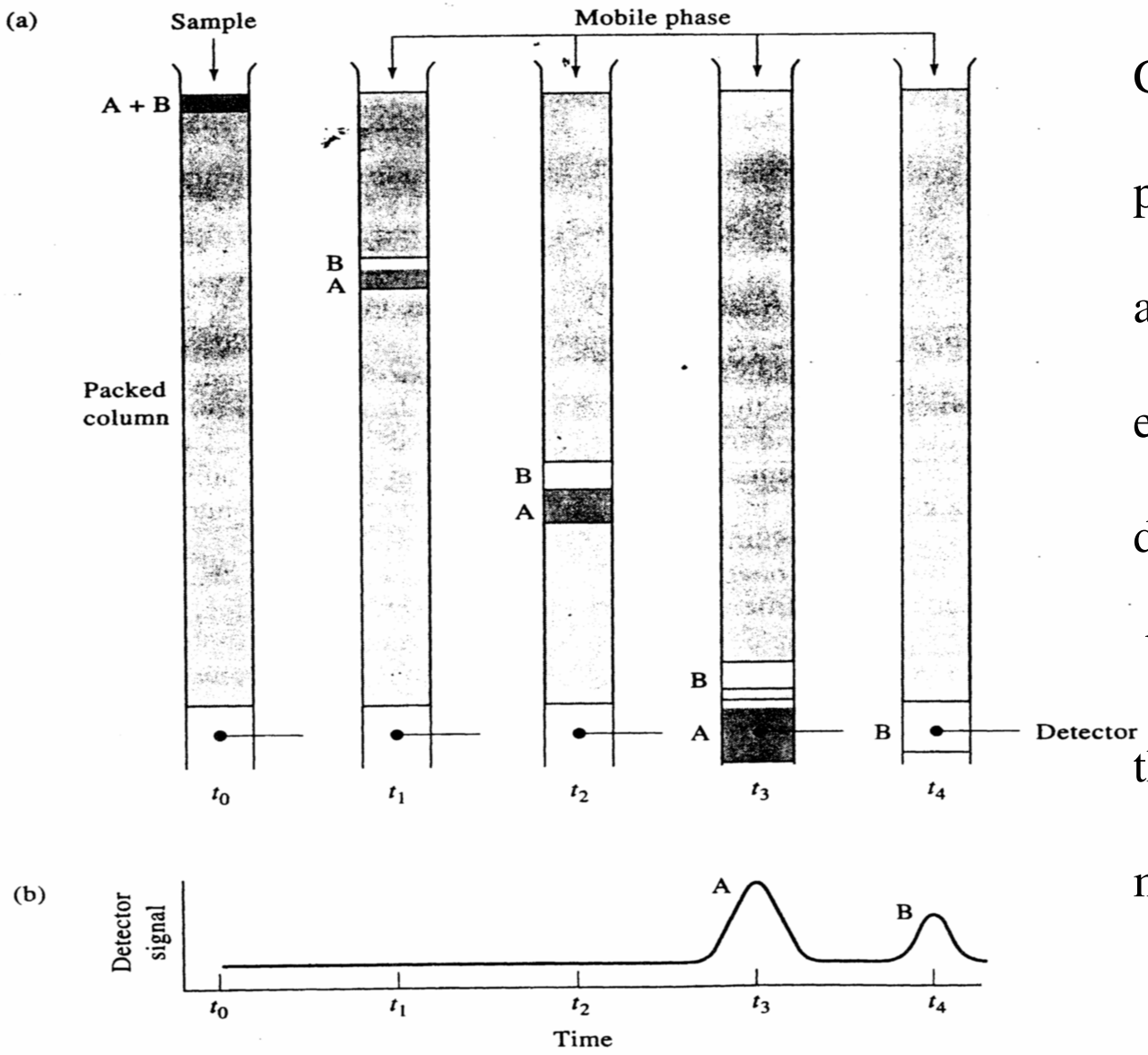
Introductory Separations

General Figure Acknowledgements

- Colin F. Poole's- "The Essence of Chromatography", Elsevier Science, 2003.
- C.F. Poole and S. A. Schuette, "Contemporary Practice of Chromatography" Elsevier Science, 1984.
- C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier Science, 1991.
- D.A. Skoog, F.J. Holler and T.A. Nieman, "Principles of Instrumental Analysis", 5 th. Ed., Harcourt Brace & Co., 1998.
- D.C. Harris, "Quantitative Chemical Analysis", 5 th. Ed., W.H. Freeman, 1999.
- D.G. Peters, J.M. Hayes and G.M. Hieftje, "Chemical Separations and Measurements" W.B. Saunders Co., 1974.
- B.L. Karger, L.R. Snyder and C. Horvath, "An Introduction to Separation Science", John Wiley and Sons, 1973.
- R.L. Grob, Ed., "Modern Practice of Gas Chromatography", 3 rd., Wiley-Interscience, 1995.
- L.R. Snyder, J.J. Kirkland and J.L. Glajch, "Practical HPLC Method Development", 2 nd. Ed., Wiley-Inerscience, 1997.

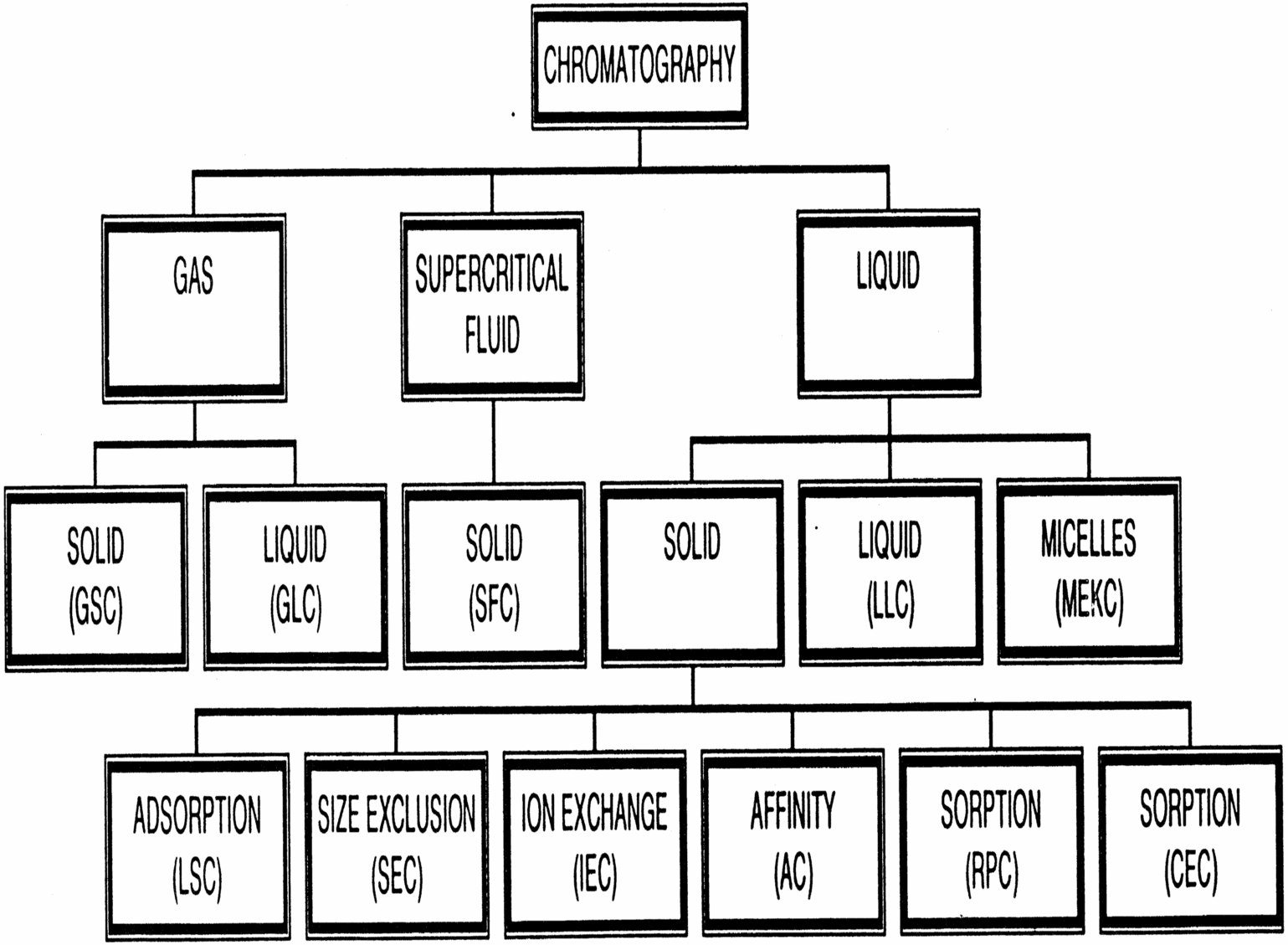
Special acknowledgment by J.D. Stuart to Joshua Denette, Senior Chemistry Dept. Intern who helped with the figure scanning and preparation of this PowerPoint Presentation.



Chromatography is a physical process in which analytes are separated from each other because of their different migration rates between the mobile and the stationary phase as they move through the column.

Picture copied from Skoog, Holler and Nieman, "Principles of Instrumental Analysis", 1998, p. 676.

Various Column Chromatographic Methods. GC, SFC and LC



From C.F. Poole, "The Essence of Chromatography", 2003, Elsevier Scientific, p. 5

Some Basic Calculations

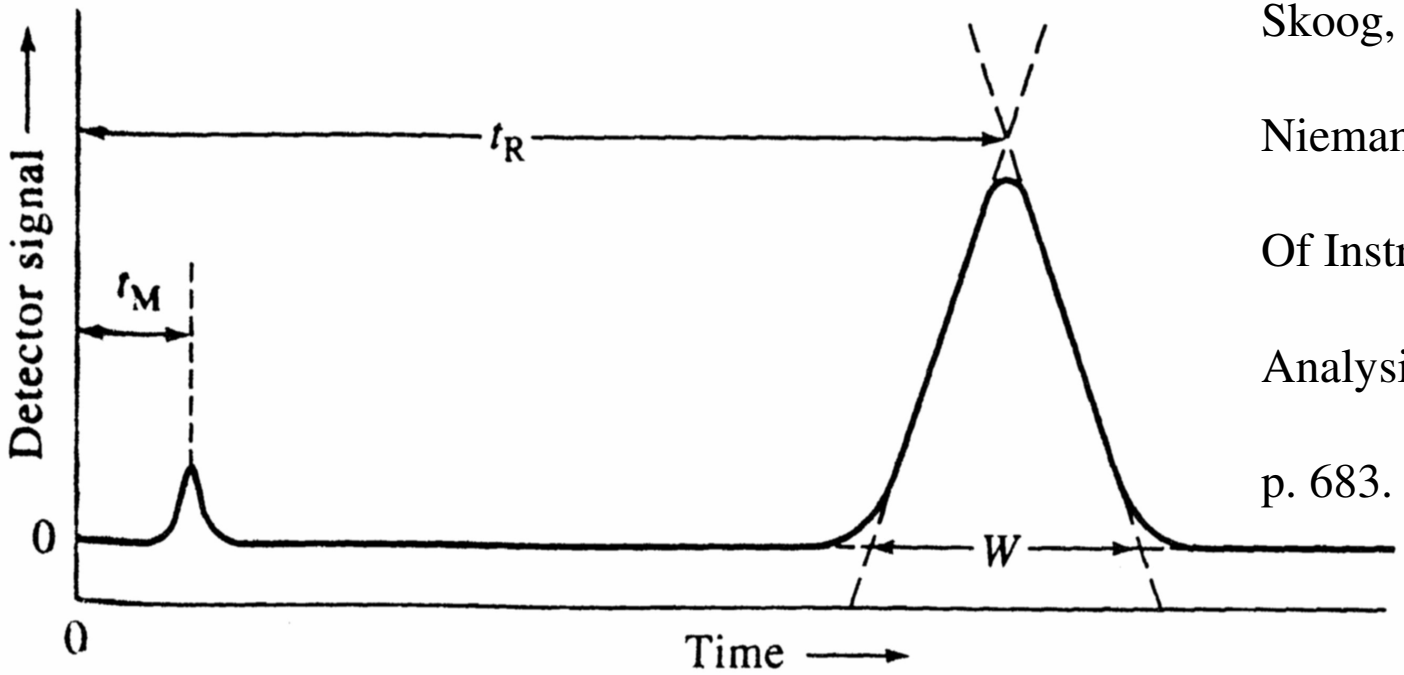


Fig. Copied from Skoog, Holler and Nieman, "Principles Of Instrumental Analysis", 5 th. Ed p. 683.

t_M = retention time of an unretained peak (or solvent front)

t_R = retention time of a retained peak.

$t_R' = t_R - t_M$ = corrected retention time

$k' = (t_R - t_M) / t_M$ = capacity factor

Selectivity Factor or $\alpha = k'_b / k'_a = K_b / K_a$ **(Distribution Constants)**

Resolution or $R_s = [(t_r)_b - (t_r)_a] / W_{ave.}$

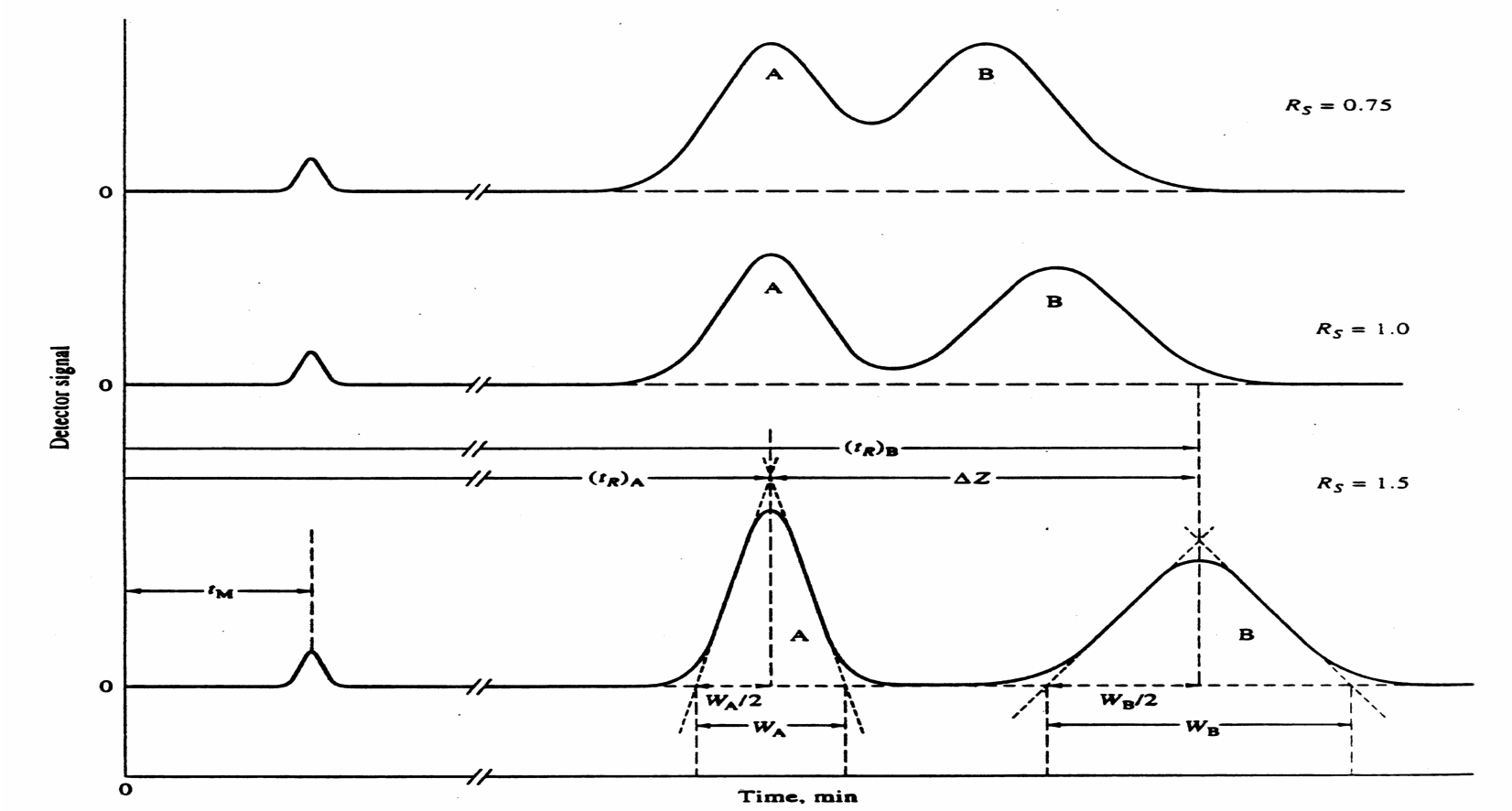
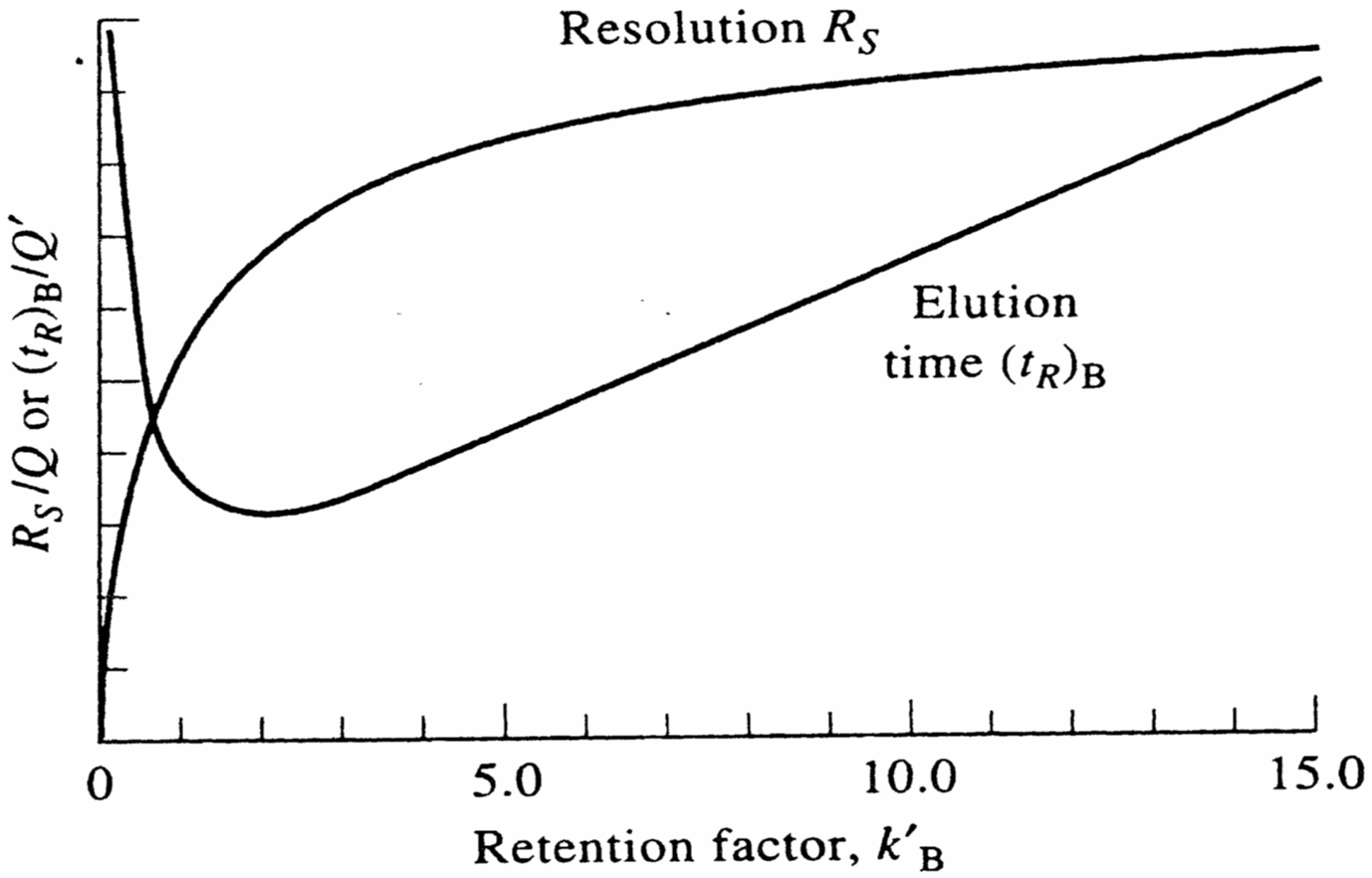


Figure 26-11 Separations at three resolutions. Here, $R_s = 2\Delta Z/(W_A + W_B)$.

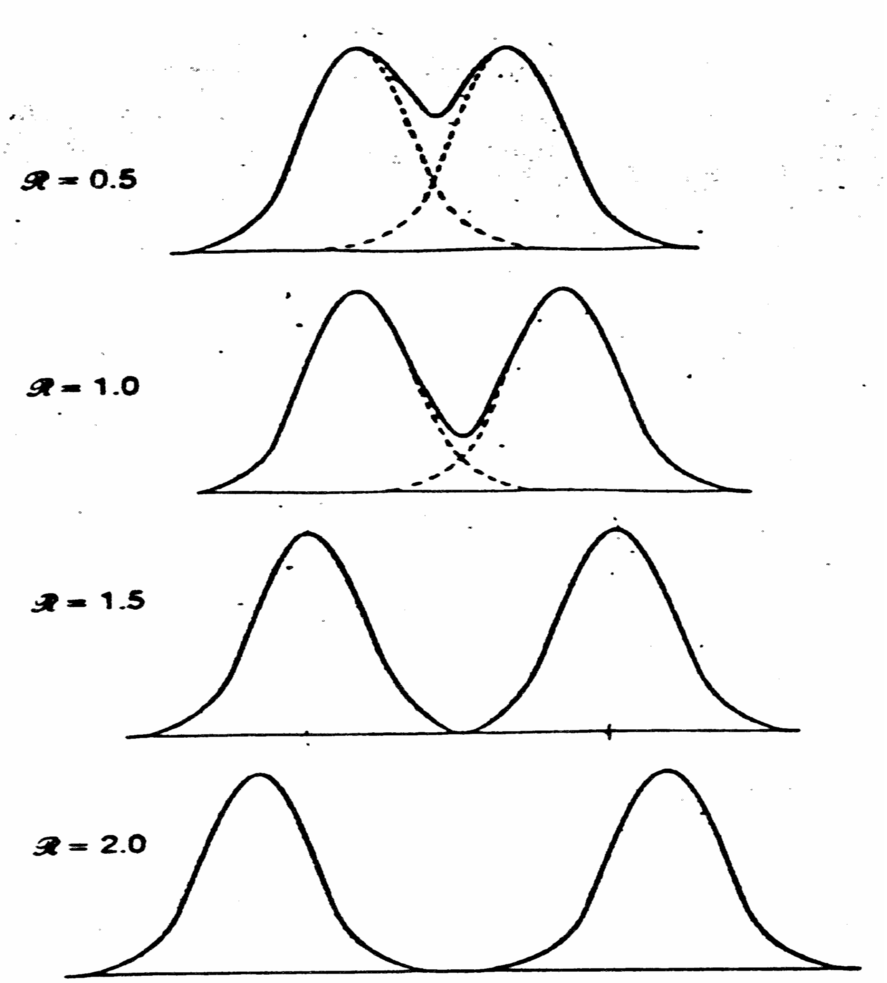
Copied from Skoog, Holler and Nieman, Principles of Instrumental Analysis, 1998, p. 688

Resolution and Elution Time as a function of Retention Factor, (why k' of 2-5 is best). Work for reasonable resolution in an acceptable time. As in life, chromatography always is a compromise!



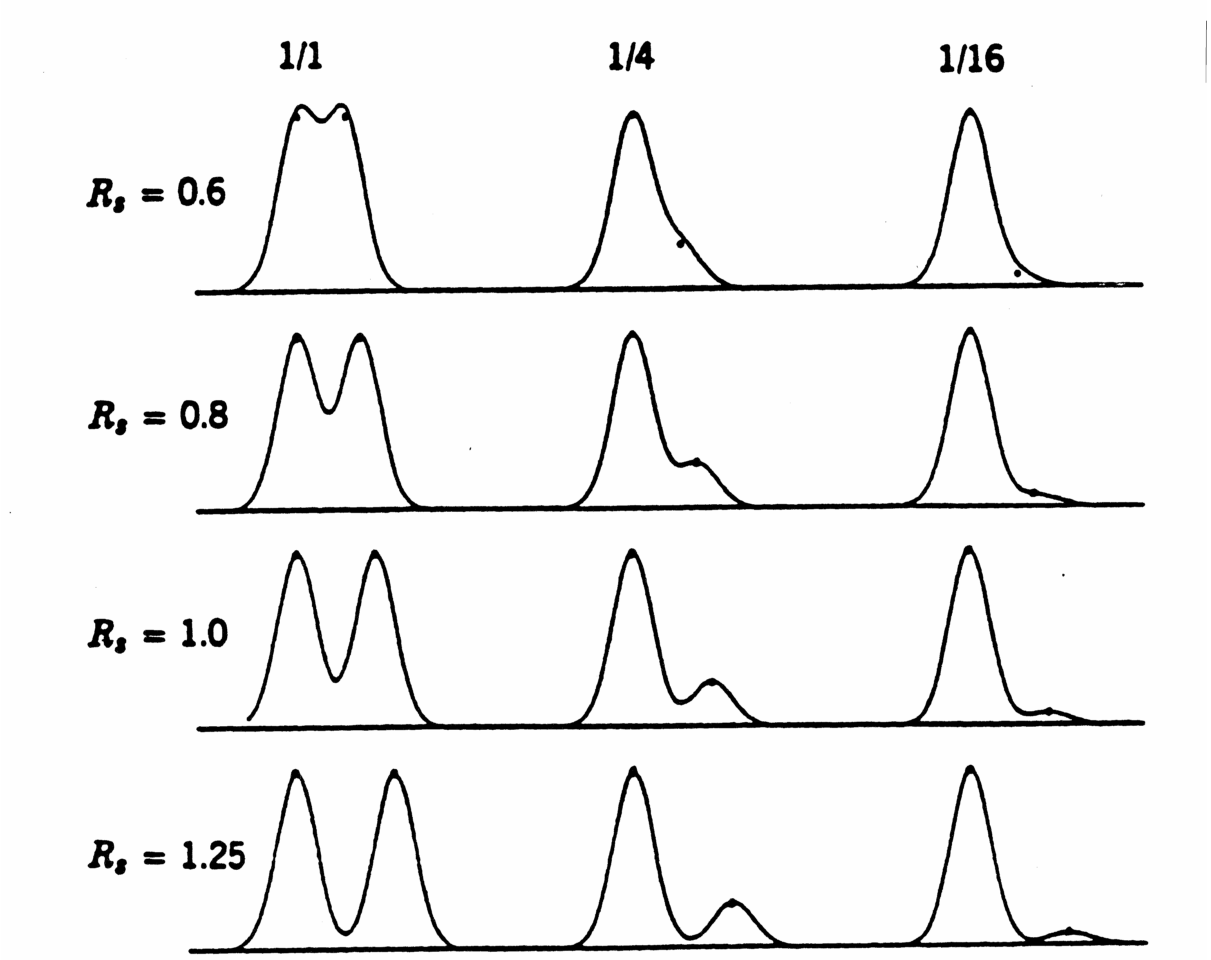
From Skoog, Holler and Nieman, "Principles of Instrumental Analysis", 1998, p. 691.

Showing Resolutions of 0.5, 1.0, 1.5 (baseline) and 2.0 for two peaks.



From D.G. Peters, J.M. Hayes and G.M. Hieftje, "Chemical Separations and Measurements", 1974, p.542.

Standard resolution curves for the separation of two closely eluting peaks,
As a function of different resolutions and different relative peak areas.



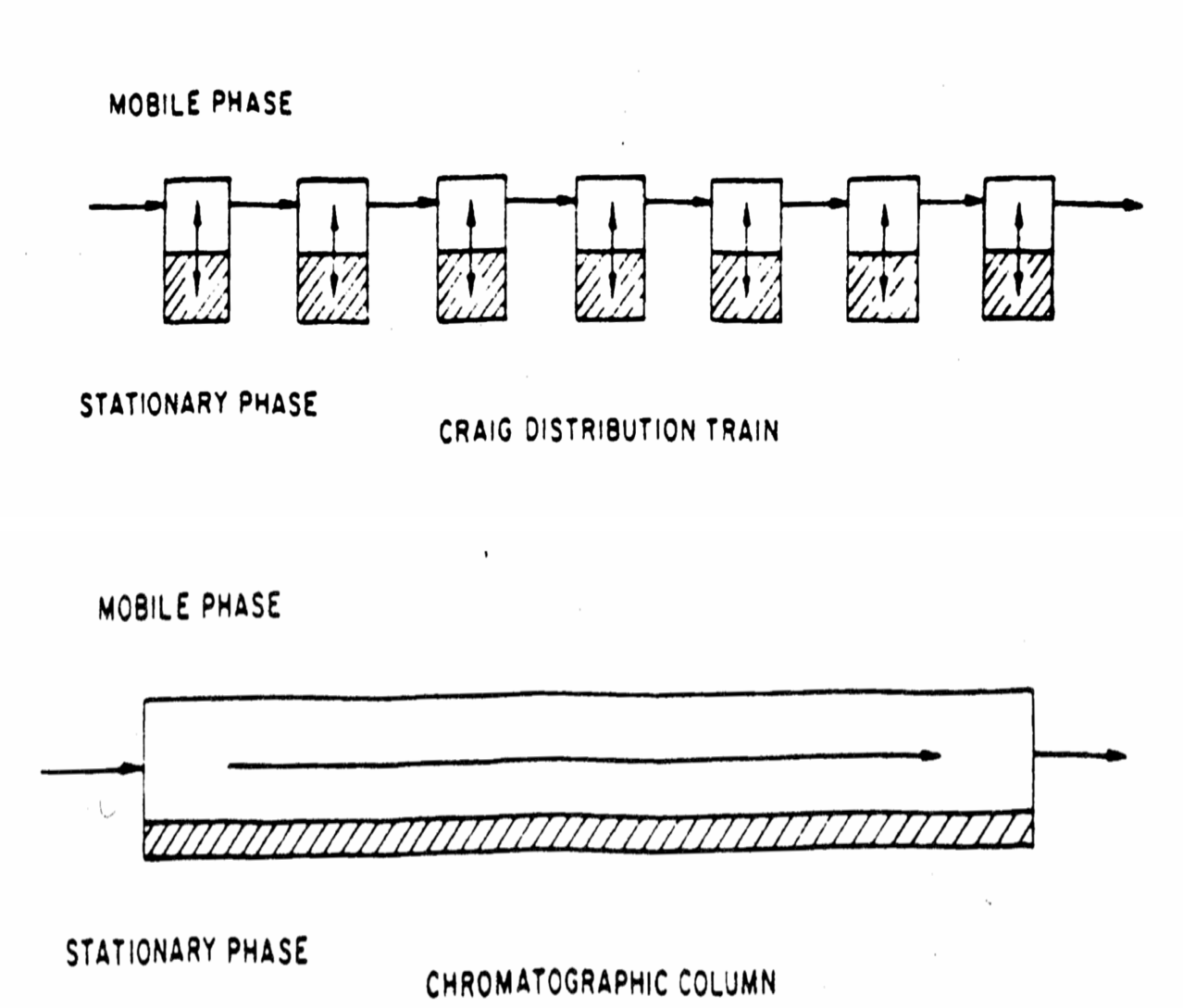
From C.F. Poole, “The Essence of Chromatography”, 2003, p. 52

Plate Theory of Chromatography

Martin and Synge theorized that chromatographic column was divided into a number of theoretical plates.

- In each plate there existed an instantaneous partition of the analyte between the mobile and stationary phase.
- That an analytes distribution constant, K_D was the same in each plate and independent of conc.
- That the mobile phase flow stopped in each plate where an instantaneous equilibrium was established.

How Counter Current Extraction conceptually could represent a chromatographic column



From B.L. Karger, L.R. Snyder and C. Horvath, "An Introduction to Separation Science", Wiley, 1973, p.111

Advantages of the Plate Theory

- Allows for the simple calculation of the the efficiency of a column by the measurement of its theoretical plates:

$$N = (t_R/\sigma)^2 = 16(t_R/w_b)^2 = 5.545(t_R/w_{0.5h})^2$$

and $HETP = L/N$

RESTEK CORPORATION

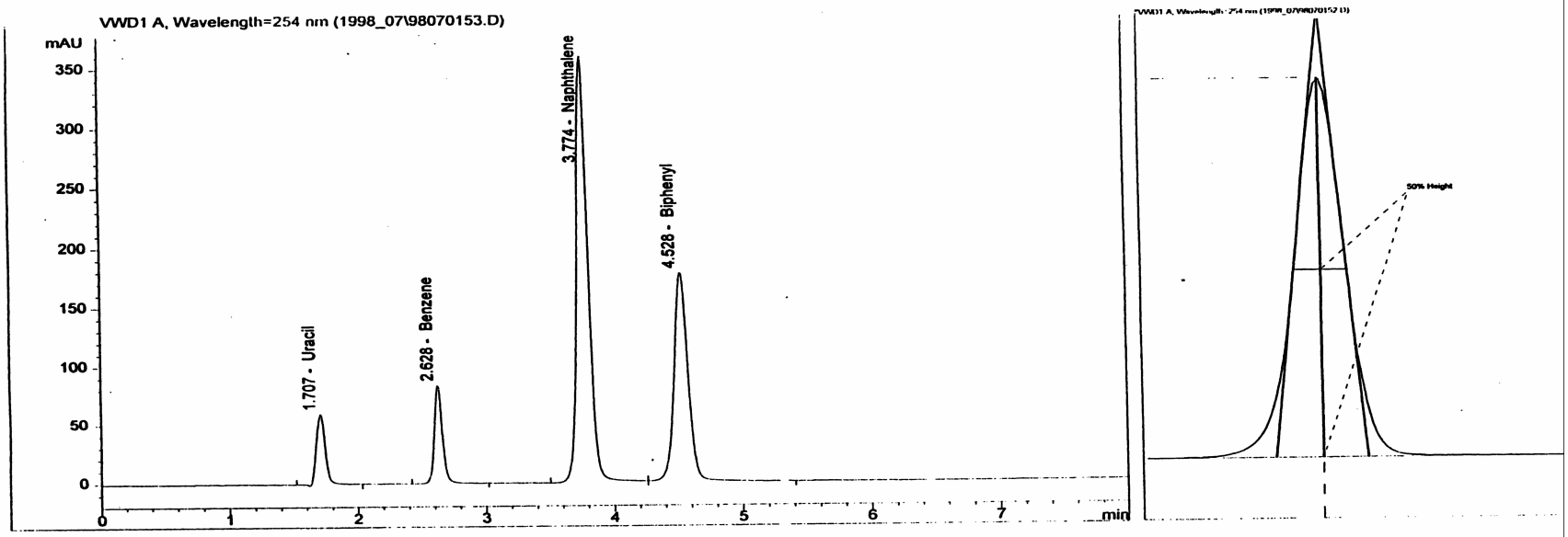
Quality Assurance Report

QA Analyst: LP Tested: 7/28/98

C-18.

Column Type : ~~CHBSS~~ IBD
Part Number : 55109
Serial Number: 98070153P
Lot Number : 079803R

Column Length : 150 mm
Inside Diameter: 4.6 mm
Particle Size : 5 µm
Pore size : 100 Å



Mobile Phase : Methanol 80% Water 20%
Flow : 1.00 ml/min Sample Amount: 5.00 uL
Pressure : 81 bar Wavelength : 254 nm

#	Compound Name	Ret. Time	Area	Area%	Width
1	Uracil	1.707	278	6.5	0.075
2	Benzene	2.628	368	8.6	0.067
3	Naphthalene	3.774	2260	53.1	0.096
4	Biphenyl	4.528	1353	31.8	0.116

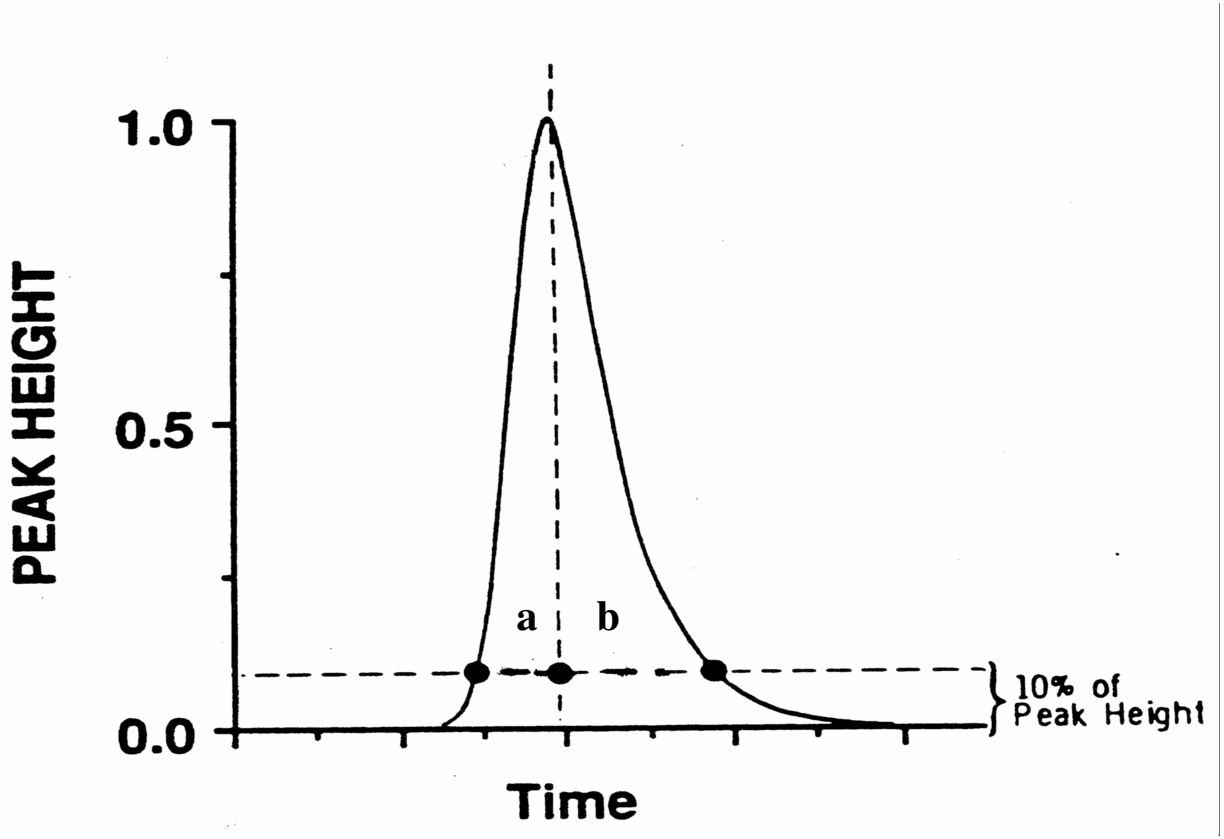
Performance results for Peak #4

Retention time : 4.53 min
k' : 1.66
Peak Width (1/2 height) : 0.11 min
Asymmmetry at 10% : 0.93
Plates/Column : 9677 n
Plates/Meter : 64512 n/m

Restek Corporation 110 Benner Circle, Bellefonte, PA 16823-8812 Phone: 814-353-1300 / 800-356-1688 Fax: 814-353-1309

Restek Corp. Bellefonte, PA., LC column calibration sheet. Showing how to calculate plate count by hand.

How the Asymmetry factor is measured. A_s = b/a at 10% peak height.
The peak depict with b >> a is a tailing peak and would have As > 1.2.



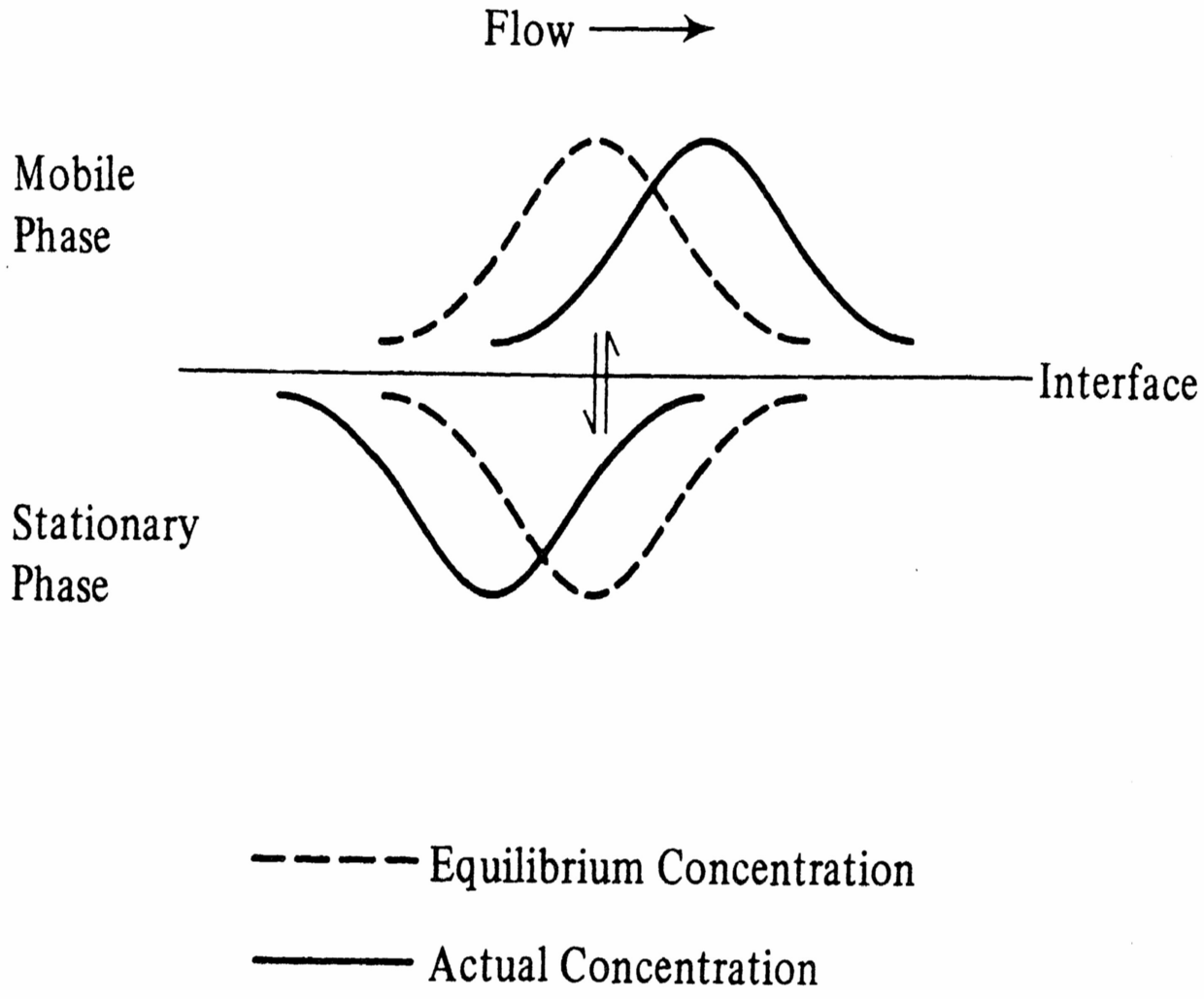
From C.F. Poole, "The Essence of Chromatography", Elsevier Scientific, 2003, p. 50.

Disadvantages of the Plate Theory

- The chromatographic column is not physically divided into distinct plates or volumes.
- There is not an instantaneous partition of the analyte between the mobile and stationary phase
- That the distribution constant is constant only over a narrow concentration range.
- That the flow rate of the mobile phase is continuous and not discontinuous.

But most important it did not the explain the effects of band broadening due to such experimental changes as mobile phase velocity, particle size and film thickness.

Showing the effect of local non-equilibrium band broadening.
Dashed lines: equilibrium conc. Profile; Solid lines: shows rate affected process.



H.H. Bauer, G.D. Christian and J.E. O'Reilly, "Instrumental Analysis", 1978, Allyn and Bacon, p. 634.

Enters the Rate Theory of Chromatography

J.C. Giddings insight, with contributions from others such as Van Deemter, explained hyperbolic function of HETP to μ (cm/sec) as

General Equation $HETP = A + B / \mu + C \mu$

Where the A term represents the contributions from packed columns eddy diffusions (due to unequal pathways).

Where the B term is the longitudinal (back and sideways diffusion of the solute in the mobile phase.

And where the C term(s) represent the slow rate of mass transfer of the solute as it travels through the column.

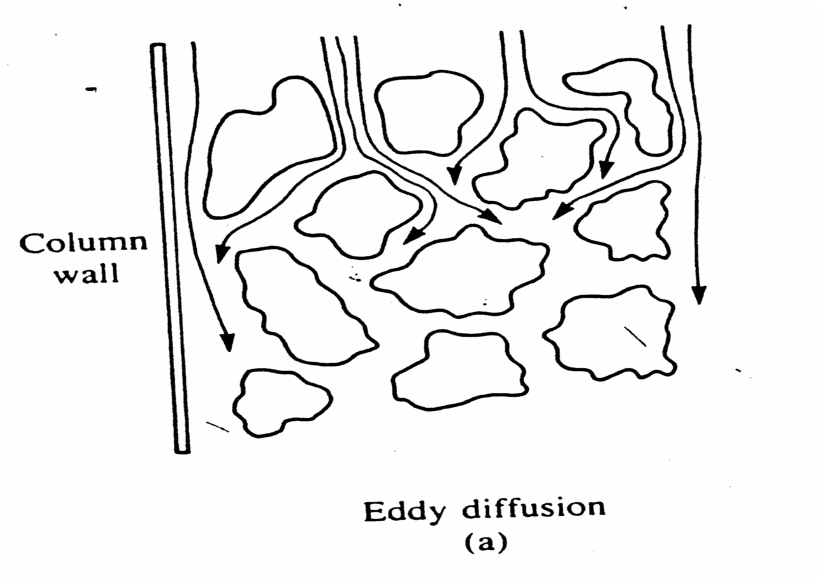
TABLE 26-3 Kinetic Processes That Contribute to Peak Broadening

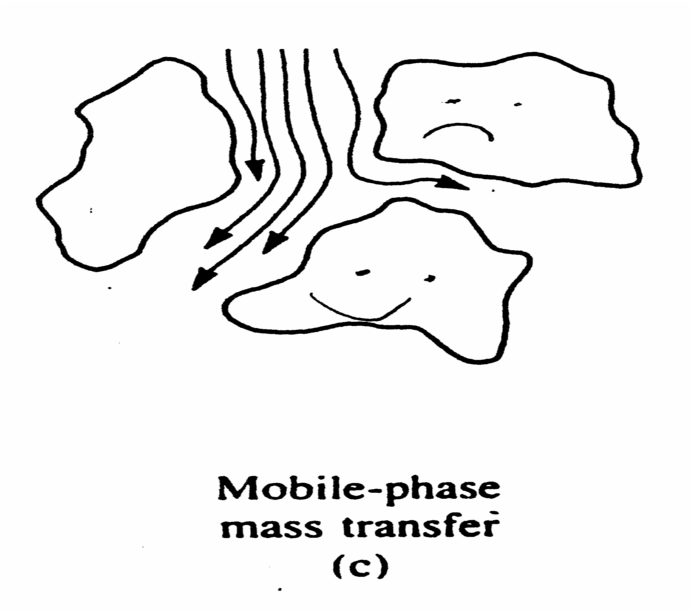
Process	Term in Equation 26-19	Relationship to Column* and Analyte Properties
Multiple flow paths	A	$A = 2\lambda d_P$
Longitudinal diffusion	B/u	$\frac{B}{u} = \frac{2\gamma D_M}{u}$
Mass transfer to and from liquid stationary phase	$C_S u$	$C_S u = \frac{f_S(k')d_f^2}{D_S} u$
Mass transfer in mobile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

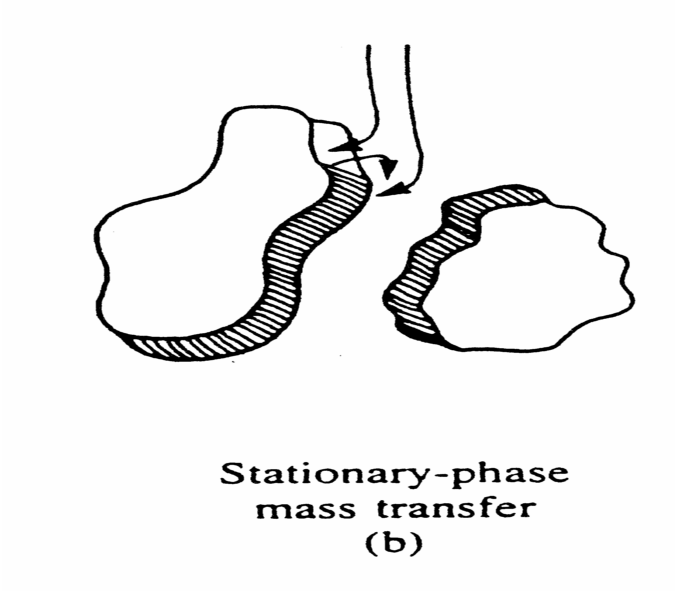
*u, D_S, D_M, d_f, d_p, k' are as defined in Table 26-2.
$f(x)$ = function of x.
λ, γ: constants that depend on the quality of the packing.
B: coefficient of longitudinal diffusion.
C_S, C_M: coefficients of mass transfer in stationary and mobile phases, respectively.

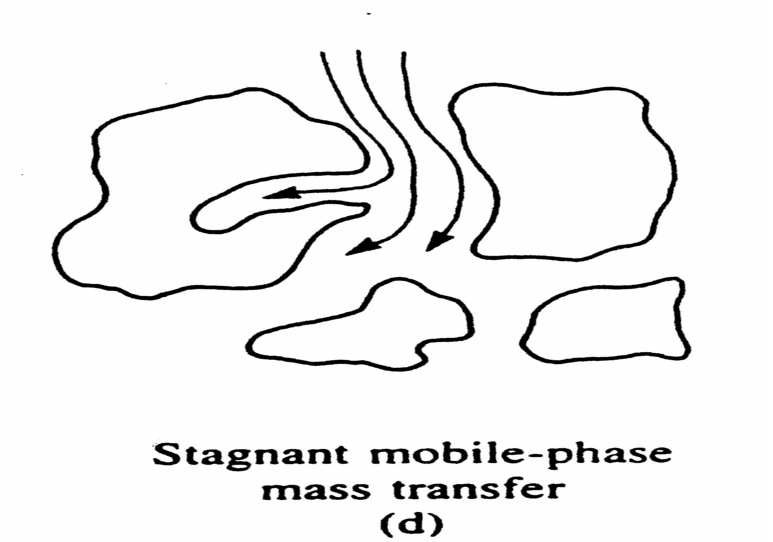
From Skoog, Holler and Nieman, "Principles of Instrumental Analysis, 5 th. Ed,. 1998, p.685.

How the A, C_l , C_m , and C_{sl} can cause chromatographic band broadening.



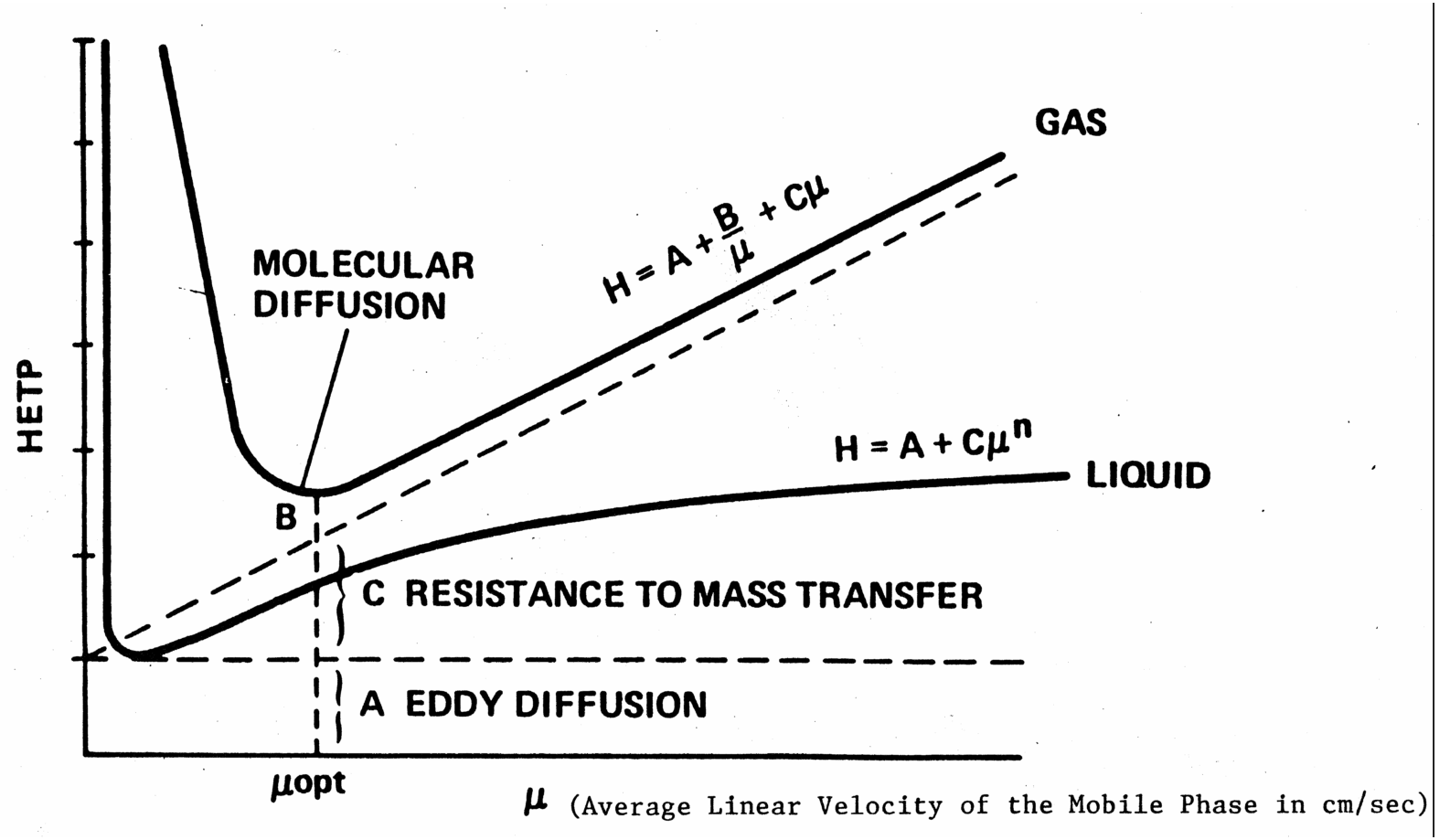






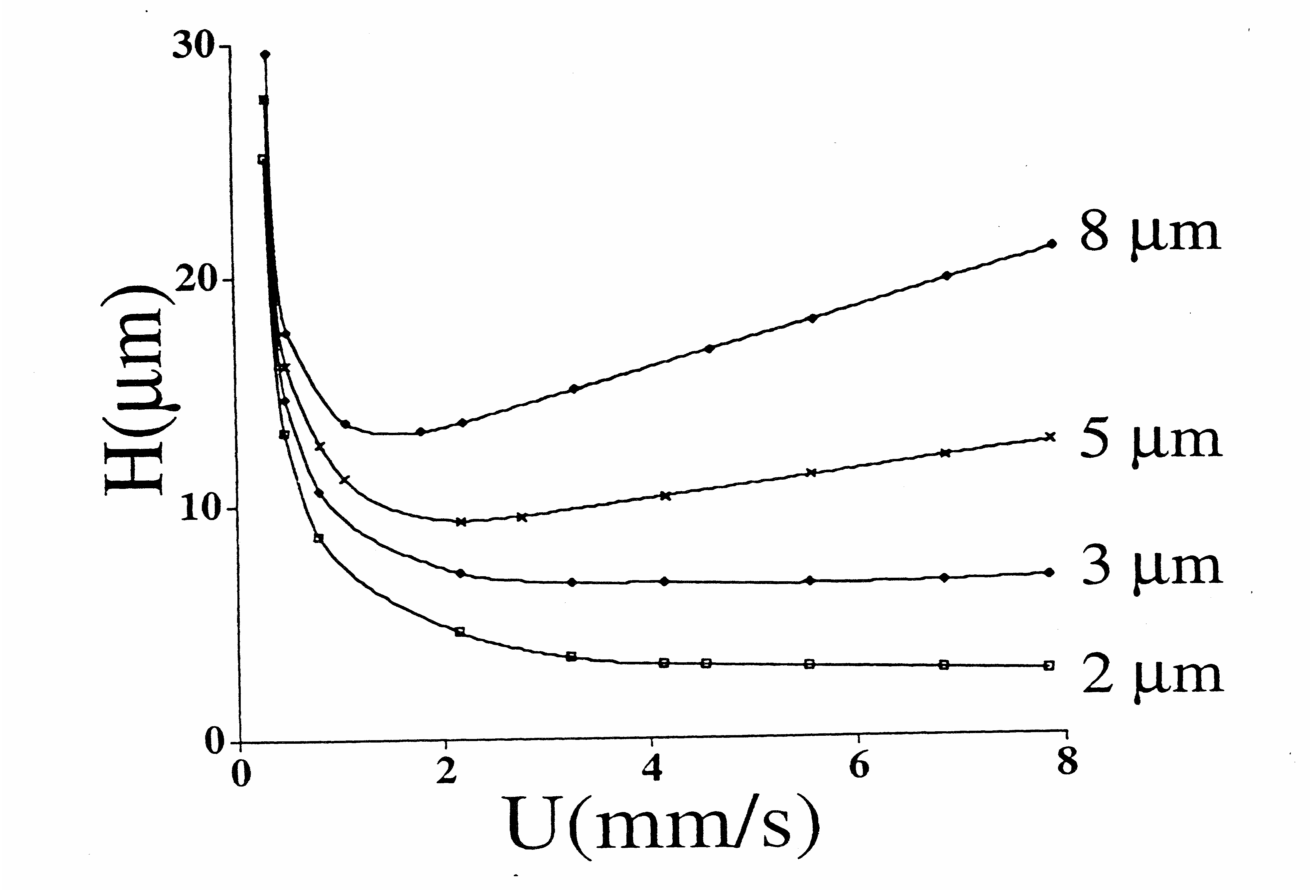
From H.H. Willard, L.L. Merritt, Jr., J.A. Dean and F.A. Settle, Jr.,
"Instrumental Methods of Analysis", 1988, Wadsworth Publishing Co., p.526

General van Deemter Plot. Graph of column plate height on mobile phase velocity

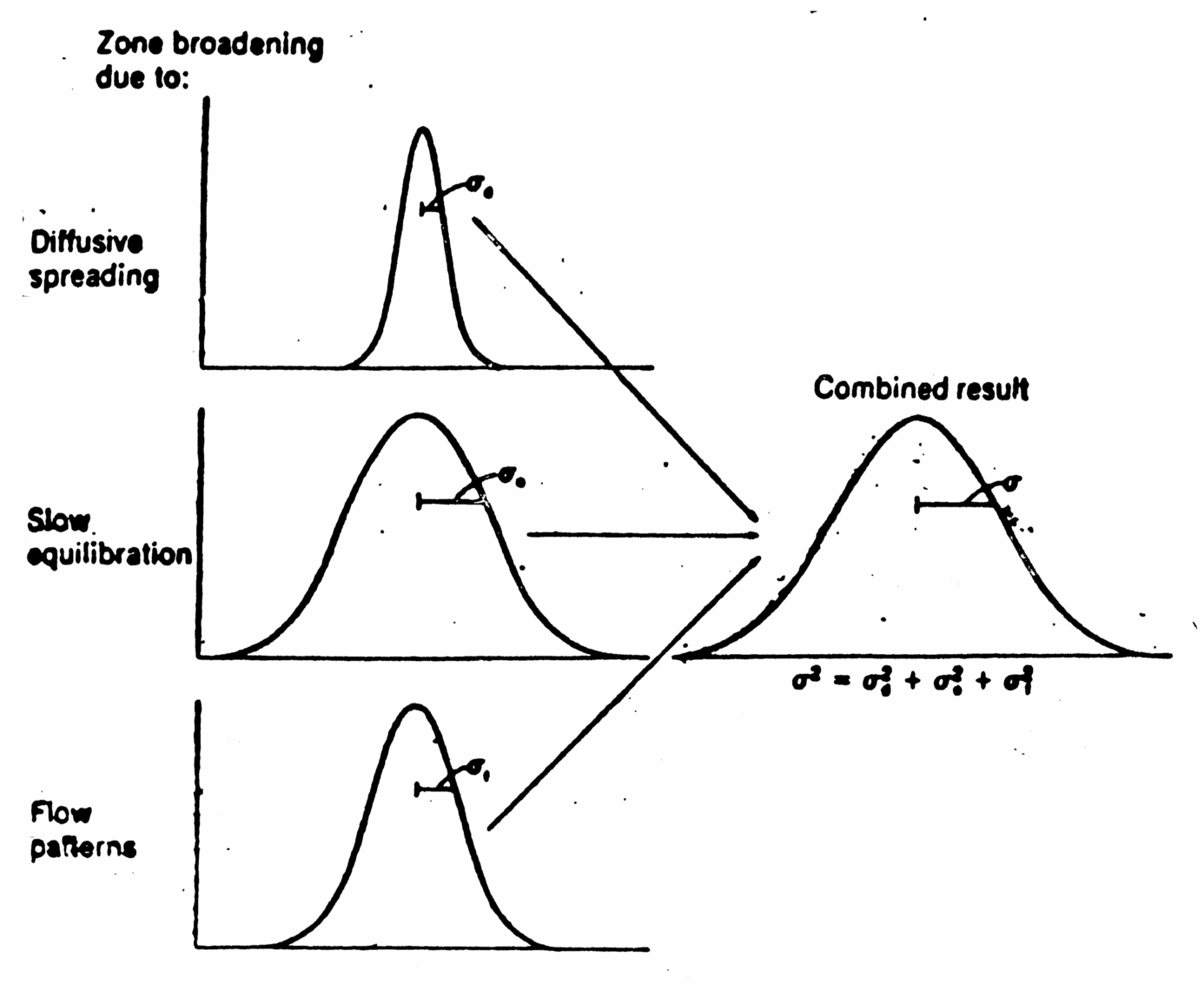


Copy and then adapted from LC training notes of Waters Inc., Milford MA.

Van Deemper Plot of Plate Height vs. Mobile Phase Velocity in Liquid Chromatography as a function of different stationary phase particle diameters.
Note the almost constant plate height vs. increase mobile phase velocity for 2- and 3-µm particles.

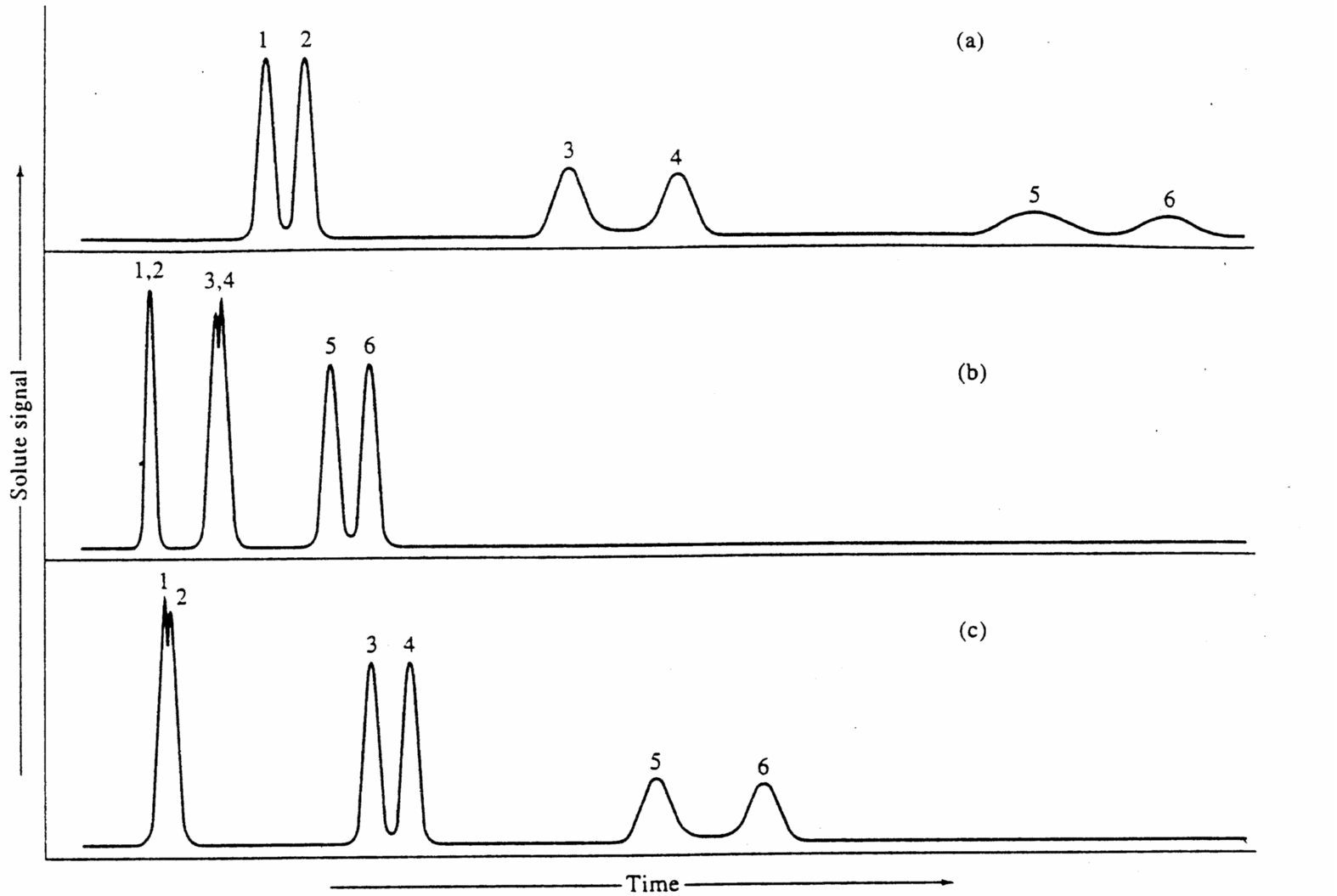


From C.F. Poole, "The Essence of Chromatography", Elsevier Science, 2003, p. 38.



From D.G. Peters, J.M. Hayes and G.M. Hieftje, “Chemical Separations and Measurements”, 1974, p.529.

Showing the General Elution Problem in Chromatography. How to overcome it?



From Skoog, Holler and Nieman, Principles of Instrumental Analysis, 1998, p. 693

Different Mobile Phase flow profiles though a tube: Laminar, Turbulent and Plug Flow.

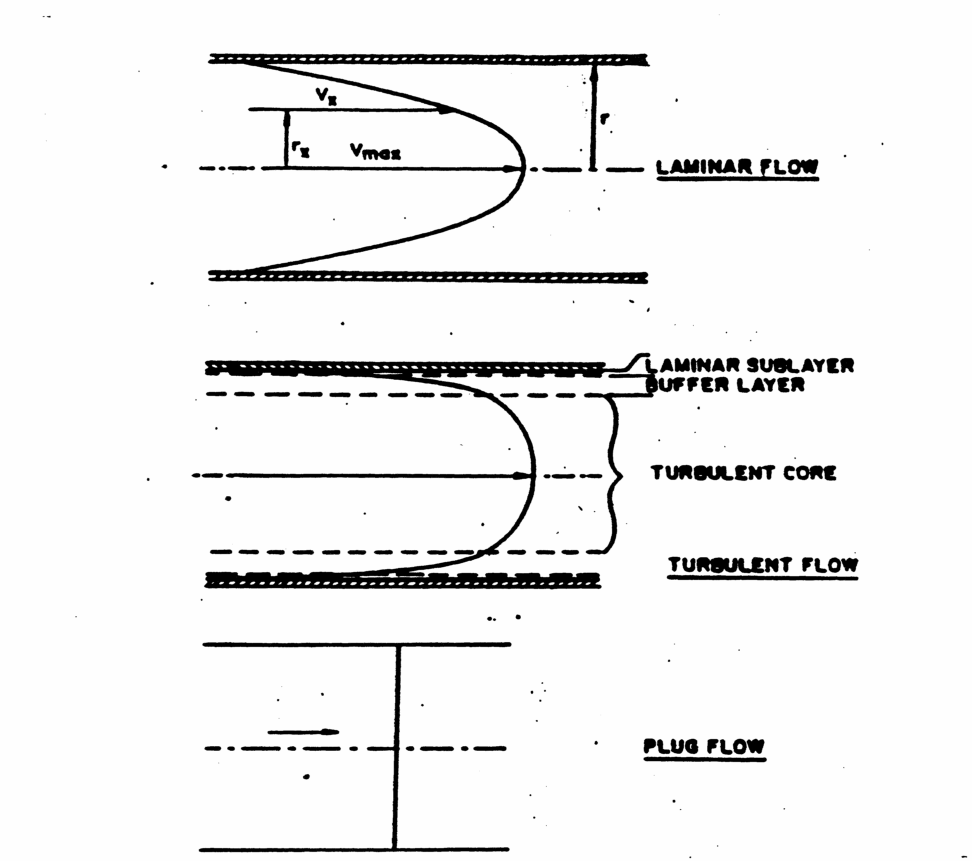


Figure 3.7. Flow profiles in tube flow. The parabolic flow profile is characteristic for laminar flow. The turbulent flow profile is flatter because of radial mixing. Plug flow is unattainable in practice but often represents a convenient model.

From B.L. Karger, L.R. Snyder and C. Horvath, "An Introduction to Separation Science", Wiley, 1973, p.87.