Objectives for our Discussion of Separations

Basics

1. Define, understand, and use key terms such as retention time, retention factor, selectivity, and resolution.
2. Predict changes in separation metrics upon a change in conditions (e.g., column length, particle size, flow rate, etc.).

Retention

1. (GC/LC) Predict the effect of a change in conditions on relative retention (e.g., change in stationary phase, mobile phase, flow rate, etc.).
2. (GC/LC) Use chemical and physical reasoning to explain the basis of retention, given a specific scenario (e.g., why is benzyl alcohol less retained than benzene in RPLC?).

Peak Broadening

1. (GC/LC) Predict the effect of a change in conditions (e.g., mobile phase type, temperature, particle size, etc.) on peak properties (e.g., height, width, retention time).
2. (GC/LC) Use chemical and physical reasoning to explain the basis of peak broadening, given a specific scenario (e.g., why is the peak for anthracene broader than the peak for benzene under RPLC conditions?).

GC Retention

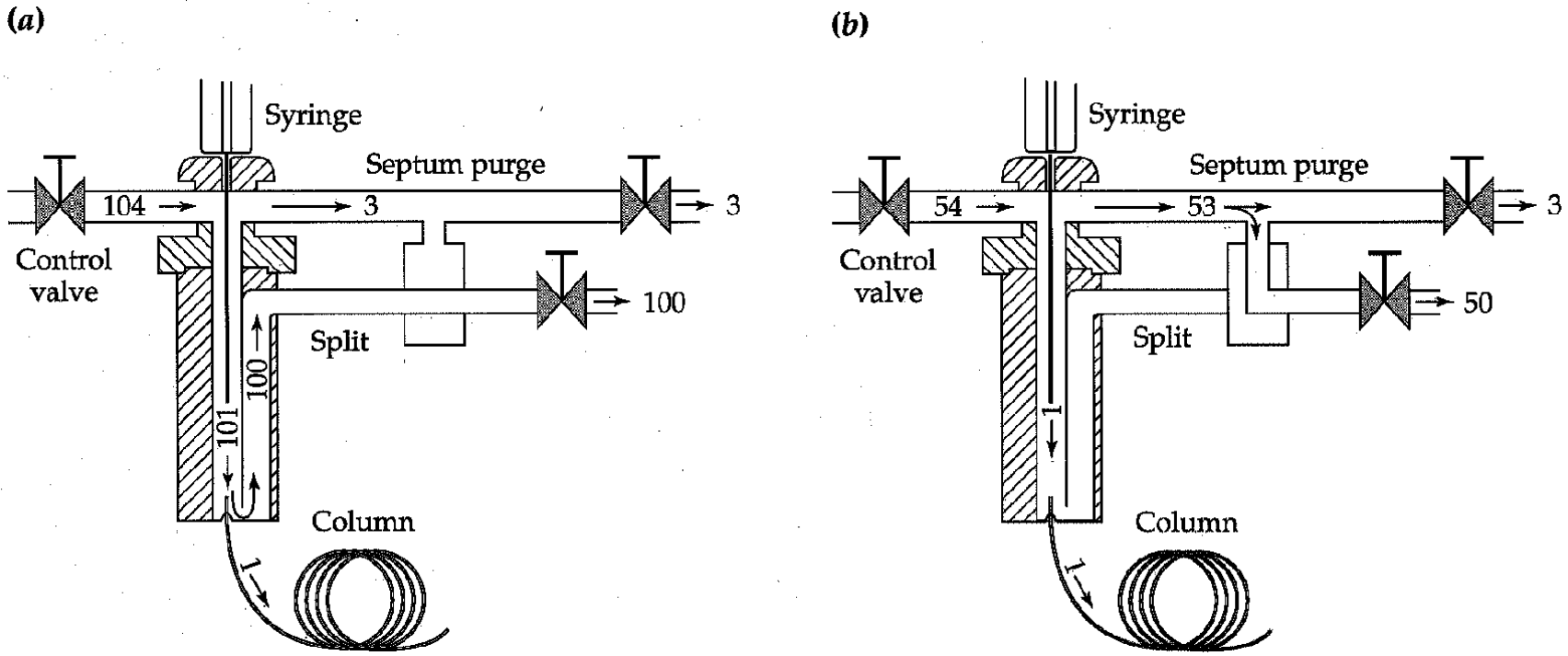


FIGURE 15.3 ▲

Schematic illustration of the structure and operation of a sample splitter.

(Left) Split configuration. The apparatus is enclosed in an oven at a temperature higher than that of the capillary column. The sample is injected, vaporized, and carried toward the top of the column by the carrier gas. The needle valve allows a precise adjustment of the fraction of sample that enters the column while the larger part is driven out through an exhaust. **(Right)** Splitless configuration. With the valve closed, the injector acts as a typical injector for a packed column, in which all of the sample flows to the column. The splitter shown also includes a **septum purge** valve to eliminate volatiles that arise from septum outgassing and decomposition. [Redrawn from HP 5890 Tutorial, courtesy of Hewlett-Packard]

TABLE 27-3 Some Common Liquid Stationary Phases for GLC

Stationary Phase	Common Trade Name	Maximum Temperature, °C	Common Applications
Polydimethyl siloxane	OV-1, SE-30	350	General-purpose nonpolar phase, hydrocarbons, polynuclear aromatics, steroids, PCBs
5% Phenyl-polydimethyl siloxane	OV-3, SE-52	350	Fatty acid methyl esters, alkaloids, drugs, halogenated compounds
50% Phenyl-polydimethyl siloxane	OV-17	250	Drugs, steroids, pesticides, glycols
50% Trifluoropropyl-polydimethyl siloxane	OV-210	200	Chlorinated aromatics, nitroaromatics, alkyl substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids, alcohols, ethers, essential oils, glycols
50% Cyanopropyl-polydimethyl siloxane	OV-275	240	Polyunsaturated fatty acids, rosin acids, free acids, alcohols

Skoog, Holler, et al. (2007). Principles of Instrumental Analysis, Thomson Brooks/Cole.

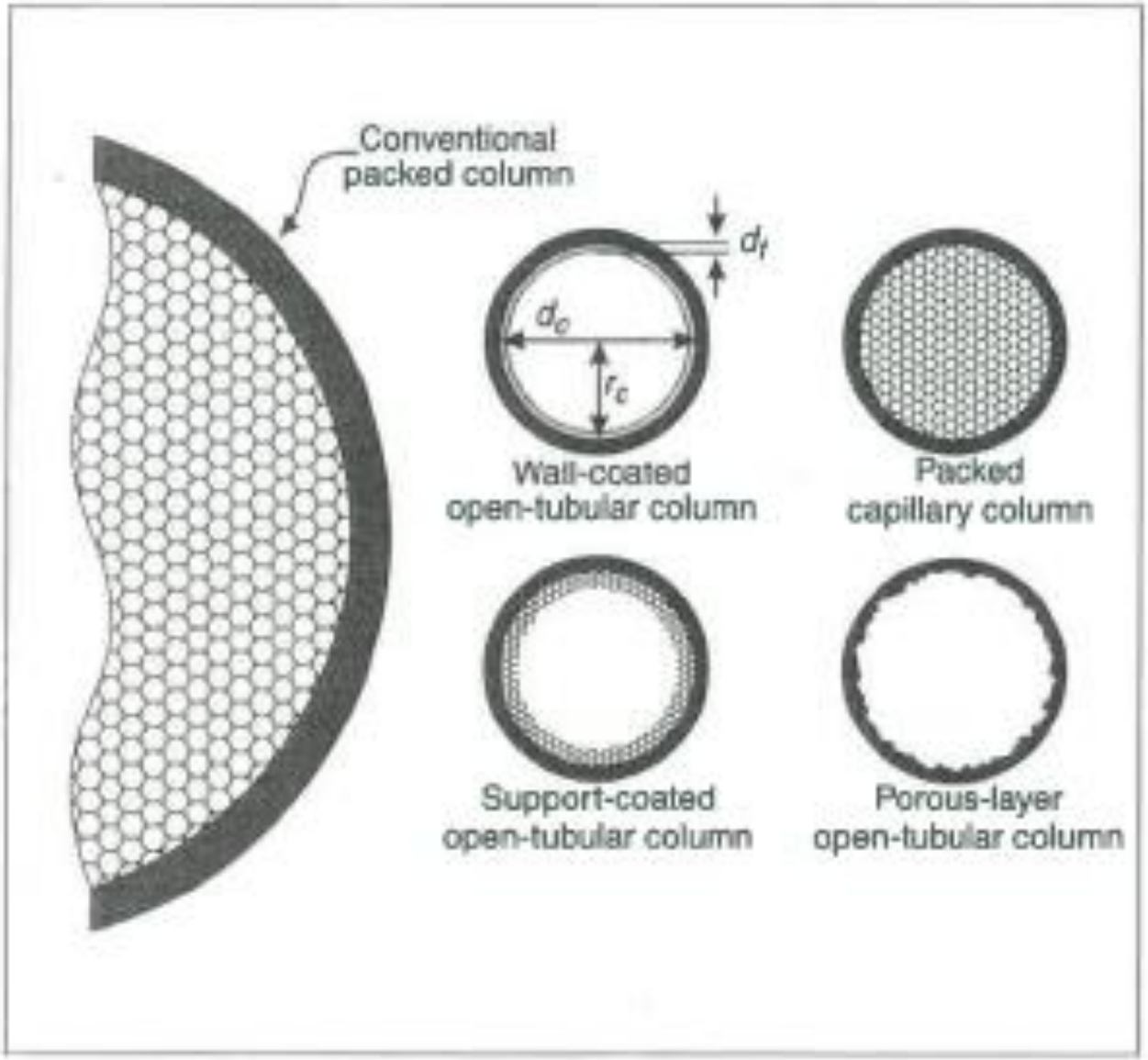


Figure 25. Types of open-tubular GC columns compared to a packed column. Drawn to scale for 0.53–mm i.d. open-tubular and 2–mm i.d. packed columns.

Hinshaw, J. V.; Ettre, L. S. *Introduction to Open Tubular Gas Chromatography*; Advanstar Communications: Cleveland, 1994.

Column Type	Open Tube		Open Tube		Open Tube/Packed Bed	
Column Diameter	Narrow ~ 0.1-0.3 mm		Narrow ~ 0.25-0.32 mm		Wide > 0.5 mm	
S.P. Film Thickness	Thin ~ 0.2 microns		Thick ~ 1-2 microns		Thick - 2-5 microns (open tube only)	
		Why		Why		Why
Efficiency	Best		Good		Poor	
Separation speed	Fast		Moderate		Slow	
Analyte Capacity	Poor		Good		Best	
Preferred Application	High speed, high resolution separations		Low boiling point compounds		Anybody can do it	
	High boiling point compounds		Trace analysis		Low boiling point compounds	
	Thermally unstable compounds					

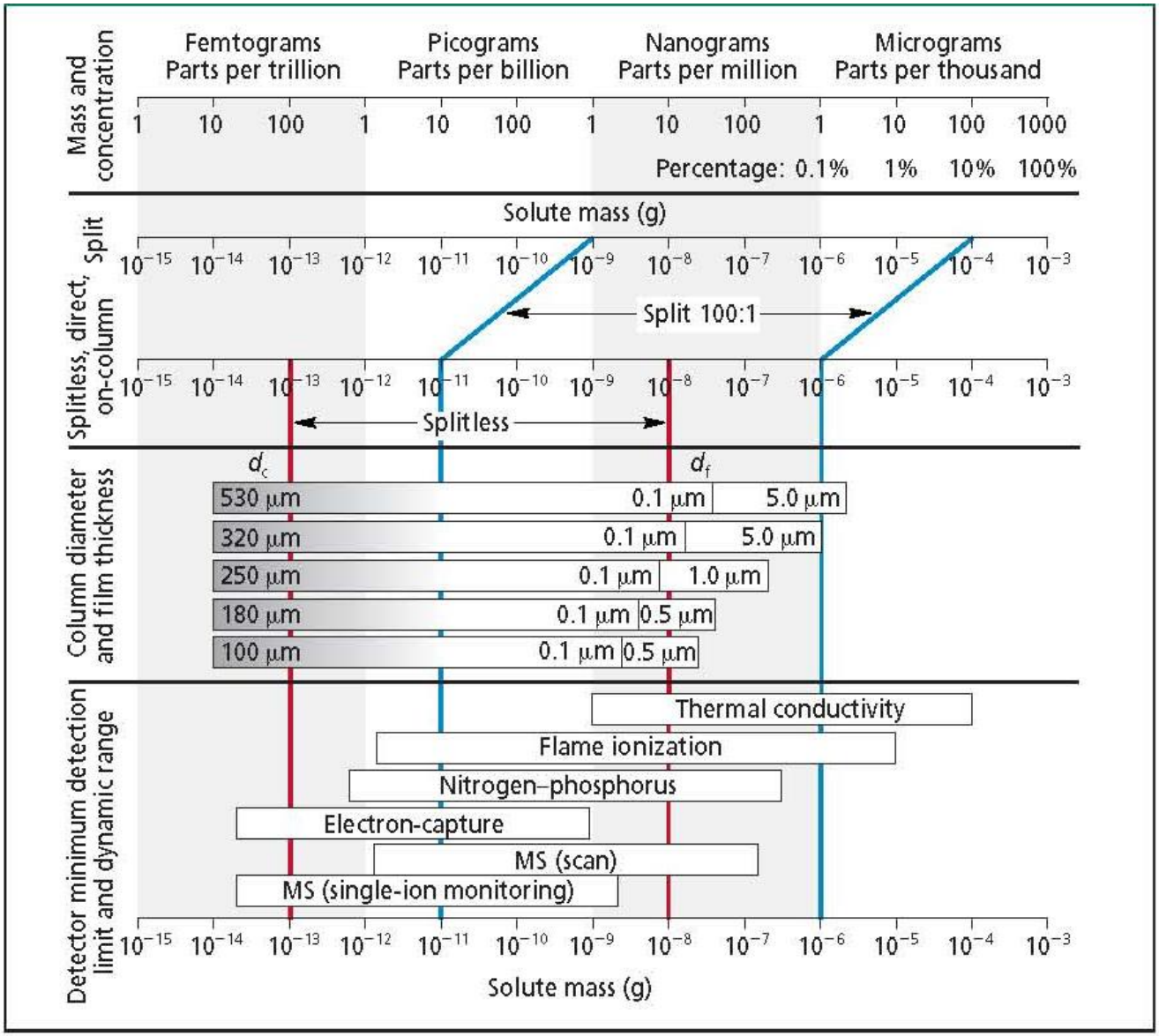


Figure 1: GC dynamic range nomogram. Concentrations are expressed in grams per microliter. Column data are from references 2 and 3 and manufacturers' information. Detector data are from reference 4 and manufacturers' aggregated 1999 specifications. Individual detector models will vary — always obtain exact specifications from the manufacturer.

Hinshaw, J. LCGC North Am. 24 (11), 2006

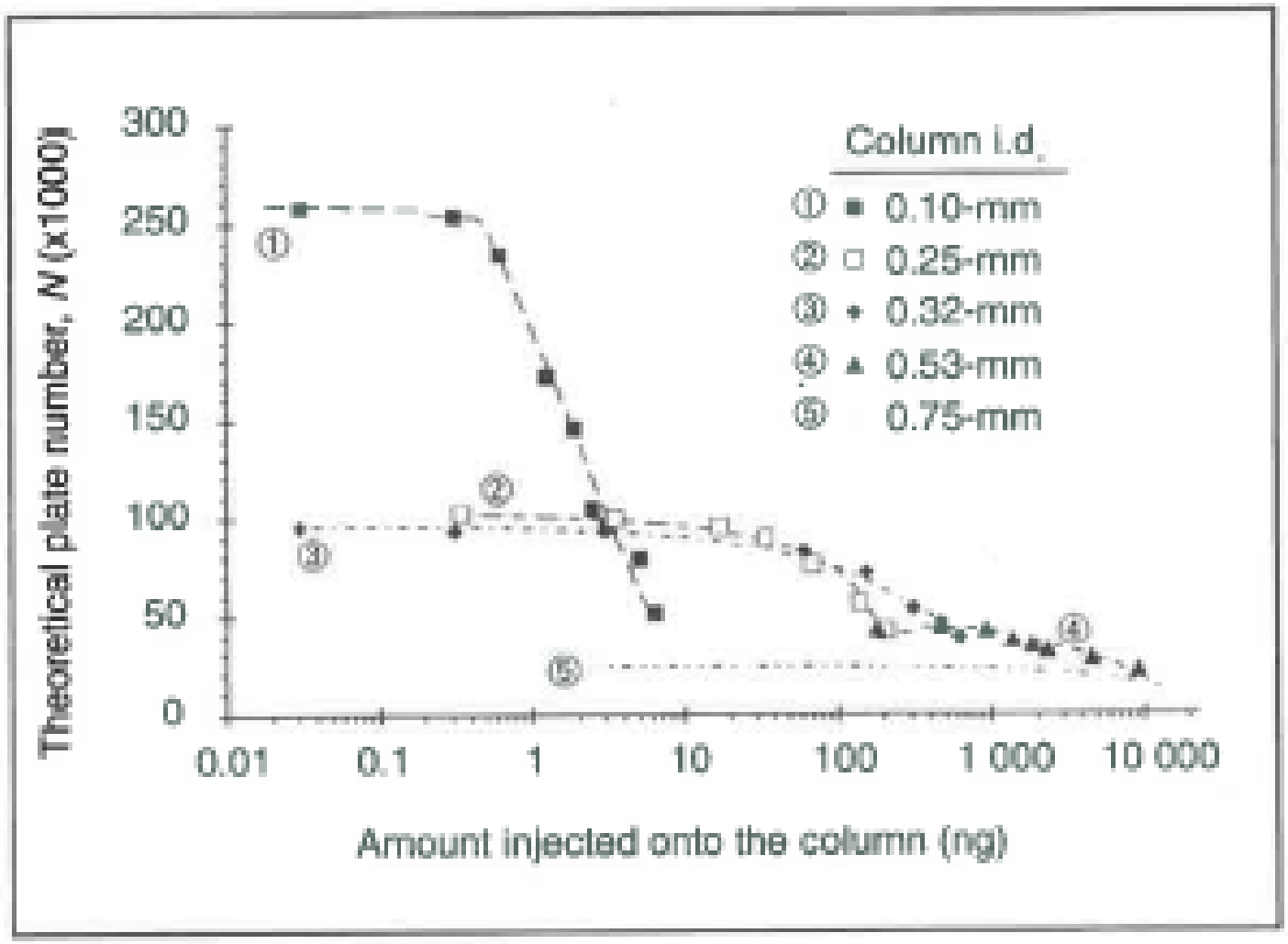


Figure 32. Number of theoretical plates calculated for n-undecane as a function of the injected sample amount. The column i.d. is indicated in the figure. All data refers to 25–m column lengths. See Table 13 on page 100 for the analytical conditions[75].

Hinshaw, J. V.; Ettre, L. S. *Introduction to Open Tubular Gas Chromatography*; Advanstar Communications: Cleveland, 1994.

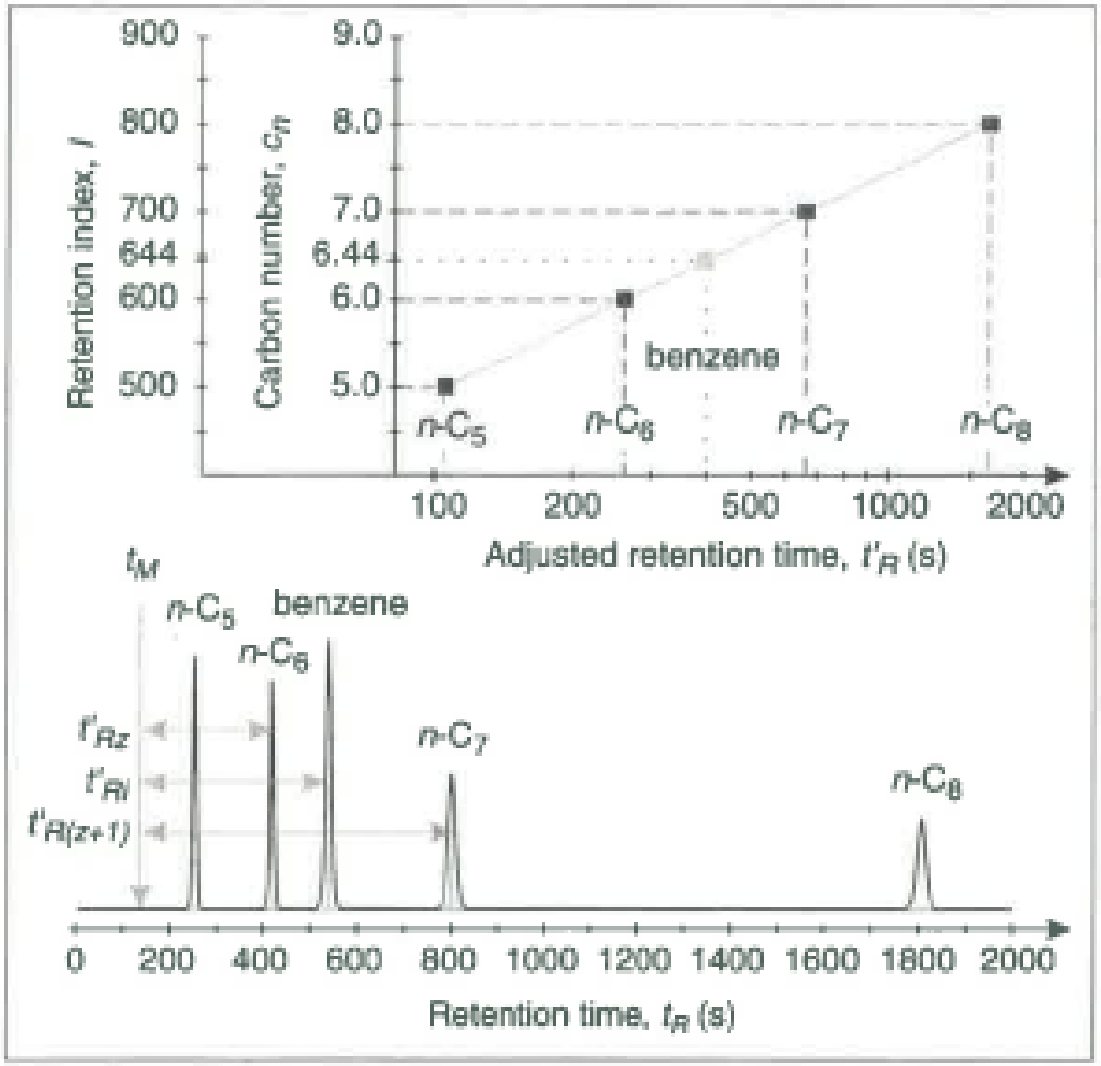


Figure 10. Calculation of the isothermal retention index. Solute i is benzene; its retention index is calculated as 644. Data refer to a 0.25-mm i.d. open-tubular column coated with methylsilicone phase and operated at 60 °C. See Table 6 on the next page for numerical data.

Hinshaw, J. V.; Ettre, L. S. *Introduction to Open Tubular Gas Chromatography*; Advanstar Communications: Cleveland, 1994.

$$I = 100\left(z + \frac{\log t'_{R\,i} - \log t'_{R\,z}}{\log t'_{R\,(z+1)} - \log t'_{Rz}} \right)$$

Peak	t_R (s)	t_R' (s)	I
t_M	147.9	—	—
n-C_5	251.8	103.9	500
n-C_6 (z)	410.0	262.1	600
benzene (i)	543.3	395.4	644
n-C_7 (z+1)	809.2	661.3	700
n-C_8	1816.8	1668.9	800

Table 6. Data for Figure 10.

Example: Calculating the retention index. Table 6 lists the data from Figure 10. Taking the adjusted retention times (t_R') for *n*-C_6, *n*-C_7, and the analyte (*i*), we can calculate the retention index, *I*:

$$I = 100 \times \left(6 + \frac{\log(\,395.4\,) - \log(\,262.1\,)}{\log(\,661.3\,) - \log(\,262.1\,)} \right) = 644$$

Hinshaw, J. V.; Ettre, L. S. *Introduction to Open Tubular Gas Chromatography*; Advanstar Communications: Cleveland, 1994.

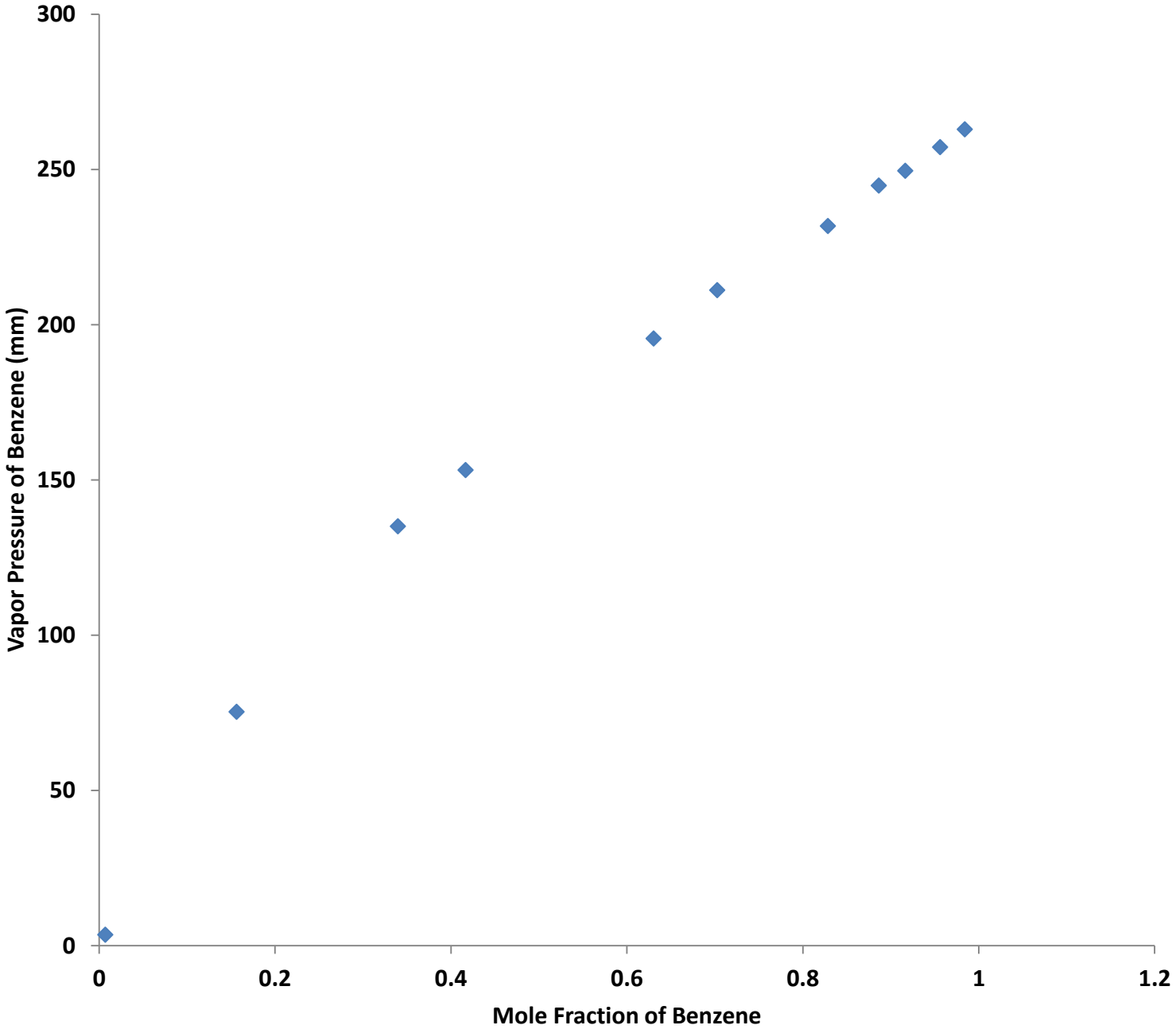

300
250
200
150
100
50
0
Vapor Pressure of Benzene (mm)
0
0.2
0.4
0.6
0.8
1
1.2
Mole Fraction of Benzene

TABLE 3.17

TYPICAL FIGURES TO TEST THE VALIDITY OF THE ADDITIVITY OF GROUP RETENTION INDICES

(*a*) Sample retention indices[a]

No.	Solute	Retention index		
		I^b	I^c	I^d
1	1-Pentene	483	402	553
2	*n*-Propanol	513	662	1132
3	Allyl alcohol	504	672	1203
4	Propyl acetate	653	737	1032
5	Allyl acetate	638	737	1085
6	Butyl methyl ether	594	640	782
7	Ethylene glycol dimethyl ether	607	683	999
8	*n*-Butanol	620	759	1243
9	1,4-Butane diol	900	1170	2100
10	*iso*-Pentane	475	475	475
11	2-Butanone	552	648	891
12	3-Methyl-2-butanone	619	708	1008
13	Methylal	476	545	788
14	Methoxymethylal	654	745	1094
15	Dimethoxymethylal	826	937	1372

Littlewood, A. B. (1970). Gas Chromatography. New York, Academic Press.

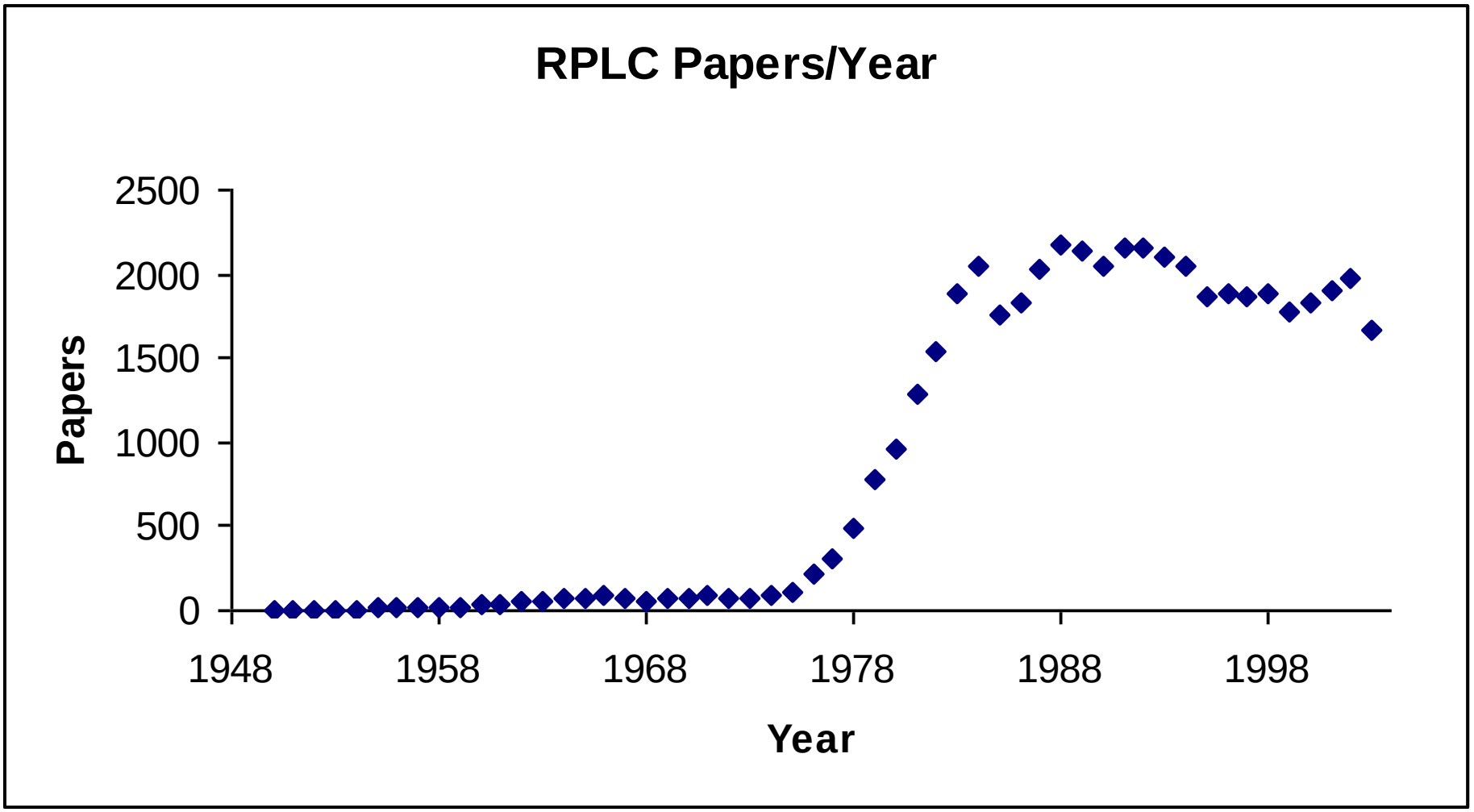
LC Retention in Three Parts

LC Retention – Part I
Context for and Empirical Knowledge of RPLC

Complementarity of GC and LC

Column types (physical) and phases (chemistries)

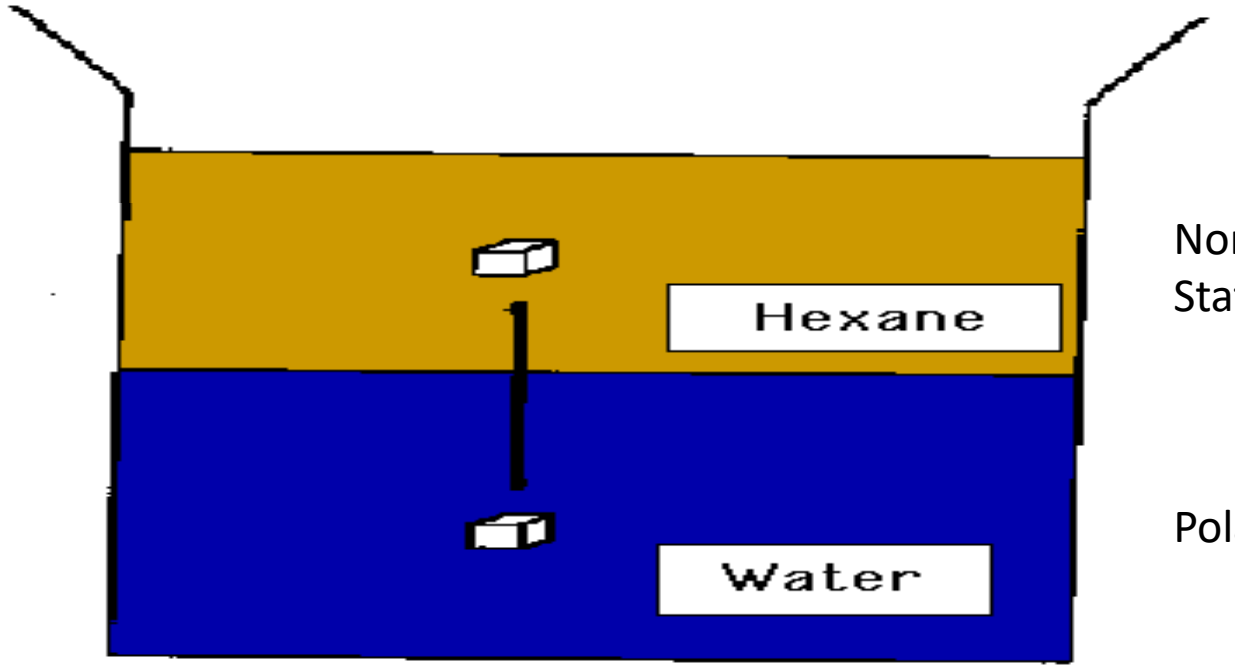
History of RPLC



Partition Model of RPLC

$$K = \frac{[A]_s}{[A]_m}$$

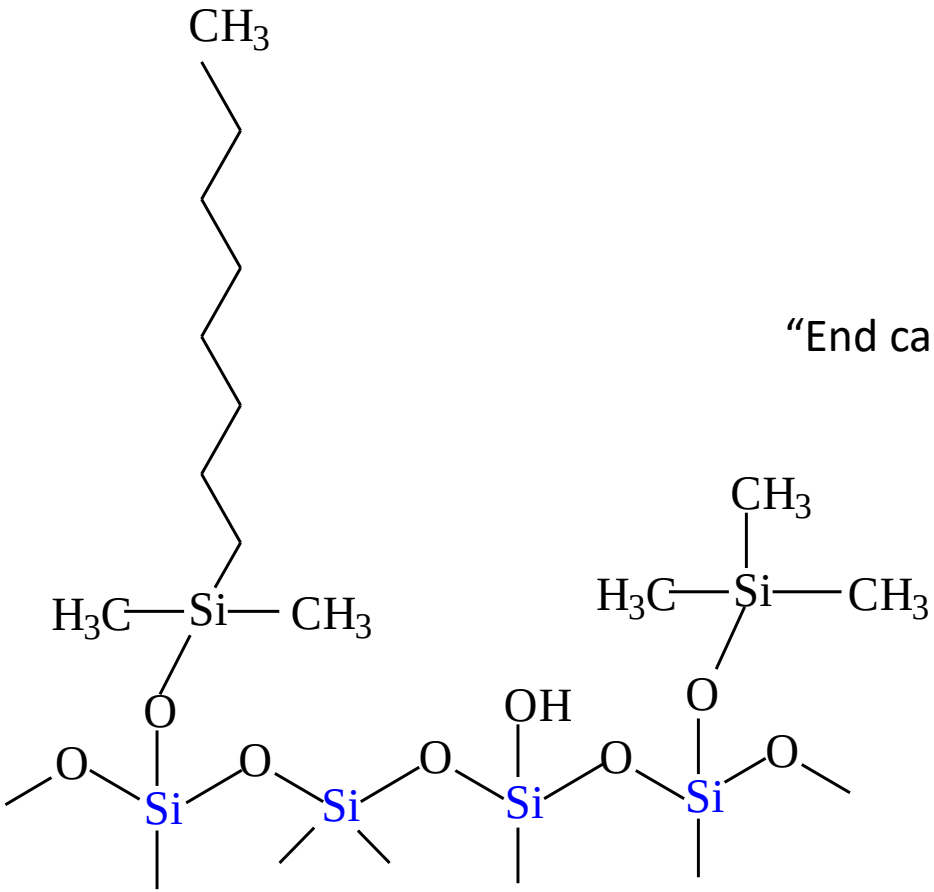
$A_m \rightleftharpoons A_s$ (K)



Non-Polar
Stationary Phase

Polar Mobile Phase

Conventional Bonded Phases



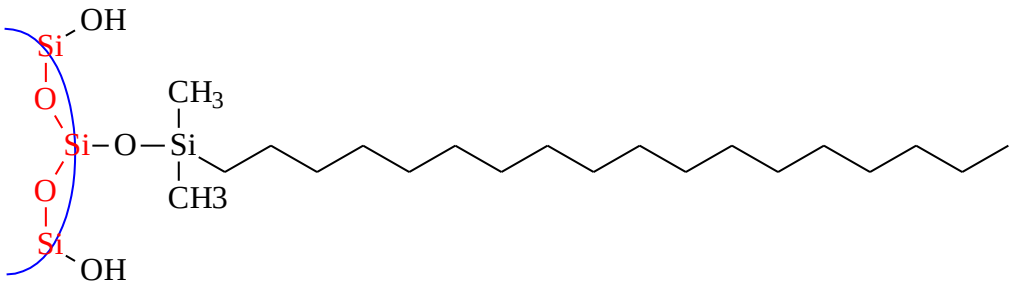
"End cap"

Siloxane Bond

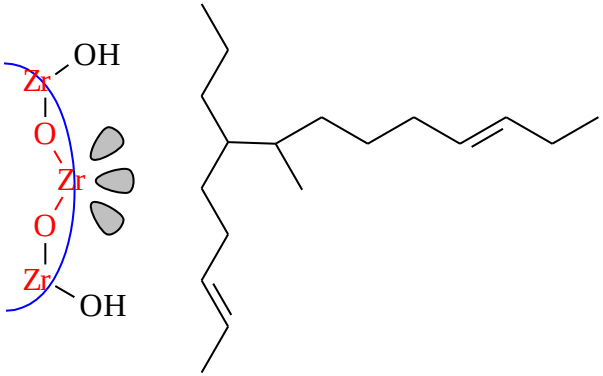
Endcapped octylsilane bonded phase

Typical silanes are C_8 and C_{18} (octadecylsilane = ODS)

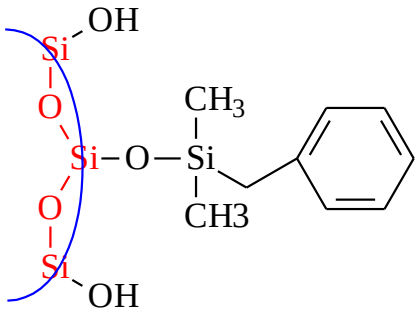
Structures of Stationary Phases



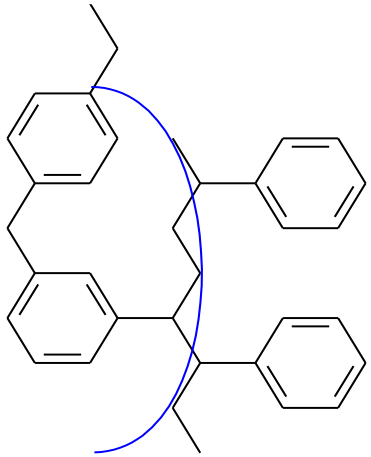
C18-SiO_2



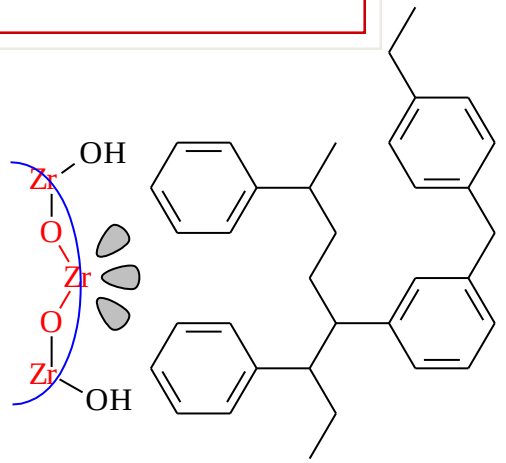
PBD-ZrO_2
PBD=Polybutadiene



Phenyl-SiO_2



PRP-1
PRP=Polystyrene-Divinylbenzene



PS-ZrO_2
PS=Polystyrene

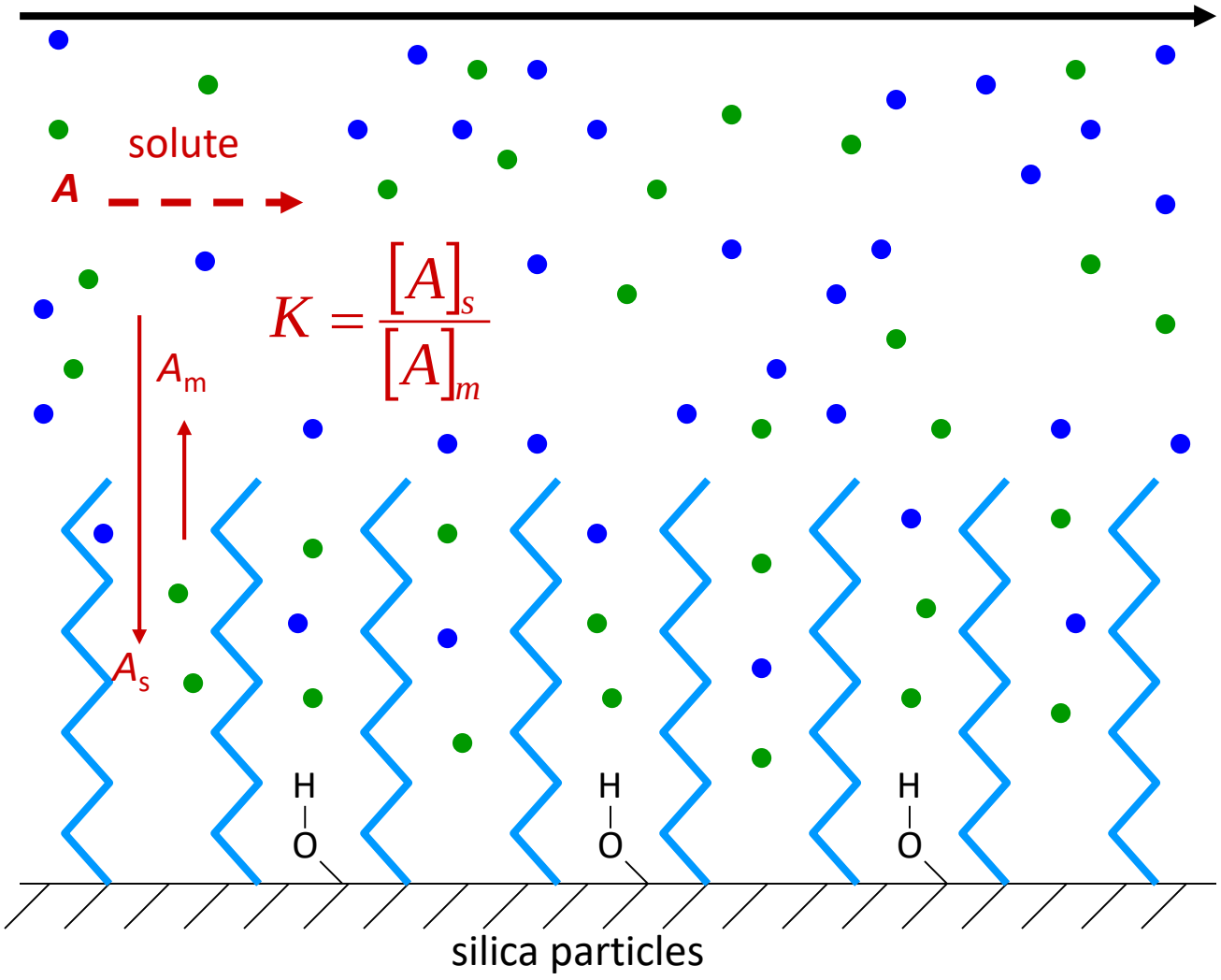
RPLC Retention

Polar
Mobile Phase

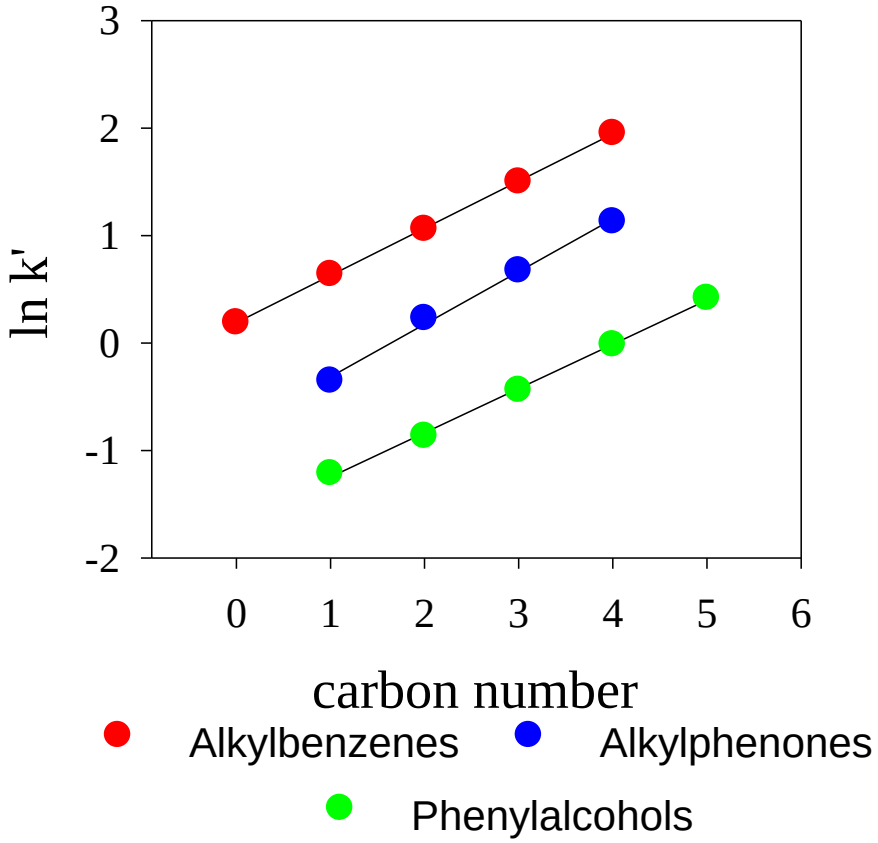
e.g. MeOH +H_2O

Non-polar
Stationary Phase

e.g. C_8 hydrocarbon



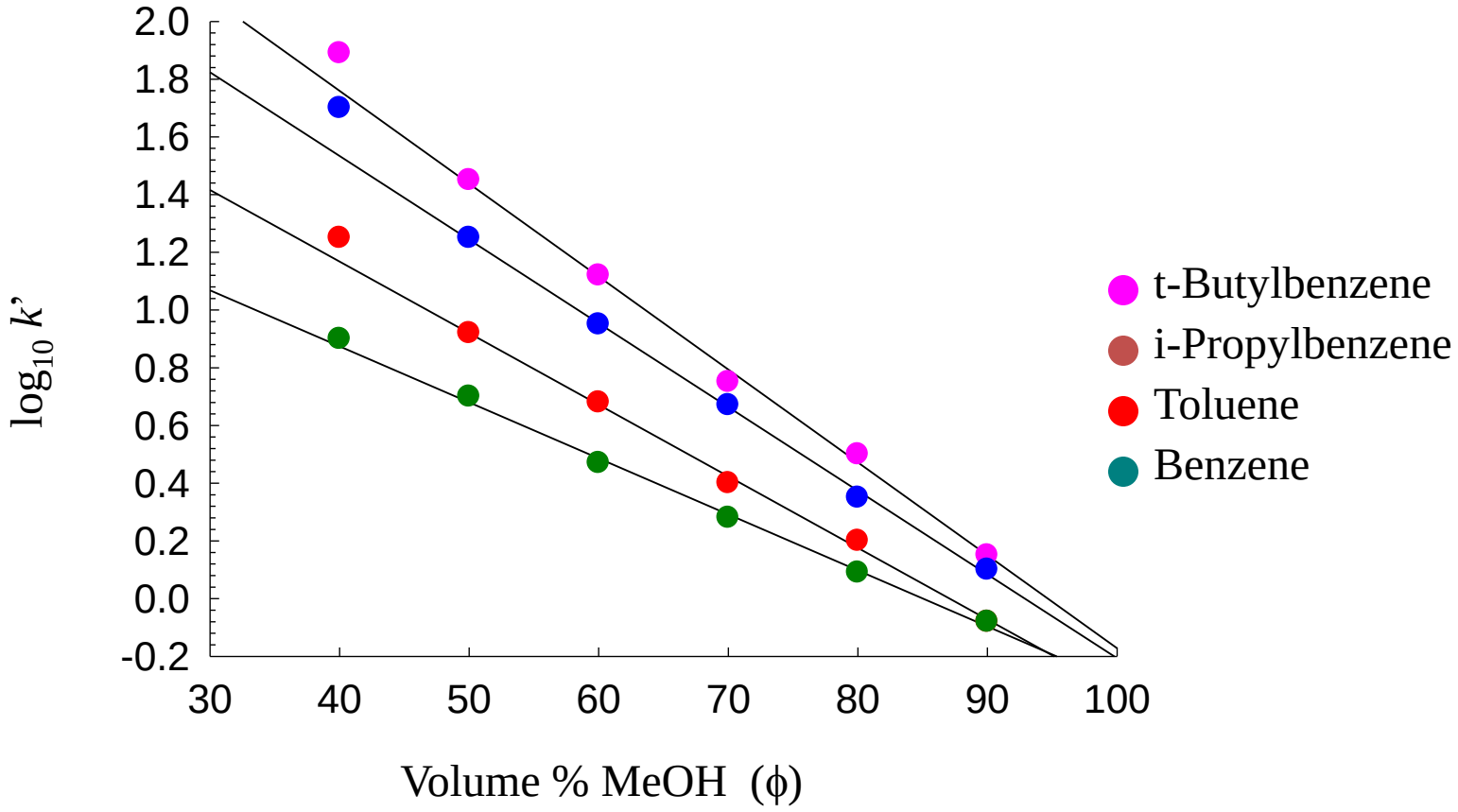
Reversed Phase Characteristics



$$\ln k' = A + Bn_{CH_2}$$

Martin Eqn.

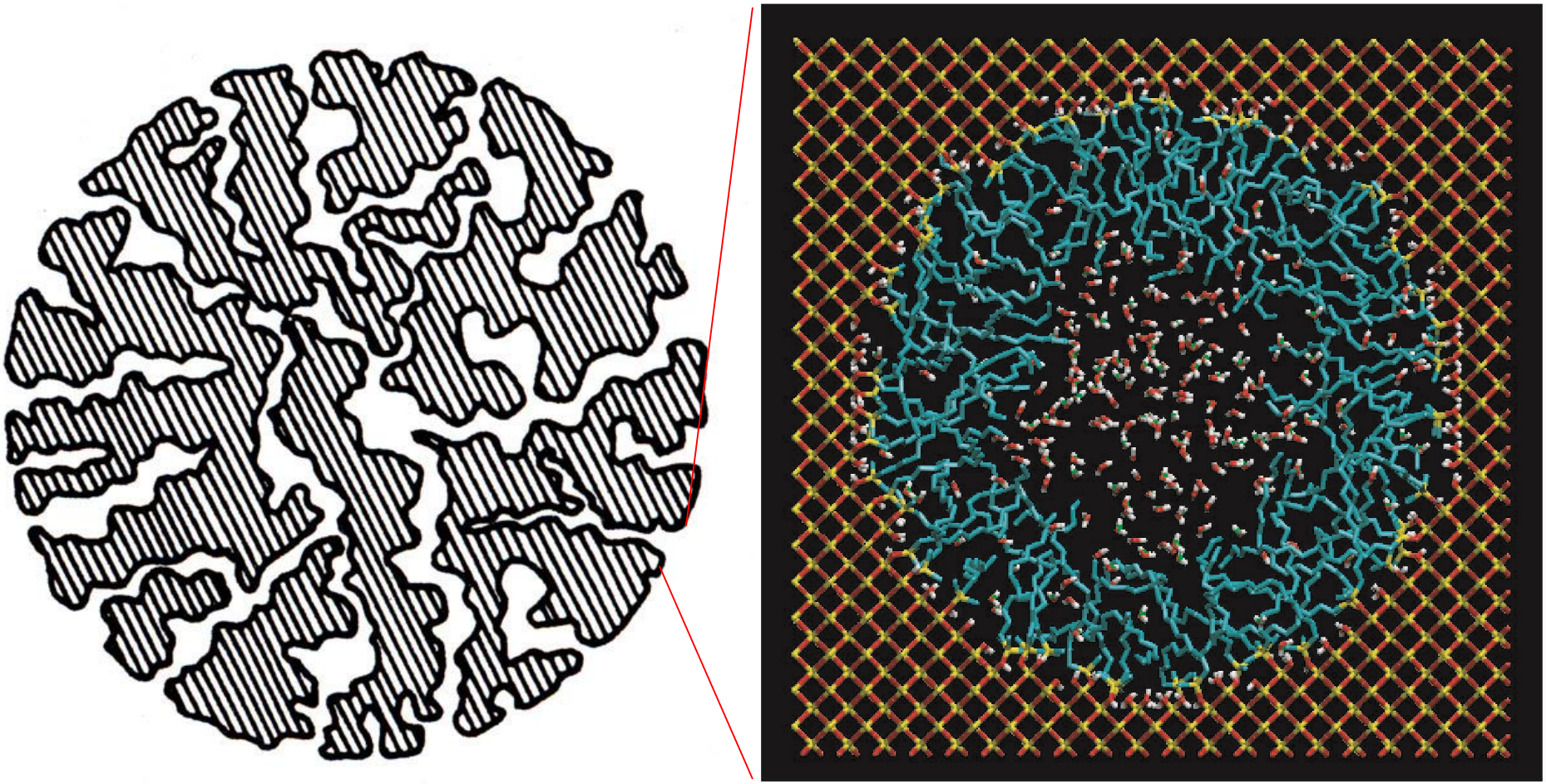
Effect of Mobile Phase Modifier in RPLC



$$\log k' = \log k'_w - S^*\phi;\ LSST\ (Snyder\ Eqn.)$$

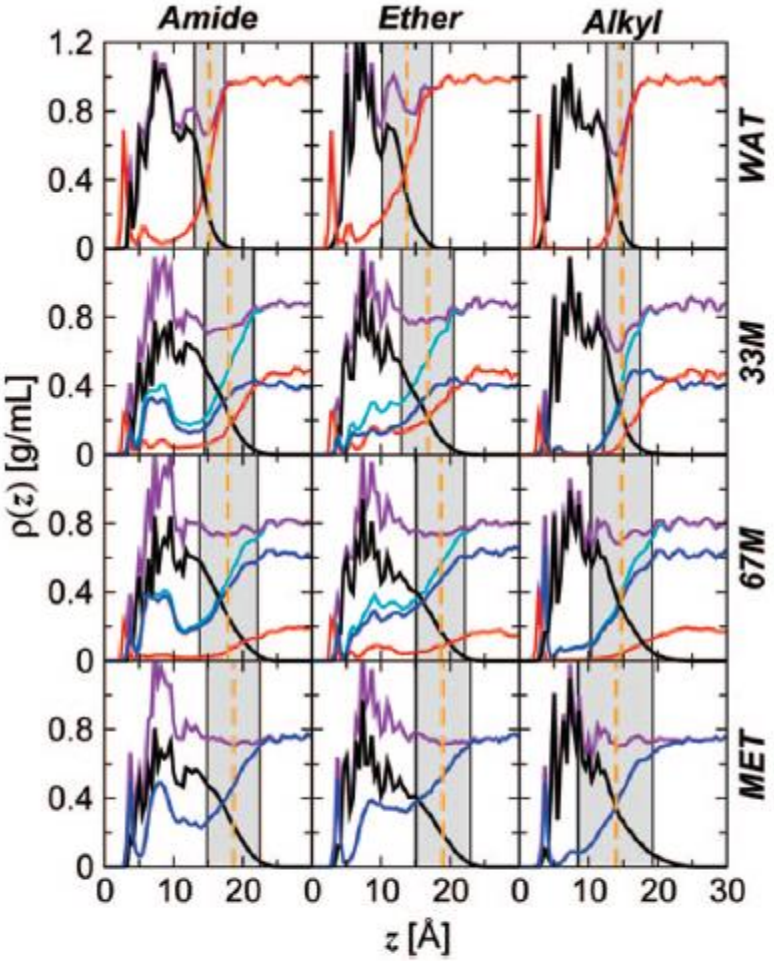
Water is the weakest solvent in RPLC. Organic is stronger solvent.

LC Retention – Part II
Thermodynamics of RPLC



PWC 4101-06 Fig. 2.5 Pore structure of a stationary phase particle. 60-80 A pores 200-300 m^2/g.

Rafferty, Siepmann, Schure, A-Chem, ca. 2007



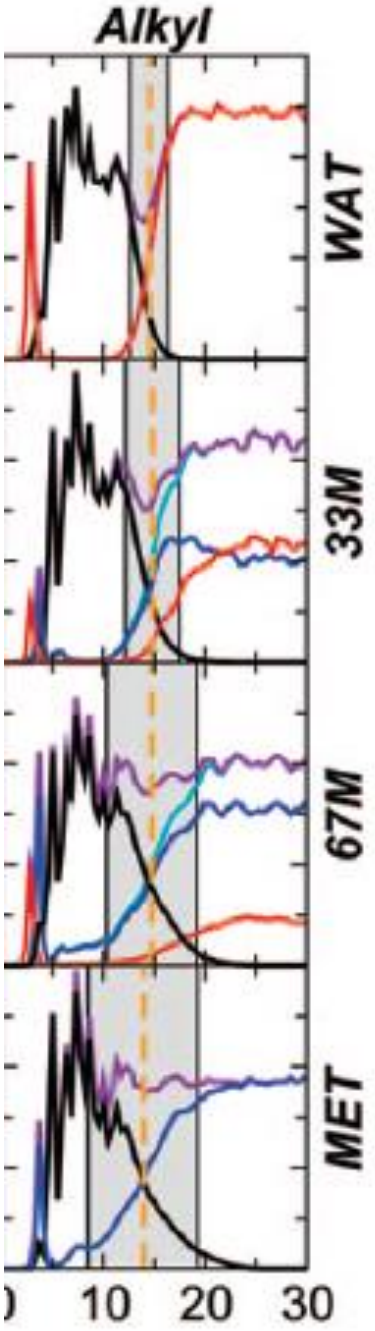


Figure 3. Bonded phase (black, excluding methyl side chains), water (red), methanol (blue), combined solvent (cyan), and total (violet) density profiles. Vertical dashed orange lines denote the Gibbs dividing surface, and the shaded gray area represents the 10−90 interfacial region as defined by the overall solvent density. These and subsequent profiles were computed using a bin width of 0.45 Å.

Rafferty, J. L.; Siepmann, J. I.; Schure, M. R. *Anal. Chem. (Washington, DC, U. S.)* ***2008, 80, 6214-6221.***

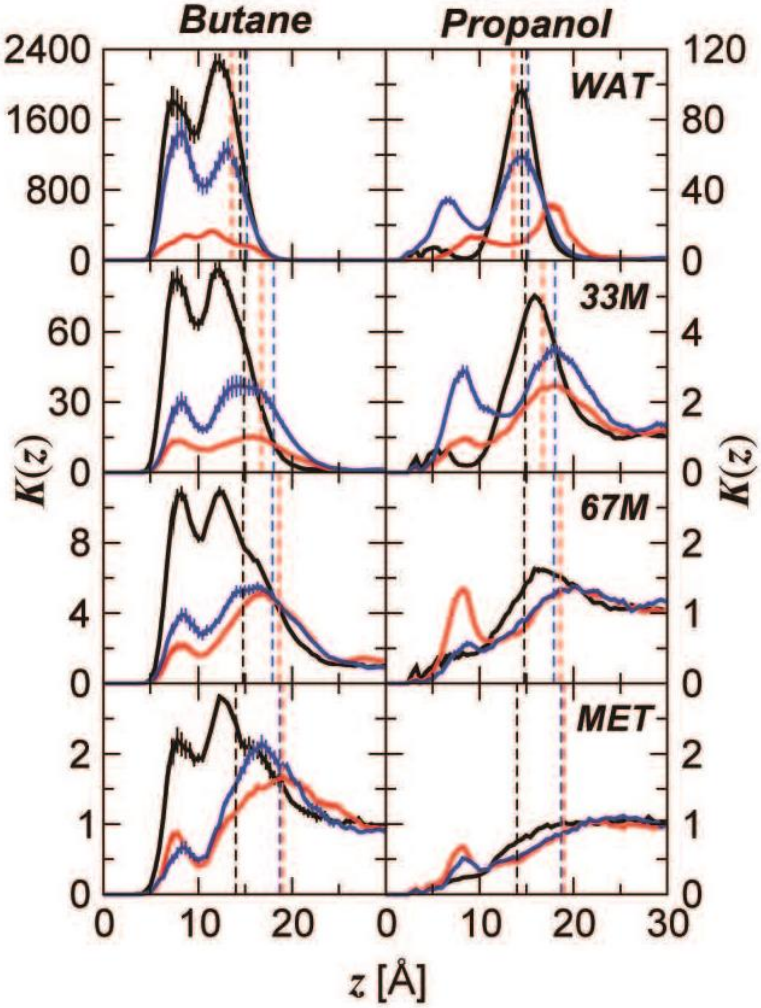
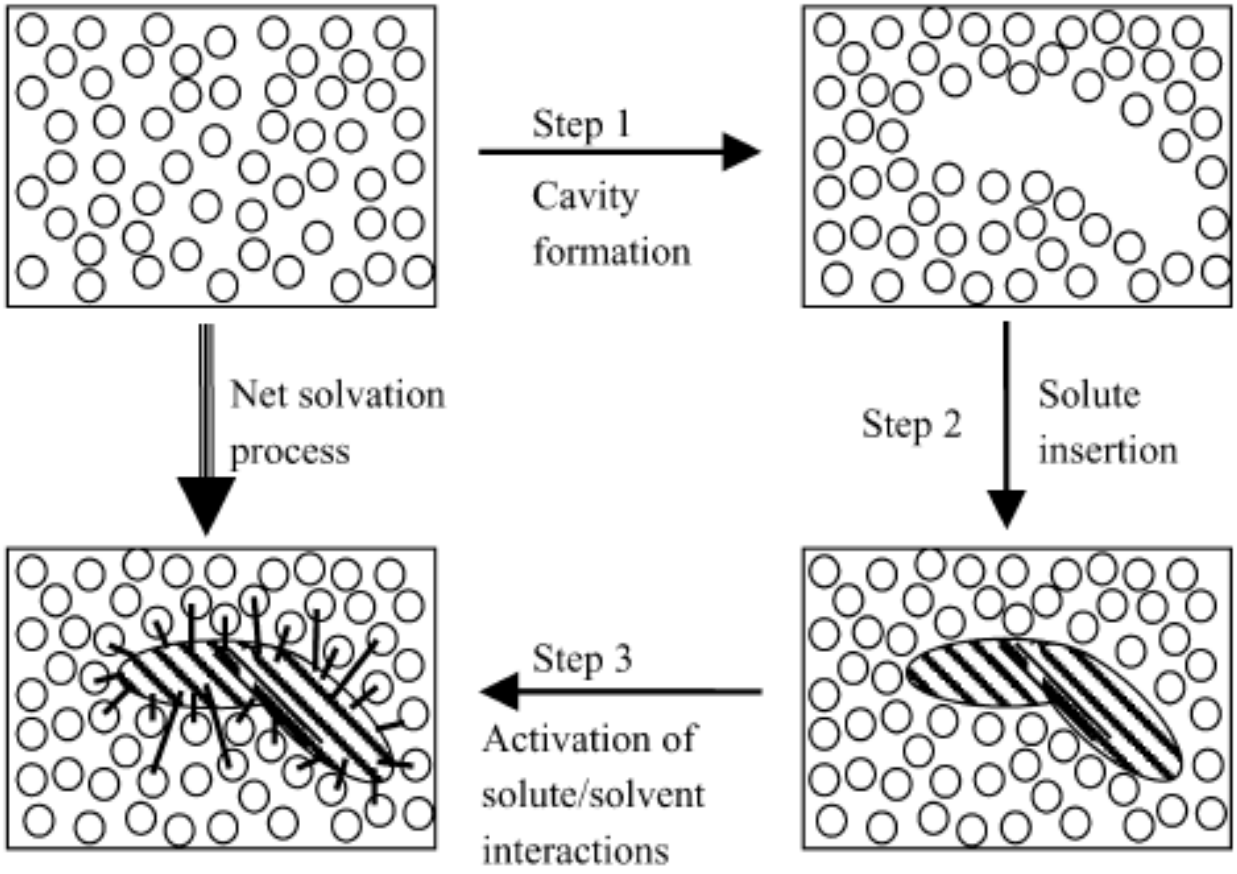


Figure 8. Distribution coefficient profiles for *n*-butane and 1-propanol computed for the transfer from the solvent phase to the box containing the stationary phase (blue, red, and black profiles for amide, ether, and alkyl phase, respectively). Dashed vertical lines denote the GDS.

Rafferty, J. L.; Siepmann, J. I.; Schure, M. R. *Anal. Chem. (Washington, DC, U. S.)* ***2008, 80, 6214-6221.***


Step 1
Cavity
formation
Net solvation
process
Step 2
Solute
insertion
Step 3
Activation of
solute/solvent
interactions

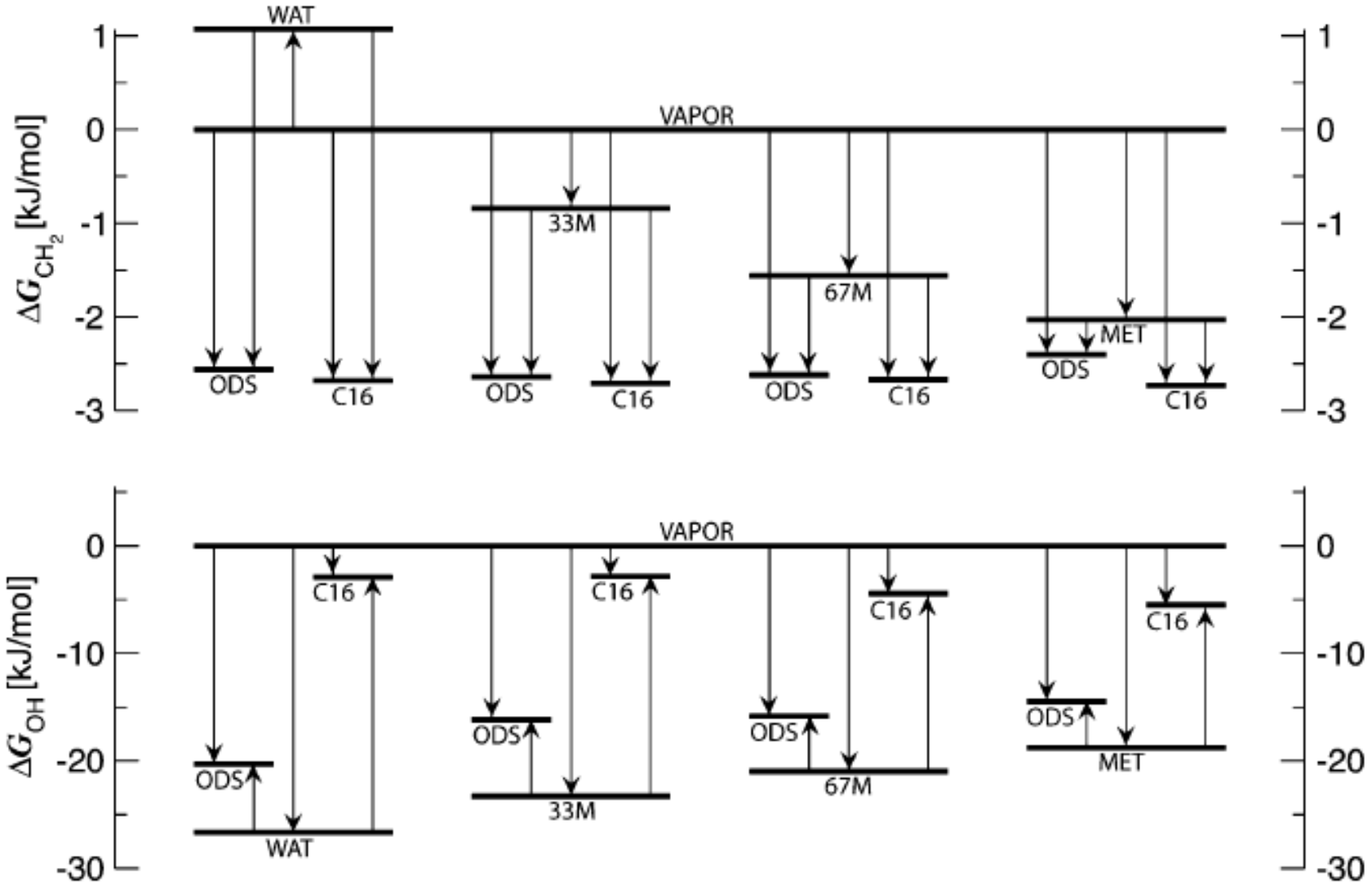


Figure 5. Thermodynamic driving forces for retention. The incremental free energies of transfer for methylene and hydroxyl groups into the stationary phase (ODS), the bulk *n*-hexadecane phase (C16), and the bulk mobile phase (WAT, 33M, 67M, MET) are shown with respect to the ideal gas reference phase (VAPOR)

Rafferty, J. L.; Zhang, L.; Siepmann, J. I.; Schure, M. R. *Anal. Chem. (Washington, DC, U. S.)* ***2007, 79, 6551-6558.***

Table 3.4 Air–Hexadecane, Air–Water, and Hexadecane–Water Equilibrium Partitioning of Hexane, Benzene, Diethylether, and Ethanol: Free Energies, Enthalpies, and Entropies of Transfer, as well as Partition Constants Expressed on a Molar Base (i.e., mol $\cdot L^{-1}$phase 1/mol $\cdot L^{-1}$phase 2)

Phase 1/Phase 2 Compound (i)	$\Delta_{12}G_i$ ($kJ \cdot mol^{-1}$)		$\Delta_{12}H_i$ ($kJ \cdot mol^{-1}$)		$T\Delta_{12}S_i$ ($kJ \cdot mol^{-1}$)	$\Delta_{12}S_i$ ($kJ \cdot mol^{-1}$)	K_{i12} [a]
Air/Hexadecane							
Hexane	4.2	=	31.0	–	26.8	89.9	2.2×10^{-3}
Benzene	4.9	=	30.4	–	25.5	85.6	1.7×10^{-3}
Diethylether	0.8	=	25.2	–	24.4	81.9	8.7×10^{-3}
Ethanol	–2.5	=	16.3	–	18.8	73.3	3.3×10^{-2}
Air/Water							
Hexane	–28.3	=	32.0	–	60.3	202.3	6.5×10^{1}
Benzene	–14.1	=	29.7	–	43.8	147.0	2.1×10^{-1}
Diethylether	–11.2	=	46.8	–	58.8	194.6	6.6×10^{-2}
Ethanol	3.1	=	52.6	–	49.5	166.3	2.0×10^{-4}
Hexadecane/Water							
Hexane	–32.5	=	1.0	–	33.5	112.4	3.0×10^{4}
Benzene	–19.0	=	1.3	–	20.3	68.1	1.3×10^{2}
Diethylether	–12.0	=	21.6	–	33.6	112.8	7.7×10^{0}
hanol	5.6	=	36.3	–	30.7	103.0	6.4×10^{-3}

[a] Eq. 3-13 with const. = $\overline{V}_1 / \overline{V}_2$; molar volumes at 25°C and 1 bar: $\overline{V}_{ideal\ gas} = 24.73\ L \cdot mol^{-1}$, $\overline{V}_{hexadecane} = 0.293\ L \cdot mol^{-1}$, $\overline{V}_{water} = 0.018\ L \cdot mol^{-1}$

Environmental Organic Chemistry by RP Schwarzenbach, PM Gschwend, & DM Imboden, 2nd edition, Wiley-Interscience, 2003.

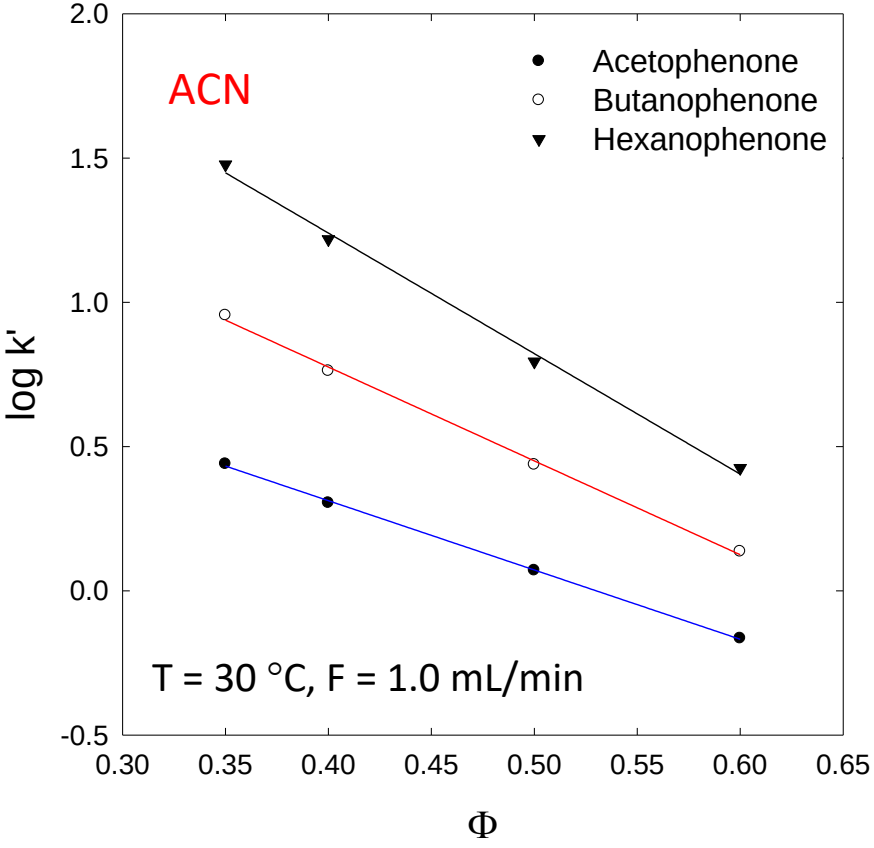
LC Retention – Part III
Practical RPLC

Solvent	Strength ε°	Viscosity η(mPa s)	Refractive index n_D^{20}	UV cutoff (nm)	Boiling point (°C)	Dipole π^*	Acidity α	Basicity β
Fluoroalkane FC-78	−0.19	0.4	1.267	210	50			
n-Pentane	0.00	0.23	1.3575	195	36			
n-Hexane	0.00	0.33	1.3749	190	69			
Isooctane	0.01	0.50	1.3914	200	99			
Cyclohexane*	0.03	1.00	1.4262	200	81			
Cyclopentane	0.04	0.47	1.4064	200	49			
Carbon tetrachloride	0.14	0.97	1.4652	265	77			
p-Xylene	0.20	0.62	1.4958	290	138	0.81	0.00	0.19
Diisopropyl ether	0.22	0.37	1.3681	220	68	0.36	0.00	0.64
Toluene	0.22	0.59	1.4969	285	111	0.83	0.00	0.17
Chlorobenzene	0.23	0.80	1.5248	290	132	0.91	0.00	0.09
Benzene	0.25	0.65	1.5011	280	80	0.86	0.00	0.14
Diethyl ether	0.29	0.24	1.3524	205	34.5	0.36	0.00	0.64
Dichloromethane	0.30	0.44	1.4242	230	40	0.73	0.27	0.00
Chloroform	0.31	0.57	1.4457	245	61	0.57	0.43	0.00
1,2-Dichloroethane	0.38	0.79	1.4448	230	83	1.00	0.00	0.00
Triethylamine	0.42	0.38	1.4010	230	89	0.16	0.00	0.84
Acetone	0.43	0.32	1.3587	330	56	0.56	0.06	0.38
Dioxane	0.43	1.54	1.4224	220	101	0.60	0.00	0.40
Methyl acetate	0.46	0.37	1.3614	260	56	0.55	0.05	0.40
Tetrahydrofuran	0.48	0.46	1.4072	220	66	0.51	0.00	0.49
tert. Butylmethyl ether	0.48	0.35	1.3689	220	53	0.36	0.00	0.64
Ethyl acetate	0.48	0.45	1.3724	260	77	0.55	0.00	0.45
Dimethyl sulphoxide	0.48	2.24	1.4783	270	189	0.57	0.00	0.43
Nitromethane	0.49	0.67	1.3819	380	101	0.64	0.17	0.19
Acetonitrile	0.50	0.37	1.3441	190	82	0.60	0.15	0.25
Pyridine	0.55	0.94	1.5102	305	115	0.58	0.00	0.42
Isopropanol	0.60	2.3	1.3772	210	82	0.22	0.35	0.43
Ethanol	0.68	1.20	1.3614	210	78	0.25	0.39	0.36
Methanol	0.73	0.60	1.3284	205	65	0.28	0.43	0.29
Acetic acid	High	1.26	1.3719	260	118	0.31	0.54	0.15
Water	Higher	1.00	1.3330	<190	100	0.39	0.43	0.18
Salt solutions, buffers	Highest							

* Becomes solid at 350 bar!

Linear Solvent Strength Characterization

$$\log k' = \log k'_w - S\phi$$

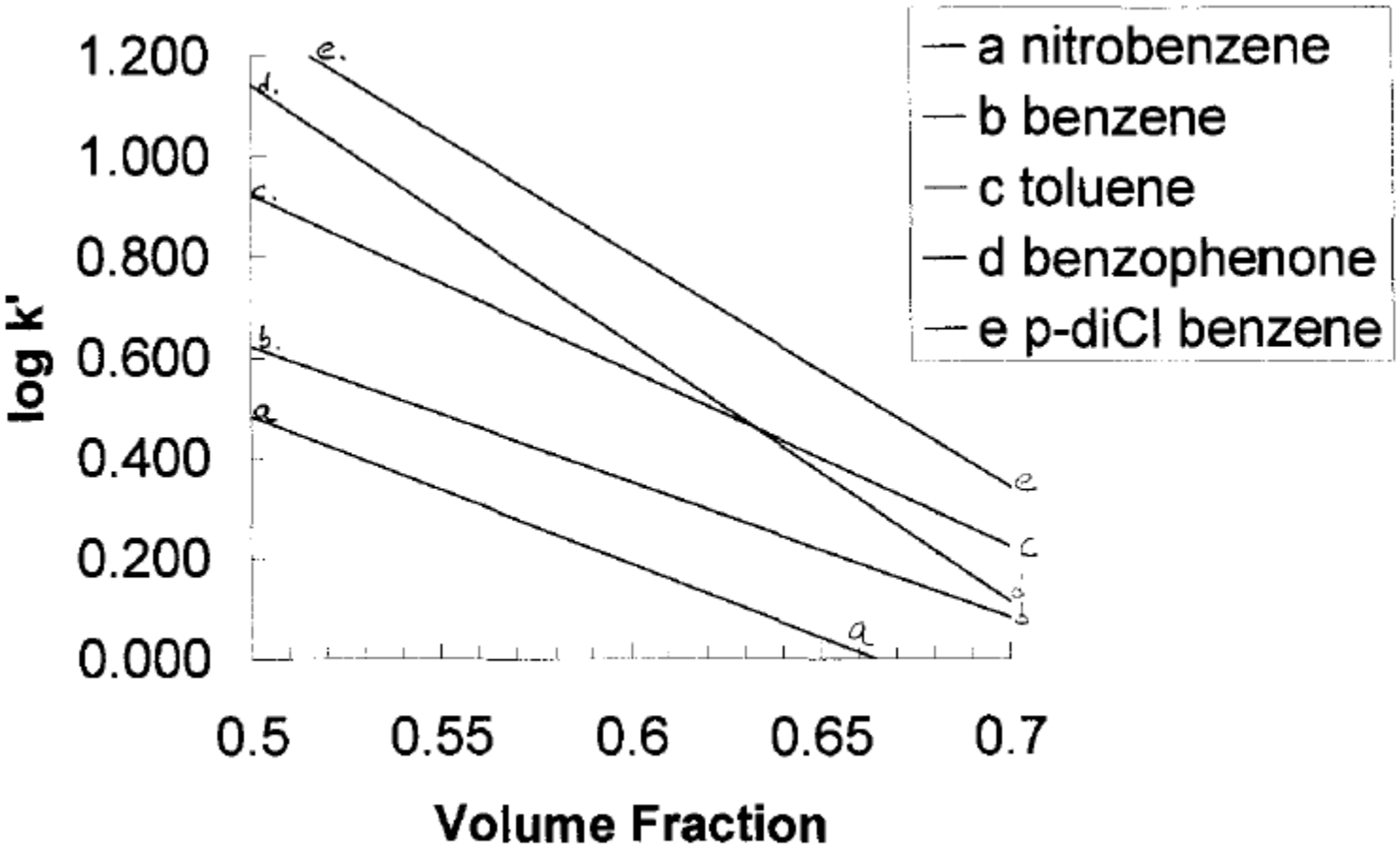


Calculated S Values

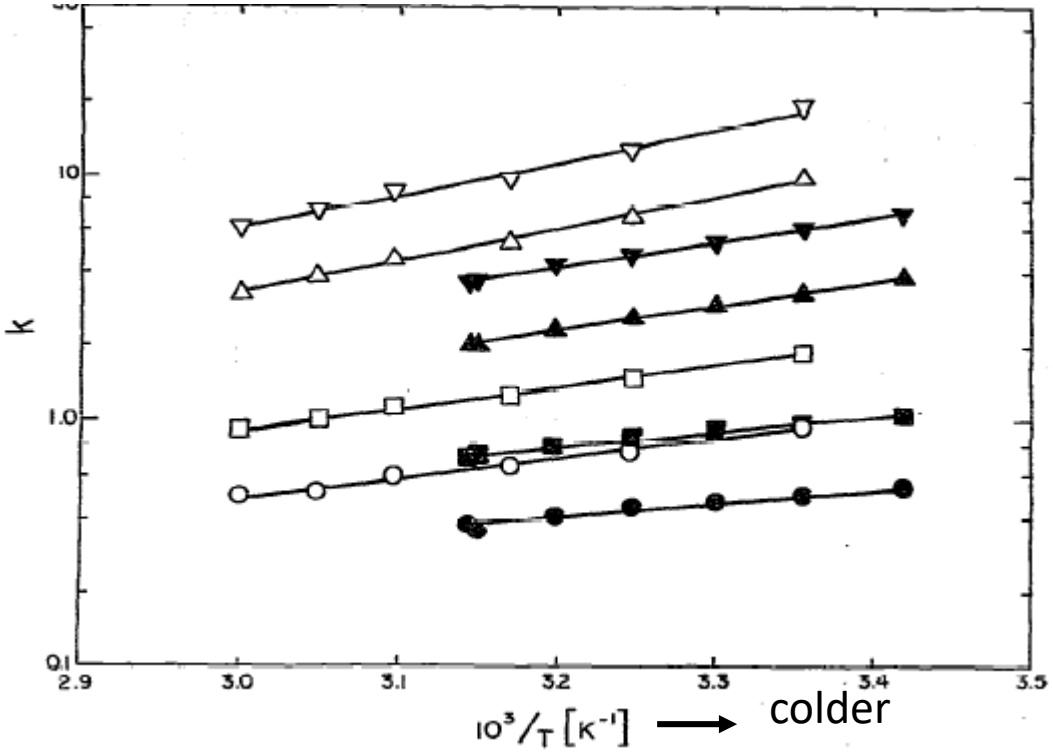
	ACN	CH_3OH	THF
AP	2.40 ± 0.04	2.60 ± 0.01	2.76 ± 0.07
BP	3.25 ± 0.10	3.53 ± 0.01	4.11 ± 0.13
HP	4.18 ± 0.18	4.52 ± 0.02	5.70 ± 0.24

The highly crosslinked C8 phase behaves as a typical reversed phase material

Effect of Volume Fraction of Methanol

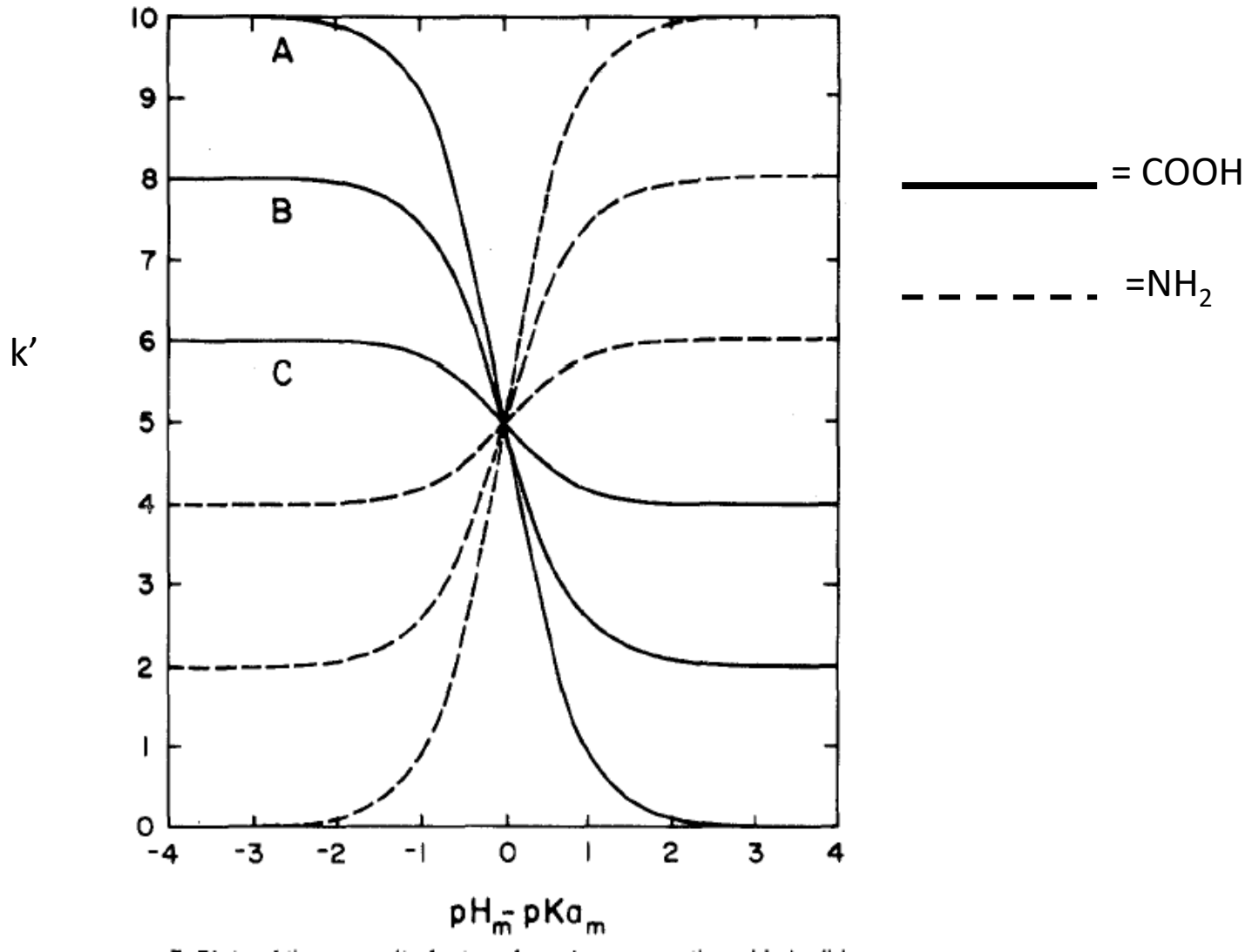


Effect of Temperature

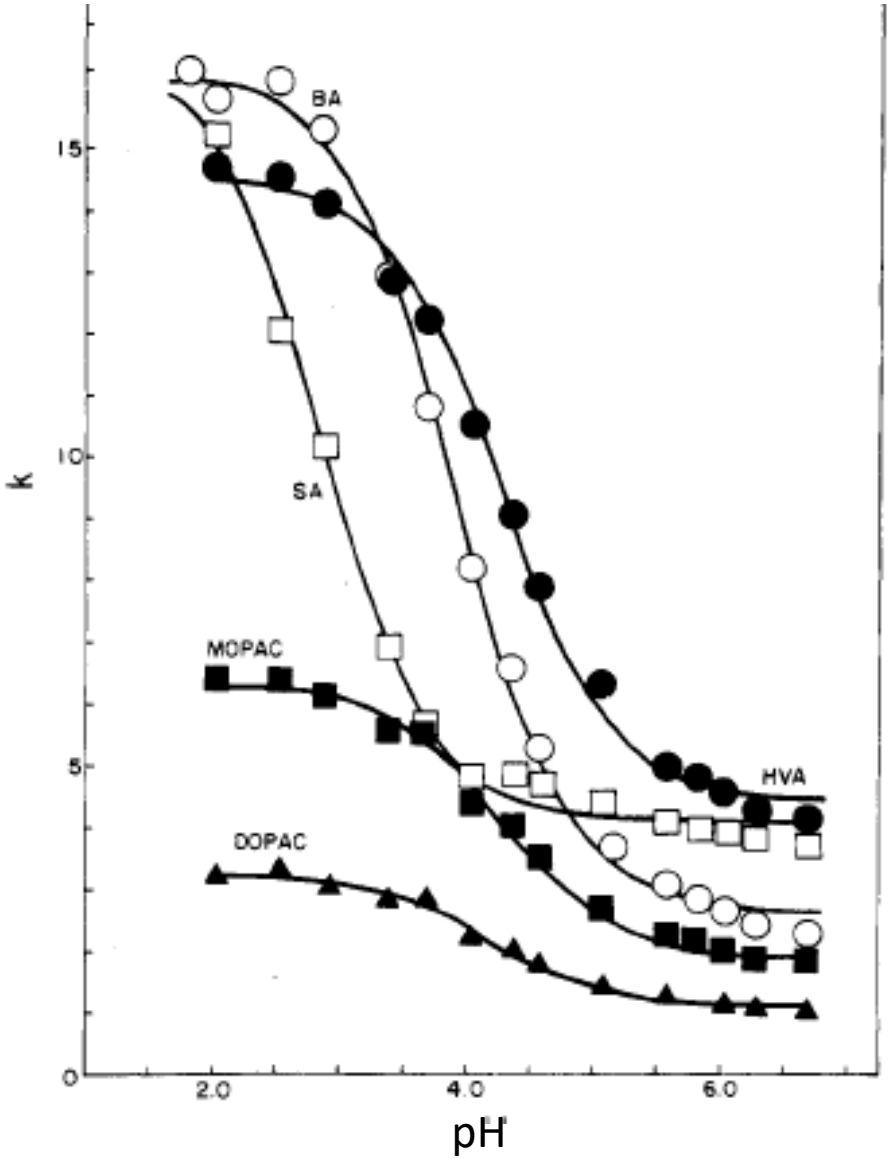


50 °C = 2-3 in k’

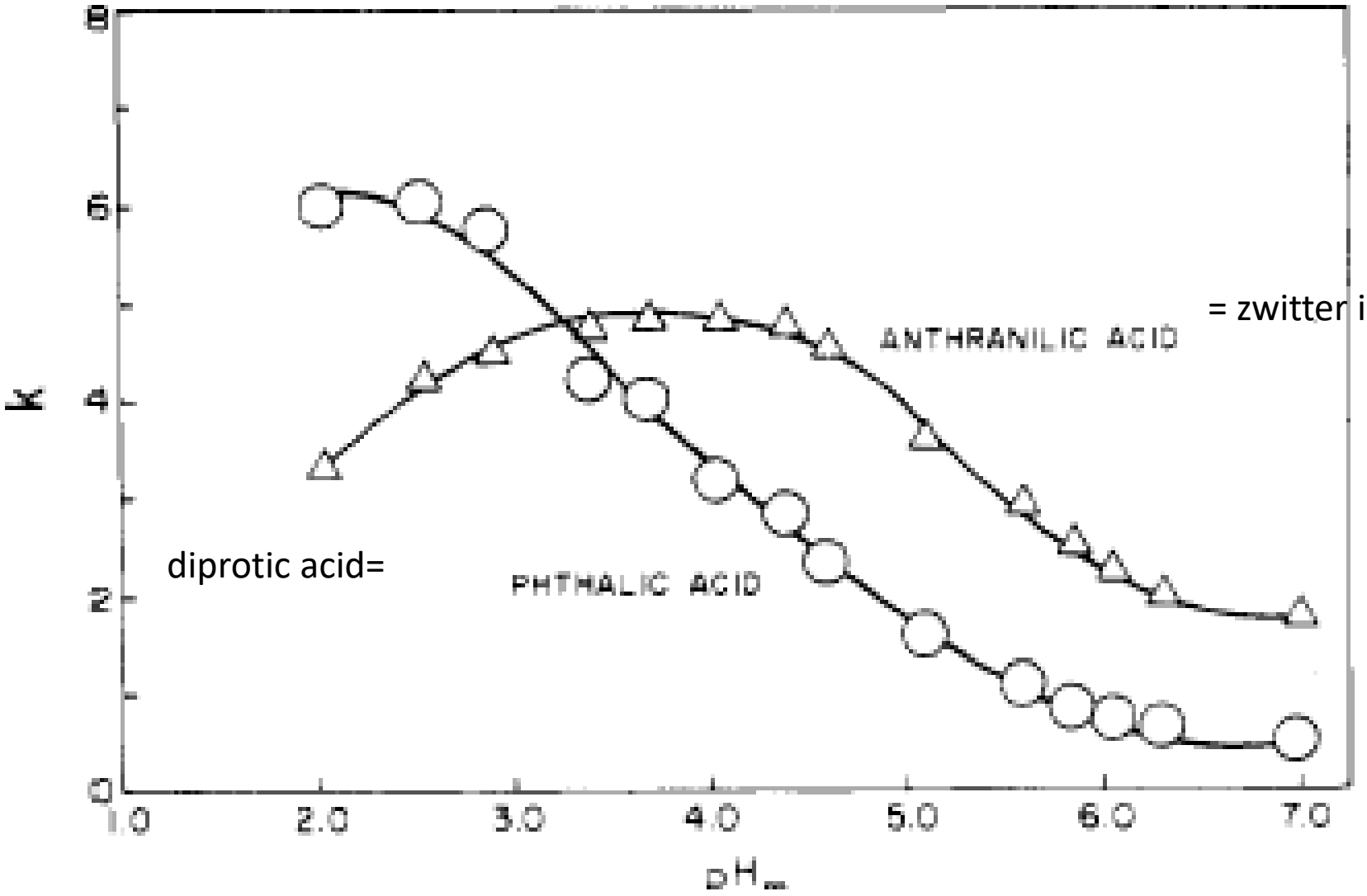
Effect of pH on k’



pH Effect on Monoprotic Acids



pH Effect on Diprotic and Zwitter Ions

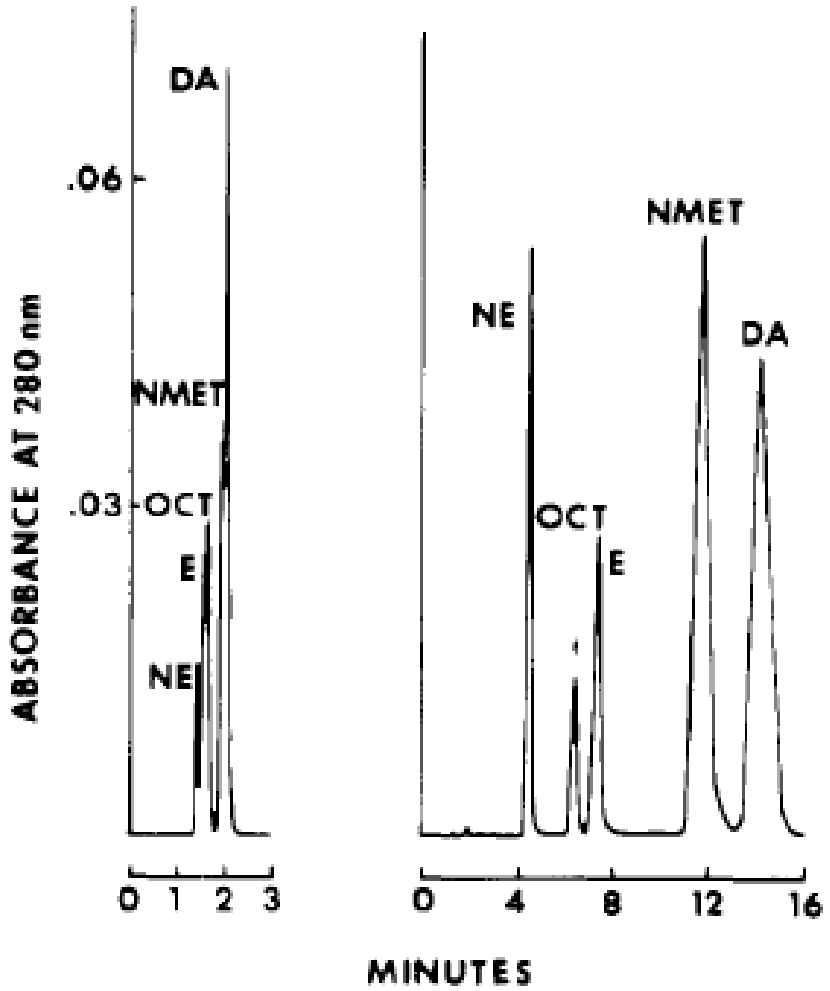


= zwitter ion

diprotic acid=

Effect of ion-pairing on RP retention

Effect of Adding 3mM Octylsulfate on Catecholamines



Typical RP-Product Line

Several Pore Sizes

⇒ 60 Å, 120 Å, 200 Å, 300 Å

Several Particle Sizes

⇒ 3µm, 5µm, 10µm, 15µm, 20µm

Several Bonding Types

⇒C1, CN, C4, Phenyl, C8, C18, C30

Several Bonding Chemistries

⇒ end capped, non end capped, polar embeded,

aqueous compatible

No Metal Impurities

Properties of RP-Packings

⇒**Hydrophobicity**

surface area

bonding type

bonding chemistry

⇒**Silanophilic Activity**

bonding chemistry

⇒**Shape Selectivity**

bonding chemistry

⇒**Polar Selectivity**

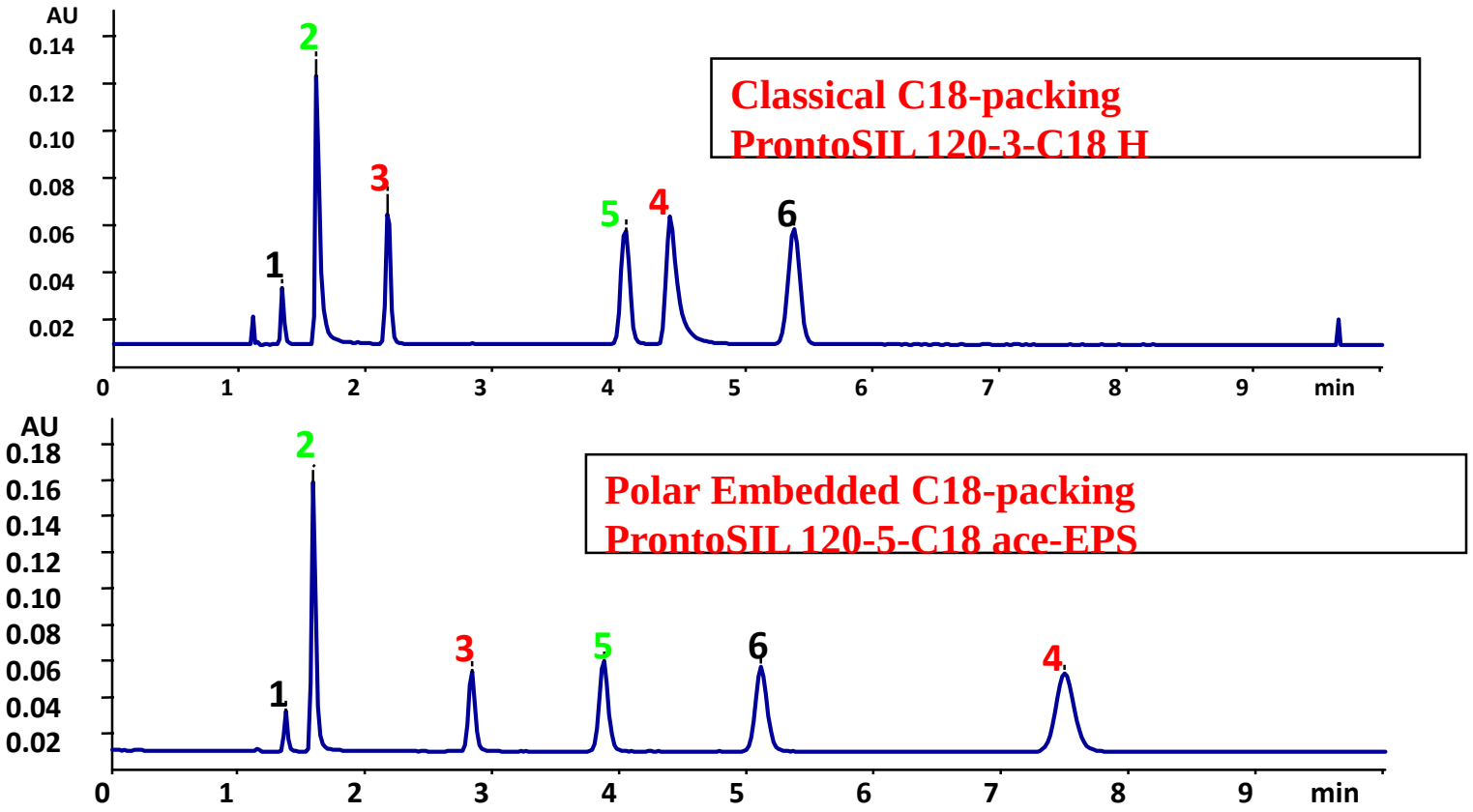
bonding chemistry

⇒**Metal Content**

manufacturing of the silica

Comparison of Selectivities

Acidic, **basic** and neutral compounds



Eluent:	ACN/50mM Phosphate buffer pH 3.2 65:35 (v/v)
Flow:	1.0 ml/min
Temperature:	ambient
Injection:	5 µl
Detection:	UV 254 nm
Sample:	**1** Uracil, **2** Pyridine, **3** Phenol, **4** p-Butylbenzoic acid, **5** N,N-Dimethylaniline, **6** Toluene

Peak Broadening in Separations in Four Parts
Stoll, CHE380

Objectives for our Discussion of Separations

Basics

1. Define, understand, and use key terms such as retention time, retention factor, selectivity, and resolution.
2. Predict changes in separation metrics upon a change in conditions (e.g., column length, particle size, flow rate, etc.).

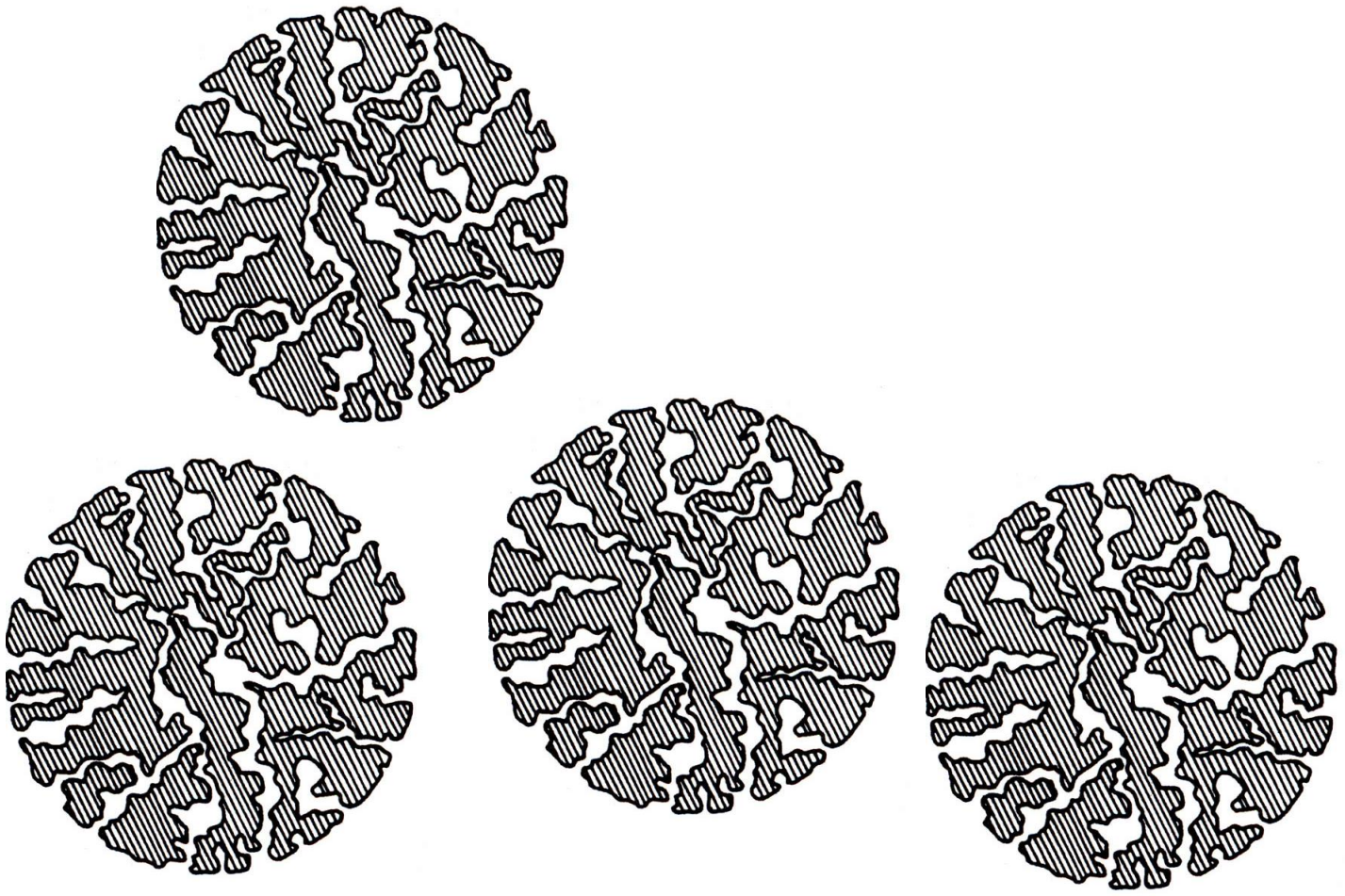
Retention

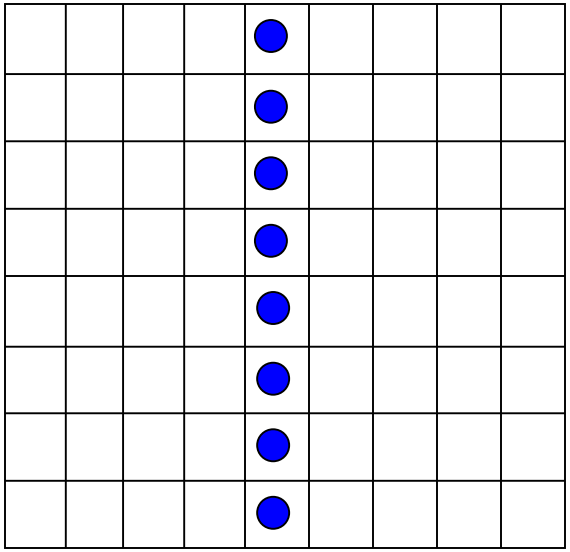
1. (GC/LC) Predict the effect of a change in conditions on relative retention (e.g., change in stationary phase, mobile phase, flow rate, etc.).
2. (GC/LC) Use chemical and physical reasoning to explain the basis of retention, given a specific scenario (e.g., why is benzyl alcohol less retained than benzene in RPLC?).

Peak Broadening

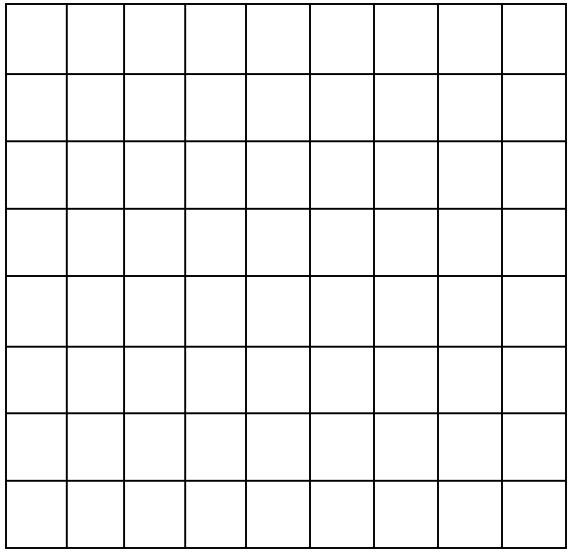
1. (GC/LC) Predict the effect of a change in conditions (e.g., mobile phase type, temperature, particle size, etc.) on peak properties (e.g., height, width, retention time).
2. (GC/LC) Use chemical and physical reasoning to explain the basis of peak broadening, given a specific scenario (e.g., why is the peak for anthracene broader than the peak for benzene under RPLC conditions?).

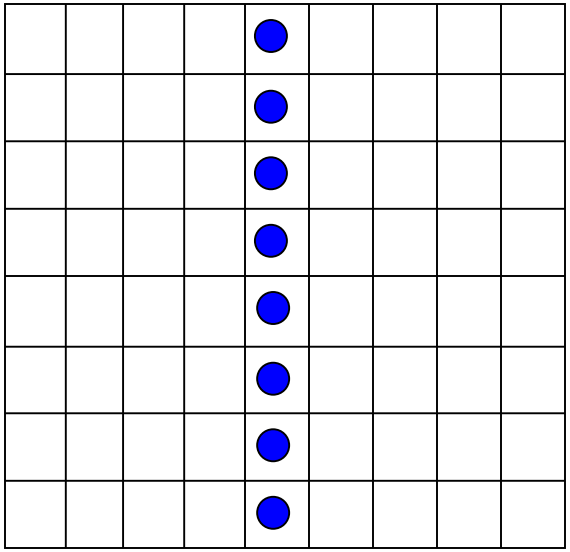
Part I – Diffusion, Convection, and the Physics of Flow through Chromatography Columns



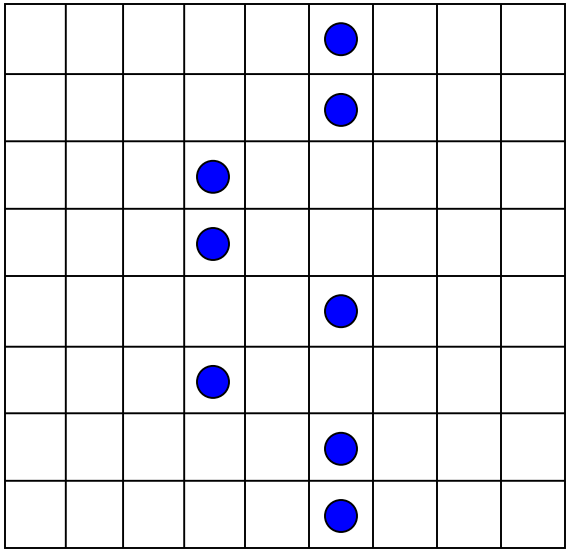


Initial Condition

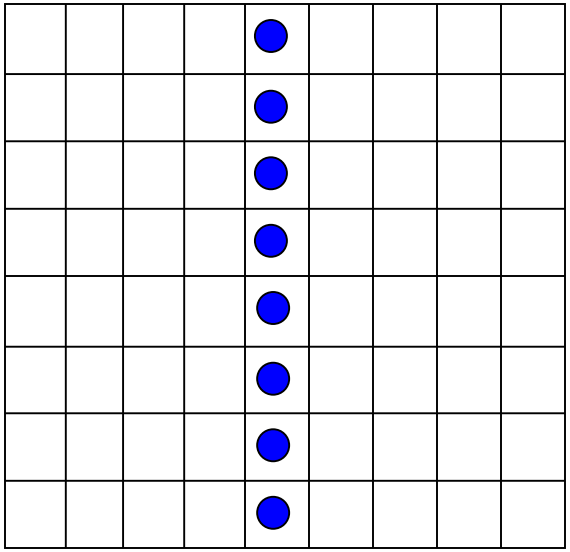




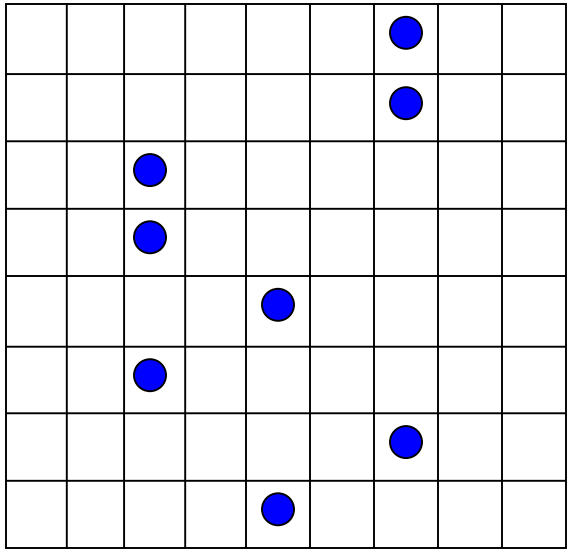
Initial Condition



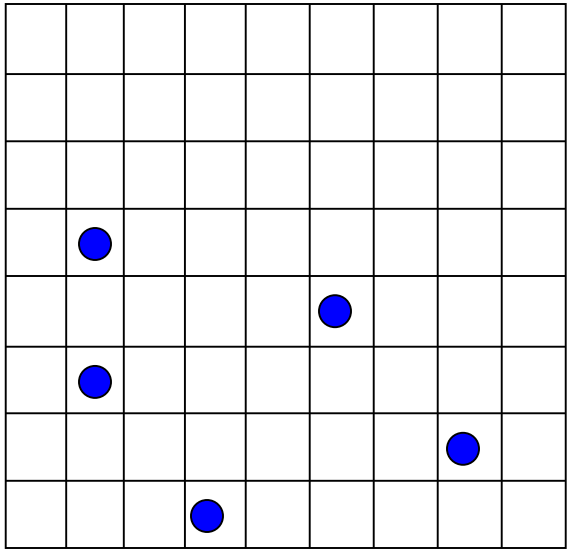
After Step 1



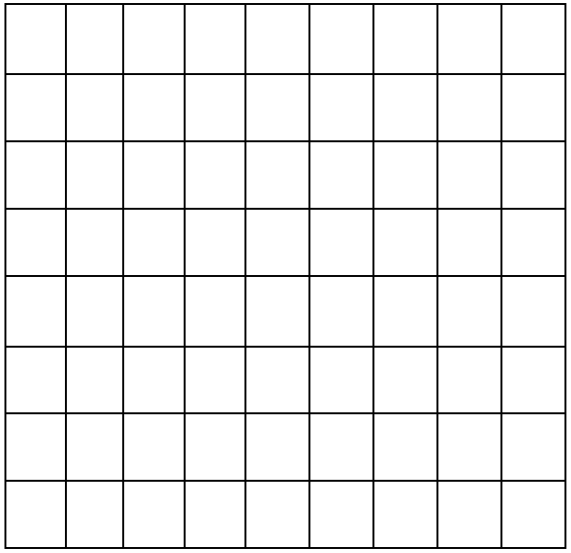
Initial Condition



After Step 2



After Step 3



After Step 4

INVESTIGATIONS ON THE THEORY OF ,THE BROWNIAN MOVEMENT

BY

ALBERT EINSTEIN, PH.D.

EDITED WITH NOTES BY

R. FÜRTH

TRANSLATED BY

A. D. COWPER

1905

WITH 3 DIAGRAMS

Fig. 1.4. An x, y plot of a two-dimensional random walk of $n = 18{,}050$ steps. The computer pen started at the upper left corner of the track and worked its way to the upper right edge of the track. It repeatedly traversed regions that are completely black. It moved, as the crow flies, 196 step lengths. The expected root-mean-square displacement is $(2n)^{1/2} = 190$ step lengths.

Berg, H., *Random Walks in Biology. 1993, Princeton: Princeton University Press.*

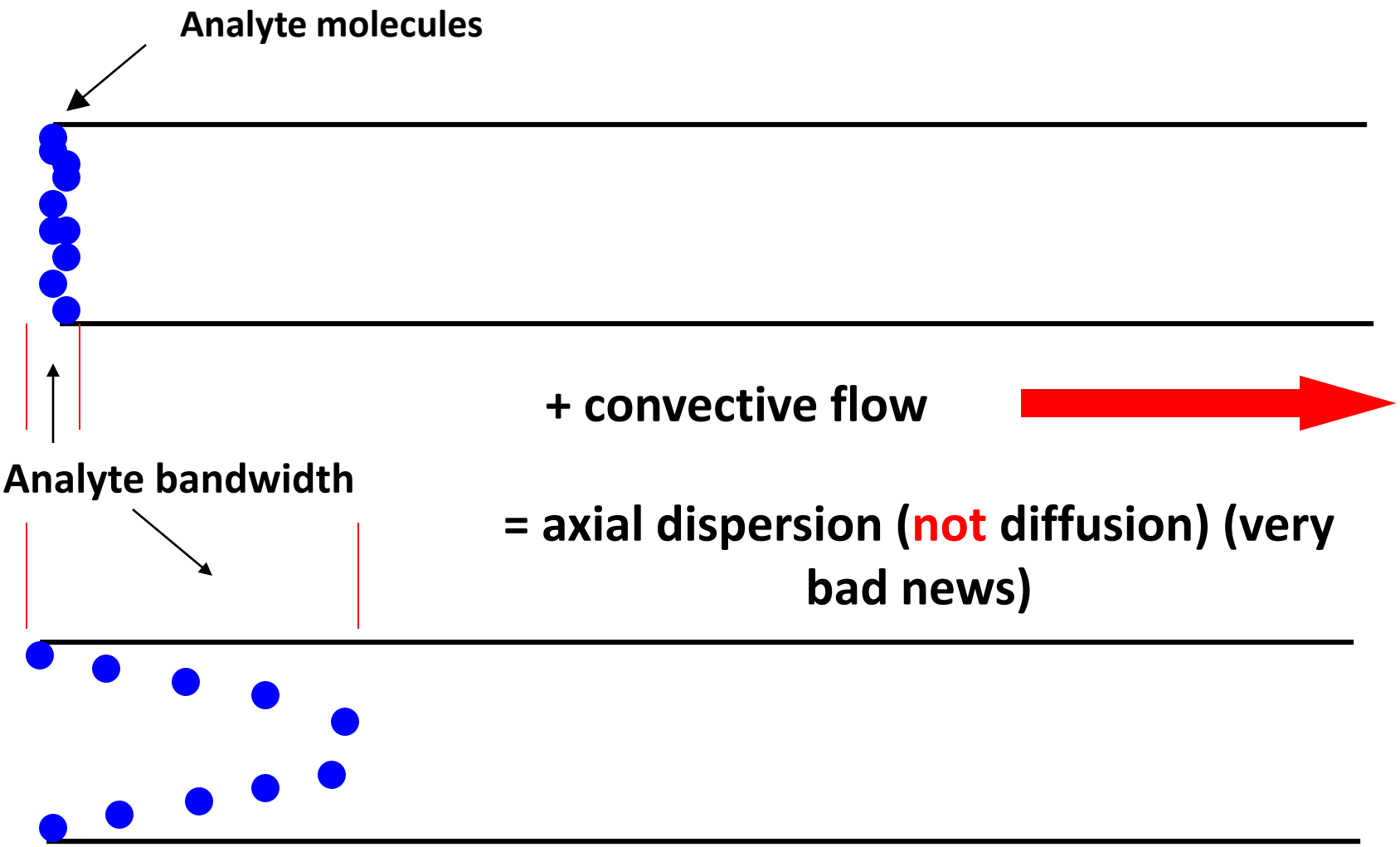
Part II – Peak Broadening in Gas Chromatography

The Equation for H in Open Tubular Chromatography (LC or GC, no particles)

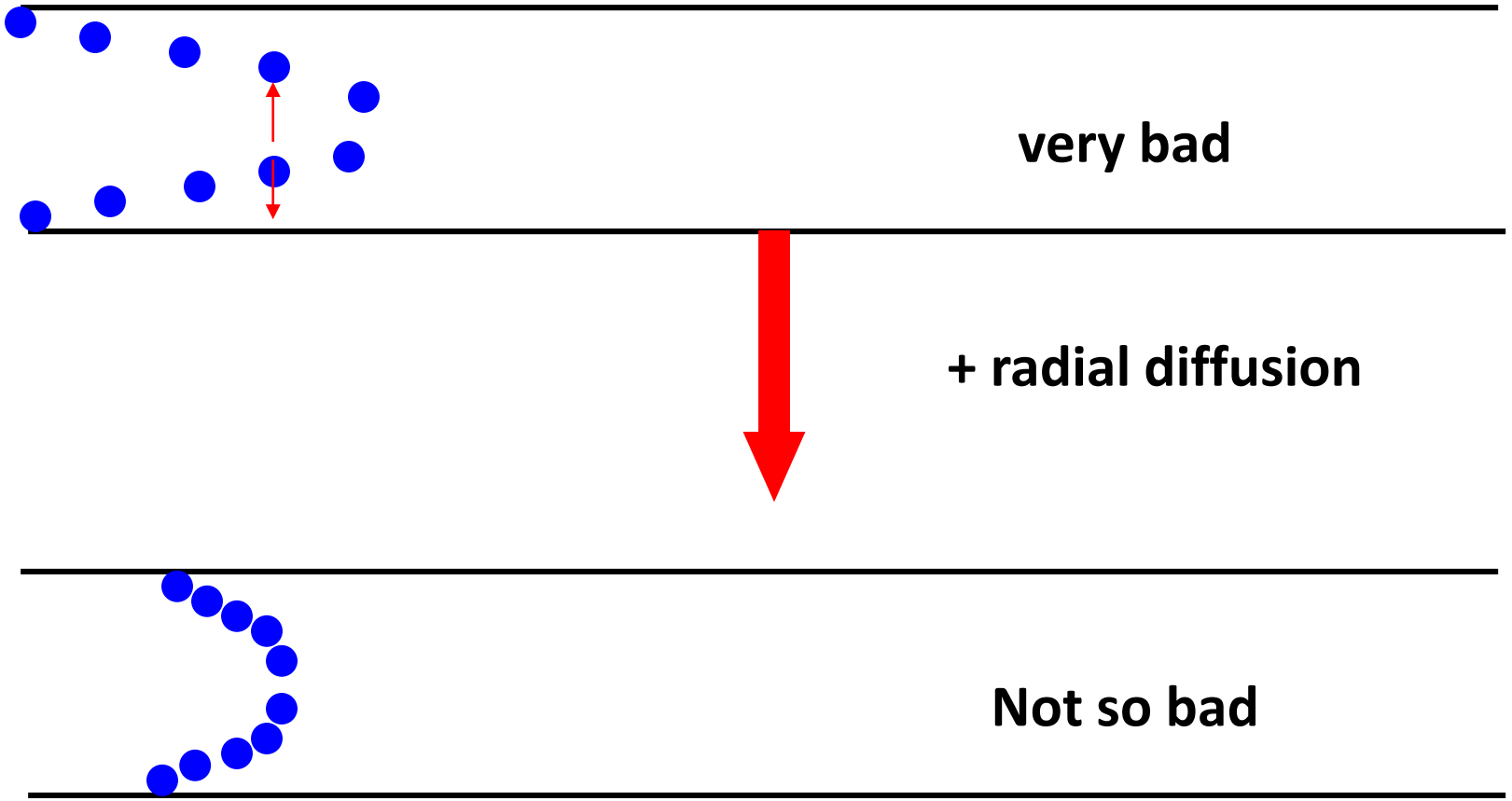
$$H_{total} = \frac{\left(1+6k'+11k'^2\right)}{\left(1+k'\right)^2}\frac{R^2 u_m}{D_m} + \frac{2D_m}{u_m} + \frac{2}{3}\frac{k'}{\left(1+k'\right)^2}\frac{d_f^2 u_m}{D_s}$$

$u_m = L/t_m$ – mobile phase velocity (cm/s)
D_m = diffusion coefficient of the analyte in the mobile phase
R = radius of the open tubular column
d_f = thickness of the stationary phase film
D_s = diffusion coefficient of the analyte in the stationary phase

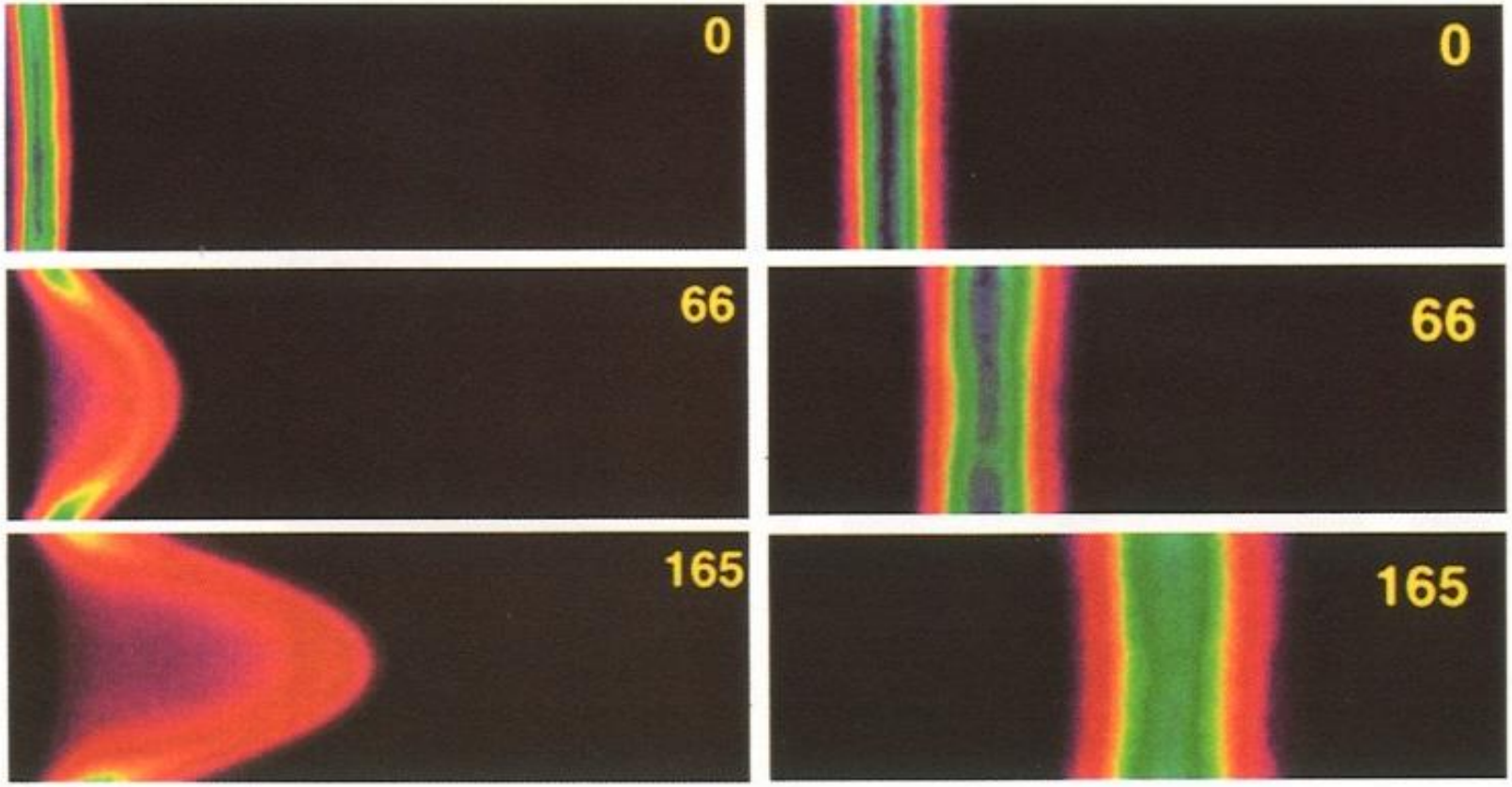
Effect of convective (laminar) flow on analyte bandwidth in an open tube



…but, radial diffusion saves the day

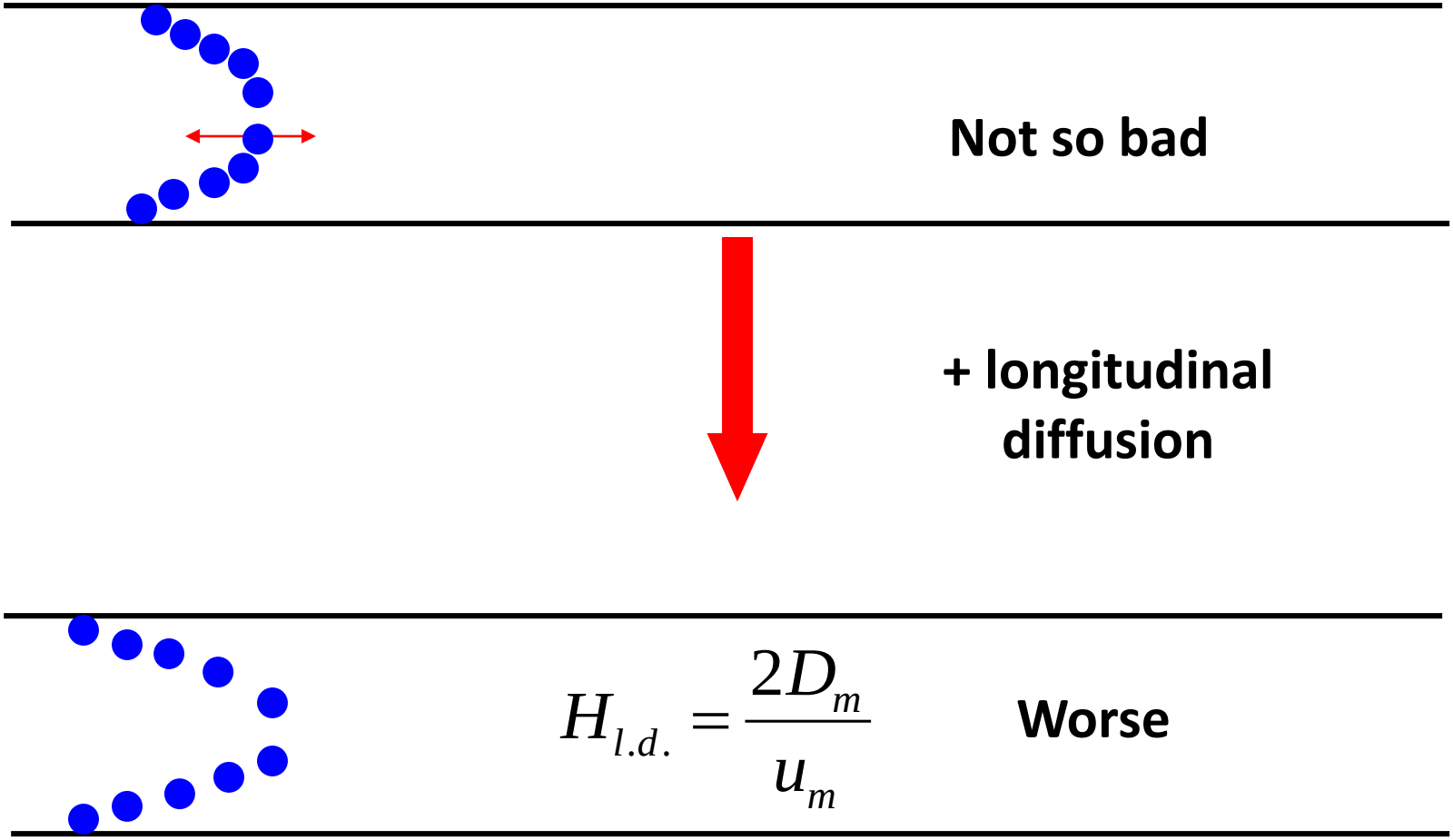


$$H_{axial} = \frac{\left(1+6k'+11k'^2\right)}{\left(1+k'\right)^2}\frac{R^2 u_m}{D_m}$$

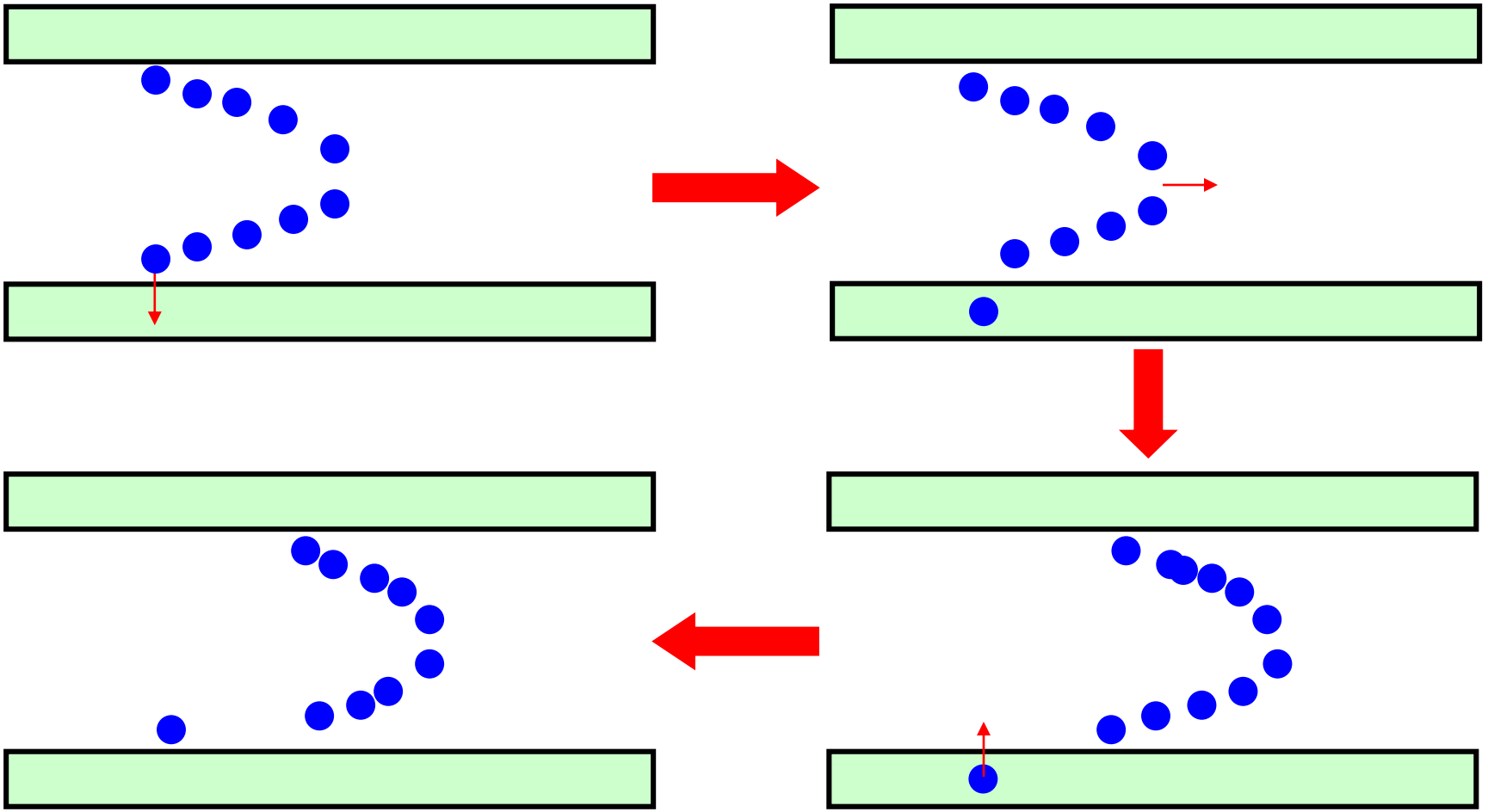


Color Plate 30 **Velocity Profiles for Hydrodynamic and Electroosmotic Flow (Section 25-6)** A fluorescent dye was imaged inside a capillary tube at times 0, 66, and 165 ms after initiating flow. The highest concentration of dye is represented by blue and the lowest concentration is red in these images in which different colors are assigned to different fluorescence intensities. [From P. H. Paul, M. G. Garguilo, and D. J. Rakestraw, *Anal. Chem.* **1998,** *70,* 2459. See also D. Ross, T. J. Johnson, and L. E. Locascio, *Anal. Chem.* **2001,** *73,* 2509.]

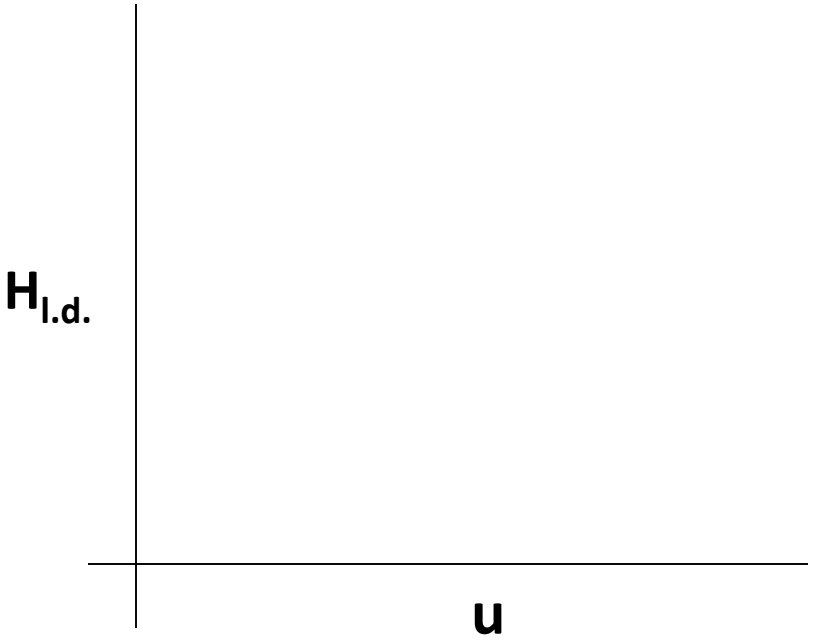
longitudinal diffusion adds to the problem

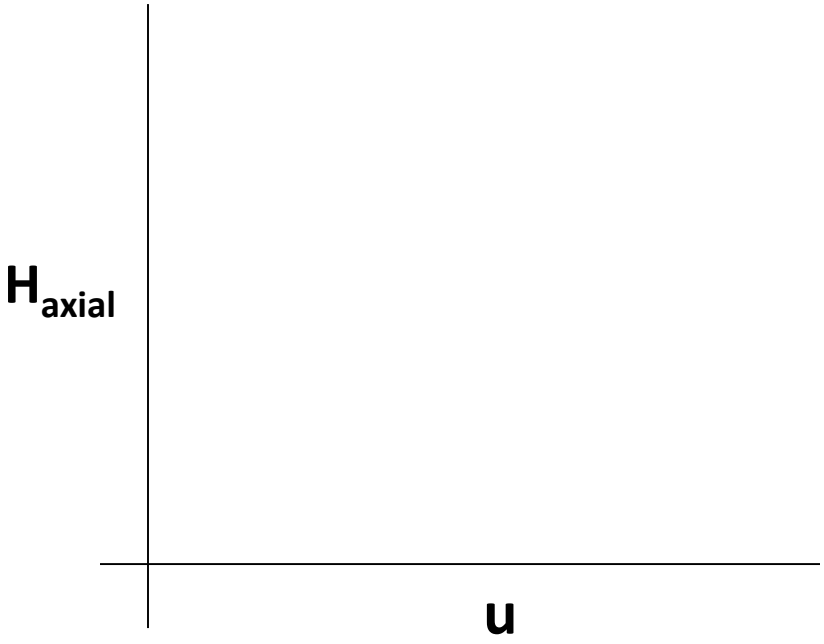


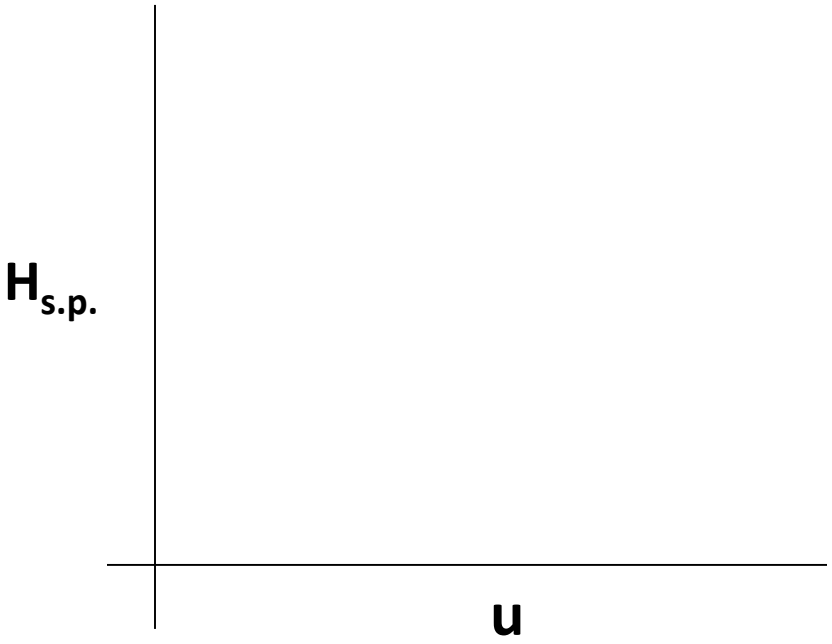
And finally…slow diffusion out of the stationary phase…

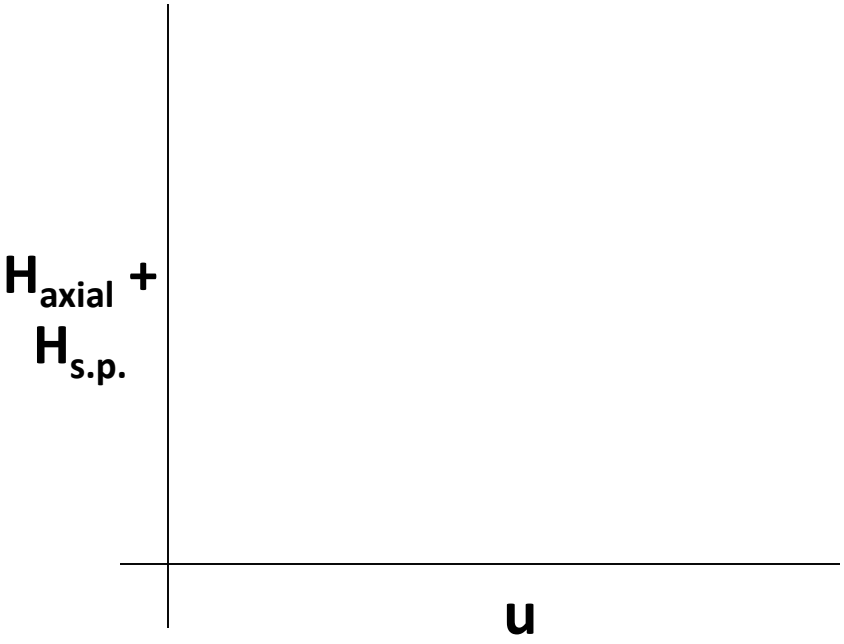


$$H_{s.p.} = \frac{2}{3}\frac{k'}{(1+k')^2}\frac{d_f^2 u_m}{D_s}$$

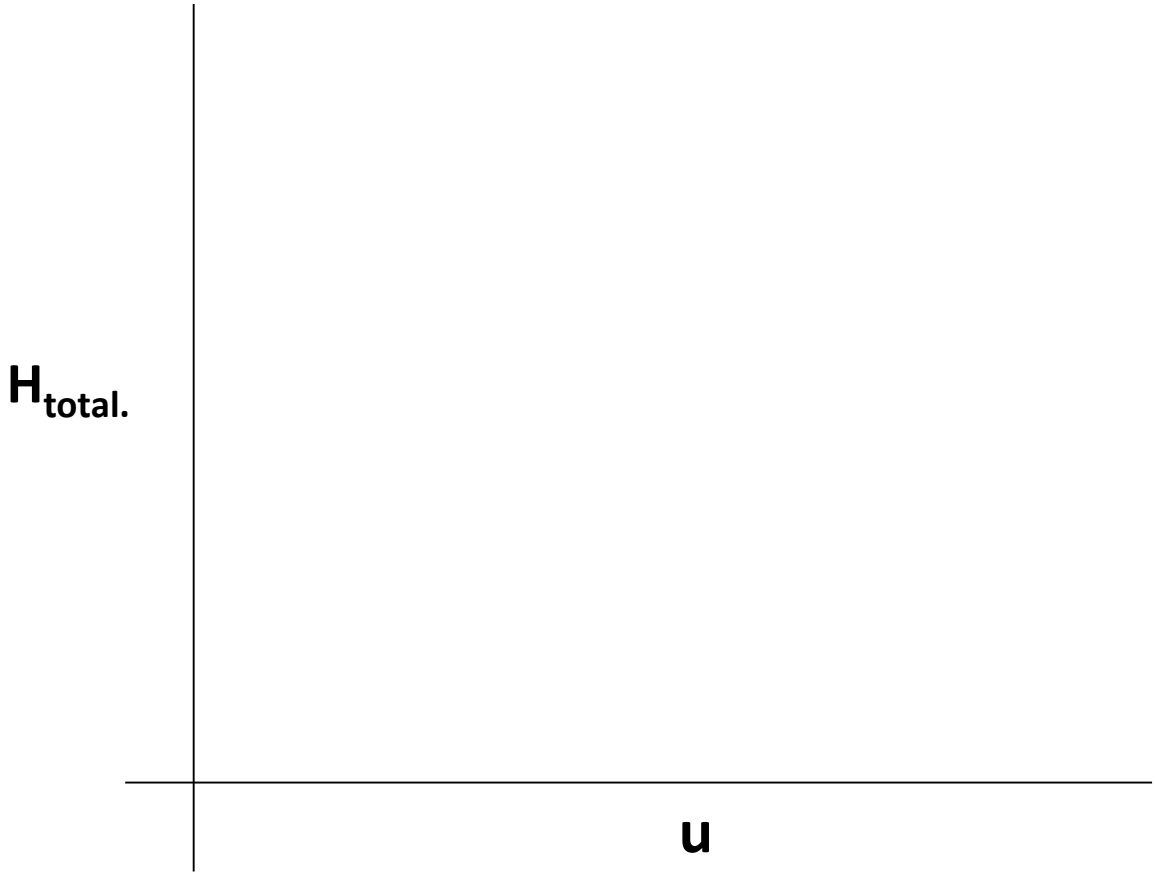

$H_{l.d.}$
u


H_{axial}
u


$H_{s.p.}$
u


H_{axial} +
$H_{s.p.}$
u

How Does H Depend on u??



For Open Tubular Columns

	To Make H Small…	Practical Consequence/Problem
H_{axial}		
$H_{l.d.}$		
$H_{s.p.}$		

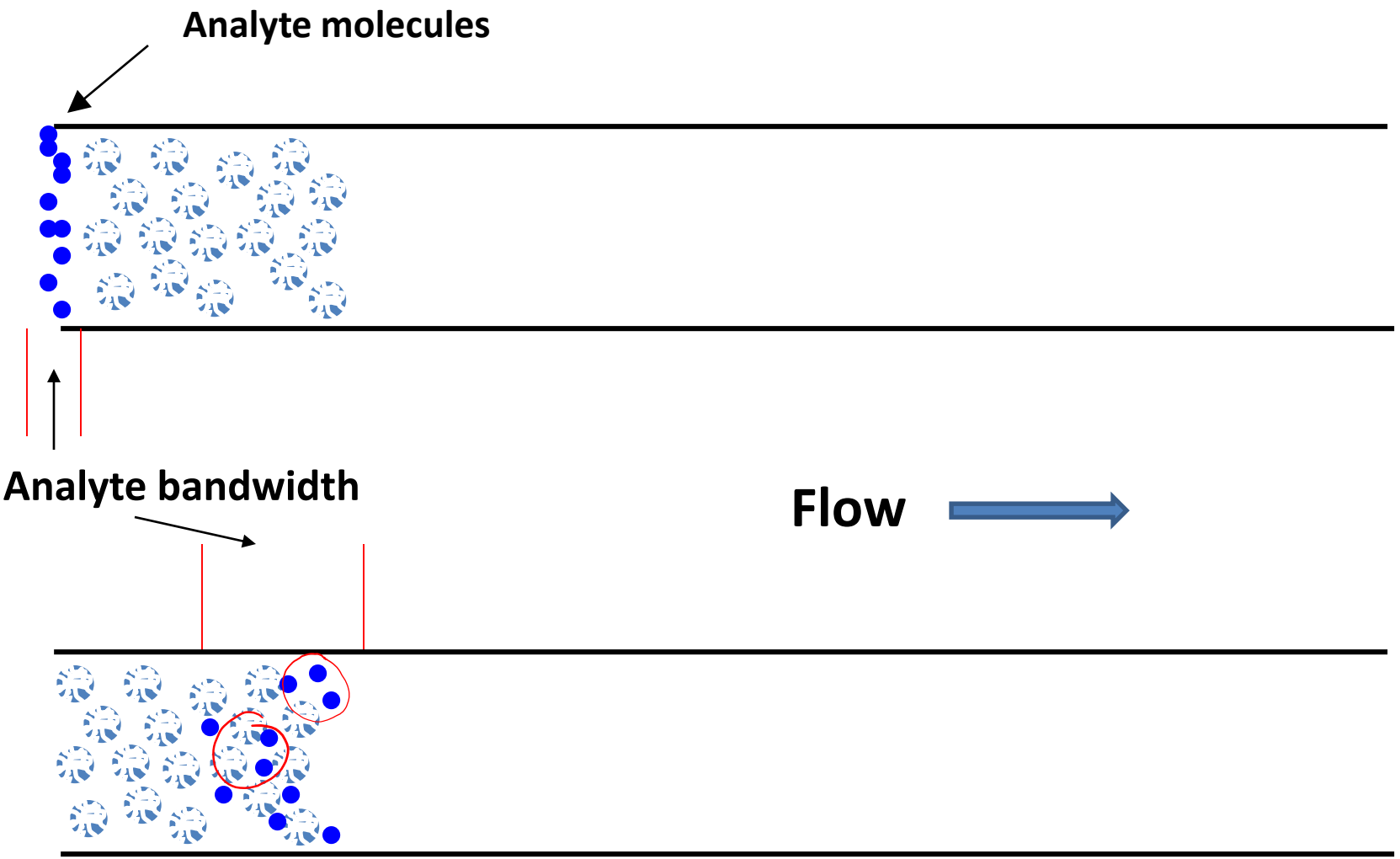
Suppose you have are running an open tubular GC column using helium as the mobile phase, and you switch to nitrogen because you've run out of helium. Draw two van Deemter curves for these two situations on one graph. Now, assume you are working at a mobile phase velocity that is $2*u_{m,opt}$ for the helium curve. Draw a chromatogram that shows two peaks separated in the helium case with a resolution of 1.5. Now, on the same graph, draw a chromatogram for the nitrogen case. For each of these, be as quantitative as you can with the information provided.

Part III – Peak Broadening in Liquid Chromatography

For Open Tubular Columns

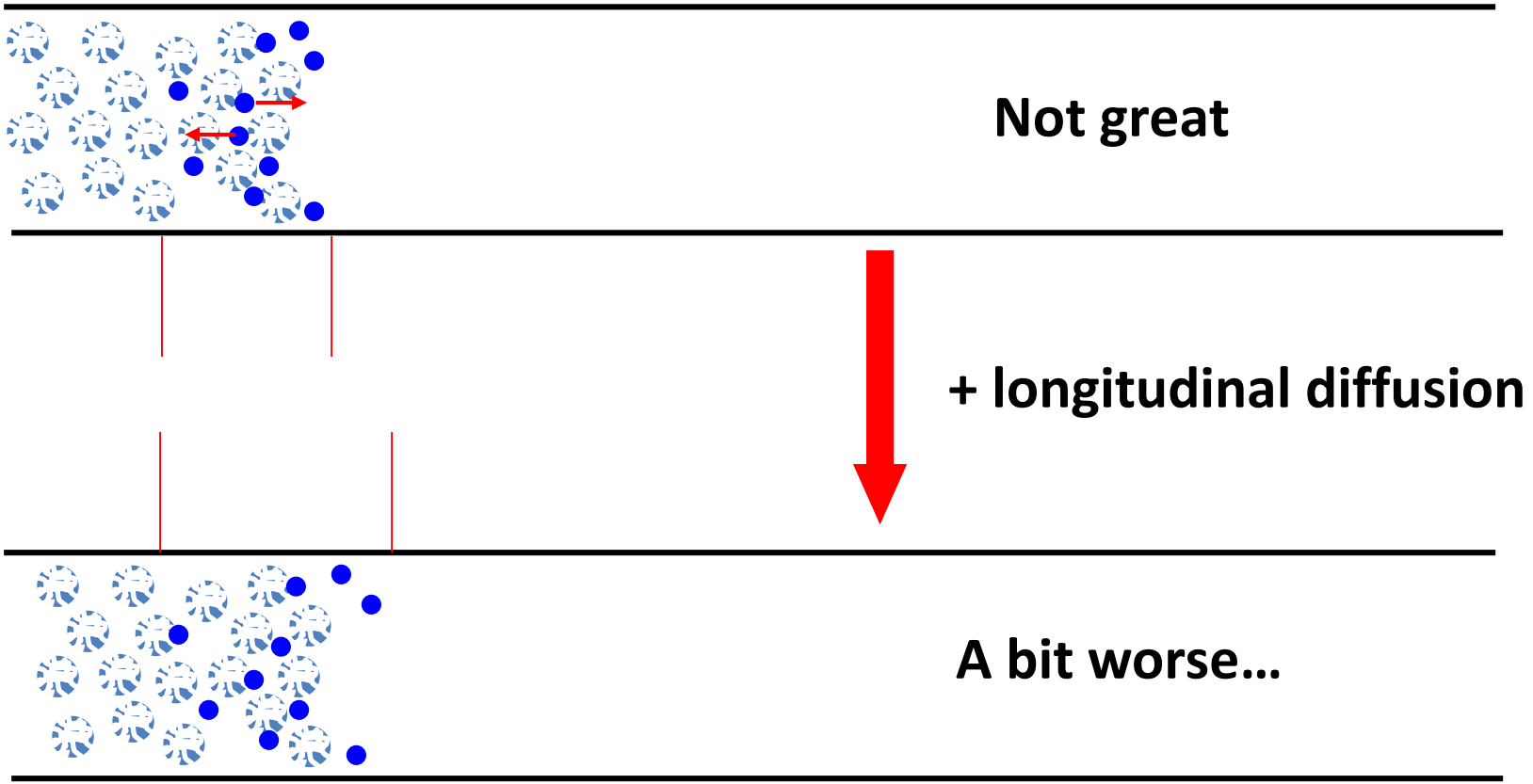
	To Make H Small…	Practical Consequence/Problem
H_{axial}		
$H_{l.d.}$		
$H_{s.p.}$		

A-term Broadening in a Packed Bed – the 'Multi-Path' Term



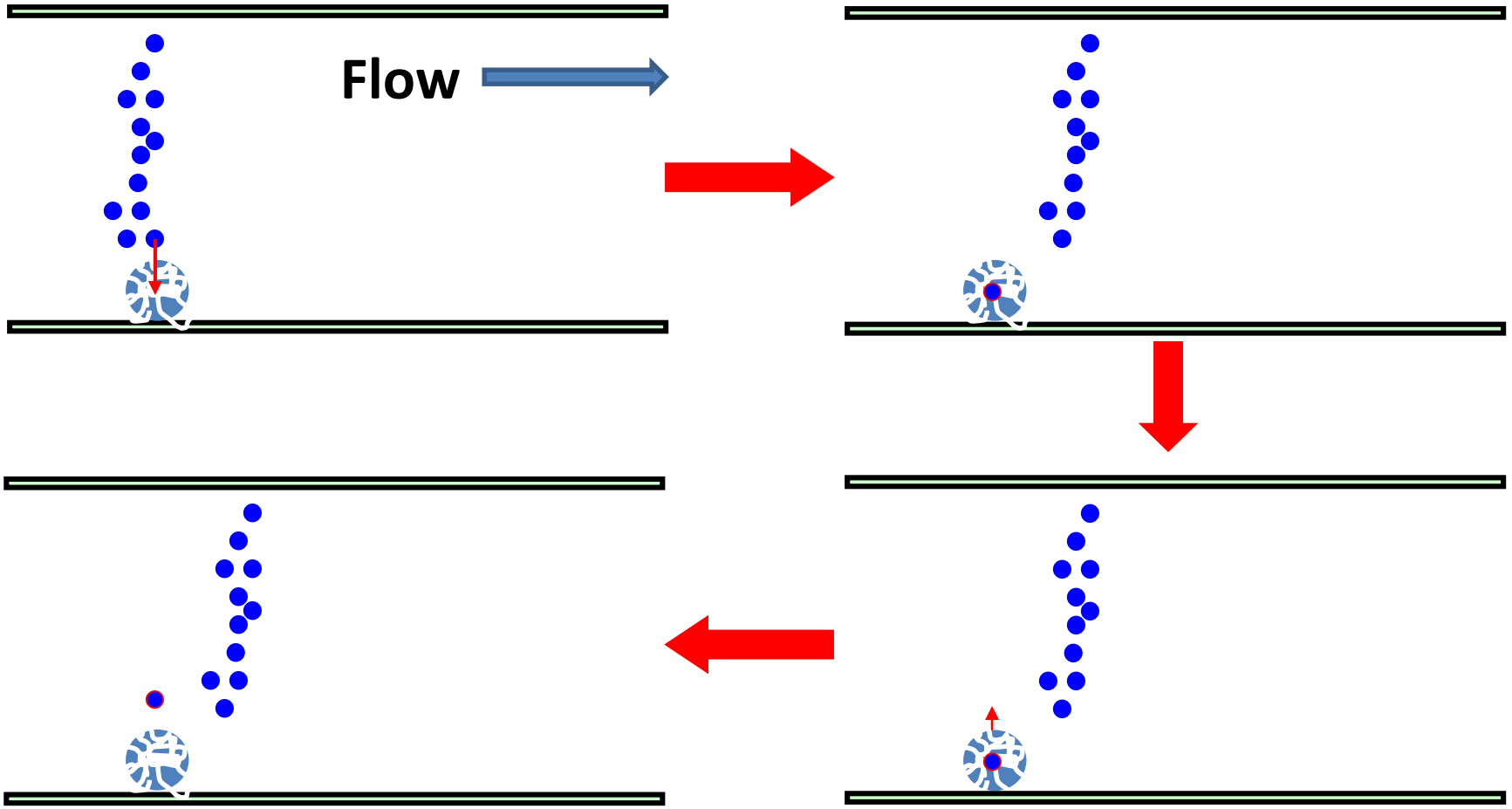
$$H_A \propto d_p$$

Longitudinal diffusion is another problem…



$$H_B \propto \frac{D_m}{u_e}$$

And finally…slow diffusion out of the stationary zone…



$$H_C \propto \frac{u_e d_p^2}{D_m}$$

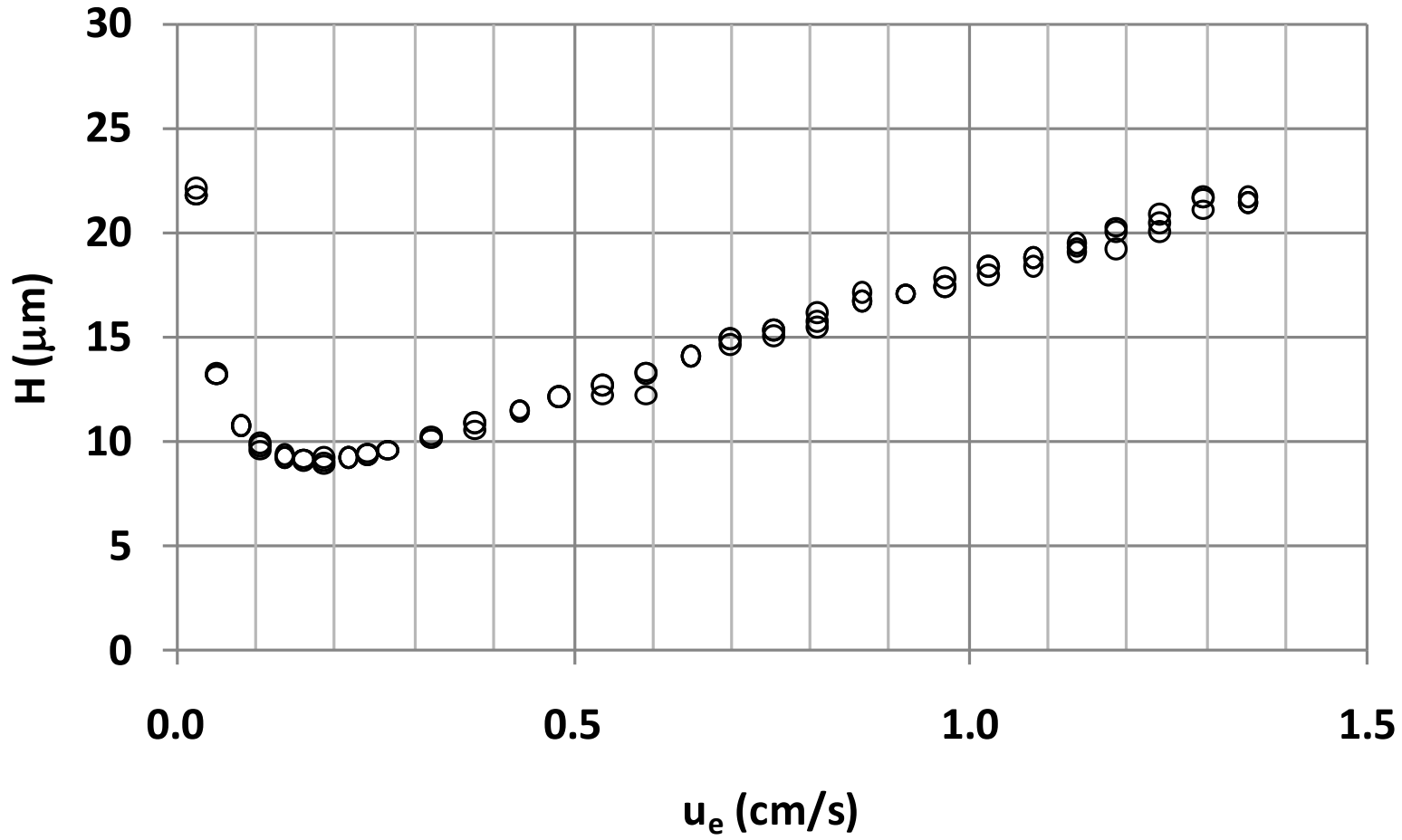
How to Make H Small??

	To Make H Small…	Practical Consequence/Problem
H_A		
H_B		
H_C		

An important fundamental observation: Peaks that are more retained by a column come out of the column with a larger peak width.

An important fundamental question: What is the physical basis for this?

1. Suppose you have a RPLC column packed with 5 μm particles. You make the measurements needed to construct the van Deemter curve shown below. How will the curve change if you do the same experiment with a column packed with 2.5 μm particles?



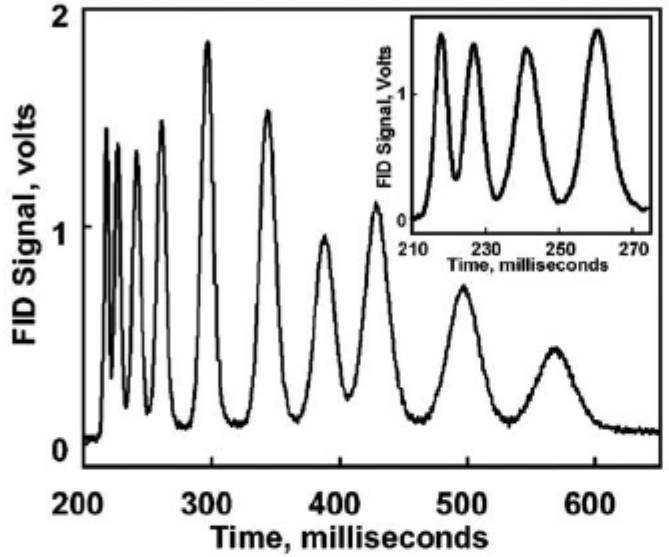
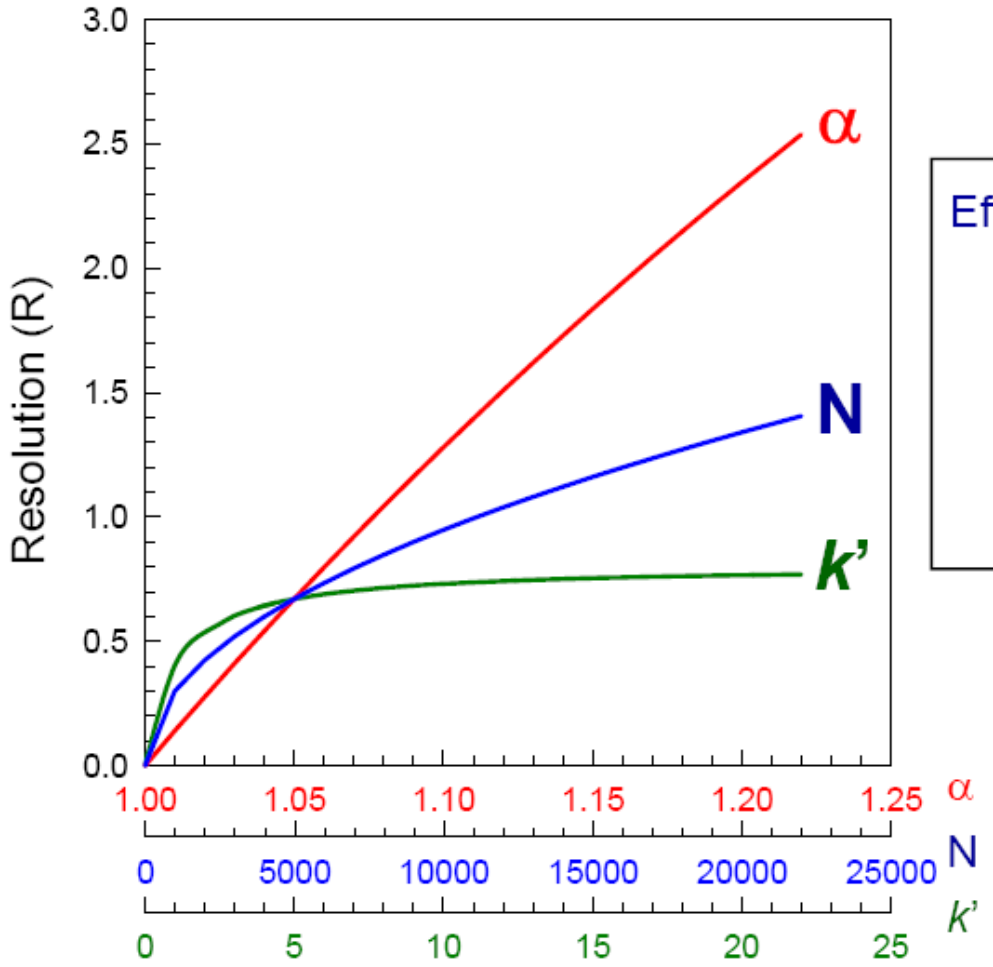


Figure 8. A 10-component separation using a 2-m column with a column absolute head pressure of 145 psi (~943 cm/s average linear flow velocity, ~21 mL/min volumetric flow rate at the column outlet). All other parameters utilized for the 1-m experiment outlined in Figure 3A (inlet pressure, oven temperature, initial injection volume, valve 2 delay time of 2.5 ms, etc.) held constant. Retention order: methanol, acetone, hexane, benzene, toluene, chlorobenzene, anisole, propylbenzene, isobutylbenzene, and butylbenzene.

Part IV – Optimization of Separations

Column Type	Open Tube		Open Tube		Open Tube/Packed Bed	
Column Diameter	Narrow ~ 0.1-0.3 mm		Narrow ~ 0.25-0.32 mm		Wide > 0.5 mm	
S.P. Film Thickness	Thin ~ 0.2 microns		Thick ~ 1-2 microns		Thick - 2-5 microns (open tube only)	
		Why		Why		Why
Efficiency	Best		Good		Poor	
Separation speed	Fast		Moderate		Slow	
Analyte Capacity	Poor		Good		Best	
Preferred Application	High speed, high resolution separations		Low boiling point compounds		Anybody can do it	
	High boiling point compounds		Trace analysis		Low boiling point compounds	
	Thermally unstable compounds					

Contributions to Resolution



Efficiency Selectivity Retention

$$R = \frac{\sqrt{N}}{4} \quad \frac{\alpha - 1}{\alpha} \quad \frac{k'}{k'+1}$$

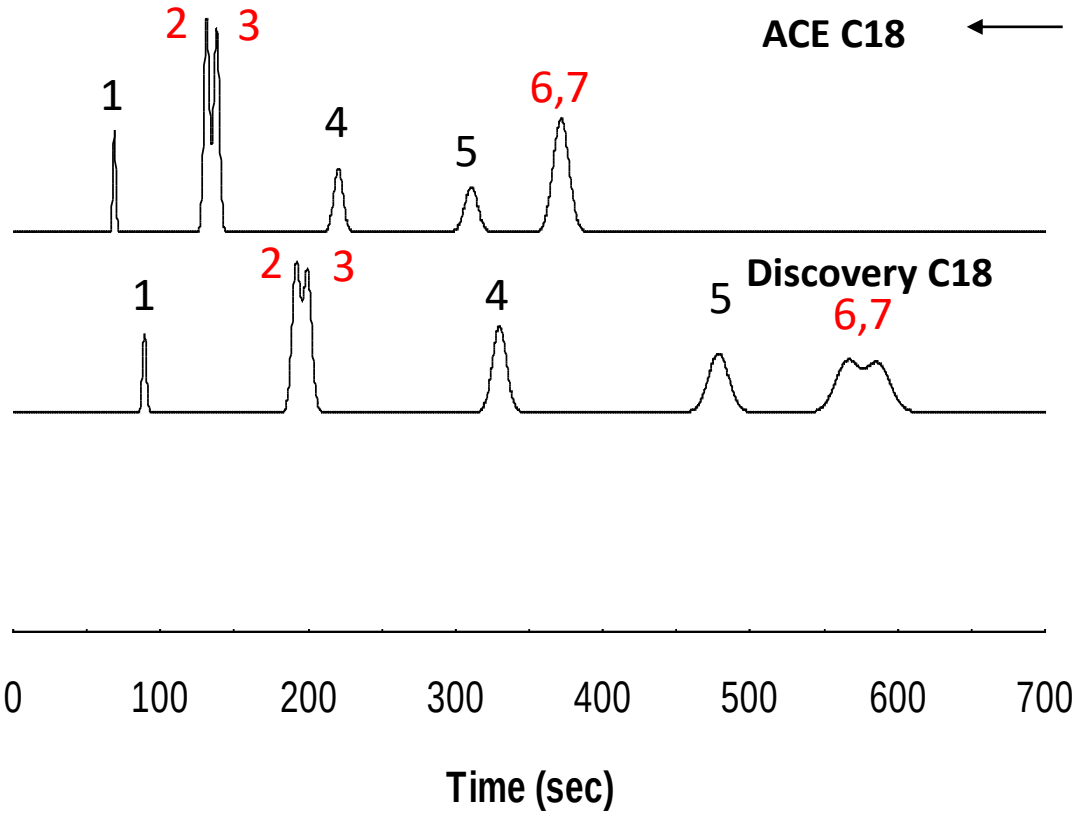
❖ **Small changes in selectivity (α) have the greatest impact on resolution.**

Mao, Y. Ph.D. Dissertation, Department of Chemistry, University of Minnesota, 2000.

The Beauty of Snyder's Model

- Quantitative comparison of selectivity for two columns by a single parameter:

$$F_s = \{12.5(H_2 - H_1)^2 + 100(S_2 - S_1)^2 + 30(A_2 - A_1)^2 + 143(B_2 - B_1)^2 + 83(C_2 - C_1)^2\}^{1/2}$$

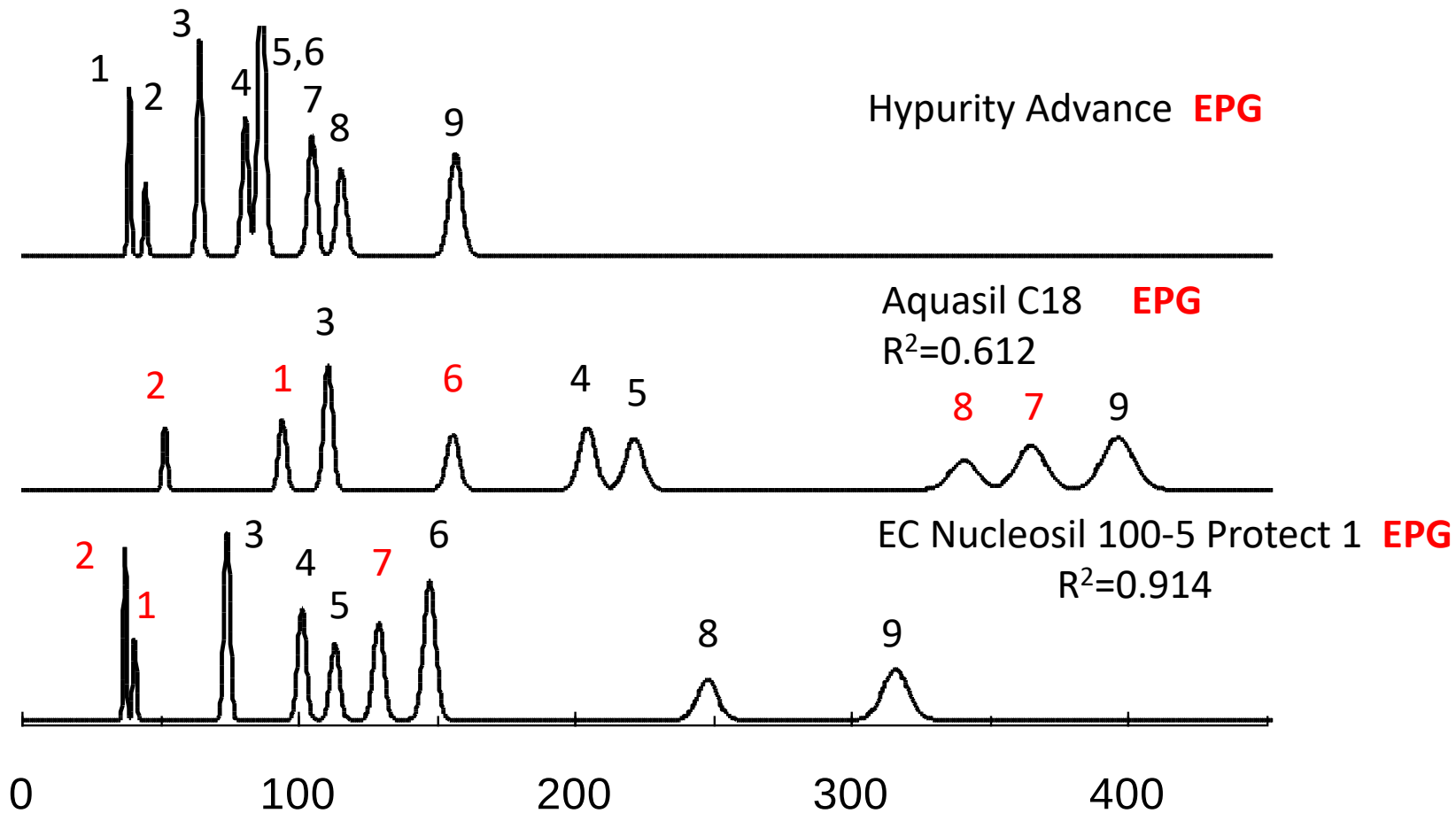


(1) 4-nitrophenol;
(2) 5-phenylpentanol;
(3) anisole;
(4) toluene;
(5) cis-chalcone;
(6) trans-chalcone;
(7) mefenamic acid.

Experimental conditions:
50% acetonitrile/pH 2.8 buffer;
35 ◦C; 1.0 ml/min

N. S. Wilson, J. W. Dolan., L. R. Snyder, P. W. Carr, L.C. Sander *J. Chromatogr. A* 2004 **961**, 217-236.

Selectivity Comparison of Three EPG Phases

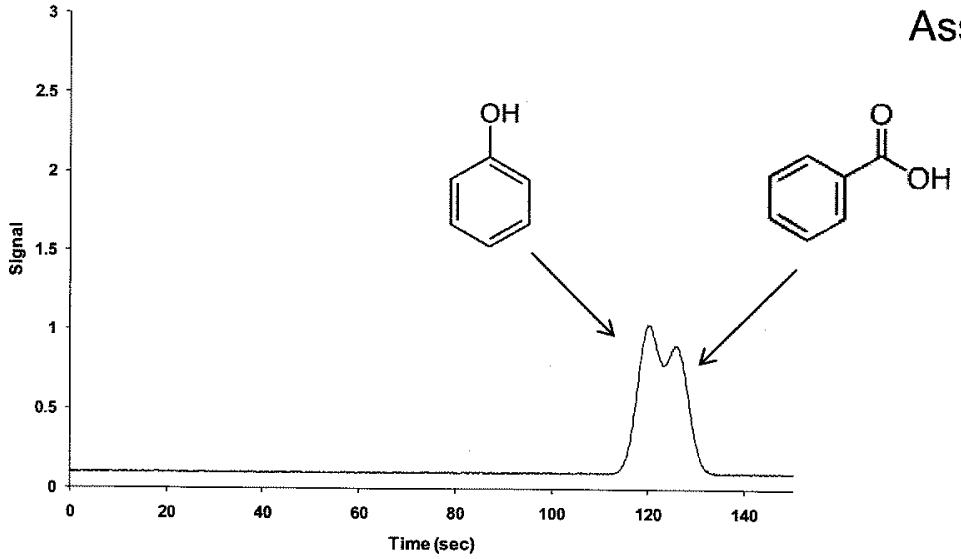


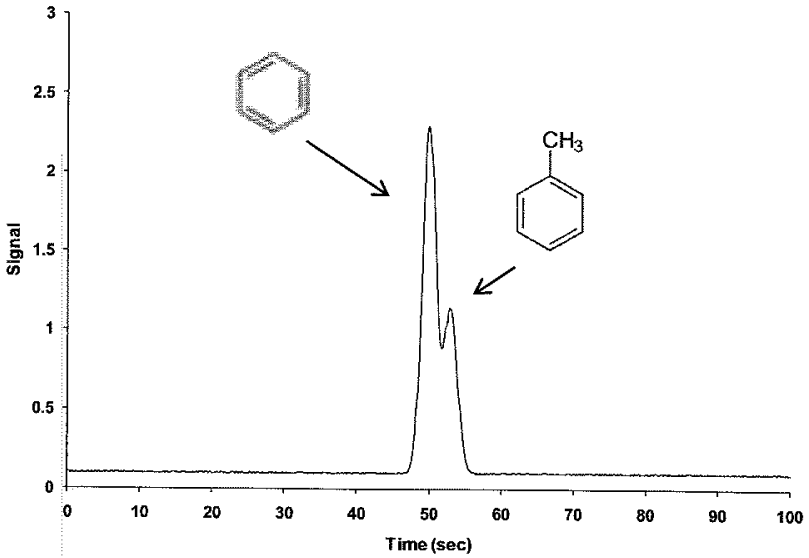
1) Amitriptyline;
2) N,N-dimethylaccetamide;
3) Acetophenone;
4) Toluene;
5) 5-phenylpentanol;
6) Trans-chalcone;
7) 4-n-butylbenzoic acid;
8) Cis-chalcone;
9) Mefenamic acid

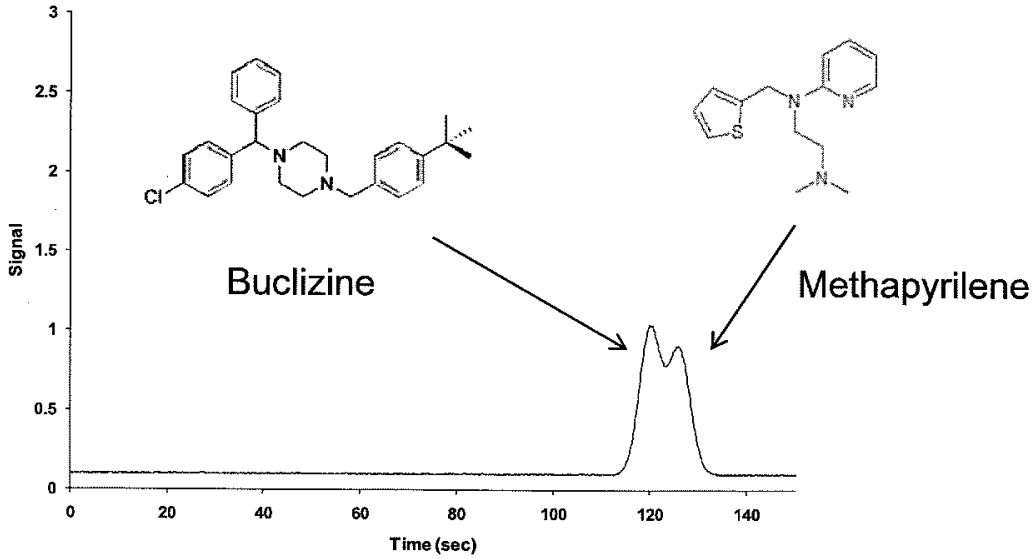
Experimental conditions:
50% acetonitrile/pH 2.8 buffer;
35 ◦C; 1.0 ml/min

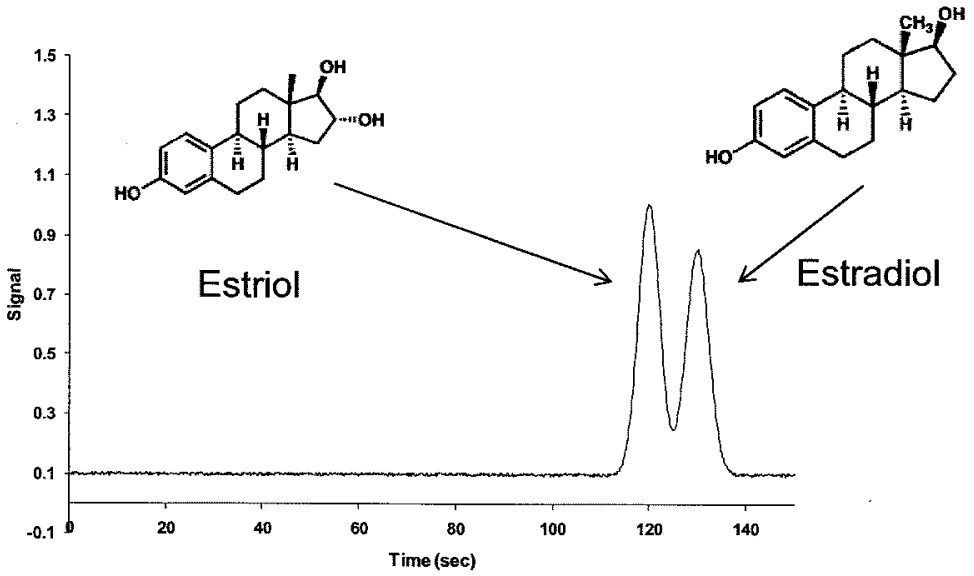
Y. Zhang, Pittcon 2008

Assume t_m = 25 sec. in all cases, and the m.p. velocity = 2 * $u_{e,opt}$






Buclizine
Methapyrilene
Signal
Time (sec)


Estriol
Estradiol
Signal
Time (sec)