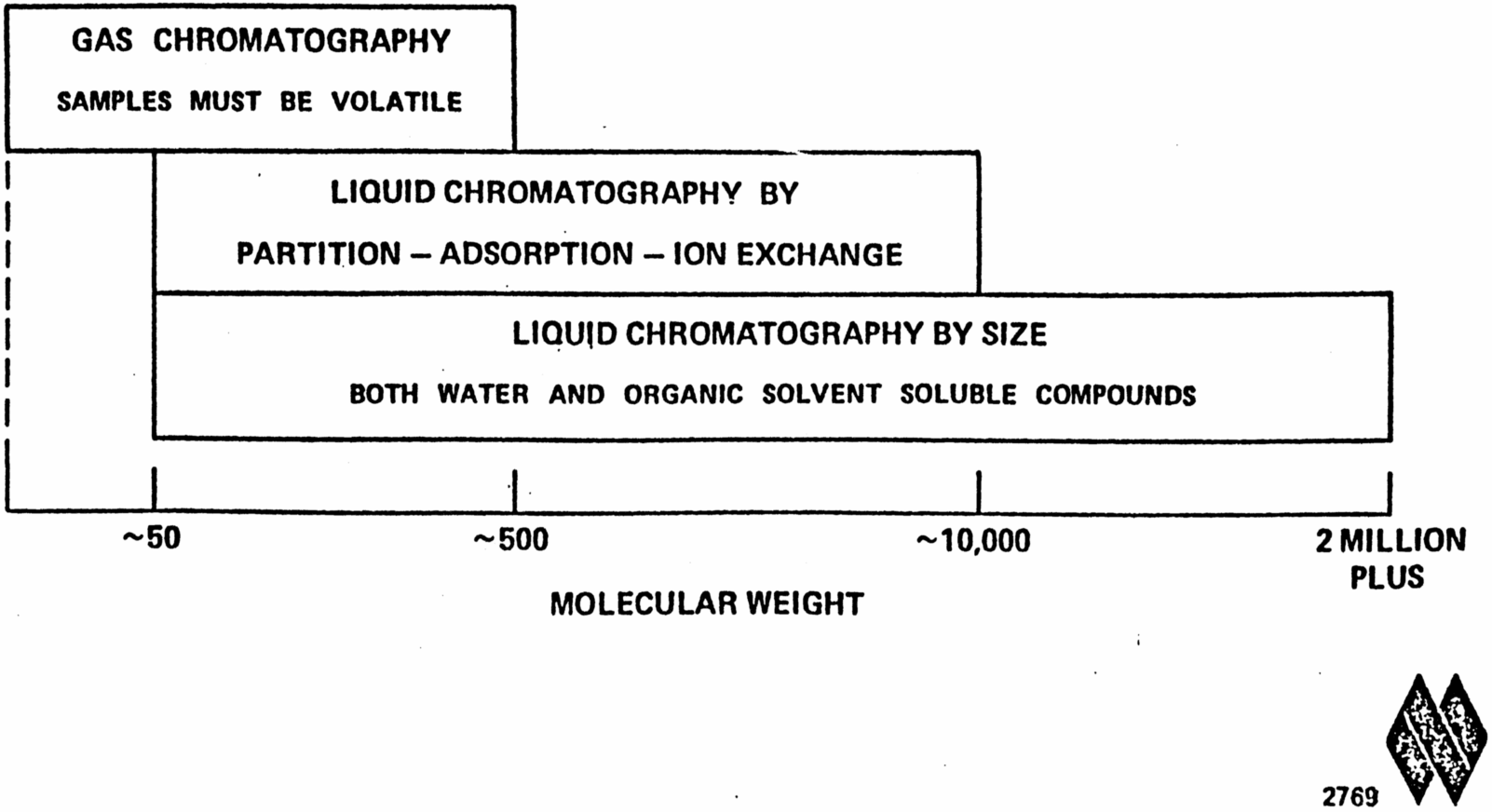
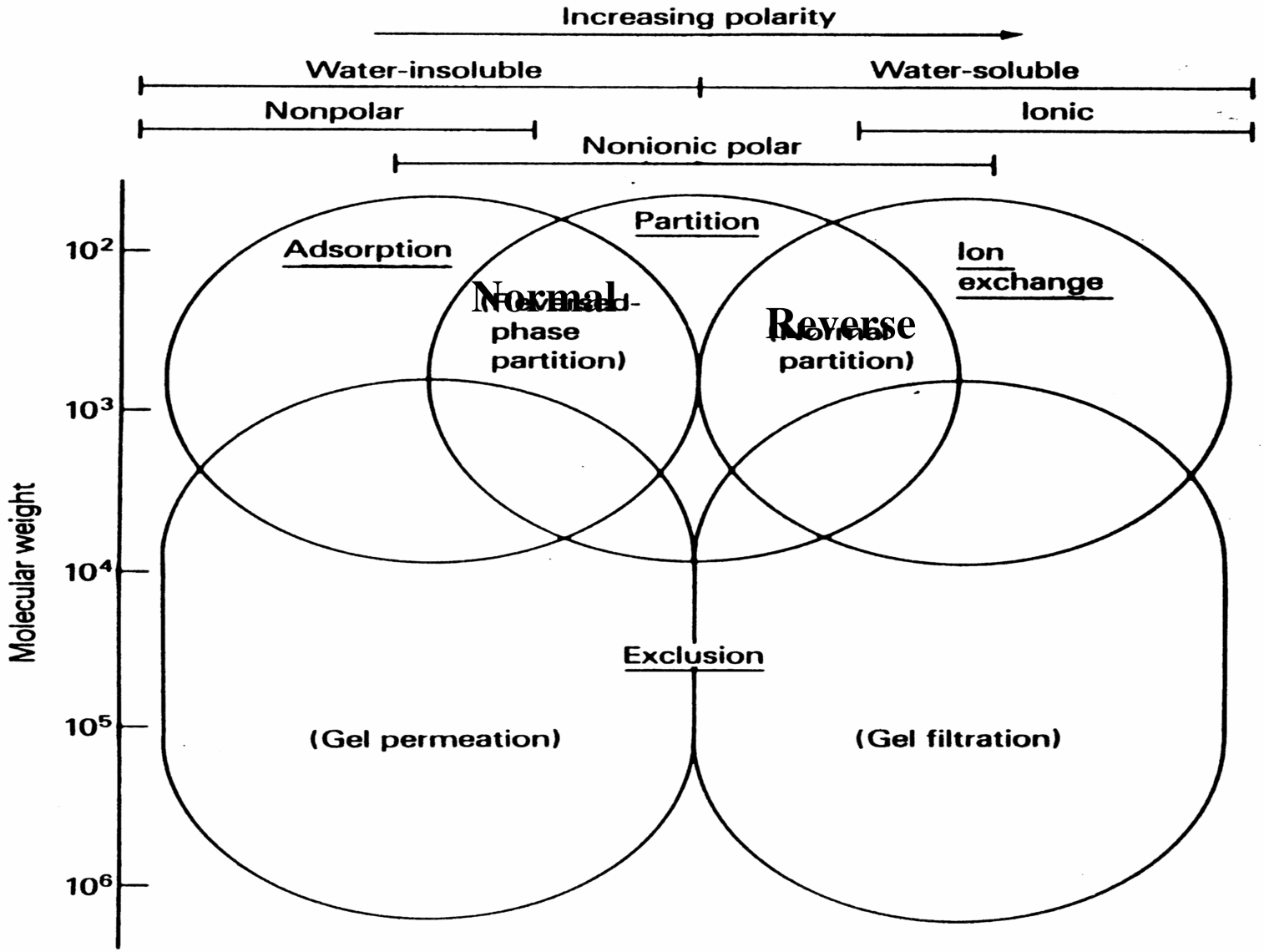
COMPARISON OF CHROMATOGRAPHY BY MW



Handout from a Waters Inc. Training Slide

Types and Range of Applications for Liquid Chromatography



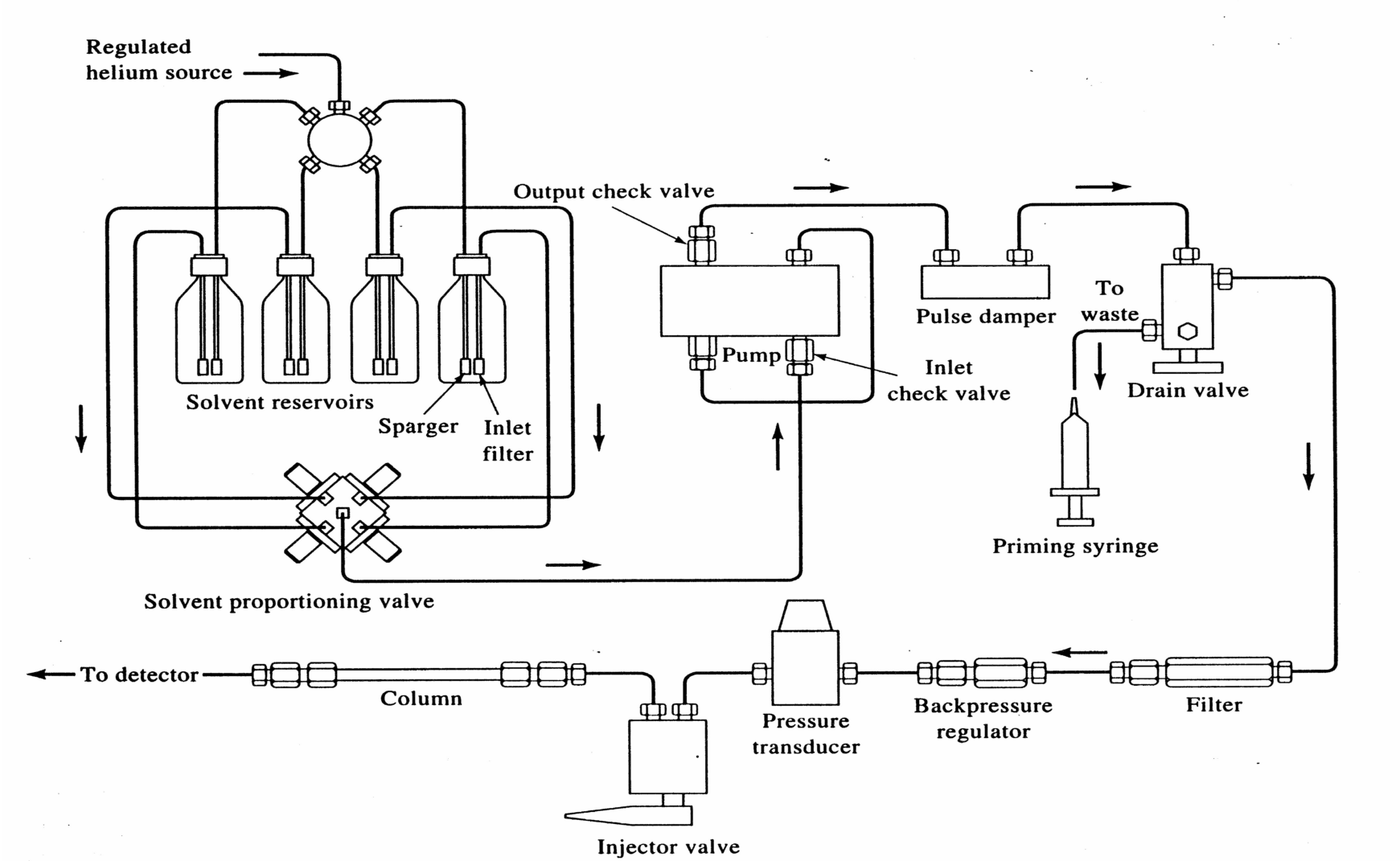
From Skoog, Holler and Nieman, “Principles of Instrumental Methods, 5 th. Ed., p. 726.

TABLE 28-3 Typical Applications of Partition Chromatography

Field	Typical Mixtures
Pharmaceuticals	Antibiotics, Sedatives, Steroids, Analgesics
Biochemical	Amino acids, Proteins, Carbohydrates, Lipids
Food products	Artificial sweeteners, Antioxidants, Aflatoxins, Additives
Industrial chemicals	Condensed aromatics, Surfactants, Propellants, Dyes
Pollutants	Pesticides, Herbicides, Phenols, PCBs
Forensic chemistry	Drugs, Poisons, Blood alcohol, Narcotics
Clinical medicine	Bile acids, Drug metabolites, Urine extracts, Estrogens

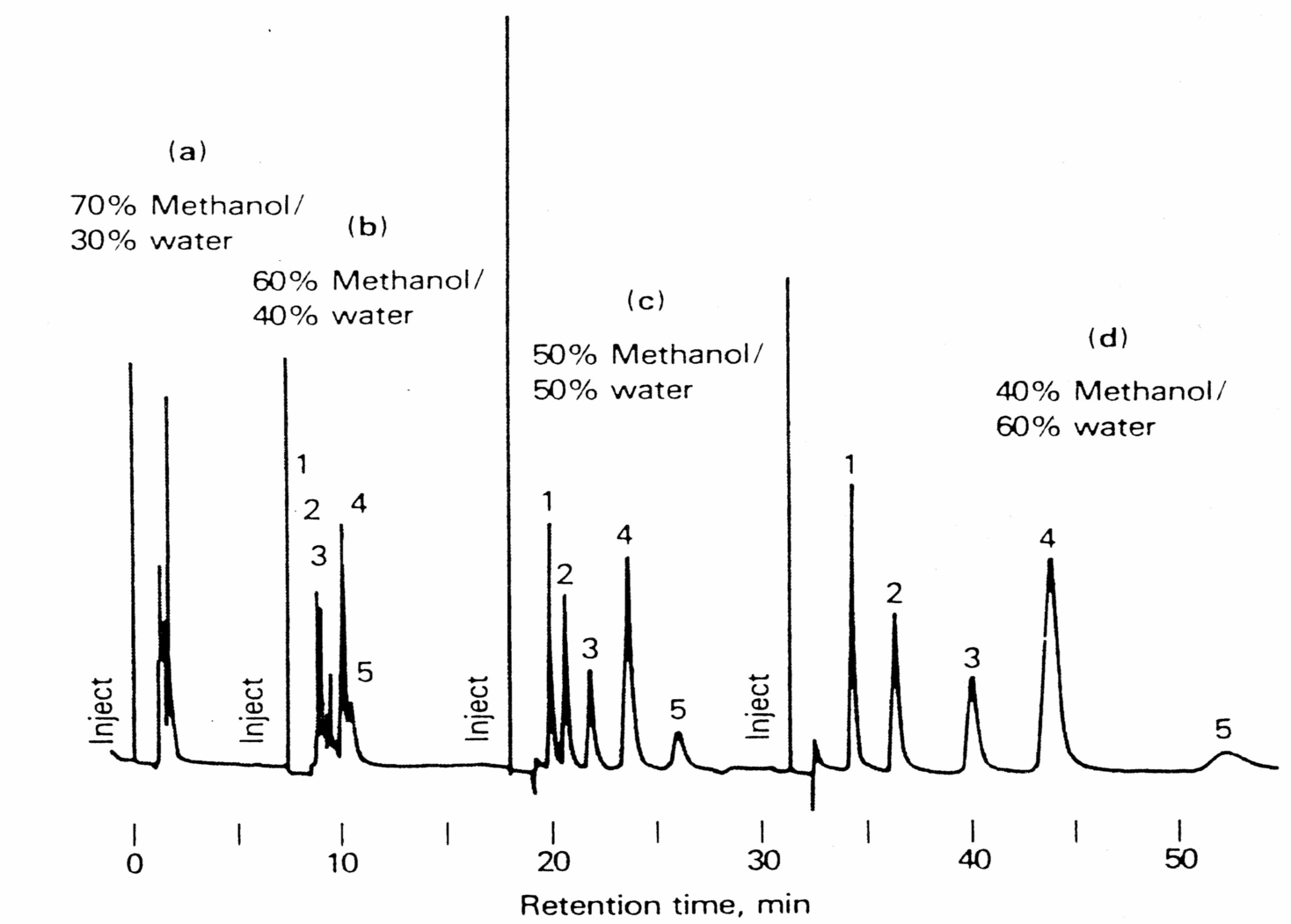
From Skoog, Holler and Nieman, "Principles of Instrumental Analysis", 5 th. Ed., p. 746.

General Schematic of a High Performance Liquid Chromatograph (LC)



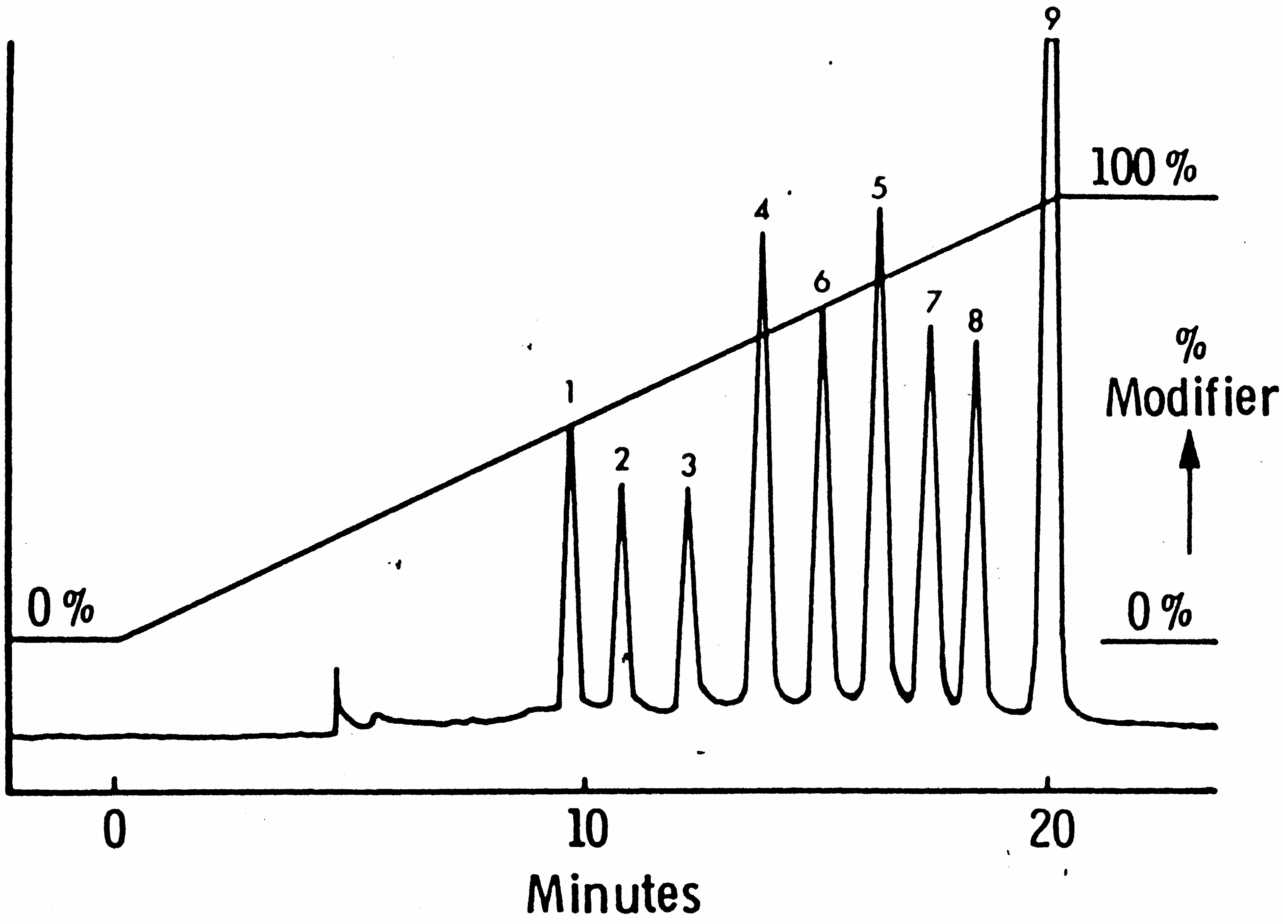
From Skoog, Holler and Nieman, "Principles of Instrumental Methods, 5 th. Ed., p. 729.

The effect of mobile phase solvent strenght in reversed phase HPLC



From Skoog, Holler and Nieman, "Principles of Instrumental Analysis, 5 th. Ed., p. 692.

Example of a linear gradient to improve peak resolution in Liquid Chromatography. Often a 20- min. linear gradient is a good place to start.



From C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier Scientific, 1991, p. 488.

Example of More Complex Gradient for more Involved Separations

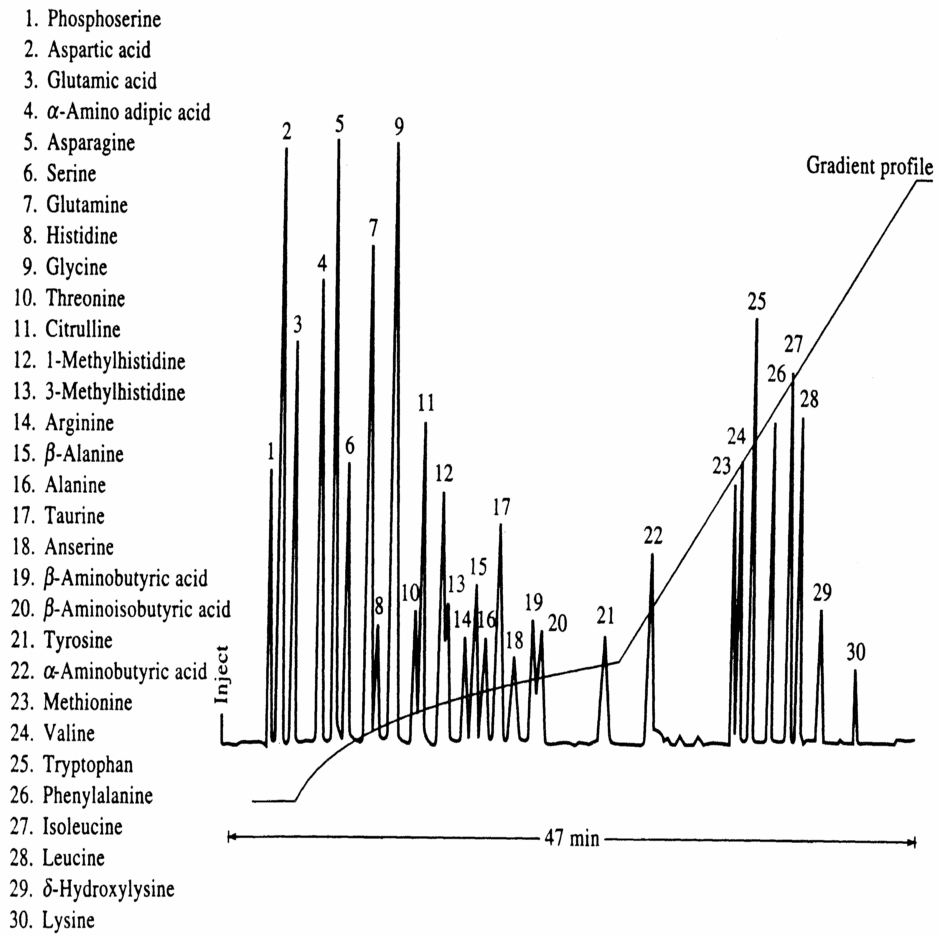


Figure 28-18 Chromatogram of orthophthalaldehyde derivatives of 30 amino acids of physiological importance. Column: 5 μm C_{18}, reversed-phase. Solvent A: 0.05 M Na_2HPO_4, pH 7.4, 96:2:2 $CH_3OH/THF/H_2O$. Fluorescence detector: excitation 334 nm; emission 425 nm.

From Skoog, Holler and Nieman, "Principles of Instrumental Analysis, 5 th. Ed., p. 747.

Common Liquid Chromatographic Detectors

Detector Name	Mode of Operation	Approx. Min. Detection Level	Linear Dynamic Range
Absorbance Detector (Fixed, Variable, Diode Array)	UV-Vis absorption of chromophore	0.1- 10 ng (depends on absorptivity)	10^4 - 10^5
Refractive Index (RI)	Change in the R.I. caused by solute	0.1 - 1.0 μg/mL	10^4
Evaporative Light Scattering (ELSD)	Change in the light scattered	1-100 ng	10^3-10^4
Fluorescent Detector	Measure of the Fluorescent Emission	1- 10 pg.	10^6
Conductivity Detector	Change in the conductivity caused by solute	0.1-10 ng/mL	10^4
Electrochemical Detector	Current as a result of an applied voltage	10 - 100 pg.	10^4
Mass Spectrometric	Monitoring mass/charge ratio as a result of ionization ESI, APCI, FAB	1 pg- ng	10^2 - 10^6

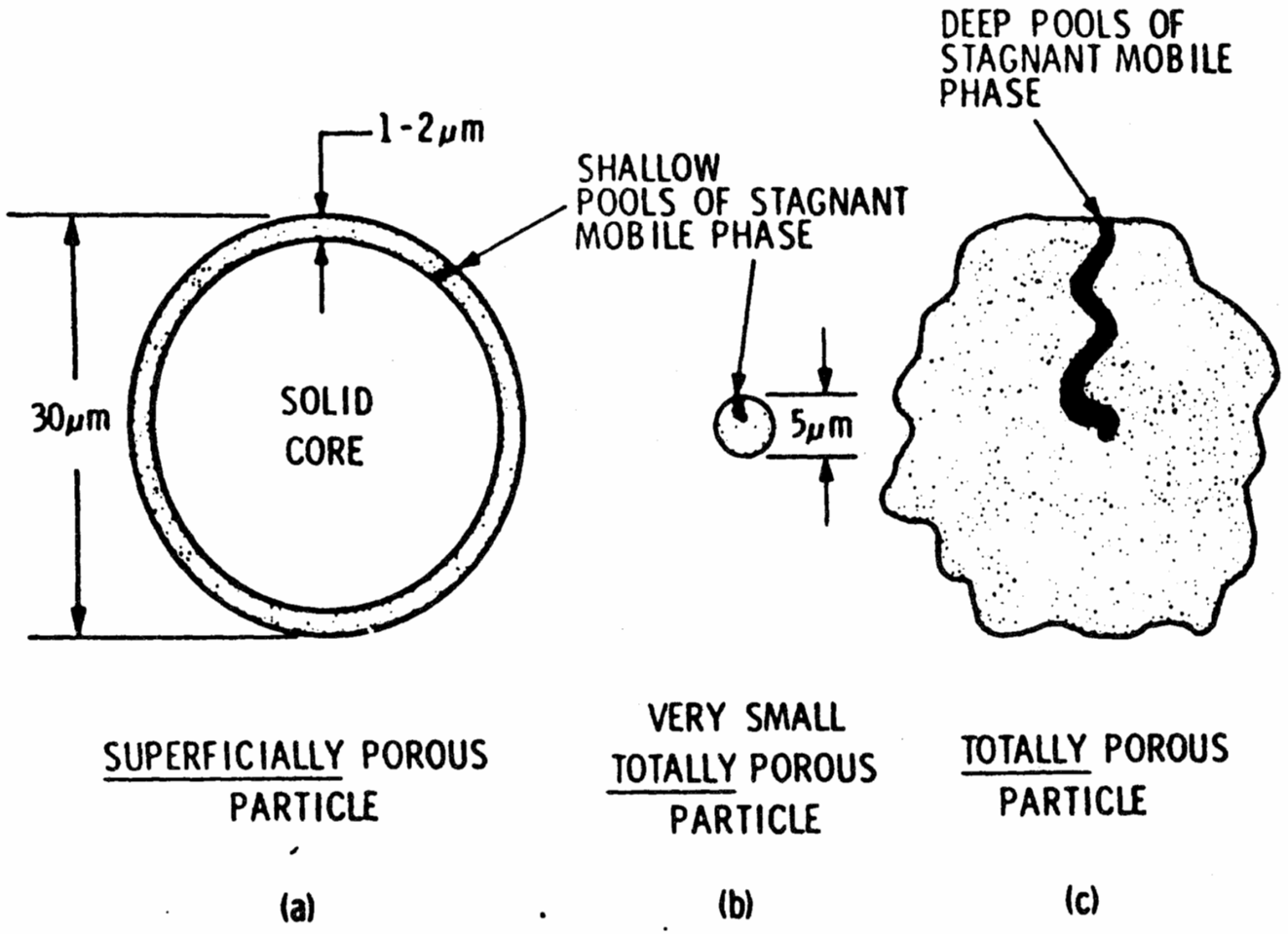
Adapted from various sources including, C.F. Poole "Essence in Chromatography", p. 455-487, and Skoog, Holler and Nieman, "Principles of Instrumental Analysis", 5 th. ed., Harcourt Brace and Co. , p. 733-739.

Typical Liquid Chromatography Columns

Column Name	Inner Diameter	Column Length	Flow Rate	Injection Vol.	Rel. Loading Capacity
Semi Prep.	**10-200 mm**	**5-50 cm**	**10-1000 mL/min**	**1-10 mL**	**100,000**
Conventional	**4-5 mm**	**5-25 cm**	**0.8-2.0 mL/min**	**5-50 µL**	**10,000**
Narrowbore	**2 mm**	**5-10 cm**	**0.1-0.3 mL/min**	**1-5 µL**	**1,000**
Microbore	**1 mm**	**5 cm-25cm**	**0.010-0.050 mL/min**	**0.2-0.5 µL**	**250**
Packed Capillary	**100-500 µm**	**5-25 cm**	**1-10 µL/min**	**0.1 µL**	**50**
Open Tubular (Nano LC)	**50-100 µm**	**1-25 cm**	**100-300 nL/min**	**1-3 nL**	**1**

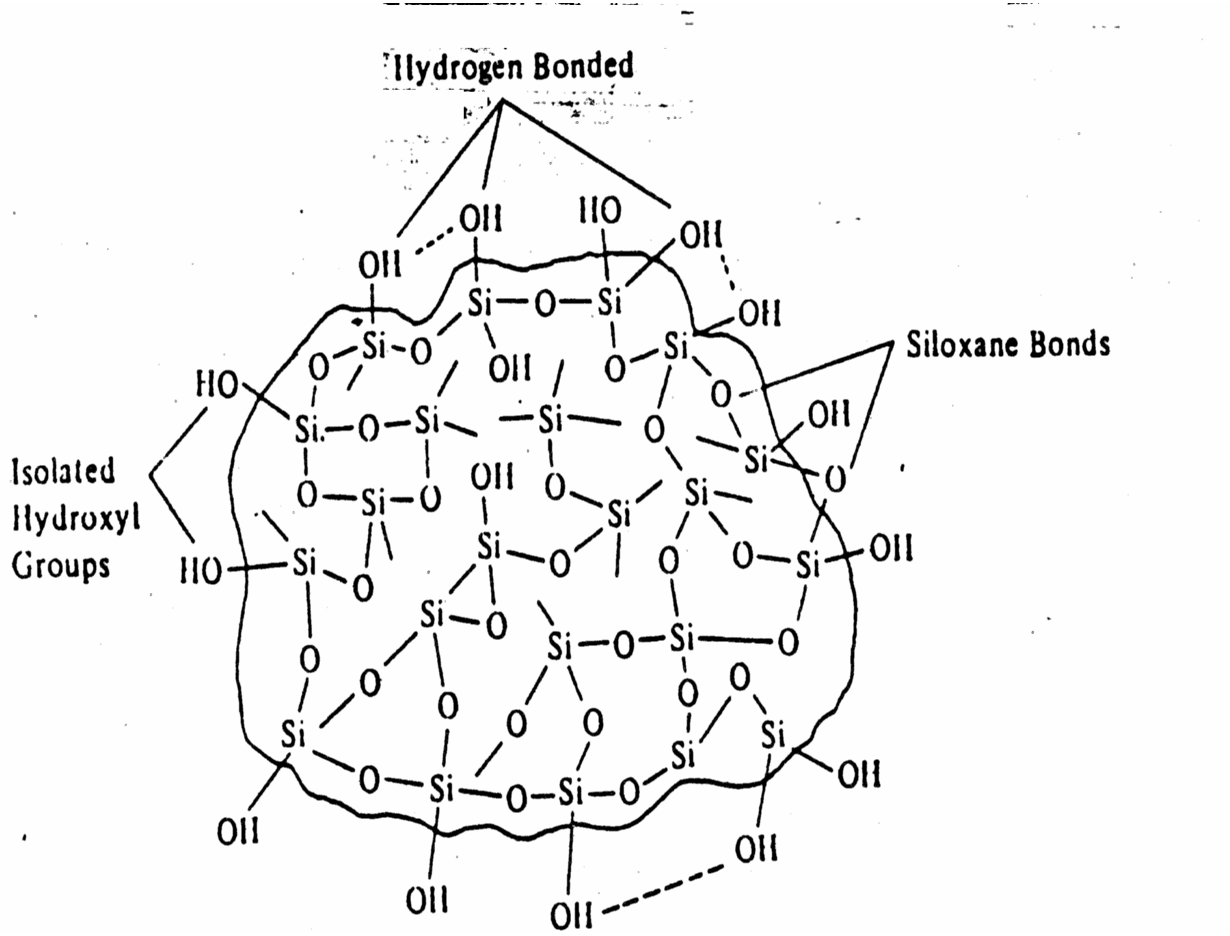
Adapted from K.B. Tomer, M. A. Moseley, L.J. Deterding, C.E. Parker, "Review- Liquid Chromatography Mass Spectrometry" in **Mass Spectrometry Reviews** (1994) 13, 432 and from C.F. Poole, "Chromatography Today", Elsevier, 1992, p. 63. (Note these are older references and it is an ever developing fields).

Depiction of larger, superficious (or pellicular) packing compared to the fully- porous, spherical (man-made) silica and the larger, irregular (natural) silica particles.



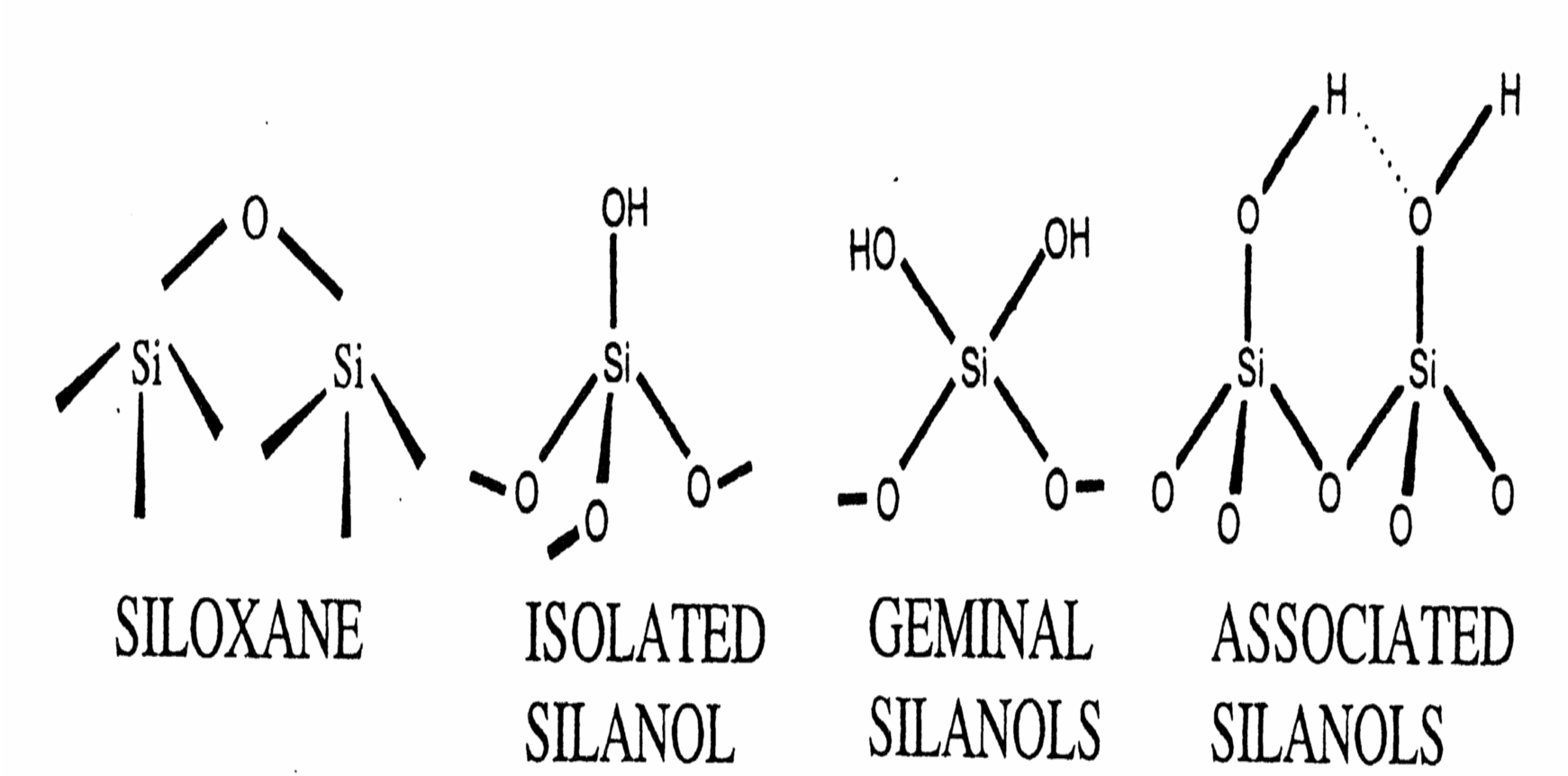
From C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier, 1991, p. 315.

Depiction of the surface of a porous silica particle.



H.H. Bauer, G.D. Christian, J.E. O'Reilly, "Instrumental Analysis", 1978, Allyn and Bacon, p. 657.

Depiction of the Functional Groups on the Silica Surface



From C.F. Poole, "The Essence of Chromatography", Elsevier Scientific, 2003, p. 276

Porous Silica as a Liquid Chromatographic Stationary Phase

Advantages

- **Mechanically strong.**
- **Porous silica particles can be made using different synthetic methods giving a range of different particles sizes and pore diameters.**
- **The available free surface silanol groups allow ready bonding.**

Disadvantages

- **Silica back-boned is stable only between the pH range of 2-8.**
- **Above pH 8 the silica backbone dissolves.**
- **Below a pH of 2 the bonds of the different groups to the silanol surface become cleaved (acid hydrolyzed).**
- **Difficult to remove impurities and control surface activity.**

(However certain new specially prepared silica bonded phases are reported to be able to be used over the range of pH of 1 to 12).

STRUCTURES OF SILOXANE BONDED PHASES

Functional Group	Structure	Applications
Alkyl	$-CH_3$	Reversed phase
	$-C_4H_9$	
	$-C_8H_{17}$	
	$-C_{18}H_{37}$	
Phenyl	$-C_6H_5$	Reversed phase
Cyano	$-(CH_2)_3CN$	Normal and reversed phase
Amino	$-(CH_2)_3NH_2$	Normal, reversed phase and weak anion exchanger
Diol	$-(CH_2)_3OCH_2CH(OH)CH_2(OH)$	Normal phase and size exclusion
Amide	$-(CH_2)_3CONHCH_3$	Size exclusion
Sulfonic Acid	$-(CH_2)_3SO_3H$	Strong cation exchanger
	$-C_6H_4SO_3H$	
	$-(CH_2)_3SO_3H$	
	$-(CH_2)_3C_6H_4SO_3H$	
Carboxylic Acid	$-(CH_2)_3OCH_2COOH$	Weak cation exchanger
	$-(CH_2)_3COOH$	
	$-(CH_2)_3C_6H_4CH_2COOH$	
Dimethylamine	$-(CH_2)_3N(CH_3)_2$	Weak anion exchanger
Quaternary Amine	$-(CH_2)_3N^+(CH_3)_3$	Strong anion exchanger

From C.F. Poole, "The Essence of Chromatography", Elsevier Scinetific, 2003, p. 285.

Effect of chain length on retention in Reversed-Phase HPLC, of C-1, C-8 and C-18.

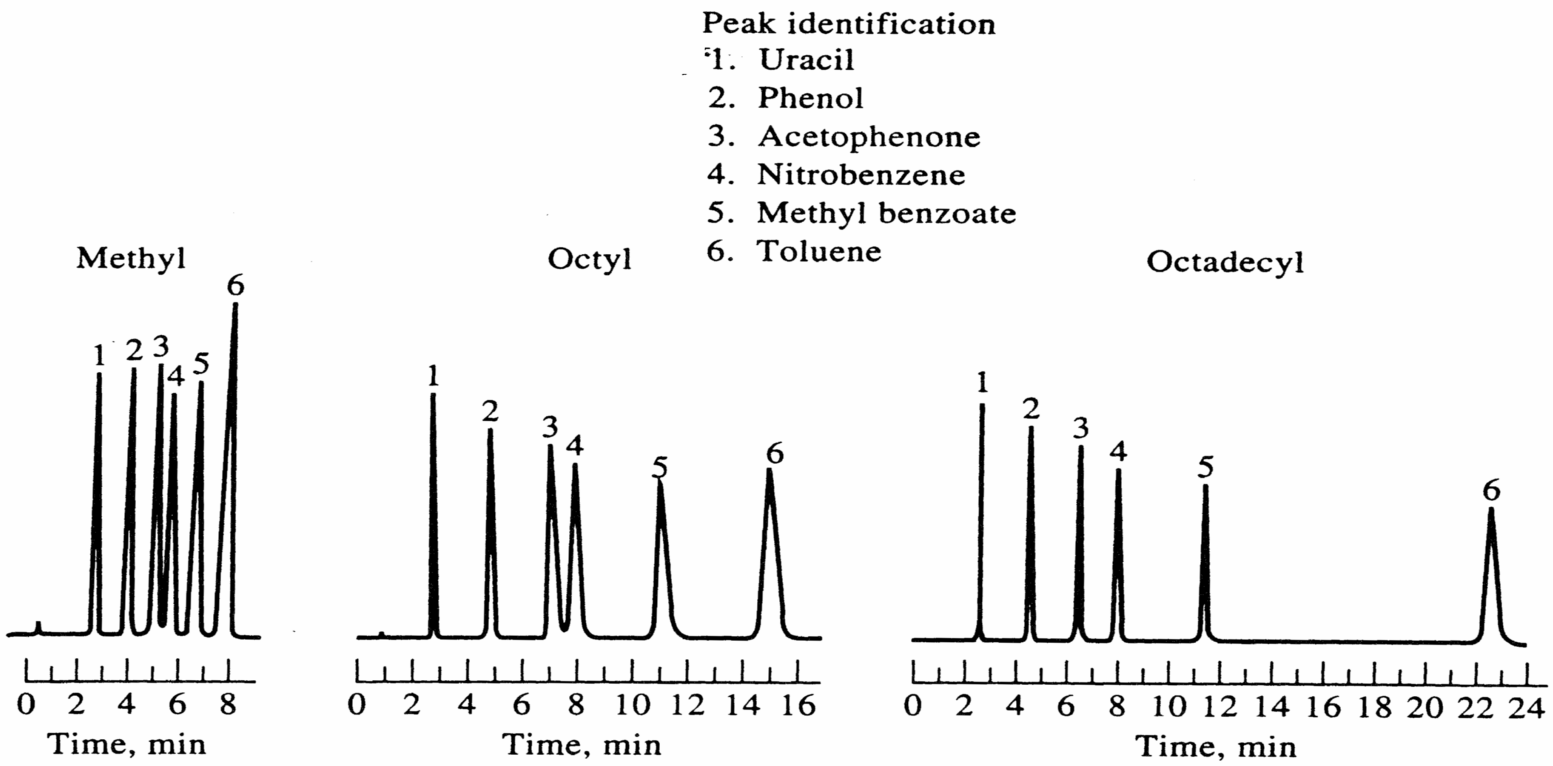
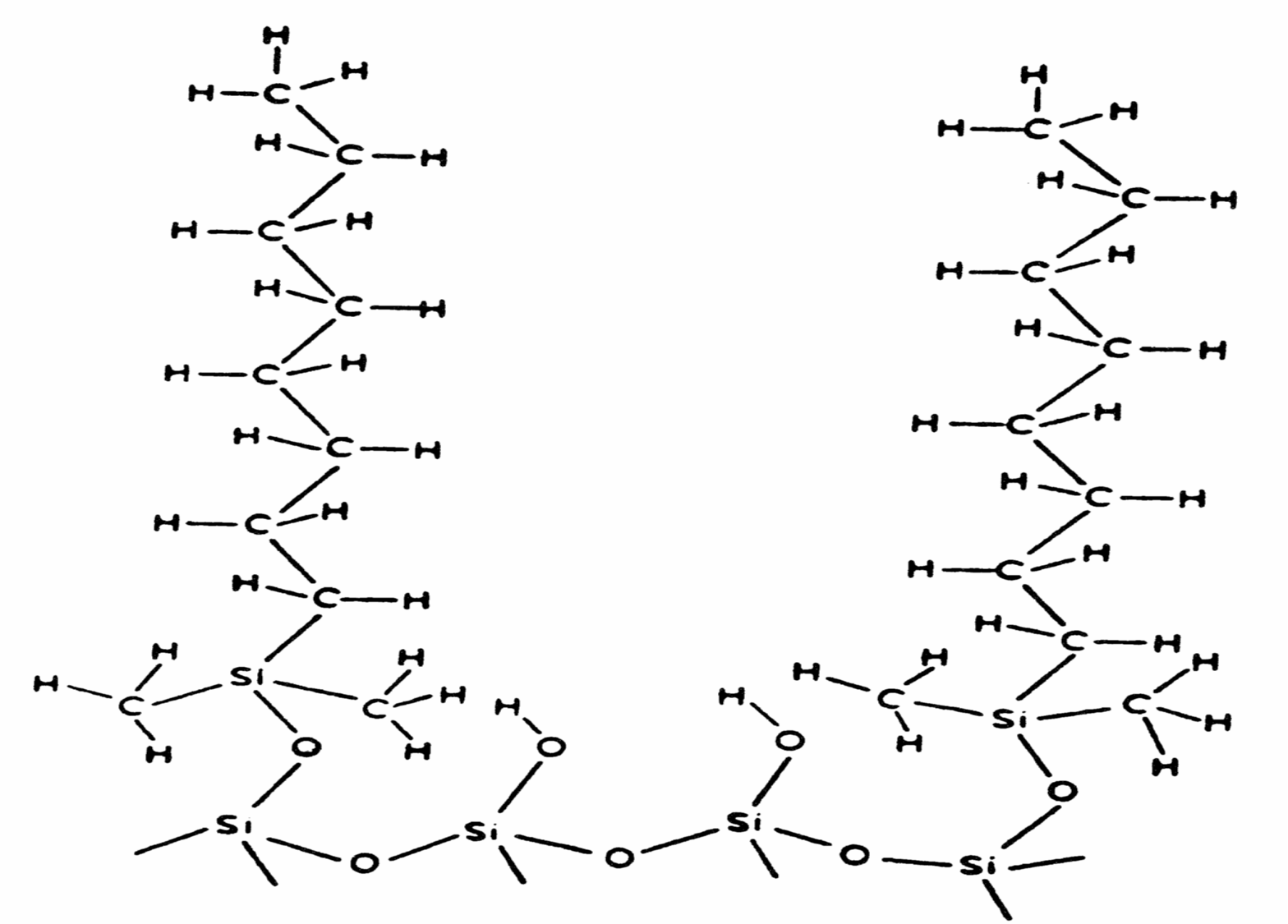


Figure 28-15 Effect of chain length on performance of reversed-phase siloxane columns packed with 5-μm particles. Mobile phase: 50/50 methanol/water. Flow rate: 1.0 mL/min.

From Skoog, Holler and Nieman, "Principles of Instrumental Analysis", 5 th. Ed., p. 741.

Steric Factors allow for only 50% coverage with C-8 functional groups.



From C. Horvath and W.R. Melander, "Reversed-Phase Chromatography" in "High-Performance Liquid Chromatography-Advances and Perspectives", vol. 2, 1980, C. Horvath, ed. Academic Press, p. 137.

Showing how Free Hydroxyl Groups can be sterically protected with bulky side groups.F

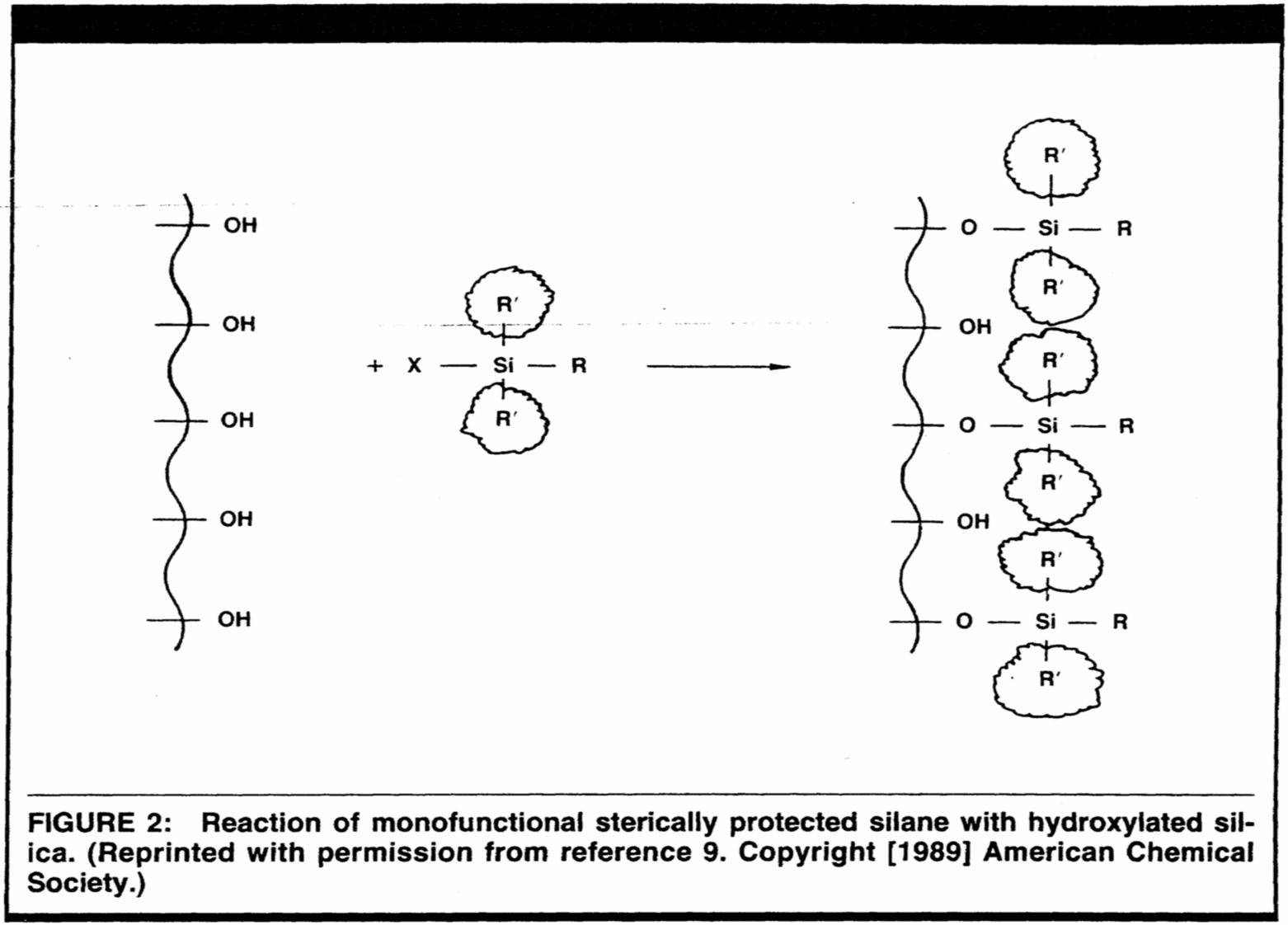
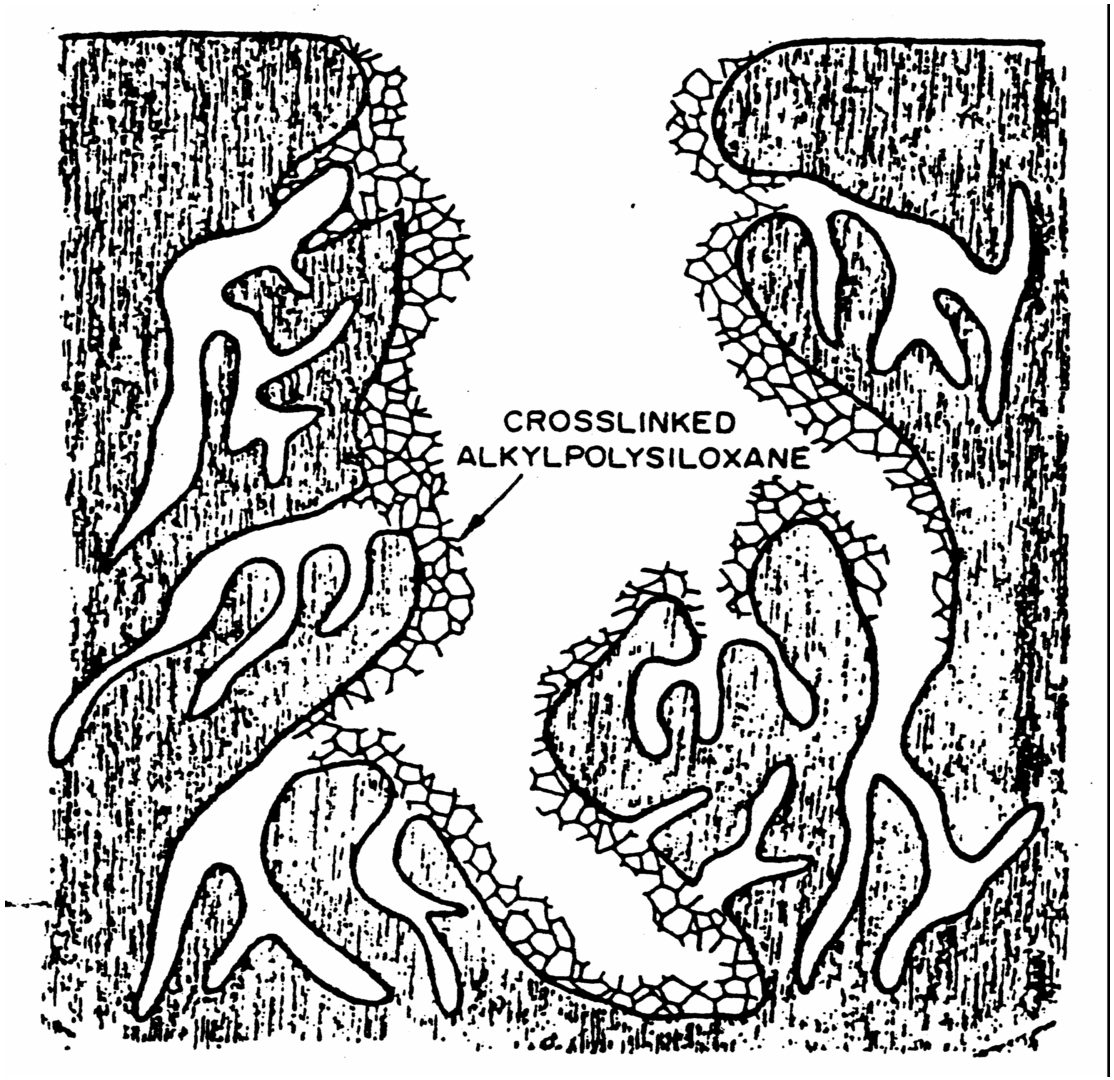


FIGURE 2: Reaction of monofunctional sterically protected silane with hydroxylated silica. (Reprinted with permission from reference 9. Copyright [1989] American Chemical Society.)

From J.L. Glajch and J.J. Kirkland, *LC.GC Magazine*, 1990, 8 (2), Fig., 1, p.

Illustration of how an octyl (C-8) and octadecyl-silane (C18) phase may coat the internal surface of silica pores.



From C. Horvath and W.R. Melander, "Reversed-Phase Chromatography" in "High-Performance Liquid Chromatography-Advances and Perspectives", vol. 2, 1980, C. Horvath, ed. Academic Press, p. 138.

Effect of a Second Silation or End-Capping to remove more of the free Hydroxyl Groups.

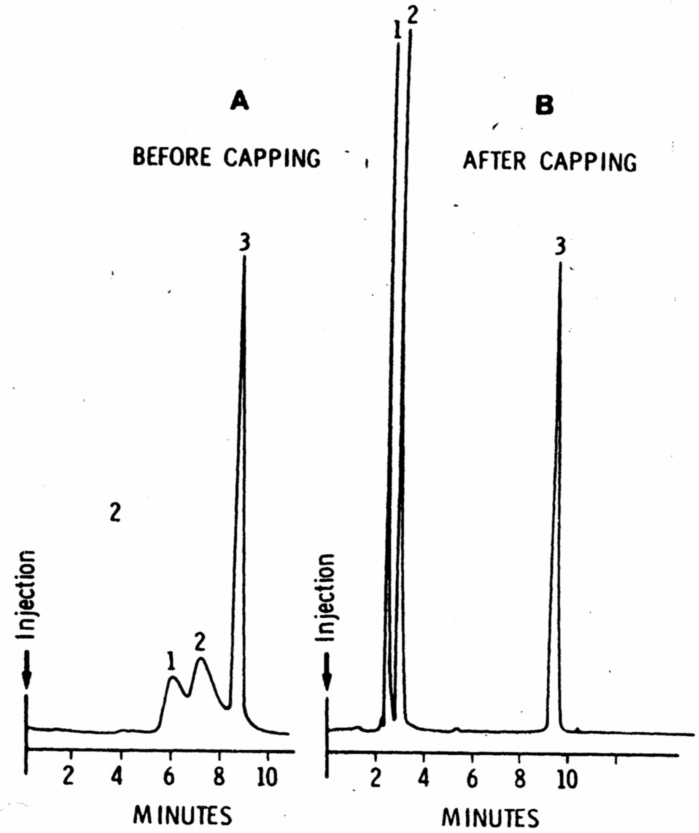
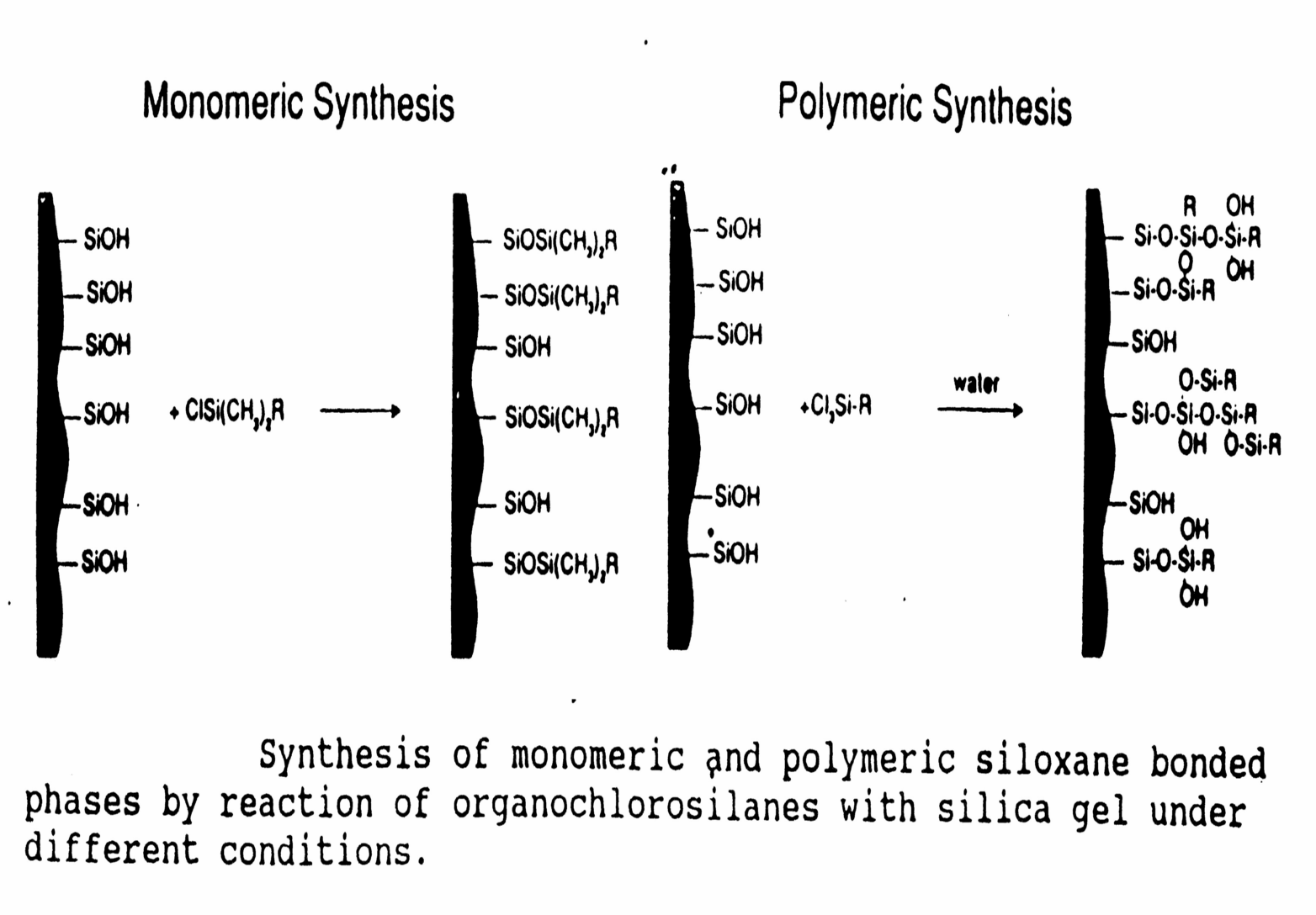


Figure 4.5 The influence of endcapping on peak shape and retention of some PTH-amino acids using a reversed-phase separation system. Peak identification: 1 = PTH-histidine, 2 = PTH-arginine and 3 = PTH-valine.

From C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier, 1991, p. 329.

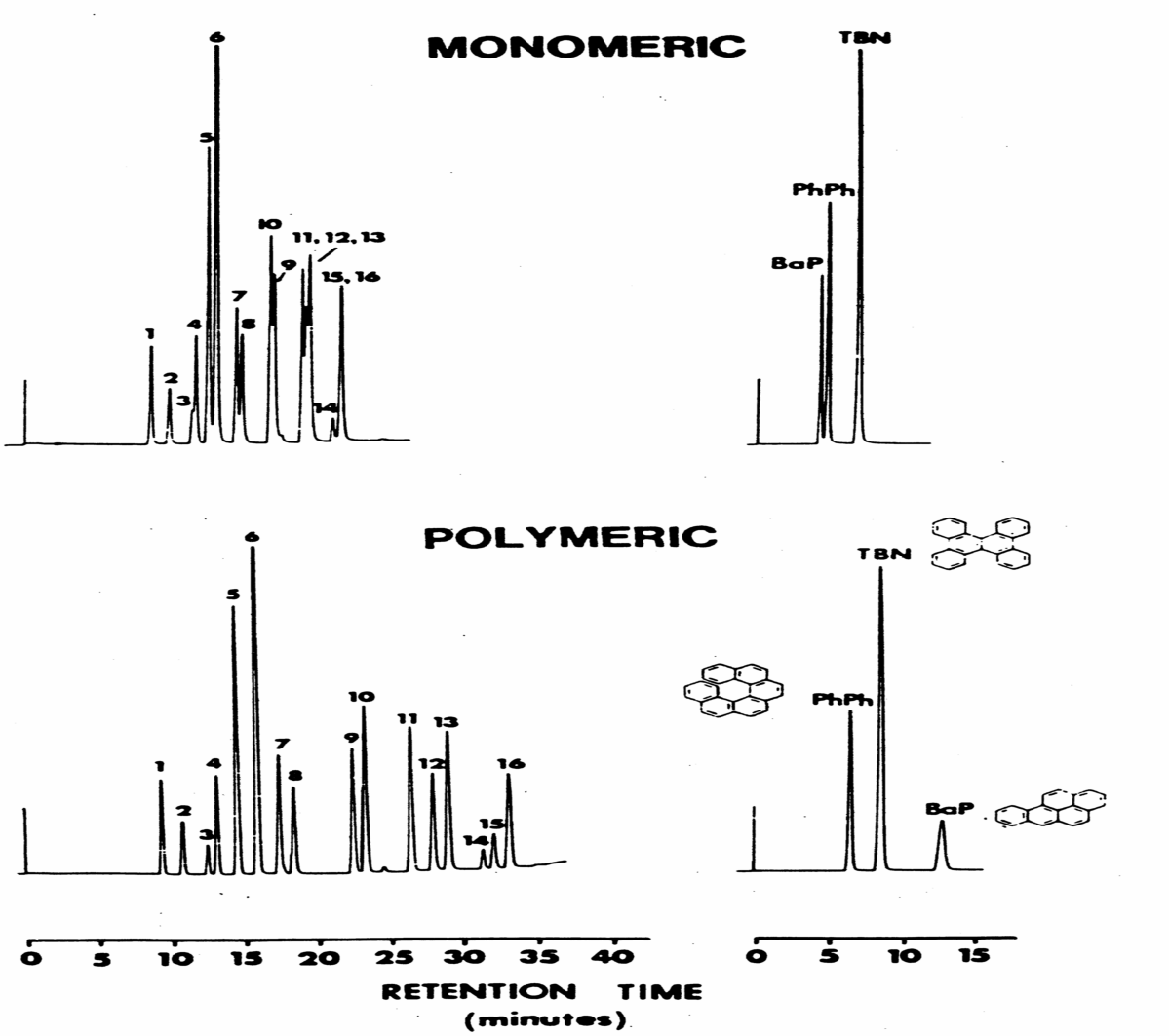
Monomeric vs. Polymeric bonding of a silica surface. Different Carbon Loads.



Synthesis of monomeric and polymeric siloxane bonded phases by reaction of organochlorosilanes with silica gel under different conditions.

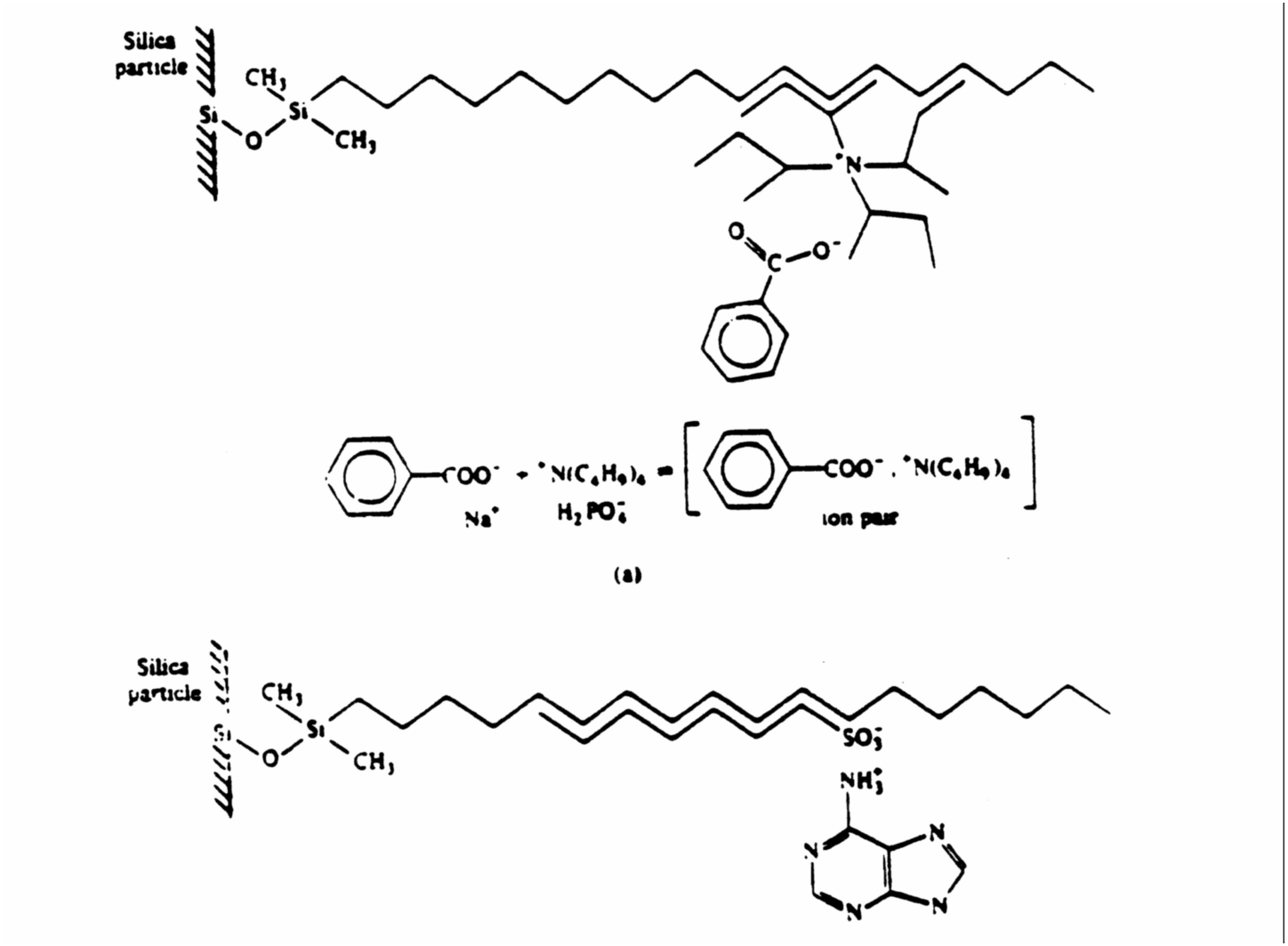
From C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier, 1991, p. 326.

A Polymeric C-18 phase has greater retention than a Monomeric C-18 Phase.



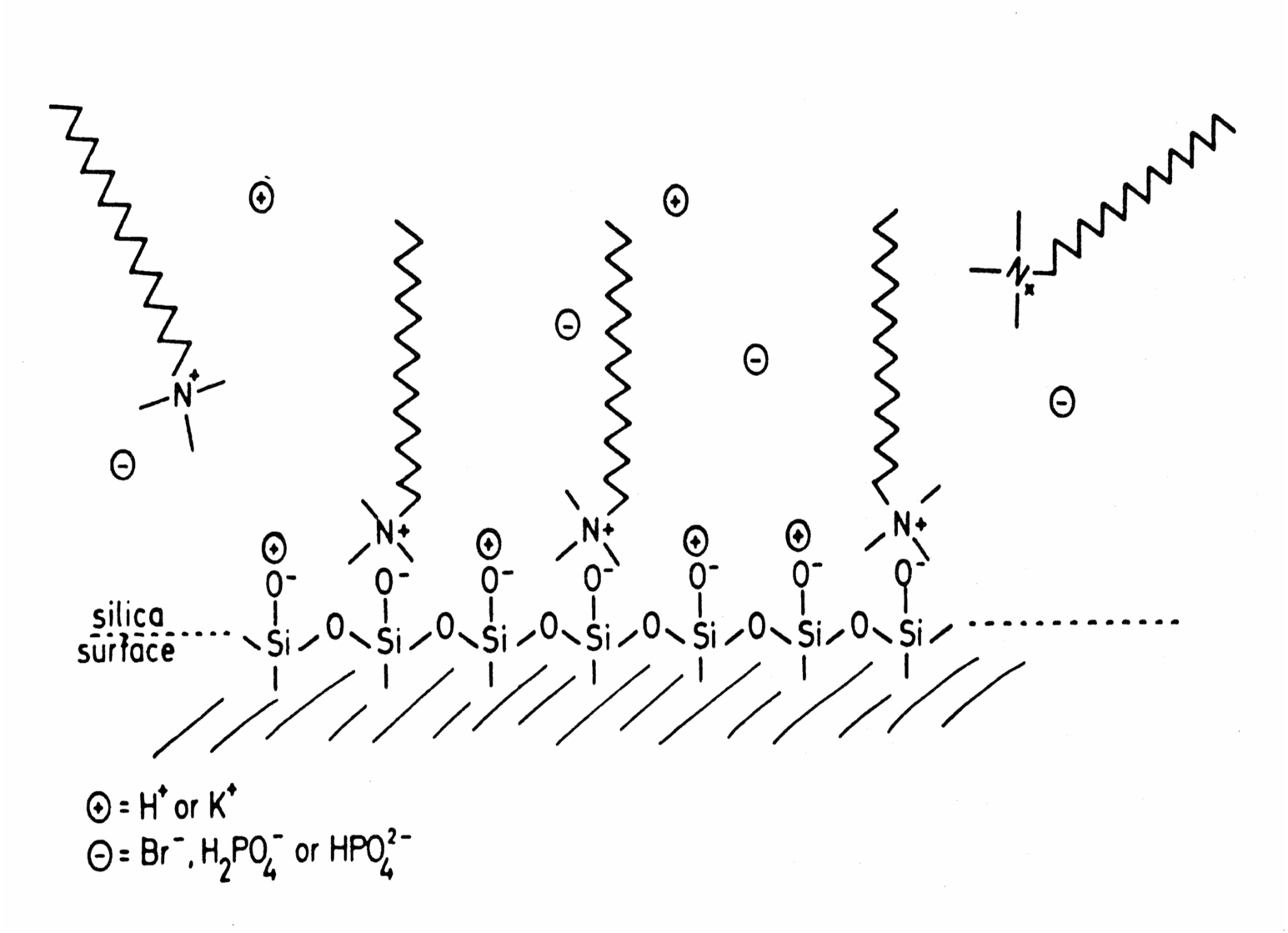
From C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier, 1991, p. 328.

Schematic of the possible "Dynamic Ionic Exchange" on C-18 bonded groups.



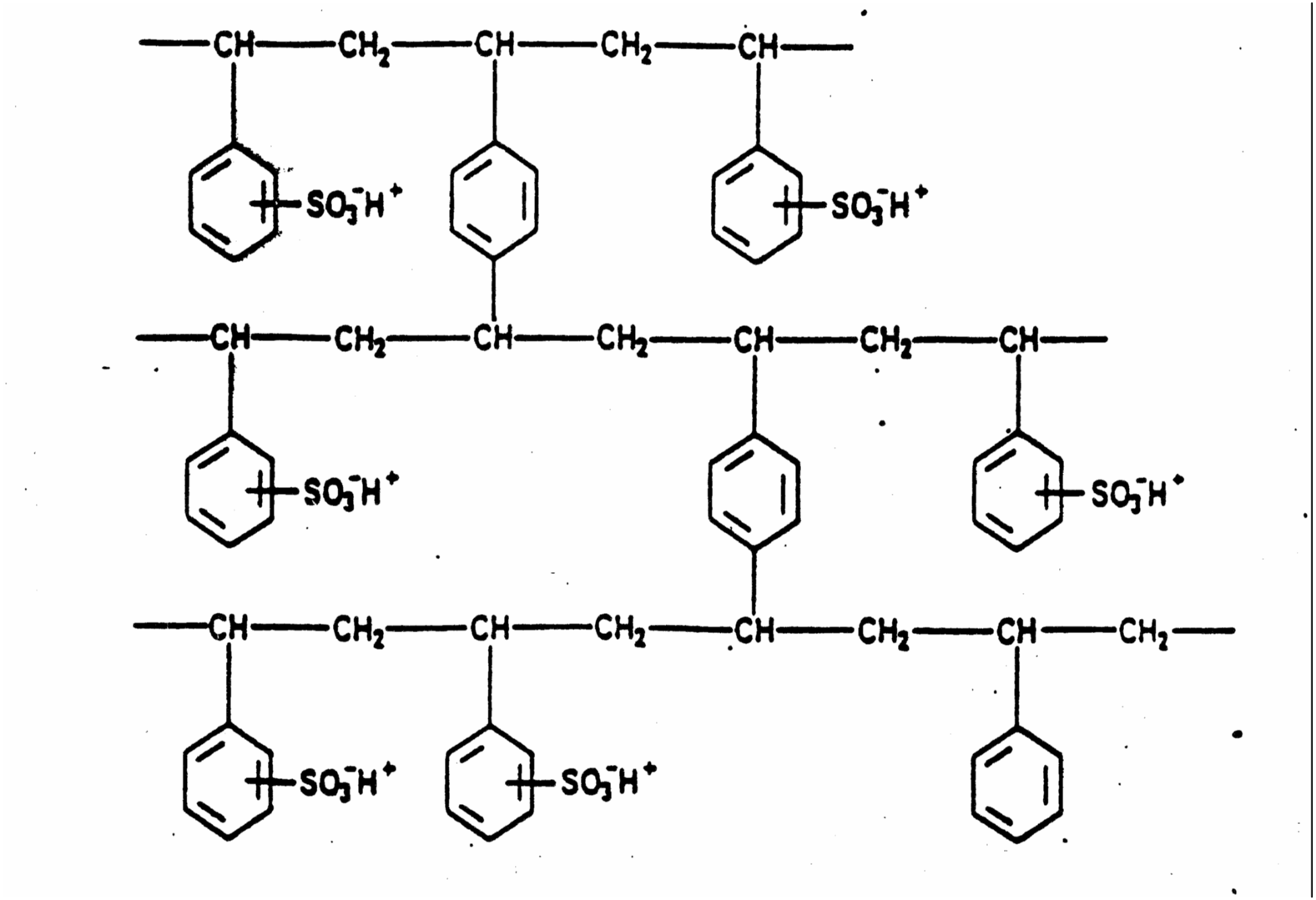
From Willard, Merritt, Dean and Settle, "Instrumental Methods of Analysis, 6 th. Ed., p. 542.

Model of a "Dynamically modified" surface silica with cetyltrimethylammonium ions.



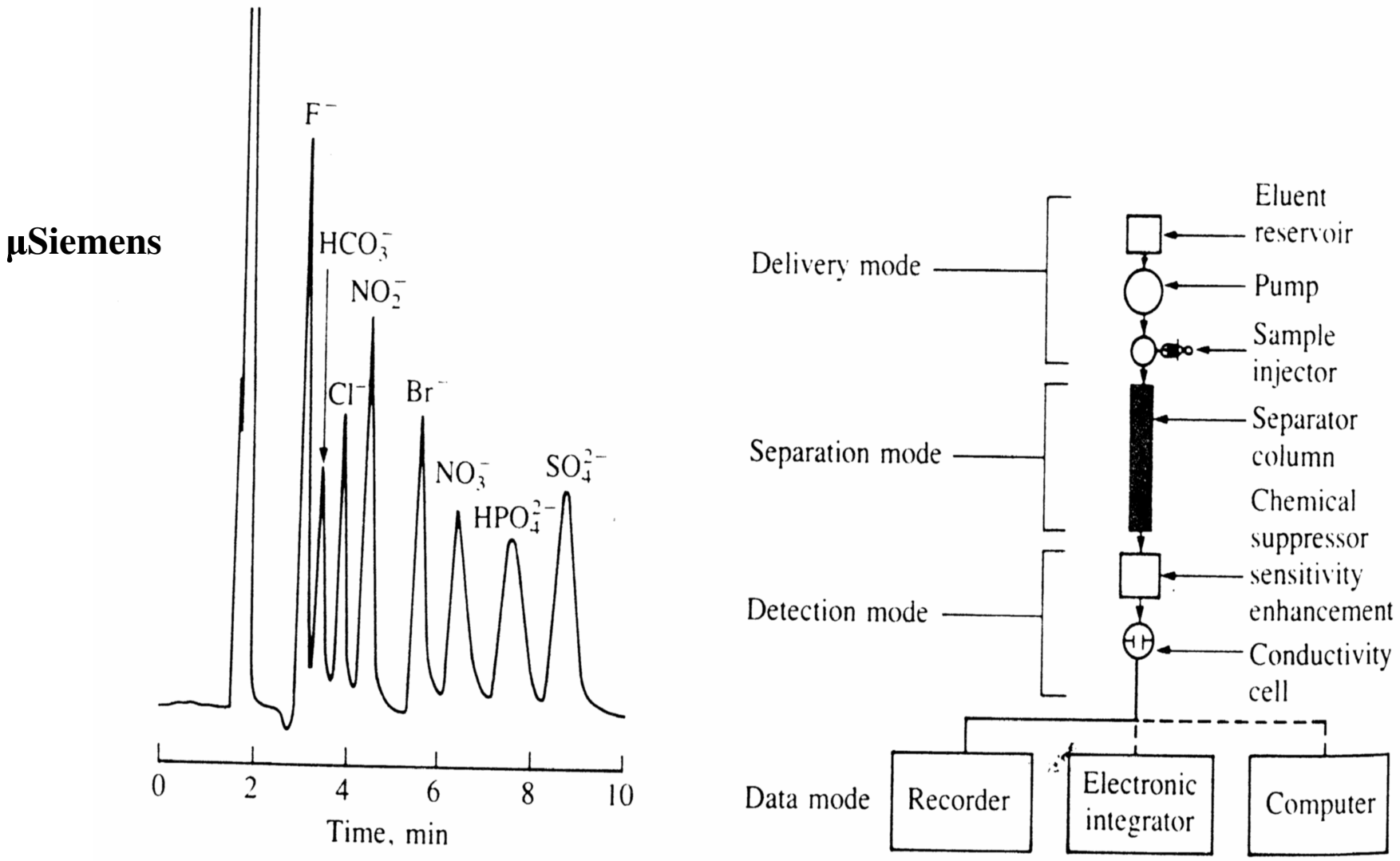
From C.F. Poole and S.K. Poole, "Chromatography Today", 1991, Elsevier, p. 392.

Illustration of a cross-linked polystyrene-divinyl benzene ion exchange resin



From Shoog, Holler and Nieman, "Principles of Instrumental Analysis, 5 th., 1998, p. 753.

Ion Chromatography- representative chromatogram and instrumentation.

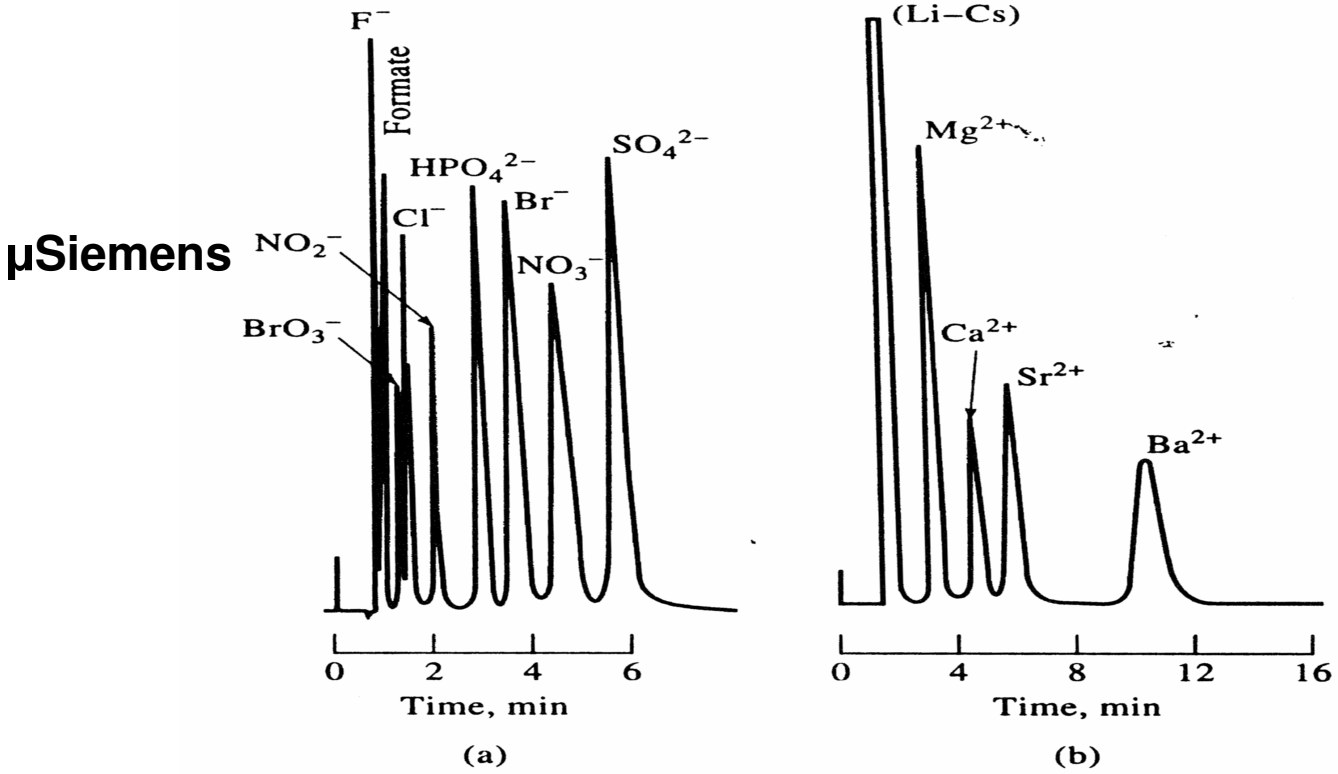


H.H. Willard, L.L. Merritt, Jr., J.A. Dean and F,A. Settle, Jr., "Instrumental Methods of Analysis" 7 th. Ed., 1988, Wadsworth Publishing Co., fig. 20.22, p. 642.

Representative separations by Ion Chromatography

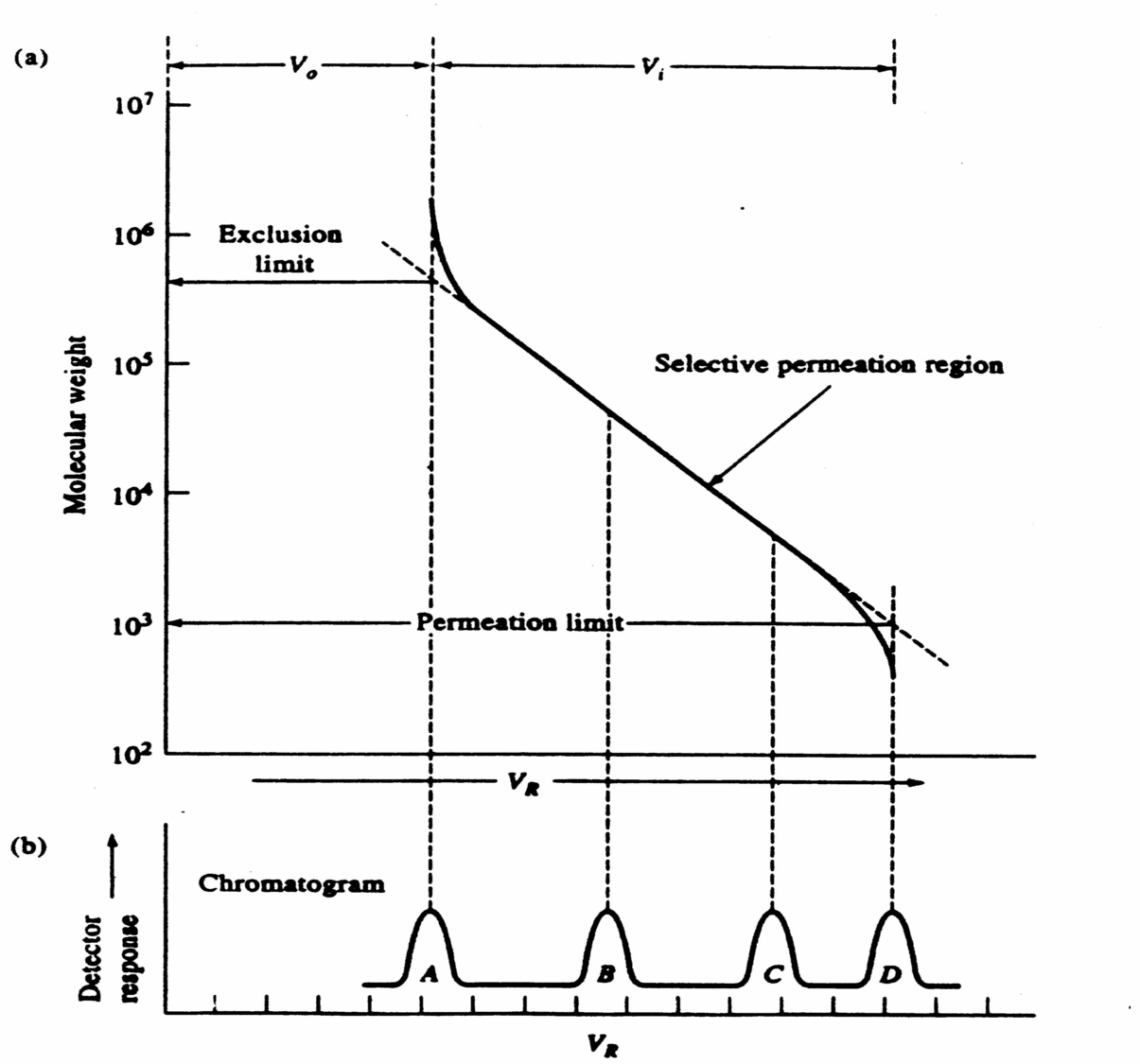
Concentrations, ppm	
F^-	3
Formate	8
BrO_3^-	10
Cl^-	4
NO_2^-	10
HPO_4^{2-}	30
Br^-	30
NO_3^-	30
SO_4^{2-}	25

Concentrations, ppm	
Ca^{2+}	3
Mg^{2+}	3
Sr^{2+}	10
Ba^{2+}	25



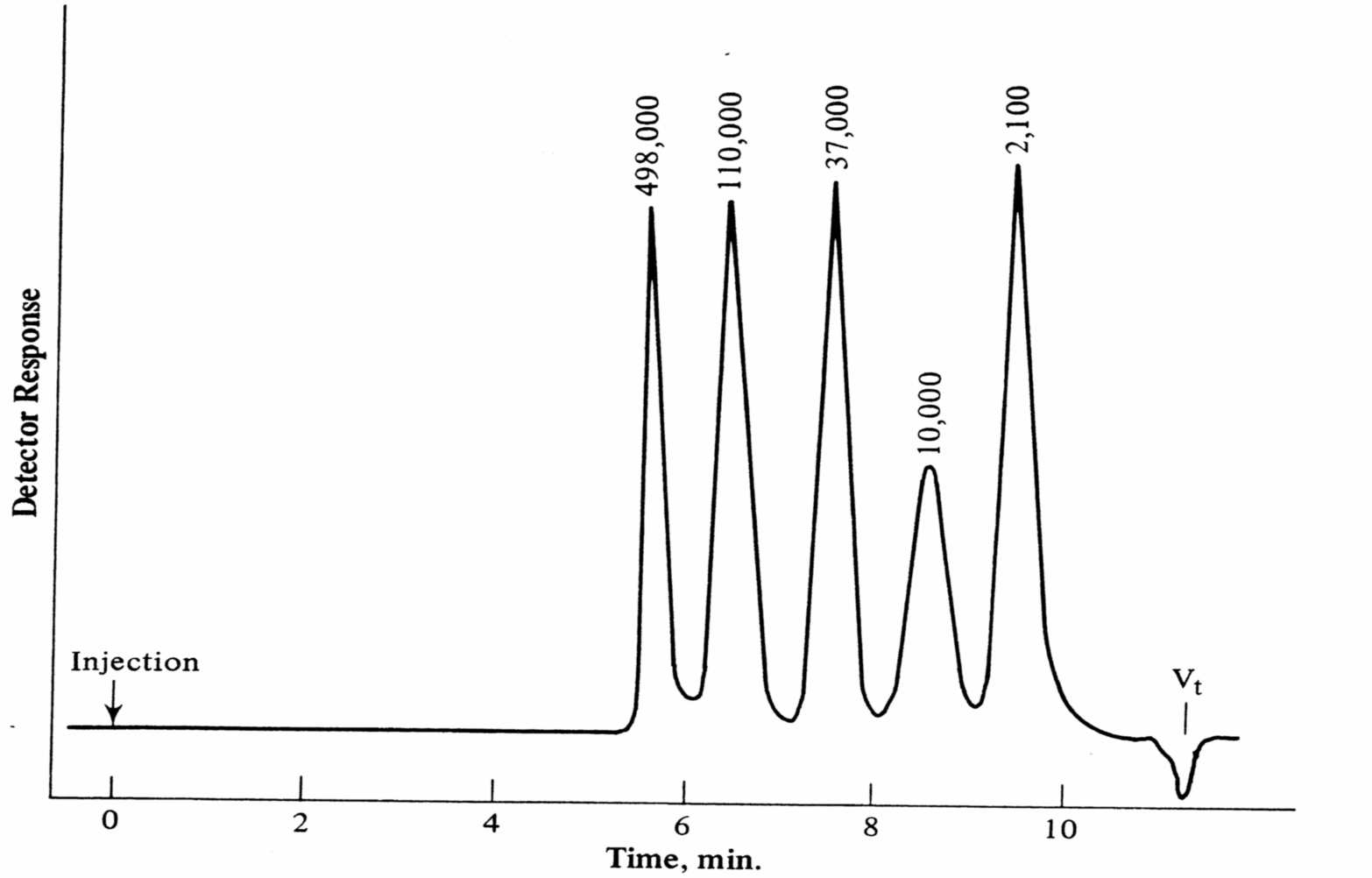
From Skoog, Holler and Nieman, “Principles of Instrumental Methods, 5 th. Ed., p. 755.

Calibration Curve for a Size-Exclusion LC separation of bio- or organic-polymers.



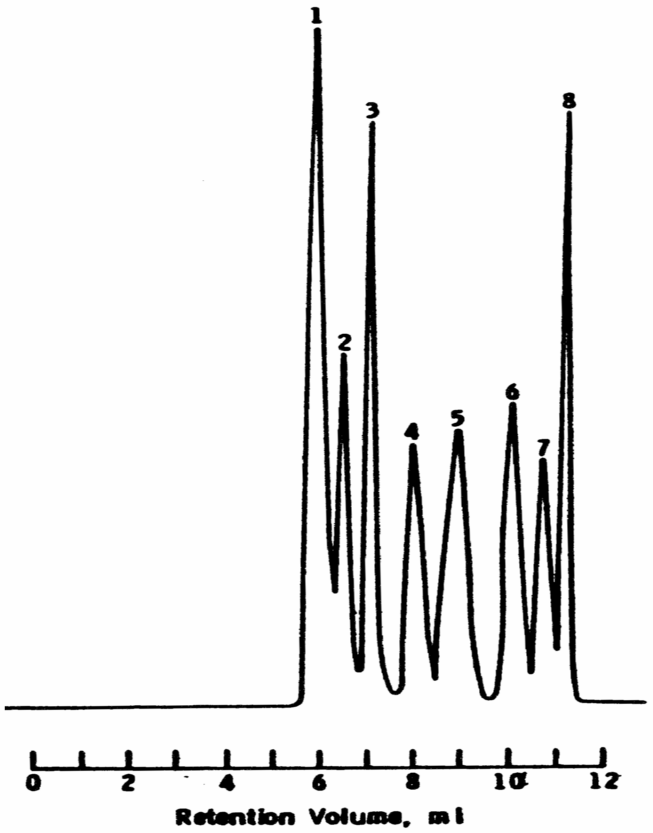
From Shoog, Holler and Nieman, "Principles of Instrumental Analysis, 5 th., 1998, p. 759.

Elusion order in Size-Exclusion Chromatography- Largest to Smallest. With a handy total permeation volume, V_t



H.H. Bauer, G.D. Christian, J.E. O'Reilly, "Instrumental Analysis", 1987, Allyn and Bacon, p. 671.

Size-Exclusion Separation of a Series of Polymer Standards



Separation of narrow molecular weight polystyrene standards on a μ-Bondagel column combination, 125, 300, 500, and 1000 Å; mobile phase methylene chloride, flow rate 0.5 ml/min. Polystyrene standards 1, 2,145,000; 2, 411,000; 3, 170,000; 4, 51,000; 5, 20,000; 6, 4000; 7, 600; and 8, benzene.

From C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier, 1991, p. 293.

Example of an aqeous size-exclusion separation of modified glutein proteins of wheat

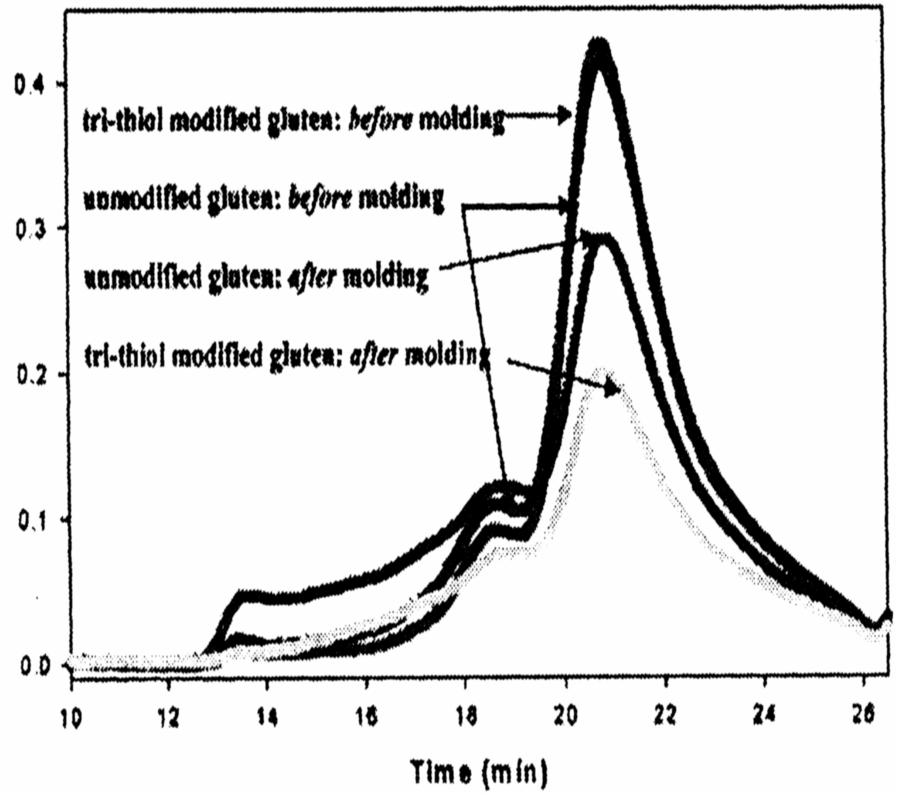


Figure 6. SE-HPLC chromatographs illustrating the function of the thiol-terminated, star-shaped molecule before and after molding. The largest peak around 21 min is believed to be a signature of the low-molecular-weight gliadins (or glutenin subunits) in the protein.

D.L. Woerdeman, W.S. Vevaverbeke, R.S. Parnas, D. Johnson, J. A. Delcour, I. Verpoest, and C.J.G. Plummer, *Biomacromolecules,* 2005, in print.