Types and Range of Applications for Liquid Chromatography

Increasing polarity

Water-insoluble   Water-soluble

Nonpolar   Ionic

Nonionic polar

Partition

Adsorption   Ion exchange

(Reversed-phase partition)   (Normal partition)

Molecular weight

10^2
10^3
10^4
10^5
10^6

Exclusion

(Gel permeation)   (Gel filtration)
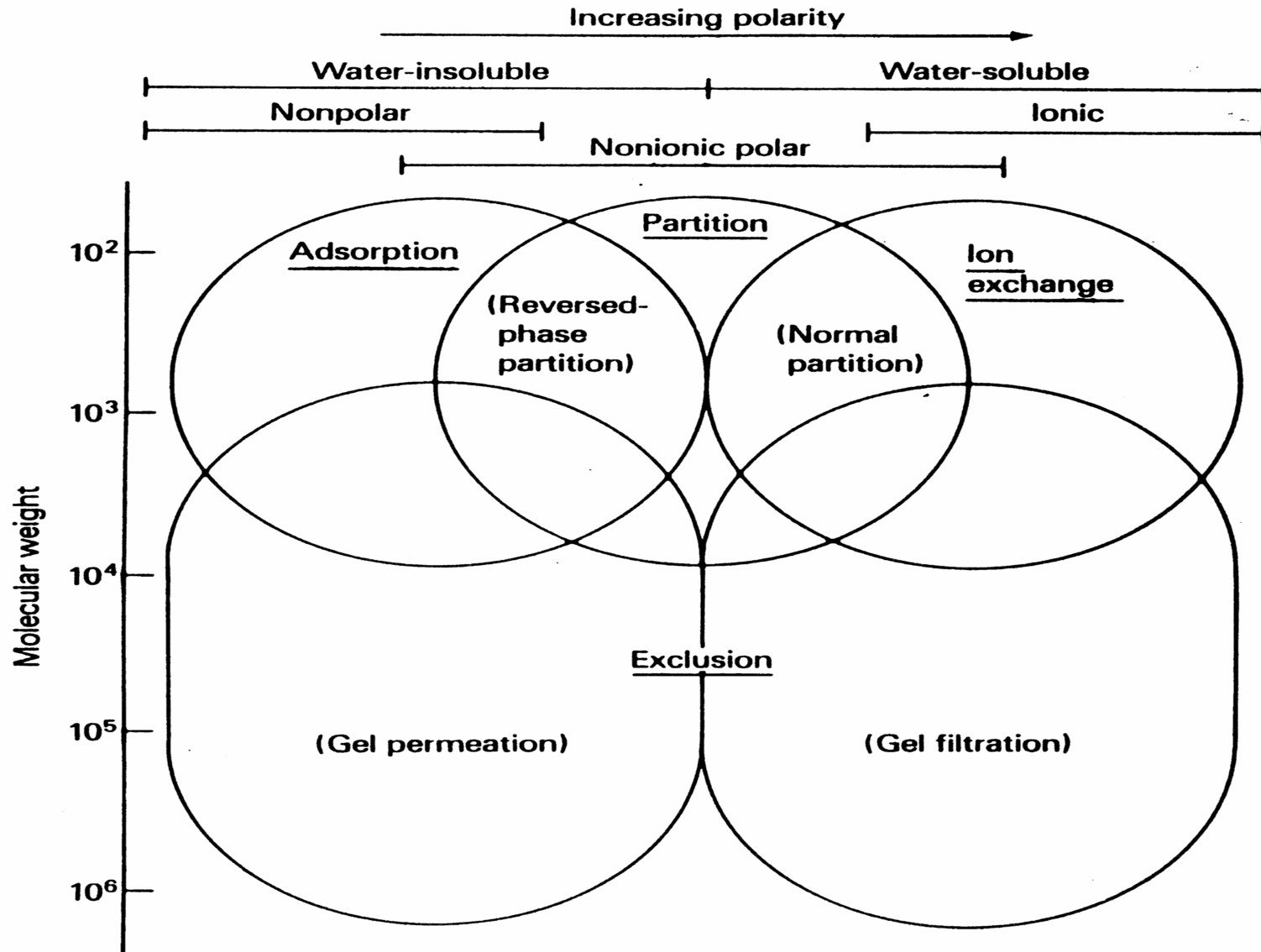
From Skoog, Holler and Nieman, "Principles of Instrumental Methods, 5 th. Ed., p. 726.
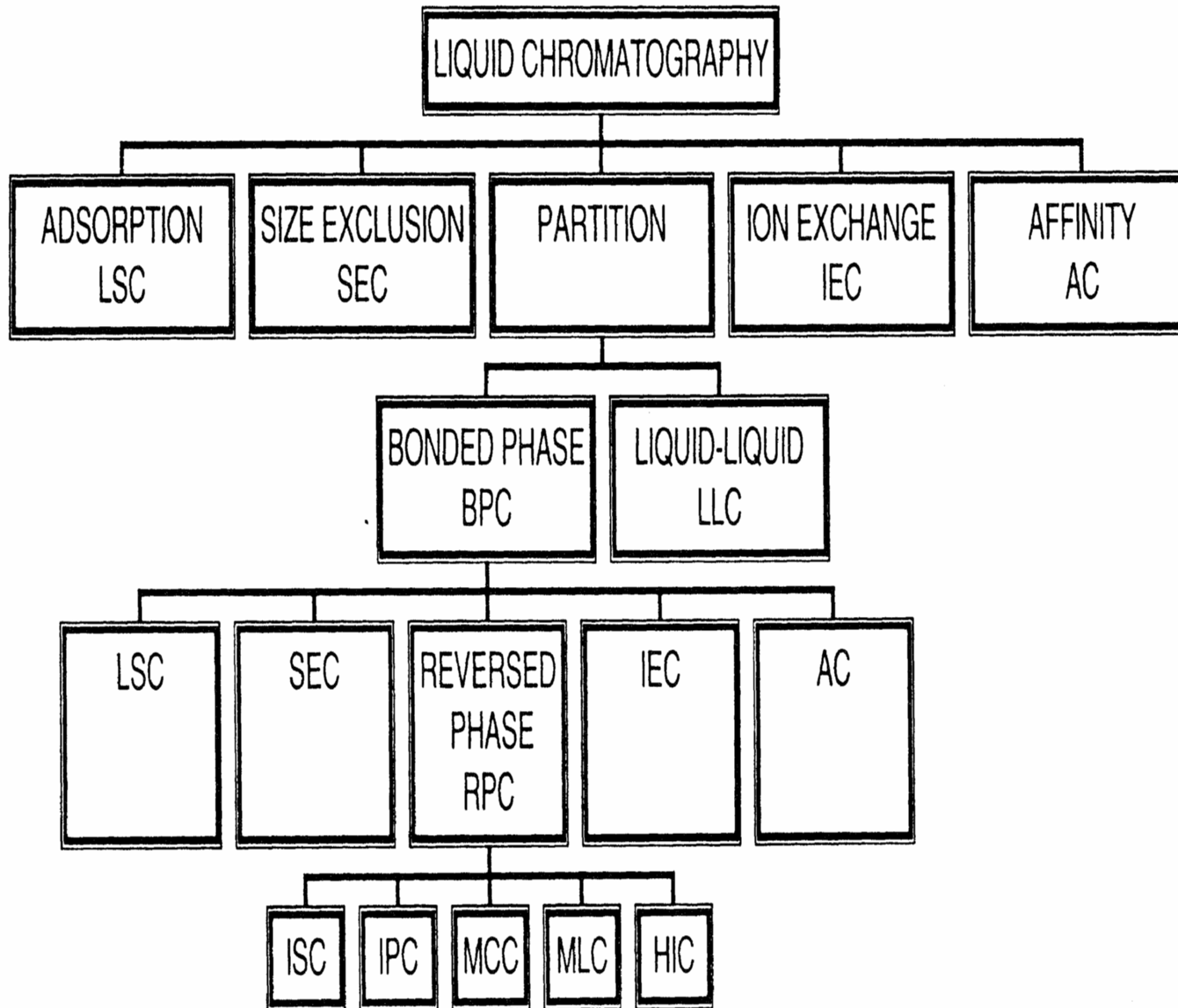
From C.F. Poole, "The Essence of Chromatography", 2003, Elsevier, p. 270.

General Schematic of a High Performance Liquid Chromatograph (LC)
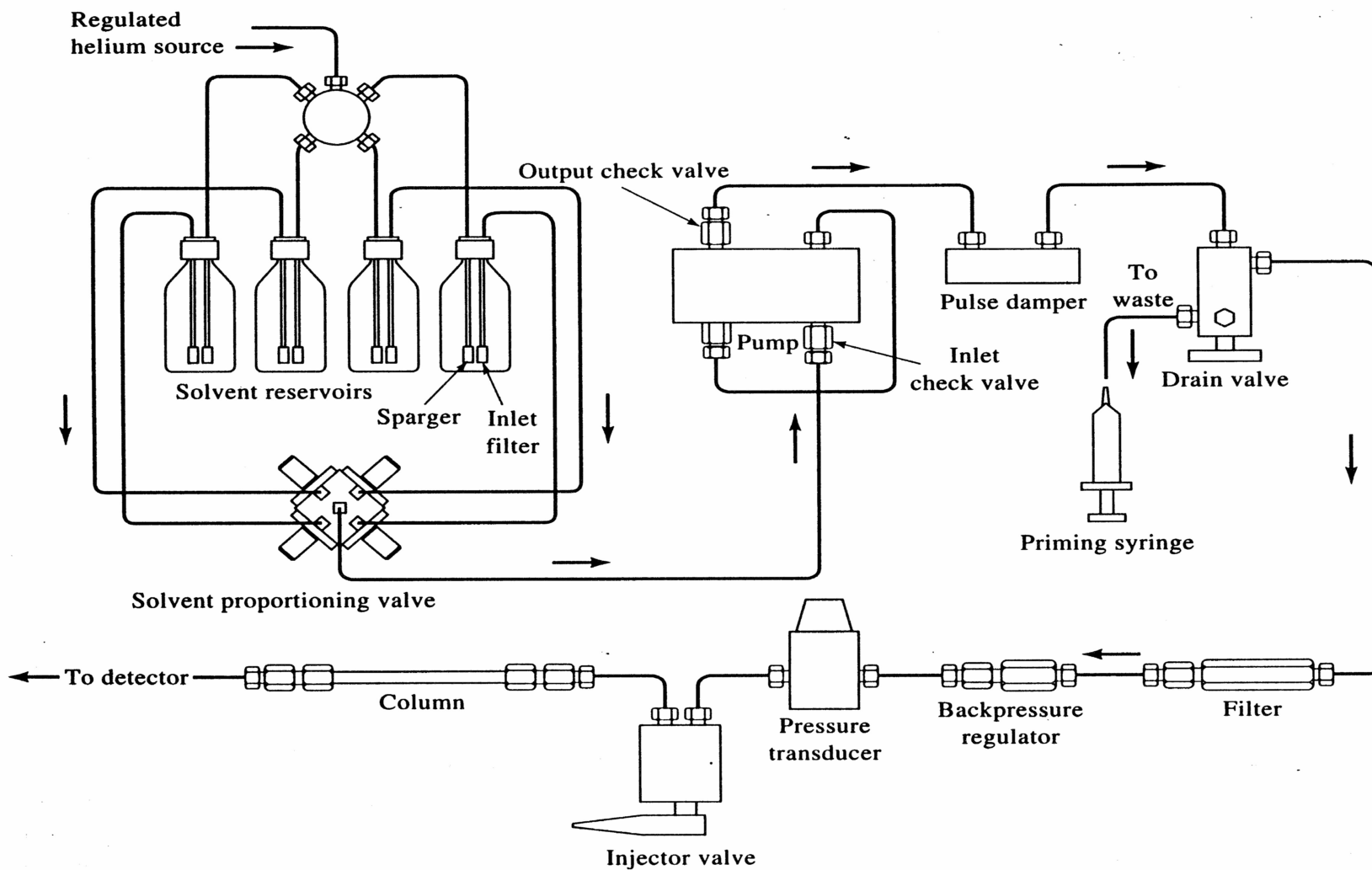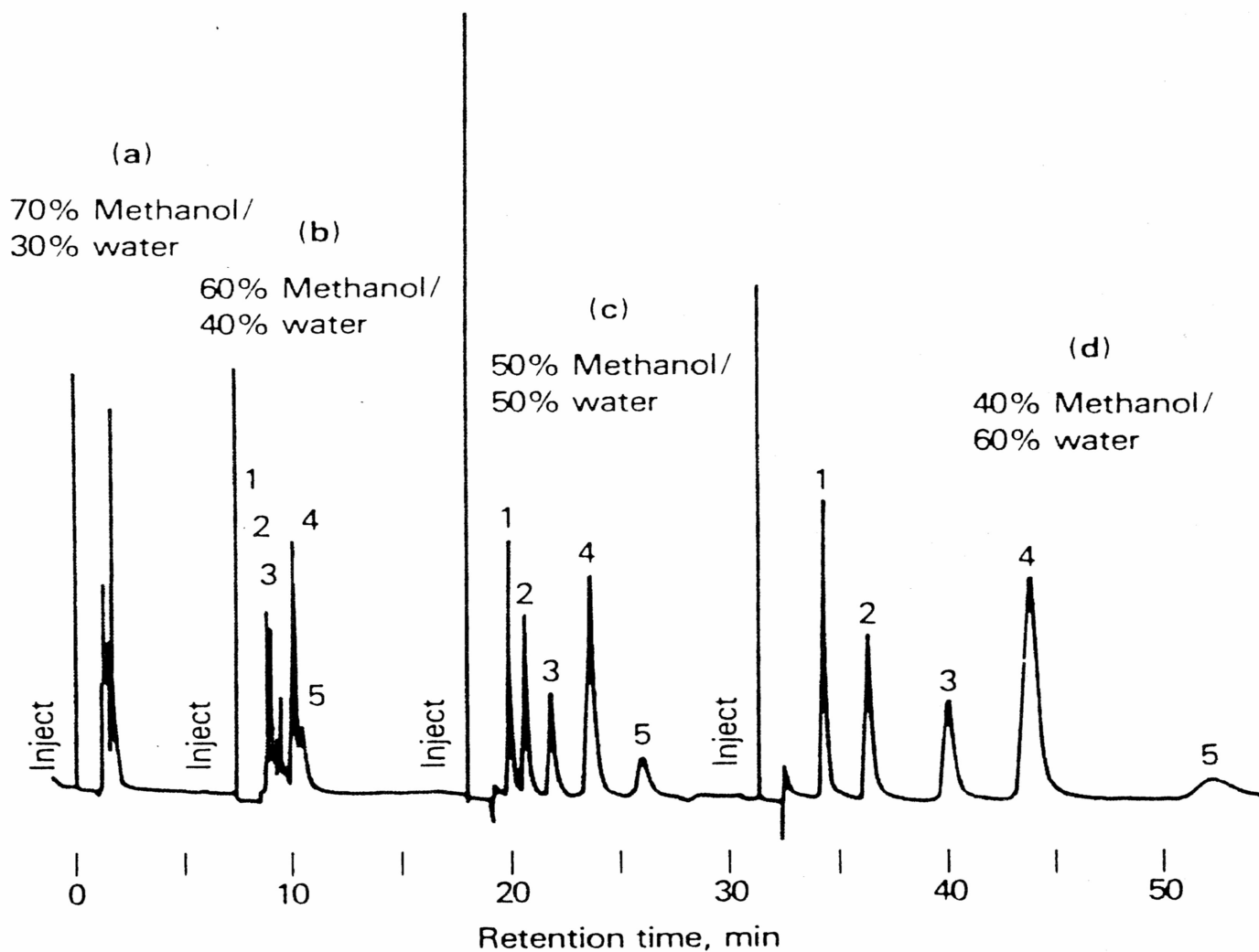

From Skoog, Holler and Nieman, "Principles of Instrumental Methods, 5 th. Ed., p. 729.

The effect of mobile phase solvent strenght in reversed phase HPLC

(a)

70% Methanol/
30% water

(b)

60% Methanol/
40% water

(c)

50% Methanol/
50% water

(d)

40% Methanol/
60% water

Retention time, min

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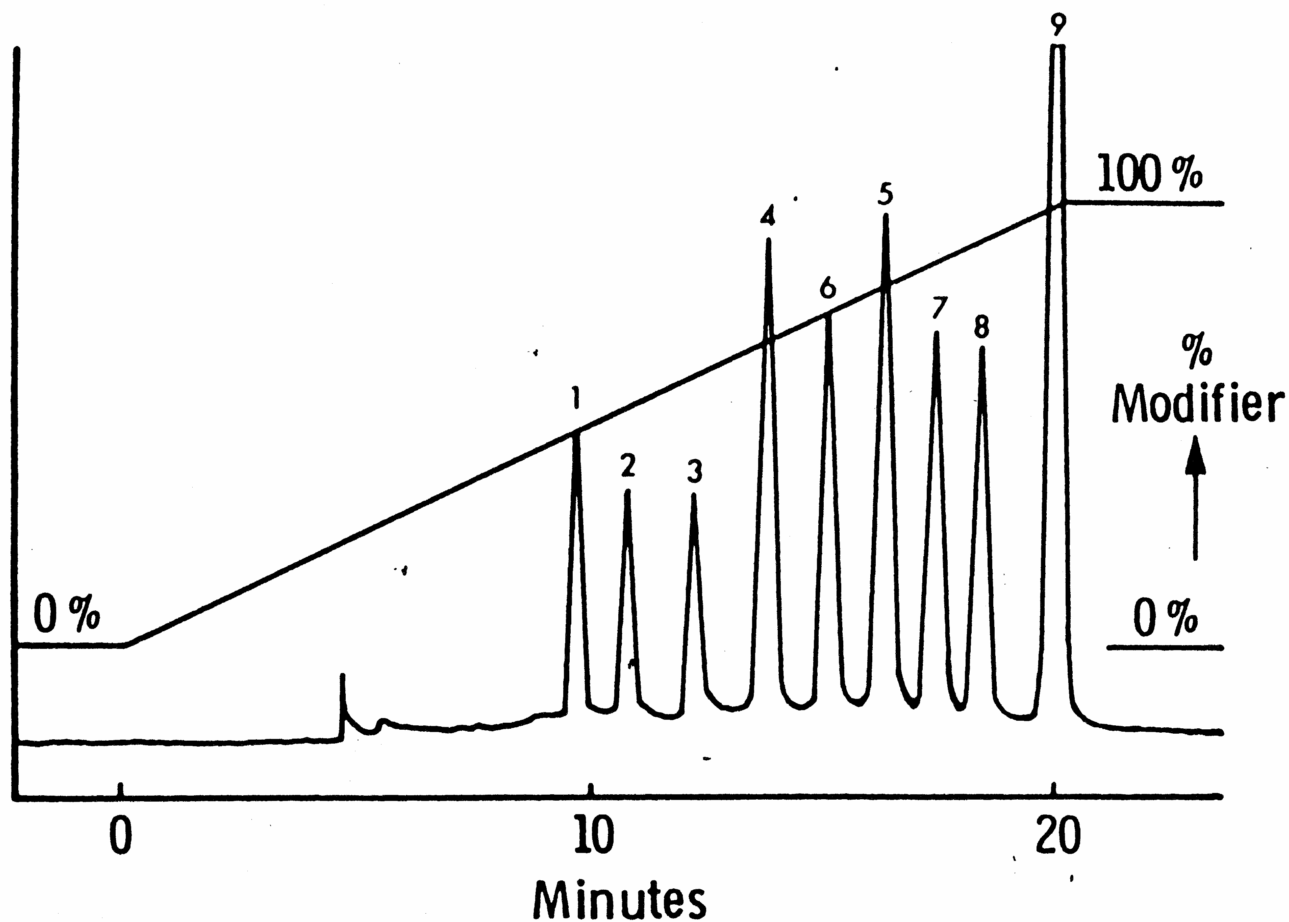
From C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier Scientific, 1991, p. 488.

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	$10^2 - 10^6$

Typical Liquid Chromatography Columns

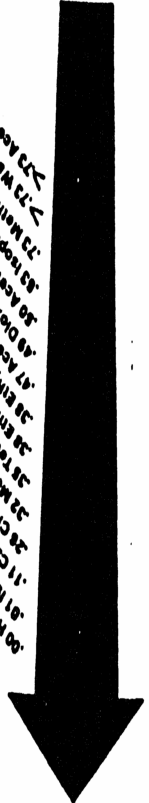| Column Name	Inner Diameter	Column Length	Flow Rate	Injection Vol.	Rel. Loading Capacity
Semi Prep.	10-200 mm	5-50 cm	10-1000 mL/min	1-10 mL	100,000
Conventional	4-5 mm	5-25 cm	0.8-2.0 mL/min	5-50 μL	10,000
Narrowbore	2 mm	5-10 cm	0.1-0.3 mL/min	1-5 μL	1,000
Microbore	1 mm	5 cm-25cm	0.010-0.050 mL/min	1-5 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

Modes of Liquid Chromatography

| Types of Compounds Separated	Mode	Stationary Phase	Mobile Phase
Neutrals Weak Acids Weak Bases	Reversed-Phase	C-18, C-8, C-4, C-2	Water/Organic Sometimes Modifiers
Bases, Acids	Paired-Ion	C-18, C-8	Water/Organic Ion-Pair Reagent
Compounds Insoluble in Water, Organic Isomers	Normal-Phase	Silica, Amino, Cyano, Diol	Organics
Ionics Inorganic Ions	Ion Exchange	Anion or Cation Exchange Resin	Aqueous/Buffer Counter Ion
High MW Compounds Polymers	Size Exclusion	Polystyrene Silica	Gel Filtration- Aqueous Gel Permeation- Organic

Reversed-Phase

Eluting Solvent Strength, $\epsilon°$

.00 Hexane
.01 Isooctane
.11 Carbon Tetrachloride
.28 Methylene Chloride
.32 Chloroform
.38 Ethyl Ether
.47 Ethyl Acetate
.49 Dioxane
.60 Isopropanol
.63 Acetonitrile
.65 Pyridine
.70 Methanol
.73 Water
>.73 Acetic Acid

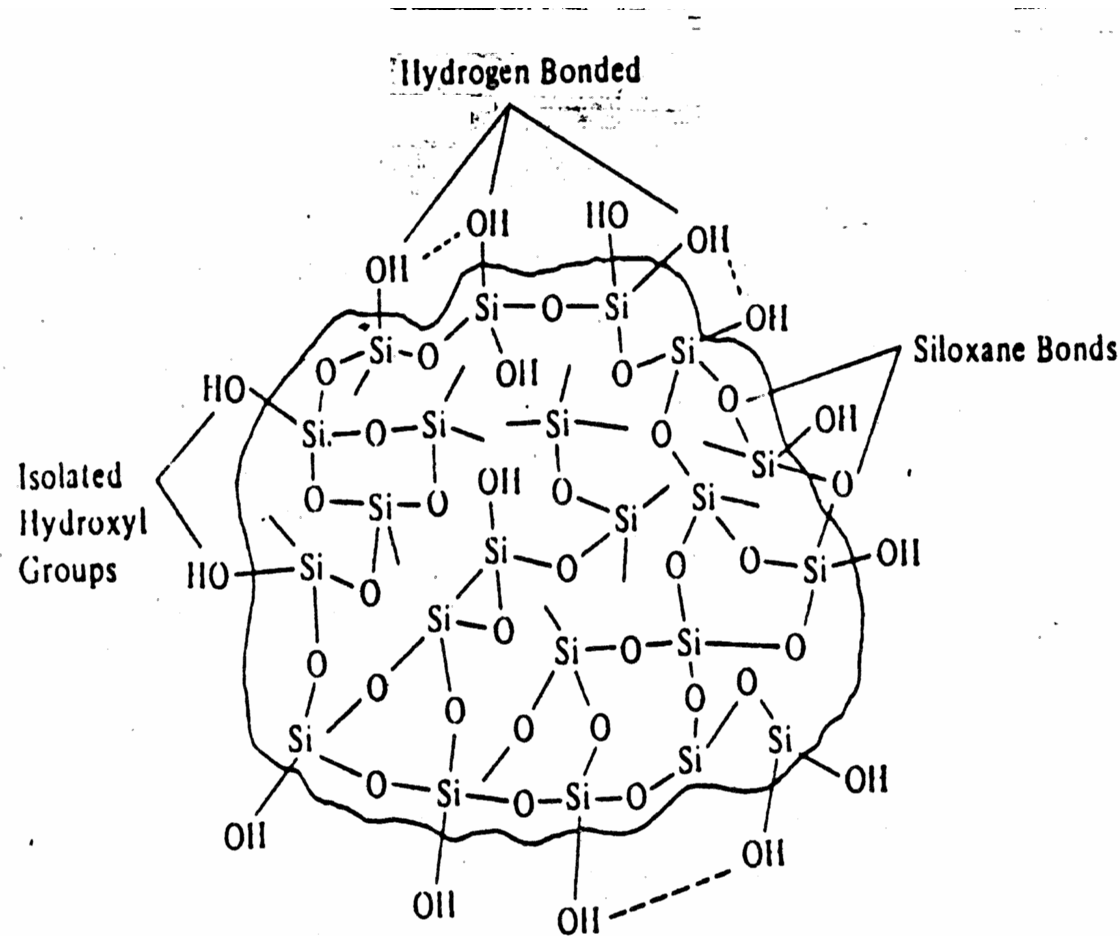**Depiction of larger, superficious (or pellicular) packing compared to the fully- porous, spherical (man-made) silica and the larger, irregular (natural) silica particles.**
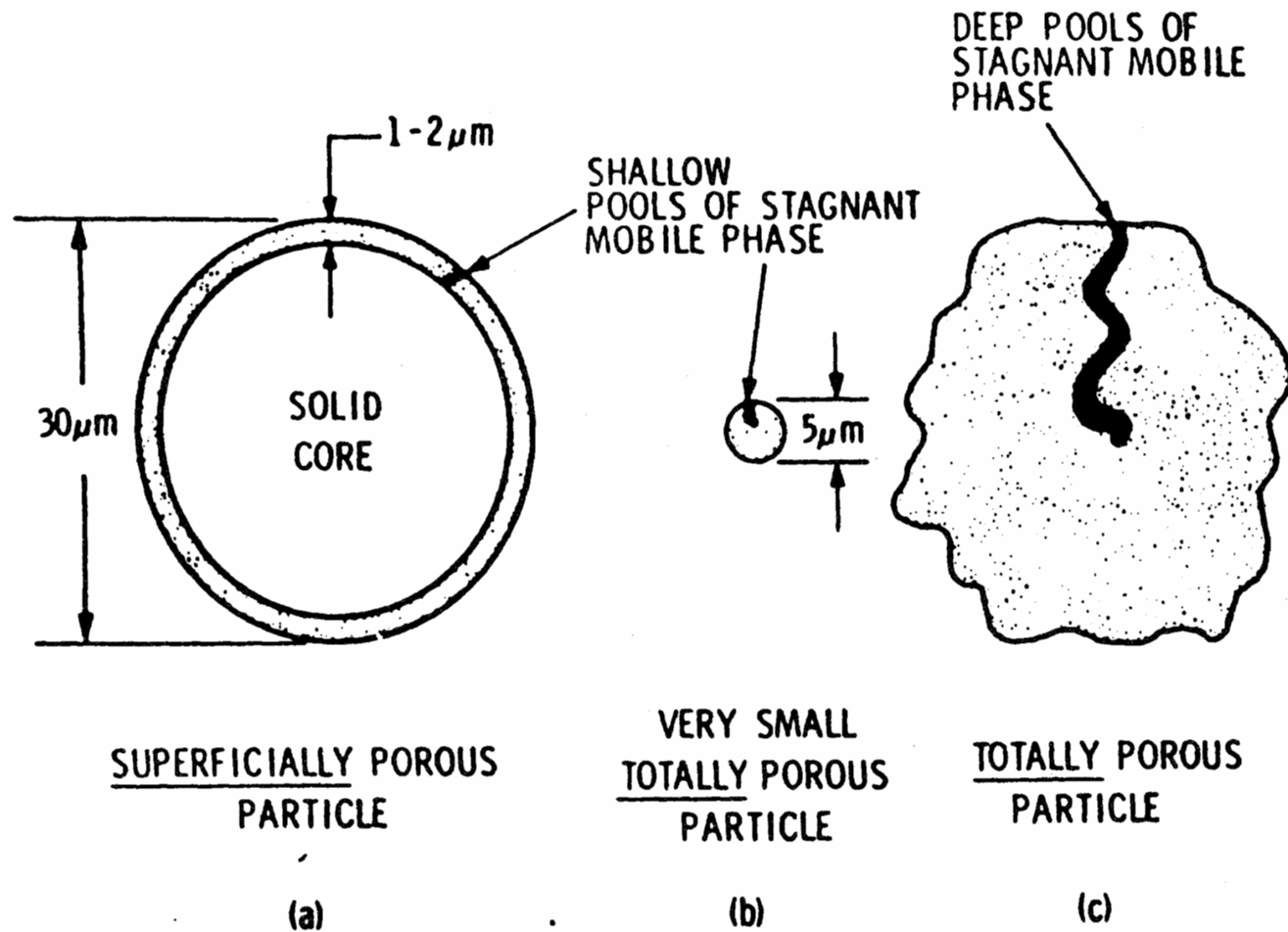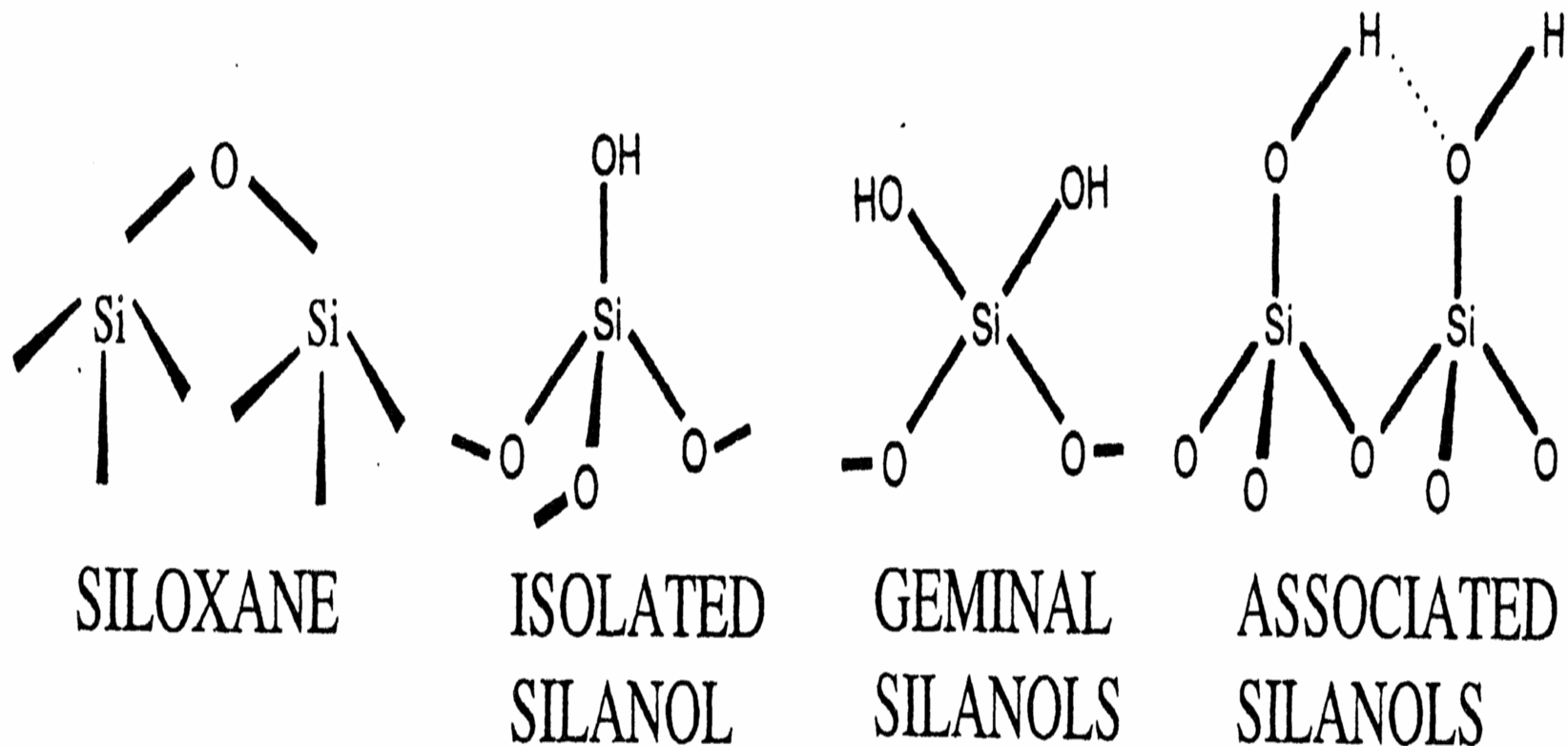

From C.F. Poole and S.K. Poole, "Chromatography Today", Elsevier, 1991, p. 315.

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

SILOXANE   ISOLATED SILANOL   GEMINAL SILANOLS   ASSOCIATED SILANOLS

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

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.

Steric Factors allow for only 50% coverage with C-8 functional groups.
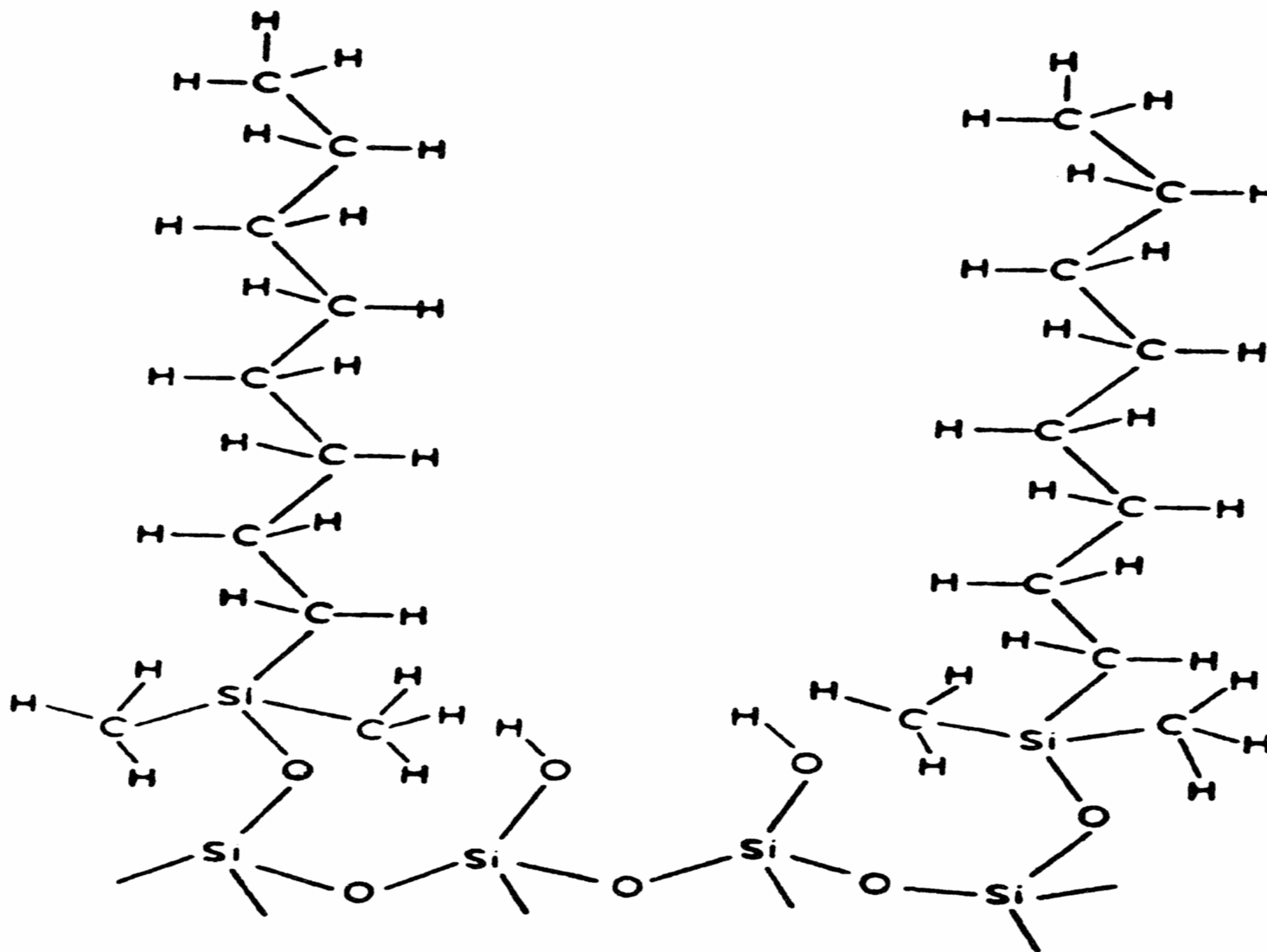
From C. Horvath and W.R. Melander, "Reversed-Phase Chromatography" in "High-Performance Liquid Chromatography-Advances and Perspectives", vol. 2, 1980, C. Horvath, ed. Academic Press, p. 137.

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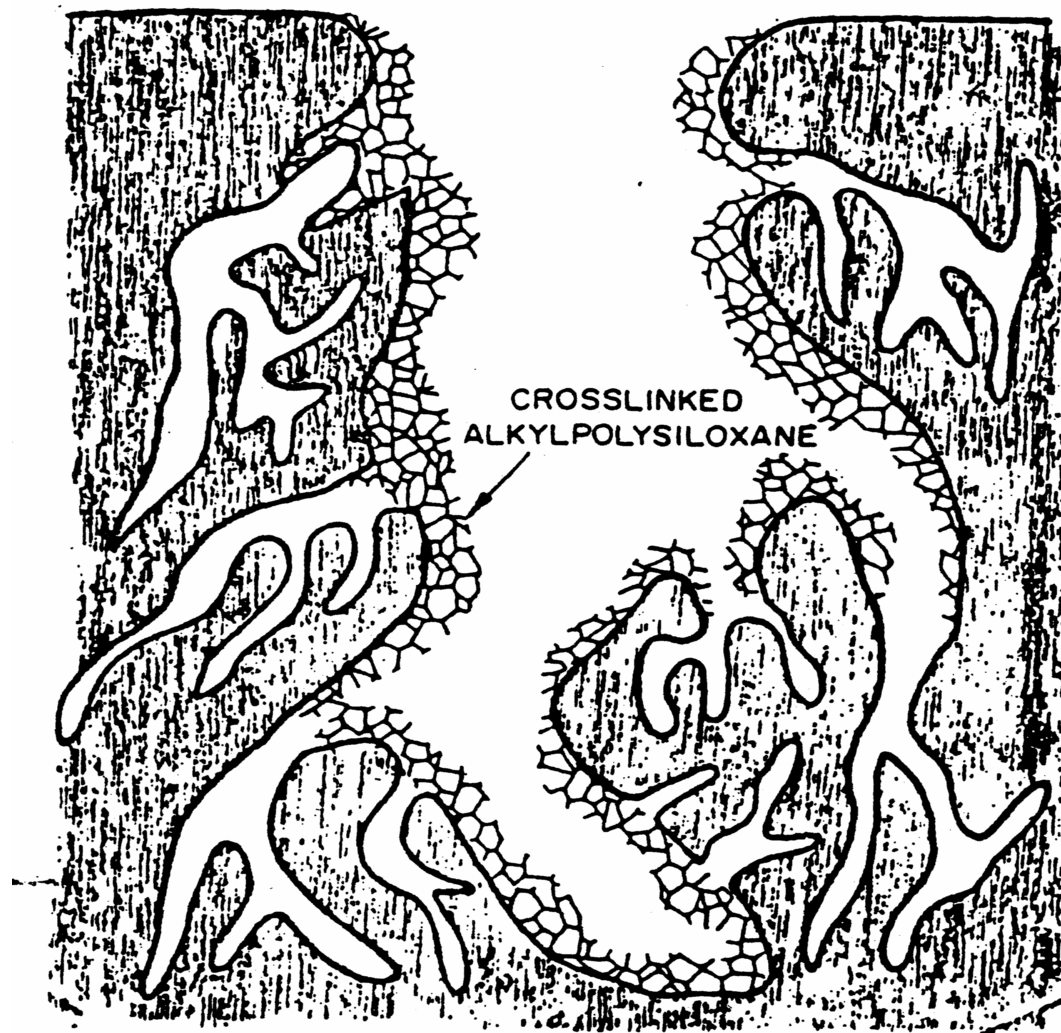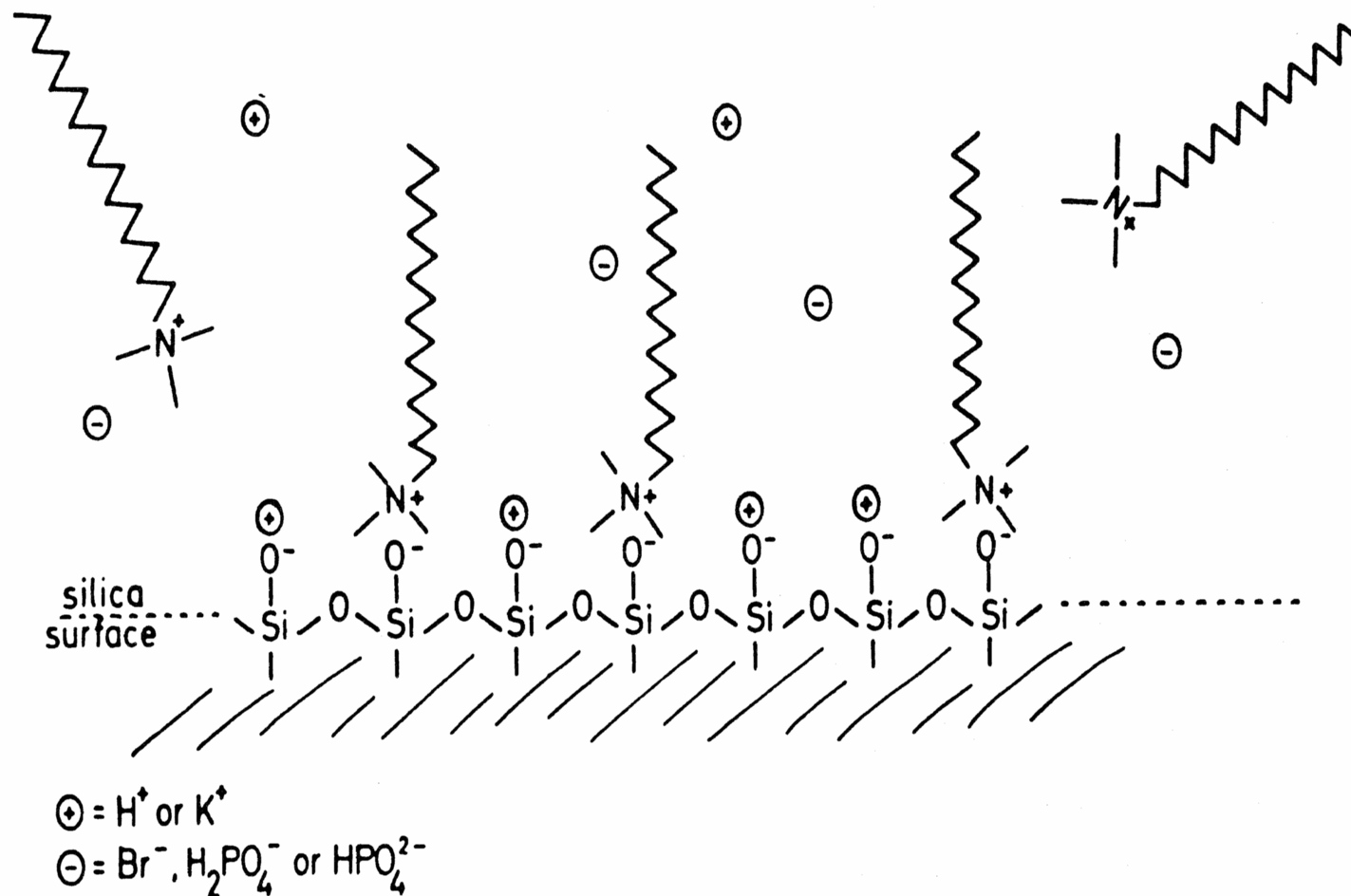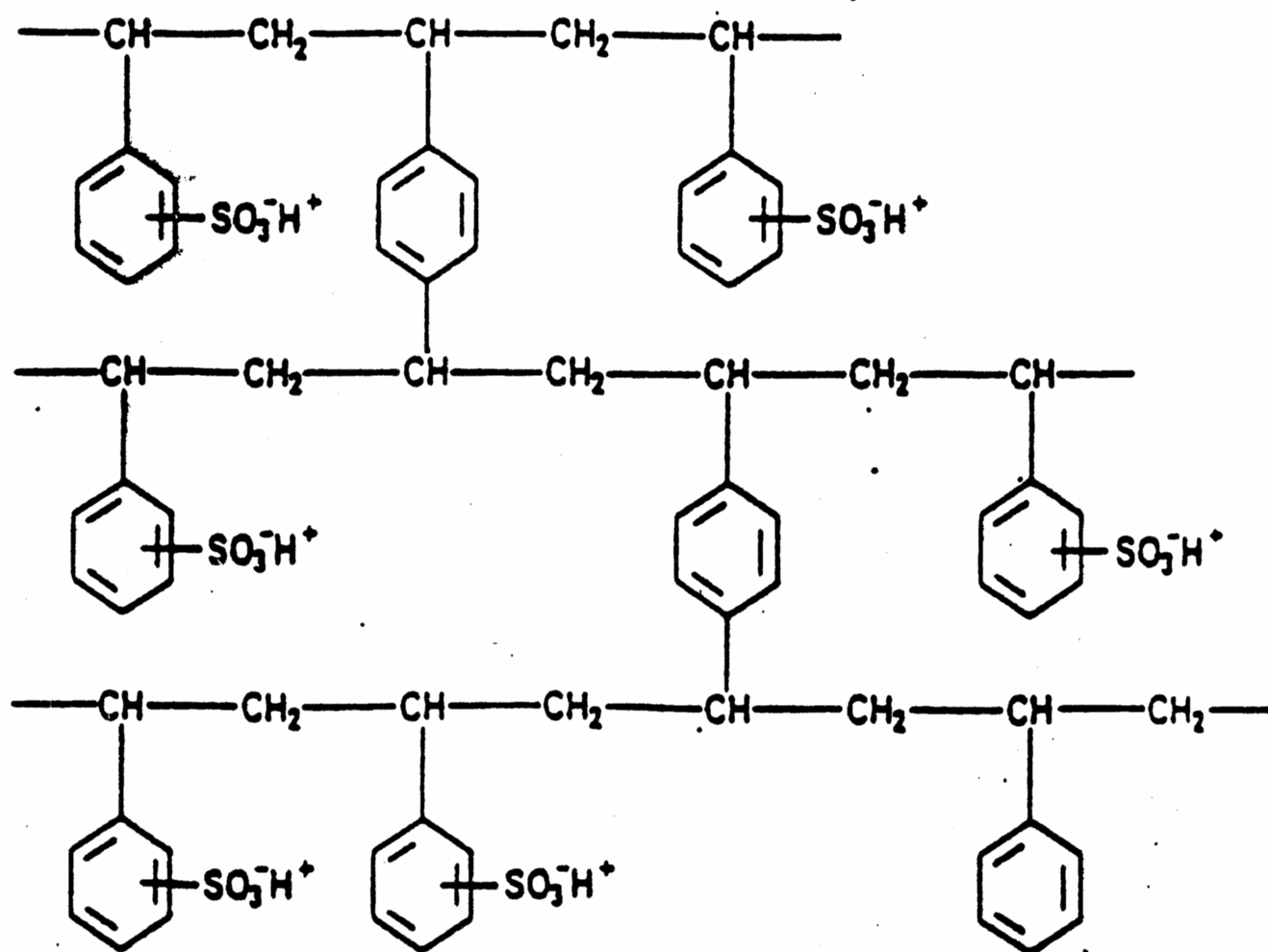

CROSSLINKED
ALKYLPOLYSILOXANE

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.

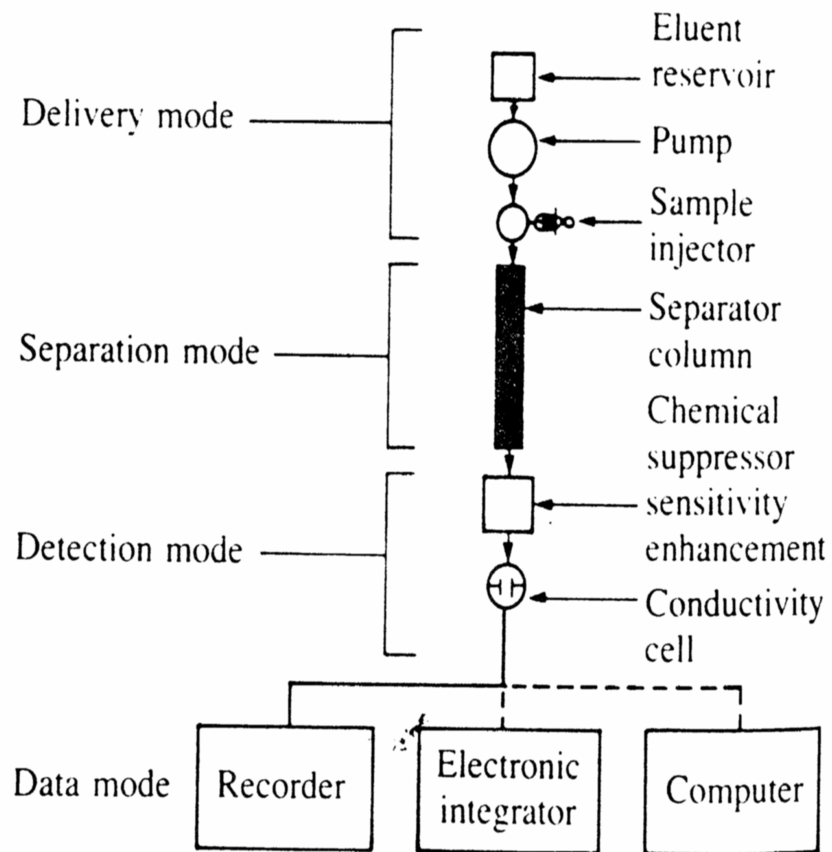**Model of a "Dynamically modified" surface silica with cetyltrimethylammonium ions.**

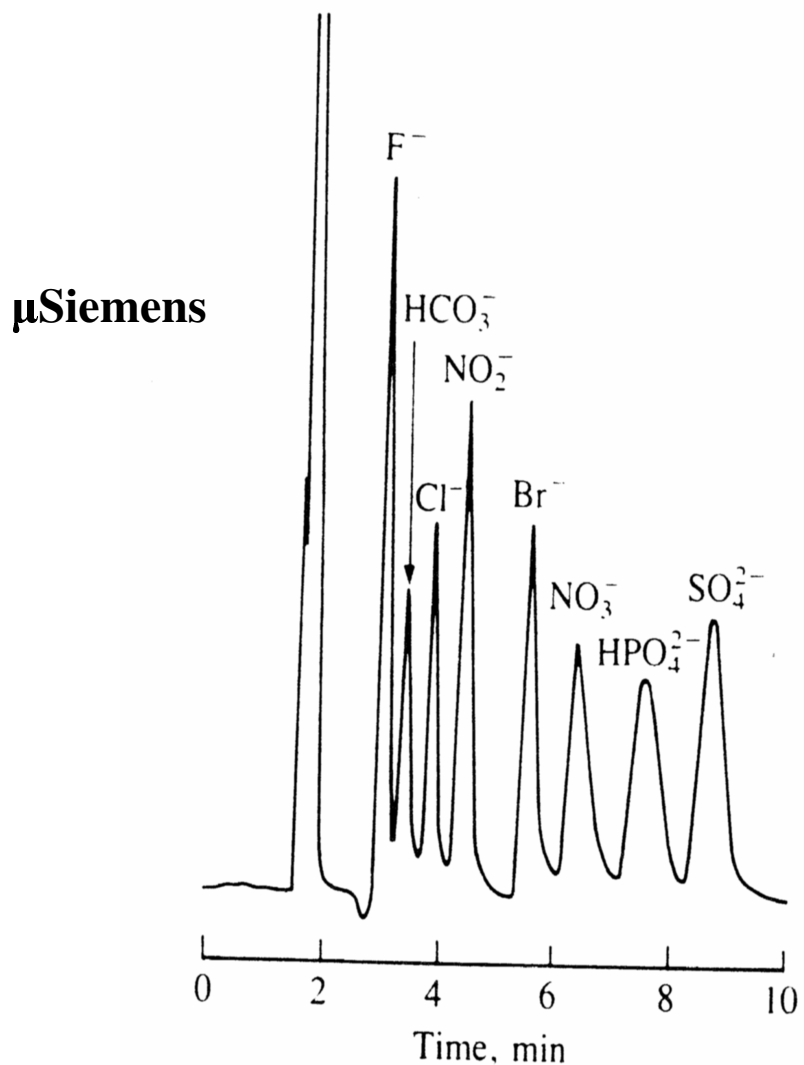From C.F. Poole and S.K. Poole, "Chromatography Today", 1991, Elsevier, p. 392.

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

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

µSiemens

(a)

(b)
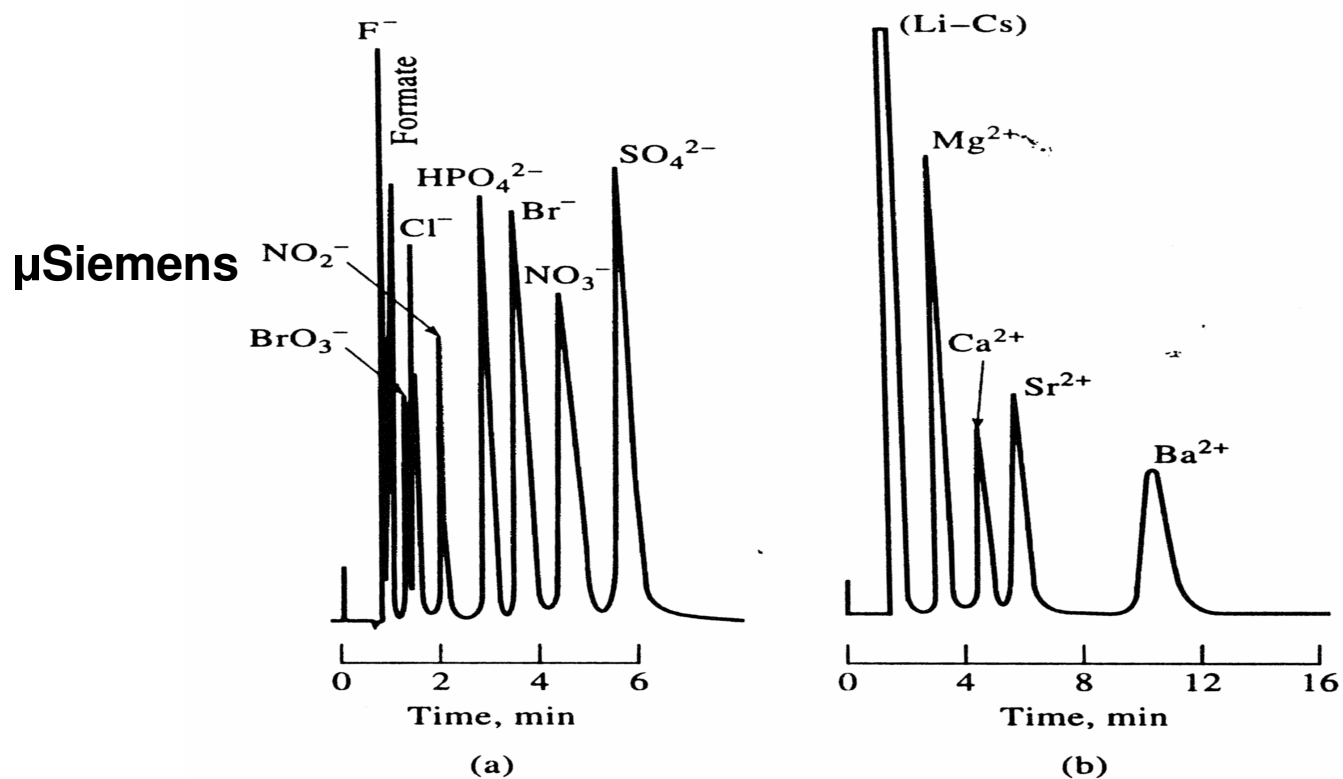
From Skoog, Holler and Nieman, "Principles of Instrumental Methods, 5 th. Ed., p. 755.

Calibration Curve for a Size-Exclusion LC separation of bio- or organic-polymers.
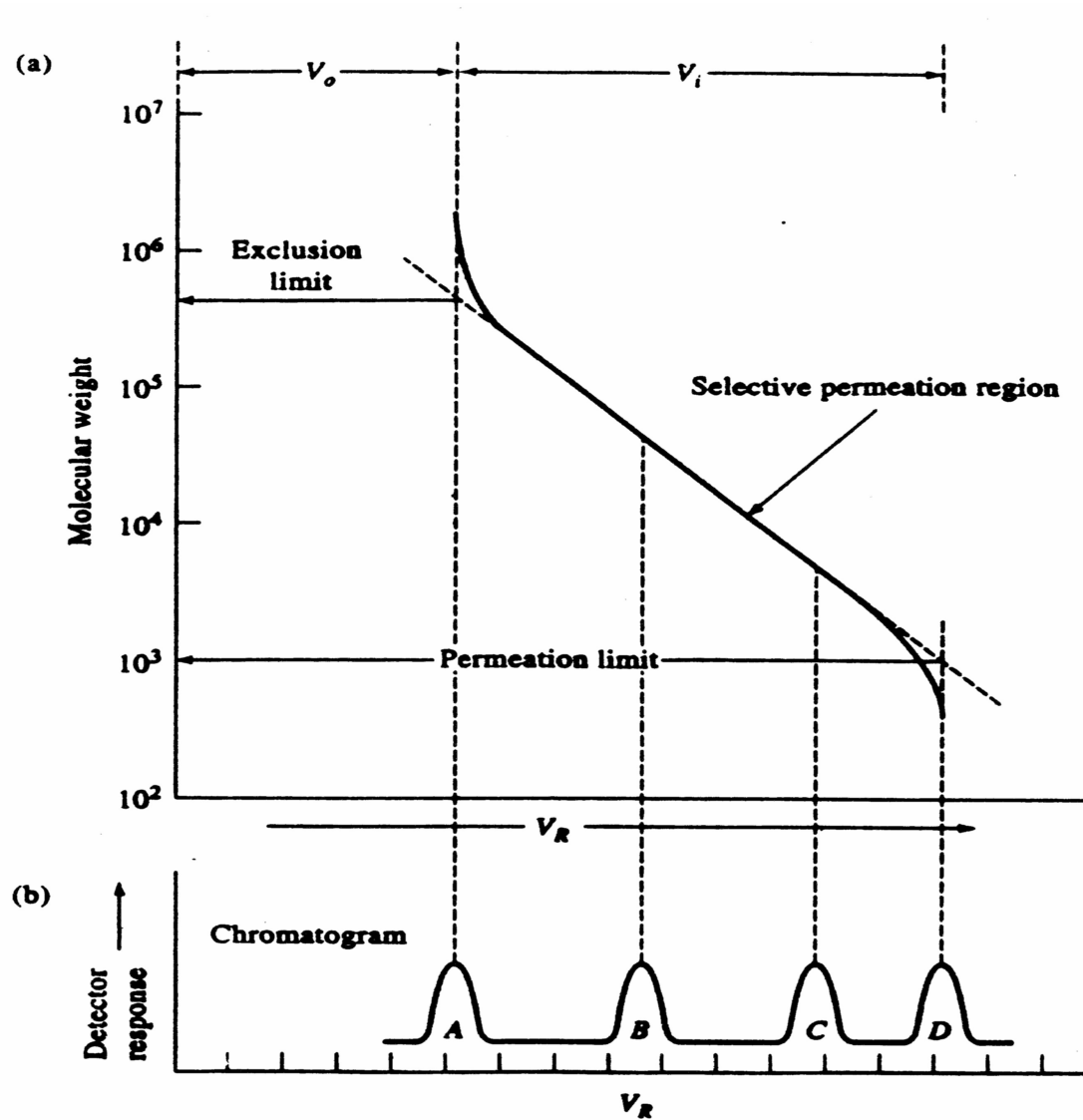
From Shoog, Holler and Nieman, "Principles of Instrumental Analysis, 5 th., 1998, p. 759.

Elusion order in Size-Exclusion Chromatography- Largest to Smallest. With a handy total permeation volume, V_t
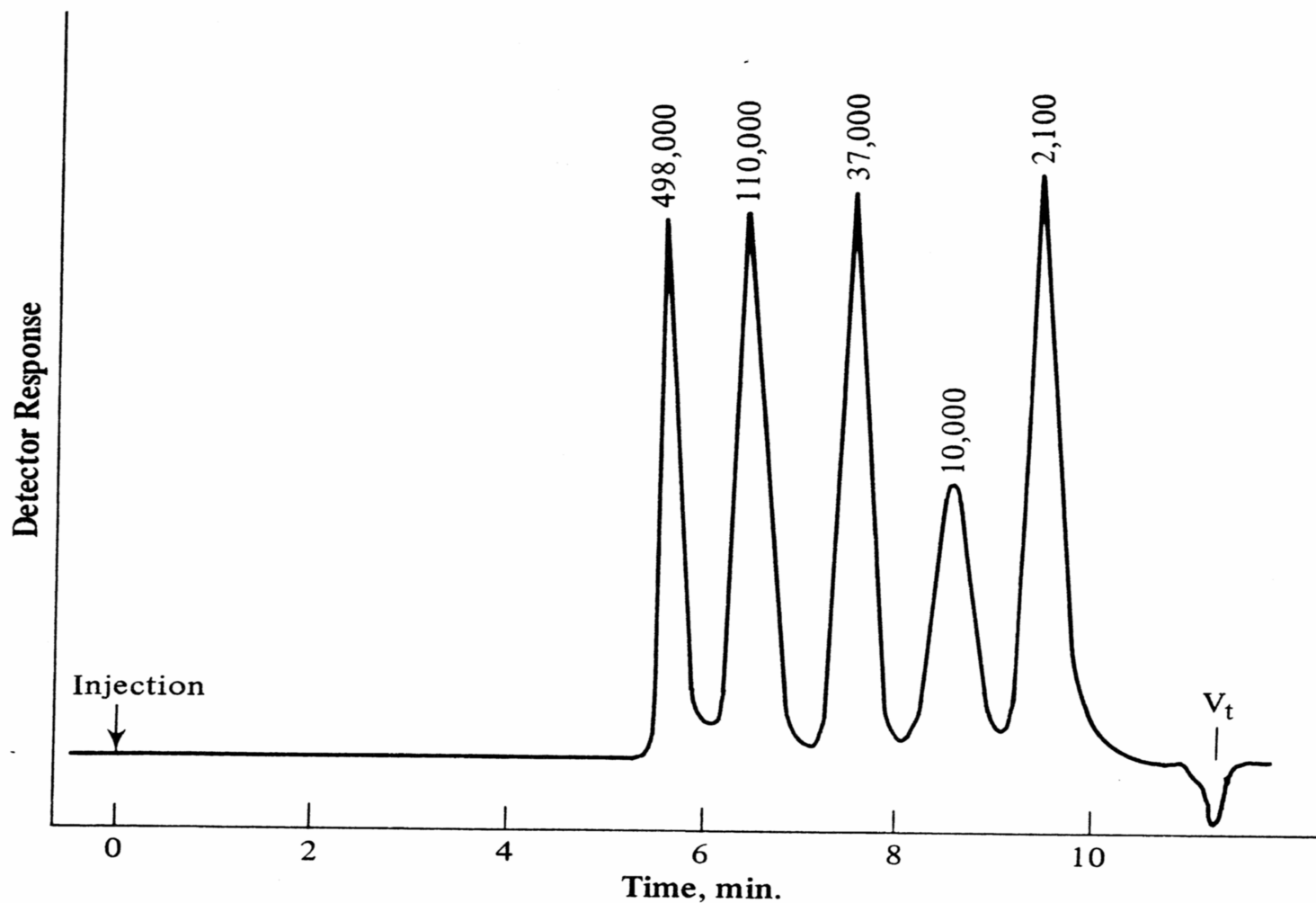
H.H. Bauer, G.D. Christian, J.E. O'Reilly, "Instrumental Analysis", 1987, Allyn and Bacon, p. 671.

Solvent strength and selectivity parameters based on Snyder's selectivity triangle.
(S_i is an empirical solvent strength parameter for reversed-phase chromatography)

Solvent	Selectivity group	Solvent strength		Solvent selectivity		
		(P')	(S_i)	x_e	x_d	x_n
n-Butyl Ether	I	2.1		0.44	0.18	0.38
Diisopropyl Ether		2.4		0.48	0.14	0.38
Methyl t-Butyl Ether		2.7				
Diethyl Ether		2.8		0.53	0.13	0.34
n-Butanol	II	3.9		0.59	0.19	0.25
2-Propanol		3.9	4.2	0.55	0.19	0.27
1-Propanol		4.0		0.54	0.19	0.27
Ethanol		4.3	3.6	0.52	0.19	0.29
Methanol		5.1	3.0	0.48	0.22	0.31
Tetrahydrofuran	III	4.0	4.4	0.38	0.20	0.42
Pyridine		5.3		0.41	0.22	0.36
Methoxyethanol		5.5		0.38	0.24	0.38
Dimethylformamide		6.4		0.39	0.21	0.40
Acetic Acid	IV	6.0		0.39	0.31	0.30
Formamide		9.6		0.38	0.33	0.30
Dichloromethane	V	4.3		0.27	0.33	0.40
1,1-Dichloroethane		3.5		0.30	0.21	0.49
Ethyl Acetate	VI	4.4		0.34	0.23	0.43
Methyl Ethyl Ketone		4.7		0.35	0.22	0.43
Dioxane		4.8	3.5	0.36	0.24	0.40
Acetone		5.1	3.4	0.35	0.23	0.42
Acetonitrile		5.8	3.1	0.31	0.27	0.42
Toluene	VII	2.4		0.25	0.28	0.47
Benzene		2.7		0.23	0.32	0.45
Nitrobenzene		4.4		0.26	0.30	0.44
Chloroform	VIII	4.3		0.31	0.35	0.34
Dodecafluoroheptanol		8.8		0.33	0.40	0.27
Water		10.2	0	0.37	0.37	0.25

From C.K. Poole, "The Essence of Chromatography", Elsevier, 2003, p. 369.

Snyder's Classification of LC Solvents into Nine Groups
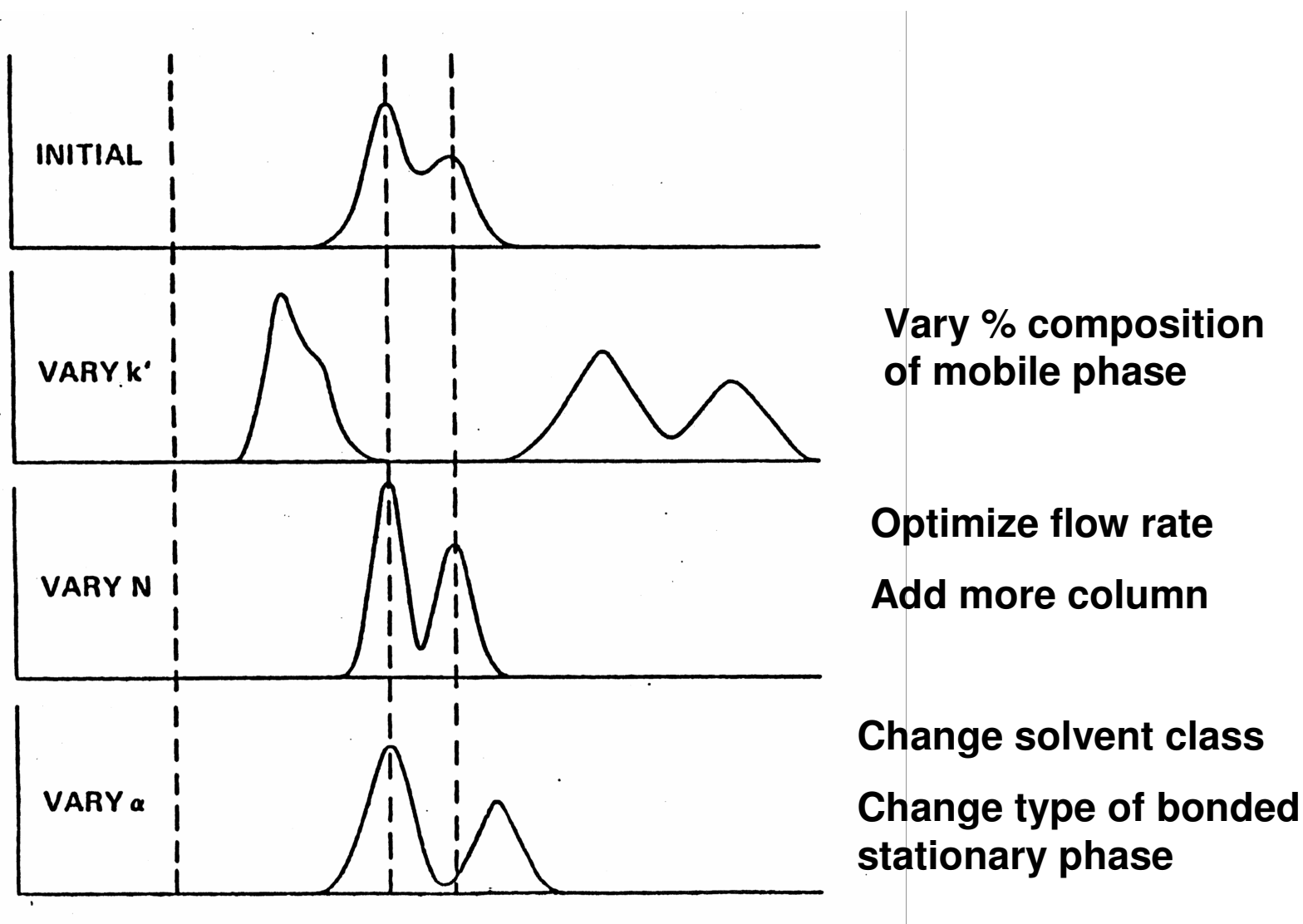
Classification of Solvents

Group	Solvents
I	Aliphatic ethers, trialkyl amines, tetramethylguanidine *(Proton Acceptor)*
II	Aliphatic alcohols *Able to either donate or accept protons, H-Bonding*
III	Pyridines, tetrahydrofuran, amides (except the more acidic formamide) *Bases*
IV	Glycols, glycol-ethers, benzyl alcohol, formamide, acetic acid
V	Methylene chloride, ethylene chloride, tricresyl phosphate *strong induced dipoles*
VIa	Alkyl halides, ketones, esters, nitriles, sulfoxides, sulfones, aniline and dioxane *permanent dipole*
VIb	Nitro compounds, propylene carbonate, phenyl-alkyl ethers, aromatic hydrocarbons *permanent dipole.*
VII	Halobenzenes, diphenyl ether
VIII	Fluoroalkanols, m-cresol, chloroform, water *Hydrogen donors*
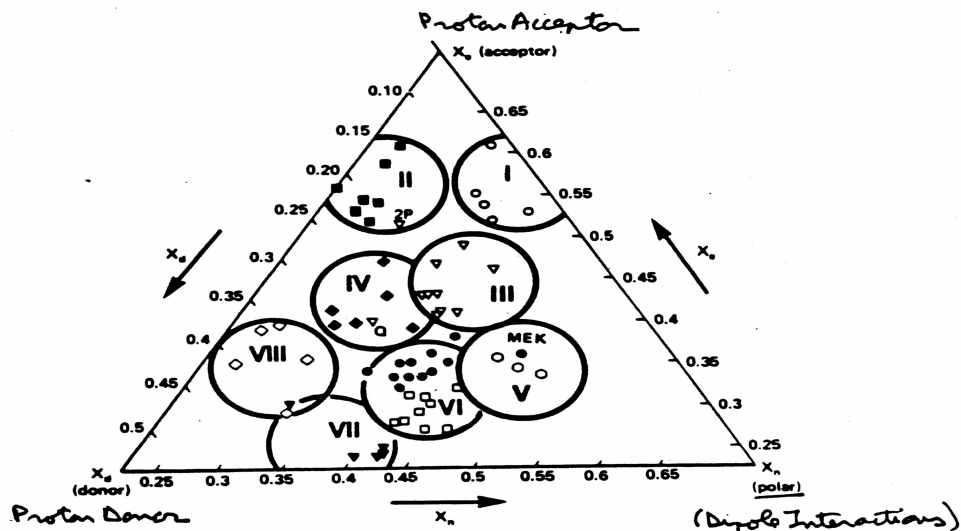
From J.J. Kirkland and L.R. Snyder, American Chemical Society Short Course, "Solving Problems in Modern Liquid Chromatography, 1974.

Steps in the Optimization of a Liquid Chromatographic Separation.

$$Rs = (k' / (1 + k')) / ((n)^{1/2} / 4) / ((\alpha - 1) / \alpha))$$

INITIAL

VARY k' — Vary % composition of mobile phase

VARY N — Optimize flow rate / Add more column

VARY α — Change solvent class / Change type of bonded stationary phase

From D.A. Skoog, "Principles of Instrumental Analysis", 3 rd. ed., 1985, p. 743.

WRIGHT'S VIEW

THIS CBS STUFF ASIDE, I'M STILL GOING TO BE A JOURNALIST WHEN I GROW UP.

WELL, I HOPE YOU'VE LEARNED HOW DEVASTATING BIAS CAN BE.

DEFINE BIAS.

A CAREFUL REVIEW OF THE FACTS...

...WHICH RESULTS IN ANY VIEWPOINT OTHER THAN MY OWN!

Palm Beach Post 2005 Int