Chapter 28: High-Performance Liquid Chromatography (HPLC)

- Scope
- Instrumentation – eluants, injectors, columns
- Modes of HPLC
  - Partition chromatography
  - Adsorption chromatography
  - Ion chromatography
  - Size exclusion chromatography

HPLC

- Most widely used separation technique
- Broad applicability – organic & inorganic
- Can be very sensitive, accurate & precise
- Suitable for separation of nonvolatile species
- Has found numerous uses in industry, clinical settings, environmental areas, pharmaceuticals, etc.

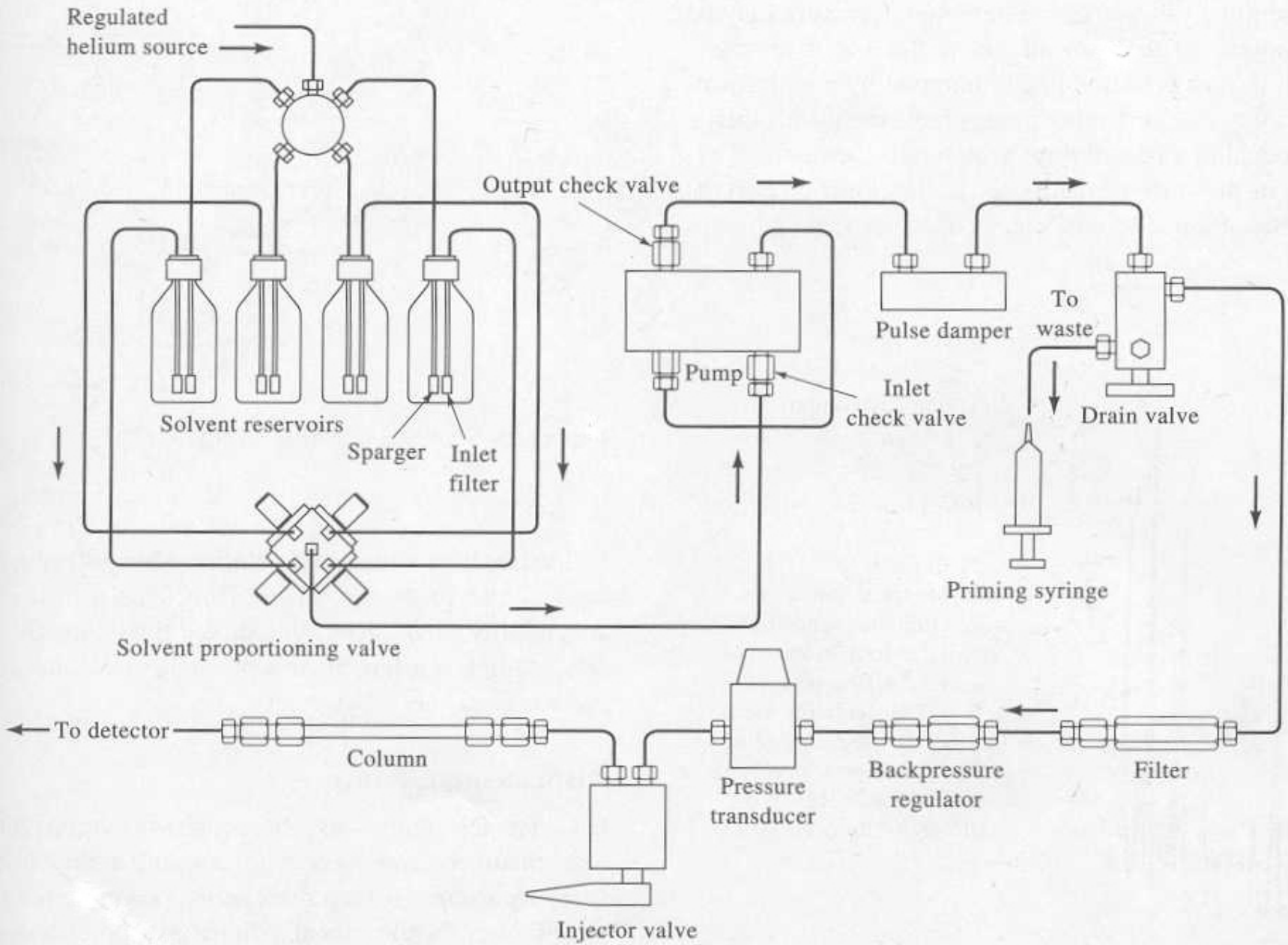


Figure 28-4 Schematic of an apparatus for HPLC. *(Courtesy of Perkin-Elmer Corporation, Norwalk, CT.)*

Modes of Separation

Partition Chromatography –

most used form of HPLC

primarily for nonionic compounds of varying polarity with low MW (< 3000)

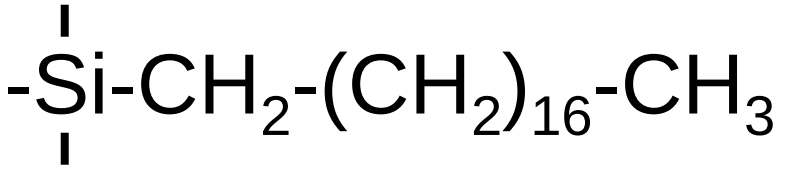
Most common form is bonded phase chrom.

using silica based packing materials functionalized by silylation (as for GC)

Early work with partition chrom. was done with polar stationary phases (like bare silica) & non-polar solutes = <u>normal phase chrom.</u>

Later bonded phases were introduced using C_{18} groups → very non-polar with polar solvents = <u>reversed-phase chromatography</u>

Today almost all partition chrom. done in reversed-phase mode with many different bonded phases (although C_{18} very popular)



18 carbon chain

Long chain acts as if it were an alkane coated on silica → analyte molecules partition into it, hence the name

In chromatogram, most polar compounds elute first because they partition into C_{18} least – like dissolves like – most non-polar compounds come out last

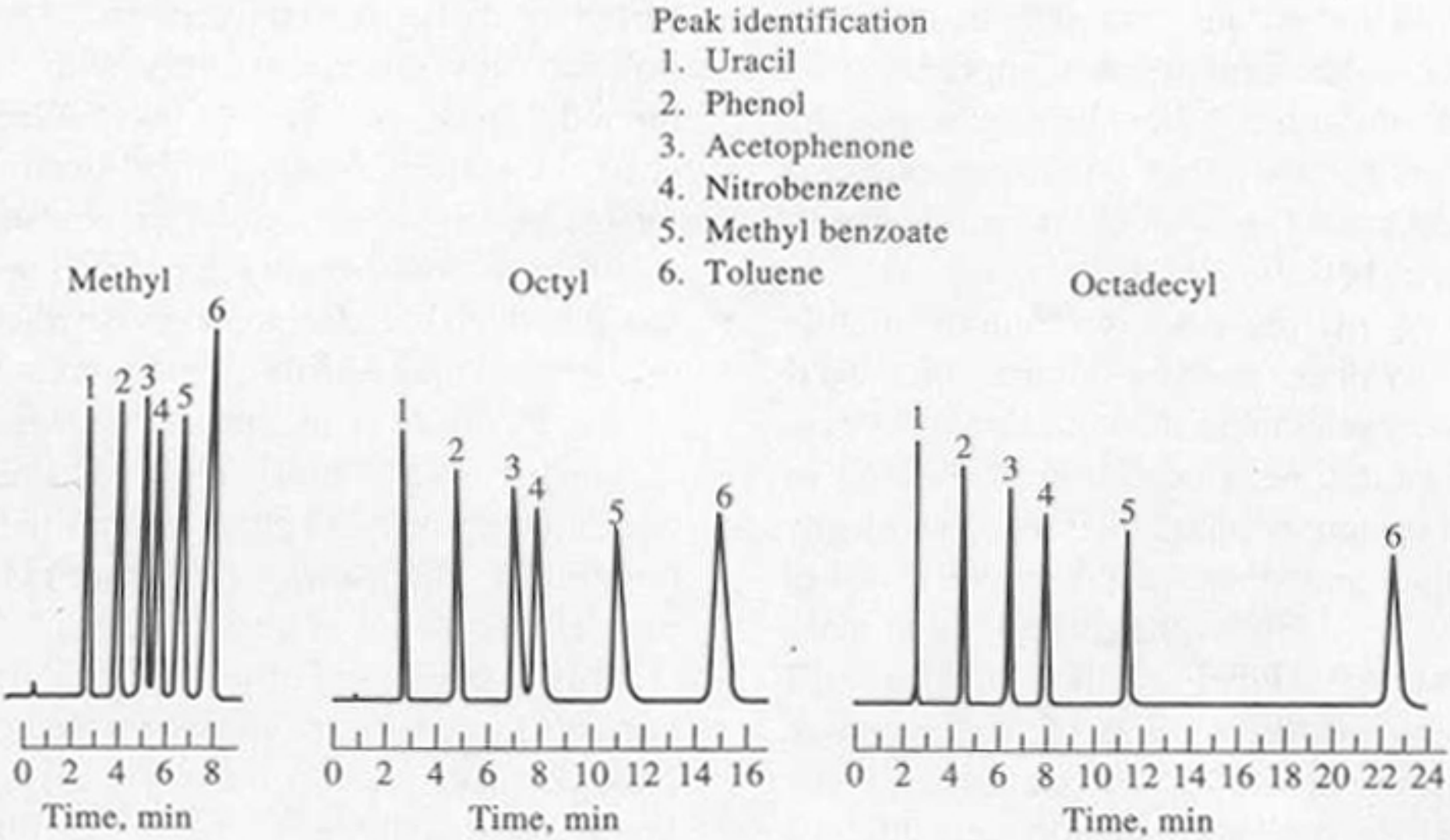


Figure 28-15 Effect of chain length on performance of reversed-phase siloxane columns packed with 5-μm particles. Mobile phase: 50/50 methanol/water. Flow rate: 1.0 mL/min.

- Besides C_{18} can have C_8, C_4, C_3, C_2, C_1 plus functionalities like cyano ($-C_2H_4CN$), amino ($-C_2H_4NH_2$), diol ($-C_3H_6O-CH_2-CHOHCH_2OH$)
- Each has different polarity
- Can also do Ion Pair Chromatography or Paired-Ion Chromatography – type of RP-HPLC used to separate ionic species
- Still partition chrom. but use a reagent like a quaternary ammonium salt $(C_4H_9)_4N^+$ to pair with analyte ions to separate by RP

Adsorption Chromatography –
bare silica or alumina to separate non-polar compounds because they adsorb to the stationary phase & are eluted by adjusting solvent strength of mobile phase – important non-linear appl.

Adsorption chrom. = normal phase chrom.

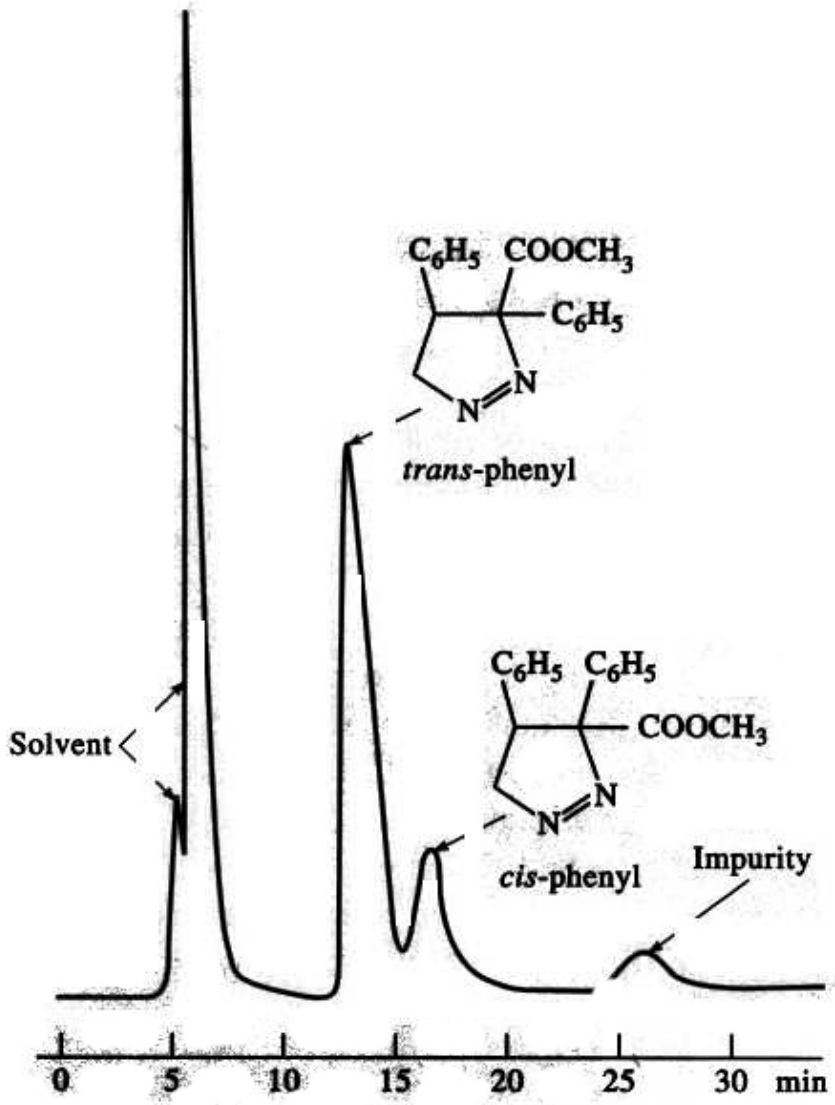


Figure 28-20 A typical application of adsorption chromatography: separations of *cis*- and *trans*-pyrazoline. Column: 100 × 0.3 cm pellicular silica. Mobile phase: 50% methylene chloride/isooctane. Temperature: ambient. Flow rate: 0.225 mL/min. Detector: UV, 254 nm.

Ion Chromatography (Ion Exchange)

Historically was developed for the

Manhattan Project (atomic bomb)

Generally not automated because of the lack of good detectors until it was reinvented in 1970's at Dow Chemical using conductivity detection & chemical suppression

Stationary phases are resin beads of styrene-divinylbenzene functionalized with cationic & anionic groups developed for water purification in 1930's

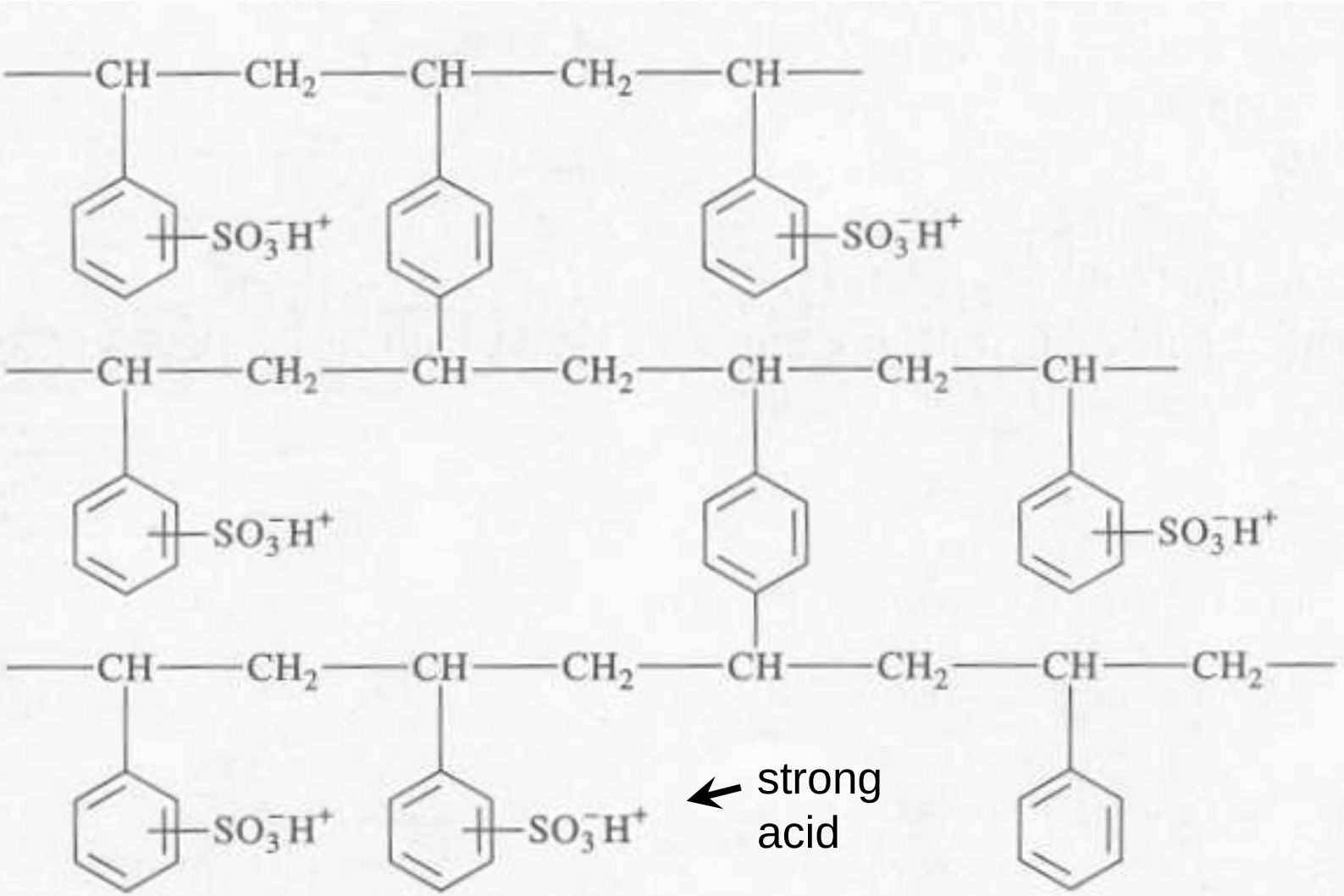


Figure 28-21 Structure of a cross-linked polystyrene ion-exchange resin. Similar resins are used in which the $—SO_3^-H^+$ group is replaced by $—COO^-H^+$, $—NH_3^+OH^-$, and $—N(CH_3)_3^+OH^-$ groups.

weak base

strong base

weak acid

Can write reactions in general format

$$\underset{\text{solid}}{x RSO_3^- H^+} + \underset{\text{solution}}{M^{x+}} \rightleftharpoons \underset{\text{solid}}{(RSO_3^-)_x M^{x+}} + \underset{\text{solution}}{x H^+}$$

Where R = polymer support (styrene divinylbenzene)

Can write equilibrium expression for exchange

$$K_{ex} = \frac{[(RSO_3^-)_x M^{x+}]_s [H^+]^x_{aq}}{[RSO_3^- H^+]^x_s [M^{x+}]^x_{aq}}$$

tells affinity of resin for M^+ compare to H^+ here or any ion

Ion Exchange Process

Analyte ions (M^{x+}) are passed thru column & retained on an ion-exchange site. The mobile phase contains some H^+ & this is increased sufficiently to cause exchange with M^{x+}.

Back to equilibrium expression

$$K_{ex} = \frac{[(RSO_3^-)_x\ M^{x+}]_s\ [H^+]^x_{aq}}{[RSO_3^-\ H^+]^x_s\ [M^{x+}]^x_{aq}}$$

Rearrange to

$$\frac{[RSO_3^-\ H^+]^x_s}{[H^+]^x_{aq}} \quad K_{ex} = \frac{[(RSO_3^-)_x\ M^{x+}]_s}{[M^{x+}]^x_{aq}}$$

During elution $[H^+]$ is high & $[RSO_3^-\ H^+]_s$ is high

Left hand side of equation essentially constant

$$K = \frac{[(RSO_3^-)_x\ M^{x+}]_s}{[M^{x+}]^x_{aq}} = \frac{C_s}{C_M}$$

K turns out to be a distribution ratio (partition)

Order of affinity for sulfonated cation exchange

$Tl^+ > Ag^+ > Cs^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$

$Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cd^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Hg^{2+}$

Ion Chromatography Detection

Basic detector is conductivity, but
others are used such as UV-vis
& atomic spectrometry (AA, AE) for metals

Measure conductivity change in effluent
when analyte passes through

Problem – use high $[H^+]$ to elute small $[M^{x+}]$
which makes it difficult to detect $[M^{x+}]$
conductivity on high background of $[H^+]$

This problem hindered development of IC
until the innovations made at Dow in 70's

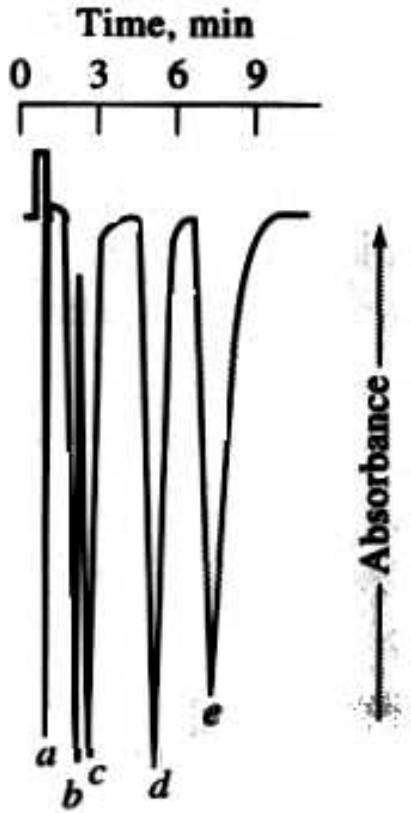


Figure 28-24 Indirect photometric detection of several anions by elution. Eluent: 10^{-3} M disodium phthalate, 10^{-3} M boric acid, pH 10. Flow rate: 5 mL/min. Sample volume: 0.02 mL. UV detector. Sample ions: (a) 18-μg carbonate; (b) 1.4-μg chloride, (c) 3.8-μg phosphate; (d) 5-μg azide; (e) 10-μg nitrate. *(Reprinted with permission from H. Small,* Anal. Chem., *1985,* 55, *240A. Copyright 1983 American Chemical Society.)*

Several ways now available to solve the conductivity problem from background ions

1) Suppressor column – Dow researchers used a second ion exchange column after the analytical column to neutralize the $[H^+]$ & remove its conductivity so M^{x+} can be easily detected (e.g. if HCl is mobile phase use resin suppressor in OH^- form R^+OH^-)

H_2O no conductivity

$$H^+Cl^- + R^+OH^-_S \rightleftharpoons H^+OH^- + R^+Cl^-_S$$

Suppressor columns must be regenerated

2) Single Column IC – no suppressor column used, instead use low capacity analytical column to keep mobile phase concentration low & therefore the conductivity low – this is coupled with the use of a special conductivity detector that can null out high background of mobile phase without suppressing conductivity

3) Other Suppressor Options – membrane, electrochemical, hollow fiber, etc.

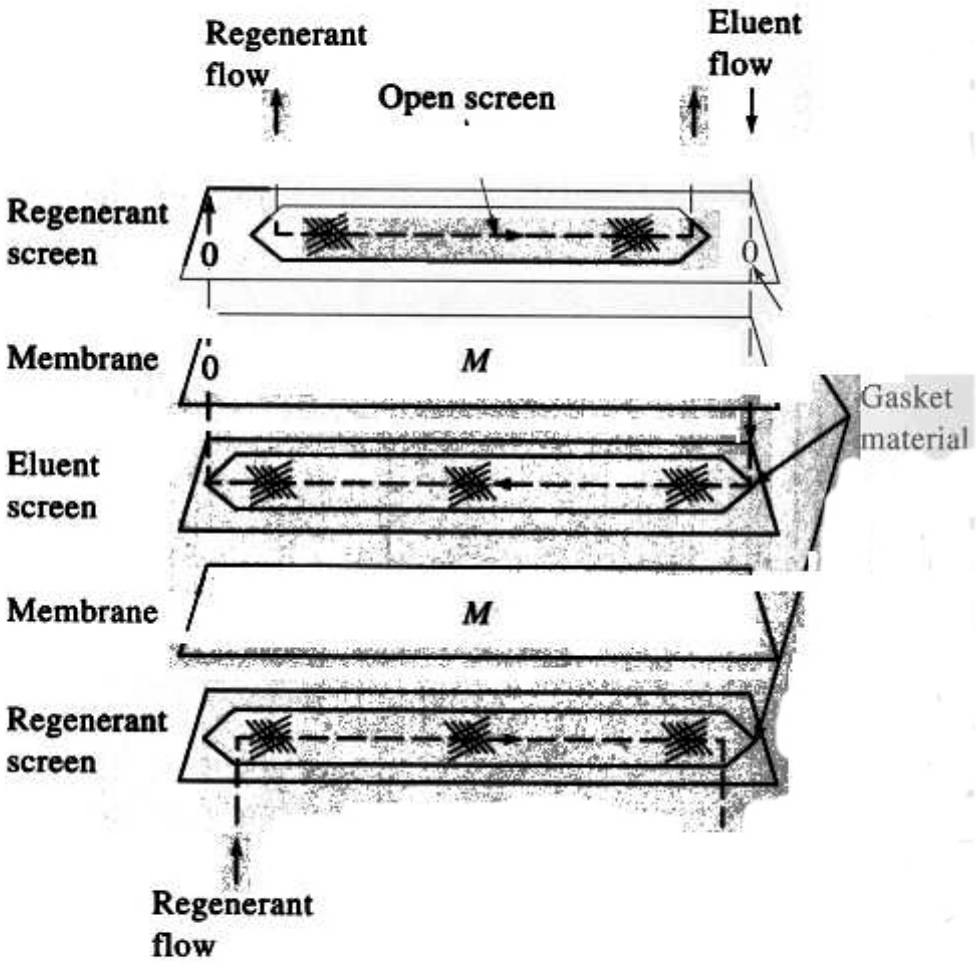


Figure 28-22 **A micromembrane suppressor.** Eluent flows through a narrow channel that contains a plastic screen that reduces the void volume and appears to increase mass-transfer rates. The eluent is separated from the suppressor solution by 50-μm exchange resins. Regenerant flow is in the direction opposite to eluent flow. *(Courtesy of Dionex Corporation, Sunnyvale, CA.)*

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

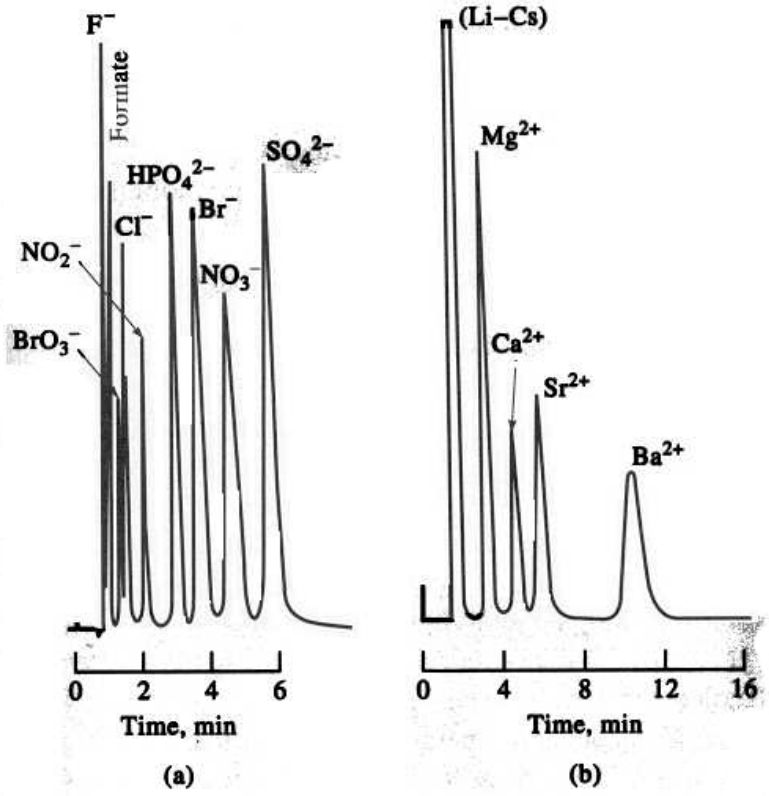


Figure 28-23 Typical applications of ion chromatography. (a) Separation of anions on an anion-exchange column. Eluent: 0.0028 M $NaHCO_3$/0.0023 M Na_2CO_3. Sample size: 50 μL. (b) Separation of alkaline earth ions on a cation-exchange column. Eluent: 0.025 M phenylenediamine dihydrochloride/0.0025 M HCl. Sample size: 100 μL. *(Courtesy of Dionex Corporation, Sunnyvale, CA.)*

Size Exclusion Chrom. (SEC)

Packings are porous polymeric (resins) or silica based materials

Two names used for the same process:

1) Gel filtration chrom. = aqueous solvent
2) Gel permeation chromatography = non-aqueous mobile phase

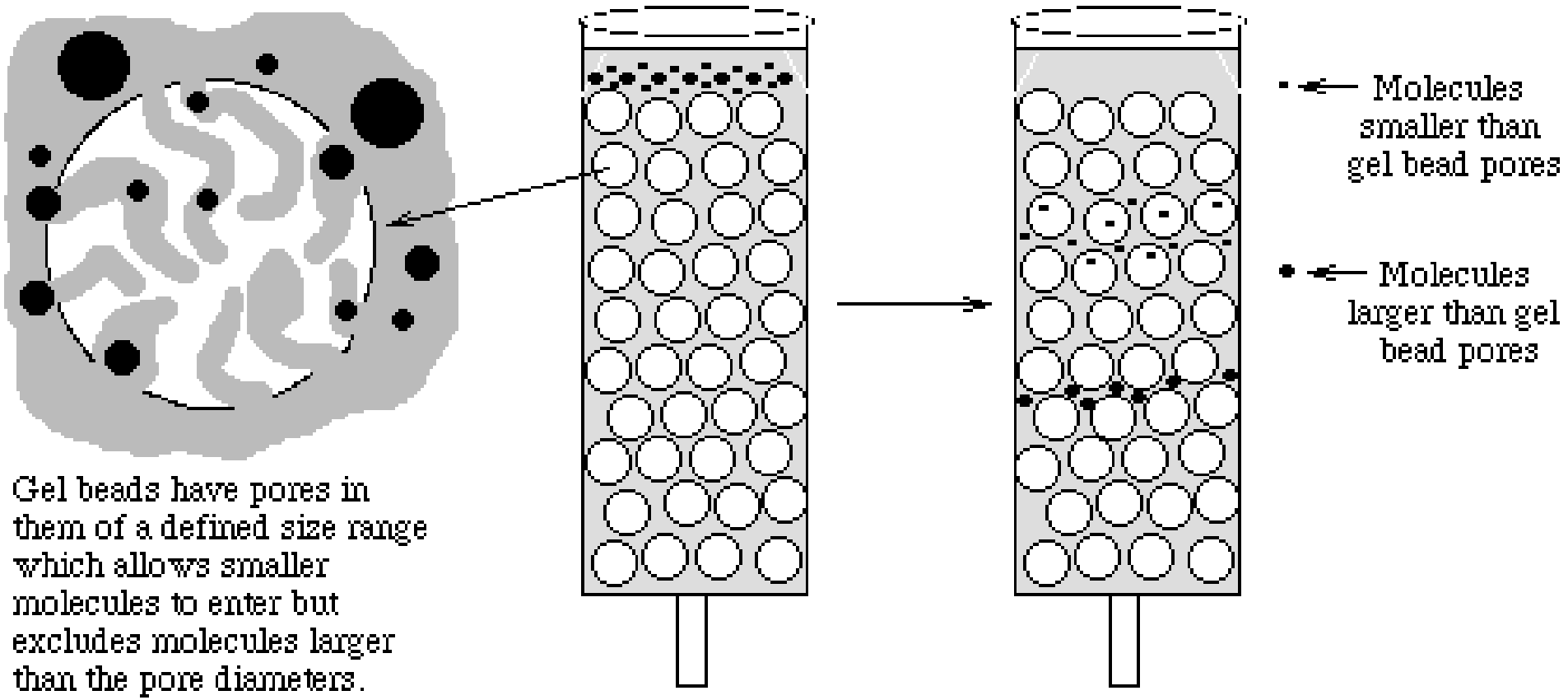
Column packing works like a molecular filter allowing small molecules access to every pore, retarding their progress – large molecules pass thru more quickly

TABLE 28-6 Properties of Typical Commercial Packings for Size-Exclusion Chromatography

Type	Particle Size, μm	Average Pore Size, Å	Molecular Weight Exclusion Limit*
Polystyrene-divinylbenzene	10	10^2	700
		10^3	$(0.1 \text{ to } 20) \times 10^4$
		10^4	$(1 \text{ to } 20) \times 10^4$
		10^5	$(1 \text{ to } 20) \times 10^5$
		10^6	$(5 \text{ to } > 10) \times 10^6$
Silica	10	125	$(0.2 \text{ to } 5) \times 10^4$
		300	$(0.03 \text{ to } 1) \times 10^5$
		500	$(0.05 \text{ to } 5) \times 10^5$
		1000	$(5 \text{ to } 20) \times 10^5$

*Molecular weight above which no retention occurs.

SEC



Gel beads have pores in them of a defined size range which allows smaller molecules to enter but excludes molecules larger than the pore diameters.

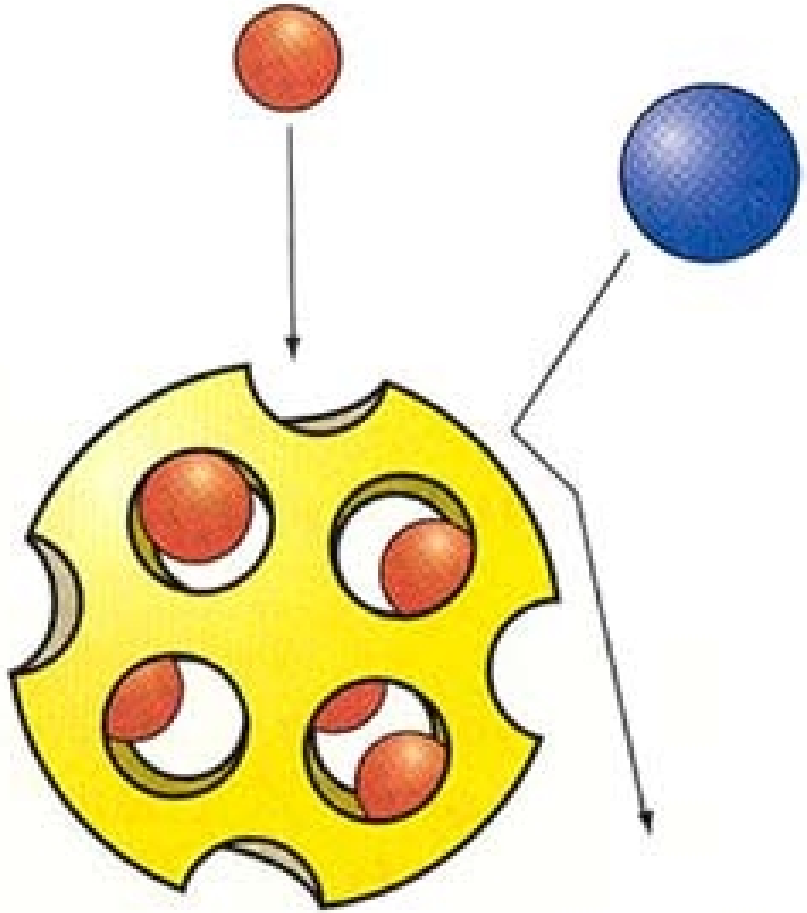
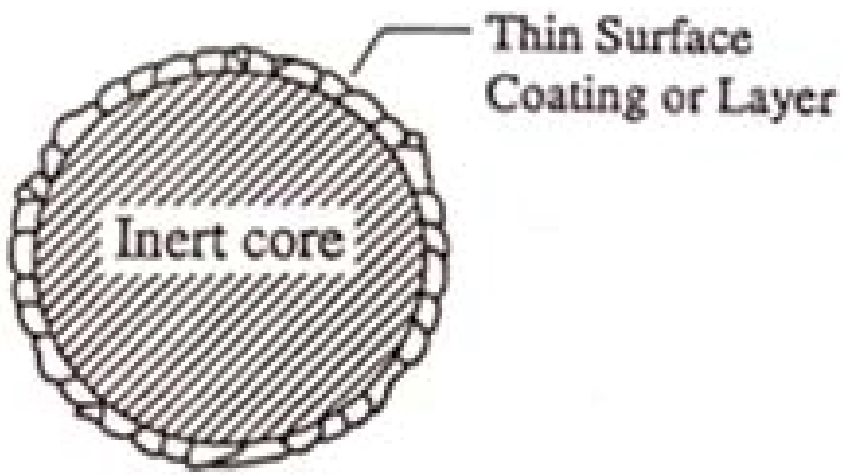


Figure **Gel filtration chromatography. (a)** Principle of the method. A resin bead is schematically represented as a "whiffle ball" (yellow). Large molecules (blue) cannot fit into the beads, so they are confined to the relatively small buffer volume outside the beads. Thus, they emerge quickly from the column. Small molecules (red), by contrast, can fit into the beads and so have a large buffer volume

Polymeric SEC packing can be thought of as a ball of yarn with pores defined by the degree of crosslinking of the polymer chains

Pellicular packings



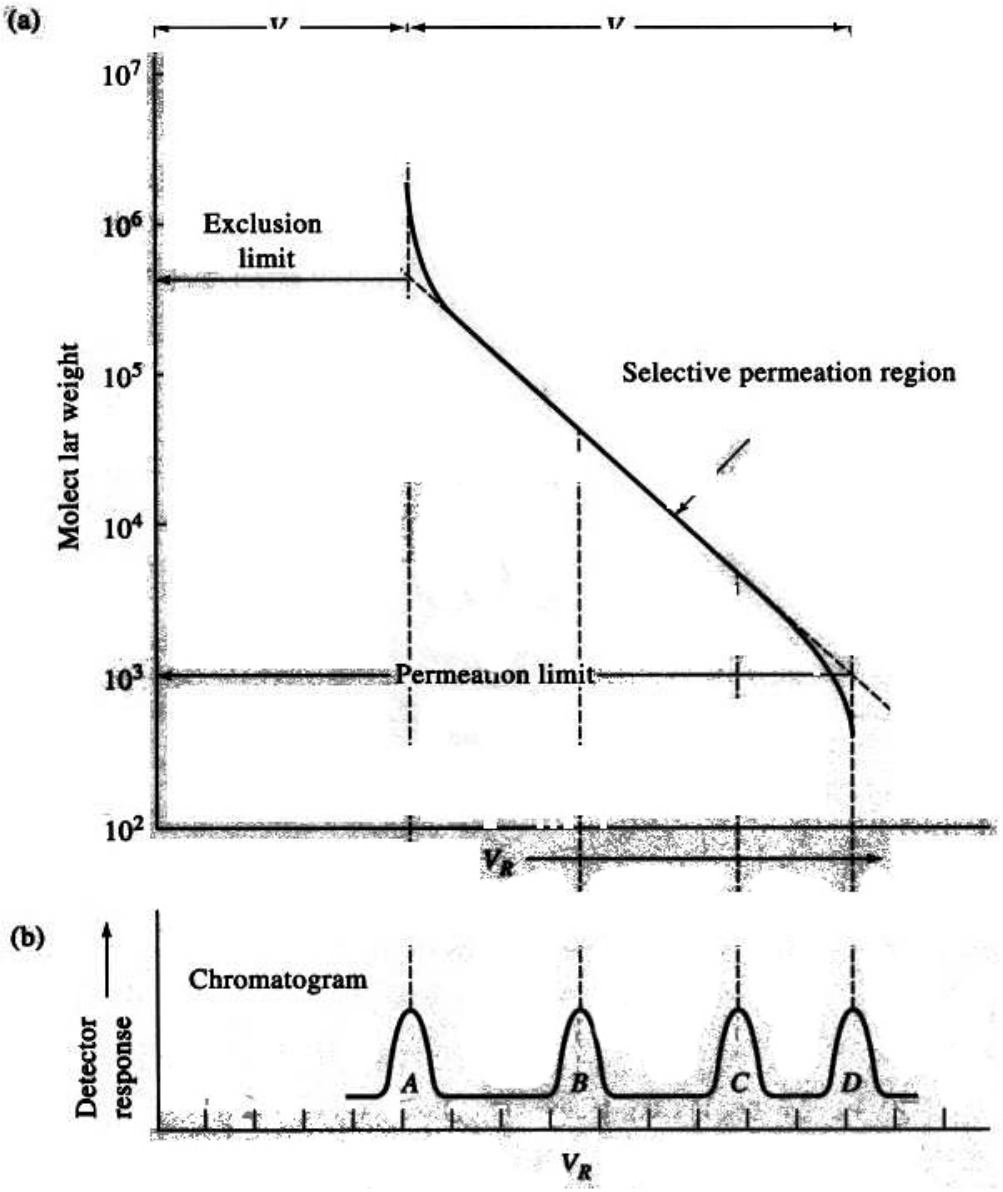


Figure 28-27 (a) Calibration curve for a size-exclusion column. (b) Chromatogram showing peak *A* containing all compounds with molecular weights greater than the exclusion limit, peaks *B* and *C* consisting of compounds within the selective permeation region, and peak *D* containing all compounds smaller than the permeation limit.