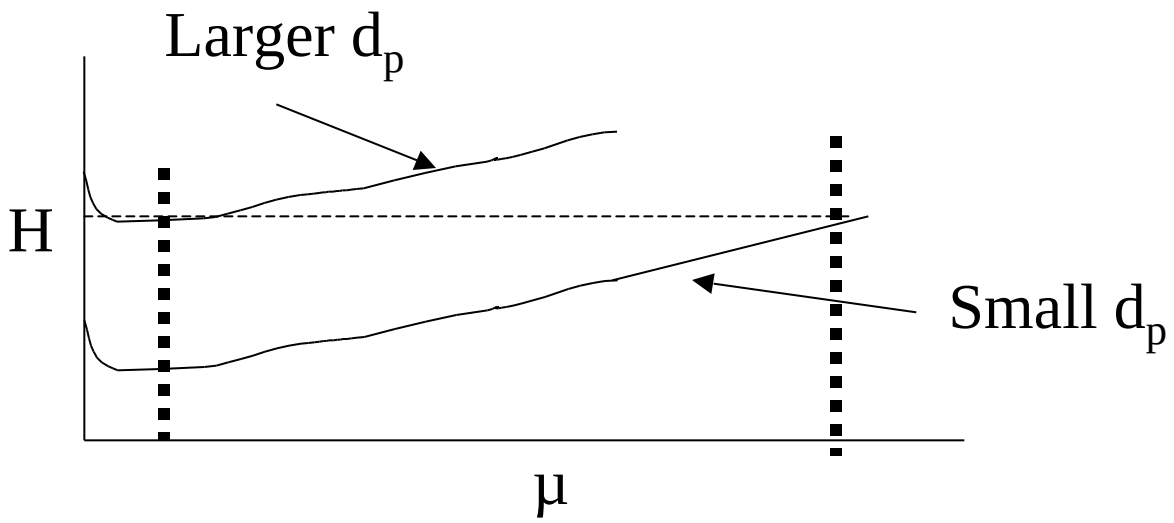
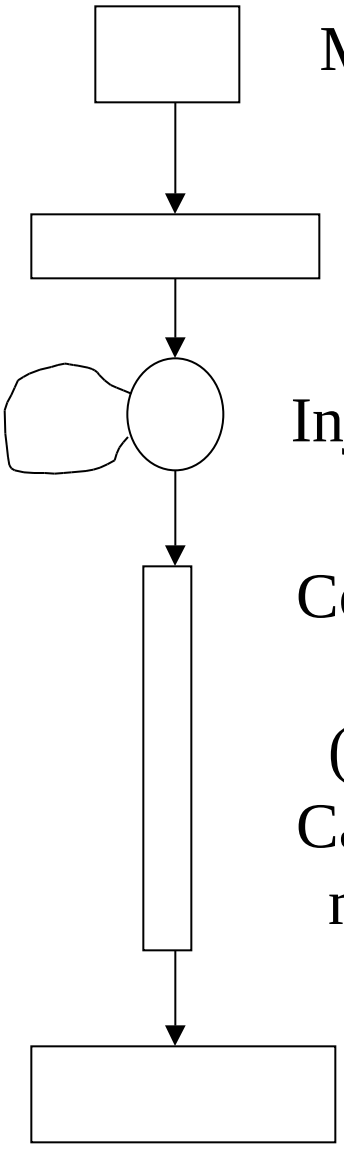
Liquid Chromatography----

Conventional---use 150-200 µm particles--glass columns---must use very slow flow rates to get enough N to achieve given separation!

Modern HPLC--use much smaller particles---3-10 µm---can use much faster flow rates--to speed analysis time---while achieving comparable or or even larger N



Components of HPLC system:



Mobile phase reservoir

Pump--capable of forcing fluid through column with small d_p

Injection valve--25-100 µl

Column---usually stainless steel 10-25 cm in length--- 4 mm i.d. (analytical columns)---
Can also have preparative columns- much wider and longer!

Detector--
- UV-Vis absorbance
- Refractive index
- Fluorescence
- Electrochemical
- Post-column rxn with one of above

Must always be concerned with dead volume of detector--don't want extra peak broadening in detector

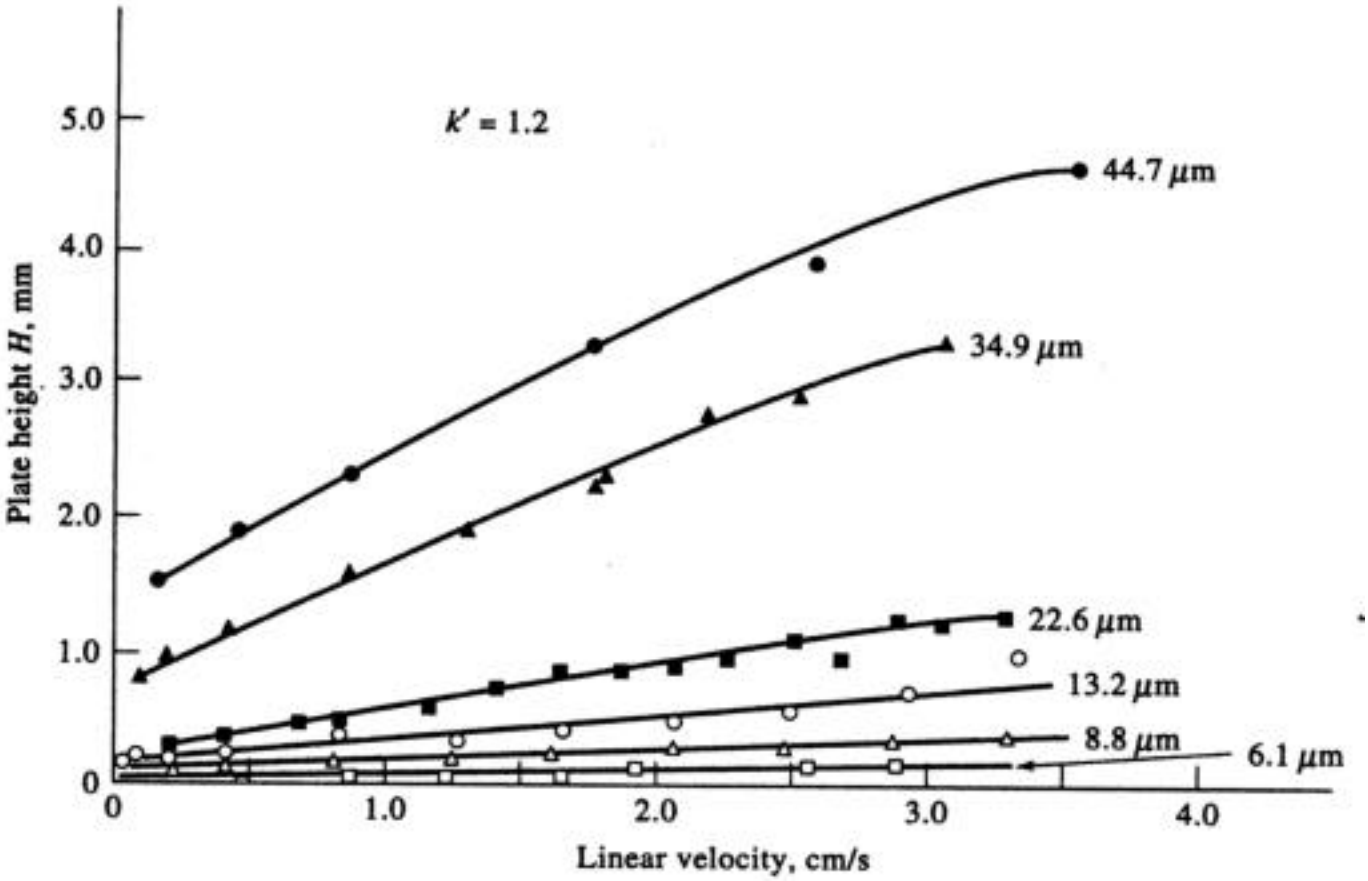


Figure 28-2 Effect of particle size of packing and flow rate upon plate height H in liquid chromatography. Column dimensions: 30 cm × 2.4 mm. Solute: N,N-diethyl-*n*-aminoazobenzene. Mobile phase: mixture of hexane, methylene chloride, isopropyl alcohol. *(From R. E. Majors,* J. Chromatogr. Sci., *1973, 11, 92. With permission.)*

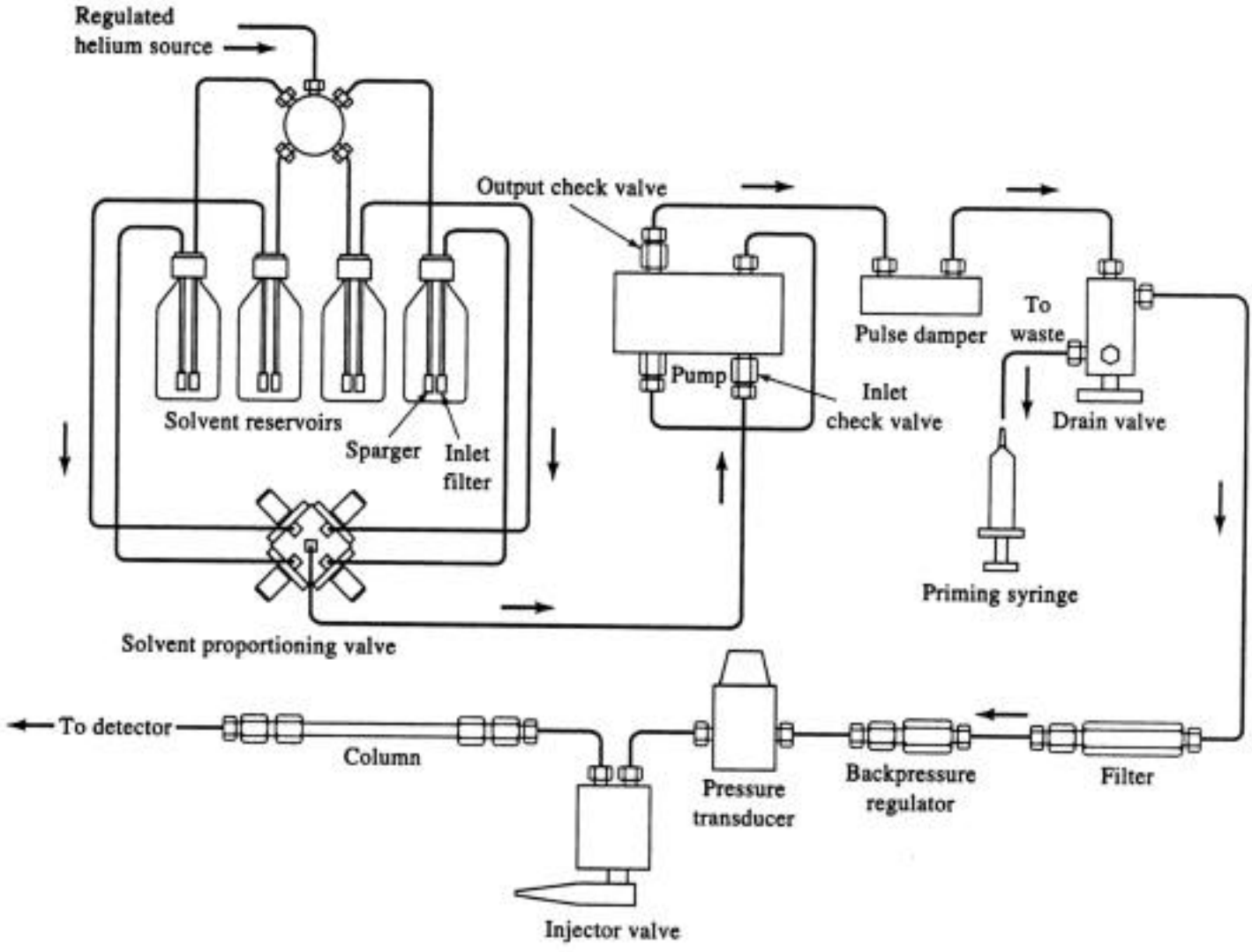


Figure 28-4 Schematic of an apparatus for HPLC. *(Courtesy of Perkin-Elmer Corporation, Norwalk, CT.)*

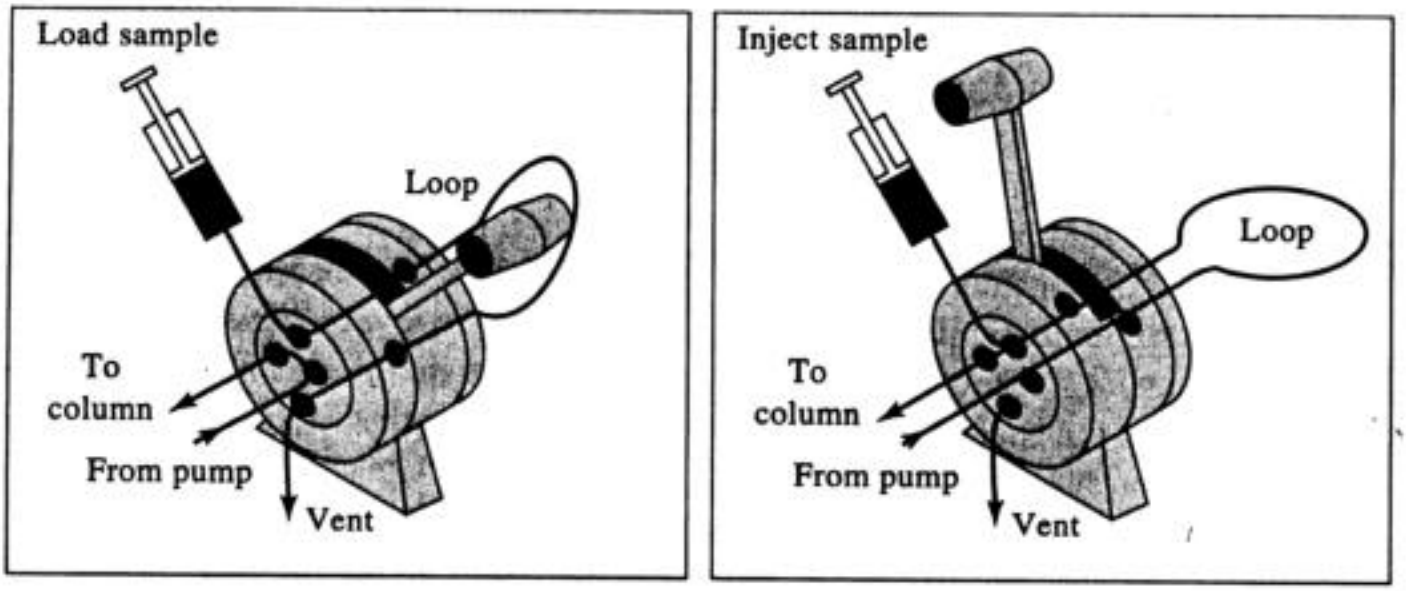


Figure 28-7 A sampling loop for liquid chromatography. *(Courtesy of Beckman Instruments, Fullerton, CA.)* With the valve handle as shown on the left, the loop is filled from the syringe, and the mobile phase flows from pump to column. When the valve is placed in the position on the right, the loop is inserted between the pump and the column so that the mobile phase sweeps the sample onto the column.

Detectors for HPLC

Key concern:- Dead Volume - when species separated on column - don't want them to mix in detector:

- Try to use detectors w/ < 10 μL volume

Column Packings

- normal phase
- Reversed-phase
- Ion-exchange
- Size exclusion

Normal phase

- Silica gel or Alumina (Al_2O_3)
- adsorb polar molecules
- use non-polar mobile phase

TABLE 28-1 Performances of LC Detectors

LC Detector	Commercially Available	Mass LOD (commercial detectors)[a]	Mass LOD (state of the art)[b]
Absorbance	Yes[c]	100 pg–1 ng	1 pg
Fluorescence	Yes[c]	1–10 pg	10 fg
Electrochemical	Yes[c]	10 pg–1 ng	100 fg
Refractive index	Yes	100 ng–1 μg	10 ng
Conductivity	Yes	500 pg–1 ng	500 pg
Mass spectrometry	Yes[d]	100 pg–1 ng	1 pg
FT–IR	Yes[d]	1 μg	100 ng
Light scattering[e]	Yes	10 μg	500 ng
Optical activity	No	—	1 ng
Element selective	No	—	10 ng
Photoionization	No	—	1 pg–1 ng

[a]Mass LOD is calculated for injected mass that yields a signal equal to five times the σ noise, using a mol wt of 200 g/mol, 10 μL injected for conventional or 1 μL injected for microbore LC.
[b]Same definition as *a*, above, but the injected volume is generally smaller.
[c]Commercially available for microbore LC also.
[d]Commercially available, yet costly.
[e]Including low-angle light scattering and nephelometry.
(From E. S. Yeung and R. E. Synovec, *Anal. Chem.*, **1986**, *58*, 1238. With permission.)

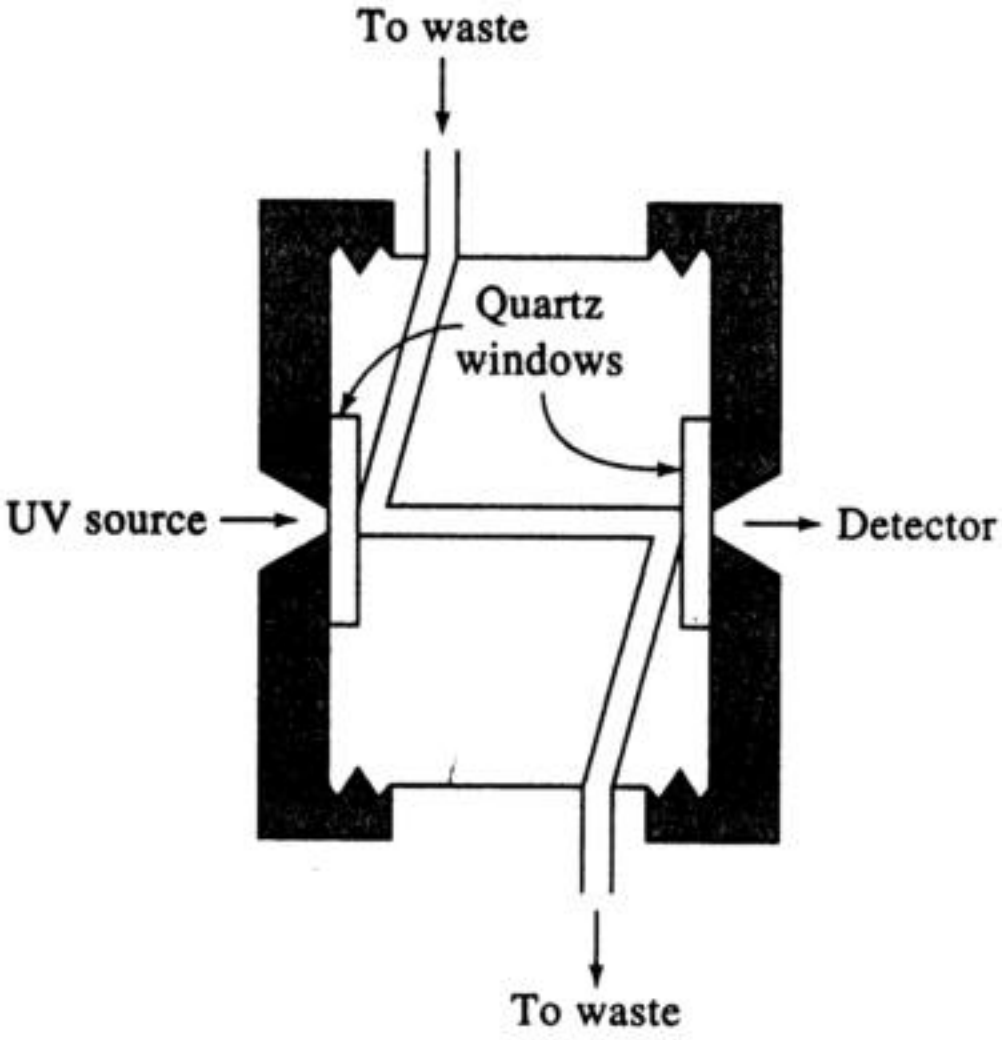


Figure 28-9 Ultraviolet detector cell for HPLC.

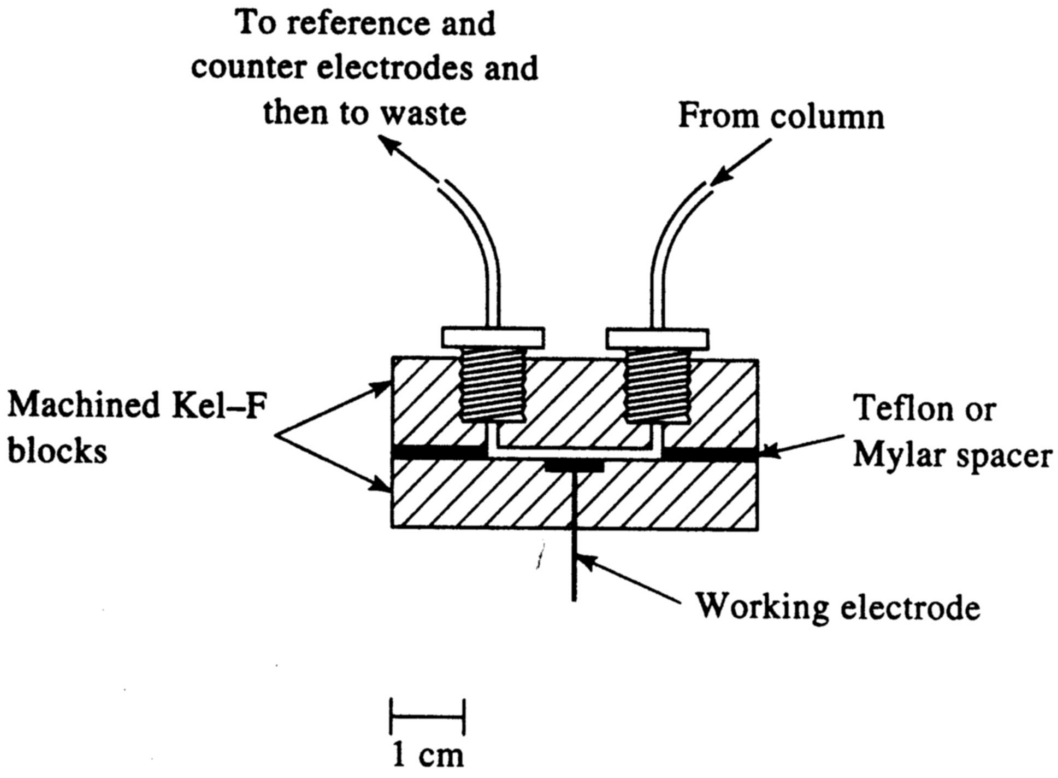


Figure 28-13 Amperometric thin-layer detector cell for HPLC.

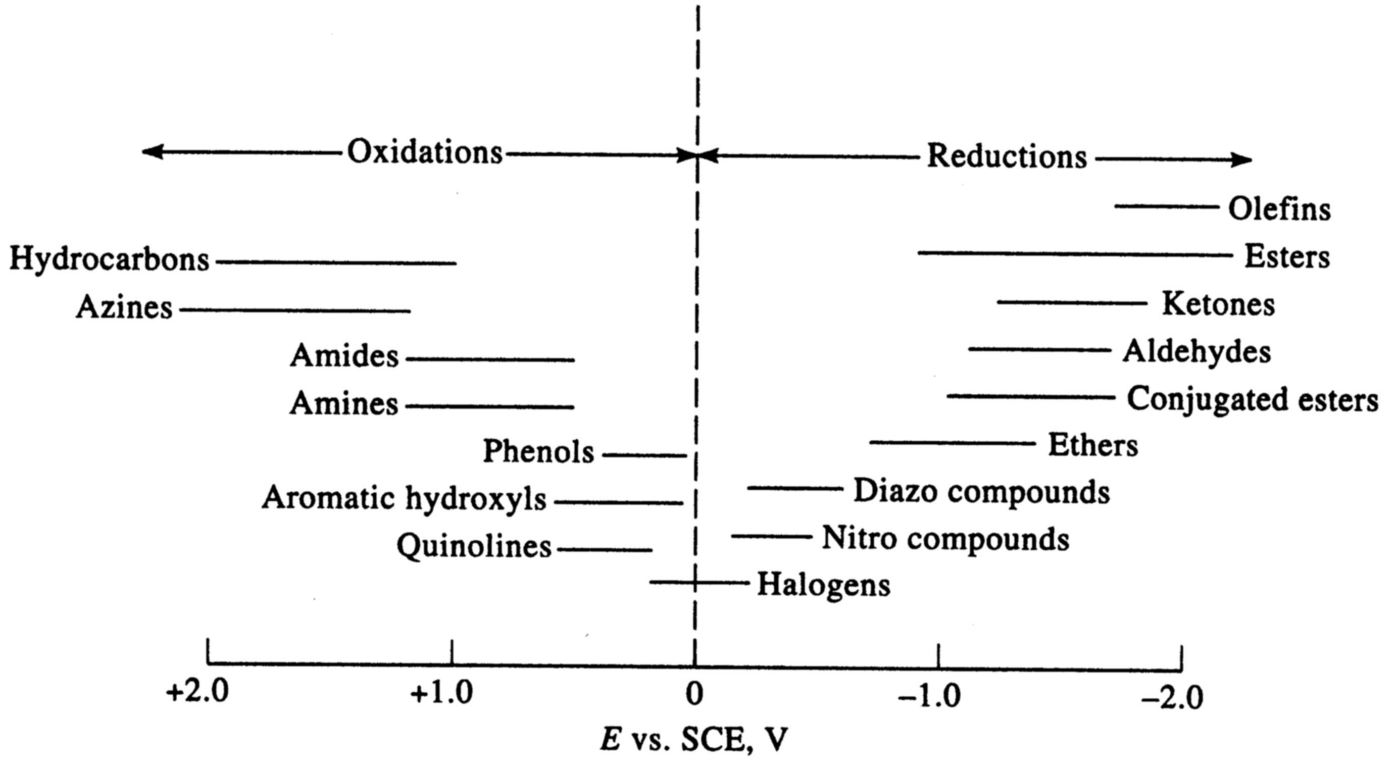
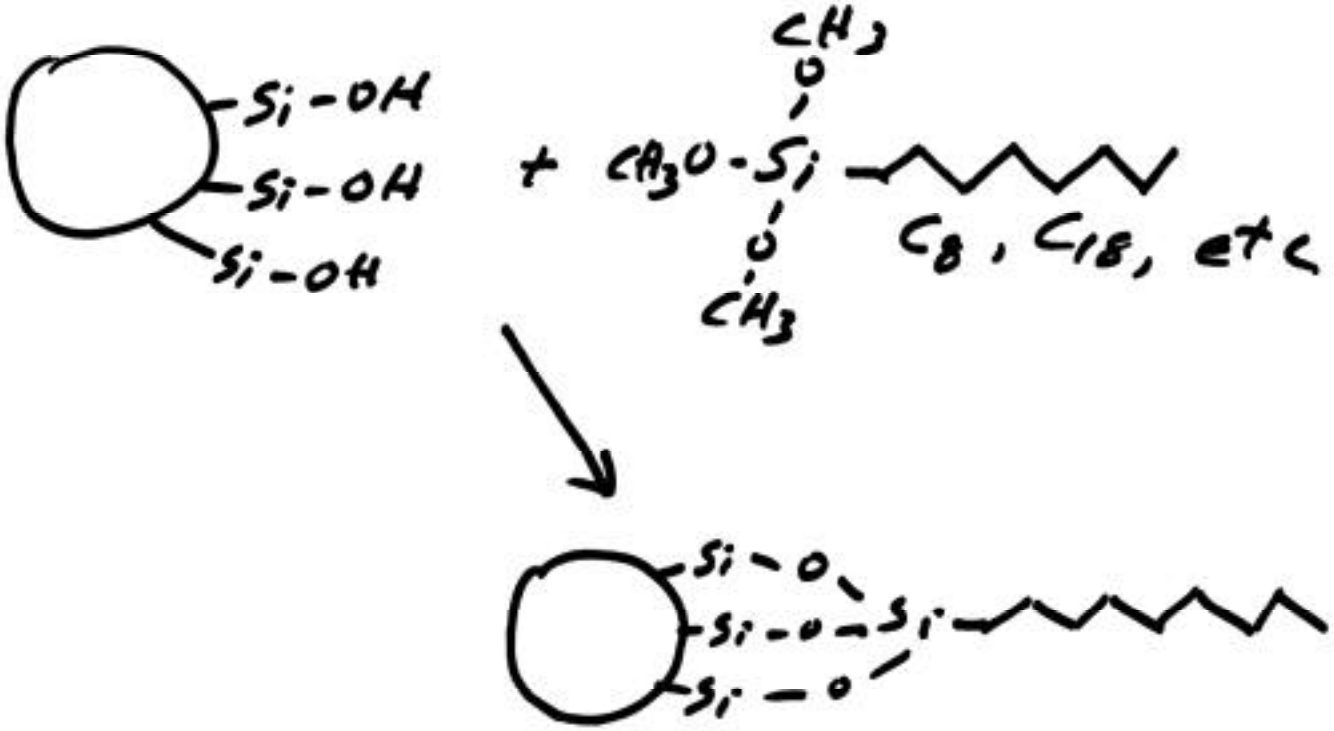
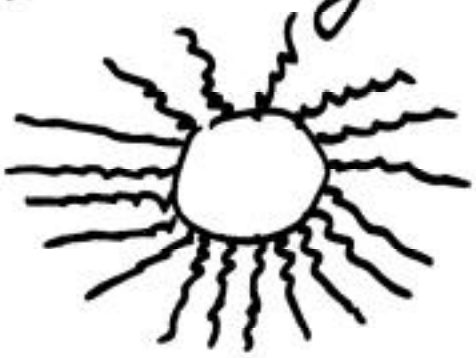


Figure 28-12 Potentially detectable organic functional groups by amperometric measurements. The horizontal lines show the range of oxidation or reduction potentials wherein compounds containing the indicated functional groups are electroactive.



Final coating - 3-5 umoles/m^2 of alkyl groups



note: not drawn to scale

particles - 3-10 um silica

monolayer of alkyl chains ≈ 30 Å for C_{18}

Can further coat alkyl layer with organic phase to create a bulk stationary phase with finite volume → yields true partition phase

* Can immobilize phenyl groups for π-π separations; chiral molecules for chiral separations

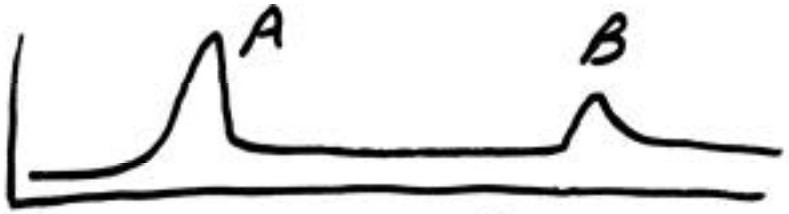
Key advantage of reversed phase systems - since most samples are aqueous-based - can inject directly onto column w/o prior extraction step.

Mobile Phases (H_2O, MeOH, acetonitrile, etc.)

- Must degas - don't want bubbles forming in column or detector
- Two modes of operation:

  Isocratic: same composition of mobile phase used throughout separation

  Gradient Elution: Since $U_R = U_m + KU_s$ use change in mobile phase composition during separation to change K values - to speed analysis time



$K_A << K_B$

$$K_B = \frac{C_s^B}{C_m^B}$$

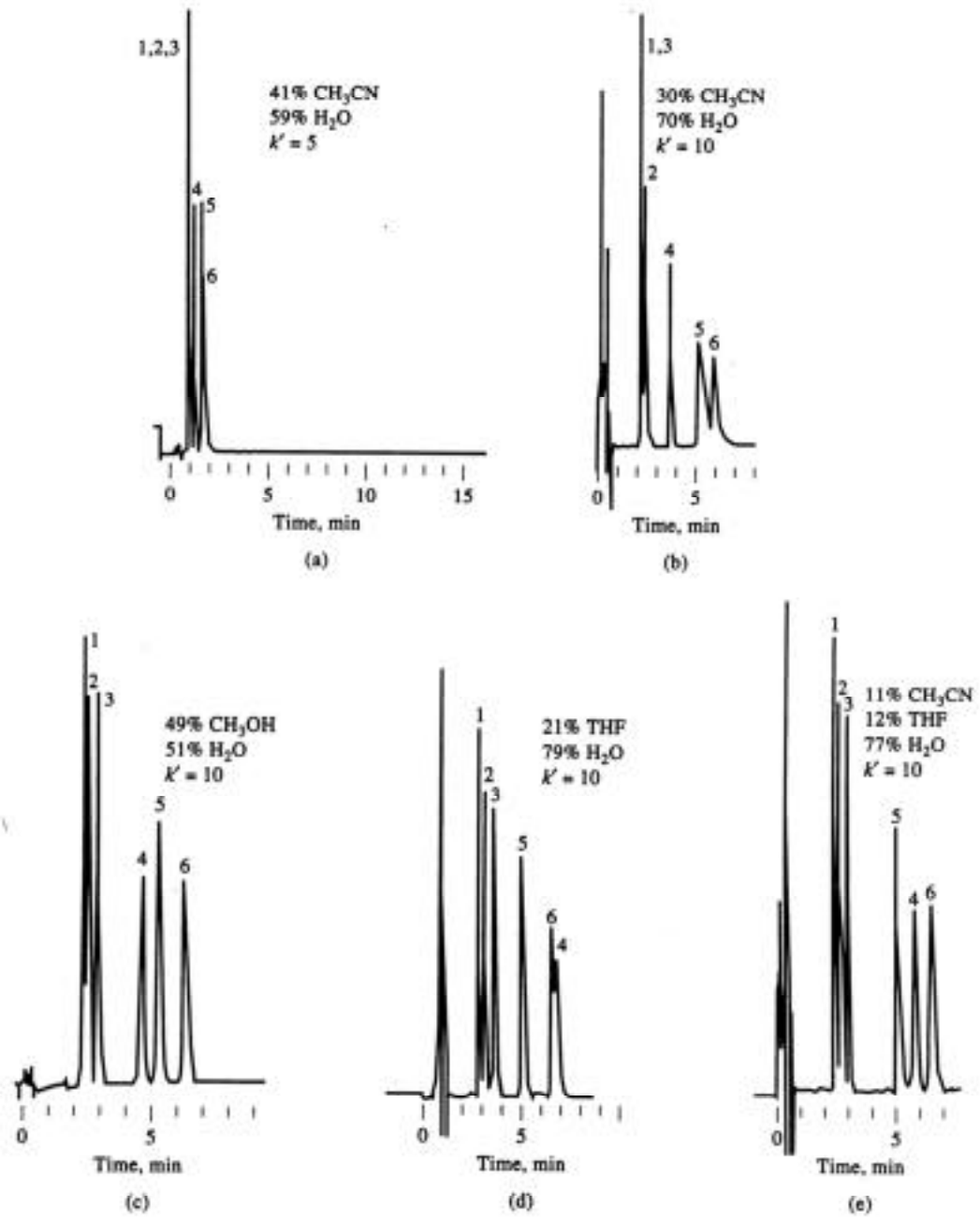


Figure 28-16 Systematic approach to the separation of six steroids. The use of water to adjust k' is shown in (a) and (b). The effects of varying α at constant k' are shown in (b), (c), (d), and (e). Column: 0.4 × 150 mm packed with 5 μm C_8 bonded, reversed-phase particles. Temperature: 50°C. Flow rate: 3.0 cm^3/min. Detector: UV 254 nm. THF = tetrahydrofuran. CH_3CN = acetonitrile. Compounds: (1) prednisone, (2) cortisone, (3) hydrocortisone, (4) dexamethasone, (5) corticosterone, (6) cortoexolone. *(Courtesy of DuPont Instrument Systems, Wilmington, DE.)*

K_B will change with polarity of mobile phase

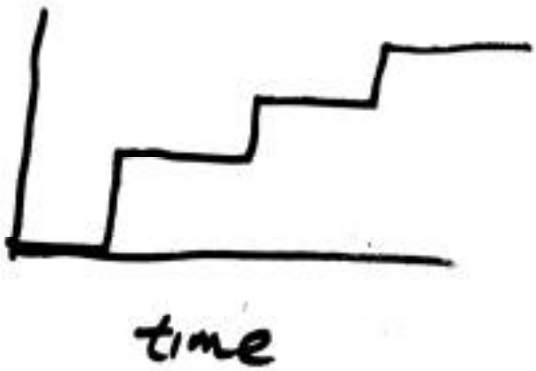
Step change in mobile phase composition

Change mobile phase solvent composition as A-elutes

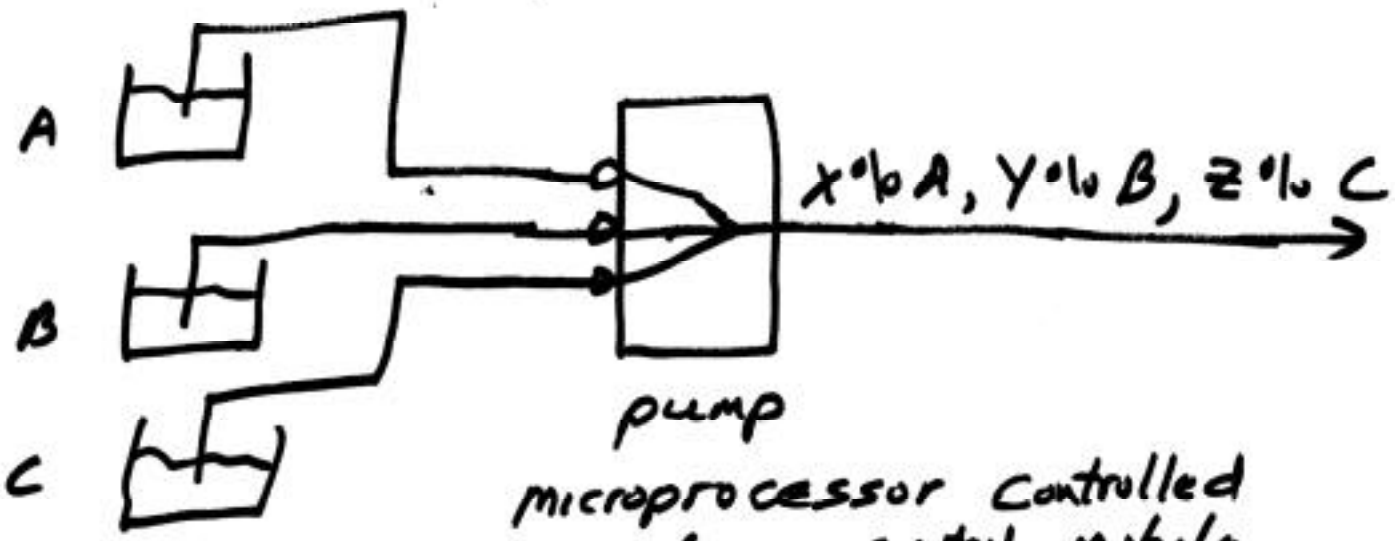
Condenses chromatogram - allows more rapid determinations for multicomponent analysis

Linear gradient

or

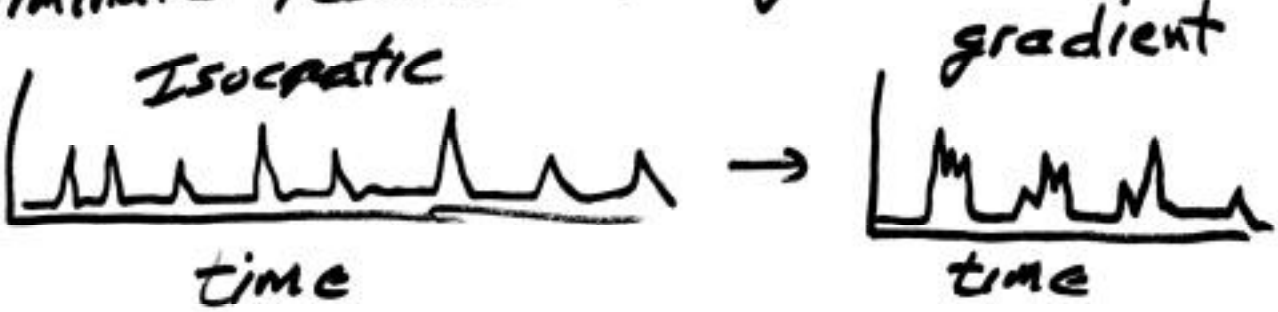


Can have binary or ternary solvent capabilities



microprocessor controlled nozzles - control mobile phase composition

Must be careful using gradient elution w/ complex samples — can eliminate resolution of given solutes



Another form of L.C. —
Planar Chromatography
a) thin layer < Reversed / normal
b) paper → Normal phase - Cellulose likes polar solutes

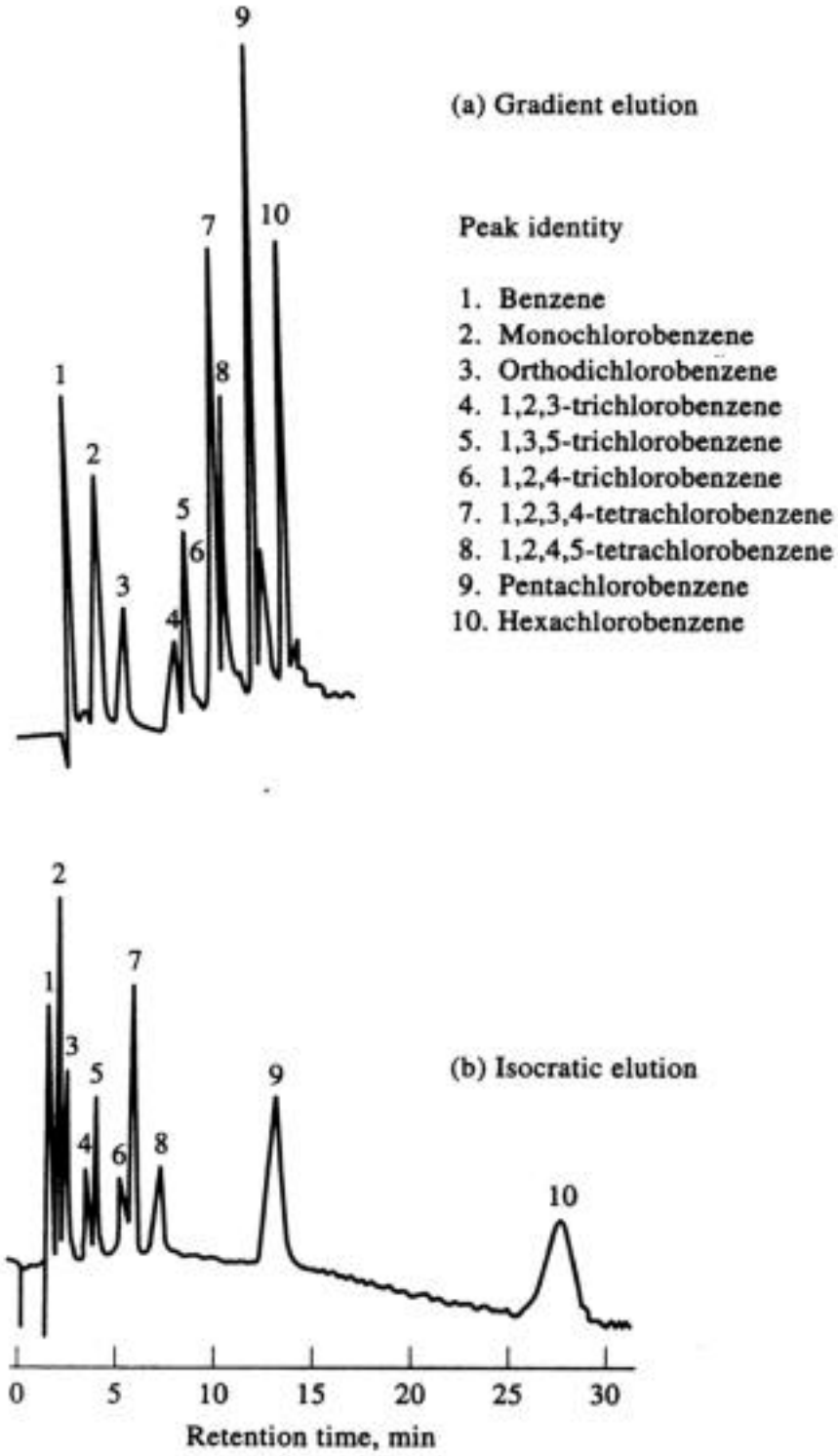
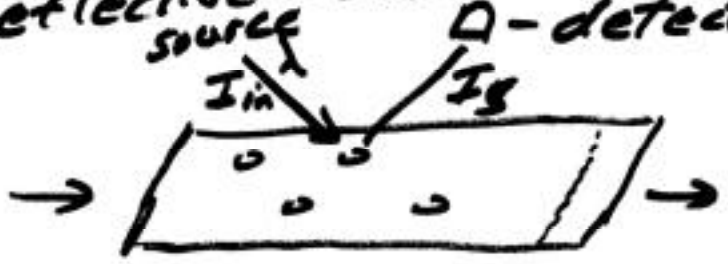


Figure 28-5 Improvement in separation efficiency by gradient elution. Column: 1 m × 2.1 mm i.d., precision-bore stainless; packing: 1% Permaphase® ODS. Sample: 5 μL of chlorinated benzenes in isopropanol. Detector: UV photometer (254 nm). Conditions: temperature, 60°C, pressure, 1200 psi. *(From J. J. Kirkland,* Modern Practice of Liquid Chromatography, *p. 88. New York: Interscience, 1971. Reprinted by permission of John Wiley & Sons, Inc.)*

HPTLC → use small diameter particles to coat thin layer plate — get more $\underline{\underline{N}}$ per given length

Can make quantitative measurements even with HPTLC — need special instrumented detectors

Reflective - absorbance:



$I_s = I_{in} - I_{abs} - I_{trans}$

↑ due to conc. of solute on spot of plate

move plate to scan —

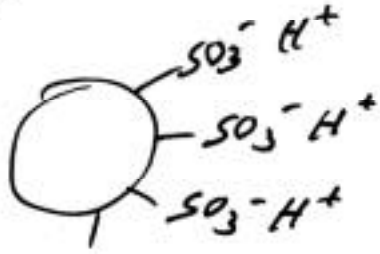
Ion-Exchange Chromatography

Cations - organic + metal ions (Na^+, Ca^{+2}, K^+)

* Anions - organic + Cl^-, PO_4^{-3}, SO_4^{-2}, Br^-, etc

Allows simultaneous multianalyte measurements of anions → no other simple way to do inorganic anions

For cations - stationary phase has negative sites (fixed)



sulfonic acid exchangers

$$R\text{-}SO_3^- H^+ + M_M^+ \rightleftharpoons R\text{-}SO_3^- M^+ + H_M^+$$

or

$$RH^+ + M_M^+ \rightleftharpoons RM^+ + H_M^+$$

selectivity coeff. $= K_{M^+/H^+} = \dfrac{[RM^+][H^+]_M}{[RH^+][M^+]_M}$

Solve for $\dfrac{[RM^+]}{[RH^+]} = \dfrac{C_S^{M^+}}{C_M^{M^+}} = \dfrac{K_{M^+/H^+}[RH^+]}{[H^+]_M} = K$

$K \propto K$ if $[RH^+] + [H^+]_M$ are kept constant.

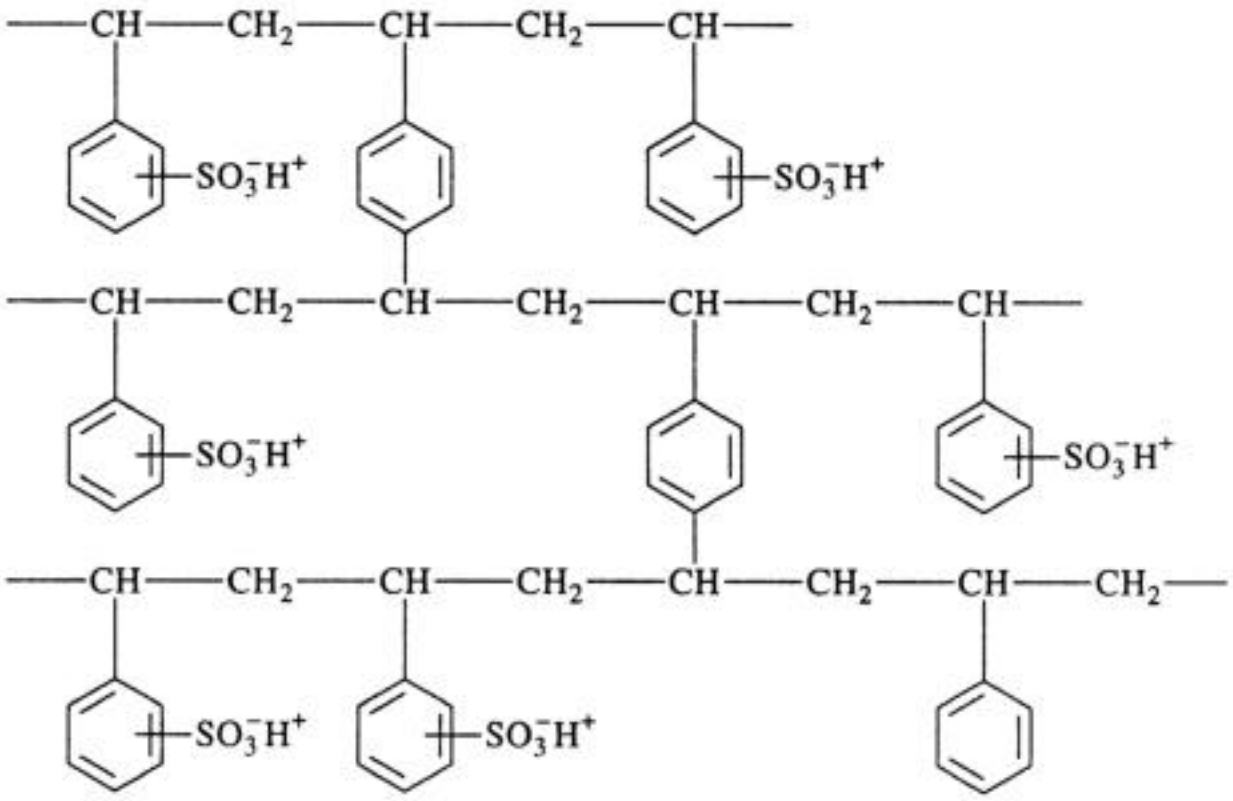


Figure 28-21 Structure of a cross-linked polystyrene ion-exchange resin. Similar resins are used in which the $—SO_3^-H^+$ group is replaced by $—COO^-H^+$, $—NH_3^+OH^-$, and $—N(CH_3)_3^+OH^-$ groups.

if resin (stationary phase) has lots of exchange sites (high $[RSO_3^-]$) compared to $[M]$ in sample - then $[RH^+]$ does not change appreciably

K_{M^+/H^+} depends on:

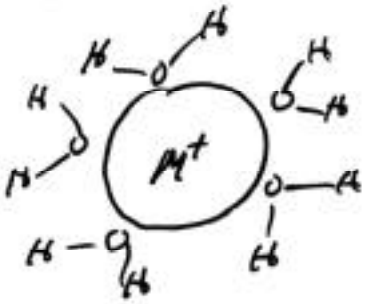
1. Charge on M^+ $\quad +3 > +2 > +1$

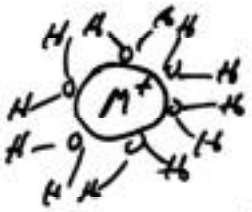
   Electrostatics

2. Ion size - K_{M^+/H^+} ↑ for larger ion

   Two reasons:

   a) smaller solvent equivalent volume for large ions



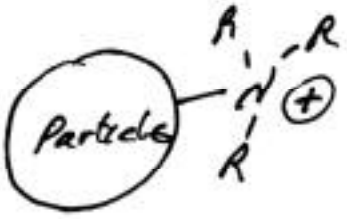


better protected by hydration sphere

   b) larger ions have greater polarizability - e^- in outer shells less influenced by nucleus

$-R$

Anion Exchangers



$$R_4N^+OH + A^-_M \rightleftharpoons R_4N^+A^- + OH^-_M$$

- For anion exchange - usually want stationary phase in OH^- form; use base solution as eluant (mobile phase)
- For cation exchange - keep stationary phase in H^+ form (RH^+), use acidic mobile phase (e.g. 0.01 M HCl)

e.g. cation chromatography

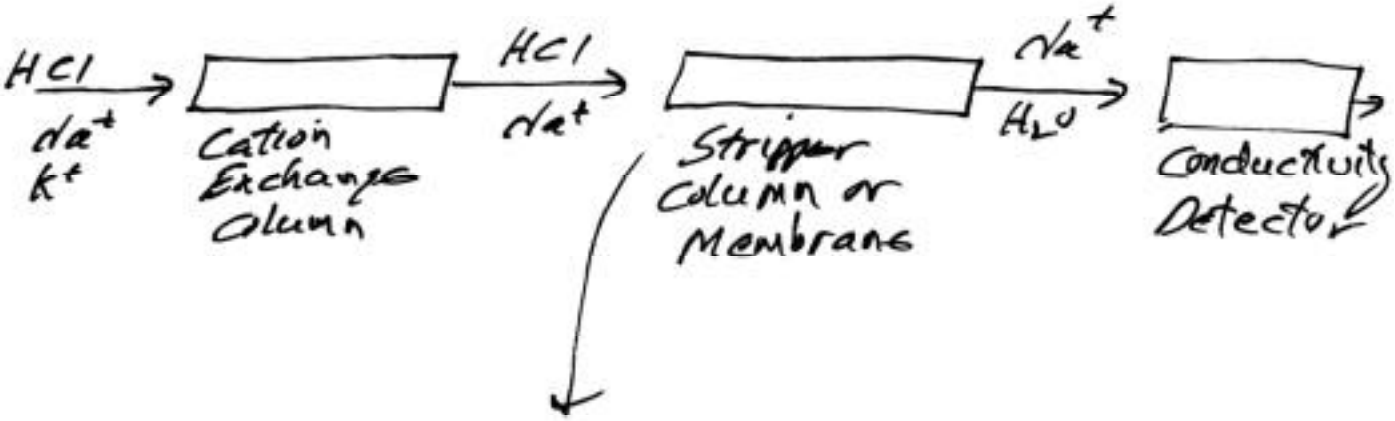
How do we detect ions eluting?

Use <u>conductivity detector</u> for inorganic ion-chromatography

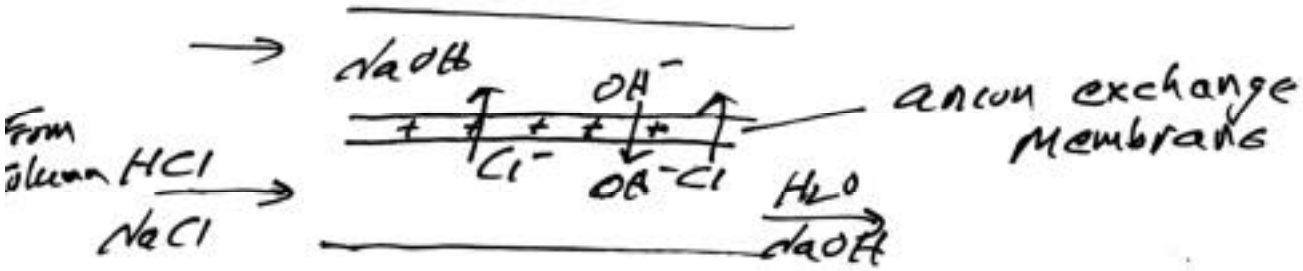
$$\text{Conductance} = \frac{1}{R_{sol}} = G$$

However – must worry about high H^+ or OH^- in mobile phase – will swamp out signal (high background conductivity of mobile phase)

Can solve with <u>Stripper</u> column or Membrane exchanger



Anion Exchanger in OH^- form



<u>Gradient Elution in Ion-Chrom.</u>

- Vary pH of Mobile phase
- Vary Salt – increase; decreases electrostatic interactions

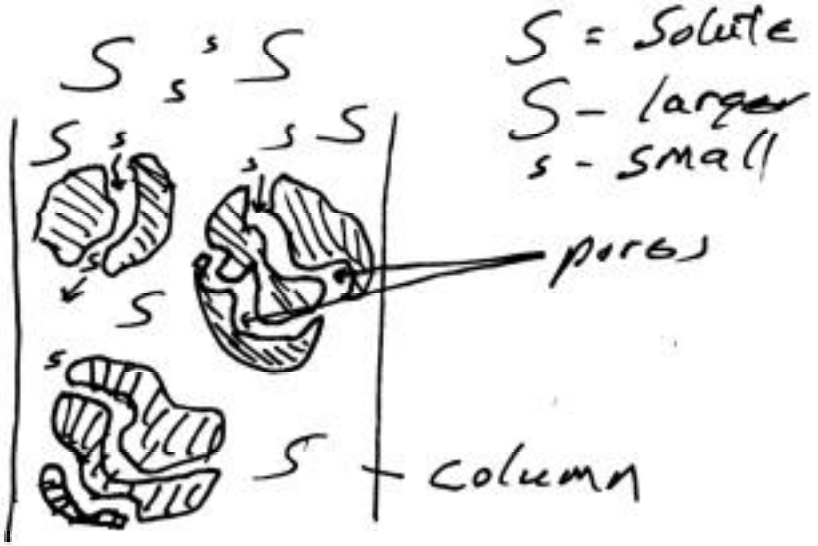
Gel Permeation/Size Exclusion Chrom

- used primarily for preparative work
- separate solutes base on size + shape - These factors determine "K"

Stationary phase: consists of porous particles - crosslinked polysaccharides - degree of cross-linking determines **pore size**

or - porous glass beads - for HPLC Type systems

Can have phases with different pore sizes



V_i = Volume of mobile phase inside pores

V_o = Volume of mobile phase outside pores

- if solute is small enough so that all V_i is accessible to it — Then:

$$V_R = V_o + V_i$$

- if S is so large it cannot enter pores at all — Then:

$$V_R = V_o = V_M$$

No retention on Column.

However - for given stationary phase - pore sizes cannot be precisely controlled

∴ you get fractionation range for solutes — from those that can't enter any V_i - to those that can enter all V_i

Fractionation range = $V_o \rightarrow V_o + V_i$

$$\therefore V_R = V_o + K V_i$$

↖ Fraction of V_i accessible to solute

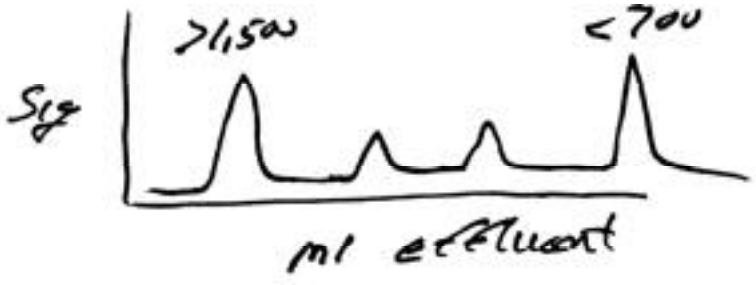
- if S is large - K is small.
- if S is small $K \rightarrow 1$

$$0 \leq K \leq 1$$

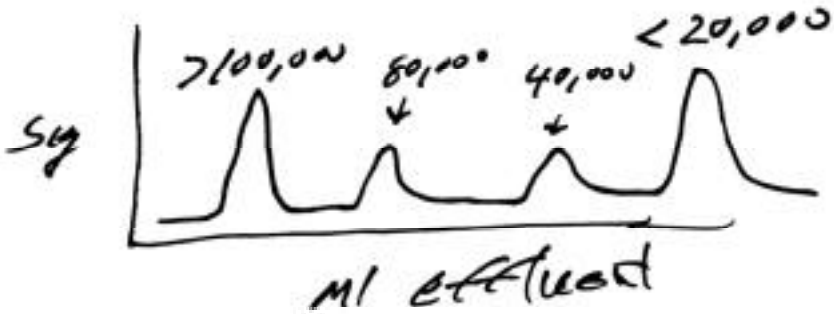
Purchase stationary phases with varying fractionation ranges -

e.g. 700 – 1500 MW
1,500 – 20,000
20,000 – 100,000
etc.

Results:



For column packed with phase with fractionation range 20,000 - 100,000



G.C. - Principles + Instrumentation

- GSC - gas-solid Chromatography
  * based on adsorption of vapor phase molecules on surface of solid support

GLC - Gas-Liquid Chromatography
  * based on gas-phase/liquid phase partitioning — stationary phase is thin film of liquid coated onto support particles - or on inner wall of glass/silica capillary tubing.

GC - only useful for volatile solutes — can derivatize solute to make it more volatile !!

See Fig. 27-1 for schematic of system - Injection system 50°C hotter than column temp.

See Table 27-1 for Types of Chromatographic Columns

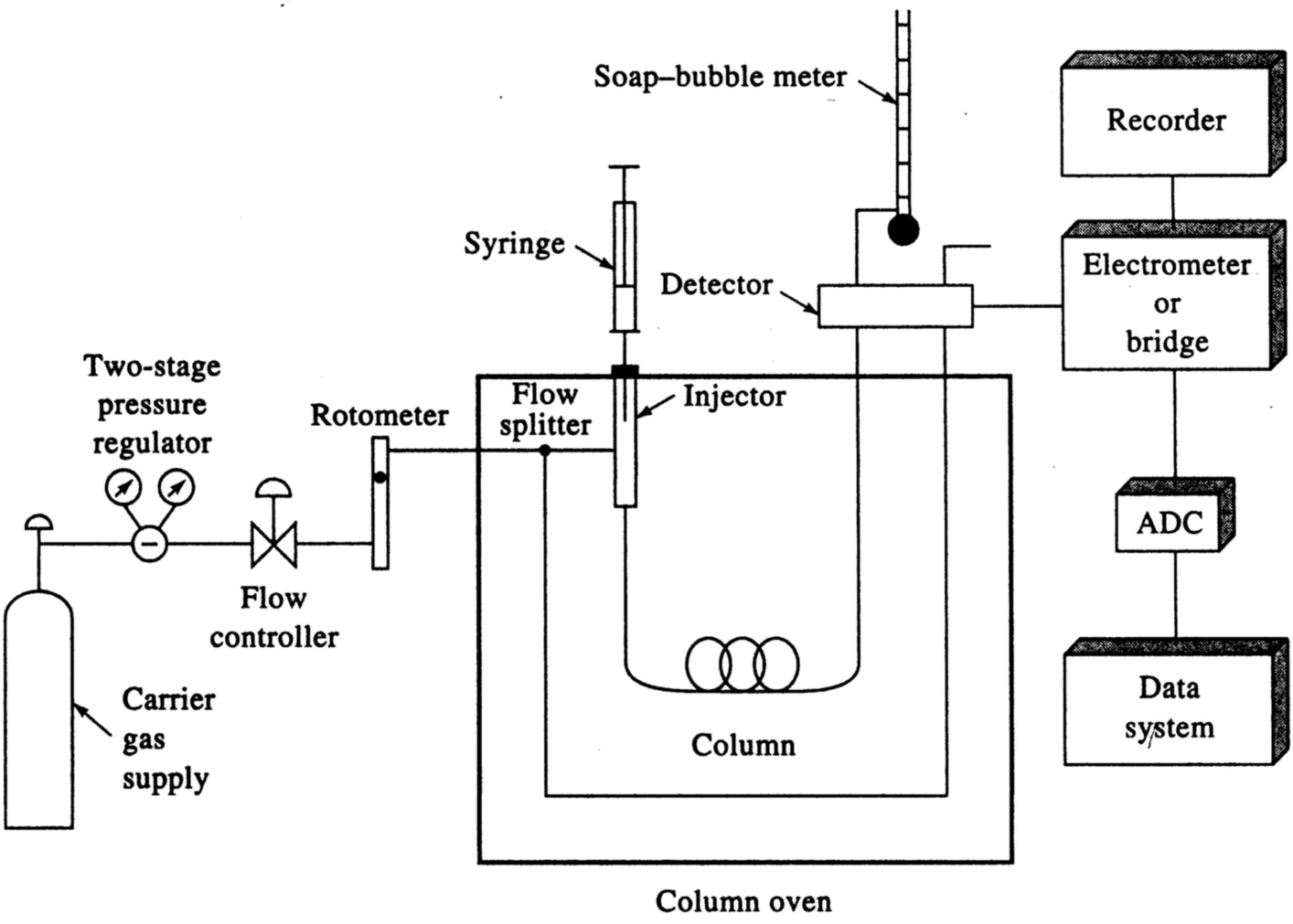


Figure 27-1 Schematic of a gas chromatograph.

TABLE 27-1 Properties and Characteristics of Typical Gas-Chromatographic Columns

	Type of Column*			
	FSOT	WCOT	SCOT	Packed
Length, m	10–100	10–100	10–100	1–6
Inside diameter, mm	0.1–0.53	0.25–0.75	0.5	2–4
Efficiency, plates/m	2000–4000	1000–4000	600–1200	500–1000
Total plates	$(20–400) \times 10^3$	$(10–400) \times 10^3$	$(6–120) \times 10^3$	$(1–10) \times 10^3$
Sample size, ng	10–75	10–1000	10–1000	$10–10^6$
Relative back pressure	Low	Low	Low	High
Relative speed	Fast	Fast	Fast	Slow
Chemical inertness	Best →			Poorest
Flexible?	Yes	No	No	No

*FSOT: Fused-silica, open tubular column.
WCOT: Wall-coated, open tubular column.
SCOT: Support-coated open tubular column.

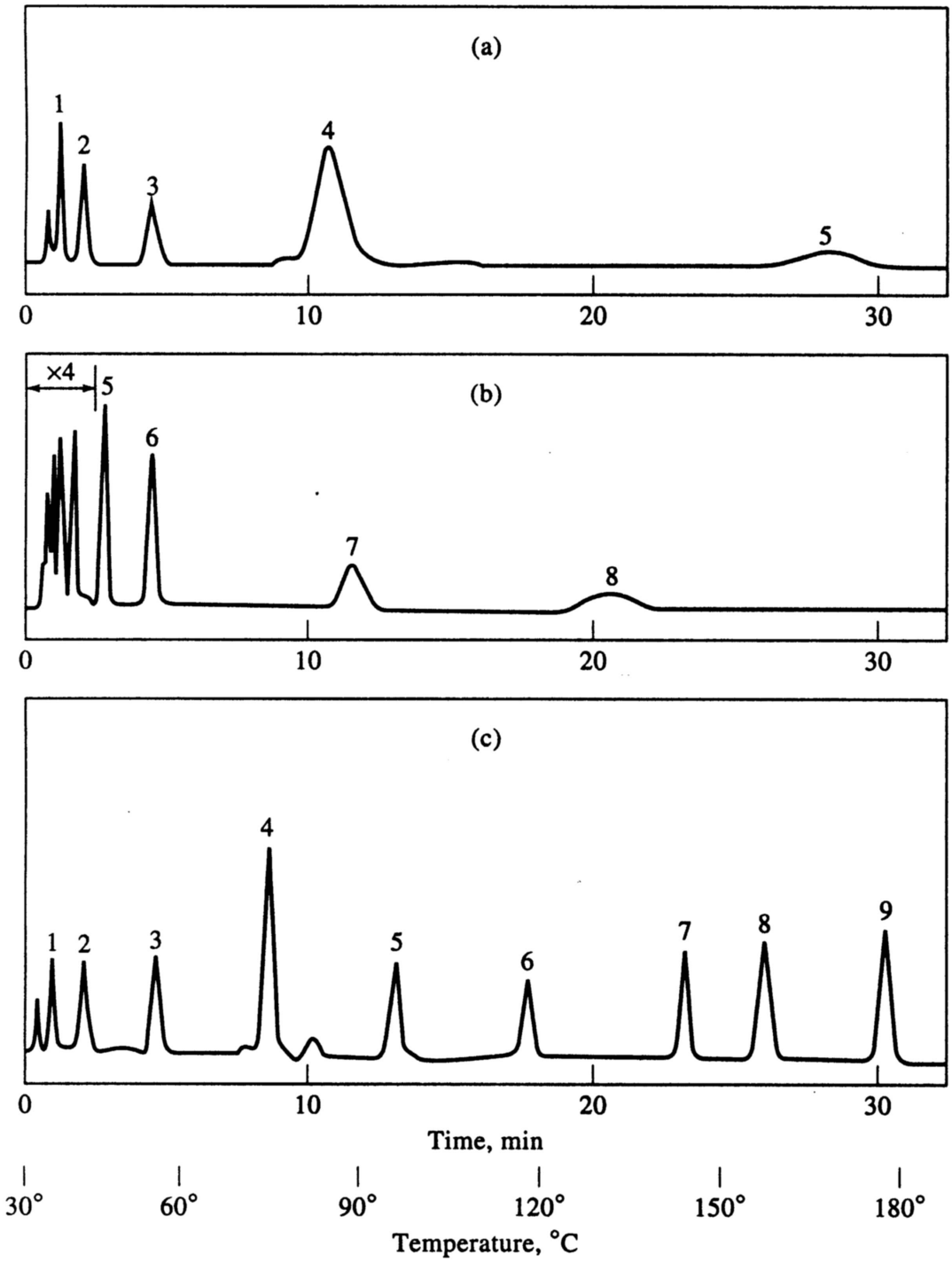


Figure 27-5 Effect of temperature on gas chromatograms: (a) isothermal at 45°C; (b) isothermal at 145°C; (c) programmed at 30° to 180°C. *(From W. E. Harris and H. W. Habgood,* Programmed Temperature Gas Chromatography, *p. 10. New York: Wiley, 1966. Reprinted by permission of John Wiley & Sons, Inc.)*

TABLE 27-2 Some Common Stationary Phases for Gas-Liquid Chromatography

Stationary Phase	Common Trade Name	Maximum Temperature, °C	Common Applications
Polydimethyl siloxane	OV-1, SE-30	350	General-purpose nonpolar phase; hydrocarbons; polynuclear aromatics; drugs; steroids; PCBs
Poly(phenylmethyldimethyl) siloxane (10% phenyl)	OV-3, SE-52	350	Fatty acid methyl esters; alkaloids; drugs; halogenated compounds
Poly(phenylmethyl) siloxane (50% phenyl)	OV-17	250	Drugs; steroids; pesticides; glycols
Poly(trifluoropropyldimethyl) siloxane	OV-210	200	Chlorinated aromatics; nitroaromatics; alkyl-substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids; alcohols; ethers; essential oils; glycols
Poly(dicyanoallyldimethyl) siloxane	OV-275	240	Polyunsaturated fatty acids; rosin acids; free acids; alcohols

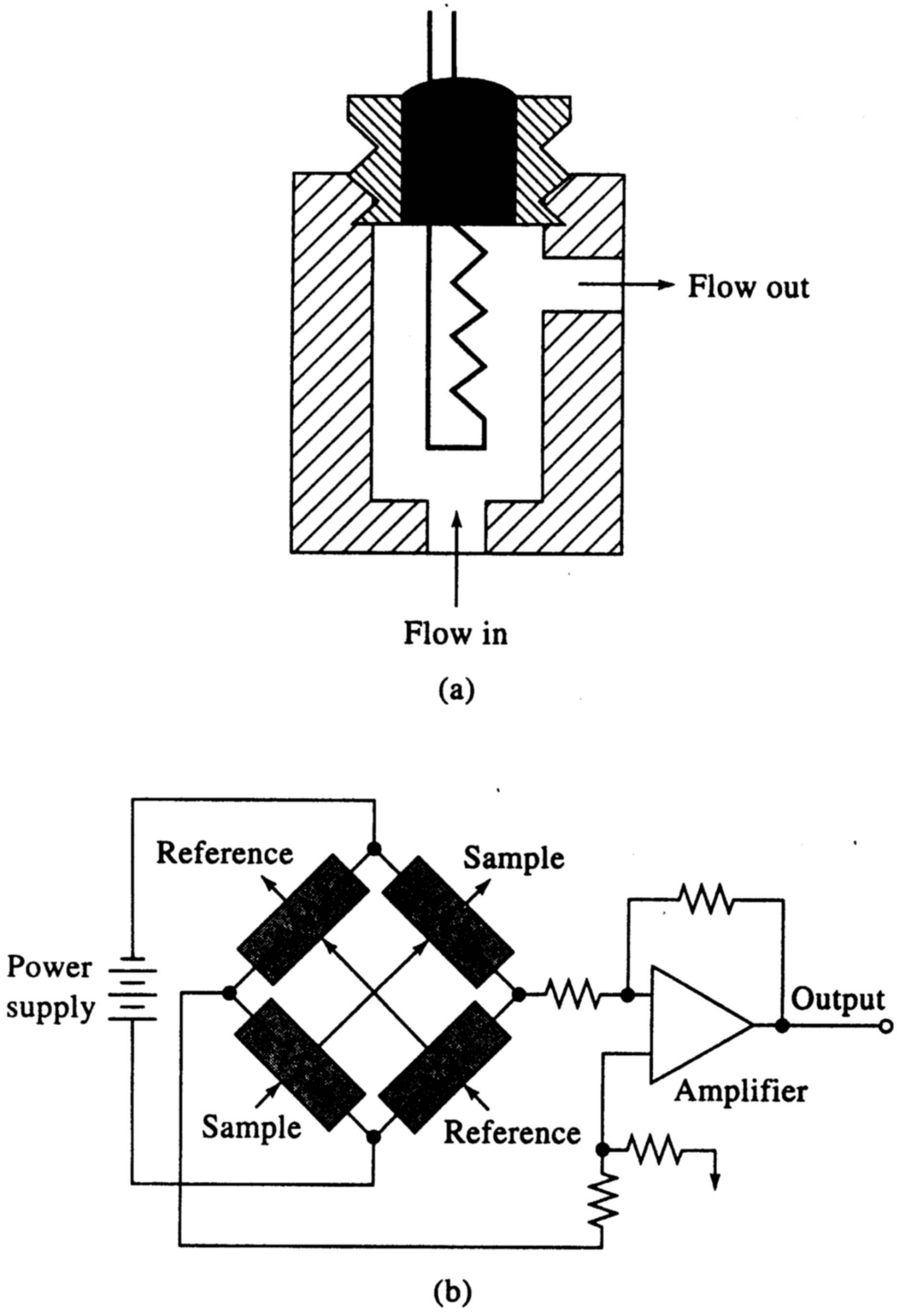


Figure 27-7 Schematic of (a) a thermal conductivity detector cell, and (b) an arrangement of two sample detector cells and two reference detector cells. *(From J. V. Hinshaw,* LC-GC, *1990, 8, 298. With permission.)*

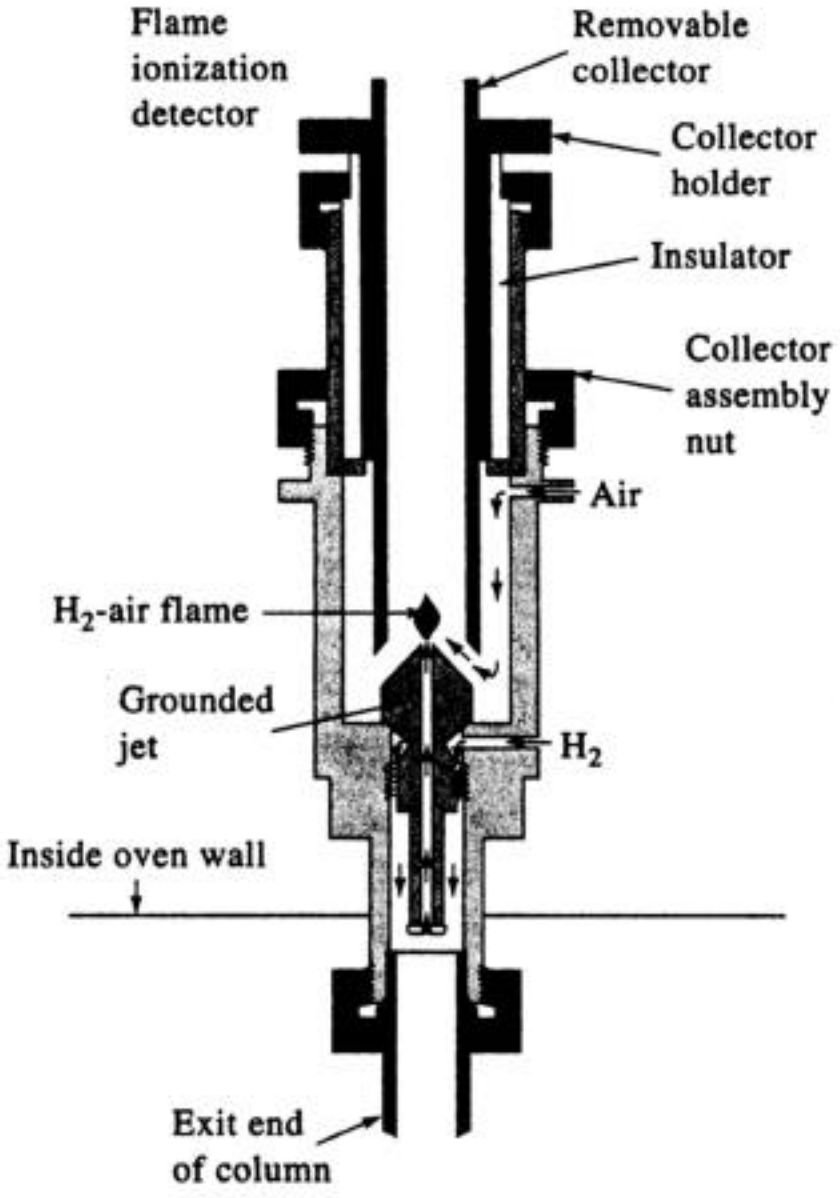


Figure 27-6 A typical flame ionization detector. *(Courtesy of Hewlett-Packard Company.)*

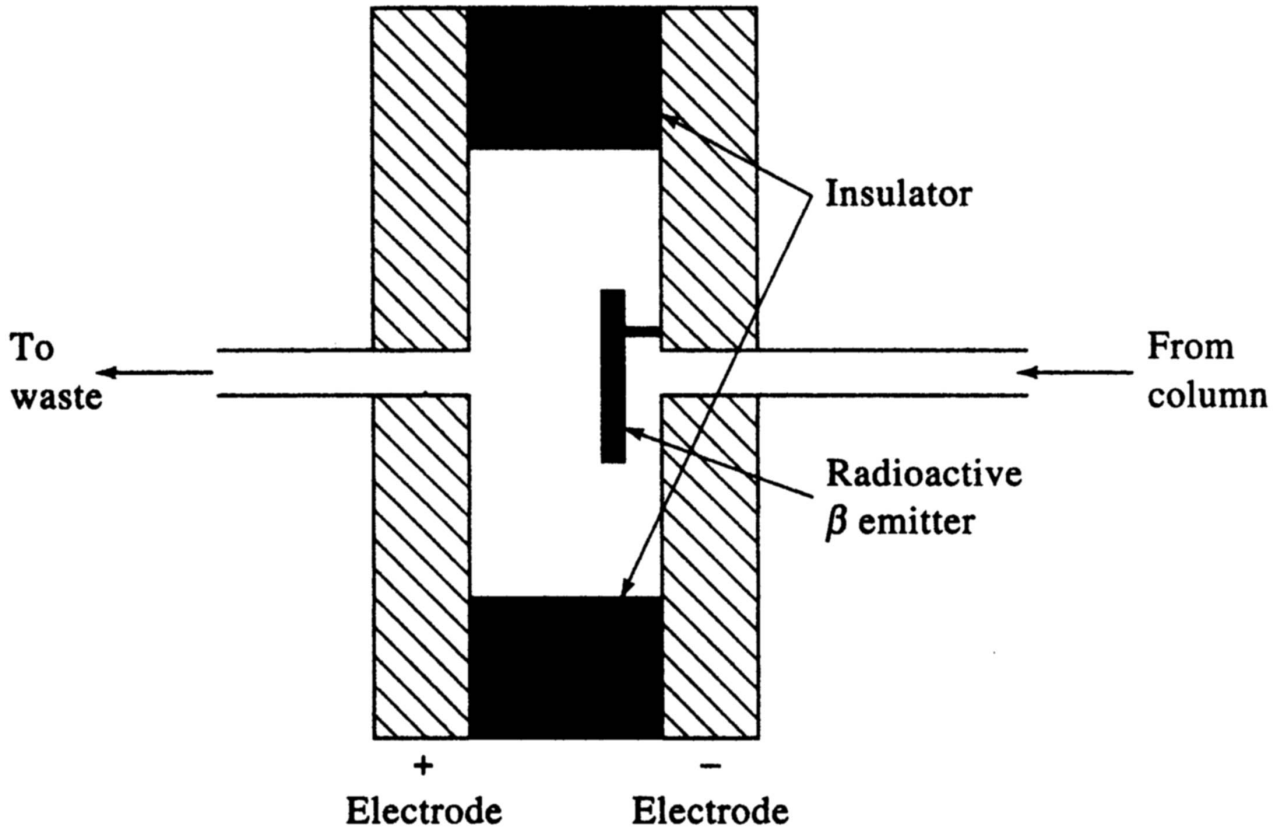


Figure 27-8 A schematic of an electron-capture detector.

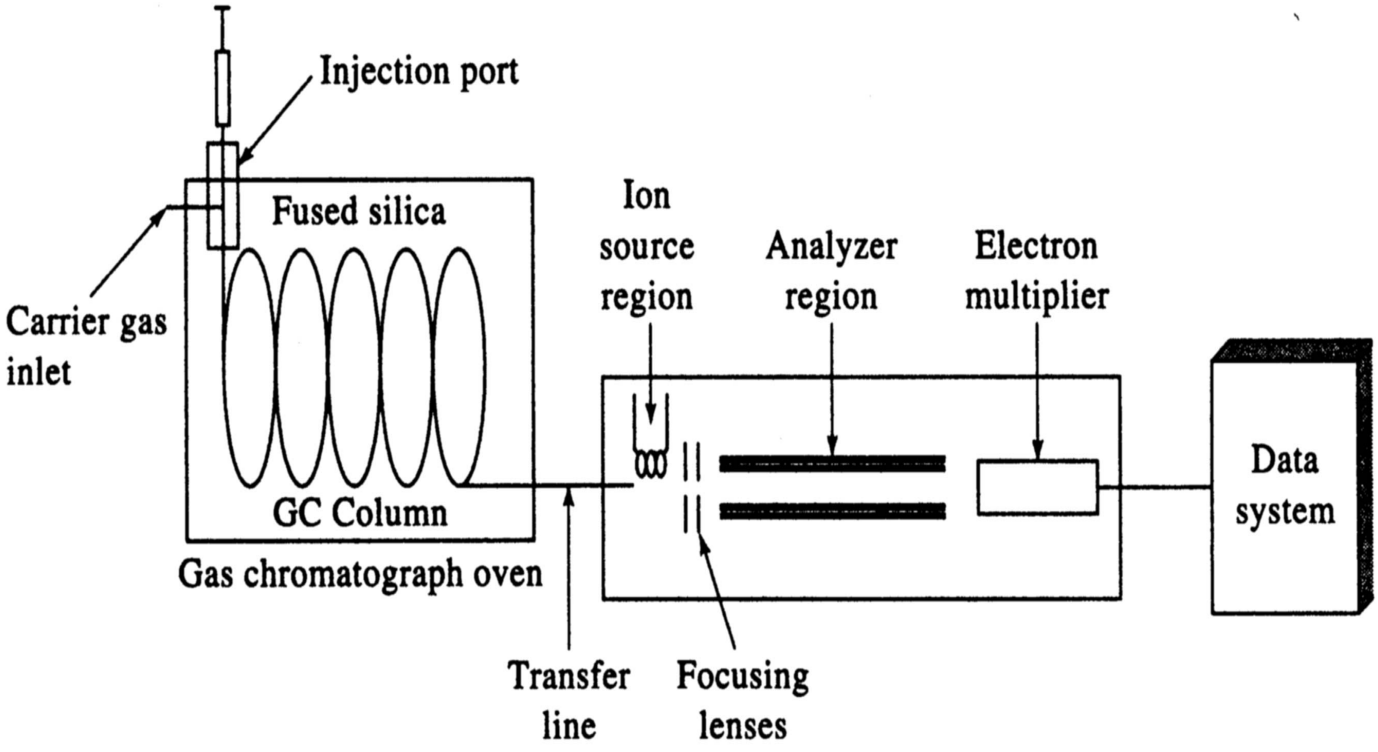


Figure 27-13 Schematic of a typical capillary gas chromatography/mass spectrometer.