Chapter 26: An Introduction to Chromatographic Separations

- Column Chromatography
- Migration Rates
  - Distribution Contstants
  - Retention Times
  - Selectivity Factor
- Zone Broadening & Column Efficiency
- Optimizing Performance
- Resolution

Intro to Chromatography

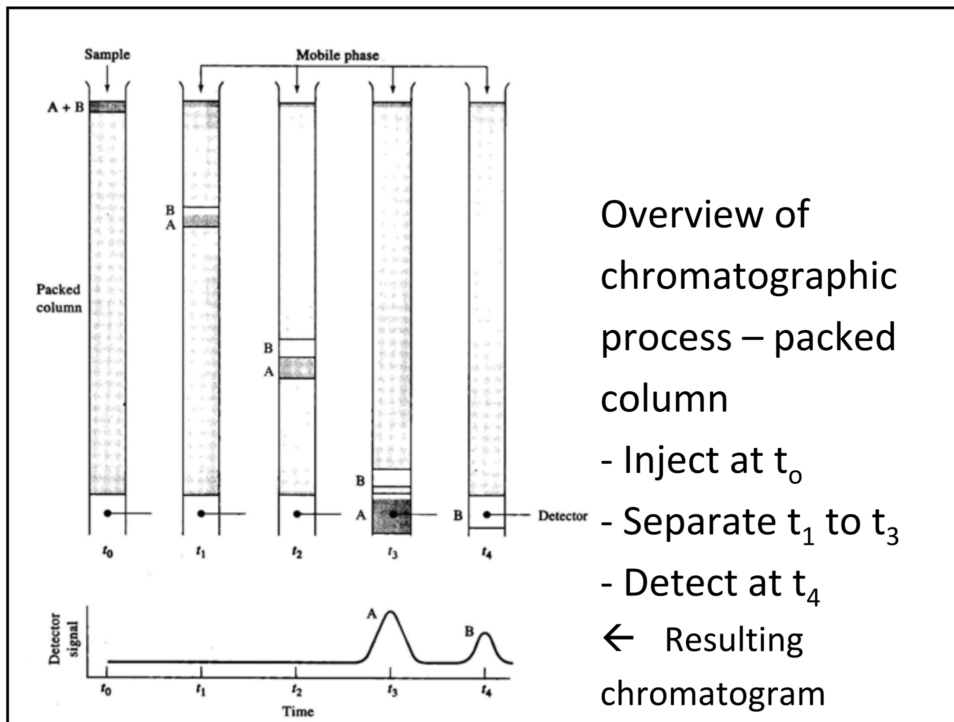
- Chromatography is a separation technique
- Many determinations involve separation followed by analysis
- Chromatography
- electrophoresis
- HPLC & GC are our primary focus
- Also discuss low pressure column chromatography & TLC (thin layer)
- All chromatographic techniques have
  - Stationary phase – solid or viscous liquid phase typically in a column
  - Mobile phase – moves sample in contact with stationary phase

TABLE 26-1 Classification of Column Chromatographic Methods

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Liquid chromatography (LC) (mobile phase: liquid)	Liquid-liquid, or partition	Liquid adsorbed on a solid	Partition between immiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Liquid-solid, or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
Gas chromatography (GC) (mobile phase: gas)	Gas-liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Gas-solid	Solid	Adsorption
Supercritical-fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

Chromatography:
sample transported by mobile phase
electrostatic or van der Waals'
some components in sample interact more strongly with stationary phase and are more strongly retained
sample separated into zones or bands

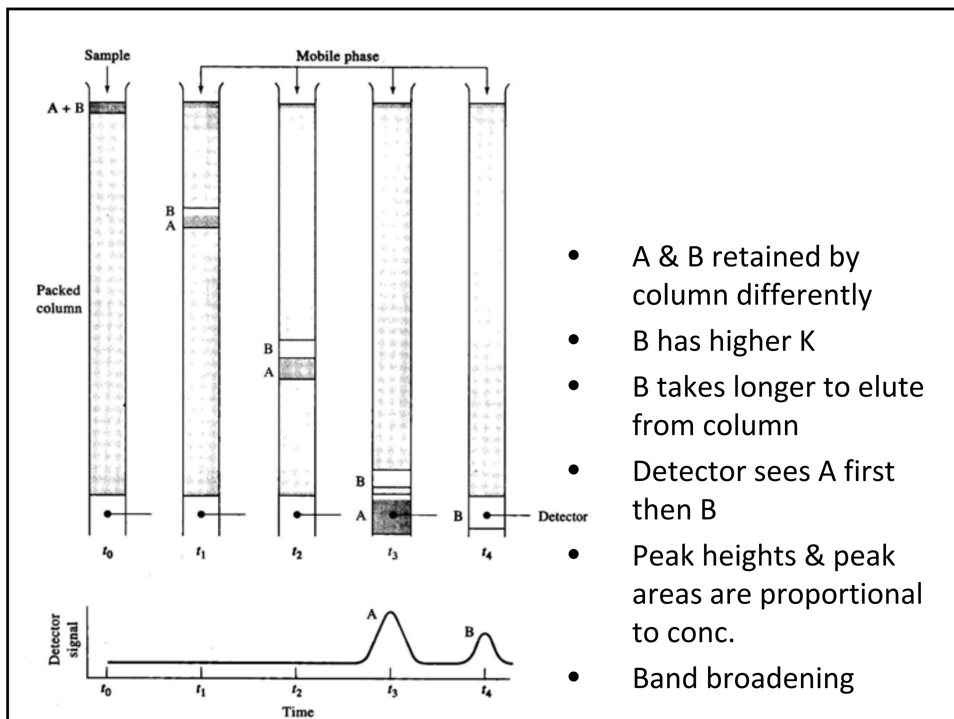
Elution Chromatography:
flushing of sample through column by continual mobile phase (eluent) addition
migration rate ∝ fraction time spent in mobile phase
Planar chromatography - flat stationary phase, mobile phase moves through capillary action or gravity
Column chromatography - tube of stationary phase, mobile phase moves by pressure or gravity



Overview of chromatographic process – packed column

- Inject at t_o
- Separate t_1 to t_3
- Detect at t_4

← Resulting chromatogram



- A & B retained by column differently
- B has higher K
- B takes longer to elute from column
- Detector sees A first then B
- Peak heights & peak areas are proportional to conc.
- Band broadening

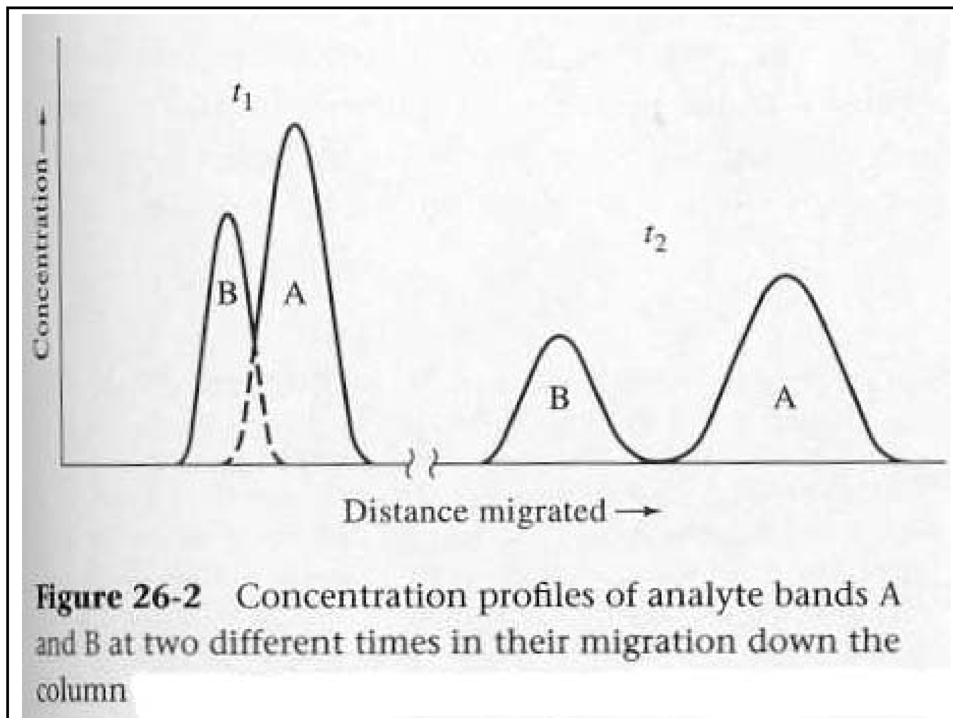
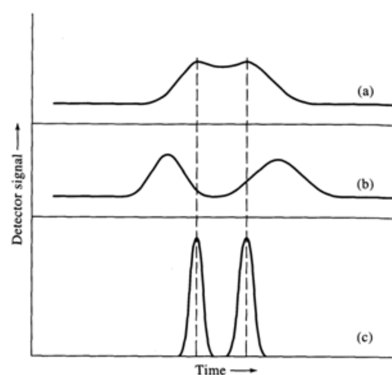


Figure 26-2 Concentration profiles of analyte bands A and B at two different times in their migration down the column

Important:
- chromatogram (concentration versus elution time)
- more strongly retained species elutes last (elution order)
- analyte is "diluted" during elution (dispersion)
- zone broadening proportional to elution time

By changing experimental conditions, non-separated bands can be separated

(A) adjust migration rates for A and B (increase band separation)
(B) adjust zone broadening (decrease band spread)



Partitioning = type of equilibrium where the analyte divides itself between two phases

For liquid-liquid extraction – two liquids

For chromatography – mobile vs. stationary phases

- Analyte A in equilibrium with two phases
- A_{mobile} $A_{stationary}$

Define a partition ratio K (or distribution constant)

$$K = \frac{C_s}{C_M}$$

where C_s & C_M are concentrations of analyte in stationary & mobile phases

- Prefer if K is constant over conc. range
- If not constant we can work in a narrow range where it is constant
- This is linear chromatography
- From now on everything is linear chromatography
- In linear chromatography a constant flow rate of mobile phase moves through column
- K is typically constant or nearly constant
- Elution = process by which analyte is flushed through the column by mobile phase (which could be a liquid or a gas)

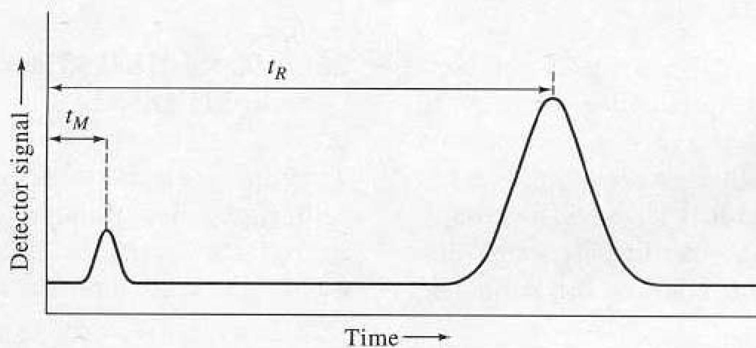


Figure 26-4 A typical chromatogram for a two-component mixture. The small peak on the left represents a species that is not retained on the column and so reaches the detector almost immediately after elution is started. Thus its retention time t_M is approximately equal to the time required for a molecule of the mobile phase to pass through the column.

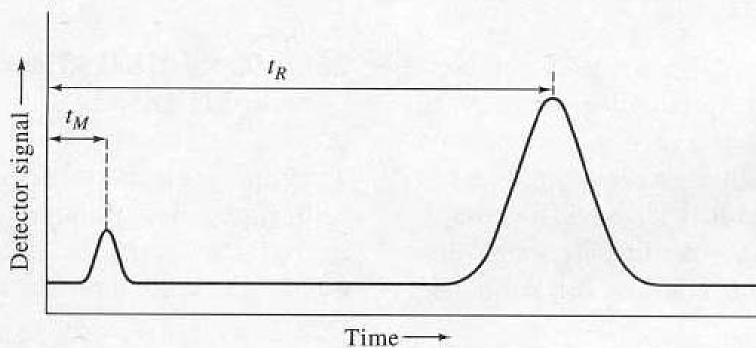
t_M = time for unretained molecule to reach detector or dead time
t_R = retention time, time for retained species to reach detector

Define $\bar{v}$ as average linear rate of <u>solute</u> migration & L as column length, then

$$\bar{v} = \frac{L}{t_R} \qquad \frac{\text{distance}}{\text{time}} = \text{velocity}$$

Similarly if define μ as average linear rate of movement of molecules of <u>mobile phase</u>

$$\mu = \frac{L}{t_M}$$



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tM = time for unretained molecule to reach detector or dead time
tR = retention time, time for retained species to reach detector

Relating retention time t_R to K (= C_s/C_M)

$\bar{\nu} = \mu$ x fraction of time analyte is in mobile phase

$$\bar{\nu} = \mu \text{ x } \frac{\text{moles of analyte in mobile phase}}{\text{number of moles of analyte}}$$

$$\bar{\nu} = \mu \text{ x } \frac{C_M V_M}{C_M V_M + C_s V_s} = \mu \text{ x } \frac{1}{1 + C_s V_s / C_M V_M}$$

Substituting $K = C_s/C_M$

Gives

$$\nu = \bar{\mu} \text{ x } \frac{1}{1 + K V_s/V_M}$$

More useful relationships - capacity factor **k'**

(comes from K) K in concentration, **k'** in moles

$$\mathbf{k'} = \frac{\text{amount of analyte in stationary phase}}{\text{amount of analyte in mobile phase}}$$

So for A → $k_A' = \frac{K_A V_s}{V_M} = \frac{n_s}{n_M}$ n = # of moles

From previous slide

$$\bar{\nu} = \mu \times \frac{1}{1 + K\, Vs/VM}$$

$$\bar{\nu} = \mu \times \frac{1}{1 + k_A'}$$

From previous equation → $\bar{\nu} = \mu \times \frac{1}{1 + k_A'}$

Can plug in $\nu = L/t_R$ & $\mu = L/t_M$

Rearrange and get $k_A' = \frac{t_R - t_M}{t_M}$

Now have k_A' in terms of something easily measured in chromatogram

Compares how long it takes a species to move through system compared to unretained species

Relative because ratio, Numerator = Net Retention

When k'A is ≤1.0, separation is poor
When k'A is >30, separation is slow
When k'A is 2-10, separation is optimum

One step further → Selectivity factor (α) describes differential migration

For two components

$$\alpha = \frac{K_B}{K_A} = \frac{k_B'}{k_A'}$$

And from chromatogram

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

Allows calculation of the resolving power of

a chromatographic system (i.e. column with A & B)

larger α = better separation

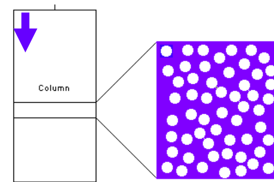
(B) Adjusting Zone Broadening:

- Individual molecule undergoes "random walk"
- Many thousands of adsorption/desorption processes
- Average time for each step with some +ve or -ve differences
- Add up to give Gaussian peak (like random errors)
- Breadth of band increases down column because morea time

- Zone broadening is affected by separation efficiency - more efficient, less broadening

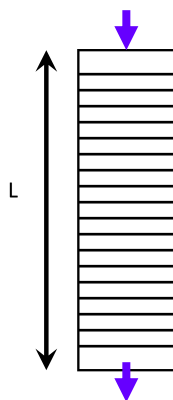
Chromatographic Plate Theory vs. Rate Theory

- Plate theory based in liquid-liquid extraction (successive extractions)
- $K = C_{org}/C_{water}$
- Chromatographic column can be thought of in the same way (only continuous process)
- $K = C_s/C_M$



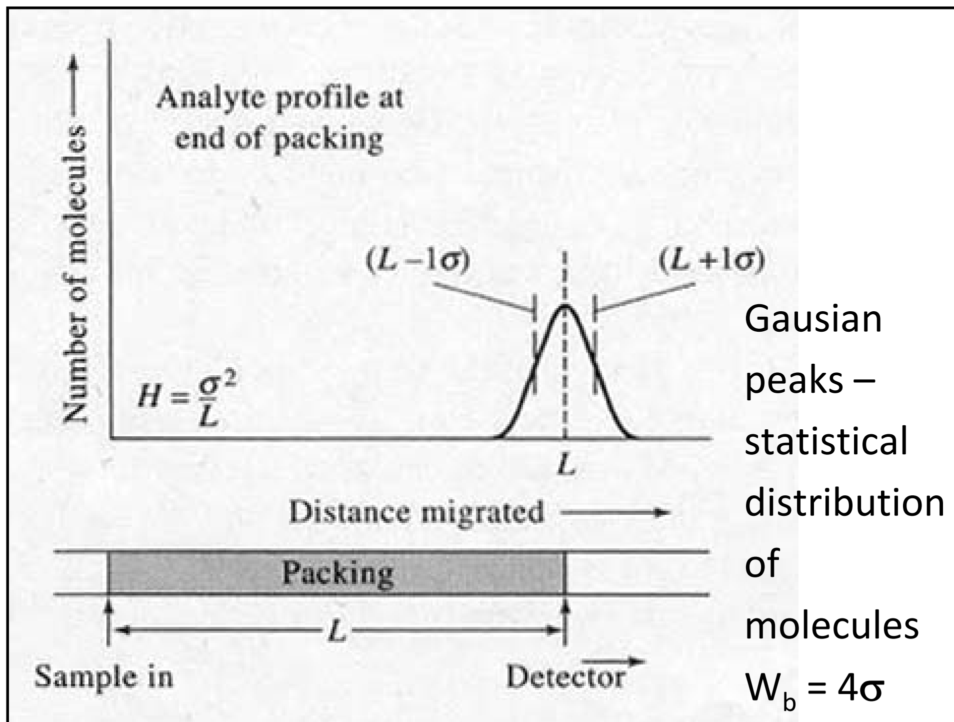
○ Stationary phase bead
■ Mobile phase (liquid)

○ Stationary phase bead
■ Mobile phase



- Divide chromatographic column up into steps or segments called theoretical plates
- The theoretical concept is that these theoretical plates are equilibrium units for $K = C_s/C_M$
- The more theoretical plates a column has, the more efficient it is
- If column length = L & N = number of plates, then H = height equivalent to theoretical plate

$L = NH$
or
$N = L/H$

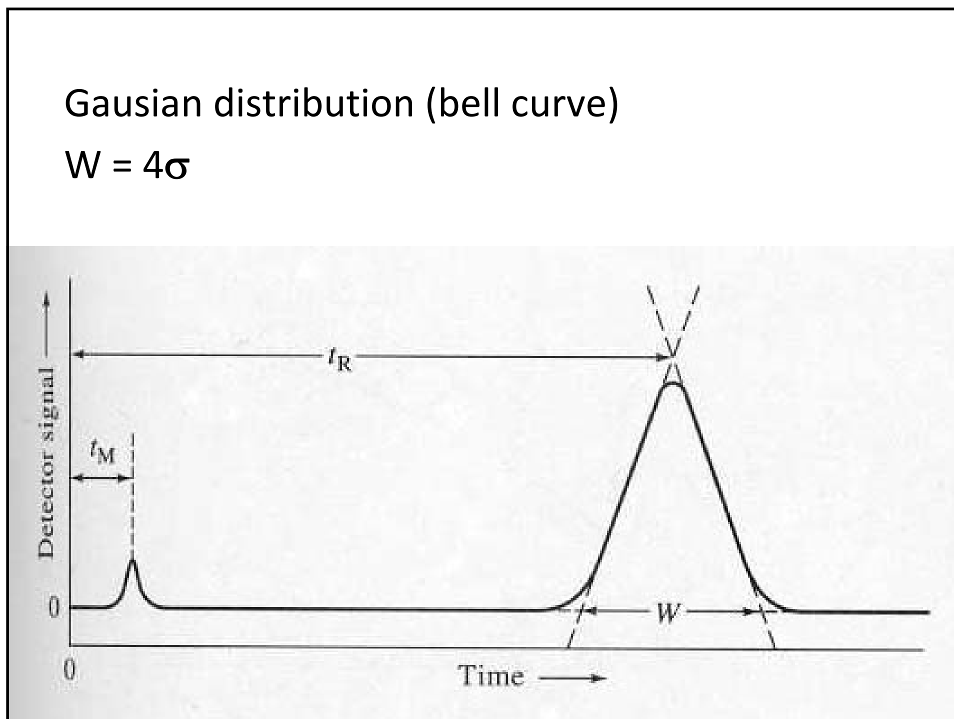


Gausian peaks – statistical distribution of molecules

$W_b = 4\sigma$

Gausian distribution (bell curve)

$W = 4\sigma$



Can derive N = number of plates

$N = 16 (t_R/W_b)^2$ W_b = base width

$N = 16 (t_R/4\sigma)^2 = (t_R/\sigma)^2$

$N = 5.54 (t_R/W_{½})^2$ $W_{½}$ = width at half height

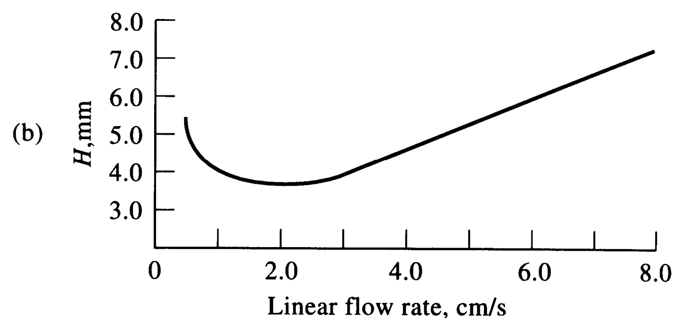
Column manufacturers use N to characterize column – N varies widely

Other Variables Affecting Peak Width (Zone Broadening):

Mobile Phase Velocity:

Higher mobile phase velocity, less time on column, less zone broadening

However, plate height H also changes with flow rate - plot of H versus u called van Deemter plot (Fig 26-8)



Rate Theory of Chromatography

$$H = H_L + H_S + H_M + H_{SM}$$

H = height equivalent to theoretical plate (as in Plate Theory)

H_L = contribution due to longitudinal diffusion

H_S = stationary phase mass transfer contribution

H_M = diffusion associated with mobile phase effects

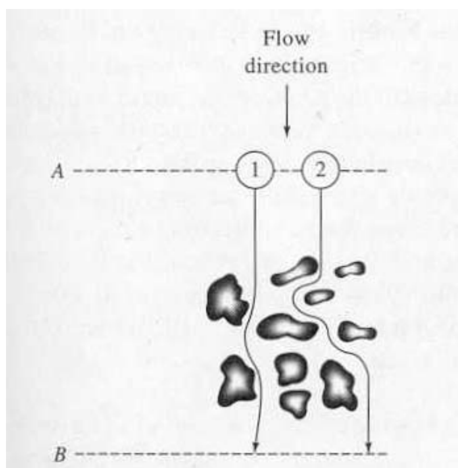
H_{SM} = diffusion into or mass transfer across a stagnant layer of mobile phase (neglect)

$$H = B/\mu + C\mu + A$$

van Deemter Equation A, B & C are coefficients, μ = velocity

1) Uneven Flow or Eddy Diffusion

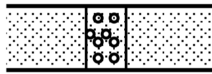
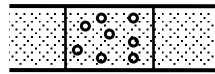
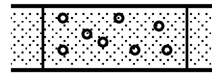
Path 1 is shorter than path 2 $H_M = A$

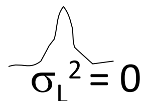
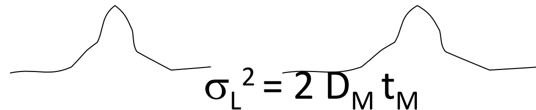


- Molecules move through different paths
- Larger difference in pathlengths for larger particles
- At low flow rates, diffusion allows particles to switch between paths quickly and reduces variation in transit time

2) Longitudinal Diffusion $H_L = (B/\mu)$

$t = 0$ $0 < t < t_R$ t_R

$\sigma_L^2 = 0$ $\sigma_L^2 = 2\,D_M\,t_M$

Variance due to longitudinal diffusion = 0 at start

Variance increases with time & diffusion coefficient D

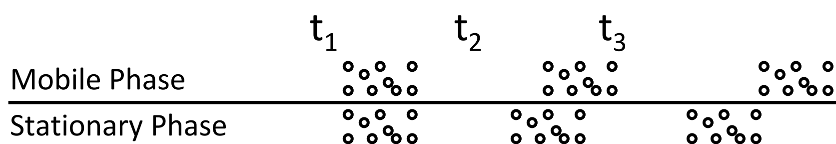
- Diffusion from zone (front and tail)
- Proportional to mobile phase diffusion coefficient
- Inversely proportional to flow rate - high flow, less time for diffusion

2) Mass transfer in & out of stationary phase

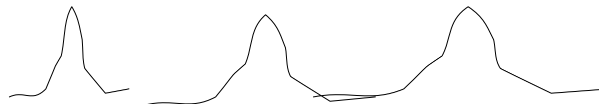
t_1 t_2 t_3

Mobile Phase

Stationary Phase



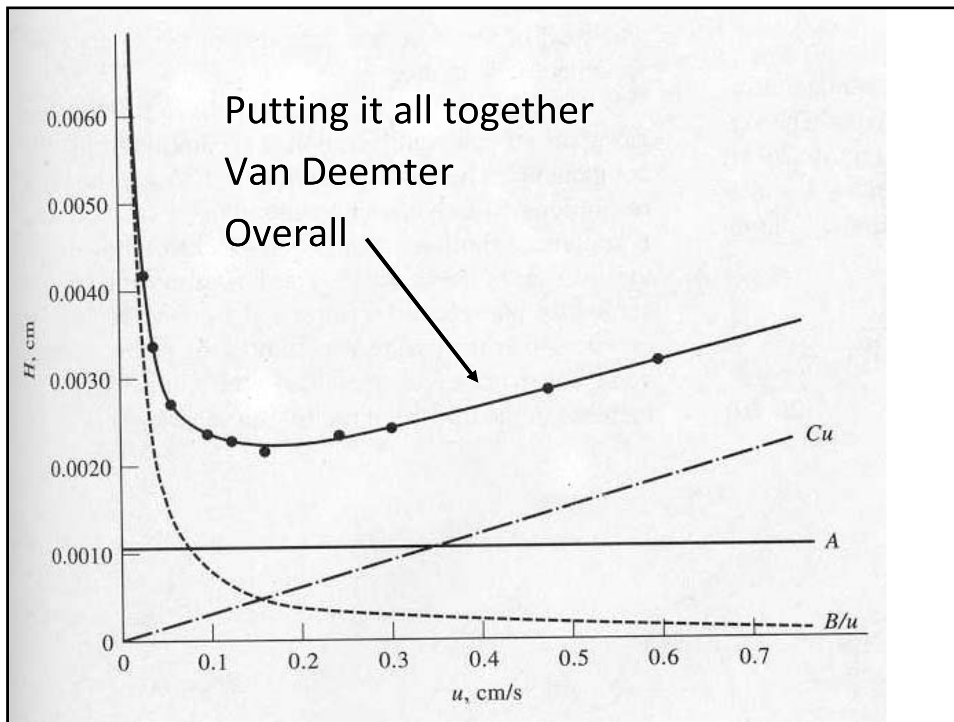
Resulting Peaks



Broadening of peaks is a function of mobile phase velocity (moving molecules faster than those in stationary phase)

Not the same as longitudinal diffusion $H_S = C\mu$

In Plate Theory condition at t_1 assumed to hold throughout



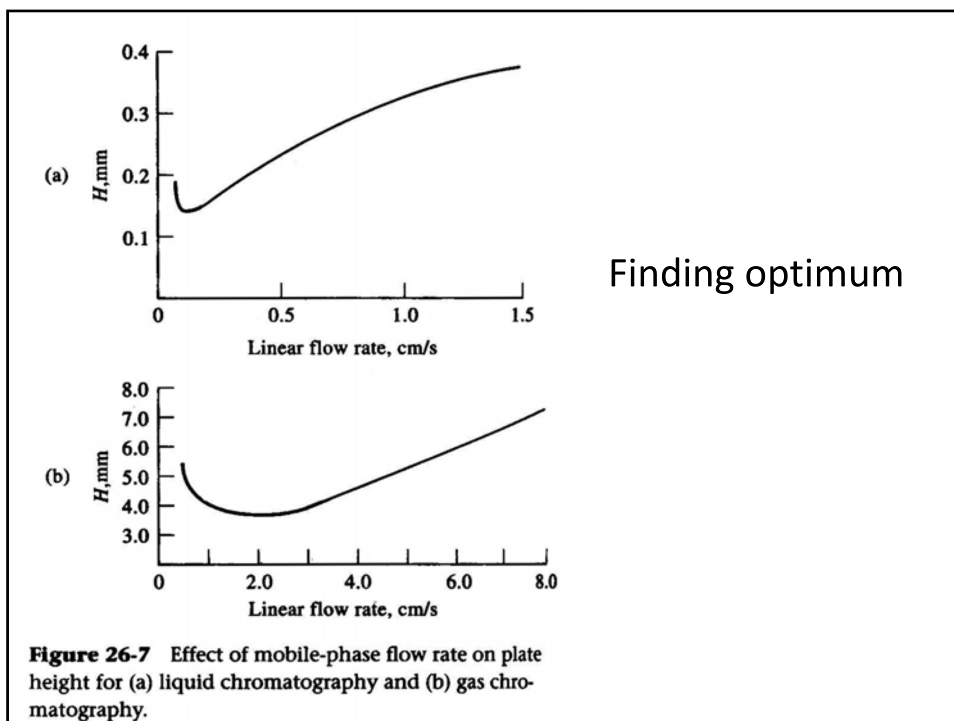


Figure 26-7 Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.

Optimizing Column Performance – seldom operate at optimum → too slow

Normally want to get required separation in shortest time, this may be at 2X μ_{opt}

Can optimize a separation by varying experimental conditions, usually goals are

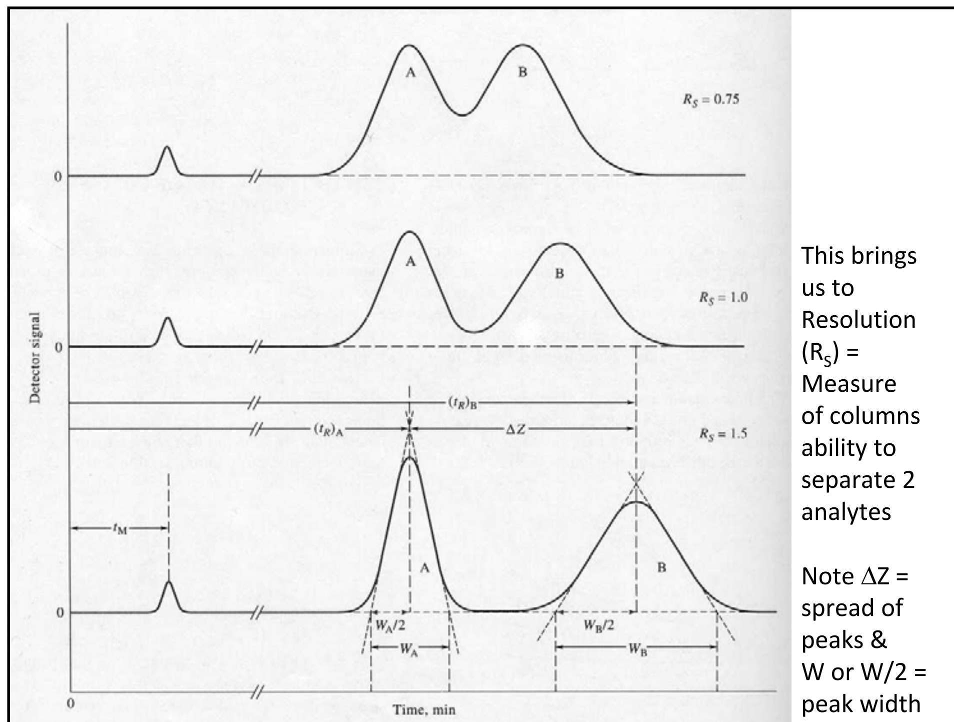
1) reduce band broadening (zone)

2) alter relative migration rates of components (allowing better separation of two components)

Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	$cm \cdot s^{-1}$
Diffusion coefficient in mobile phase	D_M	$cm^2 \cdot s^{-1}$
Diffusion coefficient in stationary phase	D_S	$cm^2 \cdot s^{-1}$
Retention factor (Equation 26-8)	k'	unitless
Diameter of packing particle	d_p	cm
Thickness of liquid coating on stationary phase	d_f	cm

TABLE 26-3 Kinetic Processes That Contribute to Peak Broadening

Process	Term in Equation 26-19	Relationship to Column* and Analyte Properties
Multiple flow paths	A	$A = 2\lambda d_P$
Longitudinal diffusion	B/u	$\frac{B}{u} = \frac{2\gamma D_M}{u}$
Mass transfer to and from liquid stationary phase	$C_S u$	$C_S u = \frac{f_S(k')d_f^2}{D_S} u$
Mass transfer in mobile phase	$C_M u$	$C_M u = \frac{f_M(k')d_p^2}{D_M} u$



This brings us to Resolution (R_S) = Measure of columns ability to separate 2 analytes

Note ΔZ = spread of peaks & W or W/2 = peak width

$$R_S = \frac{\Delta Z}{W_A/2 + W_B/2} = \frac{2\,\Delta Z}{W_A + W_B} = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

If $R_S = 1.0$ then $\Delta Z = W_A/2 + W_B/2$

and peaks touch with about 4% overlap

This is too big an error to tolerate

If $R_S = 1.5$ then about 0.3% overlap

Can lengthen column to improve resolution by increasing N → this also increases time for analysis

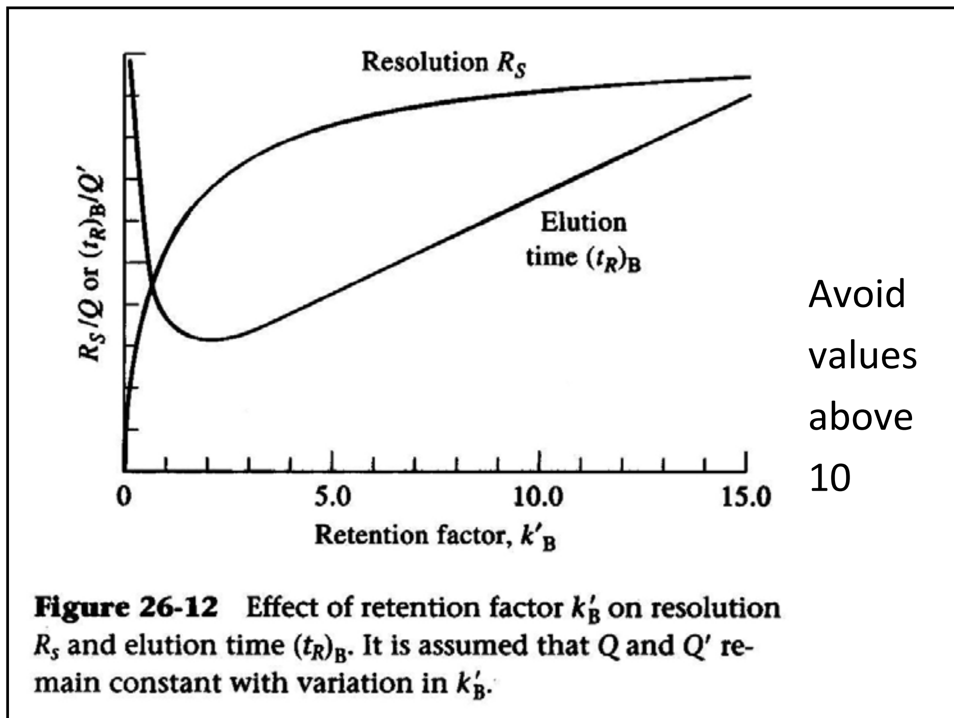
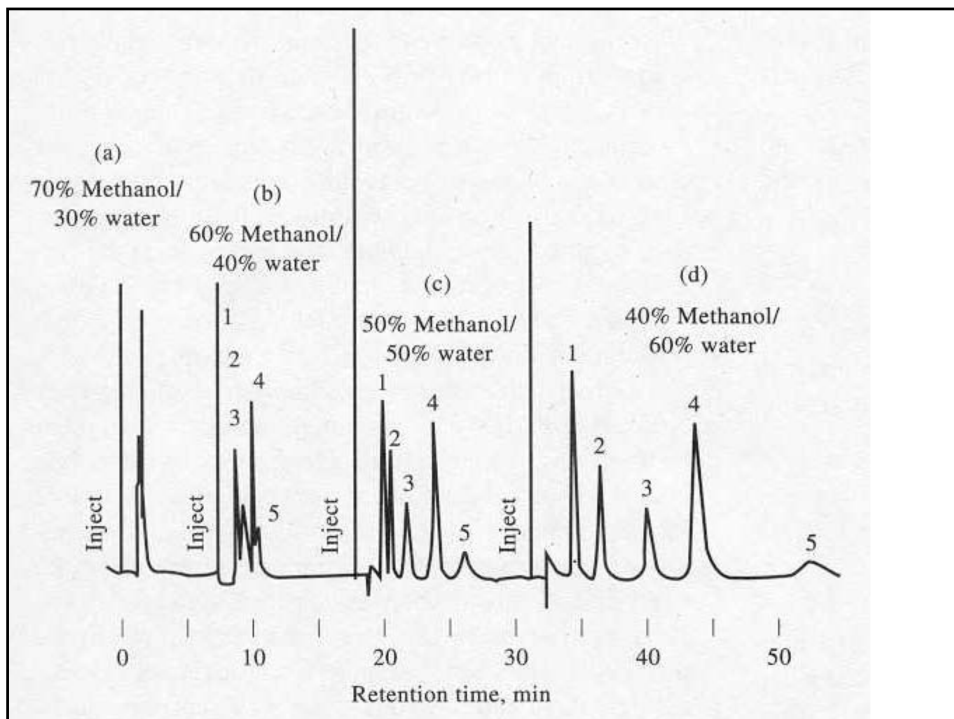


Figure 26-12 Effect of retention factor k'_B on resolution R_s and elution time $(t_R)_B$. It is assumed that Q and Q' remain constant with variation in k'_B.

Avoid values above 10



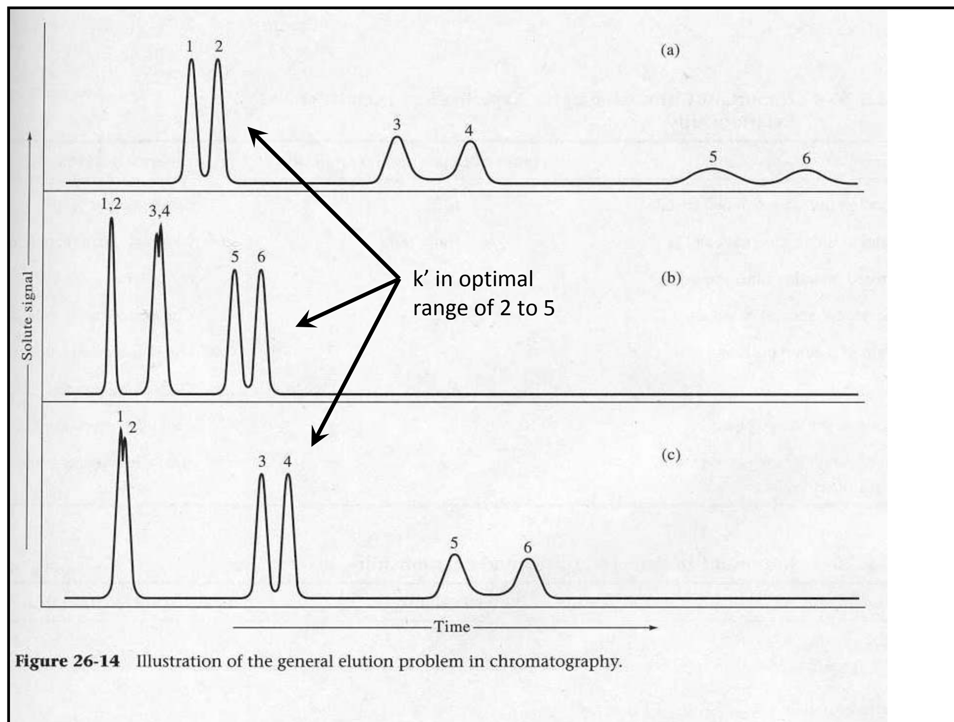


Figure 26-14 Illustration of the general elution problem in chromatography.

- Commonly found problem in chromatography
- General Elution Problem
- Solution – change conditions during chromatographic run so that k' changes
- Start with conditions for chromatogram (a), after 1 & 2 elute
- Change to conditions for chromatogram (c), after 3 & 4 elute
- Change to conditions for chromatogram (b) to get 5 & 6

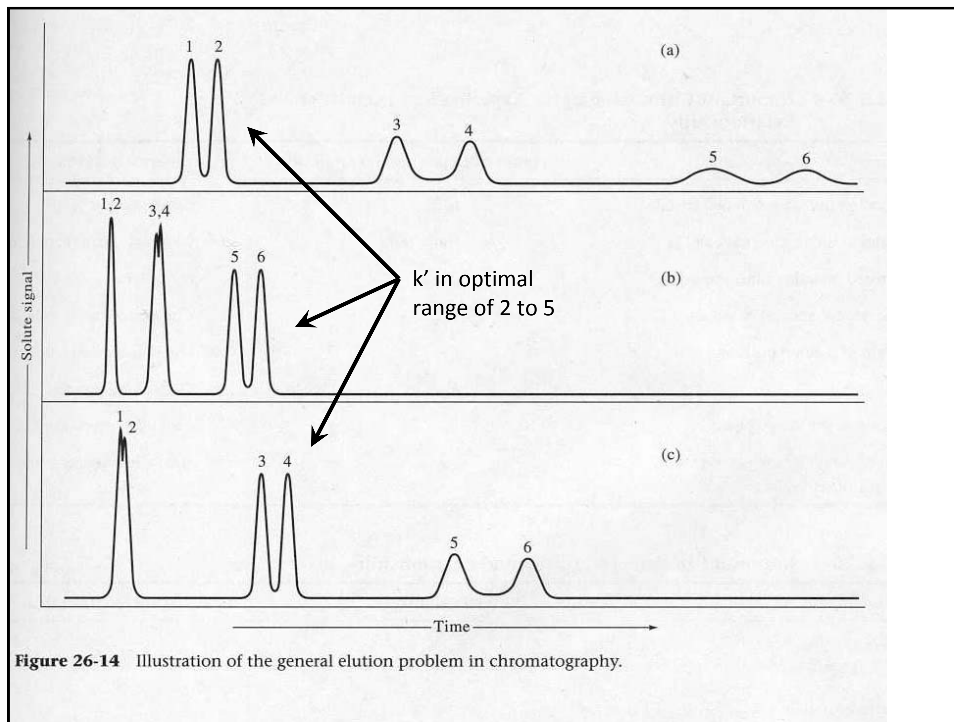


Figure 26-14 Illustration of the general elution problem in chromatography.

Since k’ is related to partitioning of solute between mobile phase and stationary phase, can easily change mobile phase

In GC do temperature programming

In HPLC do solvent programming (a.k.a. gradient elution)

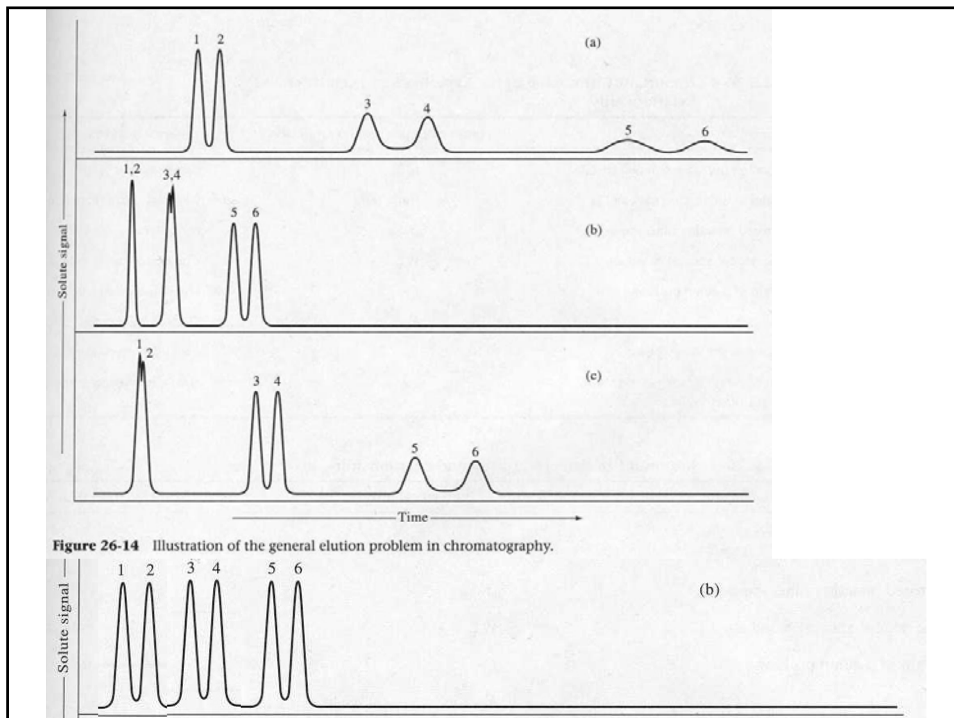


Figure 26-14 Illustration of the general elution problem in chromatography.

Homework

- 26-1
- 26-3
- 26-9
- 26-14

Chapter 27: Gas Chromatography

- Principles
- Instrumentation
- Detectors
- Columns and Stationary Phases
- Applications

Basic Principle of GC – sample vaporized by injection into a heated system, eluted through a column by inert gaseous mobile
phase and detected

Three types (or modes)

gas – solid chromatography early
(stationary phase: solid) ←
gas – liquid " ← important
(stationary phase: immobilized liquid)
gas – bonded phase " ← relatively new

An estimated 200,000 GC in use worldwide

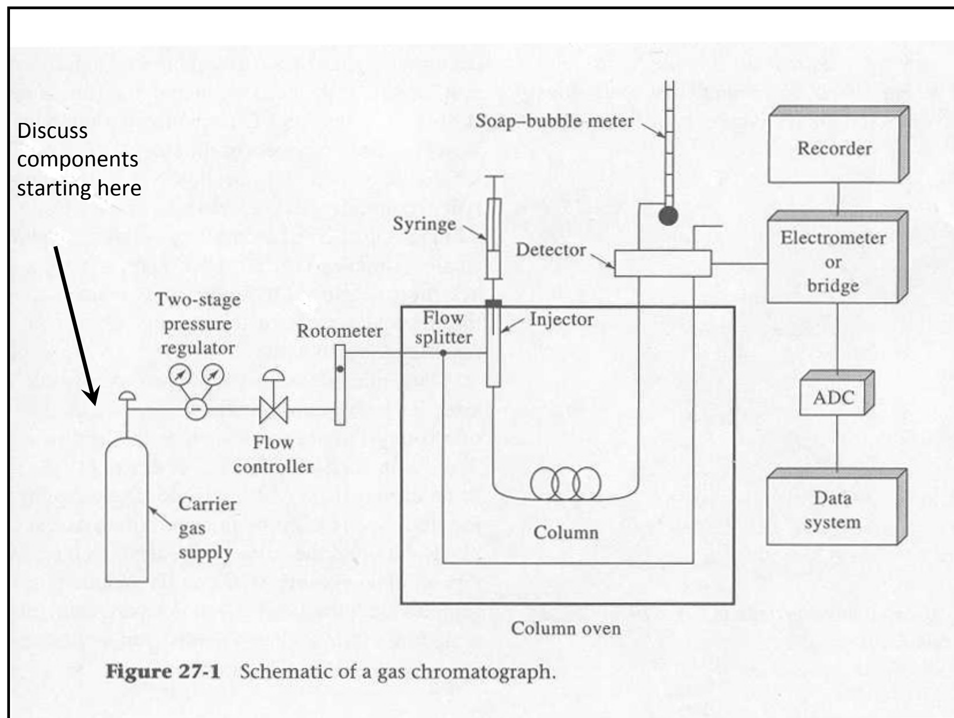


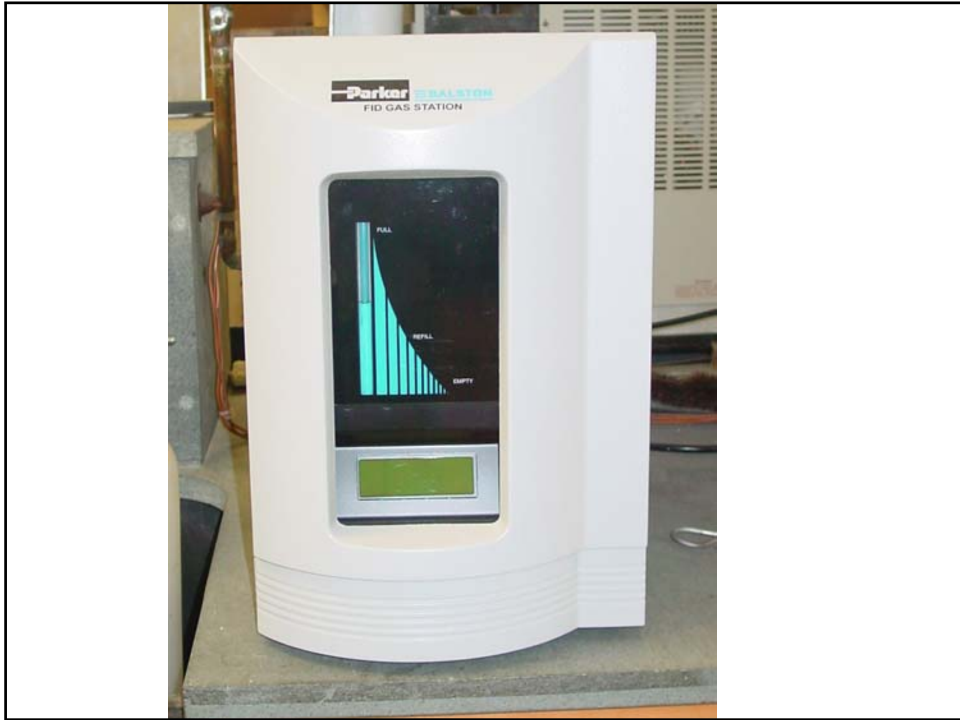
Figure 27-1 Schematic of a gas chromatograph.

Carrier gas: He (common), N_2, H_2
Pinlet 10-50 psi
F=25-150 mL/min packed column
F=1-25 mL/min open tubular column

Column: 2-50 m coiled stainless steel/glass/Teflon

Oven: 0-400 °C ~ average boiling point of sample
accurate to <1 °C

Detectors: FID, TCD, ECD, (MS)


Parker
BALSTON
FID GAS STATION
FULL
REFILL
EMPTY

SIGMA
Parker
BALSTON
HYDROGEN GENERATOR
FULL
REFILL
EMPTY
WATER LEVEL
OPERATING
CHECK SYSTEM

Carrier gases (mobile phase) – must be chemically inert He, Ar, N_2, CO_2 even H_2 and mixtures 95/5 N_2/CH_4

Often detector dictates choice of carrier gas

In GC sample doesn't really interact with carrier gas (unlike HPLC), temp controls partitioning

Often necessary to purify cylinder gas with a trap, scrubber or cartridge of molecular sieves (or buy high purity gas) O_2 ppm Hc

The move today is away from gas cylinders toward gas generators (extract pure carrier gas from air)

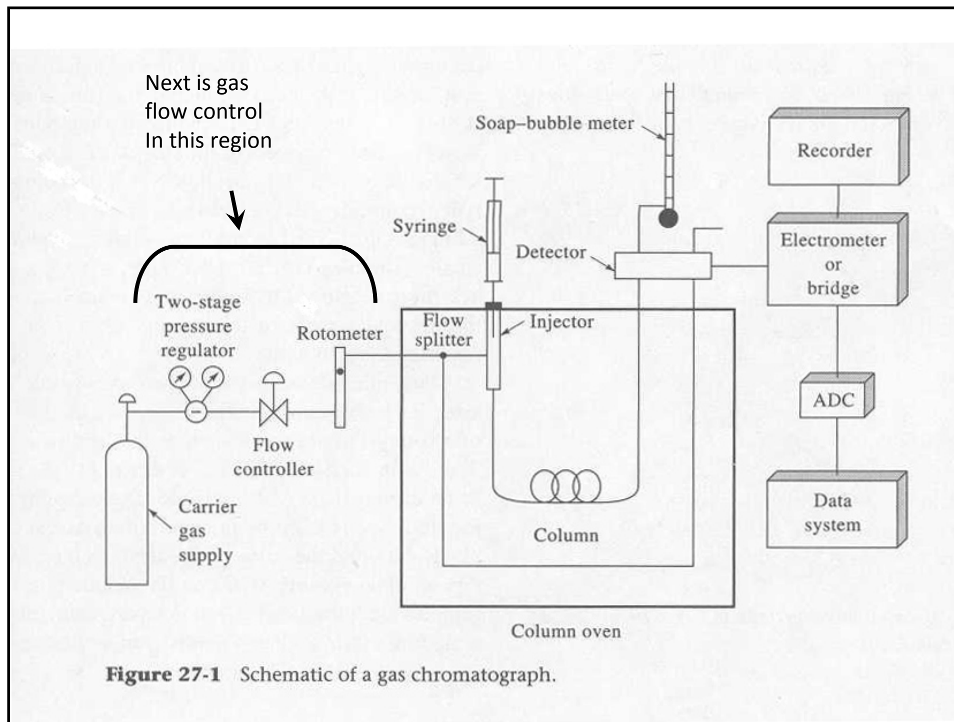


Figure 27-1 Schematic of a gas chromatograph.

Flow control – 10 to 50 psi with regulator

Regulators vary in quality, material & control, typically use a 2 stage regulator with the best material being stainless steel

Ultimately flow rate is checked by a soap bubble meter for accurate flow

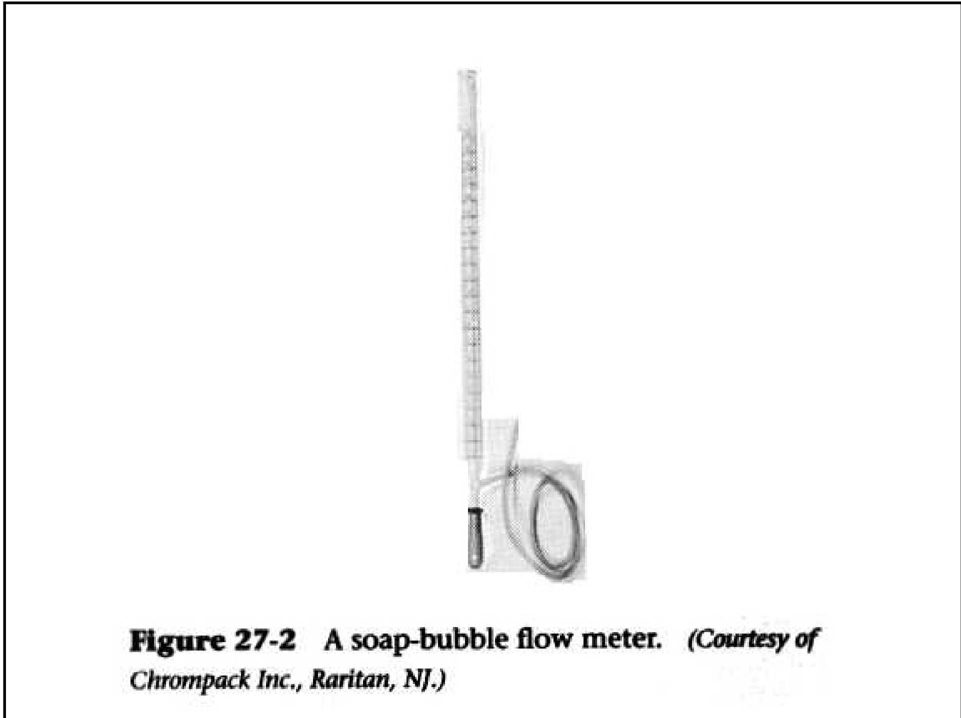


Figure 27-2 A soap-bubble flow meter. *(Courtesy of Chrompack Inc., Raritan, NJ.)*

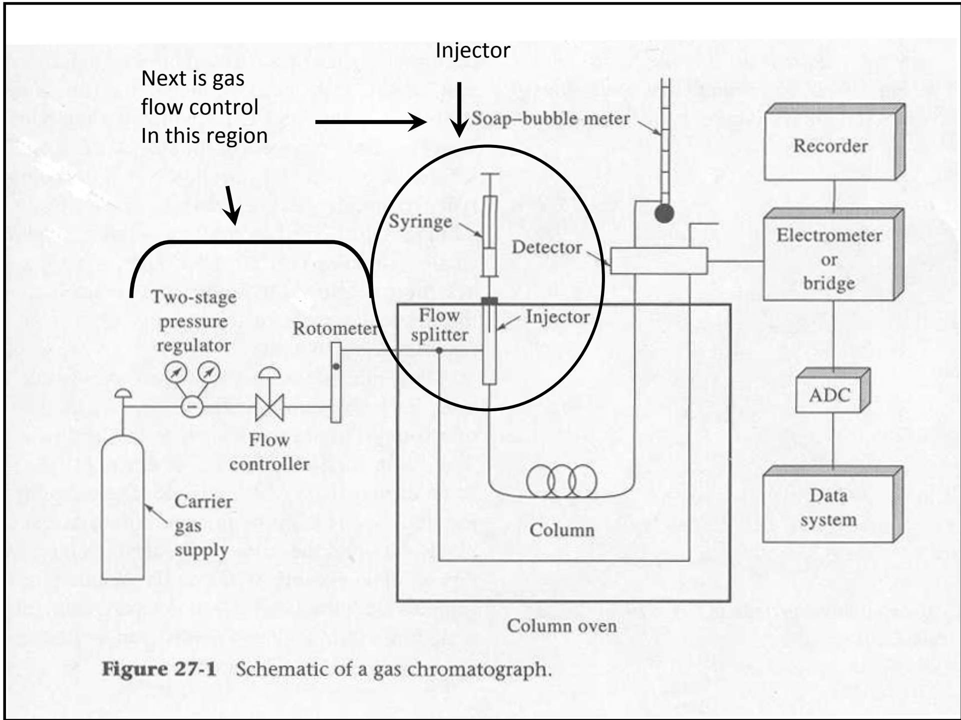


Figure 27-1 Schematic of a gas chromatograph.

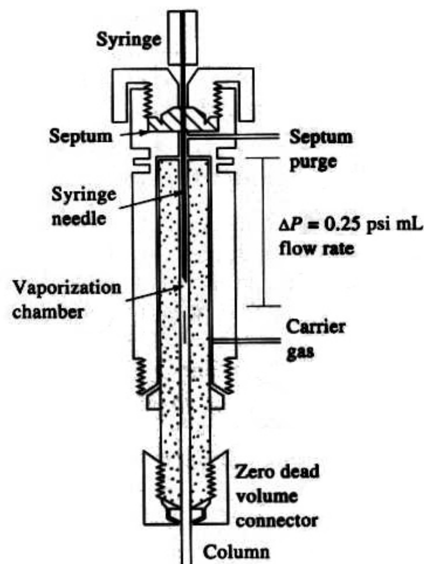


Figure 27-3 Cross-sectional view of a microflash vaporizer direct injector.

Injector – use micro syringe 99.9 % of the time injecting 1 to 20 μL, rapidly shoot in plug of sample

Old GCs had separate injection area

Today use on-column & microflash vaporizers – all have septum of synthetic rubber which is punctured by syringe

Injector usually 50 ^{o}C hotter than boiling point of sample – also hotter than column

Can use rotary injector valve (as for HPLC)

Rotary Injection Valve

Common for HPLC, rare in GC

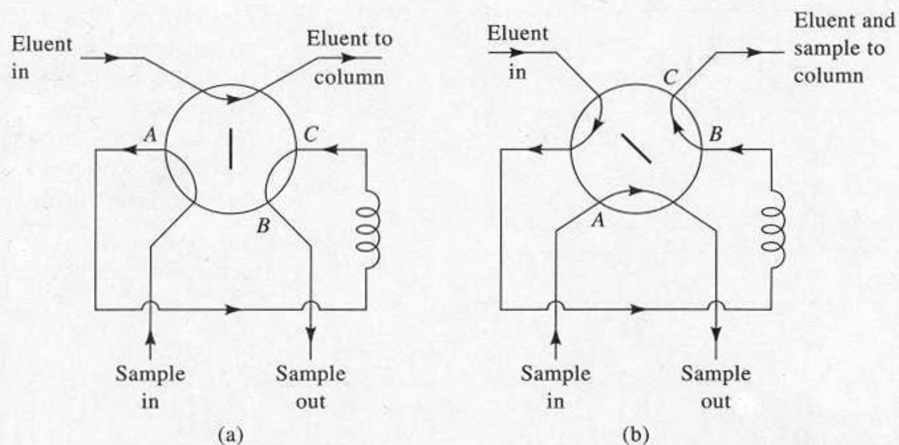


Figure 27-4 A rotary sample valve: valve position (a) for filling sample loop *ACB* and (b) for introduction of sample into column.

Split injection: routine method
0.1-1 % sample to column
remainder to waste

Splitless injection: all sample to column
best for quantitative analysis
only for trace analysis, low [sample]

On-column injection: for samples that decompose above boiling point - no heated injection port
column at low temperature to condense sample in narrow band
heating of column starts chromatography

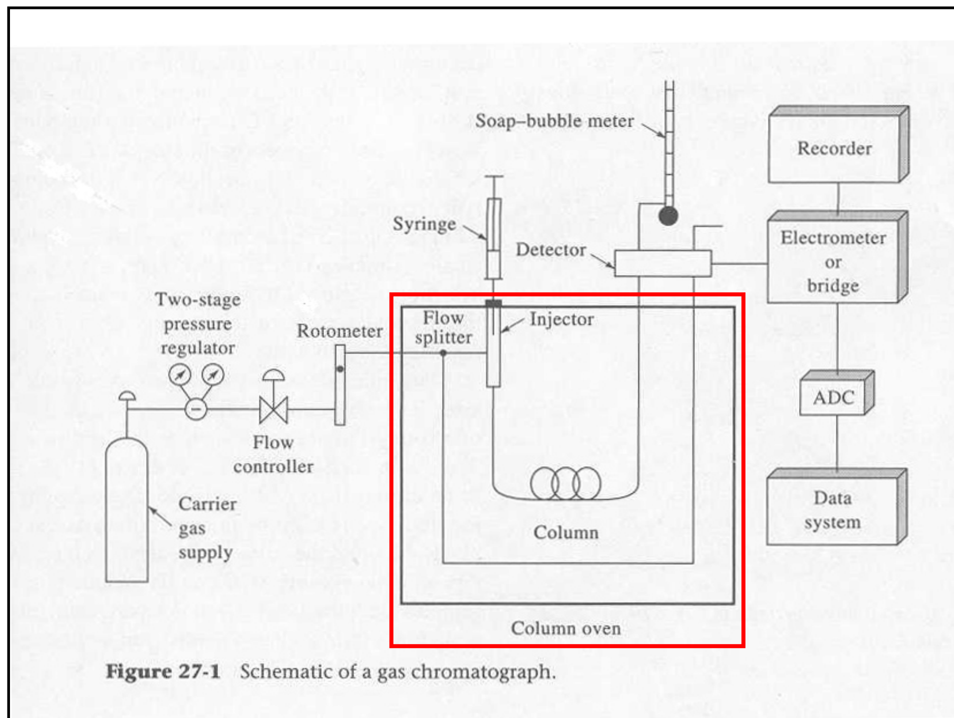


Figure 27-1 Schematic of a gas chromatograph.

Column housed in Column Oven to maintain temperature

Types – packed, open tubular, capillary

oldest ------------------ newest

Capillary columns will take over completely

Packed – tube (steel, glass, **fused silica**, Teflon) packed with material

Open Tubular – coated on walls

Capillary – coated on walls, long & narrow

Length range – 2 to 50 m (typically 30 m)

Column Concepts

In GC since mobile phase is under pressure & we operate at various temperatures

given that P V is proportional to T

Sometimes use retention volumes (V_R, V_M)

$V_R = t_R F$ for retained species t_R = retention time

$V_M = t_M F$ for unretained t_M = retention time

F = flow rate

Problem - pressure drop across a column

Pressure at head of column may be 5 atm & at end of column may be 1 atm

Need a correction factor

$$j = \frac{3[(P_i/P)^2 - 1]}{2[(P_i/P)^3 - 1]}$$

Where P_i = inlet pressure &

P = outlet pressure (atmospheric)

Detectors – dozens of detectors available

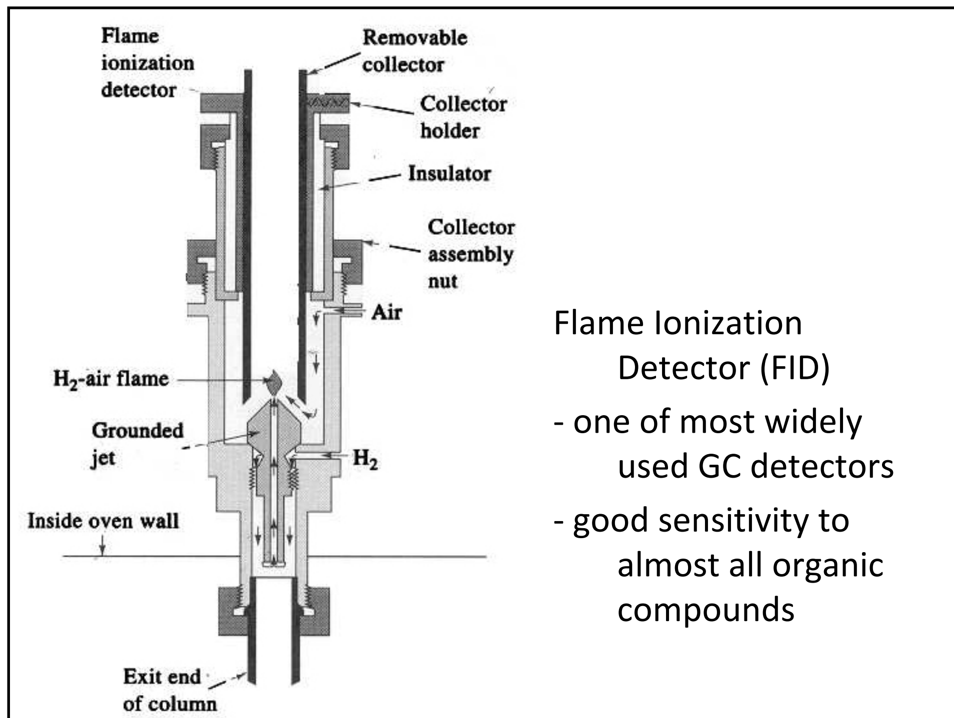
Characteristics of an ideal detector:

1) Adequate sensitivity for desired analysis (typical 10^{-8} to 10^{-15} g analyte/sec)
2) Stable – background constant with time
3) Reproducible – good precision
4) Linear response over several orders of magnitude
5) Temperature range – room temp - 400 ^{o}C

Characteristics of ideal detector: (continued)

6) Rapid response time
7) Independent of flow rate
8) Reliable
9) Easy to Use – inexperienced operators
10) Either selective or universal response
11) Nondestructive

No detector exhibits all these characteristics



Flame Ionization Detector (FID)

- one of most widely used GC detectors
- good sensitivity to almost all organic compounds

FID Basics

- column effluent mixed with air and burned in H_2 flame producing ions & electrons that conduct electricity
- a few hundred volts applied between burner tip & a collector electrode above the flame producing currents on the order of 10^{-12} amps
- amplify & measure
- signal approximately proportional to number of reduced carbon atoms in flame

FID Basics (continued)

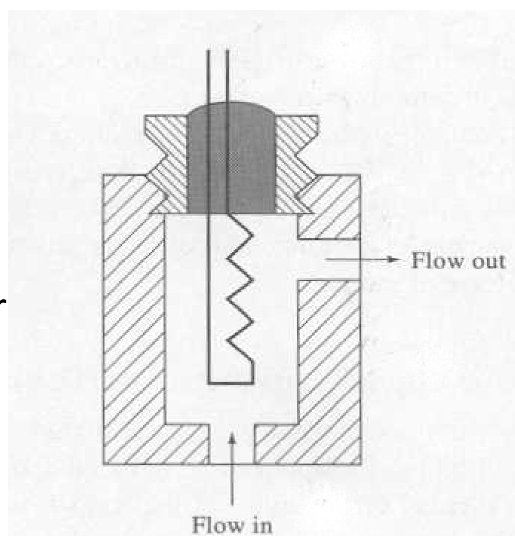
- mass sensitive rather than concentration
- insensitive to non combustible gases – H_2O, CO_2, SO_2, NO_x

FID exhibits

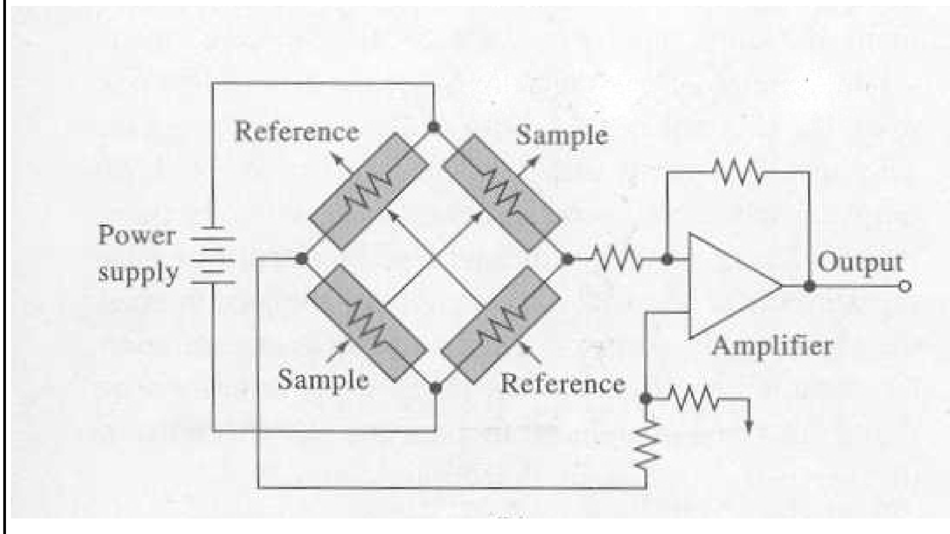
- High sensitivity (as low as 10^{-13} g/s)
- Large linear response range (10^7)
- Easy to use
- Rugged
- DESTRUCTIVE

Thermal Conductivity Detector (TCD)

- One of earliest GC detectors
- Not popular today
- Low sensitivity
- Several designs
- Use heated wire or semiconductor
- Resistance of wire changes with analyte vs carrier



TCD uses bridge circuit with
Sample & Reference Cells



TCD

- New TCDs use pulsed current to increase sensitivity & reduce drift
- Thermal conductivity of He & H_2 are about 6 to 10 times greater than most organic compounds (must use these carrier gases)
- Other carrier gases (N_2, Ar, etc) have thermal conductivities too close to organics

Advantages of TCD

- Simple → Reliable & Easy to use
- Universal response (organic & inorganic)
- Large linear dynamic range 10^5
- Nondestructive, can use in tandem
- Older instruments have built-in TCD

Disadvantages

- Low sensitivity
- Often can't use with capillary columns because amount of analyte is small

ECD

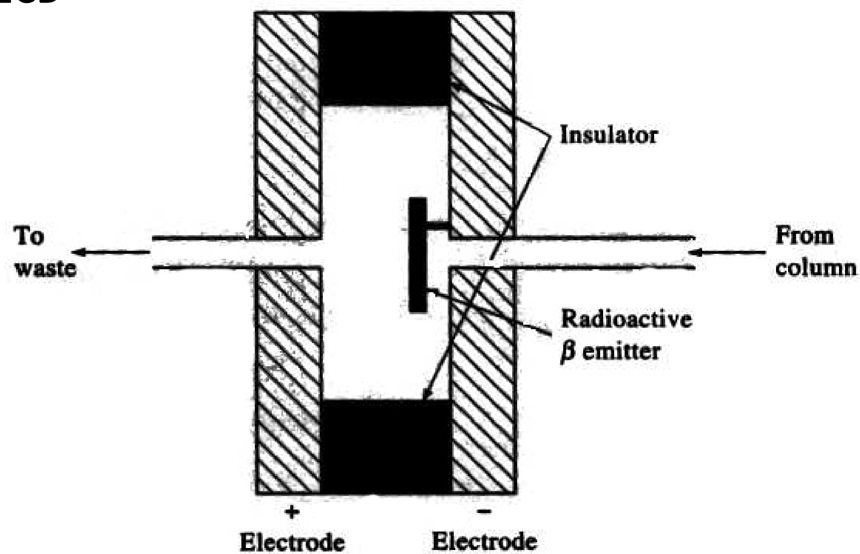


Figure 27-8 A schematic of an electron-capture detector.

Electron Capture Detector

- Sample passes over β emitter (radioactive) like ^{63}Ni foil or $^{3}H_2$ adsorbed on Pt or Ti foil
- β particles (i.e. electrons) hit carrier gas (usually N_2) causing a burst of e^- to be released & measured by electrode = standing current or constant signal
- When analyte molecule that absorbs e^- passes through, current is reduced = signal
- Response is non-linear unless pulsed

ECD Advantages

- Responds well to molecules with electronegative atoms like halogens (F, Cl, Br, I), peroxides, quinones, & nitro groups
- Insensitive to amines, alcohols, hydrocarbons
- Chlorinated pesticides are big application
- Highly sensitive
- Easy to use
- Pretty reliable, although foil can get coated
- Selective

ECD Disadvantages

- Narrow linear range
- Radioactive
- Regular wipe test
- Bake out contaminants
- Some limits to applicability because highly selective

Other Conventional Detectors

Thermionic Detector (TID)

- Selective for N & P compounds
- 500 x more sensitive than FID for P
- 50 x more sensitive than FID for N
- Bad for C
- Design similar to FID with rubidium silicate bead at 180 V vs collector → get hot plasma 600 - 800 ^{o}C
- Produces large number of ions with N & P

Flame Photometric Detector (FPD)

- Selective for P & S compounds
- Again sample goes through H_2/air flame
- Observe optical emission of HPO at 510 nm & 526 nm & S_2 at 394 nm
- Use optical filters to isolate signal
- Can also measure halogens, N, some metals (e.g. Cr, Ge, Se)

Photoionization Detector (PID)

- Column effluent irradiated with intense UV light source
- Ionizes molecules
- Measure ions with electrodes in detector cell

Unconventional Detectors

(Hyphenated Techniques)

Atomic Emission Detector (AED)

- Fairly new
- Very powerful
- Sample eluent introduced to He microwave plasma atomizing all atoms in sample
- Uses diode array detector measuring optical emission over wide spectral range (170 - 780 nm)
- Measure many elements simultaneously

GC-AED

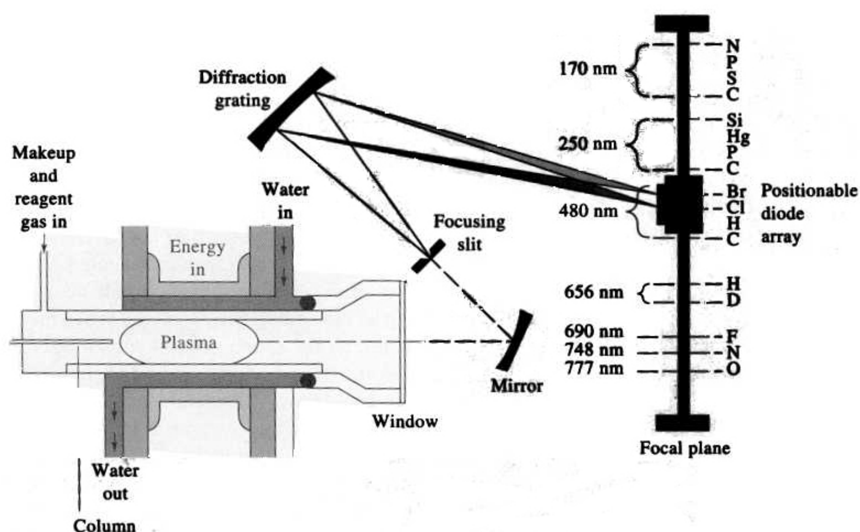


Figure 27-9 An atomic emission detector. *(Courtesy of Hewlett-Packard Company.)*

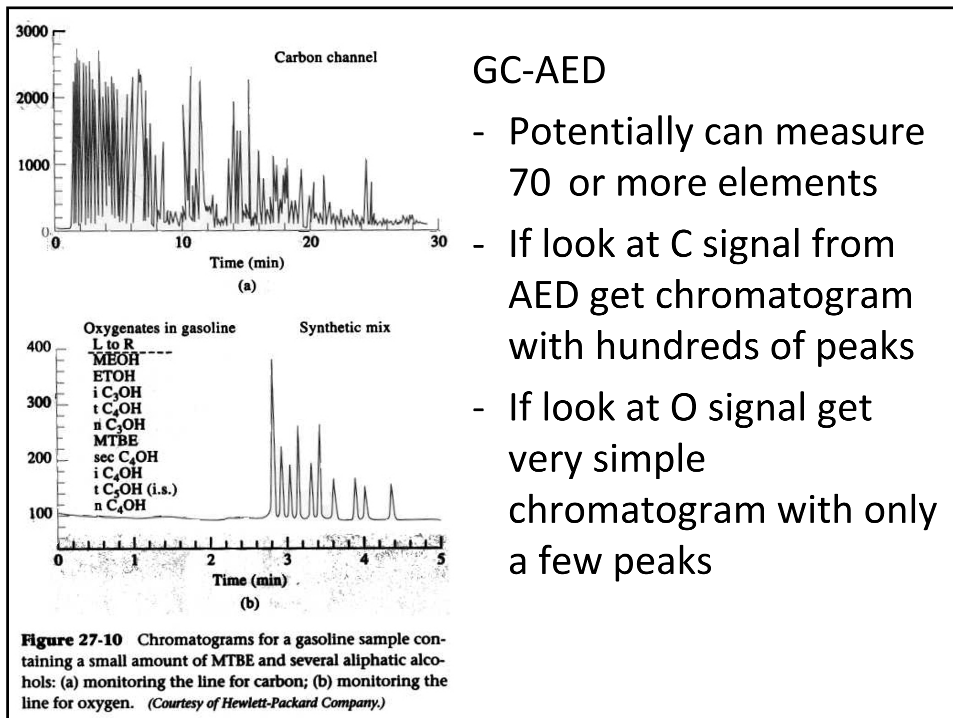


Figure 27-10 Chromatograms for a gasoline sample containing a small amount of MTBE and several aliphatic alcohols: (a) monitoring the line for carbon; (b) monitoring the line for oxygen. *(Courtesy of Hewlett-Packard Company.)*

GC-AED
- Potentially can measure 70 or more elements
- If look at C signal from AED get chromatogram with hundreds of peaks
- If look at O signal get very simple chromatogram with only a few peaks

GC – Mass Spectrometry (GC-MS)
- Already covered Mass Spec
- Interfacing GC & MS normally difficult
- GC at pressure above atmospheric while MS under high vacuum
- Need special interfaces for packed columns
  - Jet separator – discussed below
  - Membrane separator – a membrane sandwich between spiral channels, column efluent on one side under pressure, MS on other side under vacuum – relies on differential permeability of carrier gas vs analyte molecules

GC-MS Schematic

Interface less critical for capillary columns

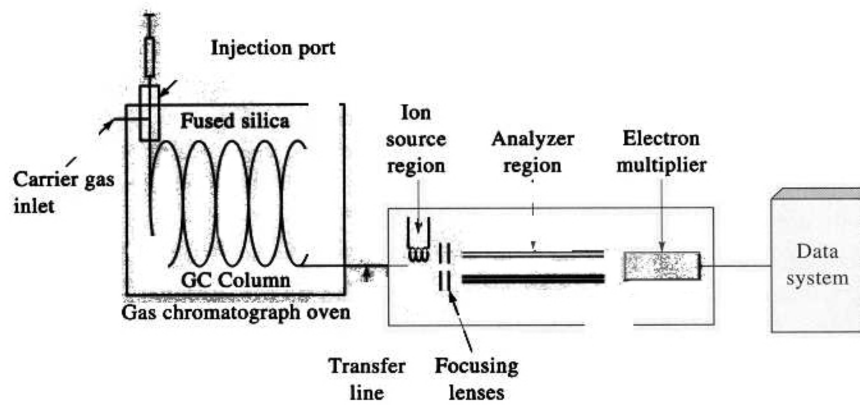


Figure 27-13 Schematic of a typical capillary gas chromatography/mass spectrometer.

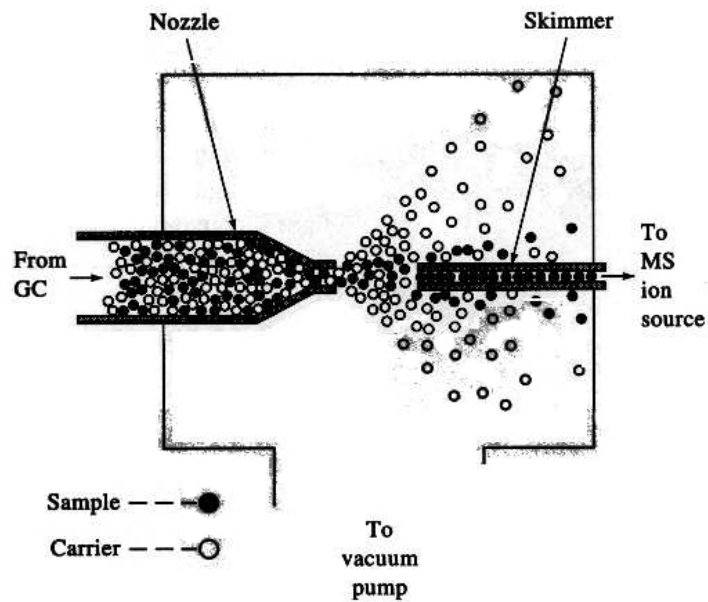


Figure 27-14 Schematic of a jet separator. *(Courtesy of DuPont Instrument Systems, Wilmington, DE.)*

Jet Separator

- Purpose is to get more analyte into MS than carrier gas
- Usually an all glass device
- Principle is that heavier atoms have greater momentum and travel a fairly straight path into the MS, lighter carrier gas molecules are deflected outward by vacuum & pumped away

Several types of Mass Specs available

- Rarely magnetic sector or time of flight
- Usually quadrapole or ion trap for GC-MS
- Less expensive
- Less maintenance
- Easy to use
- Normally use electron multiplier as detector
- All MS systems need ion source, either electron impact or chemical ionization

Three modes of operation for GC-MS

1) Spectral mode – look at mass spectrum every second or so during chromatogram - gives <u>most information</u> for research or method development
2) Total ion current – sum signal for all ions as one large signal – <u>highest sensitivity</u>
3) Selective ion monitoring (SIM) – look at certain mass/charge ratios for compounds of interest – routine analysis

GC-MS

- sensitive
- can be very selective in SIM mode
- powerful for qualitatively & quantitatively

There is also one other kind of Mass Spec

Ion Cyclotron MS which is a very high resolution, Fourier transform instrument not used for GC

GC-FTIR

- Powerful technique for identifying compounds
- Use heated light pipe 1 to 3 mm dia

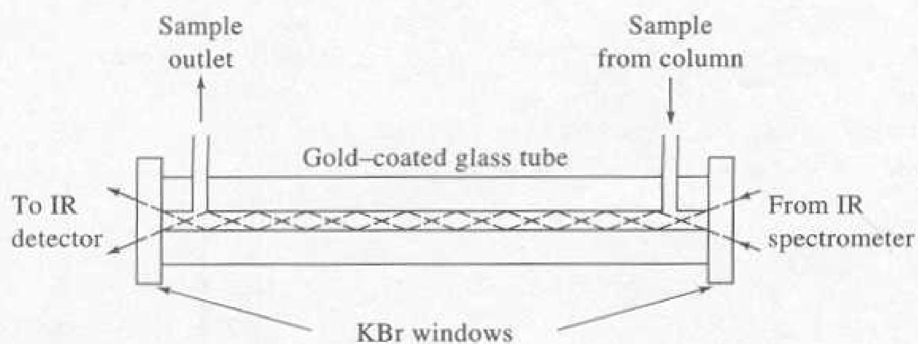


Figure 27-17 A typical light pipe for GC/IR instruments.

GC-FTIR

- Powerful technique for identifying compounds
- Use heated light pipe 1 to 3 mm dia and 10 to 40 cm long
- Heat to prevent condensation of sample
- Cool detector for sensitivity
- Gives structural information from spectrum
- Not very common

GC Columns & Stationary Phases

- Historically used packed columns
- Stationary phase coated as a thin film on a high surface area solid support
- Theoretical studies showed that unpacked columns with narrow diameters were better
- Open tubular columns first developed
- Capillary columns came later because
  - Very fragile, difficult to construct, hard to connect to GCs, small samples hard to detect, difficult to coat column walls, etc.

Packed Columns

- Tubing of metal, glass, Teflon, etc.
- 2 to 3 m long and 2 to 4 mm in dia
- Packed with diatomaceous earth (SiO_2), clay, carbon particles, glass microbeads, polymer
- Diameter 150-250 µm (60-100 mesh) 1 m^2/g
- Thin coating of liquid stationary phase

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

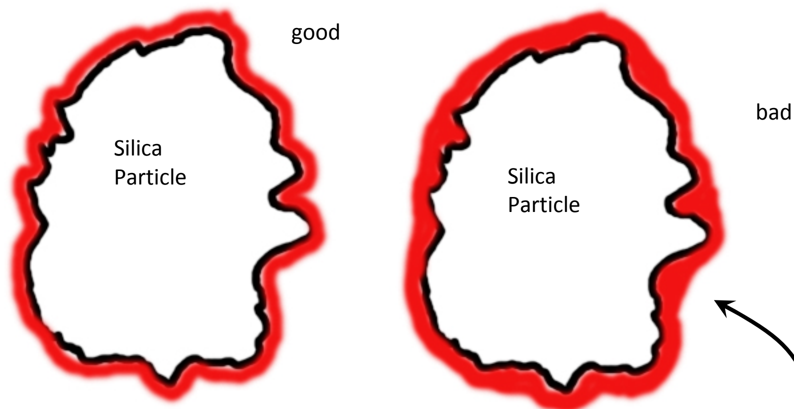
	Type of Column*			
	FSOT	WCOT	SCOT	Packed
Length, m	10–100	10–100	10–100	1–6
Inside diameter, mm	0.1–0.53	0.25–0.75	0.5	2–4
Efficiency, plates/m	2000–4000	1000–4000	600–1200	500–1000
Total plates	$(20–400) \times 10^3$	$(10–400) \times 10^3$	$(6–120) \times 10^3$	$(1–10) \times 10^3$
Sample size, ng	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.

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

Stationary Phase Coating



Don’t want stationary phase liquid coating to bleed or puddle in column – gives zone broadening & poor resolution

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Open Tubular Columns → Capillary Columns

Column evolution

Three types

Wall Coated Open Tubular (WCOT) – open glass tube with coating on wall – duh

Support Coated Open Tubular (SCOT) – open tube with particles of support material stuck to the walls

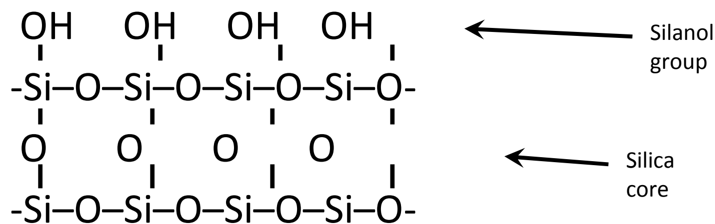
Fused Silica Open Tubular (FSOT) – WCOT made of fused silica

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

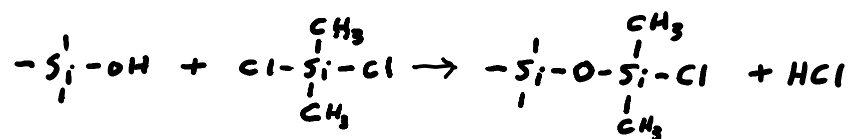
*FSOT: Fused-silica, open tubular column.
WCOT: Wall-coated, open tubular column.
SCOT: Support-coated open tubular column.

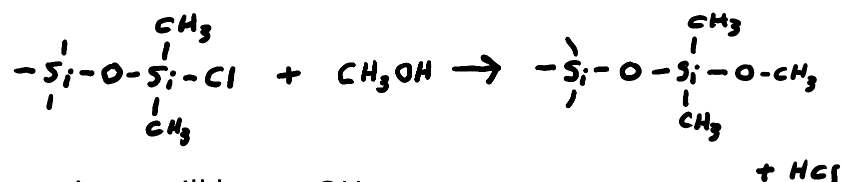
Surface chemistry – glass & silica are SiO_2 with -OH at surface



OH is a problem because it can adsorb polar substances with strong affinity causing peak tailing – must deactivate by reacting

React Si-OH groups with silane





Sometimes still have –OH groups

If silica not pure may have metal impurities M-OH
typically use high purity silica – acid wash

Same chemistry to making specialty bonded phase

Liquid coatings on stationary phase should exhibit:

1) Chemical inertness
2) Low volatility (b.p. 100 °C > max temp)
3) Thermal stability
4) Good solvent characteristics (i.e. k’ & α suitable)

Many different liquid coatings have been used or attempted for GC, only about 10 have withstood the test of time

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

Retention time of a solute depends on K (partition coefficient) which is dependent on stationary phase – must have different K's for different analytes

However, if K's too large → long retention time

if K's too small → short retention time

resulting in incomplete separation

In choosing a stationary phase use general principles such as "like dissolves like", polar groups interact with polar groups, non polar with non polar, etc.

Polar groups include –CN, –CO, –OH

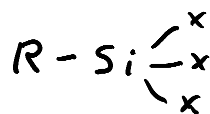
Polar analytes include alcohols, acids, amines

Non polar → hydrocarbons

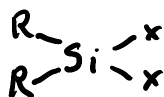
Where analyte & stationary phase match is good
→ elution order is determined by boiling points

Bonded Stationary Phases

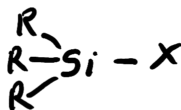
Use silylation chemistry to covalently attach stationary phase to solid support or column wall



$X = Cl$ or $-O-CH_3$ or $-O-CH_2-CH_3$



R = stationary phase functionality



Bonded Stationary Phases

Advantages

- monolayer coverage can be obtained
- reduced bleeding of stationary phase
- longer lasting
- better stability
- can be solvent washed

Chiral Stationary Phases – separating stereo-isomers is the ultimate in chromatography, separate molecules that are mirror images

Predicting retention (or identifying compounds by retention)

I) Selectivity Factors

$$\alpha = \frac{K_B}{K_A} = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} = \frac{(t'_R)_B}{(t'_R)_A}$$

If B is a standard compound & we know α, can then be able to identify compound A even if we change the the chromatographic conditions or go to another chromatograph, etc.

This is limited to specific applications where a database is available, not universally applicable

II) Retention Index (I)

Proposed by Kovats in 1958

Index based on normal alkanes

If have a mixture of 2 known alkanes & 1 unknown compound & the 2 knowns bracket unknown in t_R can then determine I for unknown & identify it

I = 100 x # of carbon atoms

Regardless of column packing, temp. or other conditions

Kovats Retention Index

Doesn't work as well for other types of compounds (Hc), but useful in some cases e.g. homologous series

Plot log adjusted retention time ($t_R' = t_R - t_M$) vs number of carbon atoms is linear

Useful in particular fields – petroleum industry, cosmetics, pharmaceuticals, etc. since have their own unique "standards"

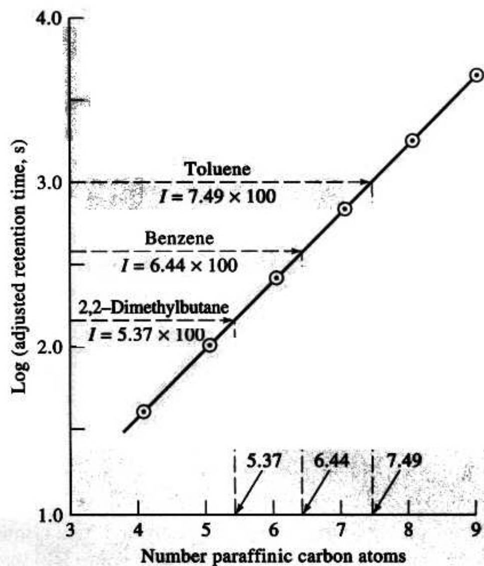


Figure 27-12 Graphical illustration of the method for determining retention indexes for three compounds. Stationary phase: squalane. Temperature: 60°C.

Note number of carbons that would be calculated for these 3 compounds based on I

Homework

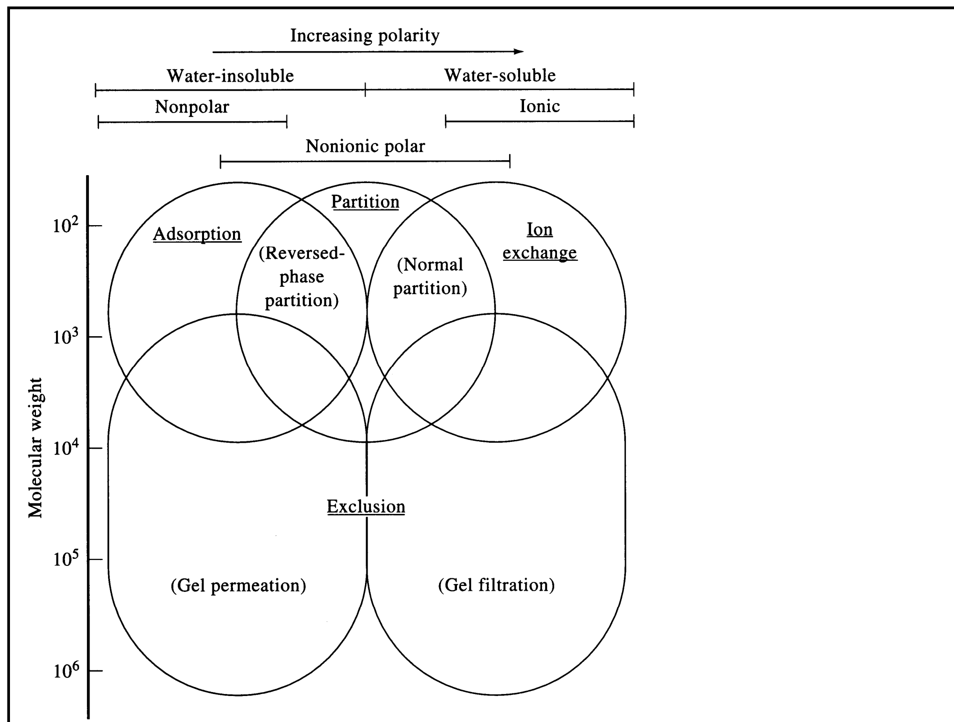
- 27-4
- 27-5
- 27-19

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.



Instrumentation for HPLC:

• For reasonable analysis times, moderate flow rate required but small particles (1-10 µm)

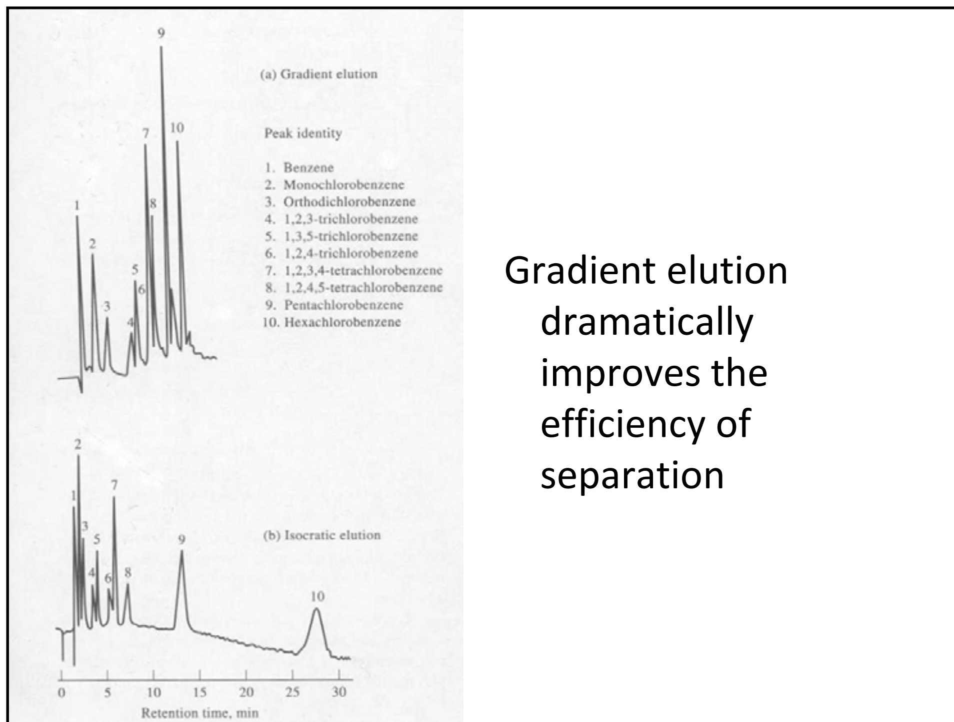
• Solvent forced through column 1000-5000 psi - more elaborate instrument than GC

• Solvents degassed - "sparging“

• High purity solvents

Single mobile phase composition - isocratic elution

Programmed mobile phase composition - gradient elution



Gradient elution dramatically improves the efficiency of separation

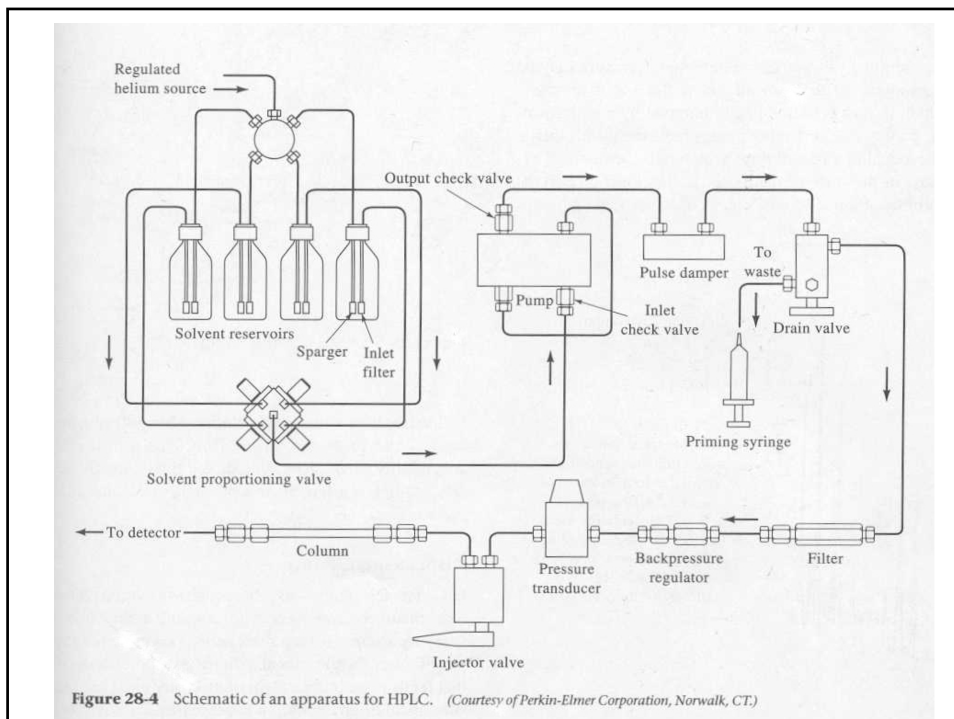


Figure 28-4 Schematic of an apparatus for HPLC. *(Courtesy of Perkin-Elmer Corporation, Norwalk, CT.)*

Solvents (mobile phase) – are stored in special reservoirs connected to the pumping system – must be free of particles that can clog components & free of bubble forming gases that get trapped in column or detector

Three basic ways to degas solvents

1) vacuum or suction filter (0.4 or 0.2 µm)
2) ultrasonicate (with vacuum)
3) He purge (sparge units often built in)

Can purchase HPLC solvents & water - still

HPLC pumping systems typically employ two reciprocating or piston pumps

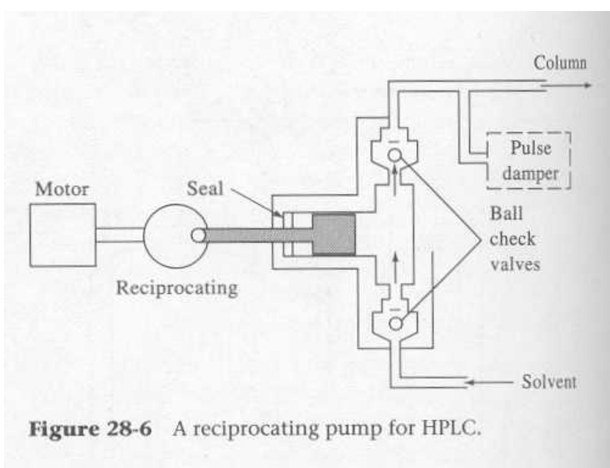


Figure 28-6 A reciprocating pump for HPLC.

Check valves & pump seals need to be replaced

Pulse-free flow is never really achieved

- Up to 10,000 psi, small internal volumes
- Produces pulsation

In GC the analyte affinity for the column is influenced by temp

In HPLC the solvent strength affects an analytes retention on column

Therefore, analogous to temp programming in GC, do solvent programming in HPLC

This is also referred to as gradient elution

HPLC sample injectors are exclusively 6 port valves that are overfilled by syringe giving extreme accuracy & precision – typical volumes are 10 to 50 μL but can be larger

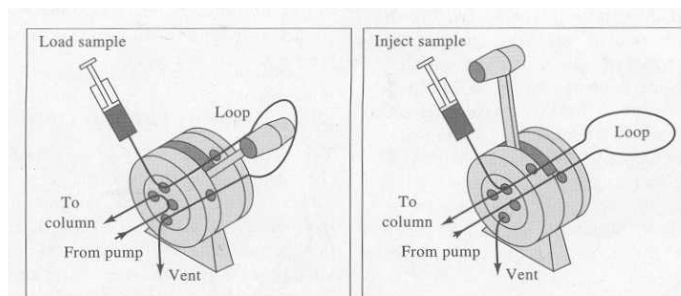


Figure 28-7 A sampling loop for liquid chromatography. *(Courtesy of Beckman Instruments, Fullerton, CA.)* With the valve handle as shown on the left, the loop is filled from the syringe, and the mobile phase flows from pump to column. When the valve is placed in the position on the right, the loop is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

Rotary Injection Valve

Common for HPLC, rare in GC

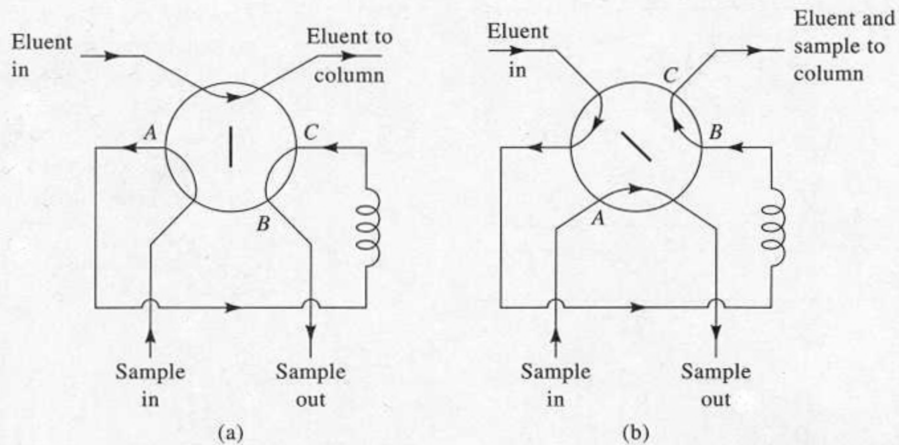
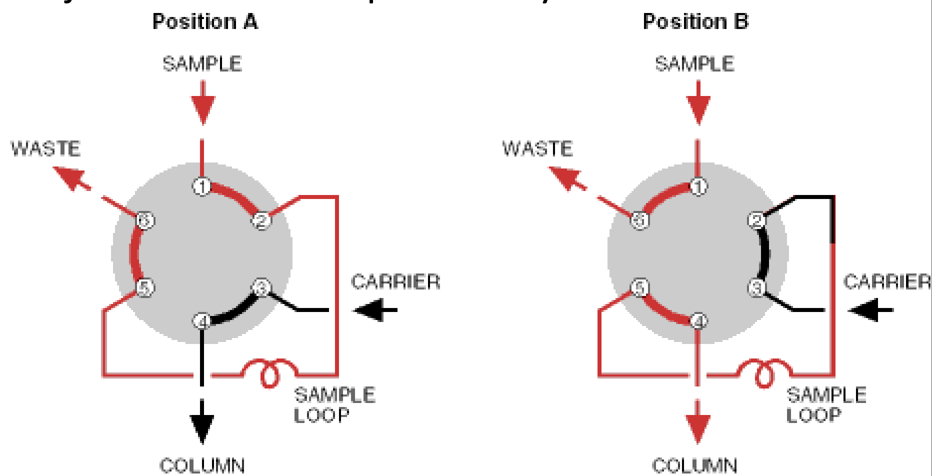


Figure 27-4 A rotary sample valve: valve position (a) for filling sample loop *ACB* and (b) for introduction of sample into column.

Injector for HPLC 6 port rotary valve

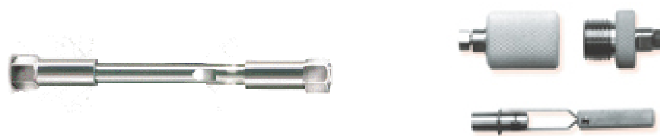


- Similar to FIA, GC
- Introduce small sample (0.1-100 μL) without depressurization
- Microsyringe/septum system (only <1500 psi)

Columns

- usually stainless steel
- can be PEEK (poly ether ether ketone)
- may cost $200-$1000 packed
- Length 10-30 cm, ID 4-10 mm
- Packings are 3, 5, or 10 μm particle size
- Most common 25 cm, 5 μ, 4.6 mm ID
- N = 40,000 to 60,000
- Normally packed under 6000 psi pressure at factory as a slurry

Guard columns are normally used before the analytical column to protect & increase lifetime of column – operator usually slurry or dry packs short guard column regularly with same or similar packing used in analytical column (old column material) – can purchase guard systems, cartridges, etc.



Detectors for HPLC

- Ideal characteristics same as GC
- Exception is temp range
- Low dead volume 1 to 10 μL

Bulk property detectors - measure property of mobile phase (refractive index, dielectric constant, density)
Solute property detectors - measure property of solute not present in mobile phase (UV absorbance, fluorescence, IR absorbance)

Most common detector is **UV-vis absorbance**

Three types

1) Filter instrument – optical filters, Hg lamp
2) Variable wavelength – monochromator
3) Diode array detector- provide spectra

Many HPLC detectors available

For universal & selective detection

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

1) Filter based UV-vis detector –Typically set at 254 nm using the most prominent band in Hg spectrum – can also use 313, 365, 334 nm and other lines as well

2) Variable wavelength detectors – use continuum source like (D_2 or H_2) & a monochromator, select any λ, less sensitive

3) PDA - D_2 or H_2 source, disperse & focus on diode array, get complete spectrum every 1 sec, powerful, expensive, less sensitive, lots of data generated

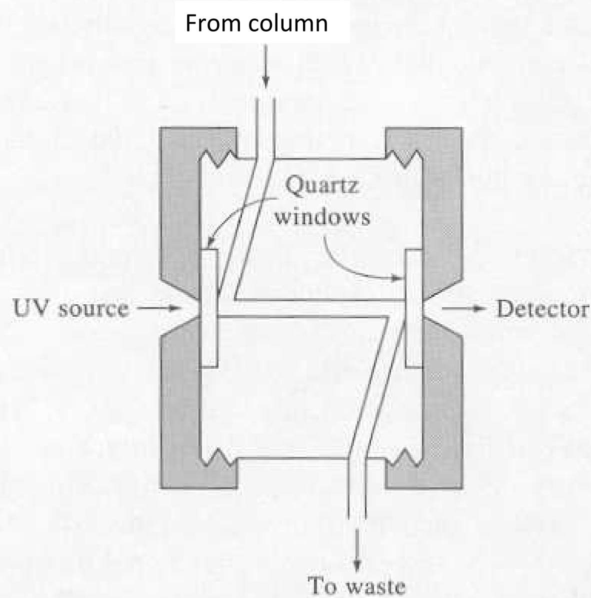


Figure 28-9 Ultraviolet detector cell for HPLC.

Cell for UV-vis detector for HPLC

- Low vol

sources: • single line (arc or hollow cathode lamp, laser)
• continuum (Xe, D2 lamp) detector:
• photodiode/photomultiplier tube
• photodiode array
Combination of separation and analysis (GC-MS, HPLC-UV-Vis) - very powerful

Diode Array Detector

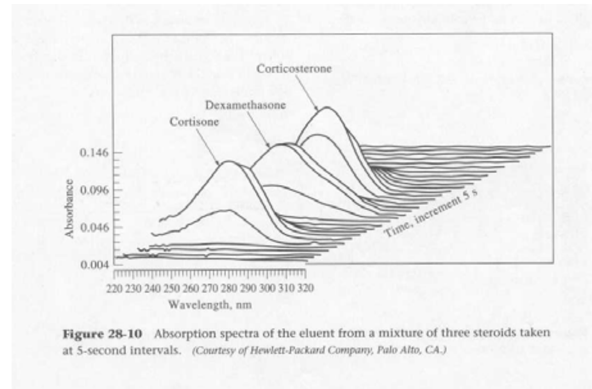


Figure 28-10 Absorption spectra of the eluent from a mixture of three steroids taken at 5-second intervals. *(Courtesy of Hewlett-Packard Company, Palo Alto, CA.)*

Fluorescence detector – normally fixed wavelength filter fluorometer excitation filter & emission filter can be changed for particular λ of interest gives selectivity based on:

- ability to exhibit fluorescence
- excitation wavelength
- emission wavelength

Variable λ monochromator based fluorescence detectors also available

Filter based detectors usually more sensitive

Refractive index detector (RI) - responds to nearly all solutes but has poor sensitivity – detects changes in refractive index as sample passes through as long as solute has different RI than solvent – analogous to TCD in GC

Electrochemical Detection

- Amperometric – fix potential & measure current (i)
- Conductometric – measure conductivity
- Coulometric – fix potential & integrate i
- Voltammetric – vary potential & measure i
- Potentiometric – measure potential

Can use 2 or 3 electrode design with Pt or carbon electrodes (glassy C or C paste)

Electrochem. detector nearly universal

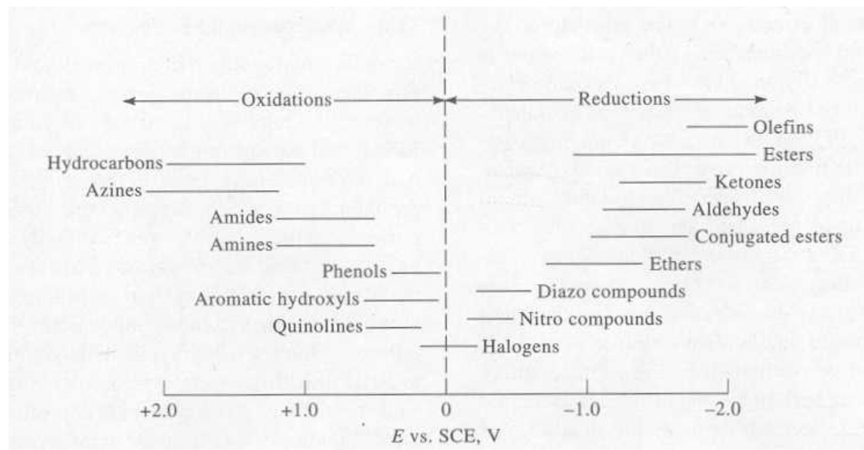


Figure 28-12 Potentially detectable organic functional groups by amperometric measurements. The horizontal lines show the range of oxidation or reduction potentials wherein compounds containing the indicated functional groups are electroactive.

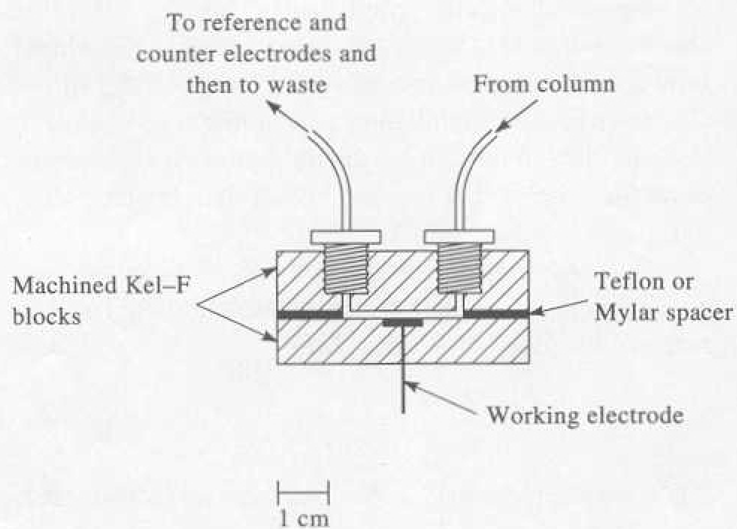


Figure 28-13 Amperometric thin-layer detector cell for HPLC.

Other HPLC detectors

- LC-MS using thermospray – new popularity (pharmaceuticals)
- Evaporative light scattering - polymers
- LC-FTIR
- LC-plasma emission or ICP-MS

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)

Partition Chromatography:
- Most popular method
- Low molecular weight (mw<3000) analytes
- Polar or non-polar
- Bonded stationary phase column (liquid chemically bonded to support particles)

3, 5 or 10 μm hydrolyzed silica particles coated with siloxanes

Normal phase HPLC nonpolar solvent/polar column

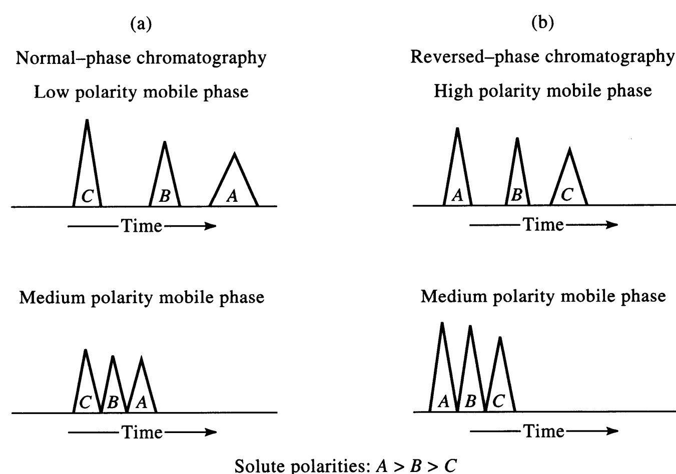
Reversed phase HPLC polar solvent/nonpolar column

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

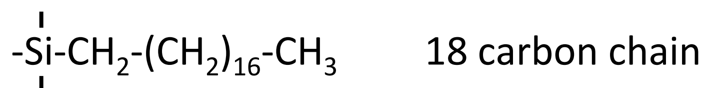
Later bonded phases were introduced using C_{18} groups → very non-polar with polar solvents = reversed-phase chromatography

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

Normal- (polar column) versus Reversed Phase (nonpolar) elution:



Reversed-phase HPLC most common (high polarity solvent, high polarity solutes elute first)



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

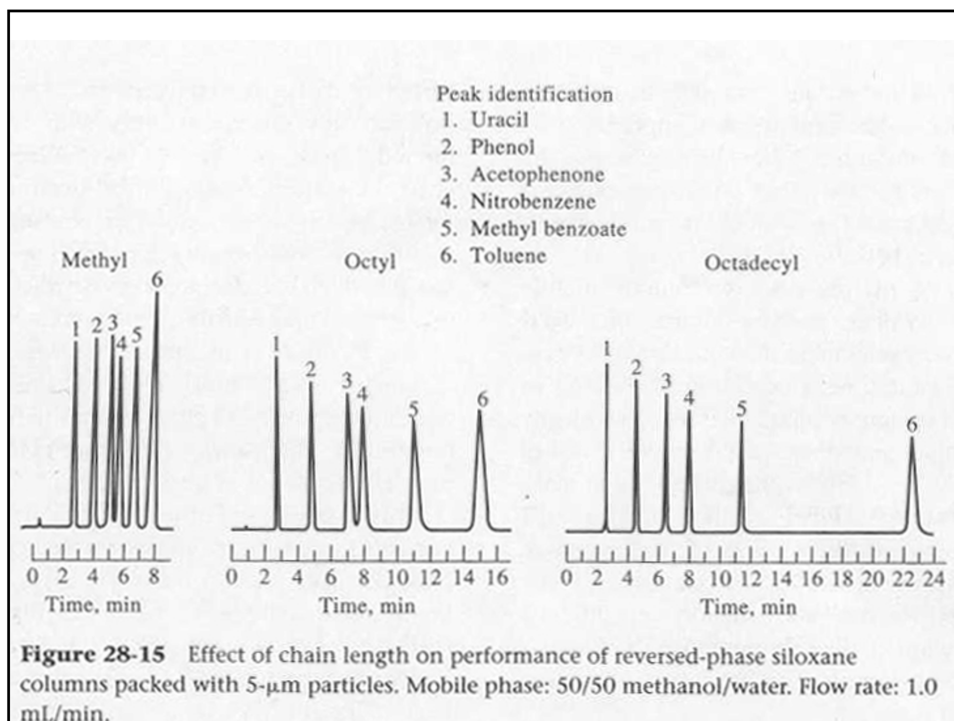


Figure 28-15 Effect of chain length on performance of reversed-phase siloxane columns packed with 5-μm particles. Mobile phase: 50/50 methanol/water. Flow rate: 1.0 mL/min.

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

Column Optimization in HPLC:
Can optimize k' and α
More difficult than GC
- in GC mobile phase just *transported solute*
- in HPLC mobile phase interacts with solute

Analyte Polarity:
hydrocarbons<ethers<esters<ketones<aldehydes<amines<alcohols

Stationary Phase Choice:
Choose column with similar polarity to analyte for maximum interaction
Reversed-phase column (nonpolar)
R hydrocarbon
Normal-phase column (polar)
R cyano (C_2H_4CN) most polar
diol ($C_3H_6OCH_2CHOHCH_2OH$)
amino ($C_3H_6NH_2$) least polar

Mobile Phase Choice:

Polar ("strong") solvent interacts most with polar analyte (solute) - elutes faster but less resolution

Strength characterized by polarity index P'

ranges from -2 (nonpolar) to 10.2 (highly polar)

in a mixture

$P'_{AB} = \phi_A P'_A + \phi_B P'_B$

fraction in mixture

In HPLC, capacity factor k' can be manipulated by changing solvent composition

best resolution/time when k' = 2-5

$k'_2/k'_1 = 10^{(P'2 - P'1)/2}$

TABLE 28-2 Properties of Common Chromatographic Mobile Phases

Solvent	Refractive Index[a]	Viscosity, cP[b]	Boiling Point, °C	Polarity Index, P'	Eluent Strength,[c] ϵ^0
Fluoroalkanes[d]	1.27–1.29	0.4–2.6	50–174	<−2	−0.25
Cyclohexane	1.423	0.90	81	0.04	−0.2
n-Hexane	1.372	0.30	69	0.1	0.01
1-Chlorobutane	1.400	0.42	78	1.0	0.26
Carbon tetrachloride	1.457	0.90	77	1.6	0.18
i-Propyl ether	1.365	0.38	68	2.4	0.28
Toluene	1.494	0.55	110	2.4	0.29
Diethyl ether	1.350	0.24	35	2.8	0.38
Tetrahydrofuran	1.405	0.46	66	4.0	0.57
Chloroform	1.443	0.53	61	4.1	0.40
Ethanol	1.359	1.08	78	4.3	0.88
Ethyl acetate	1.370	0.43	77	4.4	0.58
Dioxane	1.420	1.2	101	4.8	0.56
Methanol	1.326	0.54	65	5.1	0.95
Acetonitrile	1.341	0.34	82	5.8	0.65
Nitromethane	1.380	0.61	101	6.0	0.64
Ethylene glycol	1.431	16.5	182	6.9	1.11
Water	1.333	0.89	100	10.2	Large

[a] At 25°C.

[b] The centipoise is a common unit of viscosity; in SI units, $1\ cP = 1\ mN \cdot s \cdot m^{-2}$.

[c] On Al_2O_3. Multiplication by 0.8 gives ϵ^0 on SiO_2.

[d] Properties depend upon molecular weight. Range of data given.

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 applications

Adsorption chrom. = normal phase chromatog.

Least popular mode of separation due to: strong adsorption, surface changes over time, with pH or water content

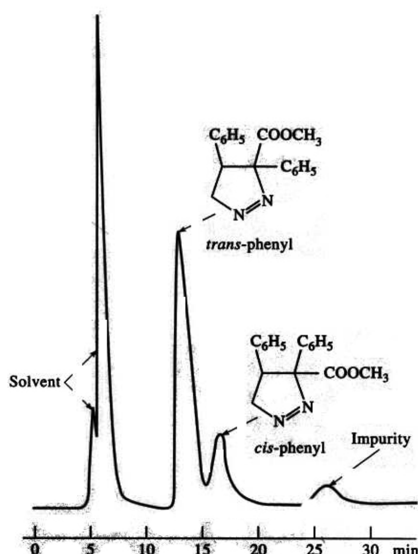


Figure 28-20 A typical application of adsorption chromatography: separations of *cis*- and *trans*-pyrazoline. Column: 100 × 0.3 cm pellicular silica. Mobile phase: 50% methylene chloride/isooctane. Temperature: ambient. Flow rate: 0.225 mL/min. Detector: UV, 254 nm.

- Sample of an application of adsorption chromatography

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

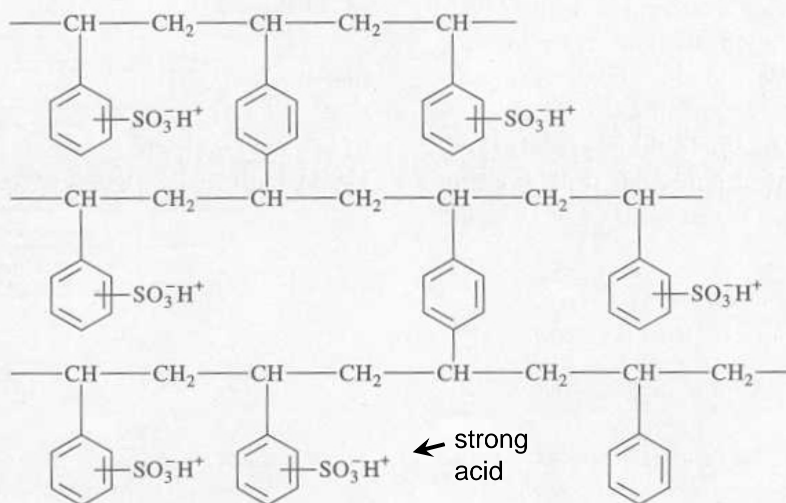
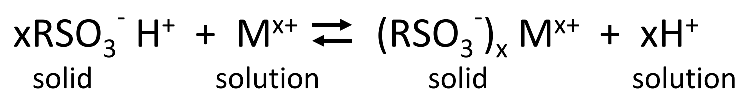


Figure 28-21 Structure of a cross-linked polystyrene ion-exchange resin. Similar resins are used in which the $—SO_3^-H^+$ group is replaced by $—COO^-H^+$, $—NH_3^+OH^-$, and $—N(CH_3)_3^+OH^-$ groups.

weak base strong base weak acid

Can write reactions in general format



$$xRSO_3^- H^+ + M^{x+} \rightleftarrows (RSO_3^-)_x M^{x+} + xH^+$$

solid solution solid solution

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

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

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

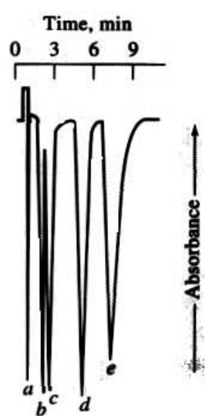
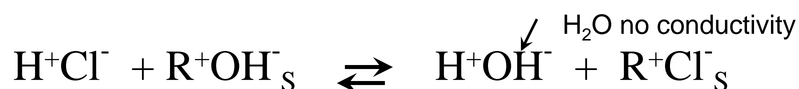


Figure 28-24 Indirect photometric detection of several anions by elution. Eluent: 10^{-3} M disodium phthalate, 10^{-3} M boric acid, pH 10. Flow rate: 5 mL/min. Sample volume: 0.02 mL. UV detector. Sample ions: (a) 18-μg carbonate; (b) 1.4-μg chloride, (c) 3.8-μg phosphate; (d) 5-μg azide; (e) 10-μg 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^-)



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.

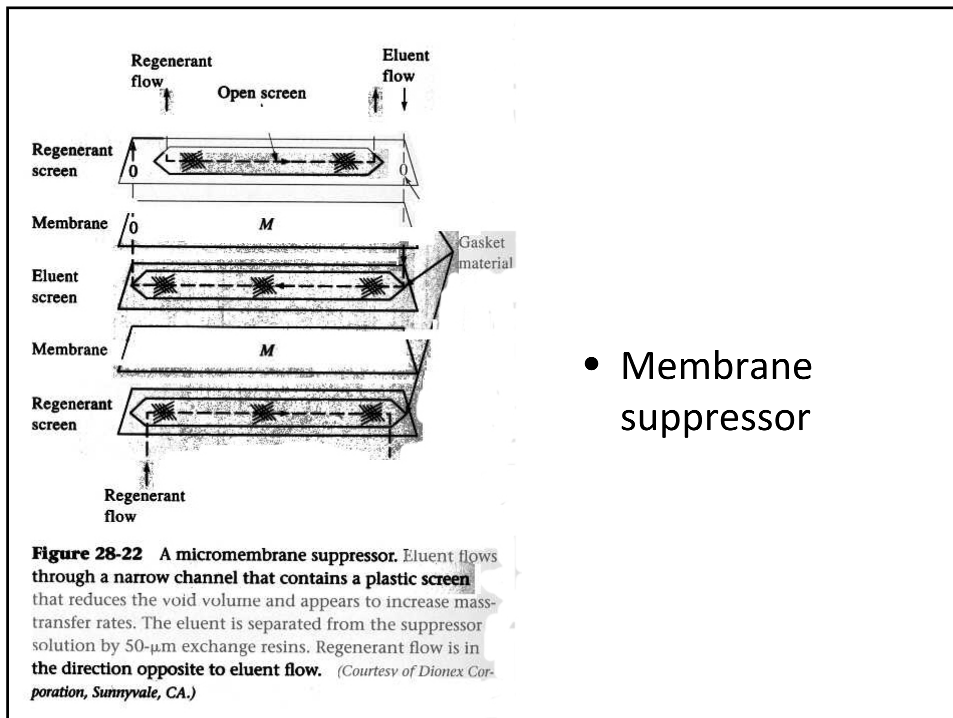


Figure 28-22 A micromembrane suppressor. Eluent flows through a narrow channel that contains a plastic screen that reduces the void volume and appears to increase mass-transfer rates. The eluent is separated from the suppressor solution by 50-μm exchange resins. Regenerant flow is in the direction opposite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

- Membrane suppressor

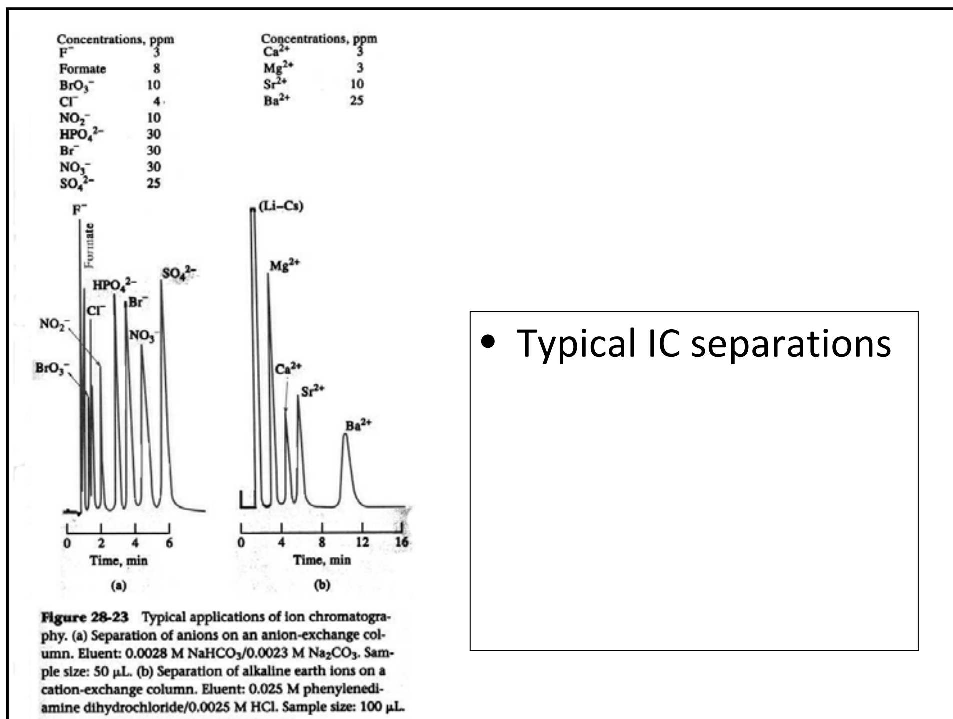


Figure 28-23 Typical applications of ion chromatography. (a) Separation of anions on an anion-exchange column. Eluent: 0.0028 M $NaHCO_3$/0.0023 M Na_2CO_3. Sample size: 50 μL. (b) Separation of alkaline earth ions on a cation-exchange column. Eluent: 0.025 M phenylenediamine dihydrochloride/0.0025 M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

- Typical IC separations

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

- Used for large mw compounds - proteins and polymers
- Separation mechanism is sieving not partitioning
- Stationary phase porous silica or polymer particles (polystyrene, polyacrylamide) (5-10 μm)

-well-defined pore sizes (40-2500 Å)

1. Large molecules excluded from pores - not retained, first eluted (exclusion limit - terms of mw)
2. Intermediate molecules - retained, intermediate elution times
3. Small molecules permeate into pores - strongly retained, last eluted (permeation limit - terms of mw)

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

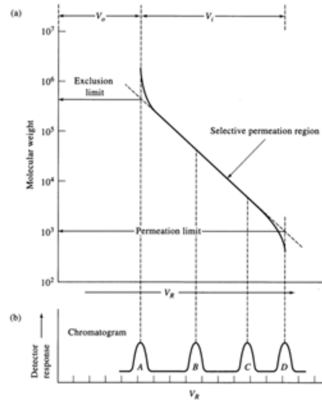
Type	Particle Size, μm	Average Pore Size, Å	Molecular Weight Exclusion Limit*
Polystyrene-divinylbenzene	10	10^2	700
		10^3	$(0.1 \text{ to } 20) \times 10^4$
		10^4	$(1 \text{ to } 20) \times 10^4$
		10^5	$(1 \text{ to } 20) \times 10^5$
		10^6	$(5 \text{ to } > 10) \times 10^6$
Silica	10	125	$(0.2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

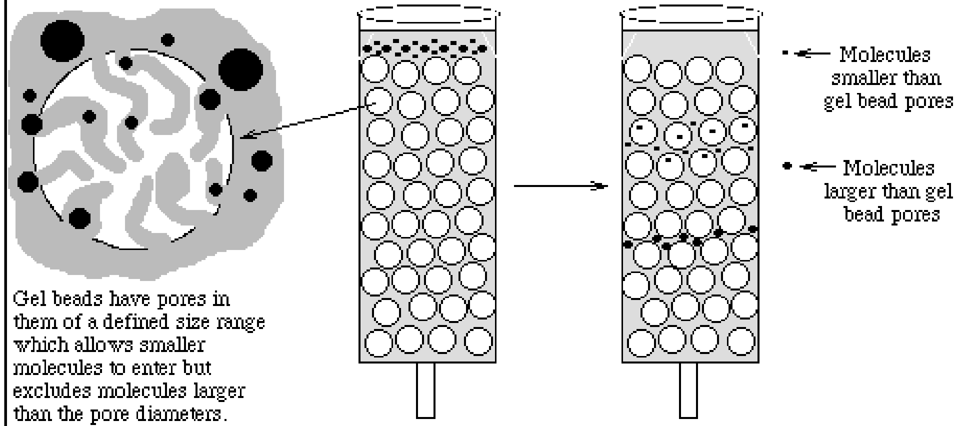
Retention related to size (and shape) of molecule

$$\underbrace{V_t}_{\text{total}} = \underbrace{V_g}_{\text{gel or solid}} + \underbrace{V_i}_{\text{inside pores}} + \underbrace{V_o}_{\text{outside pores/ free-space}}$$

V_o retention volume for non-retained (large) molecules
(V_o+V_i) retention volume for retained (small) molecules
(V_o+KV_i) retention volume for intermediate molecules ($K=c_s/c_m$)



SEC



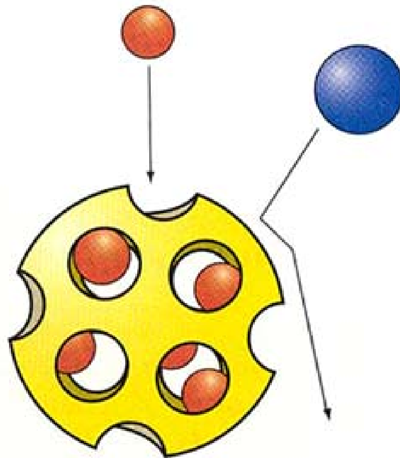
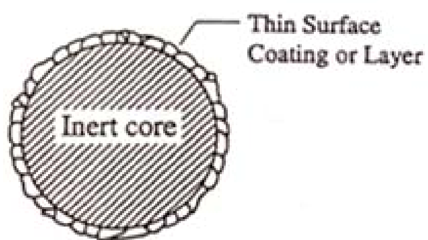


Figure Gel filtration chromatography. (a) Principle of the method. A resin bead is schematically represented as a "whiffle ball" (yellow). Large molecules (blue) cannot fit into the beads, so they are confined to the relatively small buffer volume outside the beads. Thus, they emerge quickly from the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



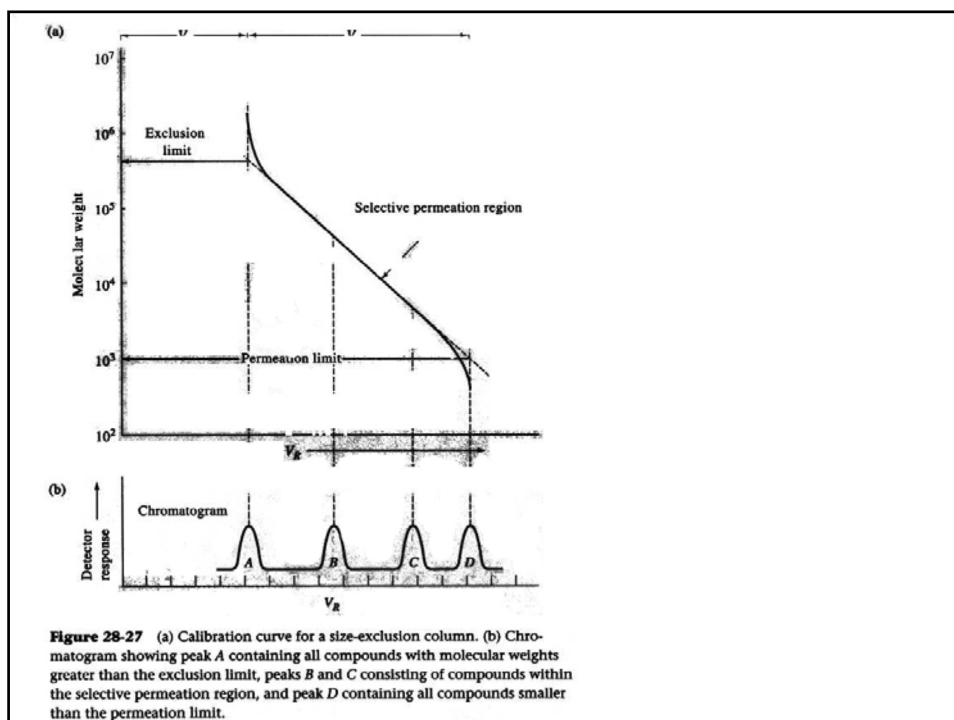


Figure 28-27 (a) Calibration curve for a size-exclusion column. (b) Chromatogram showing peak *A* containing all compounds with molecular weights greater than the exclusion limit, peaks *B* and *C* consisting of compounds within the selective permeation region, and peak *D* containing all compounds smaller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