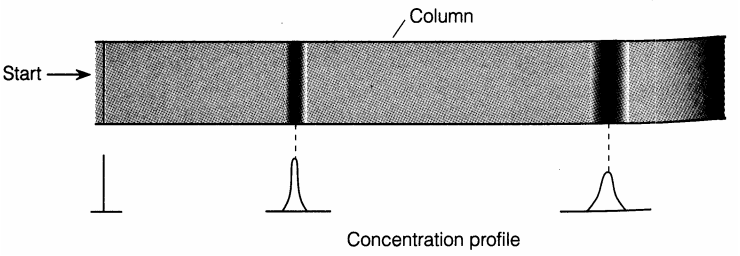
Why Do Bands Spread?



A band of solute invariably spreads as it travels through the column and emerges at the detector with a standard deviation, σ.

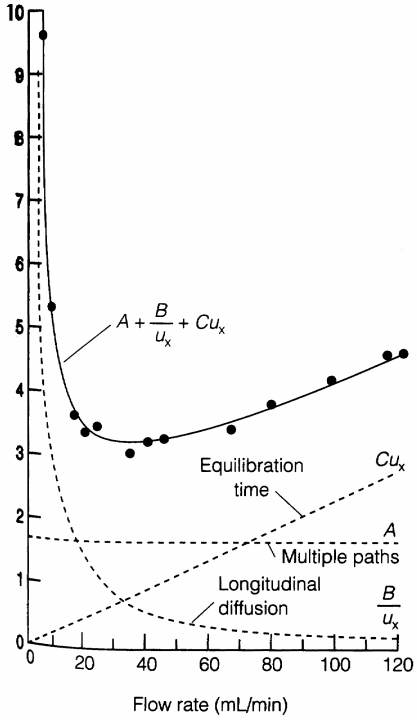


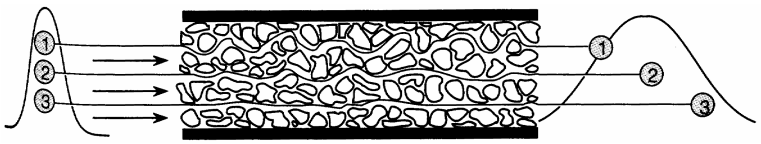
Plate height (H) is proportional to the variance (^{2}) of the chromatographic band: the smaller the plate height, the narrower the band. The **van Deemter equation** summarizes on-column effects (not including the injector and detector) that contribute to plate height.

$$H \equiv A + \frac{B}{u_X} + Cu_X$$

where A, B, and C are constants and u_X is linear flow rate

In packed columns, all three terms contribute to band broadening. For open tubular columns, the A term is zero, so bandwidth decreases and resolution increases. In capillary electrophoresis, both A and C go to zero, providing extraordinary separation powers.

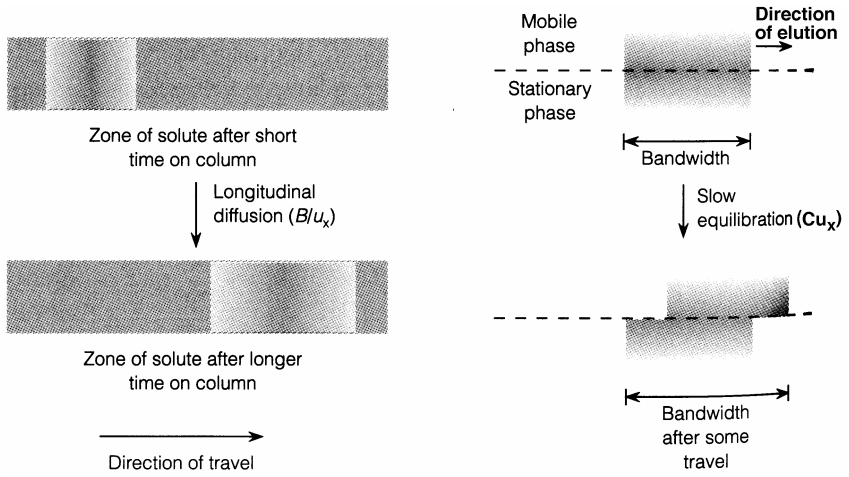
Contributions to Band Broadening as described in Van Deemter Equation



(A) deals with multiple paths the solute can travel.

The second term (B/u_X) arises from **longitudinal diffusion,** which means that solute spreads out along the length of the column.

The third term (Cu_X) comes from the finite time required for solute to equilibrate between the mobile and stationary phases.



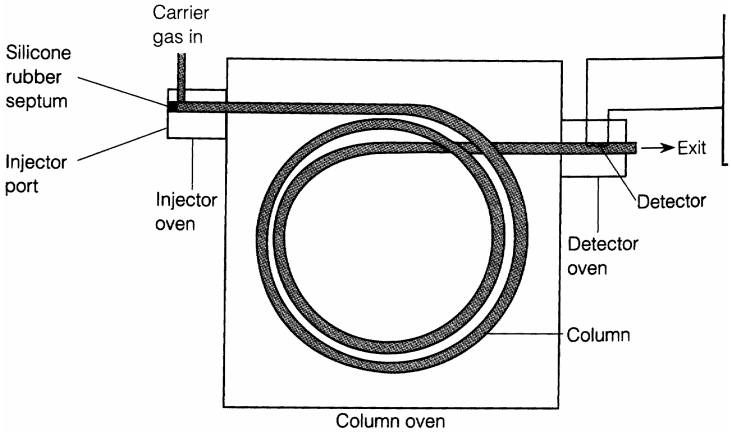
Summary of Important Chromatographic Quantities & Relationships

Experimental Quantities		
Name	*Symbol*	*Determined From*
Migration time, nonretained species	t_m	Chromatogram
Retention time, species x	$(t_r)_x$	Chromatogram
Peak width, species x	W_x	Chromatogram
Length of Column Packing	L	Direct Measurement
Flow Rate	F	Direct Measurement
Volume of Stationary Phase	V_s	Packing Preparation Data
Concentration of solute in mobile and stationary phases	C_m, C_s	Analysis and preparation data

Derived Quantities	
Name	*Calculation of Derived Quantities*
Linear Mobile Phase Velocity	$u = L/t_m$
Volume of Mobile Phase	$V_m = t_m F$
Capacity Factor	$k' = (t_r - t_m)/t_m$
Partition Coefficient	$K = \frac{k'V_m}{V_s}$
Selectivity Factor	$\alpha = \frac{(t_r)_y - t_m}{(t_r)_x - t_m}$
Resolution	$R_s = \frac{2[(t_r)_y - (t_r)_x]}{W_x + W_y}$
Resolution	$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha}\right) \frac{k'_y}{(1+ k'_y)}$
Number of Plates	$N = 16 \left(\frac{t_r}{W}\right)^2$ or $5.5\left(\frac{t_r}{W_{1/2}}\right)^2$
Retention Time	$(t_r)_y = \frac{16\, R_s^2\, H}{u} \left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1+ k'_y)^3}{(k'_y)^2}$

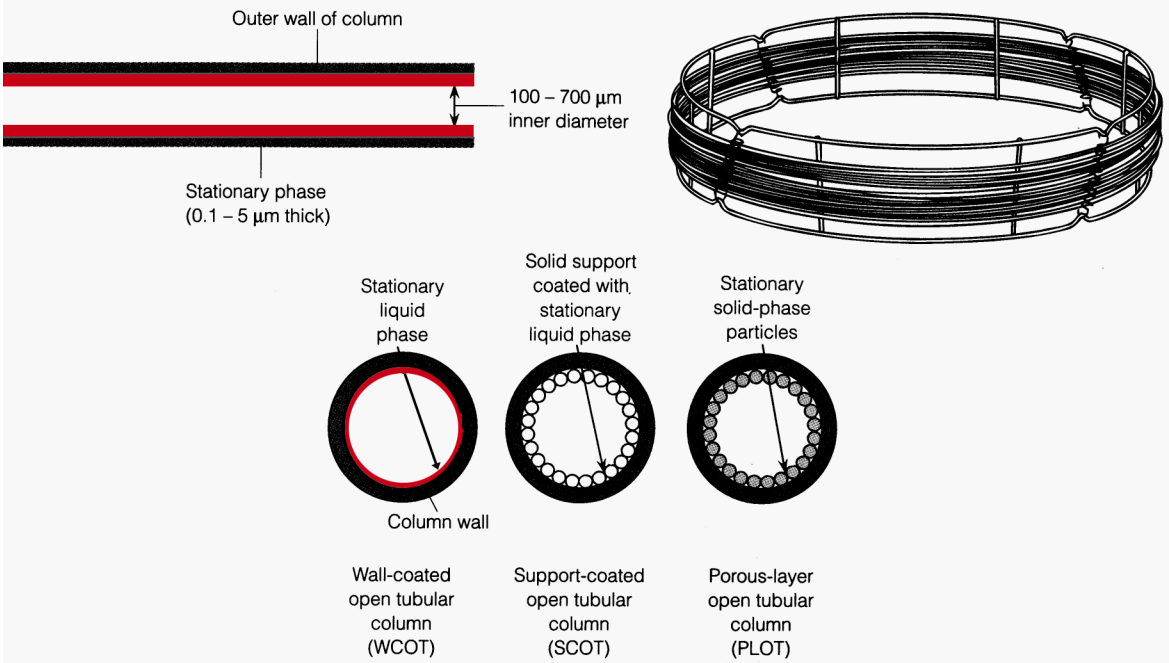
Gas Chromatography

In **gas chromatography**, a gaseous solute (or vapor from a volatile liquid) is carried by a gaseous mobile phase. In *gas-liquid partition chromatography*, the stationary phase is a nonvolatile liquid coated on the inside of the column. In *gas-solid adsorption chromatography*, solid particles that adsorb solute serve as the stationary phase.



A volatile liquid is injected through a rubber **septum** into a heated port which vaporizes the sample. The sample is swept through the column by He, N_2 or H_2 *carrier gas*, and the separated solutes flow through a detector.

The column must be hot enough to produce sufficient vapor pressure for each solute to be eluted in reasonable time.



Open-tubular Columns

The vast majority of analyses use long, narrow **open-tubular columns**

Types:

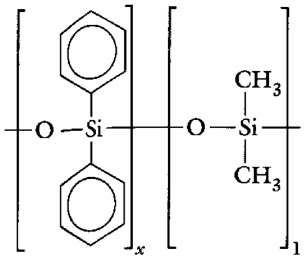
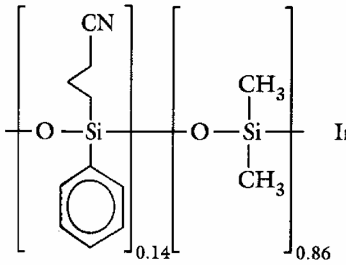
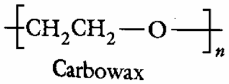
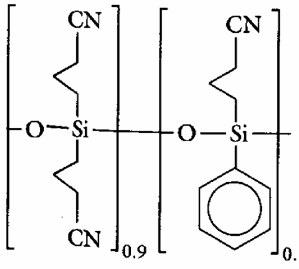
1. WCOT – liquid stationary phase on inside wall of column
2. SCOT – liquid stationary phase coated on solid support attached to inside of column
3. PLOT – solid stationary phase on inside wall of column

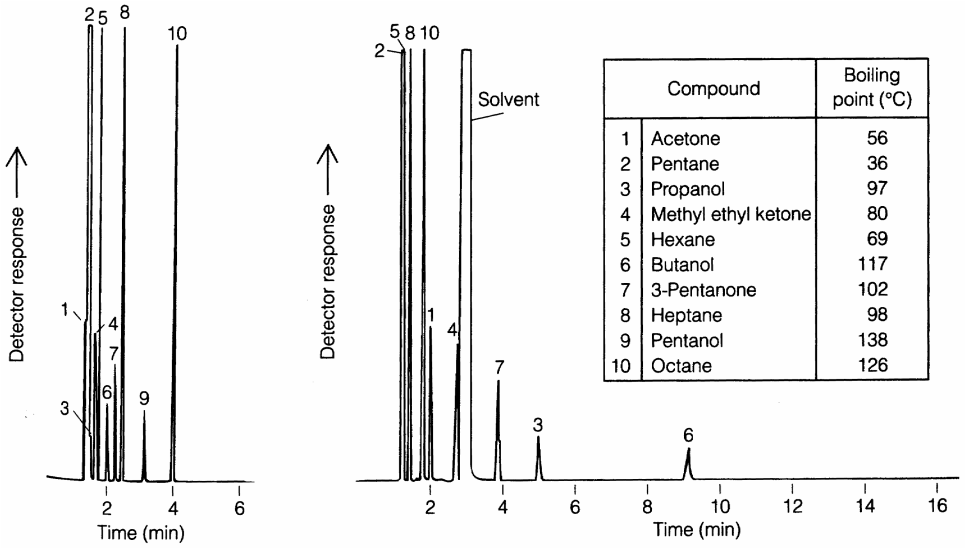
Advantages of Open Tubular Columns

Compared with packed columns, open tubular columns can provide

1. higher resolution
2. shorter analysis time
3. increased sensitivity
4. lower sample capacity

TABLE 23-1 Common stationary phases in capillary gas chromatography

Structure	Polarity	Temperature range (°C)
 (Diphenyl)$_x$(dimethyl)$_{1-x}$ polysiloxane	$x = 0$ Nonpolar	−60°–360°
	$x = 0.05$ Nonpolar	−60°–360°
	$x = 0.35$ Intermediate polarity	0°–300°
	$x = 0.65$ Intermediate polarity	50°–370°
 (Cyanopropylphenyl)$_{0.14}$ (Dimethyl)$_{0.86}$ polysiloxane	Intermediate polarity	−20°–280°
 Carbowax (ethylene glycol))	Strongly polar	40°–250°
 (Biscyanopropyl)$_{0.9}$ (Cyanopropylphenyl)$_{0.1}$ polysiloxane	Strongly polar	0°–275°



	Compound	Boiling point (°C)
1	Acetone	56
2	Pentane	36
3	Propanol	97
4	Methyl ethyl ketone	80
5	Hexane	69
6	Butanol	117
7	3-Pentanone	102
8	Heptane	98
9	Pentanol	138
10	Octane	126

The Retention Index

In the chromatogram to the left, 10 compounds are eluted nearly in order of increasing boiling point (left-most plot)

The RHS chromatogram shows that the strongly polar stationary phase strongly retains the polar solutes.

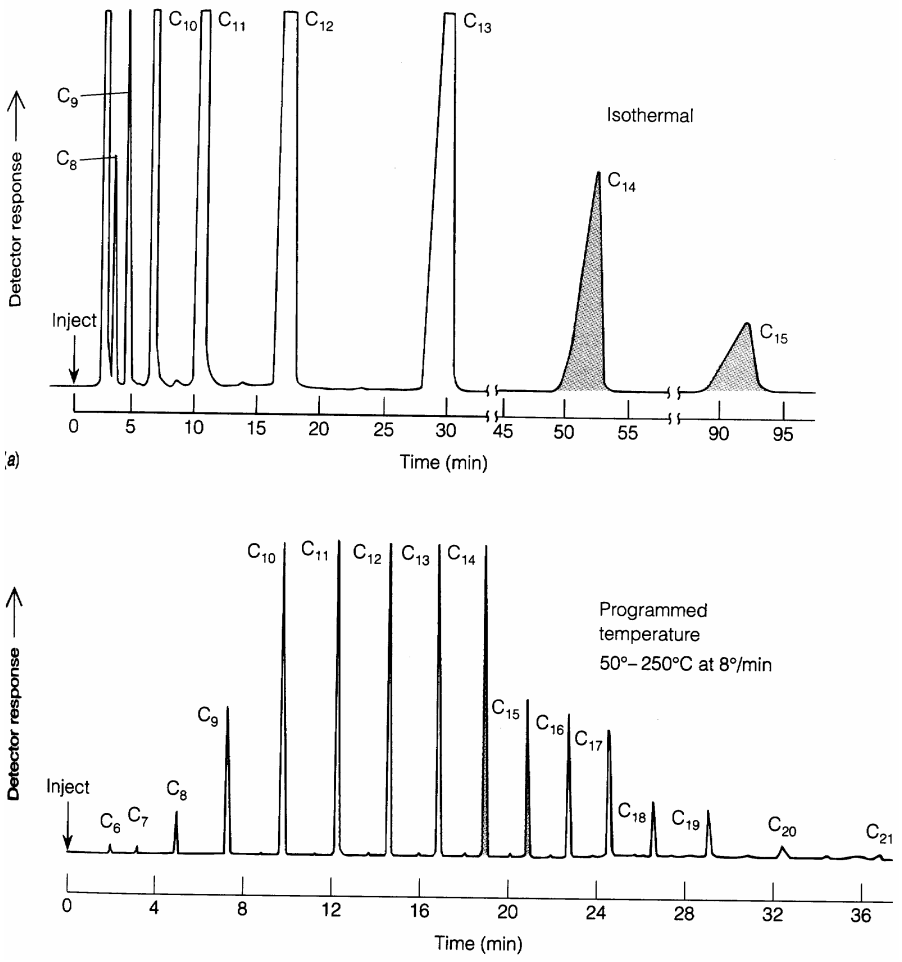
The Kovats **retention index** (I) for a linear alkane equals 100 times the number of carbon atoms (e.g., octane, I = 800). The retention index of an unknown measured on several different columns is useful for identifying the unknown by comparison with tabulated retention indexes.

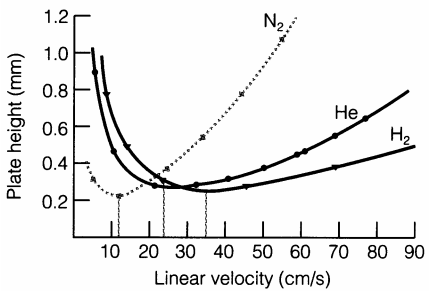
Temperature Programming

Raising column temperature

1. decreases retention time
2. sharpens peaks

When separating compounds with a wide range of boiling points or polarities, it helps to raise the column temperature *during* the separation.



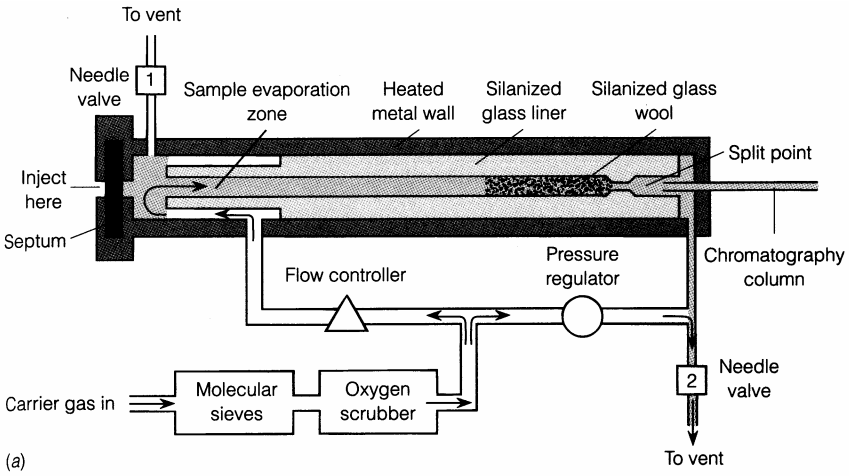


Carrier Gas

Column and detector performance depend on the identity of the carrier gas. H_2 and He give optimal resolution at higher flow rates than N_2.

Sample Injection

Liquids are introduced into a column through a rubber septum of a port leading to a glass tube inside a hot metal block. Carrier gas sweeps the vaporized sample out of the port and into the chromatography column.

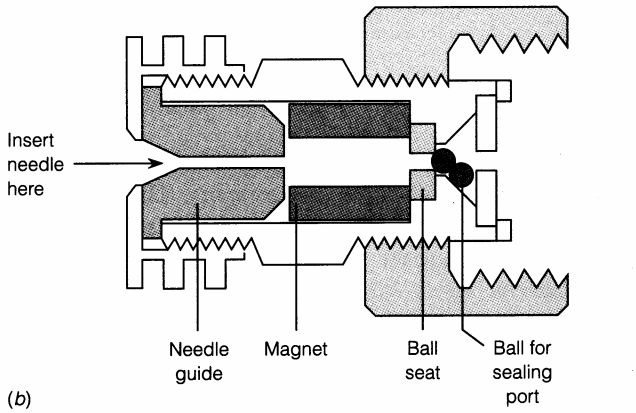


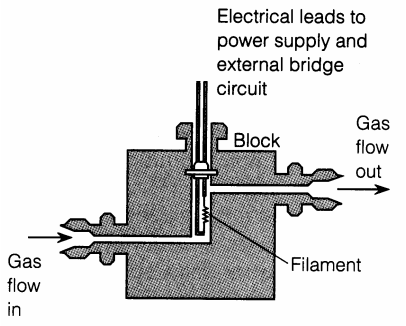
Methods for injection into open-tubular columns:

split – routine means of introducing small sample volume

splitless – best for trace levels of high-boiling solutes in low-boiling solvents; better than split injection for quantitative analysis

on-column – best for thermally unstable solutes and high-boiling solvents





Thermal Conductivity Detector

Thermal conductivity measures the ability of a substance to transport heat from a hot region to a cold region.

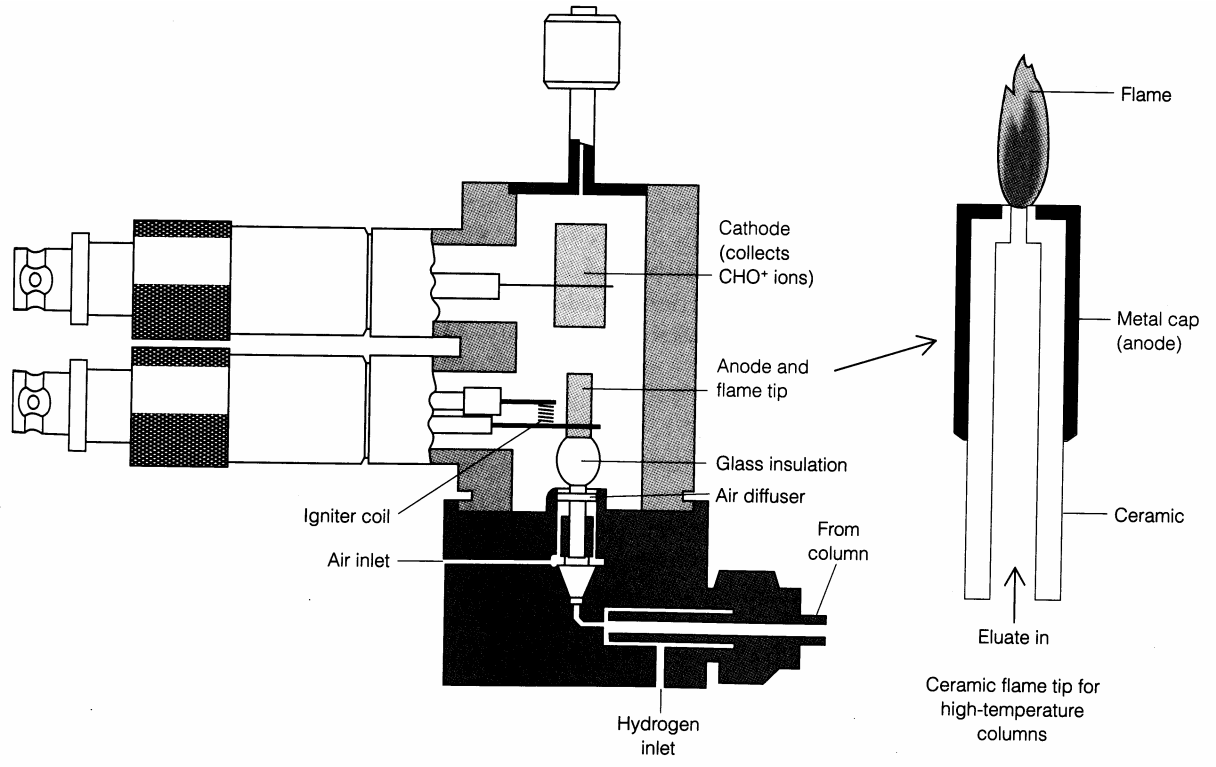
In the **thermal conductivity detector**, gas emerging from the column flows over a hot filament. When solute emerges, the thermal conductivity of the gas stream decreases, the filament gets hotter, its electrical resistance increases, and the voltage drop through the filament changes.

The detector responds to changes in thermal conductivity, so the conductivities of solute and carrier gas should be as different as possible.

Properties of thermal conductivity detector

1. 10^4 linear response range
2. H_2 and He give lowest detection limits
3. sensitivity increases with
   a) increasing filament current
   b) decreasing flow rate
   c) lower block temperature

Flame Ionization Detector



In the flame ionization detector, eluate is burned in a mixture of H_2 and air:

$$\text{sample} + H_2 + \text{air} \rightarrow CH + O \rightarrow CHO^+ + e^-$$

the CHO^+ produced is collected at a cathode above the flame

Properties of flame ionization detector

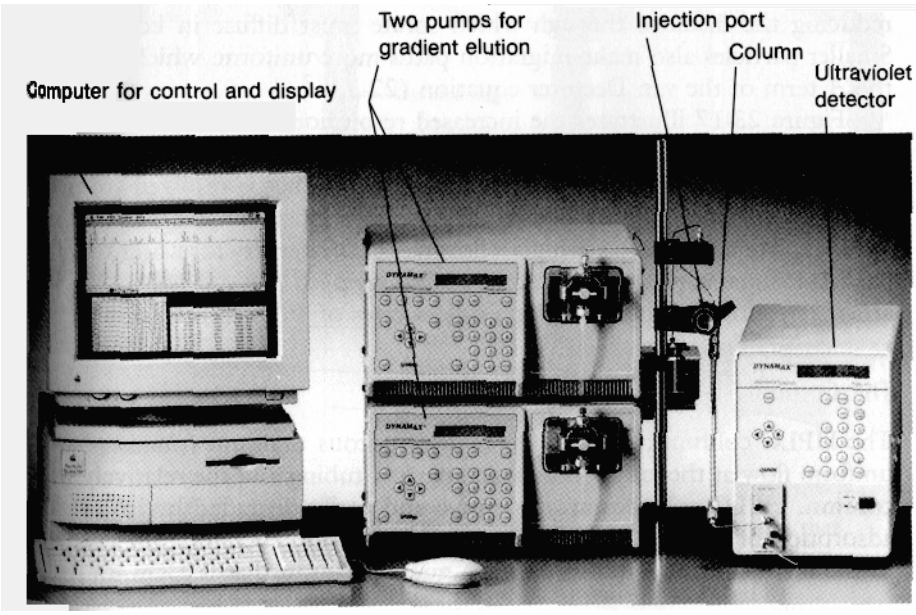
1. N_2 gives lowest detection limit
2. signal is proportional to number of carbon atoms
3. 100-fold lower detection limit than thermal conductivity
4. 10^7 linear response range

Other detectors:

FTIR - Its use provides structural information and computer matching with spectral libraries provide identification of molecular compounds through vibrational spectra.

Mass Spectrometer - A mass spectrometer is a powerful research tool and coupled to a separation technique gives an important dimension to probe research questions and industrial sample testing. Provides structure identification through mass fingerprinting (molecular weight information).

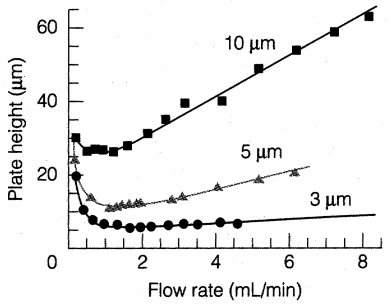
High Performance Liquid Chromatography (HPLC)



HPLC uses high pressure to force eluent through a closed column packed with micron-size particles.

Columns are 5–30 cm in length, with an inner diameter of 1–5 mm.

Common stationary phases provide 50,000 to 100,000 plates per meter.



If solute can diffuse rapidly between the mobile and stationary phases, plate height is decreased and resolution increases.

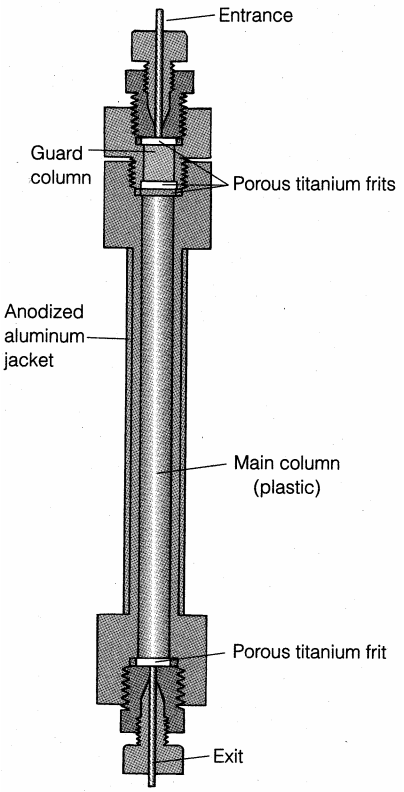
In liquid chromatography, we increase the rate of mass transfer by reducing the dimensions of the stationary phase particles, thereby reducing the distance through which solute must diffuse in both phases.

The Column

HPLC columns are expensive and easily degraded by irreversible adsorption of impurities from samples and solvents. Therefore, the entrance to the main column is protected by a **guard** column that is 1 cm long and contains the same stationary phase as the main column. The guard column collects irreversibly adsorbed solutes and is periodically replaced.

Stationary phases typically use microporous (high surface area) particles of silica to which a bonded phase is attached.

Common Polar Phases		Common Nonpolar Phases	
R = $(CH_2)_3NH_2$	Amino	R = $(CH_2)_{17}CH_3$	Octadecyl
R = $(CH_2)_3C{\equiv}N$	Cyano	R = $(CH_2)_7CH_3$	Octyl
		R = $(CH_2)_3C_6H_5$	Phenyl



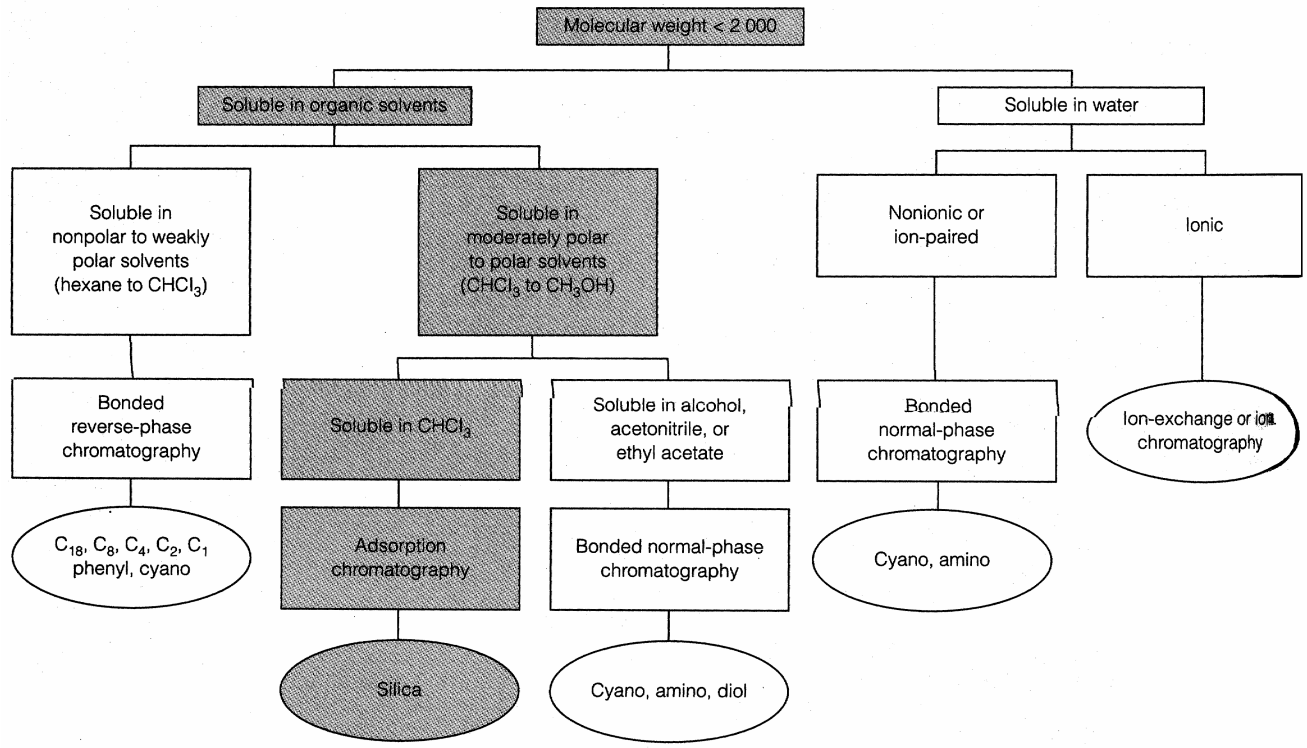
Normal-phase chromatography refers to the use of a polar stationary phase and a less polar solvent

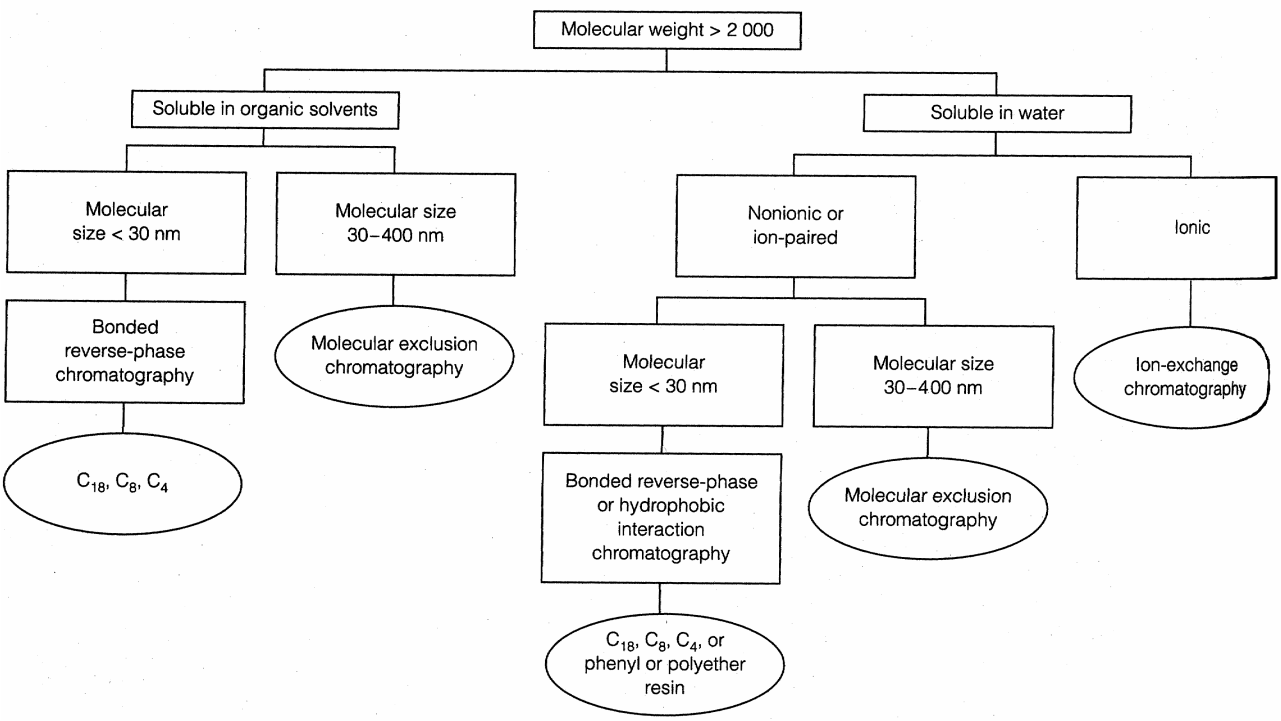
Reverse-phase chromatography refers to the use of a nonpolar or weakly polar stationary phase and a more polar solvent.

Reverse-phase chromatography is more common than normal-phase.

The C_{18} (octadecyl) stationary phase is the most widely used stationary phase in reverse-phase HPLC.

Selecting the Separation Mode in HPLC




Molecular weight > 2 000
Soluble in organic solvents
Soluble in water
Molecular size < 30 nm
Molecular size 30–400 nm
Nonionic or ion-paired
Ionic
Bonded reverse-phase chromatography
Molecular exclusion chromatography
Molecular size < 30 nm
Molecular size 30–400 nm
Ion-exchange chromatography
C_{18}, C_8, C_4
Bonded reverse-phase or hydrophobic interaction chromatography
Molecular exclusion chromatography
C_{18}, C_8, C_4, or phenyl or polyether resin

Solvents

Must be pure and degassed (removal of air bubbles)

Elution can be:

isocratic – one solvent

gradient – continuous change of solvent composition to increase the eluent strength (usually water and some organic solvent)

stepwise – discontinuous change to increase eluent strength

Detectors

An ideal detector is sensitive to low concentrations of every analyte, provides linear response, and does not broaden the eluted peaks.

Detector volumes are typically 1 – 20 μL to reduce peak broadening

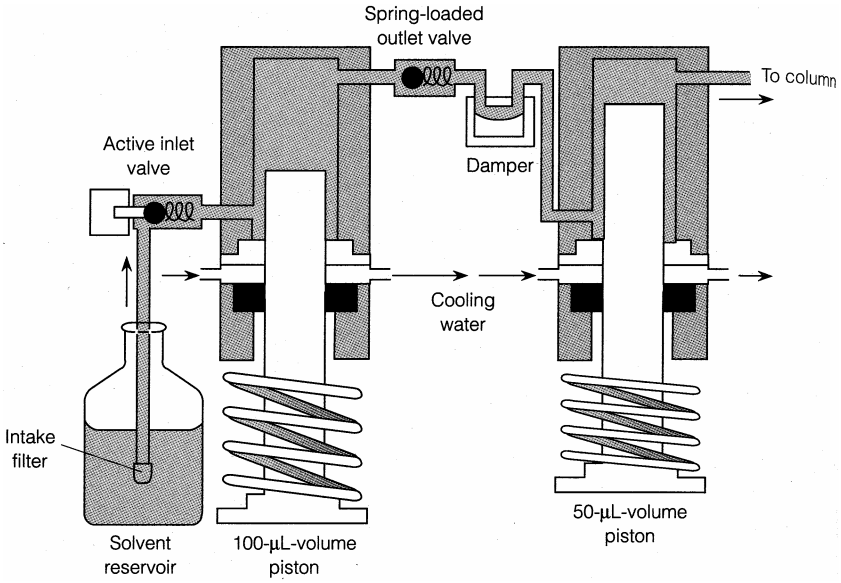
Ultraviolet - Visible detector is most common (monochromator\PMT).

Refractive index detector responds to almost every solute but its detection limit is about 1,000 times poorer than UV. Measures refractive index (n) of solution.

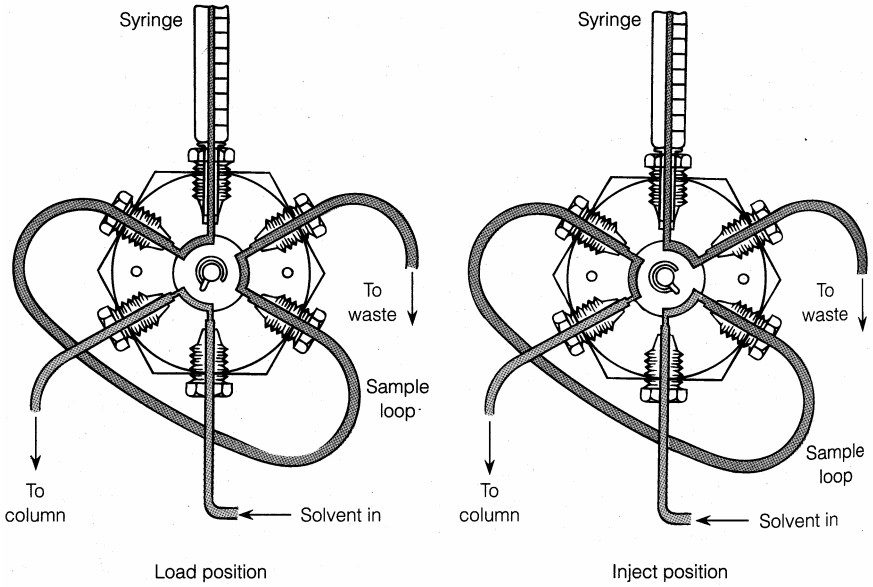
Electrochemical detector responds to analytes that can be oxidized or reduced. Very sensitive detector for ions and charged species.

Mass Spectrometer - used for some biomolecule analysis applications.

Pumps and Injector Valves



Typically HPLC instruments have two pumps which work in tandem. If a gradient mobile phase is employed in order to produce the correct ratio of the mobile phase solvents.



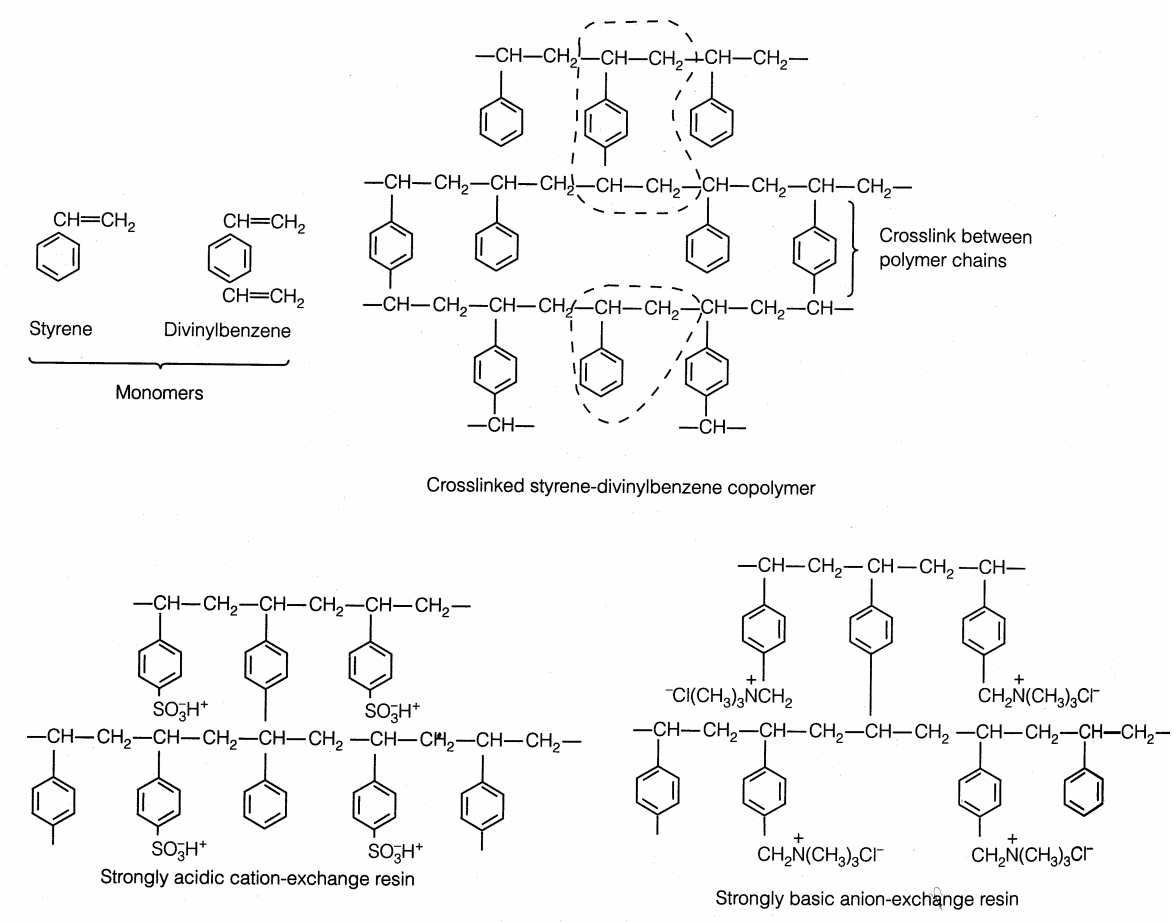
The sample injector portion shown for the HPLC is a closed system. By rotating the injector lever, the analyte is introduced to the mobile phase. The mobile phase carries the analyte on to the HPLC column where the separation begins.

Ion-Exchange Chromatography

In **ion-exchange chromatography**, retention is based on the attraction between solute ions and charged sites bound to the stationary phase.

In **anion exchangers**, positively charged groups on the stationary phase attract solute *anions.*

Cation exchangers contain covalently bound, negatively charged sites that attract solute *cations.*



Ion Exchangers

Resins are amorphous particles of organic material which contain the positively or negatively charged-sites

Ion exchangers are classified as being strongly or weakly acidic or basic

Ion-Exchange Selectivity

Consider the competition of Na^+ and Li^+ for sites on the cation-exchange resin, R^-

$$R^-Na^+ + Li^+ \Leftrightarrow R^-Li^+ + Na^+ \qquad K = \frac{[R^-Li^+][Na^+]}{[R^-Na^+][Li^+]}$$

the equilibrium constant is called the **selectivity coefficient**, because it describes the relative selectivity of the resin for Li^+ and Na^+

In general, ion exchangers favor the binding of ions of higher charge, decreased hydrated radius, and increased polarizability.

$Al^{+3} > Pb^{+2} > K^+ > Na^+ > H^+ > Li^+$

TABLE 24-1 Ion-exchange resins

Resin type	Chemical constitution	Usual form as purchased	Common trade names: Rohm & Haas	Common trade names: Dow Chemical	Selectivity	Thermal stability
Strongly acidic cation exchanger	Sulfonic acid groups attached to styrene and divinylbenzene copolymer	$\phi—SO_3^-H^+$	Amberlite IR-120	Dowex 50W	$Ag^+ > Rb^+ > Cs^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$ $Zn^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+}$	Good up to 150°C
Weakly acidic cation exchanger	Carboxylic acid groups attached to acrylic and divinylbenzene copolymer	$R—COO^-Na^+$	Amberlite IRC-50	—	$H^+ >> Ag^+ > K^+ > Na^+ > Li^+$ $H^+ >> Fe^{2+} > Ba^{2+} Sr^{2+} > Ca^{2+} > Mg^{2+}$	Good up to 100°C
Strongly basic anion exchanger	Quaternary ammonium groups attached to styrene and divinylbenzene copolymer	$\phi—CH_2N(CH_3)_3^+Cl^-$	Amberlite IRA-400	Dowex 1	$I^- >$ phenolate$^- > HSO_4^- > ClO_3^- > NO_3^- > Br^- > CN^- > HSO_3^- > NO_2^- > Cl^- > HCO_3^- > IO_3^- >$ $HCOO^- >$ acetate$^- > OH^- > F^-$	OH^- form fair up to 50°C Cl^- and other forms good up to 150°C
Weakly basic anion exchanger	Polyalkylamine groups attached to styrene and divinylbenzene copolymer	$\phi—NH(R)_2^+Cl^-$	Amberlite IR-45	Dowex 3	$\phi SO_3H >$ citric $> CrO_3 > H_2SO_4 >$ tartaric $>$ oxalic $> H_3PO_4 > H_3AsO_4 > HNO_3 > HI > HBr > HCl > HF > HCO_2H > CH_3CO_2H > H_2CO_3$	Extensive information not available tentatively limited to 65°C

SOURCE: Adapted from J. X. Khym, *Analytical Ion-Exchange Procedures in Chemistry and Biology* (Englewood Cliffs, NJ: Prentice Hall, 1974)

Molecular Exclusion Chromatography

In **molecular exclusion chromatography** (also called gel filtration or gel permeation chromatography), molecules are separated according to their size.

Small molecules penetrate into the small pores in the stationary phase, but large molecules do not.

Because small molecules must pass through an effectively larger volume in the column, large molecules are eluted first