Liquid Chromatography (Chapter 28):

Four types of high performance liquid chromatography (HPLC):

- partition
- adsorption (liquid-solid)
- ion exchange
- size exclusion or gel

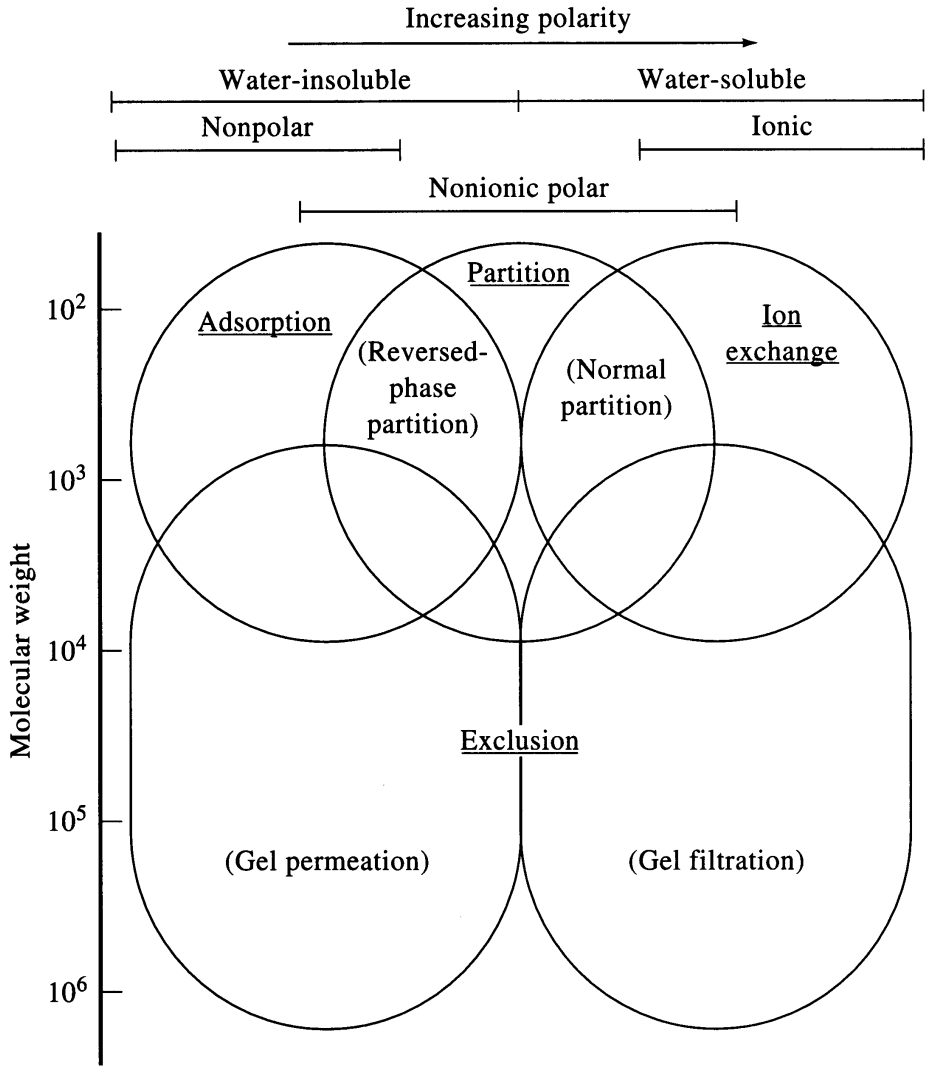


Fig 28-1:

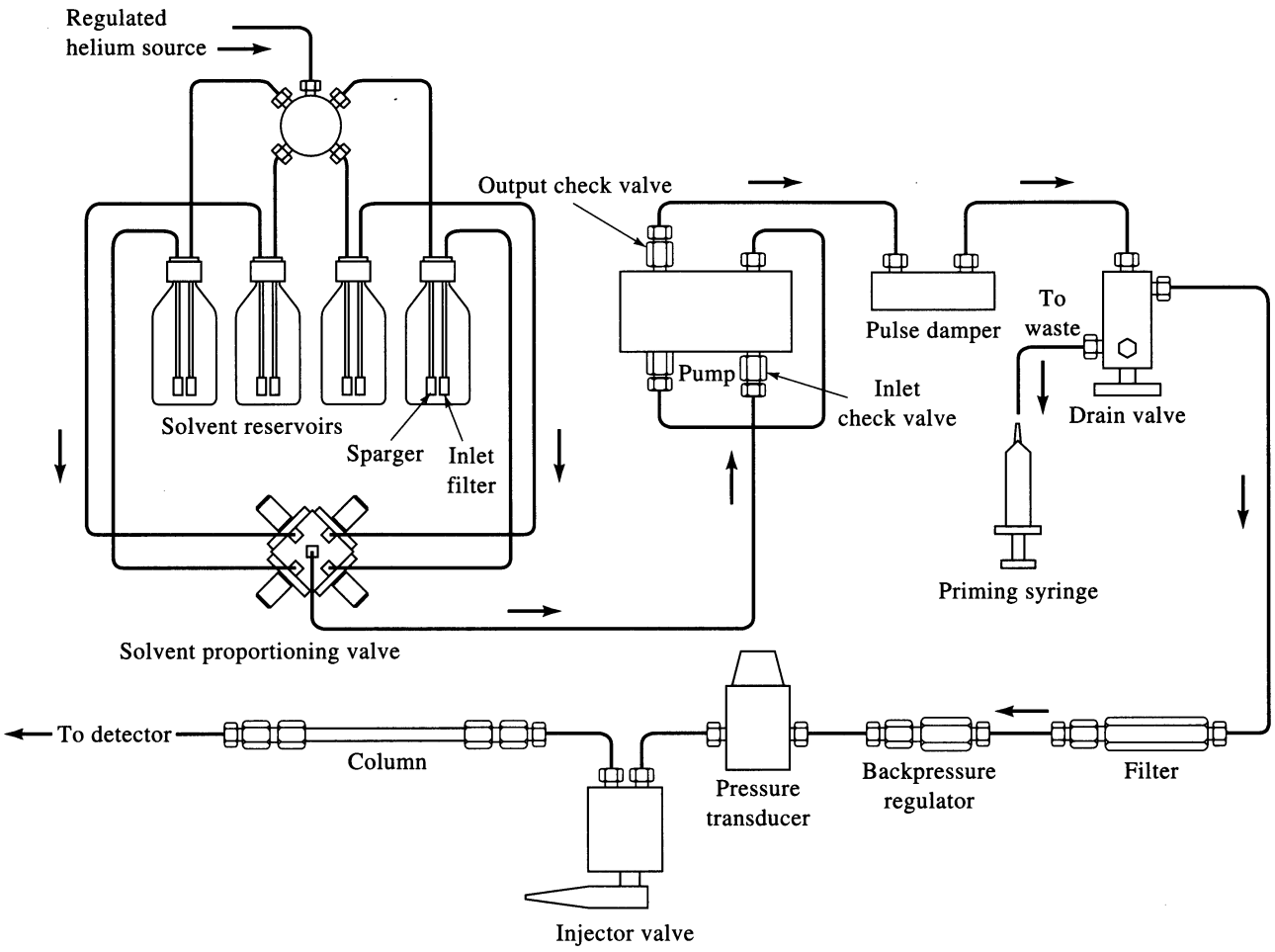
Instrumentation for HPLC:

- 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

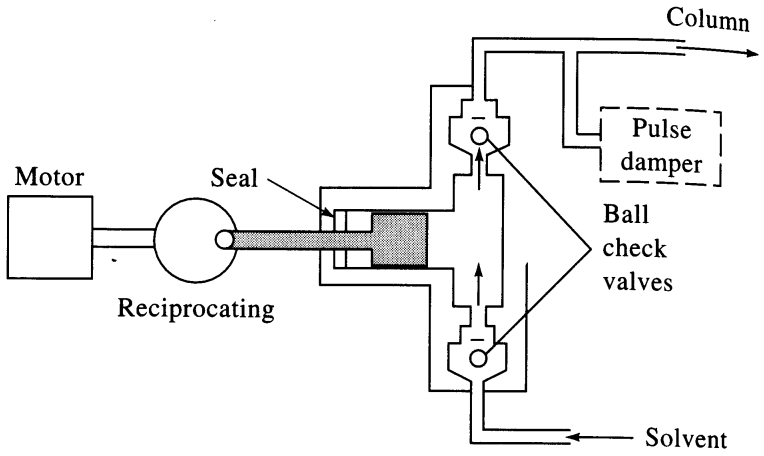
Single mobile phase composition - isocratic elution

Programmed mobile phase composition - gradient elution

Fig 28-4

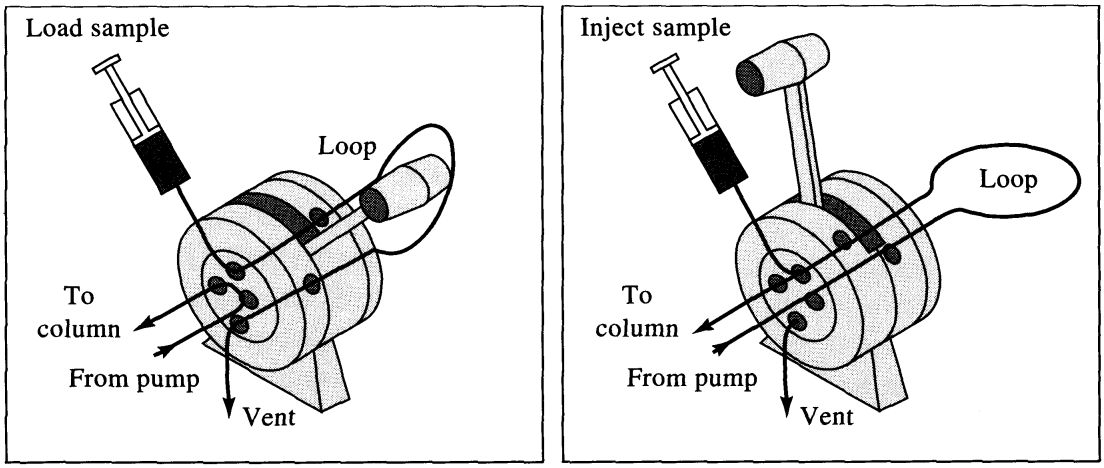


Reciprocating Pump (Fig 28-6):



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

Sample injection (Fig 28-7):



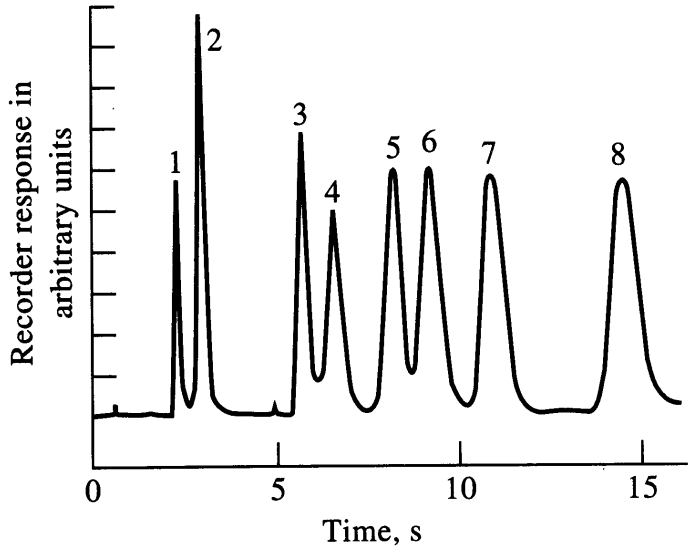
- Similar to FIA, GC
- Introduce small sample (0.1-100 μL) without depressurization
- Microsyringe/septum system (only <1500 psi)

HPLC Columns:

- Stainless steel
- 10-30 cm long
- 4-10 mm internal diameter
- 1-10 μm particle size - 40,000-60,000 plates/m

High Speed Isocratic Separation (Fig 28-8):

- 100,000 plates/m



Gradient Elution:

- Solvent polarity (composition) continuously varied or stepped

Fig 28-5

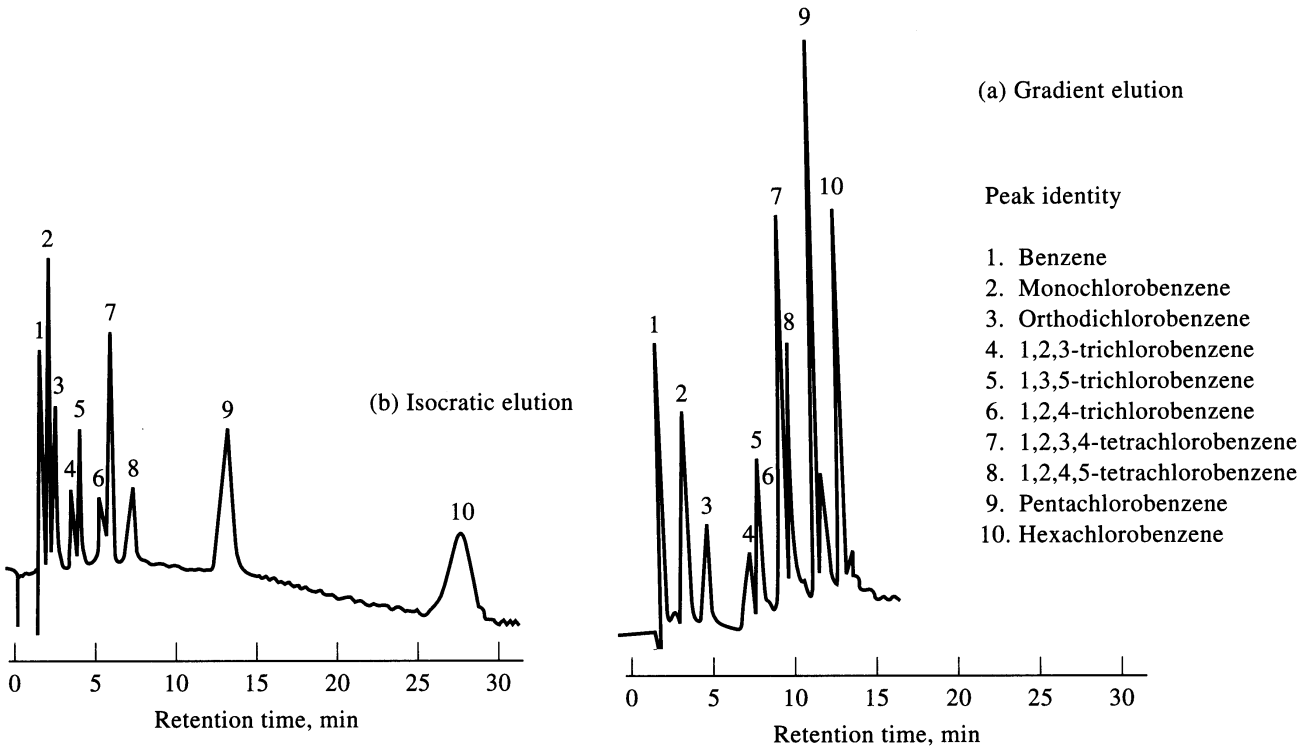
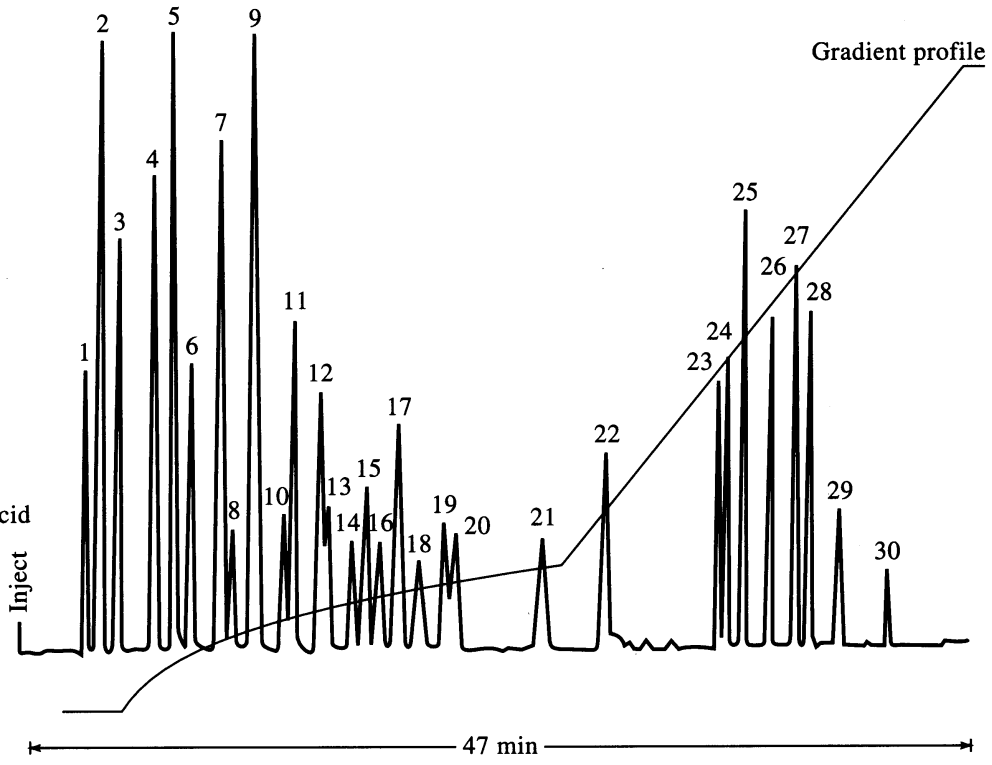


Figure 28-18:

1. Phosphoserine
2. Aspartic acid
3. Glutamic acid
4. α-Amino adipic acid
5. Asparagine
6. Serine
7. Glutamine
8. Histidine
9. Glycine
10. Threonine
11. Citrulline
12. 1-Methylhistidine
13. 3-Methylhistidine
14. Arginine
15. β-Alanine
16. Alanine
17. Taurine
18. Anserine
19. β-Aminobutyric acid
20. β-Aminoisobutyric acid
21. Tyrosine
22. α-Aminobutyric acid
23. Methionine
24. Valine
25. Tryptophan
26. Phenylalanine
27. Isoleucine
28. Leucine
29. δ-Hydroxylysine
30. Lysine



Detectors:

All properties previously discussed and

- small internal volume to reduce zone broadening

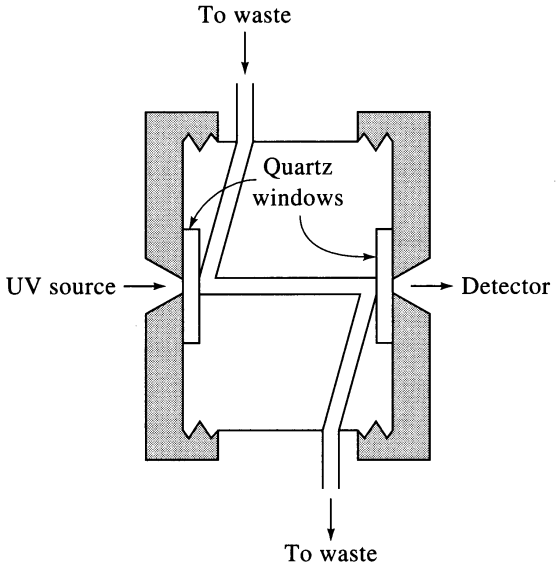
Bulk property detectors - measure property of mobile phase (refractive index, dielectric constant, density)

Solute property detectors - measure property of solute not present in mobile phase (UV absorbance, fluorescence, IR absorbance)

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

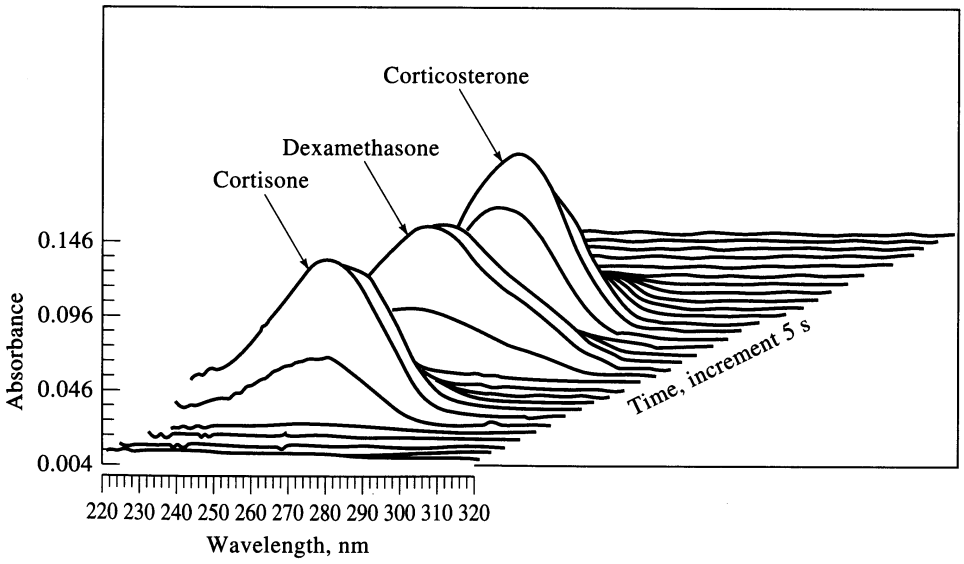
UV-Vis Detection for HPLC (Fig 28-9):



sources:
- single line (arc or hollow cathode lamp, laser)
- continuum (Xe, D_2 lamp)

detector:
- photodiode/photomultiplier tube
- photodiode array

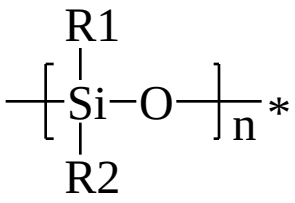
Combination of separation and analysis (GC-MS, HPLC-UV-Vis) - very powerful (Fig 28-10)



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

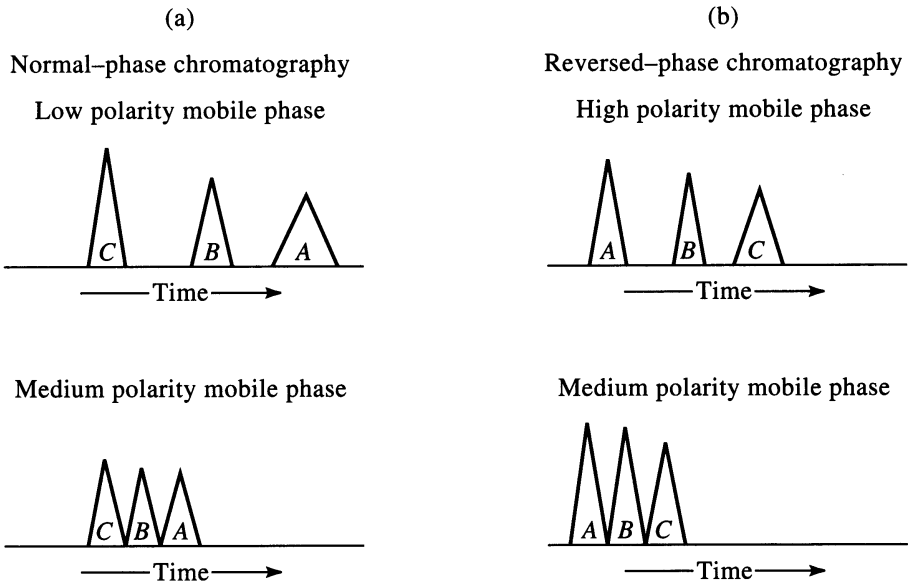


Normal phase HPLC nonpolar solvent/polar column

Reversed phase HPLC polar solvent/nonpolar column

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

Fig 28-14

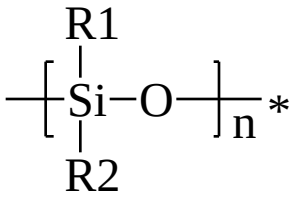


Solute polarities: $A > B > C$

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

R is C_8 or C_{18} hydrocarbon (Fig 28-15)

faster elution higher resolution



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

$$P'_{AB} = \varphi_A P'_A + \varphi_B P'_B$$

fraction in mixture

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

best resolution/time when k' = 2-5

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

Table 28-2:

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
l-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 · s · 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.

Size Exclusion Chromatography (Gel Permeation):

- 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:

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 ($K=c_s/c_m$)

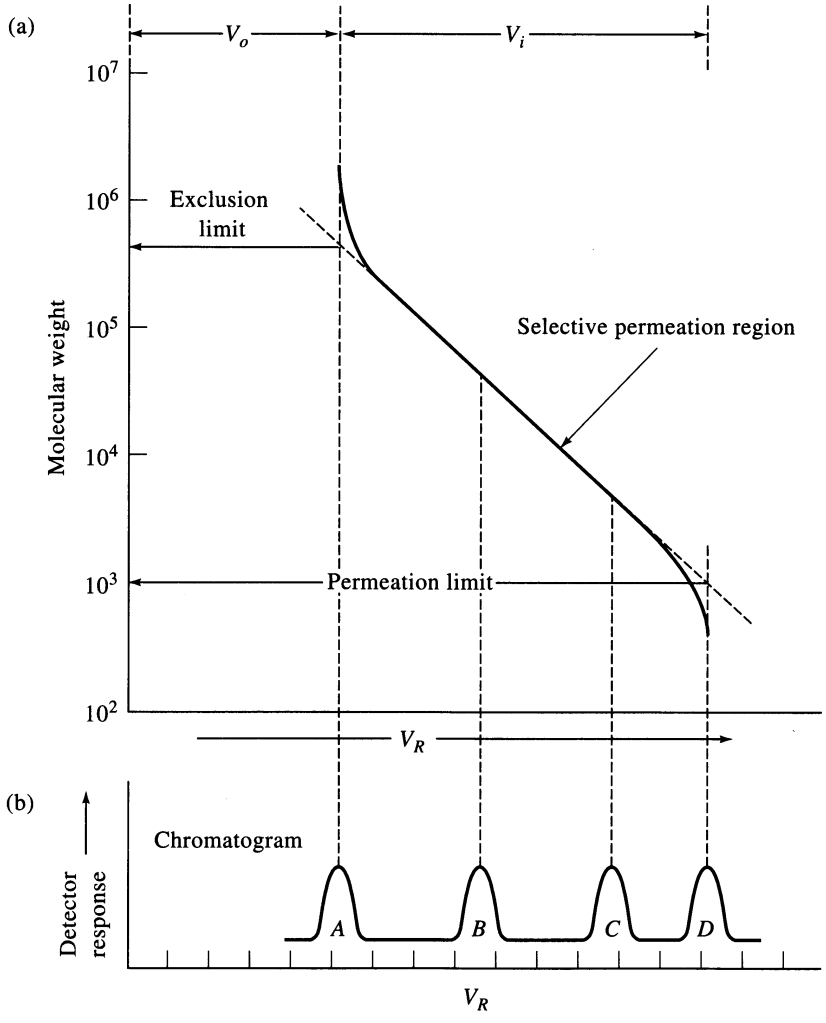


Fig 28-27

- Separation of proteins/peptides, sugars, determination of polymer molecular weight distribution