**Liquid Chromatography**

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

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

![Diagram showing comparison between Larger and Small dp](image)

In the diagram, "H" represents the height of the elution profile, and "µ" represents the flow rate, illustrating the effect of particle size on the separation performance in liquid chromatography.
Components of HPLC system:

- Mobile phase reservoir
- Pump--capable of forcing fluid through column with small $d_p$
- Injection valve--25-100 µl
- Column---usually stainless steel 10-25 cm in length---4 mm i.d. (analytical columns)---Can also have preparative columns--much wider and longer!
- Detector--
  - UV-Vis absorbance
  - Refractive index
  - Fluorescence
  - Electrochemical
  - Post-column rxn with one of above

Must always be concerned with dead volume of detector--don’t want extra peak broadening in detector
Figure 28-2  Effect of particle size of packing and flow rate upon plate height $H$ in liquid chromatography. Column dimensions: 30 cm × 2.4 mm. Solute: N,N-diethyl-$n$-aminoazobenzene. Mobile phase: mixture of hexane, methylene chloride, isopropyl alcohol. (From R. E. Majors, J. Chromatogr. Sci., 1973, 11, 92. With permission.)
Figure 28-4  Schematic of an apparatus for HPLC.  (Courtesy of Perkin-Elmer Corporation, Norwalk, CT)
Figure 28-7 A sampling loop for liquid chromatography. (Courtesy of Beckman Instruments, Fullerton, CA.) With the valve handle as shown on the left, the loop is filled from the syringe, and the mobile phase flows from pump to column. When the valve is placed in the position on the right, the loop is inserted between the pump and the column so that the mobile phase sweeps the sample onto the column.
Detectors for HPLC

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

- Try to use detectors w/ <10uL volume

Column Packings
- Normal phase
- Reversed-phase
- Ion-exchange
- Size exclusion

Normal phase
- Silica gel or Alumina (Al2O3)
- Adsorb polar molecules
- Use non-polar mobile phase
<table>
<thead>
<tr>
<th>LC Detector</th>
<th>Commercially Available</th>
<th>Mass LOD (commercial detectors)*</th>
<th>Mass LOD (state of the art)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>Yesc</td>
<td>100 pg–1 ng</td>
<td>1 pg</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>Yesc</td>
<td>1–10 pg</td>
<td>10 fg</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Yesc</td>
<td>10 pg–1 ng</td>
<td>100 fg</td>
</tr>
<tr>
<td>Refractive index</td>
<td>Yes</td>
<td>100 ng–1 µg</td>
<td>10 ng</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Yes</td>
<td>500 pg–1 ng</td>
<td>500 pg</td>
</tr>
<tr>
<td>Mass spectrometry</td>
<td>Yesd</td>
<td>100 pg–1 ng</td>
<td>1 pg</td>
</tr>
<tr>
<td>FT–IR</td>
<td>Yesd</td>
<td>1 µg</td>
<td>100 ng</td>
</tr>
<tr>
<td>Light scatteringe</td>
<td>Yes</td>
<td>10 µg</td>
<td>500 ng</td>
</tr>
<tr>
<td>Optical activity</td>
<td>No</td>
<td></td>
<td>1 ng</td>
</tr>
<tr>
<td>Element selective</td>
<td>No</td>
<td></td>
<td>10 ng</td>
</tr>
<tr>
<td>Photoionization</td>
<td>No</td>
<td></td>
<td>1 pg–1 ng</td>
</tr>
</tbody>
</table>

*Mass LOD is calculated for injected mass that yields a signal equal to five times the noise, using a mol wt of 200 g/mol, 10 µL injected for conventional or 1 µL injected for microbore LC.

Same definition as a, above, but the injected volume is generally smaller.

Commercially available for microbore LC also.

Commercially available, yet costly.

Including low-angle light scattering and nephelometry.

(From E. S. Yeung and R. E. Symovec, Anal. Chem., 1986, 58, 1238. With permission.)
Figure 28-9 Ultraviolet detector cell for HPLC.
Figure 28-13  Amperometric thin-layer detector cell for HPLC.
Figure 28-12  Potentially detectable organic functional groups by amperometric measurements. The horizontal lines show the range of oxidation or reduction potentials wherein compounds containing the indicated functional groups are electroactive.
Final coating - 3.5 mmol/m² of alkyl groups

Note: not drawn to scale

Particles - 3 - 10 μm silica

Monolayer of alkyl chains ≈ 30 Å for C₁₈

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

* Can immobilize phenyl groups for ZY or A separations; Chiral molecules for Chiral separations
Key advantage of reversed phase systems — since most samples are aqueous-based — can inject directly onto column w/o prior extraction step.

Mobile Phases (H₂O, MeOH, acetonitrile, etc.)
- Must degas — don’t want bubbles forming in column or detector

- Two modes of operation:
  - **Isocratic**: same composition of mobile phase ceased throughout separation
  - **Gradient Elution**: Since \( k = k_u + K_u \)
    - Use change in mobile phase composition during separation to change \( K_u \) values — to speed analysis time

\[
K_B = \frac{C_s}{C_B}
\]

\[
K_A \ll K_B
\]
Figure 28-16  Systematic approach to the separation of six steroids. The use of water to adjust $k'$ is shown in (a) and (b). The effects of varying $a$ at constant $k'$ are shown in (b), (c), (d), and (e). Column: 0.4 x 150 mm packed with 5 μm C8 bonded, reversed-phase particles. Temperature: 50°C. Flow rate: 3.0 cm³/min. Detector: UV 254 nm. THF = tetrahydrofuran. CH₃CN = acetonitrile. Compounds: (1) prednisone, (2) cortisone, (3) hydrocortisone, (4) dexamethasone, (5) corticosterone; (6) corteroxolone. (Courtesy of DuPont Instrument Systems, Wilmington, DE.)
$K_b$ will change with polarity of mobile phase

Step change in mobile phase composition

Condensed chromatogram - allows more rapid determinations for multicomponent analysis

Linear gradient
Can have binary or ternary solvent capabilities

\[ x\% A, y\% B, z\% C \]

A

B

C

Pump

Microprocessor controlled nozzles - Control mobile phase composition

Must be careful using gradient elution on complex samples - can eliminate resolution of given solutes

Isocratic

\[
\begin{array}{c}
\text{time} \\
\hline
\end{array}
\]

\[
\begin{array}{c}
\text{time} \\
\hline
\end{array}
\]

Another form of L.C. - planar chromatography

- Thin layer (reversed normal)
- Paper - Normal phase - cellulose likes polar solutes
(a) Gradient elution

Peak identity

1. Benzene
2. Monochlorobenzene
3. Orthodichlorobenzene
4. 1,2,3-trichlorobenzene
5. 1,3,5-trichlorobenzene
6. 1,2,4-trichlorobenzene
7. 1,2,3,4-tetrachlorobenzene
8. 1,2,4,5-tetrachlorobenzene
9. Pentachlorobenzene
10. Hexachlorobenzene

(b) Isocratic elution

Retention time, min

HPTLC → use small diameter particles to coat thin layer plate — get more N per given length

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

Reflective — absorbance:

Source

Q-detector

\[ I_s = I_{in} - I_{abs} - I_{trans} \]

\[ \text{due to conc. of solute on spot of plate} \]

Move plate to scan —

Abs

\[ \text{distance} \]
Ion-Exchange Chromatography

Cations - organic + metal ions (Na⁺, Ca²⁺, K⁺)

* Anions - organic + Cl⁻, PO₄³⁻, SO₄²⁻, Br⁻, etc

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

For cations - stationary phase has negative sites (fixed)

\[
\text{H}^+ + \text{SO}_3^- \leftrightarrow \text{H}^+ \text{SO}_3^- \\
\text{Sulfonic acid exchangers}
\]

\[
R-\text{SO}_3\text{H}^+ + M^+_m \rightleftharpoons R-\text{SO}_3^-M^+_m + \text{H}^+_m
\]

or

\[
R\text{H}^+ + M^+_m \rightleftharpoons R^-M^+_m + \text{H}^+_m
\]

Selectivity coefficient, \( K_{\text{select}} = \frac{[R^-M^+]_m}{[R\text{H}^+]} \cdot \frac{[\text{H}^+_m]}{[M^+_m]} \)

Solve for \( \frac{[RM^+_m]}{[RH^+_m]} = \frac{C_{M^+_m}}{C_{M^+_m}} = \frac{K_{\text{select}}}{K_{\text{H}^+_m} + [H^+_m]} = K
\]

\[ K \times K \leq [RH^+] + [H^+_m] \text{ are kept constant.} \]
Figure 28-21 Structure of a cross-linked polystyrene ion-exchange resin. Similar resins are used in which the $\text{--SO}_3^–\text{H}^+$ group is replaced by $\text{--COO}^–\text{H}^+$, $\text{--NH}_3^+\text{OH}^-$, and $\text{--N(CH}_3)_3^+\text{OH}^-$ groups.
if resin (stationary phase) has lots of exchange sites (high \([\text{AsO}_3^-]\)) compared to \([M]\) in sample – then \([\text{H}^+]\) does not change appreciably

\[
\frac{R_m}{\text{H}^+} \text{ depends on:}
\]

1. Charge on \(M^+\) \(+3 > +2 > +1\)
   Electrostatics

2. Ion size – \(R_m/\text{H}^+\) ↑ for larger ion

Two reasons:

a) Smaller solvent equivalent volume for large ions

b) Larger ions have greater \(\mu\)-polarizability – \(\mu\) in outer shells less influenced by nucleus
Anion Exchangers

\[
\text{Particle} \quad \text{R}^+ \text{R} \quad \text{R}^+ \text{R}^+ \text{R}^+ \text{R}^+ \text{R}^+ \text{R}^+ \text{R}^+ \text{R}^+ \text{R}^+ \text{R}^+
\]

\[
R+^{+} + H^+ \leftrightarrow R+^{+} + H^+
\]

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

E.g. Cation chromatography

![Graph with peaks for Li\(^{+}\), Na\(^{+}\), K\(^{+}\), Rb\(^{+}\), Ca\(^{2+}\)]

How do we detect ions eluting?

Use conductivity detector for inorganic ion chromatography
Conductance = $\frac{1}{R_{sol}} = G$

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

Can solve with **Stripper column or Membrane exchanger**

\[ \begin{array}{c}
\text{HCl} \\
\text{Na}^+ \quad \text{K}^+ \\
\text{Cation Exchange Column} \\
\text{Stripper Column or Membrane} \\
\text{Conductivity Detector}
\end{array} \]

Anion Exchanger in $OH^-$ form

\[ \begin{array}{c}
\text{Form solution} \quad \text{HCl} \\
\text{Na}^+ \quad \text{Cl}^-
\end{array} \]

Greatest Effect in Ion-Chrom.

- Vary pH of mobile phase
- Vary salt - increases; decreases electrostatic interactions
Gel Permeation/Size Exclusion Chrom

- Used primarily for preparative work
- Separate solutes based on size + shape - These factors determine "k"

Stationary phase: consists of porous particles - crosslinked polysaccharides - degree of crosslinking determines pore size

- Porous glass beads - for HPLC Type systems
- Can have phases with different pore sizes

\[ V_i = \text{Volume of mobile phase inside pores} \]
\[ V_o = \text{Volume of mobile phase outside pores} \]

\[ S = \text{Solute} \]
\[ S - \text{larger} \]
\[ s - \text{small} \]
If solute is small enough so that all \( V_i \) is accessible to it — Then:
\[
V_R = V_0 + V_i
\]
If \( S \) is so large it cannot enter pores at all — Then:
\[
V_R = V_0 = V_m
\]
No retention on column.

However — For given stationary phase — pore sizes cannot be precisely controlled

**: You get fractionation range for solutes — from those that can't enter any \( V_i \) — to those that can enter all \( V_i \).

\[
\text{Fractionation range} = V_0 \rightarrow V_0 + V_i
\]

\[
V_R = V_0 + K V_i
\]

If \( S \) is large —
\( K \) is small

If \( S \) is small \( K \rightarrow 1 \)
\[
0 \leq K \leq 1
\]
Purchase stationary phases with varying fractionation ranges -
E.g. 700 - 1500 MW
1500 - 20,000
to 20,000 - 100,000
etc.

**Results:**

![Diagram with peaks labeled >1500 and <700 ml eluent]

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

![Diagram with peaks labeled >10,000, 80,000, 40,000, and <20,000 ml eluent]
G.C. - Principles & Instrumentation

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

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

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

See Fig. 22-1 for schematic of system - injection system 50°C hotter than column temp.

See Table 22-1 for types of chromatographic columns
Figure 27-1  Schematic of a gas chromatograph.
<table>
<thead>
<tr>
<th></th>
<th>FSOT</th>
<th>WCOT</th>
<th>SCOT</th>
<th>Packed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, m</td>
<td>10–100</td>
<td>10–100</td>
<td>10–100</td>
<td>1–6</td>
</tr>
<tr>
<td>Inside diameter, mm</td>
<td>0.1–0.53</td>
<td>0.25–0.75</td>
<td>0.5</td>
<td>2–4</td>
</tr>
<tr>
<td>Efficiency, plates/m</td>
<td>2000–4000</td>
<td>1000–4000</td>
<td>600–1200</td>
<td>500–1000</td>
</tr>
<tr>
<td>Total plates</td>
<td>$(20–400) \times 10^3$</td>
<td>$(10–400) \times 10^3$</td>
<td>$(6–120) \times 10^3$</td>
<td>$(1–10) \times 10^3$</td>
</tr>
<tr>
<td>Sample size, ng</td>
<td>10–75</td>
<td>10–100</td>
<td>10–100</td>
<td>10–10^6</td>
</tr>
<tr>
<td>Relative back pressure</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Relative speed</td>
<td>Fast</td>
<td>Fast</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>Chemical inertness</td>
<td>Best</td>
<td></td>
<td></td>
<td>Poorest</td>
</tr>
<tr>
<td>Flexible?</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*FSOT: Fused-silica, open tubular column.
WCOT: Wall-coated, open tubular column.
SCOT: Support-coated open tubular column.
Figure 27-5  Effect of temperature on gas chromatograms: (a) isothermal at 45°C; (b) isothermal at 145°C; (c) programmed at 30° to 180°C.  (From W. E. Harris and H. W. Habgood, Programmed Temperature Gas Chromatography, p. 10. New York: Wiley, 1966. Reprinted by permission of John Wiley & Sons, Inc.)
### TABLE 27-2  Some Common Stationary Phases for Gas-Liquid Chromatography

<table>
<thead>
<tr>
<th>Stationary Phase</th>
<th>Common Trade Name</th>
<th>Maximum Temperature, °C</th>
<th>Common Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polydimethyl siloxane</td>
<td>OV-1, SE-30</td>
<td>350</td>
<td>General-purpose nonpolar phase; hydrocarbons; polynuclear aromatics; drugs; steroids; PCBs</td>
</tr>
<tr>
<td>Poly(phenylmethylidimethyl) siloxane (10% phenyl)</td>
<td>OV-3, SE-52</td>
<td>350</td>
<td>Fatty acid methyl esters; alkaloids; drugs; halogenated compounds</td>
</tr>
<tr>
<td>Poly(phenylmethyl) siloxane (50% phenyl)</td>
<td>OV-17</td>
<td>250</td>
<td>Drugs; steroids; pesticides; glycols</td>
</tr>
<tr>
<td>Poly(trifluoropropylidimethyl) siloxane</td>
<td>OV-210</td>
<td>200</td>
<td>Chlorinated aromatics; nitroaromatics; alkyl-substituted benzenes</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>Carbowax 20M</td>
<td>250</td>
<td>Free acids; alcohols; ethers; essential oils; glycols</td>
</tr>
<tr>
<td>Poly(dicyanoallyldimethyl) siloxane</td>
<td>OV-275</td>
<td>240</td>
<td>Polyunsaturated fatty acids; rosin acids; free acids; alcohols</td>
</tr>
</tbody>
</table>
Figure 27-7  Schematic of (a) a thermal conductivity detector cell, and (b) an arrangement of two sample detector cells and two reference detector cells.  *(From J. V. Hinshaw, LC-GC, 1990, 8, 298. With permission.*)
Figure 27-6 A typical flame ionization detector. (Courtesy of Hewlett-Packard Company.)
Figure 27-8  A schematic of an electron-capture detector.
Figure 27-13  Schematic of a typical capillary gas chromatography/mass spectrometer.