Chapter 26: An Introduction to Chromatographic Separations

- Column Chromatography
- Migration Rates
  - Distribution Constants
  - 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
Chromatography:
- Sample transported by mobile phase:
  - Electrostatic or van der Waals' force
- 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 $\propto$ fraction time spent in mobile phase

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

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 $t_0$
- 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
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
• $A_{\text{mobile}} \leftrightarrow A_{\text{stationary}}$

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

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

Similarly if define $\mu$ as average linear rate of movement of molecules of mobile phase

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

Relating retention time $t_R$ to $K = C_s/C_M$

\[ \bar{v} = \mu \times \text{fraction of time analyte is in mobile phase} \]

\[ \bar{v} = \mu \times \frac{\text{moles of analyte in mobile phase}}{\text{number of moles of analyte}} \]

\[ \bar{v} = \mu \times \frac{C_M V_M}{C_M V_M + C_s V_s} = \mu \times \frac{1}{1 + C_s V_s/C_M V_M} \]

Substituting $K = C_s/C_M$

Gives

\[ v = \bar{v} \times \frac{1}{1 + K V_s/V_M} \]
More useful relationships - capacity factor $k'$ (comes from K) K in concentration, $k'$ in moles

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

So for A $\rightarrow$ $k'_A = \frac{K_A V_s}{V_M} = \frac{n_s}{n_M}$ $n = \# \text{ of moles}$

From previous slide

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

From previous equation $\rightarrow$

$$\bar{v} = \mu x \frac{1}{1 + k'_A}$$

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

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

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 $\leq 1.0$, 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 ($\alpha$) 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 $\alpha$ = 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)
- $K = \frac{C_{\text{org}}}{C_{\text{water}}}$
- Chromatographic column can be thought of in the same way (only continuous process)
- $K = \frac{C_s}{C_M}$

- Divide chromatographic column up into steps or segments called theoretical plates
- The theoretical concept is that these theoretical plates are equilibrium units for $K = \frac{C_s}{C_M}$
- 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
Gaussian peaks – statistical distribution of molecules

\[ W_b = 4\sigma \]

Gaussian distribution (bell curve)
\[ W = 4\sigma \]
Can derive \( N = \text{number of plates} \)

\[
N = 16 \left( \frac{t_R}{W_b} \right)^2 \quad W_b = \text{base width}
\]

\[
N = 16 \left( \frac{t_R}{4\sigma} \right)^2 = \left( \frac{t_R}{\sigma} \right)^2
\]

\[
N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2 \quad W_{1/2} = \text{width at half height}
\]

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)

\[ H = \frac{B}{\mu} + C\mu + A \]

van Deemter Equation

A, B & C are coefficients, \( \mu = \) 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 \[ H_L = \frac{B}{\mu} \]

- \[ t = 0 \]
- \[ 0 < t < t_R \]
- \[ t_R \]

\[ \sigma_L^2 = 0 \]
\[ \sigma_L^2 = 2 D_M t_M \]

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

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>t₁</th>
<th>t₂</th>
<th>t₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary Phase</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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
Putting it all together
Van Deemter
Overall

Finding optimum

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 $2X \mu_{\text{opt}}$

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)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Usual Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear velocity of mobile phase</td>
<td>$u$</td>
<td>cm s$^{-1}$</td>
</tr>
<tr>
<td>Diffusion coefficient in mobile phase</td>
<td>$D_M$</td>
<td>cm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>Diffusion coefficient in stationary phase</td>
<td>$D_S$</td>
<td>cm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>Retention factor (Equation 26-8)</td>
<td>$k'$</td>
<td>unitless</td>
</tr>
<tr>
<td>Diameter of packing particle</td>
<td>$d_p$</td>
<td>cm</td>
</tr>
<tr>
<td>Thickness of liquid coating on stationary phase</td>
<td>$d_L$</td>
<td>cm</td>
</tr>
</tbody>
</table>

**TABLE 26-3  Kinetic Processes That Contribute to Peak Broadening**

<table>
<thead>
<tr>
<th>Process</th>
<th>Term in Equation 26-19</th>
<th>Relationship to Column* and Analyte Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple flow paths</td>
<td>$A$</td>
<td>$A = 2\lambda_d p$</td>
</tr>
<tr>
<td>Longitudinal diffusion</td>
<td>$B/u$</td>
<td>$B/u = 2\gamma D_M$</td>
</tr>
<tr>
<td>Mass transfer to and from liquid stationary phase</td>
<td>$C_{GL}$</td>
<td>$C_{GL} = \frac{f_d(k')d_p^2}{D_S}$</td>
</tr>
<tr>
<td>Mass transfer in mobile phase</td>
<td>$C_{GM}$</td>
<td>$C_{GM} = \frac{f_d(k')d_p^2}{D_M}$</td>
</tr>
</tbody>
</table>
This brings us to Resolution ($R_s$) = Measure of columns ability to separate 2 analytes

Note $\Delta Z =$ 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 $N \rightarrow$ this also increases time for analysis
Avoid values above 10

**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 $k'_B$. 

(a) 70% Methanol/30% water  
(b) 60% Methanol/40% water  
(c) 50% Methanol/50% water  
(d) 40% Methanol/60% water
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
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)
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
Discuss components starting here

**Figure 27.1** Schematic of a gas chromatograph.

**Carrier gas:** He (common), N₂, H₂

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)
Carrier gases (mobile phase) – must be chemically inert He, Ar, N₂, CO₂ even H₂ and mixtures 95/5 N₂/CH₄

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) O₂ ppm Hc

The move today is away from gas cylinders toward gas generators (extract pure carrier gas from air)
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
Next is gas flow control in this region.

Figure 27-2  A soap-bubble flow meter. (Courtesy of Chrompack Inc., Raritan, N. J.)
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 °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

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 27-4  A rotary sample valve: valve position (a) for filling sample loop ACB  
and (b) for introduction of sample into column.
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 \( PV \) is proportional to \( T \)
Sometimes use retention volumes \((V_R, V_M)\)

\[
V_R = t_R F \quad \text{for retained species} \quad t_R = \text{retention time}
\]
\[
V_M = t_M F \quad \text{for unretained} \quad t_M = \text{retention time}
\]
\( F = \text{flow rate} \)

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 \( P_i = \text{inlet pressure} \) &
\( P = \text{outlet pressure (atmospheric)} \)
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 °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 H₂ 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 – H₂O, CO₂, SO₂, NOₓ

FID exhibits
- High sensitivity (as low as 10⁻¹³ g/s)
- Large linear response range (10⁷)
- 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 & H₂ are about 6 to 10 times greater than most organic compounds (must use these carrier gases)
- Other carrier gases (N₂, 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 $\beta$ emitter (radioactive) like $^{63}\text{Ni}$ foil or $^3\text{H}_2$ adsorbed on Pt or Ti foil
- $\beta$ particles (i.e. electrons) hit carrier gas (usually $\text{N}_2$) 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 °C
- Produces large number of ions with N & P
Flame Photometric Detector (FPD)

- Selective for P & S compounds
- Again sample goes through H₂/air flame
- Observe optical emission of HPO at 510 nm & 526 nm & S₂ 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**
- 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

![Figure 27-10](Image) 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 – 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 effluent 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 most information for research or method development
2) Total ion current – sum signal for all ions as one large signal – highest sensitivity
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

![Diagram of GC-FTIR setup](image)

**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 (SiO₂), clay, carbon particles, glass microbeads, polymer
- Diameter 150-250 µm (60-100 mesh) 1 m²/g
- Thin coating of liquid stationary phase
| TABLE 27-1  Properties and Characteristics of Typical Gas-Chromatographic Columns |
|---------------------------------|----------------|----------------|----------------|
| | 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) × 10^3 | (10–400) × 10^3 | (6–120) × 10^3 | (1–10) × 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 | No | No | Poorest |
| Flexible? | Yes | No | No | No |

*FSOT: Fusion-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 |
| Polymethylsiloxane | OV-1, SE-30 | 350 | General-purpose nonpolar phase; hydrocarbons, polynuclear aromatics; drugs; steroids; PCBs |
| Poly(phenylmethylsiloxane) (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(trifluoropropylsiloxane) | OV-210 | 200 | Chlorinated aromatics; nitroaromatics; alkyl-substituted benzenes |
| Polymethylene glycol | Carbowax 20M | 250 | Free acids; alcohols; ethers; essential oils; glycols |
| Poly(dicyanoethylsiloxane) | OV-275 | 240 | Polysaturated 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

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
Surface chemistry – glass & silica are SiO₂ with -OH at surface

\[
\begin{array}{cccc}
\text{OH} & \text{OH} & \text{OH} & \text{OH} \\
\text{O} & \text{O} & \text{O} & \text{O} \\
\end{array}
\]

Silanol group

Silica core

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

---

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

<table>
<thead>
<tr>
<th></th>
<th>Type of Column*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSOT</td>
</tr>
<tr>
<td>Length, m</td>
<td>10–100</td>
</tr>
<tr>
<td>Inside diameter, mm</td>
<td>0.1–0.53</td>
</tr>
<tr>
<td>Efficiency, plates/m</td>
<td>2000–4000</td>
</tr>
<tr>
<td>Total plates</td>
<td>(20–400) × 10³</td>
</tr>
<tr>
<td>Sample size, ng</td>
<td>10–75</td>
</tr>
<tr>
<td>Relative back pressure</td>
<td>Low</td>
</tr>
<tr>
<td>Relative speed</td>
<td>Fast</td>
</tr>
<tr>
<td>Chemical inertness</td>
<td>Best</td>
</tr>
<tr>
<td>Flexible?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*FSOT: Porous-silica, open tubular column.
WCOT: Wall-coated, open tubular column.
SCOT: Support-coated open tubular column.
React Si-OH groups with silane

\[
\begin{align*}
-\text{Si}-\text{OH} + \text{Cl-Si-Cl} & \rightarrow -\text{Si}-\text{O-Si-Cl} + \text{HCl} \\
-\text{Si}-\text{O-Si-Cl} + \text{CH}_3\text{OH} & \rightarrow -\text{Si}-\text{O-Si}-\text{O-CH}_3 + \text{HCl}
\end{align*}
\]

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. \(k'\) & \(\alpha\) suitable)

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

if $K$’s too small $\rightarrow$ 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 $\rightarrow$ hydrocarbons

Where analyte & stationary phase match is good $\rightarrow$ elution order is determined by boiling points

Bonded Stationary Phases

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

\[ R - Si - x \]

\[ R - Si - x \]

\[ R - Si - x \]

\[ R - Si - x \]

\[ x = Cl \text{ or } -O-C_2H_5 \text{ or } -O-C_2H_5-C_2H_5 \]

\[ R = \text{ stationary phase functionality} \]
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)

I) Selectivity Factors by retention)

\[ \alpha = \frac{K_B}{K_A} = \frac{(t'_{R_B} - t_M)}{(t'_{R_A} - t_M)} = \frac{(t'_{R_B})}{(t'_{R_A})} \]

If B is a standard compound & we know \( \alpha \), can then be able to identify compound A even if we change 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

$$I = 100 \times \# \text{ of carbon atoms}$$

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

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

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
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

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 analyte's 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.
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**

<table>
<thead>
<tr>
<th>LC Detector</th>
<th>Commercially Available</th>
<th>Mass LOD (commercial detectors)*</th>
<th>Mass LOD (state of the art)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>Yes*</td>
<td>100 pg–1 ng</td>
<td>1 pg</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>Yes*</td>
<td>1–10 pg</td>
<td>10 fg</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Yes*</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>Yes*</td>
<td>100 pg–1 ng</td>
<td>1 pg</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Yes*</td>
<td>1 µg</td>
<td>100 ng</td>
</tr>
<tr>
<td>Light scattering</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>Photocitation</td>
<td>No</td>
<td>—</td>
<td>1 pg–1 fg</td>
</tr>
</tbody>
</table>
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 (D₂ or H₂) & a monochromator, select any λ, less sensitive

3) PDA - D₂ or H₂ 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.
- 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

Fluorescence detector – normally fixed wavelength  
filter fluorometer excitation filter & emission filter can be changed for particular $\lambda$ of interest gives selectivity based on:  
- ability to exhibit fluorescence  
- excitation wavelength  
- emission wavelength

Variable $\lambda$ 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 $\rightarrow$ 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:

![Diagram](image)

Solute polarities: $A > B > C$

Reversed-phase HPLC most common (high polarity solvent, high polarity solutes elute first)
\[-\mathrm{Si} - \mathrm{CH}_2-(\mathrm{CH}_2)_{16}-\mathrm{CH}_3\] 18 carbon chain

Long chain acts as if it were an alkane coated on silica \(\rightarrow\) analyte molecules partition into it, hence the name

In chromatogram, most polar compounds elute first because they partition into \(\mathrm{C}_{18}\) 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 C18 can have C8, C4, C3, C2, C1 plus functionalities like cyano (-C2H4CN), amino (-C2H4NH2), diol (-C3H6O-CH2-CHOHCH2OH)

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 (C4H9)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 (C2H4CN)
most polar
diol (C3H6OCH2CHOHCH2OH)
amino (C3H6NH2)
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**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Refractive Index</th>
<th>Viscosity, cP</th>
<th>Boiling Point, °C</th>
<th>Polarity Index $P'$</th>
<th>Eluant Strength $k^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoranthene²</td>
<td>1.27-1.29</td>
<td>0.4-2.6</td>
<td>50-174</td>
<td>&lt; -2</td>
<td>-0.25</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>1.423</td>
<td>0.90</td>
<td>81</td>
<td>0.04</td>
<td>-0.2</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>1.372</td>
<td>0.30</td>
<td>69</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>1-Chloronaphene</td>
<td>1.400</td>
<td>0.42</td>
<td>78</td>
<td>1.0</td>
<td>0.26</td>
</tr>
<tr>
<td>Carbox tetrachlor</td>
<td>1.457</td>
<td>0.90</td>
<td>77</td>
<td>1.6</td>
<td>0.18</td>
</tr>
<tr>
<td>i-Propyl ether</td>
<td>1.265</td>
<td>0.28</td>
<td>68</td>
<td>2.4</td>
<td>0.28</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.494</td>
<td>0.55</td>
<td>110</td>
<td>2.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>1.350</td>
<td>0.24</td>
<td>35</td>
<td>2.8</td>
<td>0.38</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>1.405</td>
<td>0.46</td>
<td>66</td>
<td>4.0</td>
<td>0.57</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.448</td>
<td>0.33</td>
<td>61</td>
<td>4.1</td>
<td>0.40</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.359</td>
<td>1.08</td>
<td>78</td>
<td>4.3</td>
<td>0.88</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1.370</td>
<td>0.43</td>
<td>77</td>
<td>4.4</td>
<td>0.58</td>
</tr>
<tr>
<td>Dioxane</td>
<td>1.420</td>
<td>1.2</td>
<td>101</td>
<td>4.8</td>
<td>0.56</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.326</td>
<td>0.54</td>
<td>65</td>
<td>5.1</td>
<td>0.95</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>1.341</td>
<td>0.34</td>
<td>82</td>
<td>5.8</td>
<td>0.65</td>
</tr>
<tr>
<td>Nitromethane</td>
<td>1.383</td>
<td>0.61</td>
<td>101</td>
<td>6.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>1.431</td>
<td>16.5</td>
<td>182</td>
<td>6.9</td>
<td>1.11</td>
</tr>
<tr>
<td>Water</td>
<td>1.333</td>
<td>0.89</td>
<td>100</td>
<td>10.2</td>
<td>Large</td>
</tr>
</tbody>
</table>

*¹At 27°C.  
²Refractive index in a common unit of viscosity is in milliP.  
³$P'$ in hexane.  
⁴$P_{AB}$ in hexane.  
⁵Properties depend upon molecular weight. Weight 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/isoctane. 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 $-SO_3H^+$ group is replaced by $-COO^-H^+$, $-NH_2OH^+$, and $-N(CH_3)_3OH^-$ groups.
Can write reactions in general format

\[ xR\text{SO}_3^- \text{H}^+ + M^{x^+} \rightleftharpoons (R\text{SO}_3^-)_x M^{x^+} + x\text{H}^+ \]

solid \hspace{2cm} solution \hspace{2cm} solid \hspace{2cm} solution

Where \( R \) = polymer support (styrene divinylbenzene)

Can write equilibrium expression for exchange

\[ K_{ex} = \frac{[(R\text{SO}_3^-)_x M^{x^+}]_s [\text{H}^+]^x_{aq}}{[R\text{SO}_3^- \text{H}^+]_s [M^{x^+}]^x_{aq}} \]

tells affinity of resin for \( M^+ \) compare to \( \text{H}^+ \) 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 \( \text{H}^+ \) & this is increased sufficiently to cause exchange with \( M^{x^+} \).
Back to equilibrium expression

\[ K_{\text{ex}} = \frac{[\text{RSO}_3^- \text{M}^{x+}]_s [\text{H}^+]_a}{[\text{RSO}_3^- \text{H}^+]_s [\text{M}^{x+}]_a} \]

Rearrange to

\[ \frac{[\text{RSO}_3^- \text{H}^+]_s}{[\text{H}^+]_a} K_{\text{ex}} = \frac{[\text{RSO}_3^- \text{M}^{x+}]_s}{[\text{M}^{x+}]_a} \]

During elution [H\(^+\)] is high & [RSO\(_3^-\) H\(^+\)]\(_s\) is high
Left hand side of equation essentially constant

\[ K = \frac{[\text{RSO}_3^- \text{M}^{x+}]_s}{[\text{M}^{x+}]_a} = \frac{C_s}{C_M} \]

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
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 $[\text{H}^+]$ & remove its conductivity so $\text{M}^{x^+}$ can be easily detected (e.g. if HCl is mobile phase use resin suppressor in OH\(^-\) form $\text{R}^+\text{OH}^-$)

\[
\text{H}^+\text{Cl}^- + \text{R}^+\text{OH}^-_S \rightleftharpoons \text{H}^+\text{OH}^- + \text{R}^+\text{Cl}^-_S \quad \text{H}_2\text{O no conductivity}
\]

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.
- Membrane suppressor

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-Å exchange resins. Regenerant flow is in the direction opposite to eluent flow. (Courtesy of Dionex Corporation, Sunnyvale, CA.)

- Typical IC separations

Figure 28.23 Typical applications of ion chromatography. (a) Separation of anions on an anion-exchange column. Eluent: 0.028 M NaHCO$_3$-0.0023 M Na$_2$CO$_3$. Sample size: 50 µL. (b) Separation of alkaline earth ions on a cation-exchange column. Eluent: 0.028 M phenylamino-amine dicyclohexylammonium dicyclohexylphosphate-0.0023 M HCl. Sample size: 100 µL.
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)
Retention related to size (and shape) of molecule

\[ V_t = V_g + V_i + V_o \]

- \( V_g \) retention volume for non-retained (large) molecules
- \( V_i \) retention volume for retained (small) molecules
- \( V_o \) retention volume for intermediate molecules (K=cs/cm)

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

<table>
<thead>
<tr>
<th>Type</th>
<th>Particle Size, μm</th>
<th>Average Pore Size, Å</th>
<th>Molecular Weight Exclusion Limit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene-divinylbenzene</td>
<td>10</td>
<td>10^2</td>
<td>700</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^3</td>
<td>(0.1 to 20) × 10^4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^4</td>
<td>(1 to 20) × 10^5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^5</td>
<td>(1 to 20) × 10^5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^6</td>
<td>(5 to &gt; 10) × 10^6</td>
</tr>
<tr>
<td>Silica</td>
<td>10</td>
<td>125</td>
<td>(0.2 to 5) × 10^4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>(0.03 to 1) × 10^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>(0.05 to 5) × 10^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>(5 to 20) × 10^7</td>
</tr>
</tbody>
</table>

* Molecular weight above which no retention occurs.
**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.