Chromatographic Methods

Prof. David Ryan
Department of Chemistry
UMass Lowell
Definitions

• **Analytical Chemistry** – qualitative and quantitative measurement of chemical species

• **Chromatography** – methods for separation and analysis of complex mixtures

• **HPLC** – High Performance Liquid Chromatography

• **GC** – Gas Chromatography
Chromatographic Methods only work for dissolved molecular species

They are not directly applicable to:

- Nanoparticles
- Carbon Nanotubes
- Particulate or colloidal species including:
  - Graphene
  - Elemental nanoparticles
  - Etc.
Introduction to Chromatography

• Chromatography is a separation technique
• HPLC & GC are our primary focus
• Also discuss low pressure column chromatography, not TLC (thin layer) or SFC
• All chromatographic techniques have
  – Stationary phase – solid or viscous liquid phase typically in a column
  – Mobile phase – moves sample in contact with stationary phase
Partitioning = type of equilibrium where the analyte divides itself between two phases

For liquid-liquid extraction – two liquids
For chromatography – mobile vs. stationary phases

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
• In linear chromatography a constant flow rate of mobile phase moves through column
• Elution = process by which analyte is flushed through the column by mobile phase (which could be a liquid or a gas)
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
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
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)
  \[ K = \frac{C_s}{C_M} \]

- Stationary phase bead
- Mobile phase (liquid)
• 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

\[ L = NH \]

or

\[ N = \frac{L}{H} \]
Gaussian distribution (bell curve)

$W = 4\sigma$
Can derive

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

\[ W_b = \text{base width} \]

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

\[ N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2 \]

\[ W_{1/2} = \text{width at half height} \]

Column manufacturers use \( N \) to characterize column – \( N \) varies widely
Shortcomings of Plate Theory

- Assumes $K$ is independent of concentration
- Assumes equilibration is rapid relative to velocity of mobile phase – not true, in reality solute may pass a plate without entering
- Assumes no longitudinal diffusion (= non ideal effect that causes band broadening)
- Does not address several factors caused by mobile phase velocity (fast or slow) Rate Theory
- Assumes discrete units or plates for equilibrium rather than a semi continuous process through the column
Rate Theory of Chromatography

\[ H = H_L + H_S + H_M + H_{SM} \]

- \( H \) = height equivalent to theoretical plate (as in Plate Theory)
- \( H_L \) = contribution due to longitudinal diffusion
- \( H_S \) = stationary phase mass transfer contribution
- \( H_M \) = diffusion associated with mobile phase effects
- \( H_{SM} \) = diffusion into or mass transfer across a stagnant layer of mobile phase (neglect)

\[ H = B/\mu + C\mu + A \]

van Deemter Equation A, B & C are coefficients, \( \mu \) = velocity
1) Longitudinal Diffusion

\[ H_L = \frac{B}{\mu} \]

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

\[ \sigma_L^2 = 0 \]

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

Variance due to longitudinal diffusion = 0 at start
Variance increases with time & diffusion coefficient D
2) Mass transfer in & out of stationary phase

<table>
<thead>
<tr>
<th></th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<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
3) Uneven Flow or Eddy Diffusion

Path 1 is shorter than path 2

\[ H_M = A \]
Putting it all together
Van Deemter
Overall
Optimizing Column Performance – seldom operate at optimum $\rightarrow$ too slow
Normally want to get required separation in shortest time, this may be at $2X \mu_{opt}$

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)
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 \( \Delta Z = \frac{W_A}{2} + \frac{W_B}{2} \)

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

General Elution Problem

Solution – change conditions during chromatographic run so that k’ changes

Start with conditions for chromatogram (a), after 1 & 2 elute

Change to conditions for chromatogram (c), after 3 & 4 elute

Change to conditions for chromatogram (b) to get 5 & 6
To Solve the General Elution Problem:

In GC do temperature programming

In HPLC do solvent programming (a.k.a. gradient elution)
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
- gas – liquid “ ← important
- gas – bonded phase “ ← relatively new

An estimated 200,000 GC in use worldwide
Figure 27-1 Schematic of a gas chromatograph.
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)
Next is gas flow control. In this region...

Figure 27-1  Schematic of a gas chromatograph.
Flow control – 10 to 50 psi with regulator

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

Ultimately flow rate is checked by a soap bubble meter for accurate flow
Next is gas flow control in this region.
Figure 27-3 Cross-sectional view of a microflash vaporizer direct injector.
Injector – use micro syringe 99.9 % of the time injecting 1 to 20 µL, rapidly shoot in plug of sample

Old GCs had separate injection area
Today use on-column & microflash vaporizers – all have septum of synthetic rubber which is punctured by syringe

Injector usually 50 °C hotter than boiling point of sample – also hotter than column
Can use rotary injector valve (as for HPLC)
Figure 27-1 Schematic of a gas chromatograph.
Column housed in Column Oven to maintain temperature

Types – packed, open tubular, capillary

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

Capillary columns will take over completely

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

Open Tubular – coated on walls

Capillary – coated on walls, long & narrow

Length range – 2 to 50 m (typically 30 m)
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 – 
  $H_2O, CO_2, SO_2, NO_x$

FID exhibits
- High sensitivity (as low as $10^{-13}$ g/s)
- Large linear response range ($10^7$)
- Easy to use
- Rugged
- DESTRUCTIVE
Thermal Conductivity Detector (TCD)
- One of earliest GC detectors
- Not popular today
- Low sensitivity
- Several designs
- Use heated wire or semiconductor
- Resistance of wire changes with analyte vs carrier
TCD uses bridge circuit with Sample & Reference Cells
TCD

- New TCDs use pulsed current to increase sensitivity & reduce drift
- Thermal conductivity of He & 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
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 $\text{e}^-$ to be released & measured by electrode = standing current or constant signal
- When analyte molecule that absorbs $\text{e}^-$ 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)
- 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
Figure 27-9  An atomic emission detector.  (Courtesy of Hewlett-Packard Company.)
GC-AED

- Potentially can measure 70 or more elements

- If look at C signal from AED get chromatogram with hundreds of peaks

- If look at O signal get very simple chromatogram with only a few peaks

Figure 27-10 Chromatograms for a gasoline sample containing a small amount of MTBE and several aliphatic alcohols: (a) monitoring the line for carbon; (b) monitoring the line for oxygen. (Courtesy of Hewlett-Packard Company.)
GC – Mass Spectrometry (GC-MS)
- Interfacing GC & MS normally difficult
- GC at pressure above atmospheric while MS under high vacuum
- Need special interfaces for packed columns
  - Jet separator – discussed below
  - Membrane separator – a membrane sandwich between spiral channels, column efluent on one side under pressure, MS on other side under vacuum – relies on differential permeability of carrier gas vs analyte molecules
GC-MS Schematic
Interface less critical for capillary columns

Figure 27-13  Schematic of a typical capillary gas chromatography/mass spectrometer.
Several types of Mass Specs available
- Rarely magnetic sector, time of flight (TOF) or ion cyclotron
- Usually quadrupole 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
- used for complete unknowns
- used for court cases/expert witness
GC-FTIR

- Powerful technique for identifying compounds
- Use heated light pipe 1 to 3 mm dia
GC-FTIR
- Powerful technique for identifying compounds
- Use heated light pipe 1 to 3 mm dia and 10 to 40 cm long
- Heat to prevent condensation of sample
- Cool detector for sensitivity
- Gives structural information from spectrum
- Not very common
GC Columns & Stationary Phases
- Historically used packed columns
- Stationary phase coated as a thin film on a high surface area solid support
- Theoretical studies showed that unpacked columns with narrow diameters were better
- Open tubular columns first developed
- Capillary columns came later because
  - Very fragile, difficult to construct, hard to connect to GCs, small samples hard to detect, difficult to coat column walls, etc.
Packed Columns
- Tubing of metal, glass, Teflon, etc.
- 2 to 3 m long and 2 to 4 mm in dia
- Packed with diatomaceous earth (SiO$_2$), clay, carbon particles, glass microbeads, polymer
- Diameter 150-250 µm (60-100 mesh) 1 m$^2$/g
- Thin coating of liquid stationary phase
<table>
<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>
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.
Figure 28-4  Schematic of an apparatus for HPLC.  (Courtesy of Perkin-Elmer Corporation, Norwalk, CT.)
Solvents (mobile phase) – are stored in special reservoirs connected to the pumping system – must be free of particles that can clog components & free of bubble forming gases that get trapped in column or detector

Three basic ways to degas solvents

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

Can purchase HPLC solvents & water - still
In GC the analyte affinity for the column is influenced by temp
In HPLC the solvent strength affects an analytes retention on column
Therefore, analogous to temp programming in GC, do solvent programming in HPLC
This is also referred to as gradient elution
Gradient elution dramatically improves the efficiency of separation.
HPLC sample injectors are 6 port rotary valves that are overfilled by syringe giving extreme accuracy & precision – typical volumes are 10 to 50 µL but can be larger.

Figure 27-4  A rotary sample valve: valve position (a) for filling sample loop $ABC$ and (b) for introduction of sample into column.
Columns
- usually stainless steel
- can be PEEK (poly ether ether ketone)
- may cost $300-$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

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>
<thead>
<tr>
<th>TABLE 28-1  Performances of LC Detectors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LC Detector</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Absorbance</td>
</tr>
<tr>
<td>Fluorescence</td>
</tr>
<tr>
<td>Electrochemical</td>
</tr>
<tr>
<td>Refractive index</td>
</tr>
<tr>
<td>Conductivity</td>
</tr>
<tr>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>FT–IR</td>
</tr>
<tr>
<td>Light scattering$^e$</td>
</tr>
<tr>
<td>Optical activity</td>
</tr>
<tr>
<td>Element selective</td>
</tr>
<tr>
<td>Photoionization</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 (D_2 or H_2) & a monochromator, select any \( \lambda \), less sensitive

3) PDA - D_2 or H_2 source, disperse & focus on diode array, get complete spectrum every 1 sec, powerful, expensive, less sensitive, lots of data generated
Cell for UV-vis detector for HPLC
- Low vol

Figure 28-9 Ultraviolet detector cell for HPLC.
Diode Array Detector

Figure 28-10  Absorption spectra of the eluent from a mixture of three steroids taken at 5-second intervals.  (Courtesy of Hewlett-Packard Company, Palo Alto, CA.)
Fluorescence detector – normally fixed wavelength filter fluorometer. Excitation filter & emission filter can be changed for particular λ of interest gives selectivity based on:
- ability to exhibit fluorescence
- excitation wavelength
- emission wavelength

Variable λ monochromator based fluorescence detectors also available

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

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

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

Electrochem. detector nearly universal
Figure 28-12  Potentially detectable organic functional groups by amperometric measurements. The horizontal lines show the range of oxidation or reduction potentials wherein compounds containing the indicated functional groups are electroactive.
Figure 28-13  Amperometric thin-layer detector cell for HPLC.
Other HPLC detectors

• LC-MS using thermospray – new popularity (pharmaceuticals)
• Evaporative light scattering - polymers
• LC-FTIR
• LC-plasma emission or ICP-MS
Figure 28-15  Effect of chain length on performance of reversed-phase siloxane columns packed with 5-μm particles. Mobile phase: 50/50 methanol/water. Flow rate: 1.0 mL/min.
Besides C$_{18}$ can have C$_8$, C$_4$, C$_3$, C$_2$, C$_1$ plus functionalities like cyano (-C$_2$H$_4$CN), amino (-C$_2$H$_4$NH$_2$), diol (-C$_3$H$_6$O-CH$_2$-CHOHCH$_2$OH)

Each has different polarity

Can also do Ion Pair Chromatography or Paired-Ion Chromatography – type of RP-HPLC used to separate ionic species

Still partition chrom. but use a reagent like a quaternary ammonium salt (C$_4$H$_9$)$_4$N$^+$ to pair with analyte ions to separate by RP
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
Sample of an application of adsorption chromatography.

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.
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 $-\text{SO}_3\text{H}^+$ group is replaced by $-\text{COO}^-\text{H}^+$, $-\text{NH}_3^+\text{OH}^-$, and $-\text{N(CH}_3)_3^+\text{OH}^-$ groups.
Can write reactions in general format

\[ \begin{align*} xRSO_3^- H^+ + M^{x+} & \rightleftharpoons (RSO_3^-)_x M^{x+} + xH^+ \\ \text{solid} & \quad \text{solution} & \quad \text{solid} & \quad \text{solution} \end{align*} \]

Where R = polymer support (styrene divinylbenzene)

Can write equilibrium expression for exchange

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

tells affinity of resin for \( M^+ \) compare to \( H^+ \) here or any ion
Ion Exchange Process

Analyte ions (M^{x+}) are passed thru column & retained on an ion-exchange site. The mobile phase contains some H^+ & this is increased sufficiently to cause exchange with M^{x+}. 
Back to equilibrium expression

\[ K_{ex} = \frac{[(RSO_3^-)_x M^{x+}]_s [H^+]_x^{aq}}{[RSO_3^- H^+]_x^{s} [M^{x+}]_x^{aq}} \]

Rearrange to

\[ K_{ex} = \frac{[RSO_3^- H^+]_x^{s}}{[H^+]_x^{aq}} \]

\[ K_{ex} = \frac{[(RSO_3^-)_x M^{x+}]_s}{[M^{x+}]_x^{aq}} \]

During elution [H⁺] is high & [RSO₃⁻ H⁺]₀ is high

Left hand side of equation essentially constant
\[
K = \frac{\left[(RSO_3^-)_x M^{x+}\right]_s}{[M^{x+}]_{aq}^x} = \frac{C_s}{C_M}
\]

K turns out to be a distribution ratio (partition)

Order of affinity for sulfonated cation exchange

\(\text{Tl}^{+}>\text{Ag}^{+}>\text{Cs}^{+}>\text{Rb}^{+}>\text{K}^{+}>\text{NH}_4^{+}>\text{Na}^{+}>\text{H}^{+}>\text{Li}^{+}\)

\(\text{Ba}^{2+}>\text{Pb}^{2+}>\text{Sr}^{2+}>\text{Ca}^{2+}>\text{Ni}^{2+}>\text{Cd}^{2+}>\text{Cu}^{2+}>\text{Co}^{2+}>\text{Zn}^{2+}>\text{Hg}^{2+}\)
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.
<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>F^-</td>
<td>3</td>
</tr>
<tr>
<td>Formate</td>
<td>8</td>
</tr>
<tr>
<td>BrO_3^-</td>
<td>10</td>
</tr>
<tr>
<td>Cl^-</td>
<td>4</td>
</tr>
<tr>
<td>NO_3^-</td>
<td>10</td>
</tr>
<tr>
<td>HPO_4^{2-}</td>
<td>30</td>
</tr>
<tr>
<td>Br^-</td>
<td>30</td>
</tr>
<tr>
<td>NO_3^-</td>
<td>30</td>
</tr>
<tr>
<td>SO_4^{2-}</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca^{2+}</td>
<td>3</td>
</tr>
<tr>
<td>Mg^{2+}</td>
<td>3</td>
</tr>
<tr>
<td>Sr^{2+}</td>
<td>10</td>
</tr>
<tr>
<td>Ba^{2+}</td>
<td>25</td>
</tr>
</tbody>
</table>

**Figure 28-23** Typical applications of ion chromatography. (a) Separation of anions on an anion-exchange column. Eluent: 0.0028 M NaHCO_3/0.0023 M Na_2CO_3. Sample size: 50 μL. (b) Separation of alkaline earth ions on a cation-exchange column. Eluent: 0.025 M phenylenediamine dihydrochloride/0.0025 M HCl. Sample size: 100 μL. (Courtesy of Dionex Corporation, Sunnyvale, CA.)
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
Gel beads have pores in them of a defined size range which allows smaller molecules to enter but excludes molecules larger than the pore diameters.
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.