SEPARATION TECHNIQUES

CH 27, 28
OBJECTIVES

Label, diagram and describe a generalized gas chromatography instrument

Compare and contrast sample preparation and injection methods

Describe typical type of chromatography, column dimensions and conditions

What conditions improve resolution and sensitivity?

What are the common detectors used for GC? How do these detectors work?
GAS CHROMATOGRAPHY

Order of decisions:
1. goal of analysis
2. sample preparation
3. detector
4. column
5. injection
Gas Chromatography

Mobile phase - carrier gas (He, N\textsubscript{2}, H\textsubscript{2})
Stationary phase - nonvolatile liquid or solid
Analyte - volatile liquid or gas
<table>
<thead>
<tr>
<th>Field</th>
<th>Typical Mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceuticals</td>
<td>Antibiotics, sedatives, steroids, analgesics</td>
</tr>
<tr>
<td>Biochemical</td>
<td>Amino acids, proteins, carbohydrates, lipids</td>
</tr>
<tr>
<td>Food products</td>
<td>Artificial sweeteners, antioxidants, aflatoxins, additives</td>
</tr>
<tr>
<td>Industrial chemicals</td>
<td>Condensed aromatics, surfactants, propellants, dyes</td>
</tr>
<tr>
<td>Pollutants</td>
<td>Pesticides, herbicides, phenols, polychlorinated biphenyls</td>
</tr>
<tr>
<td>Forensic science</td>
<td>Drugs, poisons, blood alcohol, narcotics</td>
</tr>
<tr>
<td>Clinical chemistry</td>
<td>Bile acids, drug metabolites, urine extracts, estrogens</td>
</tr>
</tbody>
</table>
Sample injection → Separation in column → detection
Sample Preparation

1. Headspace
2. Liquid Injection
3. Solid phase microextraction (SPME)

- Pierce sample septum with metal needle
- Retract fiber and withdraw needle
- Pierce chromatography septum with metal needle
- Retract fiber and withdraw needle
- Expose fiber to solution or headspace for fixed time with stirring
- Expose hot fiber to carrier gas for fixed time (column is cold)

Diagram:
- Syringe barrel
- Septum piercing needle
- Fiber attachment tubing
- Fused silica fiber with stationary liquid-phase coating
SAMPLE INJECTION

Injection types:
1. Split- most common, only >0.2-2% sample onto column
2. Splitless- trace analysis, analyte <0.01% sample
1. On column- avoid sample decomposition
SAMPLE INJECTOR
Sample injection → Separation in column → detection
**FIGURE 23-2** (a) Typical dimensions of open tubular gas chromatography column. (b) Fused-silica column with a cage diameter of 0.2 m and column length of 15–100 m. (c) Cross-sectional view of wall-coated, support-coated, and porous-layer columns.

Harris, *Quantitative Chemical Analysis, 8e*
© 2011 W. H. Freeman
COLUMN TEMPERATURE

Illustrates general elution problem.

Solution: Temperature ramp

*Raising column temperature:*
- decreases retention time
- sharpens peaks

To improve resolution, use a
- longer column
- narrower column
- different stationary phase
MOBILE PHASE CHOICE
VAN DEEMTER PLOT

\[ H \approx A + \frac{B}{u_x} + C u_x \]

A, B, and C are constant for a given column type and stationary phase:
GC- A=0
Sample injection → Separation in column → detection
Detection - analyte retention time

Use **internal standard** to account for small changes in mobile phase flow rate

\[
\frac{\text{Area of analyte signal}}{\text{Concentration of analyte}} = F\left( \frac{\text{area of standard signal}}{\text{concentration of standard}} \right)
\]

\[
\frac{A_x}{[X]} = F\left( \frac{A_s}{[S]} \right)
\]

Area of Gaussian peak = 1.064 × peak height × \( w_{1/2} \)
Detectors- ideal characteristics

1. Adequate sensitivity
2. Good stability and reproducibility
3. Linear response to analyte concentration over several orders of magnitude
4. Temperature range 20-400 C
5. Short response time independent of flow rate
6. Highly reliable and easy to use
7. Similar or predictable response to analytes
8. Nondestructive

**TABLE 27-1** Typical Gas Chromatographic Detectors

<table>
<thead>
<tr>
<th>Type</th>
<th>Applicable Samples</th>
<th>Typical Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame ionization</td>
<td>Hydrocarbons</td>
<td>1 pg/s</td>
</tr>
<tr>
<td>Thermal conductivity</td>
<td>Universal detector</td>
<td>500 pg/mL</td>
</tr>
<tr>
<td>Electron capture</td>
<td>Halogenated compounds</td>
<td>5 fg/s</td>
</tr>
<tr>
<td>Mass spectrometer (MS)</td>
<td>Tunable for any species</td>
<td>0.25 to 100 pg</td>
</tr>
<tr>
<td>Thermionic</td>
<td>Nitrogen and phosphorous compounds</td>
<td>0.1 pg/s (P), 1 pg/s (N)</td>
</tr>
<tr>
<td>Electrolytic conductivity (Hall)</td>
<td>Compounds containing halogens, sulfur, or nitrogen</td>
<td>0.5 pg Cl/s, 2 pg S/s, 4 pg N/s</td>
</tr>
<tr>
<td>Photoionization</td>
<td>Compounds ionized by UV radiation</td>
<td>2 pg C/s</td>
</tr>
<tr>
<td>Fourier transform IR (FTIR)</td>
<td>Organic compounds</td>
<td>0.2 to 40 ng</td>
</tr>
</tbody>
</table>

© 2007 Thomson Higher Education
Detectors

1. Thermal conductivity
2. Flame ionization detector (FID)
3. Electron capture
4. Mass Spectrometry (MS)
5. Others
1. Detector- thermal conductivity

Thermal conductivity detector for packed column

Low-volume thermal conductivity detector for open tubular column
2. Detector- Flame ionization

- Collector (positive electrode)
- Flame tip (negative electrode)
- Igniter coil
- Glass insulation
- Air diffuser
- Air inlet
- Hydrogen inlet
- Gas flow from column
3. Detector- Electron capture

- Anode
- Cathode
- Gas out
- Cloud of electrons and ions
- $^{63}$Ni radioactive source
- Eluent and makeup gas in
- To waste
- From column
- Electrode
- Electrode
- Insulator
- Radioactive $\beta$ emitter
4. Detector- Mass Spectrometry

- Ionization and ion acceleration
- Selection of ions
- Detection

Diagram: Gas chromatograph oven, GC column, Fused silica, Carrier gas inlet, Injection port, Gas chromatograph oven, Ion-source region, Mass-analyzer region, Electron multiplier, Data system, Transfer line, Focusing lenses.
4. Detector- Mass Spectrometry
HPLC
CH 28
CHEM 314

Solvent mixing
Sample injection

Separation in
Packed column

detection
OBJECTIVES

Label, diagram and describe a generalized HPLC instrument

Describe the effect particle size, flow rate, and temperature have on H, retention time, resolution, etc.

Why does HPLC occur at high pressures?

What are the common detectors used for HPLC? How do these detectors work?
<table>
<thead>
<tr>
<th>Field</th>
<th>Typical Mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceuticals</td>
<td>Antibiotics, sedatives, steroids, analgesics</td>
</tr>
<tr>
<td>Biochemical</td>
<td>Amino acids, proteins, carbohydrates, lipids</td>
</tr>
<tr>
<td>Food products</td>
<td>Artificial sweeteners, antioxidants, aflatoxins, additives</td>
</tr>
<tr>
<td>Industrial chemicals</td>
<td>Condensed aromatics, surfactants, propellants, dyes</td>
</tr>
<tr>
<td>Pollutants</td>
<td>Pesticides, herbicides, phenols, polychlorinated biphenyls</td>
</tr>
<tr>
<td>Forensic science</td>
<td>Drugs, poisons, blood alcohol, narcotics</td>
</tr>
<tr>
<td>Clinical chemistry</td>
<td>Bile acids, drug metabolites, urine extracts, estrogens</td>
</tr>
</tbody>
</table>
SAMPLE INJECTION

Load position

Inject position
IMPORTANT HPLC PARAMETERS

Particle size
- Reduces distance solutes must travel to interact with stationary phase (reduces $H$ and $C$)
- More uniform flow paths (reduces $A$)

Flow rate
- Van deemter plot
- At small particle size, $H$ isn’t sensitive to flow rate

Pressure
- More pressure required as particle size and column diameter decrease

Temperature
- More heating occurs when column diameter is larger
- Heating column increases flow rate and decreases retention time
PARTICLE SIZE
SMALLER PARTICLES MEANS SMALLER H
PARTICLE SIZE
SMALLER PARTICLES MEANS SMALLER H

\[ N = \frac{L}{H} \]

\[ N \approx \frac{3000 \ L \ (\text{cm})}{d_p \ (\mu\text{m})} \]
VAN DEEMTER PLOT

- Particle size $\alpha$ plate height
- For small particle sizes, $H$ is invariant with flow rate
SMALLER PARTICLE SIZE REQUIRES INCREASED PRESSURE

\[ P = f \frac{u_x \eta L}{\pi r^2 d_p^2} \]

\( P = \) pressure
\( f = \) particle shape and packing
\( u_x = \) linear flow rate
\( \eta = \) solvent viscosity
\( L = \) column length
\( r = \) column radius
\( d_p = \) particle diameter

### TABLE 24-1: Performance as a function of particle diameter

<table>
<thead>
<tr>
<th>Particle size ( d_p ) (( \mu m ))</th>
<th>Retention time (min)</th>
<th>Plate number (( N ))</th>
<th>Required pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>30</td>
<td>25 000</td>
<td>19</td>
</tr>
<tr>
<td>3.0</td>
<td>18</td>
<td>42 000</td>
<td>87</td>
</tr>
<tr>
<td>1.5</td>
<td>9</td>
<td>83 000</td>
<td>700</td>
</tr>
<tr>
<td>1.0</td>
<td>6</td>
<td>125 000</td>
<td>2 300</td>
</tr>
</tbody>
</table>

Ultra Performance Liquid Chromatography (UPLC)
Up to 1,000 bar
DERIVATIZE SILICA TO REDUCE TAILING

**Derivatization Process:**
- OH → H$_3$O$^+$ → Derivatization

**Chemical Structures:**
- C$_{18}$ stationary phase
- Silica

**Normal-phase chromatography:**
- polar stationary phase
- more polar solvent has higher eluent strength

**Reversed-phase chromatography:**
- nonpolar stationary phase
- less polar solvent has higher eluent strength

**Bidentate C$_{18}$ Stationary Phase:**
- Provides increased stability above pH 8

**Diagram:**
- Chemical structures showing derivatization process and bonding with silica.
NORMAL AND REVERSE PHASE CHROMATOGRAPHY

Normal-phase chromatography

Low-polarity mobile phase

Reversed-phase chromatography

High-polarity mobile phase

Medium-polarity mobile phase

Solute polarities: $A > B > C$
**GENERAL ELUTION PROBLEM**

*General elution problem:* For a complex mixture, isocratic conditions can often be found to produce adequate separation of early-eluting peaks or late-eluting peaks, but not both. This problem drives us to use gradient elution.
ELUTION METHODS

**Isocratic elution**: one solvent

**Gradient elution**: continuous change of solvent composition to increase eluent strength.

Example: caffeine lab
50-50 Methanol-H₂O mixture
## DETECTORS

1. **UV-Vis spectroscopy**
2. **Evaporative Light Scattering Detector (ELSD)**
3. **Mass Spectrometry**

### TABLE 28-1 Performance of HPLC Detectors

<table>
<thead>
<tr>
<th>HPLC Detector</th>
<th>Commercially Available</th>
<th>Mass LOD* (typical)</th>
<th>Linear Range † (decades)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>Yes</td>
<td>10 pg</td>
<td>3 – 4</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>Yes</td>
<td>10 fg</td>
<td>5</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Yes</td>
<td>100 pg</td>
<td>4 – 5</td>
</tr>
<tr>
<td>Refractive index</td>
<td>Yes</td>
<td>1 ng</td>
<td>3</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Yes</td>
<td>100 pg–1 ng</td>
<td>5</td>
</tr>
<tr>
<td>Mass spectrometry</td>
<td>Yes</td>
<td>&lt;1 pg</td>
<td>5</td>
</tr>
<tr>
<td>FTIR</td>
<td>Yes</td>
<td>1 μg</td>
<td>3</td>
</tr>
<tr>
<td>Light scattering</td>
<td>Yes</td>
<td>1 μg</td>
<td>5</td>
</tr>
<tr>
<td>Optical activity</td>
<td>No</td>
<td>1 ng</td>
<td>4</td>
</tr>
<tr>
<td>Element selective</td>
<td>No</td>
<td>1 ng</td>
<td>4 – 5</td>
</tr>
<tr>
<td>Photoionization</td>
<td>No</td>
<td>&lt;1 pg</td>
<td>4</td>
</tr>
</tbody>
</table>

* Mass LOD: Minimum detectable amount
† Linear Range: Number of decades

© 2007 Thomson Higher Education
1. UV-VISIBLE SPECTROPHOTOMETER
1. UV-VISIBLE SPECTROPHOTOMETER

![Graph showing absorbance vs. wavelength for different steroids: Corticosterone, Dexamethasone, Cortisone.](image)

- Absorbance is plotted on the y-axis.
- Wavelength (nm) is plotted on the x-axis.
- The graph illustrates changes in absorbance over time with increments of 5 seconds.
2. EVAPORATIVE LIGHT SCATTERING DETECTOR

1. Nebulization

2. Mobile-phase evaporation

3. Detection
COMPARING UV-VIS AND ELSD

Soluble components of a drug
3. Mass Spectrometry

Ionization and ion acceleration → selection of ions → detection