

- Separated analytes flow through a detector
→ whose response is displayed on a computer.
- The column must be hot enough
→ to provide sufficient vapor pressure for analytes to be eluted in a reasonable time.

See Figure 24-1

Open Tubular Columns

- The vast majority of analyses
 - use long, narrow open tubular columns
 - made of fused silica (SiO_2)
 - coated with polyimide (a plastic capable of withstanding 350°C) for protection from atmospheric moisture
- Open tubular columns offer
 - higher resolution,
 - shorter analysis time,
 - and greater sensitivity than packed columns

- Column inner diameters are typically 0.10 to 0.53 mm
- Column lengths are 15 to 100 m, with 30 m being common.
- Resolution is proportional to the square root of column length (from Purnell eqn in Ch 23).

- In **gas-liquid partition chromatography**,
 - the **stationary phase is a nonvolatile liquid** bonded to the inside of the column or to a fine solid support
- **The wall-coated column**
 - features a **0.1- to 5- μm -thick film of stationary liquid phase** on the inner wall of the column.

See Figure 24-1, 2

- A support-coated column
 - has solid particles coated with stationary liquid phase and attached to the inner wall.
- In **gas-solid adsorption chromatography**,
 - analyte is adsorbed directly on solid particles of stationary phase
- In the porous-layer column,
 - solid particles are the active stationary phase.

Support-coated
open tubular
column
(SCOT)

Porous-layer
open tubular
column
(PLOT)

- Support-coated columns can handle larger samples than wall-coated columns
 - because of their high surface area
- The choice of **liquid stationary phase**
 - is based on the rule "like dissolves like."
 - Nonpolar columns are best for nonpolar solutes
 - Columns of intermediate polarity are best for intermediate polarity solutes
 - strongly polar columns are best for strongly polar solutes.

- The number of theoretical plates, N , on a column is proportional to column length.
- Resolution is proportional to the square root of N and, therefore, to the square root of column length.

See Figure 24-4

- At constant linear velocity, increasing the stationary phase thickness increases retention time and sample capacity and increases resolution of early-eluting peaks with a retention factor, k , of ≤ 5 .
- Thick films can shield analytes from the silica surface and reduce tailing.

See Figure 24-5

See Table 24-1

Retention

The retention of polar and nonpolar solutes changes as the boiling point of the solute (T_{bp}) and the polarity of the stationary phase change.

See Figure 24-9

- The retention factor, k , depends on $\Delta H_{\text{vap}}^{\circ}$ and $\Delta H_{\text{mix}}^{\circ}$ of pure solute with the stationary phase liquid:

$$\ln k = \frac{\Delta H_{\text{vap}}^{\circ}}{RT} + \frac{\Delta H_{\text{mix}}^{\circ}}{RT} + \text{constant}$$

- For non-hydrogen-bonding solutes, Trouton's rule states that $\Delta H_{\text{vap}}^{\circ}$ is proportional to the boiling temperature:

$$\Delta H_{\text{vap}}^{\circ} \approx (88 \text{ J mol}^{-1} \text{ K}^{-1}) \cdot T_{\text{bp}}$$

- Combining these yields the approximation

$$\ln k \approx \frac{(88 \text{ J mol}^{-1} \text{ K}^{-1})T_{\text{bp}}}{RT} + \frac{\Delta H_{\text{mix}}^{\circ}}{RT} + \text{constant}$$

- The *Kovats retention index*, I , for a linear alkane equals 100 times the number of carbon atoms.
- For octane, $I \equiv 800$; and for nonane $I \equiv 900$.
- A compound eluted between octane and nonane has a retention index between 800 and 900 given by the equation:

$$I = 100 \left[n + (N - n) \frac{\log t'_r (\text{unknown}) - \log t'_r (n)}{\log t'_r (N) - \log t'_r (n)} \right]$$

(No need to memorize!)

See Table 24-2

Carrier Gas

- **Helium**
 - the most common carrier gas
 - is compatible with most detectors.
- Figure 24-12 shows the effect of carrier gas
 - on the separation of two compounds on the same column with the same temperature program.
- H₂ and He give better resolution than N₂ at high flow rate
 - because solutes diffuse more rapidly through H₂ and He than through N₂.

See Figure 24-12

- The main reason H_2 was not used more often in the past
→ is that concentrations >4 vol% in air are explosive.
- Impurities in carrier gas degrade the stationary phase.
→ High-quality gas should be used
→ it should be passed through purifiers to remove traces of O_2 , H_2O , and organic compounds.
- Steel or copper tubing, rather than plastic or rubber, should be used for gas lines
→ because metals are less permeable to air

- As with thermal degradation,
 - symptoms of oxidative degradation of the stationary phase include
 - i) increased baseline signal at low temperature,
 - ii) peak broadening and tailing,
 - iii) and altered retention times.

24.3 Detectors

- For **qualitative analysis**,
 - identify a peak **by comparing its retention time with that of an authentic sample** of the suspected compound.
- The most reliable way to compare retention times is by spiking,
 - in which an authentic compound is added to the unknown.
- If the added compound is identical with a component of the unknown,
 - then the **relative area of that one peak will increase**.

- **Quantitative analysis**

- based on the area of a chromatographic peak.

- In the linear response concentration range,

- the area of a peak is proportional to the quantity of that component.

- Quantitative analysis is almost always performed

- by adding a known quantity of internal standard to the unknown.

Quantitative analysis with internal standard:

$$\frac{A_X}{[X]} = F \left(\frac{A_S}{[S]} \right)$$

A_X = area of analyte signal

A_S = area of internal standard

$[X]$ = concentration of analyte

$[S]$ = concentration of standard

F = response factor

Thermal Conductivity Detector

- In the past, **thermal conductivity detectors were common** in gas chromatography
 - because they are simple and universal – responding to all analytes.
- It **is less sensitive than other detectors** used with open tubular columns
 - Thermal conductivity is **useful for packed columns**.

See Table 24-3

- Thermal conductivity measures
 - the ability of a substance to transport heat from a hot region to a cold region (Table 24-4).
- Helium
 - the carrier gas commonly used with a thermal conductivity detector.
- Helium has the second highest thermal conductivity (after H₂),
 - so any analyte mixed with helium lowers the conductivity of the gas stream.

See Table 24-4

See Figure 24-19

- Eluate from the chromatography column
 - flows over a **hot tungsten-rhenium filament**.
- When **analyte emerges from the column**,
 - the conductivity of the gas stream decreases,
 - the filament gets hotter,
 - its electrical resistance increases,
 - and the voltage across the filament changes.
- The **detector measures the change in voltage**.

See Figure 24-19

Flame ionization detector

- Eluate is burned in a mixture of H₂ and air.
- Carbon atoms (except carbonyl and carboxyl carbons) produce CH radicals,
→ which are thought to produce CHO⁺ ions and electrons in the flame.



See Figure 24-20

- Only about 1 in 10⁵ carbon atoms produces an ion,
→ but ion production is proportional to the number of susceptible carbon atoms entering the flame.

- When **no analyte** is present,
→ $\sim 10^{-14}$ A flows between the flame tip and the collector.
- When **analyte enters** the flame,
→ the current is $\sim 10^{-12}$ A.

See Figure 24-20

- Functional groups such as carbonyls, alcohols, halogens, and amines
 - yield few ions or none at all,
 - therefore the detector is **insensitive to these compounds**.
- It is also **insensitive toward noncombustible gases**
 - such as CO₂, CO, SO₂, H₂O, NO_x, and noble gases.
 - **Good for samples contaminated** with water and the oxides of sulfur and nitrogen.
- The detection limit is reduced by 50% when **N₂ is used** instead of He.
- 100-fold better detection than thermal conductivity detector.

Other detectors

- Electron capture detector → halogen containing organic compounds
- Nitrogen-phosphorus → highlights P, N
- Flame photometer → individual selected elements, such as P, S, Sn, Pb
- Photoionization → aromatics, unsaturated compounds
- Sulfur chemiluminescence → S
- Nitrogen chemiluminescence → N
- Atomic emission → most elements
- Mass spectrometer → most analytes
- Infrared spectrometer → most analytes

Analytical Chemistry

Chapter 25.

High Performance Liquid Chromatography

- This chapter discusses
→ discuss liquid chromatography.
- Chromatographers generally choose gas chromatography over liquid chromatography when there is a choice,
→ because gas chromatography is normally less expensive and generates much less waste.
- Liquid chromatography is important
→ because most compounds are **not sufficiently volatile** for gas chromatography.

25.1 The chromatographic process

- Diffusion in liquids is 100 times slower than diffusion in gases.
- Therefore, in liquid chromatography,
 - it is not generally feasible to use open tubular columns,
 - because the diameter of the solvent channel is too great to be traversed by a solute molecule in a short time.
- Liquid chromatography is conducted with packed columns
 - so that a solute molecule does not have to diffuse very far to encounter the stationary phase.

Small Particles Give High Efficiency but Require High Pressure

- Typical [particle sizes](#) in HPLC are 1.7 to 5 μm .

See Figure 25-5

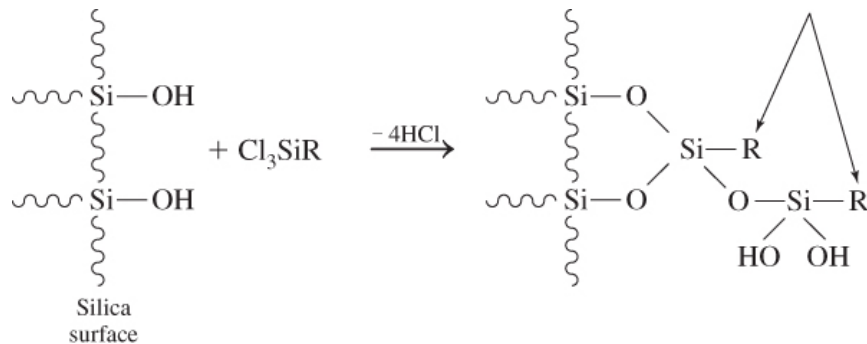
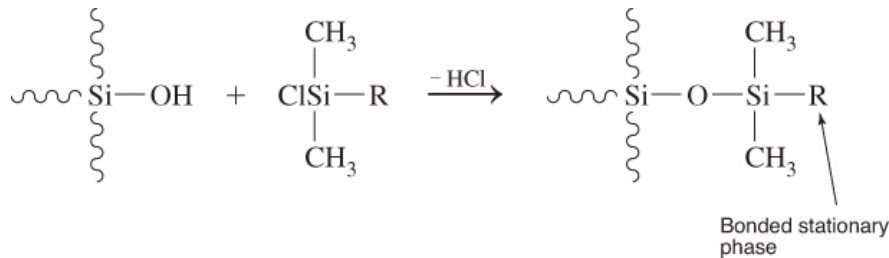
- As the size of the stationary phase particles decreases.
 - The efficiency of a packed column increases
- The peaks are sharper with the smaller particle size
 - Decreasing particle size permits us to improve resolution.

See Figure 25-2

The Stationary Phase

- The most common support
 - is highly pure, spherical, microporous particles of silica that are permeable to solvent
 - have a surface area of several hundred square meters per gram.
- Most silica cannot be used above pH 8,
 - because it dissolves in base.
- For separation of basic compounds at pH 8–12,
 - ethylene-bridged silica (see Fig 25-6) or polymeric supports such as polystyrene can be used.
 - Stationary phase is covalently attached to the polymer.

- Bare silica can be used as the stationary phase for adsorption chromatography.
- Most commonly, liquid-liquid partition chromatography is conducted
 → with a bonded stationary phase covalently attached to the silica surface by reactions such as



See Table 25-3

Spectrophotometric Detectors

- An **ultraviolet detector** using a flow cell
 - the **most common HPLC detector**,
 - because many solutes absorb ultraviolet light.

- Simple systems employ the intense 254-nm emission of a mercury vapor lamp or other discrete ultraviolet wavelengths from zinc or cadmium vapor lamps.

See Figure 25-21

Other detectors

See Table 25-5

Selecting the Separation Mode

- There can be many ways to separate components of a given mixture.
 - Figure 25-17 is a decision tree for choosing a starting point.

See Figure 25-17