Fourth Week

Gas chromatography:

mobile phase: gas

stationary phase: usually a nonvolatile liquid, but sometimes a solid

analyte: gas or volatile liquid

23-1 The Separation Process in Gas Chromatography

In **gas chromatography,**[2,3,4] gaseous analyte is transported through the column by a gaseous mobile phase, called the **carrier gas.** 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

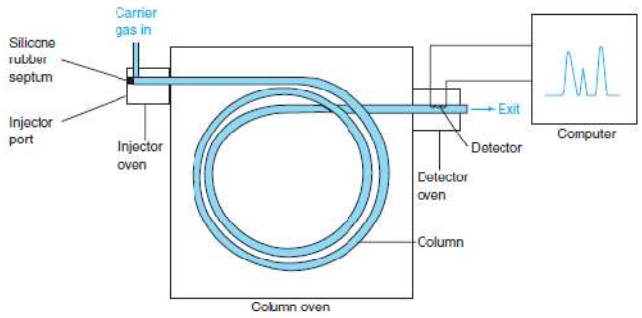


FIGURE 23-1 Schematic diagram of a gas chromatograph.

(Figure 22-6, upper right). In *gas-solid adsorption chromatography*, analyte is adsorbed directly on solid particles of stationary phase (Figure 22-6, upper left).

The choice of carrier gas depends on the detector and the desired separation efficiency and speed.

In the schematic *gas chromatograph* in Figure 23-1, volatile liquid or gaseous sample is injected through a **septum** (a rubber disk) into a heated port, in which it rapidly evaporates. Vapor is swept through the column by He, N_2, or H_2 carrier gas, and 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. The detector is maintained at a higher temperature than the column so analytes will be gaseous.

be gaseous.

Compared with packed columns, open tubular columns offer

- higher resolution
- shorter analysis time
- greater sensitivity
- lower sample capacity

Wall-coated open tubular column (WCOT): liquid stationary phase on inside wall of column

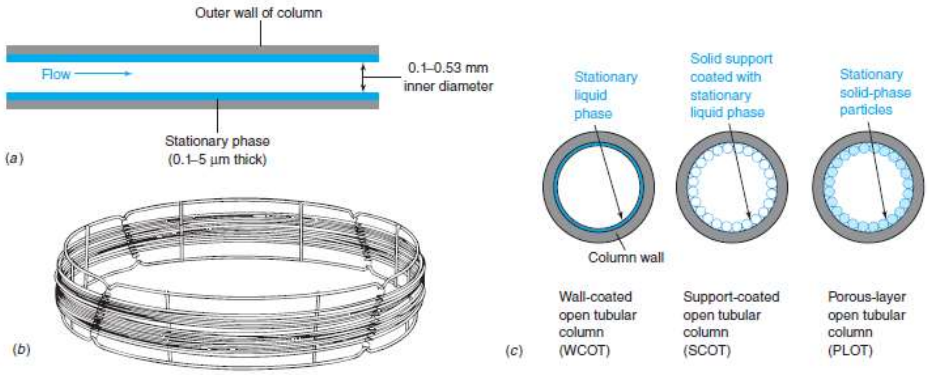
Support-coated open tubular column (SCOT): liquid stationary phase coated on solid support attached to inside wall of column

Porous-layer open tubular column (PLOT): solid stationary phase on inside wall of column

Open Tubular Columns

The vast majority of analyses use long, narrow **open tubular columns** (Figure 23-2) made of fused silica (SiO_2) and coated with polyimide (a plastic capable of withstanding 350°C) for support and protection from atmospheric moisture.[5] As discussed in Section 22-5, open tubular columns offer higher resolution, shorter analysis time, and greater sensitivity than packed columns, but they have less sample capacity.

The *wall-coated* column in Figure 23-2c features a 0.1- to 5-μm-thick film of stationary liquid phase on the inner wall of the column. A *support-coated* column has solid particles coated with stationary liquid phase and attached to the inner wall. In the *porous-layer* column in Figure 23-3, solid particles *are* the active stationary phase. With their high surface area, support-coated columns can handle larger samples than can wall-coated columns. The performance of support-coated columns is intermediate between those of wall-coated columns and packed columns.



Column inner diameters are typically 0.10 to 0.53 mm and lengths are 15 to 100 m, with 30 m being common. Narrow columns provide higher resolution than wider columns (Figure 23-4 and Equation 22-35b) but require higher operating pressure and have less sample capacity. Diameters ≥0.32 mm tend to overload the vacuum system of a mass spectrometer, so the gas stream must be split and only a fraction sent to the spectrometer. The number of theoretical plates, N, on a column is proportional to length. In Equation 22-30, resolution is proportional to $\sqrt{N}$ and, therefore, to the square root of column length (Figure 23-5).

Equation 22-30:

$$\text{Resolution} = \frac{\sqrt{N}}{4}(\gamma - 1)$$

N = plate number
γ = unadjusted relative retention

At the constant linear velocity in Figure 23-6, increasing the thickness of the stationary phase increases retention time and sample capacity and increases resolution of early-eluting peaks with a *retention factor* (Equation 22-17) of $k \lesssim 5$. Thick films of stationary phase can shield analytes from the silica surface and reduce *tailing* (Figure 22-21), but

Equation 22-17:

$$k = \frac{t_r - t_m}{t_m}$$

t_r = retention time of solute
t_m = transit time of solvent

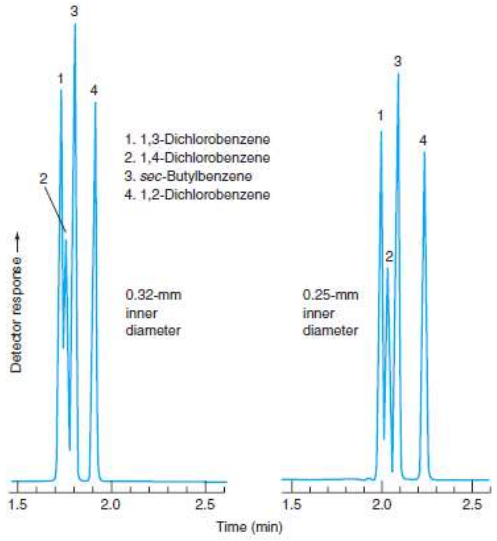


FIGURE 23-4 Effect of open tubular column inner diameter on resolution. Narrower columns provide higher resolution. Notice the increased resolution of peaks 1 and 2 in the narrow column. Conditions: DB-1 stationary phase (0.25 μm thick) in 15-m wall-coated column operated at 95°C with He linear velocity of 34 cm/s. [Courtesy J&W Scientific, Folsom, CA.]

Packed Columns

Packed columns contain fine particles of solid support coated with nonvolatile liquid stationary phase, or the solid itself may be the stationary phase. Compared with open tubular columns, packed columns provide greater sample capacity but give broader peaks, longer retention times, and less resolution. (Compare Figure 23-8 with Figure 23-3.) Despite their inferior resolution, packed columns are used for preparative separations, which require a great deal of stationary phase, or to separate gases that are poorly retained. Packed columns are usually made of stainless steel or glass and are typically 3–6 mm in diameter and 1–5 m in length. The solid support is often silica that is *silanized* (Reaction 22-36) to reduce hydrogen bonding to polar solutes. For tenaciously binding solutes, Teflon is a useful support, but it is limited to <200°C.

Teflon is a chemically inert polymer with the structure $-CF_2-CF_2-CF_2-CF_2-$.

In a packed column, uniform particle size decreases the multiple path term in the van Deemter equation (22-33), thereby reducing plate height and increasing resolution. Small particle

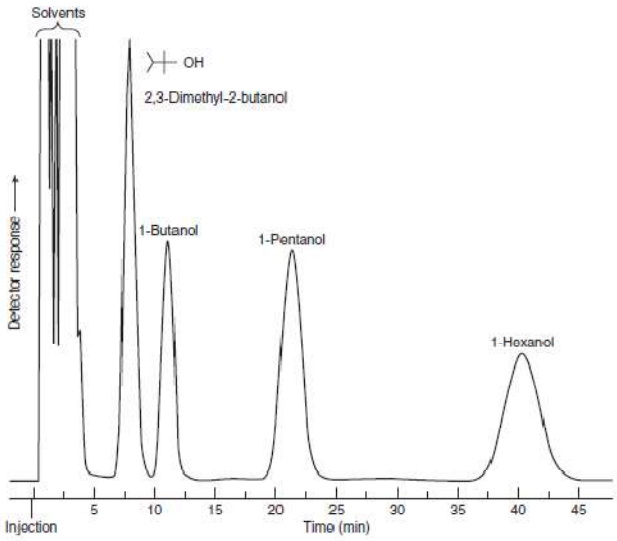


FIGURE 23-8 Chromatogram of alcohol mixture at 40°C using packed column (2 mm inner diameter × 76 cm long) containing 20% Carbowax 20M on Gas-Chrom R support and flame ionization detector. [Courtesy Norman Pearson.]

size decreases the time required for solute equilibration, thereby improving column efficiency. However, the smaller the particle size, the less space between particles and the more pressure required to force mobile phase through the column. Particle size is expressed in micrometers or as a *mesh size*, which refers to the size of screens through which the particles are passed or retained (Table 27-2). A 100/200 mesh particle passes through a 100 mesh screen, but not through a 200 mesh screen. The mesh number equals the number of openings per linear inch of screen.

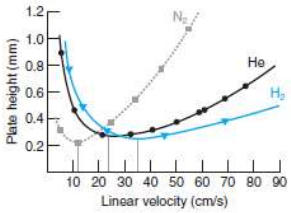


FIGURE 23-11 van Deemter curves for gas chromatography of n-$C_{17}H_{36}$ at 175°C, using N_2, He, or H_2 in a 0.25-mm-diameter × 25-m-long wall-coated column with OV-101 stationary phase. [From R. R. Freeman, ed., *High Resolution Gas Chromatography* (Palo Alto, CA: Hewlett Packard Co., 1981).]

reduce retention times of late-eluting components. At the end of a run, the pressure can be rapidly reduced to its initial value for the next run. Time is not wasted waiting for a hot column to cool before the next injection. Programmed pressure is useful for analytes that cannot tolerate high temperature.

Carrier Gas

Helium is the most common carrier gas and is compatible with most detectors. For a flame ionization detector, N_2 gives a lower detection limit than He. Figure 23-11 shows that H_2, He, and N_2 give essentially the same optimal plate height (0.3 mm) at significantly different flow rates. Optimal flow rate increases in the order $N_2 < He < H_2$. Fastest separations can be achieved with H_2 as carrier gas, and H_2 can be run much faster than its optimal velocity with little penalty in resolution.[12] Figure 23-12 shows the effect of carrier gas on the separation of two compounds on the same column with the same temperature program.

The main reason H_2 was not used more often in the past is that concentrations >4 vol% in air are explosive. Flow rates in capillary chromatography are unlikely to create a dangerous concentration of H_2. Electrolytic generators produce high-purity H_2 and eliminate the need for tanks of compressed H_2. For gas chromatography–mass spectrometry, H_2 reduces the

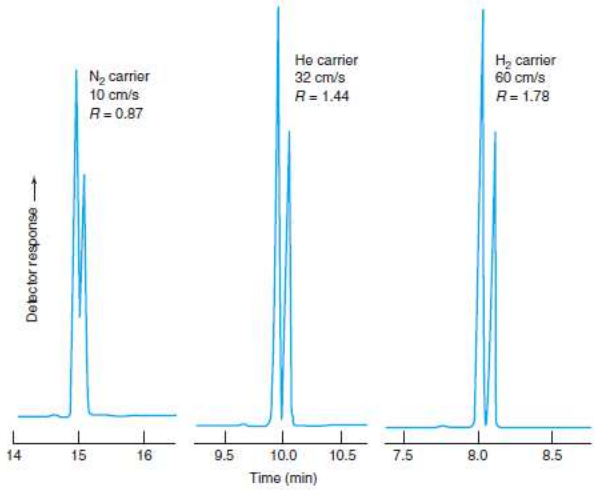


FIGURE 23-12 Separation of two polyaromatic hydrocarbons on a wall-coated open tubular column with different carrier gases. Resolution, R, increases and analysis time decreases as we change from N_2 to He to H_2 carrier gas. [Courtesy J&W Scientific, Folsom, CA.]

efficiency of a turbomolecular vacuum pump but has little effect on a diffusion pump.[13] It is possible for H_2 to react with unsaturated compounds on metal surfaces.

H_2 and He give better resolution (smaller plate height) than N_2 at high flow rate because solutes diffuse more rapidly through H_2 and He than through N_2. The more rapidly a solute diffuses between phases, the smaller is the mass transfer (Cu_x) term in the van Deemter equation (22-33). Equations 22-35a and 22-35b describe the effects of the finite rate of mass transfer in an open tubular column. If the stationary phase is thin enough ($\lesssim 0.5$ μm), mass transfer is dominated by slow diffusion through the *mobile phase* rather than through the *stationary phase*. That is, $C_s << C_m$ in Equations 22-35a and 22-35b. For a column of a given radius, r, and a solute of a given retention factor, k, the only variable affecting the rate of mass transfer in the mobile phase (Equation 22-35b) is the diffusion coefficient of solute through the mobile phase. Diffusion coefficients follow the order $H_2 > He > N_2$.

van Deemter equation:

$$H = A + \frac{B}{u_x} + Cu_x$$

Multiple paths | Longitudinal diffusion | Equilibration time

Most analyses are run at carrier gas velocities that are 1.5 to 2 times greater than the optimum velocity at the minimum of the van Deemter curve. The higher velocity is chosen to give maximum efficiency (most theoretical plates) per unit time. A decrease in resolution is tolerated in return for faster analyses.

Gas flow through a narrow column may be too low for best detector performance, so extra *makeup gas* is sometimes added between the column and the detector. Makeup gas that is optimum for detection can be a different gas from that used in the column.

Impurities in carrier gas degrade the stationary phase. High-quality gas should be used, and it should be passed through purifiers to remove traces of O_2, H_2O, and organic compounds. An oxygen indicator trap should be in line after the main purifier. Steel or copper tubing, rather than plastic or rubber, should be used for gas lines because metals are less permeable to air and do not release volatile contaminants into the gas stream. As with thermal degradation, symptoms of oxidative degradation of the stationary phase include increased baseline signal at low temperature, peak broadening and tailing, and altered retention times.

Guard Columns and Retention Gaps

In gas chromatography, a *guard column* and a *retention gap* are both typically a 3- to 10-m length of empty capillary in front of the capillary chromatography column. The capillary is silanized so that solutes are not retained by the bare silica wall. Physically, the guard column and the retention gap are identical, but they are employed for different purposes.

Guard column: accumulates nonvolatile substances that would contaminate chromatography column

The purpose of a **guard column** is to accumulate nonvolatile substances that would otherwise contaminate the chromatography column and degrade its performance. Periodically,