Nineth Week

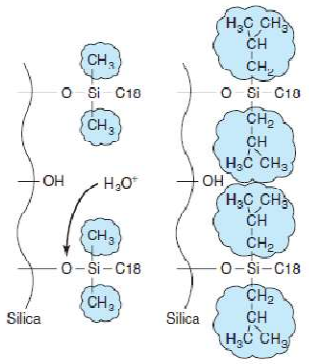


FIGURE 24-8 Bulky isobutyl groups protect siloxane bonds from hydrolysis at low pH. [From J. J. Kirkland, *Am. Lab.*, June 1994, p. 28K.]

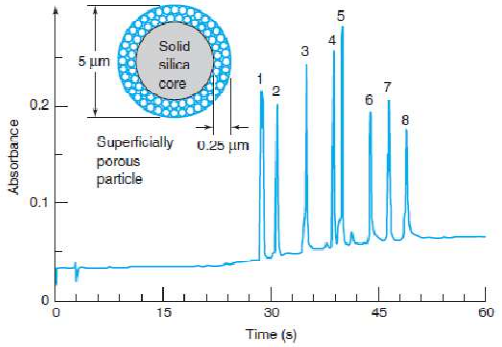
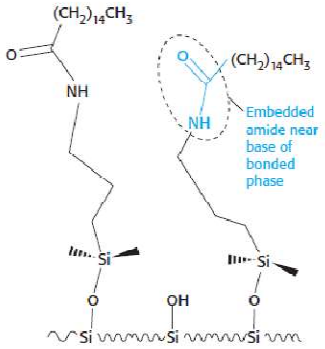


FIGURE 24-9 Rapid separation of proteins on superficially porous C_{18}-silica in 2.1 × 75 mm column containing Poroshell 300SB-C18. Mobile phase A: 0.1 wt% trifluoroacetic acid in H_2O. Mobile phase B: 0.07 wt% trifluoroacetic acid in acetonitrile. Solvent was changed continuously from 95 vol% A/5 vol% B to 100% B over 1 min. Flow = 3 mL/min at 70°C at 26 MPa (260 bar) with ultraviolet detection at 215 nm. Peaks: 1, angiotensin II; 2, neurotensin; 3, ribonuclease; 4, insulin; 5, lysozyme; 6, myoglobin; 7, carbonic anhydrase; 8, ovalbumin. [From R. E. Majors, *LCGC Column Technology Supplement*, June 2004, p. 8K. Courtesy Agilent Technologies.]



Nonpolar bonded phase with embedded polar amide group offers different selectivity from C_{18}, has improved peak shape for bases, and tolerates 100% aqueous eluent.

Another type of nonpolar stationary phase has a *polar embedded group*. The example in the margin consists of a long hydrocarbon chain with a polar amide group near its base. Embedded polar groups provide alternate selectivities from C_{18} stationary phases, improved peak shapes for bases, and compatibility with 100% aqueous phase. Other nonpolar stationary phases should not be exposed to 100% aqueous phase because they become very difficult to re-equilibrate with organic phase.

Figure 24-9 shows a rapid separation of proteins on **superficially porous particles** (also called *fused-core* particles), which consist of a 0.25-µm-thick porous silica layer on a 5-µm-diameter nonporous silica core. A stationary phase such as C_{18} is bonded throughout the thin, porous outer layer. Mass transfer of solute into a 0.25-µm-thick layer is 10 times faster than mass transfer into fully porous particles with a radius of 2.5 µm, thus enabling high efficiency at high flow rate. Superficially porous particles are especially suitable for separation of macromolecules such as proteins, which diffuse more slowly than small molecules. Figure 24-3 showed that the van Deemter curve for superficially porous particles with a total diameter of 2.7 µm and a porous layer thickness of 0.5 µm is similar to that of a conventional, totally porous particle with a diameter of 1.8 µm. The superficially porous particle enables separations similar to those achieved with 1.8-µm totally porous particles without requiring such high pressure.

Porous graphitic carbon deposited on silica[13] is a stationary phase that exhibits increased retention of nonpolar compounds relative to retention by C_{18}. Graphite has high affinity for polar compounds and separates isomers that cannot be separated on C_{18}. The stationary phase is stable in 10 M acid and 10 M base.

Pharmaceutical companies often separate the two enantiomers (mirror image isomers) of a drug because each enantiomer has a different pharmacological effect. To resolve enantiomers, optically active bonded phases, such as those shown in Figure 24-10 and Exercise 24-B, are used.[14] Figure 24-10 shows the calculated geometry of the chiral drug naproxen binding to one enantiomer of the stationary phase. Mirror image forms of the drug are designated *R* and *S*. Mirror image forms of the stationary phase are designated (*R*,*R*) and (*S*,*S*). Binding of (*S*)-naproxen to (*S*,*S*)-stationary phase is stronger than binding of (*R*)-naproxen to (*S*,*S*)-stationary phase. Therefore, (*R*)-naproxen is eluted before (*S*)-naproxen from (*S*,*S*)-stationary phase. Some other chiral stationary phases are based on substituted cellulose, on cyclic peptides with sugar substituents, and on cyclodextrins (Box 23-1).

The Elution Process

In *adsorption chromatography*, solvent molecules compete with solute molecules for sites on the stationary phase (Figure 24-11 and Color Plate 28). The relative abilities of different solvents to elute a given solute from the adsorbent are nearly independent of the nature of the solute. Elution occurs when solvent displaces solute from the stationary phase.

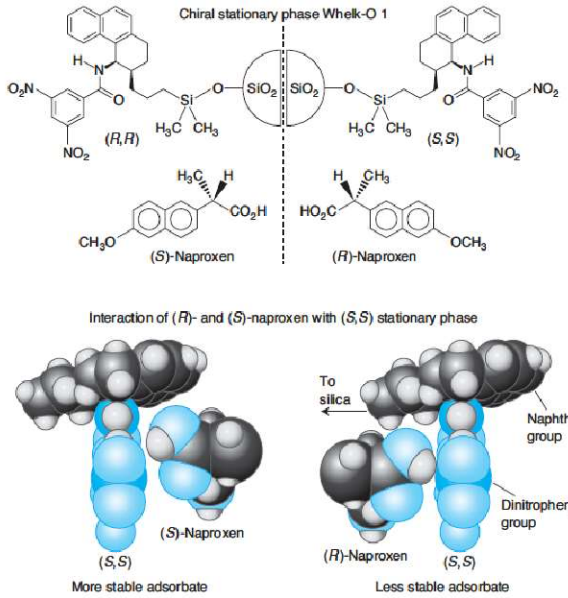


FIGURE 24-10 Interaction of enantiomers of the drug naproxen with (*S*,*S*) chiral stationary phase Whelk-O 1. (*S*)-Naproxen is adsorbed more strongly and is therefore retained longer by the column. [From S. Ahuja, "A Strategy for Developing HPLC Methods for Chiral Drugs," *LCGC* 2007, *25*, 1112.]

An *eluotropic series* ranks solvents by their relative abilities to displace solutes from a given adsorbent. The **eluent strength**, ε°, is a measure of the solvent adsorption energy, with the value for pentane defined as 0 for adsorption on bare silica. Table 24-2 ranks solvents by their eluent strength on bare silica. The more polar the solvent, the greater is its eluent strength for adsorption chromatography with bare silica. The greater the eluent strength, the more rapidly will solutes be eluted from the column.

Adsorption chromatography on bare silica is an example of **normal-phase chromatography**, in which we use a polar stationary phase and a less polar solvent. *A more polar solvent has a higher eluent strength*. **Reversed-phase chromatography** is the more common scheme in which the stationary phase is nonpolar or weakly polar and the solvent is more polar. *A less polar solvent has a higher eluent strength.* In general, eluent strength is increased by making the mobile phase more like the stationary phase. Reversed-phase chromatography eliminates peak tailing because the stationary phase has few sites that can strongly adsorb a solute to cause tailing (Figure 22-21). Normal-phase chromatography is sensitive to small amounts of

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

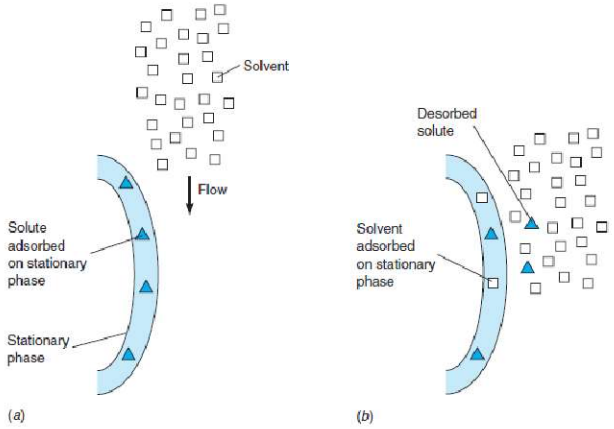


FIGURE 24-11 Solvent molecules compete with solute molecules for binding sites on the stationary phase. The greater the eluent strength of the solvent, the more easily it displaces the solute.

TABLE 24-2 Eluotropic series and ultraviolet cutoff wavelengths of solvents for adsorption chromatography on silica

Solvent	Eluent strength (ε°)	Ultraviolet cutoff (nm)
Pentane	0.00	190
Hexane	0.01	195
Heptane	0.01	200
Trichlorotrifluoroethane	0.02	231
Toluene	0.22	284
Chloroform	0.26	245
Dichloromethane	0.30	233
Diethyl ether	0.43	215
Ethyl acetate	0.48	256
Methyl *t*-butyl ether	0.48	210
Dioxane	0.51	215
Acetonitrile	0.52	190
Acetone	0.53	330
Tetrahydrofuran	0.53	212
2-Propanol	0.60	205
Methanol	0.70	205

NOTE: The ultraviolet cutoff for water is 190 nm.

SOURCES: L. R. Snyder, in High-Performance Liquid Chromatography (C. Horváth, ed.), Vol. 3 (New York: Academic Press, 1983); Burdick & Jackson Solvent Guide, 3rd ed. (Muskegon, MI: Burdick & Jackson Laboratories, 1990).

water in the eluent, but reversed–phase chromatography is not. Box 24-2 describes the solvent–stationary phase interface in reversed-phase chromatography.