17th Week

Ion-Pair Chromatography

Ion-pair chromatography uses a reversed-phase HPLC column instead of an ion-exchange column to separate polar or ionic compounds.[8] To separate cations (for example, protonated organic bases), an anionic *surfactant* (Box 25-1) such as n-$C_8H_{17}SO_3^-$ is added to the mobile

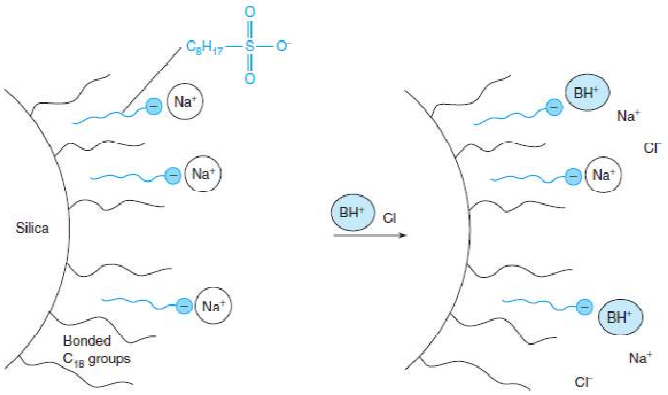

FIGURE 25-12 Ion-pair chromatography. The surfactant sodium octanesulfonate added to the mobile phase binds to the nonpolar stationary phase. Negative sulfonate groups protruding from the stationary phase then act as ion-exchange sites for analyte cations such as protonated organic bases, BH^+.

phase. The surfactant lodges in the stationary phase, effectively transforming the stationary phase into an ion exchanger (Figure 25-12). Analyte cations are attracted to the surfactant anions.[9] The retention mechanism is a mixture of reversed-phase and ion-exchange interactions. To separate anions, tetrabutylammonium salts can be added to the mobile phase as the ion-pair reagent (Figure 25-13).

Ion-pair chromatography is more complex than reversed-phase chromatography because equilibration of the surfactant with the stationary phase is slow, the separation is more sensitive to variations in temperature and pH, and the concentration of surfactant affects the separation. Methanol is the organic solvent of choice because ionic surfactants are more soluble in methanol/water mixtures than in acetonitrile/water mixtures. Strategies for method development analogous to the scheme in Figure 24-27 vary the pH and surfactant concentration with fixed methanol concentration and temperature.[10] Because of the slow equilibration of surfactant with the stationary phase, gradient elution is not recommended in ion-pair chromatography. Many ion-pair reagents have significant ultraviolet absorption, which makes ultraviolet detection of analytes problematic. Reversed-phase stationary phases with a *polar embedded group* (page 602) are a possible alternative to ion-pair chromatography for polar compounds.

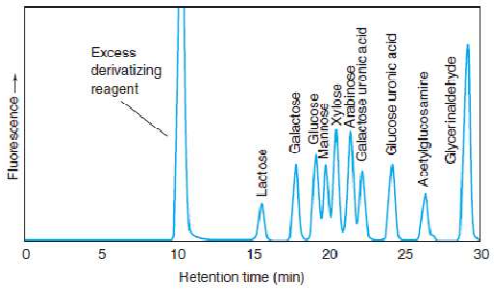

FIGURE 25-13 Separation of carbohydrates by ion-pair chromatography. Carbohydrates were *derivatized* by covalently attaching *p*-aminobenzoate (H_2N—C_6H_4—CO_2^-), which changes carbohydrates into fluorescent anions. The anions were separated on a 0.30 × 25 cm column of AQUA® C_{18}-silica,

and sensitive. Ion-pair chromatography utilizes an ionic surfactant in the eluent to make a reversed-phase column function as an ion-exchange column.

Molecular exclusion chromatography is used for separations based on size and for molecular mass determinations of macromolecules. Molecular exclusion is based on the inability of large molecules to enter small pores in the stationary phase. Small molecules enter these pores and therefore exhibit longer elution times than large molecules. In affinity chromatography, the stationary phase retains one particular solute in a complex mixture. After all other components have been eluted, the desired species is liberated by a change in conditions. In hydrophobic interaction chromatography, high concentrations of ammonium sulfate induce proteins to adhere to a hydrophobic stationary phase. A gradient of decreasing salt concentration is applied to increase the solubility of proteins in water and elute them from the column.

In capillary zone electrophoresis, ions are separated by differences in mobility in a strong electric field applied between the ends of a silica capillary tube. The greater the charge and the smaller the hydrodynamic radius, the greater the electrophoretic mobility. Normally, the capillary wall is negative, and solution is transported from anode to cathode by electroosmosis of cations in the electric double layer. Solute cations arrive first, followed by neutral species, followed by solute anions (if electroosmosis is stronger than electrophoresis). Apparent mobility is the sum of electrophoretic mobility and electroosmotic mobility (which is the same for all species). Zone dispersion (broadening) arises mainly from longitudinal diffusion and the finite length of the injected sample. Stacking of solute ions in the capillary occurs when the sample has a low conductivity. Electroosmotic flow is reduced at low pH because surface $Si—O^-$ groups are protonated. $Si—O^-$ groups can be masked by polyamine cations, and the wall charge can be reversed by a cationic surfactant that forms a bilayer along the wall. Covalent coatings reduce electroosmosis and wall adsorption. Hydrodynamic sample injection uses pressure or siphoning; electrokinetic injection uses an electric field. Ultraviolet absorbance is commonly used for detection. Micellar electrophoretic chromatography uses micelles as a pseudostationary phase to separate neutral molecules and ions, which can be concentrated by stacking. Capillary electrochromatography is essentially the same as HPLC, but the mobile phase is driven by electroosmosis instead of pressure. Capillary gel electrophoresis separates macromolecules by sieving. In contrast with molecular exclusion chromatography, small molecules move fastest in gel electrophoresis. Microfluidic devices ("lab-on-a-chip") use electroosmotic or hydrodynamic flow in lithographically fabricated channels to conduct chemical reactions and chemical analysis.