$$V_e = V_0 + K_D V_i \tag{11.83}$$

where V_e is the total eluent buffer volume, V_0 is the void volume in the column (= $V\varepsilon$), which is the volume of fluid filling the void space outside the gel particles, V_i is the total void volume inside the gel particles, and K_D is the partition coefficient.

For large molecules that do not penetrate inside the gel structure, $K_D = 0$; for small molecules that completely penetrate inside the gel, $K_D = 1$. Equation 11.83 can be rewritten as

$$\frac{V_e}{V_0} = 1 + K_D \frac{V_i}{V_0} \tag{11.84}$$

For a given solute mixture and gel beads, K_D and the V_i/V_0 ratio are fixed, and the V_e/V_0 ratio is approximately constant, irrespective of column geometry and V_e.

Gel-filtration chromatography can be used to determine the molecular weight of macromolecules. A plot of V_e/V_0 versus log (MW) yields a straight line with a negative slope that is proportional with K_D.

Affinity chromatography is based on the highly specific interaction between solute molecules and ligands attached on polymeric or ceramic beads in a packed column. The concept of affinity chromatography is described in Fig. 11.30. The matrix is usually agarose. However, polyacrylamide, hydroxyethyl methacrylate, cellulose, and porous glass can also be used as the matrix bead. Spacer arms between the matrix and ligand are usually linear aliphatic hydrocarbons. The use of spacer arms may reduce the steric hindrance generated by the matrix. Coupling between the matrix and ligand depends on the functional groups present on the matrix and ligand. Chemically reactive groups on the support matrix usually are —OH,—NH_2, or —COOH groups. If the reactive group on the matrix is an —OH group (polysaccharides, glass, hydroxyalkyl methacrylate), then cyanogen bromide (CNBr) is used as a coupling agent. The cyanogen bromide-activated agarose reacts with primary amine groups present in proteins that act as ligands. After the desired solutes are bound to the ligand, elution is achieved by changing the pH or ionic strength in the column. Ligand-solute molecule interactions in affinity chromatography

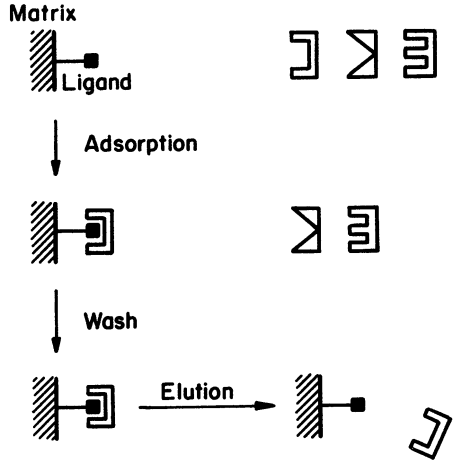

Figure 11.30. Basic principles of affinity chromatographic separations. A suitable ligand is covalently attached to an insoluble matrix. In the adsorption step, only those molecules with a specific binding site for the ligand bind to the adsorbent; molecules without the proper geometric fit pass through unaffected. In the elution step, the bound molecules are disengaged from the column and collected. (With permission, from M. L. Yarmush and C. K. Colton, in M. Moo-Young, ed., *Comprehensive Biotechnology*, Vol. 2, Elsevier Science, London, 1985.)