11.4.2. Aqueous Two-phase Extraction

Aqueous two-phase extraction is an approach under active development for the extraction of soluble proteins such as enzymes between two aqueous phases containing incompatible polymers, such as polyethylene glycol (PEG) and dextran. The phases containing PEG and dextran are more than 75% water and are immiscible. Typical aqueous phases used for this purpose are PEG–water/dextran–water and PEG–water/K-phosphate–water. PEG/dextran and PEG/K phosphate are reasonably immiscible. The partition coefficient, K_p, varies with the molecular weight (MW) of the soluble protein in the form of an exponential function.

$$K_p = e^{AM/T} \tag{11.38}$$

where M is the MW of protein, T is the absolute temperature, and A is a constant.

The partition coefficient, K_p, of many enzymes between the two phases (C_{PEG}–C_{DEX}) varies between 1 and 3.7, resulting in poor separations in a single stage. The partition coefficient can be improved by including ion-exchange resins or certain salts, such as $(NH_4)_2SO_4$ and KH_2PO_4, in one of the phases. Figure 11.12 depicts the variation of K_p of an enzyme with a concentration of KH_2PO_4 in a PEG/$(NH_4)_2SO_4$ system. More than ten-fold increases in K_p values may be achieved by increasing the concentration of KH_2PO_4 from 0.1 to 0.3 M. In PEG–salt systems, salting-out may result in protein precipitation at the interface.

Ion-exchange resins can be used to increase the value of a partition coefficient. PEG can be derivatized to yield cation or anion exchange properties and used in a two-phase

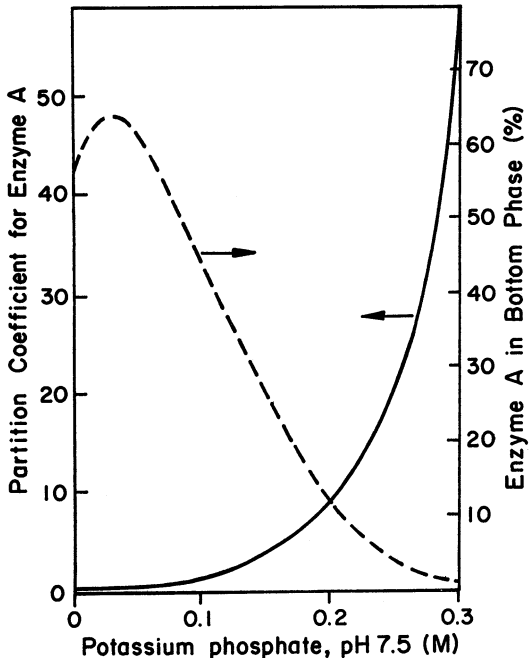

Figure 11.12. The expected behavior of an enzyme in a two-phase solution is depicted. Note the dependence of the partition coefficient of a hypothetical protein on the concentration of potassium phosphate in a polyethylene glycol 4000 ammonium sulphate system. A typical condition would be 14% PEG 4000 and 9.5% $(NH_4)_2SO_4$.