system. Affinity ligands may also be used to increase K_p values; examples are PEG–NADH and PEG–cibacron blue systems. This method is known as *two-aqueous-phase affinity partition extraction.* Also, partial hydrolysis of dextran and PEG may result in an increase in the K_p value, since lower-MW polymers may interact with proteins more effectively. By mixing two kinds of PEGs (PEG_{400} and PEG_{4000}), the K_p value may be increased by a factor of 6 for the partition of fumarase. Two-aqueous-phase extraction can also be used for the recovery of cell debris, polysaccharides, and nucleic acids. The partition coefficient for whole cells and DNA is between 100 and 0.01, for proteins it is between 10 and 0.1, and for small ions it is around 1.

After the extraction step, the phases can be separated by centrifugation or decantation, and PEG can be recovered by ultrafiltration. Figure 11.13 is a block diagram of an enzyme separation unit using the two-phase partitioning method with PEG recovery. This separation method is fast and can be operated under mild conditions of temperature, pressure, and pH. Dextran and PEG are recovered and reused, since the cost of polymers is the major economic factor.

11.4.3. Precipitation

The first step in the purification of intracellular proteins after cell disruption is usually *precipitation.* Proteins in a fermentation broth (before or after cell lysis) can be separated from other components by precipitation using certain salts. Examples include streptomycin sulfate and ammonium sulfate.

The two major methods used for protein precipitation are as follows:

1. Salting-out by adding inorganic salts such as $(NH_4)_2SO_4$ at high ionic strength.
2. Solubility reduction at low temperatures by adding organic solvents ($T < -5°$).

Salting-out of proteins is achieved by increasing the ionic strength of a protein-containing solution by adding salts such as $(NH_4)_2SO_4$ or Na_2SO_4. The added ions interact with water more strongly, causing protein molecules to precipitate. The solubility of proteins in a solution as a function of the ionic strength of the solution is given by

$$\log \frac{S}{S_0} = -K'_S(I) \tag{11.39}$$

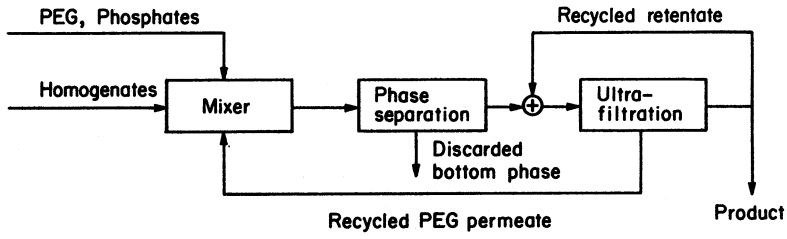

Figure 11.13. Two-phase extraction process with PEG recovery.