where S is the solubility of protein in solution (g/l), S_0 is the solubility of protein when $I = 0$, I is the ionic strength of solution, and K'_s is the salting-out constant, which is a function of temperature and pH.

The ionic strength of a solution is defined as

$$I = \frac{1}{2} \Sigma C_i Z_i^2 \tag{11.40}$$

where C_i is molar concentration of the ionic species (mol/l), and Z_i is the charge (valence) on ions.

Figure 11.14 depicts variation of the solubility of hemoglobin with inorganic salt concentrations. At high ionic strengths, the solubility of the protein decreases logarithmically with ionic strength.

Organic solvent addition at low temperatures ($T < -5°C$) causes the precipitation of proteins by reducing the dielectric constant of the solution. The solubility of protein as a function of the dielectric constant of a solution is given by

$$\log \frac{S}{S_0} = -K'/D_s^2 \tag{11.41}$$

where D_S is the dielectric constant of the water–solvent solution.

A reduction in the dielectric constant of a solution results in stronger electrostatic forces between the protein molecules and facilitates protein precipitation. The addition of solvents also reduces protein–water molecule interactions and therefore decreases protein solubility. Solvents may cause protein denaturation. However, denaturation of proteins in the salting-out method is less likely. *Solvent precipitation* can be used with salt addition, pH adjustment, and low temperature to improve precipitation. Among other protein precipitation methods, the following methods are the most widely used.

Isoelectric precipitation is the precipitation of proteins at their isoelectric point, which is the pH at which proteins have no net charge. The isoelectric point of a protein is defined as $pI = \frac{1}{2}(pK_1 + pK_2)$. When pH = p$I$, protein becomes free of charge and precipi-

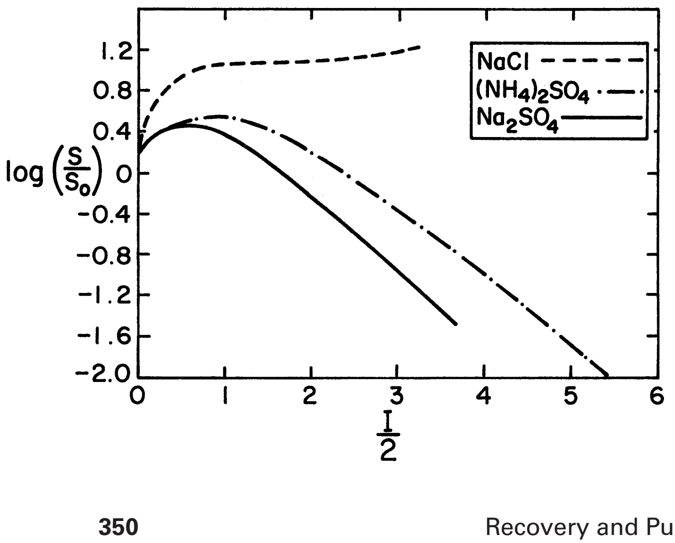

Figure 11.14. Effect of inorganic salts on solubility of a typical protein.