of such stabilizers (i.e., amino acids, polyols, sugars, and PEGs), it is obvious that specific chemical interactions are not the common stabilization mechanism. Such solutes tend to be "excluded" from the surface of the protein and therefore induce "preferential hydration" of the protein. The thermodynamics of this phenomenon is analogous to solute "surface excess" at the air-water interface. A negative surface excess means the solute is partly excluded from the interface, thereby increasing the surface tension of the solution. Indeed, there is a good correlation between those solutes that increase the surface tension of water and those that are excluded from the surface of a protein (6). The thermodynamic consequence of "solute exclusion" and "preferential hydration" is to increase the chemical potential of the protein. The first assumption in relating this thermodynamic result to cryoprotection may be stated as: if the increase in chemical potential of the native protein caused by solute exclusion is denoted, $\Delta\mu_N$, the corresponding increase for the unfolded protein is $k\Delta\mu_N$, where $k > 1$. Thus, the free energy of unfolding would be increased by the solute, which is equivalent to stabilization of the native conformation (6). The second assumption is that pharmaceutical stability, or degradation, is directly related to thermodynamic stability. Finally, it is assumed that the solution state concepts are valid throughout the freezing process, or at least valid over the period where unfolding in the absence of a cryoprotectant is both thermodynamically favored and kinetically allowed.

The extent of solute exclusion, $(\partial m_s/\partial m_p)_{T,\mu_w,\mu_p}$, can be measured, and the corresponding effect on chemical potential of the protein, $(\partial\mu_p/\partial m_s)_{T,P,m_p}$, can then be calculated (6,73–76):

$$\left(\frac{\partial \mu_p}{\partial m_s}\right)_{T,P,m_p} = -\left(\frac{\partial m_s}{\partial m_p}\right)_{T,\mu_w,\mu_p}\left(\frac{\partial \mu_s}{\partial m_s}\right)_{T,P,m_p} \tag{13}$$

where the solute exclusion parameter is the partial derivative of the molal concentration of stabilizer in the domain of the protein, m_s, with respect to the molal concentration of protein, m_p, at constant temperature, T, and constant chemical potentials of water, μ_w, and protein, μ_p. The relationship also involves the concentration dependence of the chemical potential of stabilizer, μ_s, which may be written

$$\left(\frac{\partial \mu_s}{\partial m_s}\right)_{T,P,m_p} = \frac{RT}{m_s} + RT\left(\frac{\partial 1n\gamma_s}{\partial m_s}\right)_{T,P,m_2} \tag{14}$$

The first term on the right-hand side is the "ideal solution" contribution while the "nonideal" part involves the concentration dependence of the activity coefficient of the stabilizer in water, γ_s. Since equation (14) involves the reciprocal of stabilizer concentration, it appears that the response of the chemical potential of the protein to a stabilizer, $(\partial\mu_p/\partial m_s)_{T,P,m_p}$, becomes infinite as the stabilizer concentration approaches zero. However, the molal solute exclusion parameter, $(\partial m_s/\partial m_p)_{T,\mu_w,\mu_p}$, is actually directly proportional to concentration, so the chemical potential derivative, $(\partial\mu_p/\partial m_s)_{T,P,m_p}$ is roughly independent of concentration for most of the systems studied (73–76). The factors impacting the chemical potential derivative are more transparent if we first convert from molal concentration units to mass-based concentrations (i.e., weight ratios, where the weight ratio of component "i" to water is symbolized, g_i); g_i is related to molality