may dominate in many cases. Rather, the intent is to demonstrate that the predictive power of the thermodynamic results, such as in Table 2, for selecting the "optimum" stabilizer system for a "new protein" is quite limited and this limitation should not come as a surprise.

Stabilization During Drying

As discussed earlier, all stresses that develop during freezing are still present during drying, but the normal implicit assumption is that if degradation during freezing did not occur, the freezing stresses are not sufficient to thermodynamically destabilize the protein. Thus, the critical stress during drying is normally assumed to be the removal of the water that is part of the freeze concentrate. This assumption is likely correct as long as degradation occurs during secondary drying where the timescale is similar to that for freezing. Even assuming the critical stress factor in drying is the reduction of water to low levels, thereby removing the assumed stabilizing influence of hydrogen bonding interactions between protein and water, it is not obvious that the role of a stabilizer is to replace water in the hydrogen bonding interactions and thermodynamically stabilize the native conformation. If the stabilizer were to effectively couple the internal motions of the protein to structural relaxation in the glass, thereby reducing the rate of unfolding of a thermodynamically unstable system to insignificant levels, the net result would be a freeze-dried protein in the native conformation. That is, the protein would not unfold, regardless of what the free energy of unfolding might become, and stabilization would be purely kinetic.

Many experimental studies of the relative effectiveness of various solutes in preventing drying damage can be interpreted according to either the water substitute hypothesis, which is a thermodynamic stabilization mechanism, or a purely kinetic stabilization argument based on the effective coupling of the protein motions to the glass. There are studies, however, which do not appear to be consistent with both classes of stabilization mechanisms. For example, the L-asparaginase study discussed earlier demonstrates that the water substitute hypothesis cannot always provide a satisfactory explanation of the data. Neither TMG nor PVP can hydrogen bond as water substitutes but yet are effective in preventing inactivation during drying. The catalase example discussed earlier illustrates that a high glass transition temperature for the excipient is not the critical factor in stabilization during drying. Even glucose ($T_g = 39^\circ C$) and mannitol (which frequently crystallizes) stabilize as effectively as do materials that readily form glasses with glass transition temperatures on the order of 100°C. While the catalase data do appear to be better interpreted in terms of the water substitute hypothesis than by protein immobilization in a glass, it must be acknowledged that mobility of the protein in a glass is not necessarily well measured by the difference between the glass transition temperature of the pure excipient and the sample temperature. Firstly, since the catalase formulations were 1:1 weight ratio mixtures of excipient and catalase, the glass transition temperatures of the formulations will be intermediate between the glass transition temperature of the pure excipient and the glass transition temperature of the protein. Since protein T_g values are normally quite high (63,64), the differences in formulation glass transition temperatures will be much less than the differences in excipient glass transition temperatures. Secondly, glass fragility,