unfolding in solution will tend to have the same qualitative effect during lyophilization. For example, the stability of many enzymes during freeze-thawing is altered by the presence of substrates, cofactors, and/or allosteric modifiers (18). Even for nonenzyme proteins, specific ligands can be important components of the formulation. For example, the stability of fibroblast growth factors is greatly increased in the presence of heparin or other polyanionic ligands (reviewed in Ref. 19). The *pH* and specific ligands that confer optimum stability often are known from purification protocols, preformulation studies, and/or earlier efforts at designing a liquid formulation.

However, most proteins are not adequately stabilized by specific solution conditions. Of the nonspecific stabilizers that have been tested, sugars have been shown to stabilize the most proteins during lyophilization and have been known to have this property for the longest time. To our knowledge, the first published report is the 1935 paper by Brosteaux and Eriksson-Quensel (20) in which they described the protection during dehydration/rehydration of several proteins by sucrose, glucose, and lactose. Subsequent detailed comparisons of sugars documented that usually disaccharides provide the greatest stabilization (4,8,21,22). For protection during the lyophilization cycle itself, both reducing and nonreducing disaccharides are effective. However, reducing sugars (e.g., lactose and maltose) can degrade proteins during storage via the Maillard reaction (protein browning), a process that can be accelerated at intermediate residual moisture contents (22,23). Therefore, the choice of disaccharides is essentially limited to the nonreducing sugars, sucrose and trehalose. Since, as of early 1998, trehalose has not been used in any Food and Drug Administration (FDA) approved parenteral product, sucrose is usually the first choice for commercial protein drug formulations.

Although the data are much more limited, polyvinylpyrrolidone (PVP) and bovine serum albumin (BSA) have also been shown to protect a few tetrameric enzymes, that is, asparaginase, lactate dehydrogenase (LDH), and phosphofructokinase (PFK), during lyophilization and rehydration (24,25). Another class of compounds that has been found to be useful in freeze-dried formulations are nonionic surfactants. For example, sucrose fatty acid monoester, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propa-nesulfonate (CHAPS), and Tweens have been found to increase recovery of β-galactosidase activity after freeze-drying and rehydration (26). Various surfactants have been shown to protect LDH during freeze-drying and rehydration (27). Hydroxypropyl-β-cyclodextrin, which is surface active (28,29), inhibited the inactivation of recombinant tumor necrosis factor (30), interleukin-2 (31,32), and LDH (27) during freeze-drying/rehydration.

MECHANISMS OF STABILIZATION OF PROTEINS BY SUGARS DURING DEHYDRATION

Most protein pharmaceuticals are multicomponent systems that contain protein, buffer salts, bulking agents, and stabilizers. Each component has its intended role in the formulation. For example, often a crystallizing excipient (e.g., mannitol or glycine) is chosen as a bulking agent (15). In contrast, numerous studies have documented that stabilization of a protein during dehydration requires the presence of a compound that remains at least partially amorphous. When a protein formulation is frozen, the protein partitions into the non-ice phase with other amorphous components. The interaction between the protein and these amorphous components must be maintained during the entire freeze-drying