Luckily, as water leaves the amorphous phase, T_g increases. Sample temperatures play a larger role than the duration of secondary drying for determining final water content of the lyophilized cake. Low moisture increases T_g and thus increases the temperature at which the product can be stored.

A drug solution is first frozen at the atmospheric pressure, and then, water is removed by a reduction of pressure in the lyophilizer chamber, collecting the water as ice on a condenser. Samples are placed in glass vials and frozen, either before being put in the lyophilizer or on the lyophilizer shelves. The samples contain ice crystals, unfrozen water, amorphous solids (including the therapeutic protein), and crystalline additives. Pressure is reduced, and the ice crystals sublime. This constitutes the primary drying.

It is harder to remove the unfrozen water trapped in an amorphous solid. So, after primary drying, a secondary drying stage removes that water by increasing the temperature. The final temperature of this secondary process is the key factor in determining the residual moisture in the dried cake. The pressure is kept the same for secondary and primary drying, to avoid protein collapse. The ideal result is a porous cake with little residual moisture. Porosity is important in later reconstituting the product.

Sometimes, an annealing step (in which the product is kept at a set temperature) is added before the primary drying or near the end of secondary drying to crystallize excipients. This assures that crystallization and moisture release do not happen in an uncontrolled fashion later on during shipping and storage. Phase changes (e.g., crystallization of formulation sugars) during shipping or storage can be disastrous. Moisture can even transfer from rubber vial-stoppers during storage, unless savvy formulators plan for and prevent it.

The criteria for dried-protein stability include minimum lyophilization-induced unfolding, with proteins native in the dried solid; a powder with T_g higher than the desired storage temperature; low residual moisture (<1%) in the cake; and formulation conditions (such as pH) that inhibit chemical degradation reactions unaffected by glass transition (such as oxidation). The goal is to design the fastest and most robust (acceptable quality even with variations in operating parameters) processing cycle: one that consumes the least amount of energy, does not compromise product quality, and produces a mechanically strong, rapidly insoluble cake. The cycle must be controlled for reproducibility and rapid correction of any problems that develop.

The use of FT-IR spectroscopy shows that almost all proteins except G-CSF (filgrastim) unfold during lyophilization, and while they do refold upon reconstitution, it is often necessary to add ingredients and stabilizers to keep them from unfolding in the solid state. Some of the ingredients used are listed in Table 9.4. However, the use of additives, when combined, may show unexpected interactions, synergism, enhanced instability, or even altered immunogenicity. For example, sugars (or saccharides) raise T_g and act as stabilizers. Dextran, lactose, maltose, sucrose, and trehalose are used, but the latter two are preferred. Acidic amino acids (glutamic acid, glycine, histidine, and threonine) and alkaline amino acids (arginine and lysine) are used to adjust pH besides the use of buffering systems. A nonionic surfactant (usually a polysorbate) is often added to inhibit protein aggregation in the early stage of processing, and given its innocuous nature, it is left in the formulation, while other contaminants such as the peroxides are removed. Also included in the formulations are other surfactants and bulking agents, such as mannitol and certain biodegradable polymers, mainly in low-concentration formulations.