Biological standards and reference materials are supplied for immediate use, and prior to use should be stored at the temperature indicated on the label. Once freeze-dried material is reconstituted, users must determine the stability of the material according to their own methods of preparation, storage, and use. In general, NIBSC follows the WHO policy in not setting expiry dates for freeze-dried biological standards and reference materials.

PROCESS DESIGN AND TROUBLESHOOTING

Cycle Design

The cycle design process used at NIBSC has resulted from different priorities than those facing most users of freeze-drying in the pharmaceutical or food industries. Unlike these sectors, there is little requirement for repeat batches of the same material. The normal constraints, such as the number of batches that have to be processed per week and the cost of the freeze-drying process per batch, are not considered as the highest priority. Unlike most pharmaceutical freeze-drying, our control programs must take into account that active processing occurs in a "9 a.m. to 5 p.m." weekday-only environment.

The key factors in determining successful freeze-drying are long-term stability, preservation of sufficient activity, and rapid facile reconstitution. The product residual moisture content and atmospheric composition (in terms of oxygen content) are adjuncts to this. In the WHO guidelines, there are no set limits for minimal moisture level. We have an in-house limit of $<1\%$ to reduce the likelihood of water-catalyzed hydrolytic and other degradative processes. However, in practice, the level of residual moisture is usually well below this for most of our materials.

In common with most freeze-drying operations there are three basic stages in the process—freezing, primary drying, and secondary drying.

Freezing

All constituent water needs to be totally immobilised, either by crystallization to ice or incorporation into a glass of super-concentrated biological material, before a vacuum is drawn and freeze-drying can commence. The product must be maintained at a sufficiently low temperature to be below the eutectic (T_{eu}) point(s) of the major crystallizing electrolyte present and the glass transition temperature (T_g') of the protein/carbohydrate glass for sufficient time to ensure this. NIBSC is unusual in facing different freeze-drying challenges in terms of disparate formulations with almost every fill. The start material may be any of a wide range of biologicals, by nature protein, carbohydrate, glycoprotein, nucleic acid, or a complex mixture of several different components. Indeed, we are now encountering whole-cell and whole-virus preparations where integrity is required after reconstitution. There may be a wide variety of excipients required for the preservation of the biological activity following reconstitution but which may help or hinder the freeze-drying process.

It is vital to know the composition of the material to be freeze-dried so as to determine the suitable freezing conditions. Some salts such as tris, hepes, or calcium chloride with low T_g' values may lower the glass transition to such an extent that the freezing temperature may be below the operational capabilities of the freeze drier; the lowest practical temperature achievable with our production-scale freeze driers is $-50^{\circ}C$.