Although the drying process progresses on the basis of the pre-programmed profile of shelf temperature and chamber pressure with time, there can be variation in terms of temperature across a shelf and across the chamber itself. The mapping of the variation in temperature across the freeze drier is an important part of the qualification of a new plant and should be repeated periodically, especially if problems are suspected in coolant flow path.

Formulation and Freeze-Drying Conditions

At its simplest, a product for freeze-drying might contain only water as the solvent and the biological to be dried as the solute. The establishment of suitable freezing conditions would be straightforward, and once the water had been eliminated by sublimation the product would be stable and remain active. However, in reality a variety of nonvolatile excipients will be present either to stabilize the product during the drying process or to ensure activity once the product is reconstituted. Other excipients may supply bulk, as often the quantity of the biological standard is minute and to ensure good cake formation at least 2% wt/wt solids should be maintained. These excipients will themselves influence the freeze-drying conditions and may influence the activity and stability of the product as they are concentrated on sublimation of the water ice. For instance buffers may be added to preserve biological activity, but the pH range created by these buffer components may vary due to differential crystallization during freezing, and indeed some buffer component may be volatile and so pH may shift on sublimation.

Typically we would avoid high levels of salts, such as sodium chloride and buffers (such as phosphate-based systems) that might shift pH on freezing (18). Preferred excipients would be lyoprotectants such as nonreducing sugars, especially trehalose (19), and albumin (20) or glycine as bulking agents (21).

To determine the freeze-drying parameters, a number of critical parameters are required. The first among these is the T_{eu} of formulation components that crystallize on freezing and the T_g' of those that form an amorphous state; these should be determined to set the maximum safe product temperature for primary drying and can be ascertained by one of a number of methods.

Modulated Differential Scanning Calorimetry—mDSC

The determination of T_g' of frozen liquids and the T_g of freeze-dried solids has been widely reported (22). We have recently used modulated DSC (for a review of mDSC see Ref. 23) as a routine primary tool in determining the freeze-drying conditions to apply to standards being processed. The glass transition is observed as an inflexion in the modulated reversed heating profile and the T_g' is derived by application of the manufacturer's software (24). In our experience, the use of large (100 μL) sample pans has helped to enhance what can be very weak signals. In addition clearer glass transitions can be observed at high concentrations of sample. For some samples, at least, the determined T_g' at a high concentration holds true also at the lower concentrations being freeze-dried. For example, low molecular weight heparin analyzed at concentrations of 100, 50, and 10 mg/mL (the latter being the concentration at which freeze-drying was to be performed) all gave a similar T_g' value. However, the inflexion in the DSC signal was far less distinct at the concentration at which freeze-drying was to occur (Fig. 11).