Differential thermal analysis permits more detailed analysis of thermal events comparing the heat changes in a sample against that of a reference material (often water) and can be used to determine equilibrium freezing temperature, phase changes, and onset temperature for ice melting. An advantage is that analysis can be performed in the same containers in which lyophilization will occur, but the interpretation of the profile, especially in complex formulations, may be difficult (Fig. 12B) as multiple thermal events may occur and other means will be needed to determine which are important to freeze-drying. Other thermal analysis techniques can also be used (see chap. 5).

Freeze-Drying Microscopy

Freeze-drying microscopy (FDM) mimics the freezing and thawing process under the microscope by means of a controlled temperature microscope stage with a sample mounted under vacuum in a thin film (24,28,29). It yields a collapse temperature based on visual inspection of collapse of crystal structure as the sample is gradually warmed from frozen. Although initially a home-built technology, the equipment is less expensive than DSC; cryostages are available from microscopy suppliers (such as Linkam, Epsom, U.K.) and an integrated freeze-drying microscope is also available (e.g., Lyostat, Biopharma Technology Limited, Winchester, U.K.).

Although FDM should generate T collapse values for all samples (Fig. 13), these figures are derived from the collapse in a 2 to 5 μL of sample on the slide whereas freeze-drying will be of containers possibly filled to a height of several centimeters. For this reason, broad margins for error are applied. It has been suggested to perform freezing at temperatures 2°C to 5°C below the T_g' value (24,29).

In conclusion, it is necessary to have at least one and ideally several different techniques available to get the most accurate T_g' information for use during freeze-drying cycle development (30).

Impact of Process Scale-Up

The scalability of freeze-drying cycles can be a problem as cycles devised for a pilot machine may not necessarily work in a process scale dryer with 10-fold or greater surface area, different pumps, and cooling systems. As part of the qualification of our new 12 m^2 freeze-dryer at NIBSC we adopted a strategy of investigating in the new dryer the performance of our cycles devised at laboratory scale and used successfully in smaller 1- and 6 m^2 dryers. Tests were performed when using only a single shelf and also when operating under full occupancy. Two run cycles were evaluated, one for human plasma and one for formulated influenza antigen. Both materials were frequently processed and so were typical of the operating demand on the new dryer. Following lyophilization, assessment of the product appearance and of the residual moisture content and product functional activity were made at a number of key locations across the batch. For a one-shelf run, this was the center tray and each corner tray of vials and when assessing a full load (of six shelves) the midpoint on each shelf and the midpoint and corners on top and bottom shelves were assessed. Good reproducibility across a single shelf and across a 5-shelf 20,000 run was shown for influenza antigen. However, for plasma, although moisture and activity were