It has been our practice to extend the length of the process stages well beyond the minimum time indicated from the probe-containing ampoules/vials during development studies; this helps to prevent problems during scale-up without the need for further validation runs. Although there are a number of end-point determination technologies now commercially available and indeed the smaller production dryer is fitted with a manometric pressure rise tool, we normally do not aim to modify the cycles, developed at laboratory scale, for the scale-up to manufacturing scale.

Secondary Drying

Following primary drying, secondary drying is used to remove most of the amorphous water component of the glass. The shelf temperature used is as high as can be applied to the biologicals to promote rapid drying without loss of functional activity. Given that some of our standards are labile biological factors there might well be loss of activity if too high a secondary drying temperature is used. We typically use 25°C to 30°C with a 30 μbar chamber pressure across a range of biological materials. Typically, the product temperature probes do not quite attain the shelf temperature in our experience even after prolonged secondary drying periods. Residual moisture is a particular concern for biological standards due to the requirement for a prolonged shelf life. Levels of moisture that may be acceptable for some freeze-dried products such as 2% to 5% wt/wt may not be satisfactory for reference materials and, routinely, we dry to 1.0% or less residual moisture.

There is evidence to support the view that products can be overdried. Some water may be needed to maintain the structural stability of the biological, and removal of this may lead to destabilization. Extended drying of insulin resulted in a rise in the level of degradation products detected by reverse phase high-performance liquid chromatography (HPLC) analysis, as shown in Table 3 (14). Other researchers have noted loss of activity in influenza virus with excessive drying (15). The optimum level of dryness should be determined for each material to be dried (16) where feasible, given the number of formulations to be dried.

Use of Product Temperature Probes

It is well accepted that containers housing product temperature probes are uncharacteristic of the containers that do not have probes; nucleation is enhanced by the heterogeneous surface introduced by the probe (17) and the probe leads present a heat source. Probes in containers cannot be used as an absolute indication of the conditions occurring in those containers without a

TABLE 3 Damage Caused by Overdrying of Insulin

Preparation method	Predicted degradation rate at -20^{o}C % per annum	Residual moisture (%)
Freeze-dried only	0.105	0.46
Freeze-dried then further desiccated 1 wk over phosphorus pentoxide	0.475	<0.1

Degradation rates based on Arrhenius calculations from degradation at elevated temperatures and measurement of degradation products by reverse phase HPLC.
Source: Adapted from Ref. 14.