ice fog (13), ultrasound (14–16), vacuum (11,17), and electrical (18,19). The resulting crystallization rate is dependent on the overall solution temperature at the time of ice nucleation, as well as the rate at which heat is removed after nucleation. For example, in freezing method 1 above, the vial and its contents are to be just below 0°C before a bottom corner of the vial is put in contact with dry ice. Then the vial is placed onto a shelf, the temperature of which is then gradually reduced.

While many in the past have interchangeably used the terms "cooling rate" and "freezing rate," it is important to distinguish between them. The cooling rate is the rate at which the vial is cooled. This cooling rate may affect the temperature at which ice nucleates or, more precisely, the regions of the liquid volume over which nucleation occurs. The freezing rate only applies to the postnucleation freezing which in some limited cases is irrelevant for determination of the final ice structure. A true freezing rate is either in terms of a linear front velocity for directional freezing or mass per unit time for bulk freezing operations.

As explained by Searles et al., the following terms are useful when discussing freezing for lyophilization: *primary nucleation* is the initial ice nucleation event (4). *Secondary nucleation* follows primary nucleation, and moves with a velocity on the order of mm/sec to encompass some portion of the liquid volume (20–22). Subsequent to secondary nucleation, *solidification* is completed relatively slowly as the heat of crystallization is transferred from the solidification interface through the already-solidified layer and the vial bottom to the shelf. These terms pertain to freezing by *global supercooling* in which the entire liquid volume achieves a similar level of supercooling, and the secondary nucleation zone encompasses the entire liquid volume (as in the example in the previous section). Given the design of lyophilizer shelf temperature control systems, shelf-ramp freezing is by nature slow and it will, with typical vials and fill volumes, freeze by global supercooling, which yields a low surface area. In contrast, *directional solidification* occurs when a small portion of the volume is supercooled to the point of primary and secondary nucleation. The nucleation and solidification fronts are in close proximity in space and time with the front moving into nonnucleated liquid. Many write about liquid nitrogen immersion freezing inducing less supercooling than slower cooling methods, but more accurately faster cooling results in supercooling over a smaller *volume* before nucleation than slower cooling.

Foam drying has recently gained attention as a promising method of freezing and drying that achieves high retention of activity for vaccines and proteins (23–30). It is a combination of vacuum-induced surface freezing and low-temperature vacuum boiling, which results in a low surface area foamed product.

EFFECTS ON ACTIVE INGREDIENTS

Freezing itself can adversely affect the active ingredient, and the freezing method can also affect the quality of the product through subsequent processing and until expiry. These impacts can result from the low temperature itself, acceleration of degradation reactions, crystallization of the product, product denaturation and aggregation, pH shifts, phase separation, and denaturation at the ice interface. Much of the literature on this subject concerns the stabilization