damage their protein, the subsequent freeze-drying step did. Shelf-ramp freezing and freeze-drying resulted in a similar level of damage, and polysorbate 80 in the formulation reduced the damage for both types of samples.

Freeze-concentration may bring solutes into concentration ranges where they will phase separate, causing deleterious effects on the product (9,49–55). Heller et al. demonstrated that the increased mobility during annealing facilitated phase separation in a PEG:dextran formulation. The phase separation caused unfolding of recombinant hemoglobin, and they used formulation design strategies (52) and PEGylation of the protein (54) to avoid the damage. The freezing step has also been found to be critical in lyophilization of DNA for gene therapy (56,57).

Recently Cochran and Nail published results showing a correspondence between the ice nucleation temperature and recovery of LDH protein activity (58).

It has long been known that vaccine adjuvants known as "alum" gels (aluminum hydroxide or aluminum phosphate) will be inactivated by coagulation during freezing. Recently, it has been discovered that very fast freezing as in spray-freezing into liquid nitrogen could prevent inactivation (59). Since then, Clausi et al. have found that glass-forming sugars can also prevent coagulation during freeze-drying (60), and Jones-Braun and coworkers developed liquid formulations that are stable when taken to −20°C (61).

Zhai et al. (2004) (62) and Hansen et al. (2005) (63) studied a modified herpes virus intended as a gene therapy vector. Zhai et al. found heavily formulation-dependent effects of different freezing methods on recovery of virus activity through freeze-drying. High concentrations (27%) of sucrose or trehalose achieved the best results, with lower concentrations achieving less in a dose-dependent fashion. The freezing rate studies showed that for 27% sucrose, conventional shelf freezing resulted in titers as high as higher-rate freezing. The other formulation tested in the freezing rate studies contained only 2.5% sucrose. Titer yields were lower regardless of freezing method, but in the absence of high-concentration sucrose conventional shelf freezing did very poorly compared with flash-freezing. Hansen found that with the same freeze-sensitive 2.5% sucrose formulation, flash-freezing as well as rapid thawing were required to retain potency (63).

EFFECTS ON PRODUCT MORPHOLOGY, SURFACE AREA, AND DRYING RATE

The freezing method and cooling rate during freezing have profound impacts on the morphology and surface area of the final product. These parameters can be easily modified by any of the annealing steps (intentional or accidental), and the parameters in turn determine the resistance to vapor flow (affecting the primary drying rate and temperature during drying) as well as the secondary drying rate.

Reviews of early literature on lyophilization freezing and annealing phenomena appear in recent papers by Searles et al. (3,4), parts of which will be recapitulated here. In 1925, Tammann reported that the ice crystal morphology can be strongly influenced by the nucleation temperature (64). Samples frozen at "low supercoolings" yield dendritic structures, whereas "crystal filaments" result from high supercoolings. In 1961, Rey described an annealing step for orange juice that resulted in a twofold increase in the primary drying rate (65).