+5°C to −10°C at −1°C/min, held for one hour, then cooled to −45°C at −1°C/min) alleviated the concentrated core by shifting the mechanism to "global supercooling" as described in this chapter. Annealing yielded significant reductions in primary drying time with no adverse effect on product quality.

An interesting case of inadvertent annealing is described by Wallen et al. (12) They examined a case of higher numbers of rejected vials originating from the higher shelves of a production lyophilizer. The product in question is frozen in a liquid nitrogen-freezing tunnel, in which liquid nitrogen is sprayed onto filled vials. The frozen vials are then loaded onto precooled shelves of the lyophilizer. Use of product temperature monitors found that, owing to the shelf indexing scheme and natural convection of room air, the vials loaded onto the topmost shelves were warming. This "inadvertent annealing" appears to have caused a higher collapse rate.

Blue and Yoder describe a 60% reduction in reconstitution time resulting from annealing a concentrated monoclonal antibody formulation (89).

MECHANISMS OF MORPHOLOGICAL CHANGE DURING ANNEALING

For amorphous solutes, annealing above T_g' will, by the definition of T_g', result in melting of ice into neighboring nonice regions (Fig. 1). The increased water content and higher temperature increase both the bulk mobility of and the amorphous phase and diffusional mobility of all species in that phase. The increased bulk mobility of the amorphous phase during annealing allows it to relax into physical configurations of lower free energy. Surface free energy (also known as surface tension) (γ) is defined as free energy per unit area, and consequently has units of energy per unit area (85). Therefore there will always be a driving force for a contiguous volume to reduce its surface area so long as it is not already a perfect sphere (the shape which possesses the minimum possible surface-to-volume ratio).

The structures in freeze-concentrated systems inevitably possess junctions, edges, and other departures from the perfect sphere. Each of these three-dimensional features has two principal radii of curvature. The *pressure* inside such a structure with positive radii of curvature can be shown to be

$$P = 2\gamma\left(\frac{1}{r_1} + \frac{1}{r_2}\right) + P_o$$

where γ is the surface free energy (energy/length^2), r_1 and r_2 are the radii of curvature, and P_o is the surrounding pressure. Consider a needle-shaped structure. The tip will have very small radii of curvature, and the radii of curvature for material in the body of the needle will be much greater. Using the equation above we can deduce that the pressure in the tip will be higher than the pressure in the body. Given sufficiently low viscosity, this pressure difference will (during our timescale of observation) cause *bulk flow* of material from the tip into the body, and the tip will actually retract: the needle will become more sphere-like in shape. Structures with negative internal radii, such as the concave surface at the junction of two spheres in contact, will have a *lower* pressure than the surroundings, leading to a flow of material to these areas. Sintering and accretion of high surface area ice particles into a single lower surface area structure are manifestations of this principle (90,91).