a result of freezing can be expected, with a corresponding increase in the surface concentration of protein in dried material and protein destabilization. Another possible explanation for the two apparently conflicting observation, that is, exclusion of non-antifreeze proteins from ice interface in the presence of antifreeze proteins versus destabilization of proteins by ice, is that the antifreeze proteins compete with other proteins for the interface, whereas in the absence of antifreeze proteins, other proteins might indeed be sorbed by ice surface. In either case, the use of nonionic surfactants to stabilize proteins, presumably by preventing the binding of the proteins to air/liquid surfaces, and also possibly ice/liquid interfaces, was demonstrated in many systems, e.g., in the example of recombinant human factor XIII [42].

Solute Inclusion Inside Ice Crystals

Solubility of essentially all common freeze-drying solutes in hexagonal ice is negligible, in other words, one can expect that the ice phase consists of 100% water. However, on a macroscopic scale, solution can be entrapped by growing ice crystals under certain conditions. In this section, several examples of such entrapment are discussed.

Entrapment of a solution phase by growing ice crystals depends on the freezing conditions, i.e., geometry of the crystallization front, rate of progression of the ice/solution interface, and macroscopic viscosity of the solution phase. In one study, freezing of small droplets of solutions containing sucrose, pullulan, bovine serum albumin (BSA), antifreeze glycoprotein, polyvinyl alcohol (PVA), and PEG was studied using optical refractometry [43]. Relatively diluted solutions, with the solute concentration of <5 wt.% (for sucrose) and <1 wt.% (for other solutes), were used. A concentration gradient of solute was observed at the ice/solution interface (length scale up to 200 μm) for all solutes but antifreeze glycoprotein, for which the concentration measured at the ice/solution interface, was the same as in the bulk. No incorporation of the solution phase into ice crystals was observed at the growing speed of 2 μm/s, when the ice/solution interface remained approximately planar, whereas at the higher growth speeds dendritic ice morphology was observed with a significant amount of solution trapped between the dendrites as liquid inclusions.

In a recent report, freezing behavior of ternary system water–DMSO–albumin was studied using FTIR and confocal Raman microscopy [30]. Solutions with different albumin/DMSO ratios were equilibrated at various subzero temperatures to create a two-phase (ice + freeze-concentrated solution) system. The albumin/DMSO ratios in the freeze-concentrated solution (FCS) were measured using FTIR and confocal Raman microscopy. In such a two-phase system, one would expect that the ratio would not change from the original single-phase solution, as can be shown using the temperature–composition phase diagram of a ternary system [7]. It was observed, however, that the ratio changed in a complicated manner. In particular, the albumin/DMSO ratio increased at relatively higher temperatures of -4 and -6 °C, which was interpreted as due to trapping DMSO inside ice crystals, whereas the