the nonaqueous surface, which leads to denaturation and precipitation. Thus, while proteins can tolerate high shear in a continuous liquid medium, their stability is compromised when introduced to an interface in the presence of even moderate shear forces [56]:

$$\text{amphiphilic protein} + \text{air / liquid interface} \xrightarrow{\text{shear}} \text{aggregation}.$$

The larger the surface area of the interface, the greater the possibility of aggregation and denaturation, which means that smaller droplets are subject to higher rates of aggregation because of the larger size of the interface between air and liquid. Surfactants [57] and amino acids [58] are used to lower the concentration of proteins at the air–liquid interface during spray-drying by competing to occupy the interface, thus lessening the corresponding aggregation and precipitation. Another method of reducing aggregation at the air/liquid interface is the utilization of spray-freeze-drying. Fortunately, diffusion to the liquid/ice interface during freezing is negligible as demonstrated by Webb et al. (2002), who observed that aggregation of recombinant human interferon-γ (rhIFN-γ) after spray-freezing was not significantly different from aggregation during atomization [59]. Yu et al. (2002) suggest spray-freeze-drying *directly* into liquid nitrogen through an insulated nozzle rather than spray-freeze-drying above the liquid surface, which can reduce aggregation by increasing the freezing rate of the process [60]. Yu et al. showed minimal loss of monomer bovine serum albumin (BSA; < 1 %) with rapid-freezing spraying compared to Costantino et al. (2000) who reported 2 % monomer BSA loss in their best case scenario with "traditional" spray-freeze-drying [61]. By reducing the time until freezing, the proteins do not have sufficient time to migrate to the interface and aggregate, thus reducing damage to the protein.

Another explanation for protein denaturation due to spray-drying is denaturation during the dehydration stage of processing. When water is removed from the formulation, a protein can undergo conformational changes when hydrogen bonds with water are broken as the water is removed from the system. Sugars, such as sucrose or trehalose, stabilize the proteins by forming hydrogen bonds with the proteins as the water is removed from the system, preventing the unfolding of the protein in the absence of water. The water substitution/replacement hypothesis also explains why sugars fail to prevent irreversible destabilizations when present in high concentrations [62, 63]. When high concentrations of excipients are present, excipient–excipient bonding may compete with excipient–protein hydrogen bonding and partition into sugar-rich regions and protein-rich regions, thus reducing their stabilizing capacity. Additionally, sugars and other stabilizing excipients form an amorphous glass matrix (preferably with a high T_g) which trap the protein, protecting its structure [64].

In the end, and as with all pharmaceutical formulations, different excipients will have differing effects on biopharmaceutical stability during spray-drying. Understanding the types of instability which are most likely to affect the biopharmaceutical under investigation will aid the scientist in determining the optimal type of stabilizing excipient to use for their biomolecule. As a point of comparison on