expected to have no significant amount of intermediates and it was shown that a calorimetric measurement of enthalpy for the denaturation process should be equal to that determined indirectly by the temperature dependence of the denaturation equilibrium constant, which yields the van't Hoff enthalpy (Tanford, 1968). Privalov showed that this was the case for several small globular proteins (Privalov & Khechinashvili, 1974). However, it was shown by rapid temperature jump studies that kinetic intermediates could be present and that a small globular protein such as ribonuclease A undergoes denaturation via a sequential unfolding mechanism (Tsong et al., 1971). The proposed two-state model also suggested that large unfolding of the overall protein structures often drives protein aggregation, but in fact even small localized unfolding may result in protein aggregation as shown by the IgG_2 studies where large denaturation transitions were not observed by DSC until after 37 °C.

Aggregation of mAb during unit processing operations

Freezing and Thawing

Manufacturing lots of protein DP are often stored as bulk in freeze tanks and portions thawed as needed for final fill and finishing. During this freezing process the water component of the DP starts freezing and excludes formulation excipients and protein from its environment, resulting in regions where the protein molecules and excipients are concentrated (Kolhe, Holding, Lary, Chico, & Singh, 2010). This "cryoconcentration" leads to extreme changes in solution conditions such as pH, osmolality, ionic strength, etc., which may impact protein conformation and promote aggregation. During the thawing of large frozen bulk solutions concentration gradients are also generated, which may promote aggregation. Using specially designed cryovessels, where the thawed solution is circulated during the thawing process, can mitigate some of this (Wisniewski & Wu, 1996). In addition, during the freezing process ice crystal formation can promote aggregation (Strambini & Gabellieri, 1996). Measurement of Trp intrinsic phosphorescence emission lifetime, τ, correlates with rigidity of the protein core and was used to show that perturbation of protein conformation results from the direct interaction of the protein with the ice crystal surface. The number and size of the ice crystals will determine the ice surface area available for interaction with the protein. Slow cooling rate and long-term storage can promote the formation of fewer and larger crystals resulting in decreased surface area for protein interaction with ice surfaces (Franks, 1985). The decreased surface area should also lead to smaller conformational changes of the protein resulting in less aggregation. This was demonstrated by comparing changes in τ for protein solutions seeded with an ice crystal and cooled either at 200 or at 1 °C/min. The protein solutions that were tested overall showed smaller perturbations of structure at the slower cooling rate. Frozen solutions left to anneal for 10 h at −6 °C resulted in some reversal of τ showing that maturation of the ice structure into fewer and larger crystals results in less structural perturbation.

A systematic study on the freeze-thawing of an IgG_2 mAb as a function of pH, with and without 150 mM KCl, mAb concentration, cooling and warming rates, and container type and material showed that although all the parameters studied had an impact