Aggregation, which requires bimolecular collision, is expected to be the primary degradation pathway in protein solutions. The relationship of concentration to aggregate formation depends on the size of the aggregates as well as the mechanism of association. A typical approach to minimize aggregation is to restrict the mobility of proteins in order to reduce the number of collisions.

Lyophilization with appropriate excipients may improve protein stability against aggregation by decreasing protein mobility and by restricting conformational flexibility with the added benefit of minimizing hydrolytic reactions consequent to the removal of water. The addition of appropriate excipients, including lyoprotectants, can prevent the formation of aggregates during the lyophilization process as well as during storage of the final product. A key parameter for effective protection is the molar ratio of the lyoprotectant to the protein. Generally, molar ratios of 300:1 or greater are required to provide suitable stability, especially for room temperature storage. Such ratios can also, however, lead to an undesirable increase in viscosity.

Lyophilization allows for designing a formulation with appropriate stability and tonicity. Although isotonicity is not necessarily required for subcutaneous administration, it may be desirable for minimizing pain upon administration. The isotonicity of a lyophile is difficult to achieve because both the protein and the excipients are concentrated during the reconstitution process. Excipients with protein molar ratios of 500:1 will result in hypertonic preparations if the final protein concentration is targeted for >100 mg/mL. If the desire is to achieve an isotonic formulation, then a choice of the lower molar ratio of excipient is necessary.

Determining the highest protein concentration achievable remains an empirical exercise due to the labile nature of protein conformation and the propensity to interact with itself, with surfaces, and with specific solutes.

8.7 Demonstrating equivalence of formulations

To demonstrate the similarity of formulation, the sponsor needs to show the equivalent stability of the active ingredient; this may require demonstrating the stability of the originator formulation and the stability of the originator drug substance (extracted from originator product) in the proposed biosimilar formulation.

To predict potential stability problems within a short period and to develop appropriate analytical methods, proteins are exposed to stronger-than-real stresses and various degradation products induced by the stresses are examined. The results obtained from these so-called accelerated stability studies might also be useful to predict the kinetics of the degradation processes under real handling conditions, when there are no sufficient