EFFECT OF CONTROLLED RATE FREEZE AND THAW ON BIOPHARMACEUTICALS

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Radmanovic et al.[2] determined that both freezing time and cooling time affected the level of biopharmaceutical soluble aggregates with very short and very long freezing and cooling time resulting in higher levels of soluble aggregates. In general, intermediate cooling and freezing times resulted in overall optimized biopharmaceutical product quality attributes.

Cao et al.[3] found that a freezing rate of about 1°C/min and a fast thawing rate > 10°C/min produced higher protein activity recovery, whereas fast freezing with slow thawing resulted in more severe damage to proteins. During slow thawing, additional damage to proteins is caused by the recrystallization process. Recrystallization exerts additional interfacial tension or shear on the entrapped proteins and hence causes additional damage.

Rathor et al.[4] showed that slow freezing rates can result in cryoconcentration, in which proteins and excipients form concentration gradients near the freeze front and get excluded from the ice-liquid interface.

Rathor et al. and Bhatnager et al.[5] both reported that this can further lead to pH shifts and phase separation among components, resulting in protein structural damage.

Chang et al.[6] and Strambini et al.[7] observed that fast freezing rates lead to smaller ice-crystal formation, which exposes proteins to a large ice-liquid interface. Concentration and adsorption of proteins at the surface of ice crystals along the ice-liquid interface can result in their partial unfolding, increased aggregation, and decreased biological activity. Additionally, Lashmar et al.[8] and Miller et al.[9] reported that fast freezing can entrap air in the ice. When released during thawing, the entrapped air can denature proteins as air-liquid interfaces form.

Reinsch et al.[10] revealed that only if the local redistribution of both ion concentration and pH values are known, protein product loss may be calculated and minimized by optimizing the freezing process. Concentration effects are specific for the composition of the medium and the buffer and have to be determined on a case-to-case basis.

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