Ovalbumin

Ovalbumin, a *protein* found in *egg white*, has been studied extensively for its structural properties [24]. Judge et al. demonstrate the feasibility of using bulk crystallization for the recovery and purification of ovalbumin. Ovalbumin crystals were obtained in a batch crystallization mode using ammonium sulfate. Factors influencing crystallization such as protein concentration (supersaturation, metastable region) and pH were extensively studied and evaluated. Crystallization was also performed in the presence of related proteins conalbumin (80,000 Da) and lysozyme (14, 600 Da), the obtained crystals were further washed, and protein purity determined to be >99%. These findings suggested that bulk crystallization could be applied as a purification step for bulk processing of ovalbumin; additionally the presence of impurities (related proteins) did not adversely affect crystallization as a purification step [28, 31]. Carbone et al. describe the use of a desupersaturation curve-based method to understand seeded isothermal batch crystallization of ovalbumin. Since the method utilized inexpensive instrumentation, it offers a simple and cost-effective approach for analysis and large-scale protein crystallization [10].

Lysozyme

Similar to ovalbumin, bulk crystallization has also been successfully attempted for lysozyme. As described earlier, Judge et al. discuss the effect of protein impurities on lysozyme crystal growth [29]. The findings suggest that presence of related protein impurities (avidin, ovalbumin, and conalbumin) at concentrations as high as 50% had little effect on solubility and crystal purity but the effect on face growth rates varied and was impurity specific. The effect of process parameters such as temperature and solution pH on the nucleation of lysozyme crystals has also been evaluated [30]. Hekmat et al. describe a systematic approach for the scale-up of crystallization of lysozyme from vapor diffusion-based experiments (20-µl sitting-drop volume) to batch crystallization in agitated milliliter scale vessels [22]. Quantitative phase diagrams were generated for both the 20-µl sitting-drop vapor diffusion condition and the 200-µl microtiter plates. Phase diagrams suggested that rising agitation rates, resulted in a significant reduction of the area of nucleation zone. Batch crystallization of lysozyme was performed under different conditions including 0.2–2 ml Eppendorf tubes in a laboratory rotator, an unbaffled shake flask (5-ml volume) and stirred baffled and unbaffled vessels (4-ml volume). The size of the crystals obtained in the rotated Eppendorf tubes and the stirred vessels was smaller. Large crystal aggregates were formed in the shake flask experiments, which were otherwise unsuitable for scale-up and robust process development. The use of ethanolammonium formate, a biocompatible ionic liquid acting as a crystallization aid, resulted in larger crystals in unbaffled stirred tanks [23]. Based on the crystal properties, it was concluded that ml-scale batch crystallization of lysozyme in stirred