Seeding in the context of crystallisation refers to the introduction of heterogeneous additives into a crystallising solution. A recent development of heterogeneous protein crystallisation is the design of nucleant which provides some degree of control over the protein nucleation step with its porosity and surface chemistry.[71–74] In particular, mesoporous silicates (MPS) with pore size between 2 and 50 nm have shown promising nucleation performance for a wide range of proteins.[71,75–81] In general, the pore size of MPS should be larger than the hydrodynamic or gyration diameter of the target protein.[82–84] It is reported that MPS with narrow pore size distributions between 2 and 20 nm can crystallise seven proteins which have gyration/hydrodynamic diameter smaller than the pore size.[79]

10.3.2 Scale-up and Mixing

Although high throughput screening enables the crystallisation of thousands of proteins for structural determination purposes,[85–89] only a few have been scaled up beyond the microliter scale due to the limited availability of protein as well as the difficulty of scaling up from the screening conditions.[90]

Tables 10.2 and 10.3 summarise the literature on the bulk crystallisation of 11 proteins, which include antibodies, aprotinin, insulin, lipase, L-methionine γ-lyase, lysozyme, ovalbumin, protease, rubisco, subtilisin and urease. The majority of literature report on the crystallisation of these proteins in batch stirred tank crystallisers (Table 10.2), which are simple to operate and control as compared to the tubular crystallisers that involve direct and oscillatory flow (Table 10.3). These studies demonstrate the feasibility of large-scale protein crystallisation, whose scale can reach up to 100 L as in the case of L-methionine γ-lyase.[91] In general, the crystallisations were conducted within the temperature range of 0–40 °C and the pH range of 4–10. These mild conditions mean the temperature control is relatively simple and no special construction material is required for corrosion resistance.

In large scale protein crystallisation, mixing is necessary for enhancing the mass and heat transfer within the crystalliser. The size and shape of crystals are affected by the mixing conditions, as the growth rates of different crystal faces are strongly dependent on the flow conditions.[92–96] The nucleation process is also strongly influenced by the mixing conditions. Mild agitation of protein droplets during vapour diffusion experiments delays the induction of crystallisation due to higher homogeneity of protein concentration within the droplets.[97–99] In contrast, more vigorous mixing by the impeller in a stirred tank crystalliser was found to shorten the induction time of crystallisation and lead to smaller protein crystals.[100]

More importantly, the mixing conditions have significant influence on the stability of the protein, as the denaturation of protein in high flow rate was observed in some cases.[101] The cause might be the presence of air bubbles and the resulting formation of protein foam, since some studies showed that proteins are stable under high shear rates.[102] For example, immunoglobulin-G1 monoclonal antibodies were found to be stable at 250 000 s^{-1}, whereas