of the membranes which results in unacceptably long times for filtration. Membrane fouling may be due to several factors, and the presence of protein aggregates may be a significant factor. The aggregates may be present prior to the filling, having been generated by previous unit operations such as freeze–thaw, or actually may be generated during the filtration process. Examination of the fouling deposits of albumin after filtration using field emission scanning electron microscopy (FESEM) showed two different types of fouling deposits on membrane surfaces described as cakes and aggregates (Kim, Fane, Fell, & Joy, 1992). Filtration using higher initial ultrafiltration (UF) flux showed protein aggregates whereas filtrations at lower initial UF flux showed cake formation. The "cake formation" is believed to consist of protein aggregates. These aggregates may have been generated by a rapid increase of protein concentration at the membrane surface due to the high initial convective flows. Although the FESEM was unable to detect protein deposits in the membrane pores, it was concluded that even one or two layers of protein deposits could explain the decline in flux. Such limited quantities of protein deposit are not detectable by the FESEM technique.

Cromwell et al. have discussed a nice example of the impact of UF/DF processing on a mAb (Cromwell, Hilario, & Jacobson, 2006). It was observed that the recovered sample from UF/DF processing had a significantly greater turbidity as measured at 350 nm. This increase in turbidity was hypothesized to be the result of mechanical stresses from the continuous pumping operations used during the UF/DF processing.

Filling

During filling mAb DP will be exposed to solid surfaces such as stainless steel and also the stresses imparted by pumping operations. The large-scale pumping imparts high shear to the solution resulting in the generation of stainless steel microparticles (Biddlecombe et al., 2007; Bee, Davis, Freund, Carpenter, & Randolph, 2010; Tyagi et al., 2009). Protein adsorption to the surfaces of these particle results in aggregates that are generated by surface-mediated processes since exposure to supernatant from centrifuged suspensions of stainless steel particles did not result in formation of protein aggregates (Bee et al., 2010).

The type of pumps and nozzles used for filling can have a huge impact on formation of mAb aggregates. In a rotary filling pump the protein solution actually lubricates the piston barrel and, due to the interaction with the piston hydrophobic surface and friction, the protein may adsorb and unfold. The unfolded protein may accumulate and eventually slough off the piston into the solution generating visible particulates (Figure 3.21). In one study two types of pumps were used, a rolling diaphragm pump and a radial piston pump (Cromwell et al., 2006). The mAb used in this study was known to be sensitive to frictional shear forces, and since the mAb solution would essentially lubricate the moving piston in the radial piston pump there was concern that this type of pump might generate aggregates. To ascertain if this was a problem ~1 L of the mAb solution was transferred 13 times between