in an overall smaller percentage of total protein that can unfold as the total protein concentration increases, that is, the ratio of available air–water surface area to total protein decreases.

The method used to generate the air–water interface dictates the types of aggregates that are formed. Aggregation of an IgG_1 mAb was studied at 5 and 25 °C using two agitation methods, either shaking or stirring (Kiese, Papppenberger, Friess, & Mahler, 2008). In addition, the impact of different air headspaces on mAb aggregation was also investigated. There was a large difference between horizontal shaking with a headspace and stirring in terms of the number and size of protein aggregates. In particular, shaking the solution resulted in generation of visible particulates, smaller subvisible particles (<2 mM), and soluble aggregates, whereas stirring resulted in an increase in solution turbidity attributable to generation of subvisible particles, but without production of soluble aggregates. It was suggested that these differences are due to the different stresses exerted on the solution by shaking compared to stirring. Shaking results in replenishment of the air–water interface and migration of aggregated protein into solution. Decrease of headspace by increasing the volume of agitated solution in a container decreases the amount of aggregated protein, which can be explained by the suppression of liquid movement within the closed container. Stirring results in different mechanical stresses such as local heating by friction of the stirrer and bottom of the container, and solid–water interface interactions between the hydrophobic surface of the stirrer and protein.

Use of large-scale pumps in DP unit operations

Large-scale pumps are often used to transfer DP into holding tanks or used for the actual processing step such as ultrafiltration/diafiltration and filling operations. These pumping operations can expose the mAb to mechanical stresses, which include shear and cavitation. These stresses may result in conformational changes in the mAb creating opportunities for aggregation. Although the shear itself may not impact aggregation of mAbs, the generation of air/water interfaces, exposure to solid surfaces, and pump cavitation that are associated with shear forces can be major factors in generation of aggregates. The types of pumps used have also been shown to impact aggregation processes. The following sections discuss in more detail DP unit operations that use pumps and the impact of these operations on mAb aggregation.

Filtration

Ultrafiltration/diafiltration unit operations are used as a manufacturing step to concentrate and formulate the mAb DS (Figure 3.19). In addition whenever the mAb DP is stored prior to filling the product it needs to be sterile filtered, usually through a 0.2 μm filter cassette. A common problem in these types of operations is the fouling