patient. Native-like aggregates may resemble haptens on the surface of pathogens that form organized and repetitive structures that can cross-link B cell receptors in a multivalent manner.

Aggregates can be classified according to size, reversibility, secondary or tertiary structure, covalent modification, and morphology. One class of aggregates that are of particular concern includes subvisible particles or, more specifically, aggregates ranging from 0.1 to 10 μm in size. The current United States Pharmacopeia light obscuration test for drug approval requires that the number of particulates over 10 μm is ≤6000 per container, while the number of particulates over 25 μm is ≤600 per container. Industrial researchers agree that additional analysis of subvisible particles smaller than 10 μm would support product characterization and development. Subvisible particles are too large to be analyzed by standard quality control methods such as SEC and SDS-PAGE, but too small to be visually detected. Therefore, the use of additional, less routine methods such as asymmetrical flow FFF and microflow imaging (MFI) has been recommended for extended characterization following a risk-based approach.

Product characterization should not only be limited to monitoring protein particles, but also focus on nonprotein particles. Foreign micro- and nanoparticles, for example, shed from filling pumps or product containers, are able to induce protein aggregation or nucleate the formation of heterogeneous aggregates.

7.2.3.2 Manufacturing and processing factors In addition to intended modifications, a biopharmaceutical may be chemically modified through accidental degradation in one of the many bioprocessing steps: fermentation, virus inactivation, purification, polishing, formulation, filtration, filling, storage, transport, and administration. Chemical stresses during manufacturing and storage can be caused by exposure to light or elevated temperatures and presence of oxygen, metal ions, or peroxide impurities from excipients in the formulation. Trace amounts of iron, chromium, and nickel can leach into the formulation buffer via contact with stainless steel surfaces typically used during bioprocessing and catalyze the degradation reactions.

7.2.3.3 Impurities and other production contaminants Therapeutic proteins obtained through recombinant DNA technology are produced in various cellular systems where production-linked protein impurities originate. These proteins are called HCPs, considered endogenous proteins by the immune system, and may lead to antibody formation by the standard immune mechanism. If the anti-HCP antibodies do not neutralize the biological activity of the therapeutic protein of interest, they can nevertheless have consequences in terms of general effects including skin reactions, allergies, anaphylaxis, or serum sickness. Other contaminants, such as impurities, coming from chromatographic column resins or from enzymes used for refining therapeutic proteins' purification,