phosphate-buffered solutions but not in histidine-buffered solutions at identical pH and ionic strengths. It is also important to minimize the potential presence of proteases in protein purification, either from sources intrinsic to the production process (e.g., HCPs) or from extrinsic sources of contamination (e.g., adventitious microbes).

8.5 Higher-concentration formulations

Treatments with high doses of more than 1 mg/kg or 100 mg per dose often require the development of formulations at concentrations exceeding 100 mg/mL because of the small volume (<1.5 mL) that can be given by the subcutaneous routes. For proteins that have the propensity to aggregate at higher concentrations, achieving such high-concentration formulations is a developmental challenge. Even for the intravenous delivery route where large volumes can be administered, protein concentrations of tens of milligrams per milliliter may be needed for high dosing regimens, and this may pose stability challenges for some proteins.

Protein–protein interaction in a high-concentration formulation may result in reversible self-association, which may further progress toward the formation of insoluble aggregates. At a higher concentration, the probability of one molecule bumping into another increases, enhancing the probabililty of formation of reversible oligomers such as a dimer, tetramers, etc. The formation of aggregates occurs through multiple mechanisms, including the formation of covalent linkages (e.g., disulfide exchange). Even minor conformational change in the native structure may lead to the formation of aggregates. The probability of such occurrence is greater in higher concentration.

High concentration is defined by the solution, in which a significant portion of the solution volume (≥0.1) is occupied by the solutes. Another definition of a high-concentration solution is the situation in which the molecular size and the distance between the van der Waals surfaces are on the same order of magnitude. Irrespective of the definition, high concentration refers to intermolecular distance or molecular proximity. The primary challenge in achieving high-concentration formulation is the solubility of the target protein. Solubility is controlled by its molecular property (sequence, charge distribution, etc.), as well as by the solution condition, such as pH, ionic strength, excipient concentration, etc. The solubility of a protein is defined by the maximum amount of the protein that can be present in a solution, without the appearance of any visible aggregates, precipitates, etc. A more technical definition will be the maximum amount of protein that remains in solution, following 30 min of centrifugation at 30,000*g* in the presence of cosolute. Besides solubility, there are other issues associated with the development of high-concentration mAb formulation. These include opalescence, viscosity, and aggregation. Opalescence is commonly expressed by nephelometric