higher in solution than within the red blood cell water will flow out through the cell membrane in order to balance the concentration of solutes across the membrane. The solution is hypotonic when the concentration of solutes within the red cell is greater than the solution outside the cell. This results in an influx of water often resulting in the bursting of the cell. The solution is termed isotonic when the fluid within the cell has a similar concentration of solutes as outside the cell. Usually an IV injection or infusion requires an isotonic preparation, whereas an IM or SC injection may be able to handle hypertonic and hypotonic conditions since the protein drug is not administered directly into the blood. Nonetheless, there has been concern related to disruption of tissue and possible pain on injection. In a study in rabbits, NaCl solutions ranging from 0.9% (300 mOsm) to 10% (3300 mOsm) were administered by IM and SC injections (Zietkiewicz, Kostrzewska, & Gregor, 1971). Tissue damage, as evidenced by necrosis, occurred between 3.9% (1300 mOsm) and 5.1% (1670 mOsm). In this study, 69 drugs were also evaluated for tonicity and 22 were isotonic, 23 hypertonic, and 24 hypotonic. The final conclusion from this study was that solutions up to 1300 mOsm do not cause necrosis of SC and muscle tissue. Most importantly it was concluded that for SC and IM injections the solutions do not need to be isotonic, but should avoid the critical limits of hypertonicity. It should be emphasized that this study only investigated tissue damage and did not address what the limits were for pain on injection. In another study, factors associated with muscle pain in humans were assessed using NaCl solutions of different tonicities (Brazeau, Cooper, Svetic, Smith, & Gupta, 1998). It was shown that muscle pain on IM injection only occurred for hypertonic solutions.

The tonicity of a protein formulation is modulated by inclusion of solutes, and the ionic strength of the formulation is dictated by the presence of charged solutes. One of the most used excipients for adjusting tonicity and ionic strength is NaCl. However, NaCl can hasten the corrosion of stainless steel, especially the 316L grade used in the bioprocessing of protein drugs (Ryan, Williams, Chater, Hutton, & McPhail, 2002). Thus, alternative excipients should be used for adjustment of ionic strength and/or tonicity. Sugars, which are added as stabilizers, can also be used to adjust the tonicity. If it is not possible to use less corrosive excipients such as NaCl another strategy is to use a higher grade of stainless steel alloy, which contains very little iron. Hastelloy has been used and although more expensive, the rate of corrosion of this stainless steel alloy is decreased compared to the common 316L stainless steel, which has a higher iron content (Davis, 1994; Srindhar, 1992).

Surfactants and surface-active agents

Liquid formulations of proteins and mAbs are susceptible to denaturation at air–water interfaces, which are easily generated during swirling, stirring, and agitation of the solution. The stresses on the protein due to agitation by shaking are different from those by stirring and result in different size distribution of particulates and aggregates (Luo et al., 2011). Generally, surfactants and surface-active agents are added to minimize the aggregate formation at hydrophobic air–water interfacial surfaces. The most commonly used surfactants are polysorbate 20 and 80. These surfactants may contain peroxides, which can oxidize amino acid residues that are prone to oxidation (Kishore, Kiese, et al., 2011; Kishore, Pappenberger, et al., 2011). Commercial sources of peroxide-free polysorbates