are available, but need to be handled and stored properly (Ha, Wang, & Wang, 2002). In particular, incubation of polysorbate 80 in air at high temperatures promotes peroxide formation, and removal of oxygen is recommended for long-term storage. The oxidation induced by peroxides as a result of polysorbate 20 degradation can be inhibited by antioxidants as shown in a study using an IgG_1 mAb Fab (Lam, Lai, Chan, Ling, & Hsu, 2011). In this study, it was shown that a Trp residue was oxidized as a result of the presence of increased levels of peroxide in polysorbate 20, and the decomposition of the polysorbate-involved free radicals. Addition of ethylenediaminetetraacetic acid (EDTA), catalase, and free Trp prevented oxidation. In addition to the generation of peroxides, degradation of polysorbates can also result in the generation of poorly soluble molecules such as fatty acids, and polyoxyethylene esters of fatty acids. The generation of these compounds appeared to slightly increase the subvisible particle counts in four different mAb formulations. However, overall these degradation products did not appear to impact the stability of the mAbs. A greater concern would be the decrease in effective concentration of surfactant that is needed to prevent aggregation as a result of exposure to air–water interfaces created by agitation of the liquid formulation (Kishore, Kiese, et al., 2011). It has also been shown that polysorbate 80 had several degradation products even after several months of storage, at 4 °C, whereas polysorbate 20 contained fewer degradation products than found in polysorbate 80 (Gilardi-Lorenz, 2006). Many of these impurities found in polysorbate 80 were not found in polysorbate 20, and thus polysorbate 20 may be the preferred surfactant for protein formulations. These studies prompted the recommendation that polysorbate 80 solutions should be stored at 4 °C with protection from light and air.

Antioxidants

Antioxidants have been widely used in pharmaceutical products to inhibit the oxidation process. A review and summary of antioxidants that have been used in pharmaceutical dosage forms is available (Akers, 1982). Although many of these antioxidants have been used successfully in small-molecule formulations, the unknown toxicity and potential incompatibility with protein drugs have resulted in limited use of the antioxidants in protein formulations. Several antioxidants for inhibiting Met oxidation in proteins have been used, and these include chelating agents, reducing agents, oxygen scavengers, and chain terminators. As shown previously, many oxidation degradations of proteins are catalyzed by metals, and thus chelating agents such as EDTA that bind to metals may be effective in controlling oxidation in proteins. Reducing agents such as glutathione can reverse the oxidation by reducing the oxidized product, whereas oxygen scavengers are added molecules that more readily oxidize than the residues in a protein. The latter strategy was used successfully to inhibit oxidation of Met residues in an IgG1 mAb formulation by adding the free amino acid Met (Lam, Yang, & Cleland, 1997), and Trp oxidation in a mAb Fab fragment by adding the free amino acid Trp (Lam et al., 2011). Although the addition of Trp in the case of the mAb Fab fragment was effective, a study by Wang et al. (Ji, Zhang, Cheng, & Wang, 2009) showed that the route of the Trp oxidation could govern the effectiveness of addition of Trp. Specifically Trp oxidation in a model compound, parathyroid hormone (PTH), was done using either 2,2′-azobis(2-amidinopropane) dihydrochloride