Table 1 Buffers in best-selling lyophilized biologics

Product	2012 sales (US $ billions)	Indication	Drug class	Buffer
Remicade (infliximab)	8.2	Arthritis	Monoclonal antibody	Sodium phosphate, pH 7.2
Enbrel (etanercept)	8.0	Arthritis	Fusion protein	Tris (tromethamine), pH 7.4
Herceptin (trastuzamb)	6.0	Breast cancer	Monoclonal antibody	Histidine, pH 6.0
Nutropin, Humatrope, Genotropin, Norditropin, Saizen, Serostime, Omintrope (recombinant growth hormone)	2.9	Growth hormone deficiency	Protein	Phosphate, phosphoric acid, pH 6.5 to 8.5
Avonex (interferon beta-1)	2.3	Multiple sclerosis	Protein	Phosphate, pH 7.3
Advate (antihemophilic factor)	2.2	Hemophilia	Protein	10 mM or 25 mM of each Tris (hydroxymethyl aminoethane) and L-histidine
Betaferon/betaseron	1.6	Multiple sclerosis	Protein	0.54 % NaCl, neutral pH

It is well documented that the selection of buffers, stabilizers, and bulking agents are important for stabilizing the protein during the drying process and subsequently during storage [9]. Therefore, it is critical to have a good understanding of the behavior of these formulation components during the freeze-drying process.

Freeze-drying or lyophilization is widely used to improve the long-term stability of biologics. However, freeze-drying can impose stress that adversely affects the protein if not designed properly. Freeze-drying converts liquid to a solid and generally involves the following steps: freezing, optional annealing, primary drying, and secondary drying. During each of these steps, it is important to consider and understand the stresses, the role of the formulation components and process conditions on the stability of protein and product quality. In the freezing step, the protein can experience supercooling, freeze concentration, crystallization of the formulation excipients, exposure to interfaces and sheer stresses due to formation of ice crystals, and cold denaturation, which may be deleterious to its stability [53]. In the secondary drying step, the protein experiences dehydration as the water is removed from the noncrystalline or amorphous phase.

This chapter begins by providing a general description of the types of buffers and the methods used to characterize buffer behavior during freeze-drying, including pH shifts and crystallization. The behavior of three classes of buffers (e.g., phosphate, carboxylic acids, and amines/amino acids) during freezing and thawing will be discussed next. The chapter ends with a case study that describes the impact of buffers on the stability of a lyophilized powder.