In addition to pH shifts as a result of crystallization of the buffer components, there are two other possible causes for pH changes during freezing, i.e., temperature dependence of pH, and change in an apparent pK_a as a result of decrease in polarity of the amorphous (liquid) phase due to freeze concentration. Buffers vary in their pH temperature dependence. For example, phosphate buffer demonstrates relatively minor temperature trend, with pH increasing by approximately 0.13 units (from 6.86 to 6.99) as temperature decreases from 25 to 0 °C [3]. Tris buffer is an example of much stronger temperature dependence, with a significant pH increase upon cooling, e.g., from 8.1 (25 °C) to 8.8 (0 °C) [54]. Dependence of the ionization constant (pK_a) from the media polarity is another potentially significant contributor to pH changes during freezing. Water crystallization (ice formation) results in significant decrease of water concentration in the solution coexisting with ice crystals, with a corresponding decrease in polarity due to increase in solutes concentration. In sucrose–water system, for example, dielectric constant of the 10 wt.% solution is 76.2 at 25 °C [30] while the maximally freeze-concentrated solution (sucrose concentration approximately 80 wt.%) is estimated to have dielectric constant of approximately 51 at 25 °C (graphical extrapolation using data from Mathlouthi et al. [30]). Such decrease in the dielectric constant may result in a major pK_a change, for example, the apparent pK_a of acetic acid increases significantly with decreased in polarity, from 4.76 in water (dielectric constant 78.3) to 10.32 in ethanol (dielectric constant 24.3). [4] Such media-dependent pK_a changes are highly variable between different functional groups; for example, the pK_a of an amino group ($–NH_3^+/–NH_2$ equilibria) is much less sensitive to changes in the dielectric constant of the solvent than the carboxylic group.

Further decrease in the water content by removing water during secondary drying would result in further decrease in polarity, with corresponding changes expected in the extent of proton transfer and ionization, and therefore, apparent acidity. Indeed, the changes in the apparent acidity as a function of water content were observed in a study of model trehalose–citrate system, in which the Hammett acidity function [23], was measured in amorphous lyophiles as a function of water content [21], as discussed in the next section. The study described in Govindarajan et al. [21] also demonstrated that ionization state is not fixed during the freezing stage, with proton transfer in amorphous solids taken place well below their calorimetric T_g.

The effect of buffer types on freeze-drying is described in more details below, to cover shift in the apparent “pH” during freezing and drying both in systems with buffer crystallization and in cases when buffer crystallization was avoided.

Characterization Methods

Various analytical methods have been utilized to characterize buffers in the lyophilized products to ensure the buffer functions and provide long-term stability of drug products. Mainly these methods have included direct characterization using pH electrodes [19, 20, 48, 49, 50] or indirect characterization, in which pH shifts are monitored based on crystallizing buffer components or use of pH indicators.