

been reported for glycines depending on the technique and sample tested [27]. Specifically, using a low-temperature pH probe, Larsen showed no pH change at pH 2.8 and moderate increase from 9.3 to 10.2. A possible explanation for the contradictory results may be a consequence of the difference in temperature range studied, in which Akers was working at -30°C and Larsen was working at -15°C . Furthermore, crystallization of glycine depends on the cooling rate [17] and can be inhibited by amorphous solutes, such as sucrose, as reviewed by Shalaev and Franks [41]. Presence of sucrose above certain sucrose/glycine wt. ratio, determined to 0.8, results in complete inhibition of glycine crystallization, whereas compositions with higher glycine concentration can crystallize either during cooling (if the cooling rate is less than the critical cooling rate, which also depends on the sucrose/glycine ratio), annealing, or subsequent warming.

In addition to the pH buffering properties, amino acids also may serve as lyo- and cryo-protectors [16]. For example, histidine was shown to stabilize freeze-dried recombinant factor VIII [36]. Note also that histidine forms complexes with divalent cations such as Mg, Ca, Zn, Fe [6], which can have an additional impact on stability and activity of proteins. Indeed, the complex formation between histidine and traces of Fe^{2+} might inhibit oxidative processes, whereas complexation with Zn^{2+} could compromise activity of certain metal-proteins.

Phosphate Versus Citrate Buffer: Case Study

It is commonly accepted that sodium phosphate buffer represents a stability risk for frozen solutions and lyophiles because of its high crystallization propensity and corresponding pH changes. Furthermore, it was shown that inhibition of crystallization can be achieved by using an amorphous solute, such as sucrose or trehalose, if the ratio of amorphous/crystallizable solute is above its critical ratio [41]. In many systems, such critical ratio was determined to be in the range of 1/1–5/1; for example, a formulation with 5% sucrose and 20 mM phosphate buffer is expected to remain amorphous, and the risk of buffer crystallization and corresponding pH changes could be expected to be eliminated. Indeed, it was shown recently that phosphate crystallization was inhibited by sugar [7]. However, it was also shown that the prevention of disodium phosphate crystallization does not necessarily avoid significant pH changes during freezing. Therefore, one can hypothesize that even in case when disodium phosphate remains amorphous, use of phosphate buffer may result in significant acidification during freeze-drying and corresponding destabilization of acid-sensitive molecules.

This hypothesis was tested in a case study in which sucrose lyophiles were prepared with either sodium citrate or sodium phosphate buffers adjusted to various pH values ranging from 5.5 to 7.5 before freeze-drying, and the apparent solid-state acidity of lyophilized materials was expressed using Hammett acidity function [11, 42]. The high sucrose/buffer ratio (10 wt.% sucrose/20 mM buffer in the pre-lyo solution) is expected to prevent crystallization of disodium phosphate; also, the lyophiles were confirmed to be X-ray amorphous. However, the phosphate lyophiles