Gomez et al. developed an elegant method, utilizing a low-temperature pH electrode, to measure the pH shifts during the freezing stage under far-from-equilibrium conditions [20]. The use of a low-temperature pH electrode indicated major decrease in pH, presumably due to disodium hydrogen phosphate dodecahydrate (DHPD) crystallization, at initial buffer concentration down to 8 mM. Nonetheless, the pH electrode (e.g., Inlab®cool, Mettler Toledo) could only be used at temperatures $\geq -25\,°C$. Another significant limitation of pH electrodes is that they work only in liquid media, and cannot be used to monitor changes in the acid/base relationships during drying. Therefore the utility of the low-temperature pH electrode is limited, as the apparent acidity can change in frozen solutions as they are cooled below $-25\,°C$ as well as during drying.

Indirect methods can be divided into two categories, one is based on detecting crystallization of buffer components (low-temperature X-ray diffractometry (XRD) [8, 17, 38], sub-ambient thermal analysis [10], differential scanning calorimetry (DSC) [32], scanning electron microscopy [33]), and the other monitoring changes in the ionization of ionizable groups (e.g., using pH indicators as probe molecules [34] and measuring the extent of proton transfer either in probe molecules by visible diffuse reflectance spectroscopy or in a buffer itself by Raman spectroscopy [28]).

In the case of succinic acid sequential crystallization and "pH swing" in frozen solutions, for example, low-temperature pH measurement and sub-ambient XRD proved to be excellent complementary tools in the characterization of frozen systems [45–47].

While laboratory-based low-temperature X-ray diffraction method is an essential tool for observing solute crystallization (that can cause pH shifts), it suffers from low sensitivity. For example, based on both XRD and DSC, crystallization of DHPD could not be detected at initial buffer concentrations < 190 mM. The poor sensitivity of the laboratory-based XRD method could be attributed to the low flux of the X-ray source, and the use of a point detector [8, 38]. These limitations could be overcome with the use of highly collimated and brilliant synchrotron radiation, coupled with a high-resolution 2D detector. Synchrotron XRD (SXRD) method, in addition to the increased sensitivity, provides a very rapid data collection (< 1 s) enabling time-resolved studies.

Low-temperature SXRD offers numerous advantages as follows: (a) capability to monitor phase transitions *in situ*, during the entire freeze-drying cycle. (b) Potential for obtaining quantitative information based on the intensity of the analyte peak(s). By collecting the entire diffraction data (the Debye rings), errors in net intensity measurement due to preferred orientation can be minimized. (c) Capability to determine, in real time, the effect of processing. For example, the kinetics of solute crystallization can be studied as a function of annealing time and temperature. (d) Quantification of analyte crystallinity in complex, multicomponent systems.

Varshney et al. [51] demonstrated the sensitivity of low-temperature SXRD for detecting solute crystallization in frozen sodium phosphate buffer and glycine solutions. Crystallization was detected at initial phosphate buffer concentrations down to 1 mM. In addition, the use of a high-resolution 2D detector enabled the visualization of numerous diffraction rings of the crystalline solute. The detection of DHPD was