unambiguous, based on several unique peaks, and was accomplished under far-from equilibrium conditions. The whole range of pH 1–9 at different solute concentrations of frozen glycine solutions was studied by low-temperature SXRD method to quantify complex multi-crystalline phases (with the capability to detect 0.2% crystallinity based on peak integrated intensities). Also the method was utilized for understanding the effect of annealing, primary and secondary drying on buffer crystallization. Phase transition of the phosphate buffer, partial and complete dehydration of the DHPD to amorphous phosphate buffer was shown at commercially relevant buffer concentration and freeze-drying conditions (temperatures and pressure).

Spectrophotometery and the use of pH indicator dye were demonstrated by Li et al. [28]. Authors studied the acid–base characteristics of various citrate buffer systems alone and in the presence of the pH indicator dye, bromophenol blue, in aqueous solution, and amorphous material produced after lyophilization. Fourier transform Raman and solid-state nuclear magnetic resonance spectroscopy were used to monitor the ratio of ionized to unionized citric acid under various conditions, as a function of initial pH in the range of 2.65–4.28. Ultraviolet (UV)–visible spectrophotometry was used to probe the extent of proton transfer of bromophenol blue in the citrate buffer systems in solution and the amorphous solid state. This work was extended to focus on measurement of the apparent acidity using pH indicators, with the acidity expressed as the Hammett acidity function (Equation below), and to establish relationships between the Hammett acidity function and the degradation rate of acid-sensitive molecules in the amorphous freeze-dried formulations [12]. Figure 1 below shows the main principle and example of Hammett acidity function measurement in lyophile. Where the ratio of ionized to unionized species of pH indicator dye, measured by UV–vis diffuse reflectance (DR) spectrophotometer, provides solid state acidity for lyophiles. The relationships between the Hammett acidity function and solid-state degradation were demonstrated for several small molecular weight compounds [2, 12, 17, 22, 29]. Expect that similar relationships between the Hammett acidity function and degradation rate of acid-sensitive protein molecules would be observed for freeze-dried protein formulations as well, although publications in this area are lacking.

$$\mathrm{H}_x = pK_a + log_{10}\frac{C_d}{C_p} = pK_a + log_{10}\left[\frac{F(R)_{\mathrm{d}^{\varepsilon'}\mathrm{p}}}{F(R)_{\mathrm{p}^{\varepsilon'}\mathrm{d}}}\right]$$

where C is the indicator concentration, and the subscripts “p” and “d” refer to the protonated and deprotonated indicator species, respectively. Ka is the ionization constant of the indicator, F(R)d/F(R)p is the ratio of the peak signals of the deprotonated to the protonated indicator forms determined from the DR spectra, and ε′p/ε′d is the ratio of the extinction coefficients of two species in the lyophiles. Subscript x corresponds to the charge of the basic form of the probe molecules; for sulfonephthalein pH indicators in typical pharmaceutical materials, x is equal to either -2 or -.