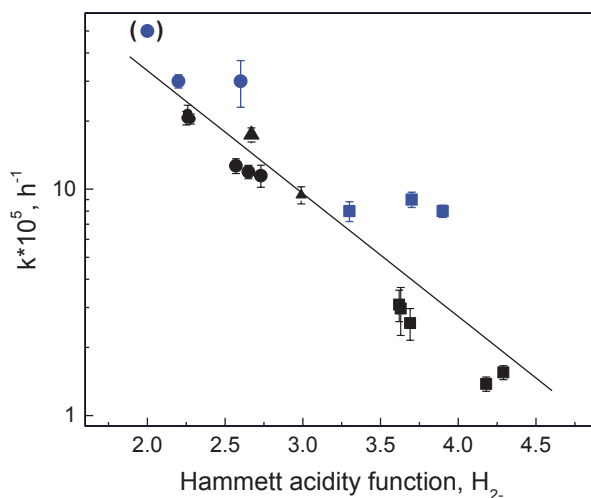


Fig. 2 Pseudo-first-order rate constants of sucrose inversion in PVP (*squares*), lactose (*triangles*), and dextran (*circles*) lyophiles as a function of Hammett acidity function. The line reflects the correlation between the rate constant (logarithmic scale) and the Hammett acidity function (*Blue symbols* are for datapoints from Chatterjee et al. [12] and black symbols from Lu et al. [29])



by lactose- and then PVP-containing formulations. The relationships between sucrose inversion rate and the Hammett acidity function are shown in Fig. 2.

An understanding of the impact of buffer and co-solutes on apparent pH is important during the design of the protein formulations since proteins are sensitive to pH, and the measurement of the Hammett acidity function would provide an essential information for development of freeze-dried proteins

Amines and Amino Acids

Alternative buffers to carboxylic acids which are known to have minor to moderate pH changes (around one pH unit change) during freezing include histidine and Tris [25]. The temperature-pH profile of 20 mM histidine has been studied and shown to have less than one pH unit increase (5.4 to 6.1) upon freezing to -30°C [25]. The crystallization behavior of histidine during freeze/thaw has been shown to be pH dependent [35]. Specifically, heating of frozen histidine solution with a pH outside the range of 5.5–6.5 results in crystallization of the buffer. Similar to histidine, the pH of Tris-HCl buffer increases by one pH unit from 7.4 to 8.5 upon freezing to -30°C . However, some pH indicator studies have shown no pH change for 10 mM Tris buffer pH 7.4 up to -40°C [13] and moderate decrease in pH during freezing of pH 9.8 [14]. Furthermore, by DSC, Tris buffer crystallization was observed during freezing or warming/annealing [10].

Other buffers which show a pH dependence on crystallization include glycine-based systems. The components of glycine buffer (glycine, glycine HCl, and sodium glycinate) have been shown to crystallize during freezing or low-temperature annealing [1, 31]. Specifically, Akers et al. showed a faster crystallization rate at pH 7 as compared to pH 3 and no crystallization at pH 10 [1]. Contrary results have