

# Spectroscopy study of freezing-induced acidity change of buffers in the presence of sugars.

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Freezing of aqueous buffer solutions leads to the formation of freeze-concentrated solutions (FCS), which can expose the local environment (bio)chemicals to extreme conditions such as low temperatures, concentration increase<sup>[1]</sup>, etc. These conditions alter the proton concentration and acidity, leading to compound degradation, including protein aggregation<sup>[2]</sup>. To overcome this problem, the use of buffers containing sugars has been proposed as a potential solution to reduce the acidity changes induced by freezing<sup>[3]</sup>. Thus, in this study, we aimed to investigate the effects of different sugars, namely monosaccharides (glucose), disaccharides (sucrose and lactose), and sugar alcohols (mannitol), on the acidity of a citrate buffer system using the molecular probe method<sup>[4]</sup>. Our results demonstrate that the addition of sugars to the buffer system can significantly mitigate the extent of acidity change induced by freezing, with sucrose exhibiting the greatest efficacy in this regard followed by mannitol and lactose. These findings are of great significance in the fields of biotechnology and medicine, where maintaining sample stability is critical for accurate and reliable analysis.

Keywords: Freezing, Freeze concentrated solution, Freeze-induced acidity change, Buffers

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