**Acidity of Frozen Solutions and Its Connection to Degradation of Enzymes during Freezing**

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**Abstract**

Constant pH is often essential for stability of many biomolecules. In solution buffers are used to maintain pH value constant. However, when an aqueous solution of a salt freezes, pH of unfrozen portion of the solution can change dramatically whether towards acidic or towards alkaline values, even if a buffer is used. During freezing the solution becomes more concentrated because compounds dissolved in the solution are expelled from a lattice of newly growing ice. This freeze-concentration often results in excessive crystallization of less soluble component of the buffer and more pronounced acidity changes than in a buffer-free solution. Such sequential buffer crystallization was shown e.g. for sodium phosphate and succinate buffer systems.1, 2 Freezing-induced acidity change was observed also in solutions of salts due to different incorporation of anions and cations into the ice lattice and consequent neutralization of a arose charge imbalance by the diffusion of the H+ or OH− from liquid into the ice.3, 4 In this study the effect of freezing-induced pH change on denaturation of model enzyme DbjA was examined. Freezing-induced pH change was monitored for various experimental conditions and correlated to loss of enzymatic activity during freezing and thawing.

**References**

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