

# Effect of freezing on the acidity of pharmaceutical excipients

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Buffers are routinely used to maintain pH constant, not only in the liquid state but also during cryopreservation or freeze-drying. However, the pH of the buffer solution can change significantly upon freezing due to the sequential precipitation of buffer components. Such large changes in acidity can dramatically affect the stability of the biomolecules <sup>[1, 2]</sup>. Therefore, studying the acidity change upon freezing is a vital step for designing freeze-dried products.

In this work, the acidity of frozen excipients (buffers and sugars), which are frequently used in pharmaceutical formulations, was determined spectrophotometrically by studying the extent of protonation of the sulfonephthalein indicators, which are used as acid-base probes and is accessed by the Hammett acidity function <sup>[3]</sup>. These indicators are present in the freeze concentrated solution giving us precise knowledge about the change in proton concentration.

Following this approach, freezing-induced acidity change of a series of the biologically used buffers (carboxylic acid, amino acid, Good's, and phosphate buffer) is reported for varied concentrations and freezing rates which helped to optimize the suitable buffer for freezing of sensitive formulations. In addition, a more detailed investigation of the crystallization of citrate buffer and PBS buffer is done via differential scanning calorimetry and optical cryomicroscopy techniques. Using this knowledge, we also found a solution to reduce the freezing-induced acidity change by introducing a buffer blend of Good's buffer and sodium phosphate buffer <sup>[4]</sup>. The comprehensive studies of these projects will be presented in detail in the presentation.

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