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EFFECT OF FORMULATION ON THERMAL PROPERTIES OF LYSOZYME, AS DETERMINED BY MODULATED TEMPERATURE DIFFERENTIAL SCANNING CALORIMETRY.

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Purpose: To thermally characterize lysozyme formulations and determine the glass transition temperature (T'_g) of lysozyme-sucrose formulation, unfolding/melting temperature (T_m) of lysozyme, and study the effect of pH and various excipients on its unfolding temperature (T_m).

Methods: Lysozyme solutions (50mg/ml) were prepared with and without various concentrations of sucrose (0-50%), mannitol (0-10%) and PEG 8000 (0-30%). DSC analysis (TA Instruments Q-100) of samples was by scanning from a temperature of 30°C-100°C with an underlying heating rate of 2°C/min and a modulation amplitude of +0.5°C every 100 seconds.

Results: The T_m for Lysozyme was 70°C in water (pH 6.85). Increasing the pH from 3.9 to 9.2 caused a decrease in T_m from 71.43°C to 66°C. Addition of increasing concentrations of lysozyme to sucrose solutions increased the T'_g of sucrose from -34°C to -23°C. Addition of sucrose (50%) and mannitol (10%) increased the T_m to 77°C and 72.50°C respectively. Addition of PEG 8000 till 20% caused a decrease in T_m . Further additions lead to complete loss of the endothermic peak suggesting unfolding of protein prior to heating.

Conclusion: Modulated temperature differential scanning calorimetry (MDSC), an extension of DSC, is a novel thermoanalytical technique which involves the application of a sinusoidal heating program to a sample and the resolution of the response into reversing and non-reversing signals, thereby enabling the deconvolution of complex and overlapping thermal processes. Increase in glass transitions suggests increased hydrogen bonding between lysozyme and sucrose that keeps them in the amorphous phase which in fact is necessary during freeze-drying of proteins. Melting endotherms for lysozyme were obtained as reversing peaks and were found to be stabilized, as shown by an increase in T_m , by use of excipients such as sucrose and mannitol. However, addition of PEG 8000 and an increase in pH caused a decrease in thermal stability of lysozyme.