PURDUE UNIVERSITY | Department of Industrial and Physical Pharmacy

Industrial & Physical Pharmacy Seminar
IPPH 69600

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"Understanding Protein Stability in Sugar Matrices"

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First Seminar

Proteins are often formulated as lyophilized products. Cryoprotectants like trehalose are used to increase the stability of proteins during lyophilization and storage. The physical state of trehalose during and after lyophilization is crucial in the stability of proteins. Literature shows presence of crystalline trehalose during annealing, and disappearance of the crystalline form during primary drying, resulting in an amorphous final product. Trehalose crystallization may result in phase separation, where there will be domains of trehalose not providing stability to protein. The purpose of this study is to investigate the impact of trehalose phase separation on the stability of BSA (Bovine Serum Albumin) using SSNMR (solid-state Nuclear Magnetic Resonance).

We studied samples containing five concentrations of trehalose that were annealed for 0-24 hours. BSA, sodium phosphate buffer (2mM, pH 7) and trehalose was frozen to -45°C, and annealed at -15°C. Primary and secondary drying was carried out for 62.5 hr at -27°C and 4hr at 30°C, respectively. Size Exclusion Chromatography (Agilent infinity 1200ii & Tosoh 2000 PWXL column) was used to analyze the monomer concentration. All SSNMR data were collected in Bruker Avance 400 using PhoenixNMRMASHX probe. 1H T1 relaxation time was lowest for 70% trehalose compared to the higher concentrations which correlates with the PXRD data as amorphous substance has low 1H T1 relaxation time. 1H T1 relaxation time increases as a function of trehalose concentration indicating generation of more crystalline domains. Difference in 1H T1 relaxation time between the protein and trehalose increased after storage for six months indicating phase separation. SEC data showed that there was no further aggregation during storage.

While SSNMR experiments showed evident phase separation, the monomer percentage remained the same. Therefore, the phase separation of trehalose in trehalose/BSA model system did not compromise the stability of BSA.