6 The Relevance of Thermal Properties for Improving Formulation and Cycle Development: Application to Freeze-Drying of Proteins

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INTRODUCTION

Although freeze-drying is the process of choice for improving the shelf life of biological products such as proteins, the process itself and the subsequent long-term storage generate several stresses responsible for protein denaturation (1–4). An appropriate choice of protective excipients is thus needed to inhibit freezing- and drying-induced damage and to preserve biological activity during processing, storage, and rehydration (1,5,6). Moreover, the active ingredient is usually present in very low concentrations (μg/mL or ng/mL) in the formulation, and excipients also act as bulking agents to increase the dry matter of the formulation and to obtain an elegant freeze-dried cake. Different molecules alone or in combination and at various concentrations can be used, making the formulation step complex, hazardous, and time-consuming and not always resulting in the highest quality product attainable. To obtain successful results when developing freeze-dried protein, the formulation has to fulfill the following five criteria (5):

- Inhibition of protein unfolding during freezing and drying
- A glass transition temperature of the freeze-dried product that is higher than the storage temperature
- Low residual water content (<3%)
- A strong and elegant cake structure
- The minimization of protein chemical degradation during storage (addition of antioxidant, conditioning in nitrogen atmosphere, etc.)

Obtaining an elegant freeze-dried cake structure requires performing the drying stages at a product temperature that does not exceed a critical value—the maximum allowable product temperature (T_{max})—usually defined as being a few degrees below the collapse temperature (T_{coll}). The (T_{coll}) is defined as the temperature above which the freeze-dried product loses macroscopic structure and collapses during freeze-drying (7–9). The thermal properties of the formulation including eutectic crystallization temperature (T_{cr}), eutectic melting temperature (T_{eut}), glass transition temperature of the maximally freeze-concentrated phase (T_g'), and ice melting temperature are used to determine the value of T_{max} and to thus design and optimize the process. Many approaches have been explored to determine the thermal properties of formulations for freeze-drying, including differential thermal analysis (DTA), differential scanning calorimetry (DSC) (6,10,11), electrical resistance analysis (ERA) (12), and freeze-drying microscopy