Thermal Analysis in Formulation of Protein Drugs

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Protein drugs, specifically monoclonal antibodies (mAbs), are at the forefront of modern therapy. Their potential is demonstrated by the fact that 5 of the 10 best selling drugs worldwide are mAbs. All of them are given parenterally as aqueous solutions. Correspondingly they are either formulated as aqueous solutions or come as lyophilisates which are reconstituted prior to application. Proteins show inherently low stability in solution; the instability pathways can be of chemical, colloidal or conformational nature.

DSC can be used to characterize the conformational stability of proteins, to some extent at domain level. The application for mAbs is however limited due to i) the typically irreversible character of the unfolding process and b) the high melting temperature well above the intended storage temperature, mostly refrigerated. Still, in a few cases a correlation between storage stability and Tm has been concluded. In formulation development DSC is frequently flanked or substituted by other analytical methods with nDSF probably being the most frequently used. nDSF utilizes the intrinsic protein fluorescence or the fluorescence of an extrinsic dye which can bind to hydrophobic sites upon heating the sample. It requires only few microliters. Furthermore, light scattering techniques are used to complement as both unfolding and aggregation can be detected. New instruments combine fluorescence and light scattering in well plate or capillary stacks format. This allows high throughput screening in early lead candidate selection and in formulation development. A case study of the use of the thermal analysis for formulation development is presented.

In the context of protein formulation development DSC is also used to analyze the glass transition temperature of the maximally freeze concentrated solution (Tg'). Tg' reflects a critical temperature during lyophilization which should not be exceeded during primary drying in order to avoid collapse. We used DSC to analyze the Tg' of pure protein solutions using highly concentrated sucrose mixtures. In various cases, Tc analysis by freeze-drying microscopy has to be used for further understanding of the formulation behavior in the course of primary drying. Furthermore the Tg' is of importance in case of long term frozen storage of bulk drug substance. Thus, thermal analysis is of high importance in early development of protein drugs enabling both high throughput screening and deeper scientific characterization.