

Effect of Temperature on Separation and Characterization of Monoclonal Antibody Using Asymmetrical Field-Flow Fractionation

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Introduction:

Thermal stability of a protein drug is an important property for formulation development. Traditionally, this has been done by differential scanning calorimetry (DSC) and long term storage at elevated temperatures. In this study, we have used the new postnova analytics AF2000 MT system to study the thermal stability of several monoclonal antibody (MAb) formulations at four different temperatures. Sample stability was monitored in terms of size distribution of aggregates. The results were compared with those from the conventional DSC method.

Method and materials:

System: AF2000 MT Mid temperature AF2000 system (Postnova Analytics)

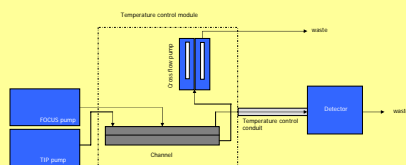


Figure 1: Picture and schematic of the AF2000 MT system

Samples:

Table1: Description of monoclonal antibody samples analyzed by AF2000 MT system

Sample	Description
1	MAb Lyo control
2	Reconstituted MAb Lyo stored at 60°C for 1 month
3	MAB Lyo stored at 60°C for 1 month
4	MAB Lyo stored at 60°C for 1 month, then reconstituted and stored at 60°C for another month

Results:

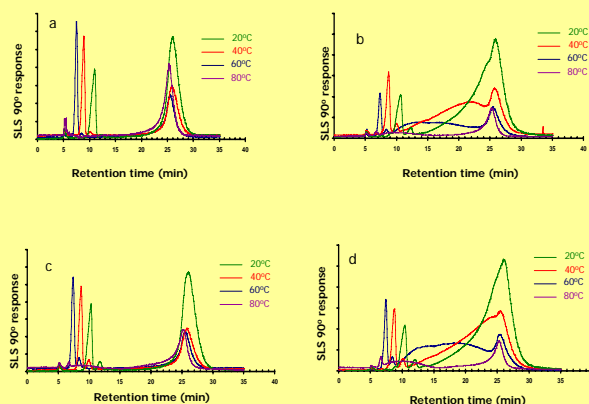


Figure 2: Fractograms of monoclonal antibody samples run at 20, 40, 60 and 80 °C
a) sample 1, b) sample 2, c) sample 3 and d) sample 4

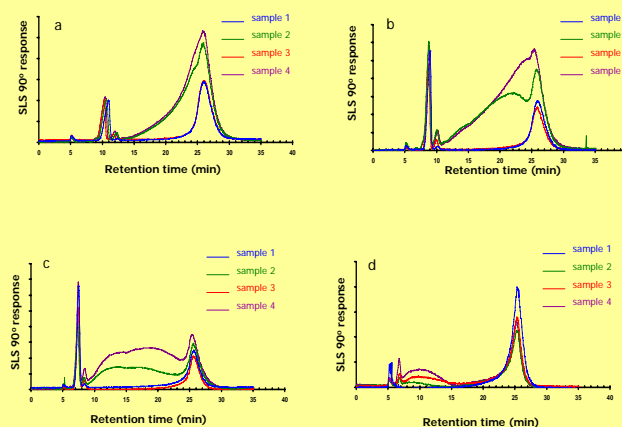


Figure 3: Comparison of fractograms of monoclonal antibody samples run at different temperatures, a) 20 °C, b) 40 °C, c) 60 °C and d) 80 °C

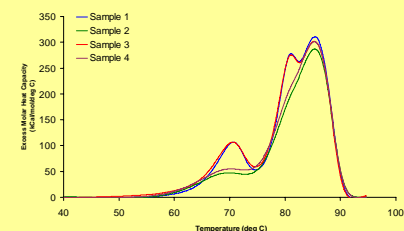


Figure 4: DSC Thermogram of monoclonal antibody samples

Discussion:

- MAb product in lyophilized formulation is more stable under thermal stressed conditions than the reconstituted liquid formulation.
- Aggregates with broader distribution are formed by the thermal stressed reconstituted liquid products (samples 2 and 4)
- Significant changes on size distribution of aggregates were observed for thermal stressed liquid product even at 20°C, whereas the lyophilized form exhibits no significant changes up to 80°C.
- Thermal stressed and no stressed samples exhibit different thermograms, but not on transition midpoint (T_m).

Conclusion:

- AF2000 MT is an excellent analytical tool in characterization of protein and protein aggregates at physiological temperature.
- The data shows that thermal stress forms different types of aggregates from the lyophilized and reconstituted MAb products.
- The data also demonstrates different degradation mechanisms for aggregates formed by the lyophilized and liquid MAB products.
- AF2000 MT provides more useful information about thermal stability of a protein formulation than the conventional DSC method.