

Denaturation and aggregation of IgG immunoglobulin

INTRODUCTION

Immunoglobulin like any protein may unfold and aggregate while heated. MICROCALVET ULTRA is the perfect fit for thermal stability analysis or formulation studies as it combines:

- A high sensitivity necessary to detect thermal event on diluted protein solutions
- Removable cells for easy cleaning (particularly significant with proteins that aggregate regularly)

EXPERIMENT

Sample : 1mg/mL IgG isotype immunoglobulin solution in a PBS (Phosphate Buffered Saline) buffer.
Reference : PBS buffer.

The sample and reference are heated in tightly closed Hastelloy vessels.

Temperature profile: from 25°C to 110°C at 1 °C/min.

RESULTS AND CONCLUSION

Two events with opposite signs were detected on the experimental data (blue dots) above 70°C. They correspond to IgG unfolding (endo) and aggregation (exo). When the concentration of unfolding IgG molecules is high enough, aggregation occurs and is so fast that the molecules become locked in aggregates before being completely denatured.

Peak separation option of Calisto data treatment software, allowed fitting these data with asymmetric Gaussian curves using a Marquard calculation routine. The grey curve corresponds to the IgG unfolding, the red curve to the aggregation. The black dots is the sum of these two events and fits quite well with the experimental data. Both events could thus be individually integrated.

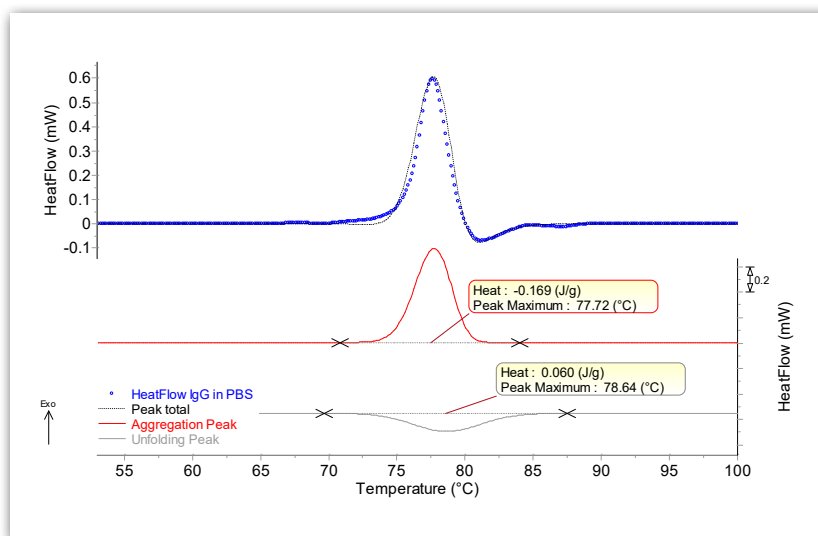


Figure 1 – Raw Heatflow vs. Temperature data superimposed with thermograms generated by peaks separation

INSTRUMENT

MICROCALVET ULTRA

-20 to 170°C



- **HIGHEST HEAT MEASUREMENT ACCURACY**
3D sensor based on Peltier elements with Joule effect calibration.
- **MODIFIABLE TEMPERATURE CONDITIONS**
for increased flexibility and replication of real life conditions.
- **CONVENIENT INTERCHANGEABLE CRUCIBLES AND CELLS**
to perform even the most demanding experiments using one instrument :
 - high pressure (1000bar) and high vacuum
 - pressure measurement and control
 - mixing experiment
- **EXTERNAL COUPLING CAPABILITY**
designed to increase your research options including manometry, BET instrumentation, gas analyzers, humidity controllers and gas panels