Thermal Stability Technology Selection Guide for Biotherapeutic Drug Development

A guide to automated biologics stability testing for ensuring product quality and supporting regulatory approvals

Waters™ | TA™ Instruments

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Introduction

Biologics or biotherapeutics, drug therapy products derived from living organisms, are transforming how we treat diseases and infections, offering greater specificity and efficacy than traditional small-molecule pharmaceuticals. Various biologics, like vaccines, are already widely used, while monoclonal antibodies (mAbs) are rapidly growing due to their versatility and ability to deactivate the target or trigger an immune response.[1] However, with greater opportunity comes greater complexity: Biotherapeutics have more complicated structures and require sophisticated characterization and manufacturing processes.

Thermal stability testing is a mandatory step in biotherapeutic development. Regulations require stability studies to prove biotherapeutics' safety and efficacy throughout their proposed shelf-life. Thermal stability also measures a therapeutic's likelihood for aggregation. Altogether, thermal data is essential for choosing a drug candidate and determining ideal formulation and storage conditions.

This guide will cover the different methods of thermal stability testing available for biologics development.

Overview of Thermal Stability Test Techniques

Short-term thermal stability testing reveals a biologics' resilience to thermal stress, predicts shelf life, and ensures quality. Information from thermal stability experiments enables scientists to make the best formulations, as well as monitor changes in their product over time.

Today, there are two widely used technologies for thermal stability testing:

Differential Scanning Calorimetry (DSC) measures the heat absorbed or released during thermal transitions, such as protein unfolding or nucleic acid melting.

Nano Differential Scanning Fluorimetry (DSF) is a fluorescence-based protein stability assay that measures protein folding through changes in fluorescence as a function of temperature.

These technologies accomplish thermal stability testing, but with significant trade-offs. Traditional DSC offers high precision and high-quality data but requires more sample per test and is slower overall. DSF offers high throughput testing, but with lower data quality and data is not as information rich as DSC data. For users seeking the data quality of DSC with the efficiency of DSF, the new Rapid-Screening DSC in the next section is the ideal solution.

[1] Johnson D. E. (2018). Biotherapeutics: Challenges and Opportunities for Predictive Toxicology of Monoclonal Antibodies. International journal of molecular sciences, 19(11), 3685.

Which DSC Suits Your Application?

DSC is considered the gold standard for evaluating the thermal stability of biomolecules with its higher precision and high-quality data. Now, biologics developers have a choice between three kinds of DSC: traditional thermal DSC, Nano DSC, and Rapid Screening DSC.

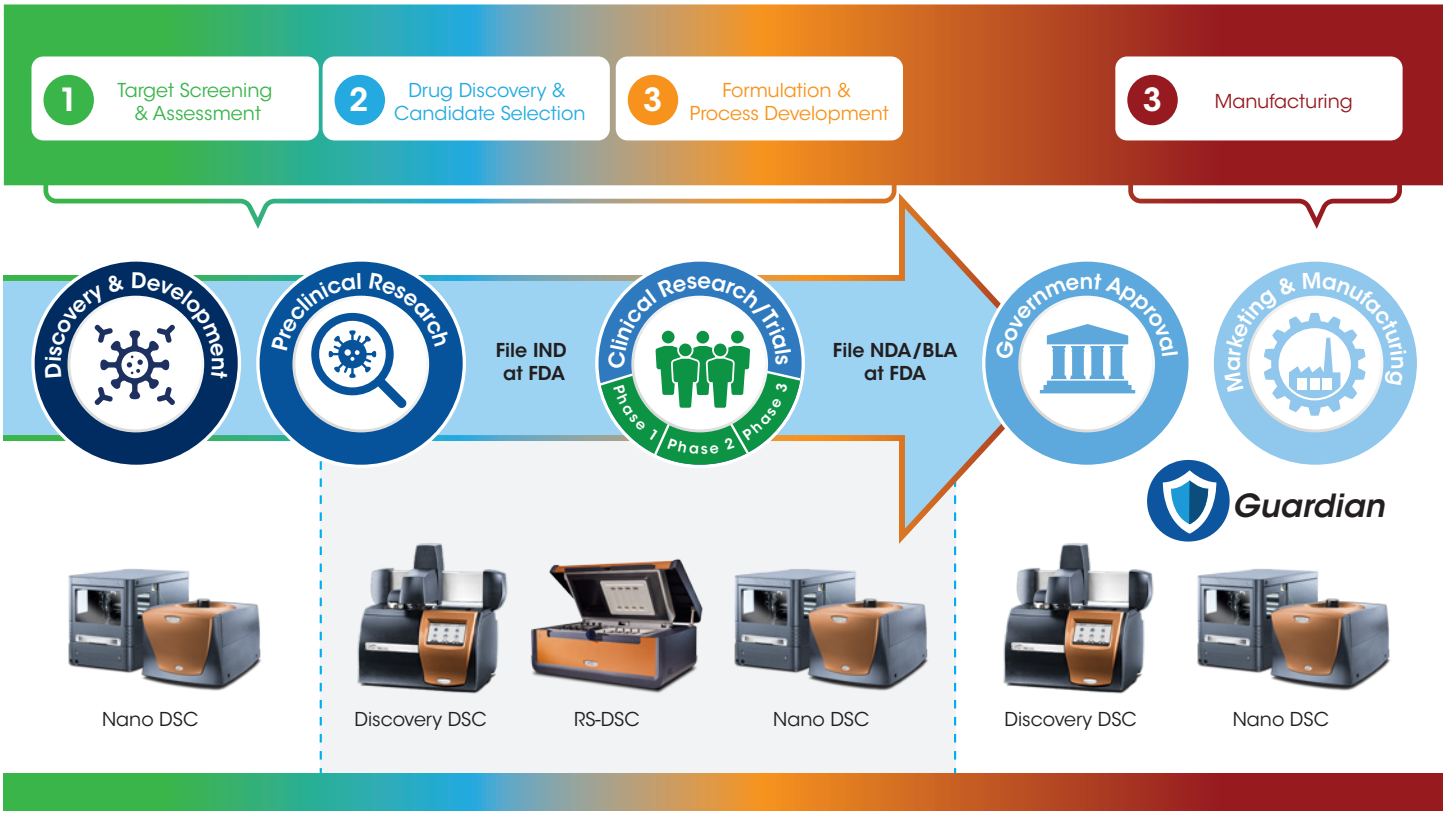
Thermal DSCs such as the Discovery DSC are ideal for small molecule characterization, including thermal stability, the presence of polymorphs, and purity. Biologics developers mainly use thermal DSC for solids testing and optimizing lyophilization.

Nano DSC is a high precision macromolecular thermal stability assay for basic research and drug discovery. Nano DSC tests liquid samples by measuring the heat of reaction from tertiary and secondary structure changes that occur when a biomolecule unfolds (protein), or melts in the case of nucleic acids and lipids.

Rapid Screening DSC is a new proprietary technology from TA Instruments that offers the benefits of high-quality DSC data paired with high-throughput, and dilution-free testing. RS-DSC is uniquely capable of testing formulation-strength concentrations while offering the complete thermal stability profile of biologics.

Thermal DSC	Nano DSC	Rapid Screening DSC
		
Mainly solids	Liquid only	Liquid only
Conc 2 mg+	Conc - 0.01 to 20 mg/mL	Conc - 5.0 to < 300+ mg/mL (molecule dependent)
<100µl; -120 to 400 °C	490 µl (Auto); -10 to 130 °C	5-11µl; 20-100 °C
Wide temp range	High sensitivity	High throughput

DSC Throughout the Biotherapeutic Workflow



Which DSC is right for your application?

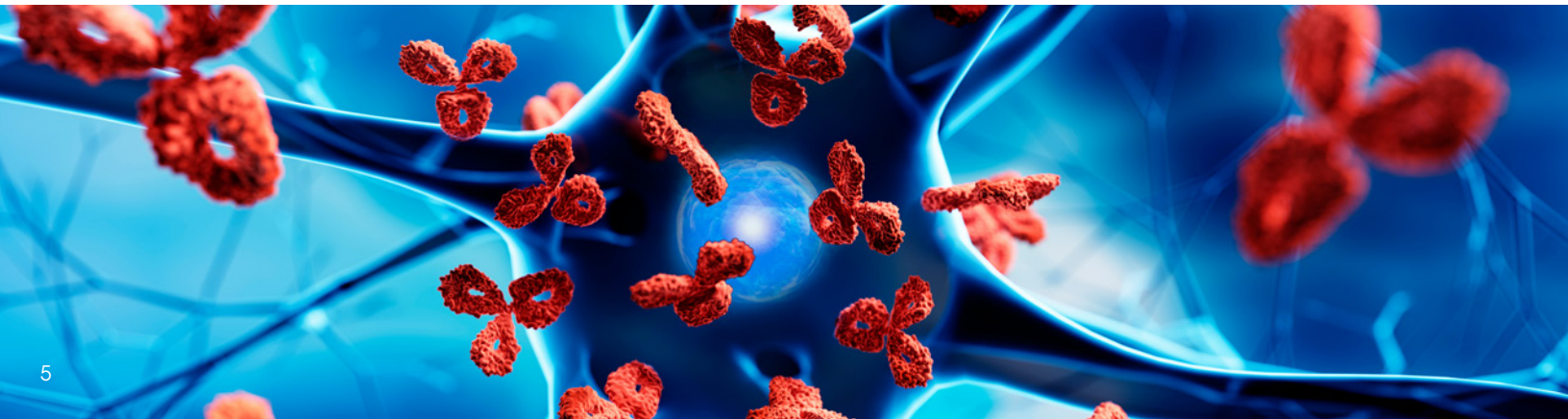
Optimizing Formulations → RS-DSC

Researchers assess the developability of antibodies by comparing their conformational stability, measured by T_{onset} and T_M, with DSC. The candidates with the highest conformational stability have the highest potential for future drug usage. Ideally, a candidate will have a T_{onset} >55 °C and a T_M of the Fab domain >65 °C. At this stage it is also important to start testing on higher concentrations that will mimic dosage concentrations, as conformational stability can be affected by the concentration of the antibody formulation.

The RS-DSC is uniquely capable of accurately measuring T_{onset} and T_M with:

- Up to 24 samples tested simultaneously
- Less sample volume required (11µL)
- Optionality to test at formulation strength (20-300+ mg/mL)

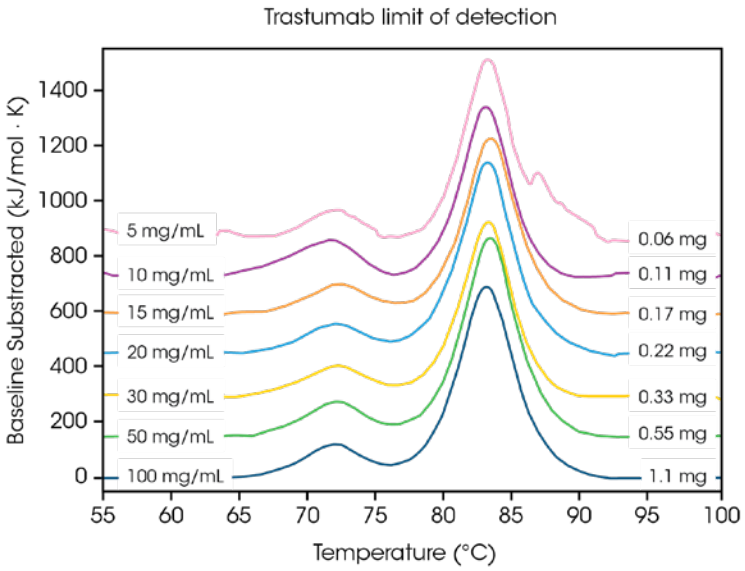
The RS-DSC helps scientists expedite formulation selection and optimization with the most efficient conformational stability testing available.



Example Experiment

To understand the concentration requirements for a multi-transition protein, the antibody Herceptin Trastuzumab was adjusted to varying concentrations in PBS and evaluated in triplicate at a scan rate of 2 °C/min (Figure o the right).

At a concentration of 100 mg/mL, two transitions are clearly visible, correlating to the unfolding of the C_H2 domain at an average Tmax,1 of 71.85 °C, and the combined Fab and C_H3 domain at an average $T_{max,2}$ of 83.01 °C [2]. No significant change in stability is observed with increasing protein concentration, ruling out concentration effects at the tested concentrations. Additionally, excellent reproducibility across calorimeters is observed.



Additional Resources

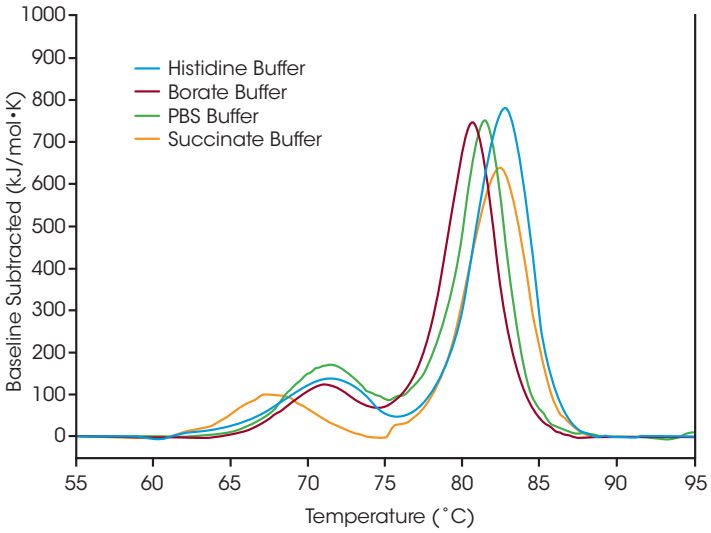
App Note: Rapid Thermal Stability Screening of High Concentration Biologic Drugs

Buffer and Excipient Optimization → RS-DSC

The effects of buffers and excipients on protein stability can be reflected in small shifts in T_{max} or in temperature changes of up to tens of degrees.

Example experiment

For example, the antibody trastuzumab was tested in four common buffer conditions, seen below. The first unfolding event is only significantly impacted by the succinate buffer which destabilizes the CH2 domain resulting in lowering the onset of unfolding and $T_{max,1}$ by about 3 °C. With respect to the main transition reflecting the Fab and C_H3 unfolding events, the histidine and succinate buffers are the most stabilizing, with a $T_{max,2}$ of 82.66 °C. The main transition is least stable in the borate buffer with a $T_{max,2}$ of 80.69 °C. Unsurprisingly, the most stabilizing buffer formulation for trastuzumab in this sample set is the histidine buffer used for final formulation of the approved drug product.

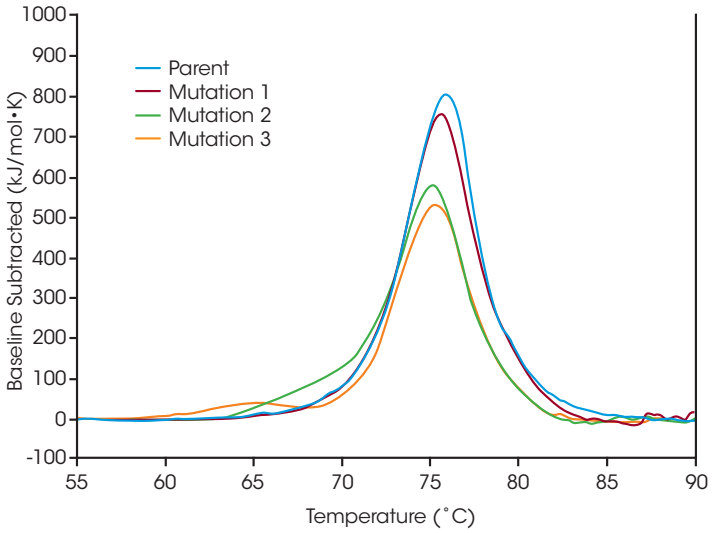


Additional Resources

See the full experiment and analysis here: Formulation Buffer Screening

Protein Mutational Analysis → RS-DSC / Candidate Selection → RS DSC

Protein mutations are a common strategy for optimizing protein structure and function, and even single amino acid modifications can have a measurable effect on overall protein stability. DSC is essential for understanding the structural impact of mutations on proteins, an important asset in biologic drug development decision making.



Example Experiment

For example, a small panel of engineered proteins were screened for changes in thermal stability resulting from single amino acid mutations in the protein sequence. A single amino acid mutation (Mutation 1) had no major effect on short-term thermal stability; however, alternative single amino acid mutations are shown to have significant impacts on the protein's stability (Mutation 2 and Mutation 3). As illustrated in the significant destabilization in Mutation 3, modification effects depend on both the site of the modification and on physicochemical properties of the new amino acid. Optimizing the desired functional benefits of sequence modification with the structural stability of the protein as a whole aids in the comprehension of the structure-function relationship and can facilitate development of advanced therapeutics.

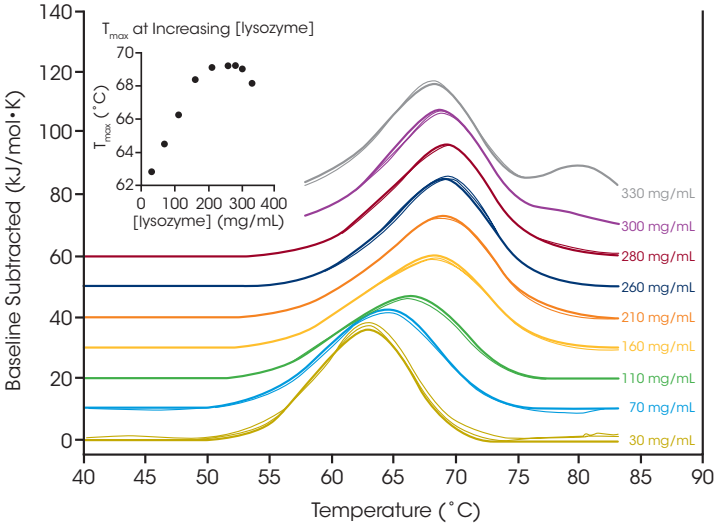
Additional Resources

See the full experiment and analysis here: Protein Mutational Analysis

Application Note: Rapid Thermal Stability Screening and Selection of Monoclonal Antibody Drug Products

Concentration Dependence → RS-DSC

With the growing success of antibody therapeutics, the pharmaceutical industry has increased interest in high concentration dosage forms that enable subcutaneous and ocular drug delivery. As such, formulations with concentrations of 50 – 150 mg/mL antibody are common and can be as high as 200+ mg/mL. Formulating proteins at high concentrations can increase susceptibility to physical instability. Conversely, some cases studies have shown enhancement in thermal stability at increased concentrations Thus, understanding of thermal unfolding and response to the solution environment at the formulation concentration of interest is a critical metric for mitigating drug product liability.



Example Experiment

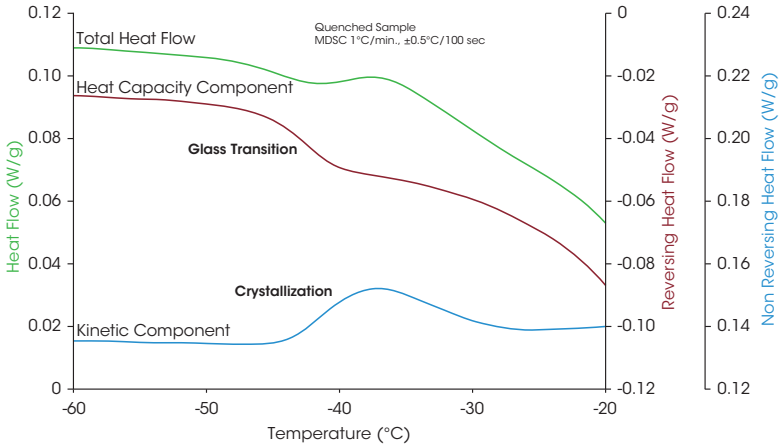
To illustrate the importance of testing at the desired formulation concentration, we evaluated chicken egg white lysozyme from 30 – 330 mg/mL in glycine buffer. With a simple single transition thermogram at low concentrations (~1 mg/mL), lysozyme is commonly used as a reference test sample for DSC. Through evaluation of protein concentrations up to 100-fold higher, we observed a concentration dependence on lysozyme stability.

Additional Resources

See the full experiment and analysis here: Concentration Dependence

Lyophilization → Thermal DSC

Comprehensive characterization is essential to optimize sample preparation, lyophilization, and product delivery. Researchers must conduct thermal analysis throughout the entire process to assess how temperature variations impact material properties.



The glass transition is the temperature at which the frozen drug product solution shifts from a glassy, rigid state to a more viscous state. The freezing phase of lyophilization is an important characteristic because staying below the glass transition temperature to prevent collapse or structure change to the product. Below the glass transition temperature, molecular mobility is also significantly reduced minimizing degradation reactions, to ensure long term stability. It is critical for researchers to identify the glass transition temperature to optimize the temperatures used at each step of the lyophilization process.

The Discovery DSC accurately measures Tg with industry-leading precision, and it can also conduct tests while modulating the sample temperature with a linear temperature ramp. Modulated DSC™ enables measurement of the sample's heat capacity as well as the total heat flow during the test.

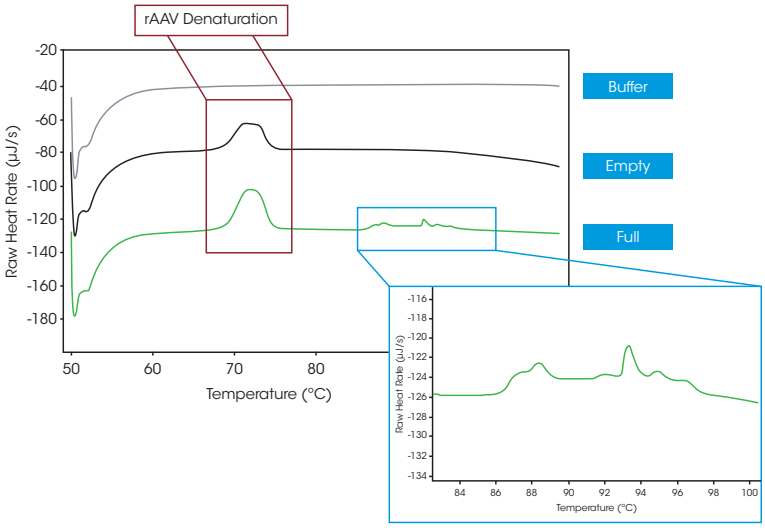
The Multi-Sample X3 DSC offers the distinct advantage of simultaneously testing three samples, providing a wealth of data in a shorter timeframe.

Additional Resources

Blog: How To Optimize Lyophilization with Thermal Analysis
Application Note: Optimization of the Freeze Drying Process Using Modulated Differential Scanning Calorimetry™

Adeno-Associated Viruses (AAVs) Gene Therapy → Nano DSC

With a growing focus on viral-based vectors for delivering life-saving gene therapy treatments, adeno-associated viruses (AAVs) are emerging as a leading gene therapy platform. AAVs are a protein-based delivery system that can efficiently traverse cell membranes, offering effective delivery but with challenges regarding encapsulation efficiency and quality control.



The Nano DSC can detect changes in intermolecular interactions that may result from changes in AAV structure due to temperature, in addition to transitions caused by contaminants, storage and buffering conditions, formulation changes, and batch to batch variability. Due to the broad range of experiments it can accommodate, the Nano DSC can provide workflow solutions at many stages of gene therapy development, from modulating drug release characteristics of the AAV to monitoring batch to batch variability during production.

Additional Resources

Application Note: Evaluating AAV Gene Therapy Vectors using Differential Scanning Calorimetry

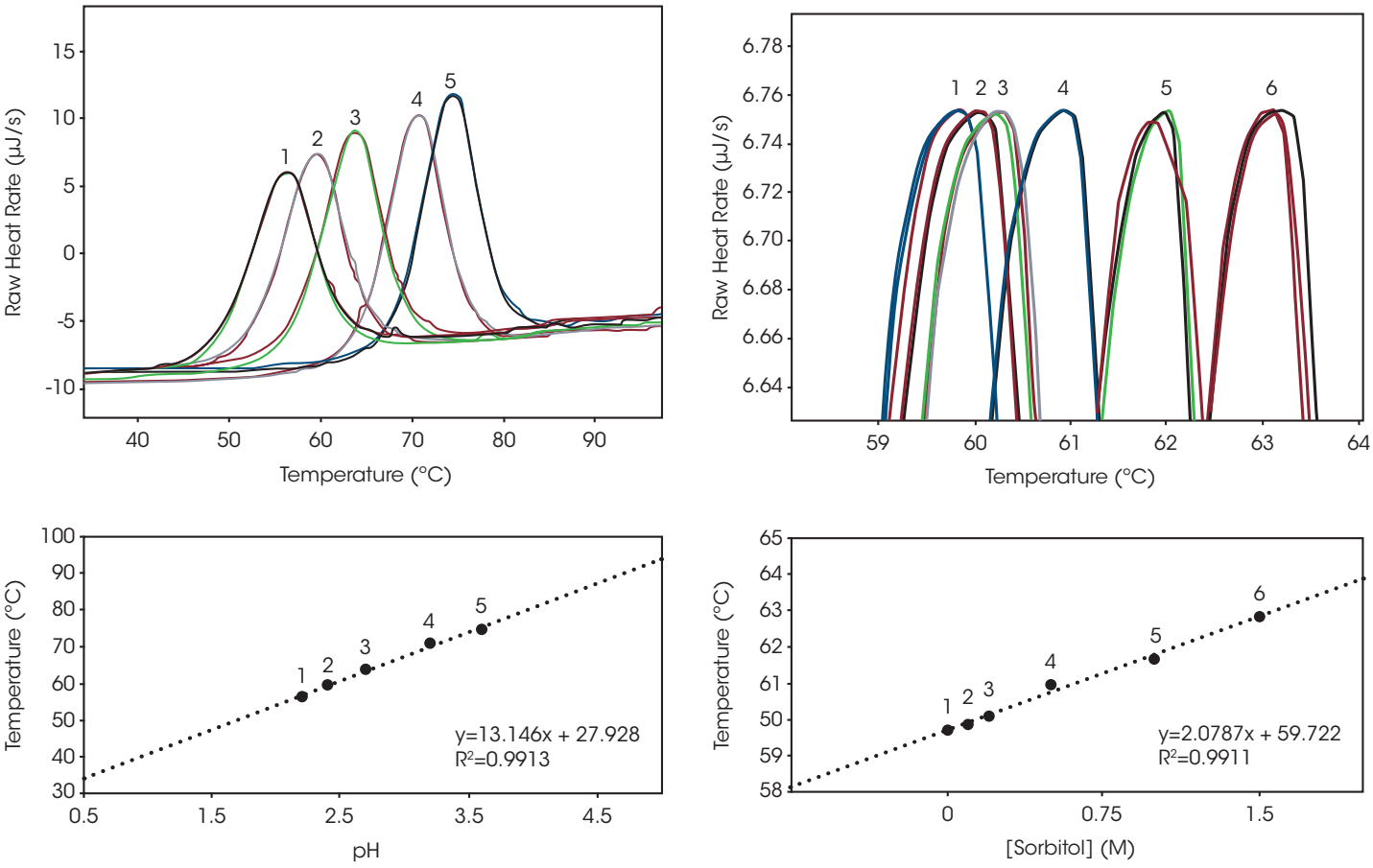
Sensitive Measurements of Small Changes in Biomolecular Structure à Nano DSC

Biological molecules require stabilization for in vitro analysis, as well as extended storage, manufacturing, and processing. Changes in their structure could unexpectedly reduce or enhance drug product quality and safety. Therefore, scientists need highly sensitive measurements of small changes in biomolecule structure and stability to optimize formulations and monitor changes in products over time.

Nano DSC offers next-level sensitivity for measuring minute changes in drug product stability. When used as part of a DOE (Design of Experiment), Nano DSC could determine ideal formulation conditions, identify changes that occur in drug manufacturing, or storage. Nano DSC also offers significant ease of use because samples do not require fluorescent labeling, fusion-tagging, or chemical digestion. When automated, the Nano DSC is an efficiency-increasing tool that provides peace of mind in decision making. The Nano DSC should be a part of every discovery, formulation and analytical R&D testing panel.

Example Experiment

Excipients, such as sorbitol, glycerol and glycols increase biomolecular structure stability by increasing solubility in aqueous environments [2]. The degree of biomolecular structure stability change that occurs when a solubilizing agent is adjusted was assessed for the lysozyme reference sample, from 0 M to 1.5 M sorbitol. Unlike the relatively large changes detected with pH adjustment, adjustment of sorbitol over the entire range was approximately 3 °C (Fig 2, Top). The plot of Sorbitol Concentration vs Tmax also displayed the proportional linear change in stability temperature (Fig 2, Bottom), like the plot of pH versus Tmax (Fig 1, Bottom). The Nano DSC resolved structure stability changes as small as 0.2 °C with excellent triplicate reproducibility (% RMS ≤ 0.24°C, Table 2).



Additional Resources

Application Note: A Novel Thermodynamic Assay for Predicting and Monitoring Biomolecular Structure Stability

Resources Library

Rapid Screening-Differential Scanning Calorimeter

- Product Demonstration Video
- **Application Note:** Rapid Thermal Stability Screening of High Concentration Biologic Drugs
- **Application Note:** Rapid Thermal Stability Screening and Selection of Monoclonal Antibody Drug Products
- **Blog:** How to Accelerate Thermal Stability Testing for High-Concentration Drugs
- **Blog:** Why DSC Testing is a Critical Step in Developing Biosimilar Drugs

Discovery Differential Scanning Calorimeters

- **Webinar:** Biophysical Characterization of Antibody Drug Conjugates Using DSC
- **Webinar:** Characterization of Amorphous Pharmaceuticals by DSC Analysis
- **Application Note:** Drug – Excipient Incompatibility with Discovery X3
- **Blog:** How To Optimize Lyophilization with Thermal Analysis

Nano DSC

- **Application Note:** Evaluating AAV Gene Therapy Vectors using Differential Scanning Calorimetry
- **Application Note:** A Novel Thermodynamic Assay for Predicting and Monitoring Biomolecular Structure Stability
- **Application Note:** Characterizing Virus Structure and Binding
- **Blog:** Evaluating Antibody Stability with Nano Differential Scanning Calorimetry

Microcalorimeters Product Brochure

Biologics Resource Hub

eBook: Must Know Analytical Techniques for Biopharma Developers

Blog: BioPharma Drug Development Workflow and Techniques

Blog: Microcalorimetry for the Biophysical Characterization of Macromolecules

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