TA Instruments

MICROCALORIMETRY: ITC & DSC

Microcalorimetry

Isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC) are powerful analytical techniques for in-depth characterization of molecular binding events and structural stability. Thermodynamic binding signatures not only reveal the strength of a binding event, but the specific or nonspecific driving forces involved. Structural stability profiles from DSC reveal strengths and weaknesses in higher order structure and define the behavior of individual domains and their interactions. The TA Instruments Affinity ITC, Nano ITC and Nano DSC provide the performance, reliability and ease-of-use required for the most demanding applications in drug discovery, protein-protein interactions, structure-function characterization and more.

Nano DSC and DSC Auto

DIFFERENTIAL SCANNING CALORIMETER

The Nano DSC has the versatility and precision for characterizing molecular stability, determining high affinity ligand binding and deconvoluting multi-domain structures. There is no other DSC with the proprietary technologies, high performance or the sample throughput of the Nano DSC and Nano DSC Auto.

Features:

- Highest sensitivity, lowest cell volume for unmatched performance
- Capillary cell design for analysis of samples that tend to aggregate or precipitate
- Built-in precision pressurizing system maintains accurate, constant pressure in the cells
- Solid-state thermoelectric elements for accurate temperature control during heating and cooling scans
- Upgradeable with industry proven HPLC grade autosampler for reliable high sample throughput

Nano DSC
TECHNOLOGY

The Nano DSC is designed for ultra-sensitive measure of heat absorbed or released by dilute in-solution bio-molecules as they are heated or cooled. The capillary cell design, solid-state thermoelectric temperature control and easy cleaning ensure the highest sensitivity and data reproducibility for a wide variety of applications.

Features:

- 300 µL active volume capillary cells for analyzing hydrophobic samples
- Easy, accurate sample loading with laboratory pipetteman
- Built-in, user-programmable pressurization system (up to 6 atm)
- Flexible data acquisition interface for easy experiment setup
- NanoAnalyze software for accurate model fitting and multi-file batch processing

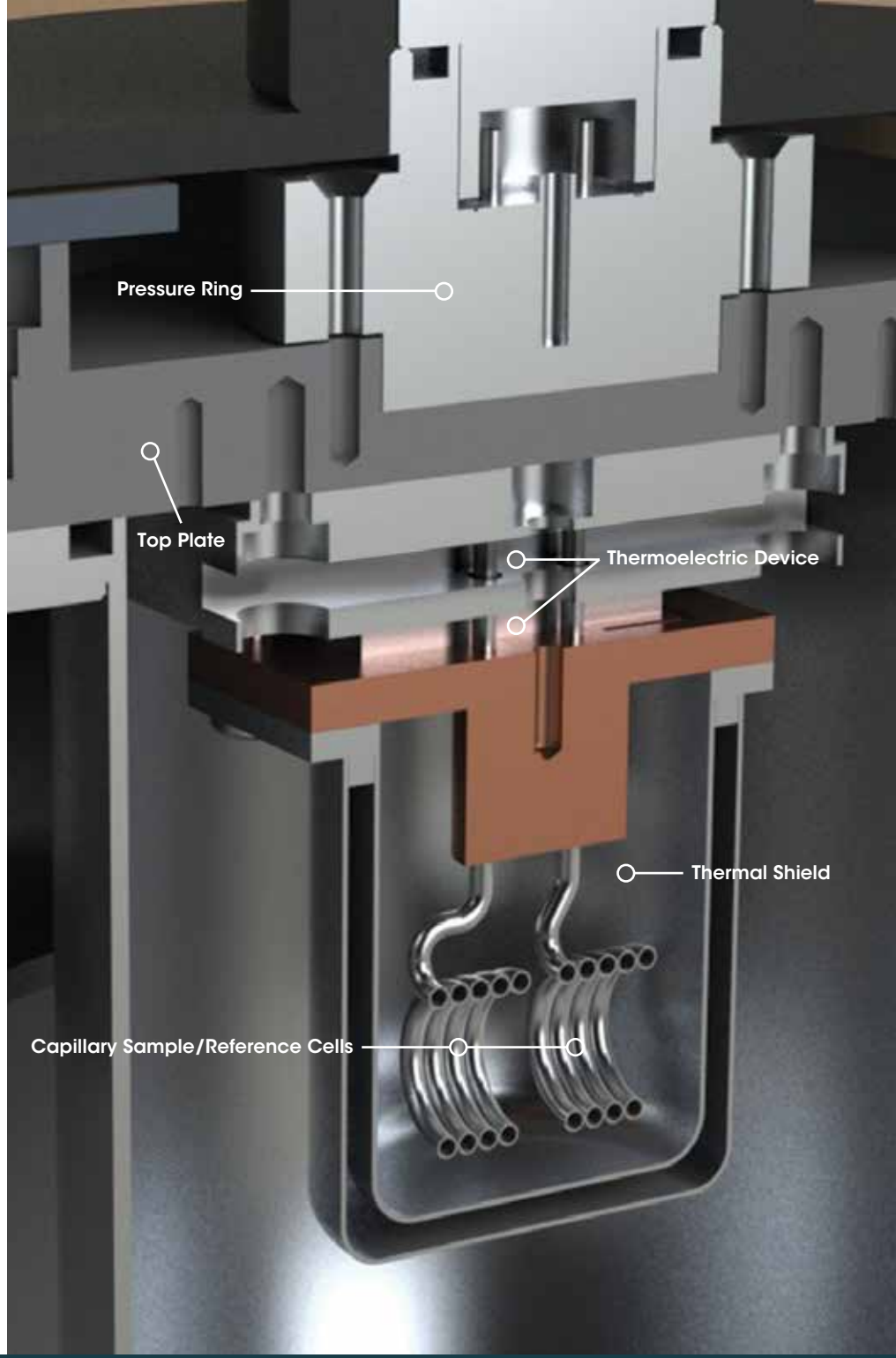
The NanoDSC is a powerful thermal scanning instrument that utilizes a 300 µL capillary cell design and solid-state thermoelectric temperature control to provide unmatched performance.

Nano DSC Capillary Platinum Cells

- Fixed-in-place capillary cells attenuate aggregation and precipitation
- Platinum cells are inert and compatible with strong acids, bases and protein cleaning enzymes
- 300 µL active cell volume minimizes sample consumption
- Sample cell loading with laboratory pipetteman is easy and ensures no trapped air bubbles

Nano DSC Solid-State Thermoelectric Temperature Control:

- Accurate, reproducible temperature control for highest sensitivity in both heating and cooling scans and unmatched baseline reproducibility
- Innovative, user-programmable built-in pressure system for complex analysis of water characteristics and molecule structure
- User-programmable scan rates for scan flexibility and highest confidence in data analysis



Nano DSC
AUTOMATION

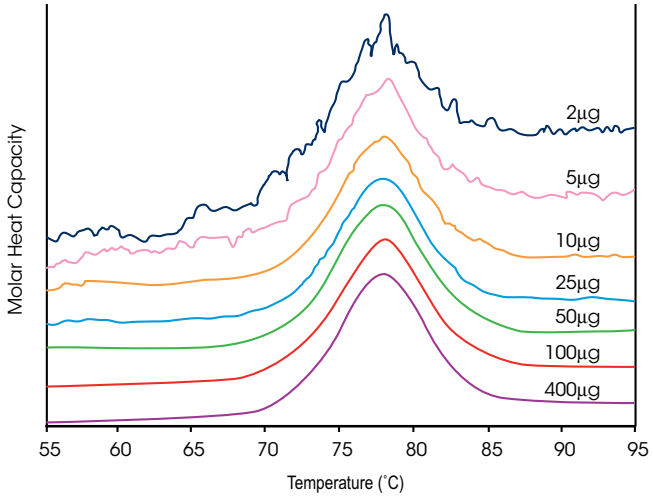
The Nano DSC Autosampler enables true "start and walk away" capability without sacrificing either sensitivity or reliability. It is an industry-proven 96-well plate autosampler that stores and delivers samples to the DSC cells. User-programmable washing routines ensure no sample carry over and the 96-well format maximizes sample throughput.

Autosampler Features:

- Industry-proven HPLC autosampler reliability
- Easy connection to the Nano DSC through autosampler interface
- Two (2) 96-well plates store samples at temperatures down to 4°C
- Four (4) wash/rinse solvent ports on the autosampler interface are user-programmable
- Two (2) exit ports enable the collection of sample and matching buffer/solvent solutions from both the sample or reference cells
- Autosampler is programmable through the Nano DSC instrument operating software

Nano DSC

APPLICATIONS



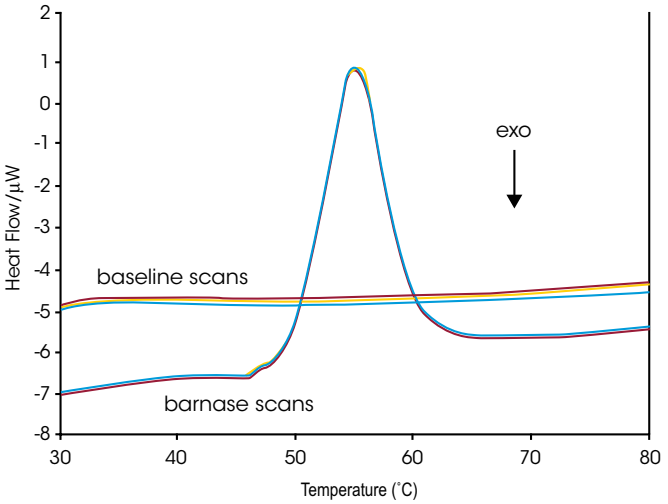
How much Protein is Required for a DSC Scan?

Determining the thermodynamic parameters of a protein by differential scanning calorimetry (DSC) using the Nano DSC requires about the same amount of protein as surface plasmon resonance or fluorescence studies. Because of the Nano DSC's extreme sensitivity and baseline reproducibility, and the sample cell's small volume (300 µL), a complete, interpretable, accurate scan can be obtained on essentially any protein of interest. The sensitivity and accuracy of the Nano DSC is demonstrated by this data. Hen egg white lysozyme (in pH 4.0 glycine buffer) was prepared at various concentrations. As little as 2 µg of lysozyme in the capillary cell is sufficient to provide quality data yielding accurate values of all four thermodynamic parameters!

Lysozyme	Calorimetric		Van't Hoff	
in cell (50µg)	ΔH (kJ mol^{-1})	ΔS (kJ K^{-1} mol^{-1})	T_m (˚C)	ΔH (kJ mol^{-1})
400	512	1.46	78.0	515
100	512	1.46	78.0	509
50	517	1.47	77.9	513
25	513	1.46	77.8	513
10	515	1.47	78.0	515
5	490	1.40	78.0	510
2	503	1.43	77.8	499

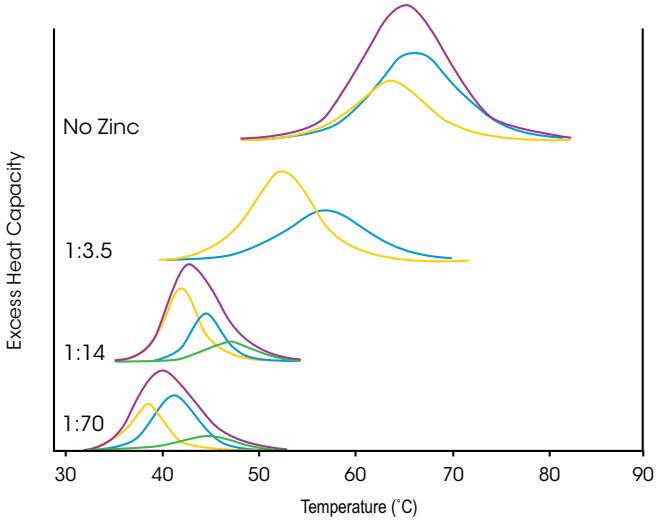
Characterization of Protein Stability

Analyzing the stability of a protein in dilute solution involves determining changes in the partial molar heat capacity of the protein at constant pressure (ΔCp). The contribution of the protein to the calorimetrically measured heat capacity (its partial Cp) is determined by subtracting a scan of a buffer blank from the sample data prior to analysis. Heating the protein sample initially produces a slightly increasing baseline but as heating progresses, heat is absorbed by the protein and causes it to thermally unfold over a temperature range characteristic for that protein, giving rise to an endothermic peak. Once unfolding is complete, heat absorption decreases and a new baseline is established. After blank subtraction, the data can be analyzed to provide a complete thermodynamic characterization of the unfolding process.



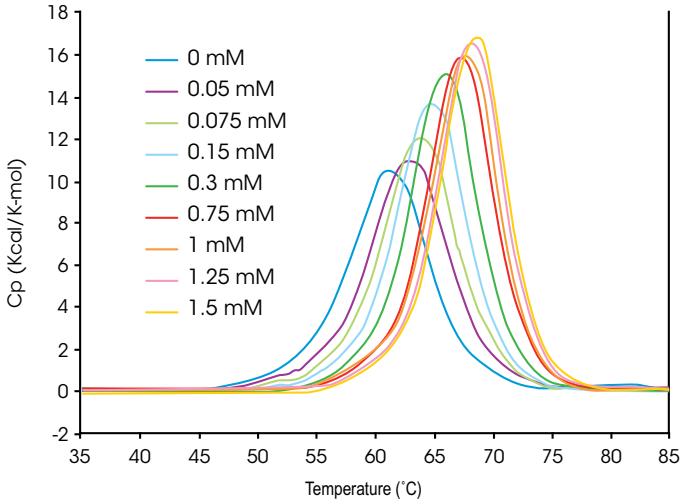
Characterization of Protein Structure

DSC can be used to characterize both the specific binding of a ligand (for example, a drug to a receptor binding site), or nonspecific binding (for example, detergents binding to hydrophobic patches on a protein surface). In some instances ligand binding, even if to a specific receptor site, results in long-range protein structural rearrangements that destabilize the entire complex. The figure shows DSC scans of Ca^{2+} saturated bovine a-lactalbumin at various protein:Zn^{2+} ratios scanned at 1 °C/min. The midpoint of the thermal unfolding of the protein decreases from 65 °C in the absence of Zn^{2+} to 35 °C at a protein:Zn^{2+} ratio of 1:70. The enthalpy of unfolding is also decreased substantially by high Zn^{2+} concentrations.



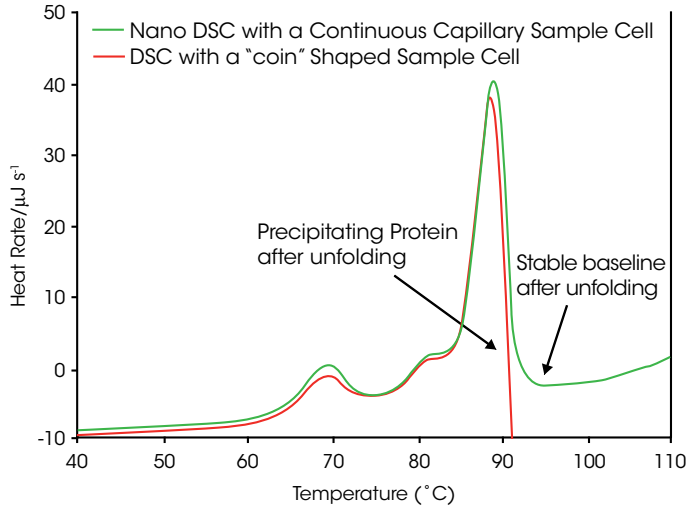
Investigation of Protein-Ligand Binding

DSC is a valuable tool for studying binding between a biological macromolecule and a ligand such as another biopolymer or a drug. Unlike ITC, DSC allows the thermodynamics that drive binding to be correlated with conformational changes in the macromolecule caused by the binding reaction. DSC is particularly useful for characterizing very tight or slow binding interactions. DSC also allows characterization of binding reactions that are incompatible with the organic solvent requirements of some ITC experiments (i.e., where ligand solubility for an ITC experiment requires concentrations of organic solvent not tolerated by the protein). The data shows DSC scans of RNase A bound with increasing concentrations of 2'-CMP, showing that the protein is stabilized by higher concentrations of the inhibitor. Essentially identical data was obtained in the presence of 5% DMSO, verifying that organic solvents are compatible with the DSC technique.



Nano DSC Capillary Cell Advantages

This figure shows two DSC scans of matched samples of human IgG1 at 0.5 mg/ml in physiological buffer. The data from the DSC with a "coin" shaped sample cell shows the easily recognizable exothermic aggregation/precipitation event at approx 89-90 °C, while the data collected on the Nano DSC with a capillary sample cell shows a stable post-transition baseline that will enable complete and accurate determinations of transition temperatures (Tm) and enthalpy (ΔH).

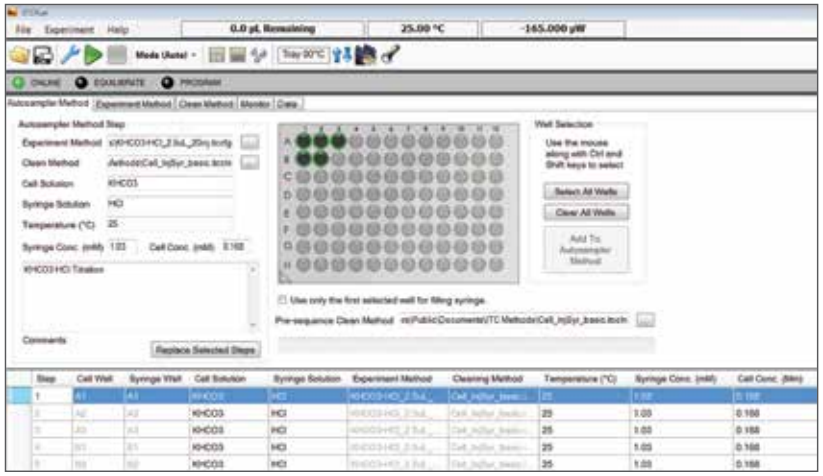


ITC & DSC
SOFTWARE

Instrument Control & Data Acquisition Software

The Affinity and Nano instruments control and data acquisition functions are executed within a Windows-compatible software interface, ITCRun or DSCRun. All experimental parameters and sample information are easily entered into an intuitive graphical user interface and can be saved as an experimental template for future use.

Real-time monitoring of the raw data as the experiment progresses allows rapid assessment of the data quality and instrument performance in individual tabs. Unique icon-controlled functions, such as immediate baseline subtraction, are always available on the display.



ITCRun & DSCRun features:

- Automatic configuration of user interface for automated or non-automated instruments
- Individual viewing tabs for real-time monitoring of instrument performance characteristics and raw data acquisition
- Easy experiment setup
- Direct autosampler programming and control for automated instruments
- Software passes all experimental parameters to NanoAnalyze™

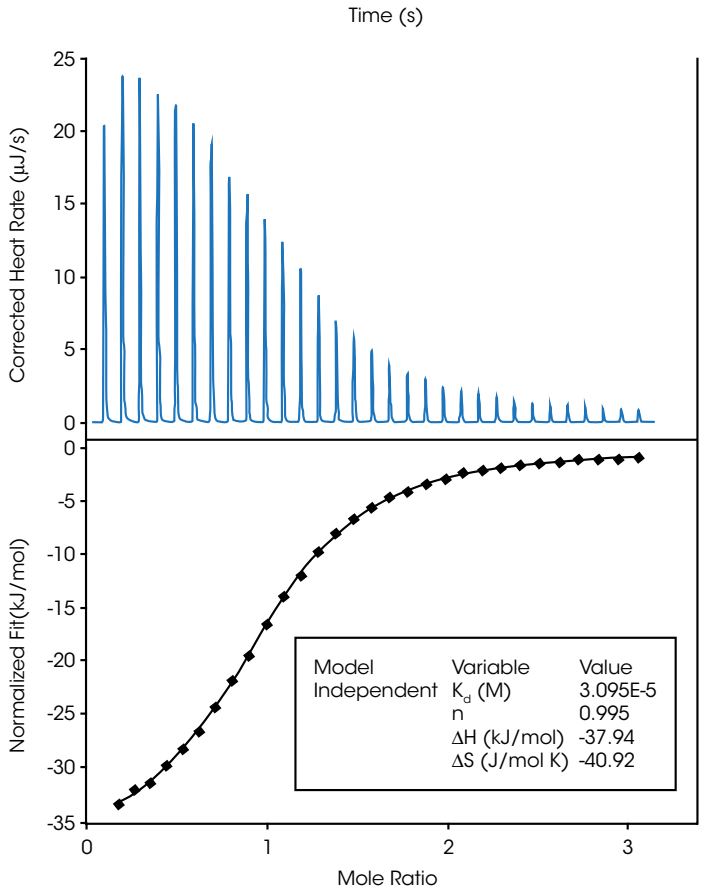
Data Analysis with NanoAnalyze™

All ITC and DSC raw data files are easily and quickly analyzed with a powerful ITC/DSC data analysis software, NanoAnalyze. Individual window tabs for each processing step guide the user through the analysis of Individual raw data files or the batch processing of multiple files.

NanoAnalyze™ features:

- Easy import of all ITC and DSC raw data files
- User selectable fitting models for ITC and DSC
- Easy set up of new fitting models
- Drag & Drop subtraction of baseline blank files
- Powerful experiment design and optimization tool
- Flexible overlay graphs for quick data comparisons
- Generates thermodynamic profile bar graph
- Easy export of all data to delimited text files
- Full-featured editing tools for preparation of publication quality images

All instrument control, data acquisition and data analysis software required for ITC and DSC data are provided with all Affinity and Nano instruments. All software updates and feature improvements are available on the TA Instruments website.



DSC
SPECIFICATIONS

Nano DSC and DSC Auto

Short-term Noise	0.015 µWatts
Baseline Stability	±0.028 µWatts
Response Time	7 seconds
Operating Temperature	-10 °C to 130 °C or 160 °C
Temperature Scan Rate	up to 2°C/minute
Pressurization Perturbation	Built-in up to 6 atmospheres
Cell Volume	300 mL
Cell Geometry	Fixed capillary
Cell Composition	Platinum
Heat Measurement Type	Power Compensation

Automation

Sample Capacity	2 standard plates x 96 wells x 1000 µL/well
Sample Tray Temperature Control Range	4 °C to Ambient
Available Wash/Rinse Buffer Ports	4 for Sample/Reference Cells; 2 for Sample Handling Syringe

References

Garbett, N., DeLeeuw, L. and J.B. Chaires. MCAPN-2010-04.
High-throughput DSC: A Comparison of the TA Instruments Nano DSC Autosampler System™ with the GE Healthcare VP-Capillary DSC™ (2010)

Demarse, N. and L.D. Hansen. MCAPN-2010-01.
Analysis of Binding Organic Compounds to Nanoparticles by Isothermal Titration Calorimetry (ITC). (2010)

Quinn, C.F. MCAPN-2010-02. Analyzing ITC Data for the Enthalpy of Binding Metal Ions to Ligands. (2010)

Quinn, C.F. and L.D. Hansen. MCAPN-2010-03. Pressure Perturbation Calorimetry: Data Collection and Fitting. (2010)

Román–Guerrero, A.,Vernon–Carter, E.J. and N.A. Demarse. MCAPN-2010-05. Thermodynamics of Micelle Formation. (2010)

TA Instruments, Microcalorimetry Technical Note MCTN-2010-02.
Advantages of Using a Nano DSC when Studying Proteins that Aggregate and Precipitate when Denatured. (2010)

TA Instruments, Microcalorimetry Technical Note, MCTN-2010-03. How to Choose an ITC Cell Volume. (2010)

Baldoni, D., Steinhuber, A., Zimmerli, W. and A. Trampuz. Antimicrob Agents Chemother 54(1):157-63. In vitro activity of gallium maltolate against Staphylococci in logarithmic, stationary, and biofilm growth phases: comparison of conventional and calorimetric susceptibility testing methods. (2010)

Choma, C.T. MCAPN-2010-01. Characterizing Virus Structure and Binding by Calorimetry. (2009)

Wadsö, L. and F. G. Galindo. Food Control 20: 956–961. Isothermal calorimetry for biological applications in food science and technology. (2009)

Baldoni, D., Hermann, H., Frei, R., Trampuz, A. and A. Steinhuber. J Clin Microbiol. 7(3):774-6.
Performance of microcalorimetry for early detection of methicillin resistance in clinical isolates of Staphylococcus aureus. (2009)

Trampuz, A., Piper, K.E., Hanssen, A.D., Osmon, D.R., Cockerill, F.R., Steckelberg, J.M. and R. Patel. J Clin Microbiol. 44(2):628-631.
Sonication of explanted prosthetic components in bags for diagnosis of prosthetic joint infection is associated with risk of contamination. (2006)

Microcalorimetry - A Novel Method for Detection of Microorganisms in Platelet Concentrates and Blood Cultures. Andrej Trampuz, Simone Salzmann, Jeanne Antheaume, Reno Frei, A.U. Daniels University of Basel & University Hospital Basel, Switzerland (2006)

Data provided by Svensson, Bodycote Materials AB, Sweden (2003)

Wingborg and Eldsater, Propellants, Explosives and Pyrotechnics, 27, 314 -319, (2002).

Schmitt, E.A., Peck, K., Sun, Y. and J-M Geoffroy. Thermochimica Acta

380 (2):175-184. Rapid, practical and predictive excipient compatibility screening using isothermal microcalorimetry. (2001)

Schmitt, Peck, Sun & Geoffroy, Thermochim. Acta, 380, 175-183, (2001).

Thermometric Application Note 22034 (2001).

Hogan, S.E. & G. Buckton. Int. J. Pharm., 207, 57-64. The quantification of small degrees of disorder in lactose using solution calorimetry. (2000)

Chemical & Engineering News, June 18, 2007, Page 31. Hongisto, Lehto & Laine, Thermochim. Acta, 276, 229-242, (1996).

Bermudez, J., Bäckman, P. and A. Schön. Cell. Biophys. 20, 111-123.
Microcalorimetric Evaluation of the Effects of Methotrexate and 6-Thioguanine on Sensitive T-lymphoma Cells and on a Methotrexate-Resistant Subline. (1992)

Bystrom, Thermometric Application Note 22004, (1990).

Thermometric Appl. Note 22024.