

TA Instruments MICROCALORIMETRY

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Lindon, UT USA

Elstree, United Kingdom

Shanghai, China

Beijing, China

Taipei, Taiwan

Tokyo, Japan

Seoul, South Korea

Bangalore, India

Paris, France

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Etten-Leur, Netherlands

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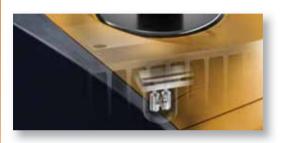
Melbourne, Australia

Mexico City, Mexico



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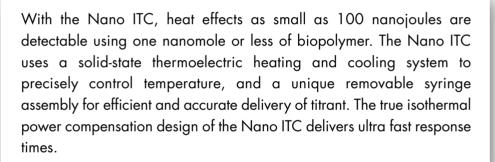


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ISOTHERMAL TITRATION CALORIMETRY



The TA Instruments Nano ITC is engineered specifically for binding and kinetics studies on purified dilute biological samples of limited availability





Specs	Standard Volume	Low Volume
Temperature Range	2 to 80 °C	2 to 80 °C
Temperature Stability	±0.0002 °C @ 25 °C	±0.0002 °C @ 25 °C
Minimum detectable heat	لبا 0.1	لر 0.05
Maximum detectable heat	5,000 µJ	3,000 µJ
Baseline Stability	±0.02 μW/hr	±0.02 μW/hr
Noise Level	2.5 nW	1.4 nW
Response Time	13 Seconds*	11 Seconds
Cell Volume	1.0 ml	190 µL
Cell Configuration	Fixed-in-place, Cylindrical	Fixed-in-place, Cylindrical
Cell Material	24K Gold* or Hastelloy	24K Gold
Injection Syringe Volumes	100 µL and 250 µL	50 µL

ITC TECHNOLOGY

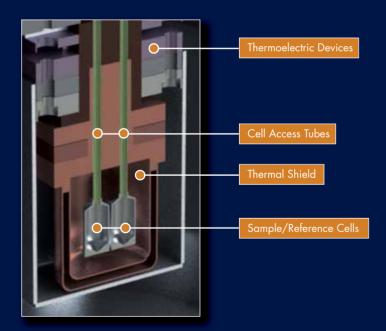
Life Science professionals know that the thermodynamic driving forces of macro-molecular interactions are critical parameters for the design of effective biomedical and pharmaceutical treatments. Calorimetry has become the method of choice for characterizing the thermodynamic driving forces of critical molecular interactions and defining molecular stabilities. Calorimetric analyses are based on accurately measuring the rate of heat absorbed or evolved when the biomolecule of interest interacts specifically or nonspecifically with another macromolecule or ligand (binding studies). The TA Instruments Nano ITC Standard Volume or Nano ITC Low Volume instruments are powerful tools to accurately and efficiently perform these important measurements.

The Nano ITC instruments are designed to improve laboratory productivity and efficiency by performing high-sensitivity analyses on nanomolar quantities of biomolecule. This is accomplished through a combination of a high sensitivity calorimeter, accurate and stable temperature control, and efficient titrant delivery.



The unique removable syringe assembly contains a mechanical paddle stirrer at the end, the speed of which is easily adjusted to accommodate the physical properties of the sample. The integrated titration assembly of the Nano ITC ensures quick-filling, simple cleaning and accurate titrations. The Nano ITC Standard Volume is available with sample cells made from 99.999 % Gold or Hastelloy C to allow for the widest range of reagent chemistry. The Nano ITC Low Volume is available with sample cell cells made from 99.999 % Gold.

The true isothermal power compensation design and the choice of sample cell volumes of the Nano ITC instruments provides the highest sensitivity and flexibility for an ultrasensitive ITC analyzing biological samples in-solution.





The Nano ITC instrument is available in two sample cell sizes. The Nano ITC Standard Volume sample cell volume is 1.0 ml. The Nano ITC Low Volume offers the lowest cell volume at 190 uL, to minimize sample consumption and at the same time provides sensitivity levels over two times better than previously achievable. Heat effects as small as 50 nanojoules are detectable in the Nano ITC Low Volume with a short term noise level of 1.4 nanowatts. Both Nano ITC instruments use cylindrical-shaped cells to make cleaning easy, solid state thermoelectric heating and cooling systems to precisely control temperature, and have the same flexible injection syringe assemblies for efficient and accurate delivery of titrant.

Advantages

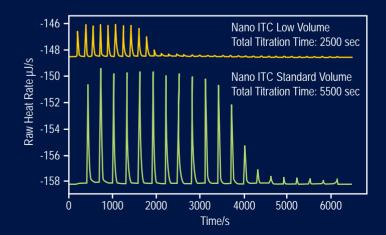
The Nano ITC Low Volume requires substantially less sample and can reduce the time required to complete a titration by one-half. The 2X improvement in sensitivity of the Nano ITC Low Volume ensures that with 80% less sample the instrument will generate accurate and reproducible results.

Nano ITC Low Volume:

Sample Cell = KHCO3; 0.36 mM Injection Syringe = HCl; 4.2 mM Injection volume = 1.4 µL Injection interval = 175 sec

Nano ITC Standard Volume:

Sample Cell = KHCO3; 0.36 mM Injection Syringe = HCl; 5.6 mM Injection volume = 5 µL Injection interval = 300 sec



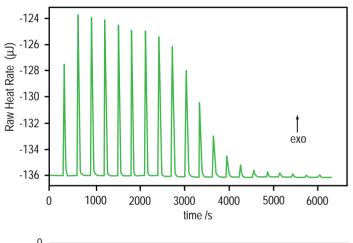


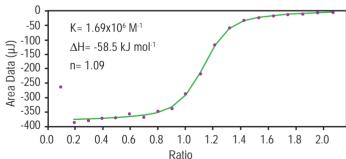


ITC APPLICATIONS

Characterizing Binding Interactions by ITC

All binding events are accompanied by the evolution or absorption of heat (a change in enthalpy, ΔH). In a single ITC experiment a full thermodynamic characterization of the binding reactions can be obtained. With the appropriate experimental design, fundamental information about the molecular interactions driving the process, as well as the stoichiometry of binding (n) and the binding constant (Ka) is generated. The first figure shows a typical incremental titration (20, 5 µL injections) of an inhibitor, 2'-CMP, titrated into RNase A; n = 1, $K_0 = 1.69 \times 10^6 M^{-1}$, and $\Delta H = -58 \text{ kJ mol}^{-1}$. The second figure shows the same experiment, plotting the individual integrated peak areas vs the ratio of the two binding molecules. As the binding sites become saturated, the amount of heat produced with individual injections decreases. The resulting titration curve reveals valuable information on the enthalpy (ΔH), entropy (ΔS) and overall Gibbs free energy (ΔG) of the reaction taking place in the calorimeter. ITC is a powerful analytical tool and considered the most sensitive assay technique for characterizing the fundamental driving forces of molecular binding reactions.

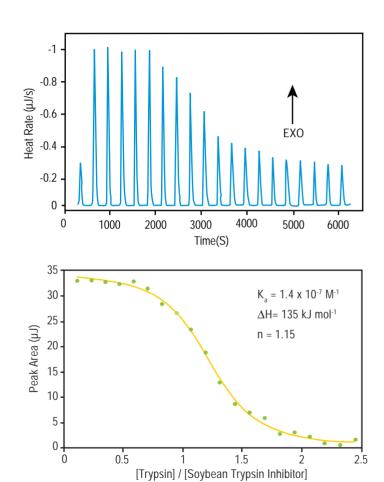




ITC APPLICATIONS

Protein Interactions

When two proteins interact and bind, conformational changes in the proteins, and rearrangement of the solvent in the vicinity of the binding site, result in the absorption or generation of heat. Quantification of this reaction heat by ITC provides a complete thermodynamic description of the binding interaction, the stoichiometry of binding, and the association constant. This figure contains the titration data of porcine pancreatic trypsin into soybean trypsin inhibitor using a Nano ITC. Twenty, 5 μ L aliquots of ligand were titrated into the sample cell while the temperature of the system was maintained at 25 °C. Top panel: The signal (heat) produced following each addition of protein to the inhibitor. Bottom panel: Integration of the heats over the time course of the experiment; the μ J in each peak are plotted against the mole ratio of the titrant to inhibitor.

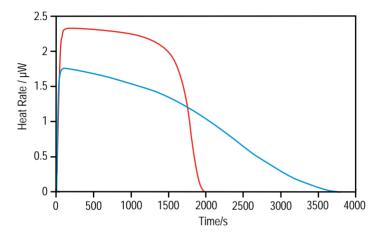


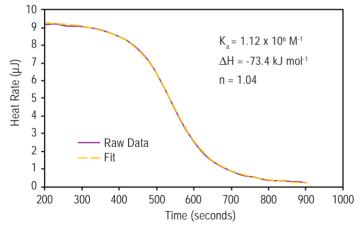
Characterization of Enzyme Kinetics

Every reaction generates or absorbs heat, so every reaction can in principle be studied by calorimetry. In practice it has been shown that representative enzymes from every EC classification can be analyzed kinetically using ITC. In addition, ITC analyses are rapid, precise, nondestructive, compatible with both physiological and synthetic substrates, and are as sensitive as spectroscopic techniques but do not require a spectroscopic label or chemical tag. Importantly, ITC analyses of enzyme kinetics are also straightforward. The figure shows the hydrolysis of a single 10 µL injection of trypsin into a solution of BAEE in the absence (blue) and presence (red) of benzamidine, a competitive inhibitor. The area under both curves (representing the total heat output for complete conversion of substrate to product) is the same either in the presence or absence of inhibitor, allowing the KM and kcat of the reaction under both conditions to be calculated, as well as the inhibition constant.

Continuous Single Injection

Continuous single injection titration is an attractive alternative to the traditional incremental titration ITC for samples exhibiting very rapid binding reactions. These continuous injection experiments can be completed in less total time than normally required for a full set of incremental titrations. This technique provides accurate determinations of stoichiometry (n) and enthalpy (ΔH) for a wide range of binding constants. Continuous injection and incremental injection experiments can be performed in both the Nano ITC standard volume and low volume instruments with no alterations in hardware or software supplied with the instruments.









DIFFERENTIAL SCANNING CALORIMETRY

The Nano DSC is specifically designed to determine the thermal stability and heat capacity of proteins and other macro-molecules in dilute solution, with the versatility and precision to perform molecular stability screening, ligand binding and pressure perturbation measurements.

The TA Instruments Nano Series calorimeters represent the highest sensitivity and unmatched performance for the investigation of biological samples.

DSC **SPECIFICATIONS**



Nano DSC Specifications

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	0.05 °C to 2°C/minute
Pressurization Perturbation	Built-in up to 6 atmospheres
Cell Volume	0.30 ml
Cell Geometry	Fixed capillary
Cell Composition	Platinum
Heat Measurement Type	Power Compensation

Automation Specifications

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

NANO DSC TECHNOLOGY

The Nano DSC differential scanning calorimeter is designed to measure the amount of heat absorbed or released by dilute in-solution bio-molecules as they are heated or cooled. Macromolecules such as proteins respond to heating or cooling by unfolding at a characteristic temperature. The more intrinsically stable the biopolymer, the higher the midpoint temperature of the unfolding transition. As these processes often exchange microjoule levels of heat, the sensitivity of the Nano DSC is critical for successful investigation of the reaction.

The Nano DSC obtains data with less sample than competitive designs and produces unmatched short term noise (±15 nanowatts) and baseline reproducibility (±28 nanowatts). Solid-state thermoelectric elements are used to precisely control temperature and a built-in precision linear actuator maintains constant or controlled variable pressure in the cell. Increased sample throughput is realized by adding on the Nano DSC Autosampler. It provides true walk-away capability for up to 96 samples. With convenient USB connectivity, built-in pressure perturbation capability and capillary cell design, the Nano DSC provides maximum flexibility with a cell design that minimizes sample aggregation and precipitation, resulting in high quality data.



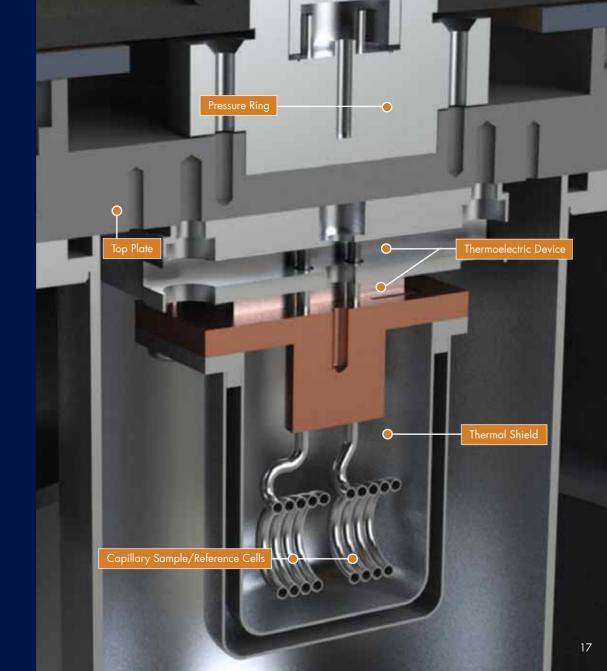
Nano DSC Capillary Cell

The capillary design of the Nano DSC provides unparalleled sensitivity, accuracy and precision. Many structurally unstable samples that show aggregation and precipitation during a scan on competitive designs can be routinely analyzed on the Nano DSC.

The Nano DSC employs solid-state thermoelectric elements to accurately and precisely control the temperature of the sample. This powerful temperature control and heat sensing architecture enables active control of both heating and cooling scans.

The unique and innovative built-in high-pressure piston and pressure ring provides the highest flexibility with user-selectable functions for standard constant pressure experiments and pressure perturbation calorimetry (PPC) experiments with no extra hardware or software accessories required.

The Nano DSC's combination of a robust capillary cell design and state-of-the-art temperature control and sensor technology provides a reliable, flexible and easy-to-use calorimeter for in-solution biological samples.

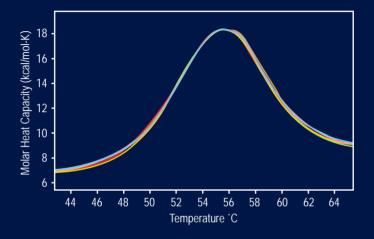


NANO DSC AUTOMATION

The Nano DSC Autosampler system enables true "start and walk away" capability without sacrificing either sensitivity or reliability. The autosampler stores samples and the matching buffers/solvents in a 96-well plate format at temperatures ranging from 4°C to ambient room temperature. Four (4) wash/rinse solvents are accessible through programmable ports on the autosampler interface. Two (2) exit ports enable the collection of sample and matching buffer/solvent solutions from both the sample or reference cell of the Nano DSC.

For molecular stability testing applications that require high sample throughput, the Nano DSC Autosampler system is a reliable sample handling system that increases the productivity of the most sensitive DSC on the market with true walk-away capability and proven reliability.

The figure shows overlapping plots of DSC scans of duplicate samples of five different Lysozyme sample concentrations when converted to Molar Heat Capacity. The Nano DSC Autosampler system produces superior data reproducibility and precision at low sample concentrations with no detectable sample-to-sample carry-over or sample degradation.

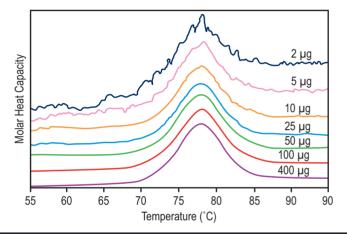




Nano DSC Applications

How much Protein is Required for a DSC Scan²

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 μ L), 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 μ g 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 (µg)	∆H (kJ mol¹1)	∆S (kJ K¹ mol¹)	T _m (°C)	∆H (kJ mol¹1)
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

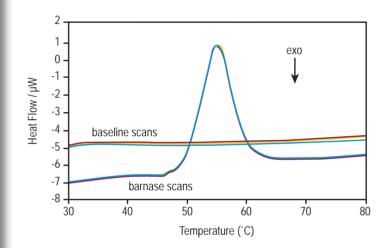
NANO DSC APPLICATIONS

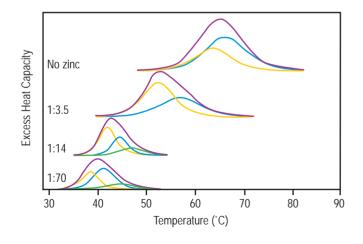
Characterization of Protein Stability

Analyzing the stability of a protein in dilute solution involves determining changes in the partial molar heat capacity of the protein at constant pressure (Δ Cp). 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 Ca²⁺ saturated bovine a-lactalbumin at various protein:Zn²⁺ ratios scanned at 1 °C/min. The midpoint of the thermal unfolding of the protein decreases from 65 °C in the absence of Zn²⁺ to 35 °C at a protein:Zn²⁺ ratio of 1:70. The enthalpy of unfolding is also decreased substantially by high Zn²⁺ concentrations.



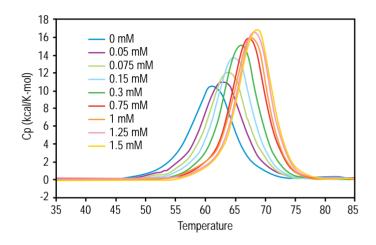


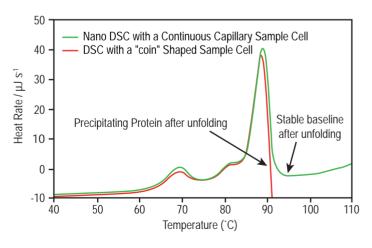
Investigation of Protein-Ligand Binding

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 thermo-dynamics 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 were 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 $\lg G_1$ 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).









TAM III SPECIFICATIONS

TAM III is the new generation, multi-channel, microcalorimetric system from TA Instruments. TAM offers maximum sensitivity, flexibility, and performance. It can be used with the most sensitive microcalorimeters and a wide variety of accessories to precisely control the experimental conditions. Up to four independent calorimeters can be used simultaneously with TAM III, to perform repetitive or different types of experiments. TAM III is totally modular and enables multiple calorimeters to be added to increase sample capacity or functionality. With the addition of a multicalorimeter holding six independent minicalorimeters, the sample throughput is substantially increased. TAM III employs patented thermostat technology to precisely control the liquid bath temperature to within 0.0001 °C, and can be operated in isothermal, step-isothermal or temperature-scanning mode.



Thermostat Specifications

Thermal Media	Oil
Calorimeter Positions	1 - 4
Temperature Range	15 to 150 °C
Accuracy	< ± 0.1 °C
Long Term Stability	< ± 100 μK/24h
Short Term Stability	< ± 10 µK (p-p)
Scanning Rate	< ± 2 °C/h (between 20 - 150 °C)

Step-wise Change of Temperature

Heating	15°C/h at 15 °C - 2°C/h at 150 °C
Cooling	15 °C/h at 150 °C - 1.5 °C/h at 15 °C

Calorimeter Specifications

CALORIMETER	SHORT TERM NOISE	BASELINE DRIFT	ACCURACY	PRECISION
Nanocalorimeter	< ± 10 nW	< 40 nW/24 h	< 2%	± 100 nW
Minicalorimeter / Multi 4ml	< ± 100 nW	< 200 nW/24 h	< 5%	± 200 nW
Microcalorimeter 20ml	< ± 100 nW	< 200 nW/24 h	< 2%	± 100 nW
Minicalorimeter/Multi 20 ml	< ± 300 nW	1 μW/ 24 h	< 2%	± 300 nW
Macrocalorimeter	<± 500 nW	6 μW /24h	< 5%	3 µW

DETECTABILITY

Solution Calorimeter	< ± 10 μK	1-4 mJ	< 0.1%	Q > 100 J:0.02%
				Q < 50 J:<±10 mJ
				$Q < 0.1 \text{ J:} < \pm 5 \text{ mJ}$



Thermal Media			Oil	
Calorimeter Positions		1 - 48		
Temperature Range		15 to 150 °C		
Accuracy		< ± 0.1 °C		
Long Term Stability		< ± 100 μK/24h		
Short Term Stability		< ± 10 μK (p-p)		
		< ± 2°C/h (between 20 - 150 °C)		
Scanning Rate		< ± 2°C/h (l	oetween 20 - 150 °C)	
Step-wise Change of	Temperature		oetween 20 - 150 °C) 5 °C - 2°C/h at 150 °C	
Step-wise Change of Heating Cooling	Temperature	1 <i>5</i> °C/h at 15	·	
Step-wise Change of Heating		1 <i>5</i> °C/h at 15	· •°C - 2°C/h at 150 °C	
Step-wise Change of Heating Cooling		1 <i>5</i> °C/h at 15	· •°C - 2°C/h at 150 °C	

TAM TECHNOLOGY

Thermal Activity Monitor

All chemical, physical and biological processes result in either heat production or heat consumption. Microcalorimetry is a versatile technique for studying this thermal activity in terms of heat, heat flow and heat capacity. TAM III offers unmatched sensitivity, long-term stability and high measuring capacity. The modular design, coupled with a wide range of accessories and auxiliary equipment, offers unrivaled flexibility.

Microcalorimetry can be completely nondestructive and non-invasive to the sample. It seldom requires any prior sample treatment nor does it limit analysis to a physical state of the sample. Solids, liquids and gases can all be investigated. Microcalorimetry does not require that a sample has a particular characteristic to enable measurement like FTIR, UV-Vis, NMR etc. Microcalorimetry is a direct and continuous measurement of the process under study. Unlike other analytical techniques that give "snapshots" of data, microcalorimetry gives real-time data continuously as the process proceeds.

Heat Flow

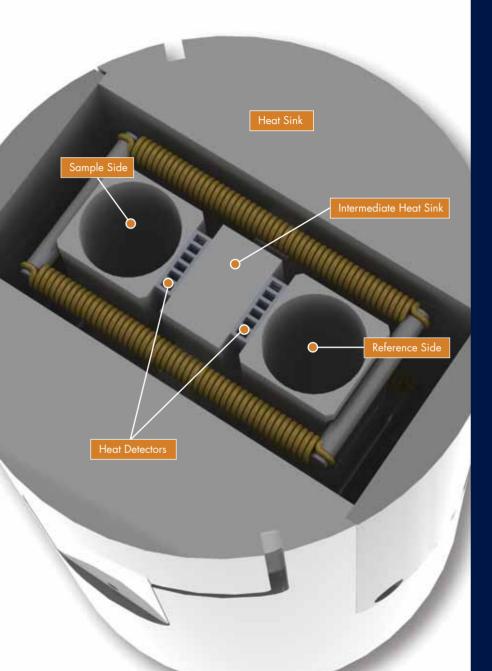
All of the calorimeters available for TAM III are of the heat flow type except for the solution calorimeter, which is a semi-adiabatic calorimeter. A heat flow calorimeter works by channeling the heat produced or consumed by a reaction in the sample through heat flow sensors comprised of thermoelectric modules. When a temperature gradient is imposed across the thermoelectric module, a voltage is created in accordance with the Seebeck effect. This voltage is proportional to the heat flow through the thermoelectric module and hence proportional to the rate of heat production or consumption by the sample. One side of the thermoelectric module is in contact with the sample and the other is kept isothermal by a heat sink which is in contact with the TAM III thermostat. Because of the excellent stability of the TAM III thermostat, even over long periods of time, TAM III maintains outstanding sensitivity. All the heat flow calorimeters are of the twin type, consisting of both a sample and a reference side. The measured property is the difference in heat flow between sample and reference. The twin principle reduces baseline noise by eliminating any small fluctuations of the thermostat.

Power Compensation

TAM III can also operate in a power compensation mode. A constant electrical power is applied to the calibration heaters of the sample and reference sides of the calorimeter. If the temperature of the sample increases or decreases due to a reaction or physical event, the heater on the sample side is compensated to keep the sample and reference at the same temperature. The power compensation mode results in a much faster response time making the calorimeters ideal for high resolution while monitoring rapid processes. (Power compensation mode is not currently available with the minicalorimeter).



Vanocalorimeter



High Performance Temperature Control and Stability

The TAM III thermostat is a liquid-based system, utilizing a mineral oil to quickly dissipate heat and minimize temperature gradients in the system. Efficient circulation of the liquid also permits precise temperature changes to be made. Temperature is controlled by a unique, patented regulation system. The average temperature fluctuation of TAM III is better than \pm 10 μ K over the range 15 to 150 °C. The drift over 24 hours is within $\pm100~\mu$ K. The unmatched stability of the TAM III thermostat contributes to a perfect environment for isothermal and temperature scanning measurements (minicalorimeter). The thermostat is controlled by the software dedicated to TAM III – TAM Assistant TM.

Isothermal

This is the classical mode for microcalorimetric experiments. The liquid thermostat is maintained at constant temperature for the duration of the experiment. Any heat generated or absorbed by the sample due to a chemical or physical process is continuously measured. Isothermal measurements give quantitative and continuous data reflecting the rate of the process under study.

Step Isothermal

Step isothermal experiments can be performed on the same sample at different temperatures. During the isothermal phases, the same signal stability and sensitivity is achieved as with conventional isothermal experiments. During the temperature transition phases, heat flow is recorded to monitor how the sample is affected by the change in temperature. This mode is useful for the study of heat capacity and temperature dependence of chemical reactions.

NANOCALORIMETER

Heat Detector System of the Nanocalorimeter

The Nanocalorimeter contains two heat detectors (thermoelectric modules) on the sample side and two on the reference side. The heat detectors are positioned on the inner side of the ampoule holder in between the ampoule holder and a surrounding heat sink (aluminum).

A foil heater is surrounding two alternate sides and the bottom of the ampoule holder and is used for calibration and power compensation. A known electrical power can be produced and controlled by TAM III.

Active heat sink control is used in order to reduce the time constant of the calorimeter and reduce the time needed to reach thermal equilibrium after a change in temperature.

Nanocalorimeter

The Nanocalorimeter is the most sensitive calorimeter for TAM III, and is typically used in the isothermal mode. It combines high sensitivity with excellent baseline stability which makes it ideal for isothermal titration calorimetry (ITC) in studying molecular interactions. The nanocalorimeter holds all closed ampoules up to 5 ml. For highest sensitivity it is used with the 1 ml titration ampoule and a similar ampoule as reference. This reference ampoule contains an inert substance, (e.g. water or sand) in order to balance the heat capacity of the two sides.





MICROCALORIMETER

Microcalorimeter - 20 ml

The 20 ml Microcalorimeter is a heat flow calorimeter of twin type. It has been designed to hold large samples, (e.g. batteries) and for experiments requiring a large gas phase above the sample. The microcalorimeter can be used with all 20 ml static ampoules and the 20 ml micro reaction system including titration facilities and control of the relative humidity during measurement.

The 20 ml Microcalorimeter is also the only calorimeter that can be used with the micro solution ampoule. This ampoule is designed for dissolution of very small amounts of solids (a few mg) in different solvents and is ideal for dissolution of slowly soluble substances. The heat of dissolution and the kinetics of dissolution can be studied.

MINICALORIMETER

Minicalorimeter – 4 ml

The Minicalorimeter is a 4 ml Microcalorimeter with a special design to reduce the space occupied by the calorimeter inside the thermostat. The reference is positioned below the sample ampoule which allows up to 48 Minicalorimeters to be positioned in the thermostat.

The Minicalorimeter is used in the Multicalorimeter and in the TAM 48 thermostat. It has been designed for increased sample throughput and is recommended for compatibility and stability testing.

Minicalorimeter – 20 ml

The 20 ml minicalorimeter is of similar design as the 4 ml minicalorimeter to allow for larger samples to be measured. The 20 ml minicalorimeter is used in a 20 ml multicalorimeter in the TAM III.





MULTICALORIMETER

Multicalorimeter - 4 ml

The TAM Multicalorimeter contains six Minicalorimeters. It is intended for use with the TAM III thermostat to increase the sample throughput. The TAM III thermostat can hold up to 24 individual calorimeters, as well as other types of calorimeters such as a Nanocalorimeter or a Precision Solution Calorimeter. These combinations offer the highest flexibility by combining high sensitivity with high sample throughput. It can be used for all applications designed for individual Minicalorimeters.

Multicalorimeter - 20 ml

The 20 ml multicalorimeter consists of three individual 20 ml minicalorimeters. The multicalorimeter occupies one position in a TAM III. It is an alternative to the 20 ml microcalorimeter offering higher sample throughput, with three individual calorimeters. This is the only calorimeter to be used with the 20 ml vacuum/pressure ampoule, in which simultaneous measurements of heat flow and pressure in the ampoule could be performed.

MACROCALORIMETER

The Macrocalorimeter is a high sensitivity large volume calorimeter. The cylindrical sample chamber will accommodate 125 mL screw-cap, glass ampoules for liquid or solid samples, as well as a wide variety of battery fixtures for accurate and reproducible static discharge and charge-discharge heat flow measurements. Similar in design to the single sample-above-reference minicalorimeter, the Macrocalorimeter has baseline stability and short term noise performance that will ensure the widest application flexibility with the possibility to match the reference with the sample for optimum performance.





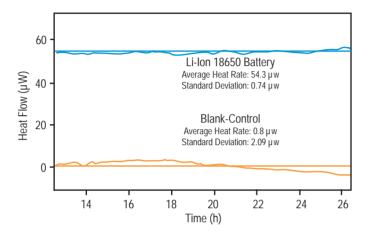
MACROCALORIMETER APPLICATIONS

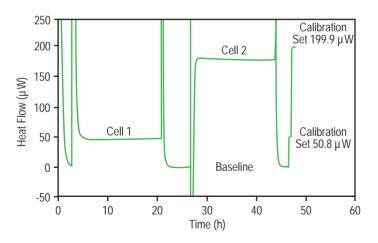
Open-Circuit Discharge Thermogram for Li-ion 18650 Battery

The open-circuit heat rate for a 2600mAh Li-lon 18650 3.7V battery (Blue) and Blank-Control (Black) at 25°C. The fit to calculate mean and standard deviation for the Li-lon 18650 Battery and Blank-Control is overlaid with each raw data set (straight line). The variation in the Blank-Control data is caused by variation in room temperature. Data and graphic analysis performed with the TAM Assistant software package.

Static Self Discharge of Batteries in Macrocalorimeter

Battery quality and stability is easily evaluated in a TAM III Macrocalorimeter by conducting an isothermal calorimetric evaluation of the heat rate during static discharge. Cell 1 shows the 50 μW heat rate generated from an intact good battery during static discharge. Cell 2 shows the 180 μW heat rate from a bad battery during a static discharge. Two calibration pulses of 50 μW and 200 μW were performed immediately following the battery testing. The reproducibility and accuracy of this data is critical when evaluating battery component compatibility, overall battery characteristics and accurate predictions of shelf life stability.





ITC ACCESSORY

Titration Calorimetry

Isothermal Titration Calorimetry requires the highest level of calorimetric sensitivity and stability, efficient titrant delivery, and a user-friendly platform which facilitates easy cleanup and rapid turnaround. Employing unique and proprietary technology, the TAM III is the ideal system for Isothermal Titration Calorimetry as well as for the study of drug effect on living cells, i.e. using microcalorimetry as a bioassay.

The TAM Isothermal Titration Calorimetry (TAM ITC) system consists of a nanocalorimeter, 1 ml removable titration ampoule with stirring facilities, and a precision syringe pump for efficient titrant delivery. Titration calorimeters with 4 and 20ml removable titration ampoules are also available. The TA Instruments nanocalorimeter is the most sensitive calorimeter available for TAM III, and can readily detect microjoule level heat flow. In power compensation mode, the response time of the calorimeter is optimized, and the temperature of the sample is held virtually isothermal.





Titration Ampoule

The removable ampoules offer a level of flexibility unmatched in the industry. Ampoules are easily removed and cleaned outside of the instrument. It allows visual inspection before and after an experiment. The open vessels of TAM ITC ampoules also allows solid suspensions, solid matrixes with attached living cells, macromolecules, etc. to be loaded into the reaction vessel. This allows ligand binding to the solid system to be measured. There is no possibility for this kind of matrix experiment to be run on fixed-cell instruments.

In TAM ITC different sizes of syringes ranging from 100 μ L to 2.5 ml are available. The injections volumes/flow are controlled by a high precision syringe pump. Each pump can support two syringes. In addition, two pumps can be attached to one titration ampoule which is useful for studying enzyme kinetics. This option is not available in competitive designs.



PERFUSION CALORIMETRY

Perfusion Ampoule

The perfusion ampoule is the simplest micro reaction system available, and is typically used with the Nanocalorimeter. A liquid or gas is perfused through the ampoule and out through an exit tube. Gases are perfused using a mass flow controller that is controlled by TAM Assistant™ software. Liquids are perfused using a peristaltic pump. The perfusion ampoule can be used to measure either the heat production rate from a flowing gas/liquid or the effect of the gas/liquid on a sample placed in the perfusion ampoule. The perfusion ampoule is available in 1, 4 and 20 ml versions.

RH Perfusion Ampoule

The RH perfusion ampoule perfuses a gas of defined relative humidity over a sample and out through an exit tube. The relative humidity is controlled using two mass flow controllers, one of which passes gas directly to the sample and the other passes gas through two humidifying chambers prior to the sample. TAM AssistantTM software controls both the relative humidity and the total flow rate over the sample. The software is also used to change the humidity in a linear ramp or stepwise. The RH perfusion ampoule is available in 1, 4 and 20 ml versions. It is possible to use solvents other than water in the humidifying chambers which allows a gas of varying vapor pressure of a solvent to be passed over a sample.





PRECISION SOLUTION CALORIMETRY

Solution Calorimetry refers to the determination of the heat of dissolution when a solid is dissolved in a liquid, or two liquids are mixed.

The TAM Precision Solution Calorimeter is a single-position, semi-adiabatic calorimeter for high precision measurements of the heat generated or consumed when a solid or liquid sample is dissolved or diluted into a solvent. The instrument is designed for highest accuracy and precision and is used in general thermodynamic investigations as well as for quantitative analytical measurements of various solid state phases. It is available with a 25 ml or 100 ml vessel, and is intended for use with the TAM III thermostat up to 80 °C.

TAM AMPOULES

TAM Ampoules are used to contain the sample in the calorimeter during measurement. The ampoules are of two basic types; Closed and Open. In the Closed ampoules, no manipulation to the sample is done during the measurement. In the Open ampoules, also referred to as the Micro Reaction System, the sample can be manipulated after insertion into the calorimeter.

Disposable Crimp Seal Ampoules

Disposable ampoules are the most convenient to use since they can be thrown away after use and no cleaning is required. The crimp seal ampoule is perfect for experiments at lower temperature ranges. They are available in 3, 4 and 20 ml sizes.

Stainless Steel Ampoules

Available in regular stainless steel or hastelloy, these ampoules are used for samples that either react with glass, are to be investigated at high temperatures, or where it is suspected that a gas will be evolved during the experiment which increases the pressure in the ampoule. Ampoule lids are screw top and are available in 4 and 20 ml sizes.

Vacuum/Pressure Ampoules

These steel ampoules in stainless are designed to hold vacuum or pressure up to 10 bar. The pressure in the ampoule can be monitored at the same time heat flow is measured. This is ideal for the study of samples generating gaseous products during a reaction. The ampoule is available in 4 and 20 ml sizes.

Heat Seal Ampoules

The heat seal ampoules are sealed by melting the glass at the top of the ampoule. These are recommended when rubber caps would be affected by gases or liquids involved in a reaction. The reactants are completely surrounded by glass. They are available for a maximum sample volume of 5 ml.

SolCal Ampoules

These ampoules are used with the Precision Solution Calorimeter and are also referred to as crushing ampoules. They are made from glass with a volume of 1.1 ml. The most common SolCal ampoule is the crushing ampoule with stopper. This is preferably used for solid sample dissolution into water. The ampoule is sealed with a rubber stopper and wax. Heat seal crushing ampoules can be used if reactivity is an issue.



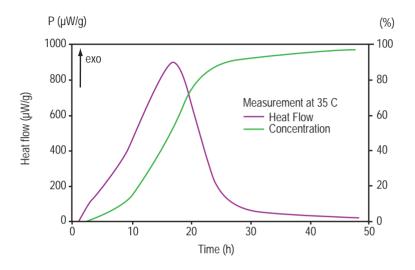
PHARMACEUTICALS APPLICATIONS

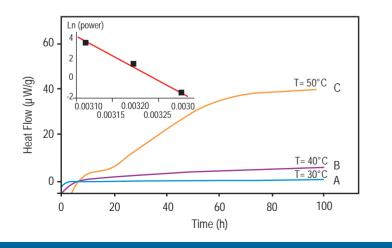
Polymorph Screening

An estimated 80% of pharmaceutical compounds exhibit polymorphism. Pharmaceutical scientists must be diligent to screen for potential polymorphic transformations which may affect the bioavailability of the compound. The selection of the appropriate crystalline form requires a thorough and systematic approach to polymorphism screening. While a lack of chemical change can pose a problem for many analytical probes, TAM can be used to successfully monitor this type of process continually over time at or near typical storage temperature. This figure illustrates the isothermal transformation of alfa-tripalmitin to beta-tripalmitin, at 35 °C over 50 hours. The yellow line shows calculated results from powder X-ray diffraction, and the orange line shows heat flow. TAM data clearly mimics the calculated data from X-ray diffraction and can do so continuously throughout the course of the reaction.

Pharmaceutical Compatibility

TAM III is an ideal screening tool for pharmaceutical compatibility trials. Like stability screening, compatibility screening can be performed at ambient temperatures and humidities without the need to dissolve or physically alter the sample prior to analysis. An experiment typically takes only a few hours as opposed to conventional HPLC which can take many weeks or months. The data shows the response of an amine-lactose interaction at different temperatures with 20% water added. The amine and lactose are very incompatible together. Only a small response was seen at 30 °C (A) with an increasing signal as the temperature of measurement is increased to 40 °C (B) and 50 °C (C). The inset Arrhenius plot confirms that all three temperature points fall on a straight line which is a strong indication that the same process is happening in all three experiments.



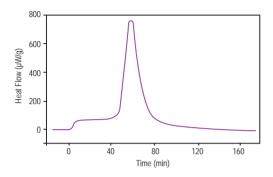


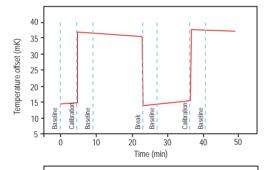
Amorphicity & Crystallinity

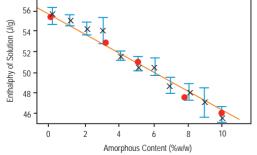
Micronization and processing can alter the surface properties of materials. Small amounts of amorphous material are often formed which may change the characteristics of the powder in a way that affects both processing and bioavailability. TAM III is an excellent tool for investigating low levels of amorphicity in a solid. The sample is loaded into an ampoule and then exposed to a solvent (usually water) vapor. The solvent lowers the glass transition temperature of the amorphous material enough to induce recrystallization, which is monitored as an exothermic response in the microcalorimeter as shown in the figure below. By integrating the curve, the amount of amorphous material in the sample can be quantified to levels below 1%.

Amorphicity & Crystallinity

Solution calorimetry is an alternative to heat flow micro-calorimetry in the assessment of amorphicity. This method works by dissolving the solid material in a solvent and measuring the temperature rise or fall in the solvent as a result of dissolution. Since amorphous and crystalline materials will have a different heat of solution, Solution calorimetry can be used to quantify the amount of amorphous material in a mixture of the two. The first figure shows the solution calorimetry data for a 5% amorphous fraction of lactose. The y-axis shows temperature offset from the bath temperature which was 25 °C. The central "break" section shows the temperature decrease as a result of dissolving the sample in water. The other two steep transitions on either side are calibrations. The second figure contains a plot of amorphous content versus enthalpy of solution, and demonstrates the ability of the solution calorimeter to effectively measure amorphicity.







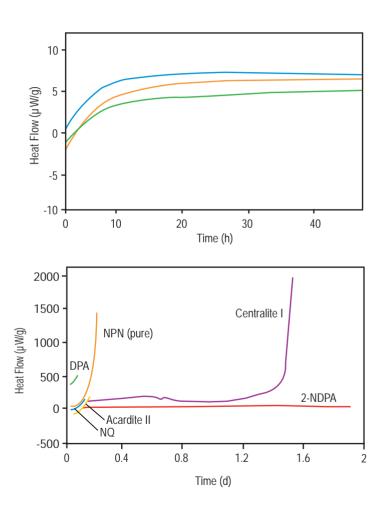
MATERIAL SCIENCE APPLICATIONS

Stability Testing-Detergent

Sodium percarbonate is manufactured in vast quantities around the world and is a major ingredient in washing powders and detergents. Unfortunately, sodium percarbonate is thermally unstable and undergoes continuous degradation. The figure contains the results of stability tests on three separate samples using the TAM III at 40 °C. This data demonstrates the relative stability of the samples based on the magnitude of the exothermic heat flow.

Stability Testing-Energetics

Some energetic materials need to be stabilized to improve the thermal stability. The degradation of these type of materials is associated with an exothermic heat flow which can be detected by TAM III. In the presence of an effective stabilizer degradation is prevented and the heat flow is low. This figure contains a comparison of a variety of stabilizers with an energetic plasticizer. The time to the onset of a pronounced acceleration is termed the induction time and is a measure on how effective the stabilizers are to prevent degradation. This data suggests the 2-NDPA stabilizer is most effective.

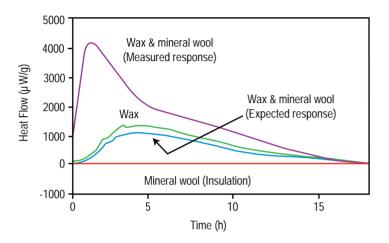


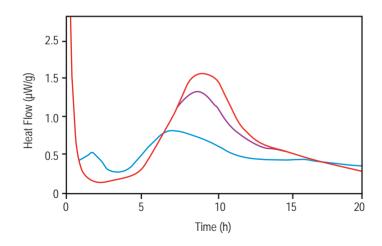
Compatibility

Compatibility testing is a kind of stability testing with reference to the constituents of a material. Microcalorimetry has proven to be particularly useful for compatibility testing; in some cases data is obtained after only a few hours. One example is the non-compatibility between wax and wool. The difference between the measured response and the expected response indicates incompatibility.

Setting Time of Cement

The TAM Air calorimeter has been shown to be excellent for diagnosis of problems related to setting time and premature stiffening of cement. The blue curve in the figure below represents an industrial cement produced with too little soluble calcium sulfate. This cement suffers from early stiffening because of the aluminate reactions at 1–1.5 hours hydration. It also suffers from low early strength, because the aluminate hydrates formed retard the strength-giving silicate hydration indicated by the unusually small silicate peak at 5-10 hours. When 0.5% (yellow curve) and 1.0% (orange curve) of calcium sulfate hemi-hydrate was added to the cement the undesired early peak disappeared, and the strength-giving silicate peak regained its normal shape. The results indicate that premature stiffening is caused by a lack of soluble calcium sulfate.





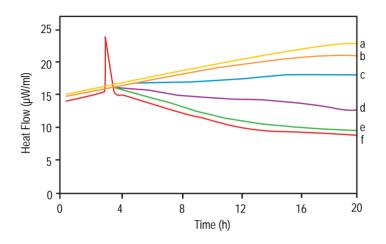
LIFE SCIENCE APPLICATIONS

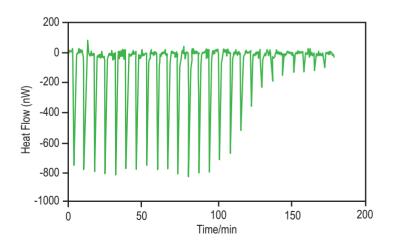
Drug Efficacy

Microcalorimetry has proven to be a sensitive and fast bioassay in cancer research to detect disorders of cellular metabolism. The figure demonstrates a direct and dose-related effect on the heat flow after injecting a variety of concentrations of the anti-cancer drug methotrexate to cultured T-lymphoma tumour cells. Dose-response curves could be calculated for different cell lines from the thermograms. The final drug concentrations were (a) 0, (b) 0.2, (c) 0.5, (d) 1.0, (e) 2.0, (f) 4.0 μ M (ref 6).

Isothermal Titration Calorimetry

Isothermal Titration Calorimetry (ITC) can be used to study molecular reaction and binding reactions in the pharmaceutical and life sciences fields. The data in this figure contains an example of how the thermodynamic properties of the binding of Insulin Growth Factor I (IGF-I) to its receptor (IGF-I-rec) can be elucidated. The microcalorimetric titration binding measurements were performed at 25 °C in saline HEPES and saline sodium phosphate buffer at pH 7.4. From this data, it is concluded that the biological response of the IGF-I-rec is due not only to the binding itself, but also to conformational changes incurred upon binding.



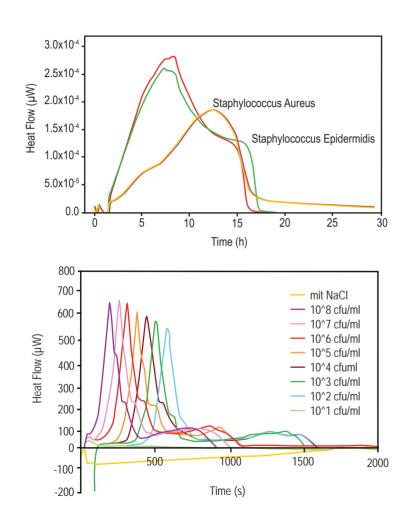


Investigation of Microbial Activity

Calorimetry has been called the "universal detector," because virtually every process involves the exchange of heat. This is particularly true for the respiration of living organisms, including bacteria. The sensitivity and versatility of the TAM system allows for the direct measurement of microbial activity in real time. As shown in the figure, the heat flow profiles of bacteria are very specific, and can easily be used to identify a particular species (staphylococcus aureus vs. staphylococcus epidermidis). In addition, the TAM analysis provides rapid detection of growth (within hours), rapid identification, and provides an excellent platform for the analysis of anti-microbial treatments. Trampuz A et al. JCM 2006; 44: 628

Microorganism Detection

Identification of microorganisms in patient blood and donated platelet concentrates is essential in clinical practice to improve patient care and safety. Currently, commercial blood culture systems (detecting microbial CO₂ production by color change) are used for microbial detection in blood and platelet concentrates. However, these techniques generally require large samples and long evaluation times. Calorimetric detection of microbial growth may be more sensitive, simple and rapid than blood culture. This data demonstrates how microcalorimetry can be used to detect metabolic activity in a platelet solution spiked with E.Coli bacteria in a variety of concentrations, over a relatively short timescale. Applying this method to all donated platelet concentrations could reduce transfusion-related infections and extend storage time.





MULTI CELL DIFFERENTIAL SCANNING CALORIMETRY

The MCDSC is a versatile, multipurpose microcalorimeter that provides continuous temperature-scanning, step-scanning or isothermal measurements on three samples and one reference simultaneously. The one milliliter sample capacity ampoules and microwatt sensitivity ensures maximum flexibility for identifying phase transitions in chemicals and biologicals, metabolic activity in cellular organisms and thermal events occurring in complex reactions of liquids and/or solids during admixing. The superior baseline stability and short equilibration time permits faster measurements and more rapid temperature changes than is possible with other isothermal calorimeters. The availability of reusable, probe-accessible and high pressure removable ampoules allows the study of materials with experimental designs that are not compatible with other microcalorimeters.

MC DSC **SPECIFICATIONS**



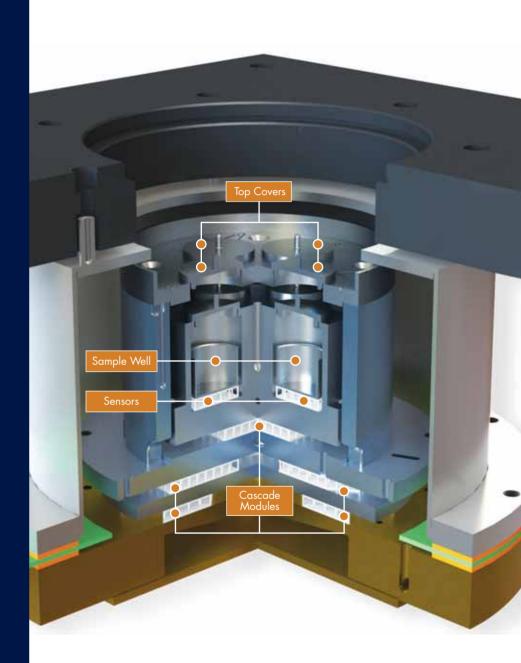
Sample Cells	<u>3</u> 1			
Reference Cells				
Temperature Range	-40 to 150 °C			
Detection Limit	0.2 μW			
Cell Volume	1 ml			
Sample Volume	up to 1 ml			
Short Term Noise Level	0.2 μW			
Baseline Repeatability	2 μW			
Scan Rate	0 (isothermal) to 2 °C/minute			
Response Time	90 second time constant or 9 minutes for 99% response			
Heat Measurement Method	Heat flux			

MC DSC TECHNOLOGY

No other DSC on the market offers more flexibility for studying thermal stability of almost any sample as a function of temperature or time. The calorimeter exploits ultra-sensitive Peltier technology for both temperature control and as sensors to detect heat effects. Peltier cascades allow precise temperature control for isothermal operation and reproducible scan rates up to 2 $^{\circ}\text{C}$ per minute. The Peltier sensors give true microwatt detection independent of scan rate. Equipped with four removable cells, this is truly a workhorse instrument.

The MC DSC runs one reference and three samples simultaneously in removable, Hastelloy ampoules sealed with O-rings to prevent loss of volatiles. With an extra set of ampoules there is no downtime for cleaning and separate ampoule sets are convenient for different user groups. The wide-mouth ampoule design allows easy cleaning and accommodates large pieces of solids and viscous liquids as well as suspensions and solutions. The O-ring seal provides a reliable, truly hermetic seal for pressures up to 15 atmospheres, sufficient to retain the vapor pressure of liquid water up to 200 °C. Hastelloy ampoules are resistant to corrosion by aggressive solvents including concentrated bases, H_2SO_4 , HCl and HNO3 and are inert to biological materials such as proteins and lipids.

The top covers are designed for easy access to the calorimeter cells which allows operation with high pressure, batch reaction, and probe accessible ampoules for a wide range of non-traditional applications.



MC DSC **AMPOULES**

Standard Ampoules	Hastelloy®, 1.0 ml volume, 11mm diameter, 5mm depth			
High Pressure Ampoules	Hastelloy®, 0.5ml volume, Kalrez® O-rings, max pressure 6000psi			
Probe Accessible Ampoules	Standard Ampoule with special connections for pressure measurements, and connecting to spectrophotometers, mass specs, and gas chromatographs.			
Batch Reaction Ampoules	Special Hastelloy® cells with two separate, sealed compartments for studying the reaction (and heat of reaction) between solids, liquids, and vapors.			



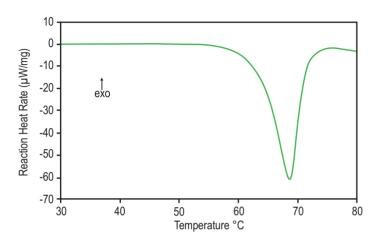
MC DSC Applications

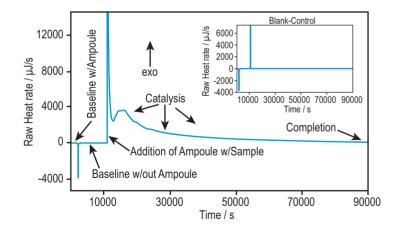
Drug-Excipient Compatibility

Because of the large sample capacity and multiple cell design, the MC DSC can be used to rapidly determine the temperature dependence of rates of drug-excipient reactions. This figure shows the results from a continuous temperature scan of 155mg of a 1:1 mixture of aspirin and magnesium stearate. The rate of the endothermic reaction indicating incompatibility becomes measurable at about 50 °C. Because of the wide-mouth (11mm) and depth (5mm) of the ampoules, whole tablets and capsules can be studied. The continuous temperature scan method is also useful for rapidly establishing useful temperatures for isothermal calorimetric measurements of stability of pure drugs and mixtures.

Biofuel Production

Determining reaction rates is a necessary prerequisite for understanding the mechanism of a chemical process. The MCDSC is a precision isothermal analysis tool, capable of analyzing both short- and long- term reaction kinetics. In the presence of a 3% w/v sodium hydroxide (NaOH) in ethanol solution, soybean oil undergoes transethylesterification to form a biofuel (biodiesel). Following the initial baseline periods (with- and without- ampoule), a sealed 1 mL ampoule containing the premixed reaction components was positioned into one of the three channels of the MCDSC equilibrated to 25 °C. The initial deflection at ~12000 s is due to the insertion of the ampoule into the MCDSC. The maximum peak for the catalysis in the reaction rate is shown at ~16000 s. Following the reaction maximum, the decreasing rate of the reaction is shown until completion. The inset shows the control experiment, whereby ethanol without NaOH was mixed with soybean oil and measured simultaneously in a second ampoule.



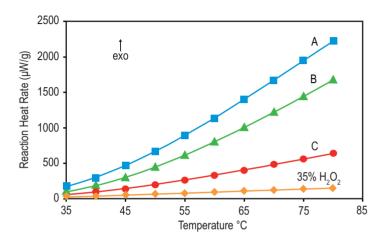


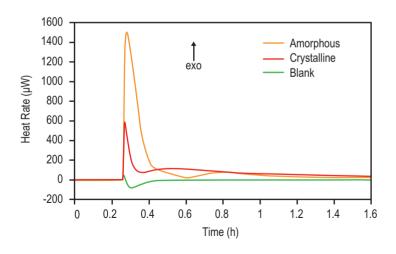
Shelf-Life Prediction by Step-Scanning

In the step-scan method, the calorimeter temperature is rapidly scanned to a programmed temperature and then held isothermal for sufficient time (30-45 min) to allow for equilibration to accurately measure the steady-state heat rate, then stepped to the next temperature for another measurement of the isothermal heat rate. Step-scan measurements are particularly useful for accurate determination of activation energies and temperatures at which reaction mechanisms change. The figure shows data collected by the step-scan method on 100 mg aliquots of three different brands of peroxide bleaches used for whitening teeth. 35% $\rm H_2O_2$ is shown for comparison. The higher the decomposition rate, the faster the product whitens teeth, but the shorter the working time. The method is applicable to reactions in a wide range of materials; for example, cleaning agents, drug-excipient mixtures, adhesives, cell cultures, and small organisms.

Polymorph & Amorphous Crystallization

The batch reaction ampoules make it particularly useful for measurement of the heat and kinetics of reactions between volatile liquids and solids. The figure shows the immediate crystallization of 30 mg of a partially amorphous lactose sample on exposure to 100% humidity. Water sorption by 30 mg of crystalline lactose is shown for comparison. The blank in dry N2 shows the exothermic heat of opening followed by the endothermic evaporation of water. The thermodynamics and kinetics of reactions such as oxidation, decomposition, hydrolysis, and curing can be determined by similar isothermal measurements in standard ampoules. Evolution of gases and changes in solution composition can be followed by adding pressure tranducers, spectral probes or mass spectrometer inlets to the probe accessible ampoules.





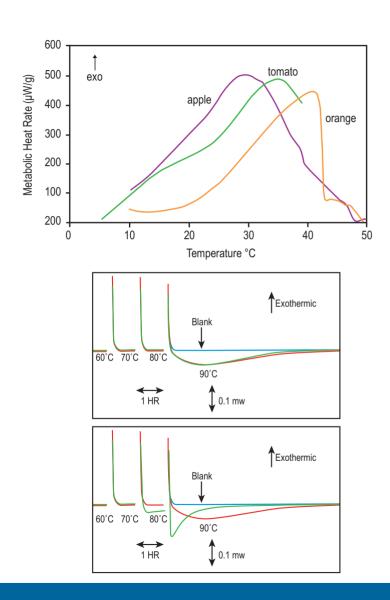
MC DSC Applications

Temperature Dependence of Metabolic Rate

In many organisms, metabolic heat rate is equivalent to the oxygen uptake rate, but heat rate is much easier to measure in cell cultures, tissues, and small organisms. Data on respiration rate as a continuous function of temperature is necessary to optimize conditions for cell cultures, for prediction of the effects of climate change on organisms and ecosystems, for selection of cultivars of crop plants for optimum productivity, and for prediction of the invasive potential of exotics. The data shows a comparison of the metabolic heat rates obtained with continuous temperature scans of 50 mg samples of leaf tissue from apple and orange trees and of a tomato cell culture. Near simultaneous measurements of metabolic heat rates and CO^2 rates as functions of temperature can be done by the step-scan method.

Characterisation of solid state drugs by calorimetry

This is an example of a drug where the enantiotropic form A spontaneously converts to form B. This experiment was conducted in the step isothermal mode of the MCDSC. Samples were loaded at 60 °C and measured for 1.5 hrs before the temperature was changed 10°C. The endothermic effect at 90 °C (Graph 1) is the conversion of form A to B, with an excellent reproducibility between duplicate samples. The shape of the curve indicates an autocatalytic mechanism consistent with nucleation and growth. The conversion went to completion in 10 hours. The second graph (Graph 2) shows the same reaction, but in this case a small amount of B was added as an impurity to A, and with this seeding the conversion started at a lower temperature, 80 °C, and at 90 °C the time to completion of the conversion was significantly reduced.

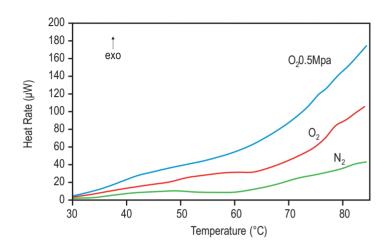


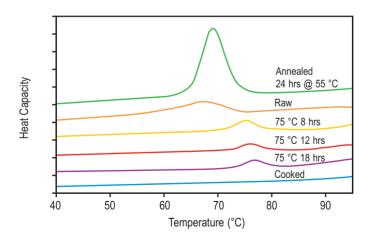
Food Processing

Determination of the temperature dependence of reaction rates is necessary for establishing proper storage, manufacturing, and compounding conditions for foods, drugs, cleaners, and industrial chemicals and products. The rates of reactions in diverse materials can be rapidly determined as a continuous function of temperature with the MC DSC. Continuous scanning is particularly useful for rapidly establishing the optimal temperatures for more definitive, but much slower, determinations of the identity of reactions and of rate laws by isothermal calorimetry and analytical methods. This figure shows data from continuous temperature scans of 0.5 g aliquots of pineapple juice concentrate under various conditions. Hydrolysis of sucrose in the juice produces glucose, a reducing sugar, which is then oxidized by oxygen and also undergoes Maillard reactions with amines in the juice to produce off-flavors and discoloration. Such data can be used to rapidly optimize process conditions.

Phase Transitions in Foods

Phase transitions occurring during cooking, freezing, drying, mixing, and storage of food products are important determinants of texture, taste, and quality. The large, wide-mouth ampoules, wide temperature range, high pressure capability, and multiple sample cells of the MC DSC make it a versatile calorimeter for both temperature scanning and isothermal studies of food products. The data shows temperature scans of whole grain rice after various treatments. The peak is due to gelatinization of crystalline starch remaining after partial cooking.





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