More worldwide customers choose TA Instruments than any competitor as their preferred thermal analysis and calorimeter supplier. We earn this distinction by best meeting customer needs and expectations for high technology products, quality manufacturing, timely deliveries, excellent training, and superior after-sales support.
QUALITY PRODUCTS

TA Instruments Microcalorimeters are manufactured in our state-of-the-art facilities in Linden, Utah or Järfälla, Sweden. Continuing on the superior standards of our predecessor companies Thermometric and CSC, all TA Microcalorimeter systems are precisely constructed by a motivated, highly-skilled workforce. The result is a combination of high performance, unparalleled quality and industry-leading delivery times.
The TA Instruments titration calorimeter offers the highest sensitivity for the investigation of biological samples. The Nano ITC is focused on the measurement of ligand binding and reaction kinetics, with increased sensitivity and lower detection limits to accommodate lower concentrations than previously possible.
The TA Instruments Nano ITC is engineered specifically for binding and kinetics studies on purified dilute biological samples of limited availability. With the Nano ITC, heat effects as small as 120 nanojoules are detectable using one nanomole or less of biopolymer. The Nano ITC uses a solid-state thermostatic heating and cooling system to precisely control temperature, and a unique removable syringe assembly for efficient and accurate delivery of titrant. The true isothermal, power compensation design of the Nano ITC delivers the fastest response time commercially available, 12 seconds.

**Specifications**

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum Detectable Heat</td>
<td>0.1 µJ</td>
</tr>
<tr>
<td>Maximum Detectable Heat</td>
<td>10,000 µJ</td>
</tr>
<tr>
<td>Low Noise Level</td>
<td>0.004 µWatt</td>
</tr>
<tr>
<td>Baseline Stability</td>
<td>0.04 µWatt/hr</td>
</tr>
<tr>
<td>Temperature Stability</td>
<td>0.005°C at 25°C</td>
</tr>
<tr>
<td>Operating Temperature</td>
<td>2 to 80°C</td>
</tr>
<tr>
<td>Cell Type</td>
<td>Fixed Conical</td>
</tr>
<tr>
<td>Sample Size</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Response Time</td>
<td>12 Seconds</td>
</tr>
</tbody>
</table>
Life Science professionals know that understanding protein interactions is critical for the design of efficient biomedical and pharmaceutical treatments. Calorimetry is rapidly becoming the method of choice for characterizing macromolecular stabilities and interactions. Calorimetric analyses are based on accurately measuring the rate of heat absorbed or evolved when the biomolecule of interest interacts with another macromolecule or ligand (binding studies) or with substrate (kinetic studies). The TA Instruments Nano ITC is a powerful tool to accurately and efficiently perform these important measurements.

The Nano ITC is 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 high sensitivity cells of the Nano ITC are made from 99.999% gold or hastelloy C to allow for the widest range of reagent chemistry. Their conical shape makes cleaning easy and facilitates efficient stirring of the solution.

The adiabatic shield of the Nano ITC is enclosed in a vacuum-tight chamber making the instrument less susceptible to environmental changes, and providing temperature stability of ±0.005°C.

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.
APPLICATIONS

Characterization of Enzyme Kinetics

The power of ITC derives from the universality of the technique: 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, non-destructive, compatible with both physiological and synthetic substrates, and are as sensitive as spectrophotometric techniques but do not require a spectroscopic label or chemical tag. Importantly, ITC analyses of enzyme kinetics are also straightforward. All enzymatic reactions generate heat, so all enzymatic reactions can in principle be directly studied by calorimetry. Figure 1 shows the hydrolysis of a single 10 µL injection of trypsin into a solution of BSA in the absence (orange) and presence (green) of bezamidine, 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 $K_i$ and $K_m$ of the reaction under both conditions to be calculated, as well as the inhibition constant.

Measuring Protein Interactions

When two proteins interact and bind, conformational changes in the proteins, and movement 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. Figure 2 contains the titration data of recombinant trypsin into soybean trypsin inhibitor using a Nano ITC. Twenty 5 µL aliquots of enzyme were injected 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: Integrations of the heat over the time course of the experiment, the µJ in each peak are plotted against the mole ratio of the titrant to inhibitor.

Characterizing Binding Interactions by ITC

All binding events are accompanied by the evolution or absorption of heat (a change in enthalpy, $\Delta H$). A full thermodynamic characterization of the binding reactions provides fundamental information about the molecular interactions driving the process, as well as the stoichiometry of binding and the binding constant. Figure 3a shows a typical incremental titration (20, 5 µL injections) of an inhibitor, 2'-CMP, titrated into RNase A; the stoichiometry of the reaction is 1:1, $K_a = 1 \times 10^6 \text{ M}^{-1}$ and $\Delta H = -65 \text{ kJ mol}^{-1}$. Figure 3b shows the same experiment, using continuous (cITC) rather than incremental titration. In this case, 2'-CMP was slowly and continuously titrated in the RNase sample over a period of 20 minutes, the approximately 1000 data points obtained provided essentially identical thermodynamic and binding parameters as the incremental titration, but in a fraction of the time. Importantly, the Nano ITC performs either incremental or cITC without any hardware or software modifications.

Characterizing Membrane Proteins and Peptides by ITC

The binding of peptides and proteins to membranes can be quickly and easily characterized by ITC, allowing determination of the binding constant ($K_a$) and reaction enthalpy. Figure 4 illustrates a simple example, the binding of cyclosporine A to dipalmitoyl phosphatidylcholine (DPPC) vesicles. Cyclosporine A is a hydrophobic 11 residue cyclic peptide used clinically as an immunosuppressive agent. Since vesicles hold proteins as potential drug carriers, the binding of hydrophobic drugs such as cyclosporine A to vesicles has relevance for clinical applications. The ITC data show that, on average, six DPPC molecules interact with each bound cyclosporine A, that the $K_a$ of binding is approximately 390 M$^{-1}$, and that the enthalpy of binding is -61 kJ/mol DPPC.
We pride ourselves in the technical competence and professionalism of our sales force, whose only business is thermal analysis, rheology and microcalorimetry. TA Instruments is recognized worldwide for its prompt, courteous and knowledgeable service staff. Their specialized knowledge and experience are major reasons why current customers increasingly endorse our company and products to their worldwide colleagues.
The TA Instruments scanning calorimeter offers the highest sensitivity and unmatched flexibility for the investigation of biological samples. The Nano DSC is specifically designed to determine the thermal stability and heat capacity of proteins and other macromolecules in dilute solution, with versatility to allow the screening of ligands and pressure perturbation measurements.
The TA Instruments Nano DSC is specifically designed to characterize the stability of a dilute sample as a function of temperature. It is a fully automated instrument, typically requiring less than one hour per sample and only nanomoles of biological material. The Nano DSC obtains data with less sample than competitive designs because of low noise (±1 nanowatt) and precise baseline repeatability. Solid-state thermoelectric elements are used to precisely control temperature, and a high pressure piston driven by a computer-controlled precision linear actuator maintains constant or controlled variable pressure in the cell. The available capillary design of the measuring cell minimizes protein aggregation and precipitation, resulting in higher quality thermal data.

**Specifications**

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Term Noise Level</td>
<td>0.015 µWatts</td>
</tr>
<tr>
<td>Baseline Repeatability</td>
<td>0.028 µWatts</td>
</tr>
<tr>
<td>Operating Temperature</td>
<td>-10 to 130°C or 160°C</td>
</tr>
<tr>
<td>Pressure Perturbation (PPC)</td>
<td>Built-in up to 6 atmospheres</td>
</tr>
<tr>
<td>Scan Rate</td>
<td>0.001 to 2°C/min</td>
</tr>
<tr>
<td>Response Time</td>
<td>5 seconds</td>
</tr>
<tr>
<td>Cell Volume</td>
<td>0.33 mL</td>
</tr>
<tr>
<td>Cell Material</td>
<td>Platinum or Gold</td>
</tr>
<tr>
<td>Cell Geometry</td>
<td>Fixed, Continuous Capillary or Cylindrical</td>
</tr>
<tr>
<td>Heat Measurement Type</td>
<td>Power Compensation</td>
</tr>
</tbody>
</table>
Differential Scanning Calorimetry

Nano DSC Technology

DSC measures the amount of heat absorbed or released by a sample as it is heated or cooled. Classical DSC instruments are designed to have broad applications, but often lack the sensitivity required for the study of biological samples. 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 micropoise levels of heat, the sensitivity of the Nano-DSC is critical for successful investigation of the reaction.

The foundation of any calorimeter is the heat flow transducer. The Nano DSC uses an innovative dual-capillary design, operating in power compensation mode. This capillary design often delays the onset of protein aggregation until after the unfolding is complete. The result is a DSC measurement of unparalleled sensitivity, accuracy and precision. In fast data, data are routinely obtained on the Nano DSC that cannot be obtained using competitive designs. In addition, the capillary cells are quickly and thoroughly cleaned because the entire internal surface of the cell can be readily flushed with cleaning solutions.

The Nano DSC employs solid-state thermoelectric elements to accurately and precisely control the temperature of the sample. Equal sensitivity is maintained in both upward and downward temperature scans, as the same elements are used for heating and cooling.

Pressure control is maintained in the Nano DSC by a built-in high-pressure piston driven by a computer-controlled linear actuator. Constant pressure is applied during the DSC experiment to obtain constant pressure heat capacity (Cp) data and to prevent bubble formation or boiling. Pressure is varied according to user-selectable functions during pressure perturbation experiments to obtain compressibility and thermal expansivity data.

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) and capillary configuration (which delays the onset of irreversible protein aggregation and precipitation), 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 the data below. Hen egg white lysozyme (in pH 4.0 glycine buffer) was prepared at various concentrations. Even 2 µg of lysozyme in the capillary cell is sufficient to provide quality data yielding accurate values of all four thermodynamic parameters!
APPLICATIONS

INVESTIGATION OF PROTEIN-LIGAND BINDING

Like ITC, DSC is a valuable approach for studying binding interactions between a biological macromolecule and a ligand such as another biopolymer or a drug. Unlike ITC, DSC allows the thermodynamics of the driving forces for binding to be correlated, at least to a degree, 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 and not included in the protein). Figure 1 shows DSC scans of α-lactalbumin 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% DMG, verifying that organic solvents are compatible with the DSC technique.

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 (Figure 2). 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. Figure 3 shows DSC scans of Ca
tabulated bovine α-lactalbumin at various protein:Zn ratios scanned at 1°C/min. The midpoint of the thermal unfolding of the protein decreases from 65°C in the absence of Zn to 35°C at a protein:Zn ratio of 1:70; the protein precipitates at higher Zn concentrations. The enthalpy of unfolding is also decreased substantially by high Zn concentrations.

PERMEABILIZATION OF MEMBRANES BY DRUGS

Some medications are specifically designed to be sparingly soluble and be slowly released in vivo over a period of weeks or months. These drugs may have low efficacy unless they can efficiently penetrate the membranes of target cells. In order to gauge the potential efficacy of drugs prior to in vivo experiments, the glassy DSC can be used to monitor changes in the phase composition of model system liposomes as the drug permeates the outer surface, the bilayer, and finally traverses the membrane and enters the liposome. DMPG liposomes at pH 7 were combined with a suspension of a very slowly-dissolving hydrophilic drug. The temperature was scanned from 25 to 60°C at 1°C/min, then reannealed slowly. The gradual change in phase composition with time (Figure 4) shows that penetration of the drug changes the physical properties of the liposome.
TA Instruments is the recognized leader for supplying innovative technology, and investing twice the industry average in research and development. Our Q Series™ Thermal Analysis and Calorimeter products are the industry standard. Many innovations are available only from TA Instruments.
TAM represents an ultra-sensitive heat flow measurement which is complementary to TA Instruments differential scanning calorimeters. Based on the pioneering Thermometric technology, TAM offers maximum sensitivity, flexibility, and productivity. It can be used with the most sensitive microcalorimeters and a wide variety of accessories to control the experimental conditions.
TAM III

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 macrocalorimeter holding six independent microcalorimeters, 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

<table>
<thead>
<tr>
<th>Thermal Media</th>
<th>Calorimeter Positions</th>
<th>Temperature Range</th>
<th>Accuracy</th>
<th>Long Term Stability</th>
<th>Short Term Stability</th>
<th>Scanning Rate</th>
<th>Step-wise Change of Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 - 4</td>
<td>15 - 150°C (90°C)</td>
<td>&lt; ± 0.1 °C</td>
<td>&lt; ± 100 µK/24h</td>
<td>&lt; ± 10 µK (p-p)</td>
<td>&lt; ± 2°C/h (between 20 - 150°C)</td>
<td></td>
</tr>
</tbody>
</table>

Calorimeter Specifications

<table>
<thead>
<tr>
<th>Calorimeter</th>
<th>Short Term Noise</th>
<th>Baseline Drift</th>
<th>Accuracy</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanocalorimeter</td>
<td>&lt; ± 10 nW</td>
<td>&lt; 20 nW/24 h</td>
<td>&lt; 1%</td>
<td>± 100 nW</td>
</tr>
<tr>
<td>Microcalorimeter / Multi</td>
<td>&lt; ± 100 nW</td>
<td>&lt; 200 nW/24 h</td>
<td>&lt; 0.5%</td>
<td>± 200 nW</td>
</tr>
<tr>
<td>Microcalorimeter (3ml)</td>
<td>&lt; ± 200 nW</td>
<td>&lt; 200 nW/24 h</td>
<td>&lt; 1%</td>
<td>± 100 nW</td>
</tr>
</tbody>
</table>

Detectability

| Solution Calorimeter | 1-4 µJ | < ± 0.1% | Q > 100 J/0.22% 0.1-9.9 µJ/24 h | Q < 10 J/0.02% 0.01-9.9 µJ/24 h |
The TAM Air is an eight channel microcalorimeter from TA Instruments designed for sensitive and stable heat flow measurements. It is the ideal tool for research and development of new formulations as well as quality control during cement and concrete manufacture and preparation. TAM Air is also ideal for other large-scale calorimetric experiments requiring sensitivity in the milliwatt range. The operating temperature range is 5-90°C. All calorimetric channels are of twin type, consisting of a sample and a reference vessel, each with a 30 ml volume. The thermostat employs a circulating air and an advanced regulating system to keep the temperature very stable (within ±0.02°C). The high accuracy and stability of the thermostat makes the calorimeter well suited for heat flow measurements over extended periods of time, e.g. weeks.

**THERMOSTAT SPECIFICATIONS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating Temperature Range</td>
<td>5 - 90°C</td>
</tr>
<tr>
<td>Thermostat Type</td>
<td>Air</td>
</tr>
<tr>
<td>Thermostat Accuracy</td>
<td>± 0.02°C</td>
</tr>
<tr>
<td>Limit of Detection</td>
<td>4 µW</td>
</tr>
<tr>
<td>Precision</td>
<td>± 20 µW</td>
</tr>
<tr>
<td>Baseline over 24 hours</td>
<td>± 40 µW</td>
</tr>
<tr>
<td>Drift</td>
<td>± 10 µW</td>
</tr>
<tr>
<td>Deviation</td>
<td>± 23 µW</td>
</tr>
<tr>
<td>Error</td>
<td>± 2.5 µW</td>
</tr>
</tbody>
</table>

**Calorimeter Specifications**

<table>
<thead>
<tr>
<th>Calorimeter Specifications</th>
<th>Short Term Noise</th>
<th>Baseline Drift</th>
<th>Accuracy</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM AIR 8 Channel Calorimeter</td>
<td>&lt; ± 2.5 µW</td>
<td>&lt; 40 µW/48 h</td>
<td>&lt; 5%</td>
<td>± 20 µW</td>
</tr>
</tbody>
</table>

---

The TAM 48 is a special version of the TAM III thermostat with unique features designed to maximize sample throughput without sacrificing data quality. Up to 48 independent minicalorimeters can be operated simultaneously with the TAM 48, to perform different experiments in each of the 48 calorimeters or to perform repeat experiments simultaneously. The design accommodates minicalorimeters in batches of 12. The TAM 48 employs patented thermostat technology to precisely control the liquid bath temperature to within 0.0001°C, and can be operated in isothermal, step-thermal or temperature-scanning mode.

**THERMOSTAT SPECIFICATIONS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermostat Type</td>
<td>Air</td>
</tr>
<tr>
<td>Thermostat Accuracy</td>
<td>± 0.02°C</td>
</tr>
<tr>
<td>Limit of Detection</td>
<td>4 µW</td>
</tr>
<tr>
<td>Precision</td>
<td>± 20 µW</td>
</tr>
<tr>
<td>Baseline over 24 hours</td>
<td>± 40 µW</td>
</tr>
<tr>
<td>Drift</td>
<td>± 10 µW</td>
</tr>
<tr>
<td>Deviation</td>
<td>± 23 µW</td>
</tr>
<tr>
<td>Error</td>
<td>± 2.5 µW</td>
</tr>
</tbody>
</table>

**CALORIMETER SPECIFICATIONS**

<table>
<thead>
<tr>
<th>Calorimeter Specifications</th>
<th>Short Term Noise</th>
<th>Baseline Drift</th>
<th>Accuracy</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minicalorimeter / Microcalorimeter</td>
<td>&lt; ± 100 nW</td>
<td>&lt; 200 nW/24 h</td>
<td>&lt; 5%</td>
<td>± 200 nW</td>
</tr>
</tbody>
</table>
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 gasses can all be investigated. Microcalorimetry does not require that a sample have 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.

**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).

**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 of 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.

**High Performance Temperature Control and Stability**

The TAM III thermostat is a liquid based system, utilizing water or 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 ±0.001°C over the range 15 to 150°C. The drift over 24 hours is within ±0.0001°C. 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™.
**Temperature Modes**

**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.

**Slow Scanning**
TAM III can be used to perform experiments under extremely slow temperature scanning conditions. This enables the calorimetric response to be monitored over a linear temperature gradient. The slow scan rate (maximum 2 °C/h) allows large samples, up to about 4 ml, to be analyzed while they are in thermal, physical and chemical equilibria. The excellent stability combined with large sample size makes TAM III far more sensitive than conventional DSC instruments. Furthermore, resolution is far greater for phase transitions. A scanning experiment can also be combined with an isothermal experiment, using as simple or as complicated a temperature profile as required.

**TAM Versatility**

The TAM III is the most versatile microcalorimetric system available. The TAM III thermostat can be configured with a variety of independent calorimeters which allow multiple experiments and maximize laboratory productivity.

- **Isothermal Calorimetry**
- **High Through-Put, Up to 48 Channels**
- **Solution Calorimeter**
- **Perfusion Calorimetry**
- **ITC Accessory**
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 calibrating 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.

The Nanocalorimeter is the most sensitive calorimeter for the 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 4 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. Thus, under normal conditions the heat flow associated with the reference ampoule will be negligible.

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 20ml 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.
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.

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.
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. The TA Instruments nanocalorimeter is the most sensitive calorimeter available for the TAM III, and can readily detect micromole 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. This is a major benefit over competitive designs, in which the sample temperature is allowed to drift during titration.

In TAM-ITC different sizes of syringes ranging from 100 µL 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.

The removable ampoules offer a level of flexibility unmatched in the industry. TAM-ITC ampoules are easily removed and cleaned outside of the instrument. Competitive designs feature fixed cells which require thorough cleaning between experiments, and preclude visual inspection. The open vessels of TAM-ITC ampoules also allows solid suspensions, solid matrices with attached living cells, micromolecules, 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 competitive fixed-cell instruments.

The TAM Isothermal Titration Calorimetry (TAM-ITC) system excels in the most demanding ITC applications such as ligand binding.
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 Assistant™ 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 Amapoules

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

Disposable Crimp Seal Ampoules
Disposable amapoules are the most convenient to use since they can be thrown away after use and no cleaning is required. The crimp seal amapoule 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 amapoules 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 amapoule. Ampoule lids are screw top and are available in 4 and 20 ml sizes.

20ml Ampoules
All 20 ml closed amapoules currently available from TA Instruments can be used in the TAM Air Calorimeter. 20 ml amapoules are available in glass, stainless steel or HDPE (plastic).

The Admix Ampoule is available to initiate reactions inside the calorimeter and is configured with or without a motor. For suspensions such as mixtures of cement/water manual stirring is recommended. For liquid systems, a motor may be used for stirring.

Heat Seal Ampoules
The heat seal amapoules are sealed by melting the glass at the top of the amapoule. 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.

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

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

Polymer 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 methods, TAM can be used to successfully monitor this type of process continually over time at or near typical storage temperature.

Figure 1 illustrates the isothermal transformation of alpha-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 minimizes the calculated data from X-ray diffraction and can do so continuously throughout the course of the reaction.

Figure 1

Pharmaceutical Compatibility

TAM III is an ideal screening tool for pharmaceutical compatibility studies. Large scale 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 HPC which can take many weeks or months. The data in Figure 2 show 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.

Figure 2

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 ampule 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 Figure 3. By integrating the curve, the amount of amorphous material in the sample can be quantified to levels below 1%.

Figure 3

Solution calorimetry is an alternative to heat flow microcalorimetry 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 graph in Figure 4 shows the solution calorimetry data for a 5% amorphous fraction of lactose. The y-axis shows temperature offset from the batch temperature which was 35°C. The central “break” section shows the temperature decrease as a result of dissolving the sample in water. The other two step transitions on either side are calibrations. Figure 4B contains a plot of amorphous content versus enthalpy of solution, and demonstrates the ability of the solution calorimeter to effectively measure amorphicity.

Figure 4
Applications - Material Science

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. Figure 5 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. Figure 6 contains a comparison of a variety of stabilizers with an energetic plasticizer. The time in the onset of a pronounced acceleration is termed the induction time and is a measure of 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 shown in Figure 7. 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 of Figure 8 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. Its 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 hemihydrate 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.

Figure 5

Figure 6

Figure 7

Figure 8
APPLICATIONS - LIFE SCIENCE

DRUG EFFICACY

Microcalorimetry has proven to be a sensitive and fast bioassay in cancer research to detect disorders of cellular metabolism. Figure 9 demonstrates a direct and dose-related effect on the heat flow after injecting a variety of concentrations of the anti-cancer drug methotrexate in cultured T lymphoma tumor 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 µM (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 Figure 10 contains an example of how the thermodynamic properties of the binding of insulin Growth Factor I (IGF-I) to its receptor (IGF-I-R) 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-R 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 Figure 11, 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 system provides rapid detection of growth (within hours), rapid identification, and provides an excellent platform for the analysis of anti-microbial treatments.

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. The data in Figure 12 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 concentrates could reduce transfusion-related infections and extend storage time.
Customers prefer TA Instruments because of our reputation for after-sales support. Our worldwide technical support staff is the largest and most experienced in the industry. They are accessible daily by telephone, email, or via our website. Multiple training opportunities are available including on-site training, seminars in our application labs around the world and convenient web-based courses.
**LOCAL OFFICES**

- New Castle, DE USA ................................. +1-302-427-4000
- Linden, Utah USA ................................. +1-801-763-1500
- Järfalla, Sweden ................................. +46-6-664-72-200
- Crawley, United Kingdom ................................. +44-1293-558300
- Shanghai, China ................................. +86-21-54263060
- Taipei, Taiwan ................................. +88-6-29838880
- Tokyo, Japan ................................. +81-3-5473-8416
- Bangalore, India ................................. +91-80-28338962
- Paris, France ................................. +33-1-20-48-04-60
- Eschborn, Germany ................................. +49-6196-410-0
- Brussels, Belgium ................................. +32-2-704-0060
- Elten-Leur, Netherlands ................................. +31-76-526-7270
- Milano, Italy ................................. +39-02-27421-265
- Barcelona, Spain ................................. +34-93-600-8500
- Melbourne, Australia ................................. +61-3-8853-0813
- Mexico City, Mexico ................................. +5255-5524-7636

**REFERENCES**

**FIGURE REFERENCES – ISOTHERMAL CALORIMETRY**

10. Thermostim Application Note 22826.