Nano Isothermal Titration Calorimeter (Nano ITC)

Getting Started Guide
Notice

The material contained in this manual, and in the online help for the software used to support this instrument, is believed adequate for the intended use of the instrument. If the instrument or procedures are used for purposes other than those specified herein, confirmation of their suitability must be obtained from TA Instruments. Otherwise, TA Instruments does not guarantee any results and assumes no obligation or liability. TA Instruments also reserves the right to revise this document and to make changes without notice.

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Important:
TA Instruments Manual Supplement

Please click on the links below to access important information supplemental to this Getting Started Guide:

- TA Instruments Trademarks
- TA Instruments Patents
- Other Trademarks
- TA Instruments End-User License Agreement
- TA Instruments Offices
Notes, Cautions, and Warnings

This manual uses NOTES, CAUTIONS, and WARNINGS to emphasize important and critical instructions. In the body of the manual these may be found in the shaded box on the outside of the page.

NOTE: A NOTE highlights important information about equipment or procedures.

CAUTION: A CAUTION emphasizes a procedure that may damage equipment or cause loss of data if not followed correctly.

WARNING: A WARNING indicates a procedure that may be hazardous to the operator or to the environment if not followed correctly.
Regulatory Compliance

Safety Standards

EMC Directive

This instrument has been tested to meet the European Electromagnetic Compatibility Directive (EMC Directive, 2004/108/EC). The Declaration of Conformity for your instrument lists the specific standards to which the unit was tested. The instrument was designed specifically as a test and measuring device. Compliance to the EMC directive is through IEC 61326-1 Electrical equipment for measurement, control and laboratory use - EMC requirements (1998).

As noted in the IEC 61326-1, the instrument can have varying configurations. Emissions may, in non-typical applications, exceed the levels required by the standard. It is not practical to test all configurations, as the manufacturer has no control over the user application of the instrument.

Immunity Testing

The instrument was tested to the requirements for laboratory locations.

Emission Testing

The instrument fulfills the limit requirements for Class A equipment but does not fulfill the limit requirements for Class B equipment. The instrument was not designated to be used in domestic establishments.

Low Voltage Directive (Safety)

In order to comply with the European Low Voltage Directive (2006/95/EC), this equipment has been designed to meet IEC 1010-1 (EN 61010-1) standards. To comply with requirements in the USA, this instrument has been tested to the requirements of UL61010a-1.
**Safety**

**Instrument Symbols**

The following labels are displayed on the Nano ITC instrument for your protection:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>This symbol indicates that you must unplug the instrument <em>before</em> doing any maintenance or repair work; AC mains power voltage is present in this system. High voltages are present in this instrument. If you are not trained in electrical procedures, do not remove the cabinet covers unless specifically instructed to do so in the manual. Maintenance and repair of internal parts must be performed only by TA Instruments or other qualified service personnel.</td>
</tr>
</tbody>
</table>

Please heed the warning labels and take the necessary precautions when dealing with those parts of the instrument. The *Nano ITC Getting Started Guide* contains cautions and warnings that must be followed for your own safety.

**Electrical Safety**

You must unplug the instrument before doing any maintenance or repair work; voltages as high as 125/250 Vac are present in this system.

**Lifting the Instrument**

The Nano ITC is not a portable instrument. In order to avoid injury, particularly to the back, please follow this advice:

**WARNING:**

*Use two people and a cart or piece of moving equipment rated to hold heavy loads to move the instrument. The instrument is too heavy for one person to handle safely.*
# Table of Contents

Getting Started Guide .......................................................... 1  

**Nano Isothermal Titration. Calorimeter (Nano ITC). Important:** ........ 3  

TA Instruments Manual Supplement ........................................ 3  

Notes, Cautions, and Warnings ................................................ 4  

Regulatory Compliance ......................................................... 5  
- Safety Standards .............................................................. 5  
- EMC Directive ................................................................. 5  
- Immunity Testing ............................................................. 5  
- Emission Testing .............................................................. 5  
- Low Voltage Directive (Safety) ............................................. 5  

Safety ................................................................. 6  
- Instrument Symbols ........................................................ 6  
- Electrical Safety ............................................................. 6  
- Lifting the Instrument ....................................................... 6  

Overview ................................................................. 9  

**Chapter 1: Introducing the Nano ITC** .................................... 9  

The Nano Isothermal Titration Calorimeter (ITC) .......................... 10  
- Applications ................................................................. 10  
- Batch/Incremental Titration ................................................. 10  
- Titration/Data Analysis .................................................... 11  
- Calculation of Equilibrium Constants ................................. 12  

System Components ......................................................... 12  
- Measuring Unit ............................................................. 13  
- Reaction Vessel ............................................................ 13  
- Syringe/Stirrer .............................................................. 14  
- Burette Assembly .......................................................... 14  

Options and Accessories ..................................................... 15  

Instrument Specifications .................................................... 15  

Unpacking/Repacking the Nano ITC ........................................ 17  

Installing the Instrument ...................................................... 17  

Inspecting the System ......................................................... 17  

**Chapter 2: Installing the Nano ITC** .................................... 17  

Choosing a Location .......................................................... 18  
- In ............................................................................. 18  
- Near ......................................................................... 18  
- Away from .................................................................. 18  
- Power Requirements ...................................................... 19
Overview

There are three ways in which a calorimeter may be designed. Heat measurements may be based on the following:

- a temperature rise measured in a system of known heat capacity, \( \Delta T \)
- the measured change in power (typically resistance heating) required to maintain a system at a constant temperature (power compensation); and
- a direct measure of the heat flowing between the system and large heat sink maintained at a constant temperature (heat flow).

Each method (\( \Delta T \), power compensation, and heat flow) has its advantages and disadvantages. The TA Instruments Nano Isothermal Titration Calorimeter (ITC) uses a differential power compensation design for maximum sensitivity and responsiveness.
The Nano Isothermal Titration Calorimeter (ITC)

The Nano ITC (shown in the figure to the right) consists of the measuring unit (calorimeter block and two non-removable reaction vessels), the burette assembly, which includes the stirring system, and a cleaning accessory. With the exception of the power on/off switch located on the back of the calorimeter unit, all functions of the Nano ITC are controlled remotely by the computer through the USB connection.

Applications

Batch/Incremental Titration

In incremental or batch titration, one of the reactants is placed in a syringe or burette external to the reaction vessel. If individual, repeated injections are made, incremental titration takes place (as seen in the example below); if only one injection is made it is batch injection calorimetry. This generic designation includes direct injection enthalpimetry (DIE).
The baseline data, \textit{i.e.} heat flow in the regions before and after each titrant pulse, shows the power required to maintain a zero temperature difference between the sample and reference cells.

The baseline in this region is a function of heating by stirring. The baseline is used to calculate the area or the heat from each pulse in the reaction vessel during the titration or batch reaction. The thermogram constructed from the integrated peak areas is then used for data analysis.

**Titration/Data Analysis**

A single titration calorimetric experiment yields heat data as a function of the ratio of the concentrations of the reactants. Titration data, in the form of heat change versus volume of titrant added, can be examined for both analytical (thermometric titrimetry) and thermodynamic (titration calorimetry) information.

Other corrections must be made to the heat data to account for heat effects associated with titrant dilution and any temperature difference between titrant and titrate solutions. These corrections are most easily accomplished by performing a blank titration experiment and subtracting the blank heat data from the experimental thermogram.

In the case of quantitative reaction of added titrant, the analysis of the thermogram is quite simple. All peak areas will be the same (with the possible exception of the last peak) and $\Delta H$ calculated from the incremental heat and the number of moles of titrant added per increment. The titrant concentration is calculated from the total heat divided by the $\Delta H$ for the reaction.
**Calculation of Equilibrium Constants**

The equilibrium constant for a given reaction may be simultaneously determined with the enthalpy change, if the magnitudes of K and $\Delta H$ for the overall reaction taking place in the calorimeter are within certain limits. The family of curves presented in the figure (a) below shows that increased overall curvature of the thermogram is generated with decreasing values of the association constant, $K_{eq}$.

Figure A above shows the effects of varying magnitudes of the enthalpy change ($\Delta H$). Figure B shows the effects of varying the equilibrium constant K.

**System Components**

The following items make up a Nano ITC system:

- Nano Isothermal Titration Calorimeter
- Power cord
- Getting Started Guide (this manual)
- Data Collection and Analysis Software
- 1 each 2.5 mL filling syringe with 16-gauge, 8-inch long needle
- 1 each 100 µL syringe
- 1 each 250 µL syringe
- 1 each burette drive
- USB cable
The components that make up the Nano ITC system are briefly described in the following sections.

**Measuring Unit**

The *measuring unit* includes the calorimeter block and two non-removable *reaction vessels* (*sample and reference cells*). *Access tubes* extend downward from inside the burette mounting cavity on the top of the calorimeter. The access tubes serve as conduits for the filling syringe, titrant delivery, and reference needle. They also provide for titrant equilibration and as a thermal barrier to the environment outside the calorimeter.

The Nano ITC utilizes a differential power compensation design. Semiconducting thermoelectric devices (TED) are used for temperature control and to detect temperature differences between the sample and reference cells. A computer-controlled PID loop uses a control heater on the sample cell to maintain a zero temperature difference between the sample and reference cells. The power required to maintain this zero difference is used as the calorimeter signal and is monitored as a function of time. If a reaction, that produces heat, occurs in the sample cell, the heat required to maintain the zero difference decreases by the amount of heat supplied by the reaction, resulting in a peak in the thermogram.

A *calibration heater* located on the outside of the sample cell is used to provide precisely controlled heat pulses for electrical calibrations, and to verify instrument performance.

The entire measuring unit is encased within an insulated air-tight canister which has been purged on a vacuum pump and filled with dry nitrogen at the factory. This is to prevent possible condensation and evaporation of moisture around the unit which would create excessive baseline noise. NOTE: Purging of the canister is not a routine maintenance operation; contact TA Instruments before proceeding.

**Reaction Vessel**

The calorimeter uses two 1.0 mL *reaction vessels*. The reaction vessels are made of 24K gold with platinum access tubes. The reference cell is constructed to match as closely as possible the thermal properties of the sample cell. Accordingly, a reference needle is placed inside the reference cell during operation to correspond to the titrant needle in the sample cell.

**CAUTION:** The purge port valve on the back of the Nano ITC should remain in the closed position at all times to maintain the integrity of the nitrogen purge.

**CAUTION:** Extreme care should be taken not to bend the syringe needle because this would impair proper stirring and possibly damage the reaction vessel.
Syringe/Stirrer

Two burette syringes are provided with 100 µL and 250 µL capacities. The only difference in dimension between the two is the inner diameter of the syringe barrel; the needles are identical in order to maintain the thermal and mechanical properties.

The titrant syringe needle also functions as the stirrer and extends down into the reaction vessel from the top when the burette is mounted. The needle is balanced for optimum stirring efficiency. It has two Teflon bushings to help dampen stirring noise and ensure that the needle spins true within the cell access tube (see the figure to the right).

Each syringe needle is equipped with a flattened, twisted paddle at the tip, which does the actual stirring of the solutions in the cell. The stirring paddle spins clear of the sides of the reaction vessel. When stirring is activated, the contents of the reaction vessel are stirred continuously until the end of the experiment or until stirring is turned off.

Stirring is controlled by a stepping motor mounted inside the calorimeter. This type of motor is used because of its very constant and adjustable speed. The motor drives the rotating shaft of the burette, which holds the titrant syringe.

Burette Assembly

The burette accurately delivers the titrant to the reaction vessel at specified volumes and intervals. The assembly also functions as the stirring mechanism for the reactants in the cell when the titrant syringe is installed. The rotating shaft on the lower portion of the burette assembly holds the titration syringe in place, and has two external o-rings which provide the friction necessary for the stir motor to rotate the shaft during operation.

See online help for details on using the burette.
**Options and Accessories**

The following items are available to complement your Nano ITC instrument:

- Computer system
- Printer
- Degassing station

**Instrument Specifications**

The tables found below contain the technical specifications for the Nano ITC.

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>Depth 38 cm (15 in.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Width 37 cm (14 in.)</td>
</tr>
<tr>
<td></td>
<td>Height 32 cm (13 in.)</td>
</tr>
<tr>
<td>Weight</td>
<td>20 kg (43 lbs)</td>
</tr>
<tr>
<td>Power</td>
<td>95 – 250 VAC, 3 amps. 50-60 Hz</td>
</tr>
<tr>
<td>Operating Environmental Conditions</td>
<td>Temperature: 15 to 30 °C</td>
</tr>
<tr>
<td></td>
<td>Relative Humidity: 5–80 % (non-condensing)</td>
</tr>
<tr>
<td></td>
<td>Installation Category II</td>
</tr>
<tr>
<td></td>
<td>Pollution Degree 2</td>
</tr>
<tr>
<td></td>
<td>Maximum Altitude: 2500 m (8200 ft)</td>
</tr>
<tr>
<td>Emissions Class</td>
<td>Class A</td>
</tr>
<tr>
<td>Temperature Range</td>
<td>0 to 80 °C</td>
</tr>
<tr>
<td>Injection Interval</td>
<td>150 s minimum</td>
</tr>
<tr>
<td>Response Time</td>
<td>15 s</td>
</tr>
<tr>
<td>Cell Volume</td>
<td>1.0 mL (24K Gold)</td>
</tr>
<tr>
<td>Precision Burette</td>
<td>100 or 250 µL</td>
</tr>
<tr>
<td>Volume Increment</td>
<td>1 to 250 µL</td>
</tr>
<tr>
<td>Stirring Rate</td>
<td>0, 150-400 rpm</td>
</tr>
</tbody>
</table>
Chapter 2:
Installing the Nano ITC

Unpacking/Repacking the Nano ITC

The instructions needed to unpack and repack the instrument are found as separate unpacking instructions in the shipping box and in the online documentation associated with the instrument control software. You may wish to retain all of the shipping hardware and boxes from the instrument in the event you wish to repack and ship your instrument.

WARNING:
Have an assistant help you unpack this unit. Do not attempt to do this alone.

Installing the Instrument

Before shipment, the instrument is inspected both electrically and mechanically so that it is ready for operation upon proper installation. Only limited instructions are given in this manual, consult the online documentation for additional information. Installation involves the following procedures:

• Inspecting the system for shipping damage and missing parts
• Connecting the Nano ITC to the TA Instruments controller computer
• Connecting USB cables

It is recommended that you have your Nano ITC installed by a TA Instruments Service Representative, call for an installation appointment when you receive your instrument.

Inspecting the System

When you receive your instrument, look over the instrument and shipping container carefully for signs of shipping damage, and check the parts received against the enclosed shipping list.

• If the instrument is damaged, notify the carrier and TA Instruments immediately.
• If the instrument is intact but parts are missing, contact TA Instruments.
Choosing a Location

It is important to choose a location for the instrument using the following guidelines. The Nano ITC should be:

**In**

- a temperature- and humidity-controlled area. Temperatures should be in range 15 to 30 °C.
- a clean, vibration-free environment, preferably on the ground floor in the building. It should be located away from pumps, motors, or other devices which produce vibrations.
- an area with ample working and ventilation space. At least 18 by 18 inches is needed for the instrument. Additional space is needed for the computer and optional printer.

**On**

- a stable work surface.

**Near**

- a power outlet. See the “Power Requirements” section on the next page.
- your TA Instruments computer.

**Away from**

- dusty environments.
- exposure to direct sunlight.
- direct air drafts (fans, room air ducts).
- poorly ventilated areas.
- noisy or mechanical vibrations.
- high traffic areas, where constant movements from passing personnel could create air currents or mechanical disturbances.
**Power Requirements**

The Nano ITC requires a grounded, single-phase power source. A three-conductor line cord ensures a safety ground. The operating voltage and line frequency were preset at the factory for 95-250 VAC, 50-60 Hz operation.

The Nano ITC and computer system should be plugged into the same surge suppressor. An isolated power line (one that is used only for electrical type instruments with no motors, compressor or heaters) is recommended. Unstable power sources may also require the use of a voltage stabilizer in order to obtain optimum performance from the Nano ITC.

Attach the instrument and all computer accessories to the surge suppressor provided.

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**Setting Up the Nano ITC**

When you have received your TA Instruments Nano ITC follow these basic steps to set it up for use. For detailed information refer to the sections that follow.

1. Unpack and inspect the instrument and all components.
2. Place the Nano ITC on a suitable bench with at least 18 by 18 inches of bench space for the instrument, along with space for the computer system and optional printer.
3. Plug the power cord into the back of the Nano ITC.
4. Setup the computer (and printer, if applicable) next to the Nano ITC.
5. Connect the USB cable between the Nano ITC and the computer system. See the next section.
6. Turn on the computer system.
7. Turn on the power switch located on the back of the Nano ITC.
8. Start the Nano ITC data collection program (ITCRun).
Connecting the Cables and Cords

Follow these steps to make the connections needed for the Nano ITC.

1. Make sure that the Nano ITC power switch is turned off.

2. Attach the power cord provided to the back of the Nano ITC. See the figure to the right. Do not plug the instrument into a power source at this time.

3. Plug the loose USB cable into the back of the instrument.

4. Plug the power cord of the Nano ITC into a surge suppressor power strip. Do not turn equipment power on at this time.

5. Connect the free end of the Nano ITC USB cable into a free USB port on the external computer system.

6. Turn the computer power on and allow the system to boot up.

NOTE: To choose a location for your instrument, see page 18 for guidelines.
Starting the Nano ITC

Once you have completely set up the calorimeter and computer system, you can start the instrument as follows:

1. Turn on the surge suppressor power switch and the computer system and monitor.

2. Turn on the power switch to the calorimeter, which is located on the back panel. The front LED will light up green when in the “on” position.

3. Start the ITCRun software on the computer. See the online help for details on the program. You are now ready to begin preparing to run an experiment.

Shutting Down the Instrument

You can leave the instrument and its associated components on when the Nano ITC will be inactive for several days.

If the Nano ITC will be inactive for more than 5 days, we recommend that you empty the cells and turn all equipment off.
Chapter 3: 
Use, Maintenance, & Diagnostics

Overview

A typical Nano ITC experiment involves the following:

- Preparing and degassing the solutions
- Preparing the sample and reference cells
- Mounting the burette
- Running the baseline
- Cleaning the calorimeter
- Performing an analysis

Each step is briefly described here. Additional information is provided in the online help supplied with the software program. It is assumed that you are familiar with standard laboratory procedures and techniques. It is critical that the Nano ITC cells be cleaned immediately at the end of each experiment. The calorimeter can be left idle for up to two days with water-filled cells at 25 °C, when not performing experiments. When the instrument is expected to remain idle longer than two days, empty the cells.
Preparing and Degassing the Solutions

Large sample molecules are often stored in buffer which will affect the final pH of the prepared solution. Dialysis is the process used to equalize the solution characteristics while retaining the large molecules. This step improves the experimental results by minimizing the enthalpies of dilution and neutralization. Do not dialyze small sample molecules; sample loss will result. All solutions used in the experiment (rinse buffer, sample titrant, and sample titrand) must be degassed prior to use. When preparing solutions for use with the Nano ITC, any solutions containing buffers and macromolecules will need to be dialyzed before use. This is a standard process that is used to equalize the pH and concentration between the sample titrant and the sample titrand.

Follow these steps to prepare the solutions:

1. Prepare a large amount of buffer as appropriate for the experiment (add the appropriate type & amount of salts). This buffer will be used as material for formulation of the titrant and titrand solutions, and in their dialysis.

2. Formulate solutions of any large-molecule sample compounds at this time using the buffer solution. (Small-molecule sample compounds will be made up in a following step).

3. Dialyze the solution(s) inside the remaining buffer. Place the sample in a dialysis bag and suspend it inside the buffer solution. Gently stir the buffer for several hours to aid in the equalization of pH and the concentrations of electrolytes. Temperature-sensitive samples may need to remain chilled during this process.

4. Small molecule samples are prepared at this time using the dialyzed buffer. Do not dialyze this solution.

5. Retain 50-300 mL of the dialyzed buffer for use later in cell rinsing and for optional blank experiments.
**Degassing Solutions**

Typically, if a solution is heated, gas bubbles will form as the solubility of dissolved gases (e.g., O2 and N2) is decreased with increasing temperature. If gas bubble formation occurs in the ITC cells during the run, the resulting data will be rather noisy since abrupt changes will result from the bubble-driven liquid displacement effects.

All solvents must be degassed prior to being placed in the ITC to minimize the possibility of gas bubble formation during the run. Pull a vacuum of 0.3-0.5 atm on the solutions for a period of 10-15 min to degas a sample.

An accessory degassing system is available from TA Instruments.
Preparing the Sample and Reference Cells

The sample cell (also referred to as the reaction vessel) contains the titrant that will be used for your experiment. The reference cell typically contains pure solvent (water in the case of aqueous sample solutions). For more information on the vessels, see Chapter 1.

Follow the instructions below to prepare the sample cells:

1. Use the filling syringe (shown in the figure to the right), to flush the sample cell several times with the same buffer solution in which the sample is prepared.

2. After flushing, remove all of the buffer and slowly load the sample into the sample cell (middle access tube) inside the Nano ITC to allow air bubbles to evacuate through the top of the cell.

   a. When liquid is just visible at the opening of the access tube, continue to gently inject, while slowly withdrawing it from the cell. This will maintain the fill level and prevent new bubbles from being introduced into the cell (see the figure to the right). When using aqueous solutions, the reference cell (side access tube) should be filled with water.

   b. Make sure that the reference needle is inserted into the reference cell access tube after filling (see the figure on the next page). The liquid should be just visible at the bottom of the conical overflow reservoir when the cell is filled.
3. Load the 100 µL syringe with the titrant taking care to remove any bubbles from the barrel of the syringe, but leaving a small, 5 to 10 µL, air gap between the plunger tip and the liquid in the barrel. Leaving an air gap is a critical step, needed to prevent signal distortion. Fill the syringe to a slight excess, 2 or 3 mm beyond the highest gradation of the barrel.

**Caution:** The signal can be distorted if there is no air gap to serve as a cushion at the plunger tip.

**NOTE:** Removal of the burette from the calorimeter is the reverse of this process.
**Loading the Burette**

When the syringe is filled with titrant, follow the instructions in this section to load the burette.

The top portion of the burette handle displays a graduated scale with an indicator showing the relative position of the syringe plunger during an experiment. The indicator must be in the fully raised position before installing a loaded syringe into the burette.

4. Insert the plunger and barrel carefully into the rotating shaft of the burette assembly. (See the upper right figure).

5. Hold the rotating shaft on the burette securely in one hand. Use the knurled knob at the base of the syringe barrel to finger-tighten the syringe into place with the other hand. A small droplet will appear at the tip of the syringe. A small droplet will appear at the tip of the syringe.

6. Wipe any excess titrant from the needle along the exterior of the barrel and the tip.
Installing the Burette Assembly

The bell-shaped upper portion of the burette assembly has three notched key slots for correct orientation in the instrument. Install the burette as follows:

7. Guide the shaft carefully, needle first, into the top opening of the calorimeter (see the figure to the right). Make sure the key slots line up with the three locking posts located in the mounting ring at the top.

8. Gently push the burette handle downward and rotate it slightly clockwise to secure the burette in place.

When the burette is installed properly, the graduations will be facing directly forward. Circular contact boards at the burette/calorimeter interface provide electrical power to the burette and enable the functional control necessary to perform a titration.

Inserting the Burette into Calorimeter
Starting the Experiment

Once the loaded burette assembly is properly loaded, you can proceed as follows:

9. Turn on the stirrer at 250 to 400 rpm.

10. Allow the system to re-equilibrate until the calorimeter heat reading is stable. See the NOTE in the left-hand column.

11. Set up the parameters for the particular experiment of interest using the ITCRun program under the Setup tab (see the online help for details).

12. Wait until a stable baseline is evident. (See the online help for details regarding baselines.) Then click on Experiment/Start or click the Go icon at the top of the toolbar to start the experiment.

13. Enter a filename when prompted. The program indicator on the Setup tab will be red when the program is active. You will be able to watch the progress of the experiment under the Monitor tab or the Data tab.

14. The titration data is automatically saved to disk at the conclusion of the experiment.

15. Evaluate the data using the NanoAnalyze software package. (See the online help for information.)

Cleaning the Nano ITC

A scrupulously clean sample cell is essential in order to obtain meaningful titration data. Because the reaction vessel is non-removable, the filling syringe may be used to repeatedly flush the cell. When a more rigorous cleaning is needed, the cleaning adapter (see the figure to the right) allows you to easily flush large volumes of fluid through the cell.

The cell should be cleaned immediately following an experiment, then rinsed with buffer to condition it for the next experiment. Fill the cells with pure deionized water between experiments to prevent contamination from drying the cell walls.

The cleaning tool is used as follows:

1. Remove the burette assembly and syringe from the top opening of the Nano ITC, and withdraw the cell contents using the filling syringe.

2. Carefully lower the shaft into the cell opening.
3. Connect the length of 1/16-inch I.D. Manosil silicone rubber tubing provided to the side port of the cleaning tool as seen in the figure below.

4. Place the free end of the tube in a beaker of clean deionized or distilled water.

5. Connect another length of tubing to the top port leading to a vacuum flask which is connected to a vacuum pump.

6. Apply a vacuum to draw the water through the system and flush the cell.

Water is drawn into the side port inlet and down the length of the outer sleeve where it exits near the top of the cell. The water then flows down the walls of the access tube and cell toward the needle opening located near the bottom of the cell. Flow continues upward to the outlet at the top of the tool and out to the vacuum flask.

The figure on the next page shows the flow of water through the apparatus.
CAUTION: Always use proper protective equipment when working with samples and cleaning fluids.

CAUTION: Thoroughly rinse all areas that come into contact with corrosive chemicals.

Sideview of Cleaning Tool in Sample Cell
CAUTION: All rinsing or flushing operations should be done with the cleaning devices described beginning on page 30. Caustic solutions should be loaded and removed with the appropriate syringe or micropipette.

Analyzing the Data

Several software programs are available for use with the Nano ITC. The table below outlines the usage for each one. For more details on these programs, see the online help.

Nano ITC

Nano ITC Programs & Functions

<table>
<thead>
<tr>
<th>Program</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITCRun</td>
<td>This program controls the operation of the Nano ITC and is used for data acquisition. The main components are:</td>
</tr>
<tr>
<td></td>
<td>• Experiment Settings</td>
</tr>
<tr>
<td></td>
<td>• Instrument Control</td>
</tr>
<tr>
<td></td>
<td>• Main Menu Functions</td>
</tr>
<tr>
<td></td>
<td>• Buret Menu</td>
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<tr>
<td></td>
<td>• Feature Tabs</td>
</tr>
<tr>
<td></td>
<td>• Monitor Tabs</td>
</tr>
<tr>
<td></td>
<td>• Data Tab</td>
</tr>
</tbody>
</table>
Maintaining the Nano ITC

Maintaining the Nano ITC consists of purging the instrument, cleaning the filter, lubricating the o-ring, and thoroughly cleaning the cells. This section provides information on these procedures.

Purging the Nano ITC

It is sometimes necessary to use a vacuum pump to remove any moisture or outgassing contaminants that have accumulated in the calorimeter shield canister over time.

Important Note: This procedure has been performed at the factory before shipment, and will only very rarely be required again in the field. It is NOT ADVISABLE.

Follow these steps to purge the instrument:

1. Remove the plastic dust cap from the purge inlet located on the back of the Nano ITC.

2. Attach a vacuum pump to the inlet.

3. Start the pump.

4. Wait until the pump has evacuated the air from the vacuum tubing then open the purge valve.

5. Run the vacuum pump for a minimum of 24 hours, but periods of two days or more are sometimes required.

6. After pumping, close the purge valve on the back of the Nano ITC.

7. Turn off the pump, close the purge valve, and remove the vacuum line.
8. Set the pressure regulator on the nitrogen tank to 3 to 8 psi. Turn on the gas and allow it to flow freely for several seconds to purge the line. Place the gas line loosely on the purge port.

9. Open the purge valve. Allow the nitrogen to fill the outer jacket (approximately five seconds).

10. Close the purge valve, then loosen the nitrogen line to relieve pressure. Try to prevent ambient air from entering the purge port across the end of the fitting. Do not shut off the flow of nitrogen, let the nitrogen flow continue. Do not pull the loosened nitrogen line completely free of the purge port.

11. Quickly (in less than one second) open and close the purge valve once to allow nitrogen to escape out of the instrument, through the purge port.

12. Repeat steps 9 through 11 at least two more times. The purge procedure is now complete.

**Step 3: Soapy Water Flush**

1. Exit from the ITCRun software. (This must be done whenever you pass large volumes of solutions through the NanoITC.)

2. Flush or aspirate 100 mL of dilute aqueous detergent solution (e.g. SDS) through the cells. Make sure to use a detergent that does not leave any residue.

**Step 4: Final Flush**

Flush or aspirate 1 L of deionized water through each cell.
Troubleshooting the Nano ITC

Minimizing Blank Corrections

There will always be blank corrections for experiments. However, minimizing the blank correction can greatly improve experiments. Even when injecting water into water there will be some heat produced due to viscous mixing. The viscous mixing heat is determined by many factors.

There are several steps that can be taken in order to minimize the dilution heats and hence the need for blank titrations. Ligand (titrant) solution should always be made up in the same dialysis buffer used for the protein (titrand). If the ligand is also a protein, it should be dialyzed in the same buffer. Less concentrated solutions also have lower dilution heats and should be used when possible.

Operating at Non-Ambient Temperatures

The Nano ITC is designed to perform over a wide temperature range. When you use the instrument at temperatures other than ambient, it is important to allow adequate time between injections for the titrant to equilibrate to the calorimeter temperature before injection. Thorough degassing of the titrant is especially important when operating above the ambient temperature.

Stirring Speeds

Adequate stirring is required in order to have rapid mixing of the titrant upon injection, but excessive stirring will result in noisy baselines. Generally, a stirring speed of 250 to 400 rpm is appropriate.
Index

A

access tube 26
accessories 15
analyzing data 33

B

baseline 30
blank corrections 36
burette 28, 30
installing 29
loading 28
removal 27

C

cautions 4
cell
  cleaning 30
cells
  flushing 26
choosing a location 18
cleaning 30
  adapter 30
components 12
conical overflow reservoir 26
connections
  electrical 20
contamination 30

D

data
  analyzing 33
degassing 25
degassing system 25
deionized water 30, 35
dialyze 24

E
electrical connections 20
electrical requirements 19
EMC directive 5
environment 18
experiment 23
  starting 30

F
filename 30
filling syringe 26
flushing cells 26

H
heat reading 30

I
inspection 17
installation 17
  electrical 20
instrument
  components 12
  electrical connection 20
  environment 18
  inspecting 17
  inspection 17
  installation 17
  installing 17
  lifting 6
  location 18
  maintenance 34
  power requirements 19
  purging 34
  repacking 17
  shut down 21
shutting down 21
starting 21
symbols 6
unpacking 17

ITCRun 30, 33

L
license agreement 3
ligand 36
location 18
location of instrument 18

M
maintaining instrument 34

N
notes 4

O
options 15

P
patents 3
power requirements 19
preparing solutions 24
purging the instrument 34

R
reaction vessels. See also sample or reference cells 26
regulatory compliance 5
repacking 17

S
safety 6
electrical 6
electrical safety 6
thermal 6
sample cell cleaning 30
shut down 21
shutdown 21
software programs 33
solutions degassing 25 preparing 24
startup 21
stirrer 30
stirring 36
syringe 28, 30
system components 12

T
TA Instruments offices 3
technical specifications 15
temperatures non-ambient 36
titrant 28
trademarks 3
troubleshooting 36
tubing connecting 31

U
unpacking 17

V
viscous mixing 36
voltage 19

W
warnings 4