Nano Isothermal Titration Calorimeter

(Nano ITC)

Getting Started Guide for Models 601000, 601001, 601002

Revision F Issued June 2014

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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Introduction

Important: TA Instruments Manual Supplement

Please click the TA Manual Supplement link to access the following 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.

MISE EN GARDE: UNE MISE EN GARDE met l'accent sur une procédure susceptible d'endommager l'équipement ou de causer la perte des données si elle n'est pas correctement suivie.

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

Un AVERTISSEMENT indique une procédure qui peut être dangereuse pour l'opérateur ou l'environnement si elle n'est pas correctement suivie.

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.

WARNING: The operator of this instrument is advised that if the equipment is used in a manner not specified in this manual, the protection provided by the equipment may be impaired.

AVERTISSEMENT: L'utilisateur de cet instrument est prévenu qu'en cas d'utilisation contraire aux indications du manuel, la protection offerte par l'équipement peut être altérée.

DANGER: High voltages are present in this instrument. Maintenance and repair of internal parts must be performed only by TA Instruments or other qualified service personnel.

DANGER: Présence de tensions élevées dans cet instrument. La maintenance et la réparation des pièces internes doivent être effectuées uniquement par TA Instruments ou tout autre personnel d'entretien qualifié.

Safety

High voltages are present in this instrument. Maintenance and repair of internal parts must be performed only by TA Instruments or other qualified service personnel.

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 appropriate care when unpacking or moving the instrument. It may be too heavy for some individuals working alone to handle safely.

AVERTISSEMENT: Soyez prudent lors du dépotage ou du déplacement de l'instrument. Il peut être trop lourd à manipuler en tout sécurité pour des personnes travaillant seules.

Table of Contents

Chapter: 1

Introducing the Nano ITC

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, (ΔT)
- 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 (ΔT, 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 ITC is available in three configurations: the Standard Volume model with 1-mL measurement cells made of either 24K gold or Hastelloy® alloy, and the Low Volume model with 190-µL gold cells. Standard Volume ITCs shipped before September 2009 were labeled "Nano ITC^{2G}". The Nano ITC incorporates second generation technology featuring enhanced baseline stability and increased sensitivity.

Instrument Models Covered in this Guide

This guide covers Nano ITC instrument models 601000, 601001, and 601002.

The Nano Isothermal Titration Calorimeter (ITC)

The Nano ITC (shown in the figure below) consists of the measuring unit (calorimeter block and two non-removable reaction vessels), the buret 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.

Figure 1 Nano ITC^{2G}.

Applications

Batch/Incremental Titration

In incremental or continuous titration, one of the reactants is placed in a syringe or buret 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 continuous injection calorimetry.

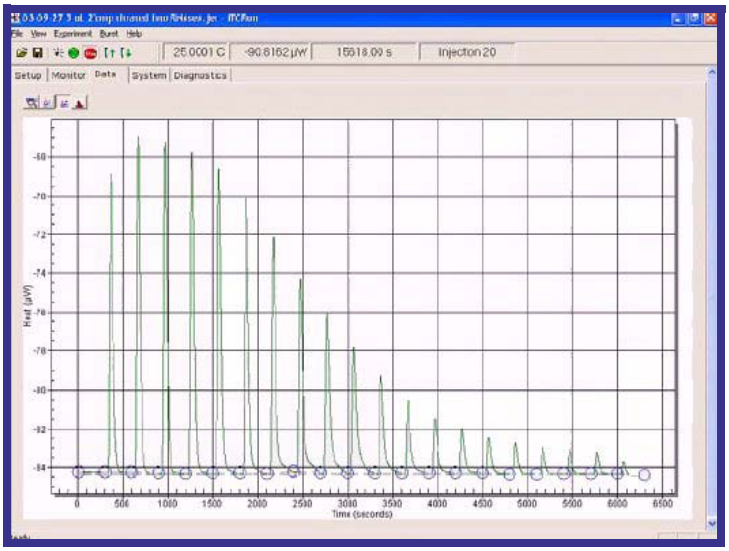


Figure 2 Incremental titrations.

The baseline data, 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 ΔH 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 ΔH 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 ΔH for the overall reaction taking place in the calorimeter are within certain limits. The family of curves presented in the figure below shows that increased overall curvature of the thermogram is generated with decreasing values of the association constant, K_{eq}.

Figure A below shows the effects of varying magnitudes of the enthalpy change ΔH. Figure B shows the effects of varying the equilibrium constant K.

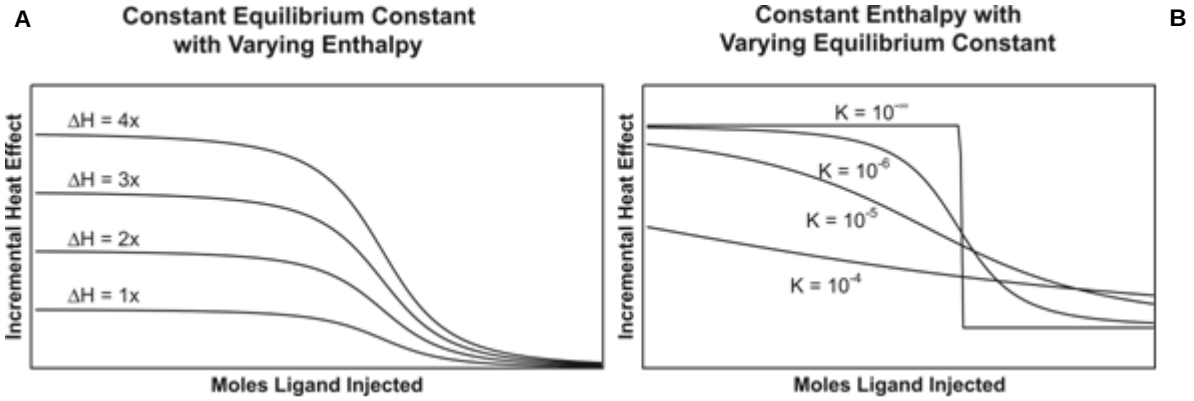


Figure 3 Calculation of equilibrium constants.

System Components

- Nano Isothermal Titration Calorimeter
- Personal computer (optionally available from TA Instruments)
- ITCRun and NanoAnalyze software
- 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 (Standard Volume ITC)
- 1 each 0.5-mL filling syringe with 16-gauge, 8-inch long needle (Low Volume ITC)
- 1 each 100-µL and 250-µL syringes (with Nano ITC Standard Volume)
- 1 each 50-µL syringe (with Nano ITC Low Volume)
- 1 each buret 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 buret 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.

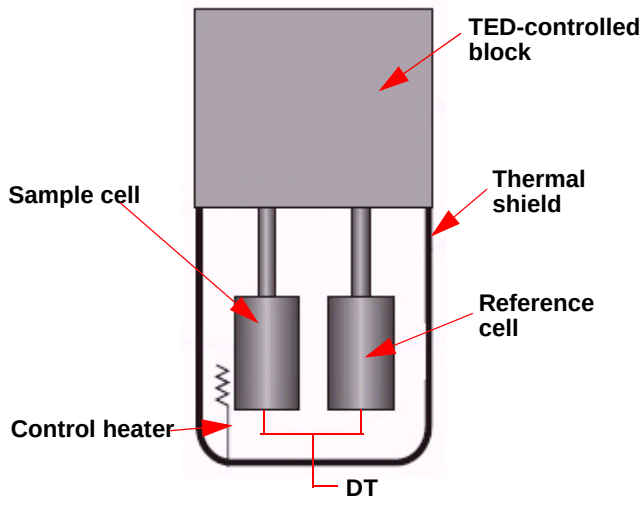


Figure 4 Nano ITC measuring unit.

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 proportional/integral/derivative (PID) control 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.

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.

MISE EN GARDE: La vanne de l'orifice de drainage située à l'arrière du Nano ITC doit toujours rester en position fermée pour maintenir l'intégrité de l'azote drainé.

NOTE: Purging of the canister is not a routine maintenance operation; contact TA Instruments before proceeding.

Reaction Vessel

The calorimeter uses two matched reaction vessels with options of 1-mL gold, 190-µL gold, or 1-mL Hastelloy®. The vessels are accessed through platinum 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.

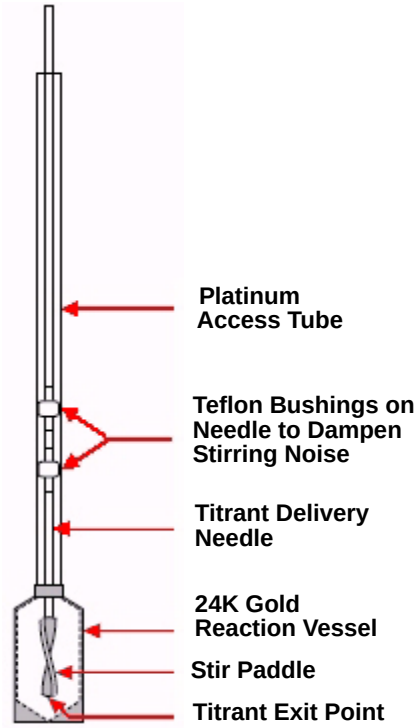


Figure 5 Sample cell assembly.

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

MISE EN GARDE: Soyez extrêmement prudent pour ne pas plier l'aiguille de la seringue, car cela pourrait altérer le mélange approprié et éventuellement endommager le récipient à réaction.

Syringe/Stirrer

Nano ITC Standard Volume systems include two buret syringes of 100 and 250 µL capacities. The only difference in dimension between the two is the inner diameter of the syringe barrel; the needles are otherwise identical in order to maintain the thermal and mechanical properties.

The Nano ITC Low Volume system includes one 50-µL syringe. This syringe uses a shorter needle. To avoid possible damage, do not use the larger syringes with the Low Volume ITC. If you feel a stiff resistance or if the buret handle does not readily slip fully into place in the ITC, do not force it. Remove the buret and check to see if the correct size syringe is installed. You can verify the feel of the normal resistance by installing a buret with no syringe in place.

The titrant syringe needle also functions as the stirrer and extends down into the reaction vessel from the top when the buret 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 below).

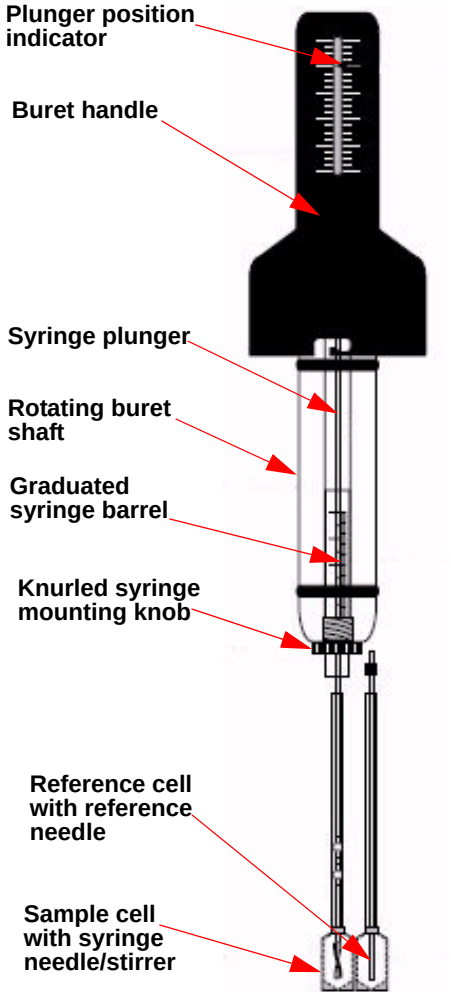


Figure 6 Orientation of buret, syringe, needles, and cells during experiments.

CAUTION: To avoid possible damage, do not use the 100 or 250 µL syringes with the Nano ITC Low Volume instrument. Syringes are shipped with warning labels which may, if desired, be affixed to the ITC by the customer to serve as a reminder.

MISE EN GARDE: Pour éviter d'éventuels dégâts, n'utilisez pas des seringues de 100 ou 250 µL avec le Nano instrument ITC à faible volume. Les seringues sont expédiées avec des étiquettes d'avertissement qui peuvent, si vous le souhaitez, être collées à l'ITC par le client pour servir de rappel.

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 buret, which holds the titrant syringe.

Buret Assembly

The buret 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 buret 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.

Options and Accessories

A degassing station is available to complement your Nano ITC instrument.

Instrument Specifications

The table found below contains the technical specifications for the Nano ITC instrument.

Table 1: Nano ITC Technical Specifications

Item/Area	Specifications
Dimensions	Depth 53 cm (21 in.) Width 35 cm (14 in.) Height 28 cm (11 in.)
Weight	37.5 lbs (17 kg)
Power	100–240 VAC, 3 amps. 50 or 60 Hz
Electrical power cord	The plug of the cord must be rated to carry at least 125% of the product current rating. The cord length must be less than 4.5 meters and must be UL or CSA approved.
Operating environmental conditions	Temperature: 15 to 30°C Relative Humidity: 5 to 80% (non-condensing) Installation Category II Pollution Degree 2 Maximum Altitude: 2500 m (8200 ft)
Emissions class	Class A
Temperature range	2 to 80°C
Injection interval	150 s minimum
Response time	13 s
Effective cell volume	Standard Volume: 1.0 mL (24K gold or Hastelloy) Low Volume: 190 µL
Sample volume range	Standard Volume: 1200 to 1500 µL Low Volume: 300 to 700 µL
Injection syringe capacity	Standard Volume: 100 or 250 µL Low Volume: 50 µL
Volume increment	0.114% of the total syringe capacity (0.06, 0.11, or 0.29 µL respectively for the 50, 100, and 250 µL syringes)
Stirring rate	150 to 400 rpm

Chapter: 2

Installing the Nano ITC

Unpacking/Repacking the Nano ITC

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

AVERTISSEMENT: Faites-vous aider par une personne pour dépoter cet appareil. N'essayez pas de le faire tout seul.

CAUTION: To avoid mistakes, read this entire chapter before you begin installation.

MISE EN GARDE: Pour éviter de commettre des erreurs, lisez tout le chapitre avant de commencer l'installation.

The instructions needed to unpack and repack the instrument are found as separate unpacking instructions in the shipping box. 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.

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 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 (if present) printer.

On

- a stable work surface.

Near

- a power outlet. See the "Power Requirements" section below.
- your 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 100–240 VAC, 50 or 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 power conditioner in order to obtain optimum performance from the Nano ITC.

Attach the instrument and all computer accessories to a single surge suppressor.

NOTE: Use a power strip to run the instrument and computer from a common power source.

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.

3 Use a power distribution strip with a voltage surge suppressor function to provide a single protected power connection for all system components.

Note for IT personnel: Since the data control and collection software depends on accurate timings, it is highly recommended to set the computer BIOS settings for performance rather than power savings. Some computer manufacturers may have different names for this. For example, some Dell computers have a setting called “C-States” that includes the C1E setting, which should be disabled. Others may call it the “Enhanced Halt State”. Other settings that should be disabled, if available, are EIST (Intel SpeedStep) and AMD’s Cool‘n’Quiet. Set the Microsoft Windows® power option to **Performance** instead of **Power Savings**. Disable automatic reboots in the Windows Update utility.

Connecting the Cables and Cords

NOTE: To choose a location for your instrument, see page 18 for guidelines.

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 below. Do not plug the instrument into a power source at this time.

3 Plug the 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.

Figure 7 Rear of Nano ITC.

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 (if present) and the computer system and monitor. Allow the computer to boot up.

2 Install the ITCRun software, following the instructions in the “Installing ITCRun” document found on the TA Instruments software CD.

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

Figure 8 LED location on Nano ITC Low Volume instrument.

4 Start the ITCRun software on the computer. 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 first thoroughly clean, then empty the cells and turn all equipment off. Exit the ITCRun software before switching off the instrument power.

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 buret
- Running the baseline
- Performing an analysis
- Cleaning the calorimeter

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 five days, clean and empty the cells.

The Nano ITC can be calibrated using standardized chemical reactions. If suitable reagents are not available, then as an alternate option perform the calibration using electrical pulses.

Calibrating Chemicals

A chemical calibration tests all aspects of the instrument including the calibration constant, the cell volume, and the injection volume. Heat is released in exactly the same location as occurs during sample titrations, and therefore is the preferred calibration method. (If chemical standards are not available, electrical pulse calibrations are generally suitable as a second choice.)

There are several standard reactions which are often used in calibrating isothermal titration calorimeters (see Brigner, L.-E. and Wadsö, I. [1991] Test and Calibration Processes for Microcalorimeters, with special reference to heat conduction instruments used with aqueous systems *J. Biochem. Biophys. Methods* 22, 101-118.). Here we will describe one: protonation of Tris base (Tris[Hydroxymethyl] Aminomethane). The Tris protonation experiment may be used to determine or verify the calibration factor value setting used in the ITCRun software.

Heat of Protonation of Tris Base

Sample Preparation

It is very important to do a thorough degassing of the water to be used for making the solutions. Use the lowest ionic content water that is available, such as what is produced by a point-of-use deionized water system. Degas this water by boiling or stirring under vacuum for a minimum of 45 minutes. Do not degas the prepared solutions, because this can result in the loss of sample. The solutions can be kept for a short time in stoppered containers. Use nitrogen or argon to fill the head space in order to exclude ambient air which contains carbon dioxide.

Prepare a solution of Tris base by dissolving approximately 0.24 g in 50 mL of distilled water. The solution will be approximately 40 mM, but the exact concentration is not important, as it is well in excess.

A 1.00 mM HCl solution is most readily prepared by pipetting 10 mL of standardized 0.1N HCl into distilled water and diluting to 1L in a volumetric flask. Alternatively, a standard solution of HCl can be purchased commercially or standardized by acid-base titration (see Skoog, D.A. and West, D.M. [1980] *Analytical Chemistry* [Saunders College Publishing], p. 228 ff). Do not degas this solution.

Experiment Setup

Experiment Parameters

Syringe size	100 µL (50 µL syringe in the Nano ITC Low Volume)
Equilibration time	200 seconds (Hastelloy: 300–400 seconds)
Time between injections	200 seconds (Hastelloy: 400 seconds)
Injection size	5 µL (Standard Volume instruments) 2.5 µL (Low Volument instruments)
Number of injections	20

1 Rinse the calorimeter cell three times with the Tris solution and then load the cell. The reference cell may be filled with degassed deionized water. Allow the cells to thermally equilibrate until the heat reading on the calorimeter is stable.

2 Load the 100 µL syringe with the 1.00 mM HCl solution, making sure to remove any bubbles from the

syringe.

3 Wipe the needle with a tissue and then screw the syringe completely into the buret drive.

NOTE: Before inserting the syringe into the buret drive, verify that the plunger indicator on the graduated handle is in the fully raised position. Otherwise, mount the buret on the Nano ITC without a syringe and click the **Buret up** icon (green arrow pointing upwards).

4 Insert the syringe and buret drive into the Nano ITC.

5 Turn on the stirrer at 250 rpm and allow the system to re-equilibrate until the heat reading on the calorimeter is stable.Then begin the experiment. Enter a file name at the prompt.

The results should be similar to those shown in the figure below.

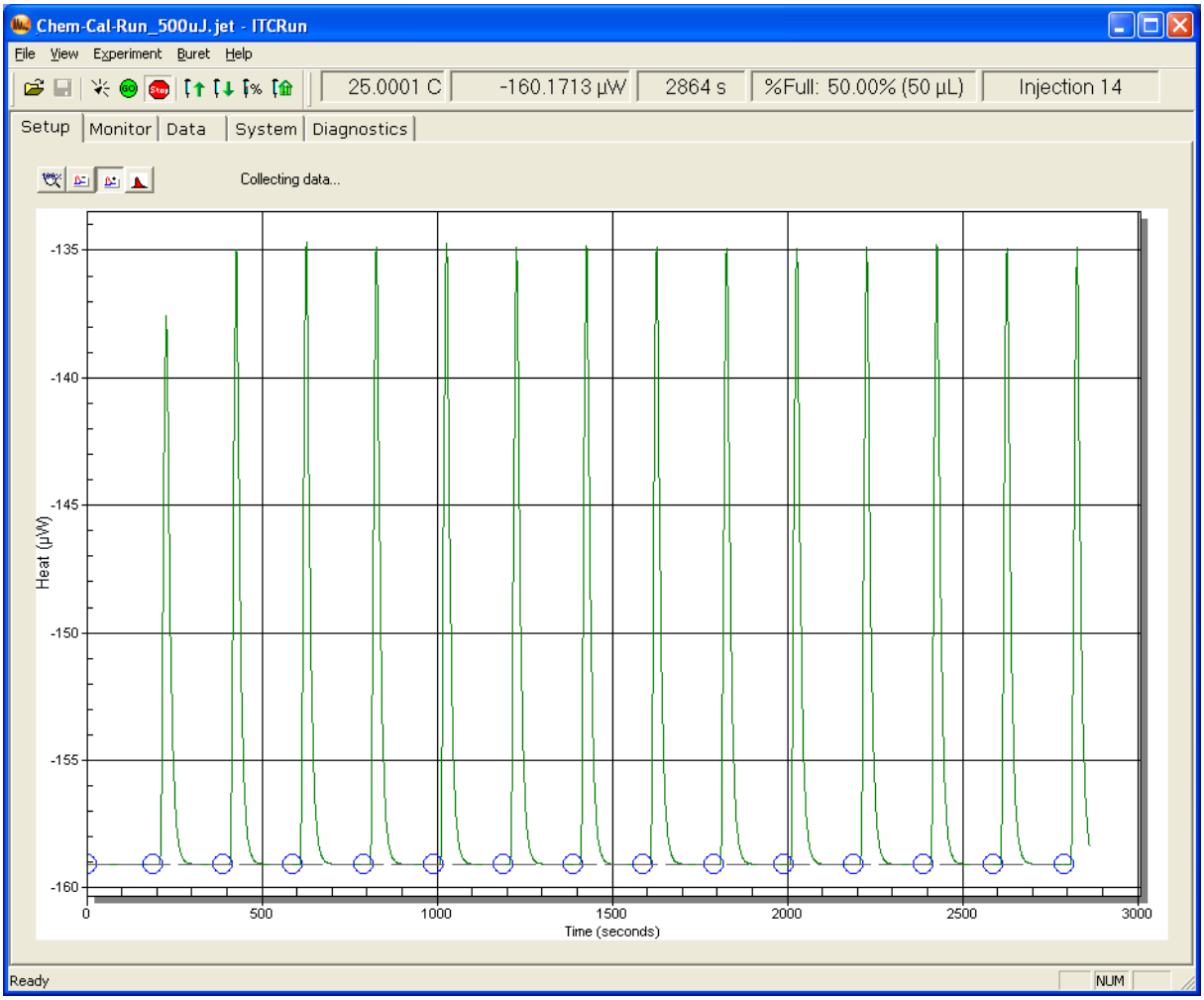


Figure 9 Example of successful calibration.

Note that each peak has the same area except for the first. Typically the first injection shows less heat than expected. This is often due to diffusion across the tip of the needle or to differences in positioning the buret drive. For 5 µL injections of 1.00 mM HCl at 25°C, the expected enthalpy is -237 µJ. The protonation enthalpy in J/mol at any temperature between 5 and 50°C is given as:

$$\Delta H_{protonation} = -49659 + 102.28T - 0.59275T^2$$

The calibration factor (C.F.) is calculated as follows:

C.F. = (Expected Heat / Measured Heat) x Existing Calibration Factor

6 Calculate an average of the C.F. for several injection peaks. In the **Settings** screen (shown below), enter the new calibration factor in the provided entry box. Calibration factors for the ITC always have a negative sign.

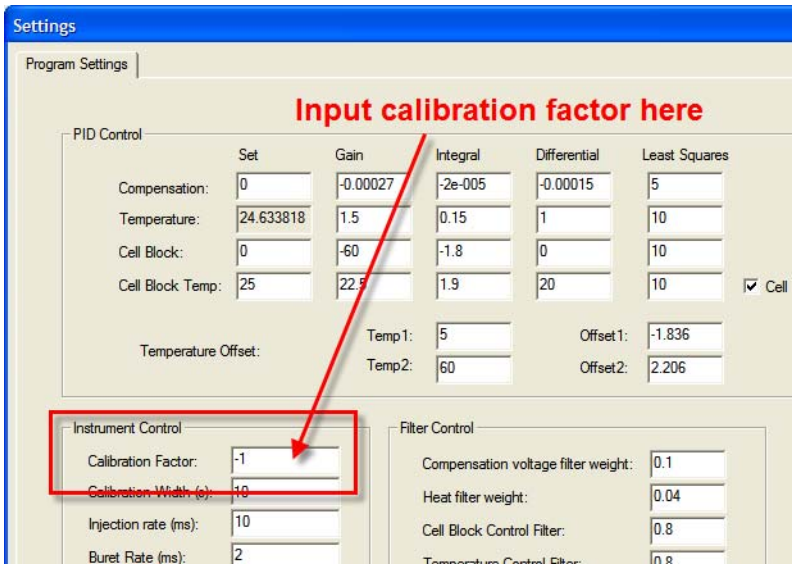


Figure 10 Calibration factor location.

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. Dialyze the samples in the buffer, when possible, to minimize blank effects. 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 should be dialyzed before use, if possible. 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 and 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., O_2 and N_2) 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 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 below) 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 next figure). When using aqueous solutions, the reference cell (right side access tube) should be filled with water. The minimum fill levels are 300 µL for the Low Volume ITC, and 1200 µL for the Standard Volume ITC. These fluid levels will rise high enough to lubricate the Teflon bushings on the syringe needle and to provide a thermal path, but they will not reach to tops of the access tubes. Use the same fill volumes for the sample and the reference cells.

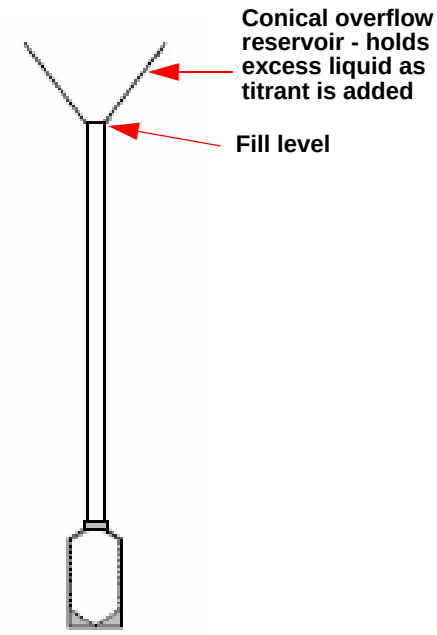


Figure 11 Side view of sample cell and access tube.

b Make sure that the reference needle is inserted into the reference cell access tube after filling (see the figure below). The liquid may be just visible at the bottom of the conical overflow reservoir when the needle is inserted.

Figure 12 Sample (center) and reference (right) cell access tubes.

3 Load the injection 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 include overly large blank effects if there is no air gap to serve as a cushion at the plunger tip.

MISE EN GARDE: Le signal peut inclure de gros effets blancs excessifs s'il n'y a pas d'entrefer pour servir d'amortisseur au bout du piston.

Figure 13 Syringe with air gap.

Loading the Buret

NOTE: Removal of the buret from the calorimeter is the reverse of this process.

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

The top portion of the buret 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 buret.

1 Insert the plunger and barrel carefully into the clear plastic shaft of the buret assembly. (See the figure below.)

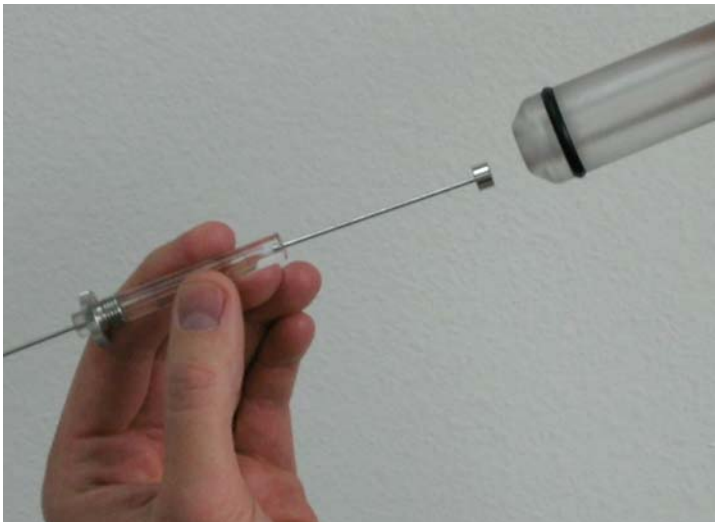


Figure 14 Plunger and barrel.

CAUTION: To avoid possible damage, do not use the 100 or 250 µL syringes with the Nano ITC Low Volume instrument.

MISE EN GARDE: Pour éviter d'éventuels dégâts, n'utilisez pas des seringues de 100 ou 250 µL avec le Nano instrument ITC à faible volume. Les seringues sont expédiées avec des étiquettes d'avertissement qui peuvent, si vous le souhaitez, être collées à l'ITC par le client pour servir de rappel.

2 Partially-filled syringes can be loaded into the Nano ITC using the following process:

- **a** Fill the syringe to a small amount greater than the starting volume. The excess volume will be displaced when the syringe is loaded into the buret handle.
- **b** Preset the buret handle to the desired starting volume. Refer to the Appendix for more details on the buret setting functions.
- **c** Install the syringe into the buret handle, and use a lint-free lab tissue to blot and remove the excess fluid that is expelled from the syringe tip.
- **d** Install the buret handle with the syringe into the Nano ITC.

3 Hold the rotating shaft on the buret 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.

Figure 15 Tightening the syringe into place.

4 Wipe any excess titrant from the needle along the exterior of the barrel and the tip.

Installing the Buret Assembly

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

1 Guide the shaft carefully, needle first, into the top opening of the calorimeter (see the figure below). Make sure the key slots line up with the three locking posts located in the mounting ring at the top. Turn the plastic barrel gently during insertion to help the needle tip self-center in the sample access tube.

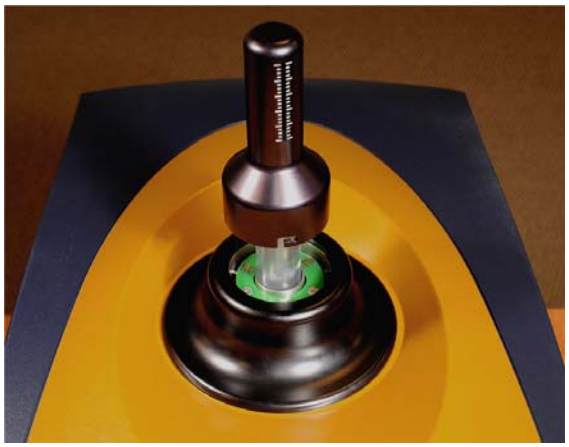


Figure 16 Inserting the buret into the calorimeter.

2 Gently push the buret handle downward and rotate it slightly clockwise to secure the buret in place. **If the buret handle does not easily slip fully into place into the Low Volume ITC, an incompatible syringe may be in place. Do not attempt to force the buret downward or damage may occur. Remove the buret handle and check the syringe type.**

Drop the buret down slowly to allow enough time for the syringe needle to come into thermal equilibration before it enters the sample cell. The **System** tab in the ITCRun software can be used to monitor this thermal settling process.

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

Starting the Experiment

Once the loaded buret assembly is installed, you can proceed as follows:

1. Turn on the stirrer at an appropriate speed. For instruments with gold cells, this is typically in the range of 250–400 rpm. Hastelloy cell instruments typically use 150–200 rpm.
2. Allow the system to re-equilibrate until the calorimeter heat reading is stable.
3. Set up the parameters for the particular experiment of interest using the ITCRun program under the **Setup** tab (see the online help for details).
4. Use the provided data entry fields for syringe and cell concentrations. There is a comment field that can be used to identify the sample chemicals, temperature, stirring speed, etc. This information will be visible when the data is loaded into NanoAnalyze software.
5. Wait until a stable baseline is evident. (ITCRun™ software includes an optional equilibration detection function which automatically starts the experiment when the desired signal stability has been established.) Then click **Experiment > Start** or click the **Go** icon at the top of the toolbar to start the experiment.
6. 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.
7. The titration data is automatically saved to disk at the conclusion of the experiment.
8. Evaluate the data using the NanoAnalyze software package. (See the online help for information.)

Analyzing the Data

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

Table 2: Nano ITC Programs & Functions

Program	Functions
ITCRun	This program controls the operation of the Nano ITC and is used for data acquisition. The main components are: • Experiment Settings • Instrument Control • Main Menu Functions • Buret Menu • Feature Tabs • Monitor Tabs • Data Tab
NanoAnalyze	• Quick, easy integration of heat rate data from Isothermal Titration Calorimeters • Graphing of both Cumulative and Peak heats • Correction for heat of dilution and blank effects • Editing of individual data points • Data import and export to other software programs • “Copy and Paste” graphs to other Windows-based programs • Creation of BindWorks compatible data files • Creation of comparison graphs using the overlay feature

Maintaining the Nano ITC

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

Cleaning Agents

Your choice of cleaning agents will depend on the samples being cleaned out of the cells. Below is a partial list of cleaning agents and their general applicability.

- General Soaking & Washing Agents: All methods should be followed with a 1 L di water rinse at room temperature
- Pepsin (0.1% solution): Used as a soaking agent to dissolve proteins. Use at room temperature.
- Liqui-Nox Detergent Soak or Wash: A good choice for most biological contamination. Has a near neutral pH and excellent rinsibility. A 1% V/V solution should work for non-precipitating samples. A 5% V/V and up to 25% V/V solution may be used for precipitating samples. Heat cell to 50 °C for a several hour soak.
- SDS Detergent Wash: Flush a dilute (1% V/V) aqueous SDS detergent solution through the cells. Use at room temperature.
- Organic Solvents: Methanol, hexane, toluene and other solvents may be used to remove lipid and some polymer contamination. Check chemical compatibility with all instrument components before trying chemicals not listed in chemical compatibility chart. Use at room temperature.
- Extremely Aggressive Cleaning Agents (use only when all else fails)
    - Chem-Solv Detergent: A solution of 2.5% V/V should be prepared immediately before use. Chem-Solvis manufactured by Mallinckrodt Baker and contains sodium hydroxide, propylene glycol, methyl alcohol, polyethylene glycol octylphenyl ether, and water.

CAUTION: Use only at room temperature. Highly caustic, use of protective devices for eyes, skin, and clothing is required.

MISE EN GARDE: A utiliser uniquement à la température ambiante. Très caustique, l'utilisation de dispositifs de protection pour les yeux, la peau et les vêtements est nécessaire.

    - Contrad-70 (Decon-90) Detergent: A solution of 25% V/V should be prepared immediately before use. Contrad-70 is manufactured by Decon Laboratories and contains dodecylbenzensulfonic acid, potassium hydroxide, sodium citrate and sodium laurel ether sulfate.

CAUTION: Use only at room temperature. Highly caustic, use of protective devices for eyes, skin, and clothing is required.

MISE EN GARDE: A utiliser uniquement à la température ambiante. Très caustique, l'utilisation de dispositifs de protection pour les yeux, la peau et les vêtements est nécessaire.

Cleaning the Sample Cell

Protocol

The Nano-ITC uses fixed-in-place gold cells. These cells must be cleaned routinely. Dirty cells contribute to cell filling problems, poor repeatability, and potential residual thermal effects in the data. Inadequate cleaning is the primary cause of most problems experienced with calorimetry. The Nano-ITC has a characteristic baseline when both cells are cleaned and filled with water.

There are three methods of cleaning the calorimetric cells.

1. The simplest is manually rinsing the cells with solution using the filling syringe.
2. The second method involves using the cleaning device.
3. The third method involves filling the cells and allowing them to soak in a cleaning solution at an appropriate temperature.

The first two methods involve some physical agitation, which is necessary to adequately clean the cells. The soaking method is generally used after the others have failed, and uses an appropriate solubilizing cleaner. The selection of a cleaning agent is crucial. All of the components of the sample to be cleaned need to be soluble in your cleaning solution. The gold cells are highly resistant to alkali solutions and partially resistant to acid solutions. The cells are filled with pure deionized water between experiments to prevent contamination from drying on the cell walls.

Sample Cell Cleaning Options

Manual Rinsing (Normal Cleaning)

Buffer/Solvent Rinse: For well-behaved (no evidence of precipitation) samples, it is best to simply syringe-rinse the cells with buffer or solvent between scans. Three or more cell volumes of rinsing are usually adequate.

Aqueous/Organic Solvent Changes: When switching between aqueous and organic solvent systems, it is important to prime the cells with methanol between solvents. Remove the solvent being used, syringe rinse the cells 5 times with methanol, then syringe rinse 5 times with the new solvent. This should prevent water-organic solvent separation and residual effects from immiscible solvent contamination.

Detergent Rinse: Rinse both cells three or more times with the full volume of a filling syringe containing a 1% detergent solution. Then rinse the sample cell with the cleaning device using 1 to 2 L water. Watch for foaming in the vacuum flask, rinse flask as needed with water before proceeding if foaming occurs. Syringe rinse the reference cell 10 times, and the sample cell 5 times with water manually, pushing the solutions up and down in the cell in order to clean the access tube thoroughly as well.

EDTA (10% solution): Used as a post cleaning rinsing agent to eliminate metal ion contamination. Use at room temperature.

Using a Cleaning Device

NOTE: Only use this method in the sample cell. Exit from the ITCRun software before passing large volumes through the Nano-ITC.

Detergent Wash: Using the cleaning device, pass one hundred to five hundred milliliters of detergent solution through the cells. Afterwards, syringe rinse the reference cell 10 times, and sample cell 5 times with water. Then pass the same volume of cold water as used in the wash through the sample cell using the

cleaning device. If the detergent is not easily rinsed, hot water may be used. Watch for foaming in the vacuum flask, rinse flask as needed with water before proceeding if foaming occurs.

Detergent Soak: Fill the cells with a general cleaning solution or detergent. Cover the cells with a damp paper towel (do not cap). Set the thermostat temperature as described for each solution below, for one hour. Do not exceed one hour unless advised to do so. Allow the cells to cool. Syringe rinse the reference cell 10 times and the sample cell 5 times with water. Continue rinsing the sample cell with 1 to 2 L of water using the cleaning device. Watch for foaming in the vacuum flask, rinse flask as needed with water before proceeding if foaming occurs.

Aggressive Cleaning

NaOH Soak: Fill the sample cell with 4 M NaOH. Do not cap the cells; place a kimwipe or 50 mL conical cap over the cell port. Thermostat the system to 65 °C for 1–24 hours. Cool the cells down to 25°C. Remove the NaOH solution from the sample cell. Syringe rinse both cells with cold water 3–5 times. Syringe rinse the sample cell with 50% formic acid 3–5 times. Using the cell cleaning device connected to the degasser, run 1–2 L of water through the sample cell. Exit from the ITCRun software before passing large volumes through the Nano-ITC.

Sample Cell Cleaning Procedure

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 below) allows you to easily flush large volumes of fluid through the cell. The Nano ITC Low Volume instrument is shipped with a cleaning tool that features a shorter needle. In both the Low Volume and Standard Volume ITC instruments, inadequate cleaning will result if the incorrect cleaning tools are used.

Figure 17 Cleaning adapter

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. Ensure that the instrument temperature is adjusted to near ambient temperatures (20–30°C) before starting the cleaning process.
2. Remove the buret assembly and syringe from the top opening of the Nano ITC, and withdraw the cell contents using the filling syringe.
3. Carefully lower the shaft into the cell opening.
4. 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.

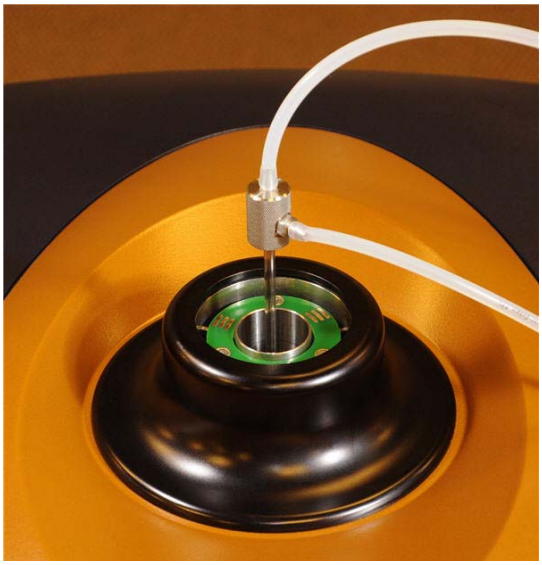


Figure 18 Tubing connected to cleaning tool.

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

6 Connect another length of tubing to the top port leading to a vacuum flask which is connected to a vacuum pump or the degassing station, set for cleaning.

7 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.

Note that the cleaning tool for the Nano ITC Low Volume instrument is shorter. Use only the correct tool for each instrument type to ensure proper operation.

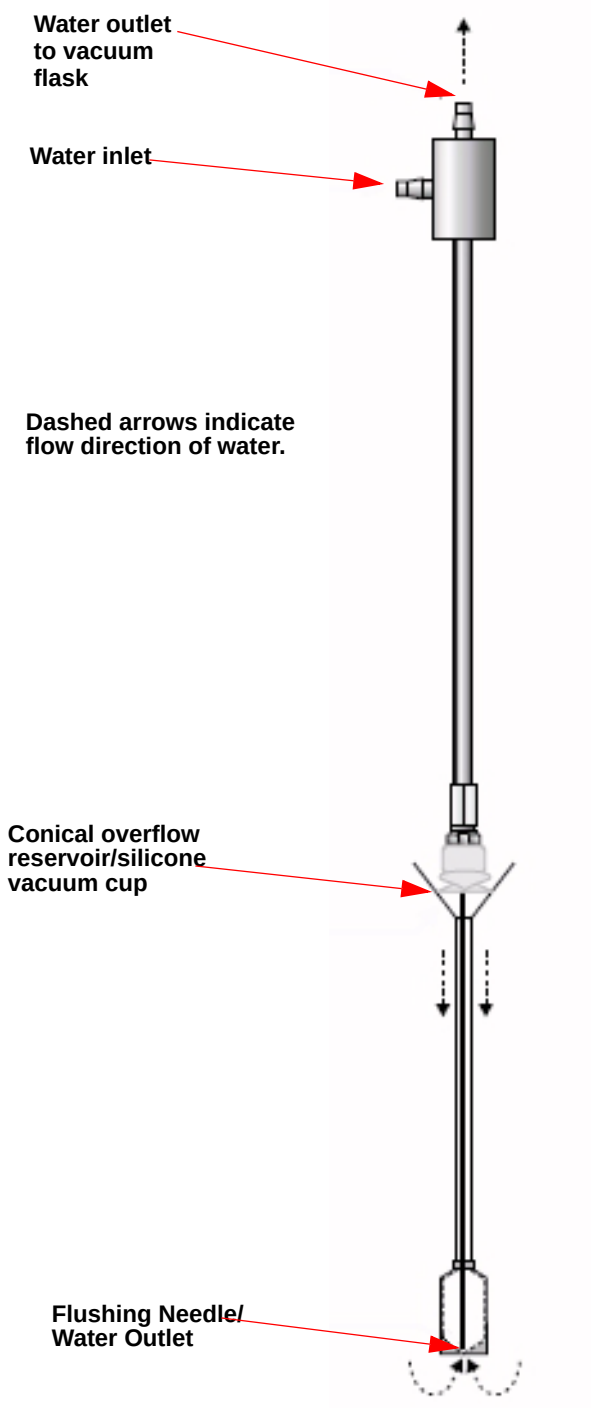


Figure 19 Side view of cleaning tool in sample cell.

CAUTION: Always use proper protective equipment when working with samples and cleaning fluids.

MISE EN GARDE: Utilisez toujours l'équipement de protection approprié lorsque vous travaillez avec les échantillons et les fluides de nettoyage.

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

MISE EN GARDE: Rincez à fond toutes les parties qui entrent en contact avec les substances chimiques corrosives.

CAUTION: All rinsing or flushing operations should be done with the cleaning devices described beginning on page 33. Caustic solutions should be loaded and removed with the appropriate syringe or micropipette.

MISE EN GARDE: Toutes les opérations de rinçage et de lavage doivent être effectuées à l'aide d'appareils de nettoyage décrits au début de la page 33. La mise en place et le retrait des solutions caustiques doivent s'effectuer à l'aide de seringues ou de micropipettes appropriées.

Lubricating the Centering O-Ring

Re-lubricate sparingly with high-vacuum silicone grease if friction becomes excessive.

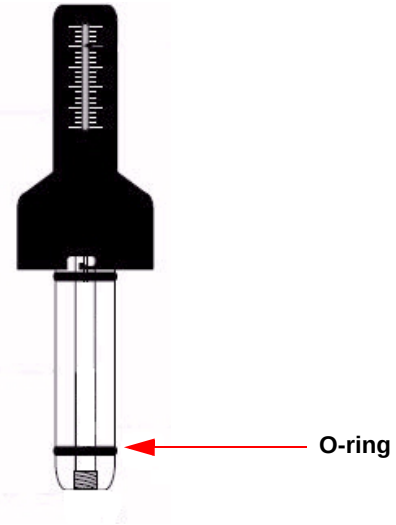


Figure 20 O-ring location.

Purging the Nano ITC

The Nano ITC features a sealed, nitrogen-filled canister surrounding the measurement cells in order to ensure stable operation. If baselines become unstable, it may be necessary to contact TA Instruments for service. Check for stability by operating the Nano ITC near ambient temperature, with the cells filled with degassed water. Stirring should be switched off and the instrument allowed to settle. The peak-to-peak noise amplitude of the baseline signal should be approximately 0.03 µWatts over a period of 10 minutes (exclusive of any drift that may be occurring).

Figure 21 Location of purge valve and port on rear of instrument.

Important Note: The canister has been vacuum-purged and back-filled with dry nitrogen gas at the factory before shipment, and will only very rarely be required again in the field. Contact TA Instruments for service if the instrument does not maintain a stable signal as described above.

Troubleshooting the Nano ITC

Minimizing Blank Corrections

There will always be blank corrections for experiments. However, minimizing the blank correction can greatly improve the experimental results. 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. The optimal stirring speed (see below) may change at different sample temperatures.

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 for instruments made with gold sample and reference cells. In Hastelloy cell instruments, the best results are often obtained with stirring speeds of 150–200 rpm. Higher speeds generally result in higher noise levels in the baseline. In Hastelloy instruments, an additional sign of excessive speed is when the baseline contains a ripple-like periodic variation.

On the other hand, stirring too slowly may result in broad titration peaks that require longer intervals between injections. Reactions that have higher enthalpies can be run at faster stirring speeds, which then allow shorter injection intervals. The best stirring speed will be a tradeoff between those various factors.

Stable Instrument Operation

It is important to monitor the operating behavior of the Nano ITC in order to be able to recognize unusual behavior that indicates abnormal conditions. The following guidelines are typical of normal operation.

The following table lists typical ranges of variation when the cells have been filled with degassed deionized water and allowed to settle with the syringe and reference needles in place, with room temperature controlled to within +/- 1 °C.

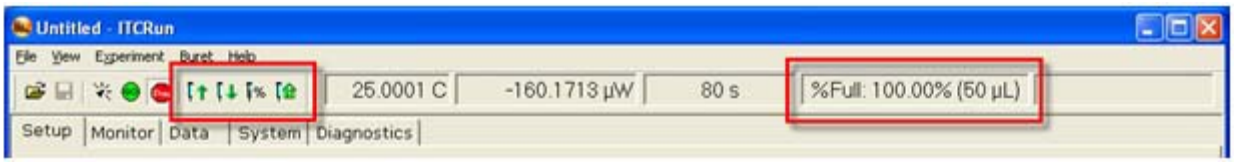
Table 3: Typical Values of System Variables During Steady Operating Conditions

ITC Run Tab	Signal	Typical Values
Monitor	Heat rate (ITC)	Variation < 0.02 µWatt (stirring off)
Monitor	Heat rate (earlier ITC model)	Variation < 0.2 µWatt (stirring off)
System	Cell block temp (ITC)	Variation < 0.000040°C
System	Compensation (V) (ITC)	± 0.0004 V (stirring 200 rpm or less)

Appendix: A

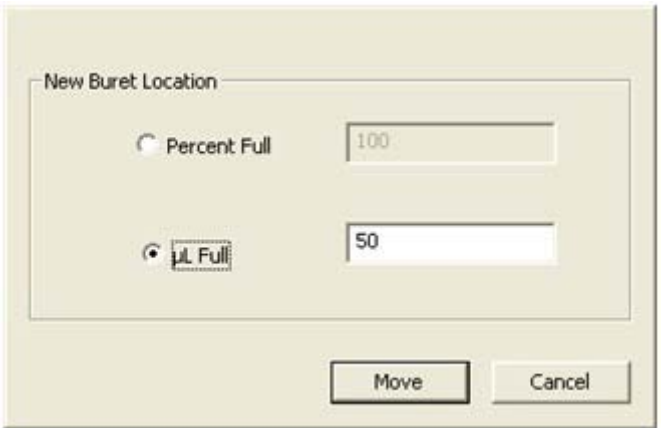
Buret Position Functions

ITCRun Software versions 1.8.7 and later include a new buret position reporting function. The buret can be preset to any position from 0 to 100% of the syringe capacity. The positioning can be performed either via a percent of the full stroke or directly in microliters.

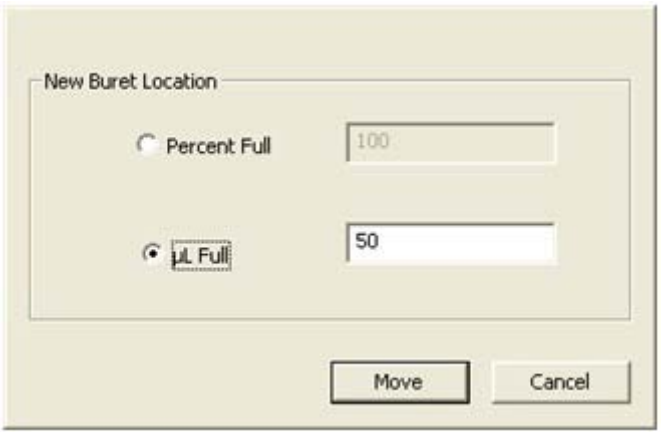


Click **Home Reset**:

The desired buret position can be entered directly in µL. (In the case if the Standard Volume ITC, be sure to set the correct syringe capacity in the **Setup** tab). Enter the desired position in terms of µL and click **Move**. The buret position field at the top of the ITCRun screen updates with the current position of the buret drive.



As an alternative, the desired position can be entered as a percent of the full capacity.



In order to ensure that the reported position of the buret drive matches the actual position, a new **Home Reset** function has been added. This moves the buret drive all the way to the bottom of the stroke in order to find a home reference, then moves the drive upwards by exactly the length of the syringe stroke. Follow the instructions on the screen, paying particular attention to removing the syringe from the buret handle before proceeding.

Click **Home Reset** to start the **Home Reset** process:

If the syringe is present in the buret drive and it contains a sample, note that the motions will empty the syringe. To avoid losing the sample, remove the buret handle from the Nano ITC, remove the syringe, then replace the buret handle. Then click the **OK**.

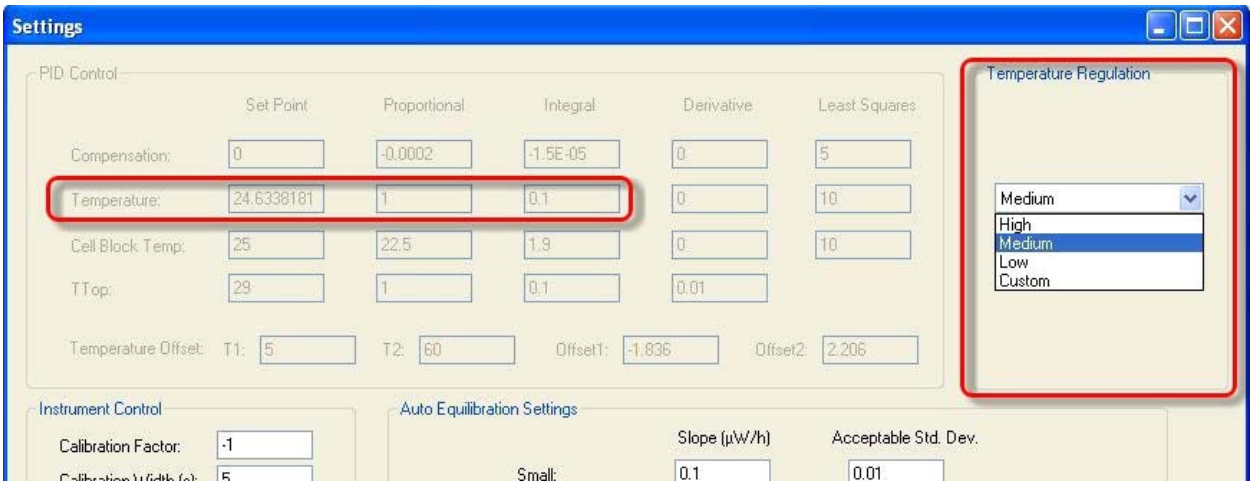
After the buret drive stops moving, it will be located at the top position and the buret position will be reported as 100% on the top of the Nano ITC screen.

Appendix: B

Temperature Regulation Control

The temperature regulation of the room environment can potentially have an impact on the baseline noise of the Nano ITC. Ideally the room temperature will be regulated within approximately 1° Celsius, with only gradual changes occurring. The instrument should also not be placed in an area that can receive direct sunlight, due to the sudden changes in the laboratory temperature that can occur on partially cloudy days.

It is not always possible to achieve the desired control over the ambient conditions, so a user-selectable setting has been provided in ITCRun software which can help to minimize the effects of the variation. A limited range of adjustment has been provided over one of the critical temperature control zones. There are three settings available to the user, labeled **High**, **Medium**, and **Low**. The selection control is provided in a drop-down menu that can be found in the Settings dialog box.



The following guidelines will help users make an appropriate selection. Make changes if there is a baseline stability issue, and use whichever setting gives the best low-noise and low-drift baseline characteristics.

High

Use this setting typically if the room temperature might experience temperature fluctuations that are wide ranging. The control is stronger at this setting and under some circumstances this may result in a small oscillation with a period of less than 10 seconds in the zone called **Temperature**. Use the System page to view the control band for the Temperature zone. If the peak-to-peak total variation in **Temperature** exceeds 200 micro degrees Celsius (0.0002°) then use a lower setting.

Medium

Medium is the default setting and it is what should generally be used unless there is a specific issue with the baseline.

Low

The Low setting may be useful in conditions where the room temperature changes are erratic rather than regular and cyclic. The temperature control is less strong in this setting and one of the stronger settings should be used instead if baseline drift occurs.

Determining the Best Setting

- Set up the instrument with degassed water or buffer in the Sample and Reference cells as well as the syringe. Set the stirring to 350 rpm for instruments with gold cells. Use 200 rpm for instruments with Hastelloy cells. Turn stirring on and allow the baseline to stabilize.
- Observe the baseline in the Monitor window for at least 10 minutes and note the baseline characteristics of peak to peak noise band and drift.
- Go to the Settings dialog box and try each of the three settings in turn. Allow at least 10 minutes of observation time and note the quality of the baseline in the Monitor page, and also the control band for the **Temperature** channel in the System page.
- If a small rapid oscillation occurs in the Temperature control band you may need to use a lower level setting for the Temperature Regulation control. This might also appear in the Monitor page as a small oscillation in the baseline.
- If the baseline slowly drifts in the Monitor page you may need to use a stronger setting for the Temperature Regulation control.

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