Cleaning Protocol for TA Instruments ITC

The ITC from TA Instruments uses fixed-in-place gold or hastelloy 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 ITC has a characteristic baseline when both cells are cleaned and filled with water.

Cleaning routines should be adapted to the samples being studied. This is not a complete list and there is not a single method that works in every case. Please use these as suggestions or a guideline. Regular cleaning after every sample will decrease the need to move to more aggressive routines.

 DO NOT insert cold or hot solutions into the cell as this could damage the sensor.

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.

The cleaning routines described can also be applied to the titration syringe. After heavy cleaning routines the syringe tip and for the Nano ITC, the Teflon® sheath, should be soaked for only mild detergents and then water.

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. Five or more cell volumes of rinses are usually adequate, followed by one buffer rinse.

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.

This is only a rinse and not a cleaning routine after running duplicates or triplicates a clean method like those listed below must be used, even if the sample is as simple as salt solutions.

Cleaning Procedures

A. Manual Rinsing – Normal Cleaning

Detergent Rinse: Both cells are rinsed 2 times with the full volume of a filling syringe containing a 1% detergent solution such as SDS, Contrad® or Micro90®. Then rinse the sample cell with 10 times or more of DI or nanopure (np)-water. When rinsing with the detergent or water manually, push 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. More difficult to remove metals such as Iron may require other chelators such as NTA.

100% Methanol or Ethanol: Suggested 1-3 x rinse when working with lipids or similar materials. Follow with a water rinse.

4% Formic Acid: Suggested cleaning *prior* to the 2% n-propanol test kit titration.

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 is easy to rinse. 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 or check with a TA employee. Use at room temperature.

B. Cleaning Device (only compatible with sample cell) It is suggested to exit from the ITCRun software before passing large volumes through the ITC.

Detergent Wash: Using the cleaning device, one hundred to five hundred milliliters of detergent solution is passed through the cells. Afterwards, syringe rinse reference cell 10 times, and sample cell 5 times with water. Then the same volume of room temperature water as used in the wash is passed through the sample cell using the cleaning device. If the detergent is not easily rinsed methanol or ethanol can be used to cut the solution. Watch for foaming in the vacuum flask, rinse flask as needed with water before proceeding if foaming occurs.

Detergent Soak: The cells are filled with a general cleaning solution or detergent. The cells are not capped but covered with a damp paper towel. The thermostat temperature is then set as described for each solution below for one hour. Do not exceed one hour unless advised to do so. The cells are then cooled. 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.

C. Aggressive Cleaning

NaOH Soak: The sample cell is filled with 4 M NaOH. The cells are not capped and a kimwipe or 50 mL conical cap placed over the cell port. The system is then thermostatted at 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 ITC.

Extremely Aggressive Cleaning Agents

Contrad-70® (Decon-90®) Detergent: A solution of 25% V/V should be prepared immediately before use. The cell can be heated to 60 °C. Contrad-70® is manufactured by Decon Laboratories and contains dodecylbenzensulfonic acid, potassium hydroxide, sodium citrate and sodium laurel ether sulfate. 1-2 L of water is required to remove this solution. NOTE! CAUTION SHOULD BE USED, USE ONLY AT ROOM TEMPERATURE, HIGHLY CAUSTIC, USE OF PROTECTIVE DEVICES FOR EYES, SKIN AND CLOTHING IS REQUIRED.

HNO_3 Soak: Prepare a solution of 2M nitric acid (HNO_3). Flush the sample cell with water and fill it with the nitric acid solution. To promote increased digestion of contaminants, temperature may be raised to

60 °C. Allow the acid to remain in the cell for several hours or overnight. Set the cell temperature back to 25°C. After the cell has cooled, remove the acid solution and flush the cell with large amounts, 1-2 L of clean DI or np water.

It may take 1-2 days after an aggressive cleaning to obtain a good water-water scan. Several rinses and fresh cell loads are typically required.