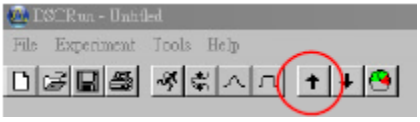
TA Instruments Nano-DSC III – Standard Operating Procedure

Contents:

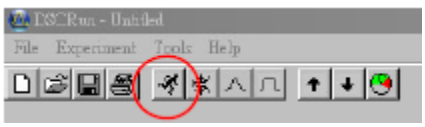
A. Setting up a conditioning run

It is recommended to condition the reference and sample cells with the buffer to be used in the experiment. Conditioning neutralizes any potential active sites on the cell surface. Conditioning scans are only necessary when changing buffer type.

1. Wash sample (S) and reference (R) cells with degassed, deionized water
2. Degas 3-5 mL (or more) of buffer for at least 10 min under vacuum
3. Rinse the cells several times with buffer, withdrawing all the buffer each time (see Appendix A)
4. Last filling: leave the buffer in the cells, cap one end of each cell (to minimize rocking of solution during heating). Remove any spills with a tissue. Screw on pressure lid (finger tight).
5. Open DSCRun software
6. Set the temperature range, scan rate and number of scans (ideally scan rapidly to 130°C)
7. Give a file name (not necessary for conditioning scan, but necessary for baseline and sample scans)
8. Pressurize the sample chamber. Set pressure to 3 atm if scanning to ≤90°C or set pressure to 6 atm if scanning above 90°C (the full temperature range of the instrument is -10 to 130°C).



9. Start run



B. Setting up a buffer baseline scan

A buffer baseline scan is necessary for analysis of the experimental results and to check baseline repeatability. Baseline repeatability scans are only necessary when changing buffer type.

1. Depressurize the DSC, remove caps and squeeze on a tissue (to remove any liquid), remove conditioning buffer from both cells, rinse cells with fresh degassed buffer, then fill cells with fresh degassed buffer and cap.
2. Pressurize sample chamber. Set scan rate and temperature range (scan at the same rate and range as sample will be scanned). Set number of scans to check baseline repeatability. Give file name.
3. Start run

C. Sample preparation

1. Dialyze sample extensively to remove low molecular weight contaminants.
2. Determine concentration of the sample. Typical concentration range is 0.1 to 0.2 mg/mL (for protein samples, full concentration can be 30 µg/mL to 1 mg/mL). Sample volume of 0.6 mL is sufficient to overfill the 0.3 mL cell volume.
3. It is preferable to ensure there are no particulates (either filter the sample or, preferably, centrifuge it).

D. Running a sample

1. Degas the sample and the reference buffer for at least 15 min
2. Ensure cells are scrupulously clean. Dirt traps minute air bubbles, which affects noise and repeatability of the scans (a 1 mL air bubble can cause a 70 mW offset at 1°C/min; baseline repeatability specification is 0.028 mW).
3. Load sample into right hand cell sample cell (marked S), buffer into left hand reference cell (marked R). Cap. Screw on pressure lid (finger tight).
4. Set temperature range, scan rate, number of scans.
5. Pressurize*
6. Start

*Before you pressurize, select the “monitor” tab in the DSCrun software to monitor the heat flow. Watch the monitor screen after pressurizing the cells. The heat flow after pressurizing should remain within ±10 µW. Any change of more than 15 µW is indicative of air bubbles in the cells. Also check the cell pressure, which should remain steady. If the pressure is observed to drop it indicates that the pressure lid has not been tightened sufficiently.

E. Cleaning the cells

Regular cleaning of the sample cell is necessary to remove adsorbed protein and other contaminants.

Regular cleaning:

1. Remove the sample
2. Load 50% formic acid solution into both cells
3. Scan from 25 to 75°C and back to 25°C.
4. At 25°C, rinse each cell with 1 L of deionized water (see Appendix B).

OR

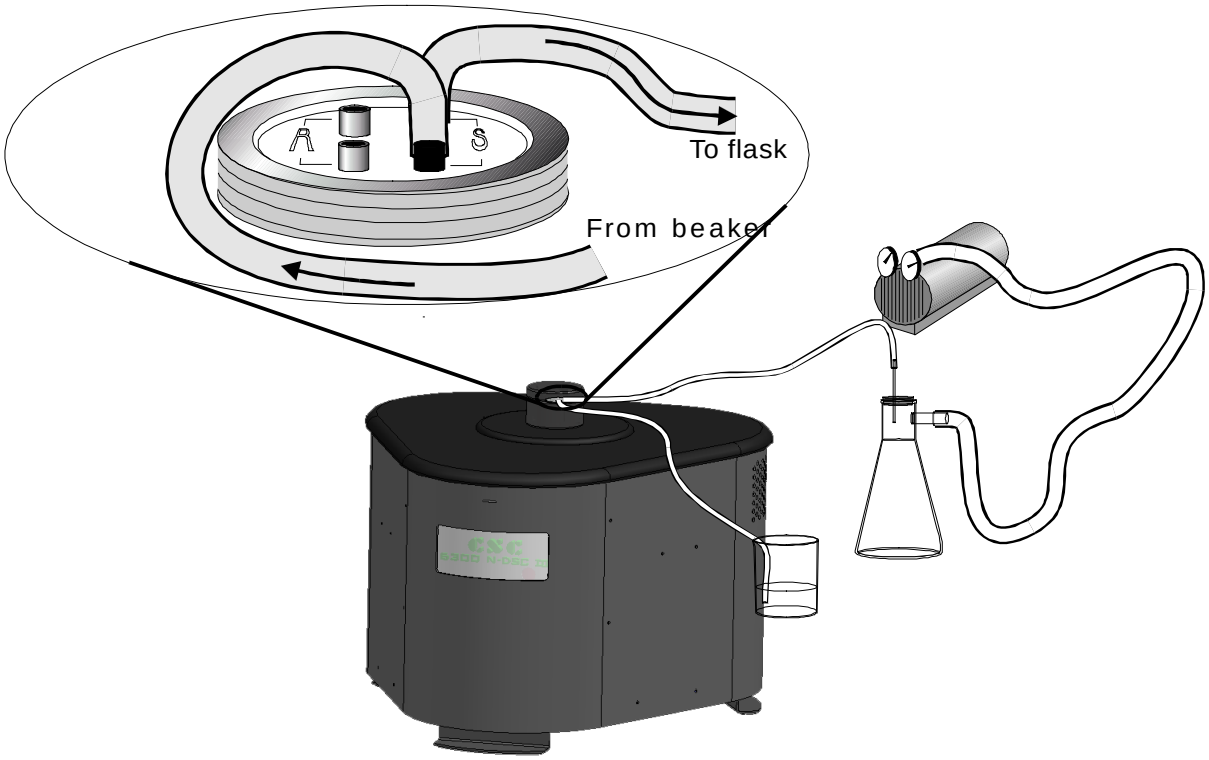
1. Remove the sample
2. Clean cells with 15% detergent/15% methanol/ 4M NaOH at 60°C for 20 minutes
3. Rinse, treat with 2M nitric acid (heat to 70°C), rinse thoroughly

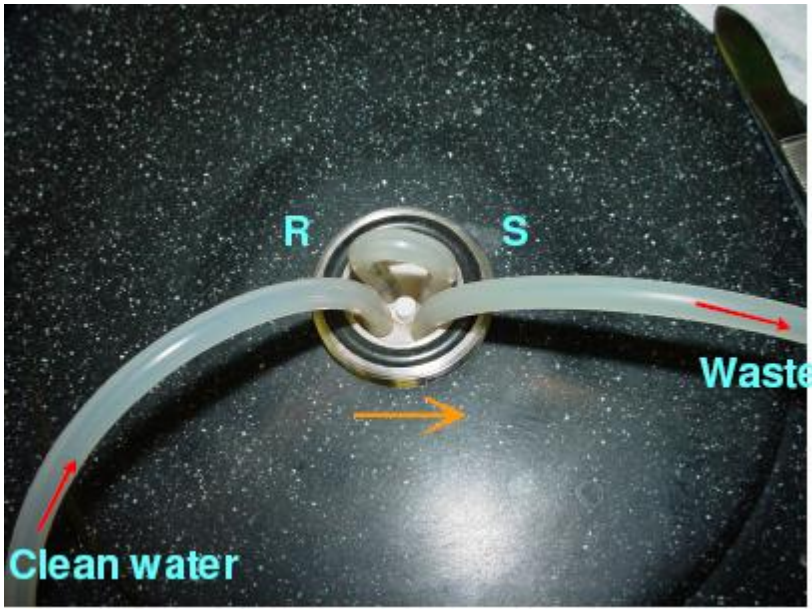
If there is excessive noise in subsequent scans, or you suspect a residue or precipitate in the cells more thorough cleaning is necessary (see Appendix C).

Appendix A: Filling the cells

1. Attach Pipetter and spare tip to cell access tubes
2. Slowly inject solution to overfill cell
3. Rock solution to remove air bubbles
4. Remove Pipetter and spare tip simultaneously for perfectly filled cell – must be able to see meniscus at top of access tubes
5. Dry any spillages in sample compartment

Appendix B: Set-up of pump for rinsing cells with large volume of deionised water





Appendix C: Extensive cleaning protocols

1. Protein Deposits: Pepsin Solution

Prepare a solution containing 0.5 M NaCl, 0.1 M Acetic Acid and 1 mg/mL Pepsin.
Place this solution in the calorimeter for a minimum of three hours, preferably at 30°C. (Use fresh pepsin each time as it eats itself up in solution.)
Flush the cells with 1-2 L of deionized water after the solution has been in the calorimeter for three hours or more.

2. Mineral Deposits

Step A: NaOH Solution
Prepare a 4.0 M NaOH solution.
Place this solution in the calorimeter and run a scan from 25 to 90 °C at 2 °C/min.
Interrupt the scan at 90 °C and let it stand at this temperature overnight.
Flush the cells with 1-2 L of deionized water after the NaOH solution has been in the calorimeter over night.

Step B: Formic Acid Solution
Prepare a 50% formic acid solution.
Place the solution in the calorimeter and run a scan from 25 to 65 °C at 2 °C/min.
Interrupt the scan at 75°C and let it stand at this temperature for 20 minutes.
Flush the cells with 1-2 L of deionized water after the 20 minutes have passed.

3. Grease or Oil

If you suspect grease or oil in the cells, follow these steps.
Place HPLC grade tetrahydrofuran in the calorimeter and run a scan from 25 to 50 °C at 2 °C/min.
Interrupt the scan at 50°C and let it stand at this temperature for 20 min.

4. Soapy Water Flush

Exit from the DSCRun software.
Flush or aspirate 100 mL of soapy water (e.g. SDS) through the cells.

5. Final Flush

Flush or aspirate 1-2L of deionized water through each cell.