

INTRODUCTION

Pressure Perturbation Calorimetry (PPC) is a technique for determination of thermal and volumetric properties of macromolecules. These properties include the partial molar thermal cubic expansion coefficient ($\bar{\alpha}$, Equation 1, [1]) and the relative partial molar volume ($\Delta V/V$, Equation 2, [2]) of the macromolecule as functions of temperature. These parameters are associated with hydration of the macromolecule [3, 4]. The method consists of measuring the heat effect, ΔQ , from applying a pressure change, Δp , to a solution of the macromolecule. $\bar{\alpha}$ and $\Delta V/V$ are then calculated by the following equations.

$$\bar{\alpha} = \left(\frac{1}{\Delta V} \right) \left(\frac{\partial V}{\partial T} \right)_p = \frac{\Delta Q}{VT\Delta p} \quad (1)$$

$$\frac{\Delta \bar{V}}{\bar{V}} = \int_{T_0}^{T_e} \bar{\alpha} dT \quad (2)$$

The Nano DSC is currently equipped with the necessary components to perform PPC without further modification. PPC is typically performed on a DSC that is capable of increasing and decreasing the pressure on a sample during a DSC temperature scan or during isothermal measurements. During a temperature scan, the pressure change must occur faster than the heating or cooling of the system (3, 4). This note is a short tutorial on the required steps to set-up an experiment on the Nano DSC with DSCRun software and then on analysis of the resulting PPC data with NanoAnalyze™ software to evaluate $\bar{\alpha}$, which denoted as TEC in the software.

PPC WITH DSCRUN

Turn on the Nano DSC, then open DSCRun and allow the system to stabilize. In the DSCRun software open the "set runtime variable" under the "Tools" menu. On the "Experimental Method" tab, check either scanning or isothermal and enter the temperature limits and scan rate, see Figure 1. If scanning, a slow rate is required because the calculations assume isothermal conditions, see the definition of $\bar{\alpha}$ in Equation 1 (4). If the scan rate is slower than the instrument feedback then it can be safely assumed isothermal during the pressure steps. A rate of 0.1 °C/min is recommended. This scan rate will typically satisfy the isothermal requirement without scanning so slowly that resolution of the heat rate is unduly sacrificed.

Choose the pressure function to be applied during the experiment, see Figure 1.

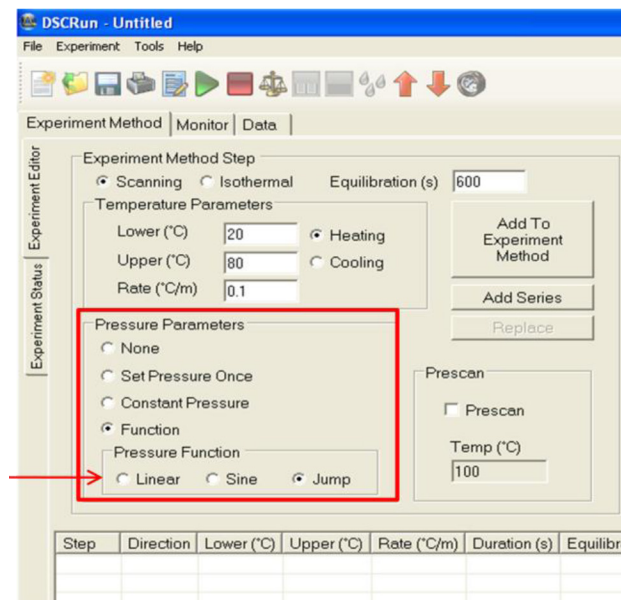


Figure 1. Screen shot of DSCRun, highlighting the different pressure parameters available.

Three different pressure control options are available, - linear, sine, or ramp (labeled jump in the software). The jump pressure function is usually used. Once the desired pressure function is selected and all the "Temperature Parameters" are set for the scan, click on the "Add To Experiment Method" button. This opens a dialog box that enables setting the "Jump Pressure Function" (see Figure 2).

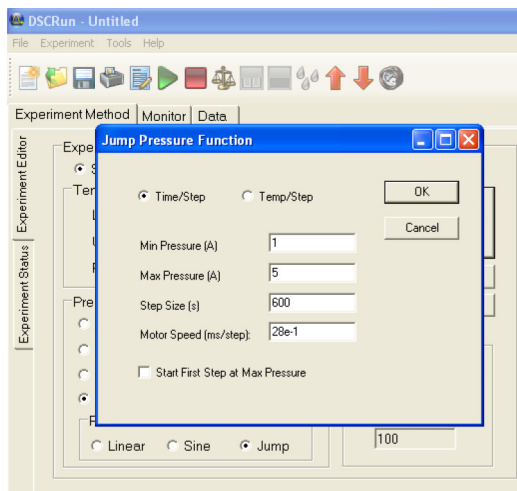


Figure 2. Screen shot of dialog box that opens after a pressure function has been selected. (A indicates atmospheres and s indicates seconds.)

Note that in scanning experiments, pressure can be designated as a function of time or temperature. The term "step" in this box refers to the pressure step. Although the instrument is capable of operating from 0 to 6 atm, relative to ambient pressure, the recommended pressure change is from 1 to 5 atm with a ramp/step time of 600 s (4). The minimum pressure must exceed a pressure that allows boiling at any temperature during the scan. For most experiments, the default motor speed of 28×10^{-1} ms/step is sufficiently slow to satisfy the requirement of isobaric conditions in Equation 1.

EXPERIMENTAL SET-UP

A water-water scan can serve as a test experiment to assess any asymmetry between the reference and sample cells. For this test, water is loaded into both the reference and sample cell. The pressure is stepped from 1 to 5 atm, starting at low pressure with time steps of 600 s while scanning at $0.1 \text{ }^\circ\text{C}/\text{min}$ from 20 to $80 \text{ }^\circ\text{C}$. During the experiment pressure is applied equally to both sample and reference cells, so any deviation of ΔQ from zero is solely due to any asymmetry between the cells.

For typical solutions of macromolecules, a concentration of 10 mg/mL should generate good data. Due to the slow scan rate, concentration requirements for PPC experiments are greater than for a normal DSC scan. The PPC experiment consists of two parts, a background run and a sample run. Background data with buffer in both reference and sample cells should be collected under identical PPC conditions as the sample. Sample data must be collected with the same buffer in the sample cell. The basis for this method and the calculations done in NanoAnalyze™ are as follows (Equations 3-5).

Equation 3

$$\frac{\Delta Q}{VT\Delta p} = \alpha_{measured} = (\alpha_{sample} - \alpha_{reference}) + \alpha_{asymmetry}$$

Equation 4

$$\alpha_{asymmetry} = (\alpha_{buffer \text{ in sample cell}} - \alpha_{buffer \text{ in reference cell}})$$

Equation 5

$$\bar{\alpha}_{macromolecule} = (\alpha_{sample} - \alpha_{reference}) = \alpha_{measured} - \alpha_{asymmetry}$$

$\bar{\alpha}$ measured is calculated from the data with sample solution in the sample cell and buffer in the reference cell and $\bar{\alpha}$ asymmetry is calculated from the data with buffer in both cells.

PPC FITTING WITH NANOANALYZE™

The data files from the background PPC run and the sample PPC run can be opened in NanoAnalyze™ without modification. When the raw data files are initially opened, a dialogue box will open with a prompt: "Analyze pressure data for Experiment Step 1?" (Figure 3) In this context, "step" indicates a temperature scan or isothermal run. This request will be made for each temperature scan or isothermal run if the box at the bottom is not checked.

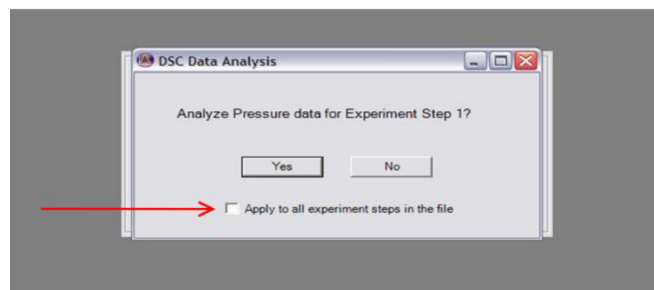


Figure 3. Dialogue box prompt when opening PPC data with NanoAnalyze™.

When the files are opened, extra data analysis tabs are automatically made accessible (Figure 4).

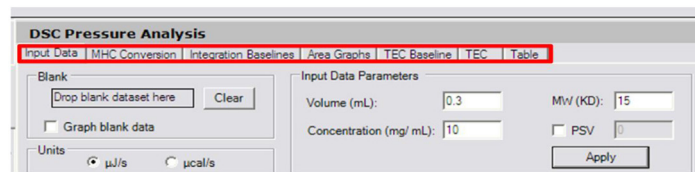


Figure 4. Screen shot highlighting the extra tabs accessible after opening PPC data on NanoAnalyze™.

In the "Input Data" tab, several key values must be entered for TEC calculations, i.e., sample cell volume, concentration, and molecular weight in kilodaltons. The data file is loaded with the "Add file" icon. The background data is dragged and dropped into the "drop baseline here" box (Figure 4). MHC (molar heat capacity), the second tab, is not used in PPC

calculations. The third tab from the left, "integration baseline", gives the integrated area under each pressure event. The baseline for integration of the heat effects (ΔQ) from the pressure changes can be either a straight-line (default) or a sigmoid (check Sigmoid Baseline box) (Figure 5).

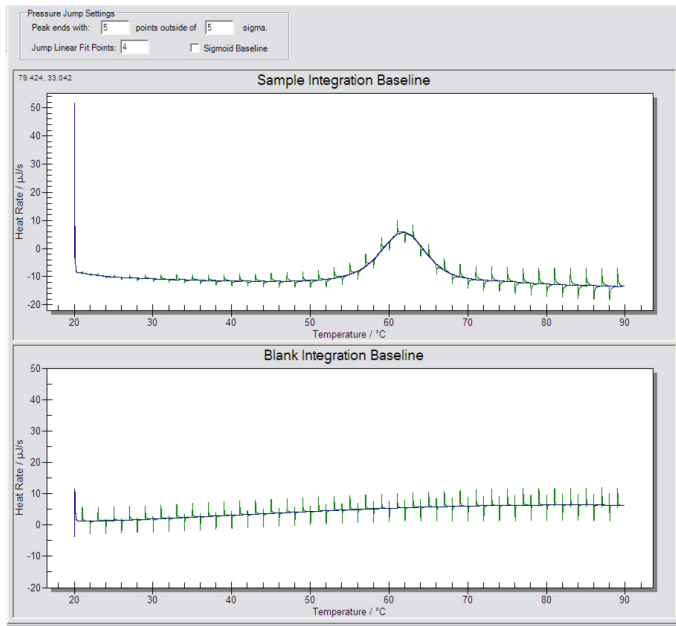


Figure 5. PPC sample and background data in the "integration baseline" window.

In the "area graphs" tab (Figure 6), background and sample areas are plotted. The baseline area data can be fit to a polynomial, labeled blank fit order in the software (as is the case with TEC Baseline and TEC tabs).

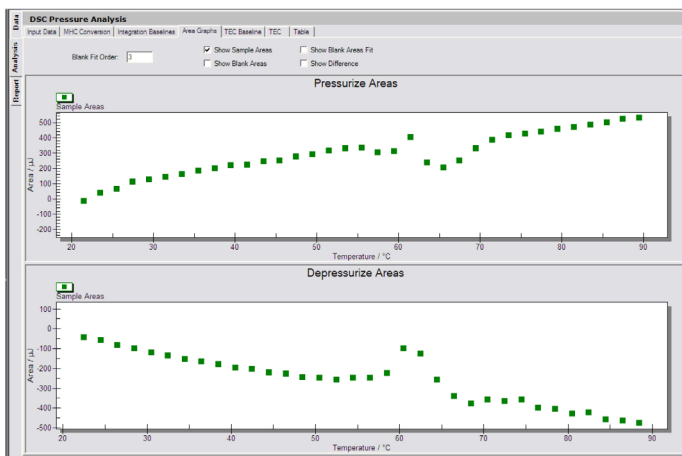


Figure 6. Area graphs window for a sample.

The "TEC Baseline" is used for calculating $\bar{\alpha}$ for pure liquids, and is not used for calculation for solutes.

The "TEC" tab plots the thermal expansion coefficients in the top window (Figure 7) and the integrated area in the bottom window. This integrated area is the relative change in volume

($\Delta V/V$), according to the relationship of Equation 2 (3).

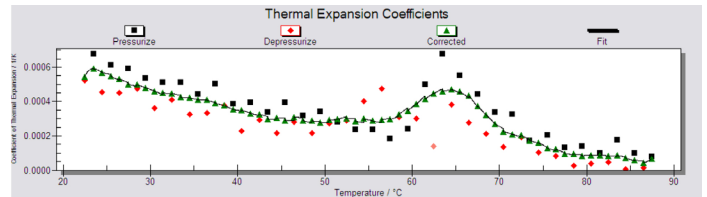


Figure 7. The TEC ($\bar{\alpha}$) for a sample.

Numerical values for all of the thermal expansion data are displayed in the "table" tab and can be copied to a clipboard and imported to another program if further manipulation is desired.

CONCLUDING REMARKS

The Nano DSC instrument is the only ultrasensitive DSC on the market that comes standard with all the necessary components built in to perform PPC analysis. In addition, NanoAnalyze™, provides all the necessary tools for complete analysis of PPC raw data files collected on the Nano DSC.

REFERENCES

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2. Mitra, L.; Smolin, N.; Ravindra, R.; Royer, C.; Winter, R. "Pressure perturbation calorimetric studies of the solvation properties and the thermal unfolding of proteins in solution—experiments and theoretical interpretation" *Phys. Chem. Chem. Phys.* 2006 8, 1249–1265.
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4. Dragan, A.I.; Russell, D.J.; Privalov, P.L. "DNA Hydration Studied by Pressure Perturbation Scanning Microcalorimetry" *Biopolymers* 2008 91, 95-101.

Colette Quinn, Ph.D. and Lee Hansen, Ph.D.

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