VP-DSC Run Parameters

The following is largely excerpted from the User's Manual. The portions highlighted in red are suggestions made by various MicroCal engineers and/or applications specialists.

Starting Temperature: The starting temperature of a scan. If the *Starting Temperature* < *Final Temperature*, then the scan will be an upscan. If not, then the scan will be a downscan.

Final Temperature: The ending temperature of a scan.

NOTE: The starting and ending temperatures for a DSC scan should allow for both pre-transition and post-transition baselines, necessary for data analysis. This is usually ~15-20°C past the T_m.

Cooling cells is a passive process so the Tm one would get from a downscan will not correspond exactly to the upscan. Also, since it is passive we [MicroCal] do not fully test the linearity of the downscan rate. People sometimes use downscan, but in practice, do not publish the data. For reversibility studies, we [MicroCal] recommend series of upscans, with cell cooling in between (the cell cooling process is relatively fast).

ScanRate: The rate at which the VP-DSC will be scanned in temperature. Upscans and downscans are distinguished from one another by their *Starting Temperature* and their *Final Temperature* and not by the sign of the scanrate. This parameter box only accepts positive values. A maximum of 90 Deg./Hr. for upscans and 60 Deg./Hr. for downscans is recommended for optimal results.

PreScan Thermostat: The amount of time to thermostat at *Starting Temperature*, prior to beginning the scan. This allows for thorough equilibration of the thermal core of the VP-DSC before beginning the scan. This parameter can affect the repeatability and the shape of the baseline. The default value of 15 minutes is recommended.

PostScan Thermostat: The amount of time to thermostat at *Final Temperature* prior to beginning the next scan. The *PostScan Thermostat* period is typically used only for cleaning purposes, in order to incubate the DSC cells at a high temperature for a specified period of time. In general, there are no performance gains to be had by specifying a post-run thermostat period during normal DSC operation, so this parameter is usually set to 0 minutes.

Filtering Period: The time period over which data samples are averaged and a data point is generated and stored. For protein transitions, a filter period in the range of 10-20 seconds is usually appropriate. For sharper transitions such as lipids, a shorter filter period of 1-5 seconds is usually appropriate.

Data File Name: The file name under which all experimental data will be saved. All VP-DSC data files will have a .dsc extension.

FB Mode/Gain: Determines the method and magnitude of cell-cell compensation used for thermal equilibration of the reference and sample cells. Each of the 4 available modes is pre-calibrated:

None (or passive) *FB Mode* means there will be no active cell-cell compensation in response to temperature differences between the reference and sample cells. Equilibration of the cells will be achieved 'passively' through thermal conduction and convection. While this method provides for the highest sensitivity (short-term noise), it also provides for the slowest response time of the DP signal. This feedback mode is appropriate for almost all protein studies (broad transitions).

Low, Mid or *High FB Mode* means there will be active compensation to the cell(s) in response to temperature differences between them. The colder cell will always receive the compensation, and the amount of compensatory heat for a given temperature difference will vary depending on the *FB Mode* chosen.

High Gain FB Mode will provide for the greatest amount of cell-cell compensation for any given temperature difference between the cells. The result will be a faster response time, but with higher short-term noise in the DP data. This mode, and its fast response are usually necessary when studying very sharp thermal transitions such as Lipids.

*Low and Mid Gain FB Mode*s will provide intermediate levels of sensitivity and response time characteristics, as compared with the *No Gain* and *High Gain* feedback modes.

Data File Comments: These comments get saved as part of the data file header and can be viewed from the data analysis program, or from any text editor such as Word or NotePad. When entering data file comments please remember that the Scan Edit Mode will be ignored. *Data File Comments* will be applied to all selected scans only, or may be applied to all scan by clicking on the *Apply Comments To All* option.

Cell Refill Parameters: The *Cell Refill Parameters* are provided to help users refill the DSC cell(s) at approximately the same temperature each time, and while the DSC is cycling. Since the VP-DSC works best while it is cycling, cell refilling must be done during the cooling cycle between scans:

1. Activate the cell filling warning by clicking on *Use Audible Fill Indicator*. This indicates that you want to be warned when the cell temperature is within the specified temperature window. (NOTE: On our computer, the warning is not audible, however, a visual reminder will pop onto the computer screen.)
2. Define the temperature window during which you want to refill the cells. Enter the desired minimum and maximum temperatures during which you want to refill. A 10°C window, well below any regions of sample transition is suggested.
3. When the cells pass into this temperature window, VPViewer will indicate that it is time to refill the cells. The warning screen continues until you clear the message (click on *OK* in message window), or until the cell temperature passes outside of the refill temperature window.
4. The easiest way to calculate the time between the start of one cycle and the next is to look at the time stamps of your series of files.

NOTE: Refill scans should be within 0.0075 C_p (mcal/min) of each other for reproducibility.

DSC Sample Related Considerations

The ScanRate, Filtering Period & FB Mode/Gain parameters are coupled:

1. Very sharp transitions may require lower scan rates to avoid shifting the transition midpoint in the direction of the scan (i.e. higher transition temperature in upscan, lower transition temperature in downscan).

2. Solutions that have multiple transitions may benefit from slower scan rates to help 'separate' the transitions in the real-time data.

3. Very dilute samples may benefit from an increase in scan rate, increasing the calorimetric sensitivity of the VP-DSC. Higher scan rates give higher absolute sensitivity.

4. Slow transitions (i.e. protein unfolding) will benefit from the passive feedback mode (feedback gain/mode = None) and will not suffer from the slow DP response time. Faster transitions (i.e. Lipids) may require the high gain feedback mode to obtain the necessary DP response time with the trade-off being a slightly higher short term baseline noise. Increasing the FB Mode/Gain, will increase resolution (helps when you need to resolve multiple transitions), but will also increase baseline noise (can be a problem if your sample concentrations are low).

5. Faster scan rates may require smaller filter periods to well resolve the transition. Slower scan rates will not require as many data points, and larger filter periods may be used.

In general,
For single domain proteins, scan rate = 60-90°C/hr, FB mode = none
For multiple domain proteins, scan rate = 60-90°C/hr, FB mode = low or mid
For lipids, scan rate = 20-60°C/hr FB mode = mid or high

Remember that you can only extract out thermodynamic parameters if the reaction is reversible. If more than 80% of the original signal is recovered upon reheating, then the reaction can be considered reversible[1].

Things to try if the reaction is irreversible:

- Decreasing the protein concentration.
- Alter the pH--should be more than 1 pKa unit away from the isoelectric point of your protein.
- Lower your scan rate.
- Decrease your ending temperature--reversibility of unfolding is strongly dependent on the upper temperature limit of the first scan[1].

1. Lopez, M.M. and G.I. Makhatadze, *Differential scanning calorimetry*. Methods Mol Biol, 2002. **173**: p. 113-9.