Starting NanoAnalyze

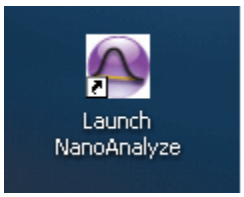


Figure 1 NanoAnalyze desktop icon.

The program opens a window on the desktop.



Figure 2 NanoAnalyze window.

Windows® XP is a registered trademarks of the Microsoft Corporation.

1 The following functions are located on the **Menu** bar:

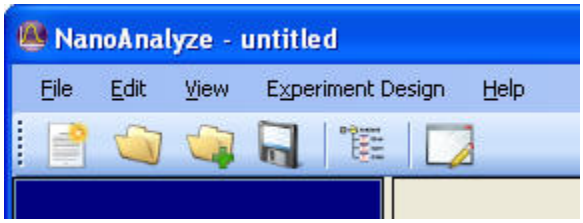


Figure 3 Menu bar.

- **File**: Provides access to file and method operations
- **Edit**: Provides access to the Model Editor, as well as other editing functions
- **View**: Shows or hides the file tree on the left side of the program window
- **Experiment Design**: Opens an experiment planning tool for ITC titrations
- **Help**: Access to Help files and program information

2 The following functions are located on the toolbar:

- **New File**: Opens a new file page in NanoAnalyze
- **Open a File**: Opens an already-existing data analysis file (***.csc** or raw data file)
- **Add a File**: Imports an additional raw data file into the file tree
- **Save File**: Saves the current state of NanoAnalyze as a ***.csc** file
- **Show/Hide File Tree**: Displays or hides the file tree
- **Model Manager**: Opens a window for managing all model fitting files

Working with Nano ITC Raw Data Files

The following describes how to open, view, analyze, and print data with Nano ITC raw data files.

Opening a File

1 Select **Open** in the **File** menu.

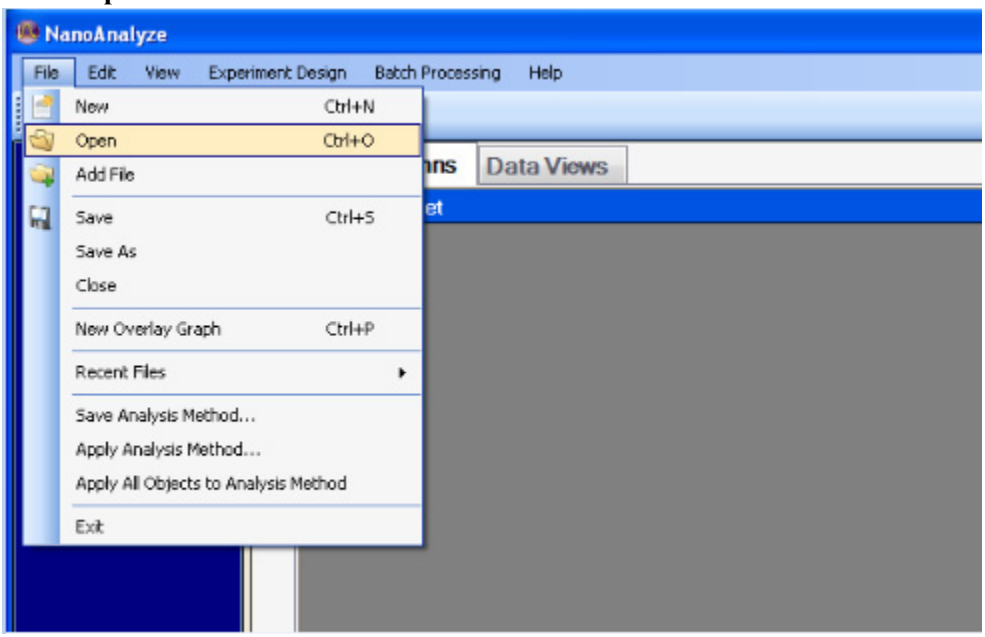


Figure 4 File > Open.

2 Use the drop-down list to select the folder that contains the ITC data files.

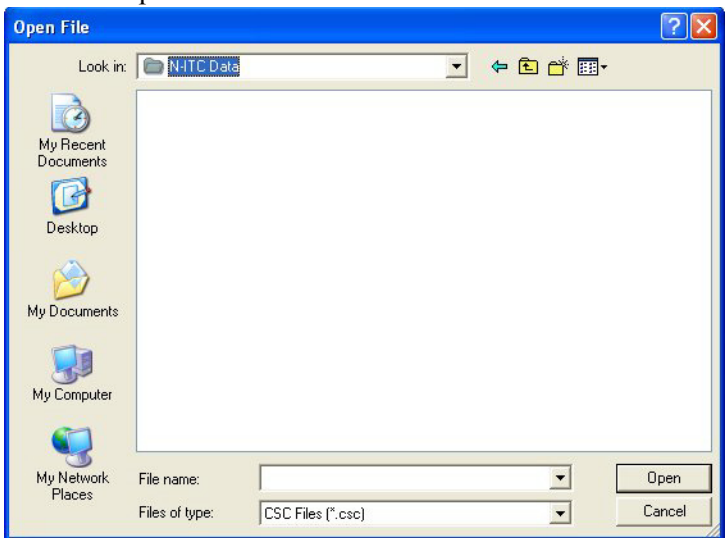


Figure 5 Select folder containing ITC data files.

3 Select data file type **ITC Files (*.jet)** from the drop-down list at the bottom of the window.

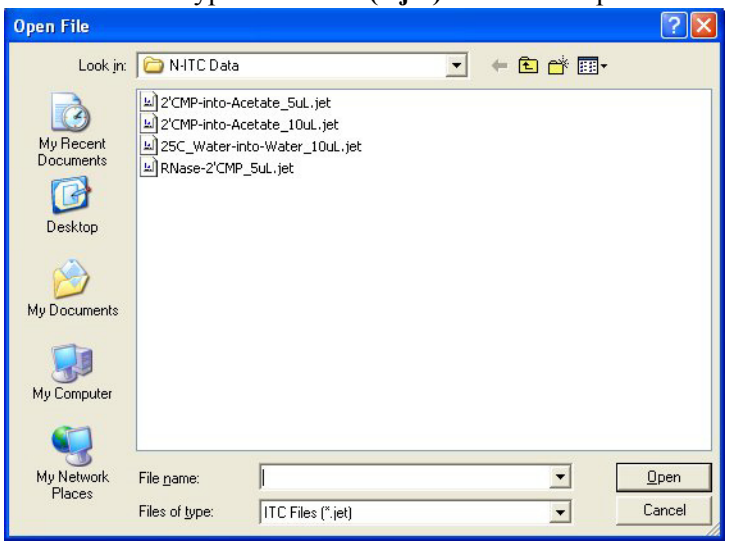


Figure 6 Select data file type.

4 Select the experiment data file that you wish to load, then click **Open**.

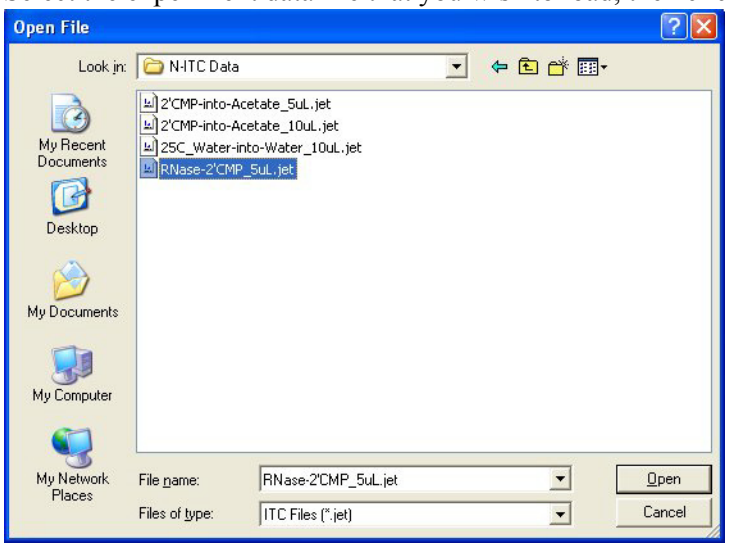


Figure 7 Select experiment data.



NOTE: Multiple files can be loaded at the same time if desired. Select the first file by clicking on the name with the mouse. Select a range by holding down the Shift key while clicking on the last file, or select any number of individual files in any order by holding down the **Ctrl** key while clicking the files with the mouse.

5 A blank experiment is often performed along with the titration. Blanks include the heat effects that result from fluid motion during the injection, along with enthalpies from dilution.Typically the syringe is loaded again with the syringe reactant; however, the sample cell is filled with the buffer that was used to dialyze the sample. Select **Add file..** from the file menu and select the blank file to be loaded. You will reference this blank experiment data file in a later step.

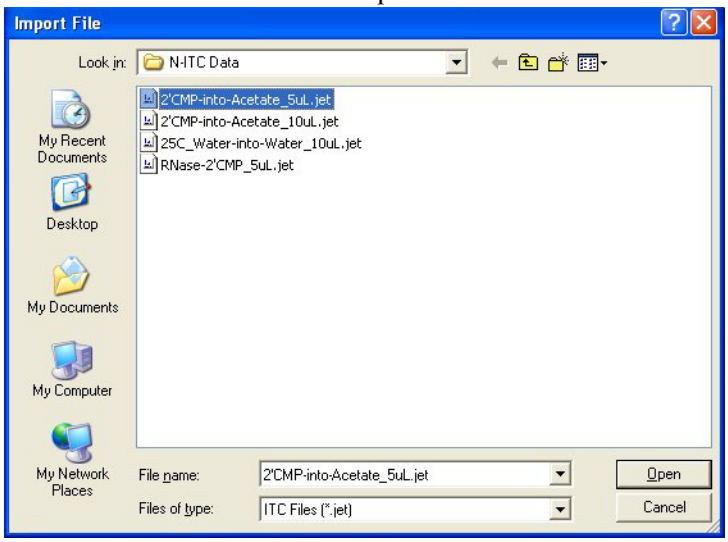


Figure 8 Select a blank experiment.

6 Click on the titration experiment file in the file tree to load the file for analysis. The data file appears in the file tree and a table of the measured heat data appears in the **Data Columns** tab on the right side of the program window. The figure below shows the appearance of the file tree after both the sample and blank data files have been brought into NanoAnalyze.

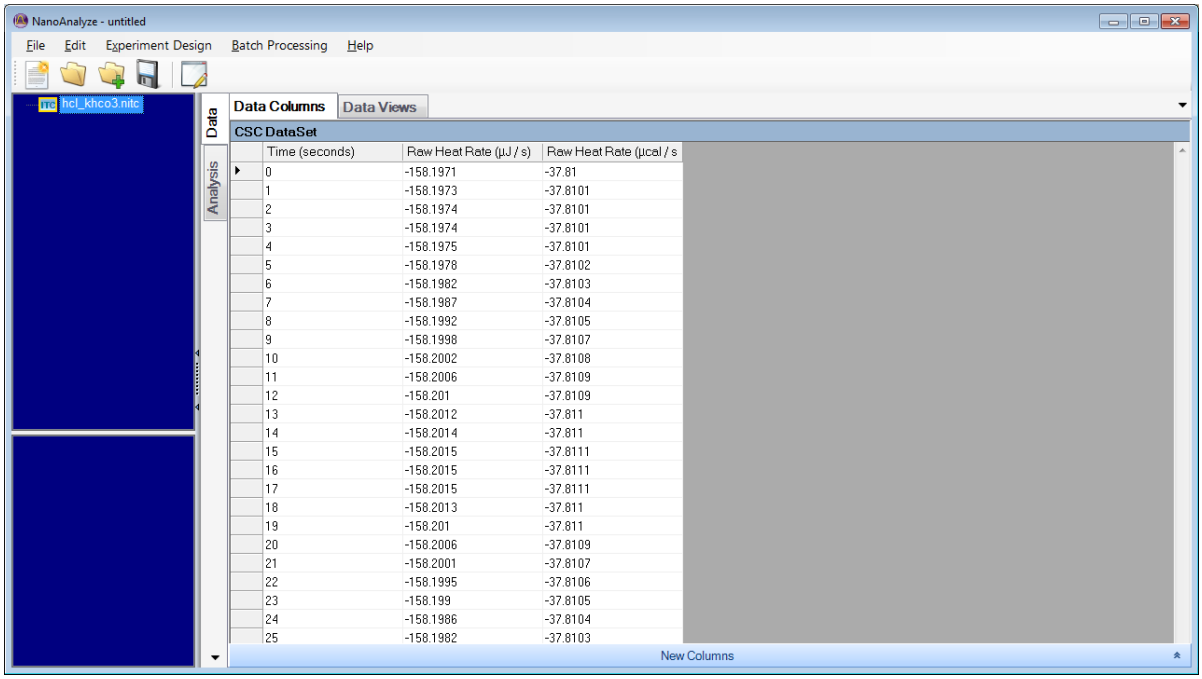


Figure 9 Data Columns.

Analyzing ITC Data

To access data analysis functions, click on the **Analysis** tab.

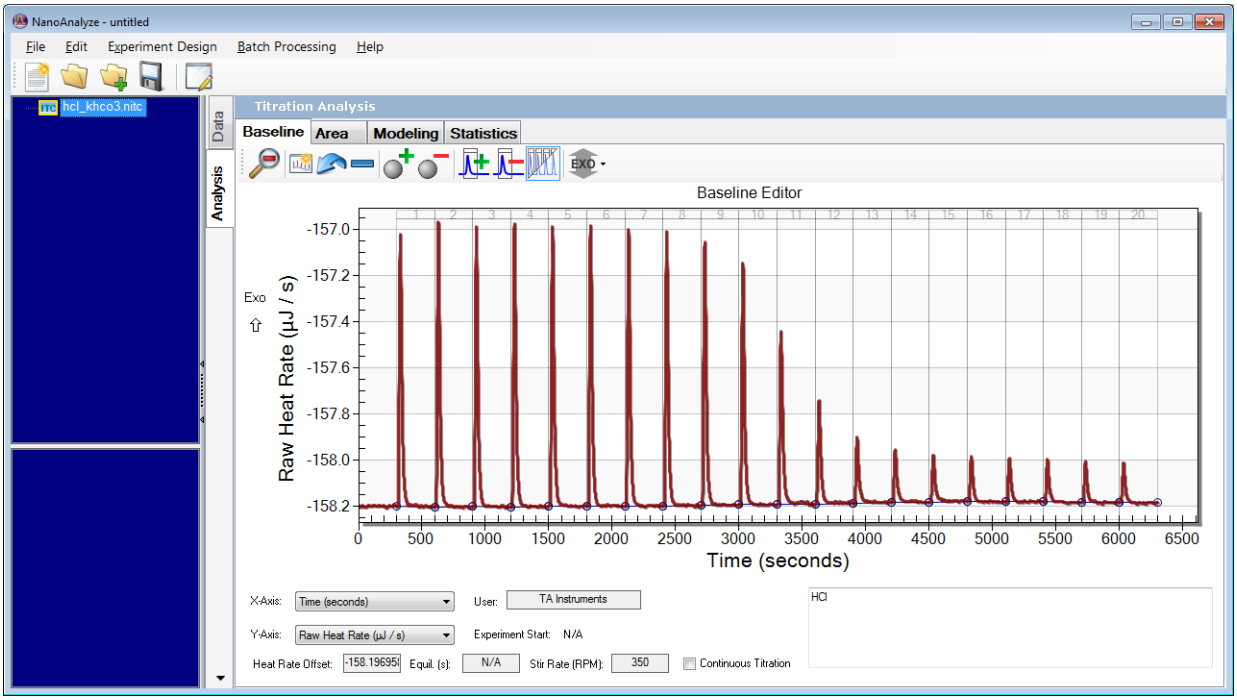


Figure 10 Analysis.

Selecting Integration Regions

Select integration regions automatically by entering the injection time table parameters.

1 Select **New baseline** by clicking the button in the toolbar in the **Analysis** tab.

The following are the available toolbar functions:

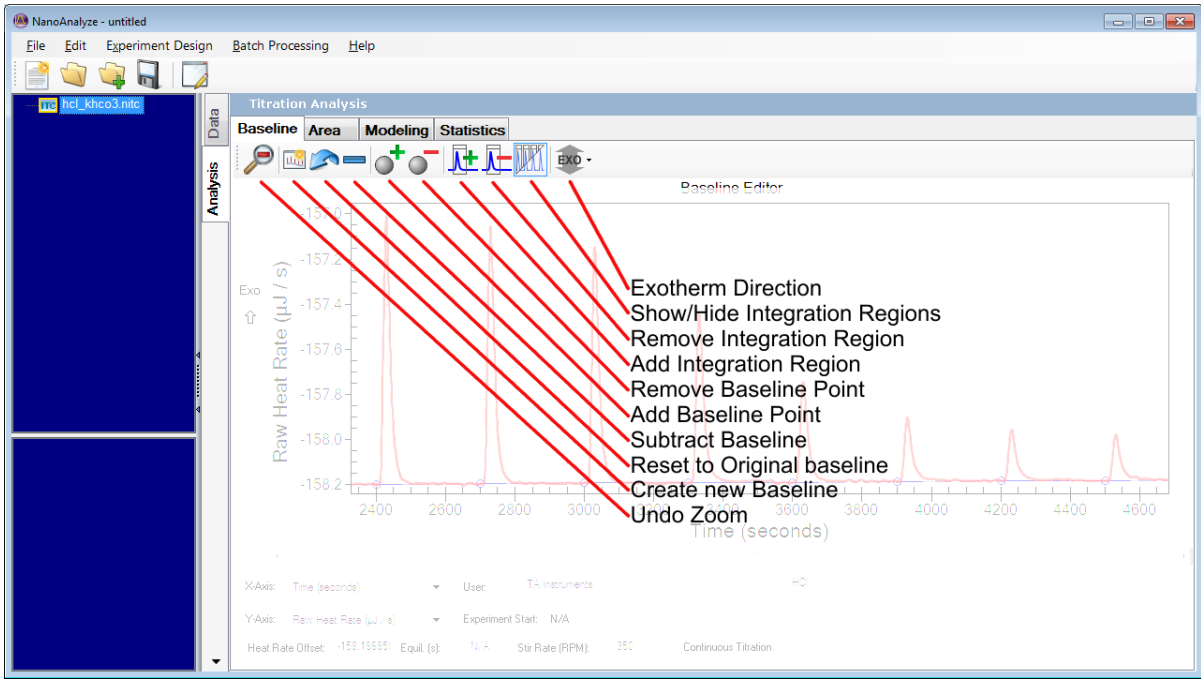


Figure 11 New Baseline.

- **Undo Zoom**: Restores a zoomed-in view to show all of the experimental data
- **New Baseline**: Sets the integration window start times and width
- **Subtract Baseline**: Subtracts the baseline from the signal data, and assigns the value of 0 microWatts to the baseline.
- **Add** and **Delete Baseline Pivot Points**: Adjusts the path of the baseline
- **Add** and **Subtract Integration Regions**: Adjusts the time intervals of the integration regions
- **Show/Hide Integration Regions**: Toggles between the two functions

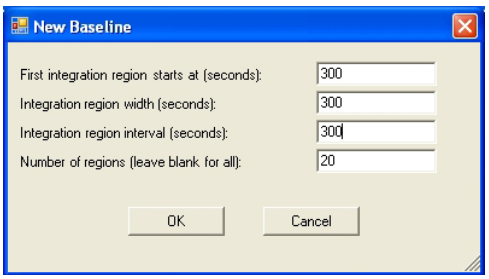


Figure 12 New Baseline dialog box.

2 Enter the following:

- Time of the first injection
- The desired width of each integration region (as a first approximation this can be set to the injection interval)
- The injection interval
- The number of injections that occurred (or leave this field blank)

3 The experiment data divides into the integration regions.

Narrow the integration regions by entering a smaller number for the integration region width. Typically the heat signal rises from the baseline at some time after the actual injection start time. Adding a few seconds to the start time aligns the integration window to the measured heat signal.

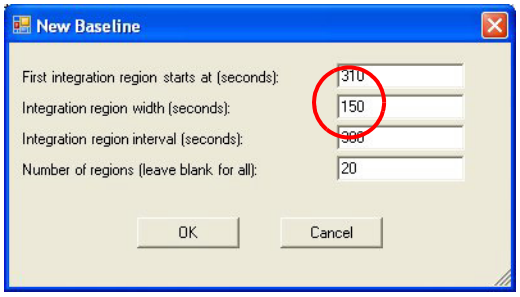


Figure 13 Integration width.

The graph now shows the narrower integration regions.

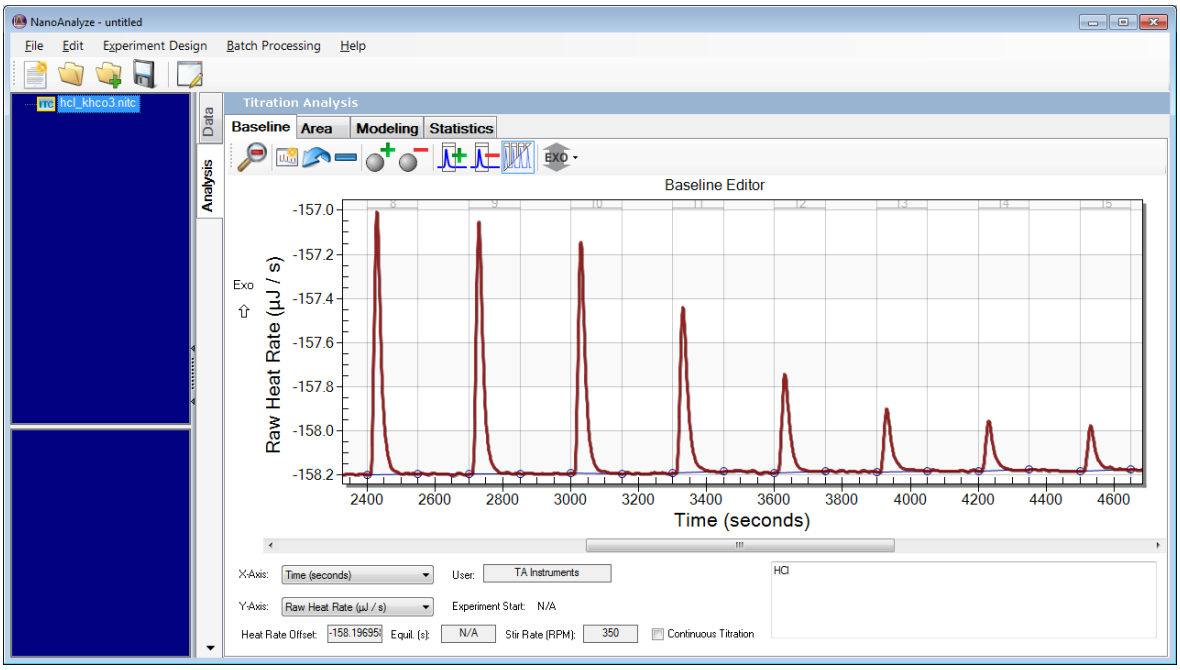


Figure 14 Baseline graph.

Examine the baseline in detail by zooming in on a selected portion of the graph.

4 Press and hold the left mouse button at one corner of the zoom region, move the mouse to define the opposite corner, and then release. The graph is redrawn to the selected region.

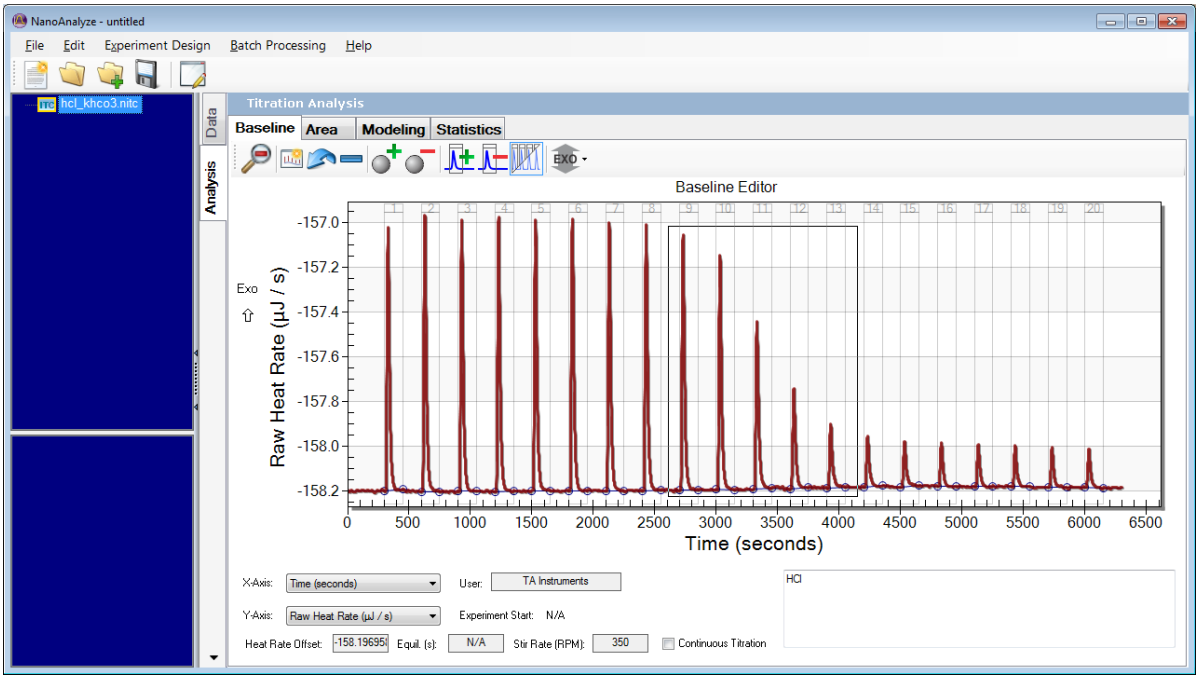


Figure 15 Baseline graph.

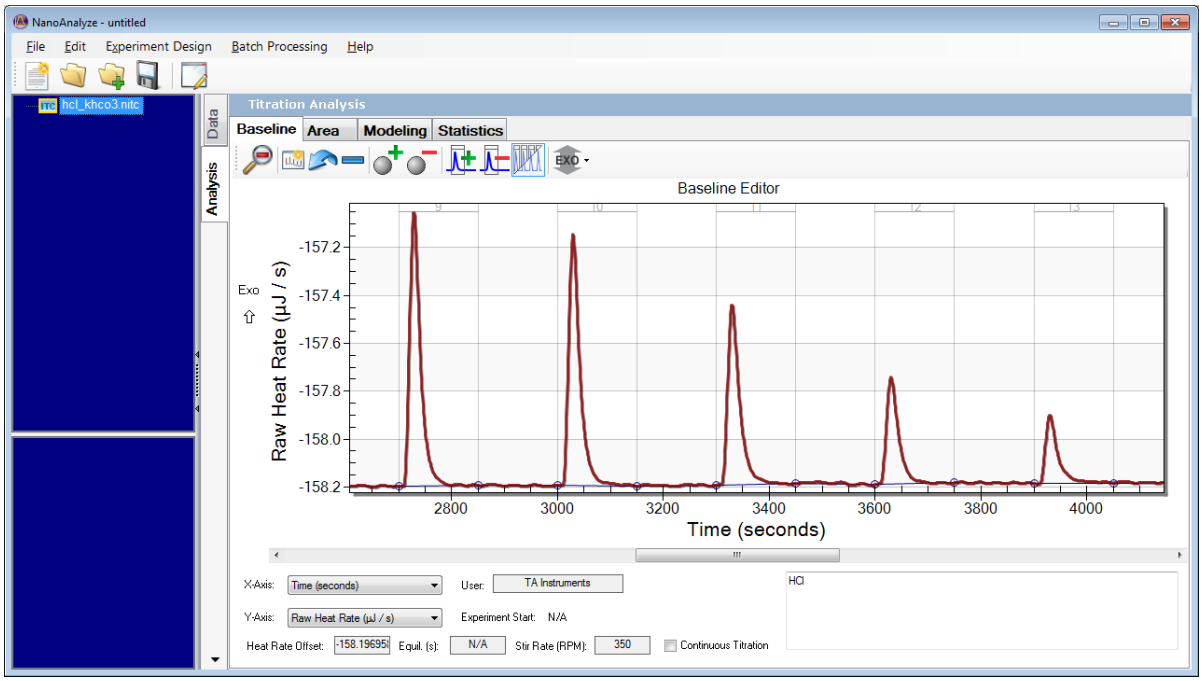


Figure 16 Baseline graph.

Integration regions can be adjusted individually:

1 Magnify the baseline region of interest, if necessary.

2 Adjust baseline pivot points if desired by moving the mouse cursor over the blue circles, then pressing and holding the left mouse button. Move the circle to the desired new position and release the button. Control handles for the start and end times are at the tops of the vertical gray lines. Adjust these times by clicking and dragging with the mouse.

The buttons above the data graph window provide additional controls for adjusting the integration regions as well as the appearance of the data display. The baseline pivot points may happen to fall on the peaks or valleys of the baseline noise. For the best results, the baseline should run through the middle of the noise band, and the integration windows should be just wide enough to include the peak without any additional baseline. The experiment data can be examined at high magnification by first zooming in, then scrolling left to right using the mouse wheel. The integration windows can be adjusted if necessary by clicking and dragging their starting and ending boundaries. The baseline can be adjusted by moving, adding, or deleting the pivot points.

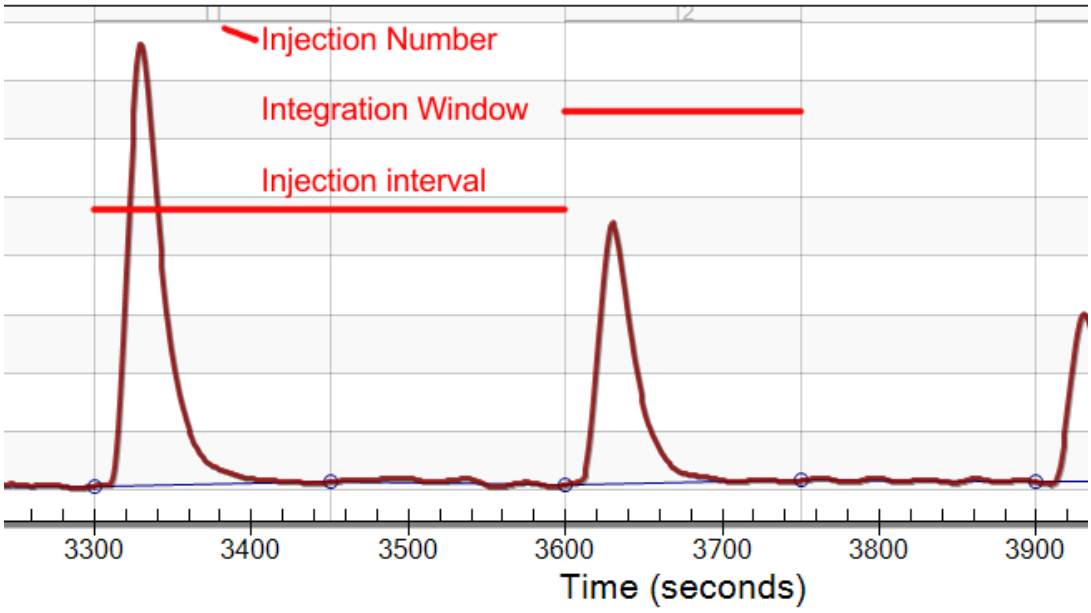


Figure 17 Baseline graph.

Integration regions are bounded vertically (heat rate axis) by the signal trace and the baseline, and bounded horizontally (time axis) by the start and end markers. Areas abve the baseline are negative (heat was liberated from the sample). Areas below the baseline are positive (heat was absorbed by the sample).

3 Select **Undo Zoom** to review the graph for any additional modifications to the integration regions or the baseline fit. Alternatively, navigate along the baseline graph by rolling the mouse wheel (if the mouse is so equipped).

Entering the Titration Data

The sample concentration data and cell volumes are automatically imported when loading a data file that was collected in ITCRun Version 1.7 or above, or DSCRun Version 3.6 and above. With data files that were collected using older program versions, enter the experimental data manually as follows (ITC example shown):

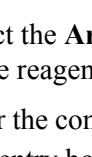
1 Select the **Area** tab and enter the reagent concentrations. **Syringe Concentration** is the concentration of the reagent that was injected during the experiment.

Enter the concentration of the reagent that was loaded into the reaction cell in the **Cell Concentration** data entry box.

2 Enter the sample cell volume of the Nano ITC, if not already present.

These volumes are individually calibrated in microliters and marked on the label at the rear panel of the instrument. Subtract 50 microliters from the printed figure to account for the displacement of the injection needle. The calculations for each injection are adjusted to account for the displacement of fluids out of the cell.

In most cases, the sample cell is completely filled before running the experiment. Do not check the box labeled **Partially Filled Cell**. Areas for each injection peak are automatically calculated.



NOTE: It is possible to perform an experiment with the sample cell only partially filled at the start of the experiment. For best results, the sample volume loaded into the cell plus the reagent volume in the syringe must be less than the sample cell volume. No fluid will be displaced from the sample cell during the experiment. In these cases, check the **Partially Filled Cell** box and enter the volume of reactant mixture that was loaded into the cell.

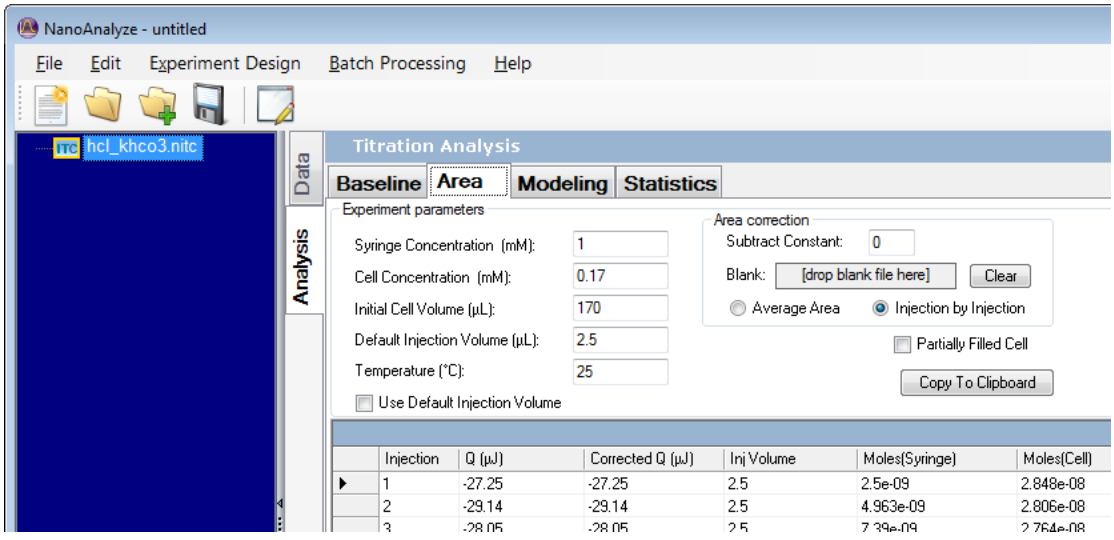


Figure 18 Area tab.

Subtracting the Blank Experiment

1 Select the blank experiment run from the file tree by moving the cursor onto the file name and holding down the left mouse button.

2 Drag the file over to the box labeled **Blank (drop blank file here)**.

3 If the blank peaks are small, they may also be inconsistent in area. In this case, select the **Average Area** option. Otherwise, when blank peaks are a substantial fraction of the titration run, this indicates that the heat of dilution may be relatively large. In this case, the peaks decrease in size during the run and the best results may be obtained by selecting the **Injection by Injection** option.

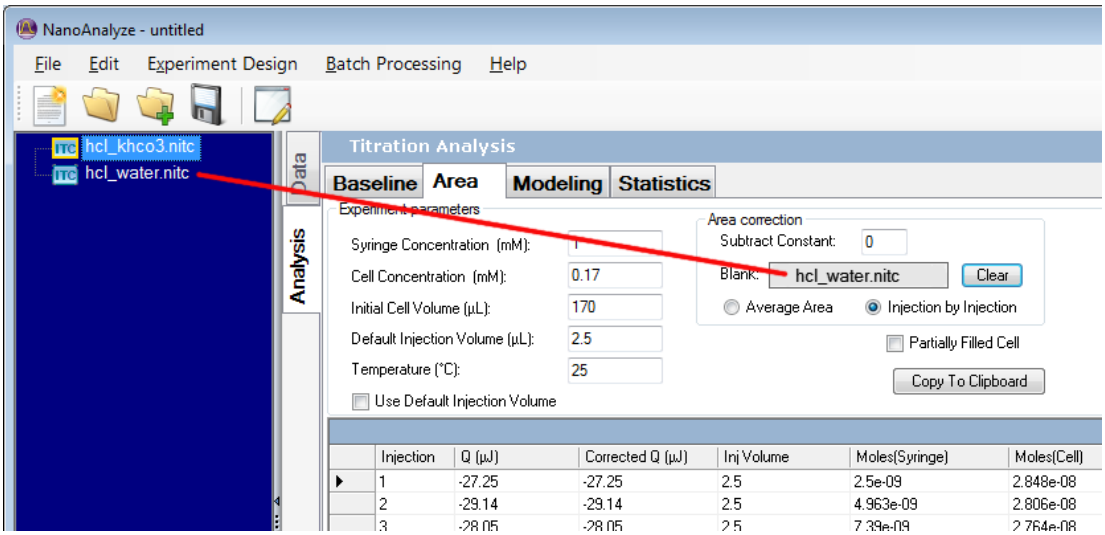


Figure 19 Drag the file to the Blank box.

Hint: If you do not have a matching blank experiment, an approximate blank correction can be made if the reaction has essentially reached saturation, meaning that the final peaks are small and have a flat trend (they are gradually approaching a limiting value). The final injections in the sample experiment may be effectively the same as blanks if the reaction has gone to completion; in some cases they can also be used to approximate the blanks of every injection in the experiment. This method applies a constant value from the **Subtract Constant** (below **Area Correction**) window to every injection in the experiment. Be sure to match the sign of Q as seen in the injection table. Enter an estimate of the final convergence value into the box labeled **Subtract Constant**.

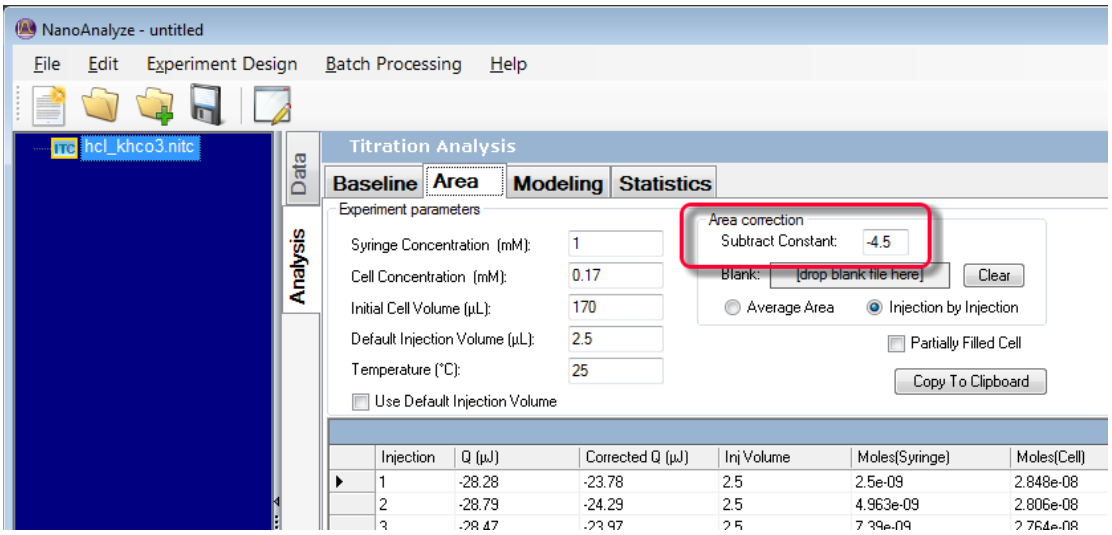


Figure 20 Subtract Constant.

Fitting a Model to Titration Data

Exporting a Previously-Fitted Model to the New Version of NanoAnalyze



NOTE: A new database for the data-fitting models was introduced in NanoAnalyze version 1.2. If you have created or modified any models while using versions prior to 1.2, please follow these instructions to ensure they remain available for use with versions 1.2 or higher. Perform these steps before installing the new version.

1. Open the existing version of NanoAnalyze and select **Edit** > **Model Editor** from the menu.
2. In the model directory tree, right-click on the customized model you wish to preserve.
3. Select **Export model...** and save the file to a directory of your choice. After exporting all desired customized models, exit NanoAnalyze and uninstall the software from your system.
4. Install the new version of NanoAnalyze and start the software. Select **Edit** > **Model Editor** from the menu.
5. Right-click the **Models** folder and select **Import Model...** From the browser window, select the model to be imported. Repeat this step for all the models you wish to import.

Fitting a Model Using the New Version of NanoAnalyze

1 Select the **Model** tab.

The data points in the graph correspond to the integrated areas of the injection peaks. The first peak is typically smaller than the following peaks due to a smaller amount of reagent injected at the first interval. (A common practice to prevent reactions from occurring during the thermal stabilizing before the experiment is to briefly blot the tip of the syringe with a clean tissue. This introduces an air bubble which acts a as a physical barrier to prevent contact between the reagents in the cell and the syringe barrel.)



Figure 21 Model tab.

2 Select a model to be used to fit the titration data.

In cases where the binding model is unknown, it may be necessary to attempt to fit according to more than one reaction model.

3 Click the control labeled **Model** to bring up a list of the available models that have been stored in the program.

4 Click the + to show the list of available models. Multiple models may be selected in this window; a typical use for this is to combine the Independent model with one of the Blank models. Click **OK** when you have selected the desired model or models.

Note that some combinations of models can have matching degrees of freedom. One wouldn't normally combine the Blank (constant) along with the Blank (linear) model; both contain a constant term and the two models would share this in an unpredictable way. The sum of the fits, however, would be mathematically valid.

Hint: In some cases, having too many degrees of freedom can slow down the fitting process. When this happens, several things can help:

- Use the slider controls to obtain an approximate fit
- Temporarily disable some variables from the fitting process
- Adjust the limits of the acceptable ranges of the variables

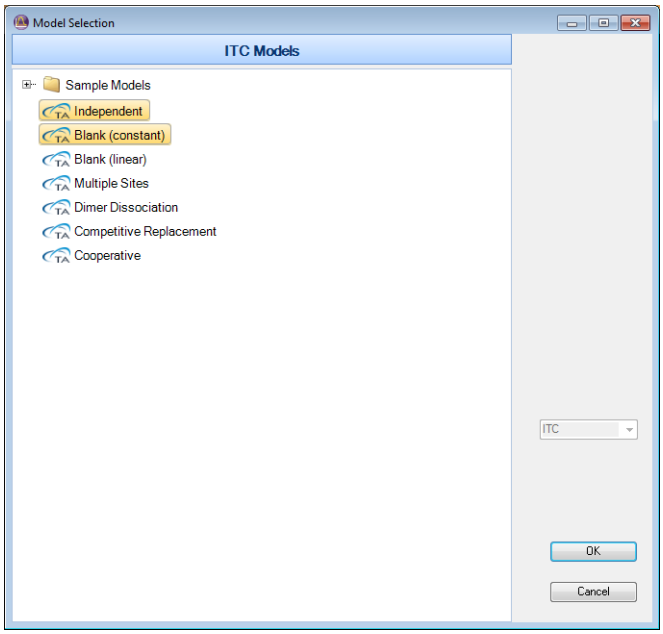


Figure 22 List of available models.

Initial values for the fit parameters are listed above the mathematical description of the model.

5 Choose the desired fitting model, then click **Select** to proceed.

6 The toolbar items allow you to perform the following:



1 Start Fitting the model(s)
2 Stop the Fitting process
3 Fit Settings
4 Variable Constraint (narrows or widens the searchable range)
5 Fit Tolerance (narrower = more precise, but also more time)
6 Stop the Fit when any local minimum is found
7 Exclude the First Data Pont from the displayed curve
8 X-Axis Option: select either Injection Number or Molar Ratio
9 Full Screen mode
10 Exclude Data Points from the curve fit
11 Include Data Points in the curve fit
12 Export Data or Image to Clipboard
13 Export Data Table or Image to File
14 Print the page
15 Save the page settings as the Default
16 Create an Overlay Graph

Figure 23 Toolbar options.

Removing Outlier Data

1 Move the mouse cursor over any data point that will not be included in the model fitting. The cursor changes into a small crosshair when the cursor is placed correctly.

2 Click the left mouse button to deselect the data point. Deselected points appear as gray dots.

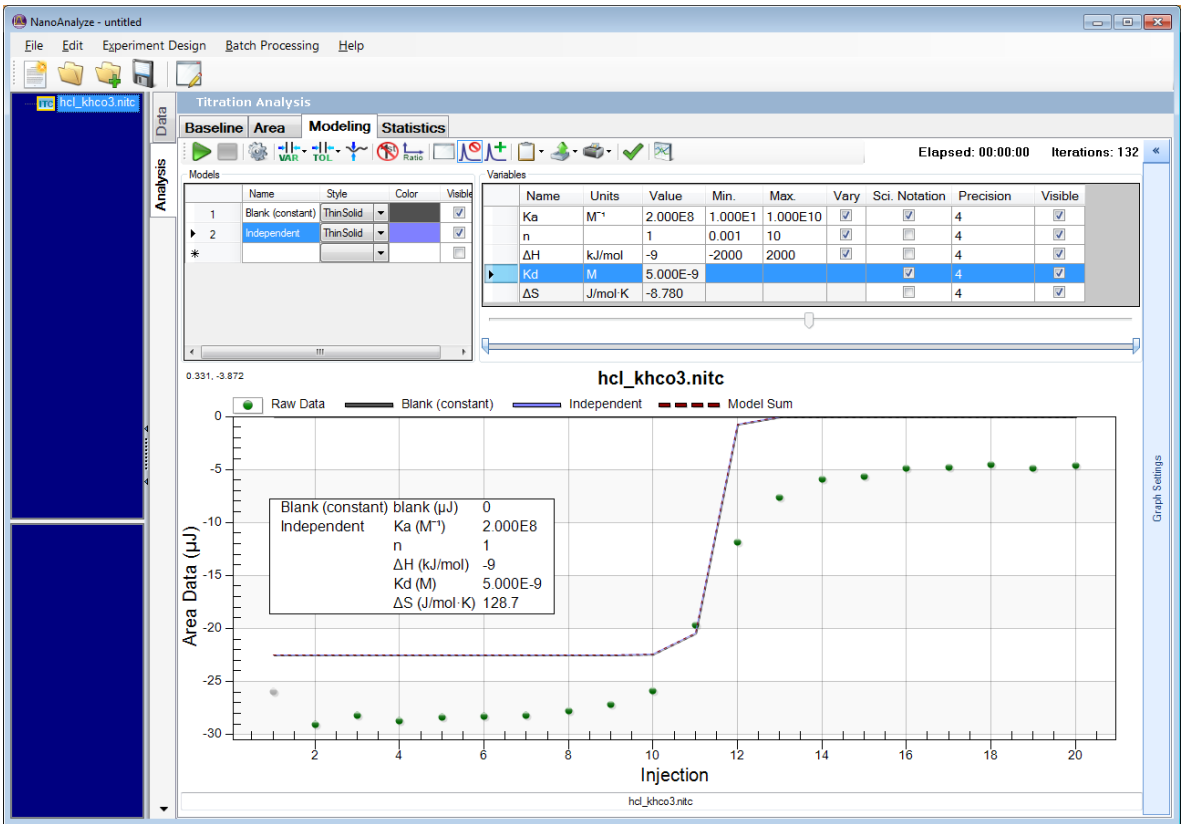


Figure 24 Deselected point.

Starting a Fit

Click **Start Fit** to begin the fitting process. The fit process runs a series of attempts, each of which improves the fit. In some cases, the time limit or the maximum permitted number of attempts expires before the fit is optimized. If this happens, simply click **Start Fit** again.

Adjusting the Model Fitting Behavior

The model is an equation used to describe the experimental data curve. At the bottom of the **Model** tab is a graph displaying the residuals (the differences between the data points and the model equation). The purpose of the model is to fit the equation to the data in such a way that the sum of the residuals is minimized, meaning that the equation curve follows the data points closely. The equation parameters are the thermodynamic variables that have been determined.

Quite often, the fitting algorithm will take a path where eventually the minimization cannot improve. This is considered a local minimum; the best possible fit is the global minimum. There is no guarantee that the first minimum found will be the global; therefore successive attempts will often deliver a better fit. NanoAnalyze software will automatically run several cycles of attempts. At any time that the cycling process halts, the best fit discovered so far will display, even if it was discovered in a previous cycle.

After the model has converged on a preliminary solution, the software runs through successive cycles of exploration of the parameters. The parameters are adjusted by a random amount, and then the fitting process starts again automatically.

1 To adjust the model fitting behavior, use the controls described below:

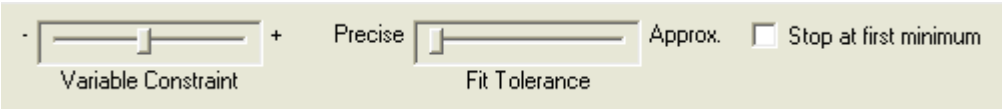


Figure 25 Adjust model fitting behavior.

- The slider labeled **Variable Constraint** controls how far the parameters randomly adjust on each cycle. The adjustment is small when the slider is adjusted to the left **(–)** and larger if adjusted to the right **(+)**. Set the controls towards the right to maximize the search area when a logical initial value and/or range of at least one of the thermodynamic variables is unknown. The value of this slider takes effect when the fit is started and when a new cycle begins after a local minimum is found.
- **Fit Tolerance** controls the magnitude of the acceptance threshold. When the threshold is met, the fitting process halts. Settings towards the left (**Precise**) require very close fits before halting. Settings to the right (**Approx.**) allow the model to halt with a relatively loose fit. If the slider is set all the way to the left, the cycling will continue until the time or count limits in the **Settings** box are exhausted.
- The **Stop at first minimum** control will stop the cycling after the next local minimum is found. This check box and the two sliders can be adjusted at any time, even if the model is running.

2 Press **Start Fit** to proceed. After an initial fit is obtained, the parameter can be checked again and the model re-fitted with all parameters enabled.

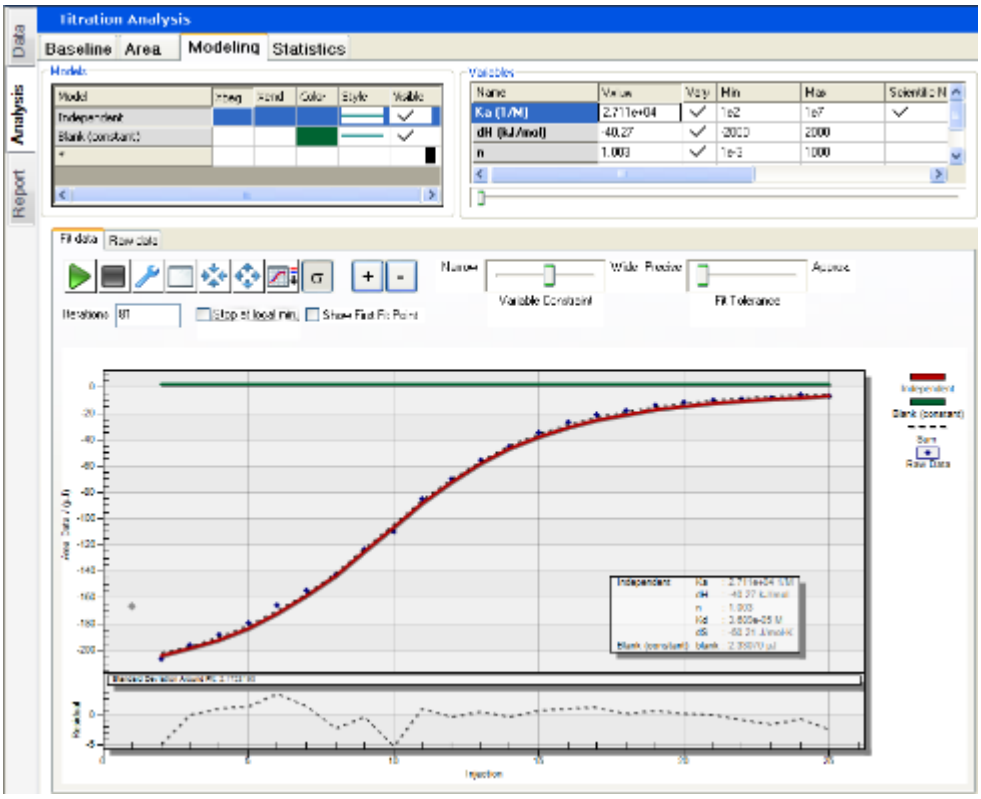


Figure 26 Start Fit.

3 After the best fit to the data is obtained, some points may be revealed as outliers. These can be deselected and the fit process started again. If the set of data points is altered by selection or de-selection, the memory of the previous best fit is discarded.

The fit parameters K (binding constant), dH (enthalpy), and n (reaction order) are listed in the upper right of the program window and in the parameter table inside the chart area. Control buttons for repositioning the parameter table and the legend are located above the chart.

To select a different font size, right-click inside the chart area and select the menu item **Font Size**.

Working with Nano DSC Raw Data Files

The following describes how to open, view, analyze, and print data with Nano DSC raw data files.

Opening a Nano DSC Raw Data File

1 Select **Open** in the **File** menu.

2 Use the drop-down list to select the folder that contains the DSC data files.

3 Select data file type **DSC Files** (***.dsc**) from the drop-down list at the bottom of the window. Files created in previous versions of DSCRun (***.scn**) can also be opened.

4 Select the experiment data file that you wish to load, then click **Open**.

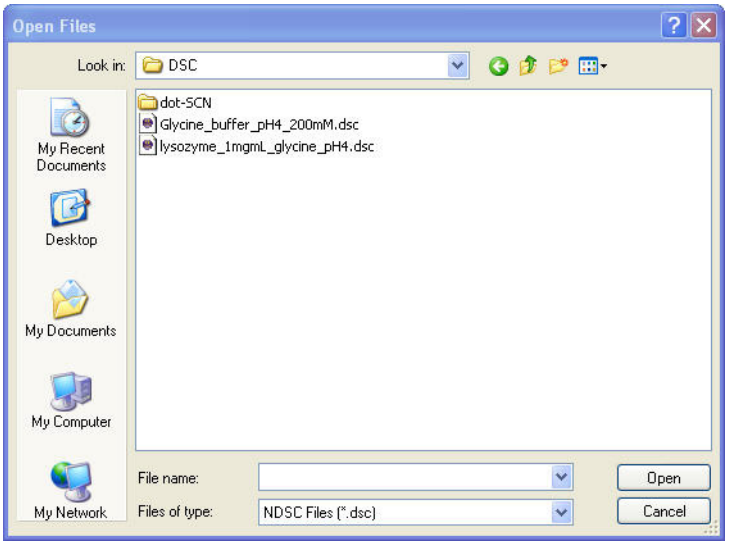


Figure 27 Select experiment data file.

NOTE: Multiple files can be loaded at the same time if desired. Select the first file by clicking on the name with the mouse. Select a range by holding down the **Shift** key while clicking on the last file, or select any number of individual files in any order by holding down the **Ctrl** key while clicking the files with the mouse.

5 When the data file has loaded and appears in the file tree, click on the titration experiment file in the file tree to load the file for analysis. The data file appears in the file tree and a table of the measured heat data appears in the **Data Columns** tab on the right side of the program window.

6 A buffer scan experiment is often performed along with the sample scan. Buffer scans include the heat effects that result from heat flow changes strictly due to an individual buffer. For a buffer scan, typically the sample cell is loaded with the buffer that was used to prepare the sample. Select **Add file..** from the **File** menu or toolbar and select the buffer scan file to be loaded. You will reference this buffer scan experiment data file in a later step.

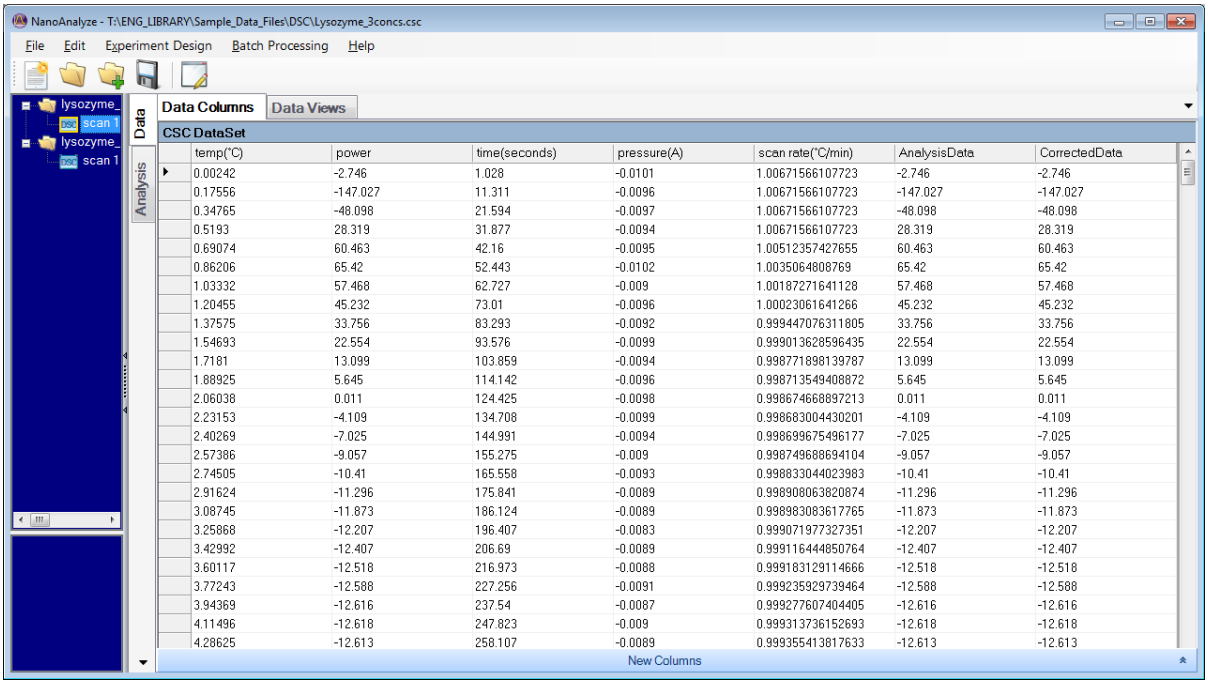


Figure 28

Analyzing DSC Data

To access data analysis functions, click the **Analysis** tab.

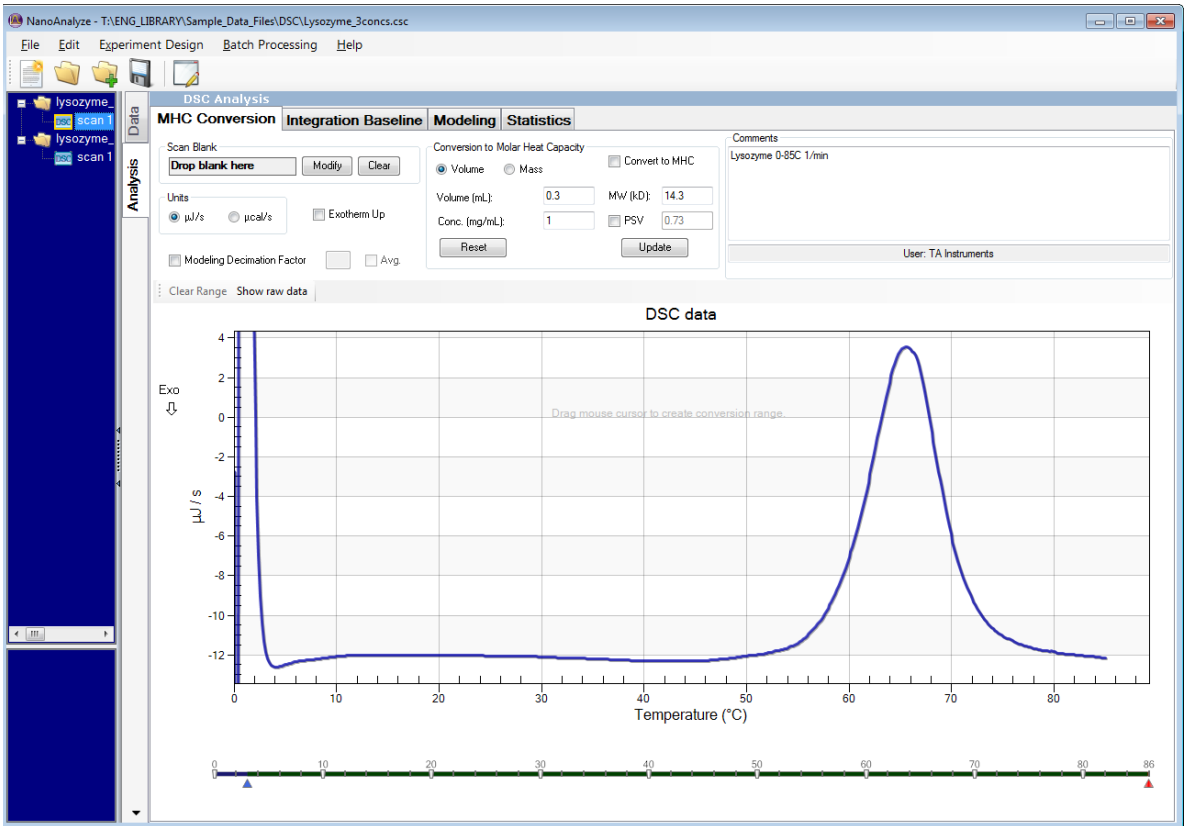


Figure 29 Analysis tab.

Automatically Subtracting the Buffer Scan

To automatically subtract the buffer scan from the sample data, perform the following steps:

1 Select the blank experiment run from the file tree by moving the cursor onto the file name and holding the left mouse button down.

2 Drag the file to the box labeled **Blank (drop blank file here)**.

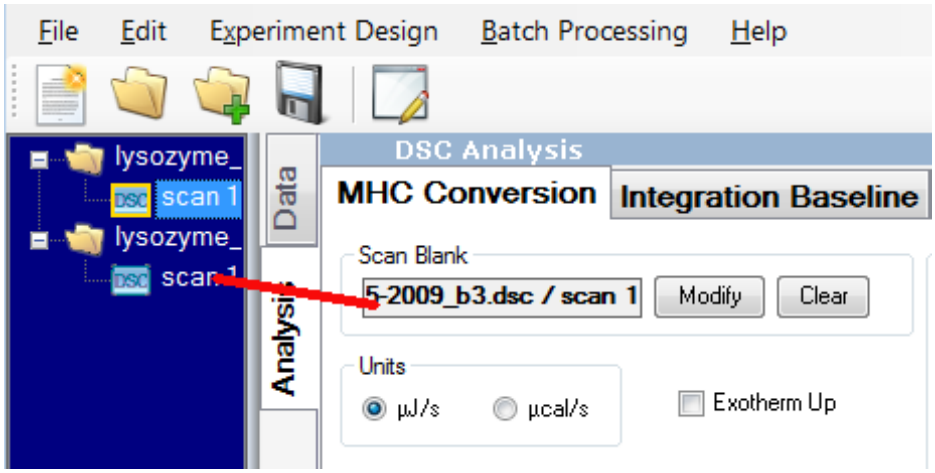


Figure 30 Drag the file to the Blank box.

3 If it has not already been entered, fill in the **Conversion to Molar Heat Capacity** information.

a Enter the volume of the sample cell in the **Volume (ml)** data entry box. This value is typically 0.30 and will always be the same number for an individual instrument.

b Enter the concentration of the sample in the **Concentration (mg/ml)** data entry box.

c Enter the Molecular Weight in kilodaltons in the **MW (KD)** data entry box.

d Click the box preceding the **PSV** (Partial Specific Volume) data entry box. A check mark appears. A PSV value of 0.73 is typical for most proteins in aqueous buffers. Adjust this value as needed for other buffer systems.

e Click the box preceding **Convert to MHC** to place a check mark in the box.

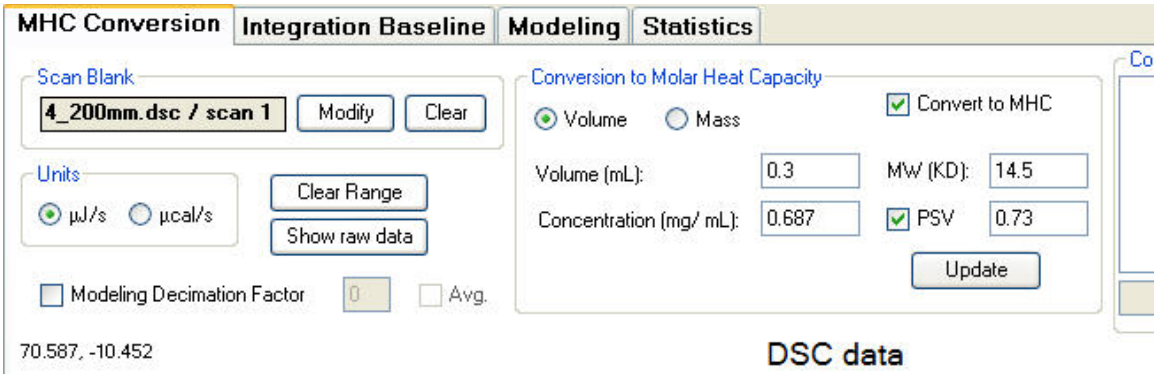


Figure 31 Convert to MHC.

f Click **Update**. The displayed data automatically transforms and the Y scale now displays in **Kcal/mol*K**.

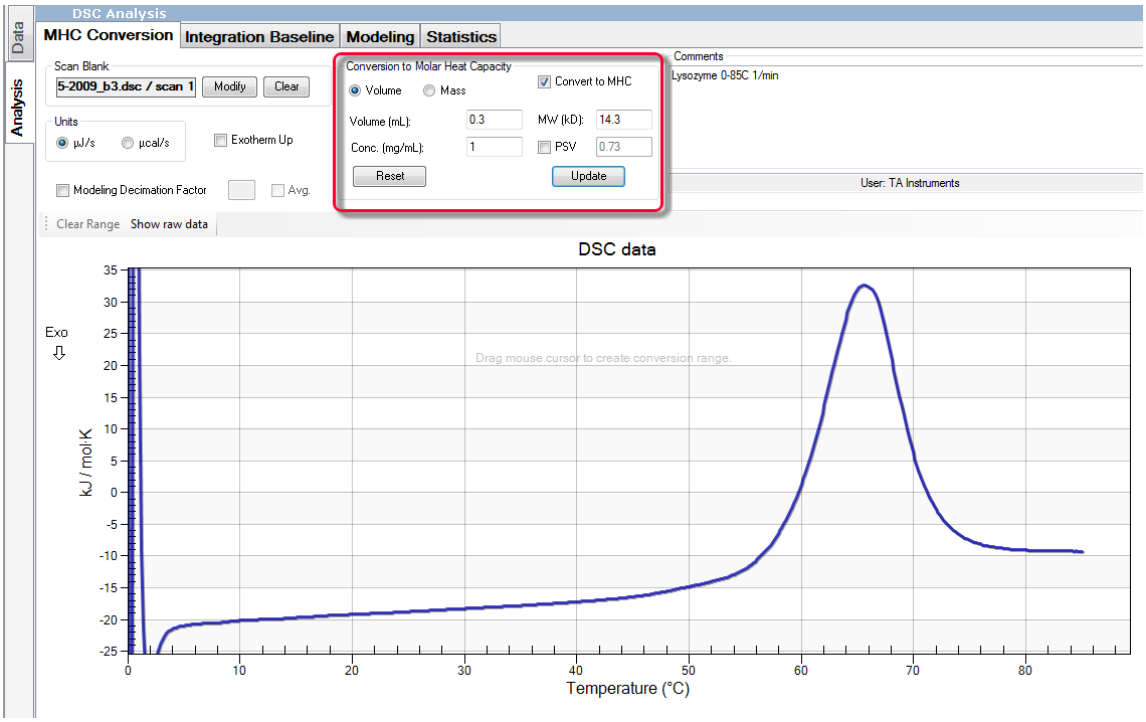


Figure 32 Y scale displayed in Kcal/mol*K.

Selecting Integration Regions

1 Set the baseline range by moving the cursor into the graph display area. The cursor becomes a plus **(+)**. Place the + on the left of the peak where you want to start the pretransition baseline, hold down the left mouse button, drag the cursor to the right to highlight the baseline range and set the post-transition baseline to be considered, and then release the mouse button.

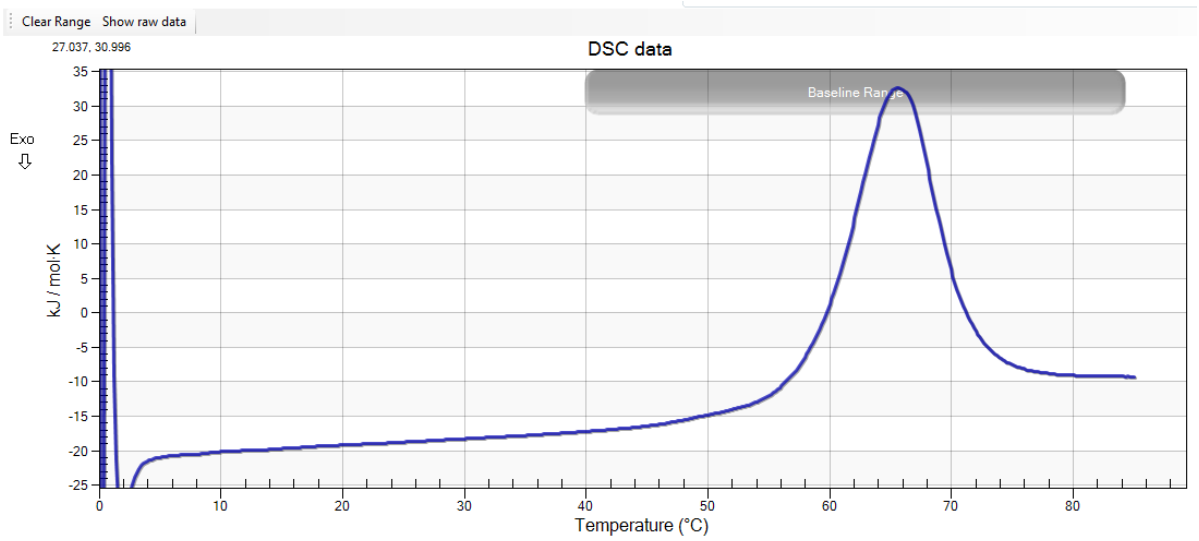


Figure 33 Highlight the baseline range.

2 Select the **Integration Baseline** tab.

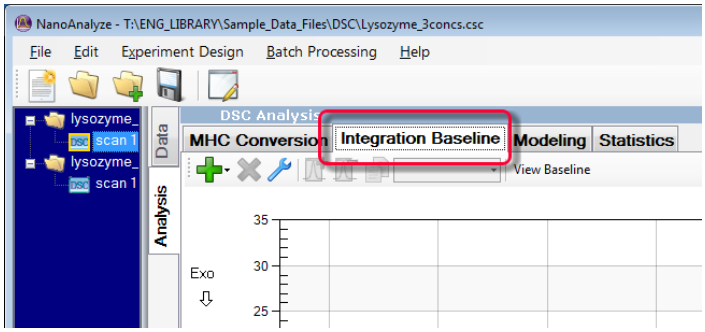


Figure 34 Integration Baseline tab.

3 The following functions are located on the **Integration Baseline** toolbar:

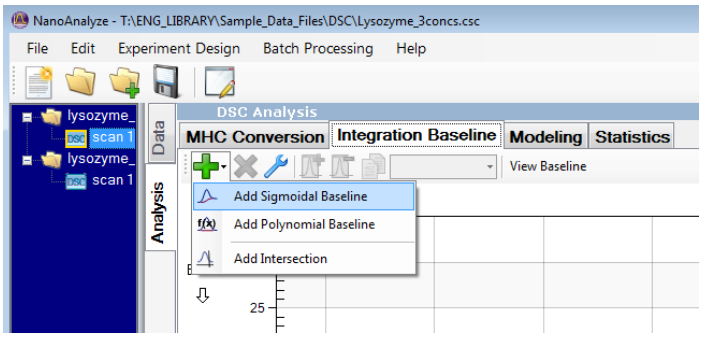


Figure 35 Integration Baseline toolbar.

- **New combination baseline object…**
- **New fit baseline object…**
- **New intersection object…**
- **Delete the selected baseline object…**
- **Settings**
- **Add Region to include in Fit**
- **Remove Region**
- **Copy Equation to Clipboard**

4 Set the pre- and post-transitional baseline by moving the cursor into the graph display area. The cursor becomes a plus **(+)**. Place the + on the left of the peak where you want to start the pre-transition baseline, hold down the left mouse button, drag the cursor to the right to highlight the baseline range and set the post-transition baseline to be considered, and then release the mouse button.

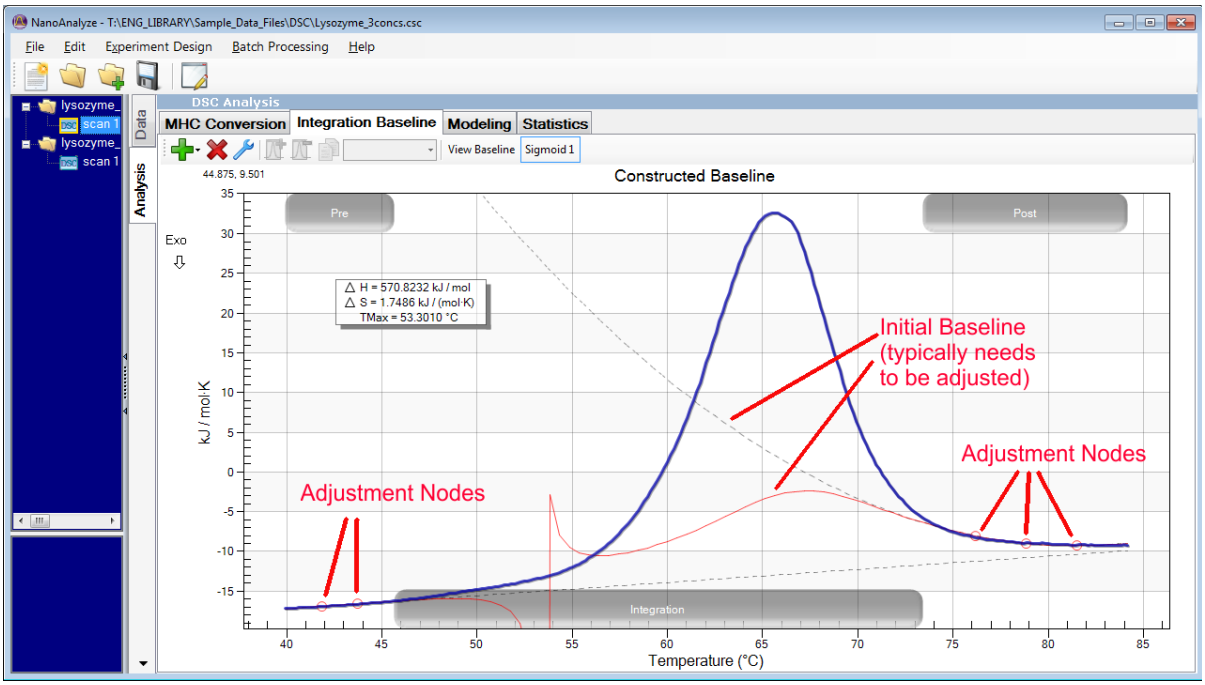


Figure 36 Set the pre- and post-transitional baseline.

5 When the mouse button is released, the preliminary baselines are fitted. The dashed lines are the Pre and Post baselines, the red solid line is the overall baseline with a smooth transition, and the gray circles represent “handles” that can be used to adjust the baseline slopes and curvature. There are two handles for a linear fit; higher order models will have additional handles.

6 Adjust the baseline fits by moving the handles within the chart. In this example, the second-order baseline on the right was adjusted to remove the curvature. Note that the handle can be moved out beyond the actual post baseline region, for ease of accurate adjustment. The enthalpy is calculated on a molar basis at this point.

7 Manually adjust the pre- and post-transitional baseline by clicking and dragging the handles to give a sigmoid curve baseline.

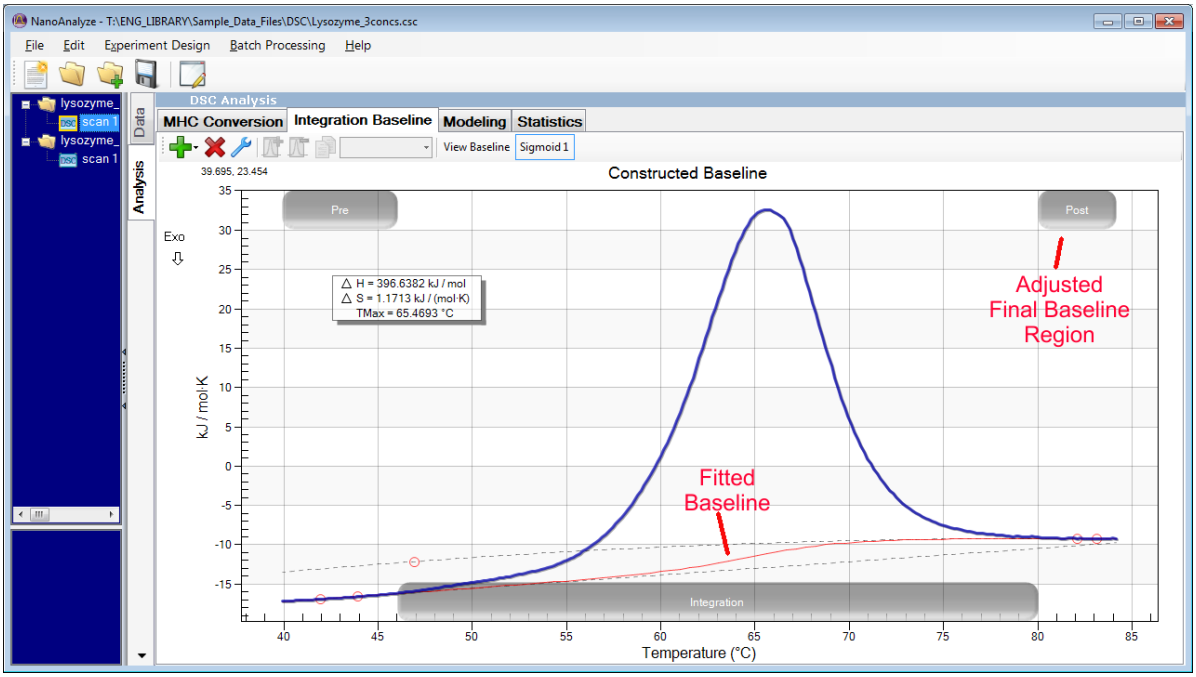


Figure 37 Manually adjust pre- and post-transitional baseline.

Fitting a Model

1 Select the **Modeling** tab. The following toolbar functions are available:

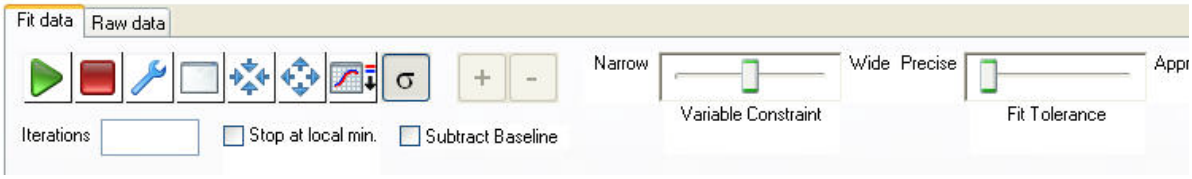


Figure 38 Modeling toolbar.

- **Start Fit**: Starts the model fitting process
- **Stop Model Fit**: Stops the model fitting process
- **Fit Settings**: Set/Adjust the model fitting parameters (Max fit time (s); Precision; Maximum iterations)
- **Graph Full Screen**: Displays graph full screen
- **Show / Hide Residual Plot**: Shows / Hides residual graph at bottom of data graph
- **Results Box Zoom In**: Expands the size of the **Results** box within the graph
- **Results Box Zoom Out**: Reduces the size of the **Results** box within the graph
- **Move Data Legends**: Moves the data legends around the outside of the graph

- **Move Results Box**: Moves Results box to alternate positions within the graph

Additional tools are available on the **Model** toolbar:

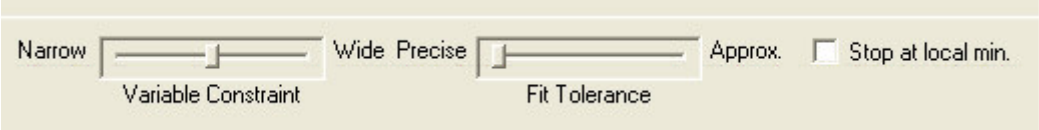


Figure 39 Model toolbar.

- **Variable Constraint**: Slide the bar toward **Narrow** to constrain variables to a narrower range and toward **Wide** to allow variables to consider a wider range.
- **Fit Tolerance**: Slide the bar toward **Precise** for a tighter fit and toward **Approx.** for loose fit.
- **Stop at local min.**: If desired, place a check mark in the box so that during the fitting, when the first local minimum is reached, the fitting iterations will stop at the minimum.

2 Click the asterisk * under **Model** to select the appropriate fit model.

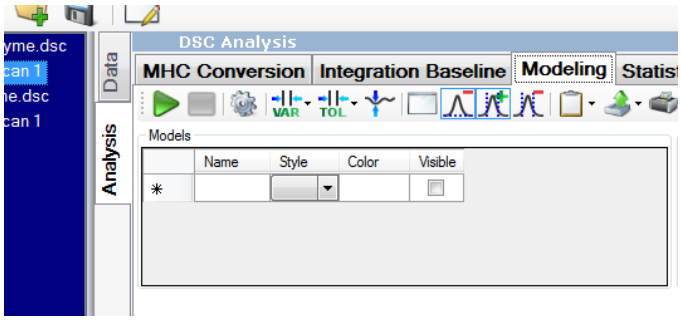


Figure 40 Click the asterisk (*).

3 Click the control labeled **Model** to bring up a dialogue box that lists the available models that are stored in the program. Click the + to show the list of available models.

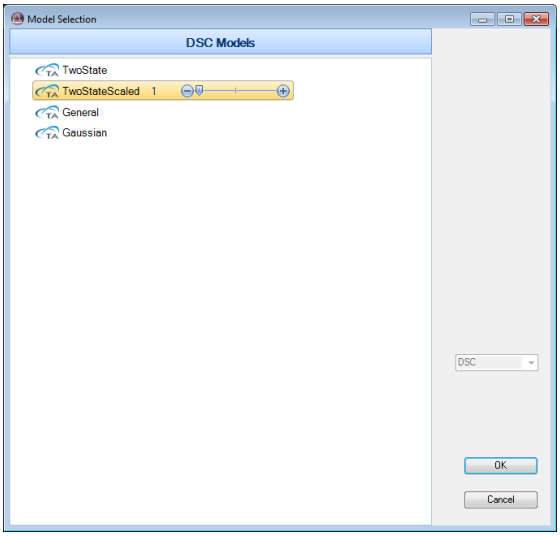


Figure 41 Model Manager.

4 Highlight the desired fitting model, then click **Select** to proceed. The red line represents the fitted model from the default values in the model.

5 Available models are:

- **Two State**: Typically used for single transition peaks
- **Two State Scaled**: Typically used for single transition peaks and particularly useful in adjusting protein concentrations in the cell
- **General**: Typically used for single transition peaks when calculation for heat capacity is required

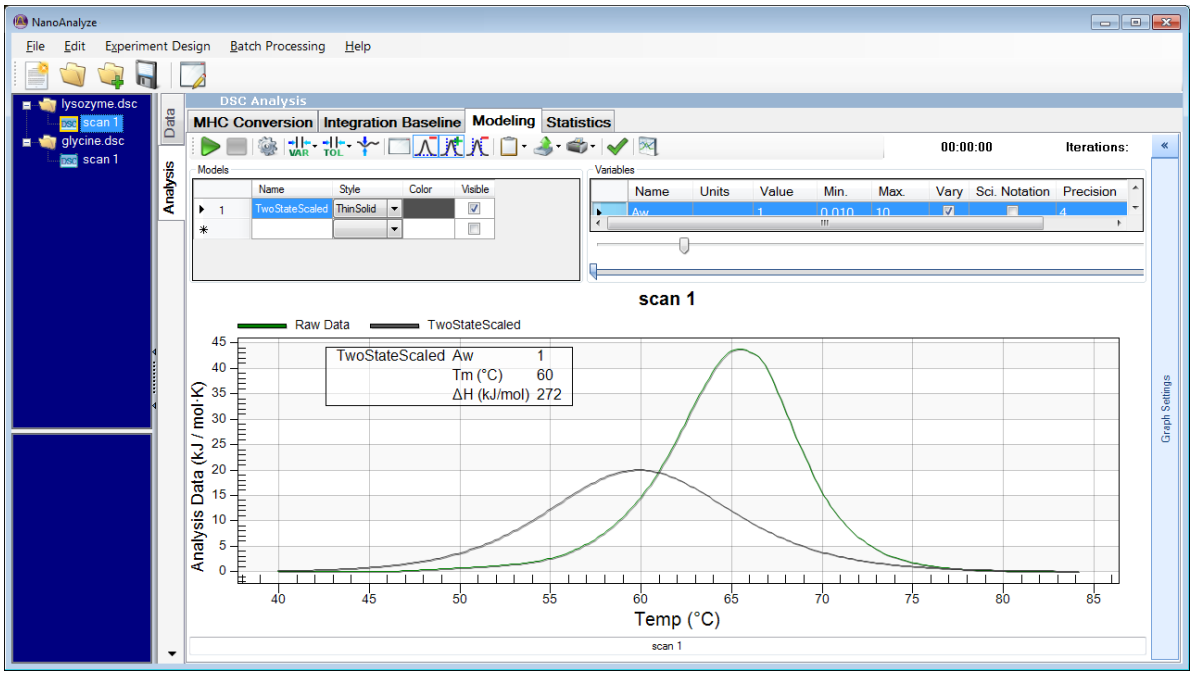


Figure 42 Models.

Multiple models may be selected in this window, and multiple instances of a model may be included. A typical use for this is to model multiple transitions. Click **OK** when you have selected the desired model or models.

Hint: In some cases, having too many degrees of freedom can slow down the fitting process. When this happens several things can help:

- Use the slider controls to obtain an approximate initial fit
- Temporarily disable some variables from the fitting process
- Adjust the limits of the acceptable ranges of the variables

Be sure to run a final automated fit with all parameters enabled. In particular, it is often helpful to impose strict limits on the available range of Tm for each model.

6 Place a check mark in the **Subtract Baseline** box.

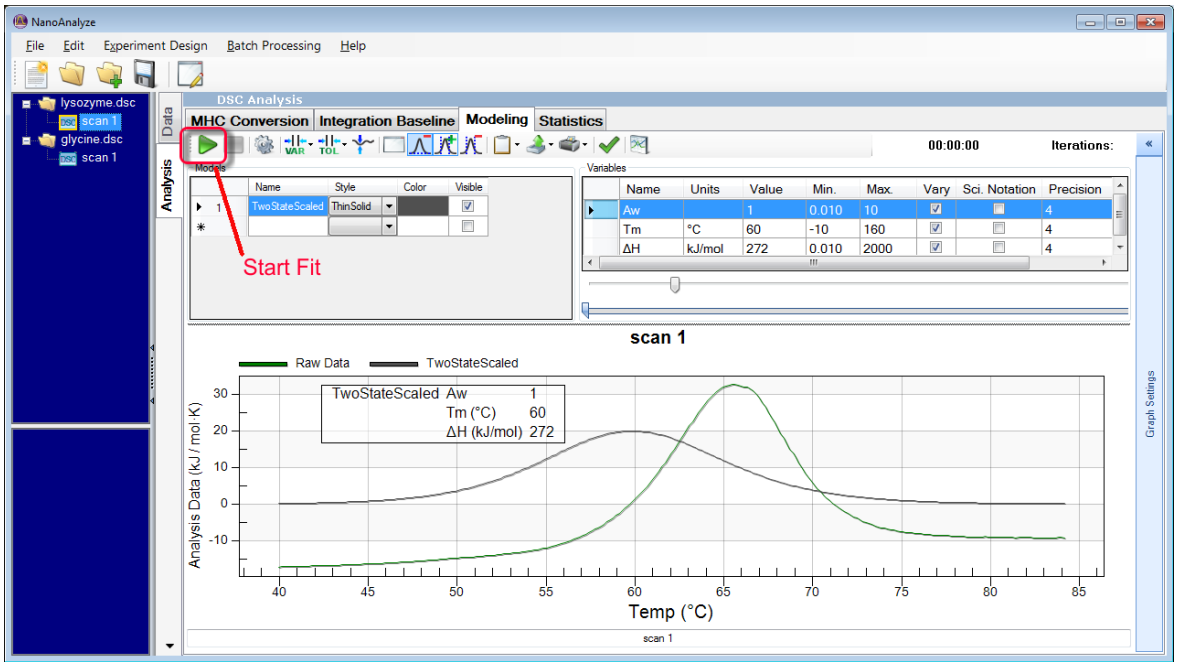


Figure 43 Select Subtract Baseline.

7 Click **Start Fit**. The red line represents the fitted model, and the calculated thermodynamic data updates in the **Variables** section of the screen.

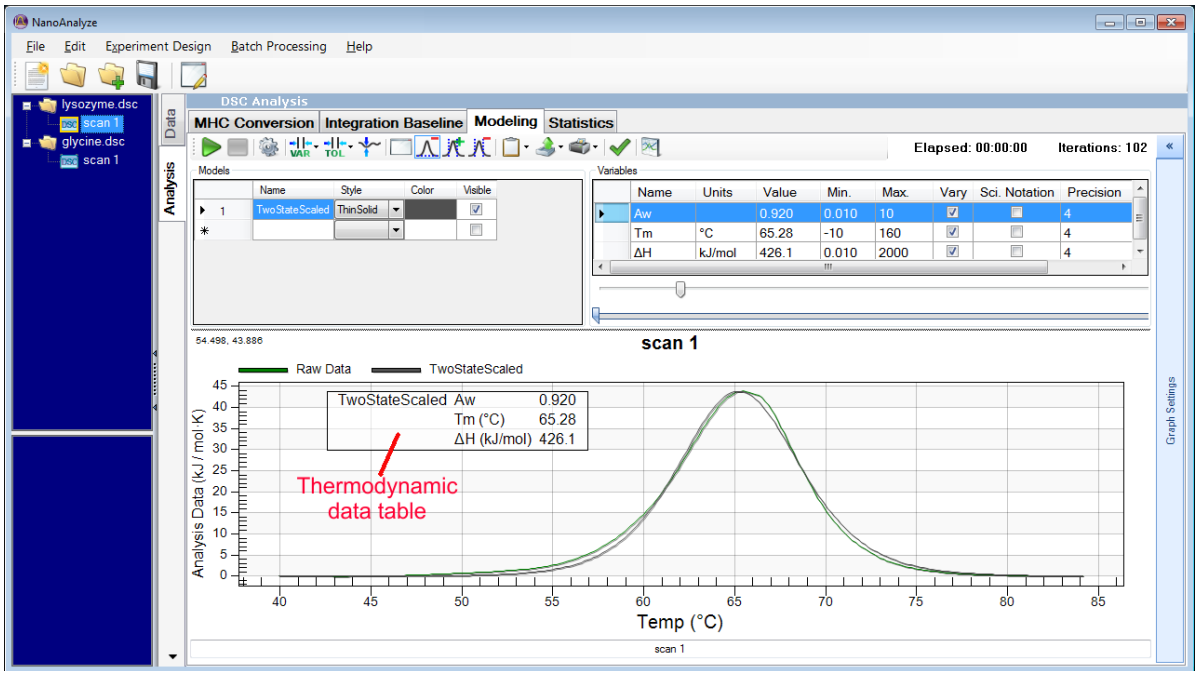


Figure 44 Thermodynamic data.

8 The final calculated data displays in the fit parameters box at the top of the data analysis window and in the data box inside the graph. To adjust any starting numbers, go back to the **MHC Conversion** and **Integration Baseline** tabs and make desired changes, then refit with the model.

9 The **Residual Plot** displays at the bottom of the fitted graph. This displays how close or far away the fitted curve is from the actual raw data.

DSC Batch Processing Mode

NanoAnalyze can rapidly perform simple analyses on groups of DSC data files with the Batch Processing feature. Basic thermodynamic values are calculated and MHC conversion is available. Batch processing provides a rapid preview of the basic DSC experimental results Tmax, ΔH and ΔS. Experiments that warrant further, more detailed attention can subsequently be modeled in the Modeling tab of NanoAnalyze. All processing parameters and initial thermodynamic results that result from batch processing are passed back to the individual data files for convenience.

1 First, load a collection of files into NanoAnalyze. Multiple files can be selected as a group by using the standard controls within the **File**>**Open** or **File**>**Add Files** dialog boxes. To open a group of files, click the first file to select it, then use Shift-Click on the final file in the set (hold the Shift key while clicking the mouse button). All the files in the range will be highlighted. Alternately, individual files can be added to the multiple selection by using Ctrl-Click for each file (hold down the Ctrl key while clicking the mouse button). Then click **Open**. Check the file tree on the left side of NanoAnalyze to verify that the desired set of files for batch processing has been loaded.

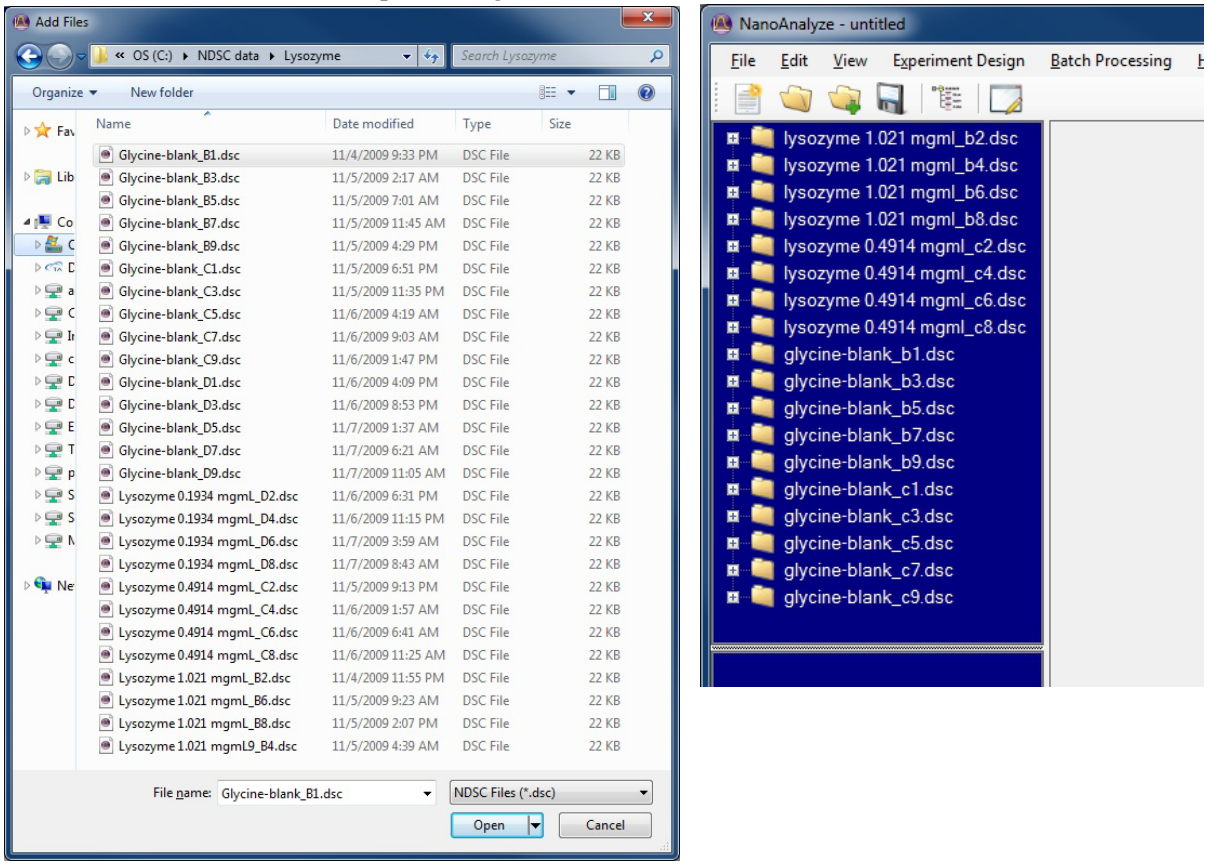


Figure 45 Load files (left figure), file tree (right figure).

2 Select **Batch Processing**>**DSC** from the menu bar.

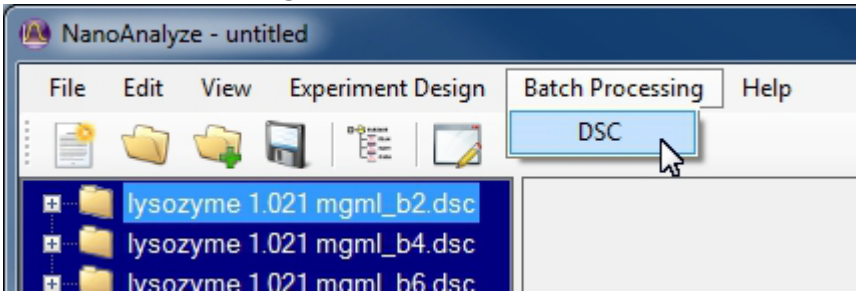


Figure 46 Entering Batch Processing mode.

The Batch Processing dialog box displays. The window is divided into three sections:

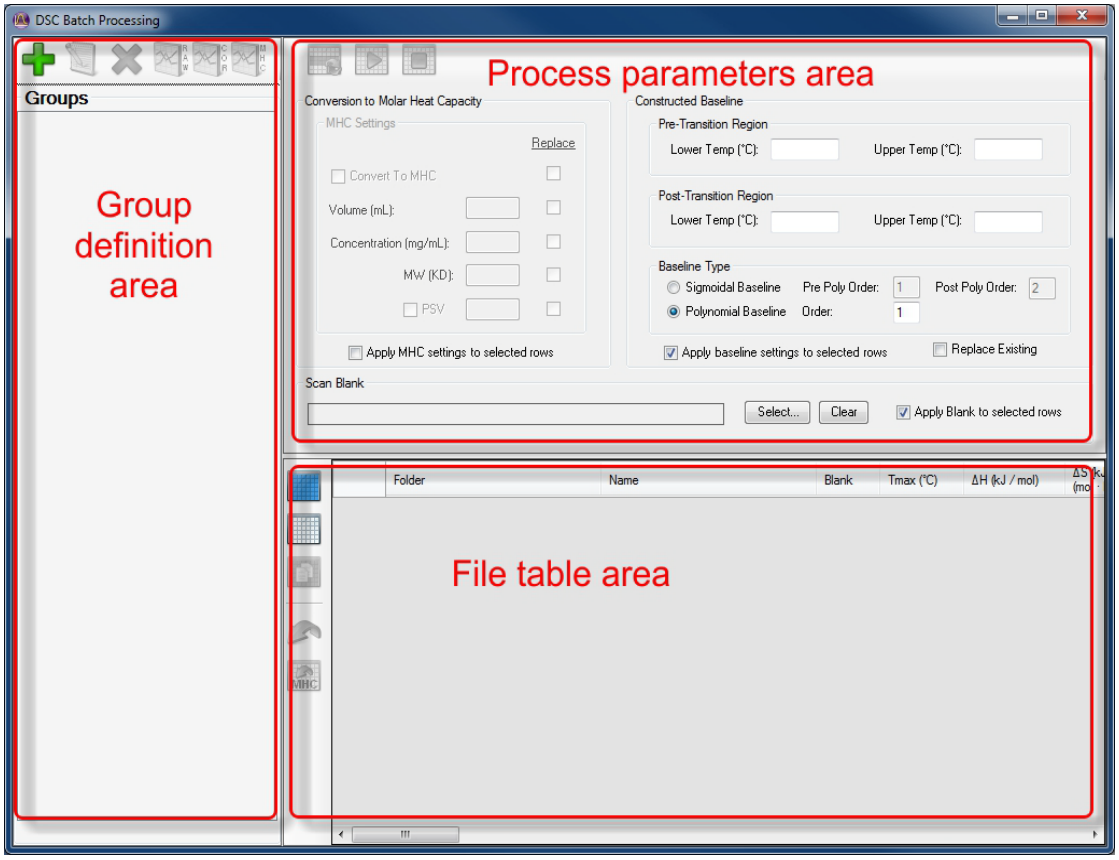


Figure 47 Batch Processing dialog box.

- The group definition area contains tools for assembling and previewing groups of files. Groups are displayed in the space below the Groups toolbar after the groups have been defined. The group is a tool to collect files which are to be processed in a similar way, completing the processing with a single click without duplicated effort. It is easy to set common parameters for the group but individual files can be assigned any unique settings as required. The results can be graphically previewed together as a set, and Overlay Graphs can be made for each group.

- The process parameters area is the data entry control to set the processing parameters for the selected file or sets of multiple files.
- The file table area displays the values of the process parameters along with the thermodynamic results from the processing. The scroll bar allows viewing of every column in the table. For convenience the file name always remains visible.



Add group



Edit group



Delete group



Create raw data overlay graph



Create corrected data overlay graph



Create corrected data overlay graph

Figure 48 Group icons

3 To assemble the groups, click the green + icon at the upper-left of the DSC Batch Processing dialog. The **DSC Group Items** dialog displays.

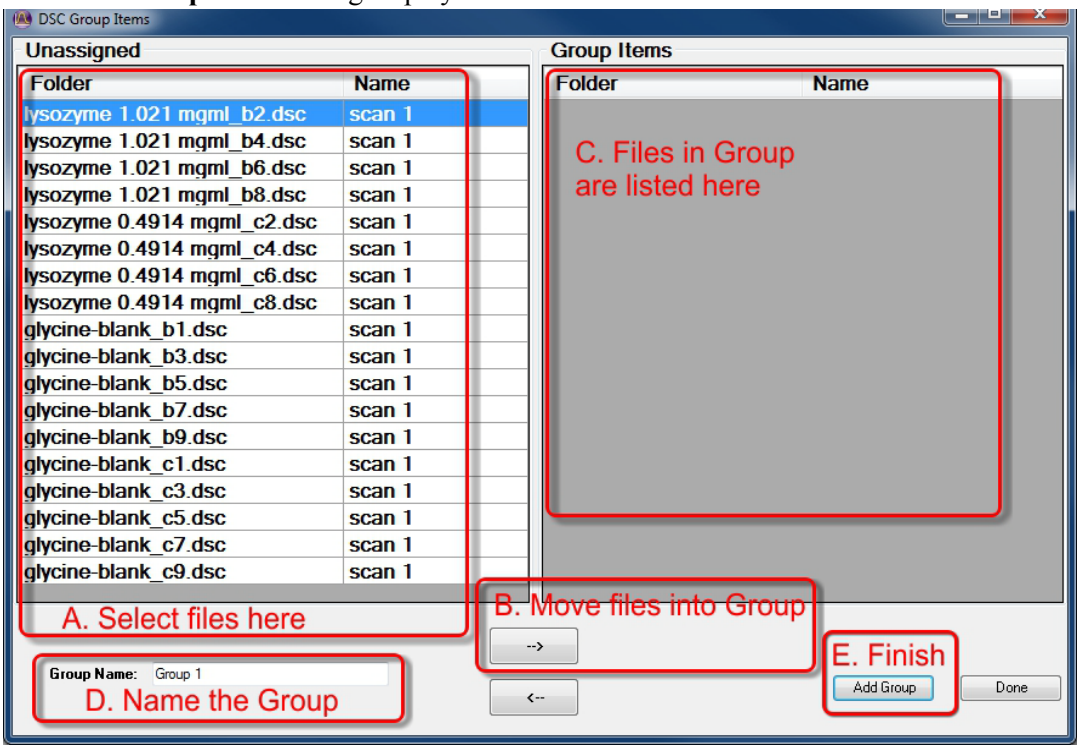


Figure 49 Group Definition dialog.

A Select the set of files that logically belongs together as a Group. The standard multiple-selection controls Shift-click and Ctrl-click are available.

B Click the right arrow button to add the selected files to the group. The labels for these files will moved from the **Unassigned** window (left side) over to the **Group** items window (right side).

C Files that have been assigned into the group will appear on the right side. If any files were accidentally added that do not belong to the group, they can be deleted by first selecting them on the **Group** side, then clicking the left arrow. The file labels will be removed from the **Group** window and will reappear in the **Unassigned** window.

Figure 50 shows a group that is partially defined and is ready to add more files.

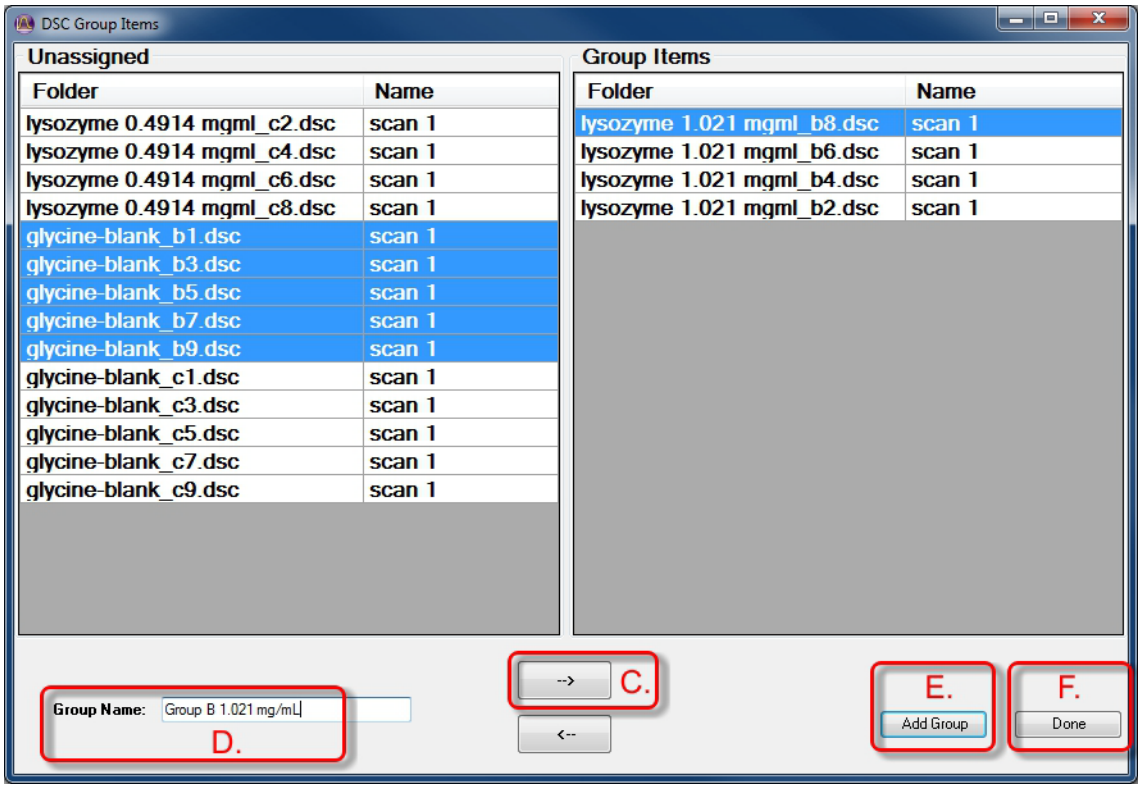


Figure 50 Defining a Group.

D If multiple **Groups** are to be created, provide a unique name for each in the entry box at the lower left (shown in Figure 50).

E When the list of files on the right side is complete and correct, complete the creation of the group by clicking **Add Group** (shown in Figure 50).

F If desired, more Groups may be defined by following the above steps A through E again. When all desired Groups are created, click **Done** on the lower-right portion of the screen.

When the desired groups have been created, click **Done** to return to the Batch Processing window. Figure 51 shows the layout of the window and the sections of related data file table editing controls.

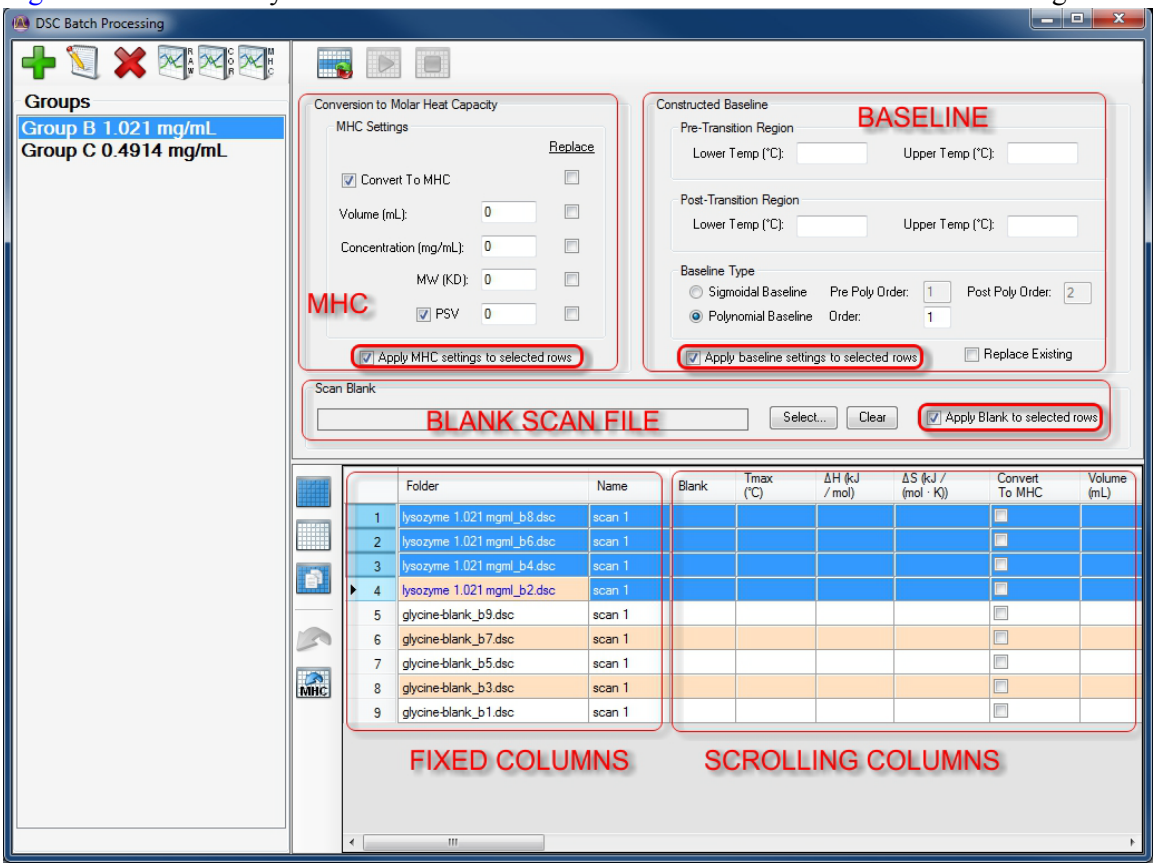


Figure 51 Batch processing controls.

4 Next, define the process parameters. The processing variables can be set up the same for all members of a Group, or with individualized settings for any member as needed. The first step is to select the lines in the table that are to be edited.

Several of the icons at the left side of the File Table Area provide functions that assist with the file selections.



Select all rows in table



Deselect all rows in table



Copy selected rows in table to Clipboard

Figure 52 Table functions.

Mouse clicks can be used along with the Shift or Ctrl keys as appropriate to assist with making the selection. Figure 51 shows four data file rows selected, as indicated by the blue highlighting.

When the desired rows have been selected, changes or additions to the process settings may be entered at the top of the page in the Process Parameters area. Sections are provided for parameters associated with blank experiment scans, molar heat capacity, and baseline. Select the **Apply** checkbox for any section where changes to settings will be made. When all the desired settings have been entered, click on the **Update table with settings** icon  .

To ensure that changes to settings are updated in the file table, check to see that all enabling settings such as the appropriate **Replace**, **Replace Existing**, or **Apply** boxes are checked.

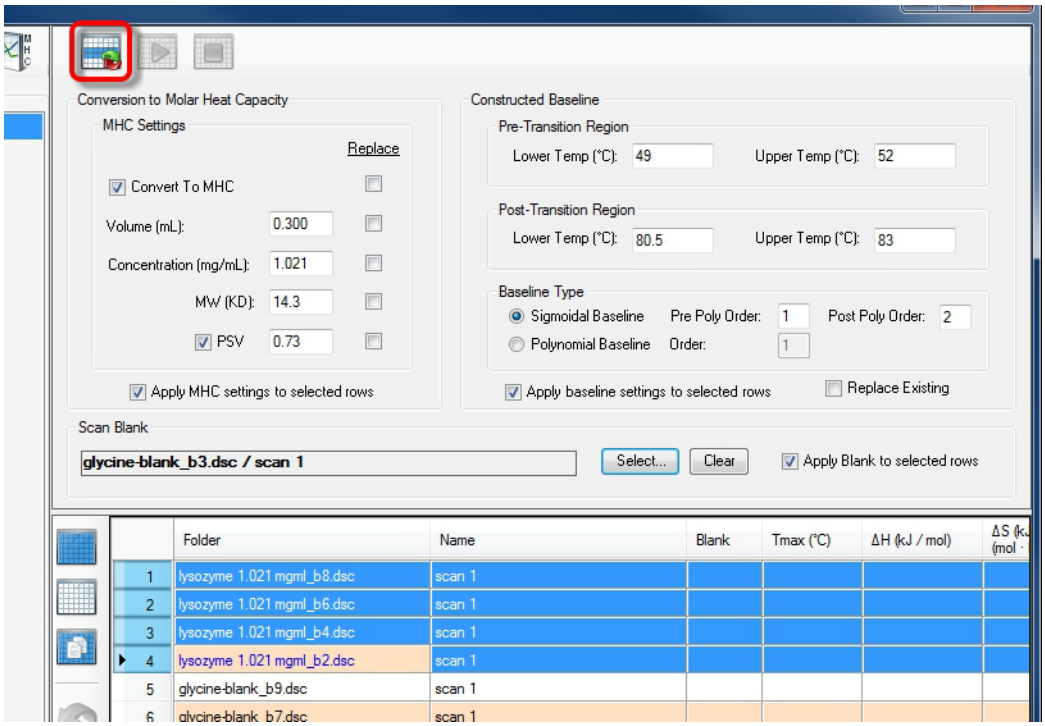


Figure 53 Entering changes to process settings.

After entering new settings into the table, every cell with altered settings will appear with a red highlight, as seen in the **Blank** column in Figure 54. Cells which are altered but not selected will appear with a yellow highlight.

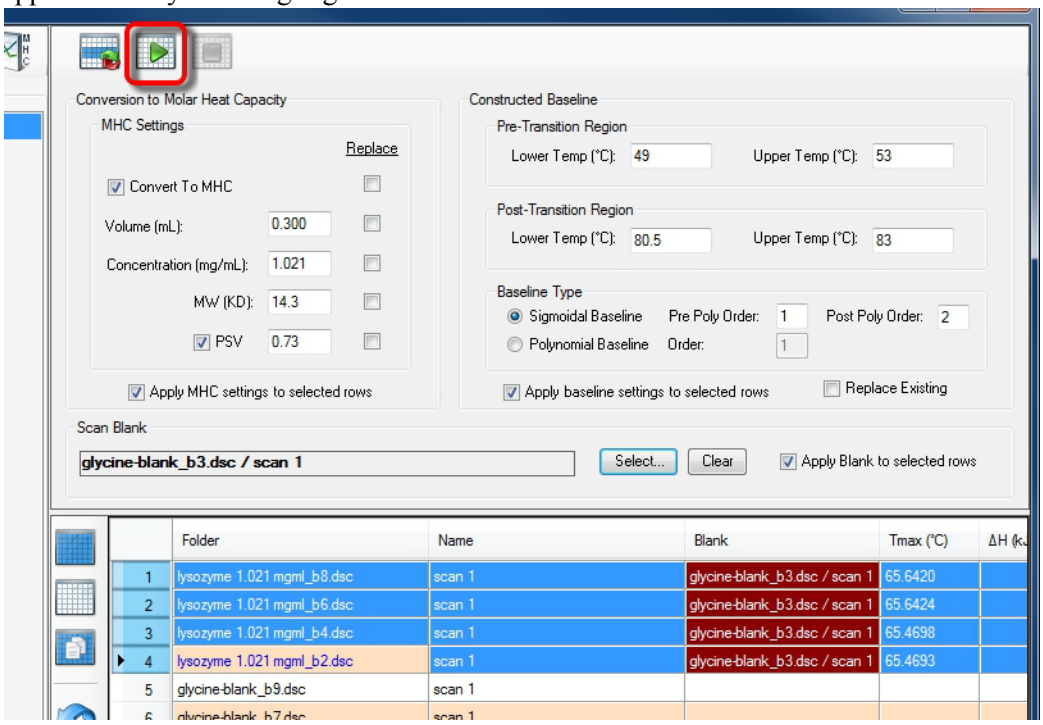


Figure 54 Files ready to process.

5 The next step is to process the data files. The button for the action **Apply table changes to the dataset**  will now be enabled; click the button to process the datasets.

Each selected data file row will be processed one by one in sequence. For each line, blank data files will be subtracted, baselines will be calculated according to the "pre" and "post" ranges, the baselines will be subtracted, and thermodynamic variables will then be calculated. The thermodynamic results will be filled into the table as seen in Figure 11.

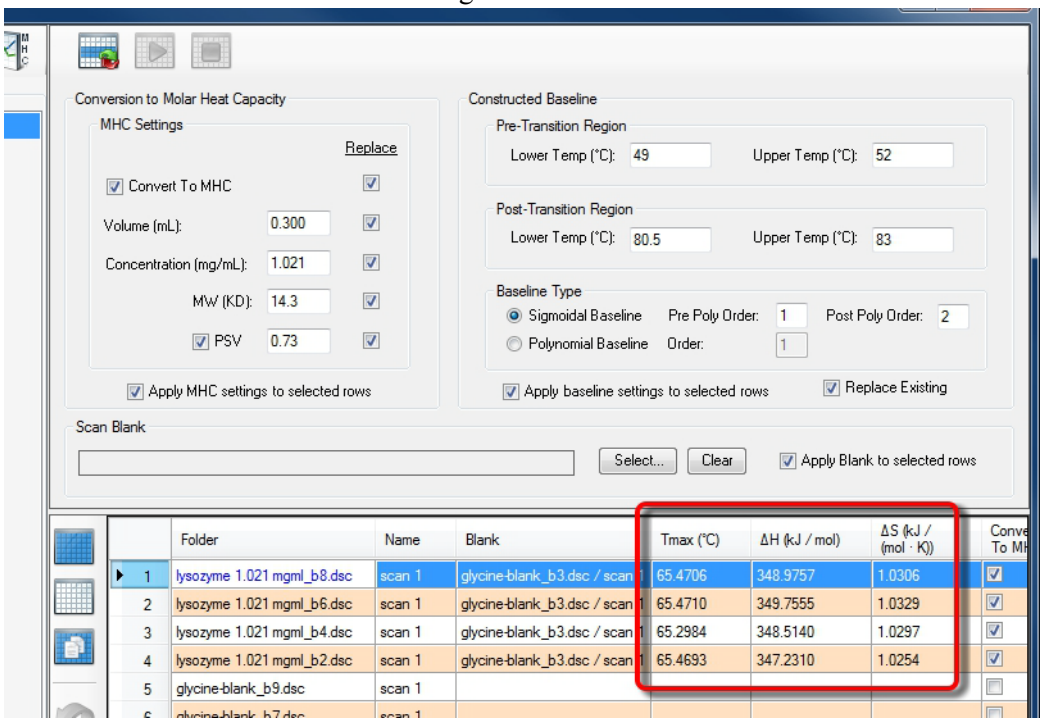


Figure 55 File processing completed.

If unexpected results arise, this may have been caused by signal noise causing changes in the details of the baseline paths. Exit batch processing mode, select the file(s) in question, and view the **Integration Baseline** window. The baseline can be shifted by moving the adjustment handles (the small open circles). When the baseline is redrawn, the thermodynamic values will be updated. If any files warrant further study, you can proceed to the **Modeling** tab.

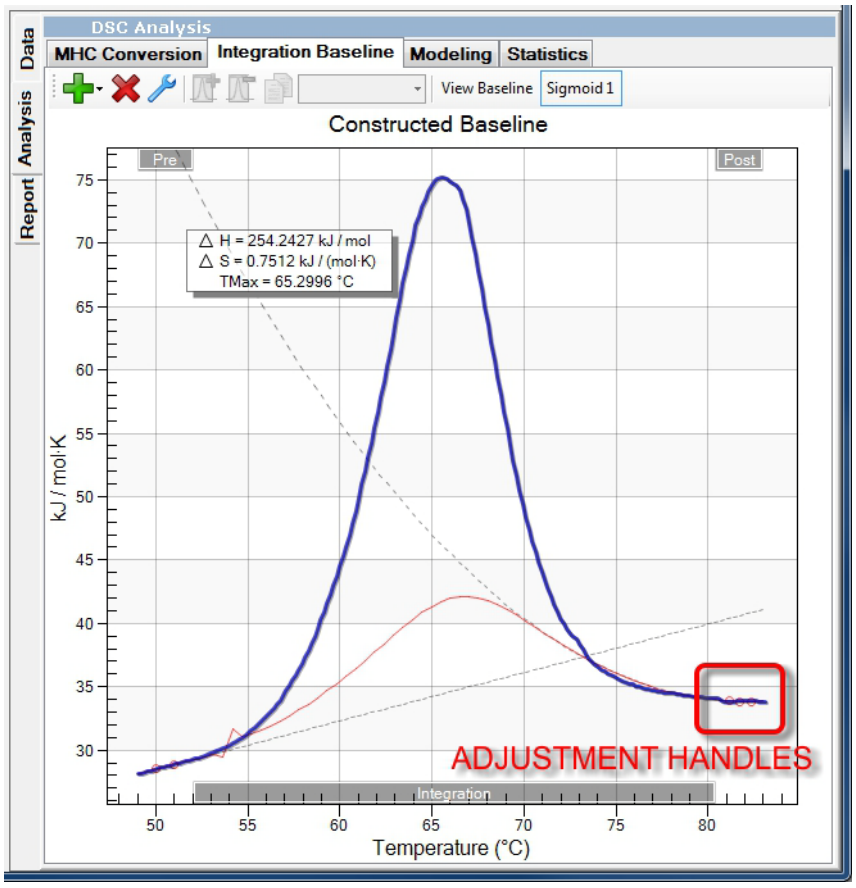


Figure 56 File processing completed.

NOTE: All data is fully synchronized between the batch processing and the standard modes of using NanoAnalyze. Any change to a data file setting made in either mode will also be present in the other mode. You can save the work on the full set of files by saving in the .CSC format from the **File** menu in the NanoAnalyze main window.

Advanced Features

Statistical Analysis of Models

Once a model has been satisfactorily fitted to a set of experimental data the parameters are considered to be an optimal fit of the chosen model to the experimental data. This is represented with an example of an isothermal titration experiment, as shown below.

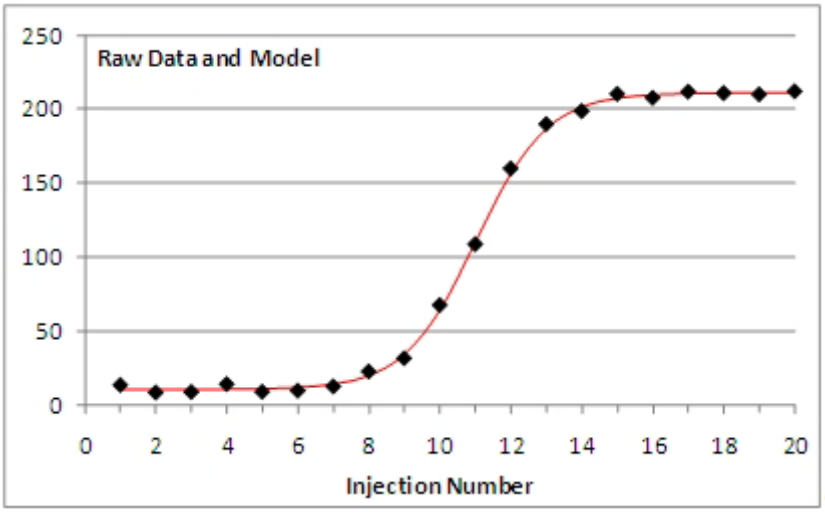


Figure 57 Isothermal titration experiment.

The **Statistics** page is used to calculate estimates of the uncertainty of the model parameters. In multiple trials, a defined amount of variation is introduced into the model and the fitting will be performed again. Each parameter will vary within a Gaussian distribution, which will be characterized.

The first step is to construct a reference set of "perfect" synthetic data points from the model curve. At the x-axis location of each experimental data point, the value of the model equation is calculated. These synthetic data points are idealized and therefore have no scatter, as seen below.

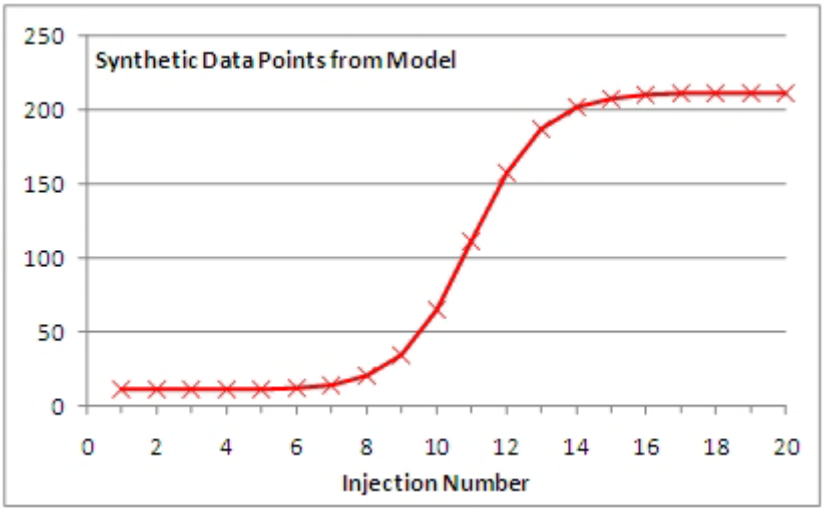


Figure 58 Idealized data points.

The next steps are to introduce a series of trials consisting of temporary perturbations to this idealized data set, re-fit the model for each trial, and record the resulting distributions of each model parameter (with the exception of the blank, if present). This repetitive process is represented below.

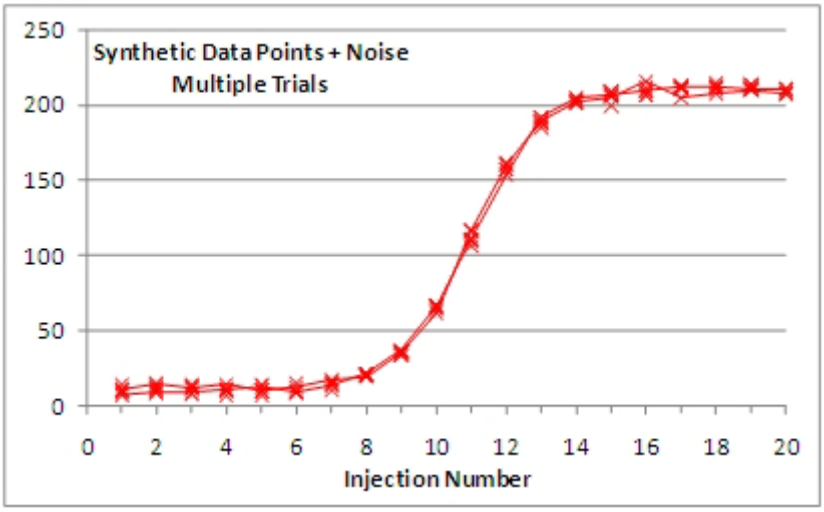


Figure 59 Multiple trials.

Random noise is superimposed onto the heat value at each of the X-axis data points in the reference set in a carefully controlled manner. Each change is randomly generated within a Gaussian distribution that has a standard deviation identical to that of the original data set. This figure is displayed on the **Modeling** page between the windows for the **Model** and the **Residuals**. Each model parameter will also tend to be normally distributed, provided that a sufficiently large number of trials have been performed.

Statistics Work Flow:

1 Fit a model (or multiple models) to the data from an experiment. Be sure that the fitting has been optimized, and that all changes to the model have been finalized before moving on to the **Statistics** page.

NanoAnalyze automatically transfers the values of the fit parameters and the standard deviation around the fit.

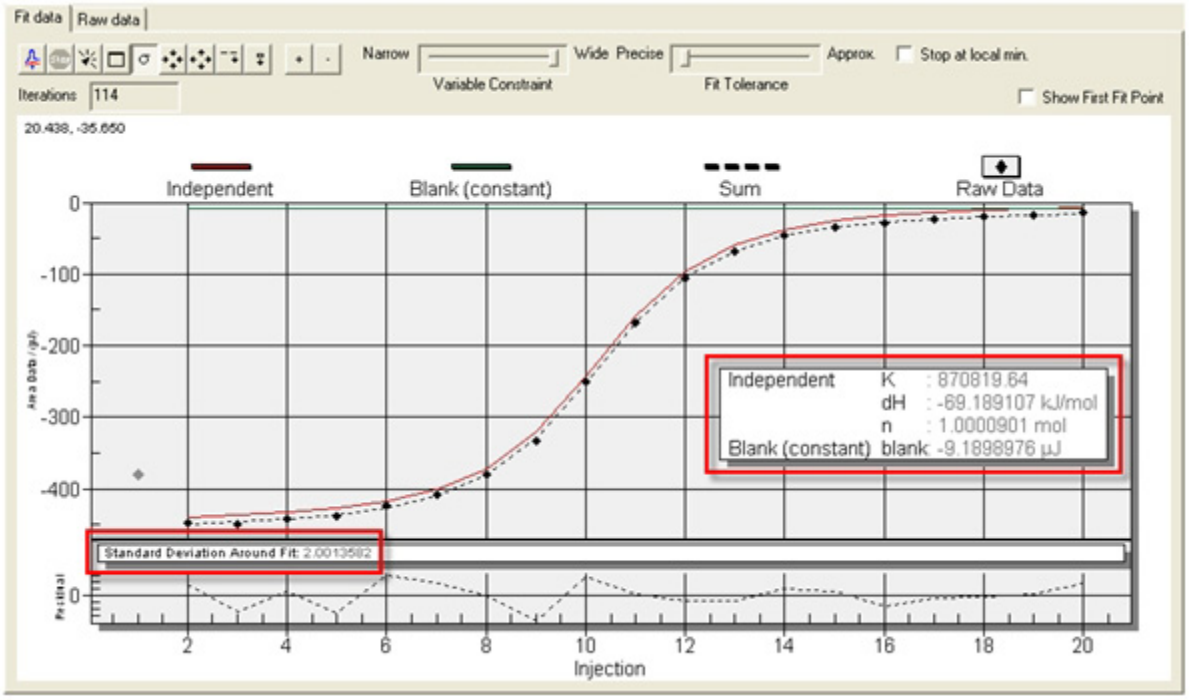


Figure 60

2 Proceed to the **Statistics** page.



NOTE: If a Blank model was used in the fit, uncheck the box next to the Blank in the **Included Models** group. Blanks should not be subjected to the random noise because this interferes with the ΔH variation, causing an incorrect distribution.

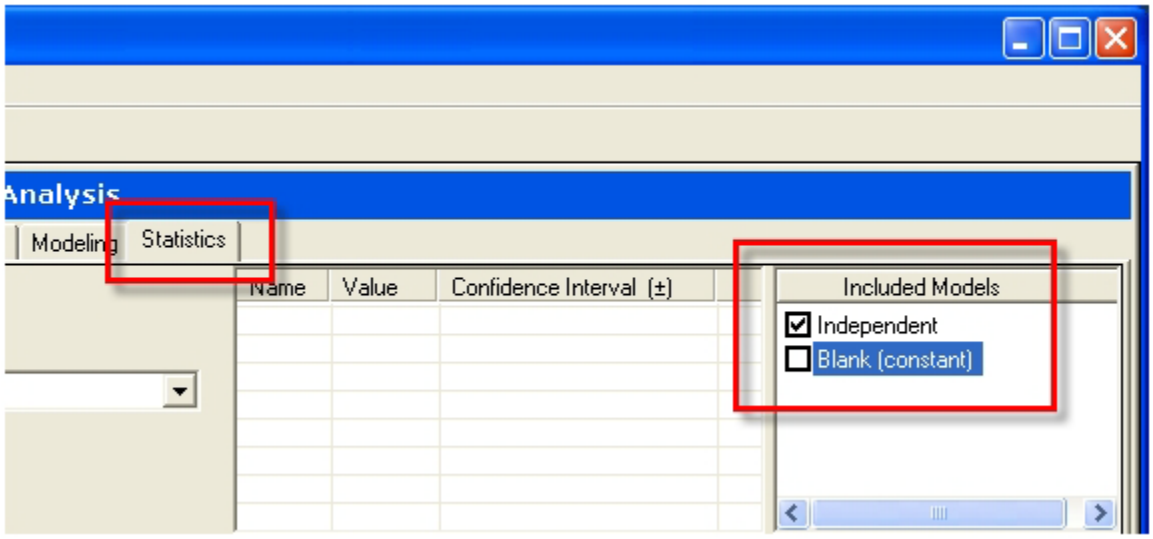


Figure 61 Statistics.

3 Select an appropriate number of trials (typically 100 to 1000) and click **Start**. The figure for **Standard Deviation** was carried over automatically from the **Modeling** page and does not need to be entered manually. The **Desired Confidence Level** and number of bars in the histogram can be adjusted either before or after running the trials. The trials can be halted with the **Stop** button; in this case the histogram and confidence levels will be adjusted according to any trials that have completed.

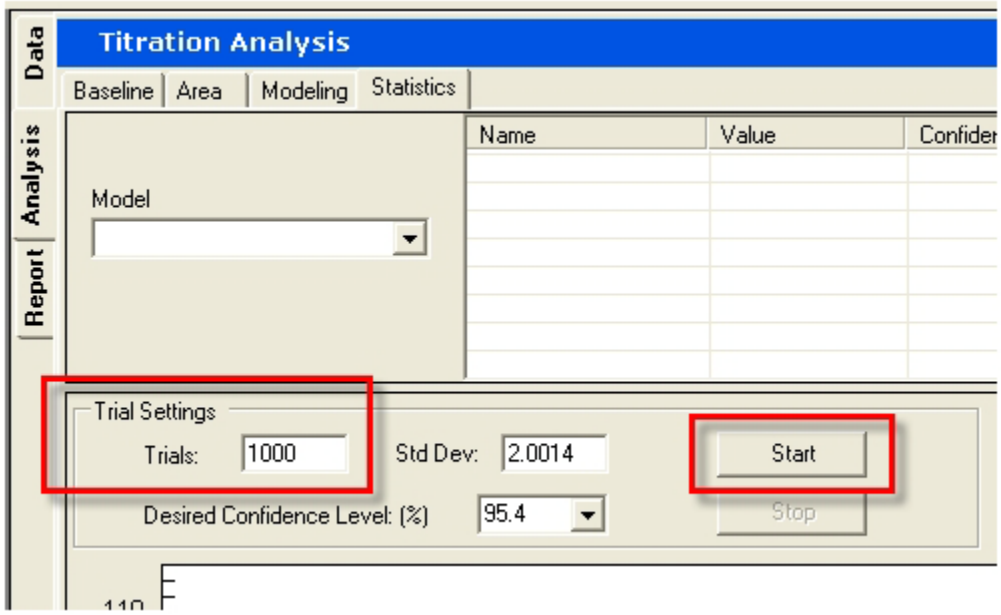


Figure 62 Select number of trials.

4 Select the desired **Confidence Level** (68.27%, 90%, 95%, or 99%), the number of bars (10 to 50), and a parameter from the list in the upper group. The histogram will be displayed in a chart, and the confidence level will be displayed in the graph table and also in the table near the top of the window. The confidence level % and the number of bars can be changed at any time. The greater the number of trials, the closer the distributions of the parameters will be to the expected Gaussian shape.

NOTE: If anything is changed in the **Modeling** tab, the **Statistics** information will no longer be valid and will be cleared.

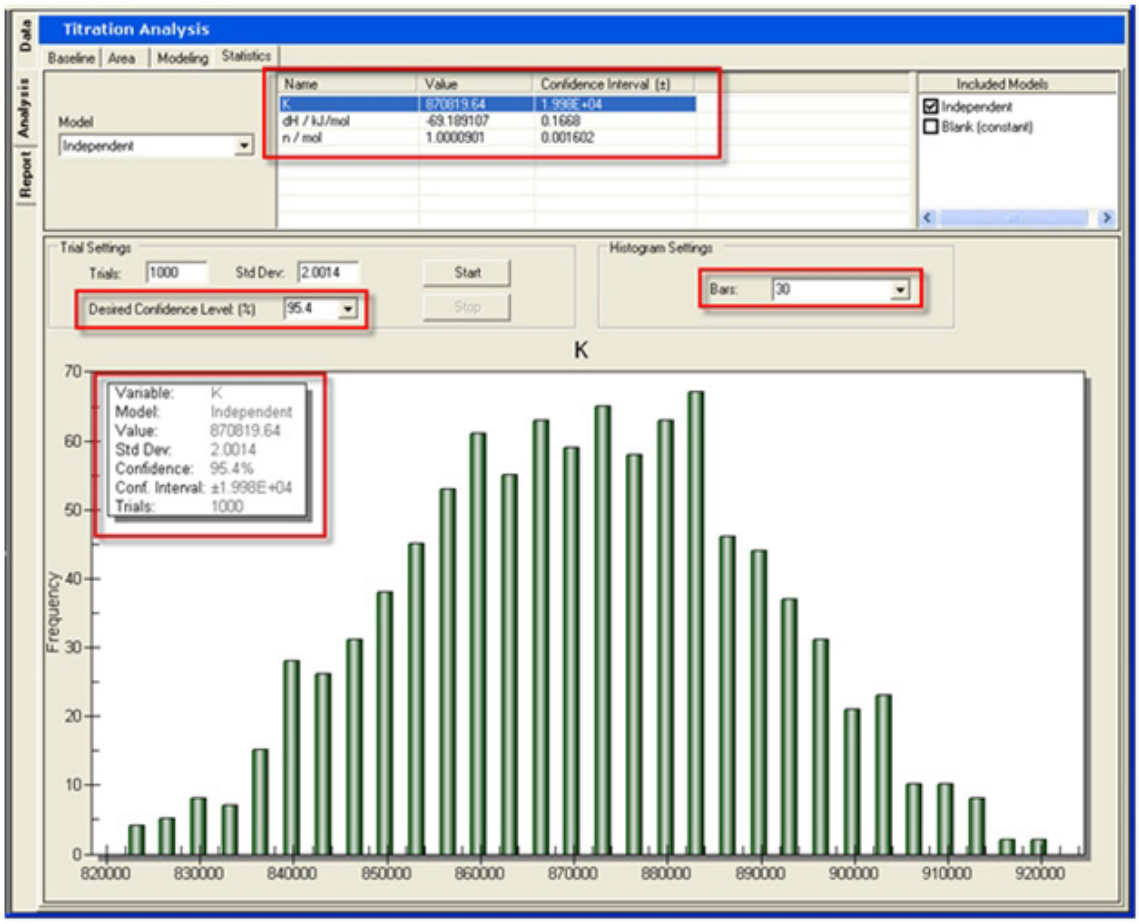


Figure 63 Select the Confidence Level.

5 The **Confidence Intervals** are multiples of the standard deviations of the distributions. An interval of +/- one standard deviation around the mean is assigned a 68.27% probability. Higher levels of confidence are associated with wider confidence intervals.

Number of Standard Deviations	Confidence Interval
1	68.27%
1.645	90%
1.960	95%
2.576	99%

The confidence intervals are based on the assumption that the standard deviations of the parameter distributions have been accurately determined. This is equivalent to an assumption that the observed distribution closely approximates the Gaussian function. However, this will not be a good assumption for small numbers of trials. The following figures illustrate examples that resulted from running 50,000 and 30 trials, respectively. Note that there is a significant bias in the case of the 30-trial example.

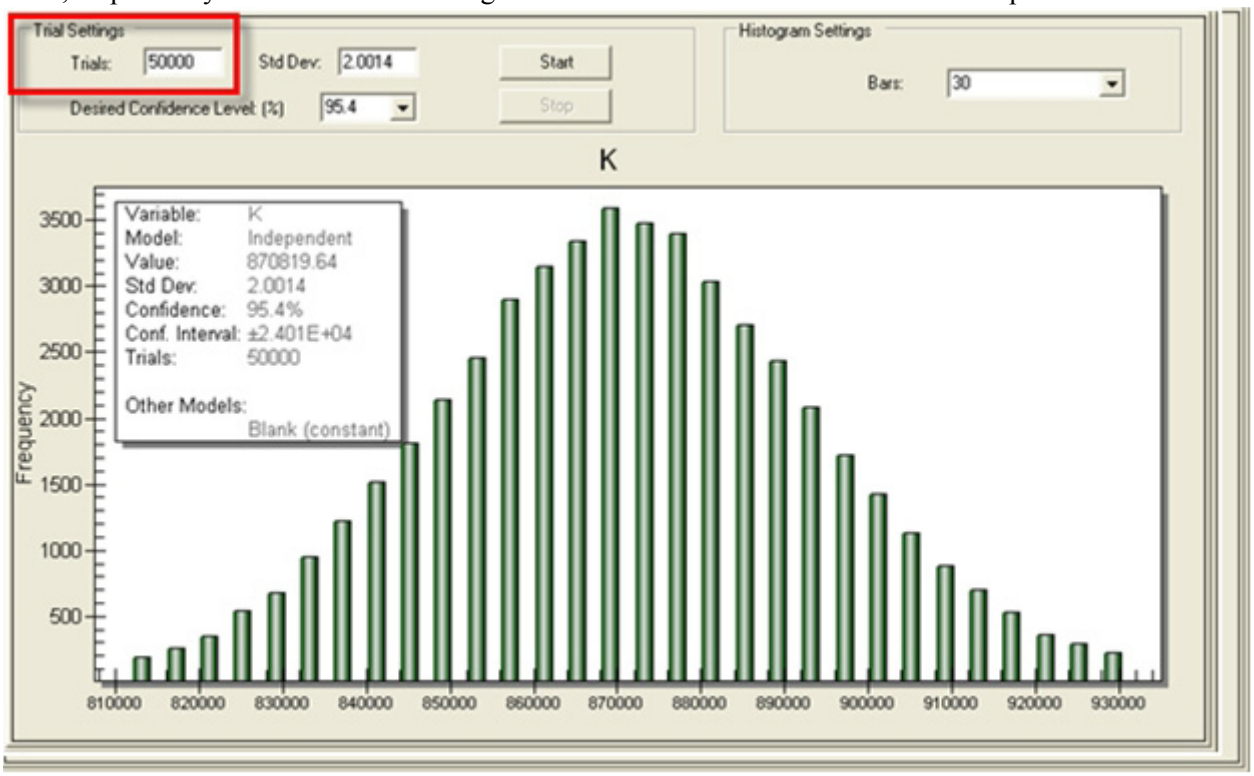


Figure 64

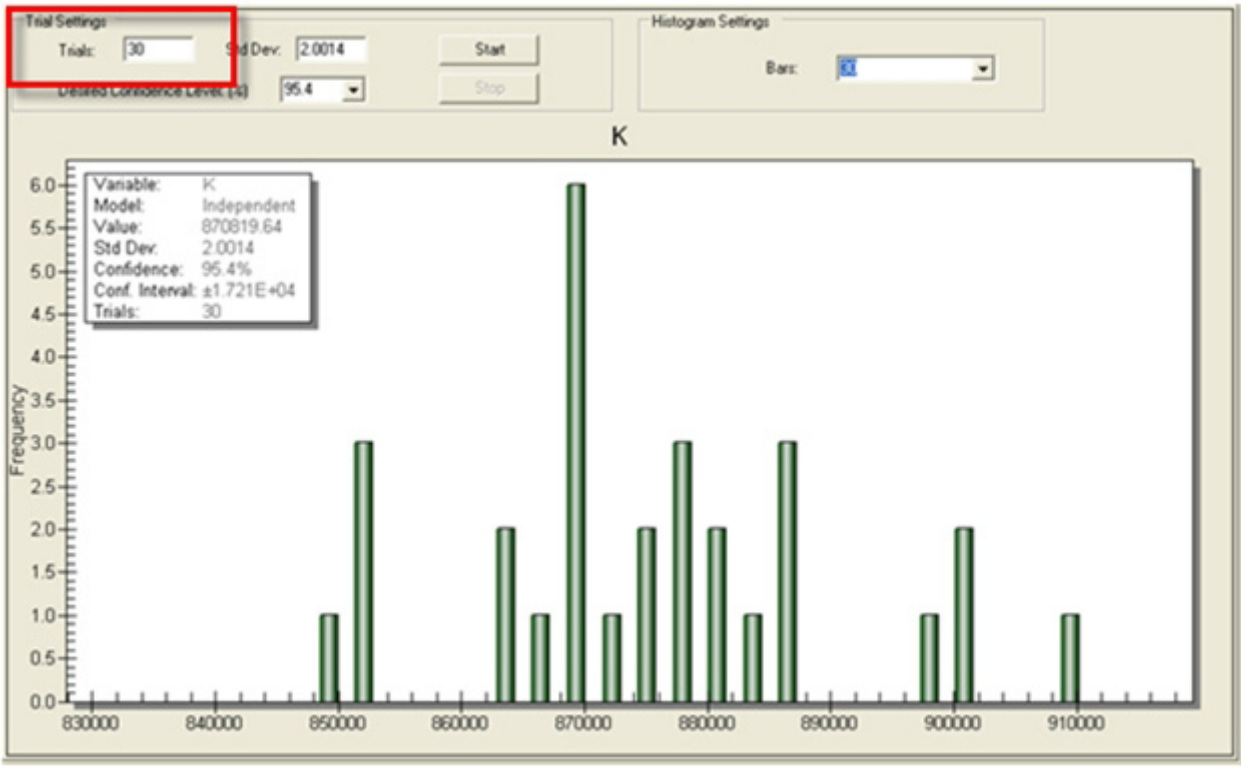


Figure 65

It is not necessary to produce a perfect-looking distribution. However, the risk of running too few trials is that the calculated confidence intervals might not accurately reflect the true potential for variation. Generally, 100 trials will be sufficient for quick investigations, and 1000 will often be sufficient for more precise work. The figure below illustrates the more consistent results that will be obtained by using a

sufficiently high number of trials. Note that with 10 trials the confidence interval results varied by nearly a factor of 6; on the other hand there tends to be little practical benefit to using more than 1000.

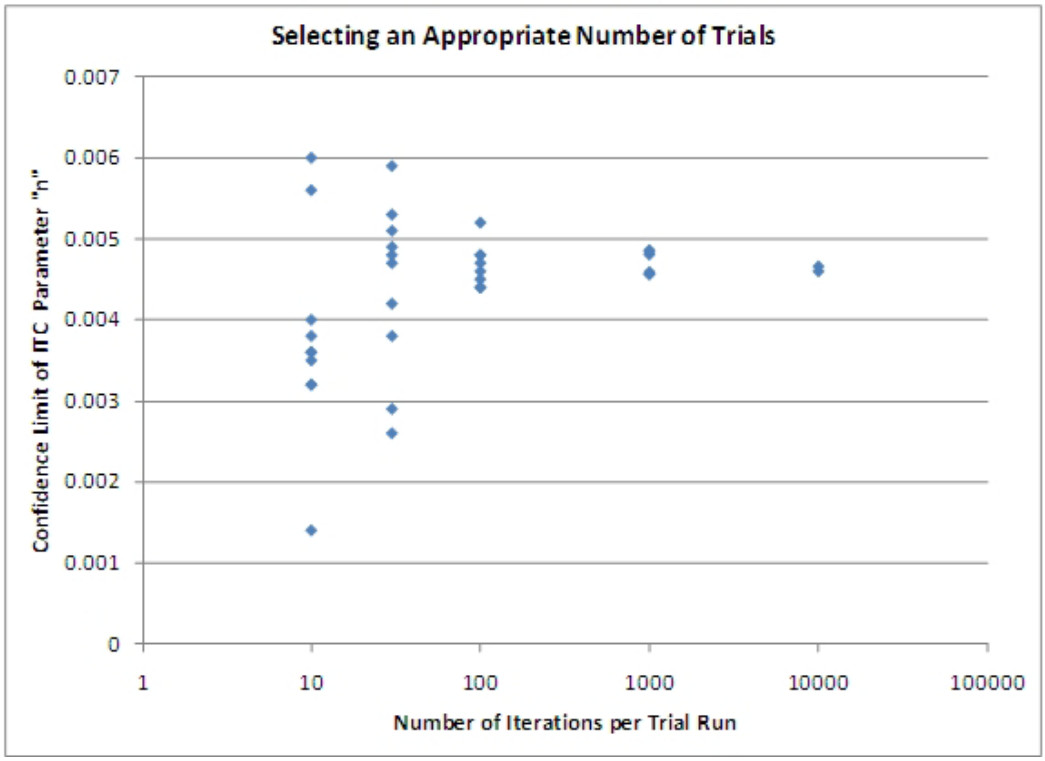


Figure 66 Selecting an appropriate number of trials.

ITC Experiment Design

Introduction

The Experiment Design module is an exploratory tool for optimizing the conditions of ITC experiments in order to maximize the quality of the resulting data. Any ITC model can be incorporated into the simulation and direct controls for all experimental parameters are provided.

An ideal and efficient ITC experiment would generate data from which it will be possible to obtain good values for n, ΔH, and K_a in only one run. This is possible when the typical S-curve of the titration is fully revealed according to these criteria:

- The curve displays a full "S" shape.
- There are multiple injection points populating the low-slope pre-inflection region.
- The inflection point is described well with multiple injections occurring in the central nearly linear section, with a moderate slope.
- There are multiple injections sampling the low-slope post-saturation section of the curve.
- The integrated enthalpy values were large enough to achieve a good signal to noise ratio, which helps insure that the results will repeatable.

The most important decisions to be made before running the titration experiment will be the selections of the sample concentrations. This requires that values are entered for the thermodynamic constants. There may be a need to develop the knowledge of the chemical system in steps. Any *a priori* knowledge of the chemical system can serve as a starting point, or perhaps an initial experiment may have been run but it delivered only partial results. The Experiment Design module makes it possible to refine the next experiment, potentially eliminating multiple trial experiments.

If K_a is high but ΔH is low, there may be mutually exclusive conditions for obtaining good measurements of both parameters within one experiment. A good measurement of K_a may require reducing the sample concentrations, however higher concentrations in a separate experiment may be needed to measure ΔH. Any information learned in either of those experiments can be updated into the model in order to help plan the other.

Using the Experiment Design Feature

To start the ITC Experiment Design module, select **Experiment Design** > **ITC** in the main menu of NanoAnalyze.

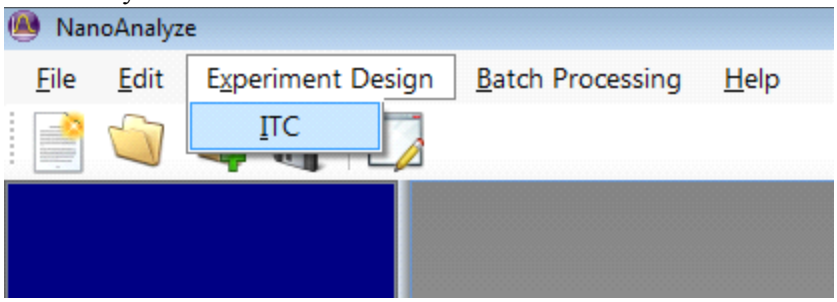


Figure 67 Starting the Experiment Design module.

Click on the indicated control to bring up the model selection dialog box. The Independent binding model will be used for this example. The default settings for the initial default values of the thermodynamic values will be in place, but these can be manually adjusted as necessary.

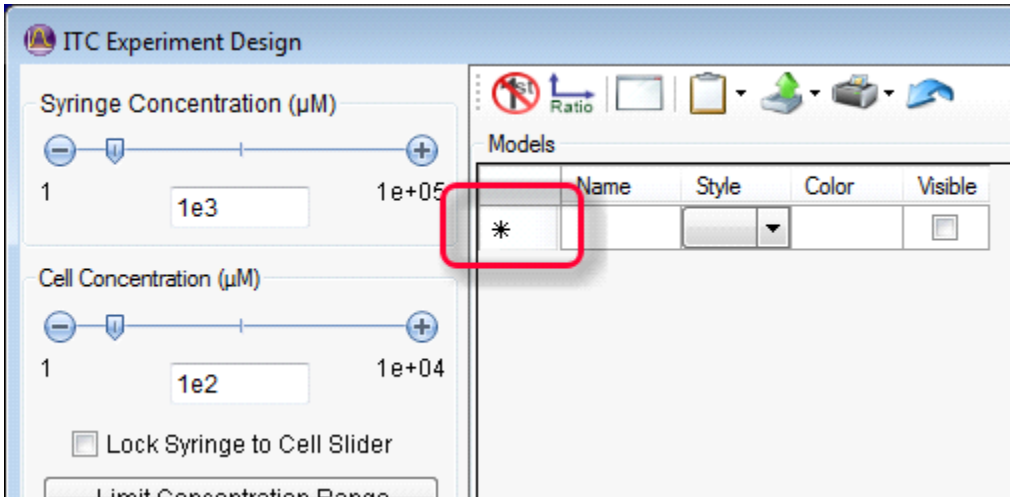


Figure 68 Selecting a Model.

The dialog box is organized into several functional groups as shown in Figure 69, and are described below.

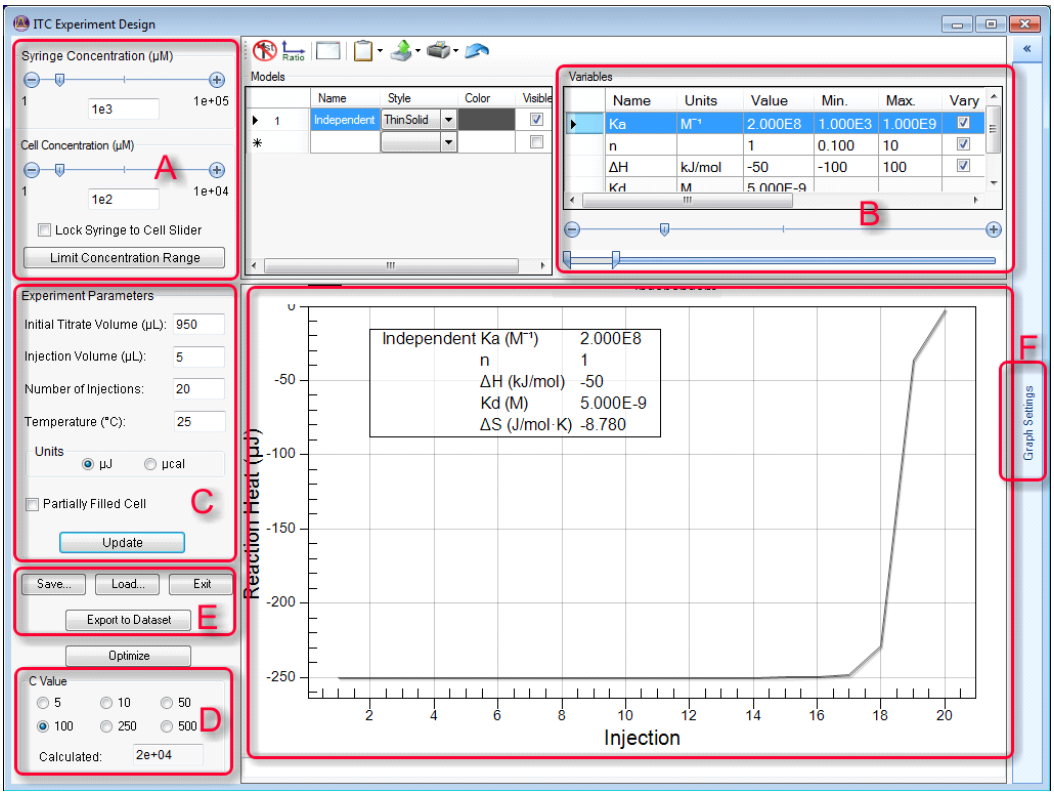


Figure 69 Controls and experiment simulation display.

A Sample concentration adjustment controls.

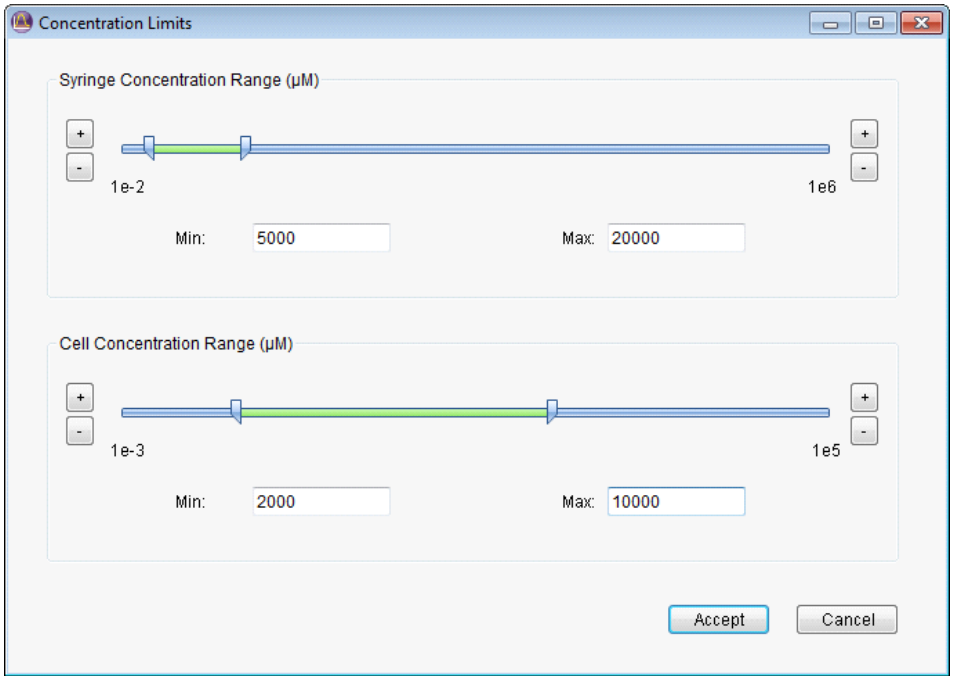


Figure 70 Sample Concentration Limits dialog box.

- Concentrations can be adjusted using the sliders, the "+" or "-" incrementing buttons, or the direct text entry boxes.
- The upper and lower limits of the sliders can be adjusted in the dialog box that is brought up by clicking **Limit Concentration Range**. Use relatively narrow limits in order to obtain fine adjustment increments in a specific region of concentrations.
- When the **Lock Syringe to Cell Slider** box is checked, both concentrations will be simultaneously adjusted (with a constant ratio) when the Cell Concentration slider is moved. The syringe concentration can always be independently adjusted via its slider control. This linked behavior can be very useful in cases of low ΔH and/or high K_a because it clearly demonstrates the trade-off between achieving good enthalpy levels that are well above the instrument noise, vs. achieving several points distributed along a moderate slope in the inflection region. If the slope at the inflection is extreme then K_a would not be accurately determined; only a lower bound would be obtained.
- Changes made to the sample concentrations will be reflected immediately in the graph.

B Thermodynamic variables adjustment controls.

C ITC instrument injection settings group.

- Change the injection settings or the desired enthalpy units in this control group.
- After making adjustments to any of the injection controls, click **Update** to show the current results in the graph.

D Whenever the independent model is loaded there will be selection buttons and indicator reading of the current value of the calculated parameter C, defined as $C = K_a \times [Mcell] \times n$. When the value of C is close to 100 the experiment is optimally designed to be able to simultaneously measure n, ΔH and K_a, for the reasons outlined at the beginning of this section.

E Experiment Design File controls. Use these to Save or Load experiment designs.

F The simulated experiment can be copied into the data file tree in the main NanoAnalyze window by using the **Export to Dataset** button.

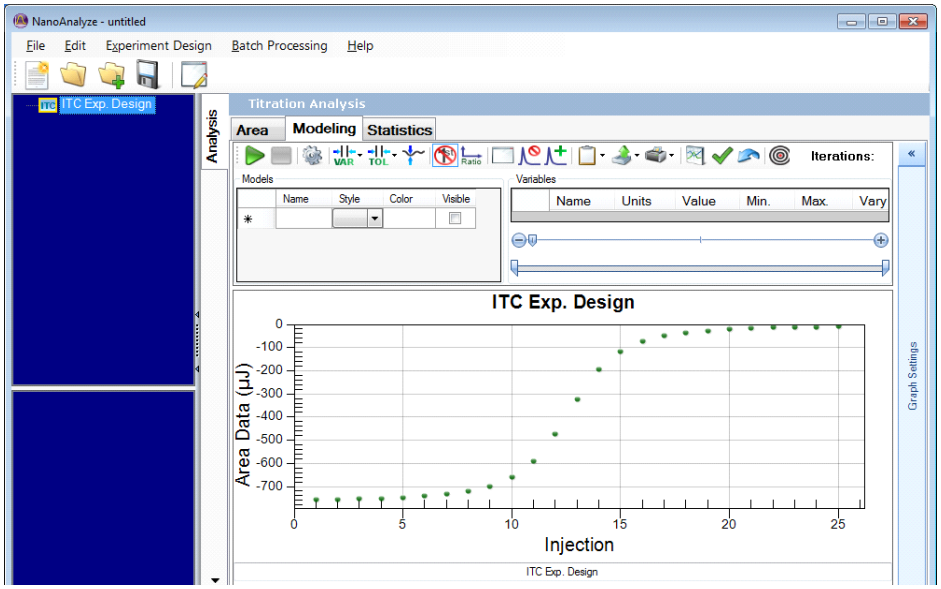


Figure 71 Experiment Design data exported to the main NanoAnalyze window.

G Experiment Simulation graph and the Graph Settings tab.

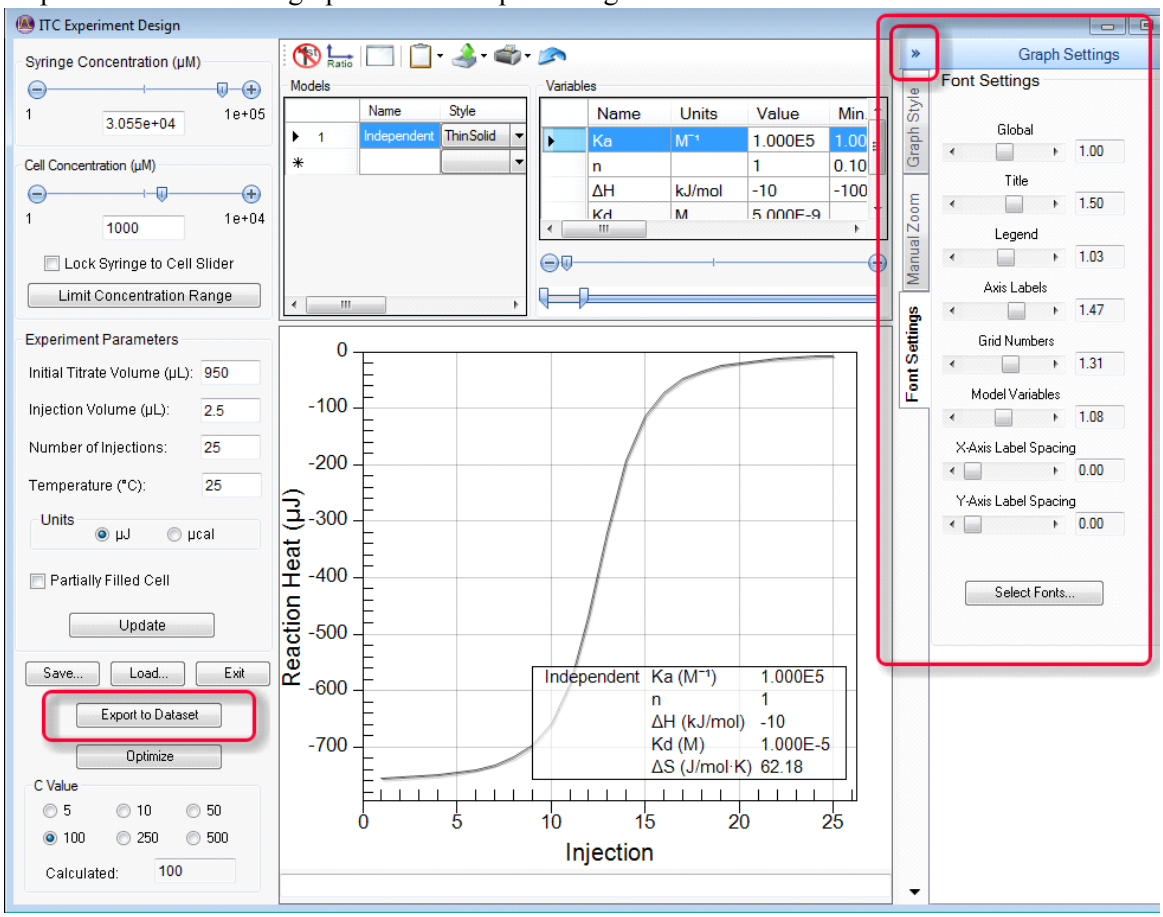


Figure 72 Showing the location of the Export button and the expanded Graph Settings tab.

- The experiment simulation with titration curve and thermodynamic values appears in this section.
- The graph appearance can be adjusted via controls that are hidden by default. They can be accessed by clicking on the **Graph Settings** border. To hide the graph settings, click on the ">>" symbol as highlighted in Figure 72 above.

Independent Model Optimizer

An experiment optimizing feature is available for the Independent binding model. There are many interacting variables which may require several attempts to optimize when using individual manual adjustments. Another alternative is to use the optimizer which makes simultaneous adjustments in order to arrive at a C value of 100. It adjusts the sample concentrations while retaining the current values of the injection settings. If either sample concentration needs to be adjusted outside of the current settings for the slider limits, it will set the ideal concentration and expand the adjustment range as necessary.

There may be external constraints which could make it impractical to use a theoretically ideal optimized design, for example acid-base titrations performed at low concentrations might risk interference from atmospheric carbon dioxide. Other C values can be selected using the option buttons as one way to explore alternative experiment designs.

The Model Editor for DSC

The Model Editor is a platform that enables the user to either create or edit preexisting models. It can be divided into three sections, Data Dependencies, Script Variables, and Logic.

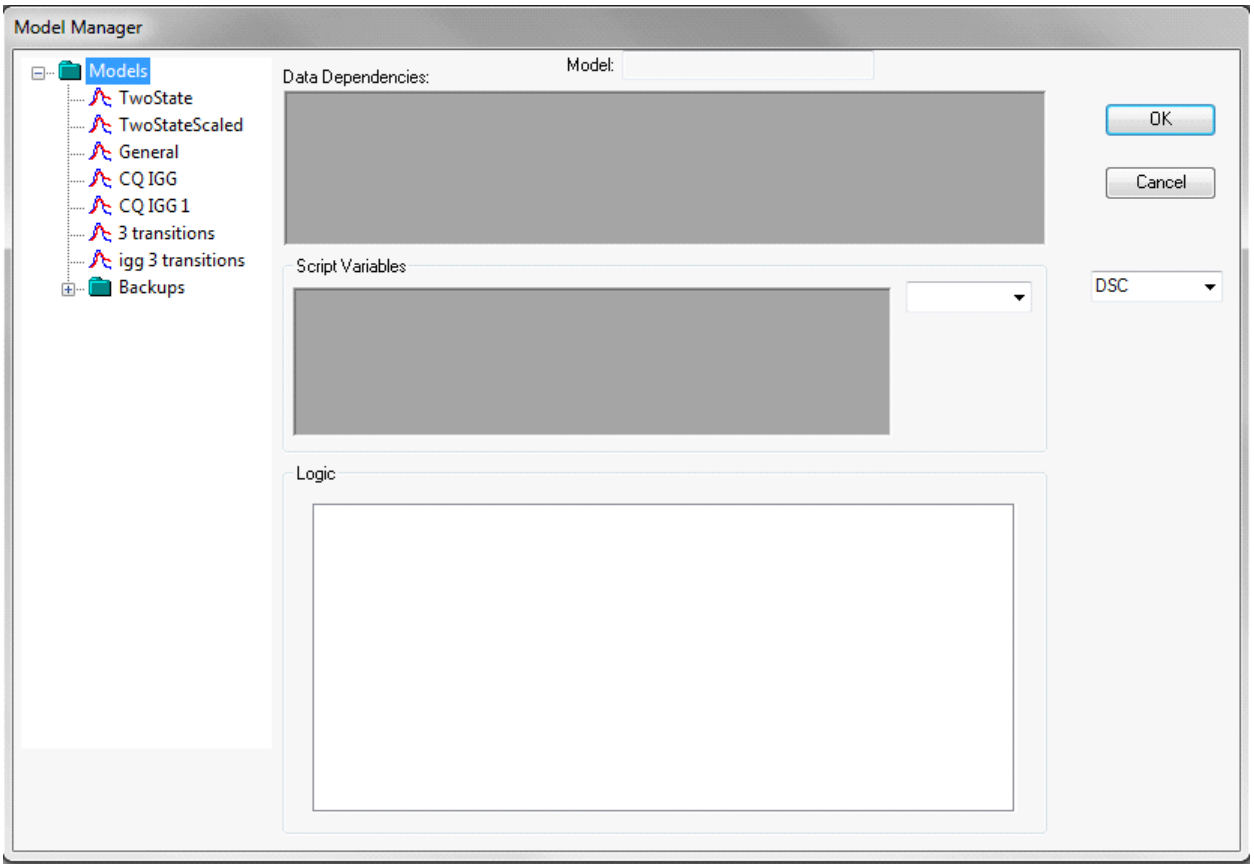


Figure 73 Model Manager.

1 Create a new model by either right-clicking on the green folder to create a model from scratch, or copy a pre-existing model by right-clicking on an existing model, making a copy, and modifying it as needed.

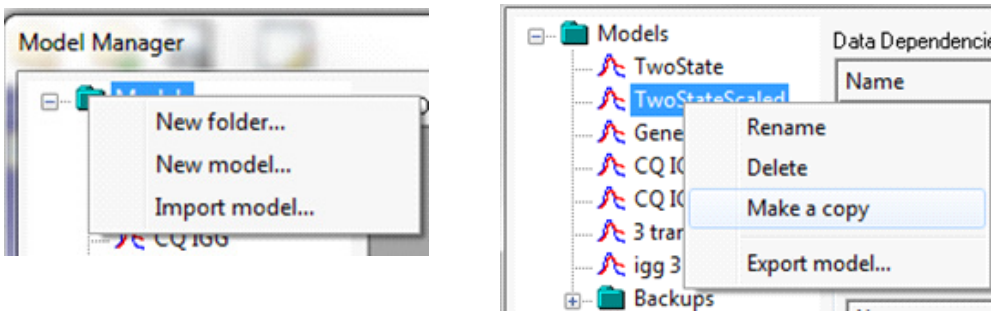


Figure 74 Create a new model.

2 Define the Data Dependencies. Data Dependencies are values that are coming from the Data portion. For the current DSC models only, the temperature in Column 1 is required. Other data columns from the data tab such as power in Column 2 can be added by left-clicking on the asterisk (*) if needed in later equations.

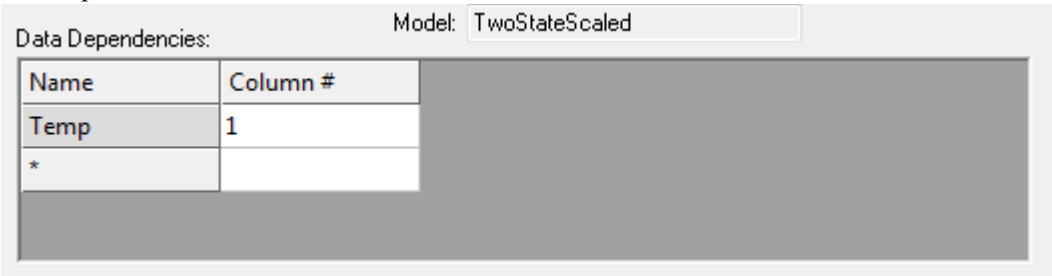


Figure 75 Define Data Dependencies.

3 Modify the Script Variables. This is the section that contains the values that are given after application of the fitting algorithm. For the two-state scaled model there are three variables that are fitted: A_w, T_m and ΔH_{VH}. The drop-down menu on the right refers to units, where default is µJ/s, and the other option is µcal/s.

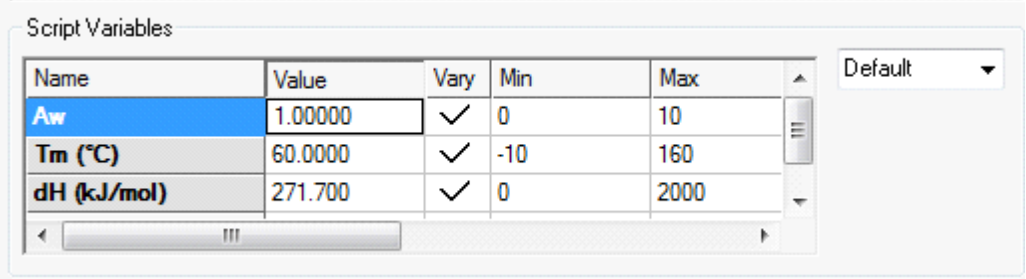


Figure 76 Define Script Variables.

4 Add additional variables, if needed. The scroll bar is used and additional variables can be added by left- clicking on the asterisk (*).

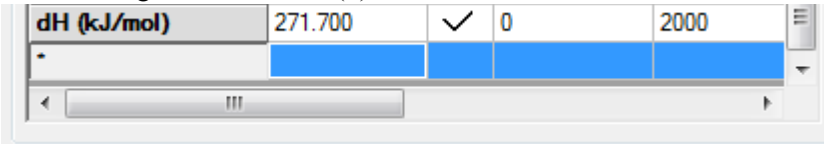


Figure 77 Additional variables.

A box displays and you will be prompted to assign a name and unit for the new variable.

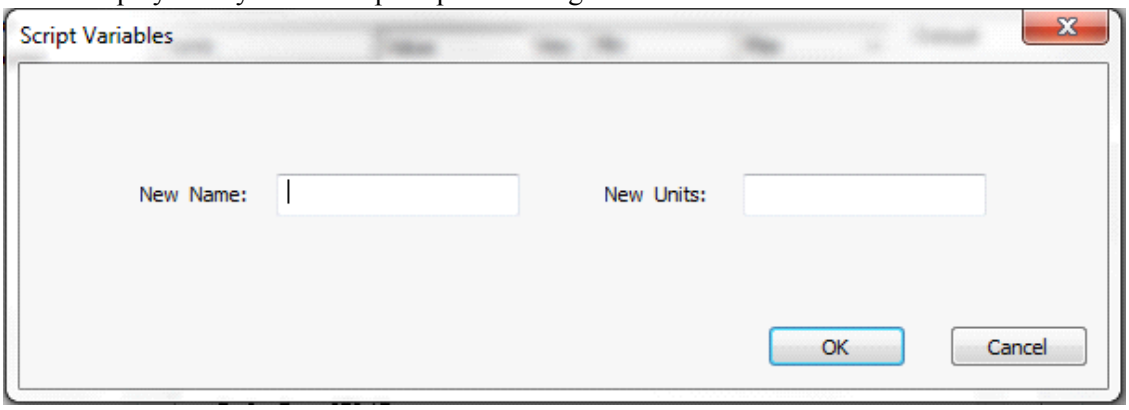


Figure 78 Assign a new name for the variable.

After the name and units have been added, the new variable displays in the Script Variable table. Generic values will populate the columns following the name.

Name	Value	Vary	Min	Max	Scientific Notation	Precision	Visible	Calculated

Figure 79 Script Variable table.

- The **Value** is the initial value prior to the application of the fitting algorithm
- Checking the **Vary** box will allow this value to float and be fitted
- The **Min** and **Max** are the constraints that will be applied.
- **Scientific Notation** and **Precision** describe how the value will be displayed.
- Checking the **Visible** box will enable the value to be displayed with the other fit parameters.
- There is also the ability to have an additional **Calculated** value that is designated after the variables are fitted. This type of calculation can be written into the **Function Definitions** tab.

5 Once variables have been added, the equation can be modified or created in the Logic section.

- The first tab is **Main Function**, which returns the equation. This is where the equation has been written in visual basic script; this language supports one equation per line. The first line, T, is the column 1 value that was defined in the Data Dependencies section. The first three lines contain equations that convert the variables units. The next two equations are from the primary literature and are written in a way that they can be solved. Another requirement is that there must be an equation that solves for Y; Y is what will be modeled. The last line is referring to the additional iterations (i).

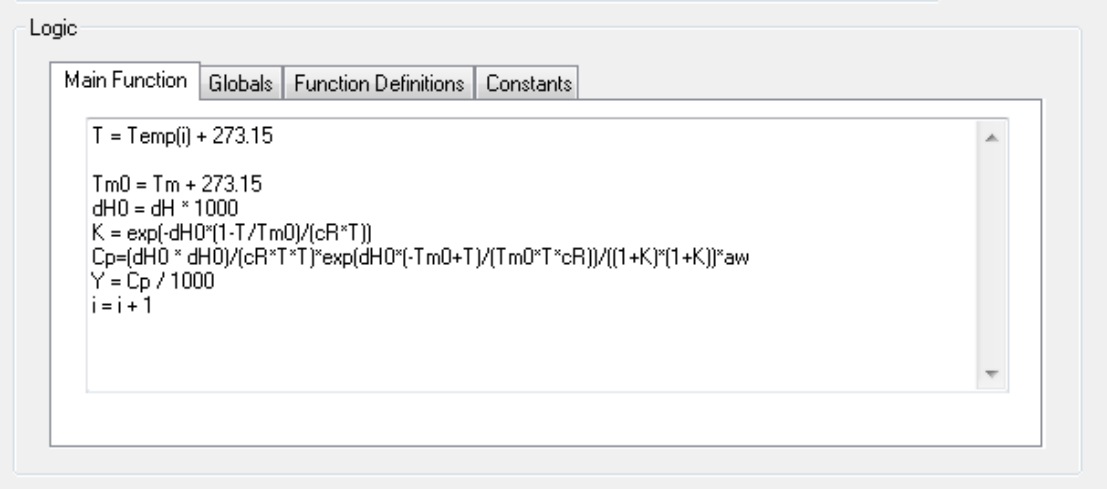


Figure 80 Main Function tab.

- The second tab is labeled **Globals**; each variable is preceded by dim (dimension, a syntax term for the variable). The variables from the equations added to the Main Function tab follow the dim T line. For the two-state scaled model Tm0 is Tm, dH0 is dH and so forth. After the last variable is added initial values can be assigned, in the figure below, 0 is the initial value for i (iteration).

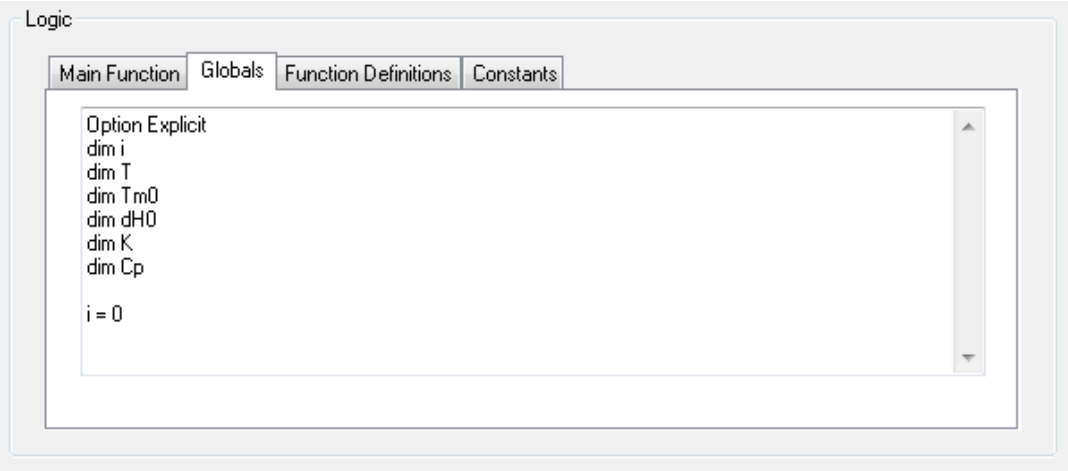


Figure 81 Globals tab.

- If the equation used contains complex equations that are not conducive to being added to the Main Function Portion they can be added to the **Function Definitions** tab. Examples include variables that are calculated after the data has been fitted.

- The last tab is labeled **Constants**. These parameters are automatically initialized whenever a model is used and they cannot be modified from within the tab. The concentration molecular weight and mass are unique and automatically updated from each experimental file. The other constants are linked to radio buttons in the **Analysis** tab, including whether or not the partial specific volume (PSV) or units in calories will be used or not.

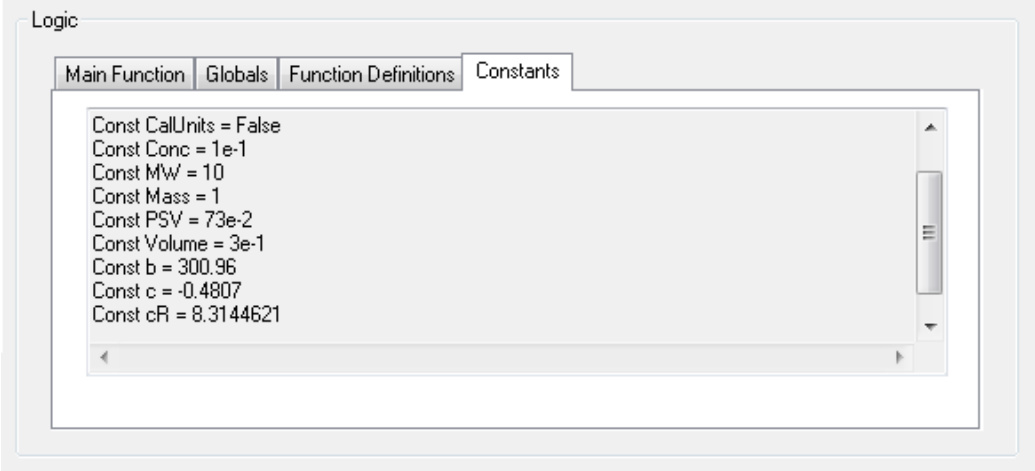


Figure 82 Constants tab.

Modifying the Two-State Scaled Model For Three Transitions

Follow the steps below to create a modified model that will load and fit three unfolding events.

1 Open NanoAnalyze and select the model manager icon.



Figure 83 Model Manager icon.

2 Expand the Models folder and right-click on the **TwoStateScaled** model.

3 Click **Make a copy** and then right-click on the new file and rename it.

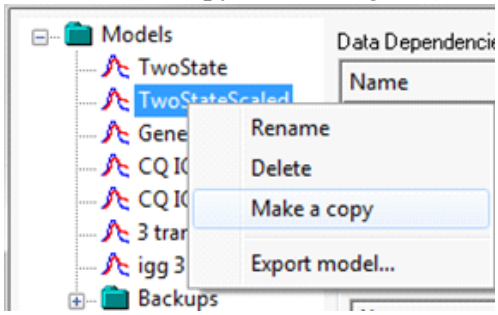


Figure 84 Models folder > TwoStateScaled > Make a copy.

4 Under the Script Variable section, rename Aw to Aw1, Tm to Tm1, and dH to dH1. This is done by right-clicking on each name. While making these changes copy the unit °C, this will be pasted later.

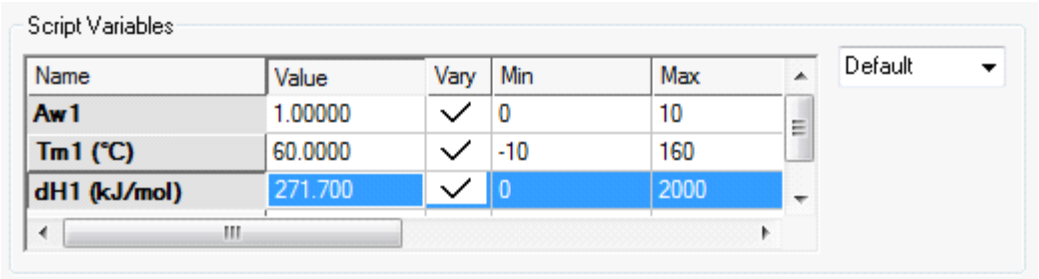


Figure 85 Script Variables table changes.

5 Add additional variables; use the scroll bar and add additional variables by left- clicking on the asterisk (*).

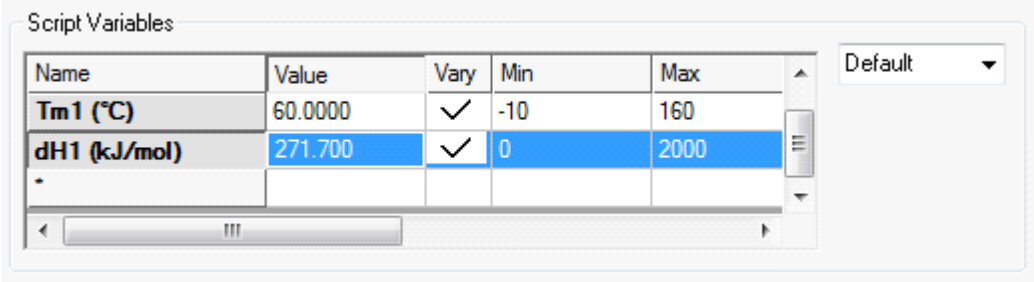


Figure 86 Add additional variables.

6 A Script Variables box displays and you will be prompted to assign a name and unit for the new variable.

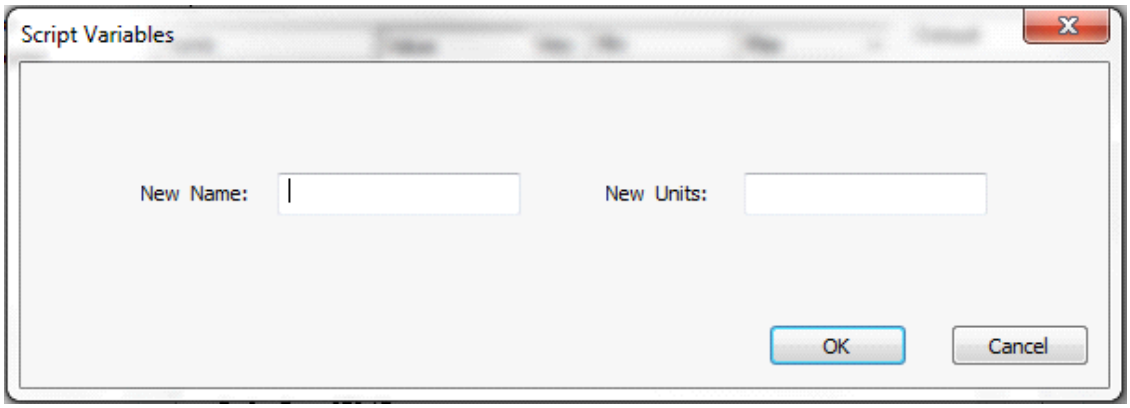


Figure 87 Assign a name and unit.

7 The variables that need to be added are Aw2, Tm2, dH2, Aw3, Tm3 and dH3. The scaling factor is unit less, and unit of the temperature is pasted, °C, and the unit for the enthalpy is kJ/mol. After the name and units have been added, the new variable will be displayed in the Script Variable table. Generic values will populate the columns following the name.

Name	Value	Vary	Min	Max	Scientific Notation	Precision	Visible	Calculated

Figure 88 Script Variable table.

8 Initial fitting values are typed into the value box and reasonable constraints are typed into the min and max boxes. Good starting places for the initial values are the default values. However, the temperature min and max should be constrained further to a reasonable range within the scan. If possible sequential temperature limits can be applied, this will increase the fitting time and decrease the probability for a redundant fit to the major peak. Precision can be set to 4 and the visible box should be checked for all variables.

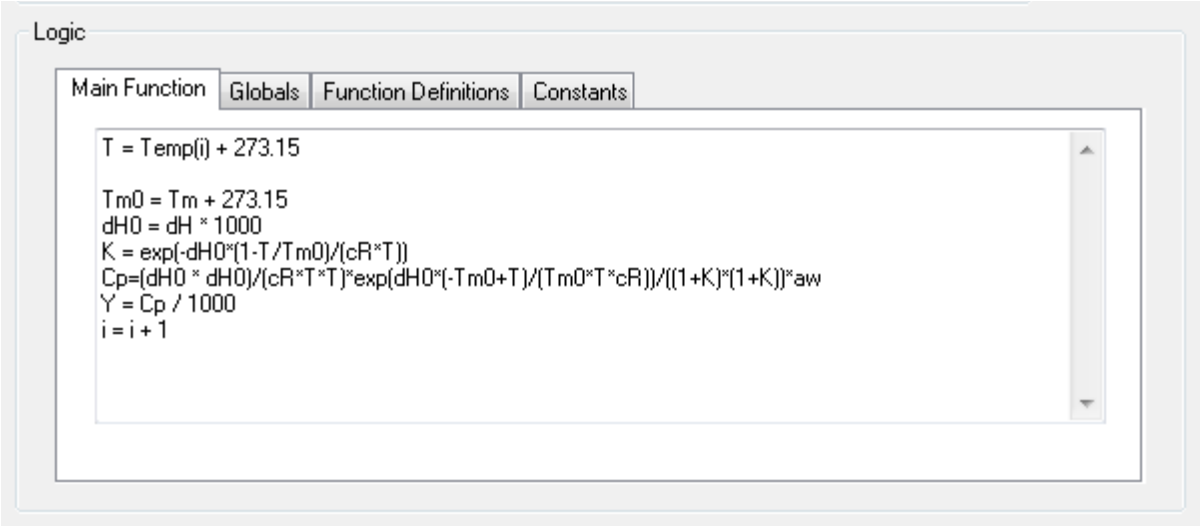


Figure 89 Main Function tab.

9 Once variables have been added, the equation can be modified. This is done in the Logic section. The first tab is **Main Function**, which returns the equation. This is where the equation has been written in visual basic script; this language support one equation per line.

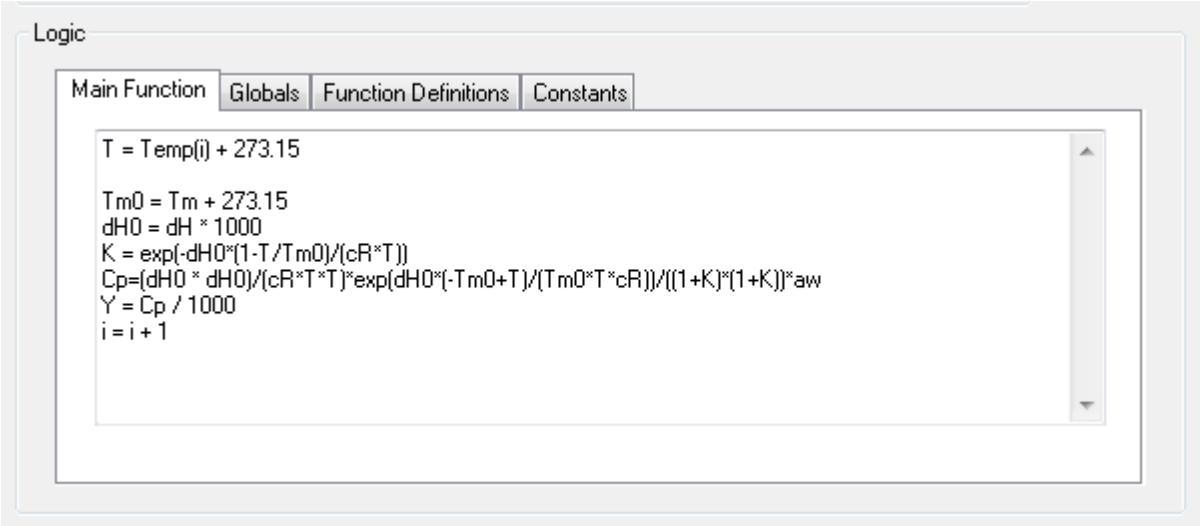


Figure 90 Main Function tab.

The four lines following adjusting the temperature to Kelvin are copied and pasted twice above the Y = Cp/1000 equation.

Each Tm0, dH0, dH, K, and Aw have additional numbers 1,2,3 following them to designate each as different variables that are associated with those added in the **Script Variable** tab.

```
Tm01 = Tm1 + 273.15
dH01 = dH1 * 1000
K1 = exp(-dH01*(1-T/Tm01)/(R*T))
Cp1=(dH01 * dH01)/(R*T*T)*exp(dH01*(-Tm01+T)/(Tm01*T*R))/((1+K1)*(1+K1))*aw1
Tm02 = Tm2 + 273.15
dH02 = dH2 * 1000
K2 = exp(-dH02*(1-T/Tm02)/(R*T))
Cp2=(dH02 * dH02)/(R*T*T)*exp(dH02*(-Tm02+T)/(Tm02*T*R))/((1+K2)*(1+K2))*aw2
Tm03 = Tm3 + 273.15
dH03 = dH3 * 1000
K3 = exp(-dH03*(1-T/Tm03)/(R*T))
Cp3=(dH03 * dH03)/(R*T*T)*exp(dH03*(-Tm03+T)/(Tm03*T*R))/((1+K3)*(1+K3))*aw3
```

Figure 91

The Y = equation is changed to include all three models and is written as Y = (Cp1+Cp2+Cp3) / 1000. The last line, i = i + 1, is left unchanged.

In the **Globals** tab the dimensions Tm0, dH0, K, and Cp are copied twice and pasted above i =0. They are then designated with numbers 1,2,3 (ie Tm01, dH01, K1, and Cp1). Dimensions i, T, and R are left unchanged as well as the initial value for i, i =0.

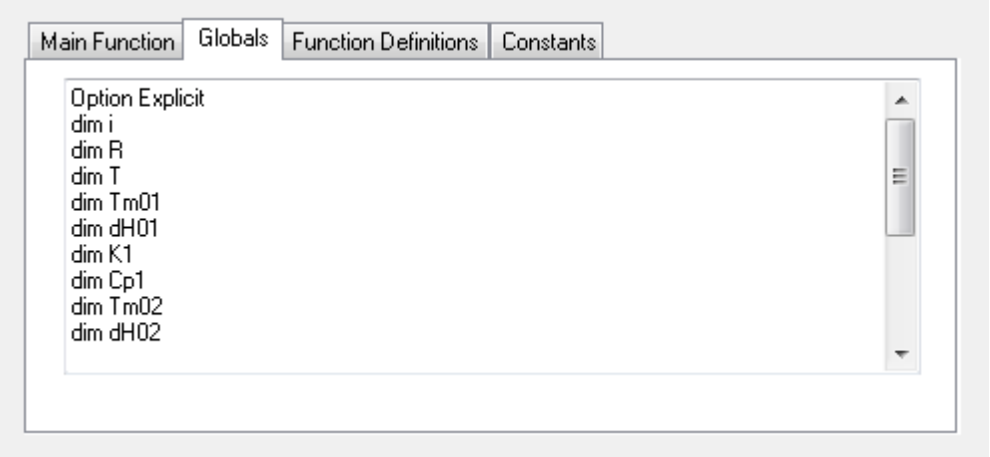


Figure 92 Globals tab.

The last two tabs, **Function Definitions** and **Constants** are not modified.

10 Click **OK**.

11 This new model can be loaded like any other in the modeling tab. If mistakes were made during the editing process an error message will display.

Pressure Perturbation Experiments

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TA Instruments, 890 West 410 North, Lindon, UT 84042, USA

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 ($\Delta\bar{V}/\bar{V}$, 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 $\Delta\bar{V}/\bar{V}$ are then calculated by the following equations.

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

$$\frac{\Delta\bar{V}}{\bar{V}} = \int_{To}^{Te} \bar{\alpha}\, dT \qquad \text{Equation 2}$$

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 **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 93. 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 ºC/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 93.

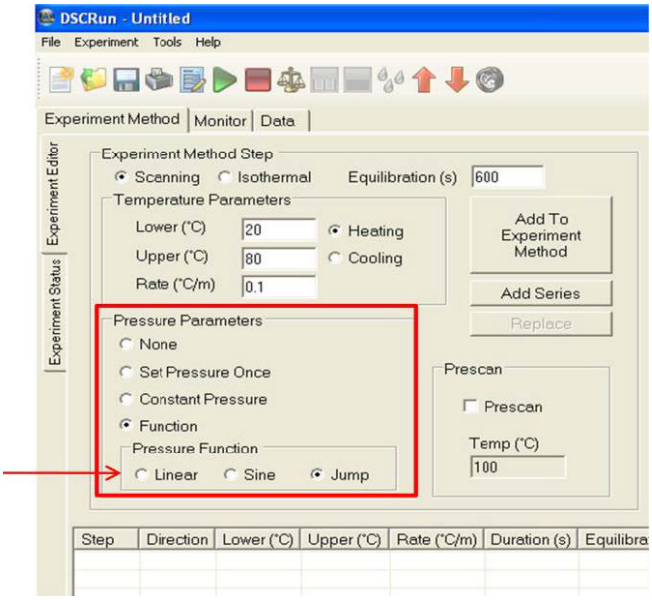


Figure 93 Screen shot of DSCRun highlighting the different pressure parameters available.

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 **Temperature Parameters** are set for the scan, click **Add To Experiment Method**. This opens a dialog box that enables setting the **Jump Pressure Function** (see Figure 94).

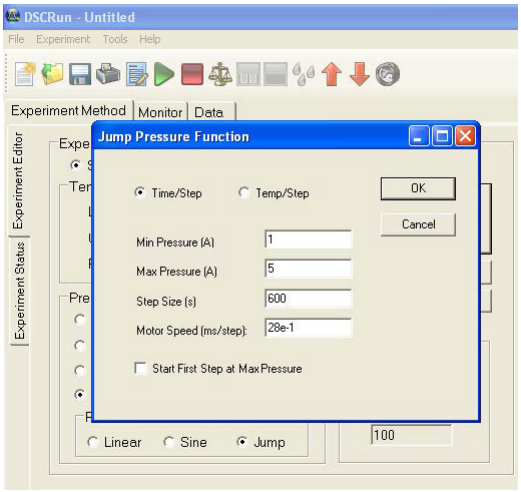


Figure 94 Screen shot of dialog box that opens after a pressure function has been selected. (A indicates atmospheres and s indicates seconds.)

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 x 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 ºC/min from 20 to 80 ºC. 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).

$$\frac{\Delta Q}{\bar{V}T\Delta p} = \alpha_{measured} = \left(\alpha_{sample} - \alpha_{reference}\right) + \alpha_{asymmetry} \qquad \text{Equation 3}$$

$$\alpha_{asymmetry} = \left(\alpha_{buffer\ in\ sample\ cell} - \alpha_{buffer\ in\ reference\ cell}\right) \qquad \text{Equation 4}$$

$$\bar{\alpha}_{macromolecule} = \left(\alpha_{sample} - \alpha_{reference}\right) = \alpha_{measured} - \alpha_{asymmetry} \qquad \text{Equation 5}$$

$\alpha_{measured}$ is calculated from the data with sample solution in the sample cell and buffer in the reference cell and $\alpha_{asymmetry}$ 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 NanoAnlayze 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 95). 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.

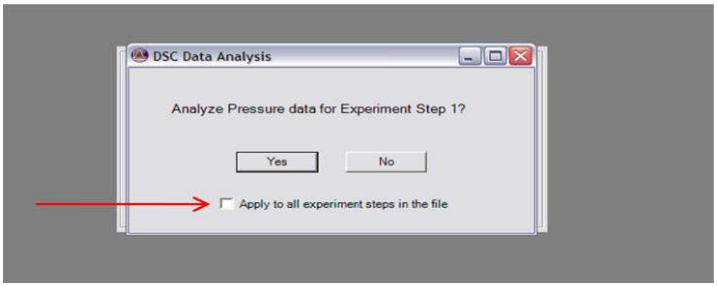


Figure 95 Dialogue box prompt when opening PPC data with NanoAnalyze.

When the files are opened, extra data analysis tabs are automatically made accessible (Figure 96).

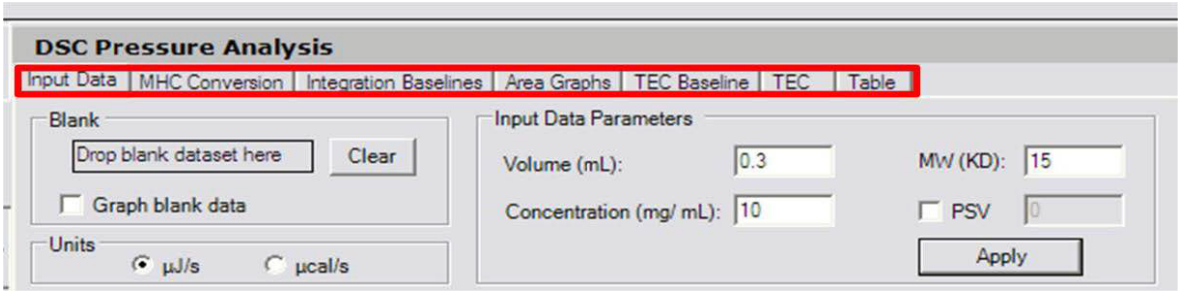


Figure 96 Screen shot highlighting the extra tabs accessible after opening PPC data on NanoAnalyze.

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 96). 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 events. 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 97).

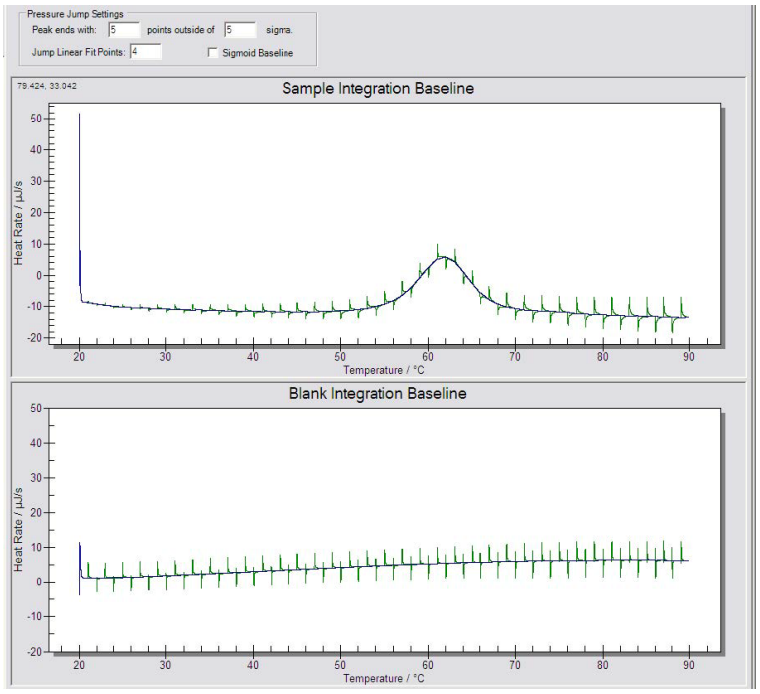


Figure 97 PPC sample and background data in the Integration Baseline window.

In the **Area graphs** tab (Figure 98), 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).

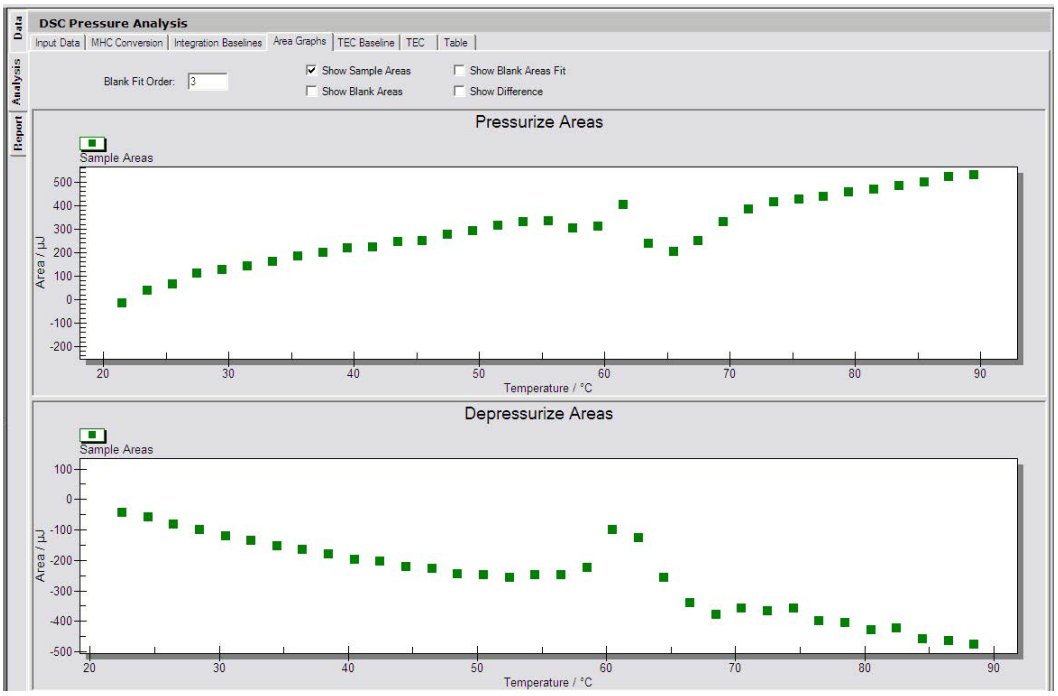


Figure 98 Area graphs window for a sample.

The **TEC Baseline** is used for calculating α for pure liquids, and is not used for calculation of $\bar{\alpha}$ 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 ($\Delta\bar{V}/\bar{V}$), according to the relationship of Equation 2 (3).

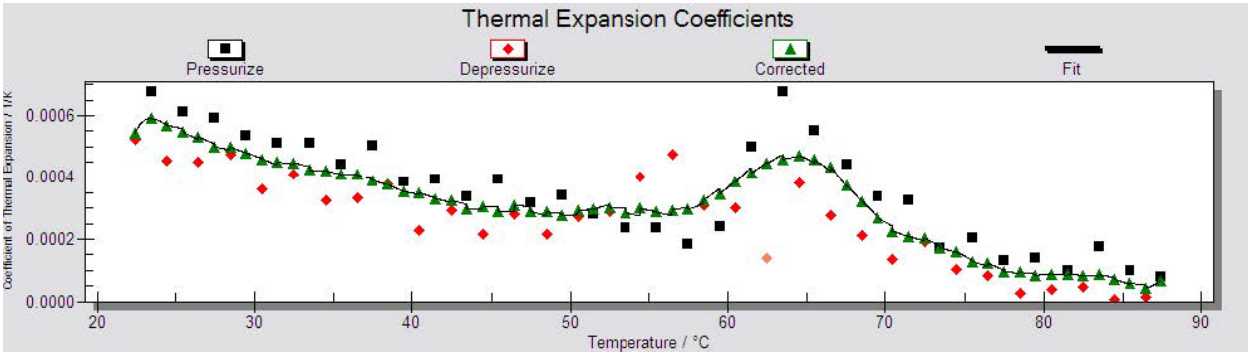


Figure 99 The TEC (α) for a sample.

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.

References

1 Randzio, S.L. "Comments on "volumetric studies of aqueous polymer solutions using pressure perturbation calorimetry . . . " [Macromolecules 34 (2001) 4130]" *Thermochimica Acta* 2003 398, 75-80.

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.

3 Rosgen, J. "Pressure-Modulated Differential Scanning Calorimetry: Theoretical Background" *Anal. Chem.* 2006 78, 991-996.

4 Dragan, A.I.; Russell, D.J. Privalov, P.L. "DNA Hydration Studied by Pressure Perturbation Scanning Microcalorimetry" *Biopolymers* 2008 91, 95-101.

*Importing Calorimeter Data into NanoAnalyze From *.txt Files*

Calorimeter data in the form of text files can be imported into NanoAnalyze for analysis. The Import function is flexible with regard to character encoding, separator characters, and column layout.

1 In the **File** menu, select **Import Text File**.

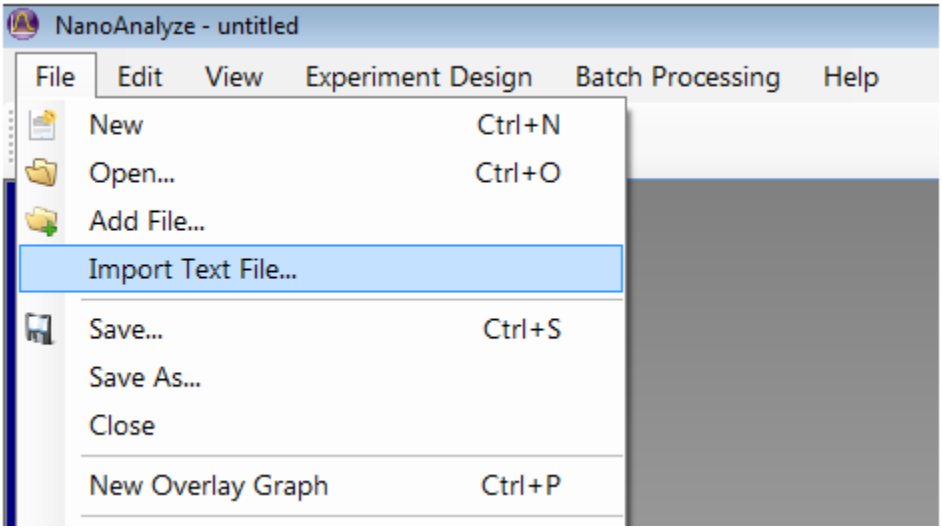
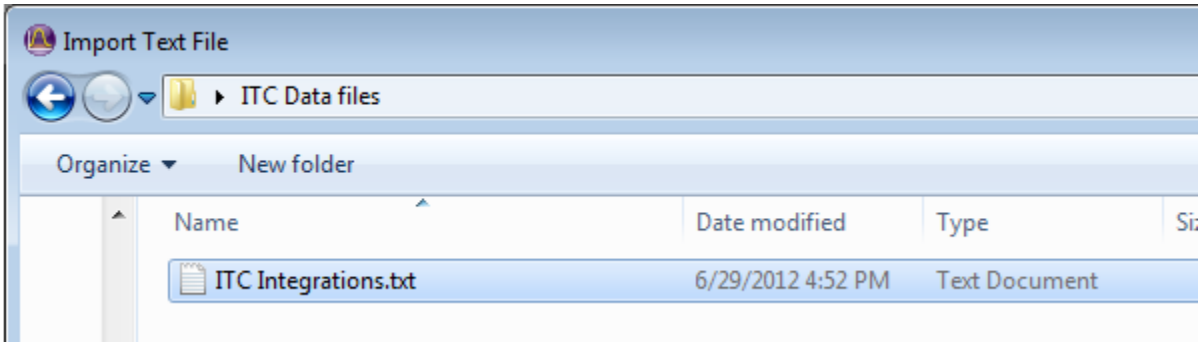


Figure 100 File > Import Test File.

2 Navigate to the folder containing the desired file and select it.



3 Several customizing settings are available to accommodate various options of the format of the text file:

- The **Encoding** selection by default will follow the currently used Regional settings in MS Windows. Other types can be selected if necessary.
- If the text contains more than one column you will need to select the appropriate Delimiter. There is typically a space or other character to separate one data field from the next. Each logical data record such as the data from one point in time, or associated with one integration must appear on its own line. Each line may contain more than one data type, for example a time stamp, a heat rate, an injection quantity, etc.
- The **Analysis Type** must be selected according to the type of experiment.

- Each **Analysis Type** has options for the categories of data that may be present.

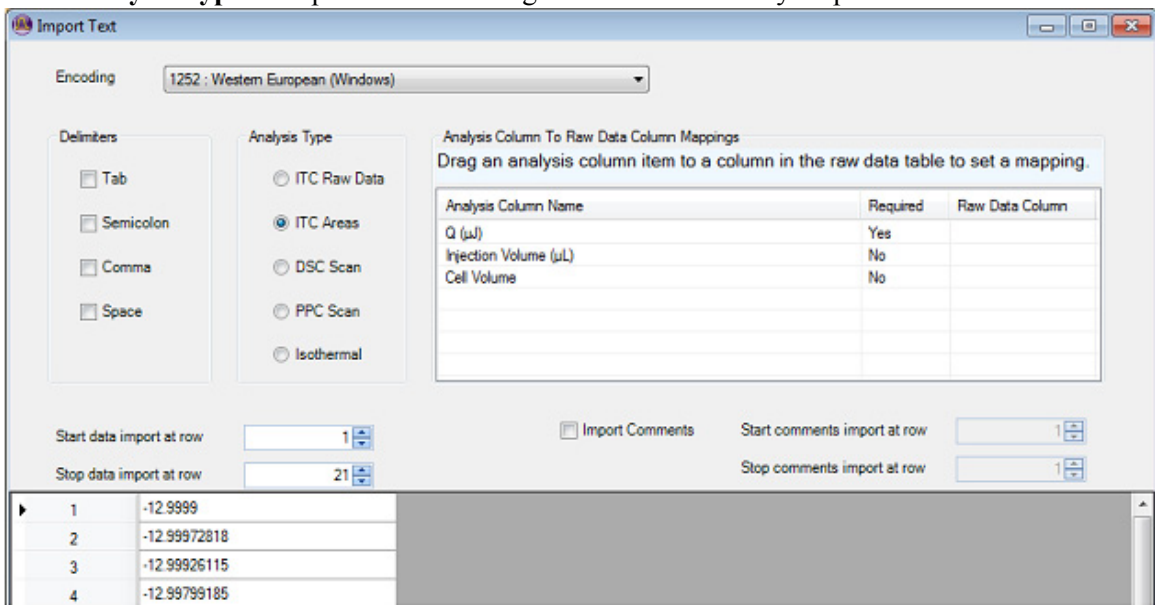


Figure 101 Import text options.

In the following example, integrations from an Isothermal Titration Calorimeter are being imported. In this case there is only one column so the Delimiter is not required. **ITC Areas** was selected. The data to be imported are heat rates, therefore the Analysis Column Name should be selected as **Q (µJ)**. Note that this is a Required field for this data type. If Injection Volume or Cell Volume data are present they may also be imported at this time.

Each column to be imported must have a name assigned. Select the appropriate line from the Analysis Column Name, and drag and drop it onto the column in the data table at the lower left of the dialog box. The text column is highlighted in blue when this has been completed.

If the file contains a header above the data table or any trailing text below the table, adjust the row numbers as appropriate in the fields for **Start data import at row** and **Stop data import at row**. If comments are present in the file they may also be imported; use the start and stop line controls as needed to define what should be imported.

When all data to be imported has been assigned (and if necessary, limited), click **Import**

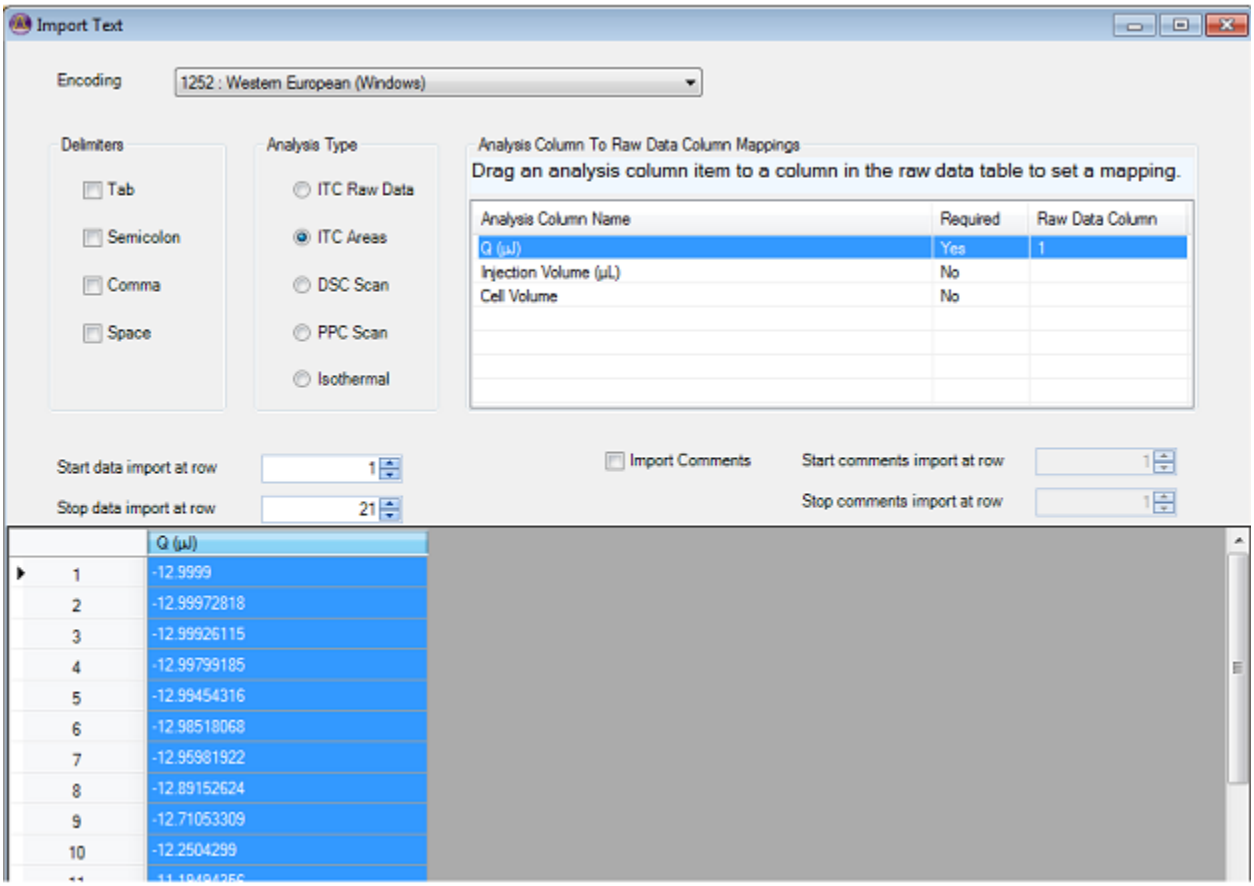


Figure 102

The data will be imported into the appropriate page in NanoAnalyze according to what type of information is represented. Raw data would go into the Data tab. In this case ITC integration areas were imported into the Analysis/Area tab.

Note that some data fields required for Modeling might not be present but they must be filled in before proceeding. Yellow highlights indicated which fields need attention.

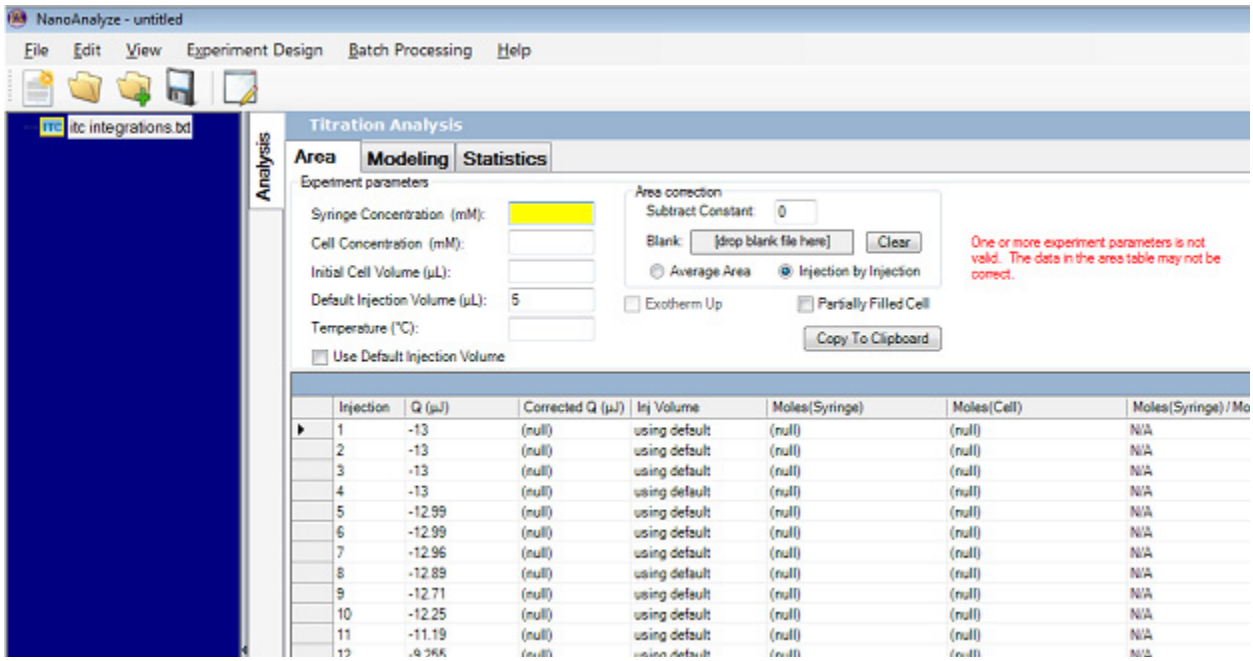


Figure 103 Yellow highlighting indicates fields that need to be filled.

When all required fields have been completed, Modeling may proceed as already described in this Guide.

Overlay Graphs

The Baseline tab also serves as an output graph formatter using the Overlay Graphs feature. Multiple data series graphs can be superimposed if desired, and publication quality charts can be formatted with a wide variety of formatting options.

1 Load a data file and go to the **Analysis** tab (from the left side group) and the **Baseline** tab (from the top group).

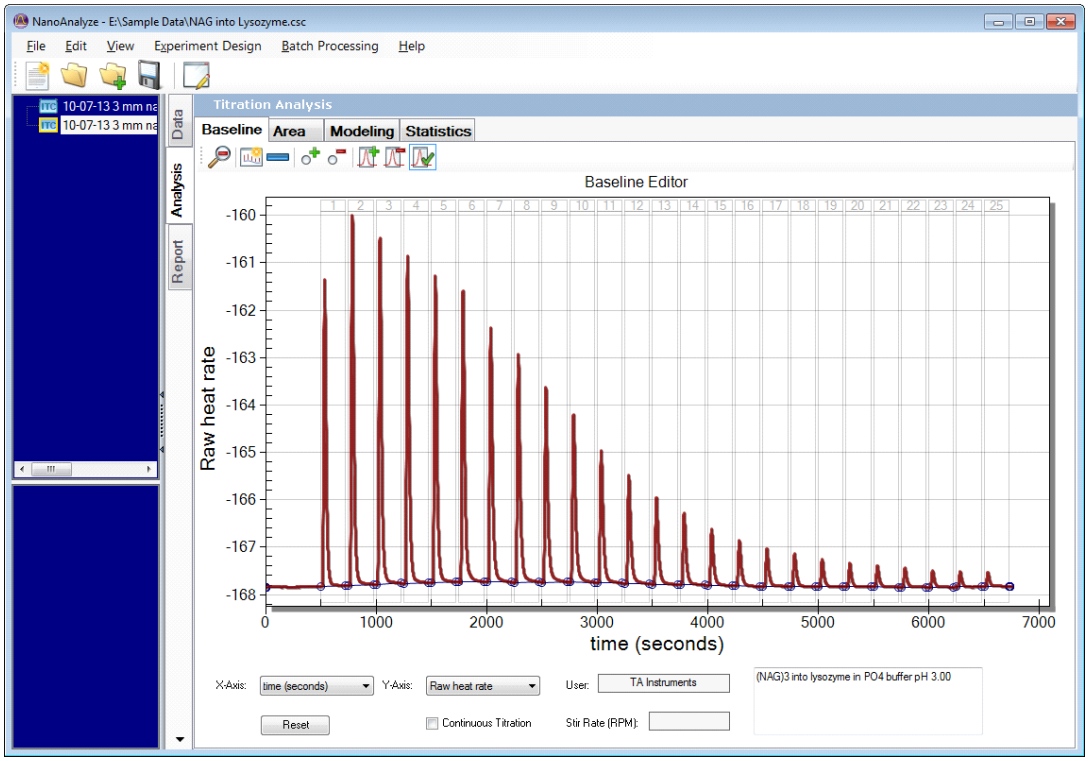


Figure 104 Load a data file.

2 Start an Overlay Graph in one of the following three ways: via **File** > **New Overlay Graph**, **Ctrl**+**P**, or by right-clicking the mouse in the Overlay Graph area at the lower left of the NanoAnalyze window.

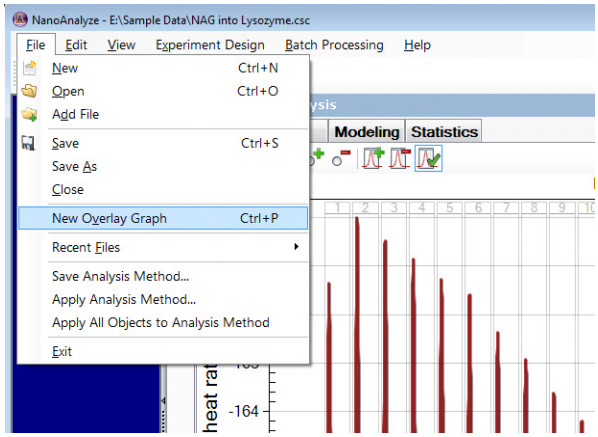


Figure 105 Creating a new Overlay Graph

Creating Publication-Quality Graphs

Drag a data file into the Primary Graph area to the right of the data file tree.

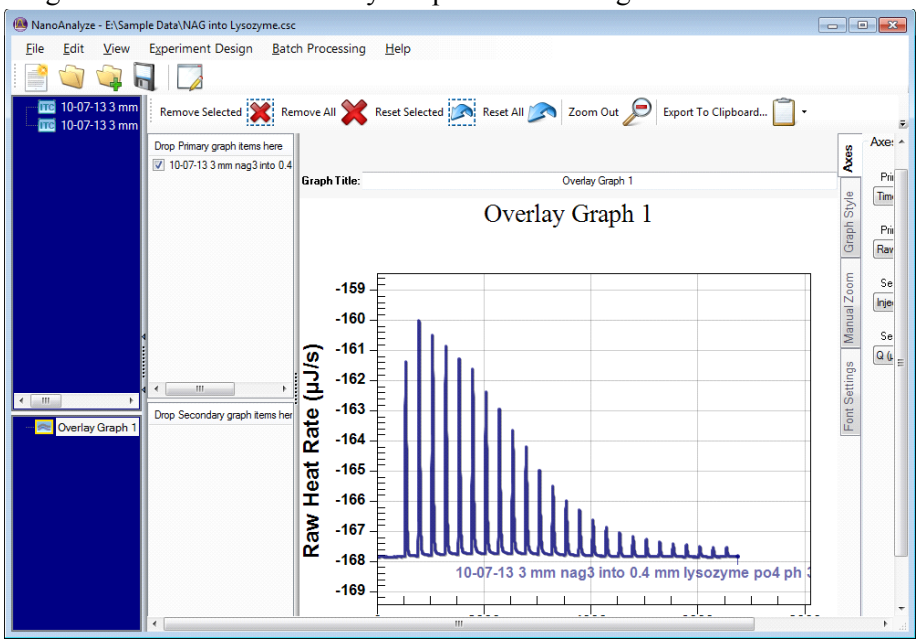


Figure 106 Create the Primary Graph.

A secondary graph can also be created by dragging a data file into the Secondary area. This can be the same data file as the Primary, and can be used to graph a different quantity.

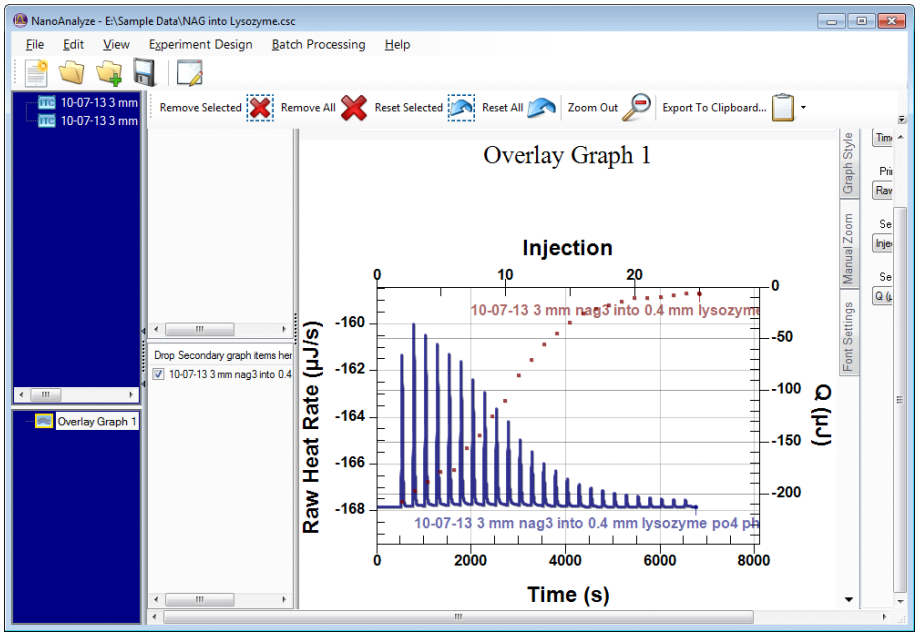


Figure 107 Create a Secondary Graph.

The Secondary graph can be separated into its own chart by using the Split Graph check box in the Graph Style control group, found at the right side of the window.

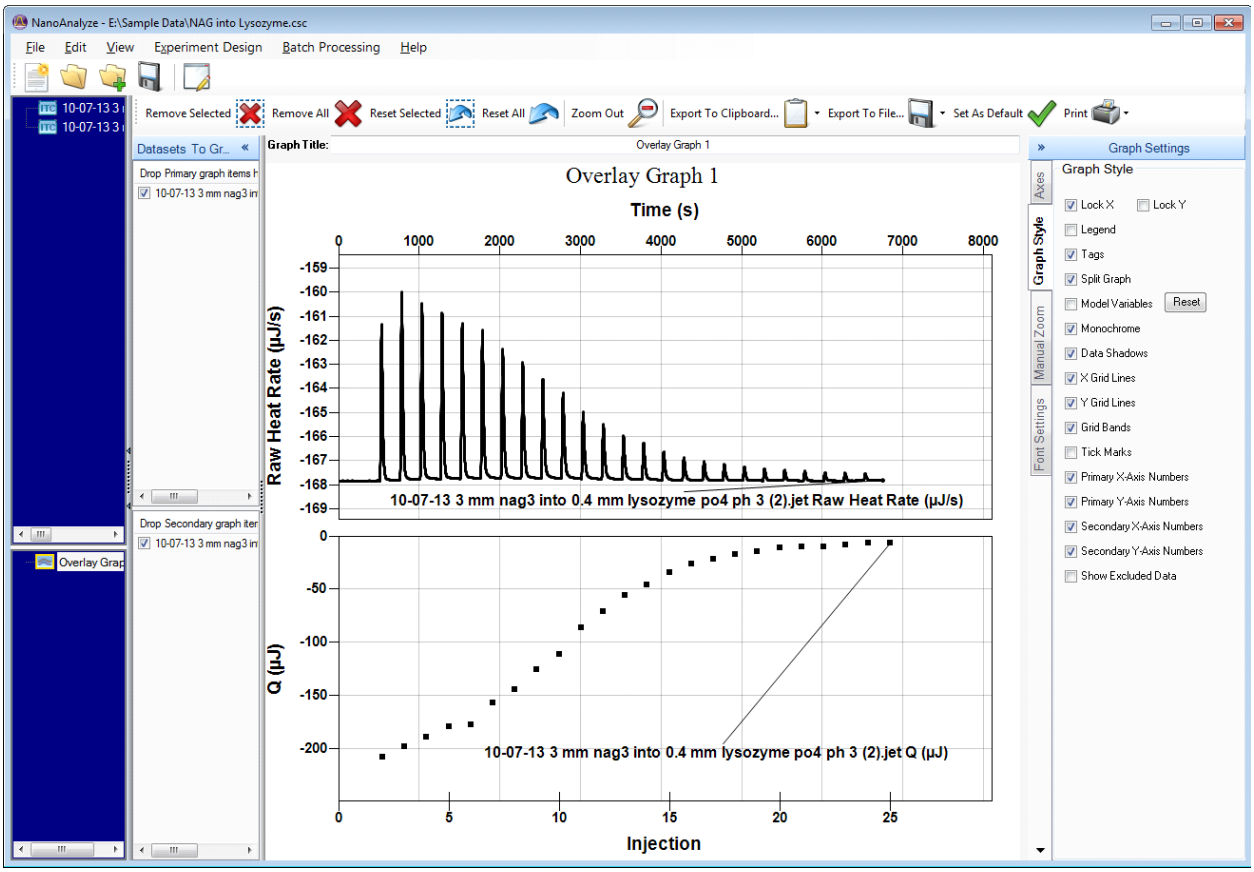


Figure 108 Split Graphs.

The controls for graph formatting are grouped into four tabs at the right side of the window.

The **Axes** tab provides independent controls for each chart, with multiple options in drop-down menus. The **Graph Style** tab contains check boxes to selectively show various chart features. The **Manual Zoom** tab applies the axis limits when the **Manual Zoom** checkbox is enabled. The **Font Settings** tab provides a global font size control along with separate controls for individual text objects.

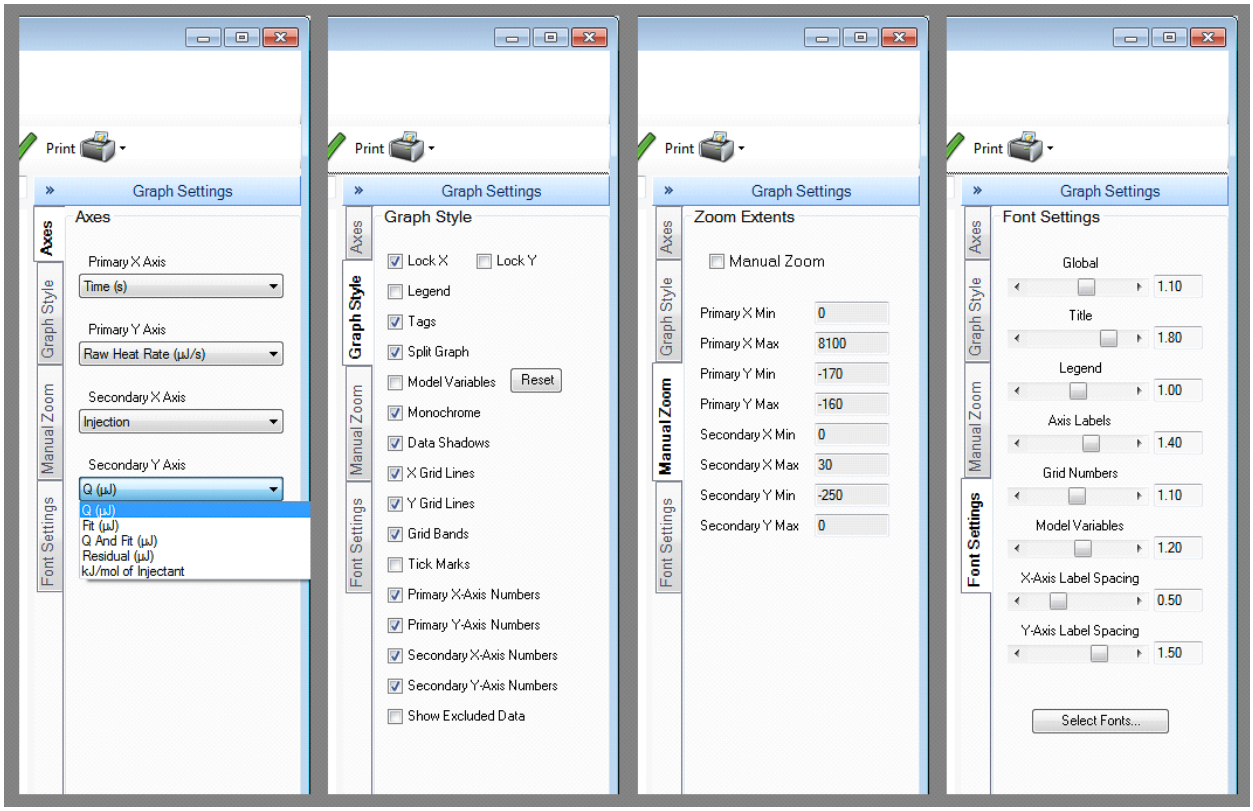


Figure 109 Graph formatting tabs.

Exporting Publication-Quality Graphs

The tool bar at the top of NanoAnalyze contains controls to allow exporting graphs in a variety of formats to either the file system or into the Windows clipboard for pasting into other applications. The export format options include the two scalable image formats EMF and WMF. There are also fixed-resolution raster output formats BMP, JPG, and PNG. Generally the highest quality images are available in the EMF and WMF formats.

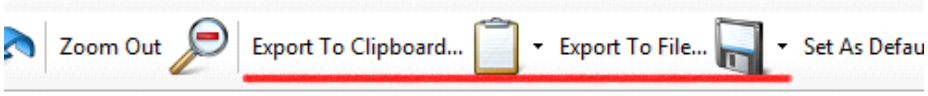


Figure 110 Export Controls.

1 Click on either **Export to Clipboard…** or **Export to File…** and select the desired image format. If you choose to create a file you will be asked to enter the file name and select a folder location.

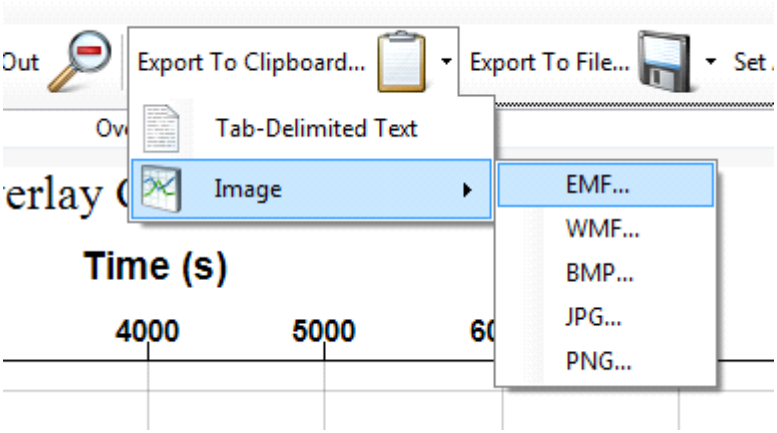


Figure 111 Selecting image export format.

2 A dialog box displays a representation of the graph. The aspect ratio, or ratio of length to height of the image, can be selected. Click **Export** to complete the graph export.

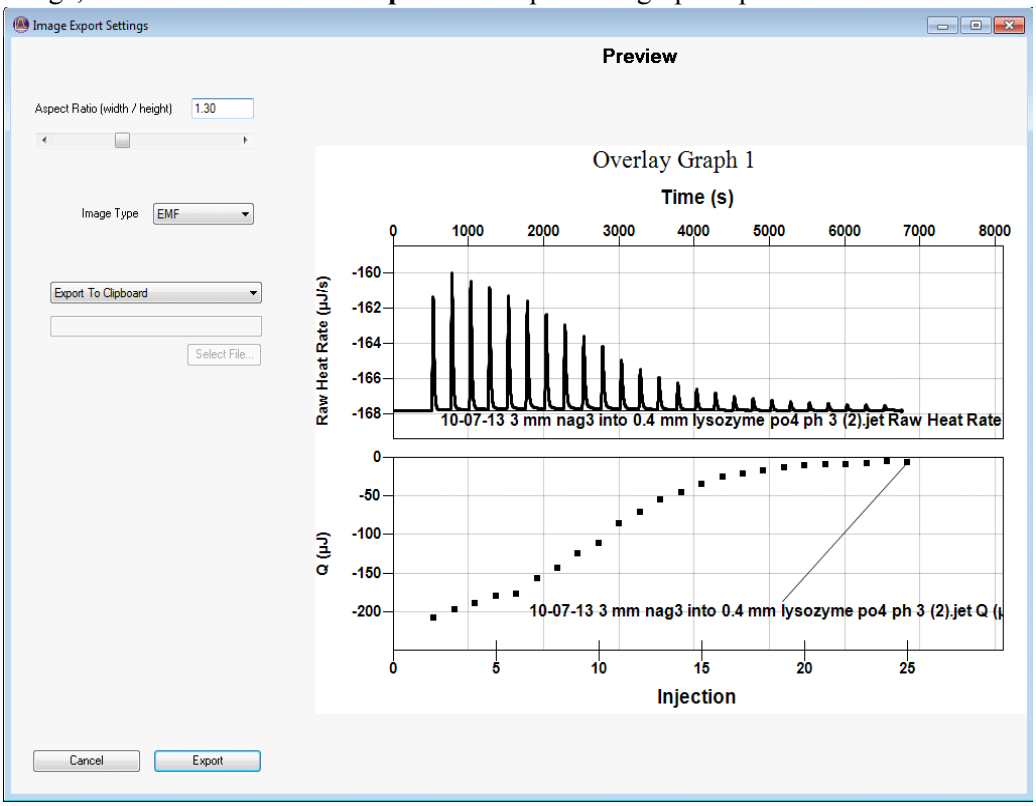


Figure 112 Image Export Settings.

Creating Graphs with Multiple Data Files

Load multiple files that you wish to compare into the file tree, and create a new Overlay Graph. Populate the graph by dragging files from the file tree over into the Primary graph data area.

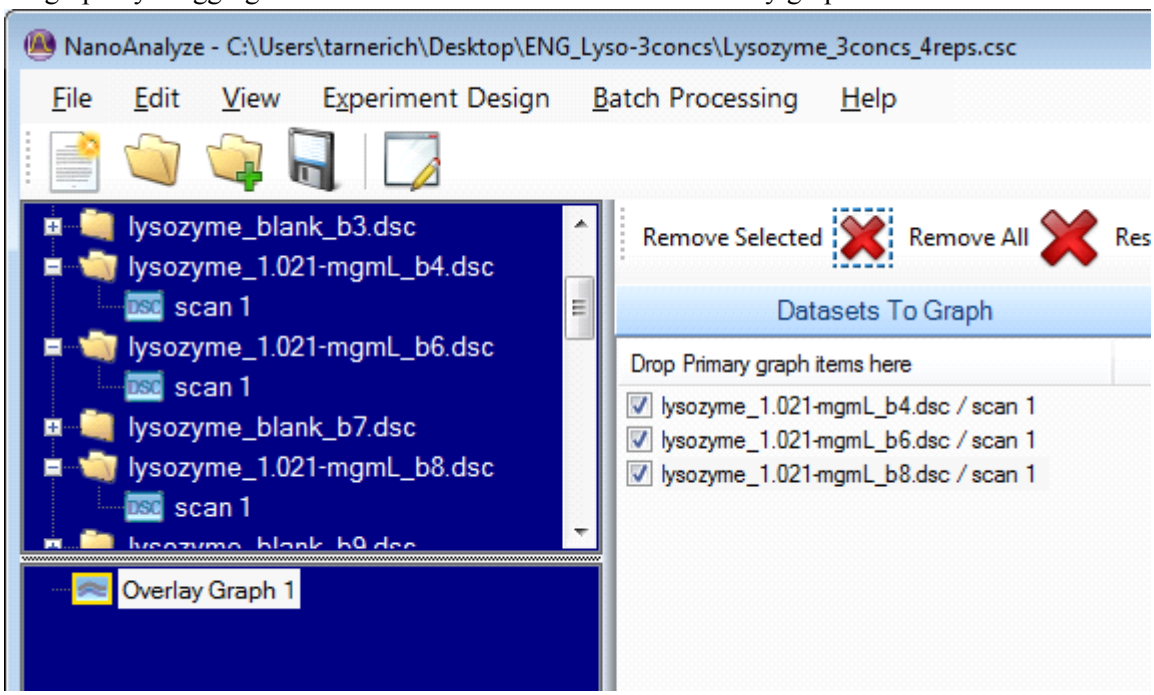


Figure 113 Populating multiple files into an Overlay Graph.

The data traces from each experiment will be graphed together. The files may be manually moved within the graph window to create a separation for visual emphasis. Note the DSC scans cannot be moved horizontally along a Temperature axis. In the Graph Style tab, select or deselect Lock X-Axis or Y-Axis as appropriate. Click on a data trace and drag to the desired position. Handles will appear on the selected data trace.

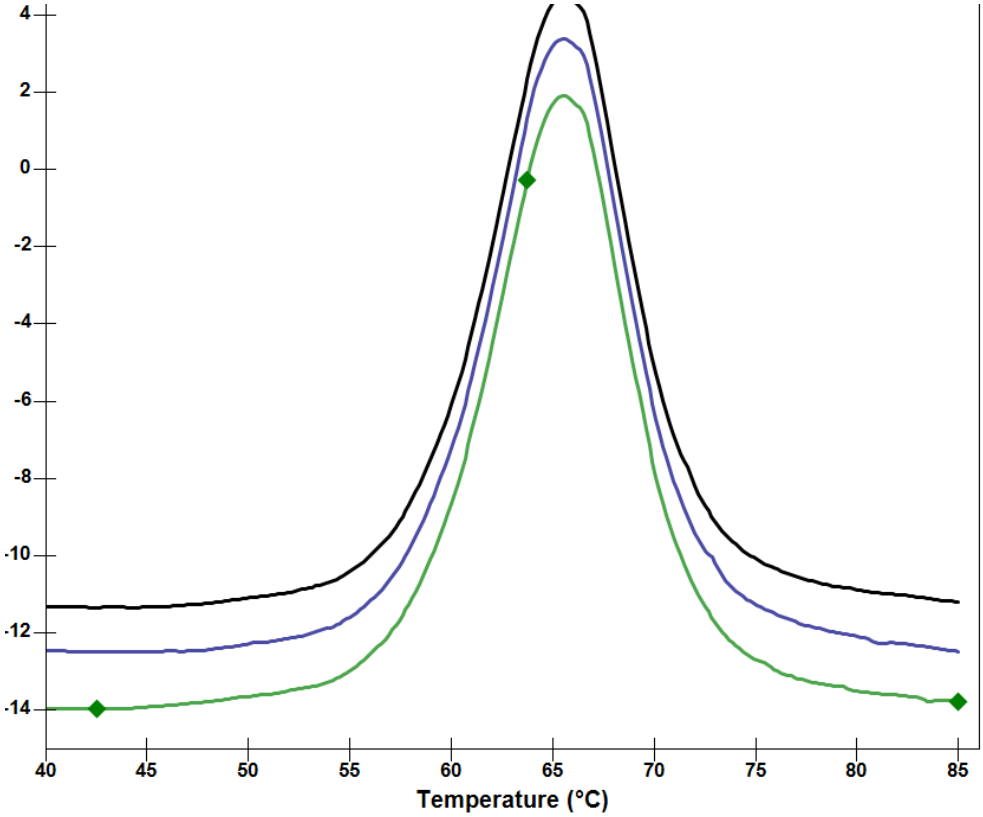


Figure 114 Selected data trace.

Exporting Data

It is frequently necessary to export instrument raw or processed data to external software applications such as word processors or spreadsheets. Data export functions have been provided in most locations within TA Instruments' software applications.

Data Export from a Table

Where data appears in a table (for example, in the NanoAnalyze **Data** viewing tab for a Nano ITC experiment or a Nano DSC scan), place the mouse cursor over the data table and right-click the mouse. A small pop-up menu appears.

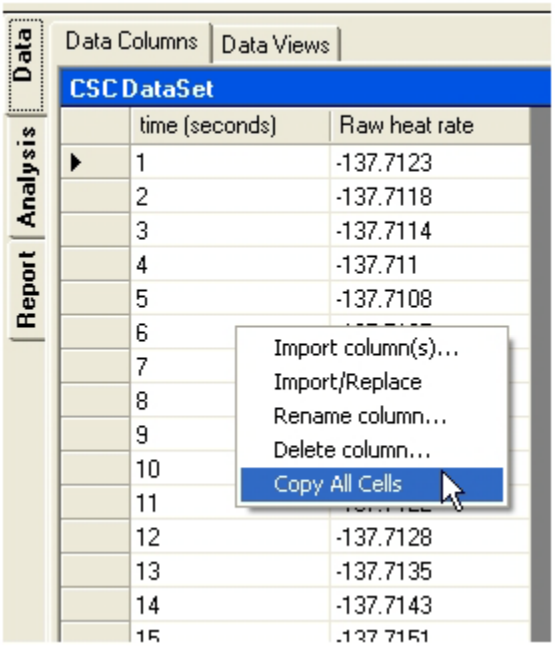


Figure 115 Copy All Cells.

Move the cursor onto and then release the button over the item labeled **Copy All Cells**. An hourglass icon may appear briefly while the data table is copied to the Windows clipboard. When the cursor returns to the normal arrow, the clipboard will contain a copy of the complete table for the entire experiment or scan. In the receiving application, use the mouse to select the desired location to paste the data. The application's **Paste** or **Paste Special** function (or the **Control-V** keyboard combination) will then create a copy of the data in the receiving application.

Copy to Clipboard Function

Some pages support data export via a dedicated control such as the **Copy to Clipboard** button seen in the **Area** tab of NanoAnalyze. The data is copied into the Windows clipboard when this button is clicked, and is ready to paste into a receiving application.

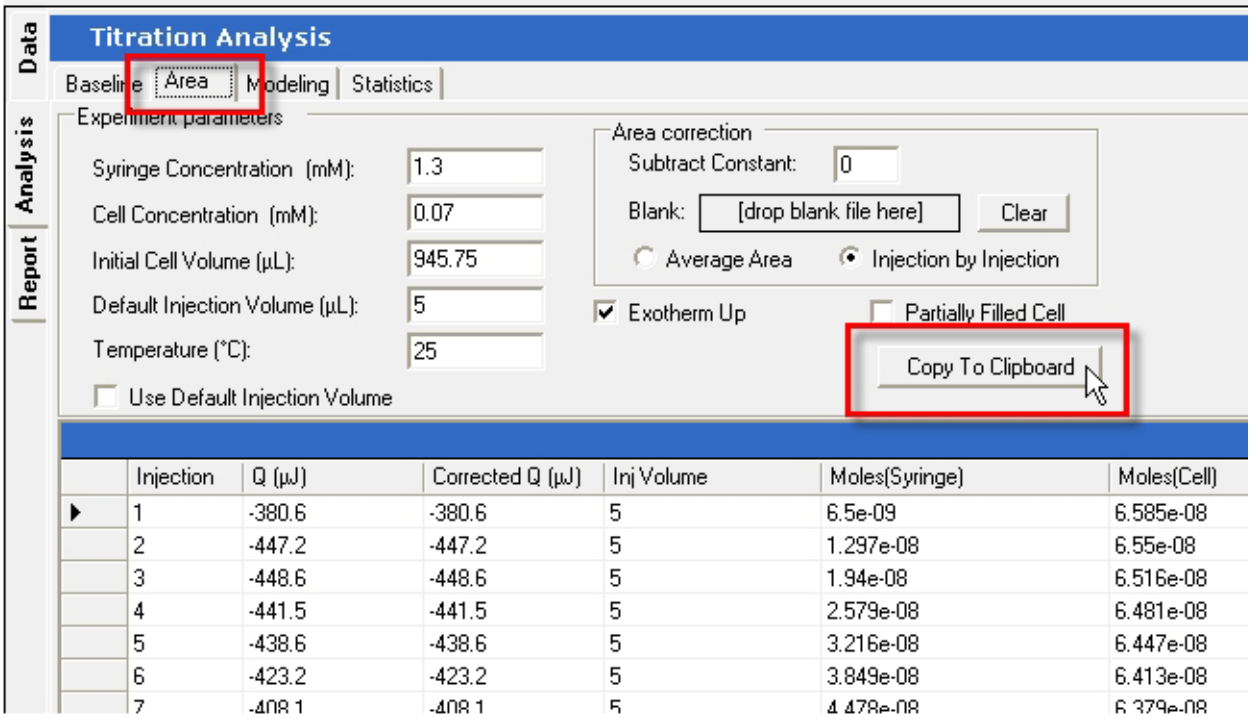


Figure 116 Area tab > Copy To Clipboard.

Export Dialog Function

Pages that display graphs have an export function that is accessed via a pop-up menu within the chart area.

1 Right-click the mouse inside the chart and select the **Export Dialog** item on the menu:

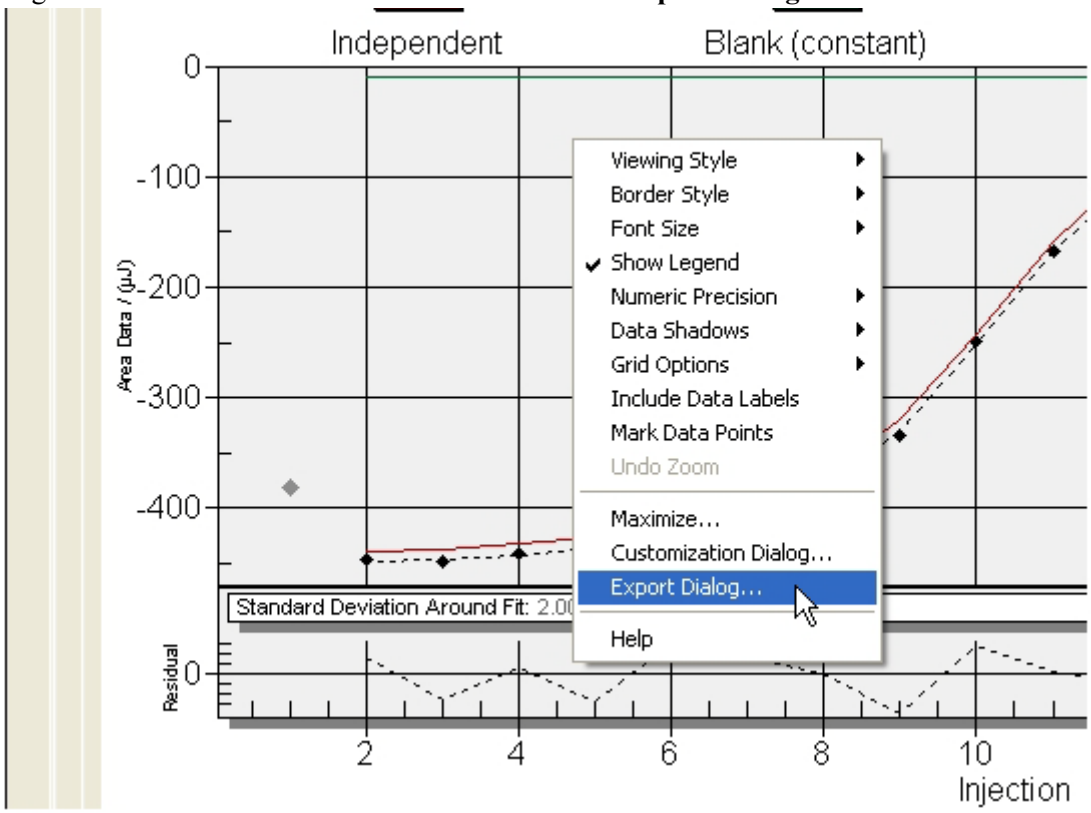


Figure 117 Export Dialog...

2 Select the **Text > Data Only** option and click the **Export** button. (Another available feature is to export an image of the graph using the Metafile, BMP, JPG, or PNG formats.)

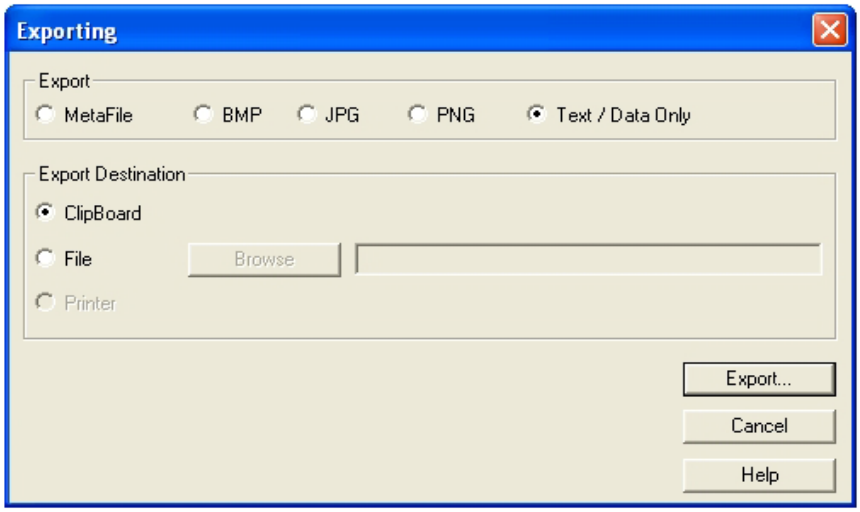


Figure 118 Select Text/Data Only.

3 For a more compact table of numeric results, select **Data** in the **Export What** group. The **List** option in the **Export Style** group creates a vertical array of data (narrow and long), while the **Table** option creates a horizontal array (wide and short). In general it is best to use the **Maximum Precision** option.

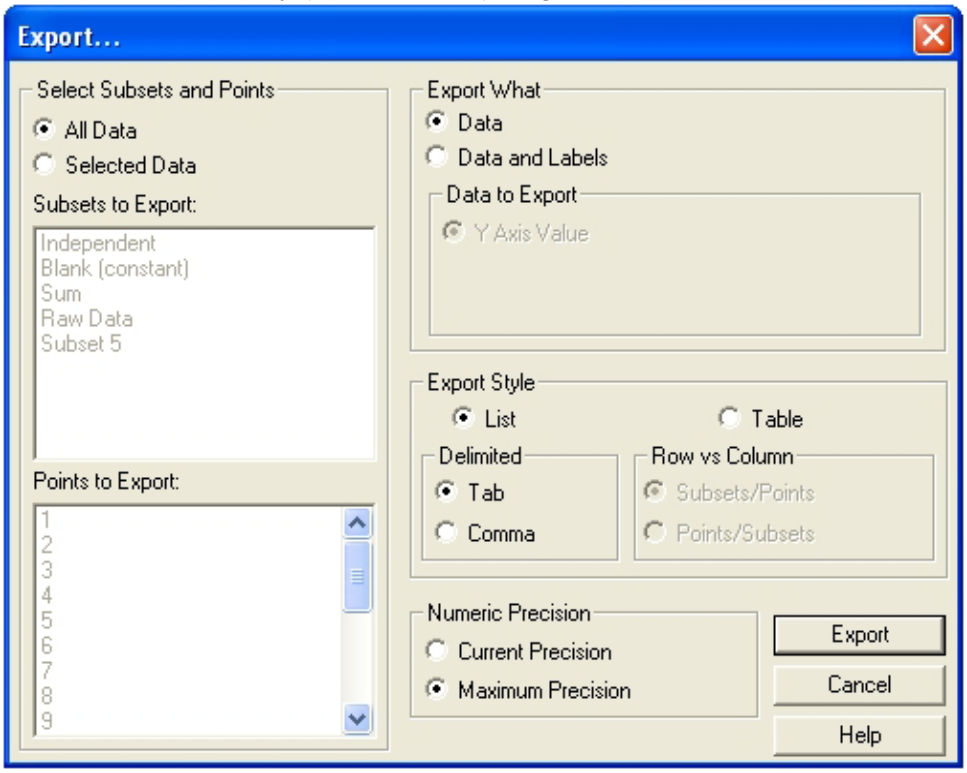


Figure 119 Export... dialog box.

When the data is copied to the clipboard, the dialog box closes.

Display Format Options

The visual appearance of TA Instruments software applications can be altered via functions available in a pop-up menu. Right-click the mouse while inside any window or tab to bring up the menu. There are appearance options for the screen area, plotting style, font, and number formats. Many of these options can be directly accessed from this menu.

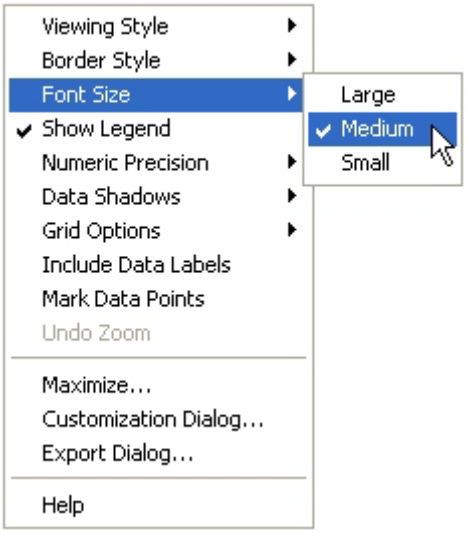


Figure 120 Formatting appearance.

A more complete set of display options can be accessed through the **Customization Dialog** menu item. Right-click on the data graph to bring up the menu:

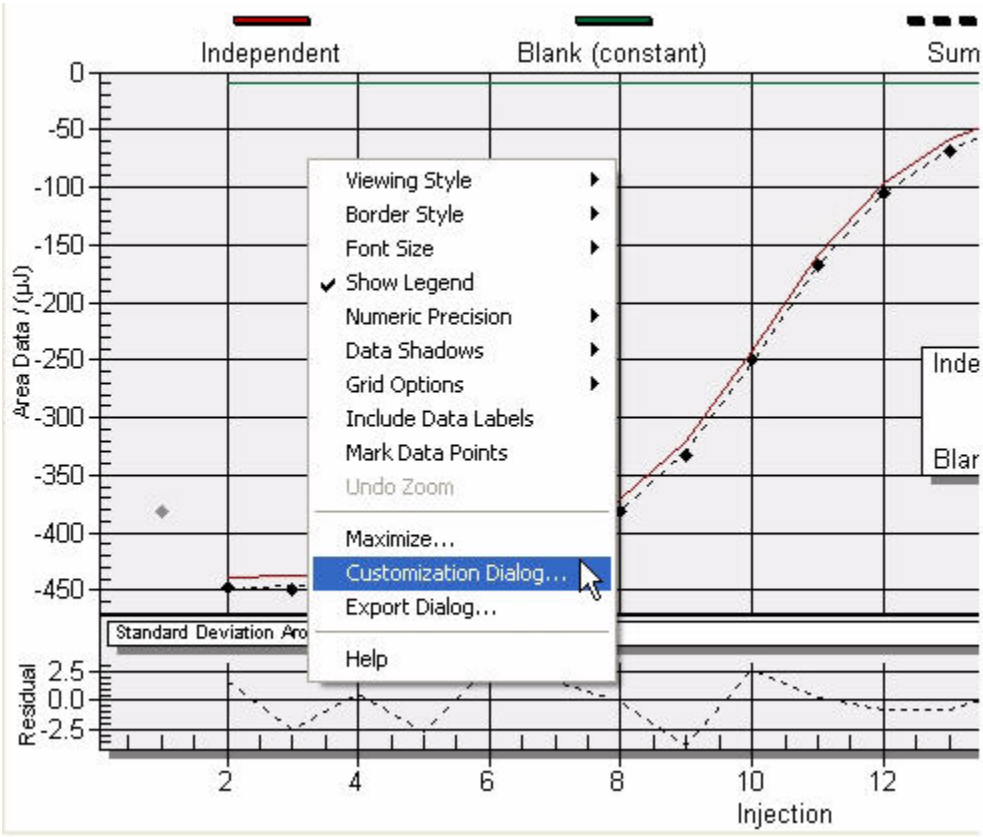


Figure 121 Select Customization Dialog...

The display controls have been grouped into tabs for easy access.

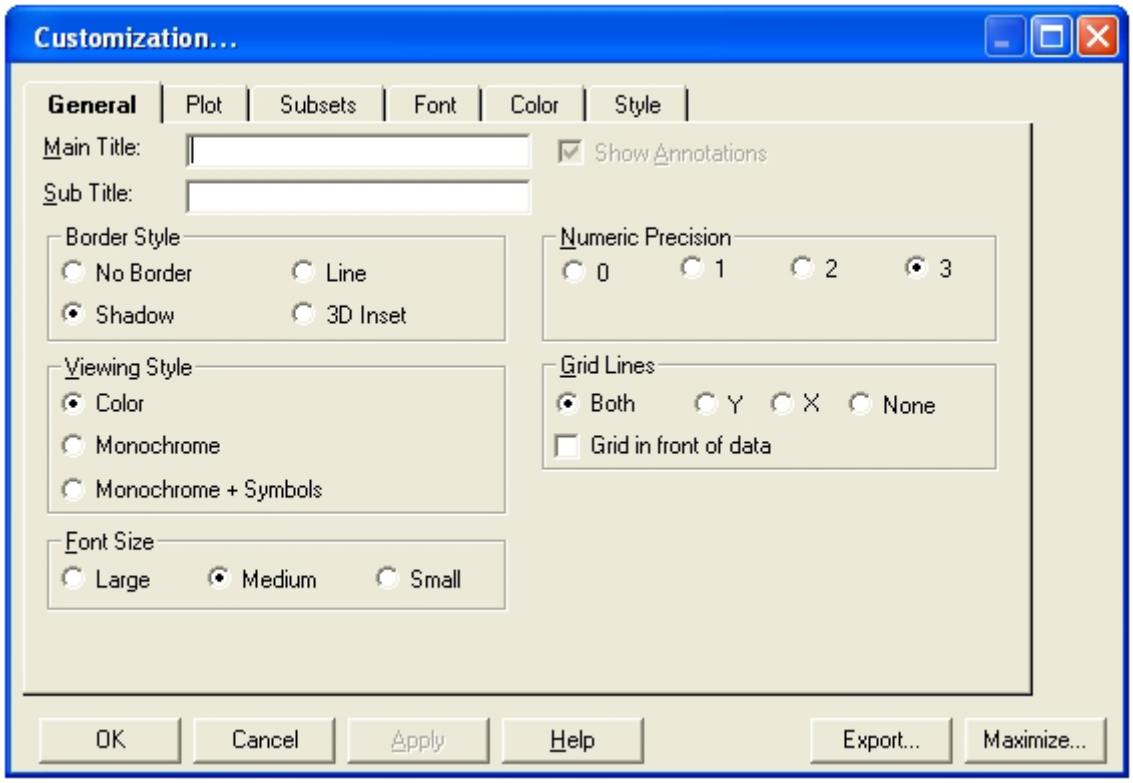


Figure 122 Customization... dialog box.

Appendix: A

Processing of Enzyme Kinetics Experiments

NanoAnalyze can process enzyme kinetics experiments using either the Michaelis-Menten or the Lineweaver-Burk methods. To get started, load a kinetics experiment data file into NanoAnalyze. An additional data file from an experiment based on the same chemical system, but with the concentrations adjusted in order to provide simple enthalpy data, can optionally be loaded as well.

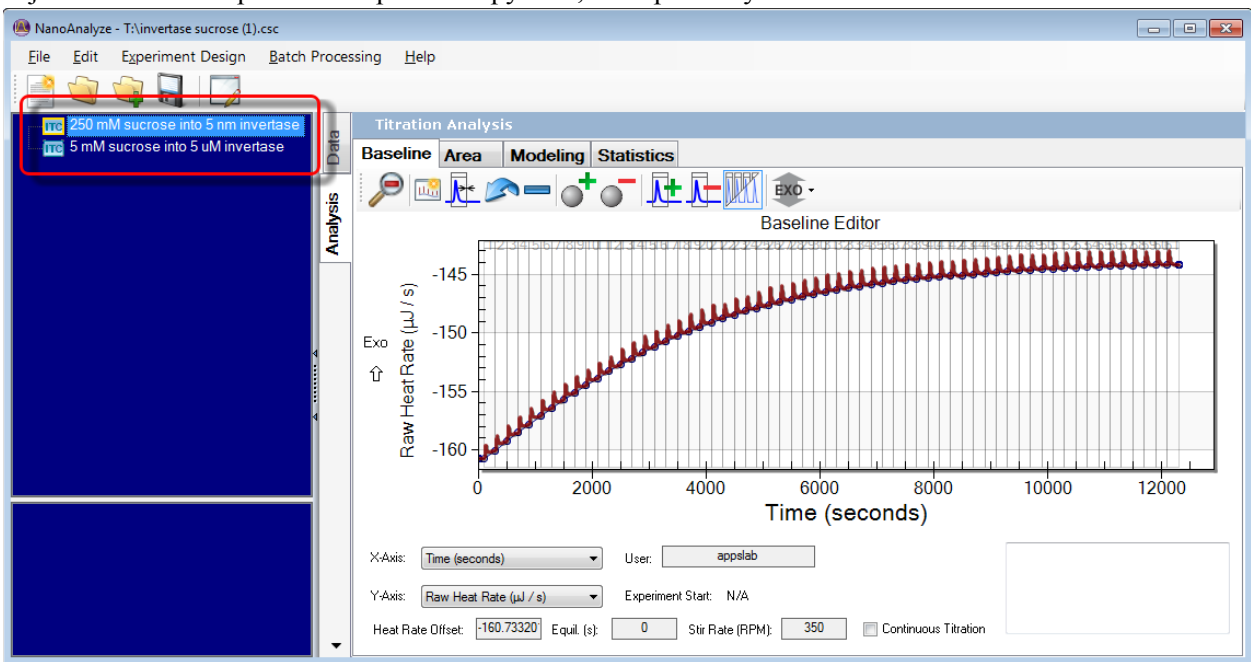


Figure 123

Now the data must be converted into the MIM format. In the File tree on the left side of the NanoAnalyze window right-click the file and select Properties.

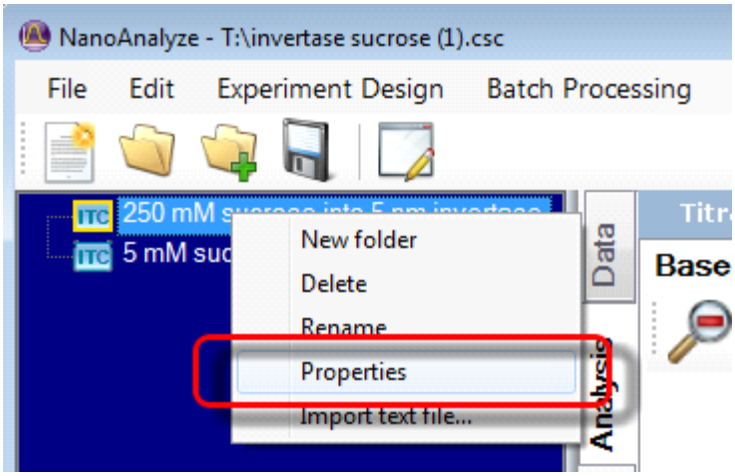


Figure 124

Select "Convert to MIM Enzyme Kinetics" and then click "Ok".

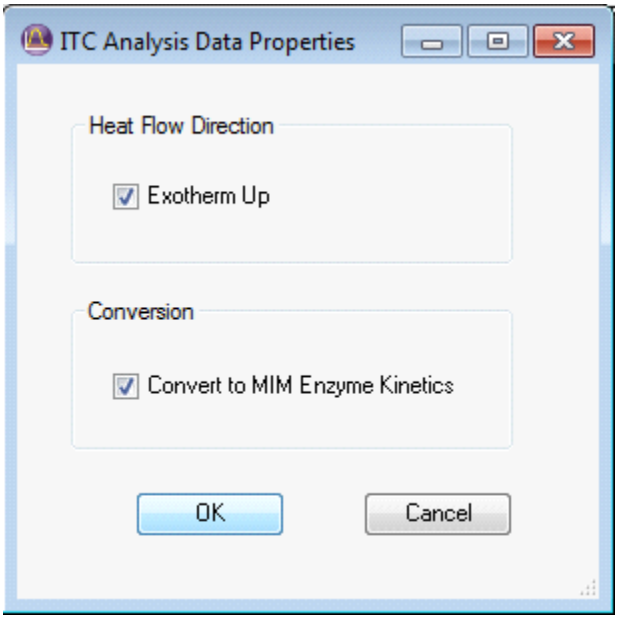


Figure 125

NanoAnalyze is now in the enzyme kinetics analysis mode. New controls and tabs for the processing steps appear above the data window. The concentration values, if they were input in ITCRun software at the time of setting up the experiment, will be carried over into NanoAnalyze. If desired, the units of concentration can be changed in NanoAnalyze software using the drop-down menu at the upper left of this page.

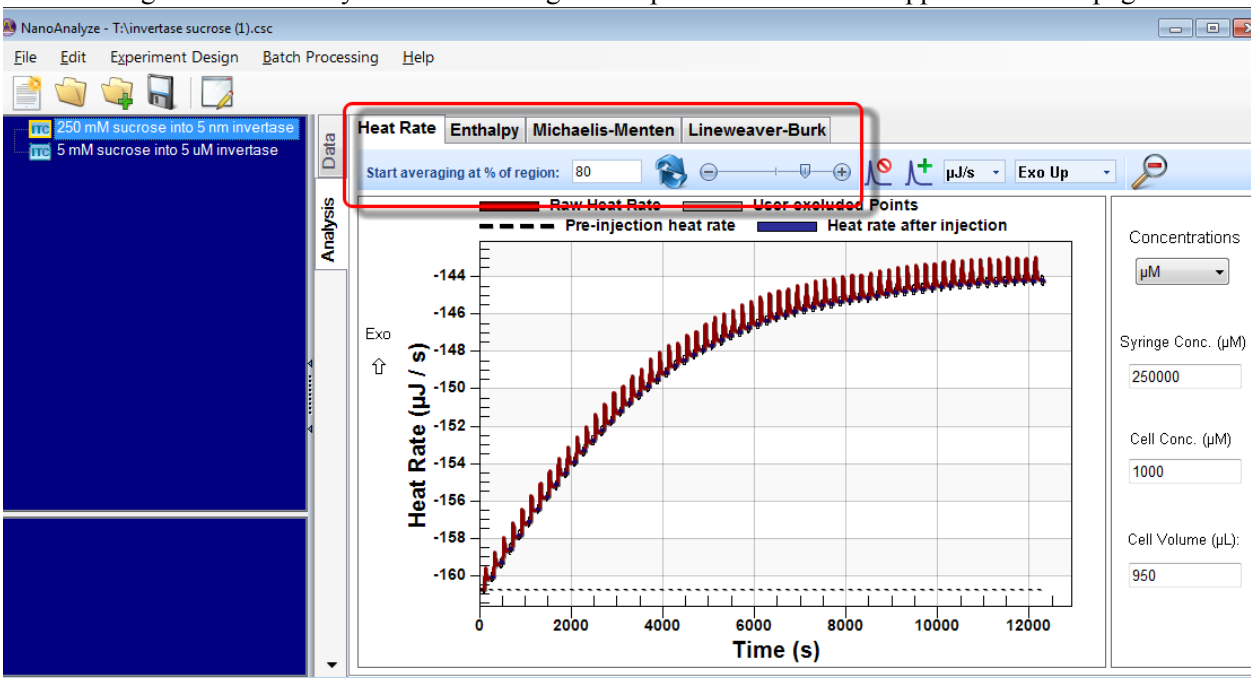


Figure 126

This zoomed-in view of the first several injections shows that analysis windows for each injection interval have been defined at the default range of the final 20% of each injection interval window.

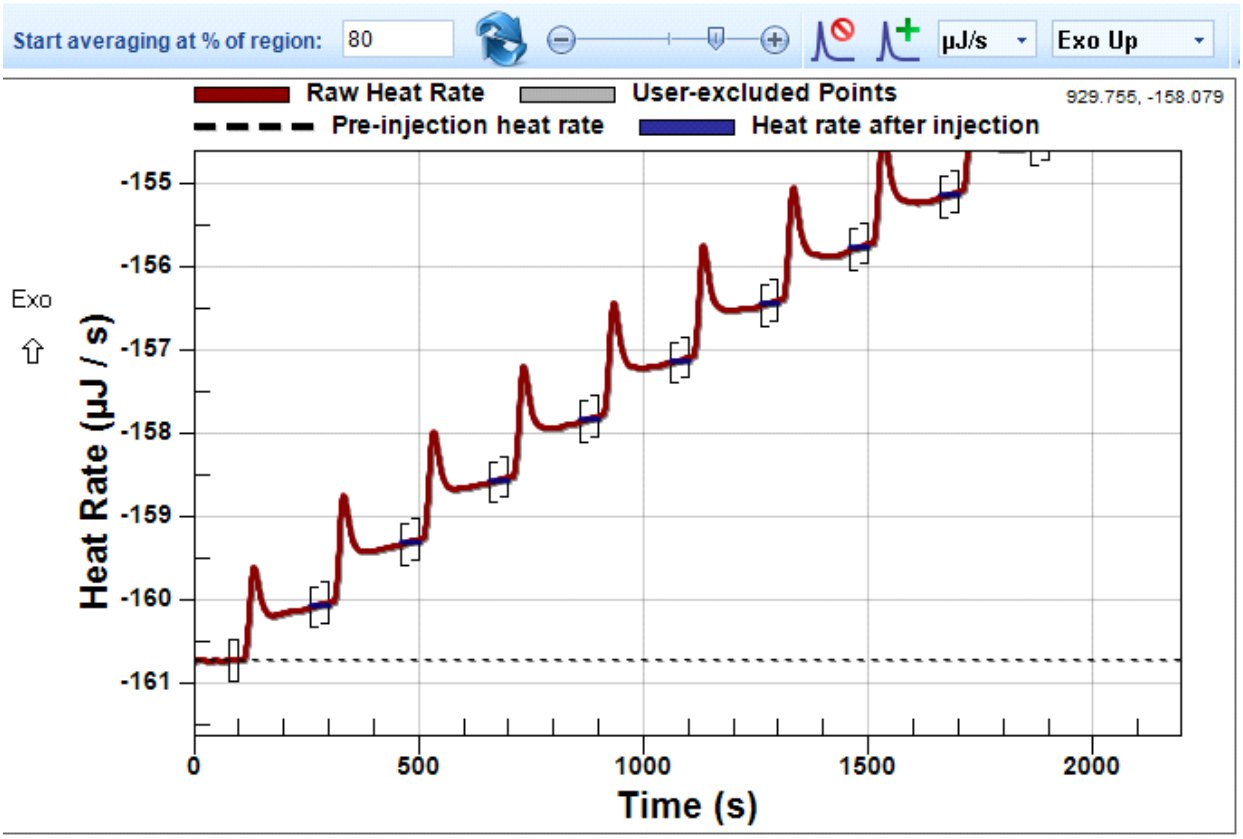


Figure 127

The widths may be adjusted if necessary, by altering the starting times for the windows. The start time can be altered by either entering a percentage figure in the text entry box (click the blue arrow icon to accept the updated number), moving the slider control, or clicking on the "-" or "+" buttons. The ends of the active

data windows are always located at the ends of their injection intervals; only the starting times are adjusted.

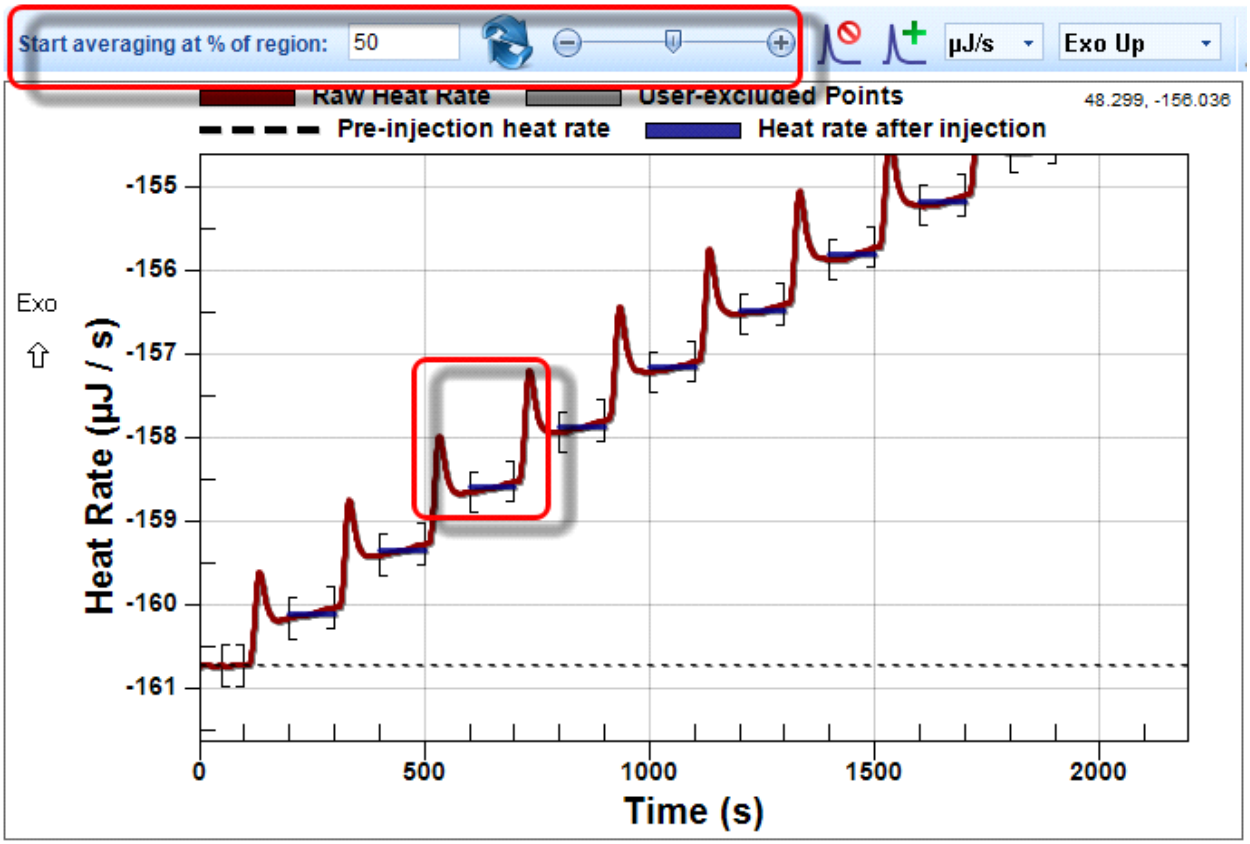


Figure 128

Proceed to the Enthalpy tab. The next step is to enter an enthalpy value for the reaction.

The value can be entered manually if this value is already known. Type the value into the entry window and click the "Apply" button. The manual entry box is highlighted in red.

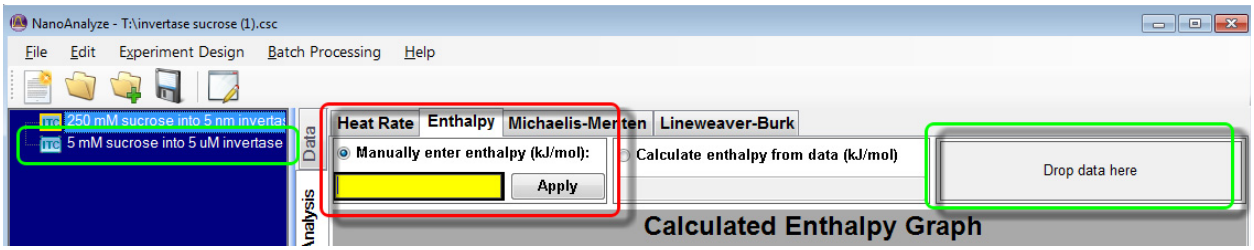


Figure 129

Another method of entering the enthalpy value is to extract it directly from an enthalpy experiment file. An enthalpy-only enzyme experiment typically uses a reduced injection amount to allow the reactions for each injection to proceed to completion within a short time interval. In the example shown below the substrate concentration was reduced by a factor of 25 in comparison to the kinetics experiment. The interval needs to be long enough to insure that the signal returns to baseline. For the enthalpy only titration, the substrate concentration is decreased and the enzyme concentration is increase. Under these conditions of low substrate and high enzyme, the enzyme will convert all of the substrate into product.

Add an enthalpy experiment into NanoAnalyze and it will appear in the file tree at the left of the program window. Click and drag the file over to the box titled "Drop Data Here". These fields are highlighted in green in the prior Figure 129. An average enthalpy value is calculated from all integrated peaks. This example exhibits the typical condition of initial peaks that have reduced areas due to the loss of some reactant from the injection syringe that occurs during the thermal stabilization of the samples in the instrument. The initial peak needs to be discarded in order to allow a correct averaging of the remaining peaks.

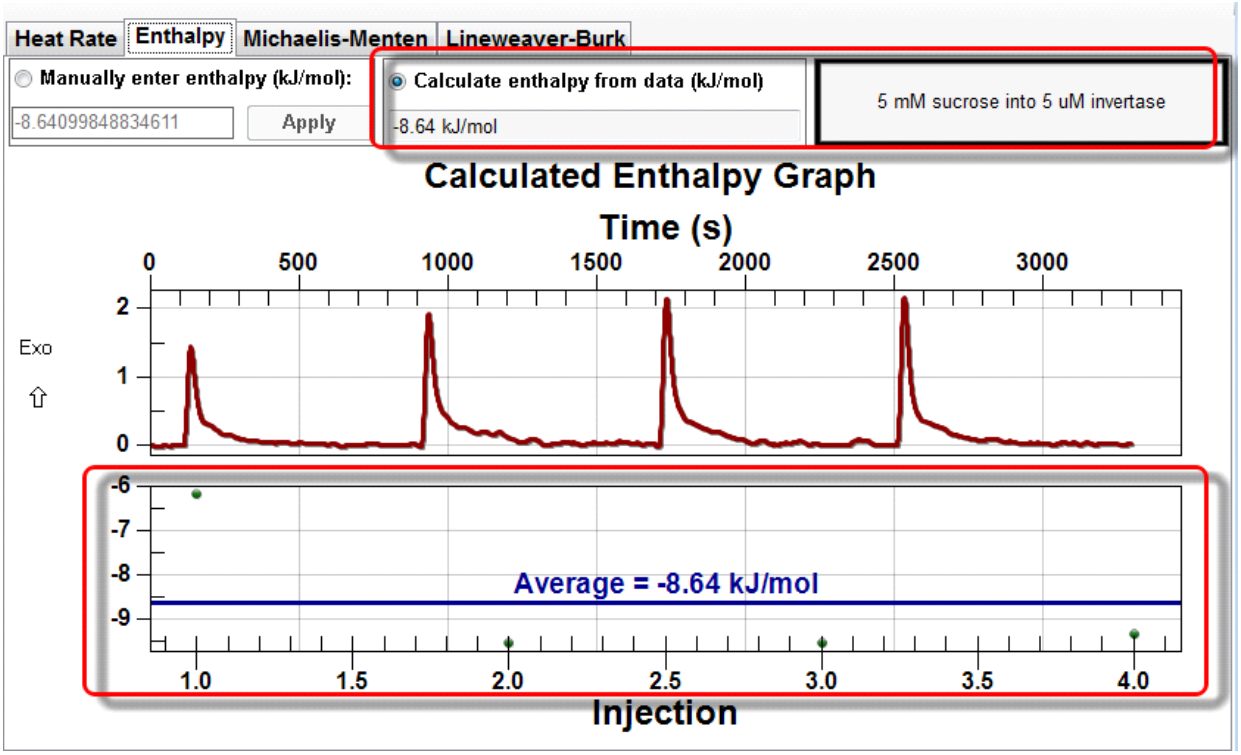


Figure 130

Individual peaks can be deselected by clicking on the appropriate green dot in the lower graph. Deselected dots show as gray instead of green. The average is now calculated on the basis of the remaining injection

peaks, and is now a visibly better representation of the correct average. The value in the calculated enthalpy window is automatically updated.

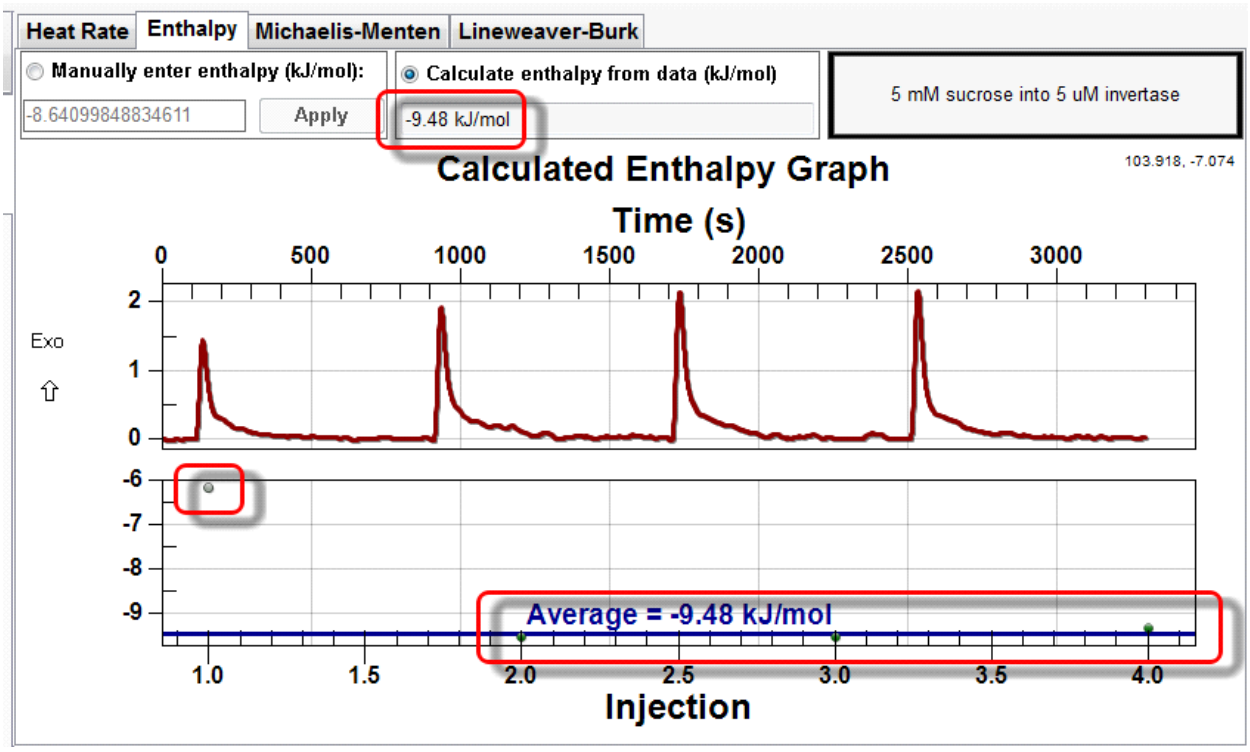


Figure 131

The kinetics calculations are now completed and ready for review. Michaelis-Menten and Lineweaver-Burk analysis methods are presented. Individual data points that might not fit the modeling function, most likely the early injection peaks, can be deselected by clicking on the colored dots. Both analysis methods are updated, and the deselected data points turn gray. There may be a difference between the calculated results of the two methods because of the nonlinear scale transformation, which effectively changes the weighting values of the data points. In the Michaelis-Menten method the weighting is equal for all data points. In the example shown below the data points for the first three injections have been eliminated in

order to allow for an improved fit of the modeling curve. Note that when the view is switched to the Lineweaver-Burk analysis method, the same points are deselected there was well.

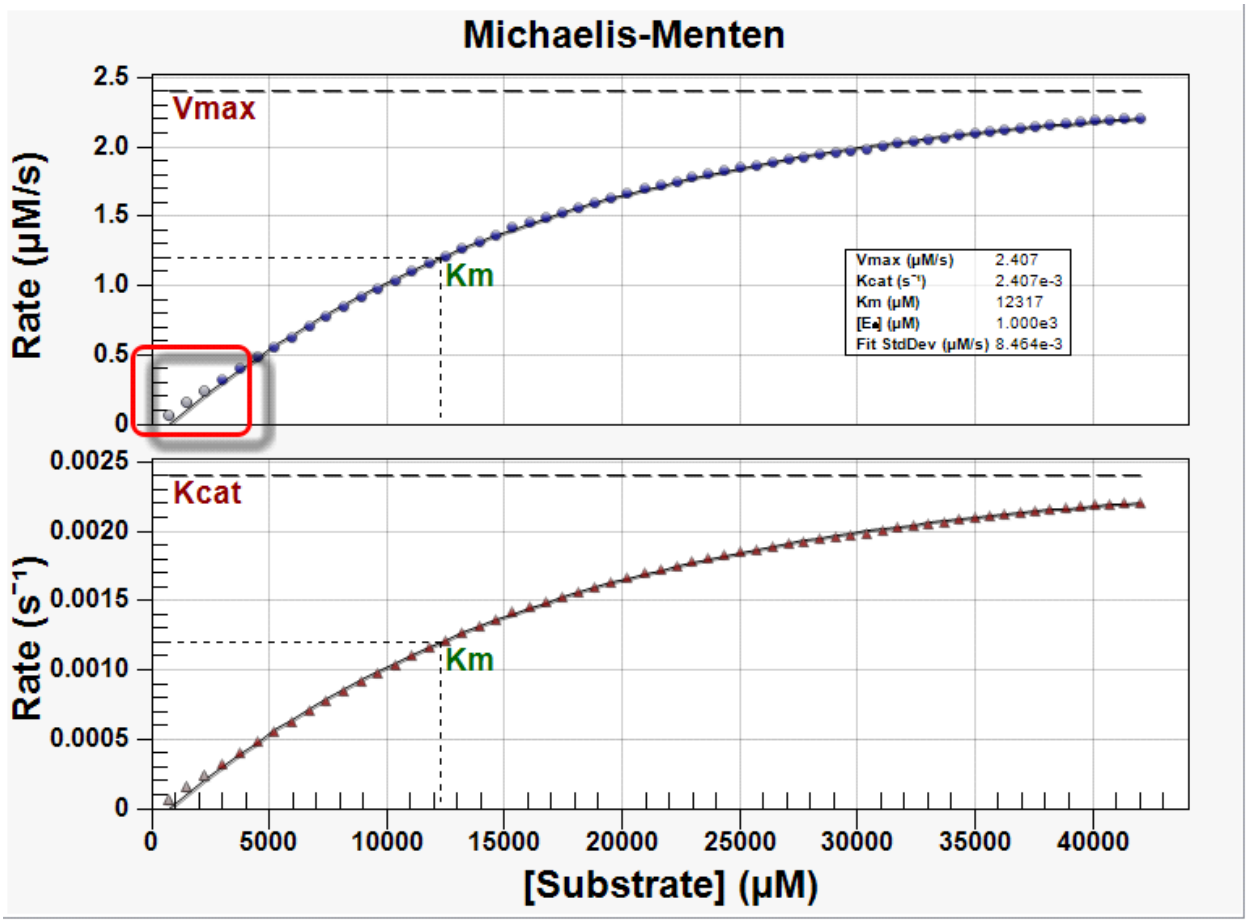


Figure 132

The Lineweaver-Burk method features a stronger weighting of the data points from the later injections, which appear on the right hand side of the chart. Note that any data points which have been eliminated in the Michaelis-Menten analysis tab are also eliminated from the data set used for the Lineweaver-Burk

analysis. Note that because Michaelis-Menten and Lineweaver-Burk have different weightings of the data points in their curve fits, it is possible for the analytical results to differ as well.

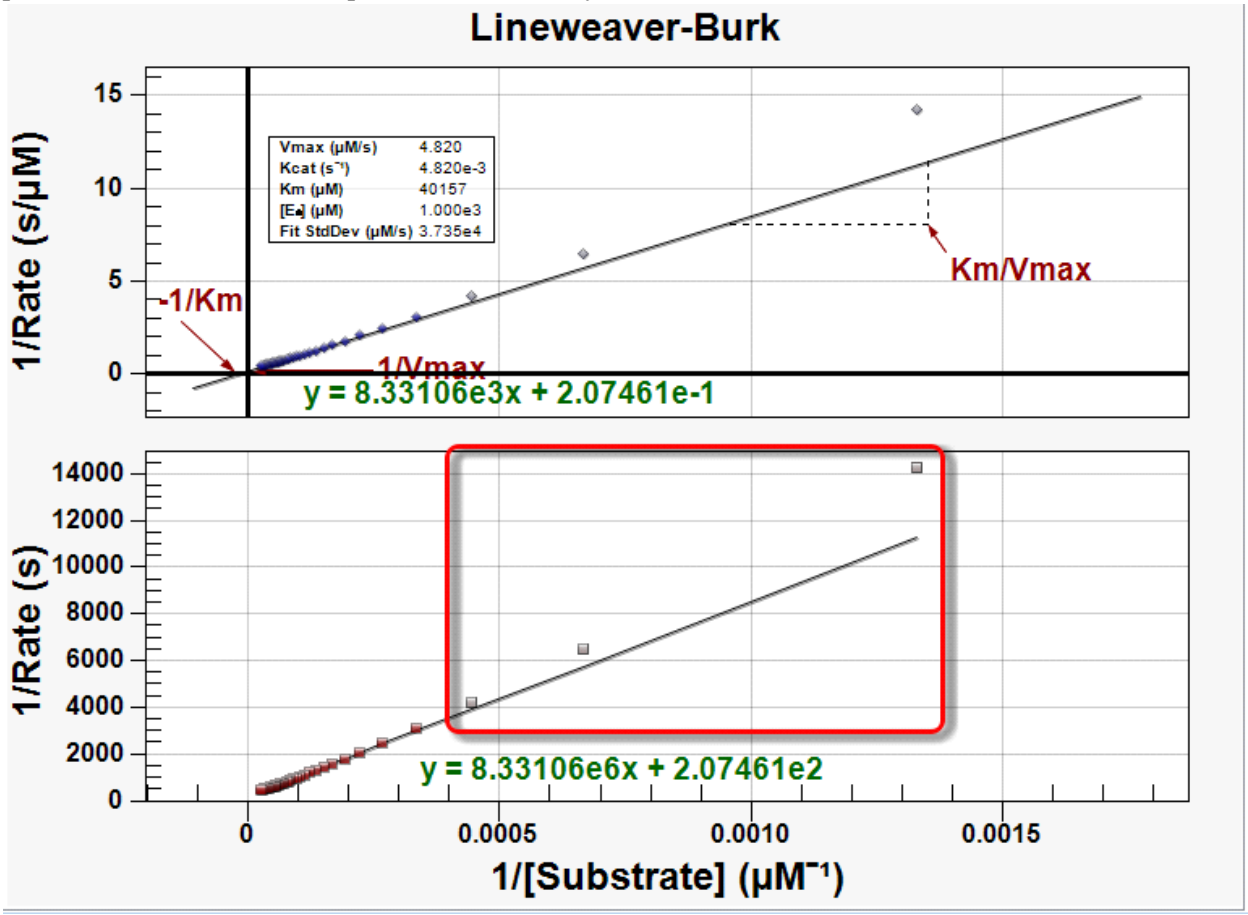


Figure 133

The numeric formats of the data fields in the results table can be adjusted if necessary. Click the Settings icon  to bring up a dialog box for setting the desired numeric formats.

Select the desired number of display digits for any date field and click the "Ok" button when done.

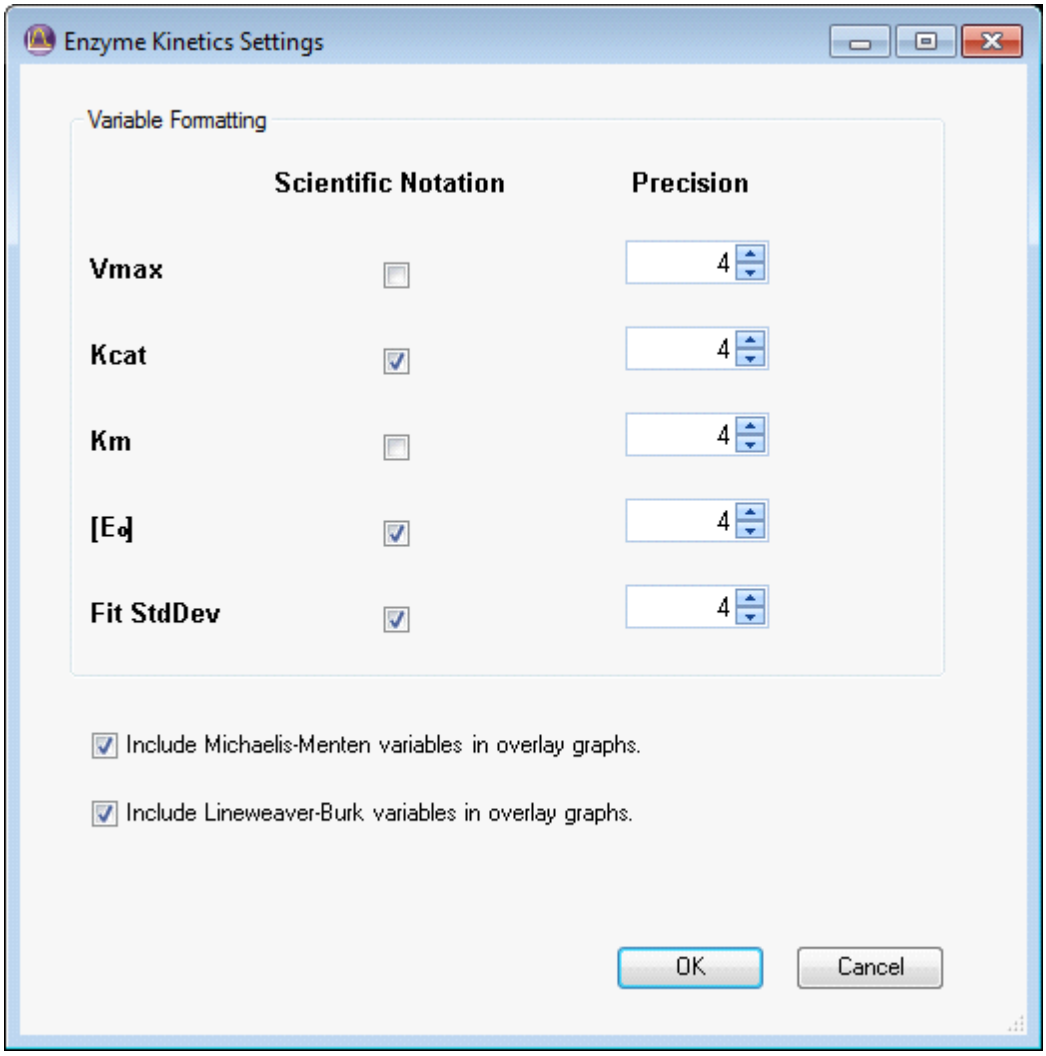


Figure 134

Appendix: B

Models Reference

ITC Binding Model Name: Independent

Main Function

```
' quadratic constants a, b, and c
Dim a As Double =  Ka
Dim b As Double = -Ka * (MolesSyringe(iteration) + MolesCell(iteration) * n) - CellVolume(iteration) / 1e6
Dim c As Double =  Ka * MolesSyringe(iteration) * MolesCell(iteration) * n
Bound = (-b - Sqrt(b*b - 4*a*c)) / (2*a)' Calculate the root using quadratic formula
If OverfillMode Then' Take loss of solution from overfill into account
   OldBound = OldBound * (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
End If
Heat = 1e9 * (Bound-Oldbound) * dh' Calculate heat
Oldbound = Bound' Save value of bound for the next iteration of modeldatapoint()
Return Heat
```

Initialization Functions

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
OldBound = 0
```

Auxiliary Functions

```
Public Function dS() As Double
  Return (dH * 1000 + R * (Temperature + 273.15) * log(Ka)) / (Temperature + 273.15)
End Function

Public Function Kd() As Double
  Return 1 / Ka
End Function
```

Global Variables and Constants

```
Dim OldBound As Double
Dim OverfillMode As Boolean
Dim Bound As Double
Dim TotalCellVolume As Double
Dim Heat As Double
```

Provided Constants

These constants are automatically created and are available for use in the model.

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Minimized Independent

Main Function

```
Dim CellVol = CellVolume(iteration) / 1e6
Atotal = MolesSyringe(iteration)
Btotal = MolesCell(iteration) * n
max = Math.Min(Atotal, Btotal) * 0.999999999999999
If OverfillMode Then
    OldAB = OldAB * (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
End If
AB = Host.Minimize(AB_Estimate, 1e-308, max, 2, AddressOf KaCalculation, Ka, Atotal, Btotal, Cell-
Vol)
AB_Estimate = AB * 0.999999999999999 ' for next iteration
Heat = 1e9 * (AB - OldAB) * dH
OldAB = AB
Return Heat
```

Initialization Functions

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
OldAB = 0
Atotal = MolesSyringe(0)
Btotal = MolesCell(0) * n
max = Math.Min(Atotal, Btotal)
AB_Estimate = (max + 1e-100) / 2.0 'Initial estimate
```

Auxiliary Functions

```
Public Function dS() As Double
    Return (dH * 1000 + R * (Temperature + 273.15) * log(Ka)) / (Temperature + 273.15)
End Function


Public Function Kd() As Double
    Return 1 / Ka
End Function
Public Function KaCalculation(ByVal parameters() As Double) As Double
   Dim AB As Double = parameters(0) ' The first parameter is the independent variable
   Dim Ka As Double = parameters(1)
   Dim Atotal As Double = parameters(2)
   Dim Btotal As Double = parameters(3)
   Dim CellVol As Double = parameters(4)
   Dim EquationLeftSide As Double
   Dim EquationRightSide As Double
   EquationLeftSide = Ka
   EquationRightSide = (AB / ((Atotal - AB) * (Btotal - AB))) * CellVol
Return EquationLeftSide - EquationRightSide
End Function
```

Global Variables and Constants

```
Dim AB As Double
Dim OldAB As Double
Dim AB_Estimate As Double
Dim Atotal As Double
```

Dim Btotal As Double

Dim OverfillMode As Boolean

Dim TotalCellVolume As Double

Dim Heat As Double

Dim max As Double

Provided Constants'

These constants are automatically created and are available for use in the model.

Private Readonly TitrantmM as Double = 0.1

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

ITC Model Name: Blank (constant)

Main Function

Return blank

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

(none)

Provided Constants

Private Readonly TitrantmM as Double = 0.1

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

ITC Model Name: Blank (linear)

Main Function

Dim XValue As Double = iteration + 1

Return slope * XValue + intercept

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

(none)

Provided Constants

Private Readonly TitrantmM as Double = 0.1

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

ITC Binding Model Name: Multiple Sites

Main Function

cellVol = CellVolume(iteration) / 1e6

St = MolesSyringe(iteration) / cellVol

Ct = MolesCell(iteration) / cellVol

' Cubic polynomial constants

```
Dim a As Double = Ka1 * Ka2
Dim b As Double = Ka1 + Ka2 + a * (Ct * (n1 + n2) - St)
Dim c As Double = 1.0 + Ct * (n1 * Ka1 + n2 * Ka2) - St * (Ka1 + Ka2)
Dim d As Double = -St
Dim roots As Double() = Host.GetCubicEquationRoots(a, b, c, d)
Xfree = roots(0)
If OverfillMode Then
    OverfillDisplacementRatio = (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
    OldQ = OldQ * OverfillDisplacementRatio
End If
 Dim Q As Double = 1e9 * cellVol * Ct * (n1 * dH1 * Ka1 * Xfree / (1.0 + Ka1 * Xfree) + n2 * dH2 * Ka2
* Xfree / (1.0 + Ka2 * Xfree))
Dim dQ As Double = Q - OldQ
OldQ = Q
Return dQ
```

Initialization Functions

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
OldQ = 0.0
```

Auxiliary Functions

```
Public Function dS1() As Double
  Return (dH1 * 1000 + R * (Temperature + 273.15) * log(Ka1)) / (Temperature + 273.15)
End Function

 Public Function dS2() As Double
  Return (dH2 * 1000 + R * (Temperature + 273.15) * log(Ka2)) / (Temperature + 273.15)
 End Function

 Public Function Kd1() As Double
  Return 1 / Ka1
 End Function

 Public Function Kd2() As Double
```

```
  Return 1 / Ka2
End Function
```

Global Variables and Constants

```
Dim OldQ
Dim cellVol
Dim St
Dim Ct
Dim Xfree
Dim OverfillMode
Dim OverfillDisplacementRatio
Dim TotalCellVolume
```

Provided Constants

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Dimer Dissociation

Main Function

```
CellVol = CellVolume(iteration)/1e6
InjVol = InjectionVolume(iteration)/1e6
Ci = MolesSyringe(iteration) / CellVol

'Calculate total dimer for cell
A = 4.0
B = -4.0 * Ci - Kd
C = Ci * Ci
DimerCell = (-B - Math.Sqrt(B*B -4*A*C)) / (2*A)
```

```
If OverfillMode Then
    LastDimerCell = LastDimerCell * (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
End If

Dim Heat As Double = 1e9 * dH *  ( (DimerSyr * InjVol) - ((DimerCell - LastDimerCell) * CellVol) )
LastDimerCell = DimerCell
Return Heat
```

Initialization Functions

```
LastDimerCell = 0

OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)

'calculate total dimer for syringe
Csyr = TitrantmM / 1e3
A = 4
B = -4.0 * Csyr - Kd
C = Csyr * Csyr
DimerSyr = (-B -Math.Sqrt(B*B - 4*A*C)) / (2*A)
```

Auxiliary Functions

```
Public Function dS() As Double
  Return (dH * 1000 + R * (Temperature + 273.15) * log(1.0 / Kd)) / (Temperature + 273.15)
End Function

Public Function Ka() As Double
  Return 1.0 / Kd
End Function
```

Global Variables and Constants

```
Dim CellVol As Double
Dim InjVol As Double
Dim Csyr As Double
Dim Ci As Double
```

Dim A As Double

Dim B As Double

Dim C As Double

Dim DimerSyr As Double

Dim DimerCell As Double

Dim Dimer As Double

Dim LastDimerCell As Double

Dim OldDimer As Double

Dim OverfillMode As Boolean

Dim TotalCellVolume As Double

Provided Constants

Private Readonly TitrantmM as Double = 0.1

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

ITC Binding Model Name: Competitive Replacement

Main Function

totalL2 = MolesSyringe(iteration) / V

totalP = MolesCell(iteration) / V

totalL1 = totalP * (L1mM / TitratemM)

' Displacement calculation (Experiment must be run in "overfill" mode.)

OverfillDisplacementRatio = (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume

LastPL1 = LastPL1 * OverfillDisplacementRatio

LastPL2 = LastPL2 * OverfillDisplacementRatio

PL1 = Host.Minimize(PL1_Estimate, 1.7e-308, max, 2, AddressOf PL1Minimization, Ka1, Ka2, totalP, totalL1, totalL2)

PL2 = (totalP - (PL1/((totalL1-PL1)*Ka1)) - PL1)

max = PL1 ' PL1 decreases with each injection

PL1_Estimate = PL1 ' Next estimate

```
Dim Heat As Double
If iteration = 0 Then
    Heat = 0.0
Else
   Heat = (((PL1-LastPL1) * dH1)+((PL2-LastPL2) * dH2)) * V * 1e9
End If
LastPL1 = PL1
LastPL2 = PL2
Return Heat
```

Initialization Functions

```
TotalCellVolume = CellVolume(0)
LastPL1 = 0
LastPL2 = 0
V = CellVolume(0) / 1000000.0
totalP = MolesCell(0) / V
max = totalP
PL1_Estimate = GetPL1Estimate(1.7e-308, max, Ka1, Ka2, totalP, totalP * (L1mM / TitratemM),
MolesSyringe(0) / V)
```

Auxiliary Functions

```
Public Function dS1() As Double
  Return (dH1 * 1000 + R * (Temperature + 273.15) * log(Ka1)) / (Temperature + 273.15)
End Function

Public Function dS2() As Double
  Return (dH2 * 1000 + R * (Temperature + 273.15) * log(Ka2)) / (Temperature + 273.15)
End Function

Public Function Kd1() As Double
  Return 1 / Ka1
End Function

Public Function Kd2() As Double
```

```
  Return 1 / Ka2
End Function

Public Function PL1Minimization(ByVal parameters() As Double) As Double
  Dim PL1 As Double = parameters(0) ' The first parameter is the independent variable
  Dim Ka1 As Double = parameters(1)
  Dim Ka2 As Double = parameters(2)
  Dim totalP As Double = parameters(3)
  Dim totalL1 As Double = parameters(4)
  Dim totalL2 As Double = parameters(5)
  Dim EquationLeftSide As Double
  Dim EquationRightSide As Double
  EquationLeftSide = Ka1 * ((totalP - (PL1 / ((totalL1-PL1) * Ka1)) - PL1) * (totalL1-PL1))
  EquationRightSide = Ka2 * (totalL2 - (totalP - (PL1 / ((totalL1-PL1) * Ka1)) - PL1)) * PL1
  Return EquationLeftSide - EquationRightSide
End Function

Private Function GetPL1Estimate(ByVal min As Double, ByVal max As Double, ByVal Ka1 As Double,
ByVal Ka2 As Double,  ByVal totalP As Double, ByVal totalL1 As Double, ByVal totalL2 As Double) As
Double
  Dim EquationLeftSide As Double
  Dim EquationRightSide As Double
  Dim MinDifference As Double = Double.MaxValue
  Dim Difference As Double
  Dim PL1 As Double
  Dim i As Integer
  Dim MinDifferenceIndex As Integer
  For i = 0 To 100
    PL1 = min + i * (max - min)  / 100.0
    EquationLeftSide = Ka1 * ((totalP - (PL1 / ((totalL1-PL1) * Ka1)) - PL1) * (totalL1-PL1))
    EquationRightSide = Ka2 * (totalL2 - (totalP - (PL1 / ((totalL1-PL1) * Ka1)) - PL1)) * PL1
    Difference = Math.Abs(EquationLeftSide - EquationRightSide)
    If (Difference < MinDifference)  Then
      MinDifference = Difference
      MinDifferenceIndex = i
    End If
```

```
  Next i
  Return min + MinDifferenceIndex * (max - min)  / 100.0
End Function
```

Global Variables and Constants

```
Dim PL1 As Double
Dim PL2 As Double
Dim LastPL1 As Double
Dim LastPL2 As Double
Dim PL1_Estimate As Double
Dim totalL1 As Double
Dim totalL2 As Double
Dim totalP As Double
Dim max As Double
Dim V As Double
Dim TotalCellVolume As Double
Dim OverfillDisplacementRatio As Double
```

Provided Constants

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Cooperative

Main Function

```
  Dim xf
  Dim z
  gB1 = b1
  gB2 = b2
  CellVol = CellVolume(iteration) / 1e6
```

```
St = (MolesSyringe(iteration) / CellVol)
Ct = (MolesCell(iteration) / CellVol)
xf = Host.ZBrent(0, St, 1e-12, gB1, gB2, St, Ct)
z = (b1 * xf * dH1 + b2 * xf * xf * dH2) * 1000
heat = 1e6 * z * CellVol * Ct / Host.ZBrentPartition(xf, gB1, gB2)
If OverfillMode Then
  b = b + heat * (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
End If
heat = heat + b
Dim deltaHeat As Double = heat - heatOld
heatOld = heat
Return deltaHeat
```

Initialization Functions

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
heatOld = 0
b = 0
```

Auxiliary Functions

(none)

Global Variables and Constants

```
Dim CellVol
Dim St
Dim Ct
Dim gB1
Dim gB2
Dim heat
Dim heatOld
Dim b
Dim OverfillMode
Dim TotalCellVolume
```

Provided Constants

Private Readonly TitrantmM as Double = 0.1

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

DSC Model Name: Two State

Main Function

```
Dim Y As Double


T = Temp(iteration) + 273.15
Tm0 = Tm + 273.15
dH0 = dH * 1000.0
K=exp(-dH0*(1-T/Tm0)/(R*T))
Cp=(dH0 * dH0)/(R*T*T)*exp(dH0*(-Tm0+T)/(Tm0*T*R))/((1+K)*(1+K))
Y = Cp / 1000.0
Return Y
```

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

```
Dim T As Double
Dim Tm0 As Double
Dim dH0 As Double
Dim K As Double
Dim Cp As Double
```

Provided Constants

Private Readonly Volume as Double = 0.3

Private Readonly Mass as Double = 1

Private Readonly Conc as Double = 0.1

Private Readonly MW as Double = 10

Private Readonly PSV as Double = 0.73

Private Readonly CalUnits as Boolean = False

Private Readonly b as Double = 300.96

Private Readonly c as Double = -0.4807

Private Readonly R as Double = 8.3144621

DSC Model Name: Two State Scaled

Main Function

Dim Y As Double

T = Temp(iteration) + 273.15

Tm0 = Tm + 273.15

dH0 = dH * 1000.0

K=exp(-dH0*(1-T/Tm0)/(R*T))

Cp=(dH0 * dH0)/(R*T*T)*exp(dH0*(-Tm0+T)/(Tm0*T*R))/((1+K)*(1+K)) * Aw

Y = Cp / 1000.0

Return Y

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

Dim T As Double

Dim Tm0 As Double

Dim dH0 As Double

```
Dim K As Double
Dim Cp As Double
```

Provided Constants

```
Private Readonly Volume as Double = 0.3
Private Readonly Mass as Double = 1
Private Readonly Conc as Double = 0.1
Private Readonly MW as Double = 10
Private Readonly PSV as Double = 0.73
Private Readonly CalUnits as Boolean = False
Private Readonly b as Double = 300.96
Private Readonly c as Double = -0.4807
Private Readonly R as Double = 8.3144621
```

DSC Model Name: General

Main Function

```
Dim Y As Double
bbCp = b
ccCp = c
Tm0 = Tm + 273.15
dHTm = dH * 1000
dCpTm = dCp * 1000
dSTm = dHTm / Tm0
acon = dCpTm - bbCp * Tm0 - ccCp * (Tm0 * Tm0)
T = Temp(iteration) + 273.15
dCp1 = acon + bbCp*T + ccCp*T*T
deltah = dHTm+acon*(T-Tm0)+(bbCp*(T*T-Tm0*Tm0)/2)+(ccCp*(T*T*T-Tm0*Tm0*Tm0)/3)
deltas = dSTm+acon*log(T/Tm0)+bbCp*(T-Tm0)+(ccCp*(T*T-Tm0*Tm0)/2)
kEQ = Exp(-(deltah-T*deltas)/(R*T))
qpar = 1 + kEQ
advh = kEQ * deltah
advh2 = kEQ * deltah * deltah
advcp = kEQ * dCp1
Temp0 = Temp(iteration) + 273.15
```

```
  dCp1 = acon + bbCp*Temp0 + ccCp*Temp0*Temp0
  deltah = dHTm+acon*(Temp0-Tm0)+(bbCp*(Temp0*Temp0-Tm0*Tm0)/
2)+(ccCp*(Temp0*Temp0*Temp0-Tm0*Tm0*Tm0)/3)
  deltas = dSTm+acon*log(Temp0/Tm0)+bbCp*(Temp0-Tm0)+(ccCp*(Temp0*Temp0-Tm0*Tm0)/2)
  kEQ = Math.Exp(-(deltah-Temp0*deltas)/(R*Temp0))
  qpar = 1 + kEQ
  advh = kEQ * deltah
  advh2 = kEQ * deltah * deltah
  advcp = kEQ * dCp1
  hav1 = (advh*advh)/(qpar*qpar)
  hav2 = advh2 / qpar
  Cp = (advcp/qpar) + (hav2-hav1)/(R*Temp0*Temp0)+(A0 + A1 * Temp(iteration)) * MW * 1000
  Y = Cp / 1000
  Return Y
```

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

```
  Dim acon As Double
  Dim T As Double
  Dim deltah As Double
  Dim deltas As Double
  Dim kEQ As Double
  Dim qpar As Double
  Dim advh As Double
  Dim advh2 As Double
  Dim advcp As Double
  Dim Temp0 As Double
  Dim dCp1 As Double
  Dim hav1 As Double
  Dim hav2 As Double
```

Dim Cp As Double

Provided Constants

Private Readonly Volume as Double = 0.3

Private Readonly Mass as Double = 1

Private Readonly Conc as Double = 0.1

Private Readonly MW as Double = 10

Private Readonly PSV as Double = 0.73

Private Readonly CalUnits as Boolean = False

Private Readonly b as Double = 300.96

Private Readonly c as Double = -0.4807

Private Readonly R as Double = 8.3144621

DSC Model Name: Gaussian

Main Function

Dim Y As Double

T = Temp(iteration)

Y = a1*exp(-((T-b1)/c1)^2)

Return Y

Initialization Functions

(none)

Auxiliary Functions

Public Function area() As Double

  Return a1*c1*1.772453851 'number is for sqr(PI)

End Function

Global Variables and Constants

Dim T As Double

Provided Constants

Private Readonly Volume as Double = 0.3

Private Readonly Mass as Double = 1

```
Private Readonly Conc as Double = 0.1
Private Readonly MW as Double = 10
Private Readonly PSV as Double = 0.73
Private Readonly CalUnits as Boolean = False
Private Readonly b as Double = 300.96
Private Readonly c as Double = -0.4807
Private Readonly R as Double = 8.3144621
```