Lesson 1: Routine ITC Data Analysis and Fitting

In this lesson you will learn to perform routine analysis of ITC data. For reasonably good data, Origin makes a very good guess on the baseline, the range to integrate the injection peaks, and the initialization of the fitting parameters. These factors can be adjusted manually, as described in the following chapters, but it is not always necessary to do so.

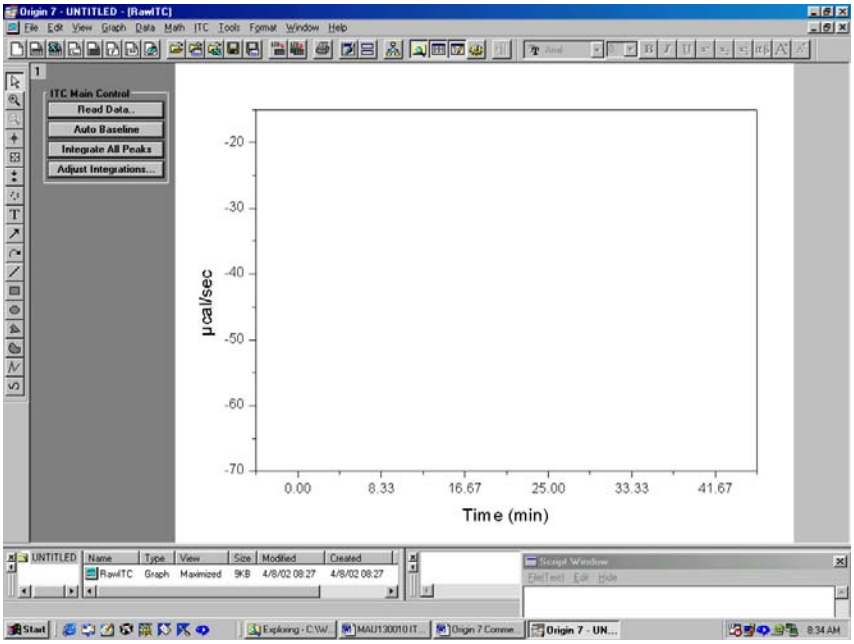
Routine ITC Data Analysis

A series of sample ITC files have been included for your use with this tutorial. A typical file is designated **RNAHHH.ITC**. This file contains data from a single experiment which included 20 injections. It is located in the **[samples]** subfolder of the **[origin70]** folder.

Note: The **.1** file extension indicates an ITC file generated with the non-Windows MicroCal data acquisition software. The **.ITC** extension indicates an OMEGA, MCS ITC or VP-ITC file generated with the Windows version of the MicroCal data acquisition software. The two file types are identical, except that the procedure for opening them differs slightly, as described below.

To open the **RNAHHH.ITC** ITC file

- Start Origin for ITC (as described in the previous section).
  The program opens and automatically displays the **RawITC** plot window. Along the left side of the window you will notice several buttons. Clicking on these buttons gives you access to many of the ITC routines.



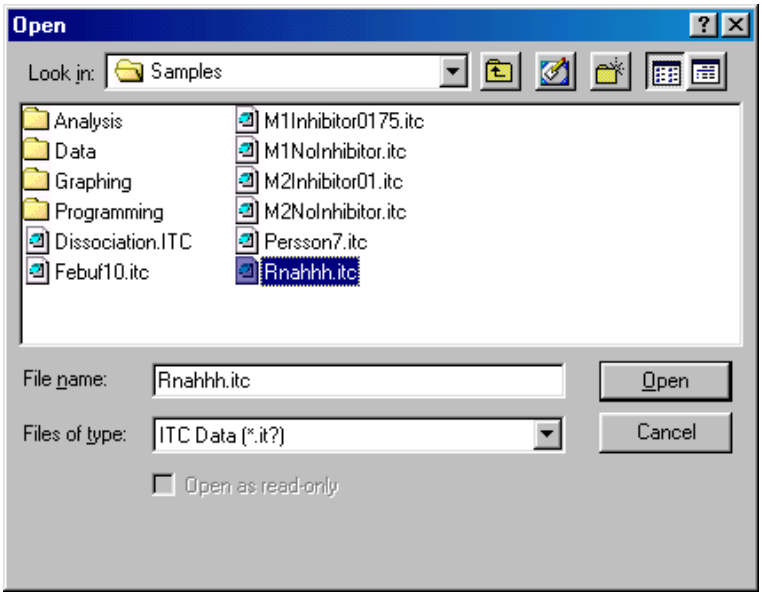
If you are not seeing the entire window as shown above, click on the Restore button  in the upper right corner of the RawITC template.

- Click on the **Read Data..** button. The **Open** dialog box opens, with the **ITC Data (*.it?)** selected as the Files of type:

- Navigate to the **C:\Origin70\Samples** folder, then select **Rnahhh.itc** from the **Files** list. PLEASE NOTE: Data file names should not begin with a number, nor should they contain any hyphens, periods or spaces. Also, Origin truncates the filenames to the first 15 characters, therefore when reading in multiple files the first 15 characters of the filename must be a unique combination to prevent overwriting the data.

  You may select a default folder for Origin to 'Look in' for a data file by selecting **File : Set Default Folder...** and entering the default path (e.g. for this tutorial you may wish to choose the path to be **C:\Origin70\Samples**).
  (Your dialog box may not show the DOS filename extension .ITC, you may view the extension by opening Windows Explorer and selecting **View : Options** and removing the check mark next to *Hide MS-DOS file extensions for file types that are registered*.)

*You may view more information about the files by clicking the **Details button**.*



- Click **Open.**

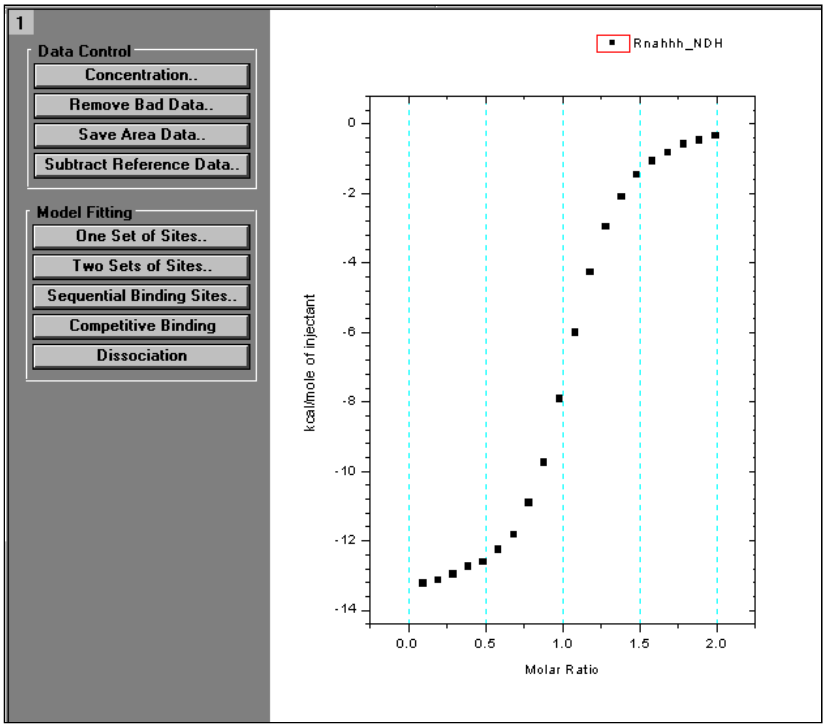
  Hint: You may prefer the shortcut method for opening files. Instead of selecting a file and clicking **Open**, simply double-click on the file name.

  The RNAHHH file is read and plotted as a line graph in the **RawITC** window, in units of µcal/second vs. minutes. Origin then automatically performs the following operations:

1) Selects **Auto Baseline** routine. Each injection peak is analyzed and a baseline is created.

2) Selects **Integrate All Peaks** routine. The peaks are integrated, and the area (µcal) under each peak is obtained.

3) Opens the **DeltaH** window. Plots the normalized area data **rnahhh_ndh**, in kcal per mole of injectant versus the molar ratio ligand/macromolecule. Note that the DeltaH window contains buttons that access ITC routines.



Each time you open an ITC raw data file series, Origin creates eight data sets[1]. These eight data sets will always follow the naming convention shown below, that is, the name of the ITC source file followed by an identifying suffix (injection number is indicated by the row number *i*).

Double click on the layer icon 1 to view the available data sets :

rnahhh_dh	Experimental heat change resulting from injection *i*, in µcal/injection (not displayed).
rnahhh_mt	Concentration of macromolecule in the cell *before* each injection *i*, after correction for volume displacement (not displayed).
rnahhh_xt	Concentration of injected solute in the cell *before* each injection (not displayed).
rnahhh_injv	Volume of injectant added for the injection *i*.
rnahhh_ndh	Normalized heat change for injection *i*, in calories per mole of injectant added (displayed in **DeltaH** window).
rnahhh_xmt	Molar ratio of ligand to macromolecule after injection *i*.
rnahhhbase	Baseline for the injection data (displayed in red in the **RawITC** window).
rnahhhraw_cp	All of the original injection data (displayed in black in the **RawITC** window).

[1] Two temporary data sets are also created; rnahhhbegin contains the indices (row numbers) of the beginning of an injection; rnahhhrange contains the indices of the integration range for the injection.

Origin creates three worksheets to hold these data sets. To open these worksheets refer to Lesson 5, which describes how to open worksheets from plotted data, copy and paste data, and export data to an **ASCII** file.

Now would be a good time to save the area data (the **RNAHHH** integration results) as a separate data file. We will use this data again in Lesson 4 when we subtract reference data.

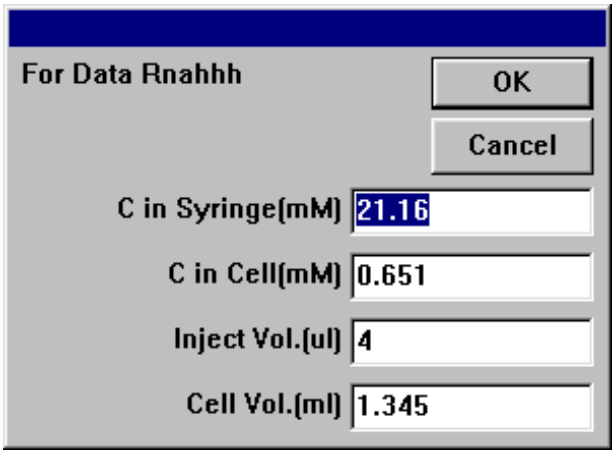
To save area data to a separate file

- Select **Window:DeltaH** to make DeltaH the active window. Alternatively, you may press and hold the Ctrl key and press the tab key to scroll through Origin's open windows.
- Click on the **Save Area Data** button
  Origin opens the File **Save As** dialog box, with **Rnahhh.DH** selected in the **File name** text box.
- Select a folder for the file and click **OK**.

  Before fitting a curve to the data, it is a good idea to check the current concentration values for this experiment.

To edit concentration values

- Click on the **Concentration** button in the **DeltaH** window.
- A dialog box opens showing the concentration values for the RNAHHH data.



Click **OK** or **Cancel** to close the dialog box.

You should always check that the concentration values are correct for each experiment. Incorrect values will negate the fitting results. If you need to edit the concentration values, simply enter a new value in the appropriate text box.

The concentration and injection volume values which appear initially are those which the operator enters before the experiment starts. The cell volume is a constant which is stored in the data collection software. This value is read by Origin whenever you call up an ITC data file.

Before you proceed to fit the data, you may want to manually adjust baselines or integration details. These procedures are discussed in Lesson 2, but here we will simply accept the computer-generated results.

Curve Fitting

Origin provides six built-in curve fitting models: **One Set of Sites**, **Two Sets of Sites**, **Sequential Binding Sites, Competitive Binding, Dissociation and Enzyme Assays**. To invoke one of these models, click on the appropriate button in the **DeltaH** window.

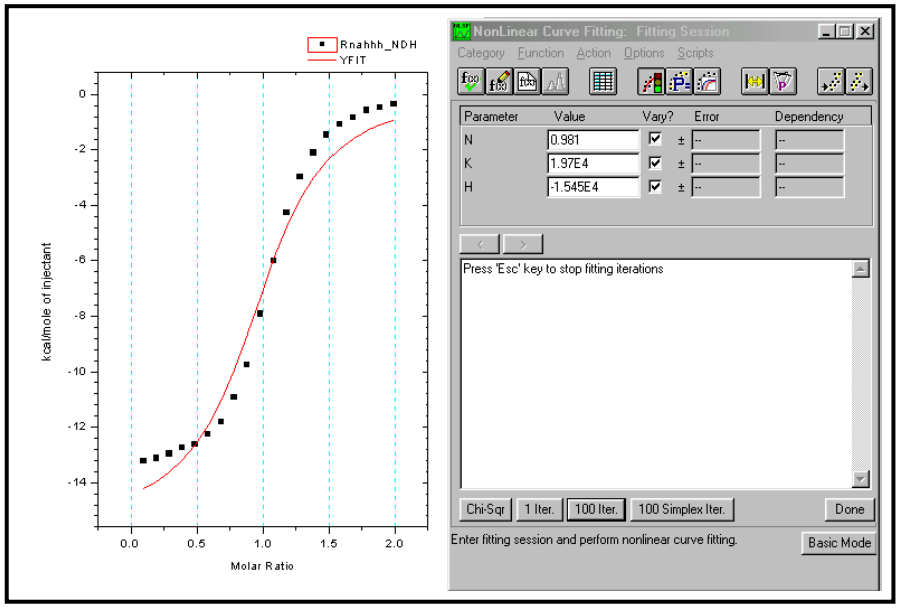
The following describes the basic procedure for fitting a theoretical curve to your data. See Lesson 7 for advanced curve fitting lessons, and the Appendix for a discussion of fitting equations.

To fit the area data to the One Set of Sites model

- Click anywhere on the **DeltaH** plot window to make it the **active window**. Or select **DeltaH** from the **Window** menu.

- Click on the **One Set of Sites** button.

  A new command menu display bar appears.
  The **Fitting Function Parameters** dialog box opens (there are two modes for the Fitting Sessions dialog box, basic and advanced, see page 58 for more information), showing initial values for the three fitting parameters for this model - **N**, **K**, and **H**.
  Origin initializes the fitting parameters, and plots an initial fit curve (as a straight line, in red, please see page 100 for a discussion of line types) in the **DeltaH** window.



- Click **1 Iter. or 100 Iter. button** in the Fitting Session dialog box to control the iteration of the fitting cycles.

  Click **1 Iter.** to perform a single iteration, **100 Iter.** to perform up to 100 iterations. It may be necessary that the **100 Iter.** command be used more than once before a good fit is achieved. Repeat this step until you are satisfied with the fit, and Chi^2 is no longer

decreasing. Note that the fitting parameters in the dialog box update to reflect the current fit.

Fitting Parameter Constraints

Each fitting model has a unique set of fitting parameters. For the One Set of Sites model these are **N** (number of sites), **K** (binding constant in M^{-1}), and **ΔH** (heat change in cal/mole). A fourth parameter, **ΔS** (entropy change in cal/mole/deg), is calculated from ΔH and K and displayed after fitting. You can use the **Fitting Session** dialog box to apply mathematical constraints to the fitting parameters. We mention this subject only in passing, for a detailed discussion see page 59 and the appendix.

To hold a parameter constant

The **Vary?** column in the **Fitting Session** dialog box contains three checkboxes, one associated with each fitting parameter. If a box is check marked, Origin will vary that parameter during the fitting process in order to achieve a better fit. To hold a parameter constant during iterations, click in the box to remove the checkmark from the checkbox.

Fitting Parameters Text

To copy and paste the fitting parameters to the DeltaH window

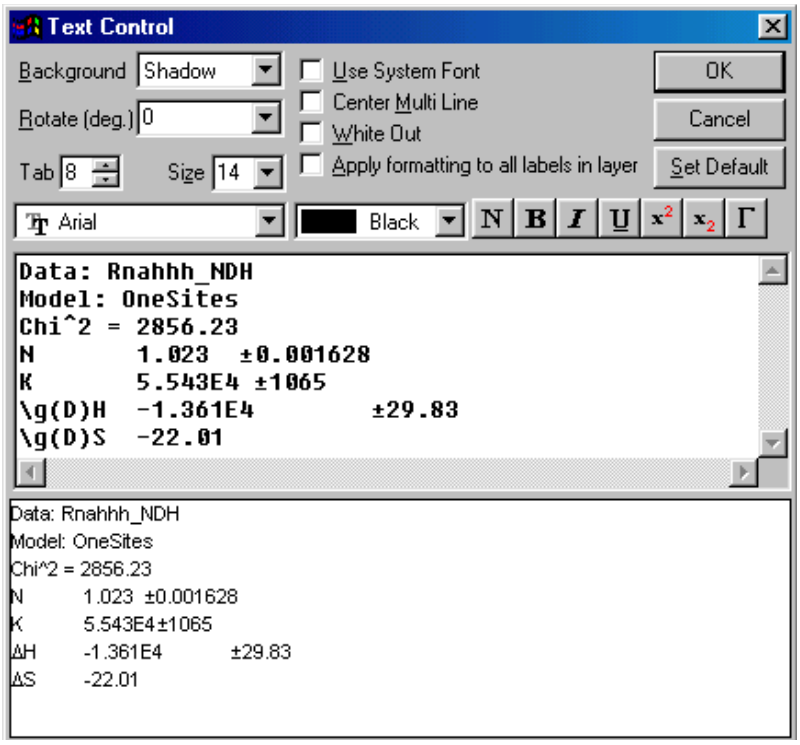
Once you have a good fit, click on the **Done..** button and the fitting parameters will be automatically pasted into a text window named Results Log and to the DeltaH window in a text label. This label is a *named object* (called **Fit.P**) that is linked to the fitting process through Origin's **label control** feature (For more information see the *Origin User's Manual* or for online help, right click anywhere in the text label and select **Label Control…** then press the F1 key).

```
Data: Rnahhh_NDH
Model: OneSites
Chi^2/DoF = 2856
N       1.02±0.0016
K       5.54E4  ±1.1E3
ΔH      -1.361E4        ±29.8
ΔS      -22.0
```

Position and format this label just as you want the fitting parameters to appear. When you paste the fitting parameters, they will replace the "Fit Parameters" label, but retain its position and style. Origin will use any text label named **Fit.P** to display the fitting parameters. To name a text label, click on the label once to select it, select **Format:Label Control**, and enter a name in the **Object Name** text box in the **Label Control** dialog box.

To format the fitting parameters text

- Right-click anywhere in the text box and select **Properties** item from the drop down menu. The Text Control dialog box will appear allowing you to format the fitting parameters text.



The Text Control dialog box is in three sections. The upper section contains various formatting options. The middle contains the text box where the desired text, with formatting options, are entered. The lower view box provides a WYSIWYG (What You See Is What You Get) display of the text entered into the middle text box.

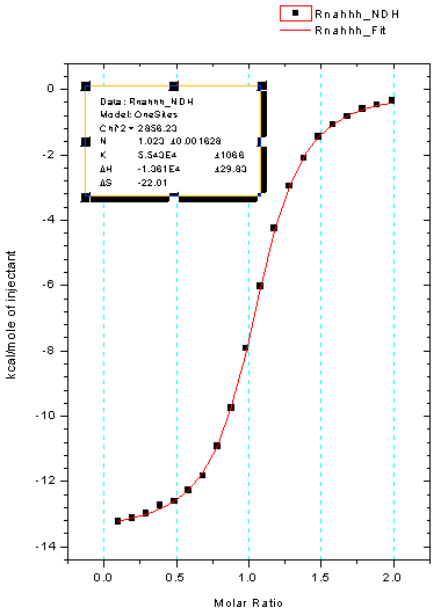
Hint: Press the F1 key while the Text Control dialog box is open for Online help and a thorough description of text formatting options.

- Use the controls to format the text.. Select **Black Line** from the **Background** drop-down list box to change the background style from shadow to border (to remove the background style altogether, select (None) from the **Background** drop down list. If the background line is not removed, it may be necessary to select **Window:Refresh**). Click **OK** when done. The **DeltaH** window redraws to show the changes in the text box.

To move the text in the plot window

- Click once on the text in the plot window.
- A colored outline appears, indicating that the text is selected and the cursor will change into an arrow headed cross.

- Slowly Click again,within the colored outline, to move the text.
- Click outside the colored outline to deselect the text.



Hint: If you click anywhere along the edge of the text background border, a colored **size box** appears around the text with various size boxes positioned around the perimeter. Click and drag on one of the small perimeter boxes to change the size of the text background frame. Origin will automatically change the font size to fit within the size of the box.

Note that any formatting changes can be saved as part of the **DeltaH** plot window template file. See page 49 for details.

To view the Results Log

When you save an Origin project, the contents of the Results Log is saved with the project.

Origin automatically routes most analysis and fitting results to the Results Log (a sub window of Origin's Project Explorer). In most cases, when results are output to the Results Log, it opens automatically (although it may be positioned out of view, docked to the lower edge of the workspace). However, to manually open (and Close) the Results Log, click the Results Log button  on the standard toolbar. Opening and closing the Results Log only controls its view state. You do not lose results by closing the log.

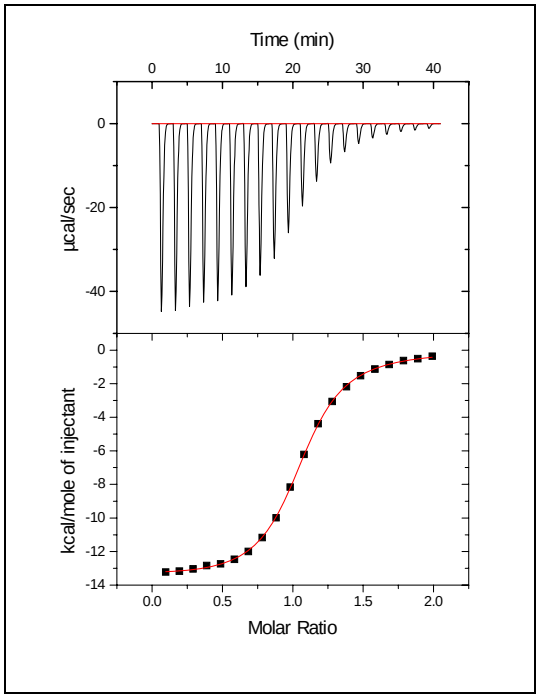
When the Results Log first opens, it displays docked to the lower edge of the workspace. You can dock it to any other edge or display it as a window in the workspace. To prevent the Results Log from docking when positioning it it as a window, press CTRL while dragging.

Each entry in the Results Log includes a date/time stamp, the window name, a numeric stamp which is the Julian day, the type of analysis performed, and the results.

Please refer to the Origin User's Manual or on-line help for more information about the Results Log and Project Explorer

Creating a Final Figure for Publication

To create a final figure for publication, select **Final Figure** from the **ITC** menu. The **ITCFINAL** plot window opens. This window contains two related graphs. The top graph shows raw data in terms of µcal/second plotted against time in minutes, after the integration baseline has been subtracted. The bottom graph shows normalized integration data in terms of kcal/mole of injectant plotted against molar ratio. The two X axes are linked, so that the integrated area for each peak appears directly below the corresponding peak in the raw data.



Note: The user should understand that the "raw data" in the upper frame of the ITCFinal template is the original raw data *after* the integration baseline has been subtracted from it. Once this subtraction has been made by creating the ITCFinal figure, there is no way to recover the original raw data except by starting a new project and calling in the raw data file again, since the subtracted data has been stored under the original filename and the original integration baseline replaced by the Y=0 baseline.

If you modify the integration data or the fit curve in the **DeltaH** window, or the raw data in the **RawITC** window, simply select **Final Figure** again to update the **ITCFINAL** window with your changes.

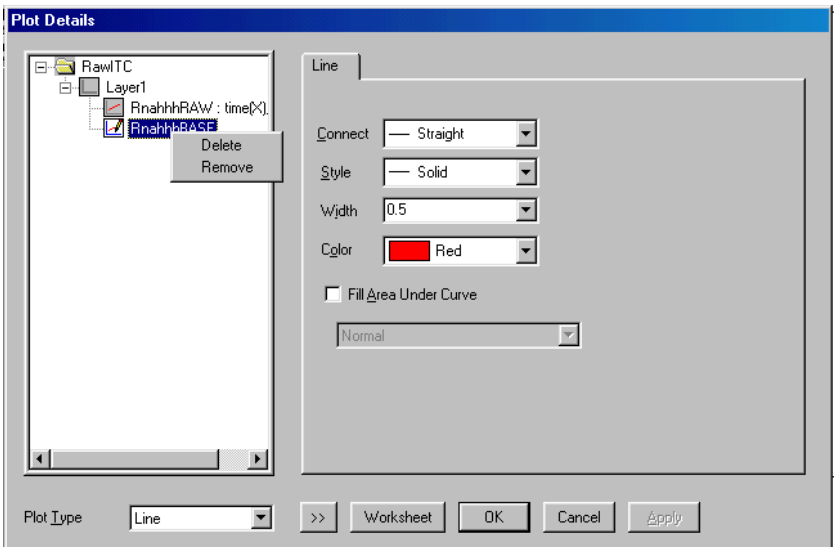
Note that the top graph in the **ITCFINAL** window still includes the integration baseline at Y = 0. You may wish to remove this baseline before printing the graph.

To remove the baseline from the raw data

Origin has drawn a baseline (rnahhhbase) for the raw data at Y = 0. If you like, you can remove this baseline as follows:

*Shortcut: Right-click anywhere inside the axis of the graph and select **Plot Details***

1. Double-click on the baseline. The **Plot Details** dialog box opens.



2. In the **Plot Details** dialog box, right-click on the RnahhhBASE data name then select Remove from the drop down menu. The baseline data are removed from the project. (Note: You may also remove the baseline from the plotted data, by double-clicking on the Layer Control button in the upper left corner of the ITCFinal window, and then move the rnahhhbase data from the Layer Contents list to the Available Data list by first highlighting it and then selecting the left-pointing arrow.)

To paste the fitting parameters to the ITCFinal window

Earlier in this lesson the fitting parameters were pasted to the **DeltaH** window. Before printing, let's copy these parameters and paste them to the **ITCFINAL** window.

1. Click on the **DeltaH** window, or select **DeltaH** from the **Window** menu. **DeltaH** becomes the active window.

Alternatively, to copy and paste, you may right-click anywhere inside the text box, select copy then right-click where you want to position the text label and select paste.

2. Click on the fitting parameters text label we had placed in the upper-left corner of the window. A colored selection square surrounds the text.

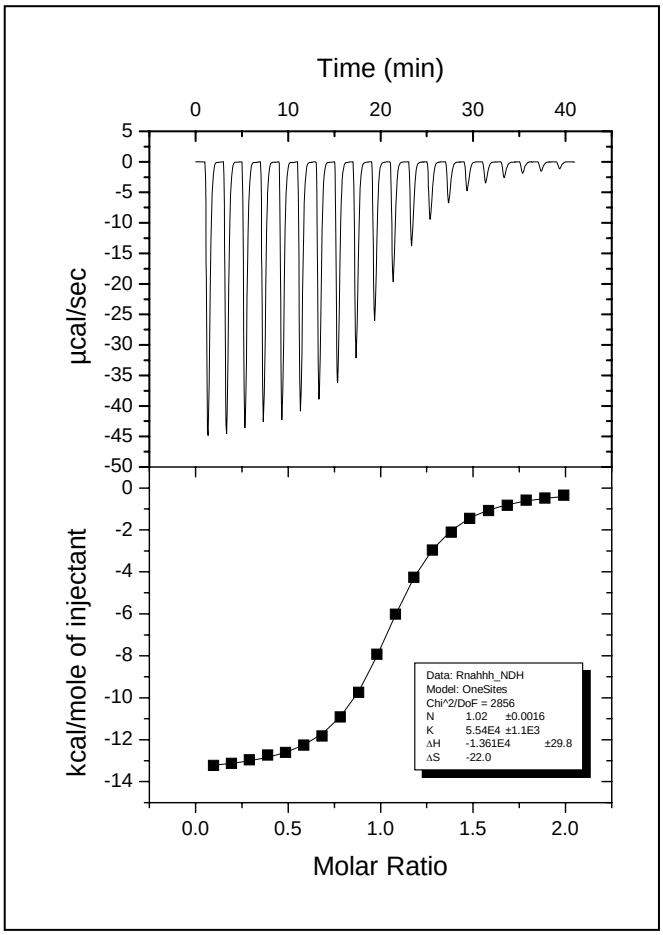
3. Select the **Edit:Copy** command.

4. Click on the **ITCFINAL** window, or select **ITCFINAL** from the **Window** menu. **ITCFINAL** becomes the active window. Click once on a position in the graph where you want the parameter box to appear.

5. Select the **EDIT:PASTE** command. The fitting parameters paste to the **ITCFINAL** window.

6. We want to position the text label next to the integration data, but first we need to reduce the size of the label. Right-click inside the text box then select **Properties…** from the drop down menu to open the **Text Control** dialog box. Select **10** (or type 10) in the Size drop-down list to reduce the point size to 8. Click **OK** to close the dialog box.

7. Click and drag the label to position it next to the integration data, as shown below:



To print the final figure

To print the page in the **ITCFINAL** window, select **Print** from the **File** menu. Before you print, make sure **ITCFINAL** is the active window. When a window is active its title bar changes from gray to blue (this can vary depending on your Windows setup, to view or change your setup select Start:Settings:Control Panel then double click on Display and click on the Appearance tab). Click on a window to make it active, or select the window from the **Window List** in the **Window** menu.

To save the project and exit

Shortcut: Click the Save Project button on the Standard toolbar.

- Choose **Save Project As...** from the **File** menu. The file **Save As** dialog box opens.
- Enter a name for the project (for example, **Lesson 1**) in the **File Name** text box. The name for the project may contain up to 255 characters and include spaces.

- Click on the **Save** button.
  The entire contents of this project (including all data sets and plot windows) are saved into a file called **Lesson 1.OPJ**.

- Choose **Exit** from the **File** menu.
  Origin closes.