Lesson 8: Autosampler Data (Optional Accessory)

The Autosampler is an optional accessory for use with the ITC to allow multiple unattended experiments. The AutoITC Origin module contains curve fitting routines that operate on multiple data files from the autosampler. If you are not familiar with curve fitting of standard ITC data, you should review the previous Lessons of this tutorial, especially Lessons 1, 2, 3 & 4, before starting this lesson.

Launching the ITC Autosampler Data Session

The routines for Auto ITC files are located on a separate window (named ARawITC) from the regular ITC routines.

- To launch the Auto DSC, double-click on the **MicroCal LLC Auto ITC** icon on the Desk Top. Or alternatively select *Start : Programs : OriginLab : MicroCal LLC Auto ITC*

When you start Origin, the program automatically opens the **ARawITC** graph window.
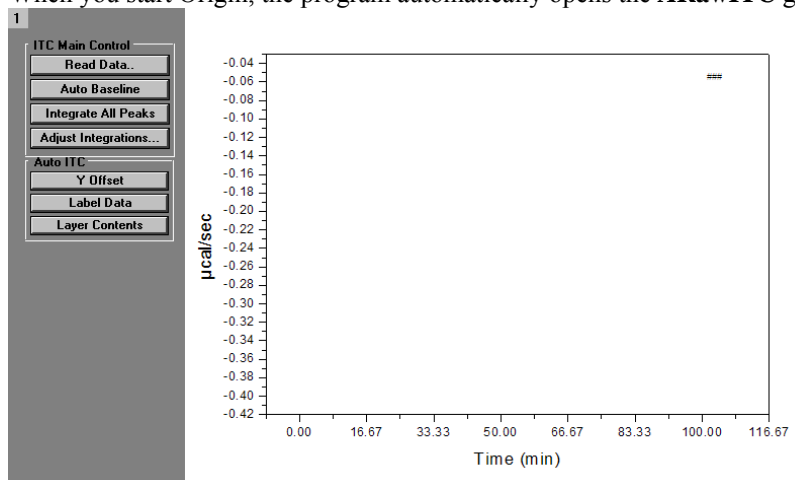
To import the data

Raw data files from the autosampler are typically a series of data files of the form FamilyName001.ITC, FamilyName002.ITC, FamilyName003.ITC, etc.. To open the file series click on the **Read Data…** button and the **Import Multiple ASCII** dialog box will open. Select the *Auto ITC Data (*.ITC)* as the **Files of type**, the upper File Name list box will list only the first file of the series (*001.ITC), but when selected all files of the same file name will be read in until the end of the series is reached.

Alternatively, you may open individual files or a selection of files similar to the normal ITC method for multiple file input. When you select the **Files of type** to be **ITC Data (*.ITC)**, then all files with the 'ITC' extension will be listed in the upper list box. You may then manually select the files to add to the lower list box and read into Origin.

The files will be imported to worksheets named
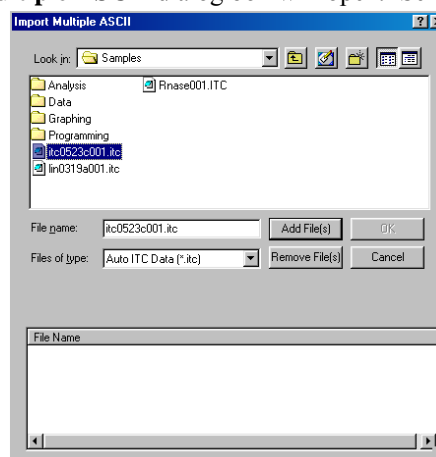
Data1Raw, Data2Raw, ...DataNRaw. The label for each worksheet will be *FileNameRaw*. The worksheets will be accessible after import through the project explorer.

| Label	Name	View	Size	Modified	Type	Created	Depende...
	ADeltaH	Maximi...	5...	6/21/2006 09...	Gr...	6/21/2006 09...	0
	ARawITC	Normal	2...	6/21/2006 09...	Gr...	6/21/2006 09...	0
itc0523c001	Data1	Hidden	5...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c001RAW	Data1...	Hidden	6...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c002	Data2	Hidden	5...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c002RAW	Data2...	Hidden	6...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c003	Data3	Hidden	5...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c003RAW	Data3...	Hidden	6...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c004	Data4	Hidden	5...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c004RAW	Data4...	Hidden	6...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c005	Data5	Hidden	5...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c005RAW	Data5...	Hidden	6...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c006	Data6	Hidden	5...	6/21/2006 09...	Wo...	6/21/2006 09...	1
itc0523c006RAW	Data6...	Hidden	6...	6/21/2006 09...	Wo...	6/21/2006 09...	1
	Results	Normal	1...	6/21/2006 09...	Notes	6/21/2006 09...	NA
MicroCal VP-ITC Autosampler Scans Summary Table	Table	Normal	2...	6/21/2006 09...	Wo...	6/21/2006 09...	0

To open serial Auto ITC (*001.ITC) files

- Click on the **Read Data..** button.
  The **Import Multiple ASCII** dialog box opens. Select *Auto ITC Data (*.itc)* as the Files of type and only ITC files that have 001 before the extension dot will be listed.

- Navigate to the C:\Origin70\Samples folder, and then select **itc0523c001.ITC** from the Files list.

Note: 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**)
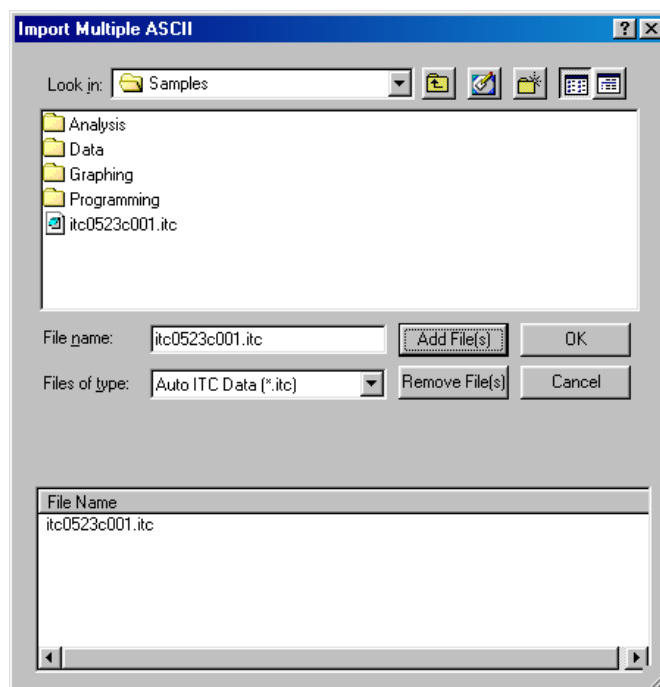
- Select **itc0523c001.ITC** file and click **Add File(s).**

- Click **OK**.

   The itc0523c001 family of files is then read in sequentially (i.e. itc0523c001, itc0523c002…….itc0523c006.). As the files are read in, the following operations take place on each data set.
   1) The data is plotted as a line graph in the **ARawITC** window.
   2) Each injection peak is analyzed and a baseline is created
   3) The peaks are integrated, and the area (µcal) between the peaks and the baseline is obtained.
   4) The normalized area data is then plotted in the **ADeltaH** window.
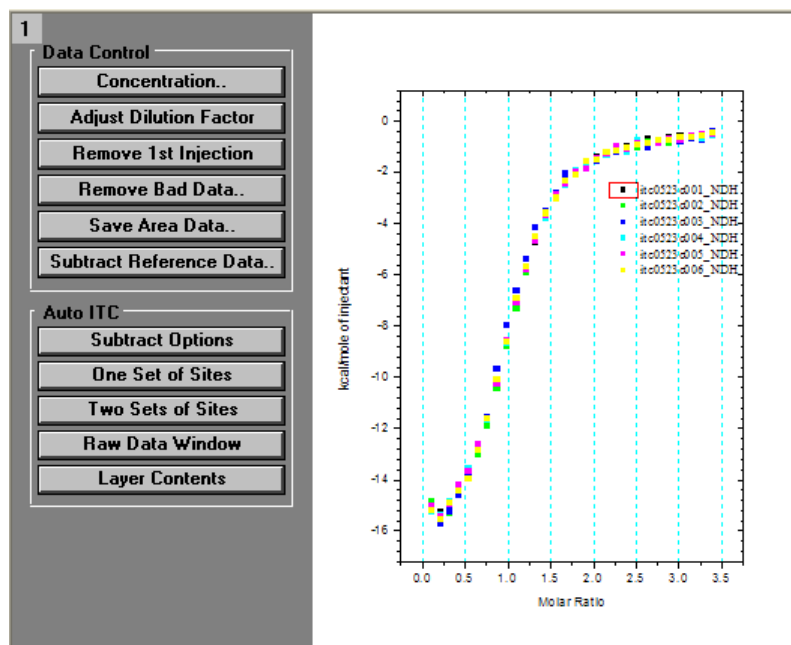
Auto ITC Buttons

Apart from the buttons associated with the standard ITC, there is a group of buttons labeled **AutoITC** that perform data manipulations on multiple ITC files. Examples of the use of each button are presented in the following pages.
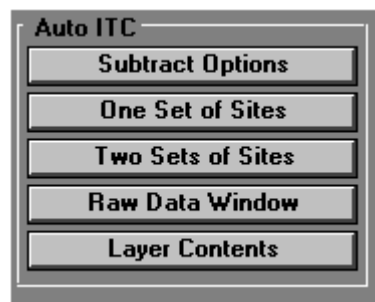
Button Name	Function
Subtract Options	When you click this button, the Control Baseline Subtraction dialog box will pop up providing 4 different means to adjust the experimental datasets to minimize the heats of dilution.
One Set of Sites	When you select this button, the One Set of Sites fitting model will be applied to all data sets plotted in the AdeltaH graph. The calculated fitting parameters will then be printed in a summary table.

Two Sets of Sites   This button will apply the Two Sets of Sites fitting model to all data sets plotted in the active layer, then prints the fitting parameters in a summary table.

Raw Data Window   This button provides a quick method to return to the ARawITC window.

Layer Contents   Opens the Layer Contents dialog box. This box displays avaiable data sets along with which data sets are being disaplayed.   User's can move datasets in and out of the displayed layer contents list.

To check and edit concentration values

The concentration values for the cell and syringe are usually entered into the data files when setting up the experiments in VPViewer. You should always check that concentration values are correct for each experiment. Incorrect values will produce erroneous fitting results.

- Select the menu item **Window : MicroCal VP-ITC Autosampler Summary Table**.
  The summary table will open and display the concentration values (mM) for the data.   If you need to edit the concentration values, enter the value in the appropriate cell, then exit the cell to make the change effective.

Filename	Worksheet	Control Run	Tray #	Well #	Syr. Conc.	Cell Conc.	Syr. Dil. Factor	Cell Dil. Factor	Comments
itc0523c001.ITC	Data1		1	4	0.1825	0.0122	0	0	
itc0523c002.ITC	Data2		1	6	0.1825	0.0122	0	0	
itc0523c003.ITC	Data3		1	8	0.1825	0.0122	0	0	
itc0523c004.ITC	Data4		1	10	0.1825	0.0122	0	0	
itc0523c005.ITC	Data5		1	12	0.1825	0.0122	0	0	
itc0523c006.ITC	Data6		1	14	0.1825	0.0122	0	0	

Select Update Concentrations in the AutoITC menu to recalculate NDH based on the new concentrations.
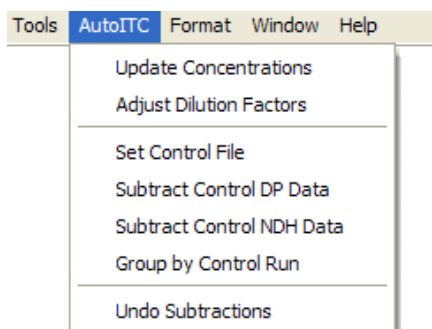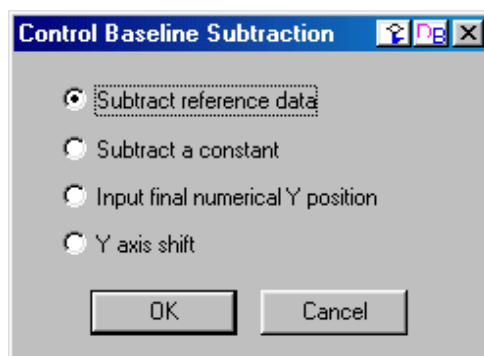

To subtract the heats of dilution effect from area data

You may use the **Subtract Options** button to adjust each integral heat data set, thereby minimizing the effects of the heats of dilution.

- Click the Subtract Options button.

The Control Baseline Subtraction dialog box will pop up.

Button Name	Function
Subtract reference data	When you click this button, the heats from a control experiment can be subtracted from all data sets that are plotted in the ADeltaH graph. The control experiment must have same or greater number of injections (data points) as any of the plotted data sets.
Subtract a constant	When you click this button, you will be prompted to enter a constant (kCal/mole) that will be used to subtract from all datasets plotted in the ADeltaH graph.
Input final numerical Y position	When you click this button you will be prompted to enter a final Y position (in kCal/mole). The end point of each plotted data set will be placed at that position and the rest dataset will be offset proportionately.
Y axis shift	When you click this button all data sets are moved out of the graph (layer), the cursor will change to the data reader tool, then the first data set will be moved back into the graph. You may click once to see the y-axis position of the data reader tool. When you click twice or press enter the end point of the data set will be moved to that y position, the data set will be removed from the graph and the next file of the series will be plotted in the graph and you may repeated the process for all data sets that were originally plotted in the graph.

Using the One Set of Sites model with multiple data sets

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

  Each data set that is plotted in the graph will be fitted to the **One Set of Sites** model and the parameters n (number of sites), K (binding constant), ΔH (enthalpy change) and ΔS (entropy change) are calculated. These parameters will then be printed in the summary table.

- Select the menu item **Window : MicroCal VP-ITC Autosampler Summary Table**.

  The summary table will open display the parameter values.

Filename	Worksheet	Control Run	Tray #	Well #	Syr. Conc.	Cell Conc.	Syr. Dil. Factor	Cell Dil. Factor	N	K	H	S	Comments
itc0523c001.ITC	Data1		1	4	0.1825	0.0122	0	0	1.07	8.39E5	-1.725E4	-29.8	
itc0523c002.ITC	Data2		1	6	0.1825	0.0122	0	0	1.07	8.45E5	-1.733E4	-30	
itc0523c003.ITC	Data3		1	8	0.1825	0.0122	0	0	1	7.71E5	-1.793E4	-32.2	
itc0523c004.ITC	Data4		1	10	0.1825	0.0122	0	0	1.05	7.81E5	-1.741E4	-30.5	
itc0523c005.ITC	Data5		1	12	0.1825	0.0122	0	0	1.05	7.98E5	-1.731E4	-30.1	
itc0523c006.ITC	Data6		1	14	0.1825	0.0122	0	0	1.04	8.09E5	-1.748E4	-30.6	

Two Sets of Sites

Using the Two Sets of Sites model with multiple data sets

To fit your data to the Two Sets of Sites model perform the same preliminary operations as described in the previous pages then:

- Click the **Two Set of Sites** button.

Each data set that is plotted in the graph will be fitted to the **Two Sets of Sites** model and the eight parameters n1, n2 (number of sites), K1, K2 (binding constants), $\Delta H1$, $\Delta H2$ (enthalpy change) and $\Delta S1$, $\Delta S2$ (entropy change) are calculated. These parameters will then be printed in the summary table.

Raw Data Window

To return to the ARawITC window

- Click the **Raw Data Window** button to return to the ARawITC window.

To edit the layer contents

- Click the **Layer Contents** button to open the layer contents dialog box. The available data column contains all the datasets in the current project. The displayed data column contains all the data sets to be plotted.

Auto Control Run Subtraction

When control runs are designated in VPViewer, the can be automatically assigned and subtracted form datasets. Use the **Control Run Options** in the AutoITC menu to control control run designation and subtraction.
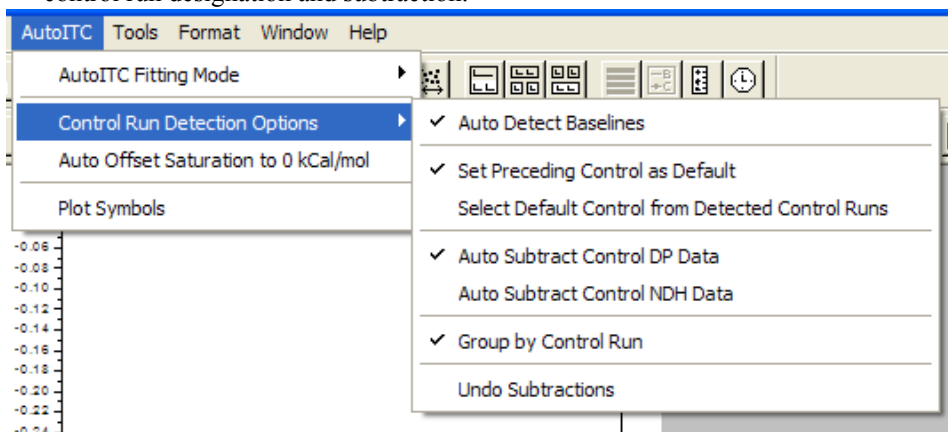
Selection	Function
Auto Detect Baselines	Activates control run detection. All designated control runs will be assigned as "Control" in the control column of the AutoITC summary table.
Set Preceding Control as Default	This option sets the previously detected control run as the control of all following data runs, until another control run is detected.
Select Default Control from Detected Control Runs	After all files are imported, user will choose the default control run to apply to all data sets from all detected control runs.
Auto Subtract Control DP Data	Automatically subtracts control run DP from data DP.
Auto Subtract Control NDH Data	Automatically subtracts control NDH from data NDH.
Group by Control Run	All data sets with the same control run will be plotted in the same window. If DP data was subtracted, data sets will be plotted in a plot called ARAWITC*ControlRun*. If NDH data was subtracted, data sets will be plotted in a graph called ADELTAH*ControlRun*.
Undo Subtractions	This option will restore all datasets to original values before subtraction.

The ARAWITC*ControlRunName* window will behave the same as the main ARAWITC plot. Operations in this window will only affect the currently plotted data. The control run is not plotted in the ARAWITC*ControlRunName*. The user may make further adjustments to the displayed data in this window. Clicking the *Integrate All Peaks* button will integrate all plotted displayed data sets, and then plot them in a window named ADELTAH*ControlRunName*.

The ADELTAH*ControlRunName* window will behave the same as the main ADELTAH plot. Operations in this window will only affect the currently displayed window. The control run is not plotted in the ADELTAH*ControlRunName* windows. All curve fittings performed through this window will only be carried out on the plotted data.

Single File Analysis

The data analysis buttons by default fit all plotted data. In the event a single file fit is required, the AdeltaH plot can be put into Single File Fitting Mode. To enter this mode, select Single File Mode from the AutoITC Fitting Mode options in the AutoITC menu.
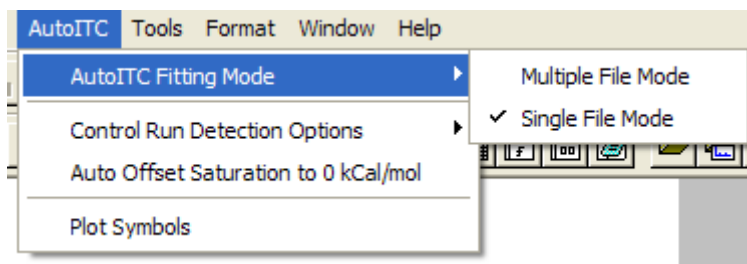
This will change the available fitting tools. The AutoITC group of buttons will be replaced with the Model Fitting group. Also, the plot will be emptied except for the active data set.
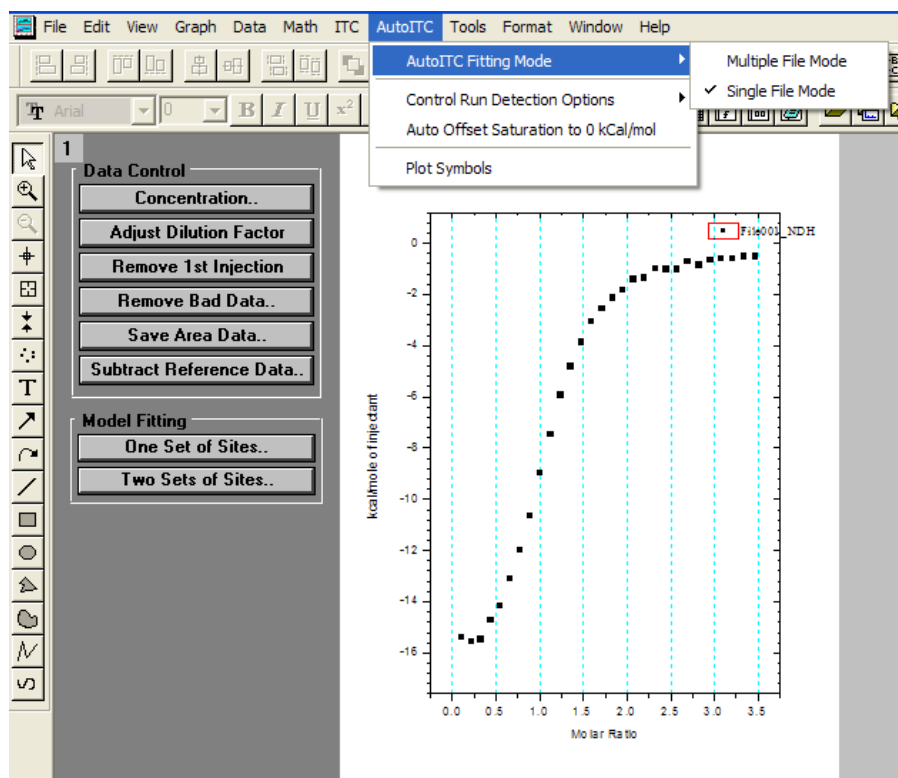
This data can then be fit using the One Set of Sites and Two Set of Sites buttons. These fits are the same as the general ITC Data Analysis (See Lessons 1 and 7).

Post Import Updating

Changes to concentrations, dilution factors, and control runs can be carried out after import. These changes can be easily done working with ITC Summary Table. To activate this window, select the menu item **Window: MicroCal VP-ITC Autosampler Summary Table.**

Filename	Worksheet	Control Run	Tray #	Well #	Syr. Conc.	Cell Conc.	Syr. Dil. Factor	Cell Dil. Factor	Comments
File001.ITC	Data1	Control	1	4	0.1825	0.0122	2	4	
File002.ITC	Data2	File001.ITC	1	6	0.1825	0.0122	2	4	
File003.ITC	Data3	Control	1	8	0.1825	0.0122	2	4	
File004.ITC	Data4	File003.ITC	1	10	0.1825	0.0122	2	4	
File005.ITC	Data5	File003.ITC	1	12	0.1825	0.0122	2	4	
File006.ITC	Data6	File003.ITC	1	14	0.1825	0.0122	2	4	

To edit Control Runs

- A control run is designated by a "Control" entry in the Control Run column. To designate any file as a control run type "Control" in this column.
- To designate a control run for an experiment, select "Set Control Run" from the AutoITC menu. This will open the set Control File dialog box. Select the Control Run from the Control Run drop down list. Only designated control runs are selectable. Select the Data File from the Data File drop down list. The Apply to All Files check box will set the control run for all non-control run files. The Apply to Rest check box will set the control run for Data File and the non-control run files in the table below the Data File row.
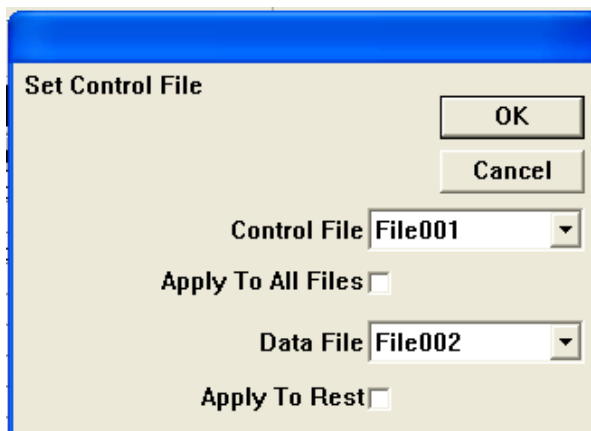
- To then perform control run subtraction, select either Subtract Control DP Data or Subtract Control NDH Data. Users can toggle the Group by Control Run option to plot the subtracted data into ADELTAH*ControlRunName* or ADELTAH*ControlRunName* plots.
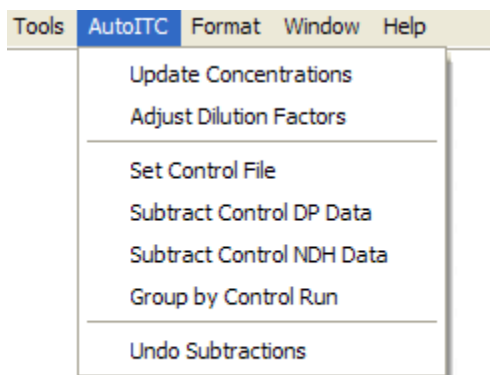
- To restore original DP and NDH data, select the Undo Subtractions option from the AutoITC menu.

To update dilution factors and concentrations

- Dilution factors can be edited using Adjust Dilution Factors option in the AutoITC menu. This will open the Adjust Dilution Factors dialog box. Enter the desired syringe and cell dilution factors. The default values will be from the first file in the summary table. Choose the data set to apply these values to or select Apply to All Samples to update all dilution factors. The Update Concentrations option must be selected from the AutoITC menu for these changes to affect NDH data.
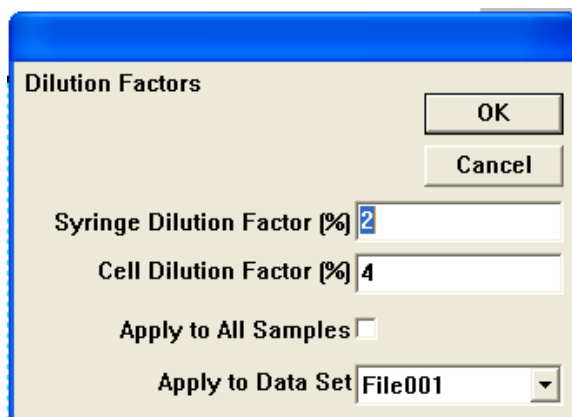
- To update concentrations, simply edit the summary table with the desired concentrations. Then choose Update Concentrations from the AutoITC menu. **Note: The internal concentration used in calculating NDH is the product of the concentration and the dilution factor:**

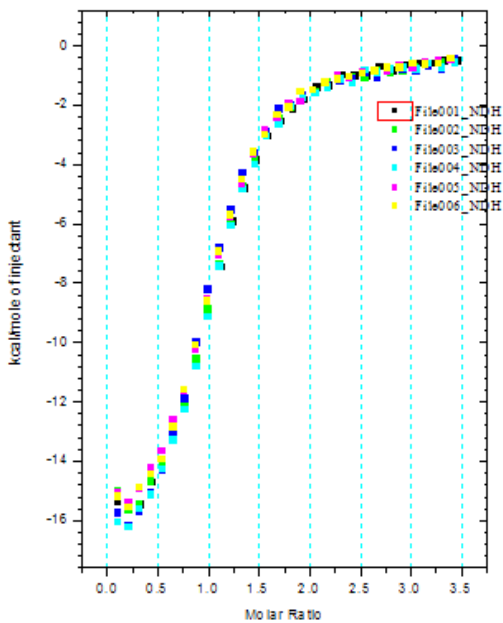**Internal Concentration = Concentration * (100 – Dilution Factor) / 100**
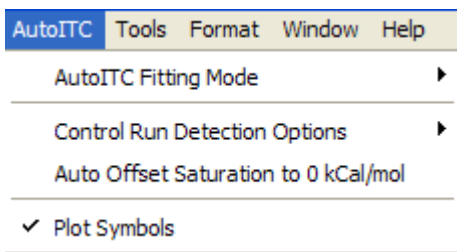
Auto Detect and Subtract Saturation

When the Auto Offset Saturation to 0 Kcal/mol option in the AutoITC menu is selected, Origin will analyze the last 5 injections of an experiment looking for saturation. If a change in peak size of less than 2% is detected, Origin will recognize this as saturation. Origin will find a mean value for the detected saturation range and subtract that value from all points. If saturation is not detected, a message will be displayed in the script window saying that no offset will occur.
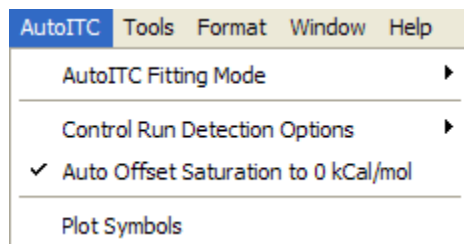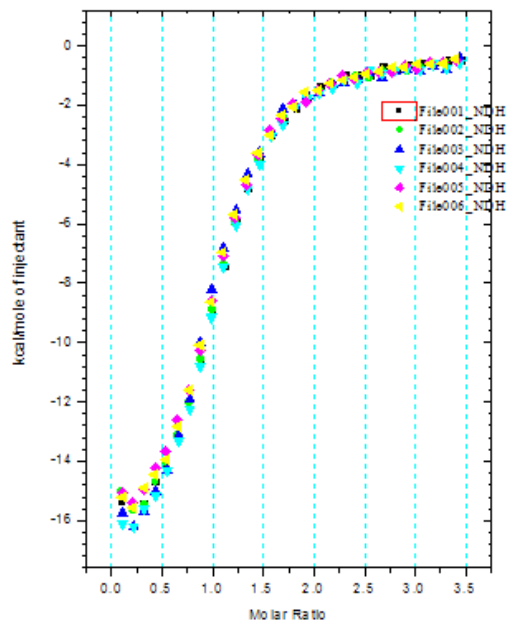

Plot Symbols

In both the ADELTAH window, datasets will be plotted in color. However, printing to a black and white printer will make the datasets indistinguishable. To make each dataset unique, the user may utilize the plot symbols option in the AutoITC menu. When utilized, symbols will be plotted instead of the default blocks. This option may be selected before or after import. Disabling this option will restore the blocks.

Blocks   Symbols