

NITPIC (MATLAB version 10.0)

USER GUIDE

This guide is distributed with software NITPIC.exe that includes a sample data set, EDTA.itc.

The content of this guide is furnished for informational use only and is subject to change without notice.

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Chapter I: Before you begin

Installation

In order to run the MATLAB based NITPIC.exe, the MATLAB Compiler Runtime (MCR) needs to be installed on your computer first. MCR is an execution engine made up of the same shared libraries MATLAB uses to enable the execution of MATLAB files on systems without an installed version of MATLAB.

The current version of NITPIC requires MCR v7.17, which can be downloaded from the following address. The version for Windows 32-bit is appropriate for the current version of NITPIC.

<http://www.mathworks.com/products/compiler/mcr/index.html>

Instructions for installing and uninstalling MCR can be found at MathWorks website. Usually the process could take ~15 min and require restarting your computer.

<http://www.mathworks.com/help/toolbox/compiler/fl2-999353.html>

NITPIC.exe does not require a systematic installation. The executable file is transferable by copy and paste to any location on your computer. Therefore, after installing MCR v7.17 successfully, you can initiate NITPIC.exe directly by clicking the icon on your computer. Please be aware that NITPIC assumes a large, high resolution display is available.

Note that a PYTHON based NITPIC version will appear soon, which will supersede the current one.

Download NITPIC and SEDPHAT

NITPIC.exe can be downloaded at this address. SEDPHAT is recommended for analyzing the processed ITC data by NITPIC, which is also available from the same URL.

<http://sedfitsedphat.nibib.nih.gov/software/default.aspx>

Download GUSSI

GUSSI is a plotting program written by Dr. Chad Brautigam (UT Southwestern Medical Center), which interfaces nicely with SEDPHAT. It can be downloaded from the link below.

<http://biophysics.swmed.edu/MBR/software.html>

Save SEDPHAT and GUSSI

After downloading, NITPIC can be saved at any location, while SEDPHAT and GUSSI need to be saved in specific directories as following.

- ❖ c:\sedfit\sedphat.exe
- ❖ c:\sedfit\GUSSI\GUSSI.exe

Chapter 2: How to use NITPIC

Importing ITC data into NITPIC

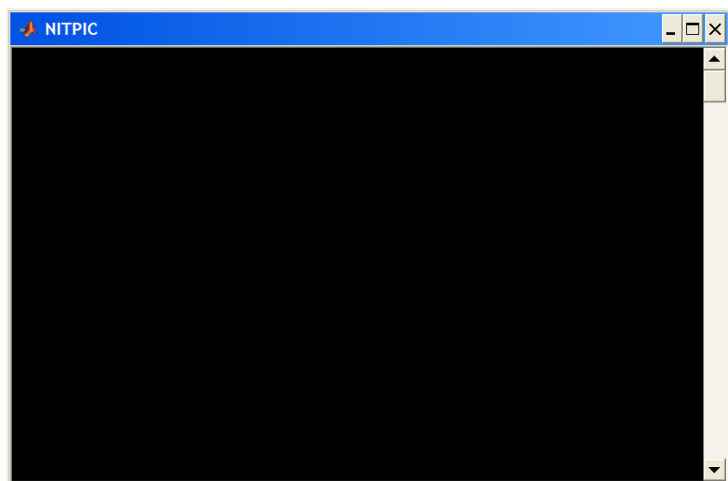
Input file

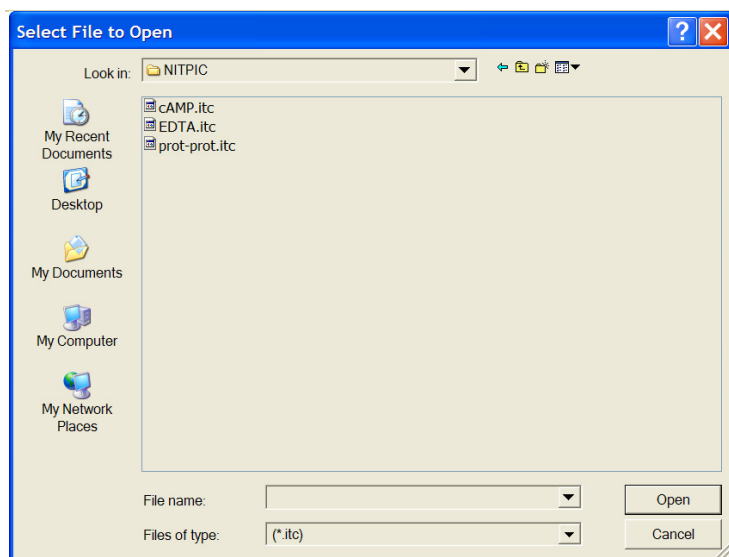
The ITC experimental file of each titration generated by MicroCal calorimeters (*.itc) is the default format for the NITPIC input file. Experimental parameters (temperature, concentrations, signal of heat, etc.) are recognized by NITPIC from this file.

Current requirements are that syringe and cell concentrations are available in the *.itc file, and that all injection have the same length. Furthermore, be aware that NITPIC assumes that at least 30% of the time between injections represents the baseline. If the time between injections are too short, it will not perform well. These requirements will be relaxed in future releases.

Loading *.itc file

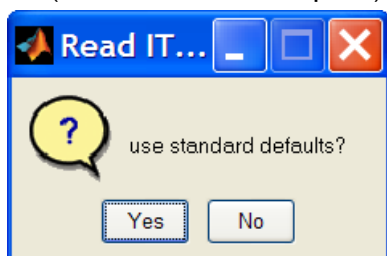
When NITPIC is initiated, a console window and a file input pop-up window appear; the latter asks the user to select which *.itc file to open.



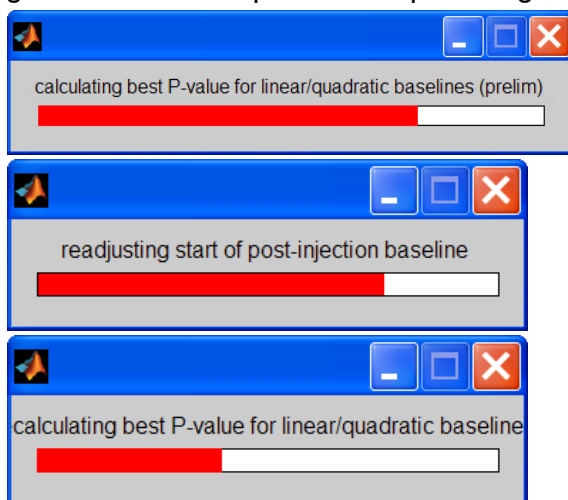


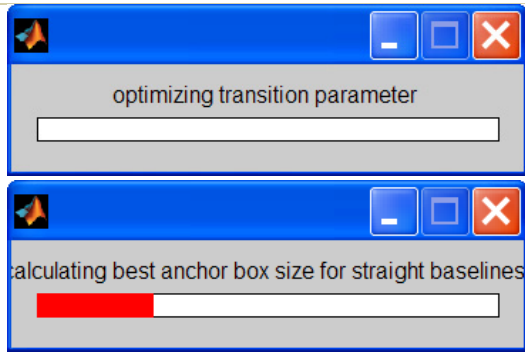
Processing the data using standard defaults

Once a specific .itc file is selected, NITPIC provides options for baseline assignment of peak integration. One can use standard defaults or click No to proceed to customize the parameters for baseline assignment (described in later chapters). In this demonstration, EDTA.itc is selected.



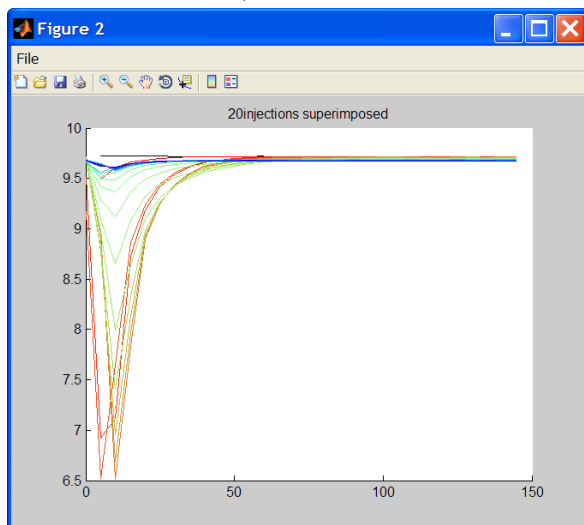
The progress bar shows the processes of peak integration using the algorithms embedded in NITPIC.



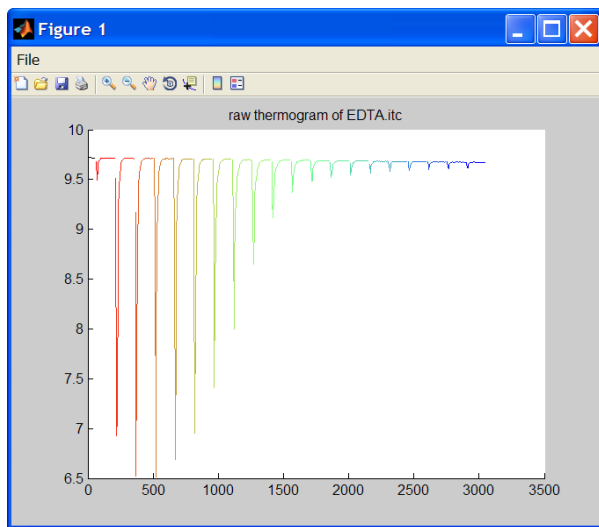


A set of windows show up to illustrate how the baseline assignment is performed.

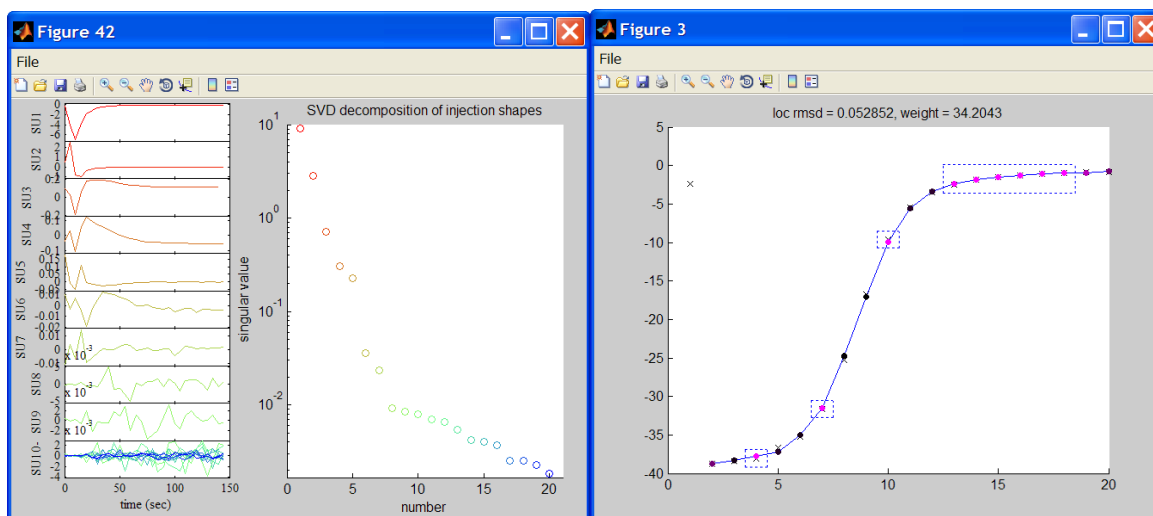
The raw ITC injection peaks are superimposed in the window of Figure 2, with the initial baseline shown as a solid black line. First injection is red, the later ones in green to blue.



In the window of Figure 1, raw thermogram of the ITC titration is shown.

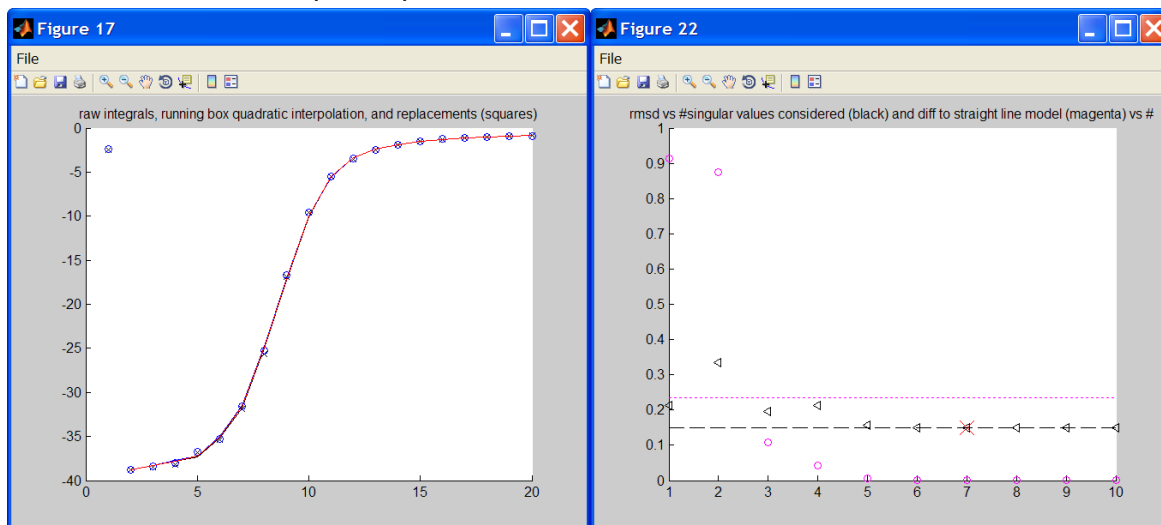


Window Figure 42 illustrates the shape components determined by singular value decomposition (SVD). By using SVD we can identify basic shape components, which is used as a filter to allow the user to focus on the major shape components. For this thermogram, the first 7 components are relevant. The later components, such as component 10, are mainly noise. Figure 3 is for internal debugging purpose.



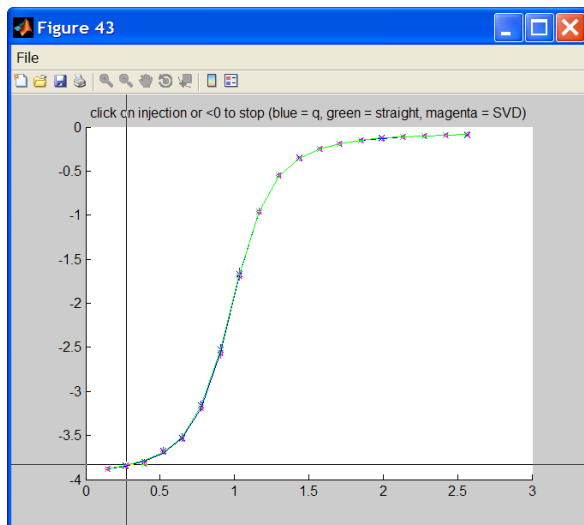
Window Figure 17 shows the resulting isotherm, with each point representing the integrated peak area.

Window Figure 22 shows the refinement of the SVD with a plot of root-mean-square-deviation (rmsd) of the isotherm vs. the number of components considered (black triangles), as well as rms difference of the truncated data from the untruncated data (magenta circles). The dotted line is the estimated uncertainty of integration (errors from extrapolations), the dashed black line is the estimated rmsd in the isotherm when all shape components are used.



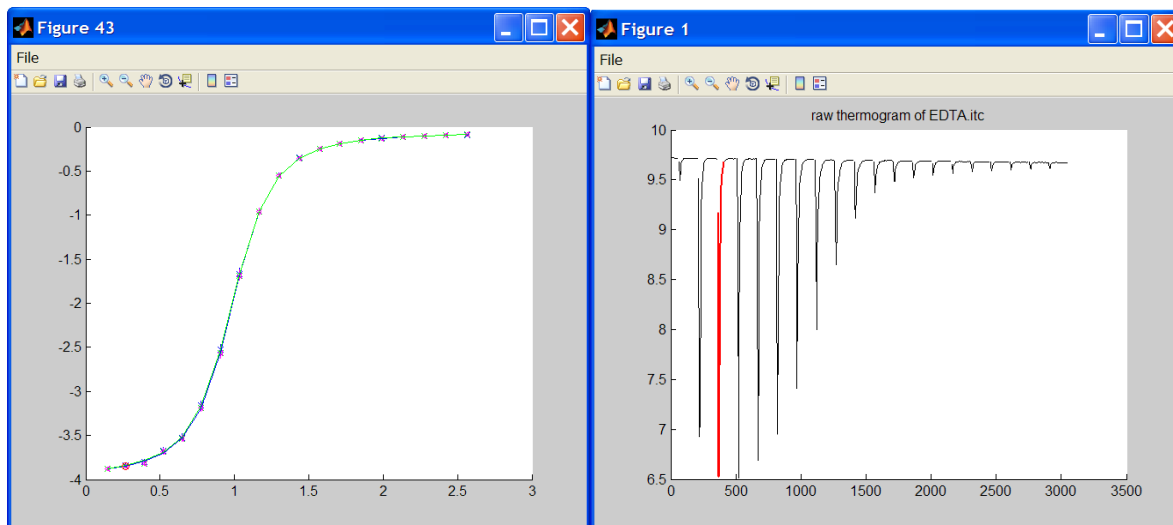
Two blank windows, Figure 100 and Figure 44 are for the illustration of peak integration for individual injections in subsequent steps.

When NITPIC finishes, it displays Figure 43 with a crosshair (the cursor). The user can move the cursor along the generated isotherm and click a data point to examine the details of the respective peak integration. One important aspect of NITPIC peak integration is the generation of error bars for the integrated peak area (demonstrated below).



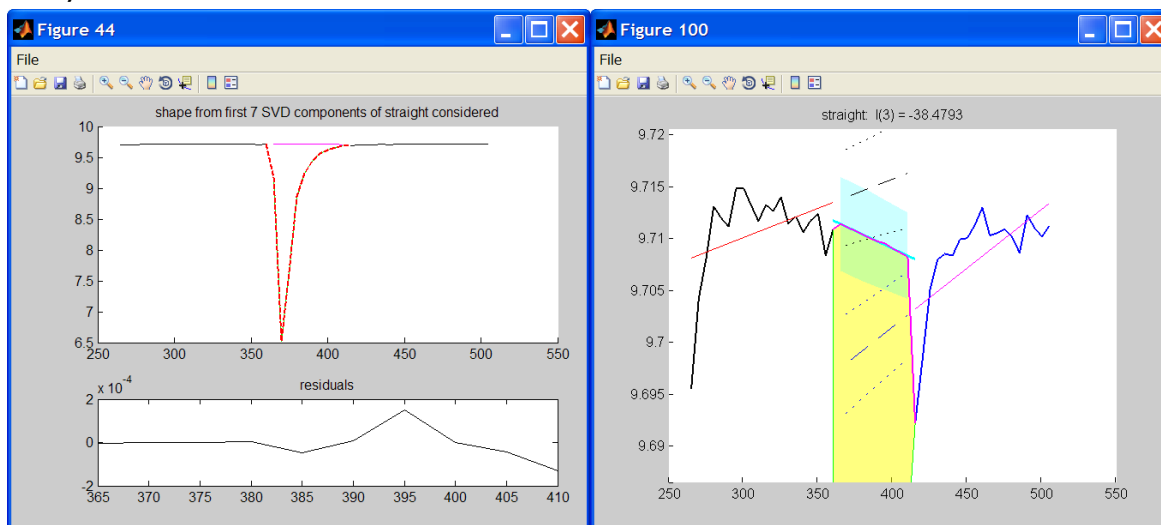
Note: Version 10.0 now creates a STOP pushbutton in Figure 43 to terminate this selection loop.

The selected injection peak is highlighted in the isotherm in Figure 43 and the thermogram in Figure 1, as a red circle and a red line segment, respectively.

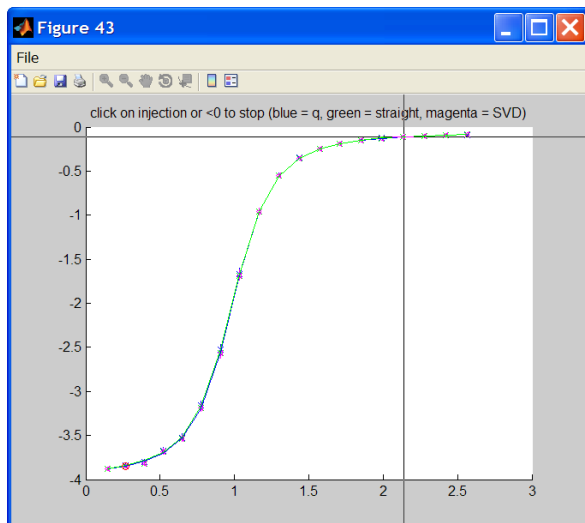


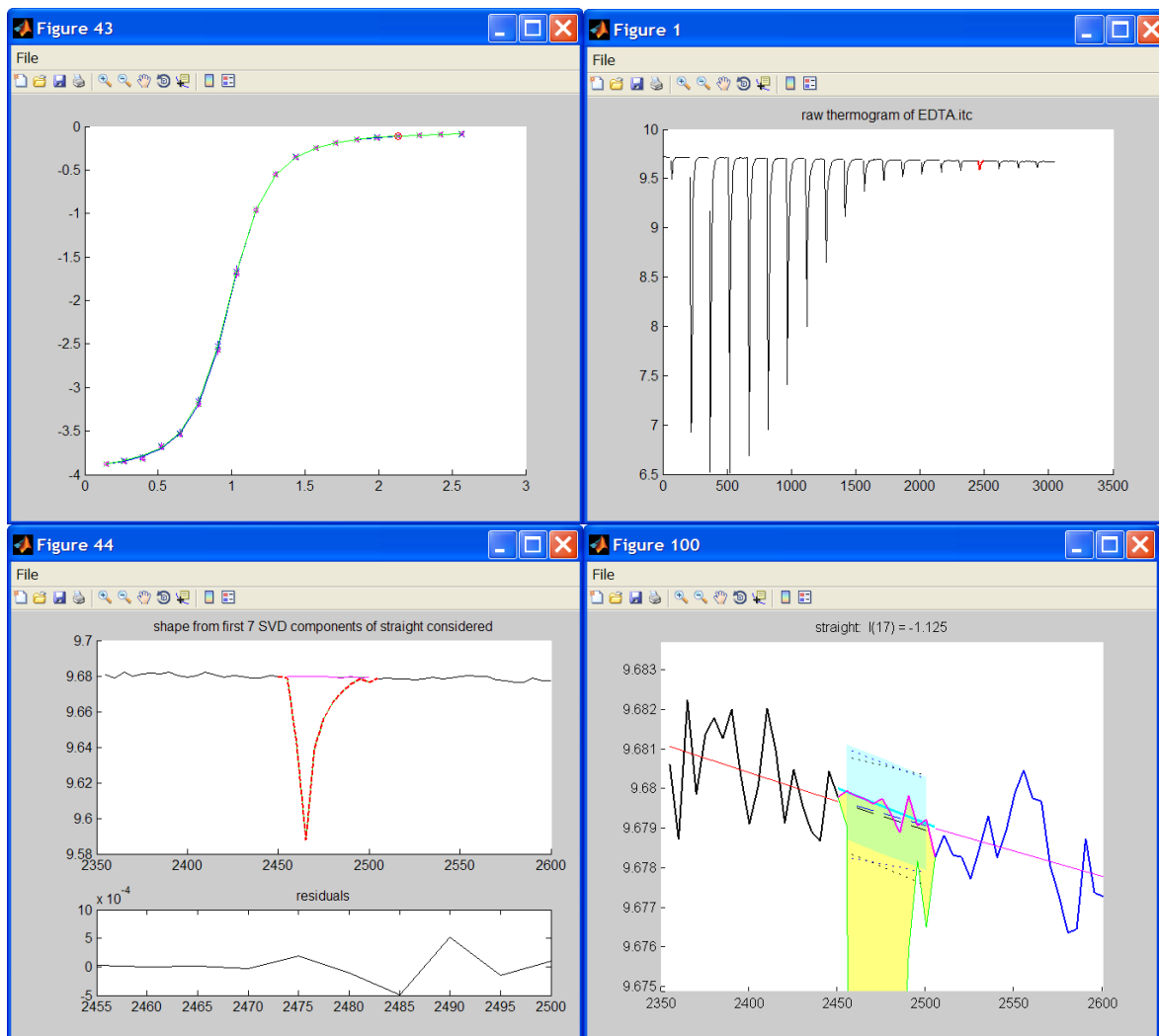
Windows Figure 44 and Figure 100 illustrate the principle of baseline interpolation. In Figure 100 solid black lines are available experimental baseline data surrounding the injection, and solid green lines are injection data. Thin solid red and magenta lines through the baseline regions are the least-squares fits to the pre- and postinjection baselines, respectively, and dashed black lines denote their extrapolations into the injection region, and dotted black lines indicate their 68% confidence range. The cyan line in the injection region is the interpolated baseline, and the area shaded in light cyan is the corresponding

confidence band, which determines the error of the peak integration. The bold solid magenta line is the best-estimate baseline during the injection. The heat generated by the injection therefore corresponds to the area between this bold solid magenta line and the green line of the injection signal, as highlighted with yellow shade.



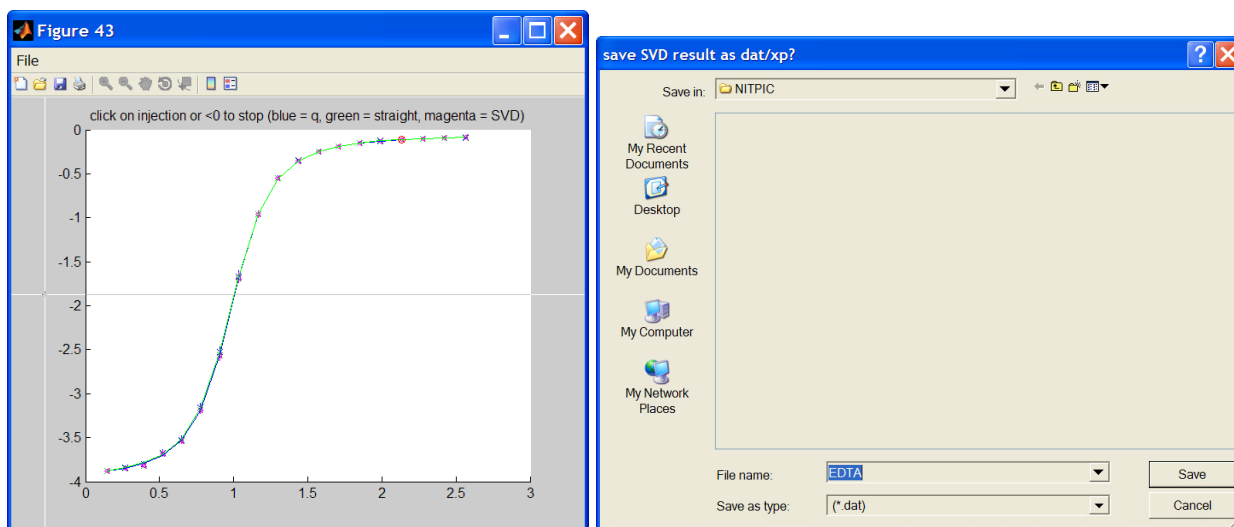
The user can examine the integration of every peak using the crosshair by selecting the corresponding data point on the isotherm in Figure 43.





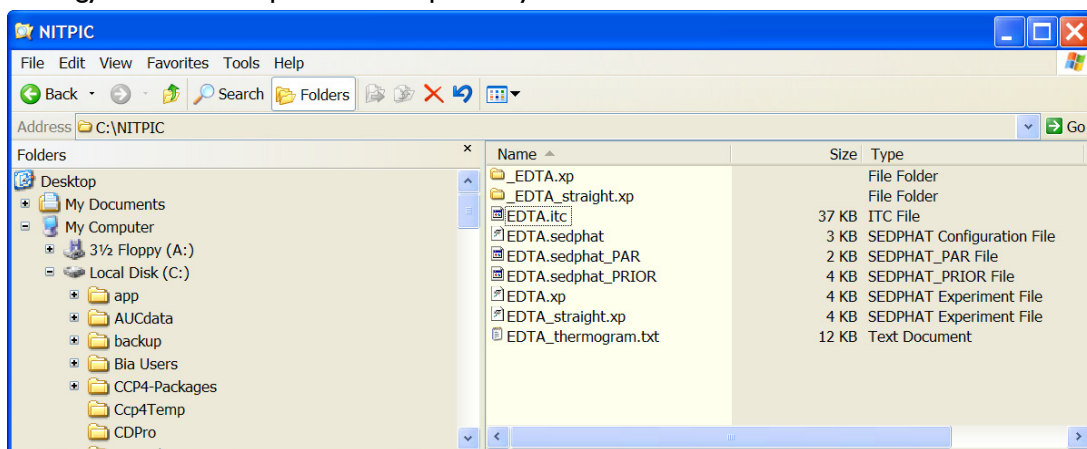
Exporting the generated ITC isotherm into SEDPHAT

After peak integration by NITPIC, you can exit the program by moving the crosshair to any place on the left region of the Y-axis in Figure 43 as shown below (or in version > 10.0 by clicking on the pushbutton “STOP”), then right click the mouse button. Next a pop-up window appears and asks the user to save the peak integration result as dat and xp files, which will be automatically loaded into SEDPHAT.

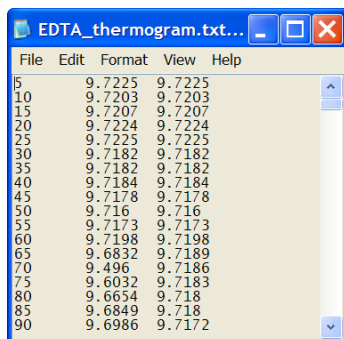


After clicking Save, you can save the data for further analysis. If SEDPHAT was installed successfully at c:\sedfit\, NITPIC automatically generates a set of files in the dedicated directory as shown below.

EDTA.sedphat is a configuration, which can be read in SEDPHAT; the two subfolders, _EDTA.xp and _EDTA_straight.xp contain the processed data and error for each data point using SVD and straight line strategy to define the peak area respectively.

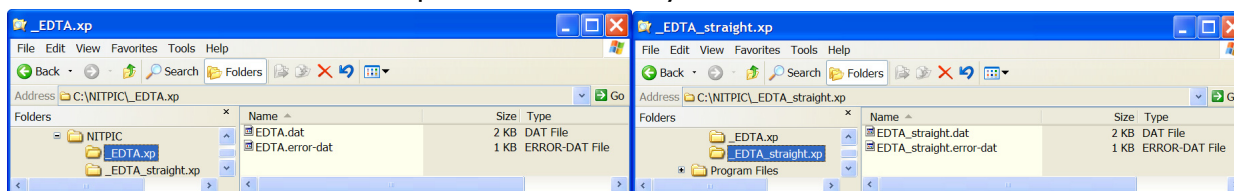
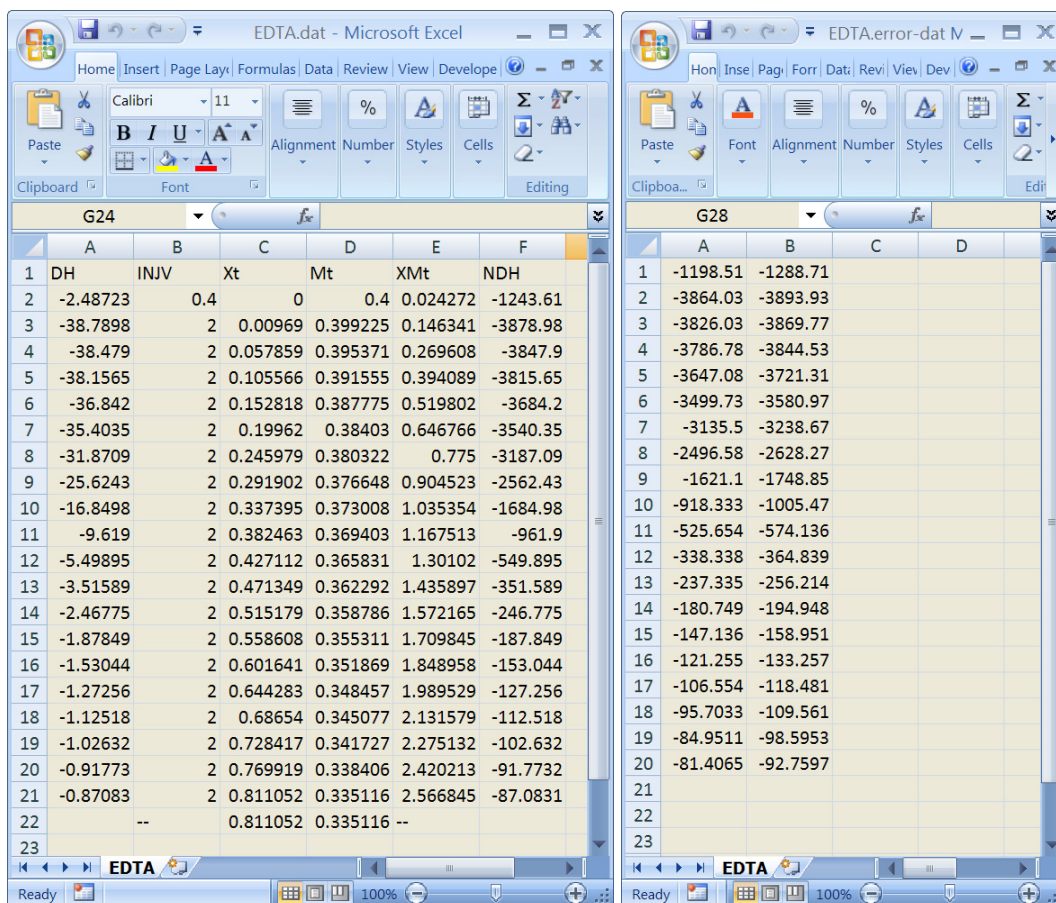


The file EDTA_thermogram.txt contains the thermogram data as well as the baseline signals.



5	9.7225	9.7225	
10	9.7203	9.7203	
15	9.7207	9.7207	
20	9.7224	9.7224	
25	9.7225	9.7225	
30	9.7182	9.7182	
35	9.7182	9.7182	
40	9.7184	9.7184	
45	9.7178	9.7178	
50	9.716	9.716	
55	9.7173	9.7173	
60	9.7198	9.7198	
65	9.6832	9.7189	
70	9.496	9.7186	
75	9.6032	9.7183	
80	9.6654	9.718	
85	9.6849	9.718	
90	9.6986	9.7172	

Each folder has two files: EDTA.dat is the standard MicroCal Origin format of exported NDH tables, and EDTA.error-dat contains the confidence limits for the peak area of each injection. In SEDPHAT, these values will be read and incorporated into the analysis.

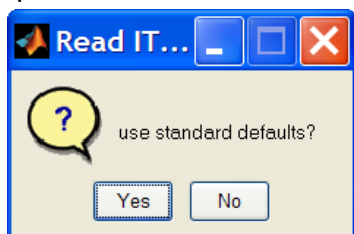



	A	B	C	D	E	F
1	DH	INJV	Xt	Mt	XMt	NDH
2	-2.48723	0.4	0	0.4	0.024272	-1243.61
3	-38.7898	2	0.00969	0.399225	0.146341	-3878.98
4	-38.479	2	0.057859	0.395371	0.269608	-3847.9
5	-38.1565	2	0.105566	0.391555	0.394089	-3815.65
6	-36.842	2	0.152818	0.387775	0.519802	-3684.2
7	-35.4035	2	0.19962	0.38403	0.646766	-3540.35
8	-31.8709	2	0.245979	0.380322	0.775	-3187.09
9	-25.6243	2	0.291902	0.376648	0.904523	-2562.43
10	-16.8498	2	0.337395	0.373008	1.035354	-1684.98
11	-9.619	2	0.382463	0.369403	1.167513	-961.9
12	-5.49895	2	0.427112	0.365831	1.30102	-549.895
13	-3.51589	2	0.471349	0.362292	1.435897	-351.589
14	-2.46775	2	0.515179	0.358786	1.572165	-246.775
15	-1.87849	2	0.558608	0.355311	1.709845	-187.849
16	-1.53044	2	0.601641	0.351869	1.848958	-153.044
17	-1.27256	2	0.644283	0.348457	1.989529	-127.256
18	-1.12518	2	0.68654	0.345077	2.131579	-112.518
19	-1.02632	2	0.728417	0.341727	2.275132	-102.632
20	-0.91773	2	0.769919	0.338406	2.420213	-91.7732
21	-0.87083	2	0.811052	0.335116	2.566845	-87.0831
22	--		0.811052	0.335116	--	
23						

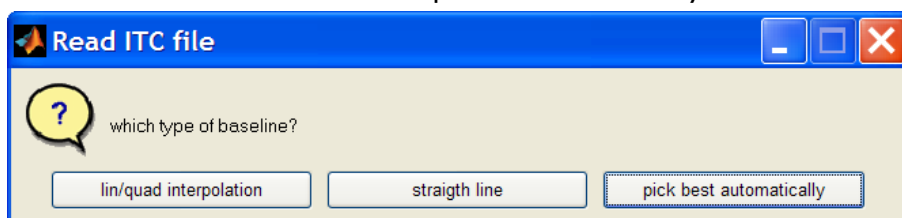
	A	B	C	D
1	-1198.51	-1288.71		
2	-3864.03	-3893.93		
3	-3826.03	-3869.77		
4	-3786.78	-3844.53		
5	-3647.08	-3721.31		
6	-3499.73	-3580.97		
7	-3135.5	-3238.67		
8	-2496.58	-2628.27		
9	-1621.1	-1748.85		
10	-918.333	-1005.47		
11	-525.654	-574.136		
12	-338.338	-364.839		
13	-237.335	-256.214		
14	-180.749	-194.948		
15	-147.136	-158.951		
16	-121.255	-133.257		
17	-106.554	-118.481		
18	-95.7033	-109.561		
19	-84.9511	-98.5953		
20	-81.4065	-92.7597		
21				
22				
23				

Processing the data using customized parameters

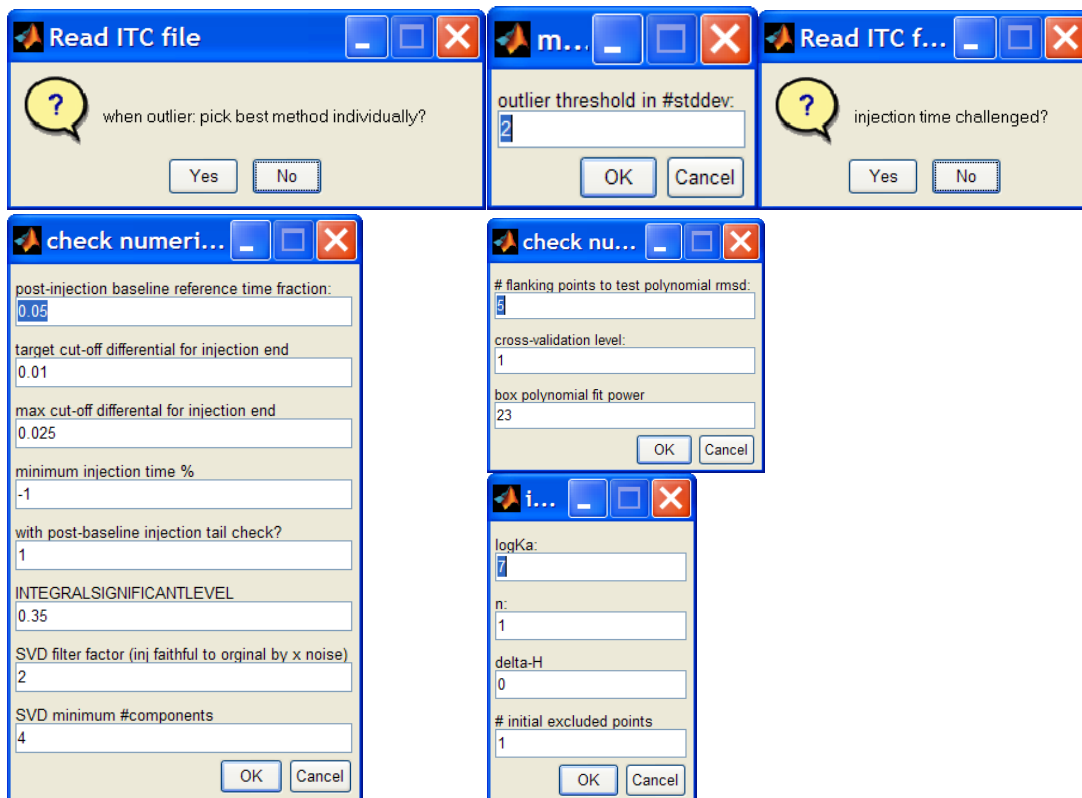
Once a specific .itc file is selected, NITPIC provides the user with the opportunity to select options:



When “No” is selected, a set of options will be provided for the user to pick. The following demonstration is based on the selection of ‘pick best automatically’.



The following windows will appear one after another to ask the user to initialize the parameters for baseline assignment.



The most important parameters for injection time control are the thresholds for “target cut-off differential”, which may be slightly increased or decreased to control the automatically assigned end-

time of the injection. (While running NITPIC, the MATLAB console window will show some control parameters that might be useful when changing the defaults.) Another control parameter for the injection time assignment is “minimum injection time”, which if set > 0 will be a lower limit for the injection time. For example, setting the “cut-off differential” thresholds very high (e.g., 0.10 and 0.05) in combination with a given “minimum injection time” (e.g., 30%) effectively allows the user to take over control of the injection time settings (in this case, the injection time is forced to be 30% of the time between injections). After defining the parameters, NITPIC will start the integration process as shown in the previous section, **Processing the data using standard defaults**.

Chapter 3: Data analysis in SEDPHAT

Loading NITPIC output ITC data

Once the integrated peak areas are saved as dat/xp files, a SEDPHAT parameter window will appear, with a default model, $A + B \rightleftharpoons AB$, which assumes a 1:1 binding stoichiometry. The parameters *incfA* and *incfB* are the incompetent fractions for species A and B respectively, which represent the inactive population unable to participate in binding. *K_a* is the equilibrium association constant in M⁻¹. *dHAB* is the enthalpic change associated with binding in kcal/mol.

A + B \rightleftharpoons AB

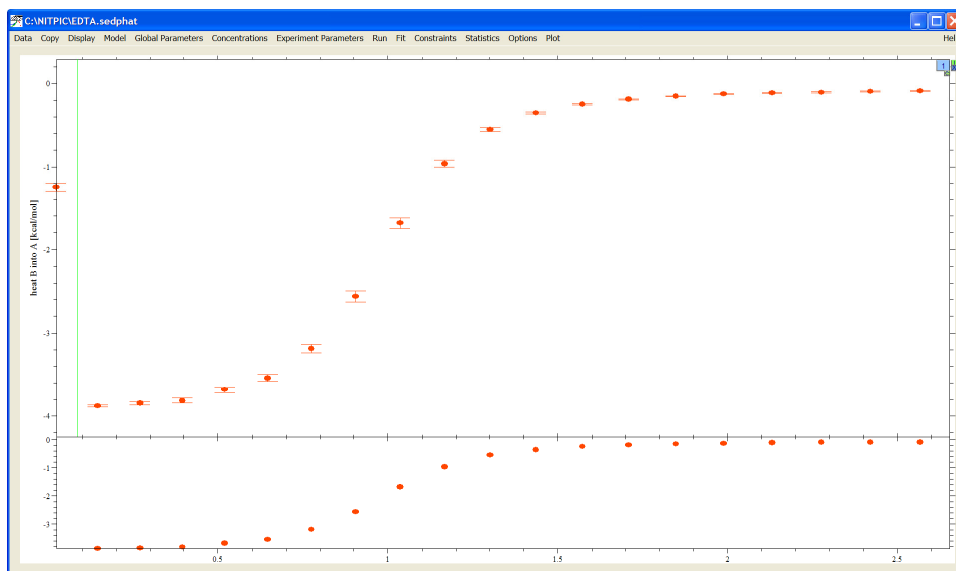
Component A (experiment 1)		Component B (experiment 1)		Complex AB	
<input type="checkbox"/> atot	1.00000	<input type="checkbox"/> btot	1.00000	<input type="checkbox"/> Mass Conservation	
<input type="checkbox"/> Ma	30000.00000	<input type="checkbox"/> Mb	45000.00000	<input type="checkbox"/> Mab	0
<input type="checkbox"/> sA	3.500	<input type="checkbox"/> sB	5.000	<input type="checkbox"/> sAB	6.500
extico a	1.0	extico b	1.0	<input checked="" type="checkbox"/> log(<i>K_a</i>)	5.07990
<input checked="" type="checkbox"/> incfA	0.05878	<input type="checkbox"/> incfB	0.00000	<input checked="" type="checkbox"/> dHAB	-3.91903
				<input type="checkbox"/> log(<i>k</i> -)	-1.00000

☒ both A,B in micromolar concentrations
☐ A in micromolar conc and B/A molar ratio

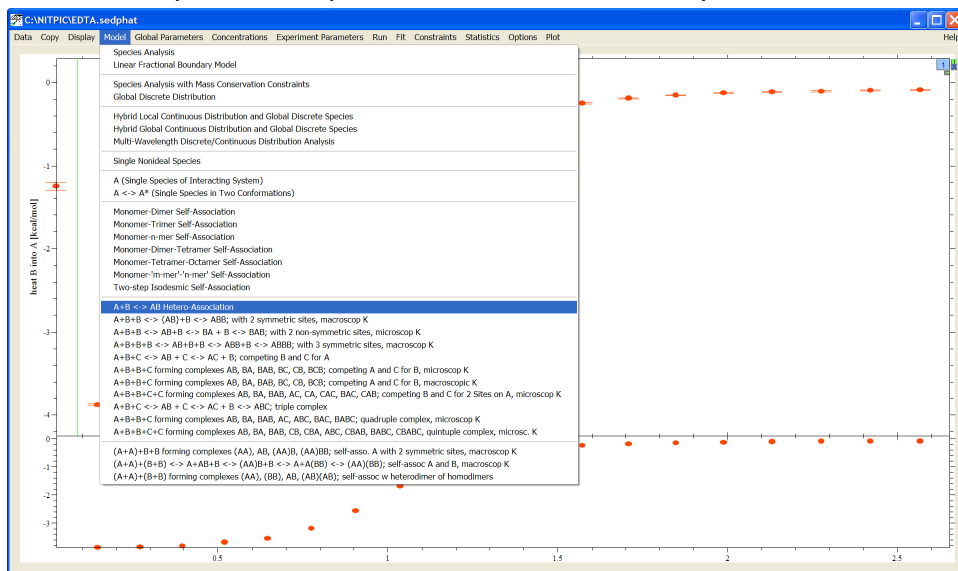
☐ add non-participating species

Cancel OK

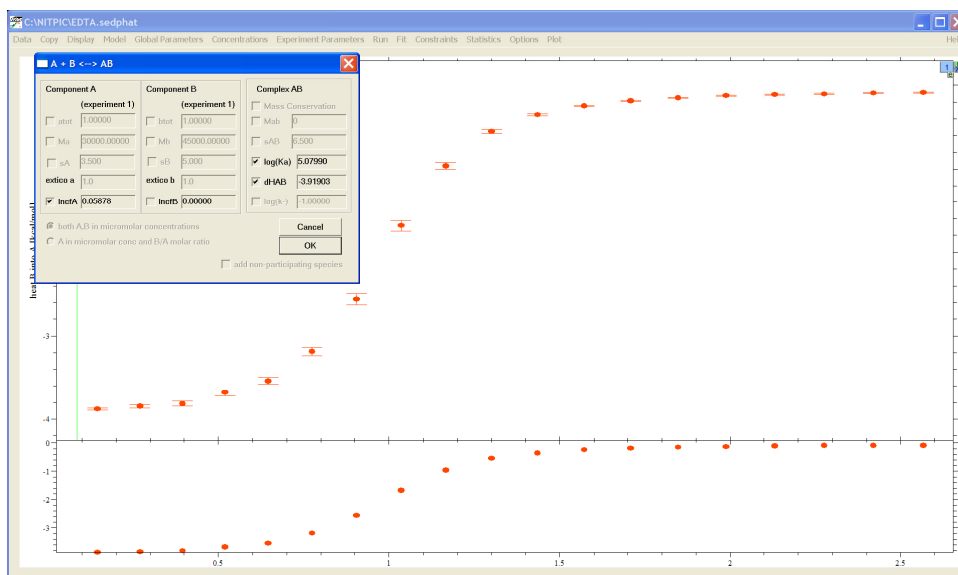
As you can see, NITPIC has filled in initial values for these parameters after performing a “courtesy” fitting of the data using this model. Click OK to proceed. SEDPHAT window with the ITC data will appear, as well as the error bars for the data points. The green solid lines define the fitting range of the data. SEDPHAT usually by default excludes the first data point due to the leakage of material from the syringe during the time of equilibration.



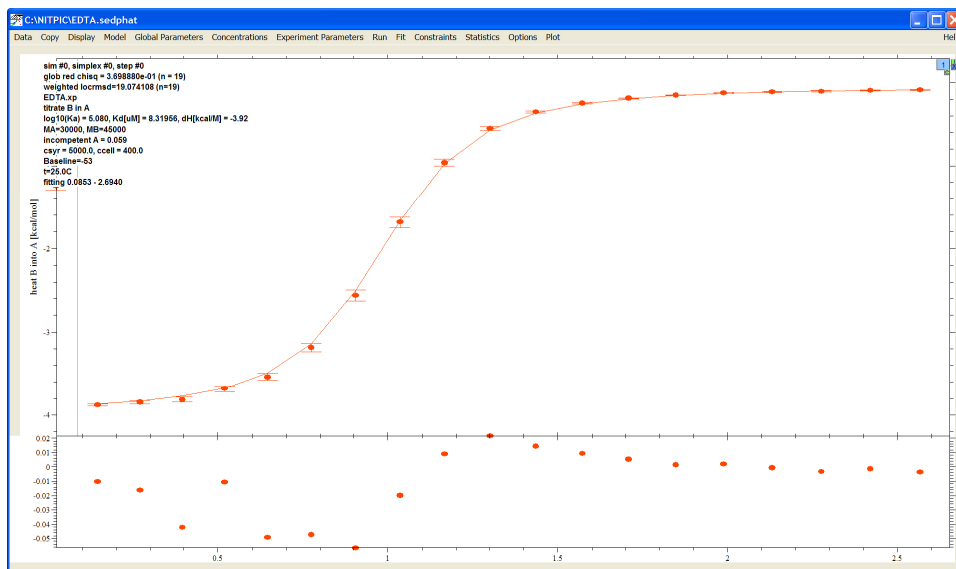
By default, SEDPHAT picks the simplest model. Other models may be selected, as shown below:



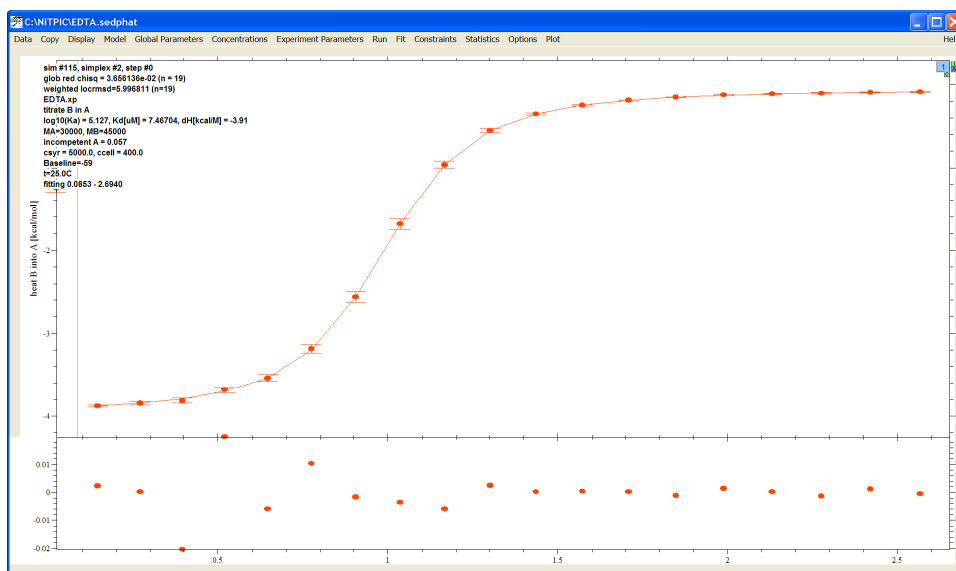
Fitting parameters can be adjusted under Global Parameters, a tab on top.



One can click Run (either global or single, since there is only one data set in this demonstration) to see what the predicted binding isotherm looks like.



Optimization can be started by clicking Fit.

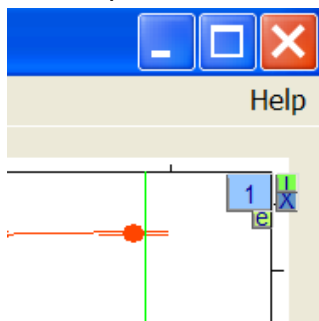


In practice, you might need to select other models to analyze the data.

Adjusting experimental parameters

The pre-input experimental parameters (temperature, concentrations, cell volume and etc) can be modified in SEDPHAT. One can click the tab on top of SEDPHAT window, Experimental Parameter or

the blue square button on the right top corner of the isotherm with number 1, representing the order number of the experimental data in the analysis. In global analysis with multiple datasets, with this number, each experimental data can be easily distinguished by the user.



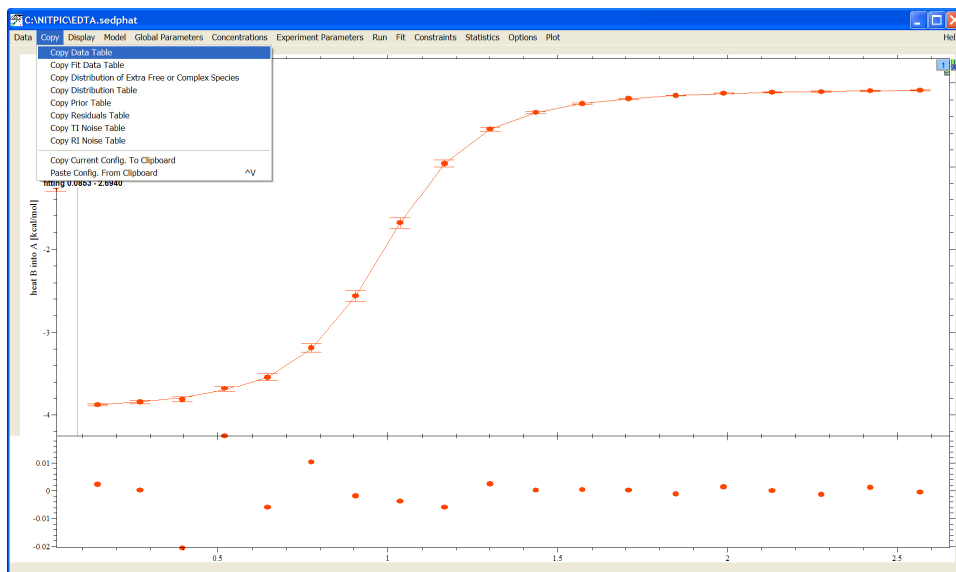
Within the window of Experimental Parameters, the user can change the values and define the titration configuration (A into B or B into A). Caution should be exercised when changing many of these values; remember that NITPIC took them directly from the raw thermogram file. Thus, parameters like Temperature or cell volume are supplied to SEDPHAT from NITPIC and are expected to be correct.

The 'Experimental Parameters' dialog box is shown. It has a blue title bar with a close button. The main area is divided into several sections. At the top, it says '(1) ITC data for ITC' and shows the file path 'C:\NITPIC\EDTA.xp (EDTA.dat ...)'. Below this is a 'Comment' field. A section with checkboxes includes 'active' (checked), 'noise' (32.1771), 'fit baseline' (checked), and 'slope' (0.000000). There are also fields for 'cell conc [uM]' (400.000), 'syringe conc [uM]' (5000.000), and 'cell volume [ul]' (206.2). A section for 'binary interaction: titration of' has radio buttons for 'B into A' (selected) and 'A into B'. Below this is a section for 'for ternary interactions:' with radio buttons for 'A into C', 'B into C', 'C into A', 'C into B', 'B into AC', 'BC into AC', 'AC into B', 'BC into A', 'A into BC', 'C into AB', and 'AB into C'. There are also fields for 'molar ratio C/A', 'molar ratio C/B', and 'molar ratio B/A'. A section for 'local incompetent A', 'local incompetent B', and 'local incompetent C' each has a 'fit' field set to 0.0000. A 'max' field is set to 1.0000. At the bottom, there are checkboxes for 'use weighted fit' (checked) and 'use for sigma of MC sims' (32.1771), and a radio button for 'use local rmsd' (selected). 'Cancel' and 'OK' buttons are at the bottom right.

Exporting data and fit for record

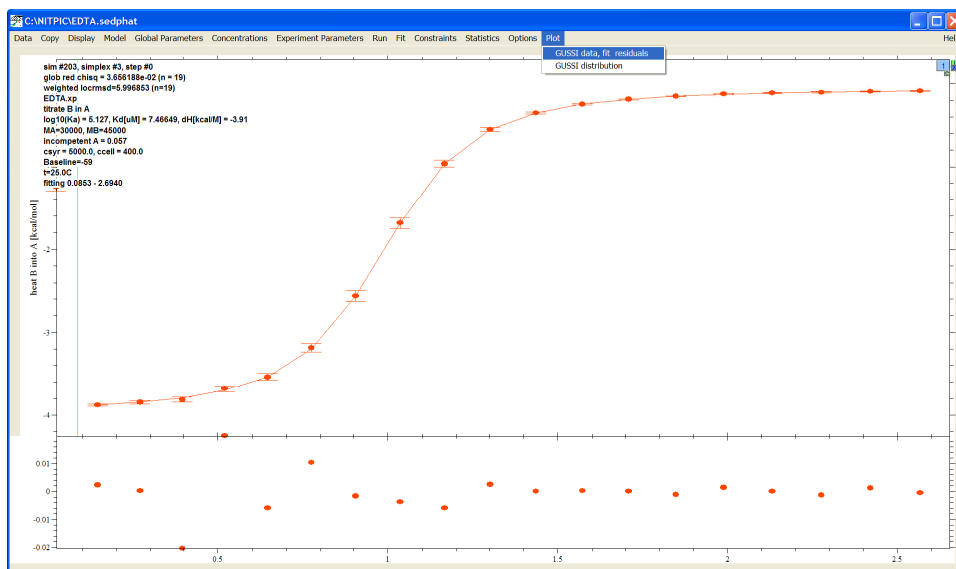
Copy data tables

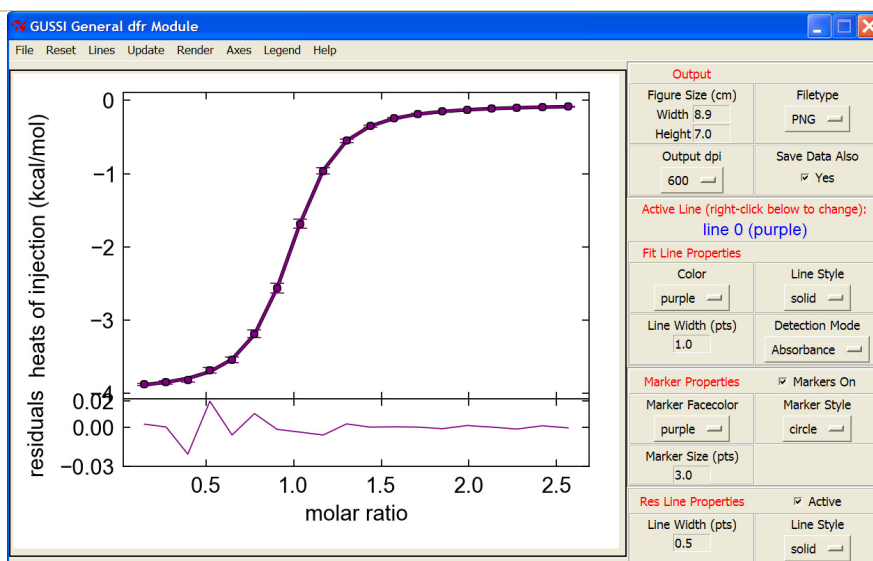
After the analysis has been finalized, one can export the data and fit to other programs for your records or for plotting. For example, to record the data (circles), one clicks on “Copy” in the tab on top, then “Copy Data Table.” The data now reside on the system’s clipboard, and should be pasted into the recording/plotting software of your choice. The fit data table and residuals table may be copy/pasted similarly.



Making plot using GUSSI

The user can easily make plot in GUSSI by clicking Plot, GUSSI data, fit residuals. From GUSSI, one can save a plot and also the data, fit data, and residuals in an easily recalled format.





Theoretical background of NITPIC is described in detail in reference (1).

Global analysis and error analysis are available

Global analysis

Detailed information regarding the analysis of ITC data using SEDPHAT can be found in the following links:

http://www.analyticalultracentrifugation.com/sedphat/experiment_parameters.htm#ITC%20data%20box

http://www.analyticalultracentrifugation.com/sedphat/isothermal_titration_calorimetry.htm

Discussions on global analysis of ITC data in SEDPHAT can be found in the references (2, 3).

Error analysis

In SEDPHAT, a variety of tools to determine the errors in the fitted parameters are available. The users are recommended to find information on the following webpage.

<http://www.analyticalultracentrifugation.com/sedphat/statistics.htm#measure%20for%20the%20goodness%20of%20fit>

Automatic error surface projection is available in SEDPHAT. Detailed information will be updated on the website soon.

Workshop

Workshops on hydrodynamic and thermodynamic analysis of macromolecules with SEDFIT and SEDPHAT are organized annually, in which SEDPHAT and NITPIC analyses are covered. For detailed information, please see the following page:

<http://www.analyticalultracentrifugation.com/workshop.htm>

References

1. Keller, S., Vargas, C., Zhao, H., Piszczek, G., Brautigam, C.A., and Schuck, P. 2012. High-precision isothermal titration calorimetry with automated peak shape analysis. *Anal. Chem.* in press.
2. Houtman, J.C.D., Brown, P.H., Bowden, B., Yamaguchi, H., Appella, E., Samelson, L.E., and Schuck, P. 2007. Studying multisite binary and ternary protein interactions by global analysis of isothermal titration calorimetry data in SEDPHAT: application to adaptor protein complexes in cell signaling. *Protein Sci.* 16:30-42.
3. <http://www.analyticalultracentrifugation.com/default.htm>
4. <https://sedfitsedphat.nibib.nih.gov/default.aspx>

Contact information

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