Kinetic Evaluation 5.3

User manual

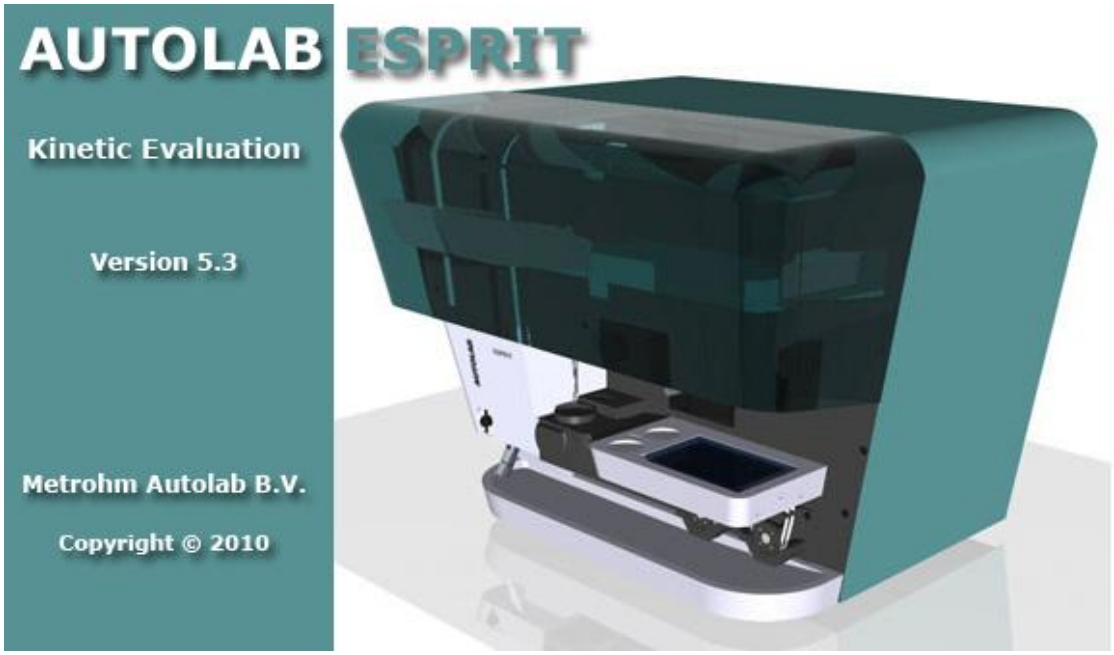


Table of Contents

Kinetic Evaluation 5.2

1 – Introduction

Kinetic Evaluation (KE) is the data analysis application that is bundled with the Autolab SPR Software.

This version of KE has been redesigned incorporating the functionality of the previous versions of KE while introducing new analysis techniques and improving the overall workflow of the system.

The remaining sections of this manual are divided into the following topics:

- **Section 2**, *Working with KE* – This section describes the components that make up the user interface of the Kinetic Evaluation application.

- **Section 3**, *Getting Started with the KE Workflow* – This section provides an example of a typical workflow with the software. This example starts with opening data files and proceeds to fitting interaction plots using the Analysis Wizard.

- **Section 4**, *Working with Data* – An overview of all the file types that the KE application can open, create, and save.

- **Section 5**, *Analyzing the Data* – Description of the available analysis tools within the KE application. The Modeling Wizard, used to create kinetic models; the Signal Processing tool, used to improve data quality; and the various Analysis Wizards, used to determine kinetic binding constants.

- **Section 6**, *Calibration* – A description of calibration curves that can be used in analyses.

- **Section 7**, *Van't Hoff Analysis* – A description of analyses versus temperature that measures thermodynamic properties.

2 – Working with KE

In this section introduces the user interface of the KE application. The navigation via various menus and toolbars will be described in the following subsections.

2.1 – User Interface Overview

Figure 2.1 is a typical screenshot of the KE application. In this section each of the numbered regions in the figure will be explained.

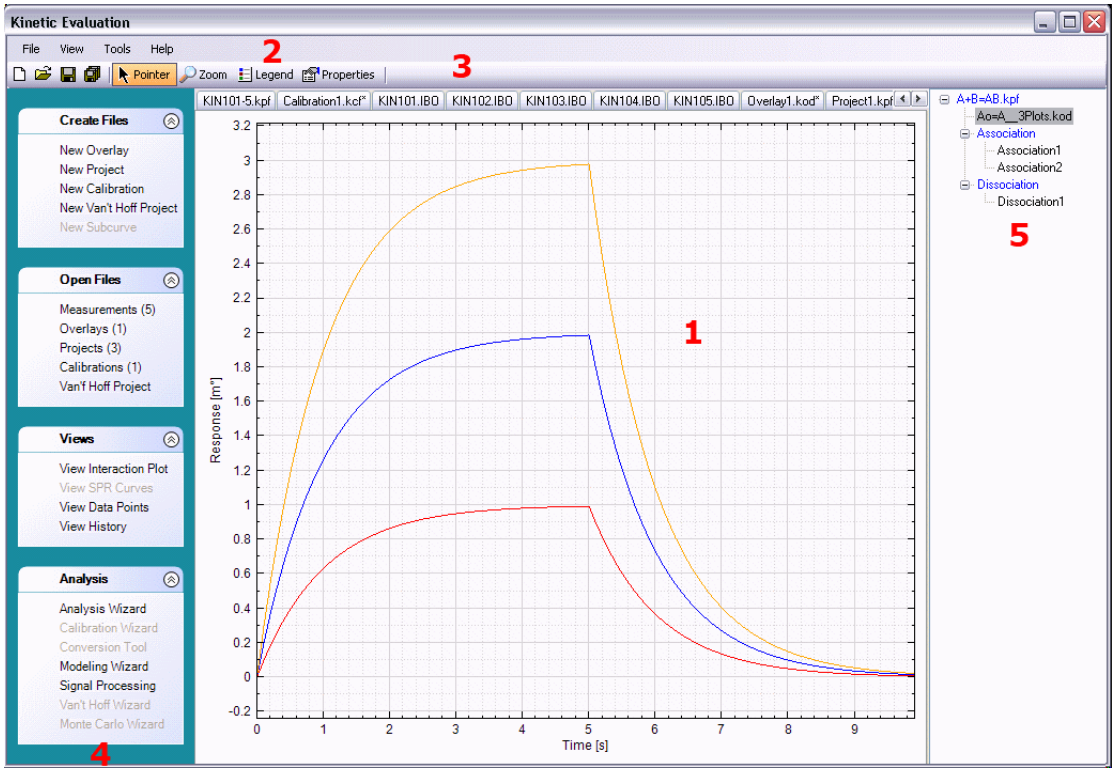


Figure 2.1 - Screenshot of the Kinetic Evaluation application

The following descriptions correspond to the numbers that appear in Figure 2.1.

1.) **Opened Files** – Files that have been opened are added as tabs. The data within these files are graphed within the tabs. For more information about the file types that KE supports, see section 4.

2.) **Main Menu** – All the functionality of the KE application is accessible via the main menu (see section 2.2 for more information).

3.) **Toolbar** – The buttons in the toolbar represent KE functionality (see section 2.3 for more information).

4.) **Activity Bar** – Provides quick access to commonly used functionality. The functions in the Activity Bar are grouped into common themes (see section 2.4 for more information).

5.) **Project Navigation Bar** – Provides a method to view the data that is stored in a Project file (see Section 2.5 for more information). This Navigation Bar is only displayed when a Project file (see section 4.5) is in focus.

2.2 – Main Menu

The main menu provides functionality to access all the features of KE. Figure 2.2 below is a screenshot of the Main Menu.

Figure 2.2 - Main Menu of KE

The following subsections describe the Main Menu items.

2.3 – File Menu

The *File* menu contains functionality that is required to work with files in the KE application. The screenshot below depicts the *File* menu.

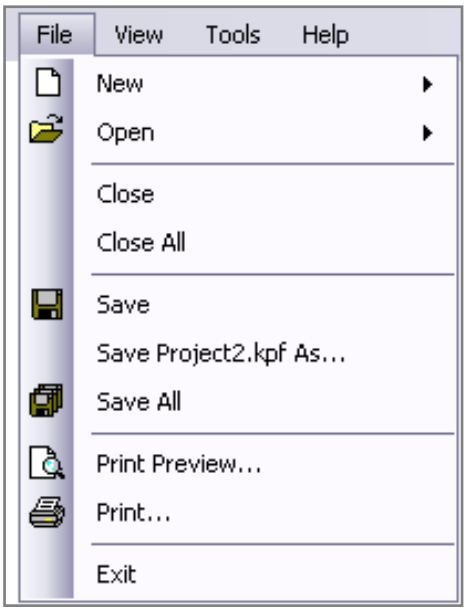


Figure 2.3 - Main Menu → File

The table below on this page provides an overview of the *File* menu items.

Table 2.1 – Overview of the File Menu Items

Menu Item	Description	Additional
New → Overlay…	Displays the Overlay Builder dialog	Figure 2.4 Section 4.4
New → Project…	Displays the Project Builder dialog	Figure 2.4 Section 4.5.1
New → Calibration…	Displays the Calibration Builder dialog	Figure 2.4 Section 4.5.2

Open → Measurement	Opens a Measurement file from disk	Figure 2.5 Section 4.3
Open → Overlay	Opens an Overlay file from disk	Figure 2.5 Section 4.4
Open → Project (including Van't Hoff)	Opens a Project file from disk	Figure 2.5 Section 4.5
Open → Calibration	Opens a Calibration file from disk	Figure 2.5 Section 4.5.2
Close	Closes the file in the visible tab	
Close All	Closes all open files	
Save	Saves the file in the visible tab	
Save As…	Saves a copy of the opened file with a new name	
Save All	Saves all open files	
Print Preview…	Opens the Print Builder Dialog	Section 4.7
Print…	Opens the Print Builder Dialog	Section 4.7
Exit	Exits the KE application	

The *File→New* menu item contains sub-menu items as shown in Figure 2.4. To access these items, click the *New* menu item and the sub items will appear.

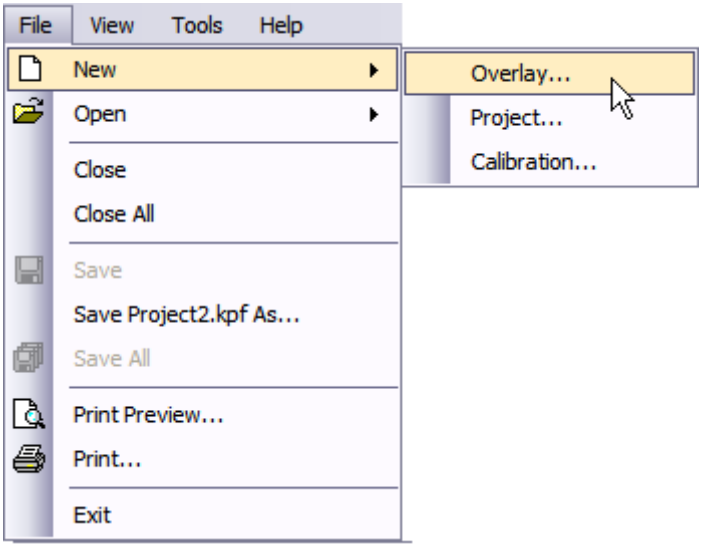


Figure 2.4 – File → New Menu

The *File→Open* menu item also contains sub-menu items (Figure 2.5).

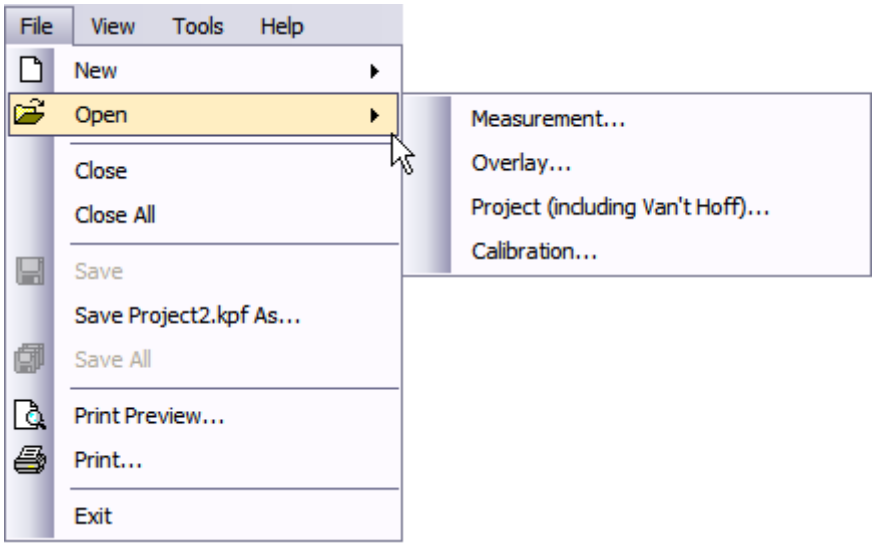


Figure 2.5 – File → Open menu

2.3.1 – View Menu

The *View* menu item contains functionality to show/hide various components of the KE application. The screenshot below shows the *View* menu.

The View menu contains different items depending on the file type being displayed. Figures 2.6, 2.7, and 2.8 are screenshots of the three different *View* menus for each file type.

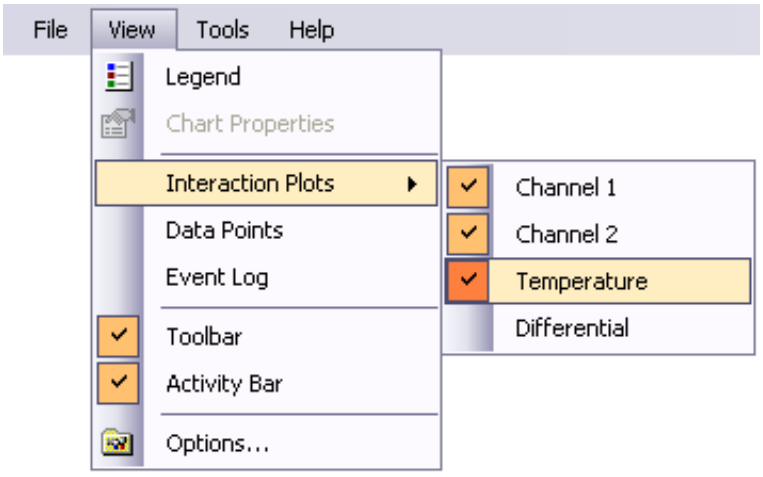


Figure 2.6 - Main Menu → View (Measurement file)

The table below provides an overview of the *View* menu items.

Table 2.2 – Overview of the View Menu Items

Menu Item	Description	Additional

Legend	Show/Hide the graph legend	Legend is not available for Analysis files
Chart Properties	Show the Chart Properties dialog	Measurement files do not have chart properties. See Section 4.6
Interaction Plots	Shows the data of the file in a graph. Measurement files have the option to show/hide plots	See Section 4.2.1
Data Points	Show a grid containing the files data points	See Section 4.2.2
Event Log / History / Summary	Depending on the file type this view shows additional data about the file	See Section 4.2.3
Toolbar	Show/Hide the Toolbar	See Section 2.3
Activity Bar	Show/Hide the Activity Bar	See Section 2.4
Options…	Opens a dialog to allow the user to reset the application settings	

The *View* menu for an Overlay file provides access to the Chart Properties as shown in the figure below.

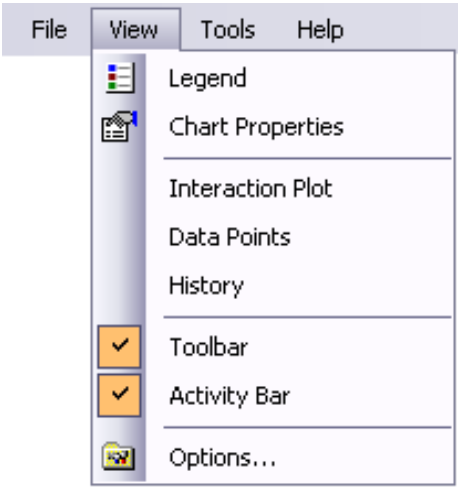


Figure 2.7 - Main Menu → View (Overlay file)

The *View* menu for an *Analysis* file.

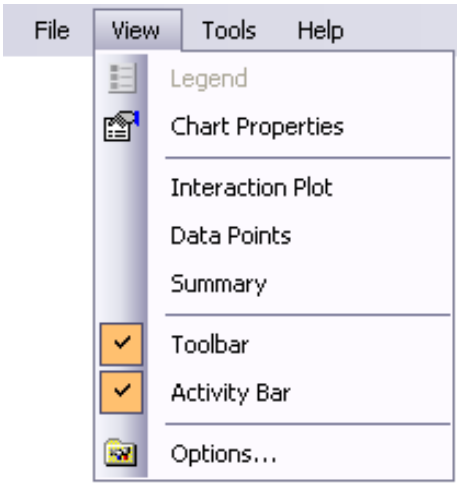


Figure 2.8 - Main Menu → View (Analysis)

2.3.2 – Tools Menu

The *Tools* menu provides access to functionality to analyze data. The screenshot below portrays the *Tools* menu.

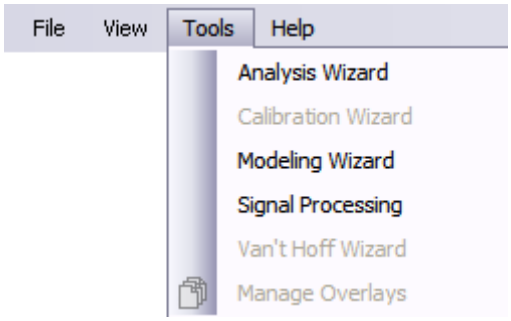


Figure 2.9 - Main Menu → Tools

The table below provides an overview of the *Tools* menu items.

Table 2.3 – Overview of the Tools Menu Items

Menu Item	Description	Additional
Analysis Wizard	Opens the Analysis Wizard dialog	See Section 5.3
Calibration Wizard	Opens the Calibration Wizard dialog	See Section 6
Modeling Wizard	Opens the Modeling Wizard dialog	See Section 5.1
Signal Processing	Opens the Signal Processing dialog	See Section 5.2
Van't Hoff Wizard	Opens the Van't Hoff Wizard dialog	See Section 7
Manage Overlays	Opens the Manage Overlay dialog	See Section 4.4.1

2.3.3 – Help Menu

The *Help* menu provides access to the KE's online help system. The screenshot below shows the *Help* menu.

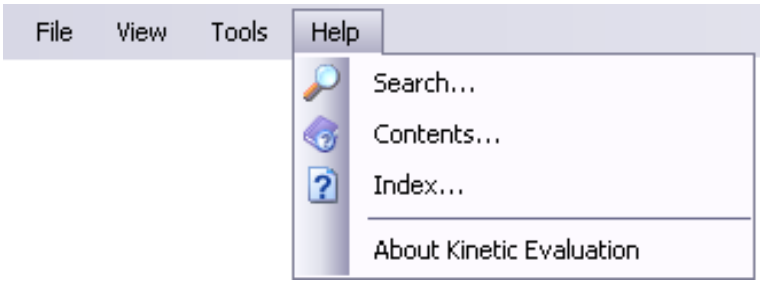


Figure 2.10 - Main Menu → Help

The table below provides an overview of the *Help* menu items.

Table 2.4 – Overview of the Help Menu Items

Menu Item	Description	Additional
Search…	Opens the online help system and shows the Search tab	
Contents…	Opens the online help system and shows the Contents tab	
Index…	Opens the online help system and shows the Index tab	
About Kinetic Evaluation	Opens the About dialog	Provides the KE 5.1 number

2.4 – Toolbar

The *Toolbar* contains buttons that launch KE functionality. The screenshot below illustrates the Toolbar.

Figure 2.11 – KE Toolbar

The following gives an overview of the items in the *Toolbar*.

Creates a new Overlay or Project file depending on which type the user chooses

Opens a Measurement, Overlay or Project file depending on which type the user chooses (see section 4.1.2)

Saves the file in the visible tab

Saves all the open files

The default mode of the graph. When moving the mouse pointed over the graph, the coordinates (x,y) will be displayed next to the mouse icon

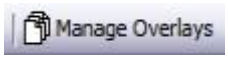
Sets the graph to Zoom-mode. Left-click and dragging the mouse pointer will zoom in on the selected region

Sets the graph to Select-mode. Left-click and dragging the mouse pointer will select an area of the graph

Shows/Hides the graph legend

Displays the Chart Properties dialog (see Section 4.6)

Creates a sub curve from the selected area of a measurement file (see Section 4.3.2)



Launches the Manage Overlays dialog (see Section 4.4.1)

2.5 – Activity Bar

The Activity Bar, as shown in Figure 2.12, is used to access commonly used KE functionality.
Activity Bar items are enabled or disabled based on the currently displayed file. If the displayed file does not support a certain action (for example you cannot run the Analysis Wizard on a measurement file) the item will be disabled.

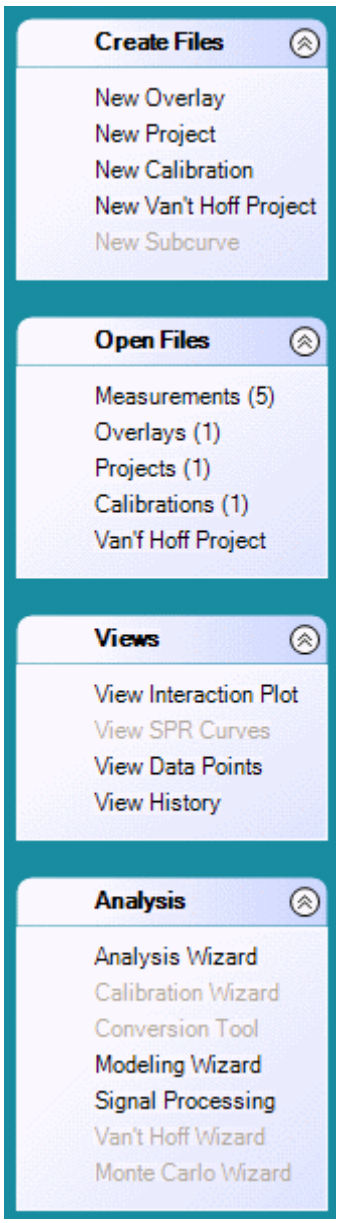


Figure 2.12 – KE Activity Bar

The table below provides an overview of the *Activity Bar*.

Table 2.5 – Activity Bar Items

Section	Action	Description
Create Files	New Overlay	Launches the Overlay Builder dialog
	New Project	Launches the Project Builder dialog
	New Calibration	Launches the Calibration Builder dialog
Open Files	Measurements	Opens a measurement file from disk. The number to the right of shows the number of opened measurement files.
	Overlays	Opens an Overlay file from disk. The number to the right shows the number of opened overlay files.
	Projects	Opens a Project file from disk. The number to the right shows the number of opened project files. Use this to open Van't Hoff Project files as well as normal Project files.
	Calibrations	Opens a Calibration file from disk. The number to the right shows the number of opened calibration files.
Views	View Interaction Plot	Shows the data from the file in a graph
	View Data Points	Shows the data points from the file in a grid
	View Event Log / History / Summary	Shows additional information about the file. Different files contain different information
Analysis	Analysis Wizard	Launches the Analysis Wizard
	Modeling Wizard	Launches the Modeling Wizard
	Calibration Wizard	Launches the Calibration Wizard
	Signal Processing	Launches the Signal Processing dialog
	Van't Hoff Wizard	Launches the Van't Hoff Wizard

Figure 2.13 shows the startup state of the Activity Bar. In this state the *New Overlay* action is disabled because a prerequisite is that there must be at least one measurement file open.

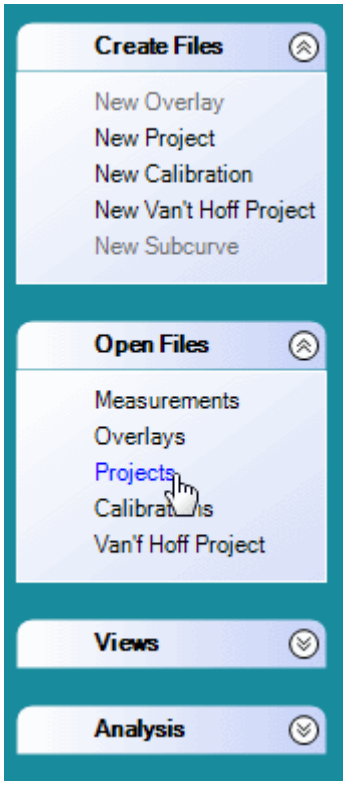


Figure 2.13 - Startup state of the Activity Bar

2.6 – Project Navigation Bar

Project files (section 4.5) can contain more than one file. Since there are multiple files there must be a way to navigate through them.

When a project file is in focus, the navigation bar will be shown on the right of the screen, as shown in Figure 2.14 below.

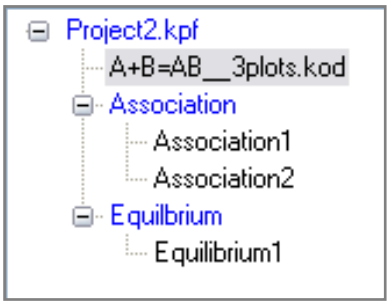


Figure 2.14 - Project Navigation Bar

The Navigation Bar provides links to the data within a project. These links in the Navigation Bar are grouped into categories.

The Overlay files will appear directly below the name of the project file. For normal projects, there is only one overlay. For Van't Hoff projects, there can be more than one overlay. In Figure 2.14, the name of the overlay file is *A+B=AB__3plots.kod*.

The next groups are the analyses that have been performed on the Overlay data. The analyses are grouped into *Association*, *Equilibrium* and *Dissociation* analysis. The project in Figure 2.14 contains two association analyses, one equilibrium and no dissociation analysis.

2.6.1 – Editing Navigation Bar Items

Editing the items in the Project Navigation Bar can be performed by right-clicking on the item to display a pop-up menu (as shown in Figure 2.15 below).
To rename an item, right-click on the item and select **Rename**. The item's name will be highlighted and the new name can be entered.

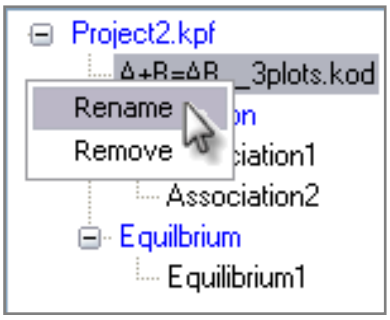


Figure 2.15 - Renaming a Project Component

2.6.2 – Removing Navigation Bar Items

It is possible to remove an item from a Project via the navigation bar. To remove an analysis (it is not possible to remove the overlay) right-click on the analysis and select **Remove** from the pop-up menu (see Figure 2.16). A confirmation will be required to complete the removal.

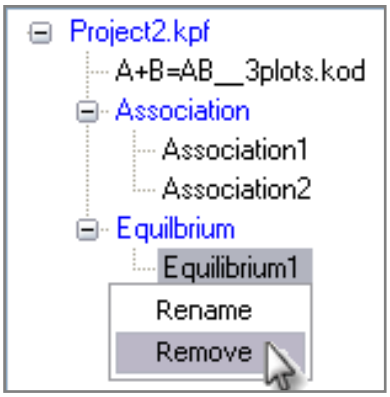


Figure 2.16 - Removing an Analysis from a Project

Note

It is not possible to remove the Overlay file from a Project.

This is done in order to guarantee that the analyses in the Project are always linked to the correct Overlay file.

2.6.3 – Reanalyzing an Analysis

To reopen one of the analyses in a Project, double-click on the analysis name and it will be reloaded in the Analysis Wizard (see section 5.3).

3 – Getting Started with the KE Workflow

This section of the user manual gives an overview of a typical session using the KE application.

The following flowchart describes the workflow in this section.

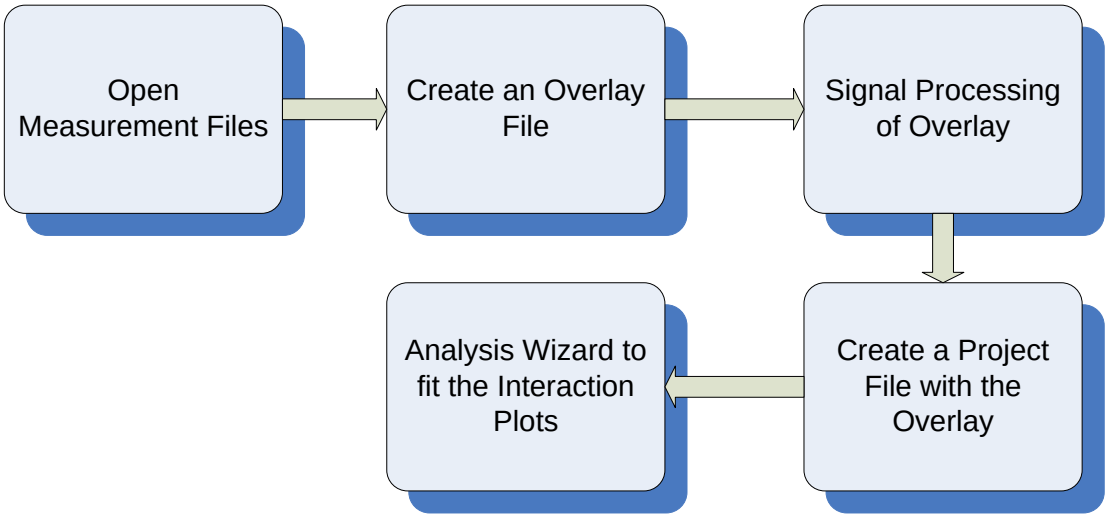


Figure 3.1 - Standard KE Workflow

3.1 – Opening Measurement Files

In this step we will be loading SPR measurement files into the KE application.

a.) Click on the **Measurements** item in the *Activity Bar* as shown in the screenshot below:

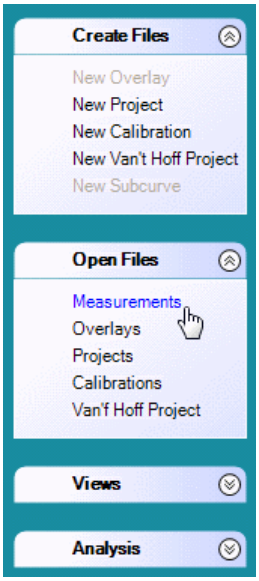


Figure 3.2 - Selecting the Measurements item

b.) The measurement files for this example are located in the**…/Autolab SPR/Data** folder. On the Open dialog change the *Files of type* to: ***Data files (*.ibo)***. Select the following data files to open: **KIN101.ibo, KIN102.ibo, KIN103.ibo, KIN104.ibo, KIN105.ibo**, and click **Open**.

c.) The five files that were opened into the KE application are shown in the screenshot below. Notice that there is now the number "5" beside the *Measurements* item to indicate the number of files opened.

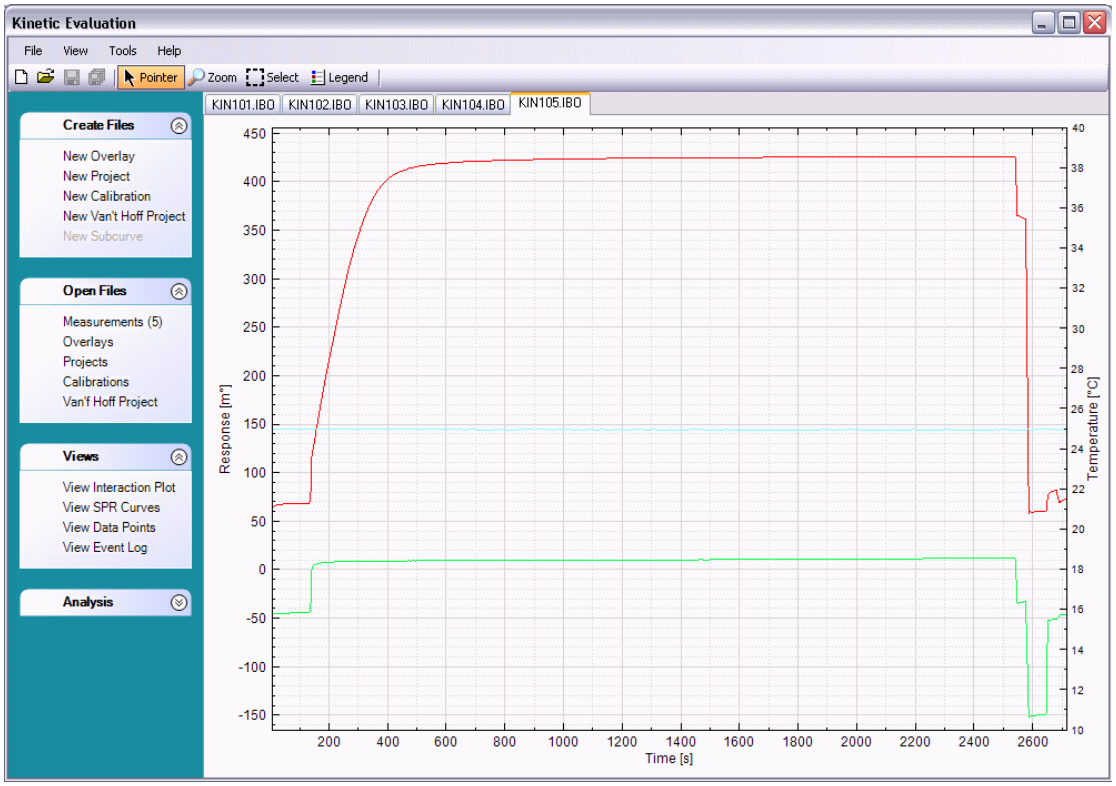


Figure 3.3 - Data Loaded in the KE Application

3.2 – Creating an Overlay

In this step the five measurement files that were opened will be used to create a new overlay file.

a.) Click the **New Overlay** item in the *Activity Bar* as shown in the screenshot below.

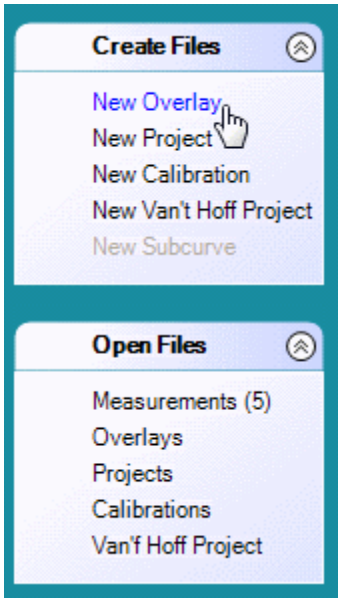


Figure 3.4 - Selecting the New Overlay item

b.) Example of a new overlay using the Channel 1 data from each of the measurement files. Select the Channel 1 checkboxes for each measurement as shown in the screenshot of the overlay builder below. Once all the Channel 1's have been selected click the **OK** button.

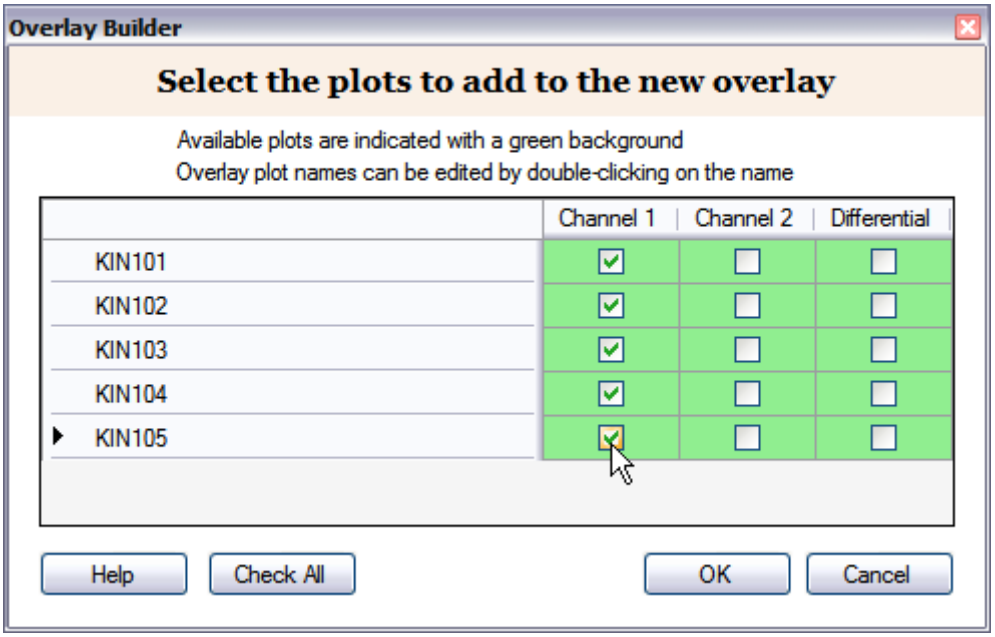


Figure 3.5 - Selecting the Plots for the New Overlay

c.) A new overlay file will be created and added. Notice in the screenshot below that there is now a "1" beside the *Overlays* item in the Activity Bar.

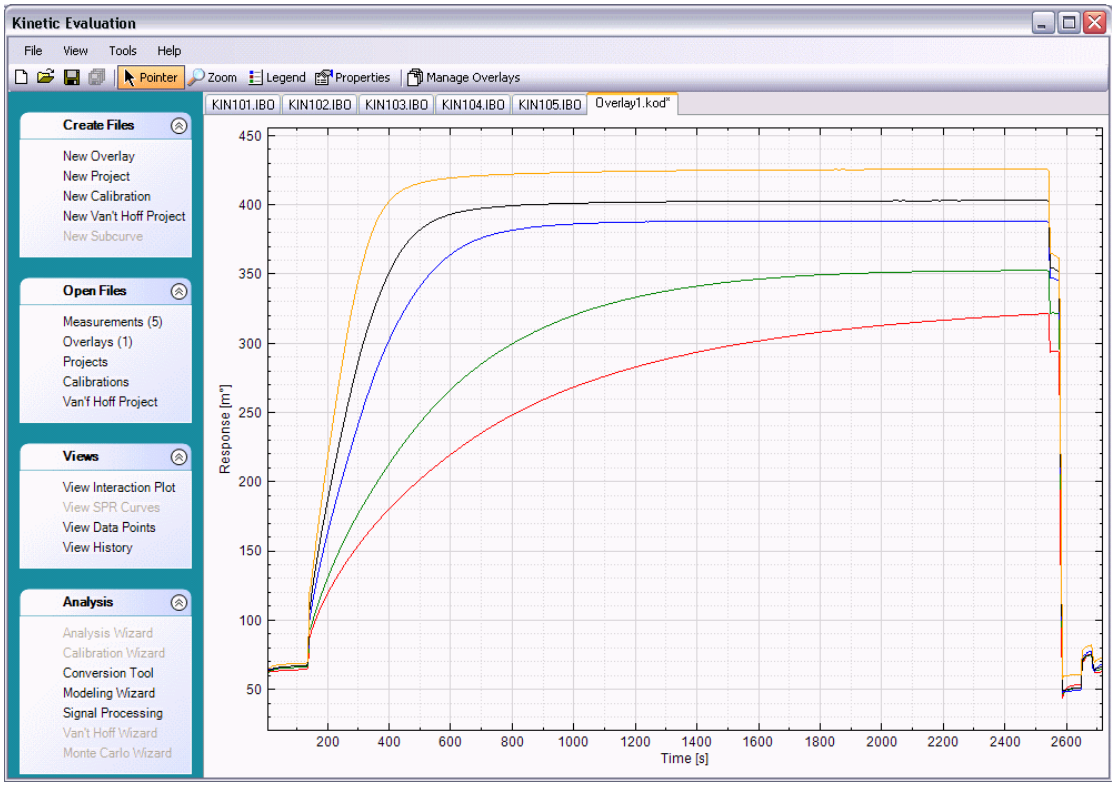


Figure 3.6 - The New Overlay

d.) You can save this overlay to disk by clicking the *Save* Toolbar icon. 

3.3 – Perform Signal Processing

The overlay created contains an offset. This can be removed by using the Signal Process feature in KE.

a.) To open the Signal Processing dialog, click the **Signal Processing** item in the Activity Bar as shown in the figure below.

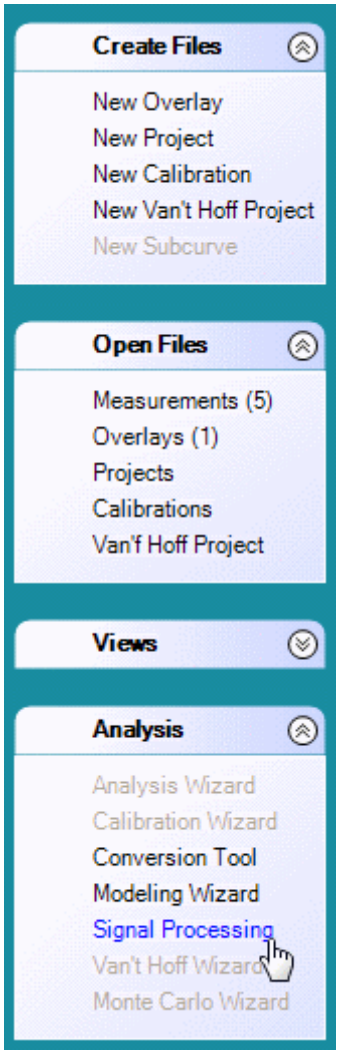


Figure 3.7 - Selecting the Signal Processing item

b.) The Signal Processing routine to remove the baseline offset is called *Zero at Average.* Select this action from the list as shown in the figure below.

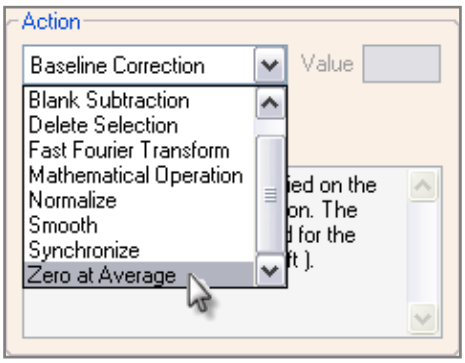


Figure 3.8 - Selecting the Zero at Average Option

c.) The Zero at Average will take the average of the selected area and subtract this amount from the entire plot. Select a region in the flat part of the overlay using

the *Selection* tool and click the Apply button. The screenshot below shows the selection of the area that will be averaged.

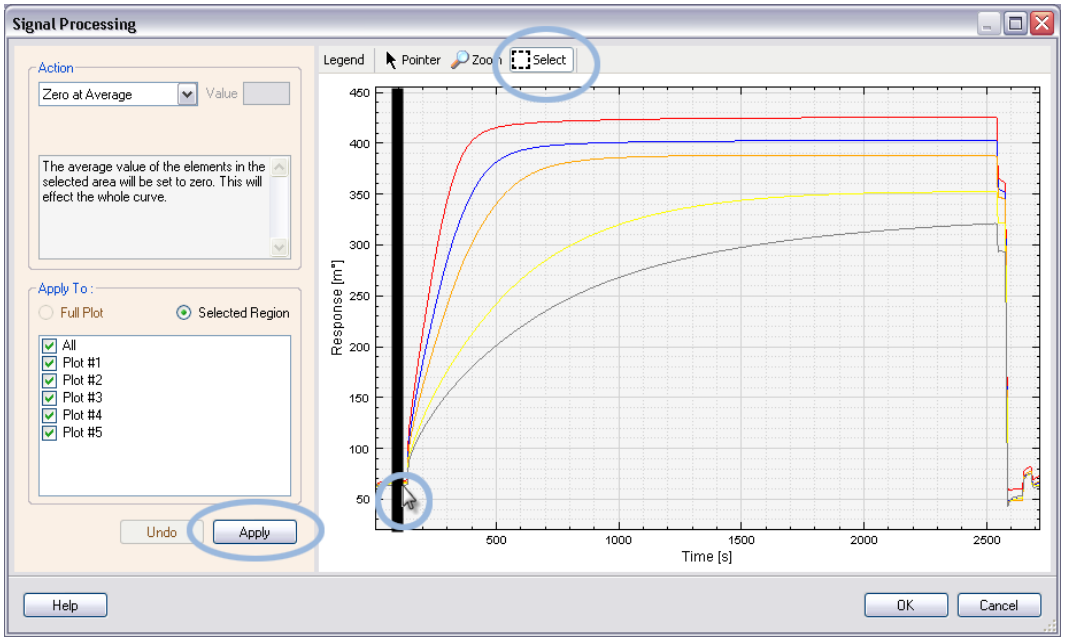


Figure 3.9 - Selecting a Region for the Average to be calculated

d.) The Overlay's baseline should be now zero, as shown in the figure below. Click **OK** to close the Signal Processing dialog.

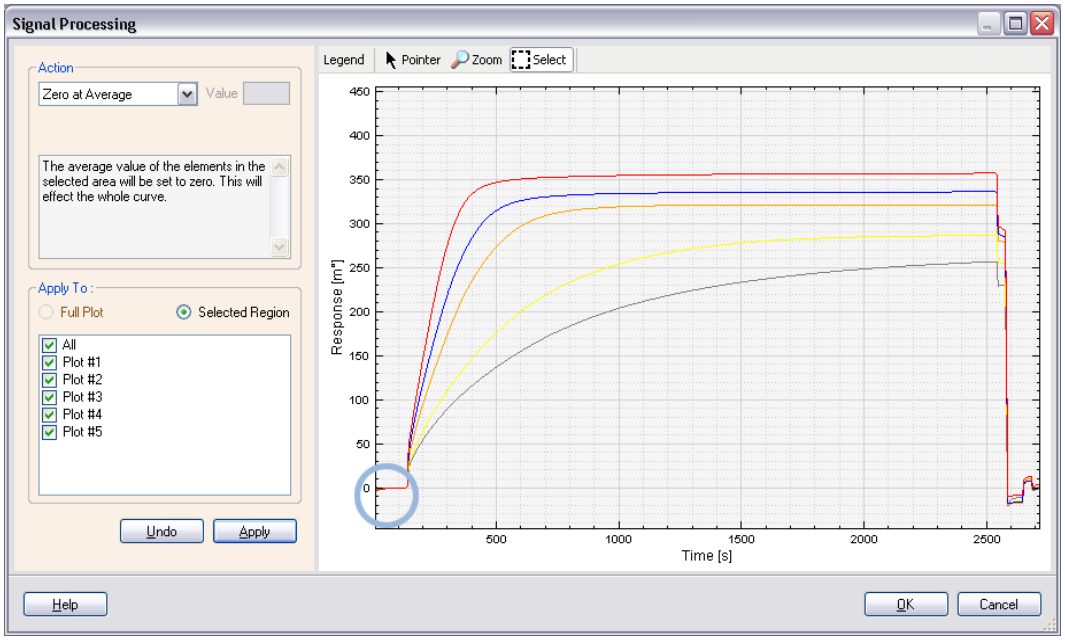


Figure 3.10 - The Corrected Baseline of the Overlay

3.4 – Create a Project File

In this step the Overlay file will be added to a new Project file.

a.) Click the **New Project** item in the Activity Bar as shown in the screenshot below.

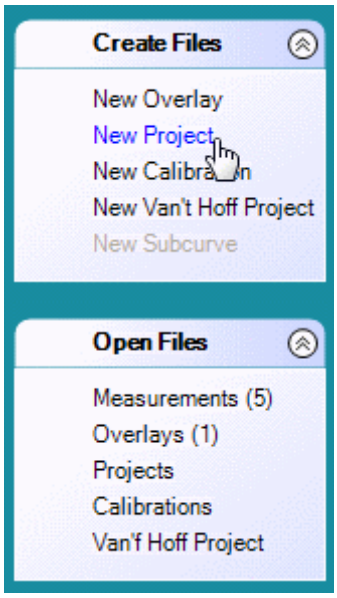


Figure 3.11 - Selecting the New Project item

b.) Select your Overlay in the dropdown box in the *Project Builder* dialog as shown in Figure 3.12. Press **OK** to create the new Project.

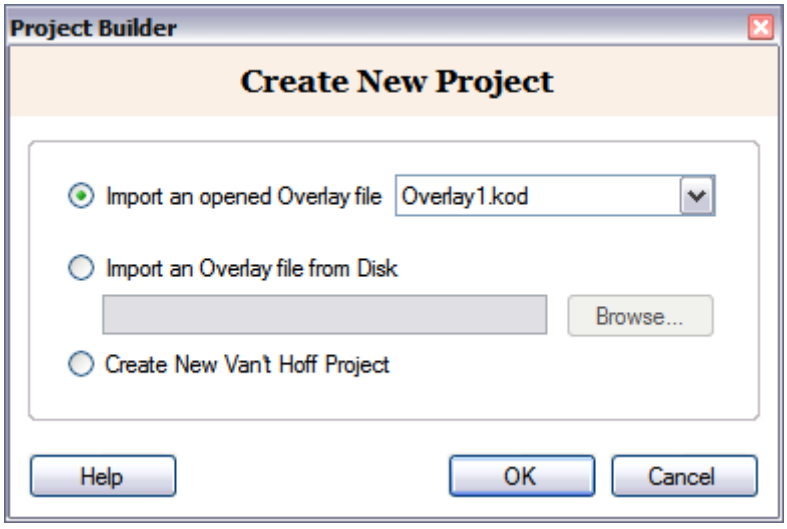


Figure 3.12 - Creating a new Project with the Project Builder

c.) The new Project will be created and displayed in the KE application as shown in Figure 3.13. A copy of the Overlay has been added to the Project. Any changes to the Overlay in the Project file will not affect the original Overlay file.

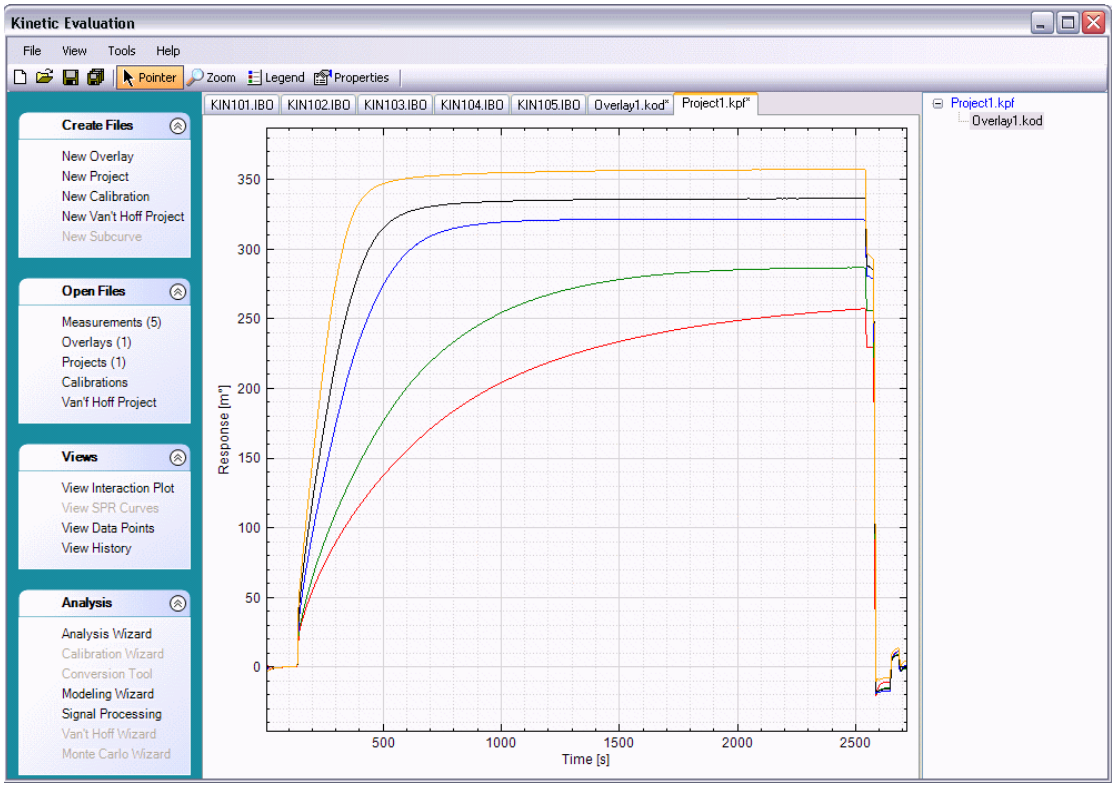


Figure 3.13 - The New Project File

3.5 – Using the Analysis Wizard to Fit the Data

Now that the Project file has been created we can use the *Analysis Wizard* to fit the data.

For this Workflow example we will be performing an *Association* analysis using the *Monophasic Integrated Rate Equation.*

a.) To launch the Analysis Wizard, select the **Analysis Wizard** item from the Activity Bar as shown in the figure below.

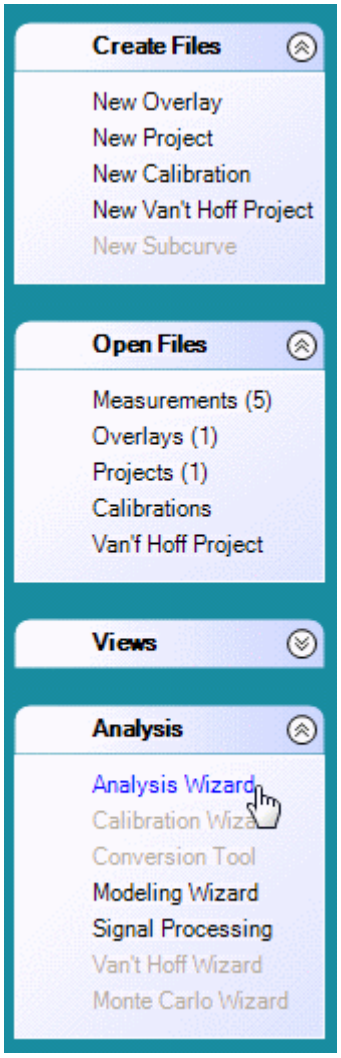


Figure 3.14 - Selecting the Analysis Wizard

b.) The Analysis Wizard is a step-by-step process that assists the user in analyzing data. The figure below gives a picture of **Step 1** which is displayed when the wizard is opened. Selected the **Association** option and click **Next**.

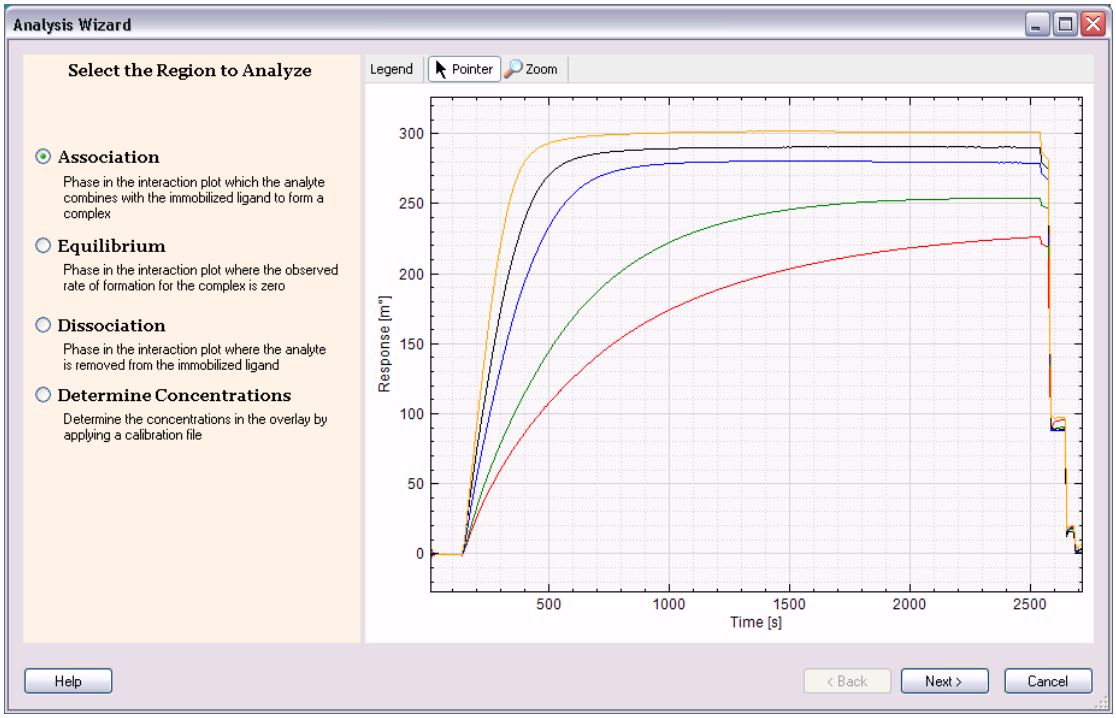


Figure 3.15 - Analysis Wizard

c.) **Step 2 of 7** - In this step the concentration for each of the measurements is entered. The screenshot below shows the values that are to be entered. Fill out the concentrations and click **Next**. Refer to Section 6 for a discussion of using Calibration Files to set the concentrations.

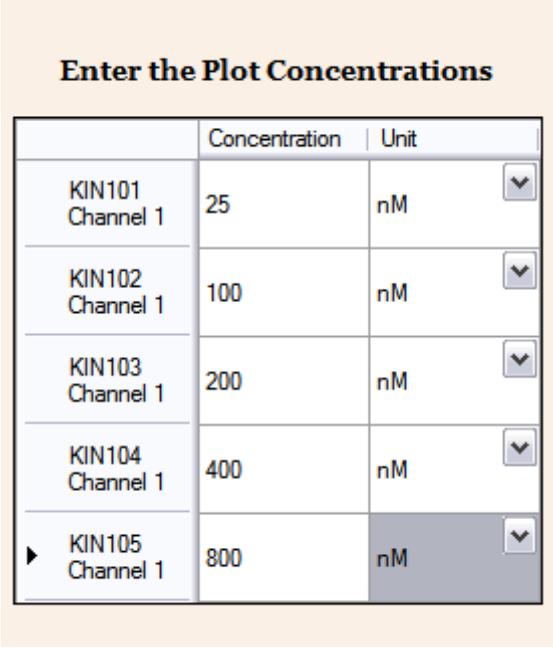


Figure 3.16 - The Concentrations for the Measurements

d.) **Step 3 of 7** – Selecting the Analysis Region. For this analysis all plots are going to use the same start and end times. Ensure that the **Same Region for all Plots** option if selected. Now select any one of the start times with a single-click and then select the **Line** tool as shown in the figure below.

Figure 3.17 – Select the Line Tool to Specify the Region

With the Line Tool selected, mouse over the graph to guide the line to the desired start point and then right-click to set the start time. The screenshot below illustrates the start time selection. Changes made via the Line Tool are reflected in the table, and vice-versa. The start and end times are shown with diamond arrows on the analysis curves.

Once the start point has been selected (the default end point can be used for this example) click the **Next** button.

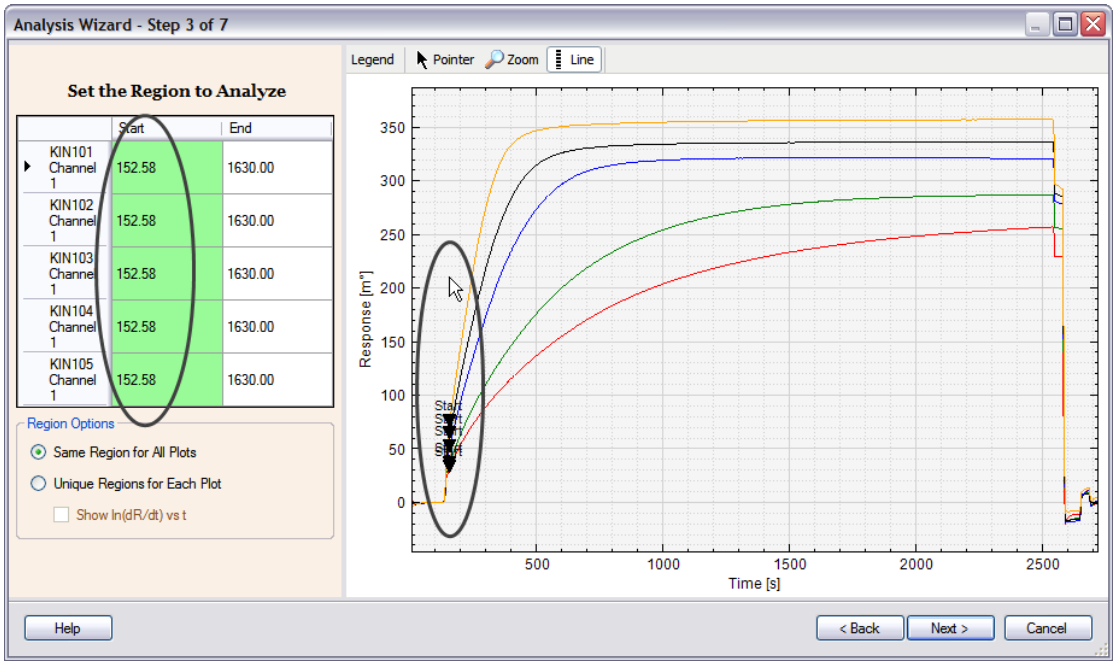


Figure 3.18 – Using the Line Tool to Select the Start Point

e.) **Step 4 of 7** – The graph will now show only the region that was selected in the previous step (see Figure 3.19). This example will be using the **Integrated Rate Equation method**. Make sure that this option is selected and click **Next**.

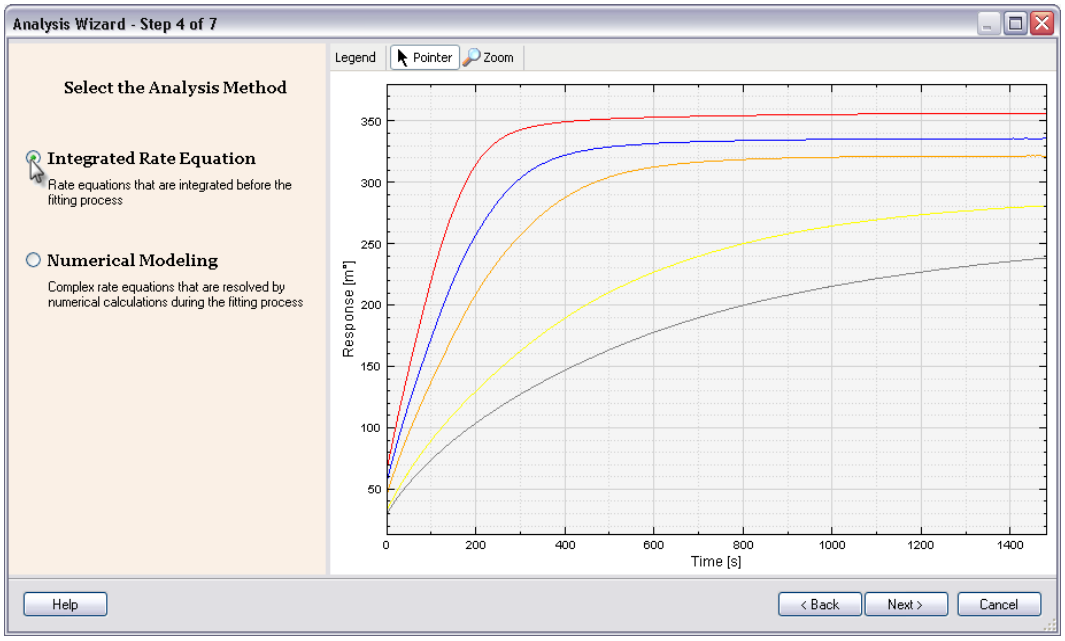


Figure 3.19 – The Graph now shows only the selected region

f.) **Step 5 of 7** – This example will be using the **Monophasic** Integrated Rate Equation. Ensure that this method is selected, as shown in the figure below, and click **Next**.

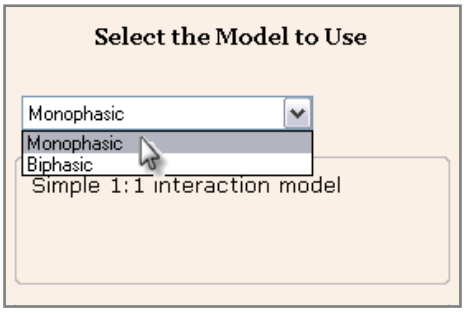


Figure 3.20 – Selecting the Monophasic Model

g.) **Step 6 of 7** – In this step of the Analysis Wizard the fitting of the model to the experimental data will take place.

It is strongly suggested that the initial fitting parameters be tested before starting the fit procedure. To test the parameters, press the **Test** button as shown in the figure below

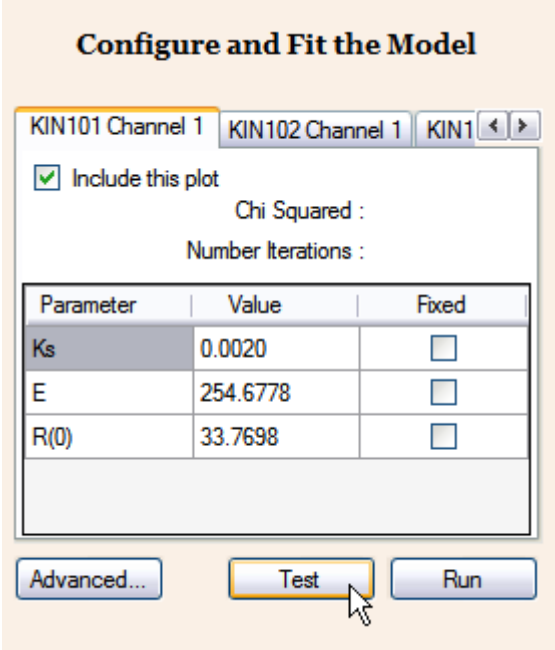


Figure 3.21 – Testing the Initial Fit Parameters

The initial fit parameters are acceptable if the model plots (green lines) are of the same order of magnitude as the experimental data. Figure 3.22 shows the initial fit plot for this example (which is acceptable).

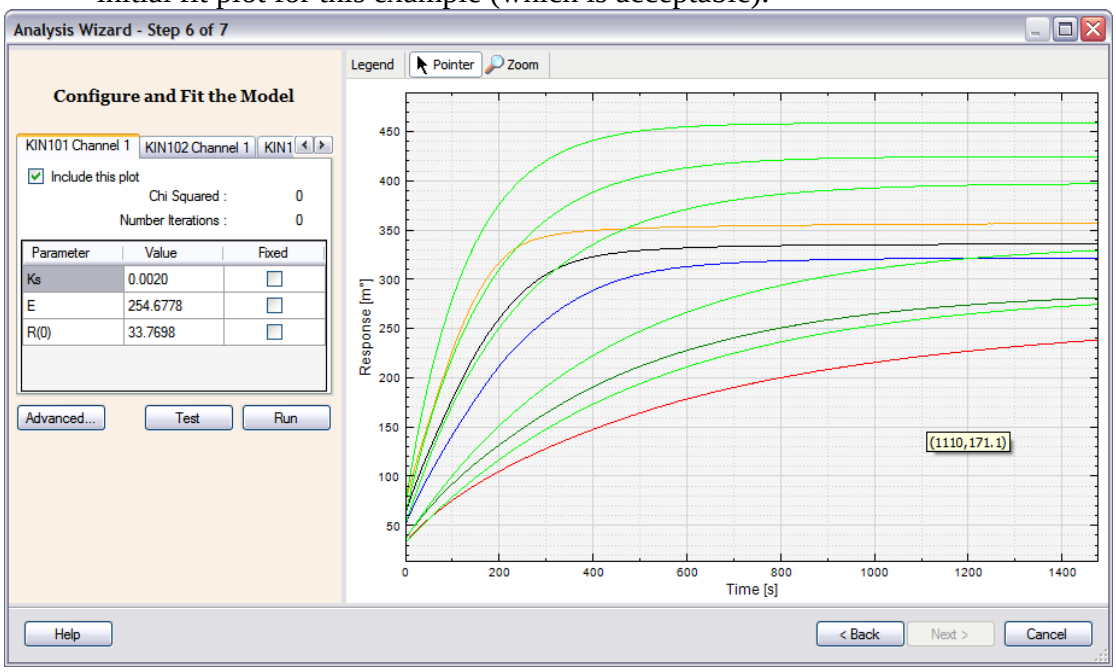


Figure 3.22 – Initial Plot of the Fit Parameters

Once the initial fit parameters have been tested, the fitting of the data can be performed by clicking the **Run** button.

During the fitting procedure the plot that is currently being fit will be shown in the tab on the left of the screen. The green model lines for the plots will be

redrawn upon each iteration of the fitting routine that improves the Chi^2 of the fit. Figure 3.23 depicts the fitting of Plot#2. A more detailed overview of the fitting procedure can be found in section 5.3.

Once the fit procedure has ended, click **Next** to proceed to the summary.

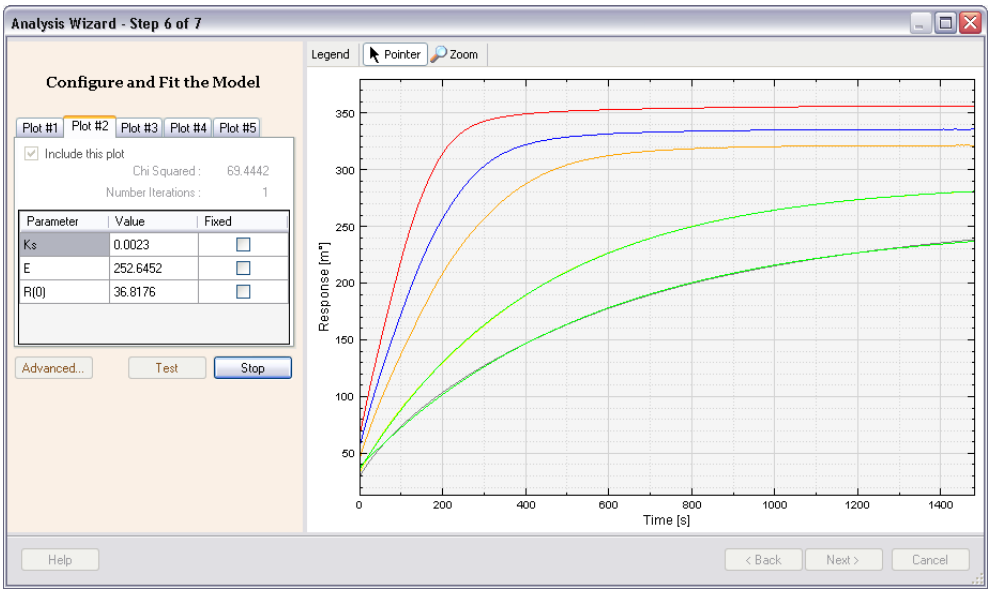


Figure 3.23 – Running the Fitting Procedure

h.) **Step 7 of 7** – The final step of the Analysis Wizard is the summary of the performed fit. The summary, as shown in the figure below, shows the general configurations that were used and the final parameter values for each plot.

This data can be copied to the clipboard by clicking the **Copy to Clipboard** button.

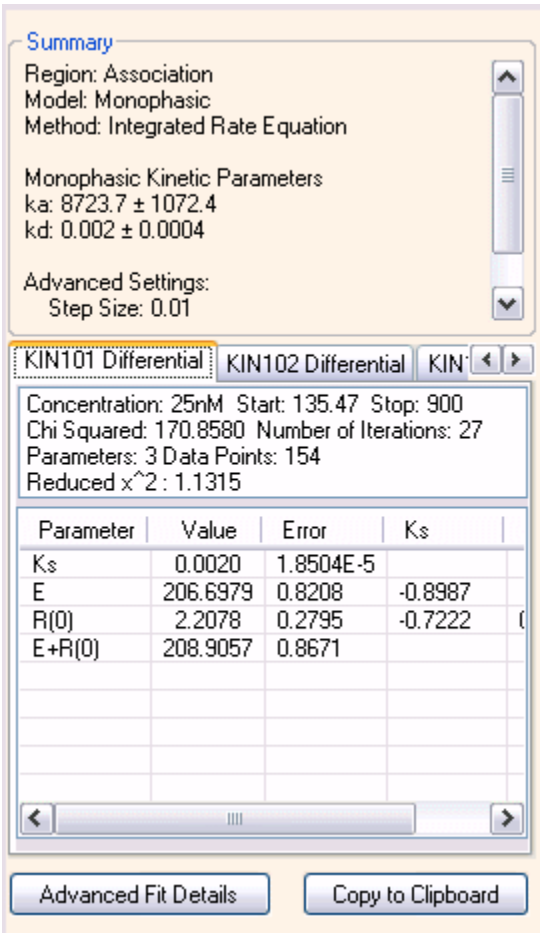


Figure 3.24 – Summary of the Fit

i.) The Analysis Wizard is now complete. Press the **Finish** button to add this analysis to the project.

j.) The analysis will be added to the Project and will be displayed in the user interface. Notice that in the Project navigation bar (see left side of the figure below) the Analysis has been added. To display the Overlay plot again right-click on the Overlay name.

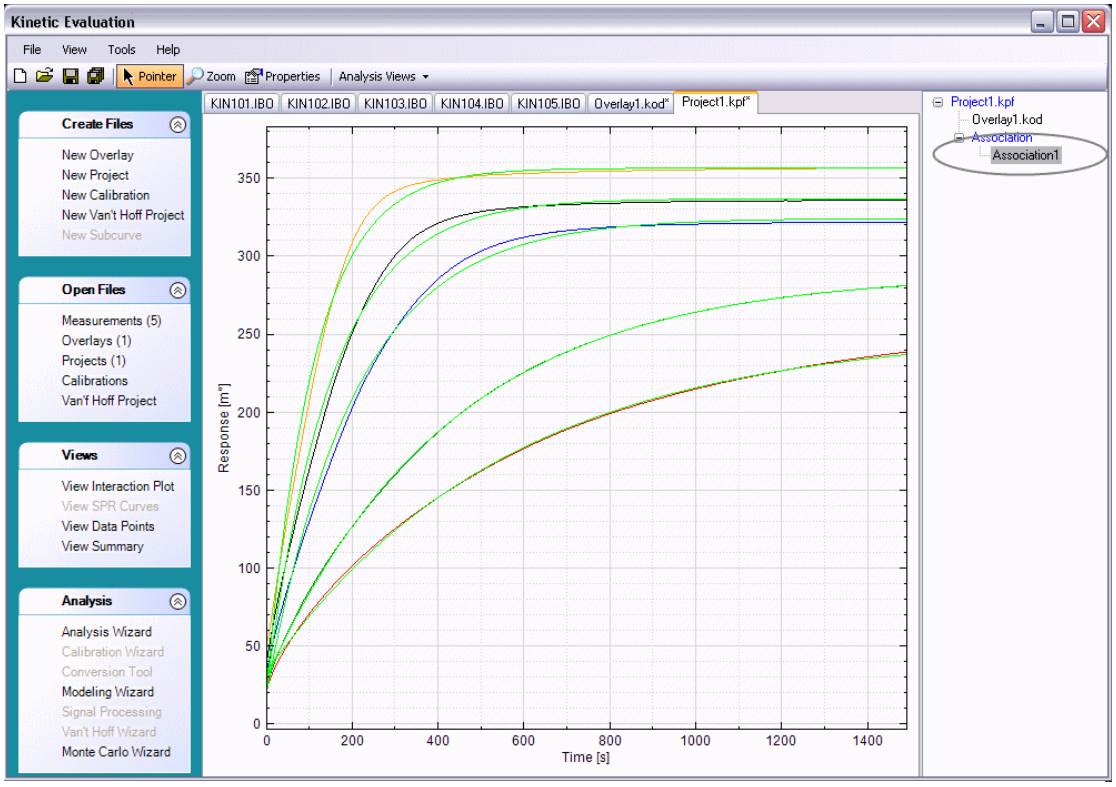


Figure 3.25 – Analysis has been added to the Project

This concludes the KE workflow demonstration. The remaining sections in the manual will go into more detail about everything that was performed in this example.

4 – Working with Data

This section of the manual provides an overview of the different file types that the KE application supports.

4.1 – Opening Files

There are three methods to open file in KE. The following subsections give an example of each.

4.1.1 – Opening a File from the Main Menu

From the Main Menu, *Measurement*, *Overlay*, *Project* and *Calibration* files can be opened. Figure 4.1 shows the menu items.

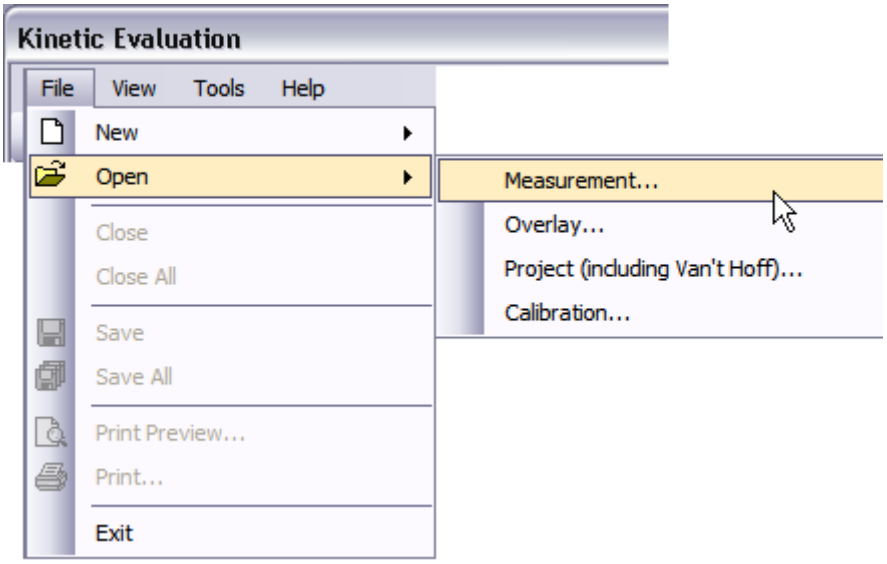


Figure 4.1 - Opening a Measurement file from the Main Menu

4.1.2 – Opening a File from the Toolbar

Files can be opened from the Toolbar by clicking the open icon as shown in Figure 4.2.

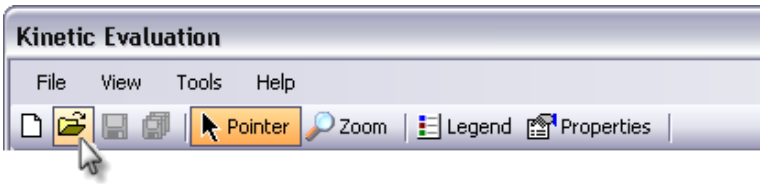


Figure 4.2 - Opening a File from the Toolbar

Different types of files can be opened (*Measurement*, *Overlay*, *Project and Calibration* files). Upon clicking the open icon in the Toolbar, a selection for what type of file to open will be made (as shown in Figure 4.3).

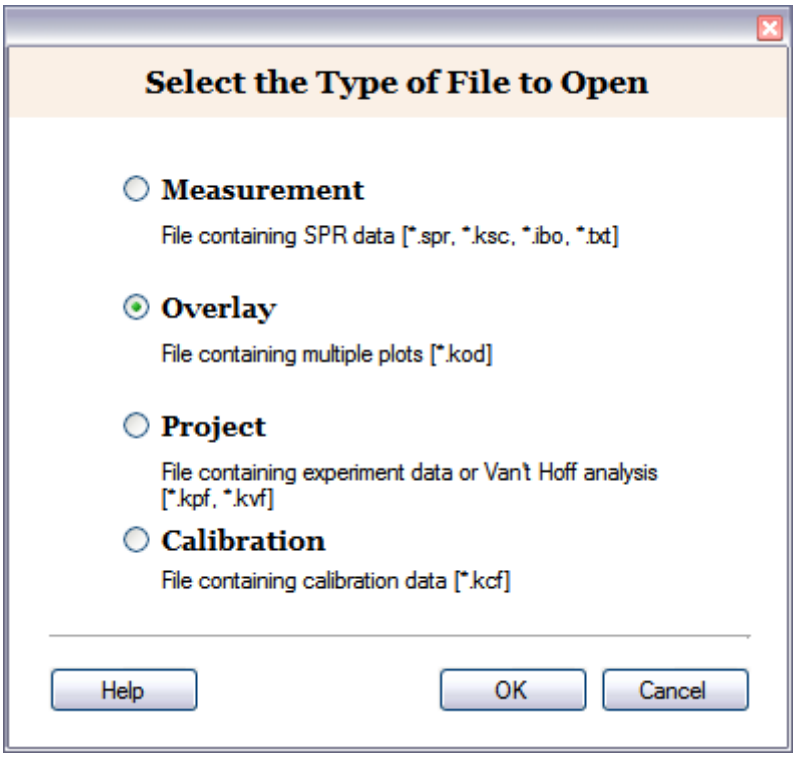


Figure 4.3 - Opening an Overlay file from the Toolbar

4.1.3 – Opening a File from the Activity Bar

The third method to open data files in KE is to click on the file type in the Activity Bar.

Figure 4.4 depicts the action of opening a Project file.

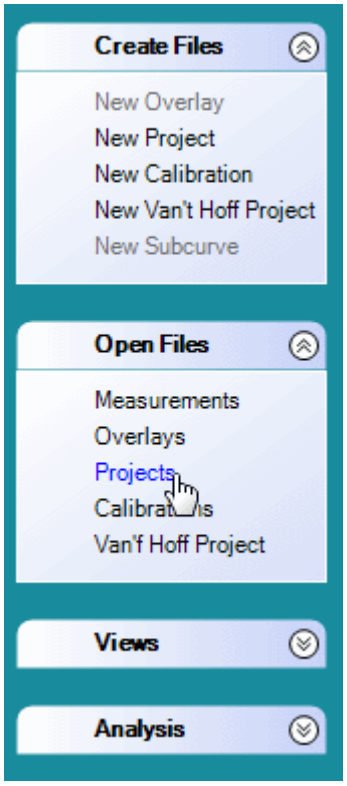


Figure 4.4 - Opening a Project file from the Activity Bar

4.2 – Viewing Files

The KE application allows the user to view all files (*Measurement*, *Overlay*, *Project* and *Calibration*) using three different views.

Changing the current view for a file can be performed via the Main Menu (see Figure 4.5) or by clicking on the desired view from the Activity Bar as shown in Figure 4.6.

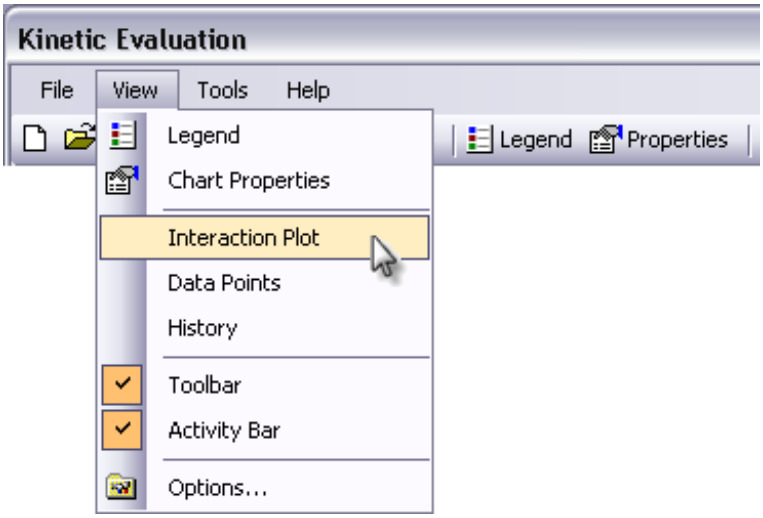


Figure 4.5 - Changing the View Mode via the Main Menu

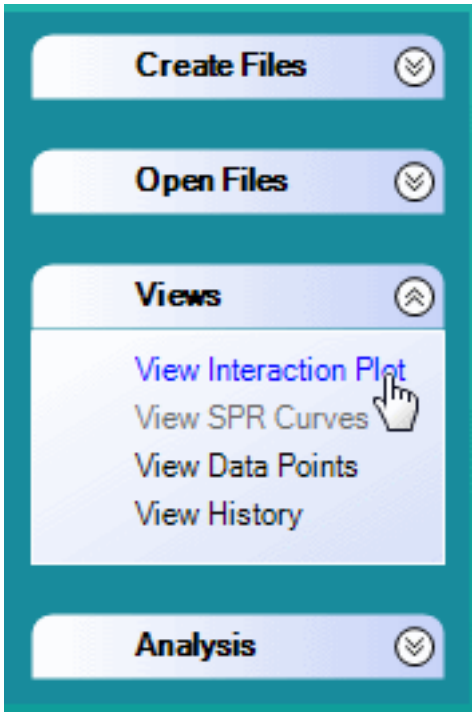


Figure 4.6 - Changing the View Mode via the Activity Bar

The following subsections give an overview of each of the views

4.2.1 – Interaction Plot View

The *Interaction Plot View* is the data plotted in a graph. This view is the default view for all file types.

Measurement files have the option to show/hide the various plots that they contain. This functionality is available via the Main Menu and is shown in Figure 4.7. By default, all components of a Measurement file are displayed (*Channel 1*, *Channel 2*, and *Temperature* or *Differential* data on the second Y Axis).

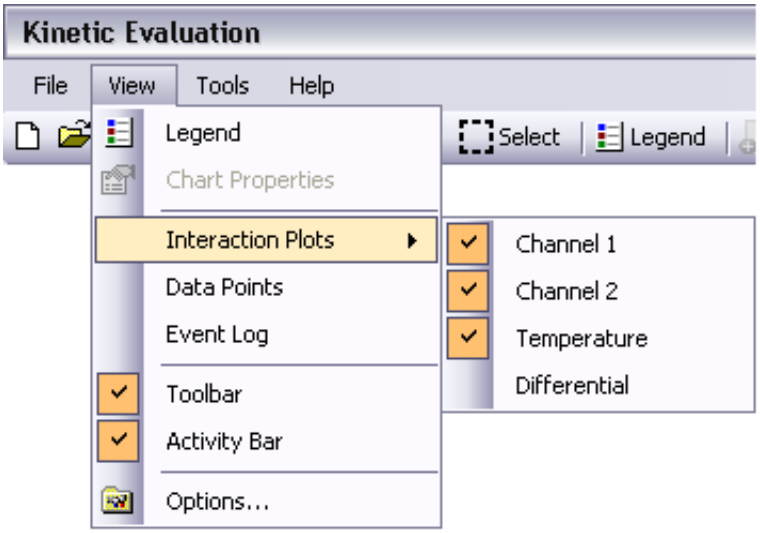


Figure 4.7 - Setting the visibility of the Measurement Plots*

4.2.2 – Data Point View

The *Data Point View* displays the data points of the plots in a grid.

Figure 4.8 shows the Data Point View of an Overlay file.

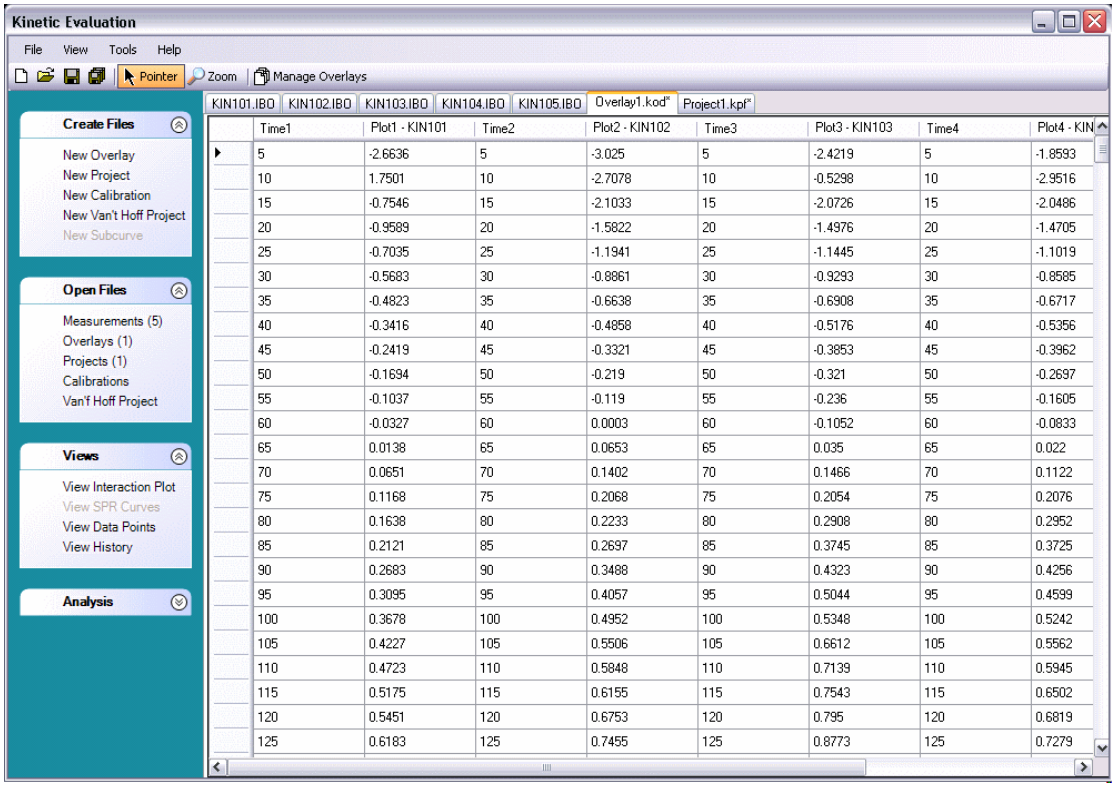


Figure 4.8 - Viewing the data points of an overlay file

* For users of the Autolab SPR - Springle software the Channel 2 and Differential plots are not available.

It is possible to copy this data to another application via the clipboard. To copy the data, follow the steps below.

a.) Click on the columns that you wish to copy (or click the square in the top-left corner to select all the data, as shown in Figure 4.9).

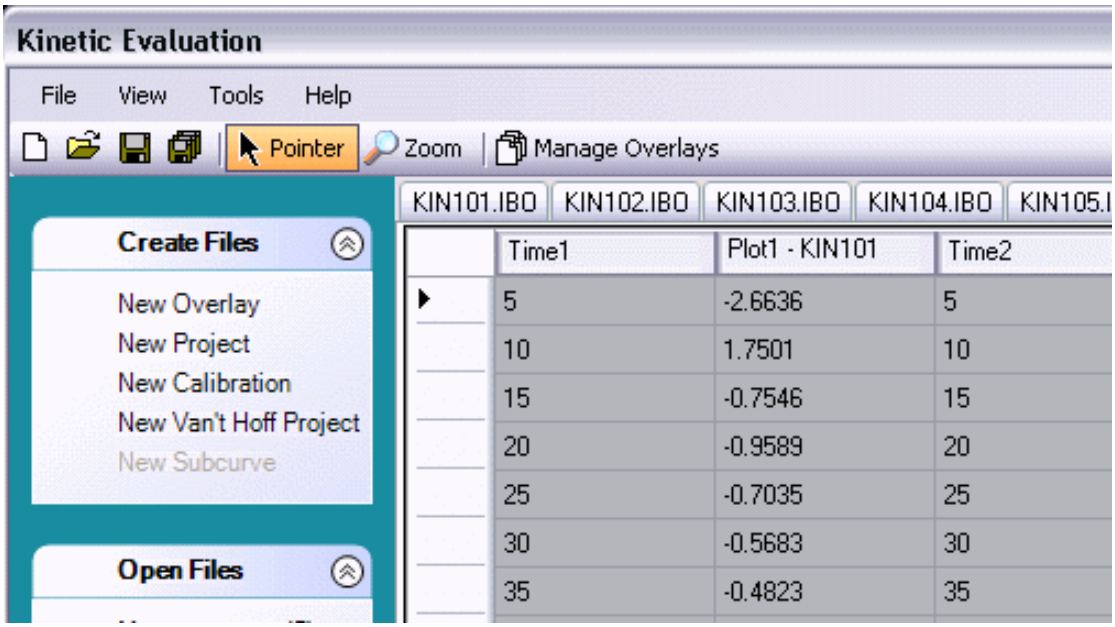


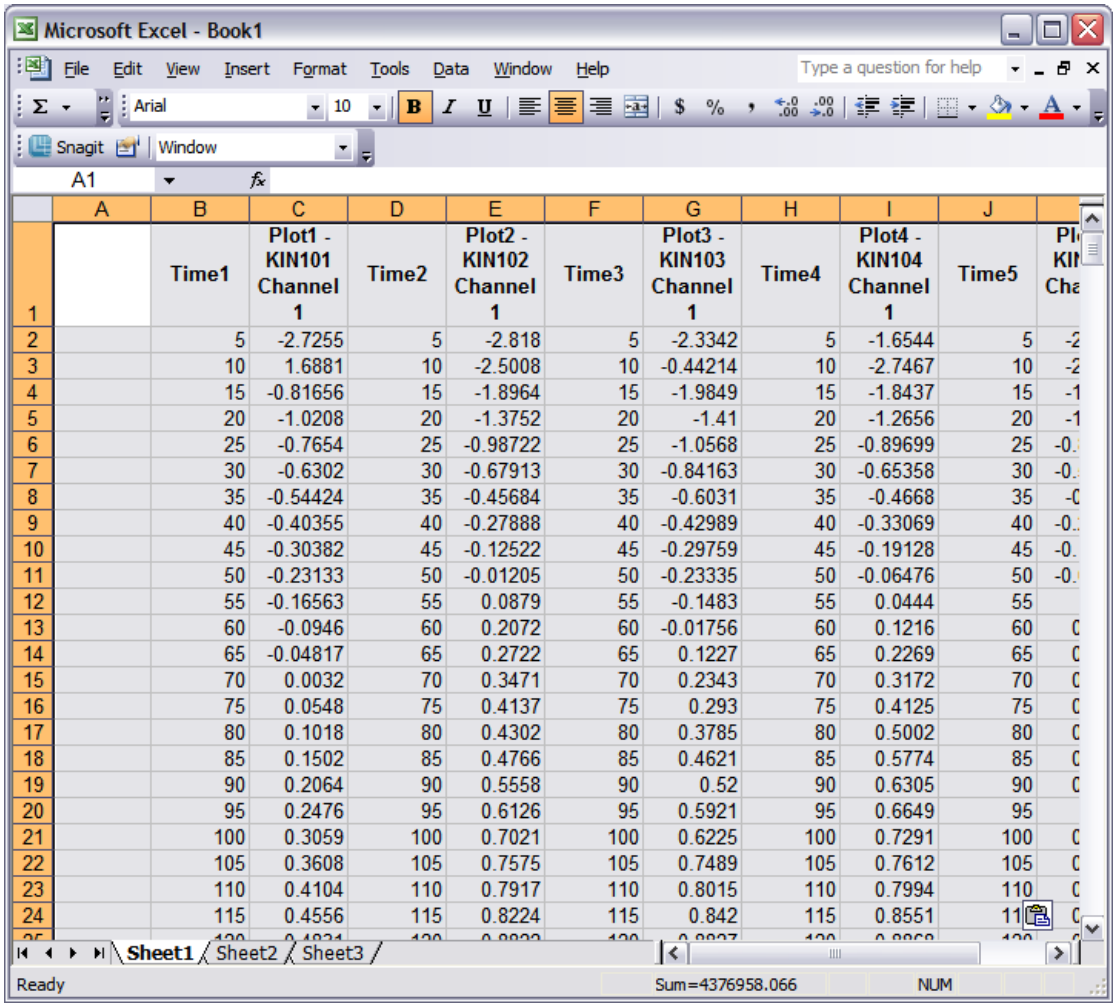
Figure 4.9 - Selecting all the data points

b.) Press **CTRL-C** on the keyboard to copy the data

c.) Open the application that you wish to copy the data to (Microsoft Excel is being used for this example).

d.) Press **CTRL-V** on the keyboard or click **File→Edit→Paste** from Excel's Main Menu.

e.) The data points from Kinetic Evaluation will now be copied into Excel as shown in the figure below.



Microsoft Excel - Book1

File Edit View Insert Format Tools Data Window Help — Type a question for help

Arial 10 B I U

Snagit Window

A1

	A	B	C	D	E	F	G	H	I	J	
1		Time1	Plot1 - KIN101 Channel 1	Time2	Plot2 - KIN102 Channel 1	Time3	Plot3 - KIN103 Channel 1	Time4	Plot4 - KIN104 Channel 1	Time5	Pl KI Cha
2		5	-2.7255	5	-2.818	5	-2.3342	5	-1.6544	5	-2
3		10	1.6881	10	-2.5008	10	-0.44214	10	-2.7467	10	-2
4		15	-0.81656	15	-1.8964	15	-1.9849	15	-1.8437	15	-1
5		20	-1.0208	20	-1.3752	20	-1.41	20	-1.2656	20	-1
6		25	-0.7654	25	-0.98722	25	-1.0568	25	-0.89699	25	-0.
7		30	-0.6302	30	-0.67913	30	-0.84163	30	-0.65358	30	-0.
8		35	-0.54424	35	-0.45684	35	-0.6031	35	-0.4668	35	-0
9		40	-0.40355	40	-0.27888	40	-0.42989	40	-0.33069	40	-0.
10		45	-0.30382	45	-0.12522	45	-0.29759	45	-0.19128	45	-0.
11		50	-0.23133	50	-0.01205	50	-0.23335	50	-0.06476	50	-0.
12		55	-0.16563	55	0.0879	55	-0.1483	55	0.0444	55	
13		60	-0.0946	60	0.2072	60	-0.01756	60	0.1216	60	0
14		65	-0.04817	65	0.2722	65	0.1227	65	0.2269	65	0
15		70	0.0032	70	0.3471	70	0.2343	70	0.3172	70	0
16		75	0.0548	75	0.4137	75	0.293	75	0.4125	75	0
17		80	0.1018	80	0.4302	80	0.3785	80	0.5002	80	0
18		85	0.1502	85	0.4766	85	0.4621	85	0.5774	85	0
19		90	0.2064	90	0.5558	90	0.52	90	0.6305	90	0
20		95	0.2476	95	0.6126	95	0.5921	95	0.6649	95	
21		100	0.3059	100	0.7021	100	0.6225	100	0.7291	100	0
22		105	0.3608	105	0.7575	105	0.7489	105	0.7612	105	0
23		110	0.4104	110	0.7917	110	0.8015	110	0.7994	110	0
24		115	0.4556	115	0.8224	115	0.842	115	0.8551	11	0

Sheet1 Sheet2 Sheet3

Ready Sum=4376958.066 NUM

Figure 4.10 - Overlay data copied in a Microsoft Excel worksheet

4.2.3 – Event Log / History / Summary View

The different file types in Kinetic Evaluation contain different details that can be displayed.

Event Log – Measurement Files

The Event Log, in Measurement files, is generated during the collection of data the Autolab SPR - Data Acquisition software. This data is read-only in the KE application. If the measurement file does not contain Event Log information then the grid will be empty.

The following screenshot shows the *Event Log* for a measurement.

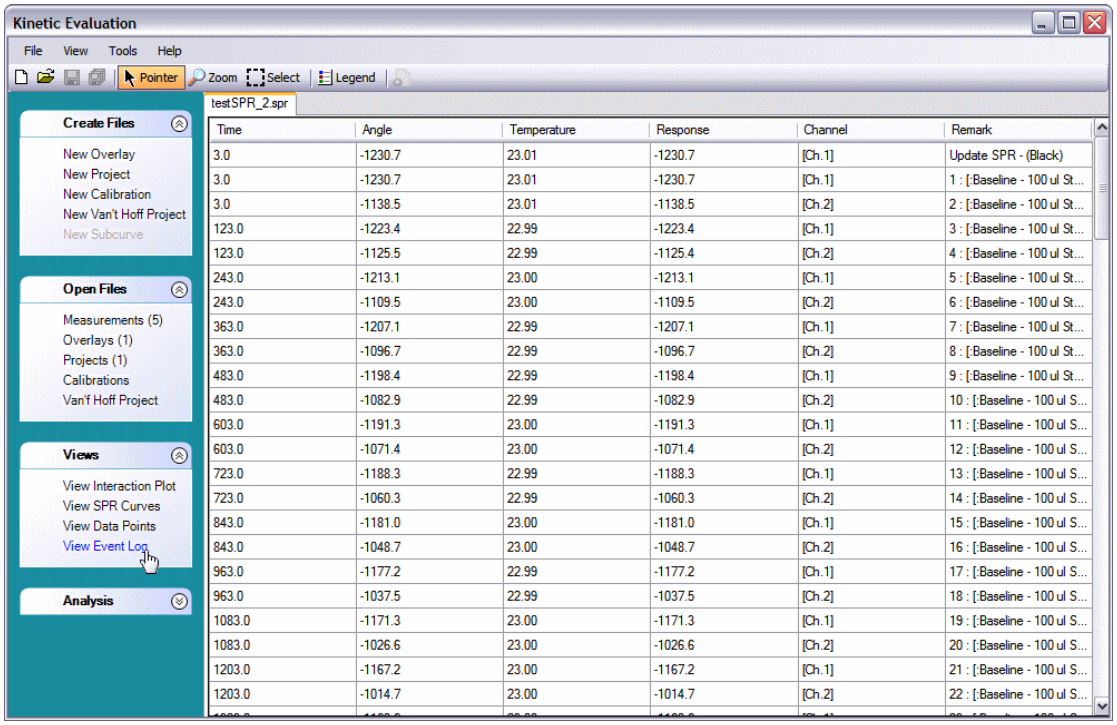


Figure 4.11 - Measurement Event Log

History – Overlay Files

Overlay files keep an internal history of the changes made to the file. These logged items can be displayed by viewing the History of the overlay file, as shown in the figure below.

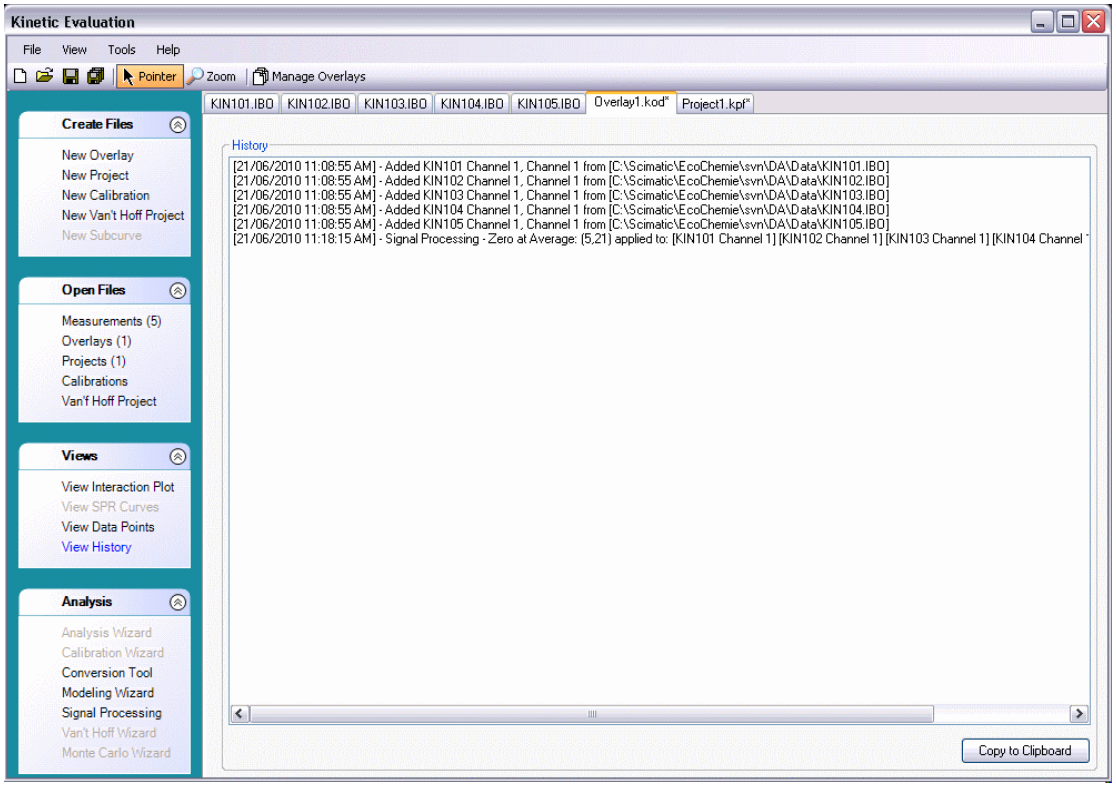


Figure 4.12 - History items of an Overlay file

The history log keeps track of what files were used to construct the Overlay file and what (if any) actions were taken on the data with the Signal Processing Tool (see section 5.2).

It is possible to copy this data to another application by clicking the **Copy to Clipboard** button.

Summary View – Analysis Files

When an analysis is performed using the Analysis Wizard (see section 5.3) an analysis is added to the project file. By changing the view of the analysis to *Summary*, it is possible to view the results of the fitting. The fitting results can also be copied to another application by clicking the **Copy to Clipboard** button.

Figure 4.13 shows the summary view for an Association analysis.

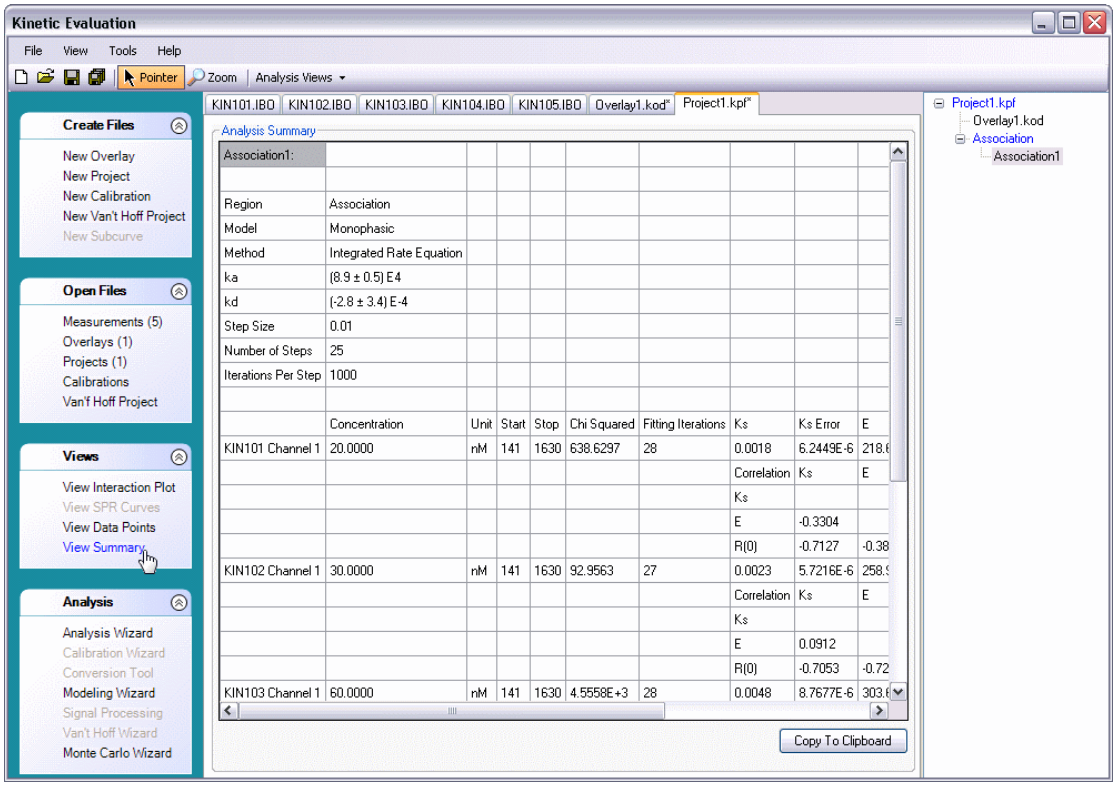


Figure 4.13 – Summary View of an Analysis

4.3 – Measurement Files

Measurement files are generated using the Autolab SPR – Data Acquisition application. KE also provides support for measurement files of different formats
KE supports the following file types of measurement files:

Table 4.1 – Supported Measurement Files

File Type	File Extension	Description
SPR file	.spr	DA measurement file (version 4.2)
KE Sub Curve file	.ksc	Partial measurement made by KE (version 5.0 or greater)
Data file	.ibo	Legacy DA measurement file (versions older than 4.2)
Text file	.txt	Exported data from Biacore or CLAMP

Within the KE application measurement files are treated as read-only files. It is not possible to change the contents of a measurement file within the KE application.

4.3.1 – Opening a Measurement File with Multiple Plots

Measurement files that are generated by other applications (i.e. Biacore or CLAMP) may contain more than one measurement per file. If a text file is opened that contains more than one measurement it is up the user to decide how KE will handle the measurements. In Figure 4.14 below the user is given the option to open the measurements as separate files (i.e. 1 file per measurement) or to directly build a new Overlay (see section 4.4) from the measurements in the file.

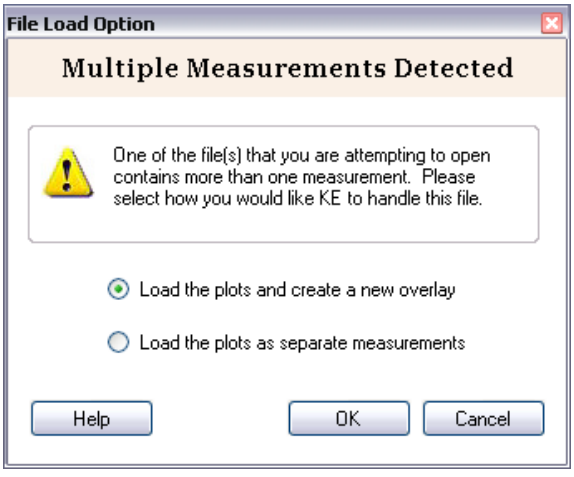


Figure 4.14 - Multiple Measurements User Prompt

4.3.2 – Creating Sub Curves from a Measurement

It is possible to select a section of a measurement and copy it into a new file. This type of file is called a *KE Sub Curve*.

The following steps show how to create a KE Sub Curve file

a) Open a measurement file

b) Change the graph mode *Selection*, as shown in Figure 4.15.

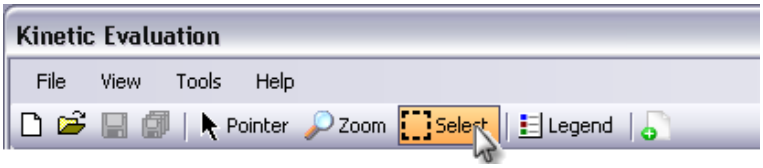


Figure 4.15 - Changing the Graph Mode to Select

c) Right-Click and drag the mouse pointer to select the region that you want to copy into a new measurement file (see Figure 4.16).

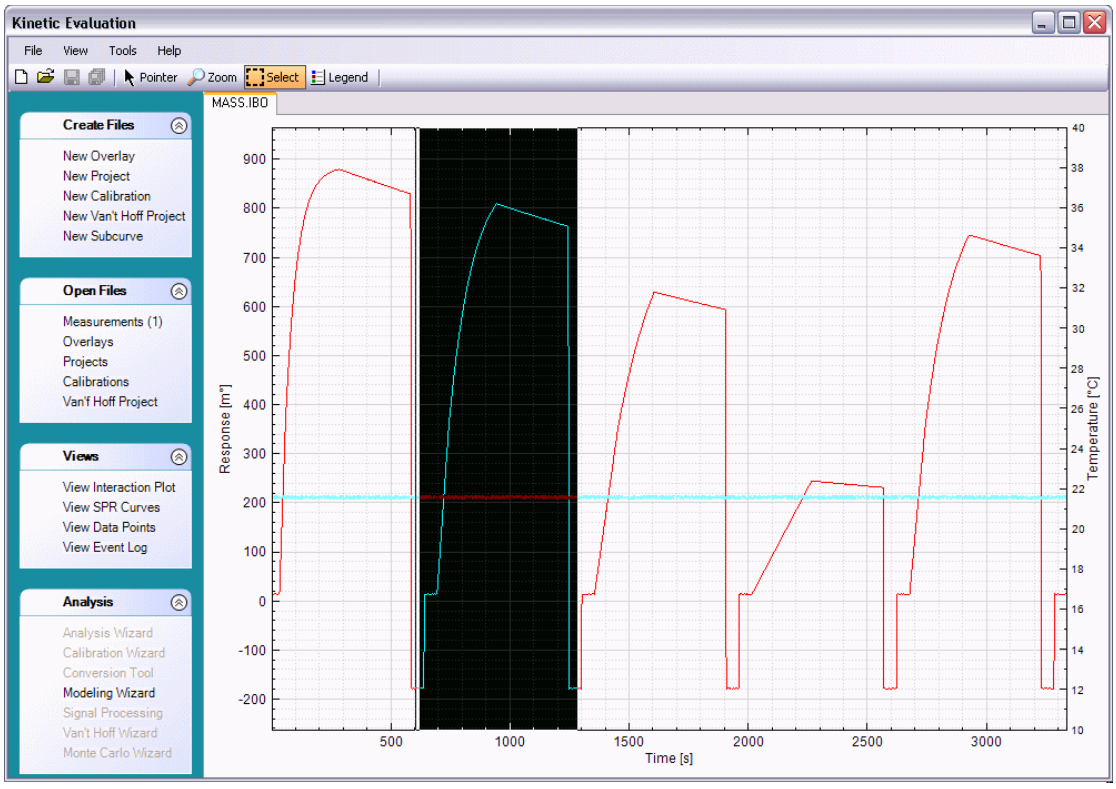


Figure 4.16 - Selected data to be copied to a sub curve

d) Once the selection has been made, Click the New Subcurve item in the Activity Bar as shown in Figure 4.17.

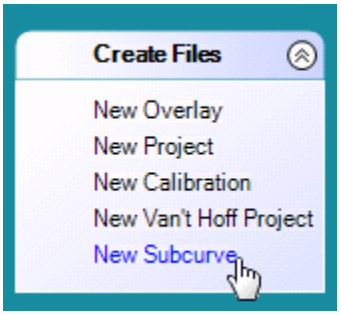


Figure 4.17 - Choosing the sub curve option

e) A new file will be created which contains the selected data. Figure 4.18 shows the new KE Sub Curve file.

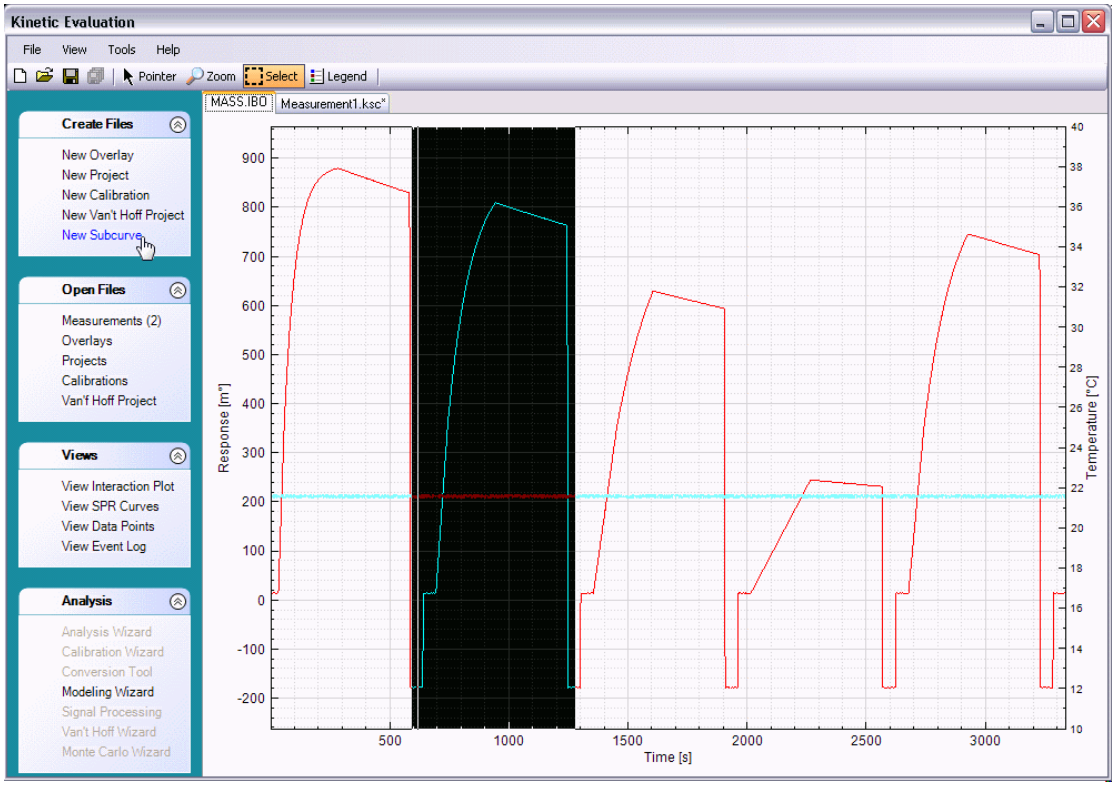


Figure 4.18 - The newly created Sub Curve

4.4 – Overlay Files

Overlay files are the result of combing multiple interaction plots (Measurement files) into one file.

Note

In order to create a new an Overlay file there must be at least one Measurement file opened.

Overlays are built with the *Overlay Builder*, which is depicted in Figure 4.19. The Overlay Builder can be opened from the Main menu (**File→New→Overlay…**) or by clicking on the **New Overlay** item in the Activity Bar.

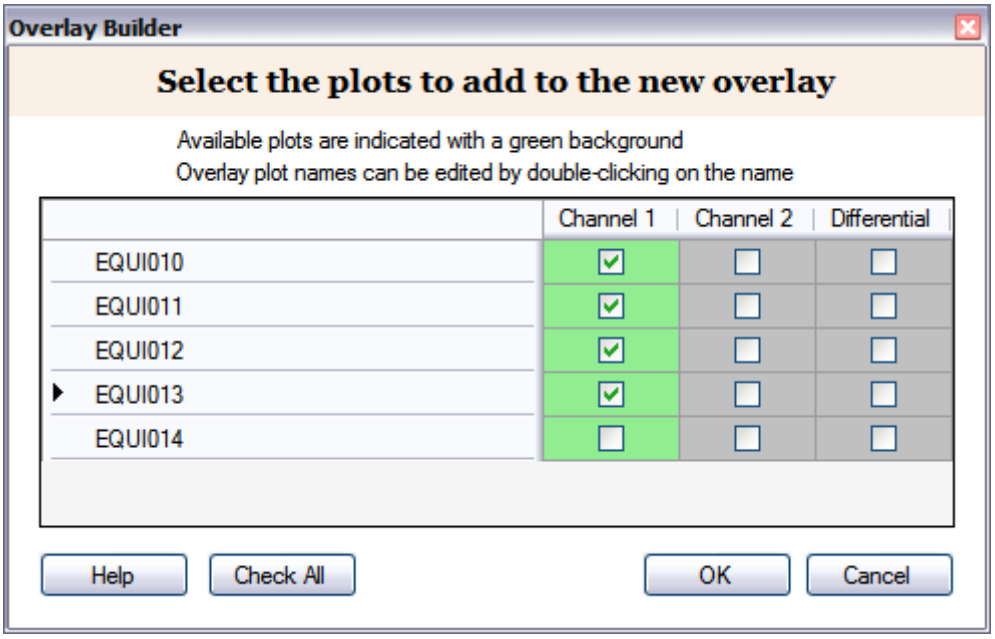


Figure 4.19 - The Overlay Builder[†]

To create a new overlay select the interaction plots (by checking the boxes) that you wish to add. Items that are present in the measurement are presented with a green background while items with gray background indicate interactions that were not located in the measurement. To select all available interaction plots, you can click the "Check All" button.

Once the interaction plots have been selected, press the **OK** button to create the new Overlay. Figure 4.20 shows the results of the selections made in Figure 4.19.

[†] For users of the Springle software the Channel 2 and Differential columns are not available.

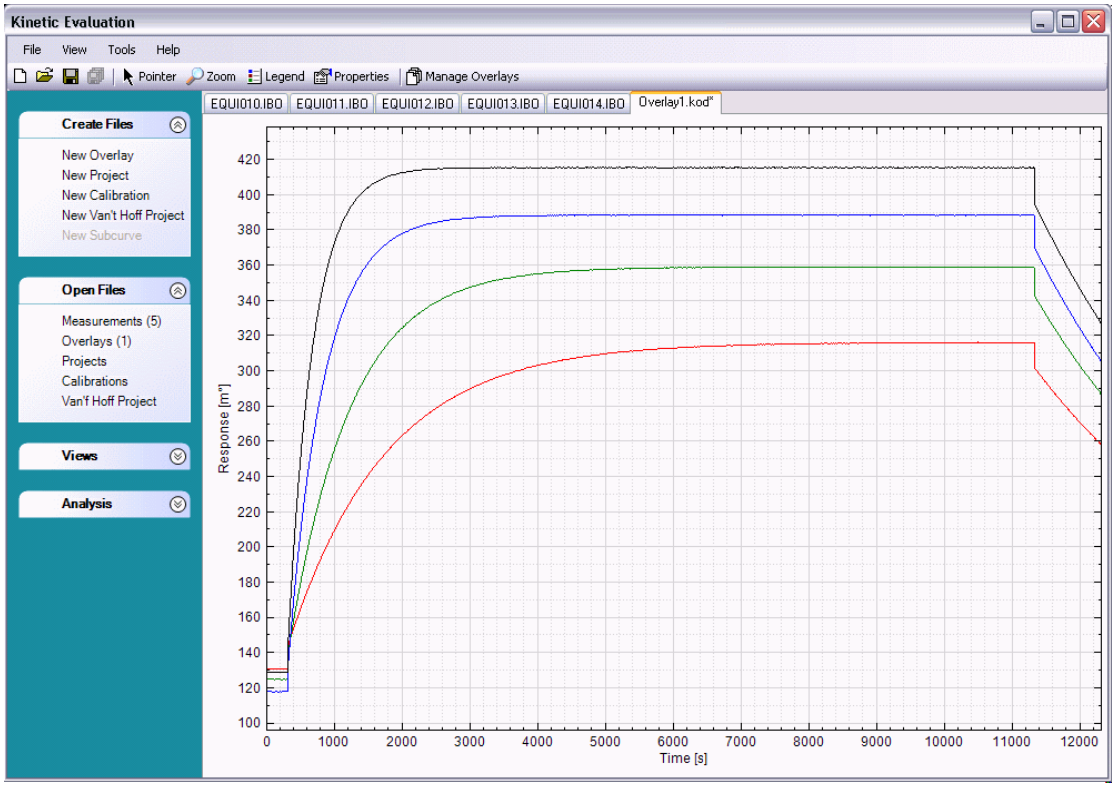


Figure 4.20 - The new overlay file

The new Overlay file can be saved to disk by clicking **File→Save**, or click the save icon in the Toolbar.

In general, any files that are opened in KE that contain unsaved data will appear with a "*" at the end of their name (i.e. the new Overlay name is *Overlay.kod**).

4.4.1 – Overlay Manager

Once an overlay is created, it is possible to add or remove measurements from the overlay before it is saved. Clicking on the **Manage Overlay** button on the Toolbar, or ***Tools → Manage Overlays***, to the launch the Manage Overlay Dialog.

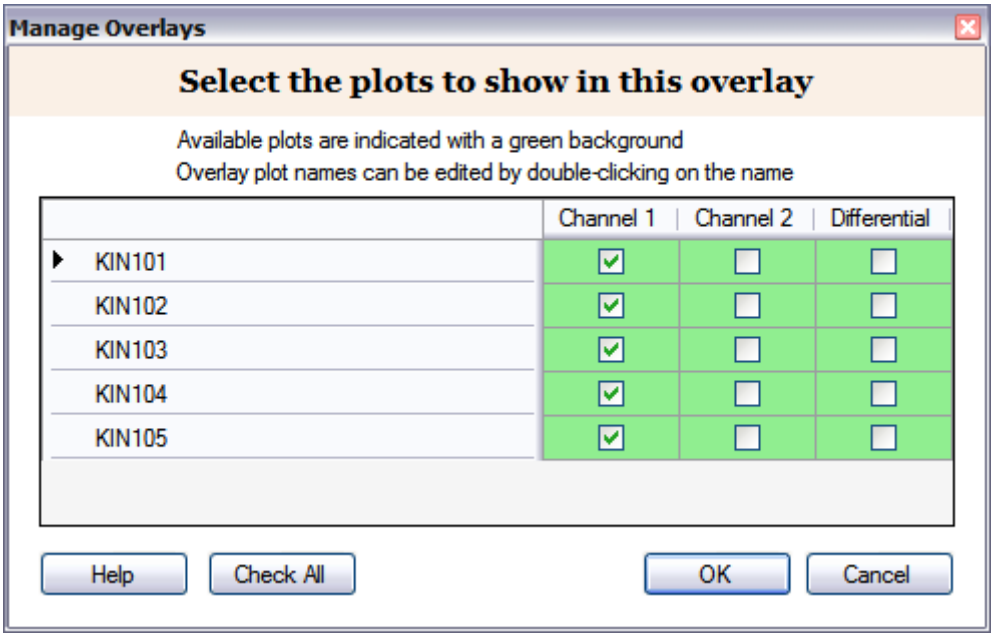


Figure 4.21 – Overlay Manager dialog before the Overlay is saved

The check boxes will allow the user to add or remove measurements from the overlay.

Note

It is possible to change plot names in the Overlay Manager by Double-clicking on a plot name.

Once the overlay is saved, each measurement is saved as a single element in the overlay. Figure 4.22 shows the Manage Overlay dialog after loading and overlay from disk.

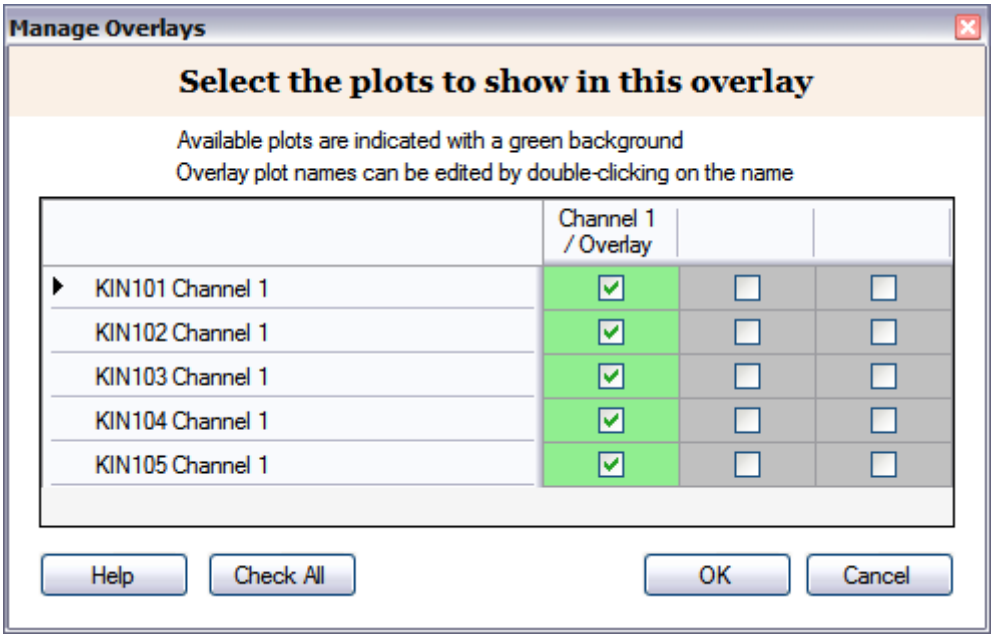


Figure 4.22 – Overlay Manger dialog after the Overlay is saved

4.5 – Project Files

Project files are a new concept since KE version 5.0. The purpose of a project file is to store all analyses that were performed on an Overlay into one file.

4.5.1 – Creating a Project File

To create a new Project file, click **File→New→Project…** or click on **New Project** in the Activity Bar. The following dialog (Figure 4.21) will be shown to assist the user in selecting how to create the new overlay.

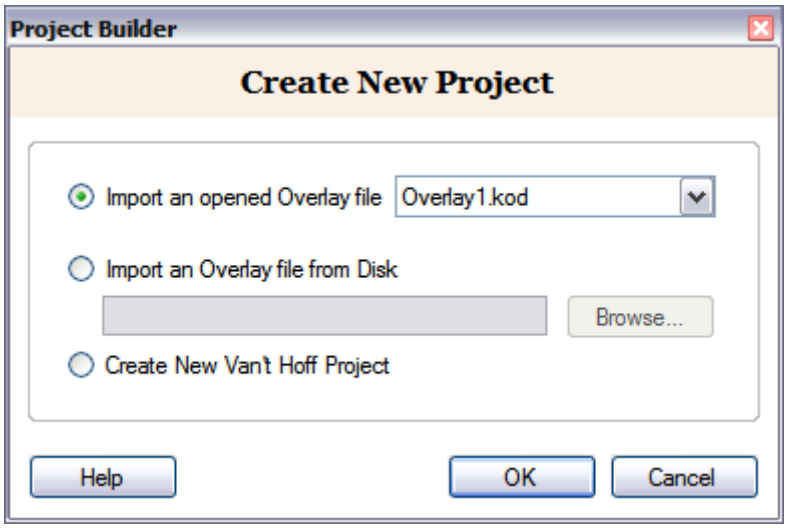


Figure 4.23 - Options to Create a new Project

If there are Overlay files opened in KE, they can be selected in the combo box and imported into the new Project. If there are no Overlay files opened then it is possible to import an Overlay file from disk.

Van't Hoff Projects are a special form of projects that support temperature based analyses. These projects are discussed in section 7.

Upon clicking the **OK** button on the *Project Builder* dialog, a new project file is displayed, as shown in the Figure 4.24.

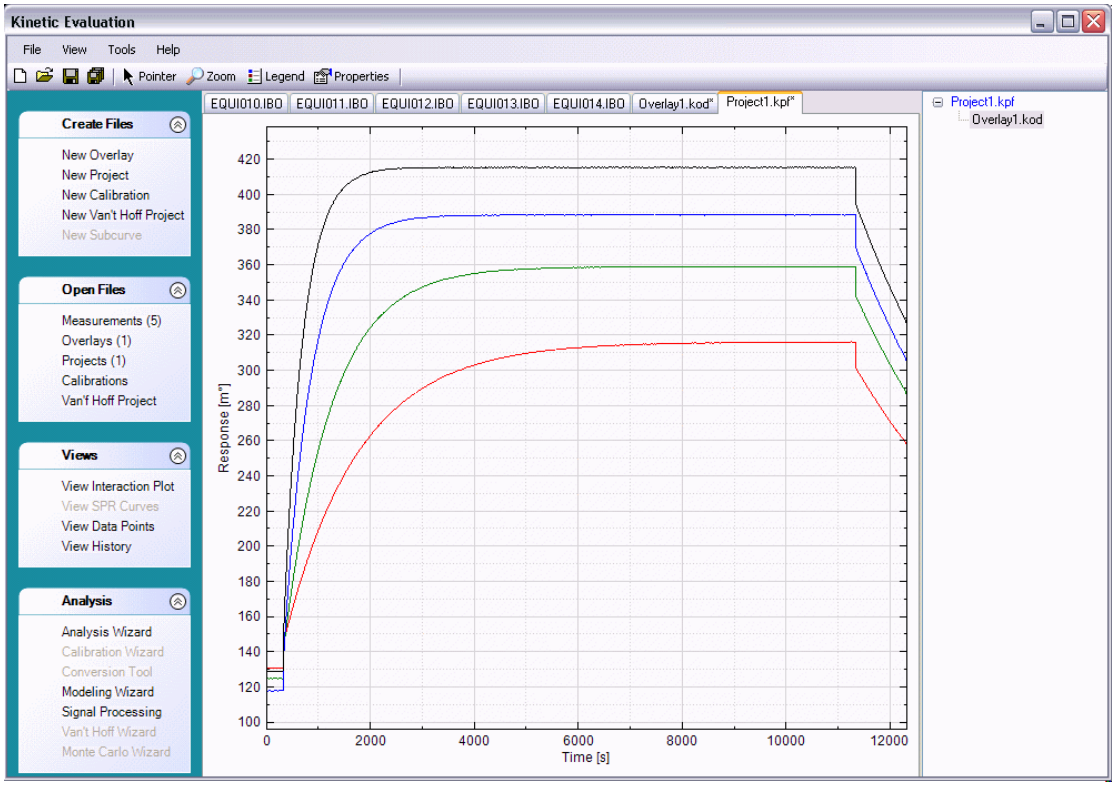


Figure 4.24 - The new project file

When a new Project is created, the *Project Navigation Bar* (see section 2.5) is displayed on the left-hand side of the screen. This navigation bar tool is required because Project files can contain multiple sets of data.

4.5.2 – Performing an Analysis

Analysis files are created by the Analysis Wizard which is described in section 5.3. Analysis files are a part of a Project file and cannot be saved separately. The reasoning for this restriction is to guarantee that the analysis is always sorted with the Overlay file (also stored in the Project file) was used for the analysis.

The result running the Analysis Wizard is that a new analysis will be added to the project. The project now handles three different types of analysis – *Association*, *Equilibrium* and *Dissociation* analysis.

4.5.3 – Navigation of a Project File

Project files contain one Overlay file and analysis files. It is possible to view the different components of the Project file by clicking on them in the Project Navigation Bar. Figure 4.25 shows the Project Navigation Bar in which an analysis named *Associaton1* is selected.

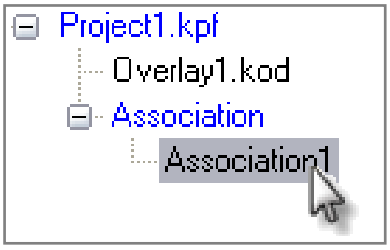


Figure 4.25 - Changing the Visible Plot

4.5.4 – Types of Project Files

In KE 5.1, there now exists three types of project file. The preceding section has discussed a default project file. One can also create Calibration projects and Van't Hoff projects. The differences in these project types are detailed in Table

Table 2.1 – Overview of the File Menu Items

Project Types	Purpose	Allowed Analyses	Number of Overlay Files	Additional
Project	Data analysis	Association Dissociation Equilibrium	One Overlay File only	Section 5.3
Calibration	Calibration	Equilibrium	One Overlay File only	Section 6
Van't Hoff	Temperature based analysis	Van't Hoff	Multiple overlays allowed	Section 7

4.6 – Changing Chart Properties

It is possible to change to look of the graph for Overlay and Project files. By clicking **View→Chart Properties** or the Properties button in the Toolbar will launch the Chart Properties dialog as shown in Figure 4.26 below.

Note

It is not possible to change the plot properties of a Measurement file. The KE application handles Measurement files as Read-Only documents.

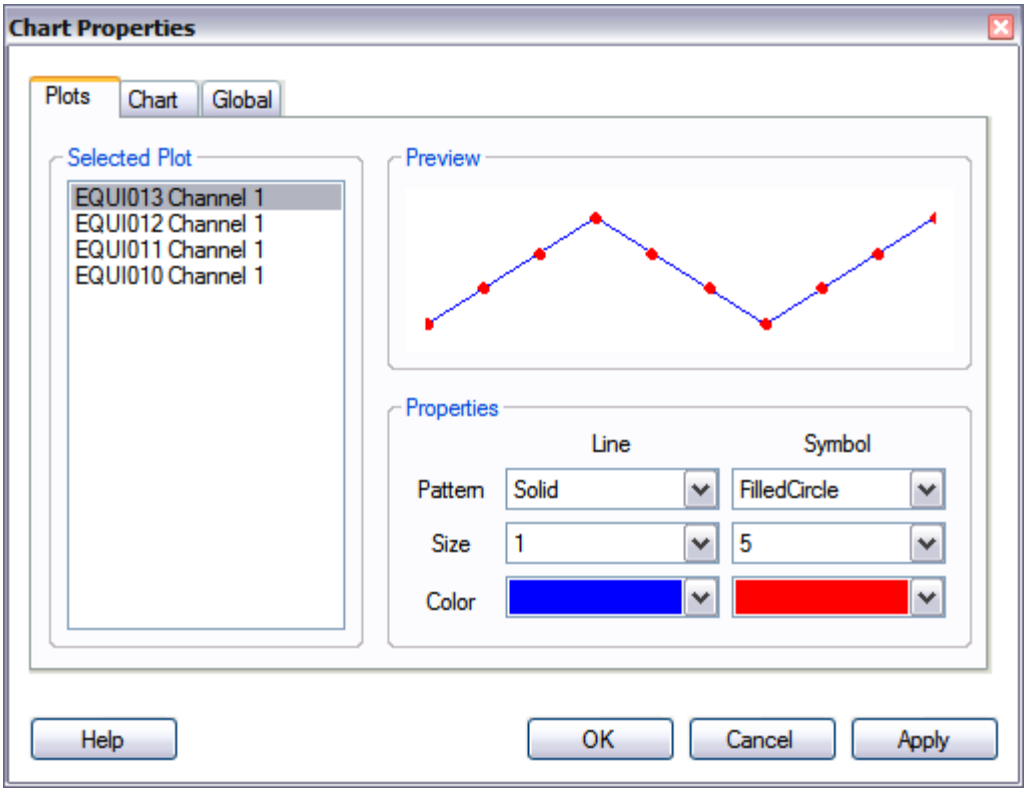


Figure 4.26 - Chart Properties Dialog

The first tab in the Chart Properties dialog is *Plots*. To change one of the plots in the graph, click on its name from the list of plots.

Note

It is possible to edit the properties of multiple plots at once. Select multiple plots by holding down the SHIFT button while clicking on the plot names.

The Line Pattern, Size and Color can be changed by using the dropdown lists on this dialog.

It is also possible to display Symbols on the data points in the plot by selecting a Symbol Pattern (by default, Symbol Pattern is set to none).

The next tab in the Chart Properties dialog is for the *Chart* itself. As shown in Figure 4.27, the Title, Vertical & Horizontal Axis can be configured here.

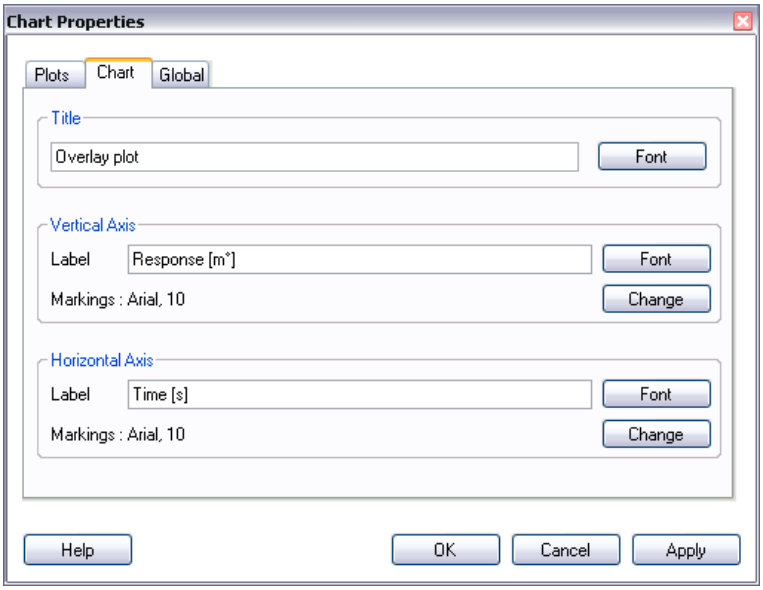


Figure 4.27 - Chart Properties

The last tab in the Chart Properties dialog (see Figure 4.28) contains the configurable items that are global (the same) for all opened files.

The Border and Background colors can be changed while the Vertical and Horizontal gridlines can be shown or hidden.

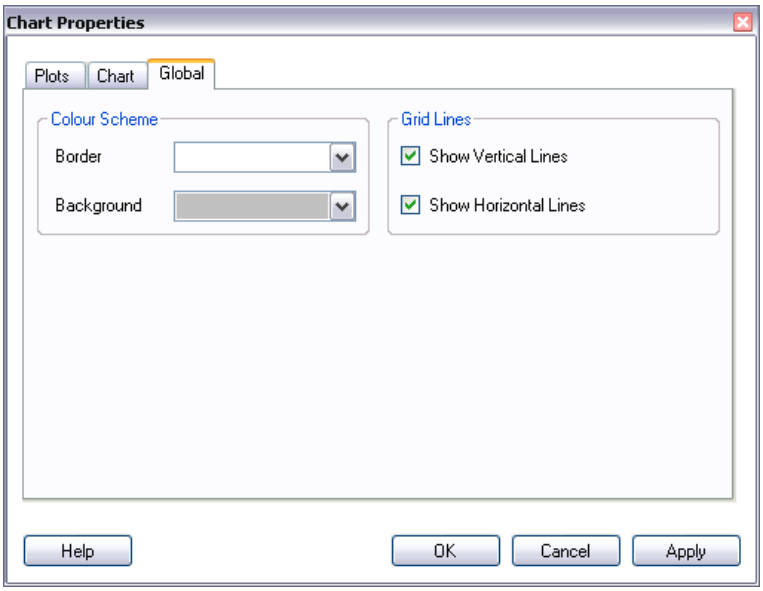


Figure 4.28 - Global Properties

4.7 – Printing Data

In KE it is possible to print the graphs and the files details of the graphs.

To print a file, click the **File→Print** menu item and Figure 4.29, will be displayed.

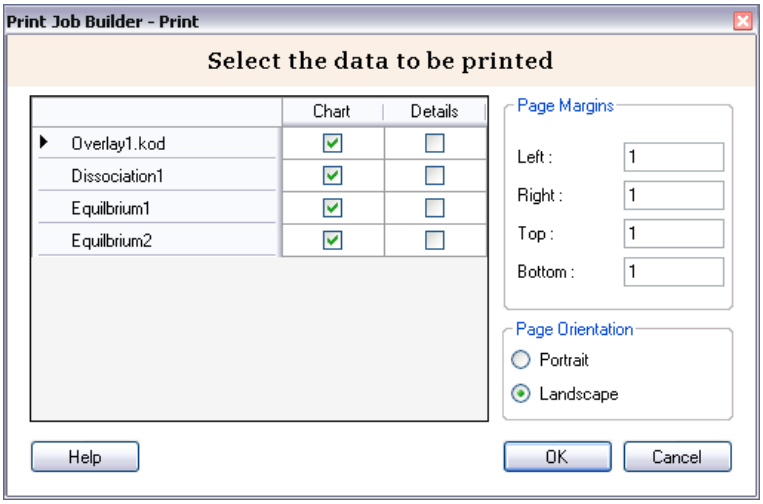


Figure 4.29 - Print Dialog

From this dialog it is possible to configure the items that are to be printed. Figure 4.29, depicts a project file with all of its graphs to be printed. To print file details click the appropriate check box in the *Details* column.

The *Page Margins* and *Page Orientation* can be also configured from within this dialog. It is also possible to preview a file by clicking **File→Print Preview** in the main menu. Figure 4.30 shows the print preview of an Overlay file.

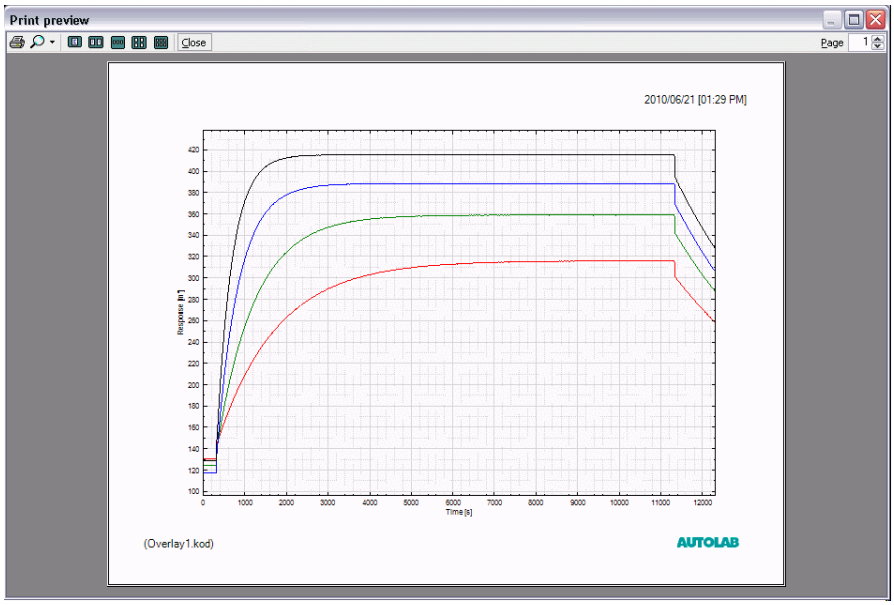


Figure 4.30 - Print Preview Dialog

5 – Analyzing the Data

This section of the manual provides an overview of the different features that are available in the KE application to analyze data.

5.1 – Modeling Wizard

The Modeling Wizard is used to construct Rate Equations that are used by the Numerical Integration method in the Analysis Wizard (see section 5.3).

The process to construct a rate equation is performed in five steps. The following subsections go over each step in detail.

5.1.1 – Starting the Modeling Wizard

The Modeling Wizard can be opened from the Main Menu (section 2.2) **Tools→Modeling Wizard** or from the Activity Bar (section 2.4) **Modeling Wizard**.

Upon opening the Modeling Wizard the dialog in Figure 5.1 will be displayed.

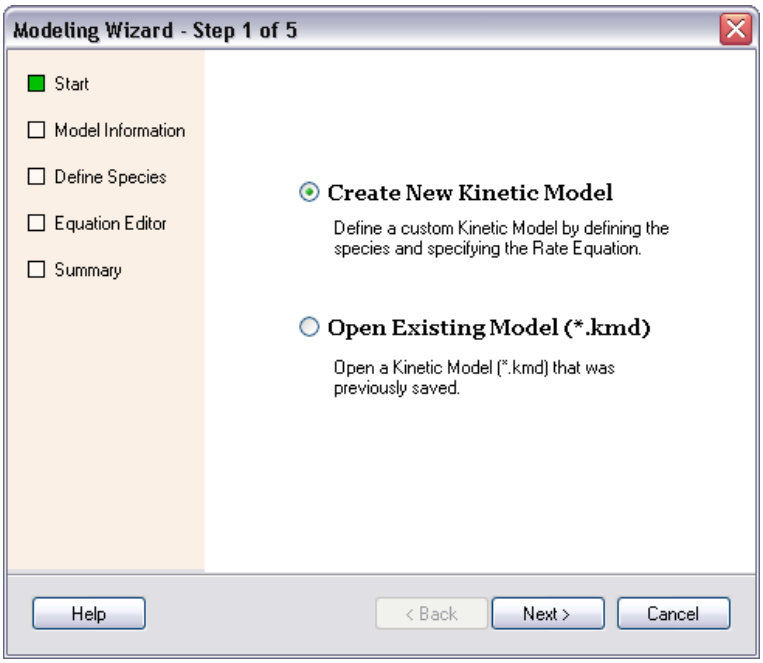


Figure 5.1 – Creating a model

In Step 1 of the Modelling Wizard there are two options to choose from, *Create a new kinetic model* or *Open Existing Model* from disk.
Select the **Create New Kinetic Model** and click **Next**.

5.1.2 – Configuring the Modeling Information

Step 2 of the Modeling Wizard is shown in Figure 5.2. In this step the information about the model is entered. Every model is given a name and an optional description.

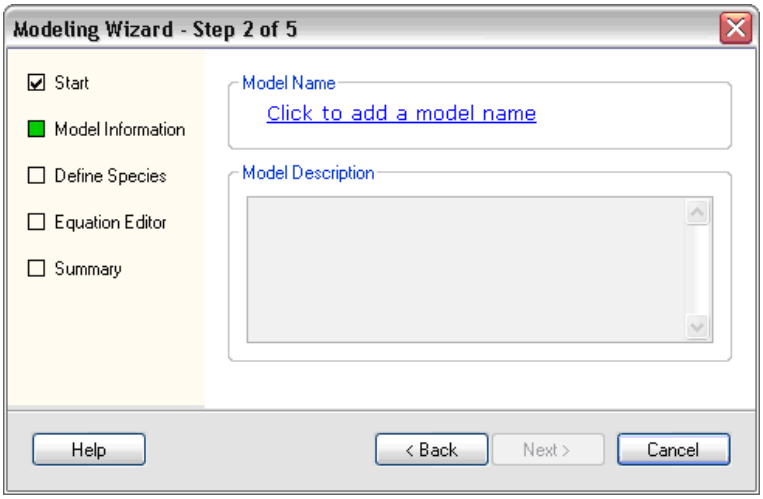


Figure 5.2 – Model Information Step

To give the model a name, click the text **Click to add a model name**. To add a description to the model, click in the Model Description text box and enter the text.

Figure 5.3 shows an example of a model name and description entered.
Add your model name and description and click **Next**.

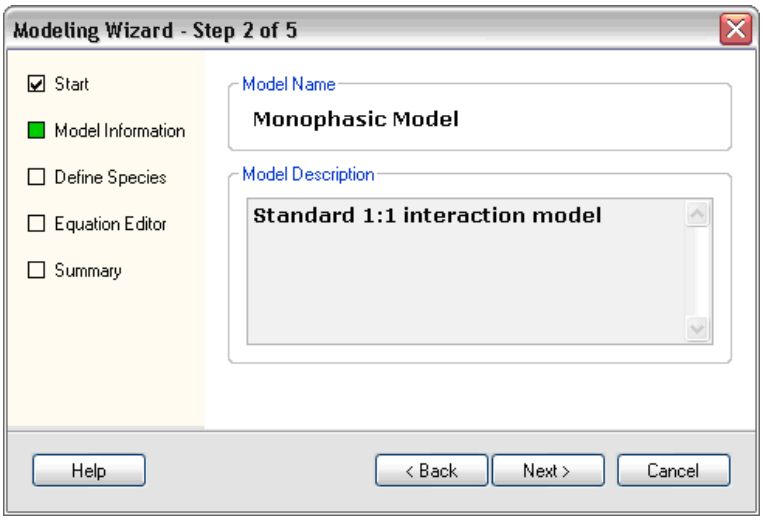


Figure 5.3 – Information for the Model

5.1.3 – Defining the Species

In Step 3 of the Modeling Wizard the species that are used in the rate equations will be defined.

Enter in each unique species for the rate equation, as shown in Figure 5.4. Once you have entered in your species click the **Next** button to proceed.

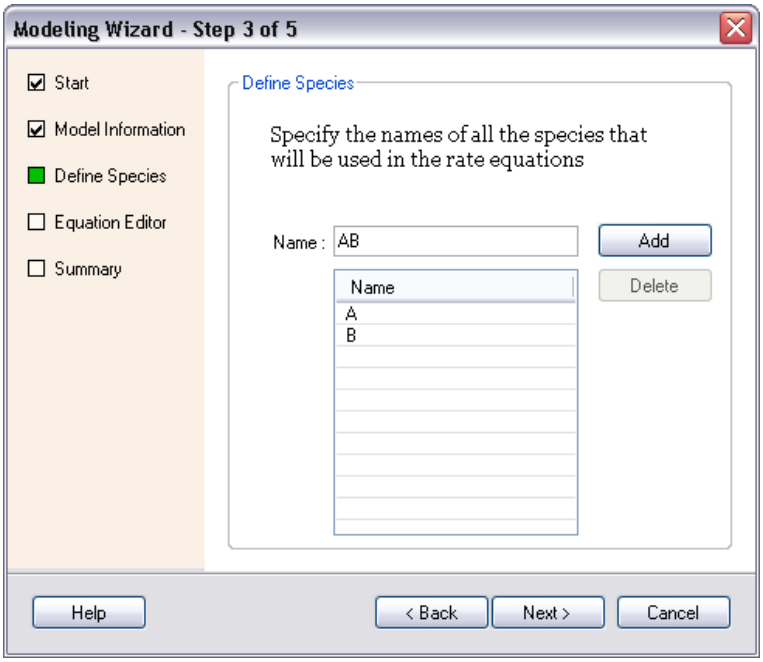


Figure 5.4 – Defining the Species

Define Species Only Once

If the rate equation for the model is made up of multiple equations, for example:

$$A_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A + B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} AB$$

Define each species once even though they may be used in several equations (A in the rate equation above is the product of the 1st rate equation and is the analyte in the second part of the rate equation.

5.1.4 – Creating the Rate Equations

In step 4 of the Modeling Wizard it is time to construct the rate equation. Use the dropdown controls on the dialog, as shown in Figure 5.5, to configure the species for the rate equation.

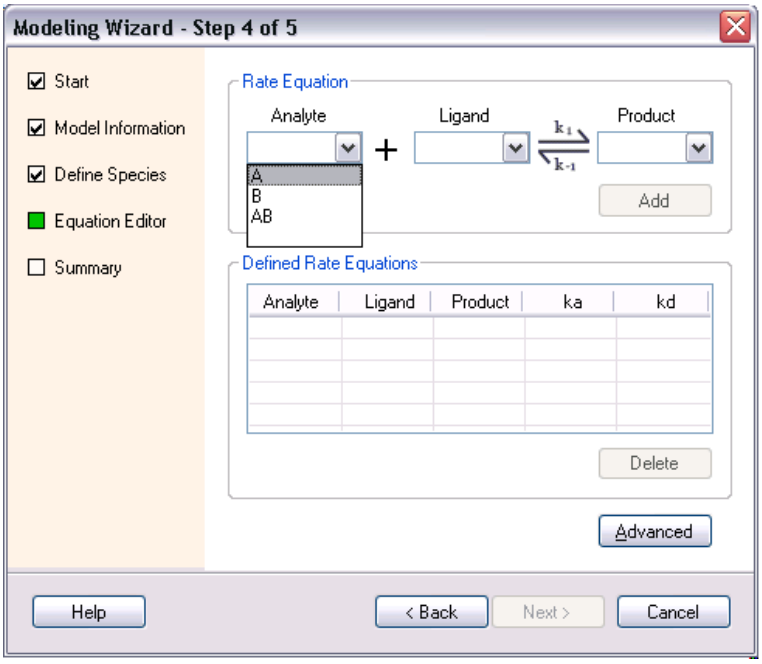


Figure 5.5 – Creating a rate equation

Once the *Analyte*, *Ligand* and *Product* have been defined, click the **Add** button to add the rate equation to the model.

Note

A valid rate equation must consist of at least an Analyte and a Product.

Figure 5.6 shows that the model has one rate equation defined for it. If you wish to remove a rate equation from the model then select the equation from the defined equations (click on it to highlight it) and press the **Delete** button.

Once you have configured your rate equation, click **Next** to proceed to the next step.

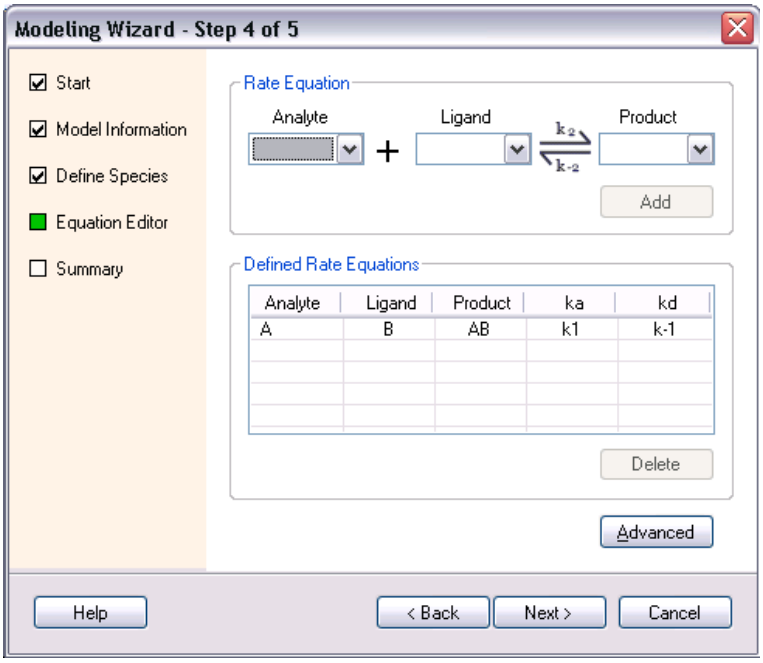


Figure 5.6 – Completed rate equation

5.1.5 – Summary of the Model

The last step of the Modeling Wizard is the summary of the completed model. To make changes to the model click the **Back** button to return to the step that requires changing.

Figure 5.7 shows the summary for the Monophasic model that was constructed in this example.

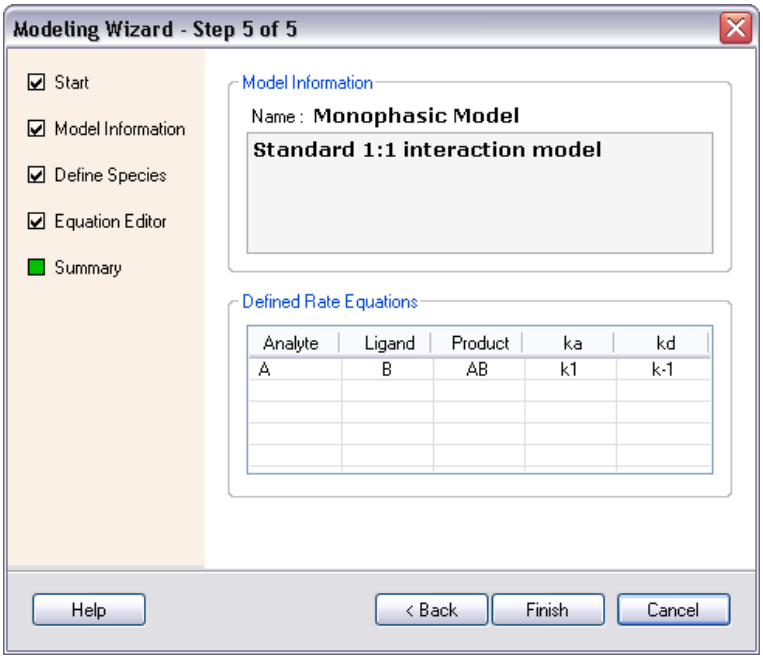


Figure 5.7 – Summary of the newly created model

To end the modeling wizard, click the **Finish** button. Before the wizard closes the user will be prompted to save the model.

5.1.6 – Editing an Existing Model

To edit a previously saved model, start the Modeling Wizard and select the **Open Existing Model** option in the first step of the wizard (as shown in Figure 5.8). The selected model will be loaded into the wizard and all the model information will be automatically entered into the wizard.

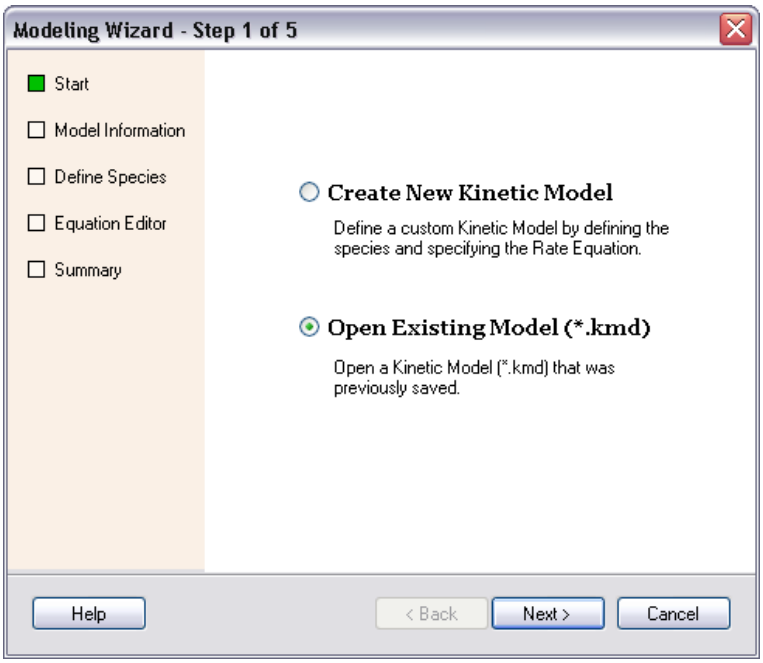


Figure 5.8 – Opening a Model from Disk

5.1.7 – Advanced Settings

Certain models can have more than one product that contributes to the SPR signal. For example, in the set of equations that describe a conformal change reaction,

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} AB^*$$

both AB and AB* can contribute to the SPR signal. To indicate to the model that both AB and AB* are final products, click on the "Advanced" button in step 4 as shown in Figure 5.9.

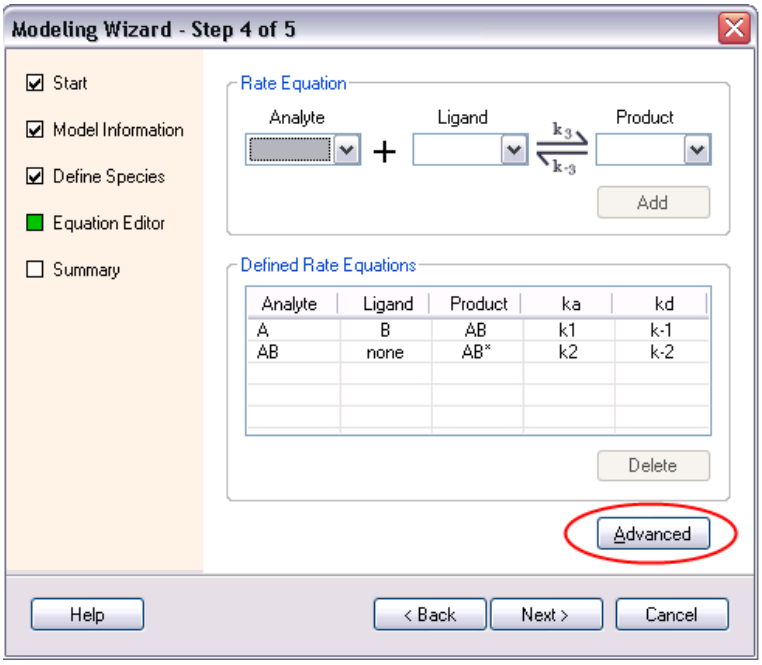


Figure 5.9 – Advanced model configuration

Clicking the "Advanced" button will show an additional dialog where the signal weights can be entered. In Figure 5.10, the signal weights for AB and AB* are set to be equal, so that each of AB and AB* contribute the same to the SPR signal in the model.

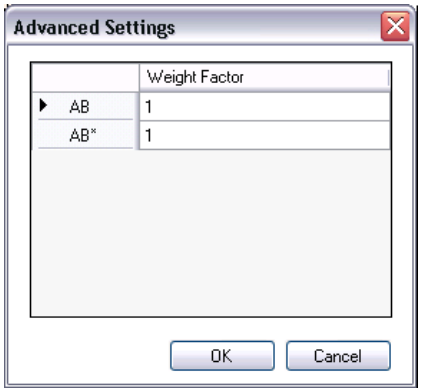


Figure 5.10 – Setting the signal weights

5.2 – Signal Processing

It is possible to improve data quality in the KE application by using the Signal Processing tool. Signal processing can be performed on Overlay files only. Overlays that are a separate file (*.kod) or part of a Project file (*.kpf) can be used in the Signal Processing tool.

The Signal Processing dialog can either be opened by clicking from the *Main Menu* **Tools→Signal Processing** or clicking on the **Signal Processing** item in the *Activity Bar* as shown in Figure 5.11.

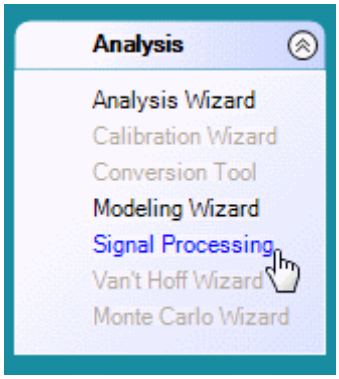


Figure 5.11 – Selecting Signal Processing

Once the Signal Processing dialog has been opened it will appear as shown in Figure 5.12.

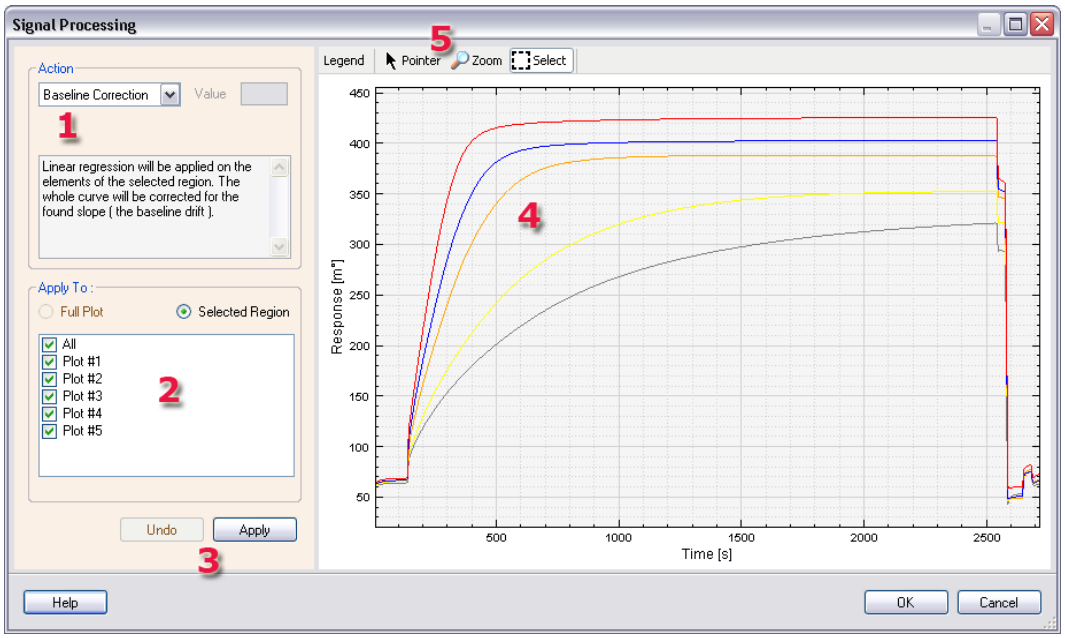


Figure 5.12 – Signal Processing Dialog

The following descriptions correspond to the numbers that appear in Figure 5.12.

1. **Actions** – Select which signal processing technique to perform on the data. The following subsections will explain each of the available actions.

2. **Apply To** – This section is used to specify which interaction plots are to be included in the signal processing technique and how the technique will be applied. There are two options for how the technique will be applied, it can process the entire plot or only a portion of the plot that is selected via the *Select* mode (click the Select button and with the right mouse button down drag the mouse to select a region).

3. **Apply/Undo** – Once the technique and the plots that will be processed have been configured, press the **Apply** button to run the action. If the action did not

perform the desired effect then this action can be undone by pressing the **Undo** button. There is no limit to the amount of times an action can be applied.

4. **Overlay Data** – The overlay data is updated upon each press to the Apply button. All signal processing changes can be viewed in this graph.

5. **Graph Toolbar** – The figure below depicts the tools that are available for the Signal Processing dialog.

Figure 5.13 – Signal Processing Toolbar

The Toolbar contains the following items:

Table 5.1 – Toolbar Items

Toolbar Item		Description
Legend	-	Show/Hide the legend for the Overlay
Pointer	-	This is the default mode of the graph
Zoom	-	Left-click and dragging the mouse pointer on the graph will zoom in on the selected region
Select	-	Left-click and dragging the mouse pointer will select a region for the signal processing action to process

The following subsections will give an overview of each of the Signal Processing techniques.

5.2.1 – Blank Subtraction

The *Blank Subtraction* action subtracts the selected plot from the other plots that are selected in the *Apply To* section of the Signal Processing dialog. Blank Subtraction can execute on a selected region or the full plot.

To use the Blank Section action, select the plot that you wish to use as the blank (as shown in Figure 5.14). This plot will then be subtracted from the other selected plots.

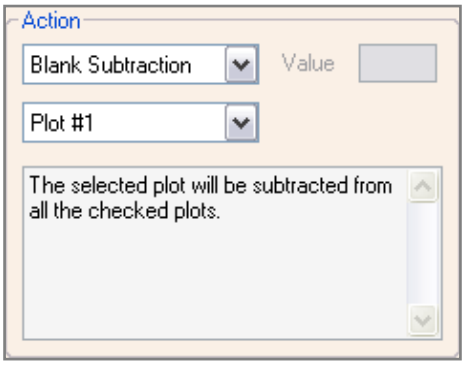


Figure 5.14 – Blank Subtraction Options

The following screenshots show the effect of using the Blank Subtraction action. The effect of the blank subtraction is can be seen in Figure 5.16 as the Response intensity has been reduced.

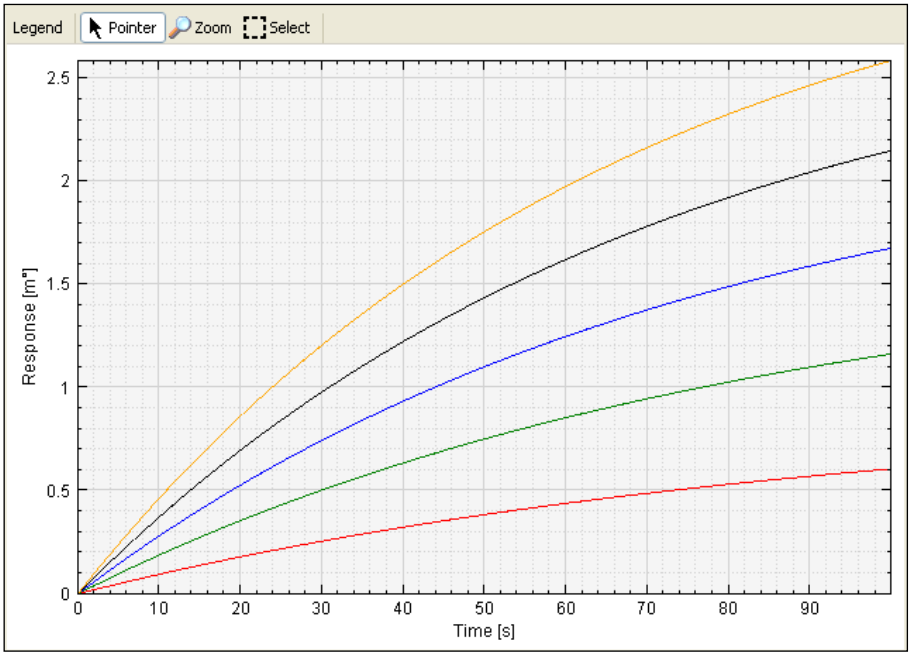


Figure 5.15 – Overlay before Blank Subtraction

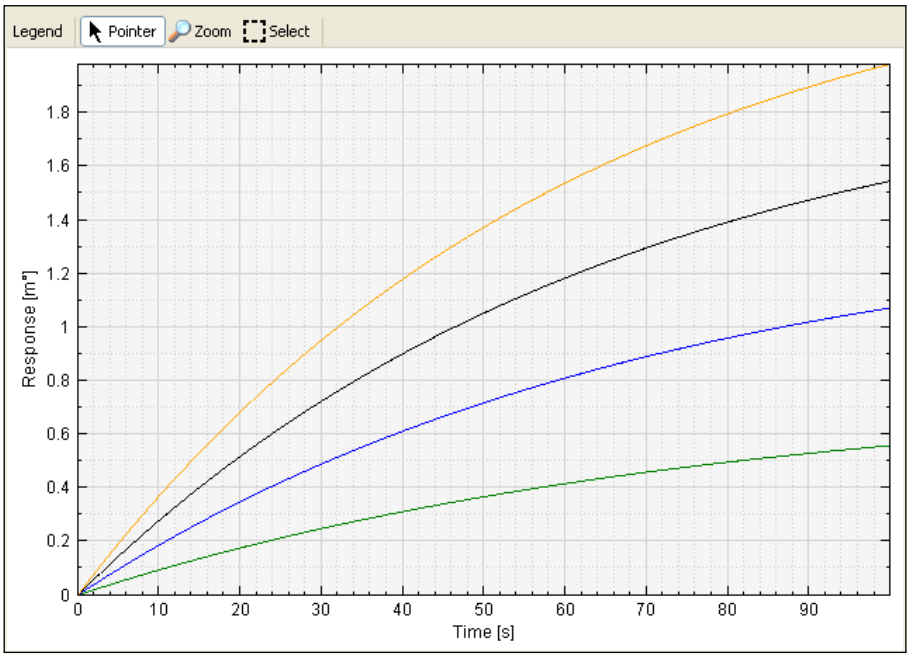


Figure 5.16 – Response Intensity is reduced after Blank Subtraction

5.2.2 – Delete Selection

The Delete Selection action deletes the selected region of the plot. This action is available only for a selected region and cannot be used with the *Full Plot* option.

The selected region of the plot is removed while any plot information before and after the deleted region is retained. The data that remains after the deleted region is moved to the end of the plot that is still remaining before the deleted region.

The following two figures are before and after screenshots of the Delete Section action.

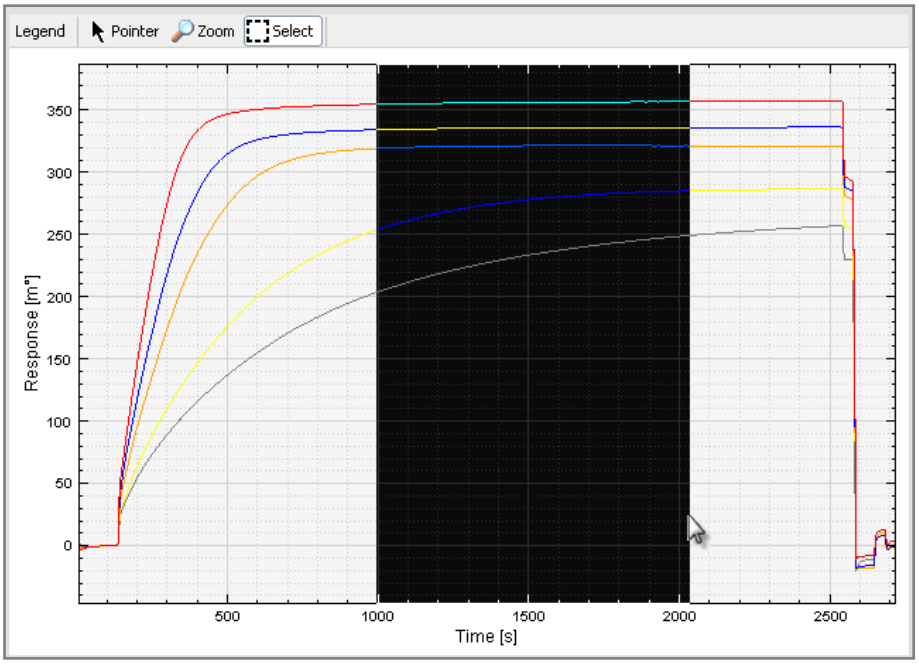


Figure 5.17 – Select a Region to be Deleted

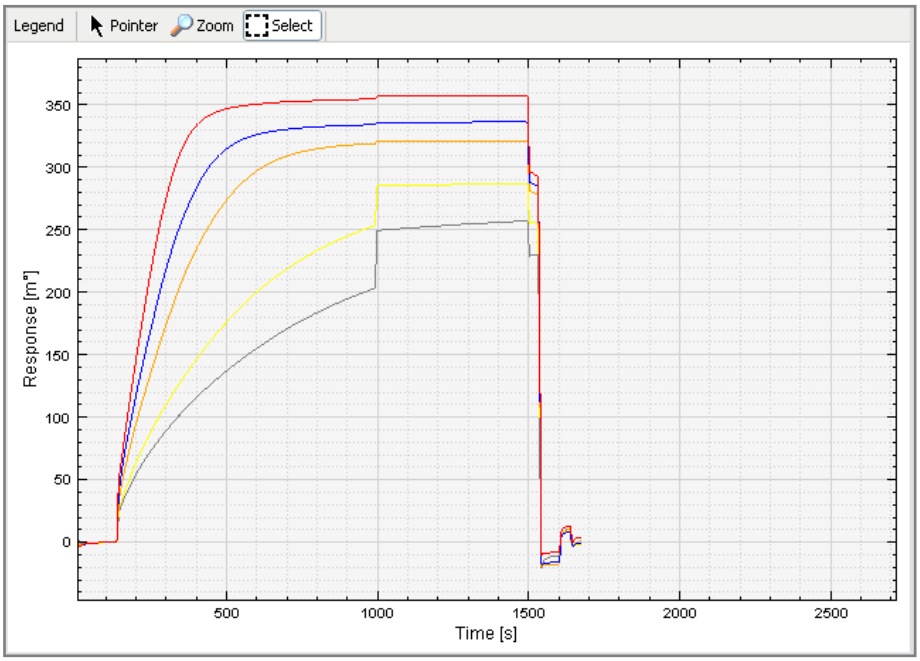


Figure 5.18 – Result of the Delete Selection Action

5.2.3 – Fast Fourier Transform (FFT)

The *Fast Fourier Transform* (FFT) action is a filtering technique used to reduce high frequency interference in the plots. This action can be applied to a selected region or to the full plot(s).

The Figure 5.19 shows plots that contain high frequency noise.

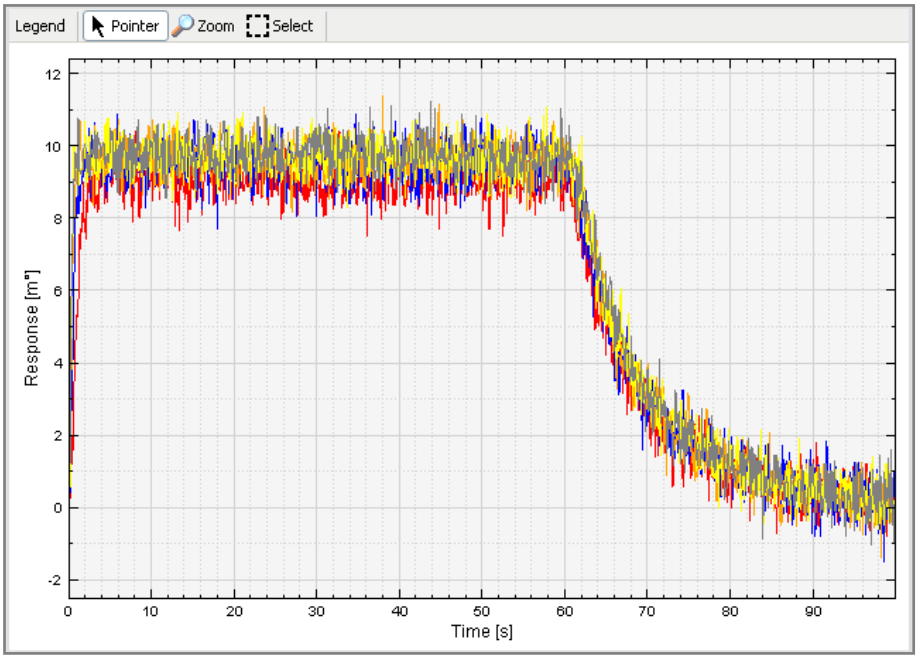


Figure 5.19 – Noisy Data

To improve the quality of the plots, select the Fast Fourier Transform action and click the Apply button.
There is an intermediate step in the FFT action. The user must select the region of the frequency plot they wish to retain for each plot that is being filtered. In the frequency plot the data at the lower frequency is what is to be retained. Select the area of the graph that contains intensity above the baseline. Figure 5.20 depicts this selection.

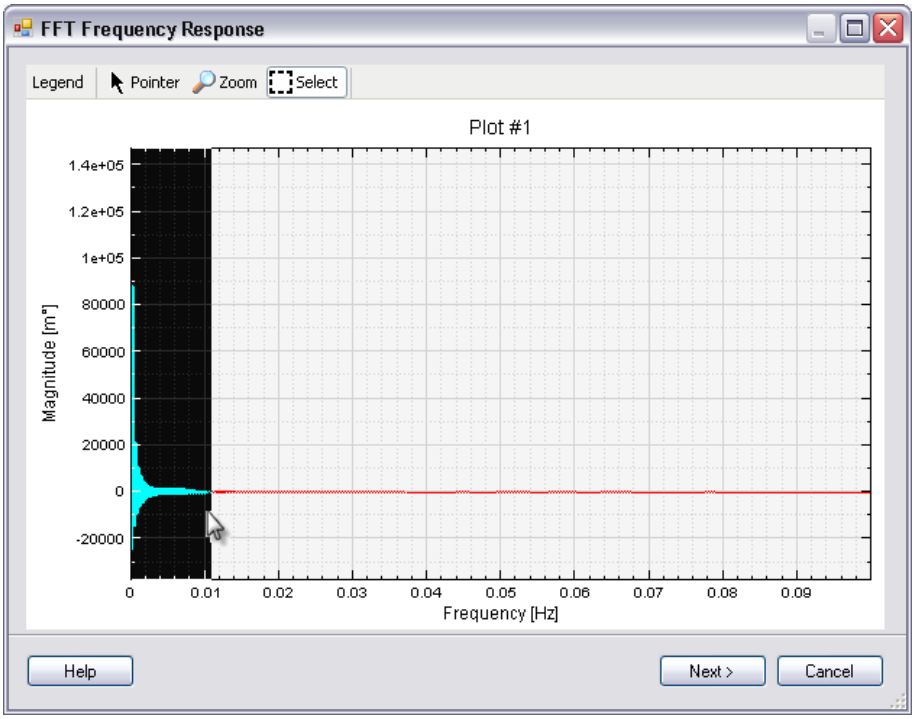


Figure 5.20 – Region of the spectrum to retain

After selecting the region to retain for each plot the filtered plots will be presented as shown in Figure 5.21.

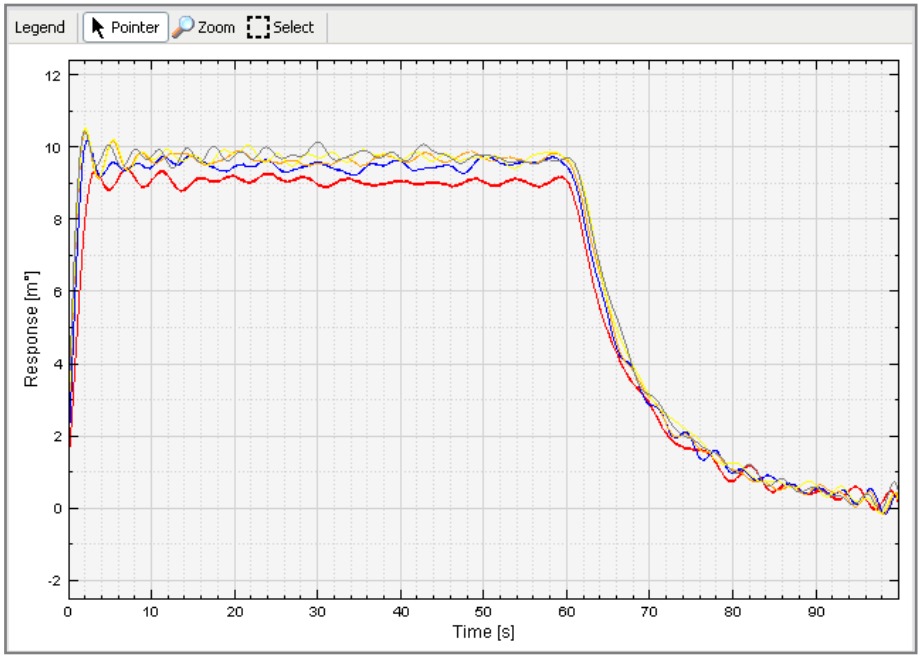


Figure 5.21 – Result of the Fast Fourier Transform

5.2.4 – Mathematical Operation

The *Mathematical Operation* action performs the operation that is selected by the user on the selected plot(s). The available operations, as shown in the figure below, are *Addition*, *Division*, *Multiplication* and *Subtraction*.

To use this action, select the operation that you wish to perform and enter in the *Value* to assign to this action. Next select the plots that this action will be applied to and select the region of the plot(s) that will be affected (it is also possible to use the *Full Plot* option).

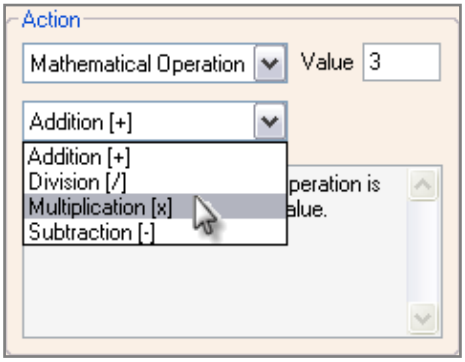


Figure 5.22 – Mathematical Operation Options

Figure 5.23 and Figure 5.24 shows the result of multiplying one of the plots in the Overlay by three.

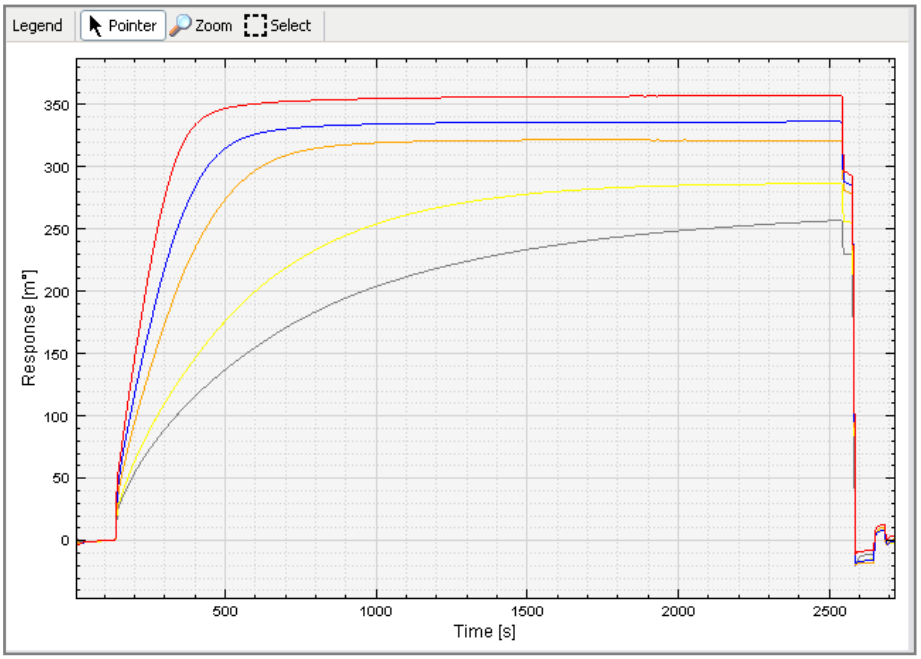


Figure 5.23 – Overlay before the Mathematical Operation is applied

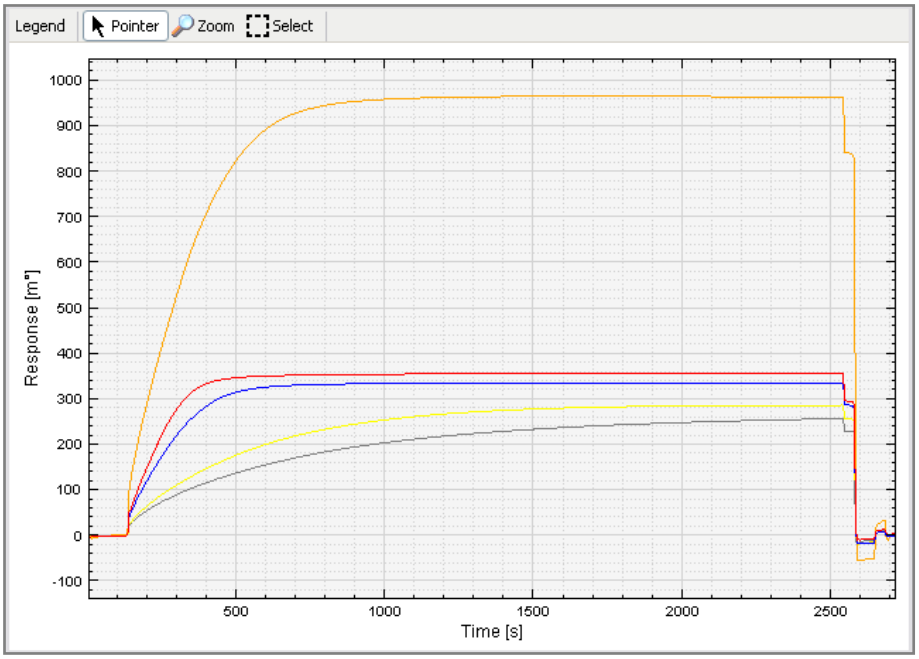


Figure 5.24 – Multiplication of a Plot by Three

5.2.5 – Normalize

The *Normalize* action subtracts the offset of the first Response data point (y axis) from the rest of the plot. This technique is useful to quickly remove an offset from the data. This action is only available to the *Full Plot*.

Figure 5.25 and Figure 5.26 show the effect of executing the Normalize action.

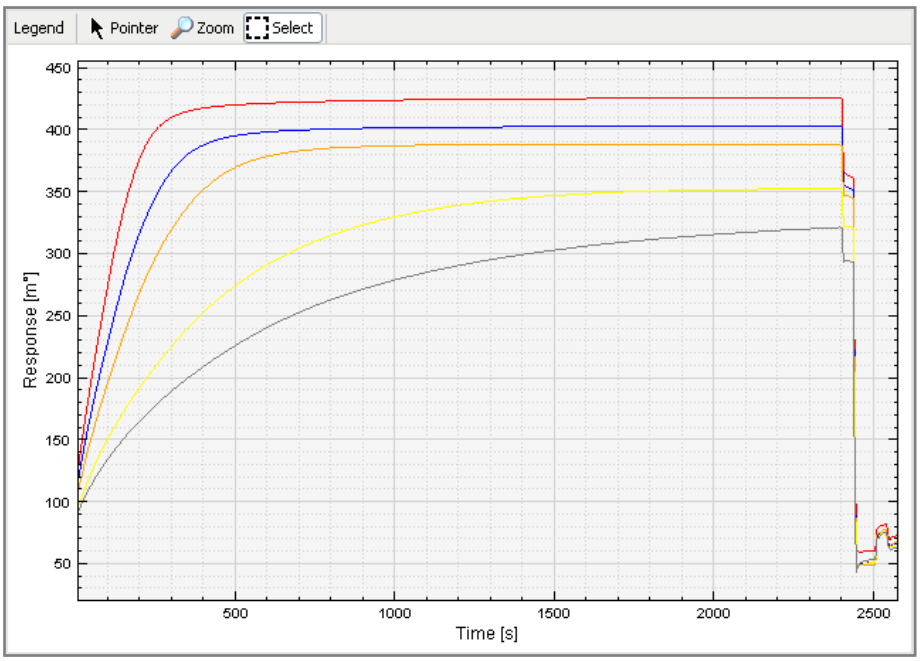


Figure 5.25 – Overlay before Normalize Action

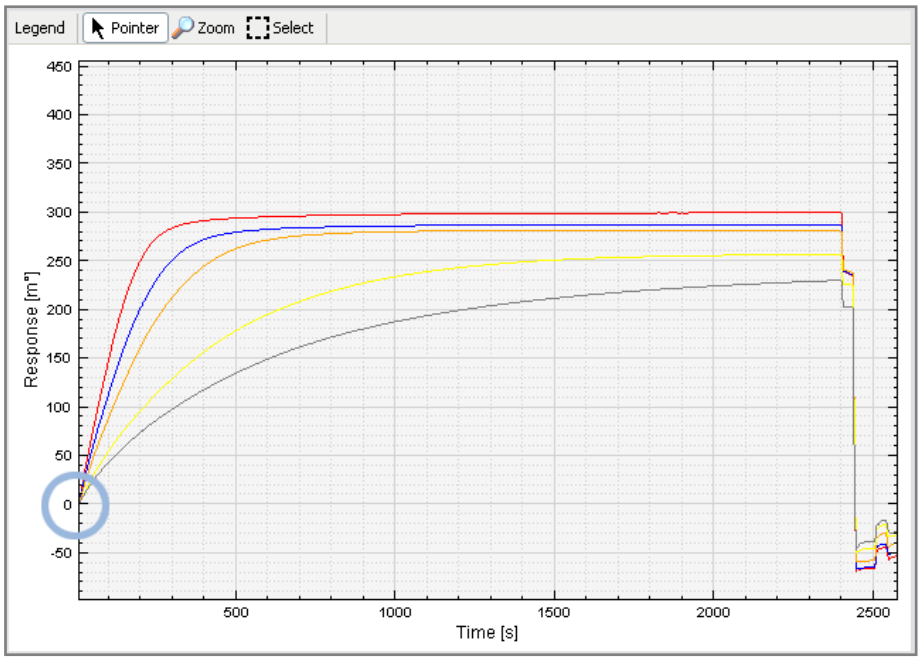


Figure 5.26 – Overlay after Normalize Action

5.2.6 – Smooth

The *Smooth* action performs a Savitzky-Golay smoothing filter on the selected data. This action can be executed on the full plot or the selected region within the plot(s).

To perform the Smooth action select *Smooth* from the list of actions, as shown in Figure 5.27. The polynomial used in the filter is set by the user, either 2nd or 4th order (default: 2nd Order).

The number of points in the smoothing window is defined by the equation 2(*m*+1) where the value of *m* is determined by the user via the *Value* dropdown list (see Figure 5.27).

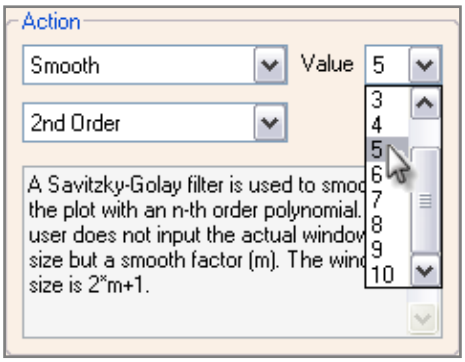


Figure 5.27 – Smooth Options

The screenshot Figure 5.28 is before and Figure 5.29 is after the *Smooth* action.

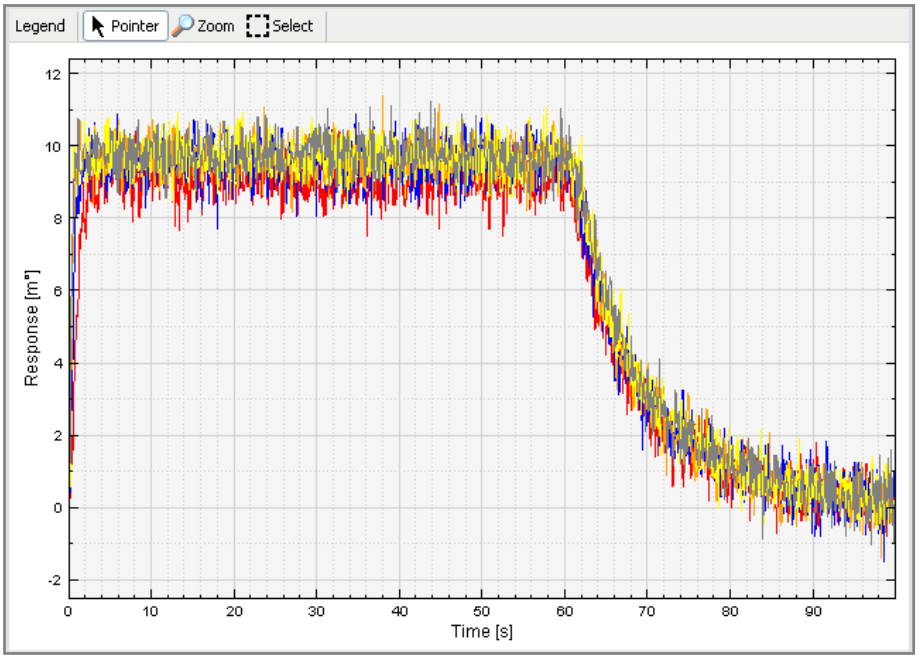


Figure 5.28 – Overlay before Smoothing

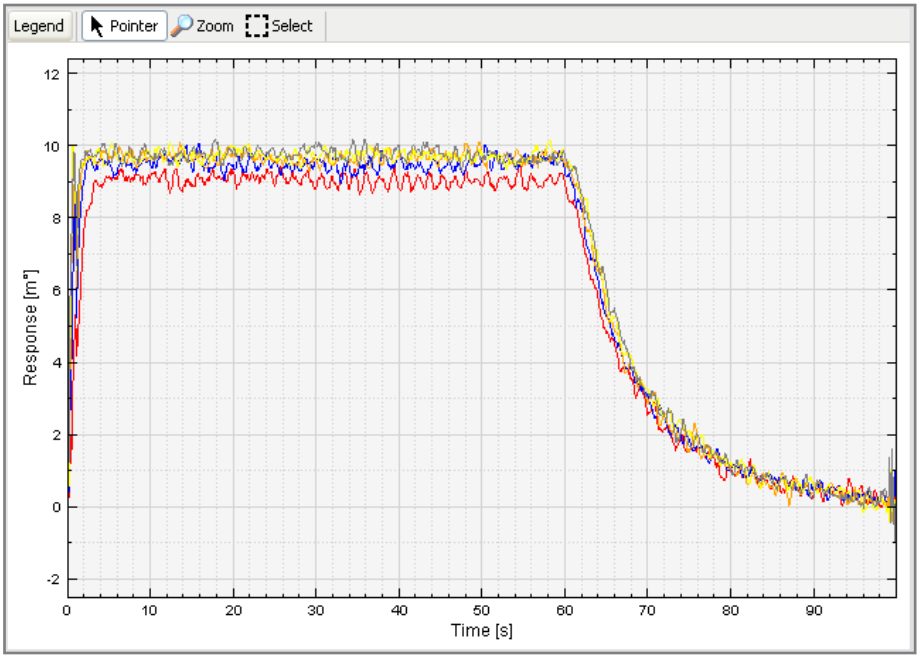


Figure 5.29 – Result of the Smooth Action

5.2.7 – Synchronize

The Synchronize action removes the selected region between plots so a common starting time can be achieved.

Typical data in which this action should be applied is shown in Figure 5.30. Notice that the starting times of the association regions do not start at the same time point.

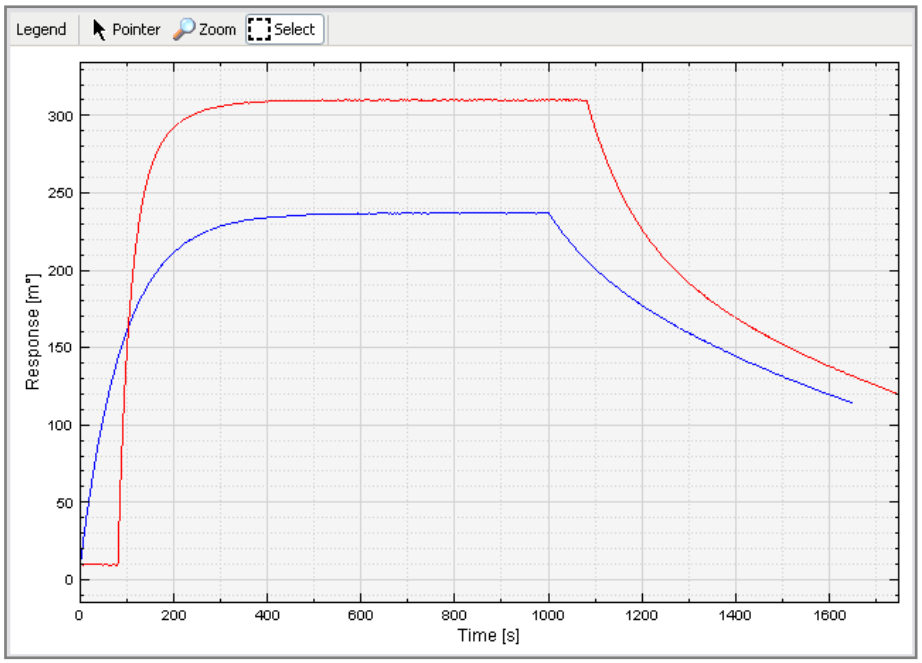


Figure 5.30 – Association Regions starting at Different Times

To synchronize the starting times, *Zoom* in on the starting points of the plots and use the *Select* tool (in the Signal Processing Toolbar) to select the region to synchronize. Figure 5.31 depicts this selection.

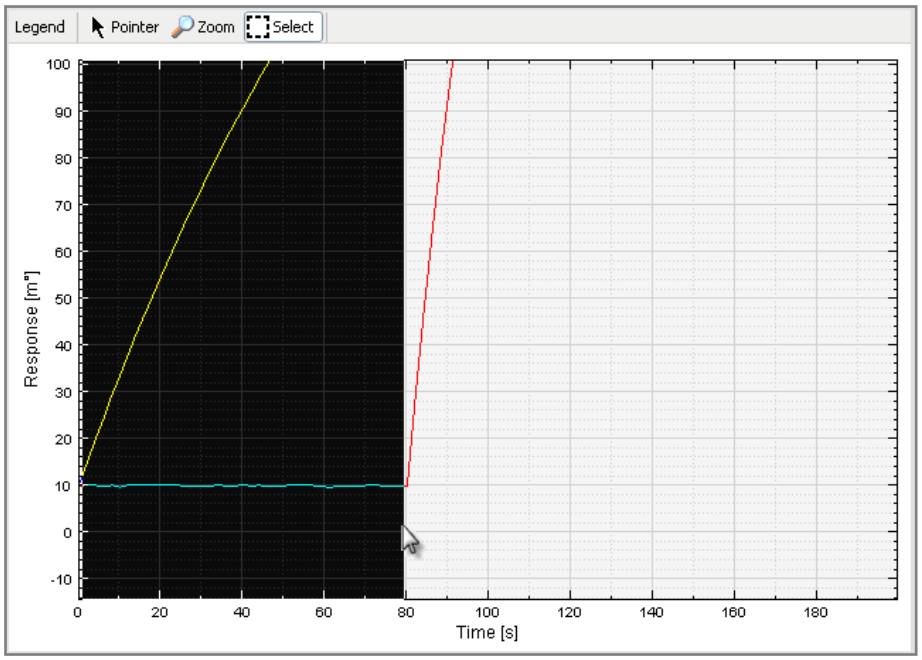


Figure 5.31 – Selecting the Region to Synchronize

Ensure that the Synchronize action is selected and the plot to be synchronized is selected in the list of plots.

The following figure depicts the result of the synchronization.

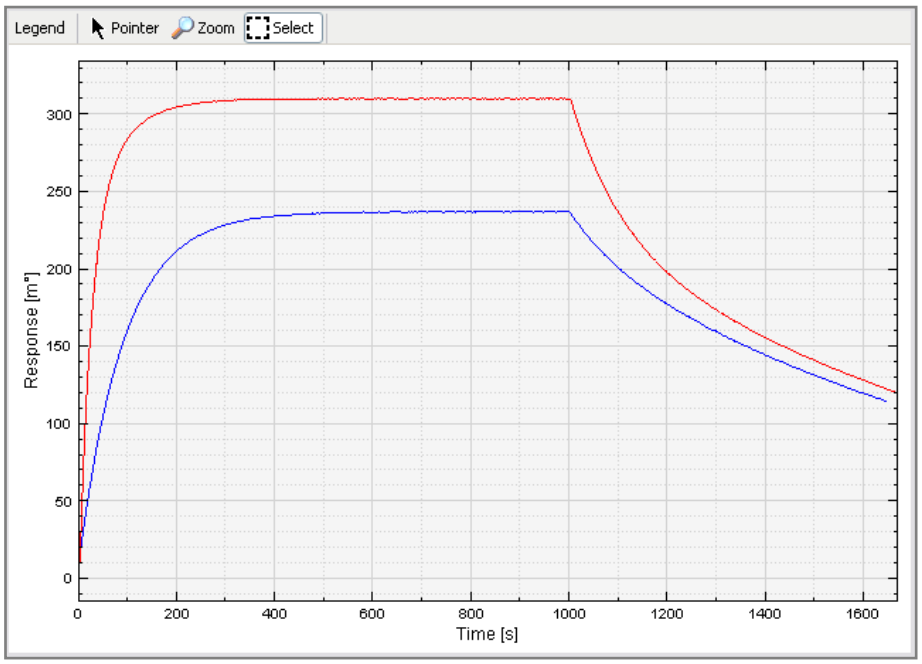


Figure 5.32 – Result of the Synchronize action

5.2.8 – Zero at Average

The *Zero at Average* action averages the value of the data in the selected region and subtracts this value from the entire plot. The user must select a region before using this action as the *Full Plot* option is not possible.

The following Figure 5.33 and Figure 5.34 are before and after screenshots of the *Zero at Average* action. Note that the plots have been zoomed into a region to clearly demonstrate the action.

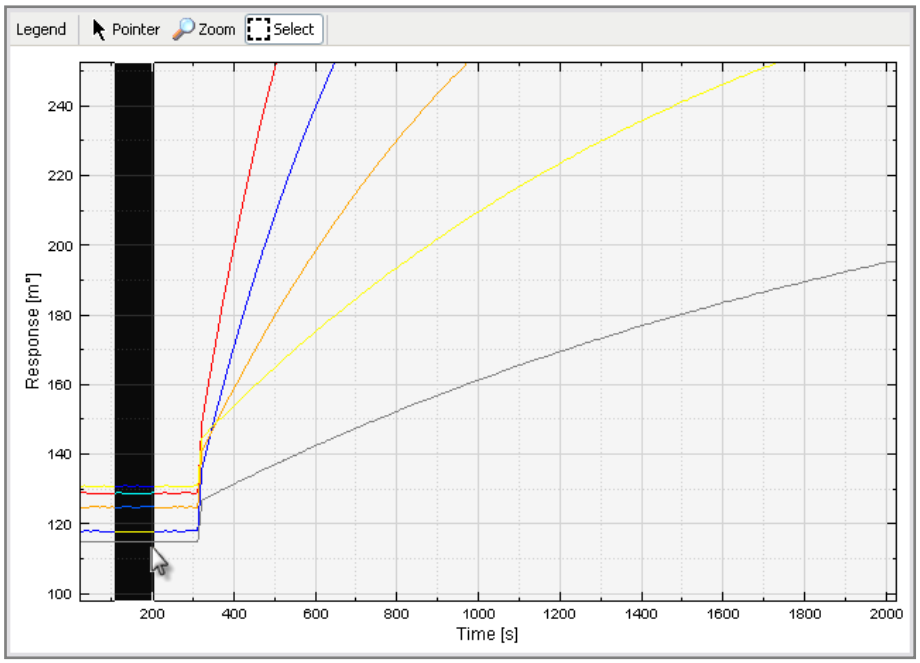


Figure 5.33 – Zoomed in and Selected Region to Zero

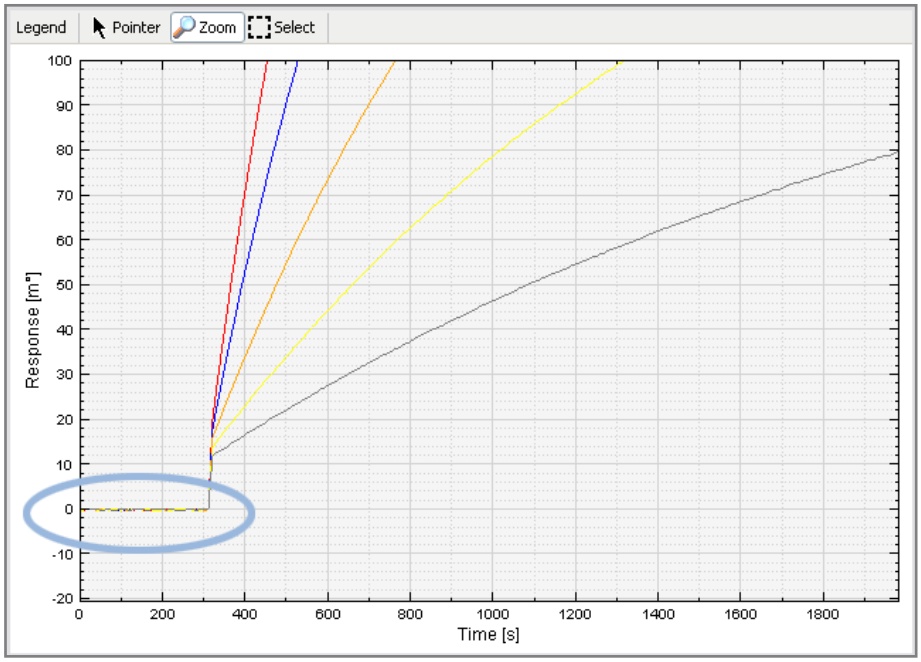


Figure 5.34 – Result of the Zero at Average action

5.3 – Analysis Wizard

The *Analysis Wizard* is used to determine the kinetic binding rates of the interaction plots that are measured with the Autolab SPR Data Acquisition software.

This section gives an overview of the features of the Analysis Wizard. A step-by-step example of how to use this wizard is given in the *Getting Started with the KE Workflow* section of the manual (section 3.5).

The Analysis Wizard can only be opened when a Project file (section 4.5) is created or a saved Project file is opened from disk.

To launch the Analysis Wizard click **Tools→Analysis Wizard** from the *Main Menu* or click on the **Analysis Wizard** item in the *Activity Bar* as shown in Figure 5.35.

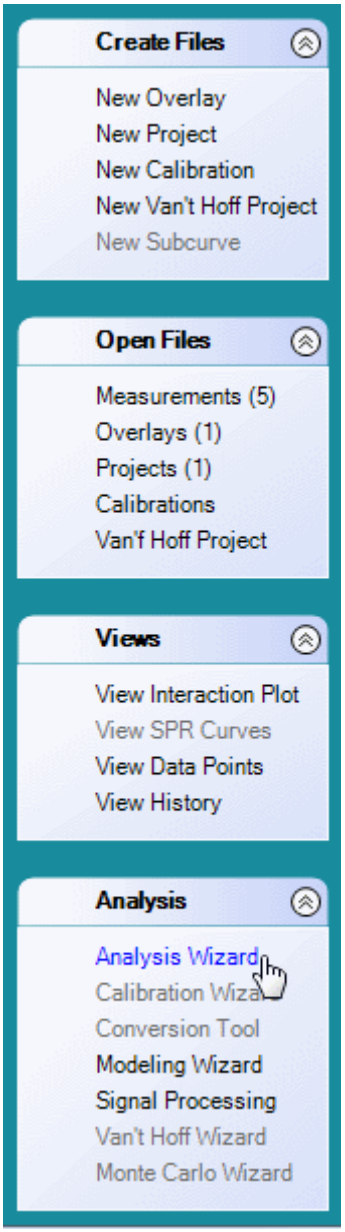


Figure 5.35 – Launching the Analysis Wizard from the Activity Bar

The screenshot below (Figure 5.36) depicts the first step that is shown when the Analysis Wizard is opened.

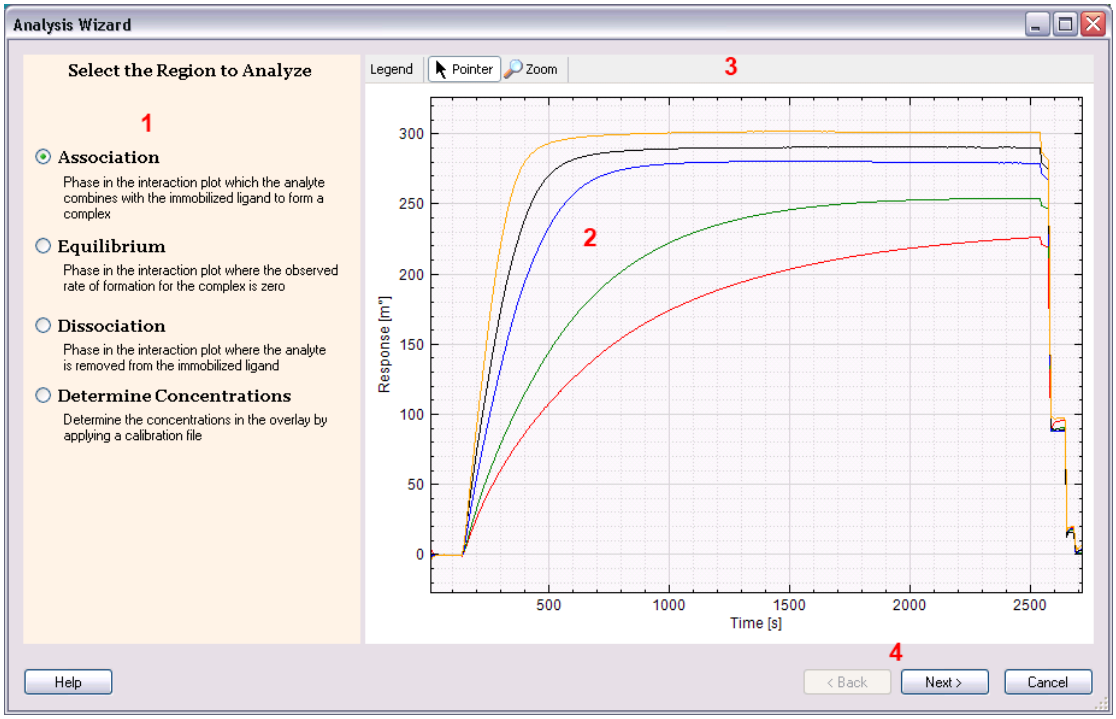


Figure 5.36 – The Analysis Wizard

The following descriptions correspond to the numbers that appear in Figure 5.36.

1. **Action Area** – This region of the Analysis Wizard is where all the user options and configurations take place. Each step in the wizard will present a new panel.

2. **Overlay Data** – The Analysis Wizard uses the Overlay data from a Project file. This graph will display the Overlay plots and eventually will show only the region of the plots that is selected by the user to analyze (section 5.3.3).

3. **Graph Toolbar** – The figure below depicts the tools that are available for the Analysis Wizard dialog. The *Plots* toolbar option is not available until the end of the Analysis Wizard process (see section 5.3.7).

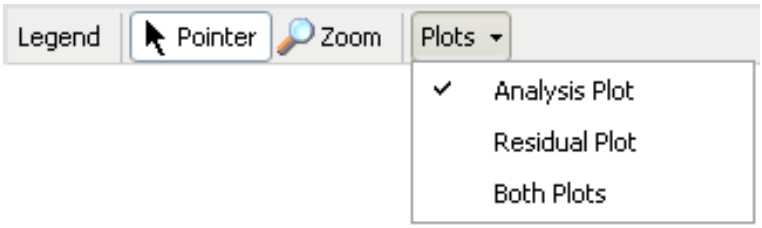


Figure 5.37 – Analysis Wizard Toolbar

4. **Navigation Buttons** – To move step-by-step through the Analysis Wizard use the **Next** button. To move to a previous step in the wizard

use the **Back** button. To quit the wizard at anytime use the **Cancel** button.

The following subsections provide a detailed overview of each step in the Analysis Wizard.

5.3.1 – Selecting the Region to Analyze

The first step in the Analysis Wizard is to specify which type of analysis is to be preformed. Figure 5.38 shows the first step of the wizard.

The available analysis options are:

- **Association** – Region in the Overlay where the analyte (injected sample) combines with the immobilized ligand (sample on the gold disk) to form a complex. This is the region in the graph that has a positive slope.

- **Equilibrium** – Region in the Overlay when there are no more ligand locations remaining for the analyte bind with. This is the region in the graph where the slope of the line approaches zero.

- **Dissociation** – Region in the Overlay where the analyte is removed from the immobilized ligand. This region of the graph has a negative slope.

- **Determine Concentrations** – The concentrations of an overlay can be set from a previously calculated Calibration curve (See Section 6 for creating Calibration curves). Section 5.3.13 details how to apply a calibration curve to an overlay to determine the concentrations.

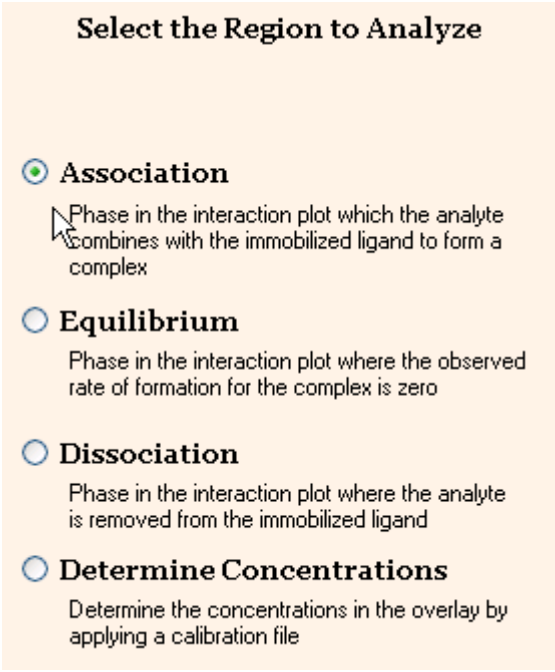


Figure 5.38 – Select the Region to analyze

Note

Equilibrium Analysis contains different steps in the Analysis Wizard. The Equilibrium Analysis process is described in the following sections:

a.) 5.3.8
b.) 5.3.9
c.) 5.3.10
d.) 5.3.11
e.) 5.3.12

Determine Concentrations contains different steps in the Analysis Wizard. These steps are described in the following sections:

a.) 5.3.13
b.) 5.3.14
c.) 5.3.15
d.) 5.3.16

5.3.2 – Specify the Concentrations

The second step of the Analysis Wizard is to specify the concentrations of the interaction plots.

As shown in Figure 5.39, the concentration value is entered and the unit of the concentration is specified.

Note

If the concentration of the interaction plot is for example 100 nM then enter in 100 and select the nM units. Do not enter 100e-9 unless the M unit is selected.

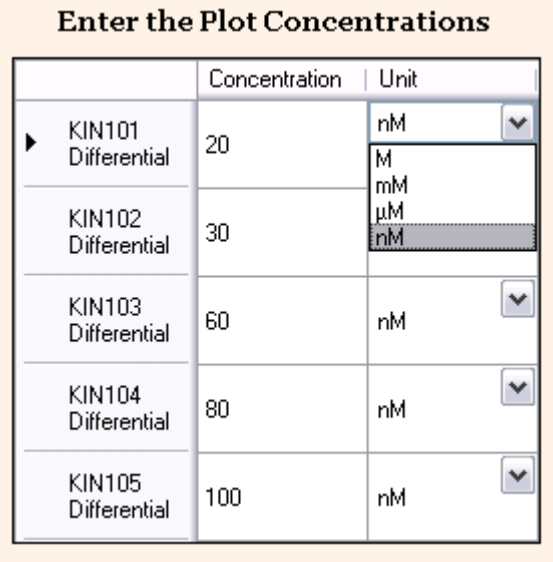


Figure 5.39 –Specifying the Concentrations

It is possible that the concentrations were already supplied before running the Analysis Wizard. One such method to determine the concentrations is to run the Analysis Wizard and select "Determine Concentrations" from a Calibration curve. This process is detailed in section 5.3.13.

If the concentrations were determined from a Calibration curve, the user will get a warning.

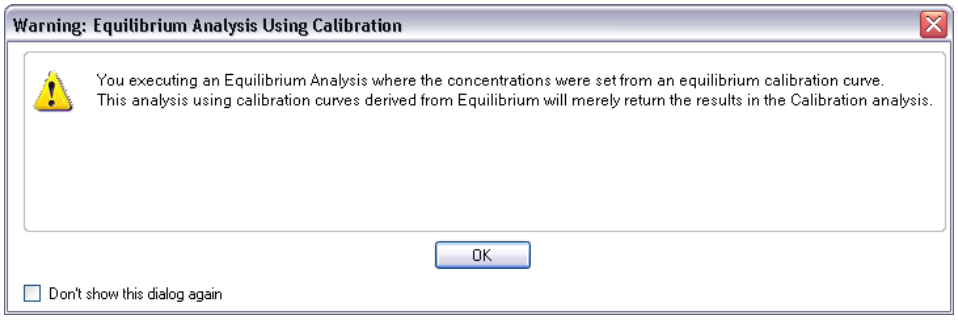


Figure 5.40 -- Equilibrium concentration warning

Because the concentrations were determined from a Calibration curve, and the calibration curve uses an equilibrium analysis (see section 6.1), running the equilibrium analysis will simply return the same analysis from the calibration analysis.

5.3.3 – Specifying the Start/End Times of the Analysis

The next step in the Analysis Wizard is to specify the start and end times for the type of analysis region that was selected.

In general the following guidelines should be followed when selecting the start/end times:

- *Association* – The start time is the start of the positive sloped curve while the end time is where the slope levels off towards zero.

- *Equilibrium* – Use the same starting point as Association and the end time will be the time right before the slope changes to a negative value (this is the start of Dissociation).

- *Dissociation* – The start value is the start of the region with a negative slope while the end region is where the slope levels off to zero.

To select the start and end times the following instruction dialog will be presented to the user.

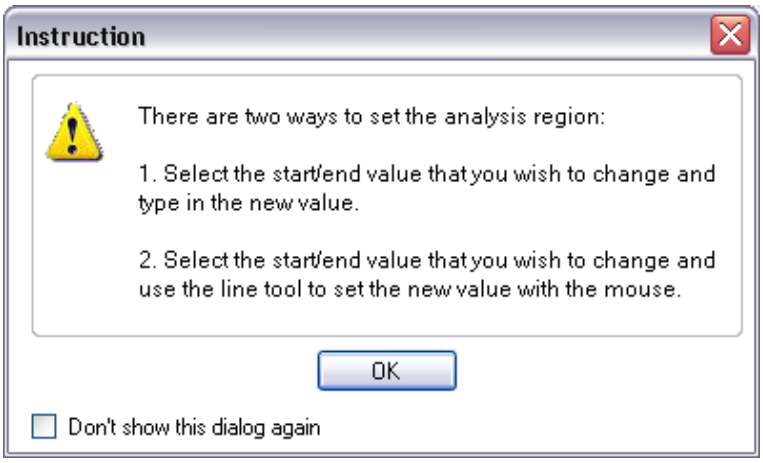


Figure 5.41 –Instructions for Selecting Start/End Times

This dialog (Figure 5.41) explains that there are two methods of setting the analysis times. If you don't require this message it can be removed from the wizard by clicking the **Don't show this dialog again** checkbox and clicking **OK**.

The first method is to manually enter the times. To enter a value, double-click the desired cell and enter in the time. Figure 5.42 shows the end time for *KIN101 Channel 1* being manually entered.

There are two Region Options to select on the dialog. The default, **Same Region for All Plots**, will copy your entered time to all interaction plots. The **Unique Regions for Each Plot** will allow the user to enter custom start/end times for each plot.

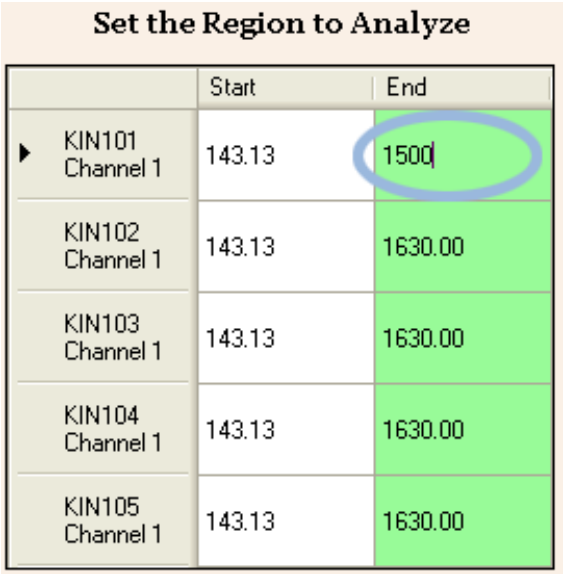


Figure 5.42 – Changing the End Point Manually

The second method to change the analysis times is to use the graph's Line Tool to visually set the times.

The steps to use this method are:

- Click (single click) on the time that you wish to change
- Click on the graph Line Tool (Line button in the toolbar) to activate it.
- Drag the mouse pointer over the graph to select the desired time
- Click (single click) to set the time

Figure 5.43 highlights the items that are used in this method.

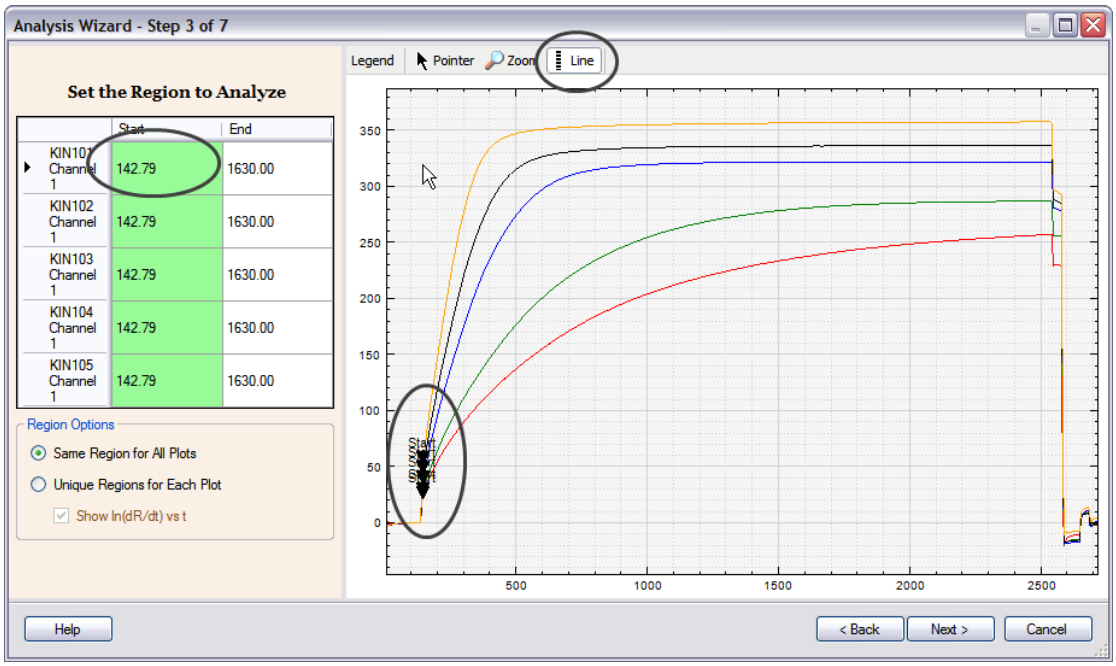


Figure 5.43 – Using the Line Tool to Select the Start Time

It is possible to obtain additional information about the association starting point of the curve. Selecting **Unique Regions for Each Plot** and Show **ln(dR/dt) vs. t** will overlay the logarithm of the derivative versus time, and can be useful to see mass transport effects. This option is only available when unique regions are used for each plot. The user has to set each starting and ending point for each plot. The overlay of the derivative curve is shown in Figure 5.44.

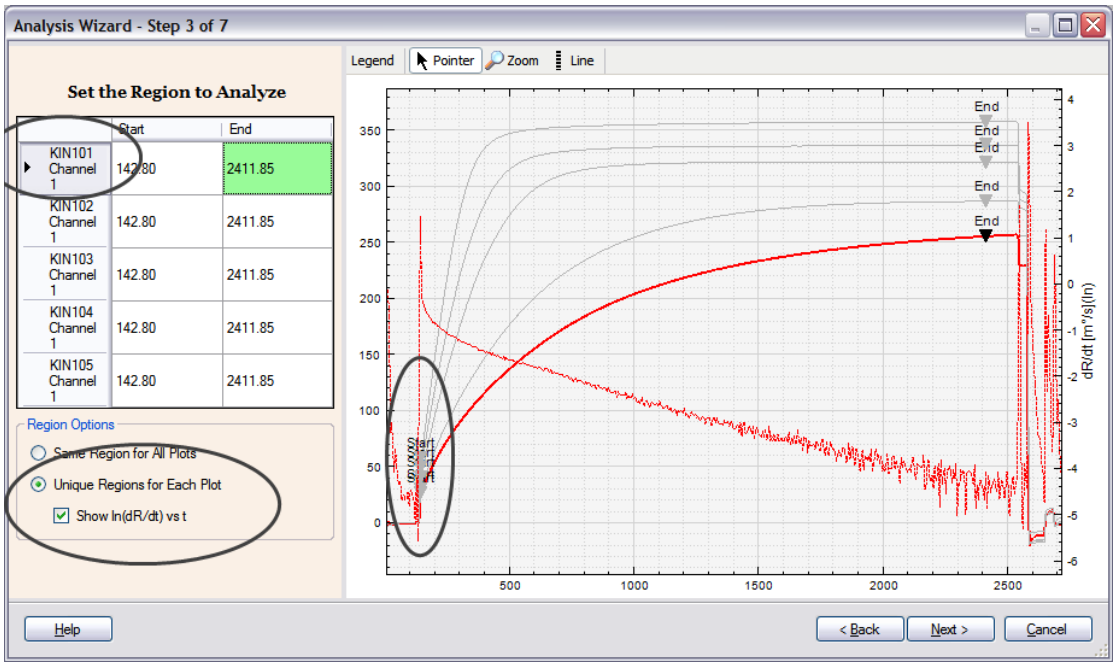


Figure 5.44 – Showing the derivative of the curve to aid in setting the Start Time

5.3.4 – Selecting the Analysis Method

The next step in the Analysis Wizard is to select the method that will be used for the fitting of the data. Figure 5.45 depicts a screenshot of this step.

Note

This Analysis Wizard step is not required for Equilibrium Analysis.
For Equilibrium Analysis, skip to section 5.3.8.

The current Kinetic Evaluation application provides two analysis methods:

Integrated Rate Equation – These equations are pre-integrated before they are used in the fitting routine. Due to this pre-integration step this model cannot handle complex rate equations. The available integrated rate equations are *Monophasic* and *Biphasic*. It is not possible for the user to create custom integrated rate equations.

Numerical Modelling – This is a more complex method in which the rate equations are integrated numerically during the fitting process. The benefit of having the rate equation numerically integrated is that more complex models can be used. It is possible for users to construct rate equations to use with the Numerical Modelling method (see section 5.1 for more details).

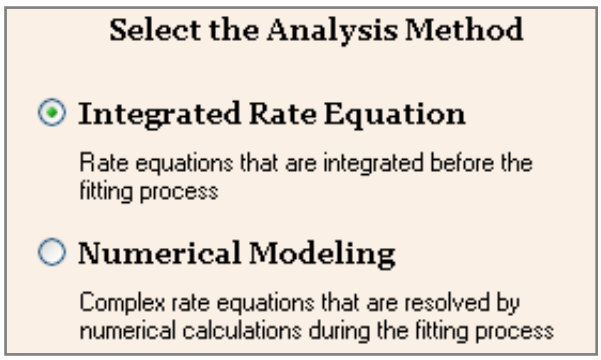


Figure 5.45 – Selecting an Analysis Method

5.3.5 – Selecting the Model to Use

The next step in the Analysis Wizard is to specify which model to use. This step differs depending on which analysis method was selected in previous step (see section 5.3.4).

Figure 5.46 is a screenshot of the models that are available for the Integrated Rate Equation method. The model is specified by selecting it from the drop-down list. A brief description is displayed for the currently selected model.

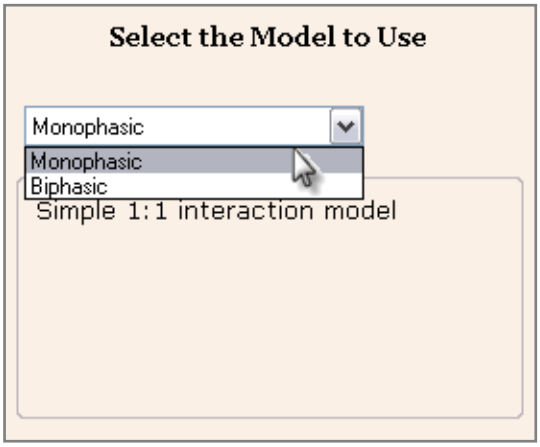


Figure 5.46 – Selecting the Integrated Rate Equation

If you have selected Dissociation analysis, a third model option will appear, **Rebinding Monophasic**, as shown in Figure 5.47. This is a monophasic model that incorporates terms related to rebinding[*].

[*] See, for example, Schuck and Minton, "Analysis of Mass Transport-Limited Binding Kinetics in Evanescent Wave Biosensors" *Analytical Biochemistry* 240, 262–272 (1996)

For the fit to the monophasic rebinding model to converge, it is necessary for the user to supply some input parameters, B_{max} and K_A/K_{tr} (see the Schuck and Minton paper for definitions). B_{max} can be determined from an equilibrium analysis, and if equilibrium analyses have been performed, it can be imported from an opened equilibrium analysis by using the combo selection box. The box is not shown if equilibrium analyses have not been performed. K_A/K_{tr} is typically a small number << 1.

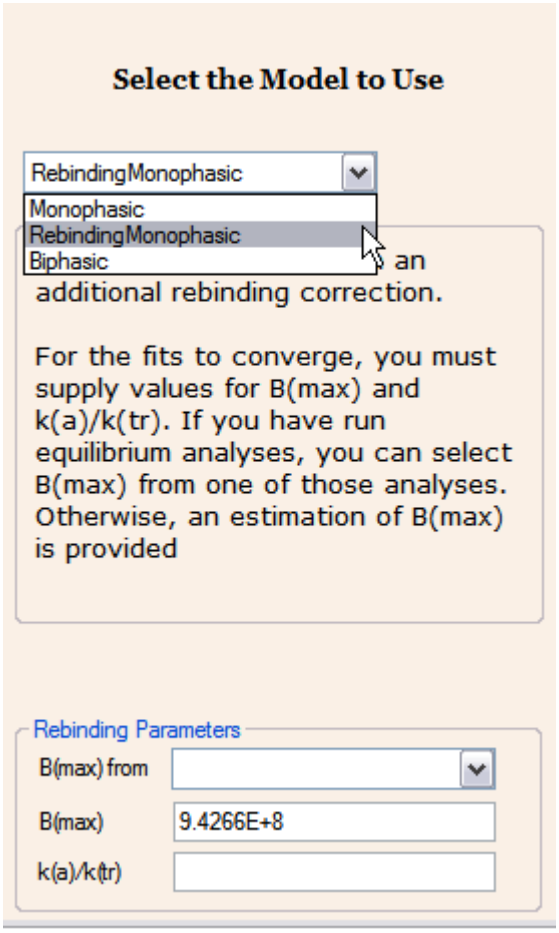


Figure 5.47 – Selecting the Integrated Rate Equation

Selecting a model for the Numerical Analysis method is similar to the previous process except the user can also load a custom model from disk.

The models in the drop-down list, as shown in Figure 5.48, are the models that are located in the Autolab SPR Models folder (**..\Autolab SPR\Models**). When a model is selected the model is displayed and the description of the model is shown.

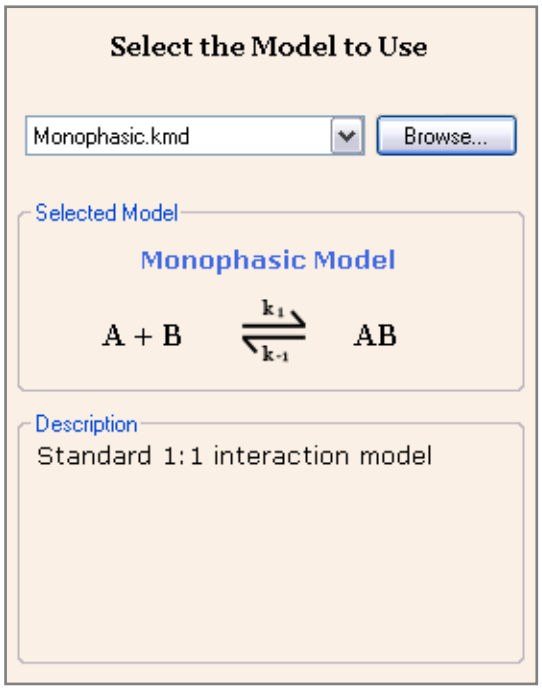


Figure 5.48 – Selecting the Numerical Analysis Method

To select a custom model use the **Browse** button to select a Kinetic Model Description file (*.kmd).

If the selected model does not already exist in the standard modelling folder then a prompt will be given to copy the selected model. Figure 5.49 shows this user prompt.

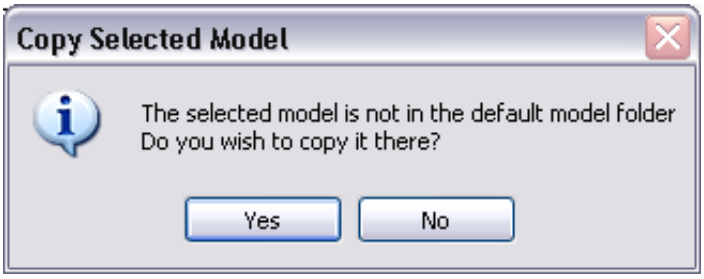


Figure 5.49 – Prompt to Copy a Model to the Default Model Folder

5.3.6 – Configuring and Fitting the Model

The next step in the Analysis Wizard is the configuration of the fitting parameters.

On first open of this step the information dialog, shown below, will be shown to explain that for best results use the **Test** button to ensure that the initial parameters are in range of the experimental data. *In range* means the model plots should be in the same order of magnitude as the experimental data.
If you do not wish to see the information dialog the next time the wizard is run click the **Don't show this dialog again** checkbox.

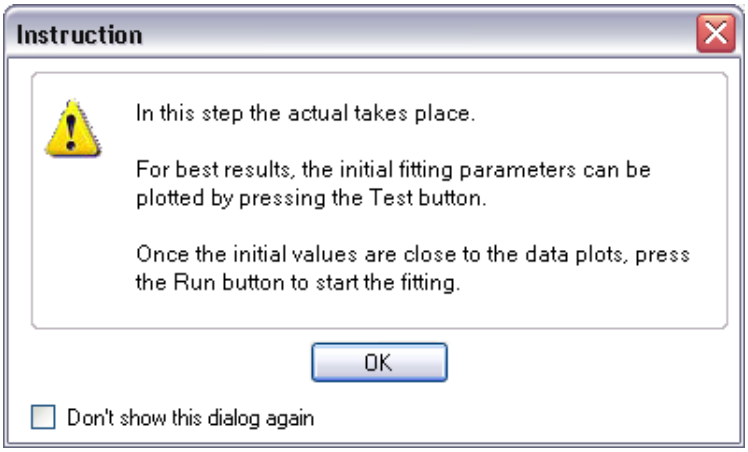


Figure 5.50 – Additional Instructions for Fitting Data

The configuration of the fitting parameters for the Integrate Rate Equation method (see Figure 5.51) and the Numerical method (see Figure 5.52) are quite similar. The difference between the two methods is that the Integrate Rate Equation method uses a separate set of fitting parameters for each interaction plot while the Numerical Modelling method uses one set of parameters for all plots.

Numerical Modelling uses the Global Analysis technique in which all of the plots are fit at the same time to the same set of parameters values. The benefit of using global analysis is that it ensures that the selected model does in fact fit to the experimental data because the binding constants are the same in all interaction plots (only the concentrations differ). The initial values of the parameters are shown in Figure 5.52.

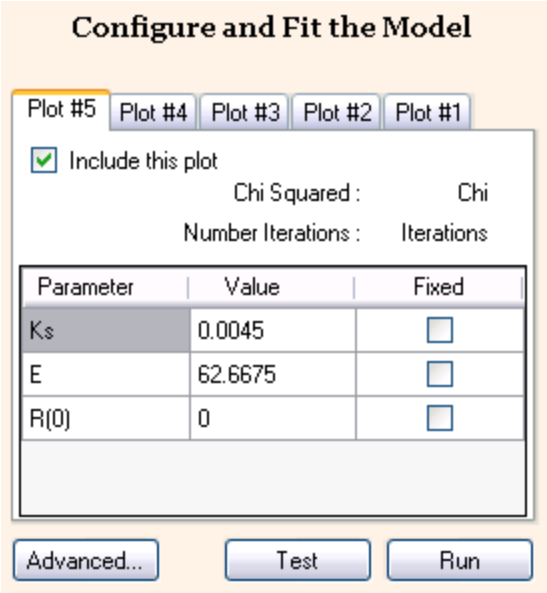


Figure 5.51 – Integrated Rate Equation Parameters

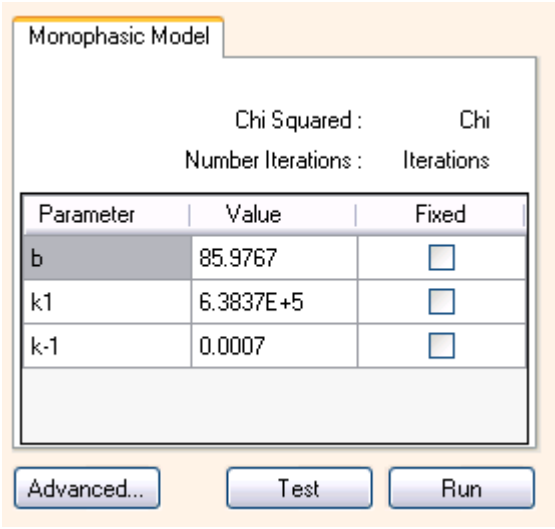


Figure 5.52 – Numerical Analysis Parameters

In certain numerical models, such as mass transport, the forward and reverse rate equations may need to be kept equal. The fit can be forced to fix one parameter equal to the value of another parameter. This can be accomplished by setting the value of the second parameter "=" sign and the name of the first parameter to which it should be fixed. This is shown in Figure 5.53.

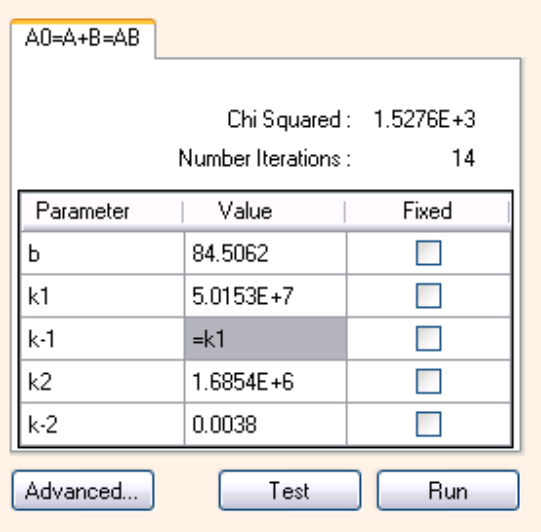


Figure 5.53 – Fixing a numerical fitting parameter

In this case, the fit will be evaluated with K-1 always having the whatever value the fit finds for K1.

To start the fitting process, after the initial parameters have been tested, click the **Run** button. During the fitting process the model plots (green lines) will be updated on the graph for each successful improvement in fitting to the experimental data.

The **Advanced** button is used to configure the parameters of the fitting routine. The Advanced Setting dialog, shown in Figure 5.54, consists of the following parameters.

- **Step Size** – Step size between iteration
- **Number Steps** – Number of steps with defined step size
- **Max. Iterations** – Maximum number of iterations

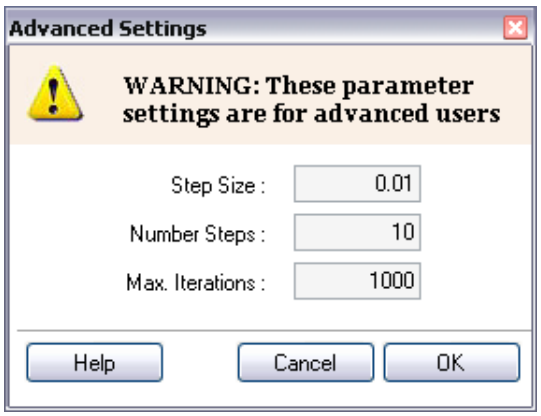


Figure 5.54 – Advanced Fitting Parameters

5.3.7 – Viewing the Analysis Summary

The last step of the Analysis Wizard is a summary of the process that was executed.

The figure below is a screenshot of the summary step for an Integrated Rate Equation analysis. The final parameters for each interaction plot are listed as well as the final modelling plot (green plots).

To copy this data to another application use the **Copy to Clipboard** button.

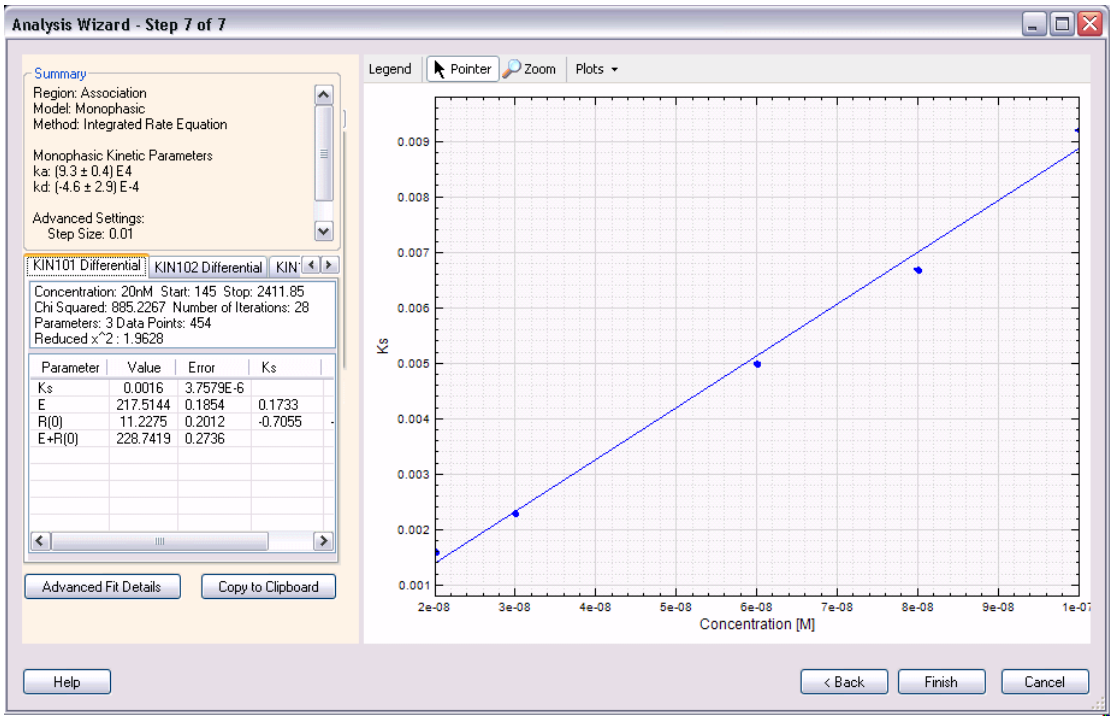


Figure 5.55 – Summary for the Integrated Rate Equation Fit

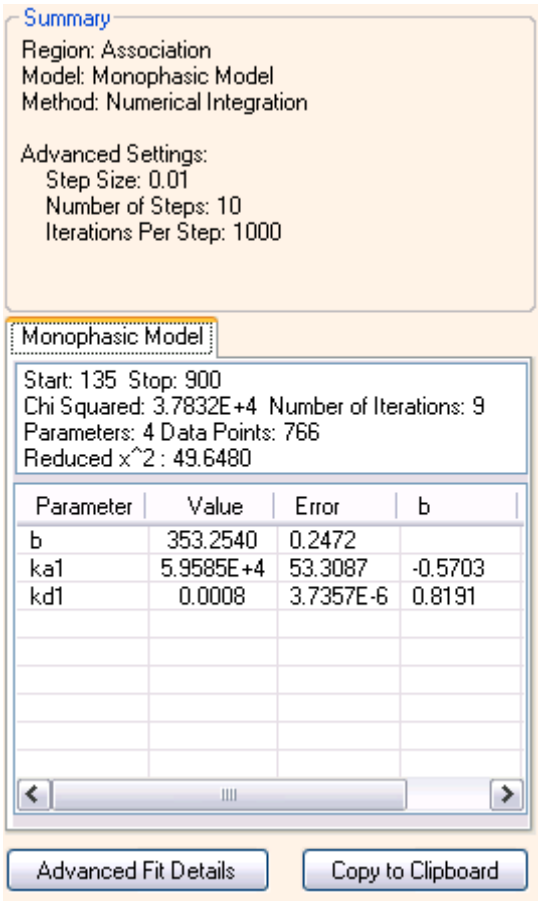


Figure 5.56 – Summary for the Numerical Analysis Fit

Once fitting procedure has been completed it is possible to view the goodness of fit of the model using a Residual plot. To view the Residual plot, click **Plots→Residual** from the Toolbar as shown in the figure below.

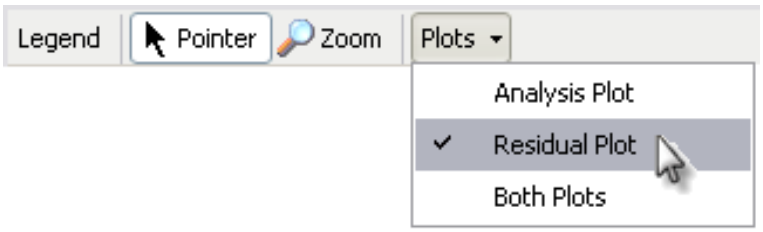


Figure 5.57 – Displaying the Residual Plot

The Residual plot shows the difference between the model and experimental data. A model that is a good fit will have a residual plot where all the data points are randomly scattered around zero.

Figure 5.58 is a screen shot of a Residual plot for the example data. From this plot we can see that the model selected does not fit the data. The next step would be to repeat the analysis process with the *Biphasic* model.

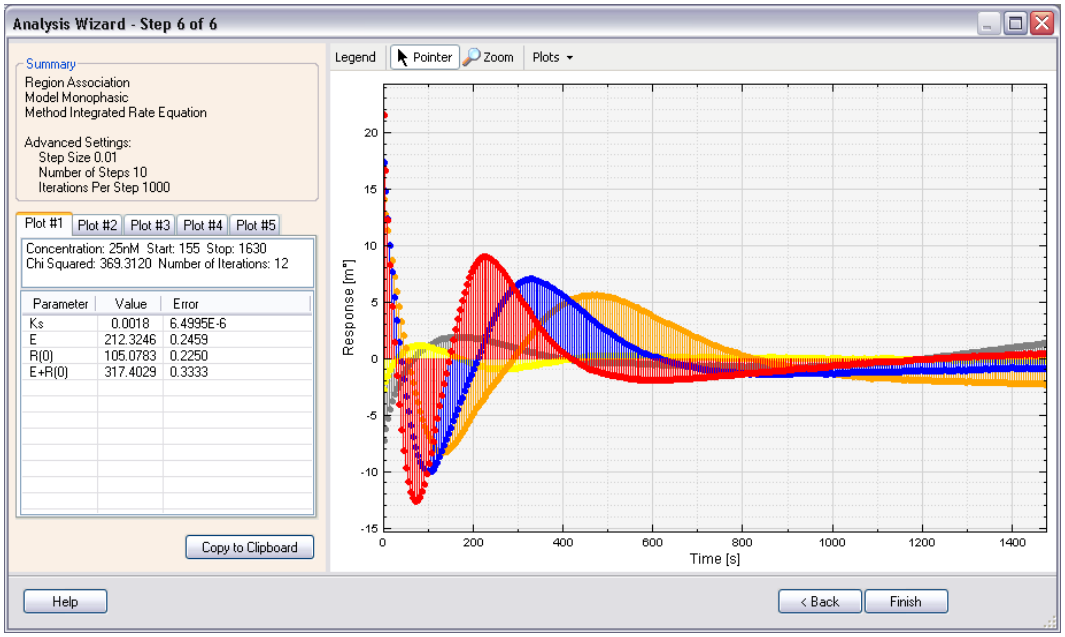


Figure 5.58 – Residual Plot

The following figure is of an analysis in which the model fits the experimental data well. Notice that the residual plot in randomly scattered around zero.

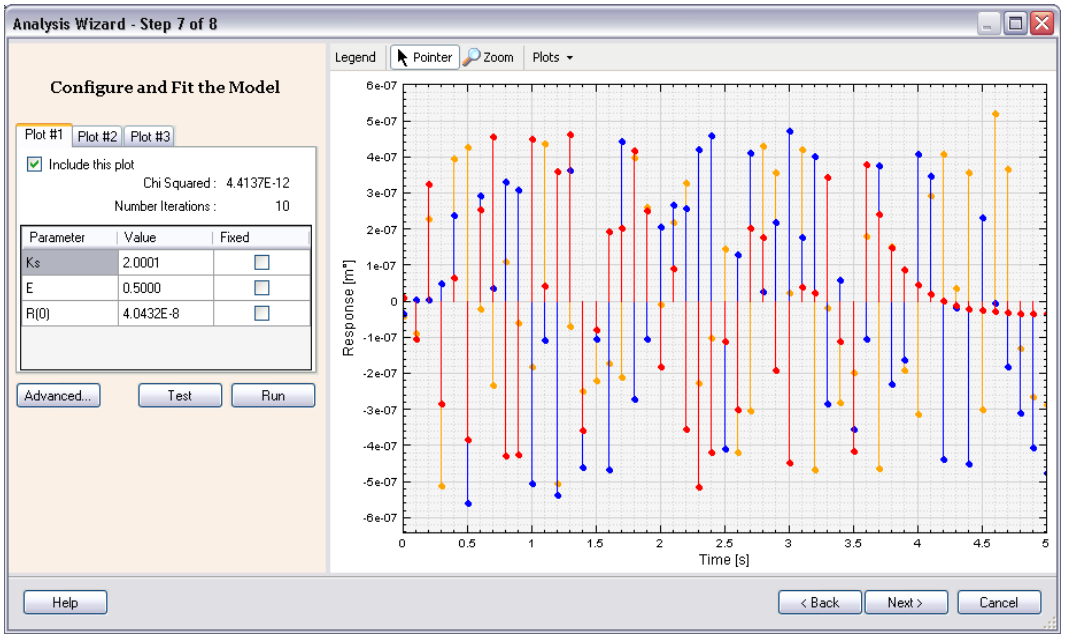


Figure 5.59 – Example of a Well Fitting Model

The summary data will be saved with the analysis and added to the Project once the **Close** button on the wizard is clicked. The summary of the analysis will be still available by clicking on the *Main Menu* **View→Summary** or by selecting the **View Summary** option in the *Activity Bar*.

5.3.8 – Equilibrium Analysis

Equilibrium analysis uses a number of different steps from either Association or Dissociation analysis.

Note

This section is for Equilibrium Analysis Only.

5.3.9 – Equilibrium Analysis: Plots at Equilibrium

The first distinction follows immediately after setting the start and end points for the plots. In step 4, each plot is fitted to see if it has reached equilibrium. First, a dialog discussing the fit appears, as shown in Figure 5.60.

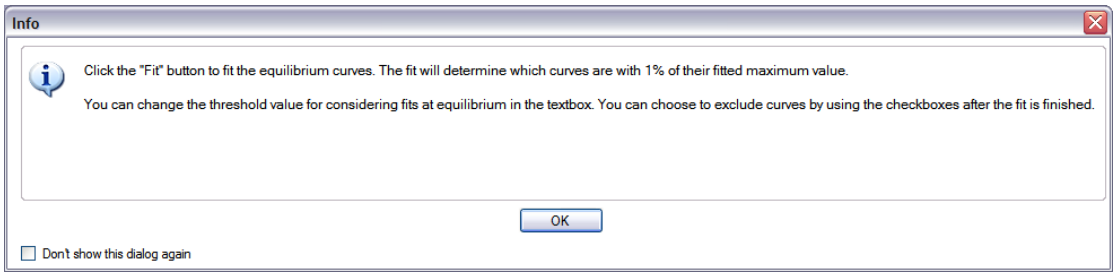


Figure 5.60 – Dialog indicating a fit for equilibrium will occur

Once the dialog is dismissed, the user has the option to test each plot to determine if it is at equilibrium. The data table first shows the maximum value of R_{eq} determined from the data. The maximum value as estimated from a fit is not yet shown. To fit the data, click on the **Fit** button. This is shown in Figure 5.61. Each curve will be considered at equilibrium if:

- Its maximum value from the data and from the fit are within the threshold value (in Figure 5.61, that is 1%),
- Or the maximum value from the data is greater than the fit maximum value.

The fit threshold can be changed in the text box if necessary.

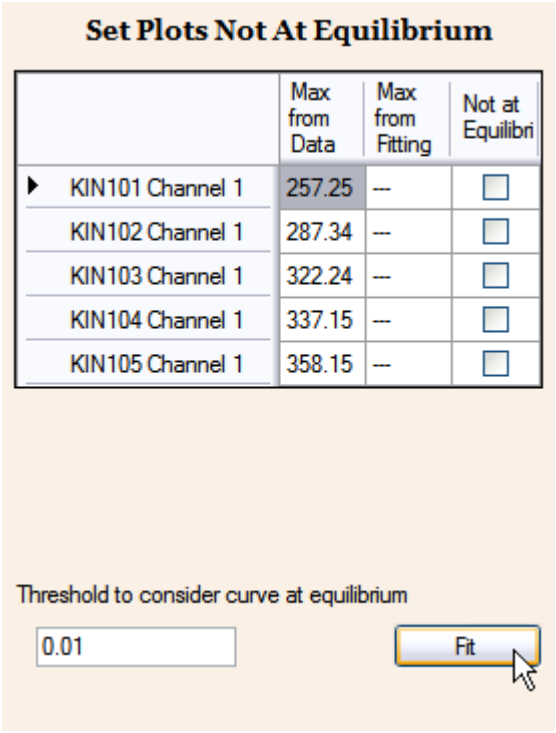


Figure 5.61 – Table of equilibrium plots before fitting for equilibrium

Once the **Fit** button is clicked, each curve will be fit for equilibrium and the results presented, as shown in Figure 5.62. In the figure, the maximum from the fit is shown in the column next to data column. If the fit indicates that the plot is not at equilibrium, the **Not at Equilibrium** checkbox will automatically be checked. This is shown in the area marked **1** in Figure 5.62.

Furthermore, the extrapolated fits will be shown in the area marked **2** in Figure 5.62. This is shown to aid the user in deciding the goodness of fit. If the user believes the plot is not at equilibrium, the user can check the **Not at Equilibrium** checkbox to use the value from the fit instead of the value from the data.

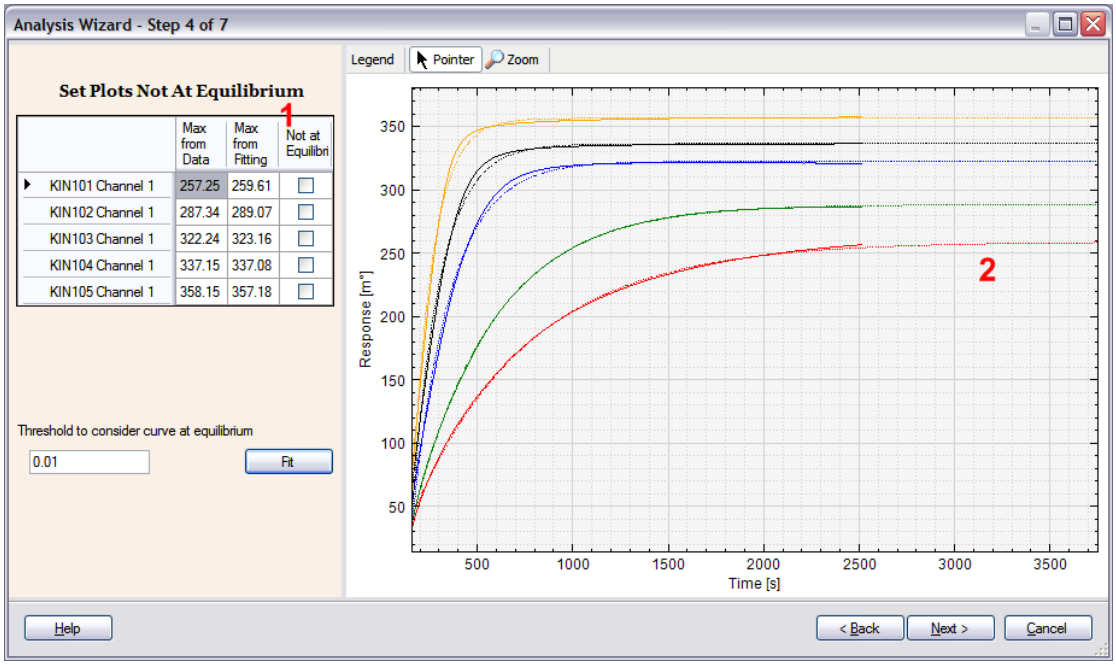


Figure 5.62 – Equilibrium via extrapolation

5.3.10 – Equilibrium Analysis: Outlier Analysis

Step 5 of an Equilibrium analysis will attempt to identify outlier points on the equilibrium plot. Outliers are identified by using a Lorentz fit as described in the paper by Motulsky and Brown*. Standard fitting techniques assume that the data points are Gaussian distributed around the true values. The Gaussian distribution has large "tails" and the fits are pulled by these tails to towards outliers. The Lorentz fitting technique replaces the assumption that the data points are Gaussian distributed with the assumption that the data points are Lorentz distributed. Because the Lorentz distribution has narrower "tails," it is not affected by outliers to the degree that the Gaussian fit is. This technique allows for more robust identification of Outliers.

On entry into Step 5, a dialog box is shown that indicates if the Lorentz fit identified any outliers. This dialog is shown in Figure 5.63.

* Motulsky and Brown, "Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate", *BMC Bioinformatics*, 7:123 (2006)

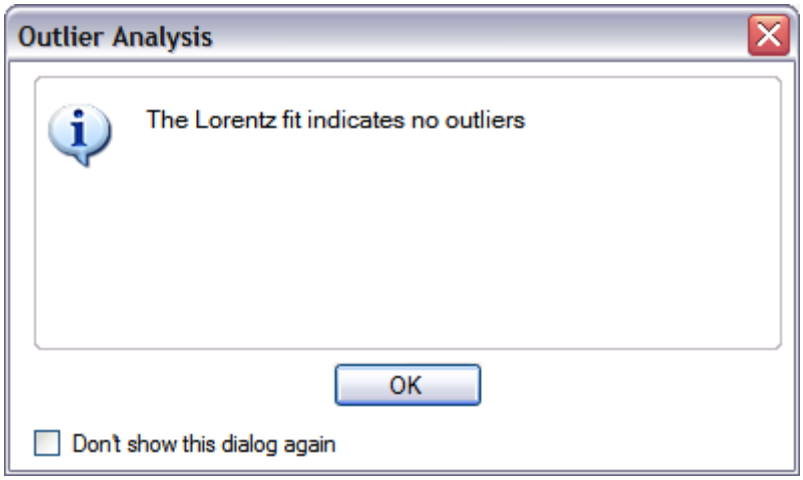


Figure 5.63 – Dialog box indicating the presence or absence of outliers

Figure 5.64 shows the results of the outlier analysis. The blue curve shows the fit using the Lorentz analysis. The red curve is the standard Gaussian fit. In the figure, the two curves are nearly identical, indicating that although the second data point might appear to be an outlier, it is not.

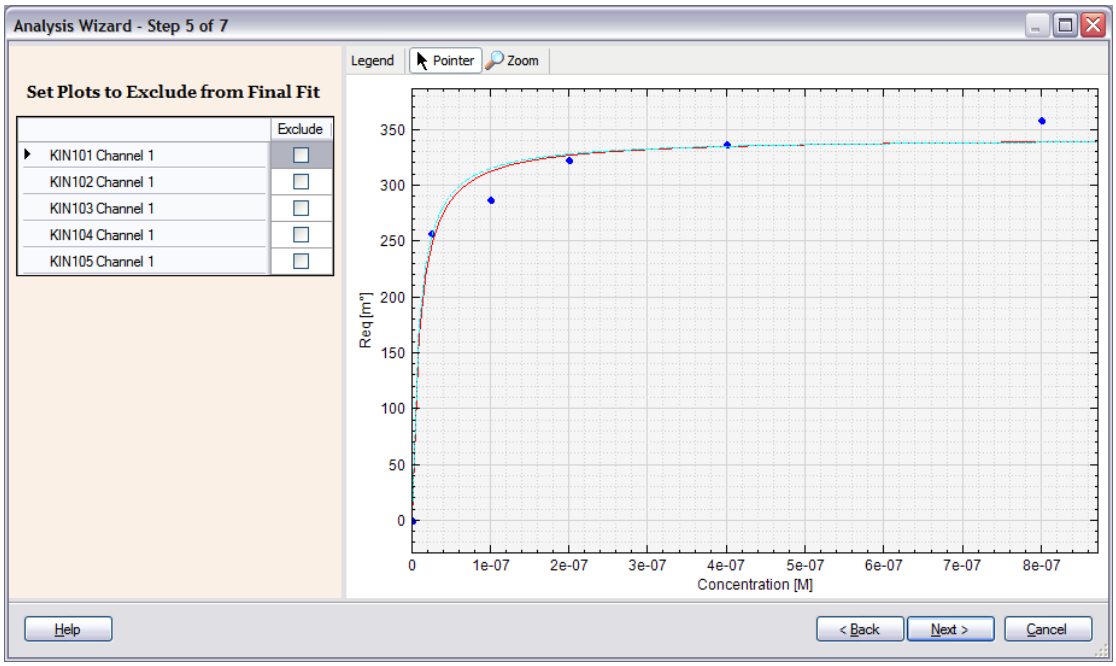


Figure 5.64 – Outlier analysis showing the Lorentz fit (blue) and the standard Gaussian fit (red)

The table on the left allows the users to manually exclude data points that the user believes are outliers. When a data point is excluded, the Lorentz curve (blue) remains the same, while the Gaussian fit (red) is updated to ignore the excluded points. Figure 5.65 shows the plot with two excluded points (the red “X” data points).

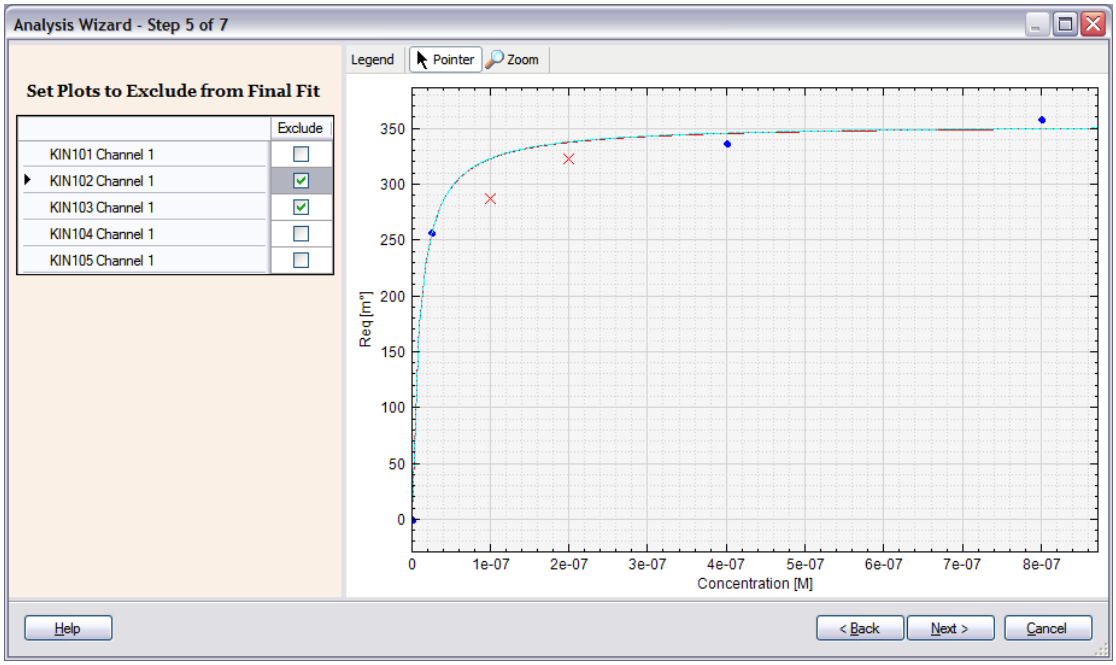


Figure 5.65 – Outlier analysis with two excluded data points

5.3.11 – Equilibrium Analysis: Analysis

The last two steps of the Equilibrium Analysis do not require user interaction. Once the concentrations and start/end times are configured a Response at Equilibrium (R_{eq}) vs. Concentration graph will be plotted.

By nonlinear fitting of the data in the R_{eq} vs. Concentration graph, as shown in Figure 5.66, the affinity constant (K_D) and B_{max} values can be determined.

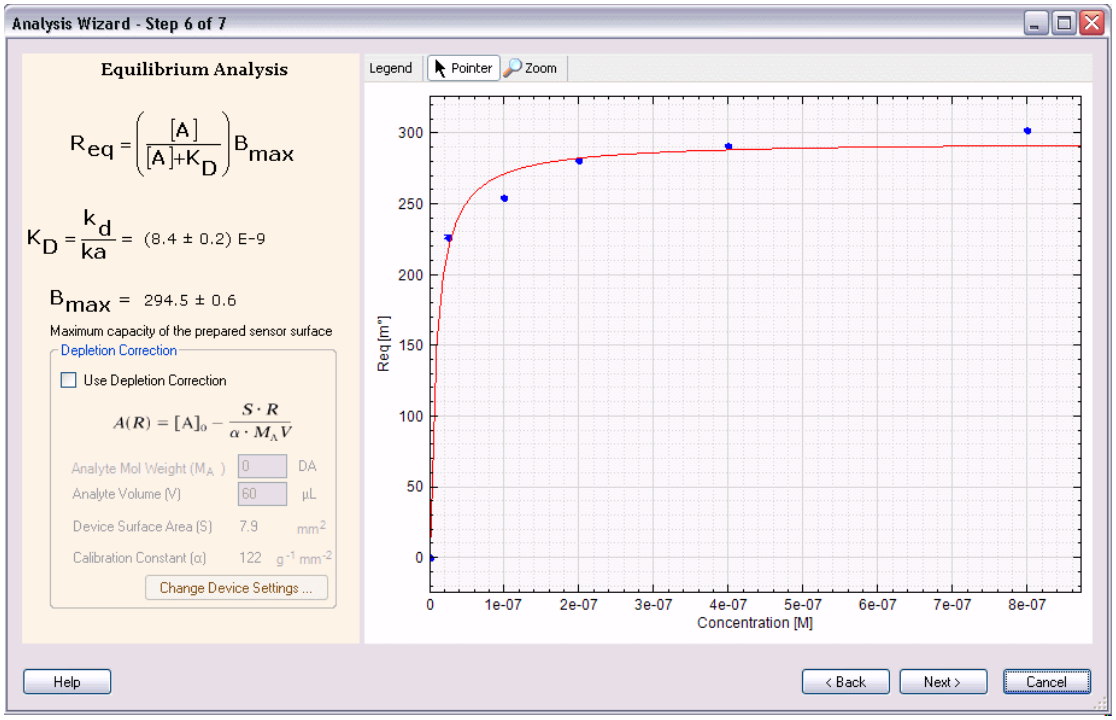


Figure 5.66 – Equilibrium Analysis

The user can modify the results of the fit by making a depletion correction[*]. The depletion correction depends on the analyte molecular weight and the volume of the analyte in the Autolab SPR. By default, the analyte volume is 60 µl for the Autolab SPR. The effect of the depletion correction is shown in Figure 5.67. Here, a depletion correction was applied that changed both K_D and B_{max}.

The standard way to apply a depletion correction is to first adjust all of the concentrations according to the formula:

$$A(R) = [A]_0 - \frac{S\,R}{\alpha\,M_A V}$$

where S is the device surface area, α is the calibration constant, M_A is the analyte molecular weight and V is the analyte volume.

However, this would involve modifying the measured data. Instead, KE uses the fully integrated equilibrium equation[†]

$$R_{eq} = \frac{1}{2\beta}\left\{([A_0] + R_{\max}\beta + K_D) - \sqrt{([A_0] + R_{\max}\beta + K_D)^2 - 4[A_0]R_{\max}\beta}\right\}$$

$$\beta = \frac{S}{\alpha\,M_A V}$$

[*] See, for example, de Mol, N.J and Fischer, M.J.E, "Kinetic and Thermodynamic Analysis of Ligand-Receptor Interactions: SPR Applications in Drug Development" *Handbook of Surface Plasmon Resonance*. Ed. Richard B.M. Schasfoort and Anna J. Tudos. Cambridge: RCS Publishing, 2008. 123-172

[†] See equation 23 of Edwards, P.R., *et al*, "Second-Order Kinetic Analysis of IAsys Biosensor Data: Its Use and Applicability" *Anal Biochem*, **263**, 1-12 (1998).

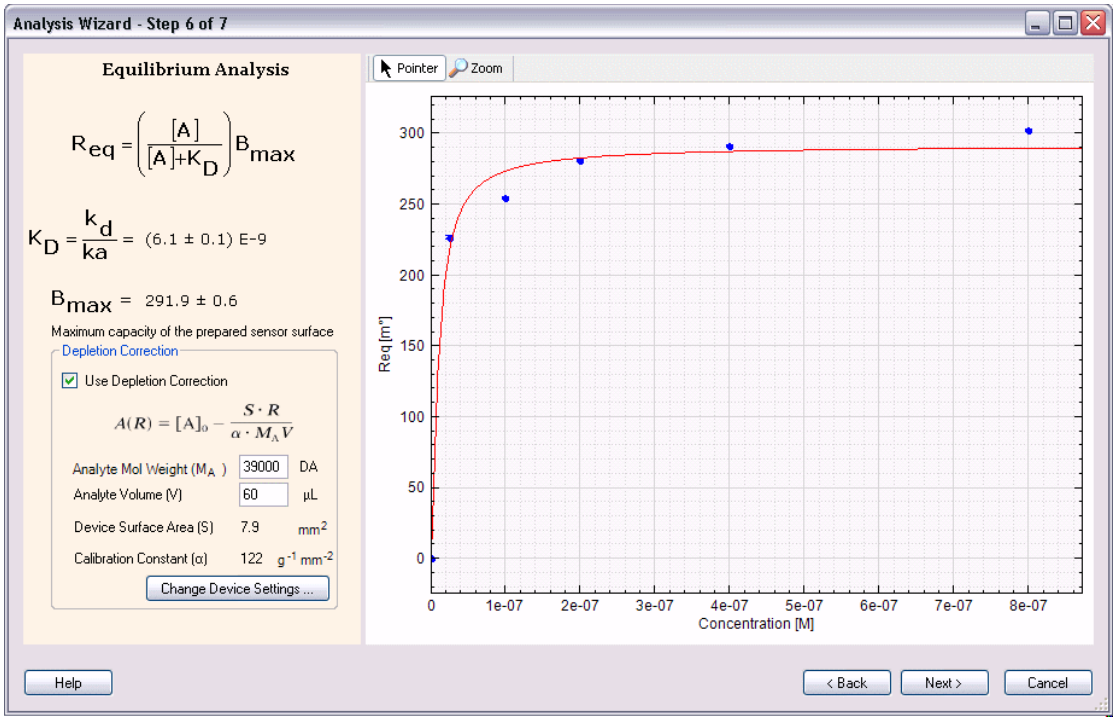


Figure 5.67 – Equilibrium analysis after applying a depletion correction

5.3.12 – Equilibrium Summary

The last step in the Equilibrium Analysis is the summary.

> **Note**
>
> This section is for Equilibrium Analysis Only.

The Equilibrium Analysis summary step, as shown in the figure below, shows the fitted data on the R_{eq} vs. Concentration graph and the data points used to construct the graph. The affinity constant (K_D) and the B_{max} value are also presented.

The data on the summary step can be transferred to another application by clicking the **Copy to Clipboard** button.

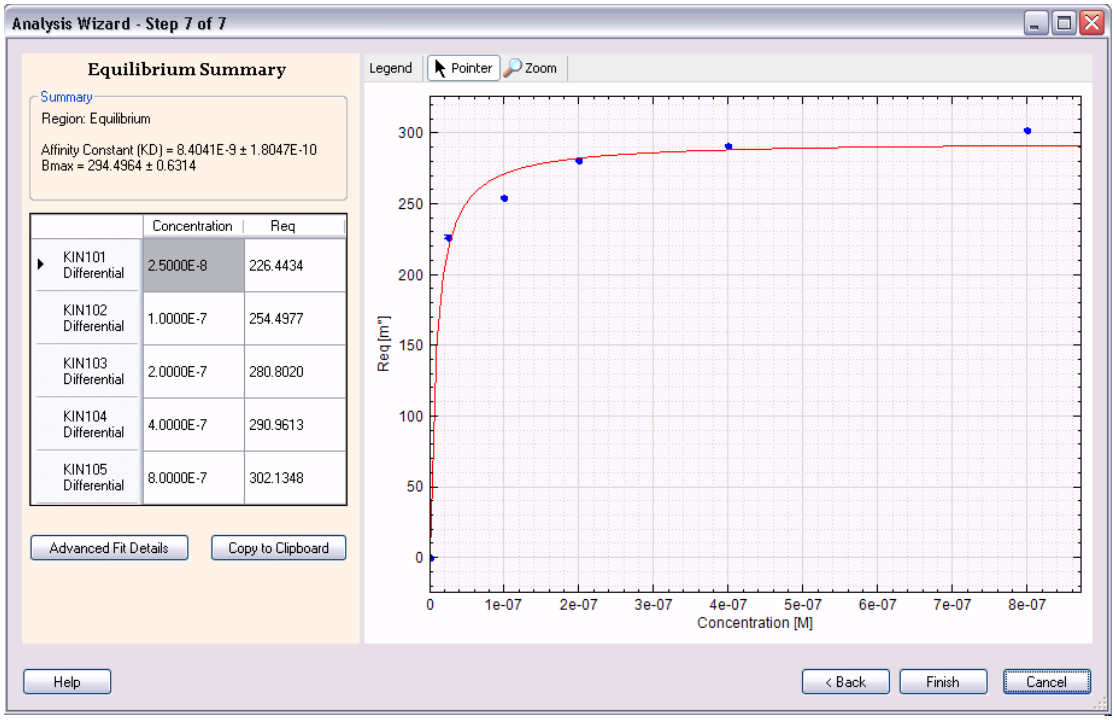


Figure 5.68 – Equilibrium Summary

The summary data will be saved with the analysis and added to the Project once the **Close** button on the wizard is clicked. The summary of the analysis will be still available by clicking on the *Main Menu* **View→Summary** or by selecting the **View Summary** option in the *Activity Bar*

5.3.13 – Determine Concentrations

The analysis wizard can be used to set the concentrations of a set of measurements by applying a concentration Calibration curve. Creating a Calibration curve is described in Section 6.

To calculate the concentrations for a set of measurements from a Calibration, select "Determine Concentrations" on the first panel of the Analysis Wizard, as shown in Figure 5.69.

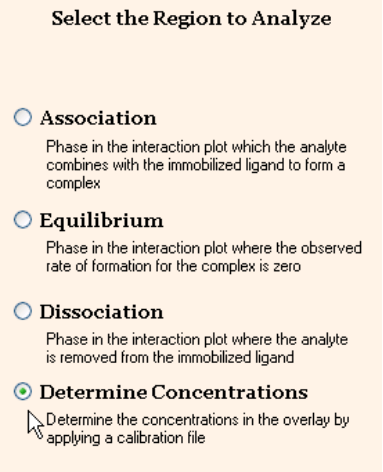


Figure 5.69 – Select Determine Concentrations

The analysis wizard will proceed in four steps. Each step is similar to a step in the Equilibrium analysis, but the steps occur in a different order. Because setting the concentrations from a calibration curve uses an equilibrium analysis, the user should not use this method to set the concentrations if they then want to perform an equilibrium analysis (see section 5.3.2). Doing so will simply return the equilibrium analysis that was initially used to create the calibration curve.

5.3.14 – Setting the Region to Analyze for Determining the Concentrations

The concentrations are determined by fitting each of the measurements via an equilibrium analysis. Therefore the next step is to set the region to fit, as shown in Figure 5.70

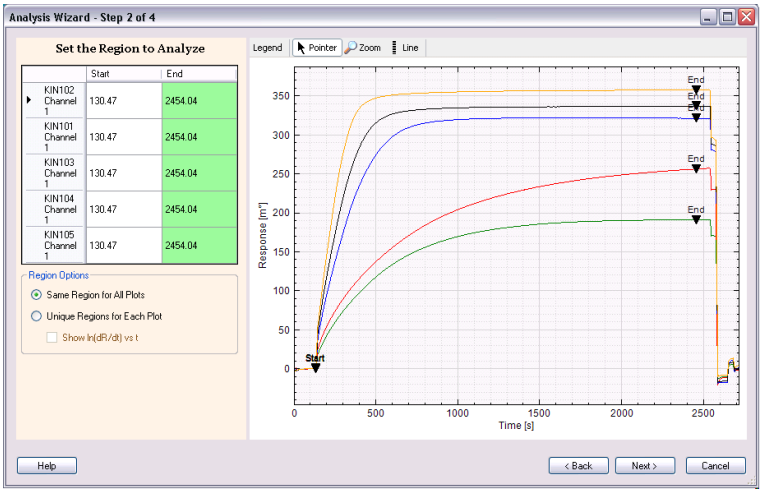


Figure 5.70 – Set the Start/End points for determining the concentrations

5.3.15 – Determine plots not at equilibrium

The next step is to determine which plots are not at equilibrium. By clicking the “Fit” button as shown in Figure 5.71, the plots will be fit to a set of equilibrium curves. However, it is not required to try to determine if the curves are at equilibrium; if the “Max from Data” valid, the user can elect to continue to the next step by clicking the “Next” button.

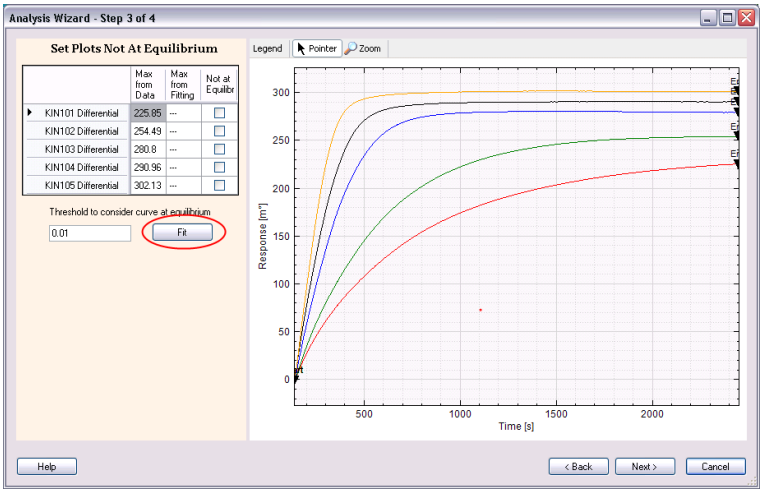


Figure 5.71 -- Apply Equilibrium fit to data

If the user decides to fit the plots, the plot panel will show the data along with a regression curve, as shown in Figure 5.72.

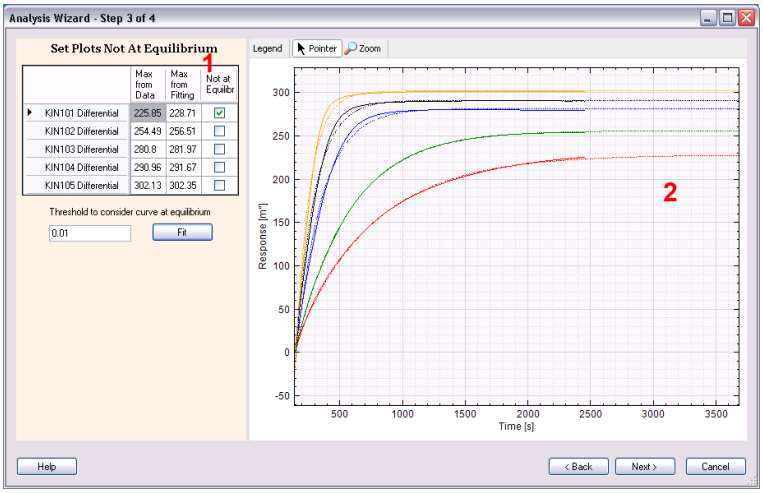


Figure 5.72 -- After Equilibrium fit

Similar as was shown in the Equilibrium Analysis (Figure 5.62), the “Determine Calibrations” analysis will show plots not at equilibrium. The **Not at Equilibrium** checkbox will automatically be checked. This is shown in the area marked **1** in Figure 5.72. Furthermore, the extrapolated fits will be shown in the area marked **2** in Figure 5.72. This is shown to aid the user in deciding the goodness of fit. If the user believes the plot is not at equilibrium, the user can check the **Not at Equilibrium** checkbox to use the value from the fit instead of the value from the data. In Figure 5.72, only “KIN101 Differential” (the red plot) is considered to be Not at Equilibrium.

5.3.16 – Apply the Calibration file

The final step of determining the concentrations is to apply the calibration curve to the equilibrium data. This process is shown starting in Figure 5.73.

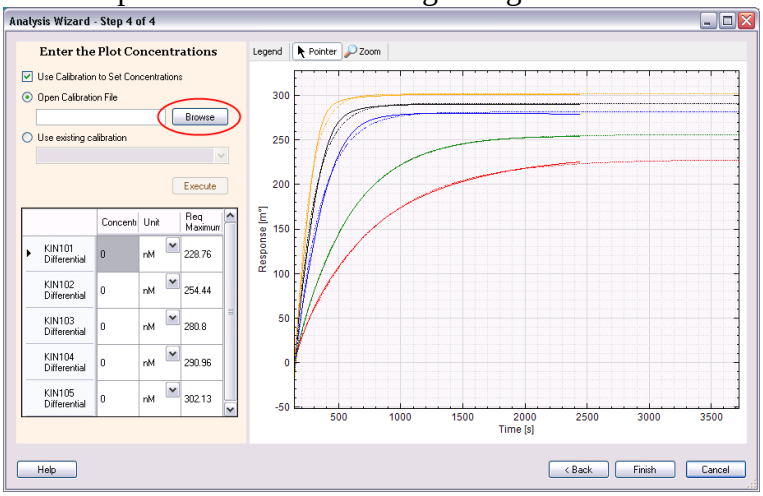


Figure 5.73 -- Applying the Calibration curve

Initially, the user must select a calibration to use to calculate the concentrations. The user can select to use

- A Calibration file (.kcf) loaded from disk, as shown in the circled "Browse" button
- A previously loaded Calibration, which will be shown in the "Use existing calibration" combo-box.

Once a calibration is selected, the calculation can be executed, as shown in the circled "Execute" button in Figure 5.74.

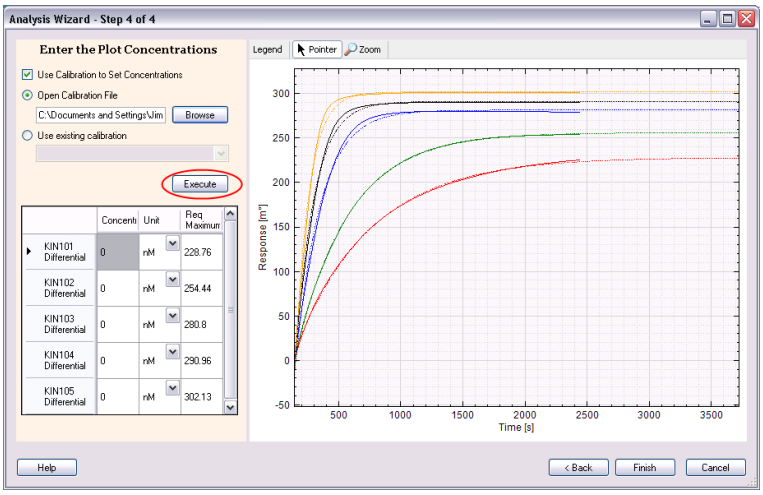


Figure 5.74 -- Loading a Calibration File in preparation to execute the calibration

Once the concentration calculation is completed, the calculated concentrations will be shown in the table (circled in Figure 5.75). In addition, the calibration curve will be shown, with blue data points for the original calibration curve data, and pink points

for the calculated concentrations. This plot is shown in more detail in Figure 5.76 after the “Legend” button in the Toolbar was pressed.

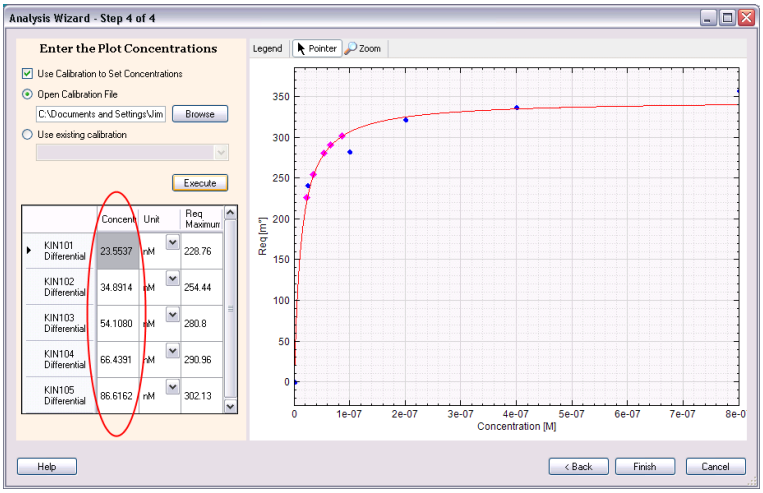


Figure 5.75 -- Calibration after calculating the concentrations

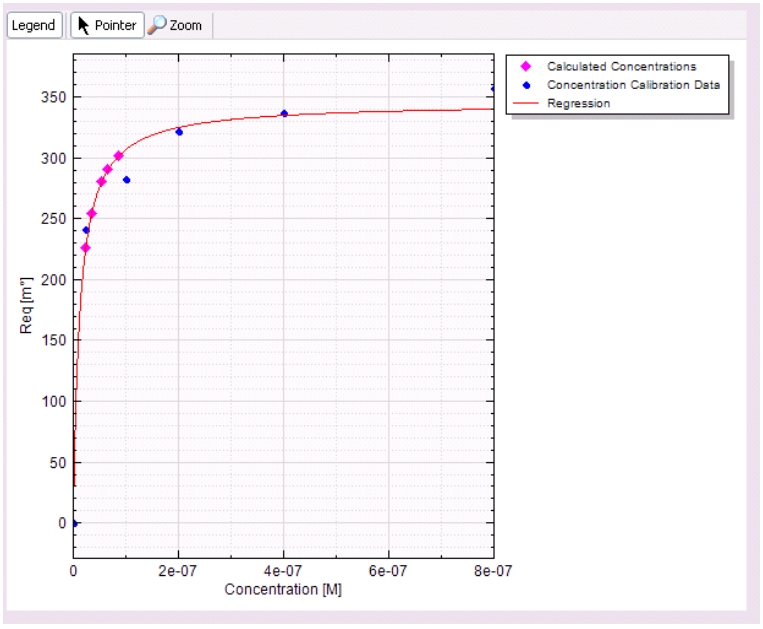


Figure 5.76 -- Concentration curve showing the calculated concentrations after the “Legend” button was pressed

It is possible that a given calibration curve cannot be applied to the current data. This situation can occur when the $R_{equilibrium}$ of the extrapolated data from Step 3 is greater than the B_{Max} of the calibration curve. In this case, the warning dialog shown in Figure 5.77 will appear.

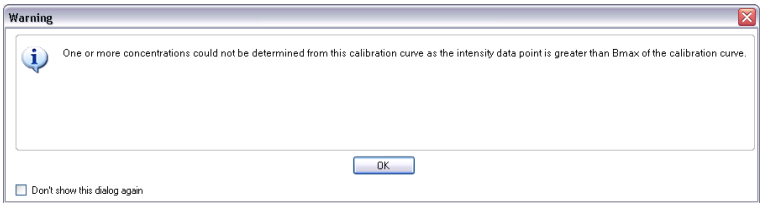


Figure 5.77 -- Warning for invalid concentration calculation

This inconsistency is shown in Figure 5.78. The $R_{equilibrium}$ for "KIN105" is 357.41, which exceeds the B_{Max} of the calibration curve (which is roughly 340). In this case, it is not possible to calculate a concentration for "KIN105", and that entry is empty in the table.

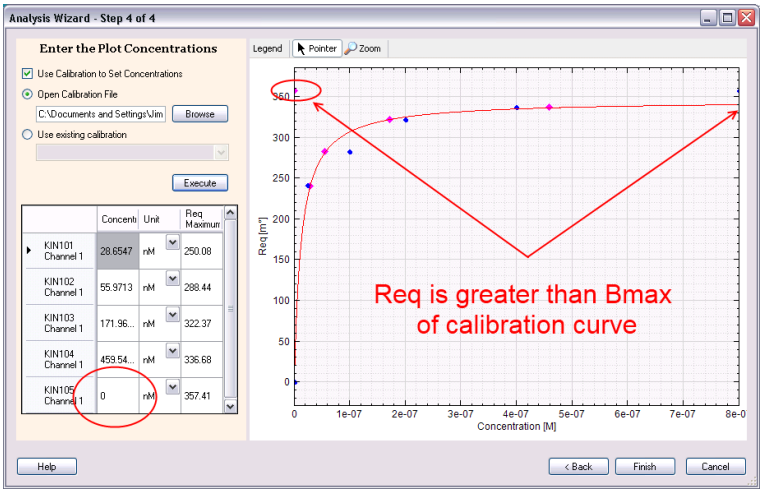


Figure 5.78 -- Missing concentration

At this point, the user should load a different calibration curve and execute a new concentration calculation.

6 – Calibration Analysis

Calibration analysis is a special kind of equilibrium analysis. It is used to derive a calibration curve that can be applied to Association or Dissociation measurements to determine the concentration values.

6.1 – New Calibrations

To begin a calibration, select **New Calibration**, either from the activity bar as shown in Figure 6.1, or from the File menu. This will start the process to create a new calibration project.

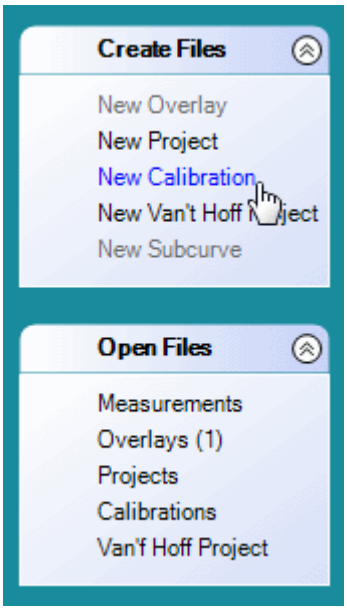


Figure 6.1 – Creating a calibration project

Once the **New Calibration** is selected, the Calibration Builder dialog box is shown. The Calibration builder is shown in Figure 6.2. Just like in the Project builder, the user can select to add either an opened overlay file, or to import an overlay from a file on disk.

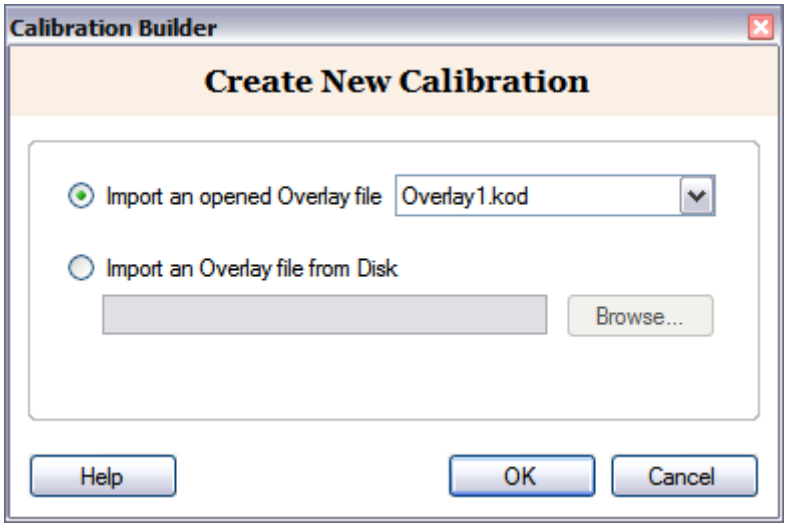


Figure 6.2 – The Calibration Builder dialog.

6.2 – The Calibration Wizard

Once the Calibration Builder is completed, a new calibration project is created. This project is shown in Figure 6.3 and it is very similar to a default project. However, only *Signal Processing* and the *Calibration Wizard* are available for calibration projects.

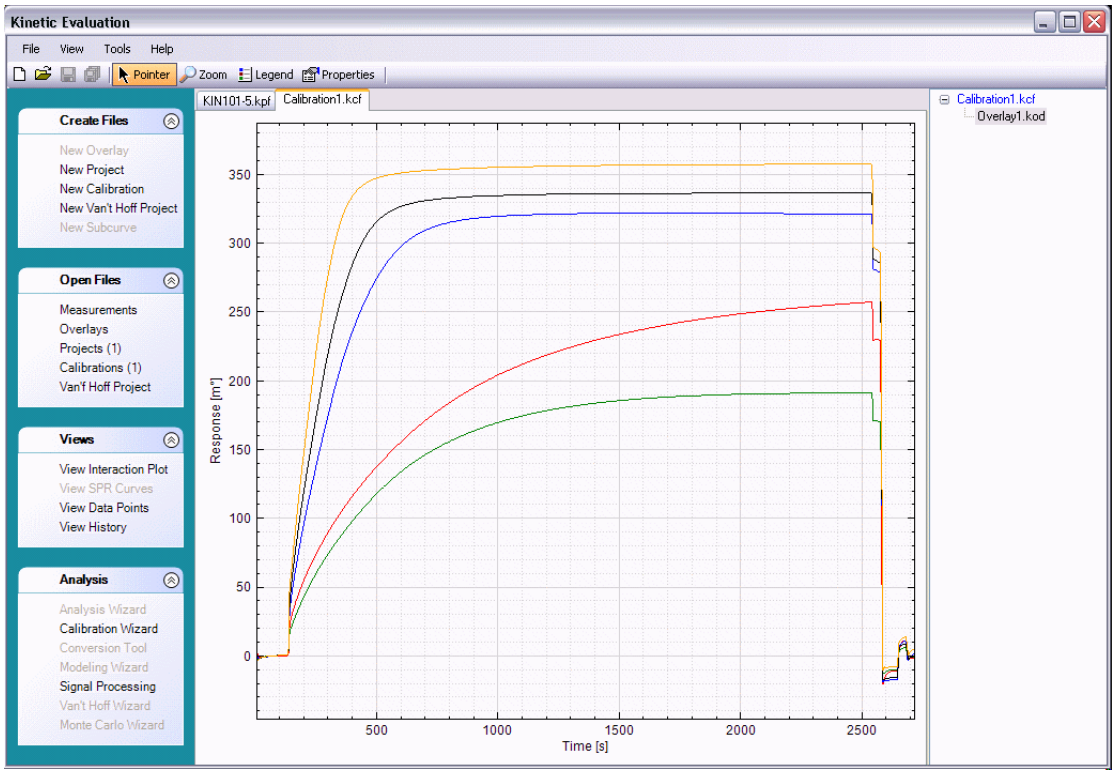


Figure 6.3 – A newly created calibration project

To launch the Calibration Wizard, select **Calibration Wizard** from the Activity bar as shown in Figure 6.4.

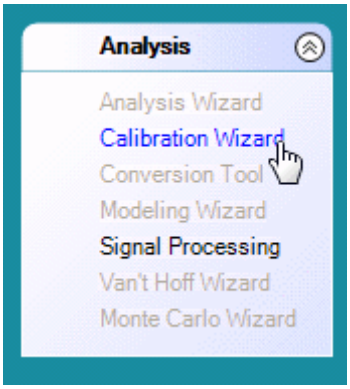


Figure 6.4 – Launching the Calibration Wizard from the Activity bar

The Calibration Wizard is similar to the Analysis Wizard (see section 5.3) but it only runs a simplified equilibrium analysis.

6.3 – Specifying the Concentrations

As with the Equilibrium analysis, the first step is to set the concentrations, as shown in Figure 6.5.

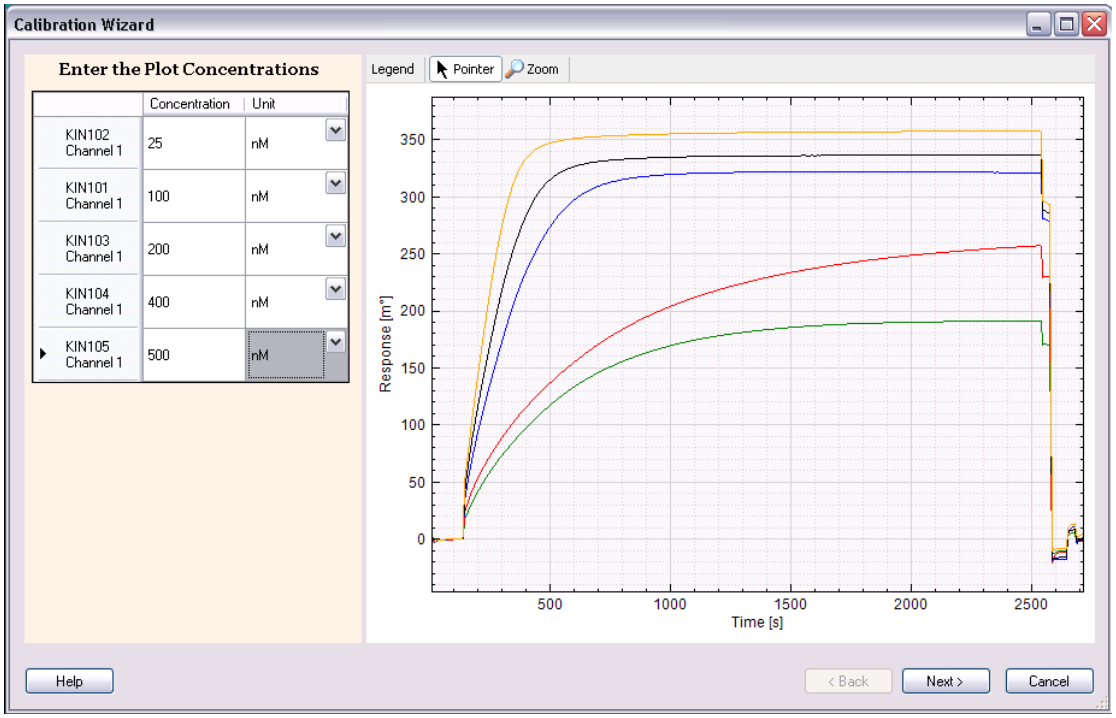


Figure 6.5 – Plot Concentrations for the Calibration Wizard

6.4 – Specifying the Start/End Times of the Analysis

Step 2 is to set the plot ranges, as shown in Figure 6.6. This procedure is the same as procedure for a regular analysis.

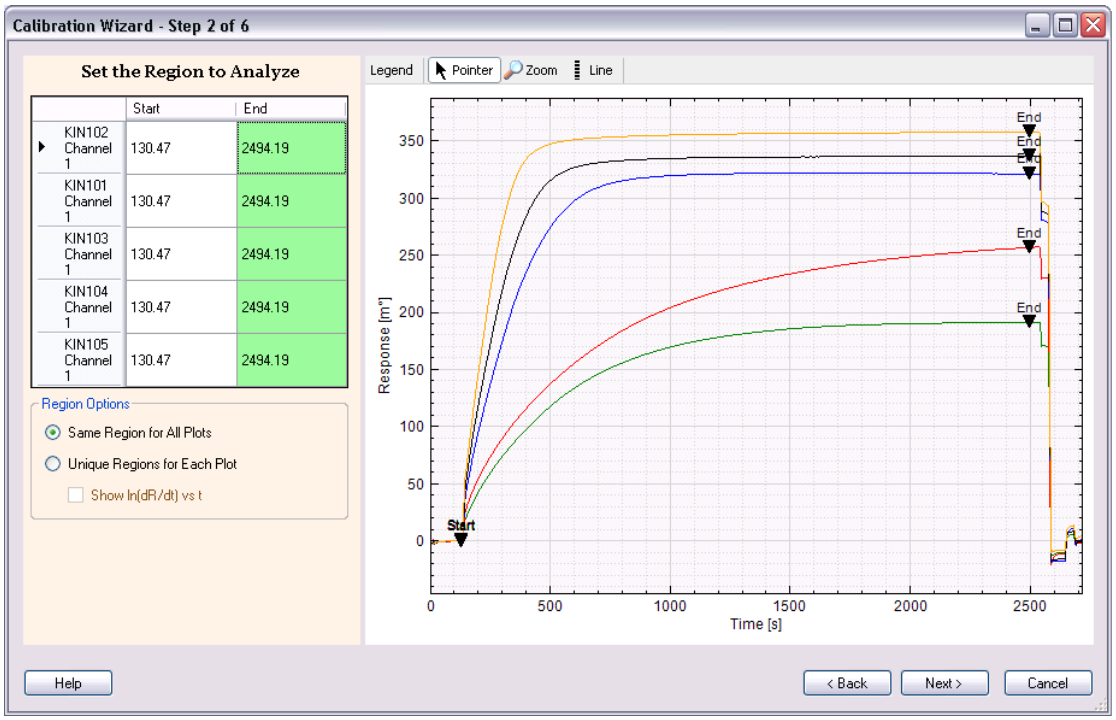


Figure 6.6 – Setting the plot ranges for a Calibration project

6.5 – Extrapolation to Equilibrium

Step 3, as shown in Figure 6.7, determines any plots that are not at equilibrium by fitting the data. The fit is accomplished by pressing the **Fit** button. Any plots not at equilibrium will be checked in the **Not at Equilibrium** column. The fit extrapolations are shown in the plot area.

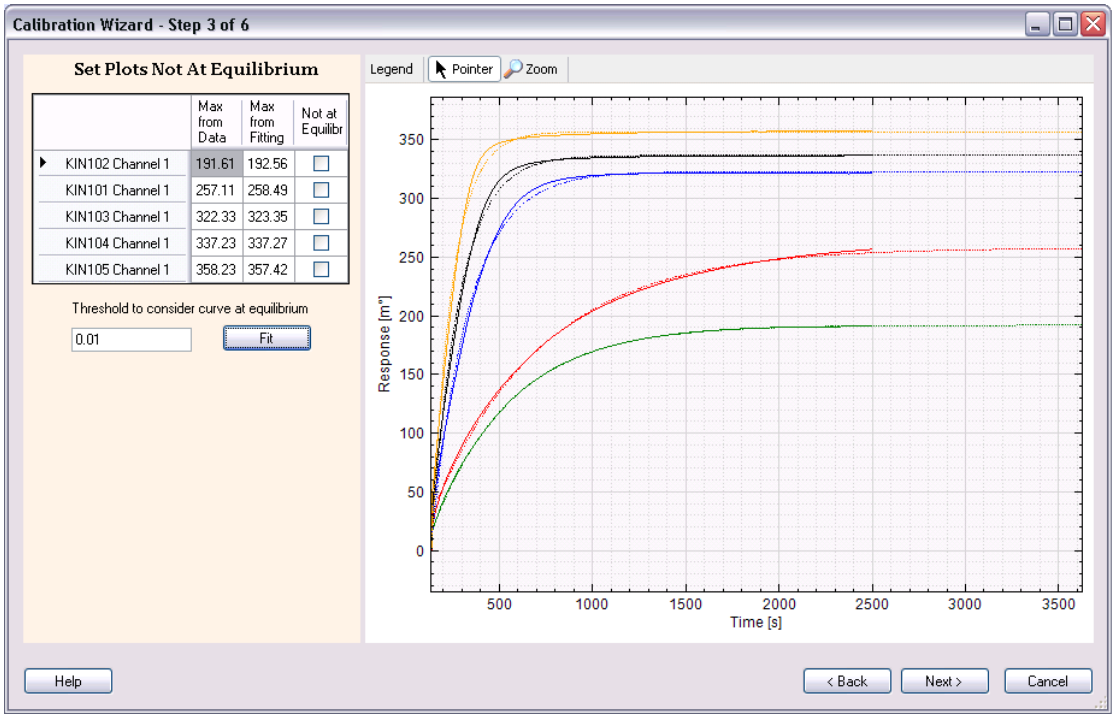


Figure 6.7 – Equilibrium extrapolation for a Calibration project

6.6 – Outlier Analysis

The calibration analysis looks for outliers in exactly the same way that the Equilibrium analysis looks for outliers, using the Lorentz fit. The outlier analysis is shown in Figure 6.8.

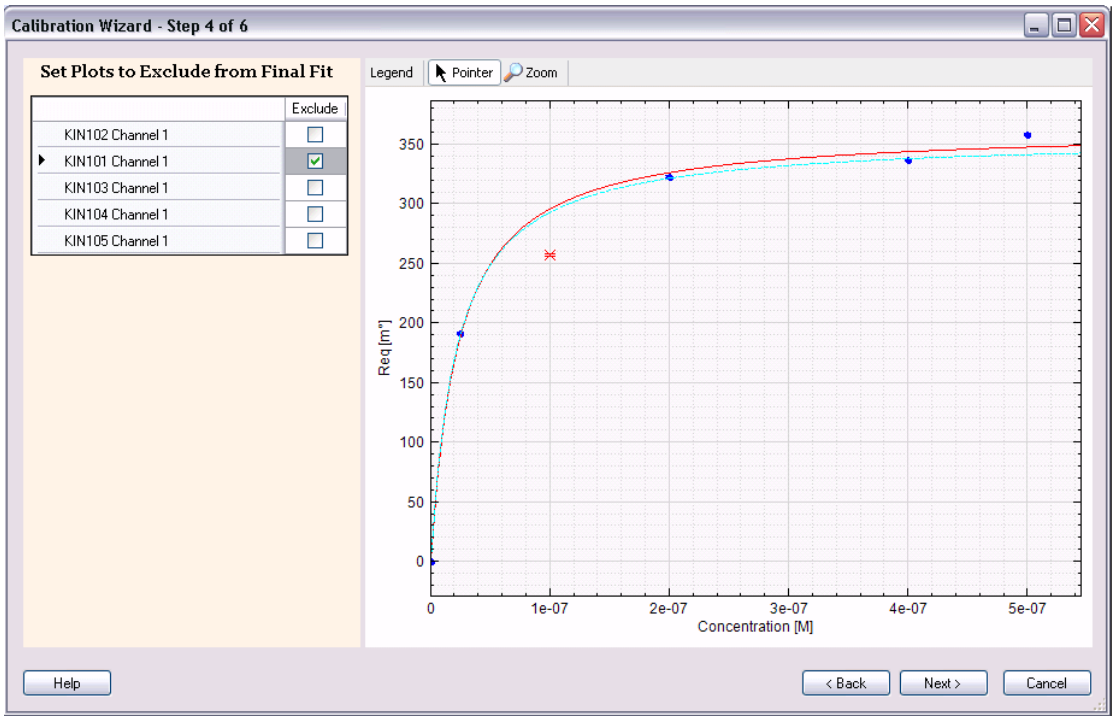


Figure 6.8 – Outlier analysis for a Calibration project

6.7 – Equilibrium Fit

Step 4 is the Equilibrium fit for the Calibration project. Figure 6.9 shows the fit to the data.

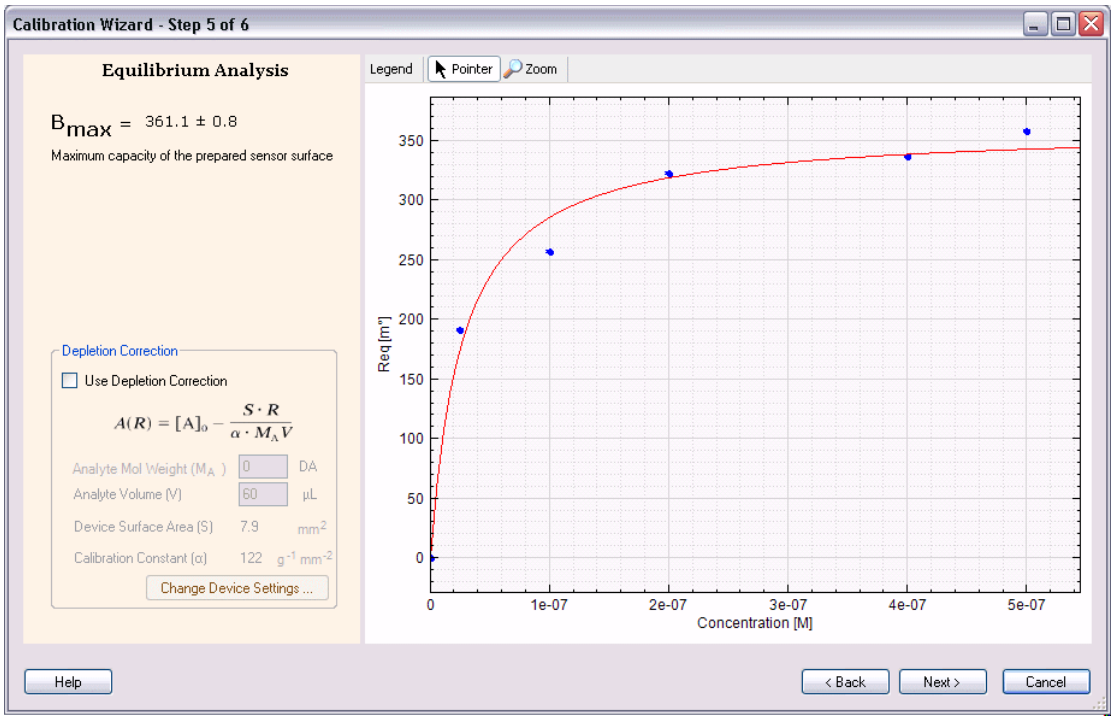


Figure 6.9 – The Equilibrium analysis for a Calibration

6.8 – Summary

Figure 6.10 shows the summary for the Calibration analysis, with the fit values for B_{max} and K_D. These values can be extracted to the clipboard if necessary.

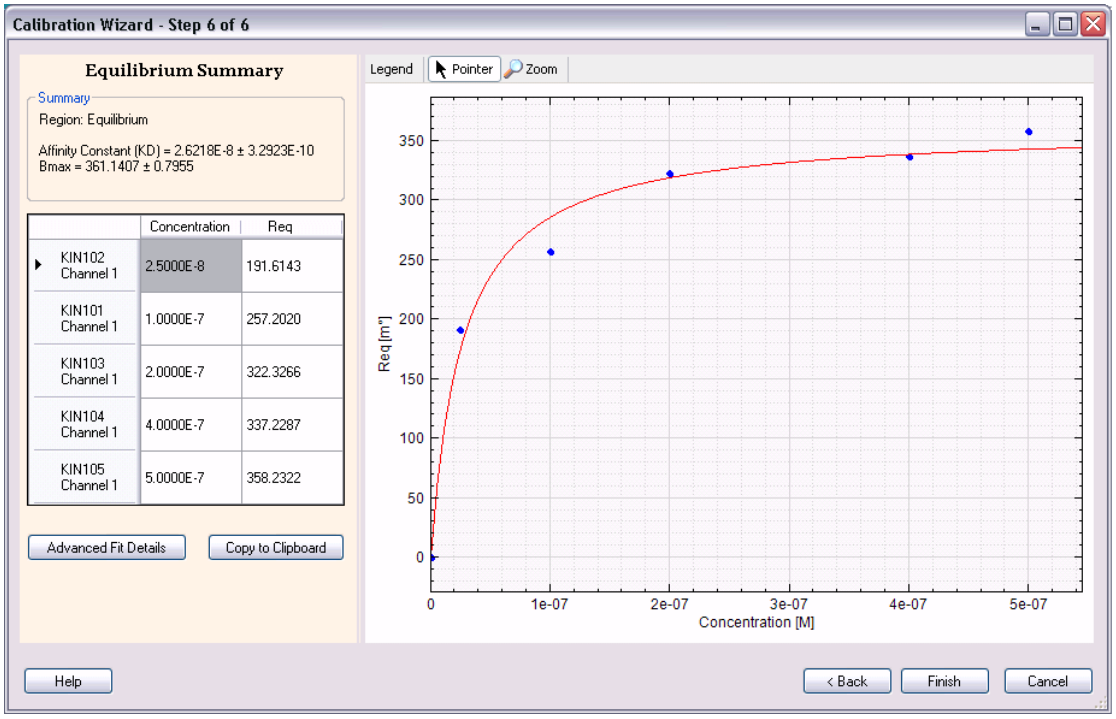


Figure 6.10 – Summary of a Calibration Analysis

Figure 6.11 shows the Calibration project in the main KE window. In the right panel is the project navigator; here we have a Calibration file with the extension *.kcf*, the overlay from which the calibration was derived, and the one calibration analysis under the node *Calibration*.

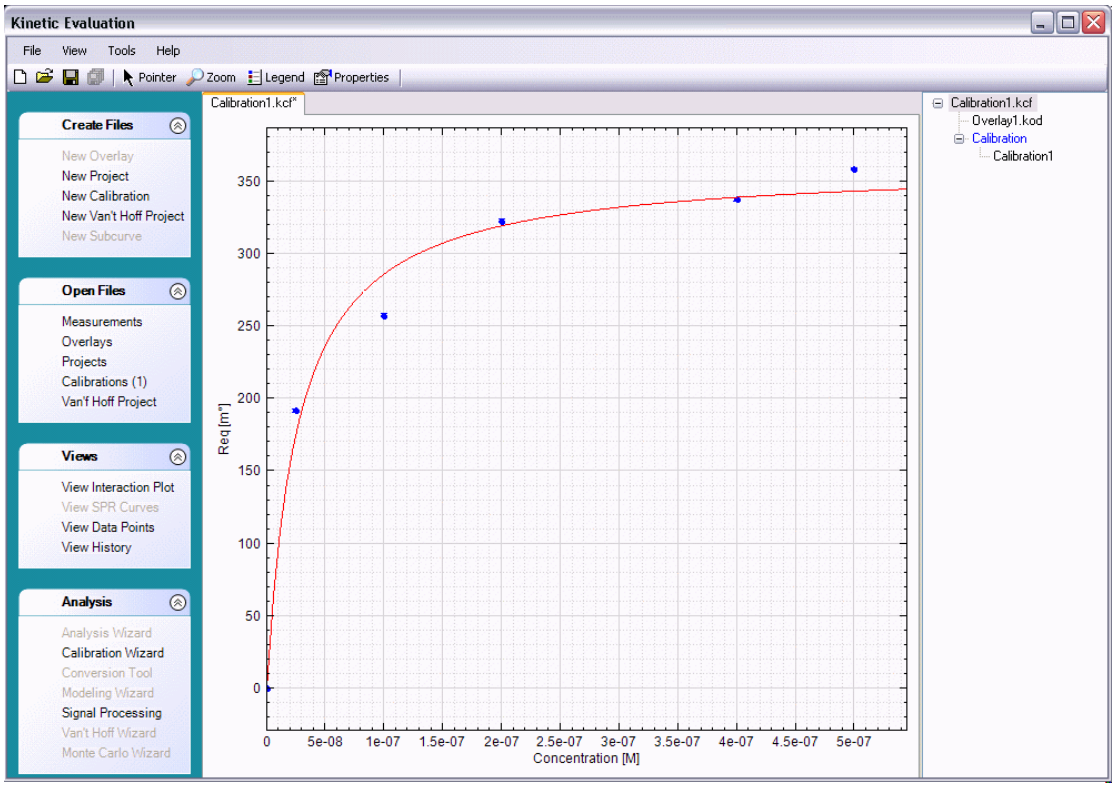


Figure 6.11 – A Calibration Project with one Calibration Analysis

6.9 – Using Calibration Files

As discussed in section 5.3.2, one can use calibration files saved to a .kcf file to set the concentrations of an Association or Dissociation analysis.

7 – Van't Hoff Analysis Wizard

7.1 – Introduction

Van't Hoff analysis is a technique that links changes in the affinity constant (K_A) with respect to temperature to the thermodynamic properties ΔH, ΔS and ΔC_p (Delta Enthalpy, Delta Entropy and Delta Heat Capacity).

The integrated Van't Hoff equation is:

$$\ln K_A = -\frac{\Delta H°(T°)}{RT} + \frac{\Delta S°(T°)}{R} - \frac{\Delta C_P}{R}\left[\left(\frac{T - T°}{T}\right) - \ln\left(\frac{T}{T°}\right)\right] \quad \text{(Equation 7.1)}$$

In Equation 7.1 the logarithm of the affinity constant is related to (1/T) and the constants ΔH°, ΔS° and ΔC_p, where each constant is evaluated at a reference temperature T° (typically 25° C). Since there are three free parameters, at least four measurements of K_A at different temperatures are required to over –constrain the three fitted constants. This requirement means that the user should measure R_{eq} versus Time for five concentrations, at four separate temperatures (typically in the range 10° – 40° C), for a total of at least 20 measurements.

7.2 – Creating a Van't Hoff Project

A Van't Hoff Project is created through the *New Project* builder. Select **New Project** from the Activity bar, or *File→New Project (Including Van't Hoff Projects) ...* from the File Menu. These actions will launch the New Project builder, as shown in Figure 7.1

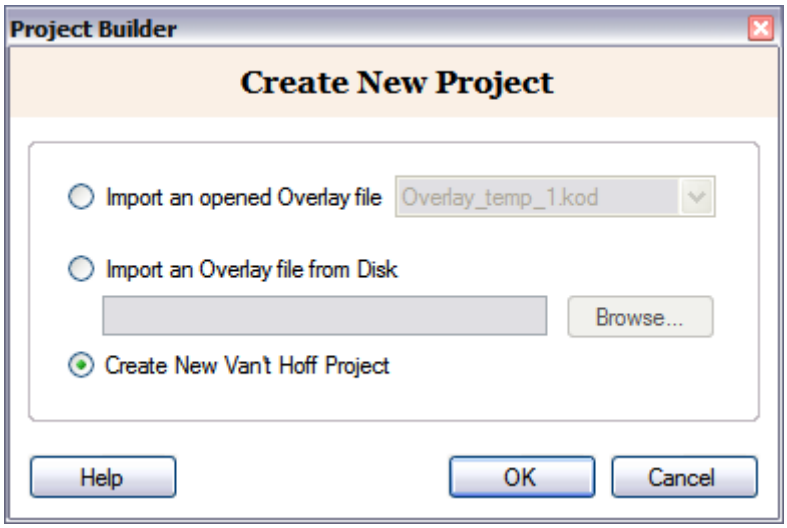


Figure 7.1 – The New Project builder with the Van't Hoff option selected.

Select the **Create New Van't Hoff Project** radio button and click **OK**. This action will launch the Overlay importer.

7.3 – Importing Overlays

The Van't Hoff analysis requires at least four overlays. Each overlay corresponds to multiple measurements (each taken at different concentrations), with each set of overlay measurements taken at the same temperature.

The Van't Hoff Project Builder is shown in Figure 7.2. The Van't Hoff Project Builder will allow the user to import multiple Overlay files. These overlay files will either be already open in KE (as shown in the figure), or can be imported from disk. Van't Hoff Projects are the only projects that allow multiple overlay files to be added to the same project file.

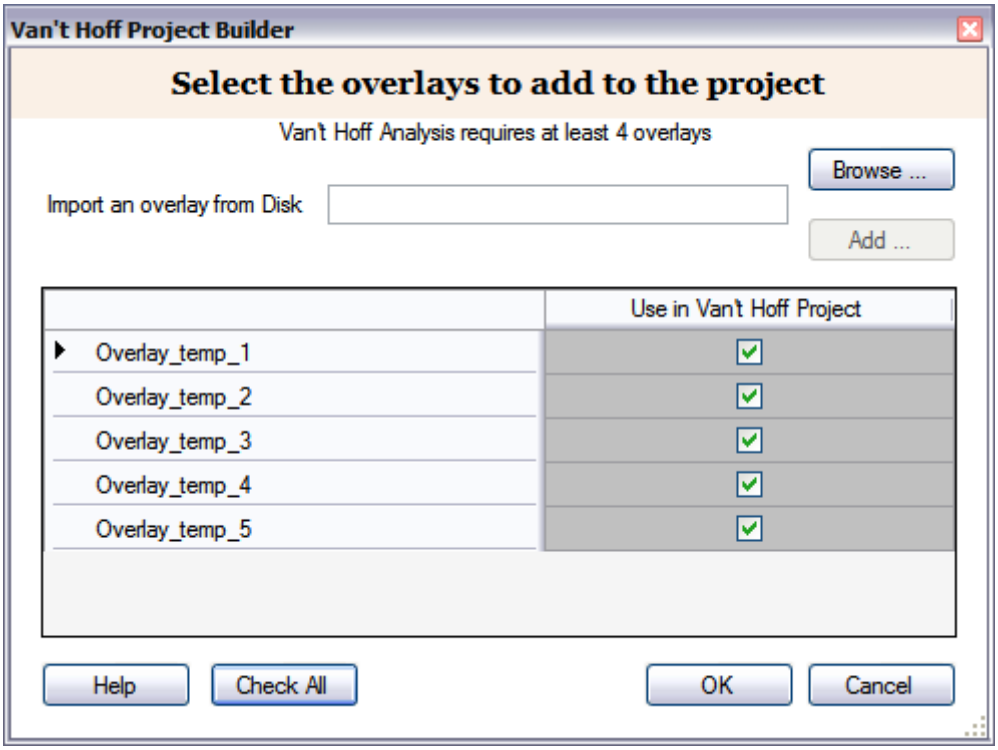


Figure 7.2 – The Van't Hoff Project Builder, used to import multiple overlay files to a Van't Hoff Project.

Once the overlay files are selected, the user can import the overlay files into the Van't Hoff project by clicking **OK**. The Van't Hoff project will then be displayed in the main KE window. The Project Navigator shows the new Van't Hoff project (VantHoffProject1.kvf) with the five imported overlays. Each overlay corresponds to measurements taken at a single temperature. The Van't Hoff project is the only project type where multiple overlay files can be imported into one project.

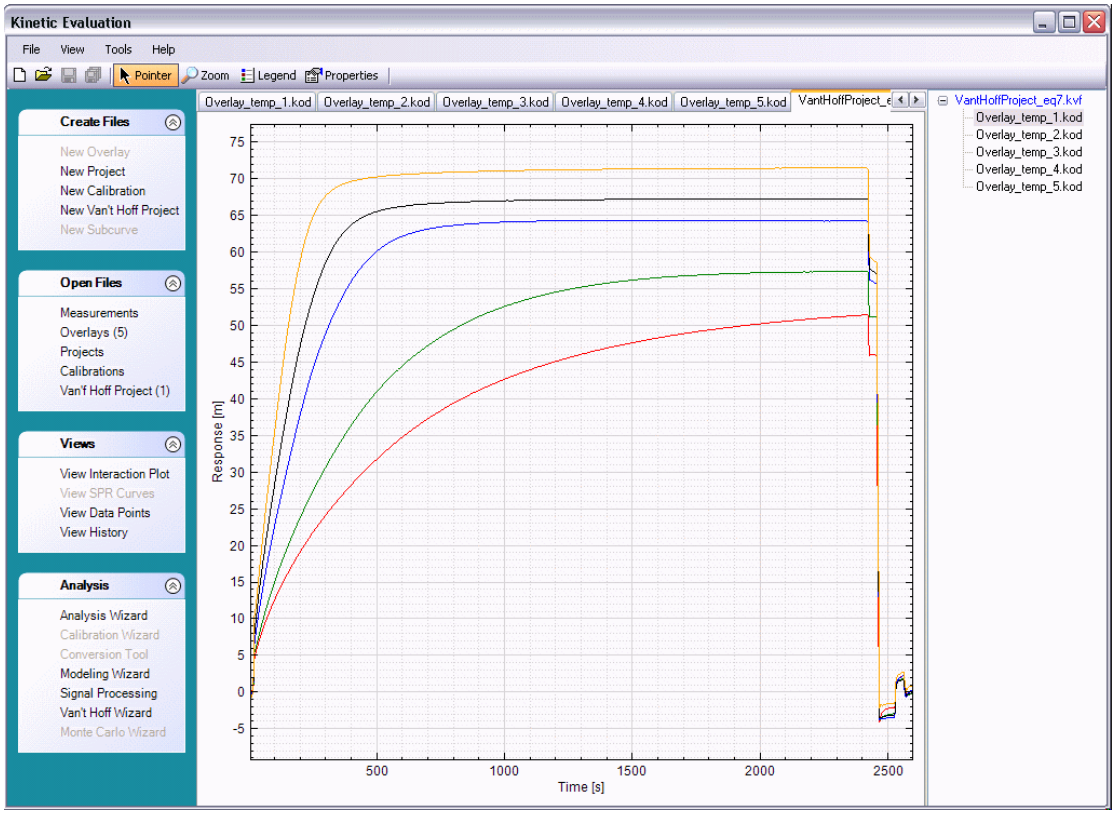


Figure 7.3 – The Van't Hoff Project in the main KE window.

At this point, one could execute Signal Processing on each of the overlay files, if necessary. Signal Processing is discussed in Section 5.2.

7.4 – Saving and Loading Van't Hoff Projects

Van't Hoff Projects are saved with a .kvf file extension. These project files can be loaded from the *Open Files* Activity bar by selecting *Projects* or from *File→Open→Project (including Van't Hoff Projects)…* By default, the Open file dialog will default to regular Project files with a *.kpf* file extension. The user can change this to the *.kvf* file extension as shown in Figure 7.4 to load Van't Hoff Project files.

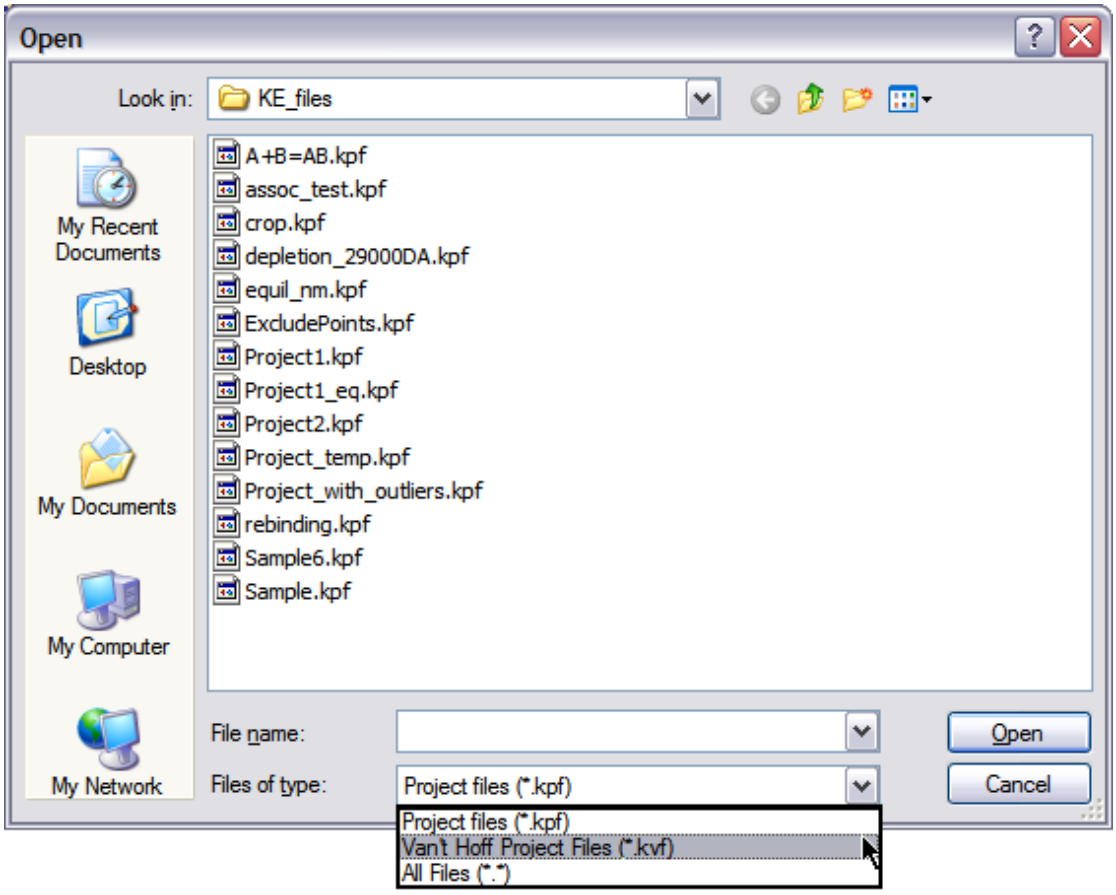


Figure 7.4 – Selecting Van't Hoff Project Files (*.kvf) to load in the Open File dialog.

7.5 – Van't Hoff Equilibrium Analysis

The Van't Hoff analysis uses affinity data in the calculation. To provide the affinity information, each Overlay file must go through an Equilibrium analysis using the Analysis Wizard, as discussed in Section 5.3.8.

To launch the Analysis Wizard, select it from the Activity Bar, as shown in Figure 7.5.

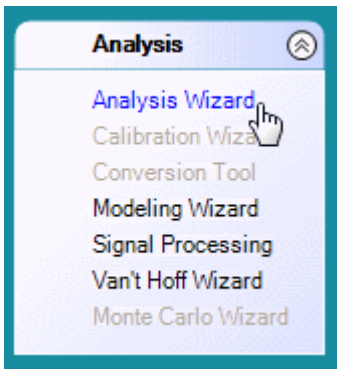


Figure 7.5 – Selecting the "Analysis Wizard"

This action will launch the Analysis Wizard, which is discussed in detail in Section 5.3. There is one significant difference between a default Analysis and one in the Van't Hoff project. Because the user can import multiple overlay files in a Van't Hoff analysis, the first panel of the Analysis wizard will allow the user to select the overlay to analyze from a drop-down combo box, as shown in Figure 7.6.

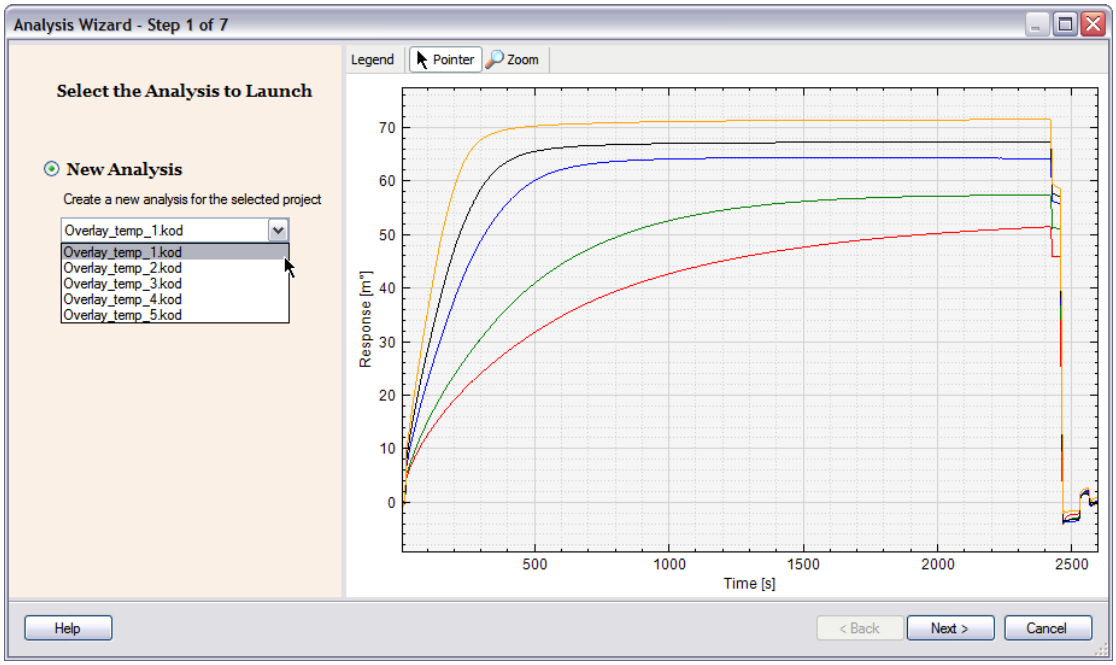


Figure 7.6 – Selecting the overlay for a new analysis in a Van't Hoff Project

For the second and subsequent analyses, the wizard will either allow the user to create a new analysis from a selected overlay file, or to open a previously-created analysis, as shown in Figure 7.7.

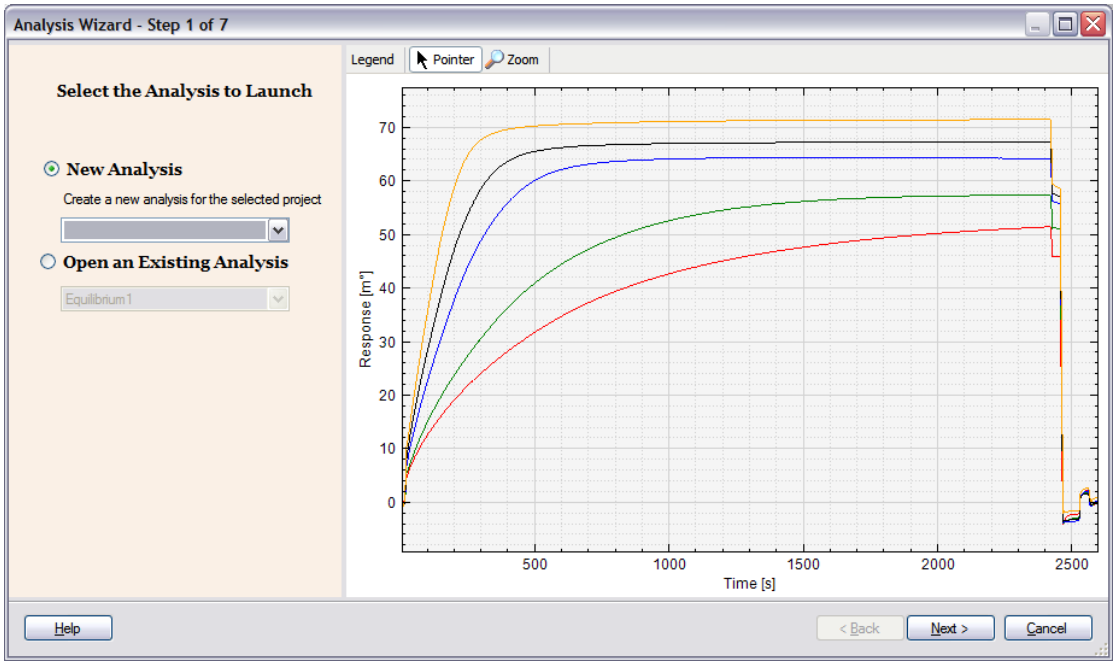


Figure 7.7 – Selecting to either create a new analysis or open an existing analysis in a Van't Hoff Project.

The equilibrium analysis process must be repeated for each overlay file in the Van't Hoff Project file. Figure 7.8 shows the results for the project after this process is complete. There are seven overlay files and seven equilibrium analyses.

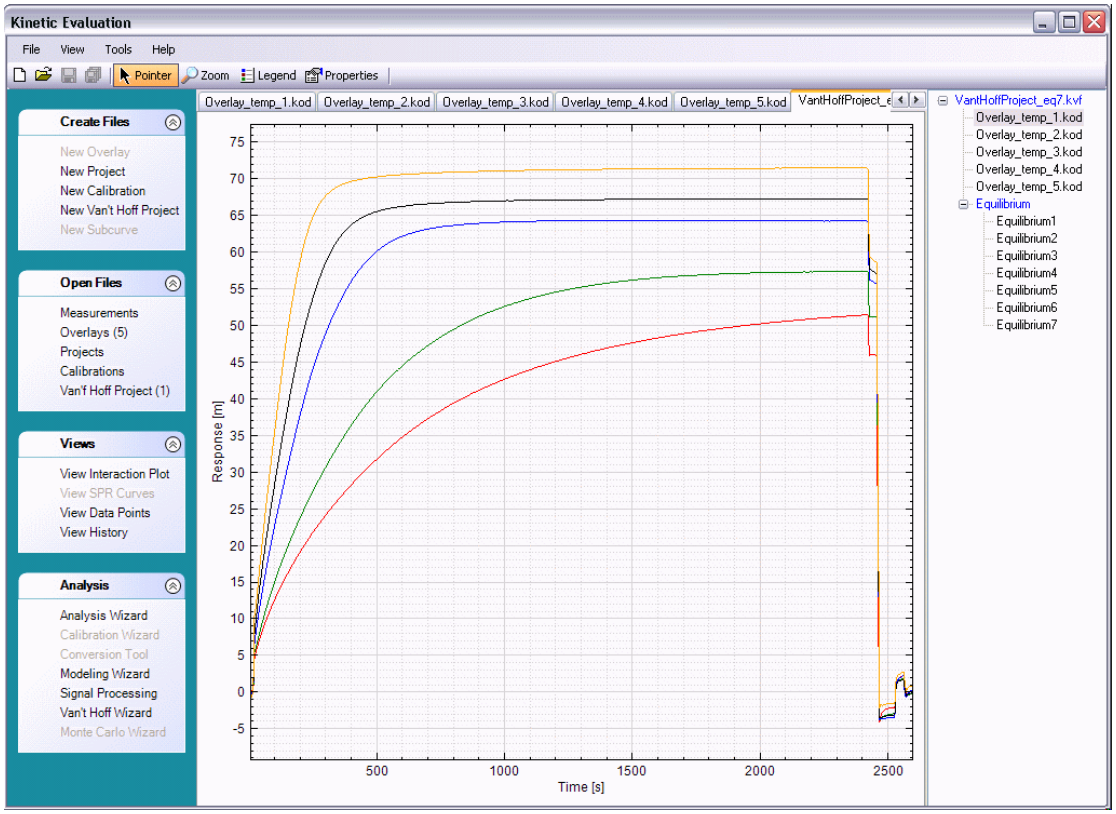


Figure 7.8 – The Van't Hoff Project in the main KE window after all the equilibrium analyses are complete. The project navigator shows seven overlay files and seven analyses.

7.6 – Van't Hoff Analysis Wizard

Once the requisite equilibrium analyses are completed, the user can launch the Van't Hoff Wizard, by selecting **Van't Hoff Wizard** from the Activity bar. This is shown in Figure 7.9.

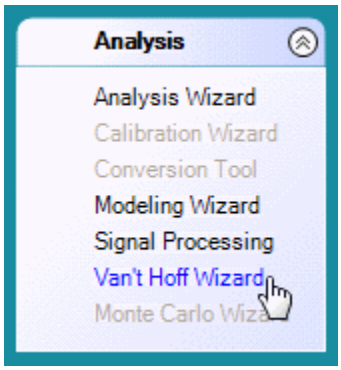


Figure 7.9 – Launching the Van't Hoff Wizard from the Activity bar.

Step 1 of the Van't Hoff Wizard is to select the K_A fits that will be used in the Van't Hoff Analysis. Figure 7.10 shows the first panel of the Van't Hoff Wizard. On the left is a table of the Equilibrium Analyses. The first column of the table shows the temperature of measurement.

The temperature is the average of the temperature measurements in each of the measurement in the given overlay. If the standard deviation of the temperature average is greater than the *Temperature Variation Threshold*, the analysis will not be used by default. This means that the *Include* column will be unchecked.

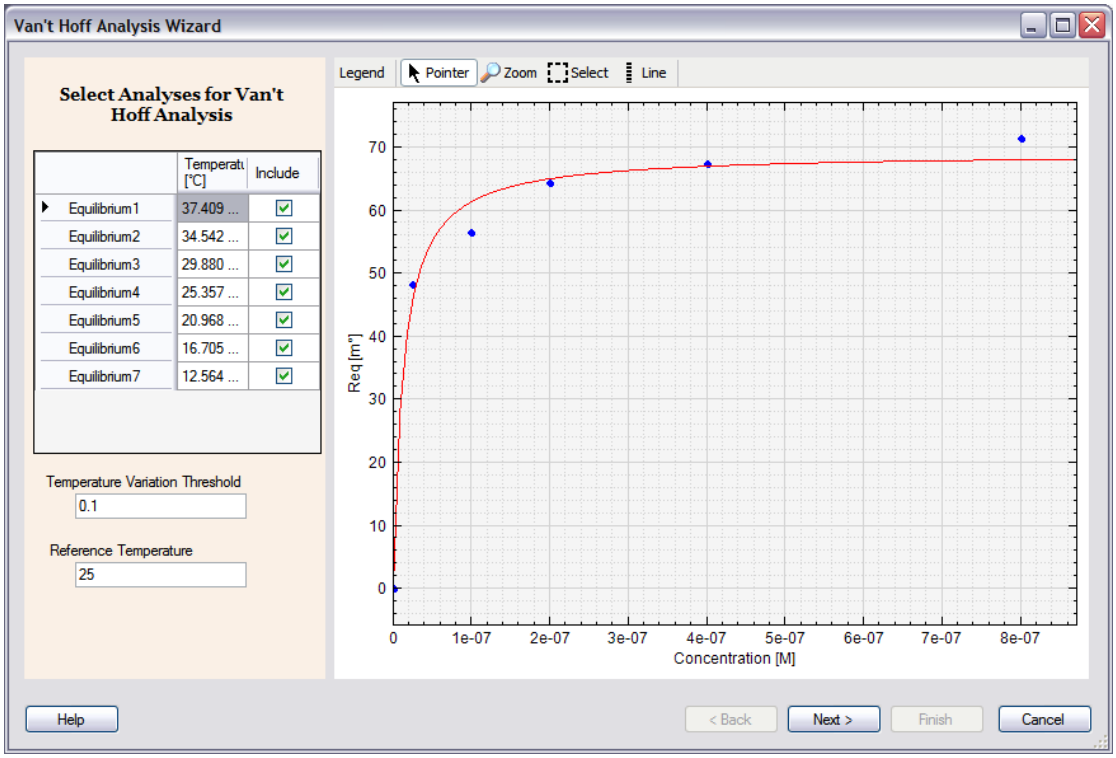


Figure 7.10 – Initial panel of Van't Hoff Wizard.

If the user adjusts the *Temperature Variation Threshold*, the wizard will automatically exclude measurements whose standard deviation is larger than the threshold. In Figure 7.11, the threshold has been set to an arbitrary low number, and all the analyses have been automatically excluded.

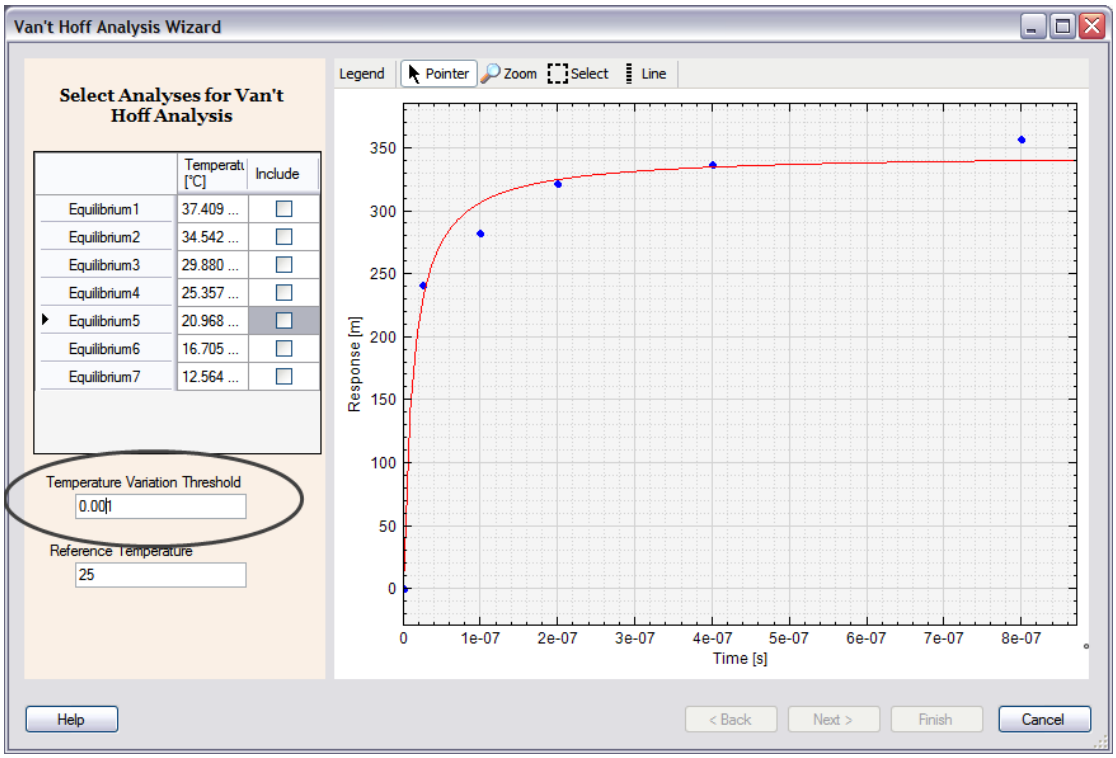


Figure 7.11 – Adjusting the Temperature Variation Threshold may exclude overlays whose temperature standard deviation shows too much variation.

Because the Van't Hoff Analysis requires four K_A data points to over-constrain the three constants in the fit, if the user includes less than four analyses, the *Next* button will be disabled, as shown in Figure 7.12.

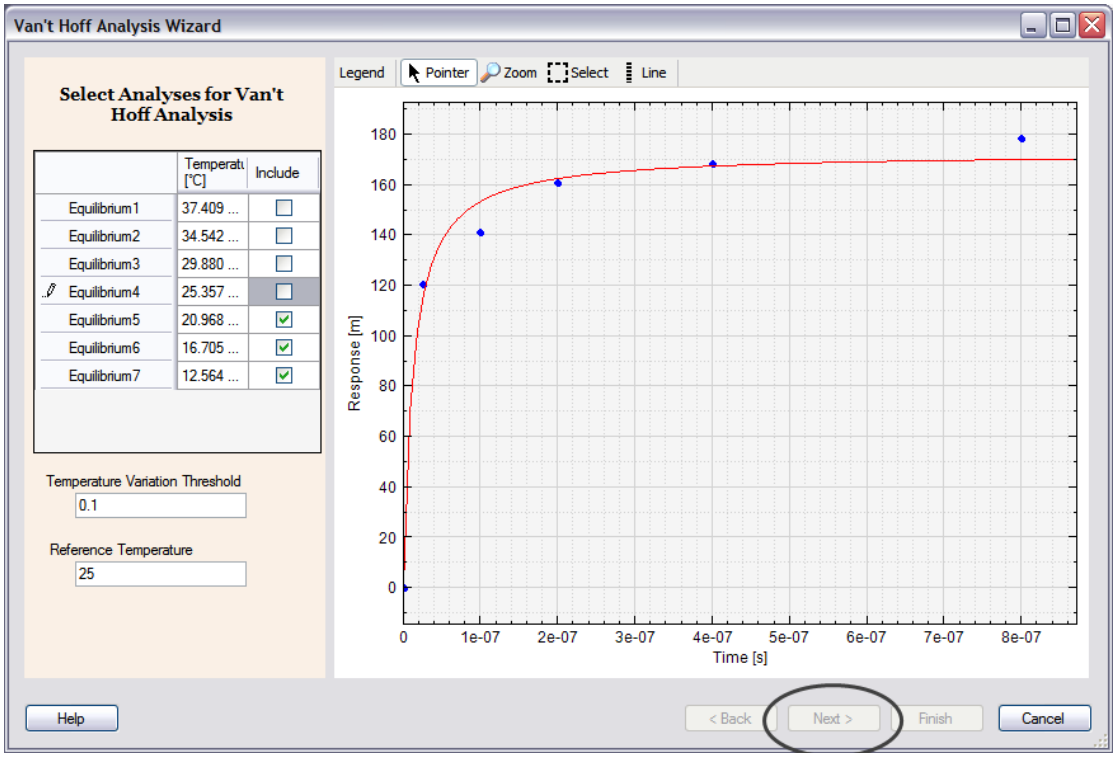


Figure 7.12 – Van't Hoff analysis requires four equilibrium analyses. Including less than four will disable the "Next" button.

Step 2 of the Van't Hoff analysis is to do the actual fit. Click on the **Next** button to show the second panel of the Van't Hoff Wizard. This panel is shown in Figure 7.12.

The results of K_A versus (1/T) are plotted in the graph window. The results for the fit to Equation 7.1 are shown in the summary area. The results for $\Delta H°$, $\Delta S°$ and ΔC_p, as evaluated at the reference temperature T°, can be copied to the clipboard for importing into other programs.

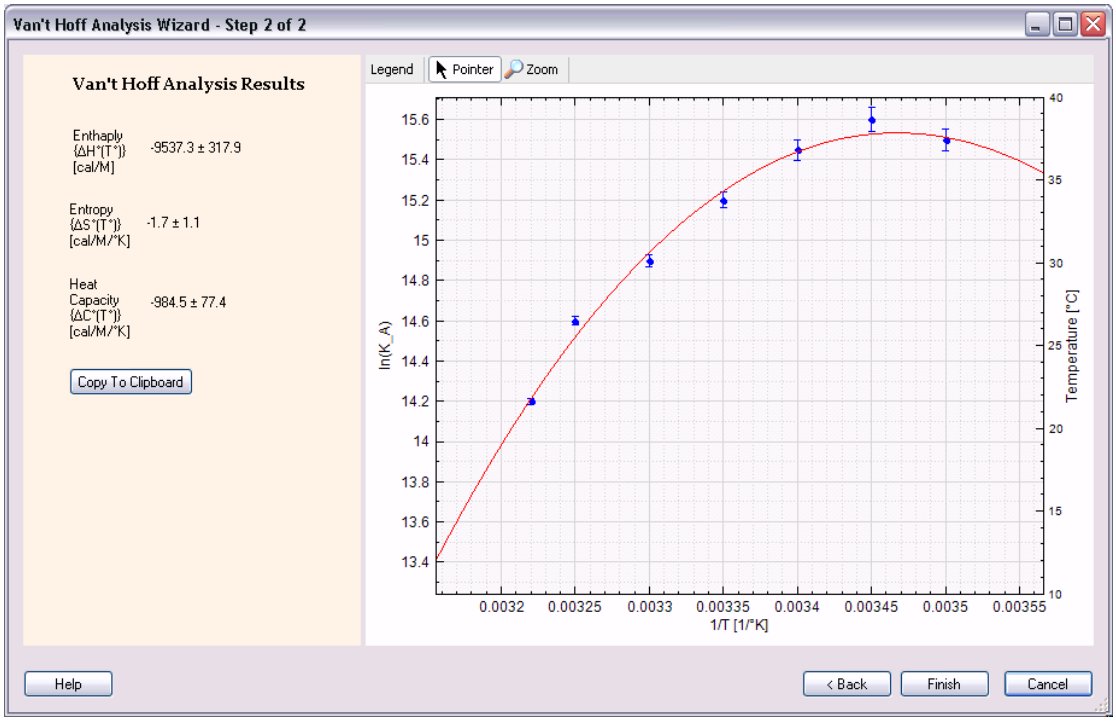


Figure 7.12 – The results of the Van't Hoff Analysis.

Once the analysis is complete, the Van't Hoff Project can be saved.

8 – Monte Carlo Wizard

8.1 – Introduction

Monte Carlo is a technique that uses pseudo-random numbers to vary the fit. This technique is used to cross-check the results of the fit for consistency, and also to validate the error estimates for the fit.

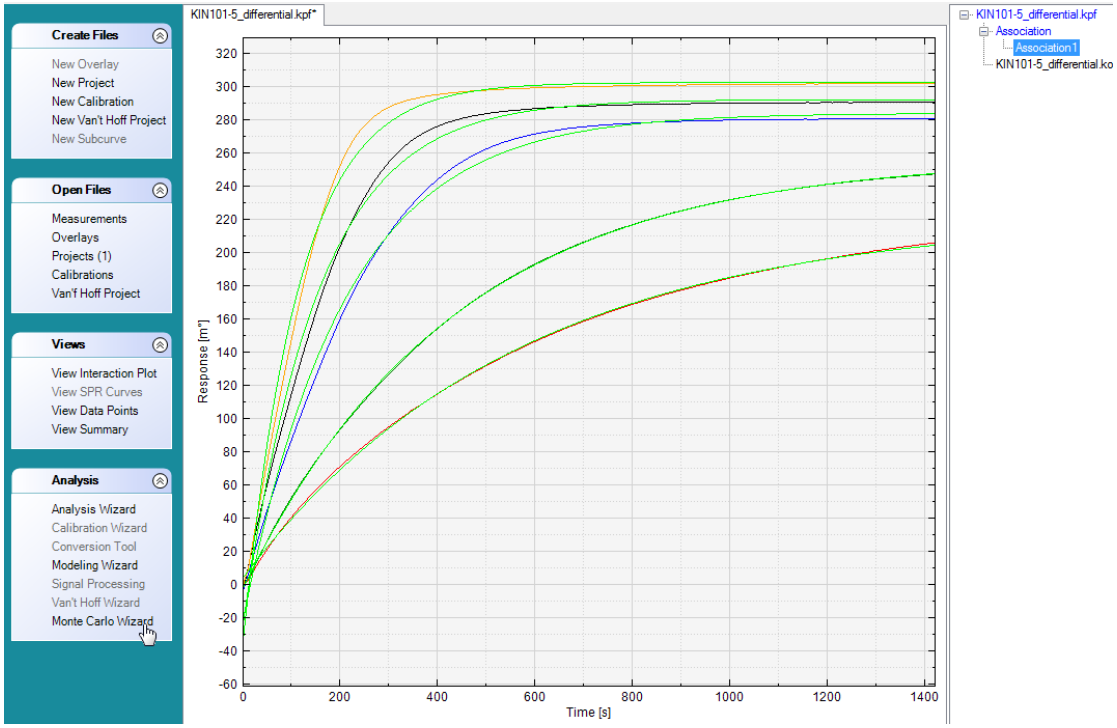
Under the Monte Carlo technique, the fit is run multiple times under differing conditions. The result of each "run" of the fit is then compared to the original fit.

Kinetic Evaluation has a Monte Carlo Wizard that can generate two types of Monte Carlo simulations:

1. Vary initial parameters: for each run of the Monte Carlo, the starting points of the fit are varied by a percentage fraction of the fit values. The fits are then tested to see if the fit converges to the same final fit values.
2. Add noise to the data: for each run of the fit, a random amount of "noise" is added to each data point. Because the data is modified, the fit will typically converge to a different result. However, the average value of all of the Monte Carlo fits should be consistent with the original fit value.

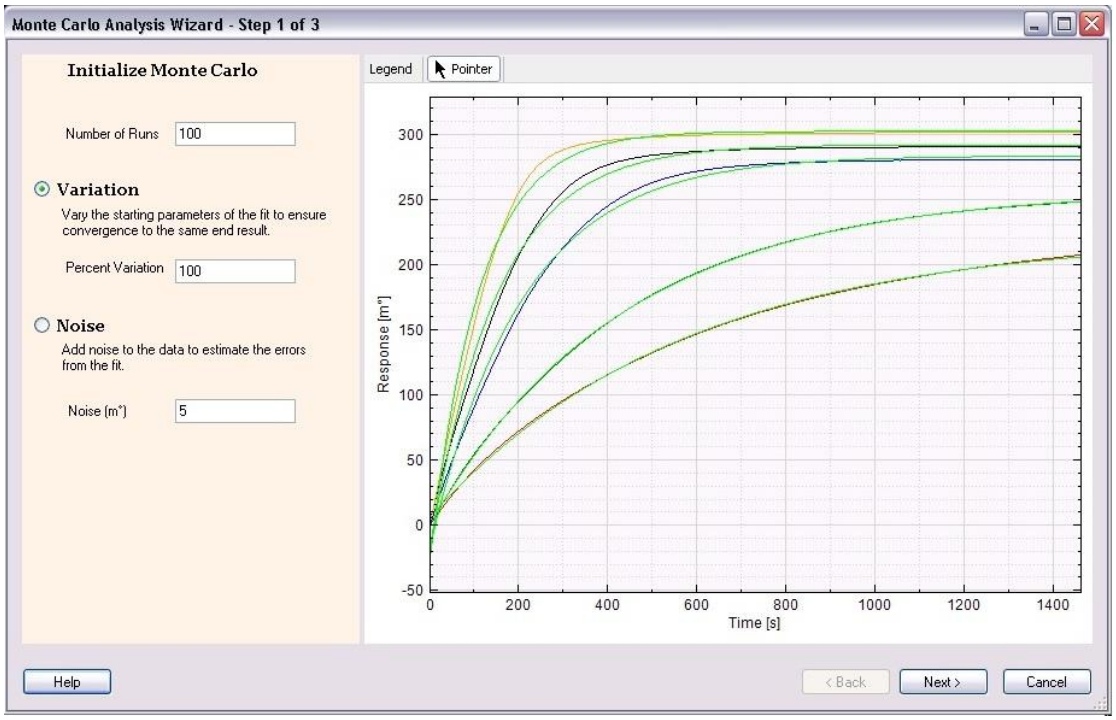
8.2 – Running the Monte Carlo Wizard

To run the Monte Carlo wizard, select an existing analysis within a project. Select "Monte Carlo Wizard".



8.1– Selecting the Monte Carlo Wizard

Selecting “Monte Carlo Wizard” will launch the Monte Carlo Wizard.



8.2 – The Monte Carlo Wizard

The wizard has the two options discussed above. Each of these options will be discussed below in the following sections. One feature common to both types of

Monte Carlo runs is the number of runs that will be executed for the Monte Carlo. The default number of runs for integrated equations is 100. For numerically integrated equations, the default is 25. The numerically integrated equations use a lower default as they are more computationally intensive. The user can change the default number of runs.

8.2.1 Monte Carlo Analysis of Numerically Integrated Fits

If the user has selected a Monte Carlo analysis of a fit that uses numerical integration, they will see an initial warning:

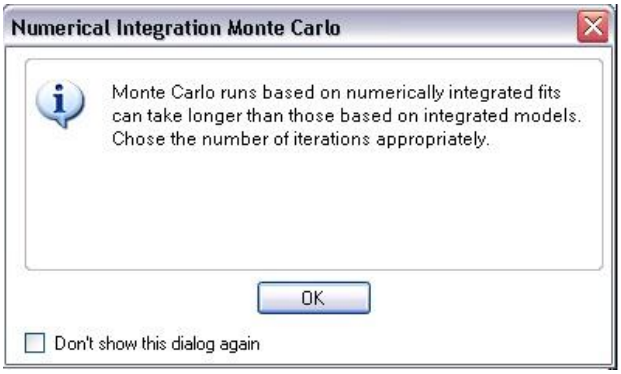


Figure 8.3 – Numerical Integration warning

If the original fit was run against a numerical model that cannot be found by KE, the user will see this warning

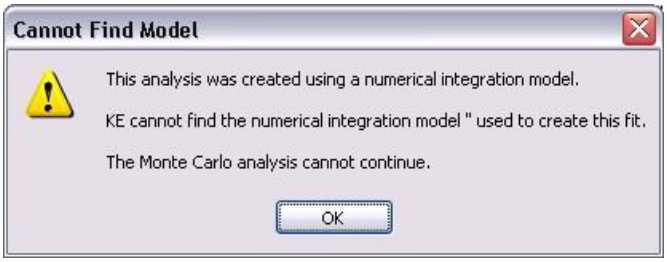


Figure 8.4 – Cannot find model

The Monte Carlo wizard cannot continue. The user should refit the data using the Analysis wizard before attempting to use the Monte Carlo wizard.

8.3 – Run the Monte Carlo

The second panel of the Monte Carlo wizard is shown in Figure 8.5. The Wizard shows one histogram for each parameter in the fit. In this example, there are three floated parameters in the fit, Ks, E and R(0). For each run of the Monte Carlo, an entry will be added to the histogram for each parameter.

The table on the left shows one tab for each plot that is fit. In the table are listed the three parameters of the fit, along with the original fit value, the average of all Monte Carlo runs, and the standard deviation of the Monte Carlo runs.

Once the user clicks the “Start” button, the Monte Carlo will commence.

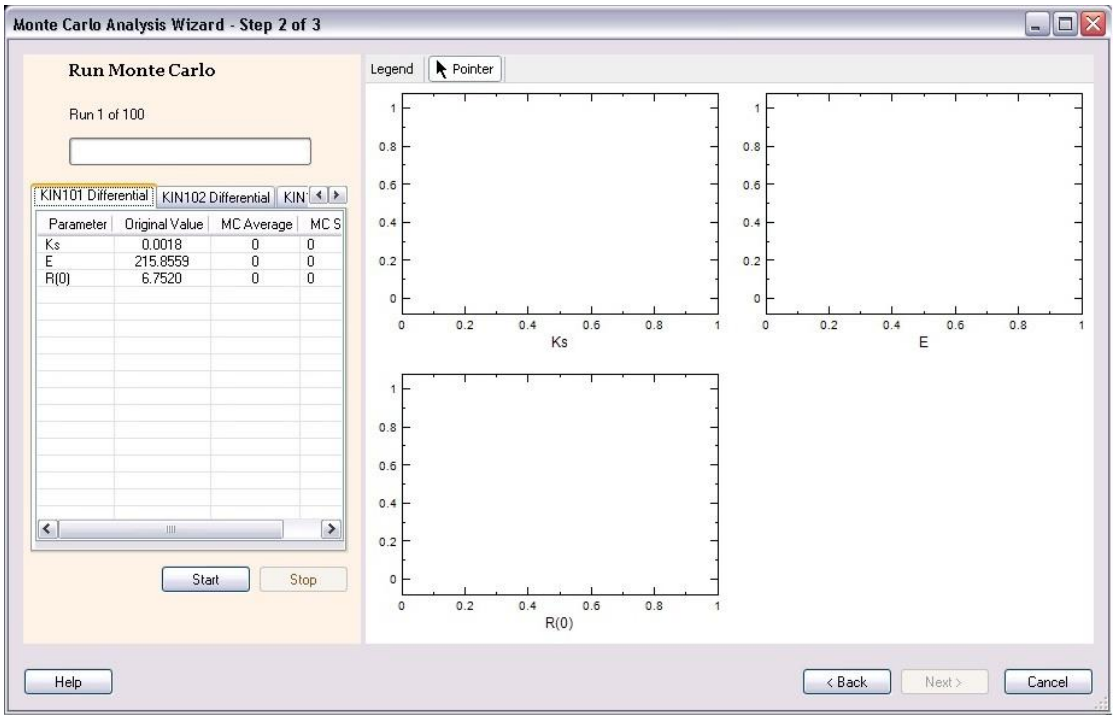


Figure 8.5 – Start of running the Monte Carlo

Once the user clicks “Start” the Monte Carlo runs begin. There is a progress bar that indicates the number of completed runs. While the Monte Carlo is running, all buttons except the “Stop” button are disabled.

As the runs progress, the table of the Monte Carlo average and standard deviation are updated. The histograms also update as a Monte Carlo run is completed. This progress is shown in Figure 8.6.

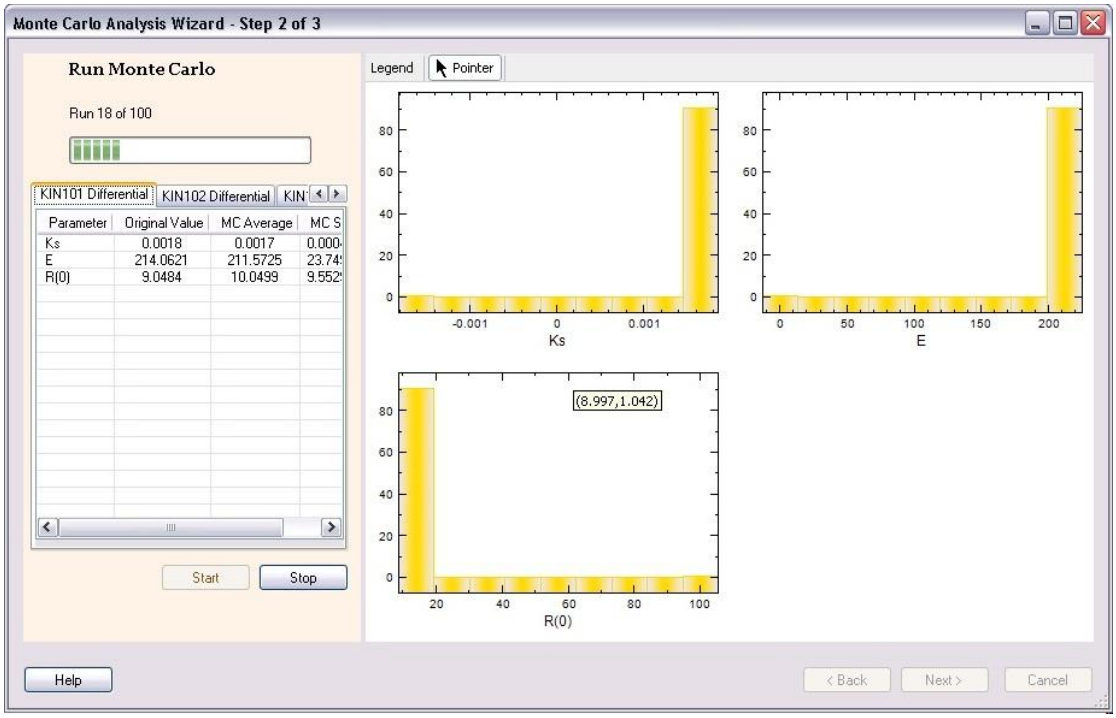


Figure 8.6 – The Monte Carlo wizard when the Monte Carlo is running

Figure 8.7 shows the second panel of the Wizard when all runs of the Monte Carlo have finished. A Gaussian plot with the average and standard deviation of the parameter values is overlaid on each histogram.

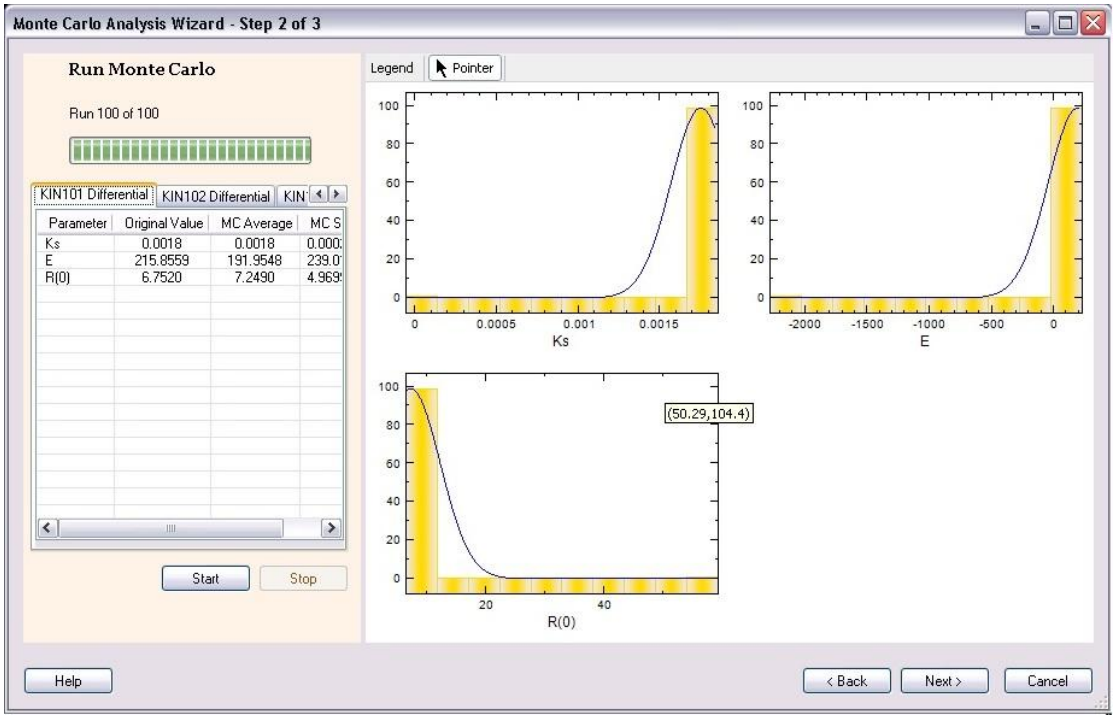


Figure 8.7 – The Monte Carlo run completed

8.3.1 – Vary the Start Parameters

The first option for the Monte Carlo is to vary the starting point of the fit. In this option, the parameters that are used as starting points for the fit are varied by the specified percentage (although they are not varied negative). The fits are then run to determine if the final parameters that are determined from the fit are the same as from the original fit.

This technique allows the user to determine if the original fit found a "local minimum" of the fit, or if the original fit converged to an absolute minimum. If all of the varied parameter runs end up converging to the same set of final parameters, the user has confidence that the reported parameters are the best fit for the given model. It is important to realize that the presence of only one set of final parameters does not mean that the chosen model is a good fit to the data. It only means that there is one set of parameters that work for that model.

This technique is not a true Monte Carlo because the actual data is not modified.

8.3.2 – Add Noise to the Data.

The second option for the Monte Carlo is to use a random number generator to add a random amount of noise to the actual data. Here the data points are changed. The noise is selected from a Gaussian distribution with a standard deviation equal to the "noise" factor input by the user.

Figure 8.8 shows the result of a "noise" Monte Carlo where 10 millidegrees of noise was added to the data. The fits show that the Monte Carlo average is very similar to the original fit values. The standard deviation of the Monte Carlo values is larger than the estimated uncertainties on the original fit due to the addition of the 10 millidegrees of noise in the data.

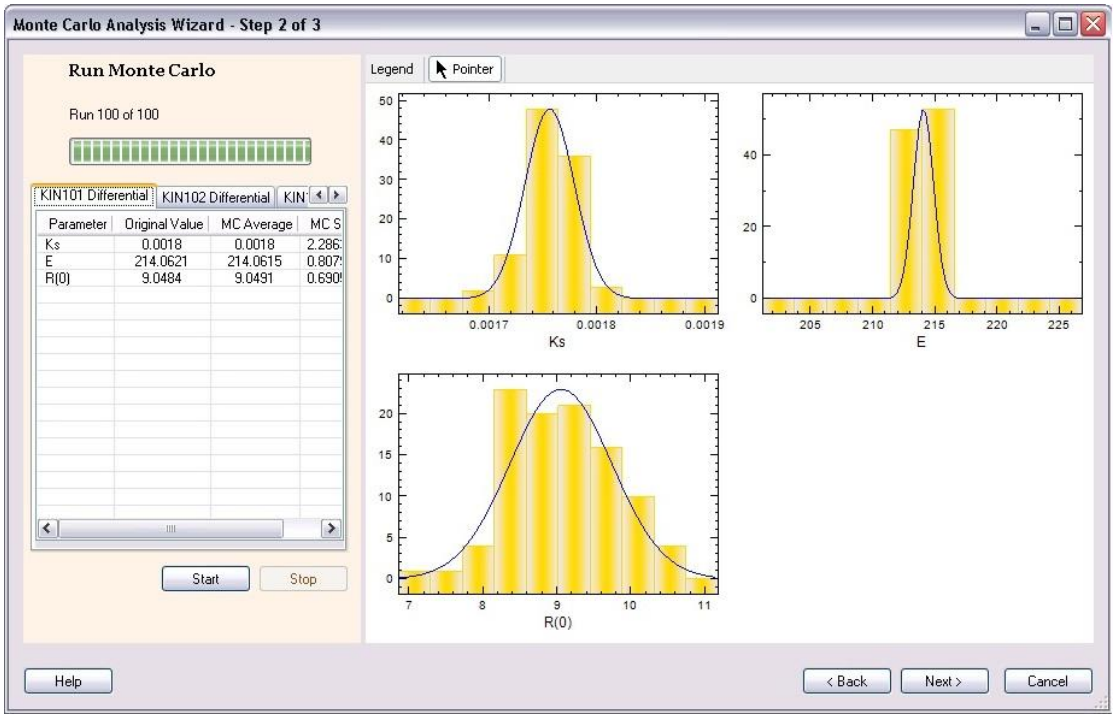


Figure 8.8 – Monte Carlo results for "noise" fit

8.4 – Monte Carlo Summary

The final panel of the Monte Carlo wizard (shown in Figure 8.9) summarizes the Monte Carlo run.

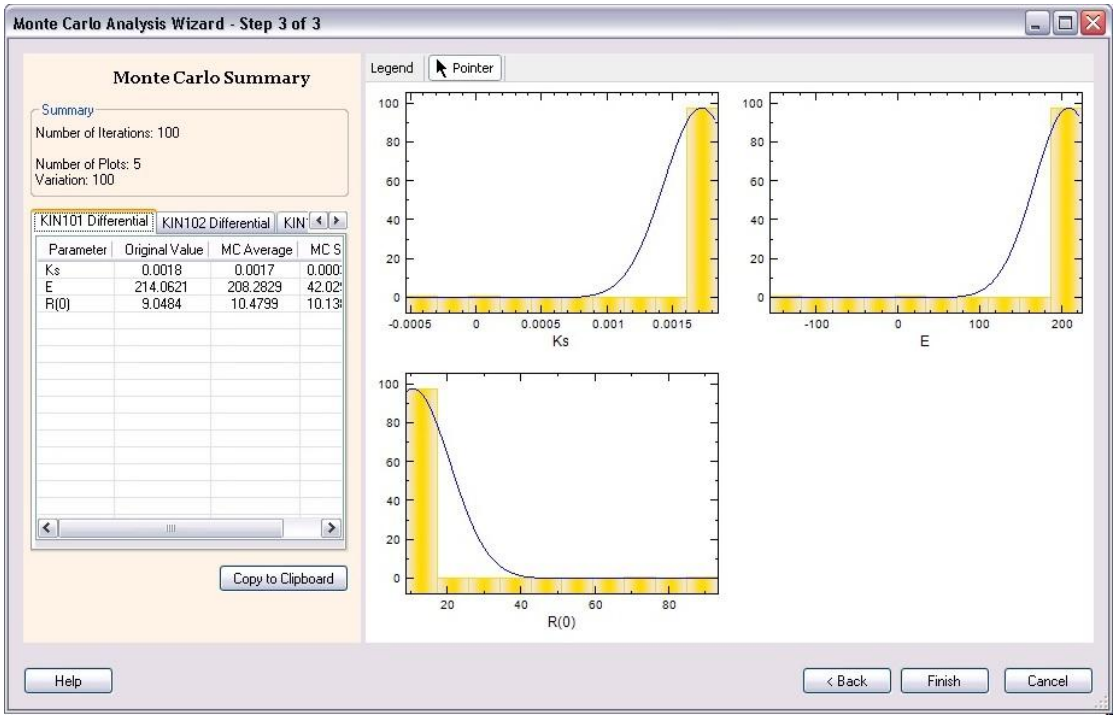


Figure 8.9 – Monte Carlo Summary Panel

The user can copy the results of the Monte Carlo runs to the clipboard. These results can be pasted into a text editing program or a spreadsheet such as Microsoft Excel. The summary will show the Chi Squared for each run of the fit, and the parameter values.

	A	B	C	D	E
1	Number of Iterations: 100				
2					
3	Number of Plots: 5				
4	Variation: 100				
5					
6	KIN101 Differential				
7	Fit ChiSq	Ks	E	R(0)	
8	366.5493	0.001782	217.5192	4.56916	
9	366.5493	0.001782	217.5192	4.56916	
10	366.5493	0.001782	217.5192	4.56916	
11	366.5493	0.001782	217.5192	4.56916	

Figure 8.10 – "Copy to Clipboard" results

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Kanaalweg 29/G
3526 KM Utrecht
The Netherlands

www.metrohm-autolab.com