

## Exploratory Enzyme Inhibition Analysis

Enzyme inhibition data is analyzed with the Exploratory Enzyme Kinetics option in SigmaPlot. The direct linear plot, secondary plots and a numeric report are created to help determine if Michaelis-Menten kinetics are satisfied and to elicit the type of inhibition. This analysis provides excellent qualitative and quantitative information prior to fitting multiple candidate inhibition models using the Enzyme Kinetics module.

**Keywords:** enzyme kinetics, inhibition, direct linear plot, secondary plots, competitive inhibition, mixed inhibition, uncompetitive inhibition, noncompetitive inhibition.

### Introduction

The Exploratory Enzyme Kinetics analysis in SigmaPlot is used to obtain qualitative and quantitative information about the enzyme inhibition mechanism. It creates a direct linear plot, secondary plots and a numeric report. A simple direct linear plot for six substrate and three inhibitor concentrations is shown in Figure 1.

Direct Linear Plot

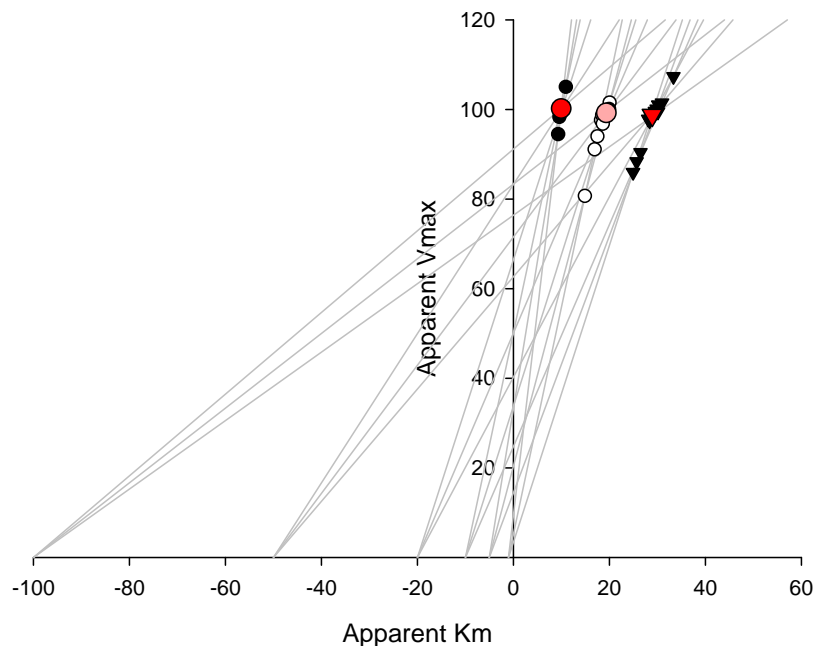


Figure 1. A direct linear plot showing lines defined by substrate and velocity data, intersections of those lines as black and white symbols and the medians of those intersections as alternating dark and light red symbols. The direction that the medians progress in this plot with increasing inhibitor concentration defines the type of inhibition.

The Exploratory EK analysis is designed to work in conjunction with the Enzyme Kinetics module or with data entered into a SigmaPlot worksheet.

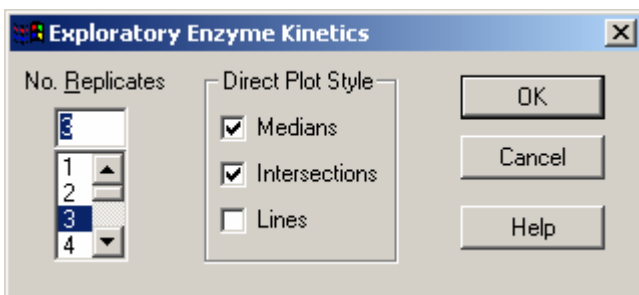
### An Exploratory EK Analysis

Enzyme inhibition data entered directly in a SigmaPlot worksheet is shown in Figure 2. There are six groups of three replicate velocity values corresponding to the six inhibitor values in column 2.

	1-[Substrate]	2-[Inhibitor]	3-v11	4-v12	5-v13	6-v21	7-v22	8-v23
1	2.0000	1.0000	14.1000	13.6990	14.9930	11.7560	12.1860	11.6650
2	5.0000	2.0000	28.7920	25.1240	30.3710	27.0510	26.4780	26.3700
3	10.0000	5.0000	42.8150	41.6450	40.5550	39.7870	38.6250	38.5550
4	20.0000	10.0000	65.0410	61.1880	56.7120	56.9340	50.5170	54.7240
5	50.0000	15.0000	75.6180	71.2600	80.6160	72.4800	72.1540	68.0710
6	100.0000	20.0000	84.3210	87.0180	78.4780	71.7030	74.4400	79.0400
7								
8	0							

Figure 2. An enzyme inhibition data set entered into a SigmaPlot worksheet. Two of six groups of replicate velocity values are shown.

Running Exploratory Enzyme Kinetics displays the dialog



The number of replicates is selected (this is not necessary if the analysis is run on an EK module worksheet) and then the type of plot is selected. For data sets like this one with relatively large number of substrate and inhibitor values, the Lines option is not selected since it clutters the graph and obscures the intersection and median information. Click Ok to run it. Two graph pages and a report are created.

The graph pages are shown side-by-side in Figure 3 below.

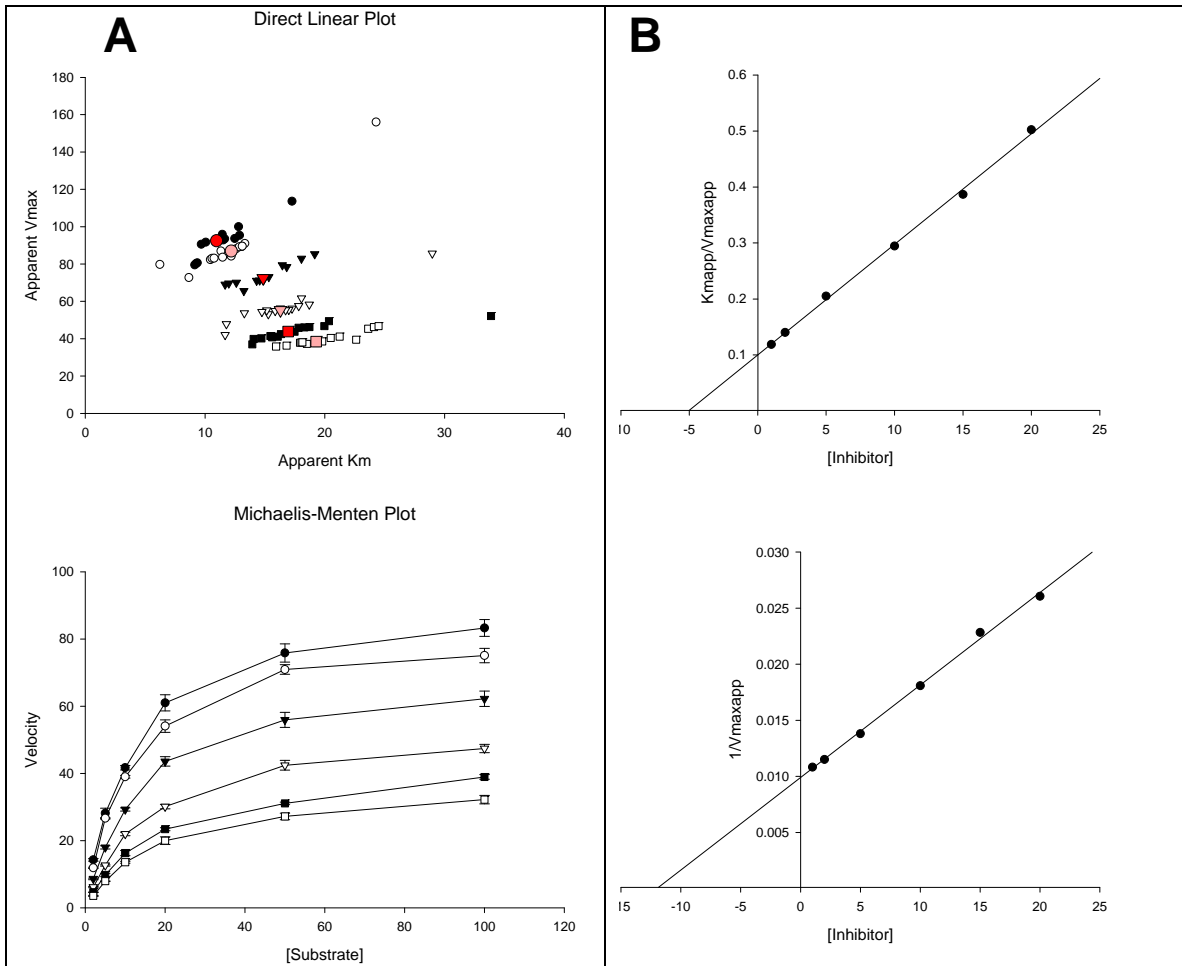


Figure 3. Two graph pages are produced by Exploratory Enzyme Kinetics. A - the direct linear and Michaelis-Menten plots. B – the two secondary plots.

These plots strongly suggest mixed inhibition since the medians progress diagonally down and to the right in the direct linear plot. Also, straight lines fit the secondary plots data very well. The two inhibition constants for mixed inhibition are the intercepts of these lines with the inhibitor axis. From the upper graph  $K_{ic} = 5.1$  and from the lower  $K_{iu} = 12.0$ .

The numeric report provides median values for the direct linear and secondary plots and inhibition constant estimates from the secondary plot linear regressions – Figure 4.

	1	2	3	4	5	6	7	8	9
1	[Inhibitor]	Kmapp	Vmaxapp	Kmapp/Vmaxapp	1/Vmaxapp	Intersections	3rd quad Intersections	Kic	Kiu
2	1.0000	10.9600	92.4600	0.1186	0.0108	15.0000	0.0000	5.0690	11.9900
3	2.0000	12.1900	86.9600	0.1402	0.0115	15.0000	0.0000		
4	5.0000	14.8600	72.4500	0.2052	0.0138	15.0000	0.0000		
5	10.0000	16.3000	55.3300	0.2945	0.0181	15.0000	0.0000		
6	15.0000	16.9400	43.7900	0.3868	0.0228	15.0000	0.0000		
7	20.0000	19.2800	38.3700	0.5024	0.0261	15.0000	0.0000		
8									

Figure 4. The Exploratory EK report.

## A Partial Competitive Inhibition Example

The Michaelis-Menten plot for simulated enzyme kinetics data is shown in Figure 5A. The direct linear plot in Figure 5B has a median trajectory that moves more-or-less horizontally from left to right suggesting a competitive inhibition (a slight decrease in apparent  $V_{max}$  can be visualized so there is a possibility that this is mixed inhibition).

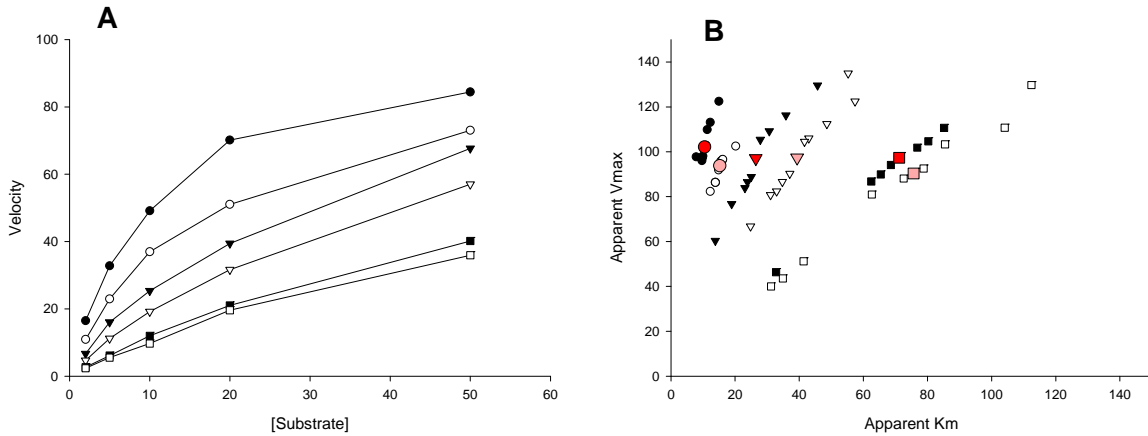


Figure 5. Michaelis-Menten (A) and direct linear plots (B) for simulated data.

The secondary plots in Figure 6 give additional information. The regression line for the apparent  $1/V_{max}$  plot in Figure 6B has a slight positive slope with an inhibitor axis intercept that yields a very large inhibition constant  $K_{iu} = 1345$ . As seen below this is much larger than the inhibition constant  $K_{ic} (=K_i) = 1.85$ . This slope is probably not different from zero in which case the inhibition mechanism is competitive.

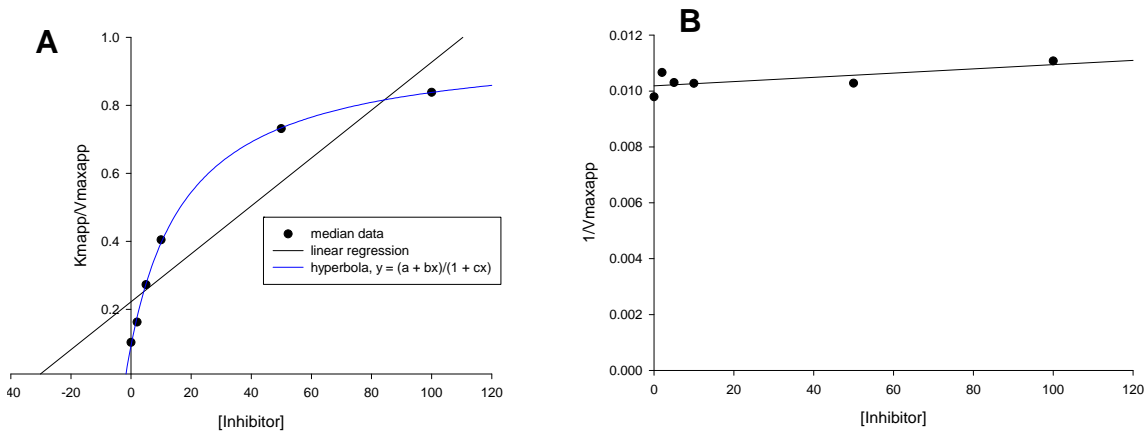


Figure 6. Secondary plots. The apparent  $K_m/V_{max}$  data in Figure 6A is fit with a hyperbolic function which intersects the inhibitor axis at  $-K_{ic} = -1.85$ .

The straight line generated does not fit the apparent  $K_m/V_{max}$  data well. The SigmaPlot hyperbolic function "Rational, 3 Parameter I" fit this data very well ( $R^2 = 0.999$ ). This suggests that the inhibition mechanism is partial since partial inhibition results in hyperbolic secondary plots (hyperbolic inhibition is another name for partial inhibition).

The partial competitive inhibition parameters can be computed from the hyperbolic fit in Figure 6A as  $K_i = 1.85$  and  $\alpha = 10.5$  (we are using the Enzyme Kinetics module parameter terminology where ( $K_i = K_{ic}$  and  $\alpha K_i = K_{iu}$ ). This compares well with the error-free simulation values  $K_i = 2.0$  and  $\alpha = 10.0$ .

A question remains as to whether the inhibition mechanism is competitive or mixed. Analysis of the initial velocity data with all equations in the Single Substrate – Single Inhibition section of the Enzyme Kinetics module produced the equation comparison shown in Table 1. The table is sorted by the Akaike criterion AICc. It separates candidate equations into groups. The competitive (partial) and mixed (partial) equations clearly form one group.

The remaining equations have AICc values nearly 100 units or more higher and therefore can be removed from further consideration. The competitive (partial) equation has an AICc value 2 units less than the mixed (partial) equation and, given this data set, is the best candidate. Though the 2 unit AICc difference is considered to define a difference between equations it is not a large difference, so if determining the mechanism type is important then collecting additional data is warranted.

Rank by AICc	Equation	R <sup>2</sup>	AICc	Sy.x	Runs Test	Converg.
1	Competitive (Partial)	0.98375	204.778	3.00676	pass	Yes
2	Mixed (Partial)	0.98379	206.845	3.02051	pass	Yes
3	Noncompetitive (Partial)	0.95465	297.117	5.02213	pass	Yes
4	Competitive (Full)	0.93093	332.741	6.16233	fail	Yes
5	Mixed (Full)	0.93093	334.985	6.19806	fail	Yes
6	Noncompetitive (Full)	0.90242	363.845	7.32470	fail	Yes
7	Uncompetitive (Full)	0.86781	391.170	8.52549	fail	Yes
8	Uncompetitive (Partial)	0.86952	392.241	8.51920	fail	Yes

Table 1. Comparison of Enzyme Kinetics Module single substrate-single inhibitor equation fits to simulated data.

The excellent fit of the competitive (partial) equation to this data is shown in Figure 7 by the Lineweaver-Burk plot from the Enzyme Kinetics Module.

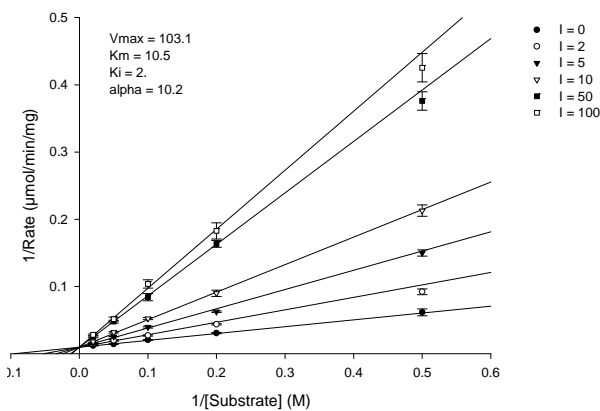


Figure 7. Lineweaver-Burk plot of the competitive (partial) equation fit to simulated data. Very good inhibition parameter estimates were obtained for the realistic 7% constant percentage error used.

## **Other Analyses**

One can read the article: "Exploratory Enzyme Kinetics Help" for other analysis examples.