# Enzyme Kinetics Analysis Report

Jozef Fülöp

March 2023

## 1 Introduction

Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzymes. This report analyzes two sets of experimental data on enzyme kinetics, one with an inhibitor and one without an inhibitor. Calculates the  $V_{max}$ ,  $K_M$  and  $K_I$  values.

## 2 Input data

(a) Reaction without inhibitor

[S](mmol/l)	[I](mmol/l)	V (µmol/L/min)	
1	0	0.67	
2	0	1.13	
3	0	1.48	
4	0	1.74	
5	0	1.98	
(b) Reaction with inhibitor			

[S](mmol/l)	[I](mmol/l)	V (µmol/L/min)
1	1	0.05
2	1	0.10
3	1	0.15
4	1	0.16
5	1	0.18

Table 1: Reaction rates with and without inhibitor

### **Equations**

The Michaelis-Menten equation is given by:

$$V = V_{max} \frac{[S]}{K_M + [S]} \tag{1}$$

The Lineweaver-Burk equation is given by:

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{1}{K_M} \cdot \frac{1}{[S]} \tag{2}$$

The Michaelis-Menten equation can be derived from the Lineweaver-Burk equation by multiplying both sides by V and rearranging the terms:

$$V = \frac{V_{max}}{1 + \frac{K_M}{[S]}} \tag{3}$$

The Michaelis-Menten equation can be used to calculate the  $K_I$  value, in noncompetitive inhibition, as follows:

$$V = \frac{\frac{V_{max}}{1 + \frac{[I]}{K_I}} \cdot [S]}{K_M + [S]}$$

$$(4)$$

#### **Plots**

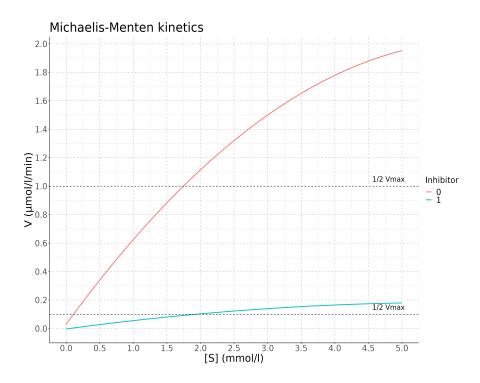


Figure 1: Michaelis-Menten kinetics plot showing the relationship between substrate concentration and reaction rate with and without inhibitor. The plot also includes fitted values and  $1/2\ V_{max}$  lines for both datasets.

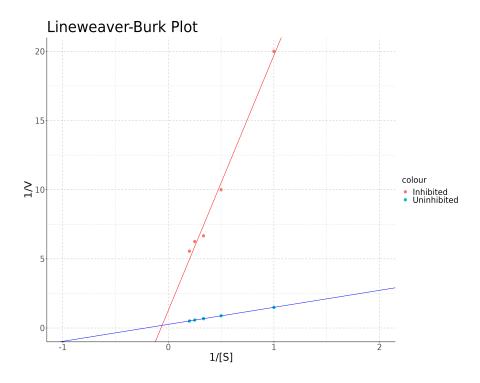


Figure 2: Lineweaver-Burk plot showing the reciprocal of substrate concentration (1/[S]) versus the reciprocal of enzyme velocity (1/V) for inhibited (red) and uninhibited (blue) enzyme reactions. The plot includes two linear regression lines with slopes and intercepts calculated from the data.

## 3 Uninhibited Reaction

The first set of data is from an uninhibited reaction. The data was fit to the Michaelis-Menten equation  $V = V_{max} \frac{[S]}{K_M + [S]}$ , where  $V_{max}$  is the maximum velocity of the reaction, [S] is the substrate concentration, and  $K_M$  is the Michaelis constant. The fitted parameters are as follows:

- $V_{max} = 3.89 \pm 0.08 \text{ mmol/l}$
- $K_M = 4.88 \pm 0.18 \, \mu \text{mol/L/min}$

Table 2: Model summary without inhibitor

	Estimate	Std. Error	t value	$\Pr(> t )$
$V_{max}$	3.892276	0.0846582	45.97636	0.0000227
$K_M$	4.881717	0.1828702	26.69498	0.0001153

Table 3: Confidence interval for Vm and Km for uninhibited reaction

	2.5%	97.5%
$V_{max}$	3.640811	4.185819
$K_M$	4.340821	5.518560

### 4 Inhibited Reaction

The second set of data is from an inhibited reaction. The data was fit to the Michaelis-Menten equation  $V = V_{max} \frac{[S]}{K_M + [S]}$ , where  $V_{max}$  is the maximum velocity of the reaction, [S] is the substrate concentration, and  $K_M$  is the Michaelis constant. The fitted parameters are as follows:

- $V_{max} = 0.39 \pm 0.10 \text{ mmol/l}$
- $K_M = 5.71 \pm 2.30 \; \mu \text{mol/L/min}$

Table 4: Model summary with inhibitor

	Estimate	Std. Error	t value	$\Pr(> t )$
$V_{max}$	0.3945488	0.0974732	4.047765	0.0271504
$\overline{K_M}$	5.7125880	2.2981536	2.485729	0.0888187

Table 5: Confidence interval for  $V_{max}$  and  $K_M$  for inhibited reaction

	2.5%	97.5%
$V_{max}$	0.2437008	3.270304
$K_M$	2.5583919	74.920253

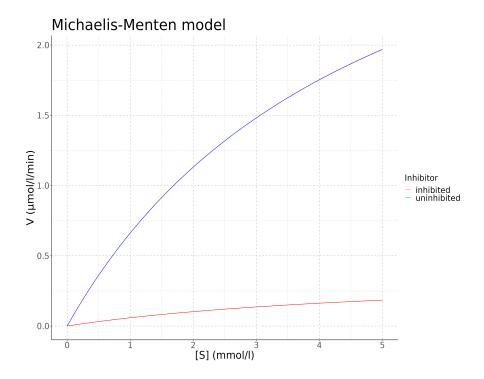


Figure 3: Michaelis-Menten model for enzyme kinetics, showing the substrate concentration [S] (mmol/l) on the x-axis and the reaction rate V ( $\mu$ mol/l/min) on the y-axis. The blue line represents the reaction rate without inhibitor, while the red line shows the reaction rate with inhibitor. The legend indicates whether the inhibitor is present or absent. The plot illustrates the effect of an inhibitor on the enzyme activity, with the inhibited reaction rate curve shifted downwards compared to the uninhibited reaction rate curve.

# 5 Non-competitive inhibition

The data was fit to the same Michaelis-Menten equation as before, but with an

additional term to account for the inhibitor: 
$$V = \frac{\frac{V_{max}}{1 + \frac{[I]}{K_I}} \cdot [S]}{K_M + [S]}$$

where [I] is the inhibitor concentration and  $K_I$  is the inhibition constant. The fitted parameters are as follows:

- $V_{max} = 0.39 \pm 0.10 \, \mu \text{mol/L/min}$
- $K_M = 5.71 \pm 2.30 \text{ mmol/l}$
- $K_I = 0.10 \pm 0.00 \text{ mmol/l}$

Table 6: Model summary for non-competitive inhibition

	Estimate	Std. Error	t value	$\Pr(> t )$
$V_{max}$	3.8955119	0.0767059	50.78505	0
$K_M$	4.8887879	0.1656901	29.50562	0
$K_I$	0.1017605	0.0036314	28.02254	0

Table 7: Confidence interval for non-competitive inhibition

	2.5%	97.5%
$V_{max}$	3.7222470	4.0877450
$K_M$	4.5155594	5.3052748
$K_I$	0.0932454	0.1104207

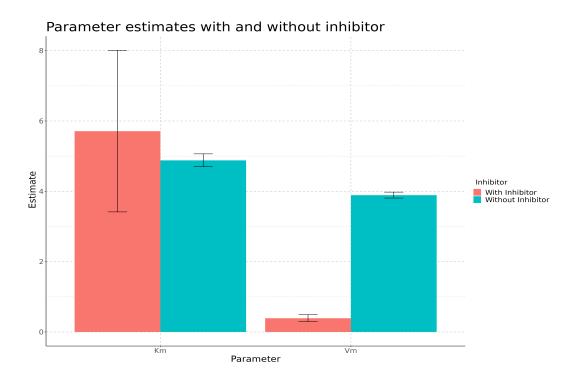


Figure 4: Bar plot showing parameter estimates with and without inhibitor for the  $V_{max}$  and  $K_M$  parameters. Error bars represent the standard error of the estimates.