Kinetic Characterization of the enzyme and study of substrate inhibition

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Background

The experiment involves the kinetic characterization of an enzyme, which can be determined by calculating the values of Km and Vmax.

The Lineweaver-Burk plot is a graph of 1/V vs 1/[S], which can be used to determine the values of Km and Vmax.

Eisenthal and Cornish-Bowden suggested a new approach to determine Km and Vmax, which involves plotting a hypothetical graph of Vmax vs Km at a constant [S] and V.

Inhibition of enzyme-catalyzed reactions can occur through three main mechanisms: competitive, non-competitive, and mixed inhibition.

The effect of inhibitors on the kinetic parameters of the enzyme can be expressed mathematically using the equations for competitive, non-competitive, and mixed inhibition.

Procedure


A total of 5µL of the sample was taken and added to 50µL of freshly prepared and diluted sucrose solution and 545µL of distilled water/CuSO4 concentration 1/CuSO4 concentration 2.
The mixture was incubated at 30°C for 5 minutes.
After the incubation, 200µL of alkaline DNS was added to the mixture.
The mixture was further incubated at 90°C for 5 minutes.
Next, 200µL of 50mM sodium acetate buffer with pH 4.8 was added to the mixture.
A blank was prepared with 50µL of distilled water in place of sucrose.
The absorbance readings of all the samples were taken at 540nm using a spectrophotometer.
The Lineweaver-Burk plot was plotted for all three cases.
The equipment used, such as the spectrophotometer, water bath, cuvettes, pipettes and tips, and Eppendorfs, were properly cleaned and maintained before and after use.
The reagents used, including 1M sucrose solution, alkaline DNS, 50mM sodium acetate buffer with pH 4.8, 100IU/ml invertase enzyme, CuSO4 (two concentrations), and distilled water, were of high quality and freshly prepared.

Observation

Sucrose conc.	Set 1 (water)	Set 2 (Inh 1)	Set 3 (Inh 2)
0	0	0	0
50	0.03	0.008	0.015
100	0.033	0.011	0.058
150	0.018	0.034	0.023
200	0.035	0.065	0.006
250	0.043	0.069	-0.003

Reaction rate calculation

Set 1 (water)

C1 (mM)	Abs. for C2	ε (mM^-1 cm^-1	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	v = ΔC/t	
	0	0	14.2	1	1000	0.000	0	5	0.00
	50	0.03	14.2	1	1000	2.113	48	5	9.58
	100	0.033	14.2	1	1000	2.324	98	5	19.54
	150	0.018	14.2	1	1000	1.268	149	5	29.75
	200	0.035	14.2	1	1000	2.465	198	5	39.51
	250	0.043	14.2	1	1000	3.028	247	5	49.39

Set 2 (Inh 1)

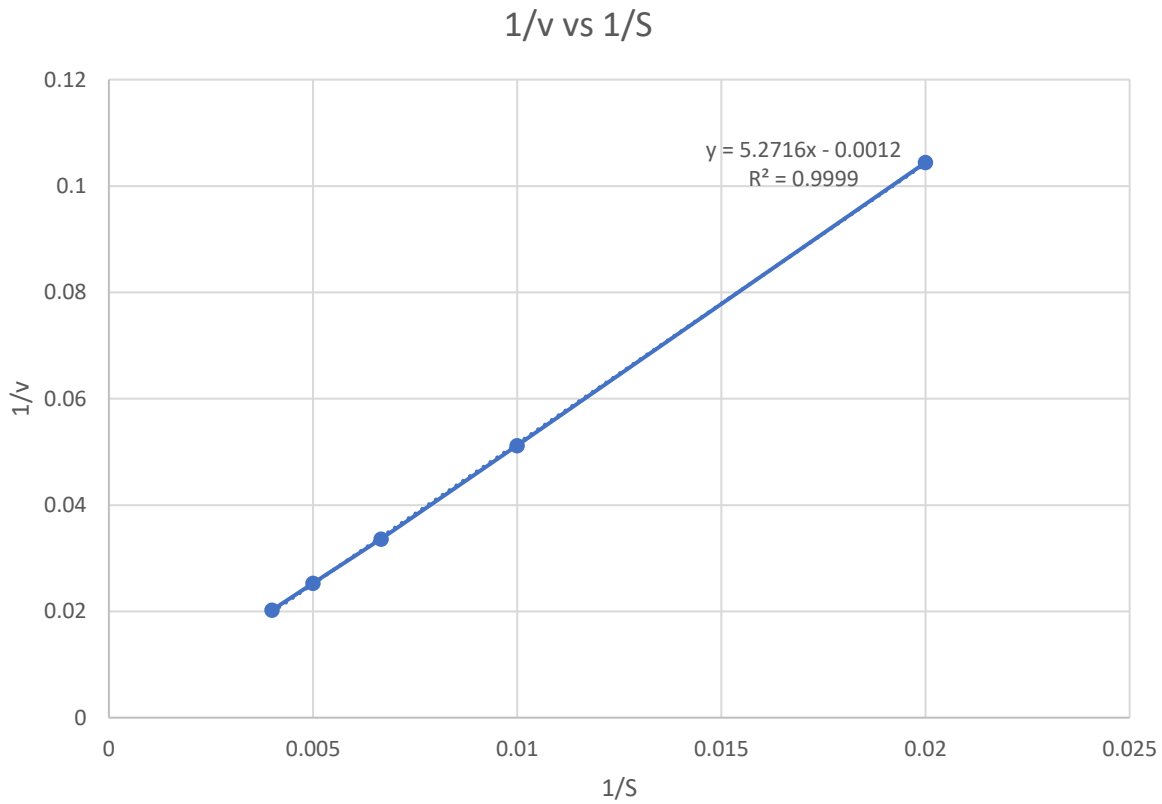
C1 (mM)	Abs. for C2	ε (mM^-1 cm^-1	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	v = ΔC/t	
	0	0	14.2	1	1000	0.000	0	5	0.00
	50	0.008	14.2	1	1000	0.563	49	5	9.89
	100	0.011	14.2	1	1000	0.775	99	5	19.85
	150	0.034	14.2	1	1000	2.394	148	5	29.52
	200	0.065	14.2	1	1000	4.577	195	5	39.08
	250	0.069	14.2	1	1000	4.859	245	5	49.03

Set 3 (Inh 2)

C1 (mM)	Abs. for C2	ε (mM^-1 cm^-1	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	v = ΔC/t	
	0	0	14.2	1	1000	0.000	0	5	0.00
	50	0.015	14.2	1	1000	1.056	49	5	9.79
	100	0.058	14.2	1	1000	4.085	96	5	19.18
	150	0.023	14.2	1	1000	1.620	148	5	29.68
	200	0.006	14.2	1	1000	0.423	200	5	39.92
	250	-0.003	14.2	1	1000	-0.211	250	5	50.04

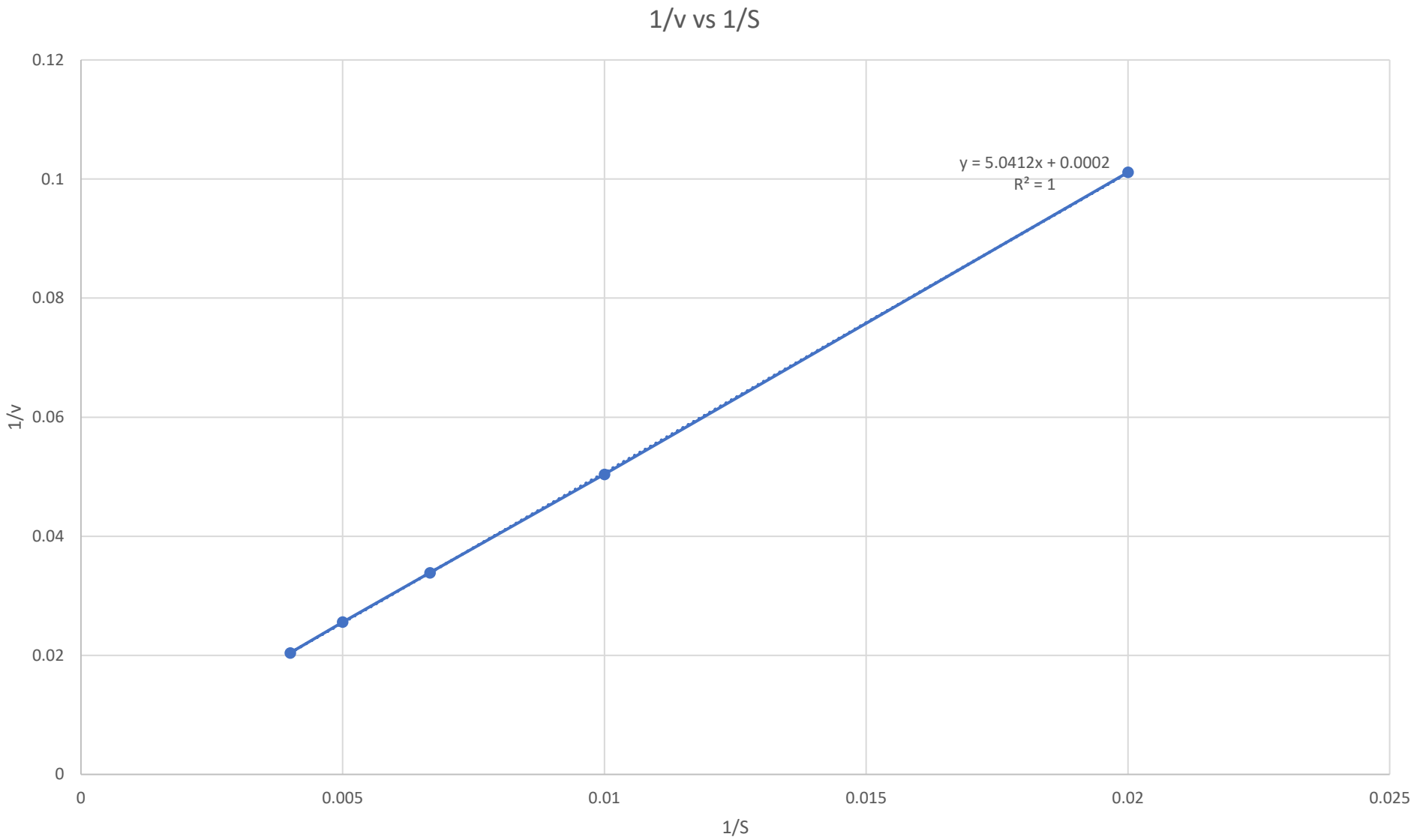
Without Inhibitor

Set 1 (water)	Without Inhibitor									
C1 (mM)	Abs. for C2	ε (mM^-1 cm^-1	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	v = ΔC/t	1/[S]	1/V
0	0	14.2	1	1000	0.000	0	5	0.00		
50	0.03	14.2	1	1000	2.113	48	5	9.58	0.020	0.104
100	0.033	14.2	1	1000	2.324	98	5	19.54	0.010	0.051
150	0.018	14.2	1	1000	1.268	149	5	29.75	0.007	0.034
200	0.035	14.2	1	1000	2.465	198	5	39.51	0.005	0.025
250	0.043	14.2	1	1000	3.028	247	5	49.39	0.004	0.020


1/v vs 1/S
y = 5.2716x - 0.0012
R² = 0.9999
0.12
0.1
0.08
0.06
0.04
0.02
0
1/v
0
0.005
0.01
0.015
0.02
0.025
1/S

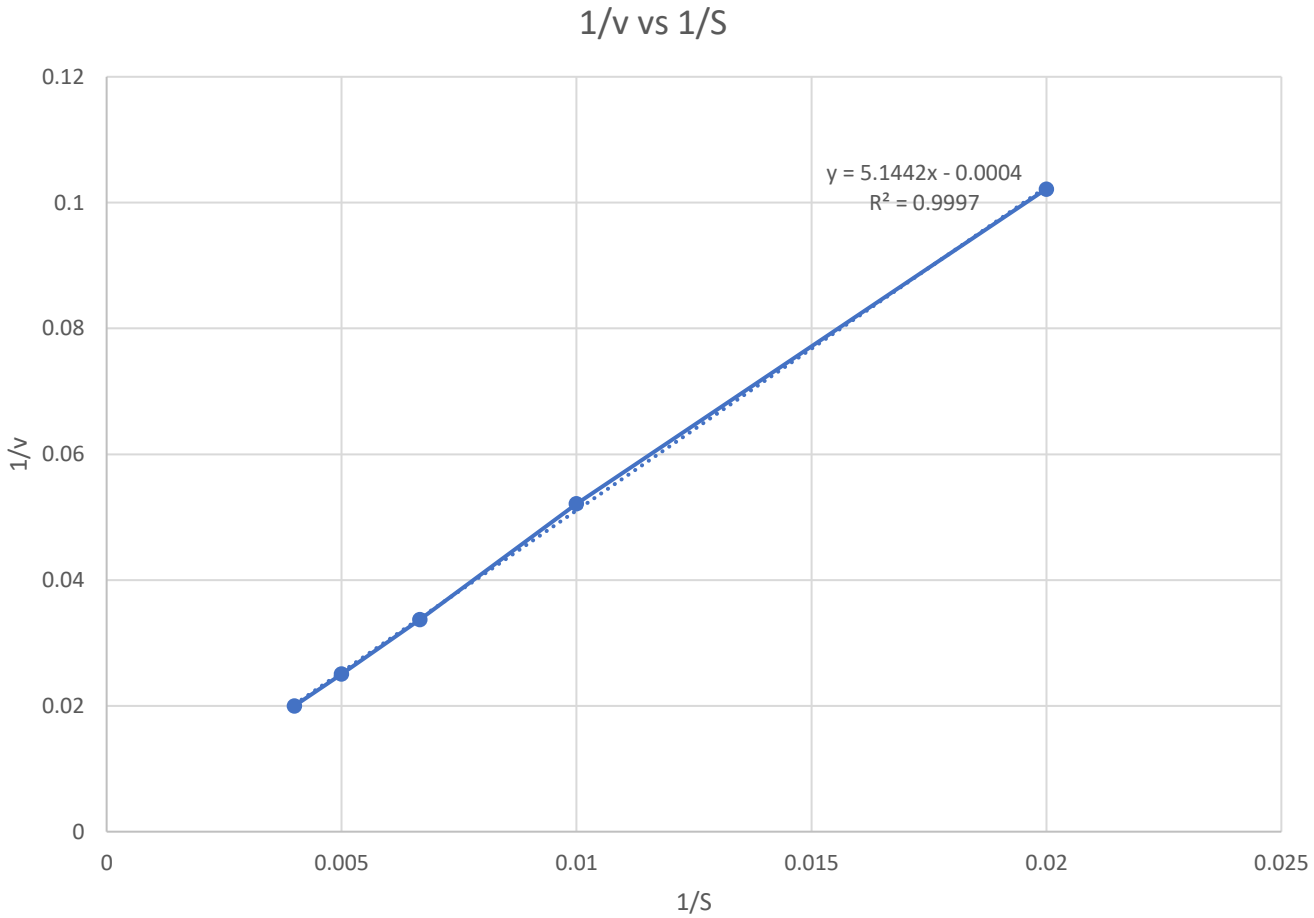
With Inhibitor (1mM)

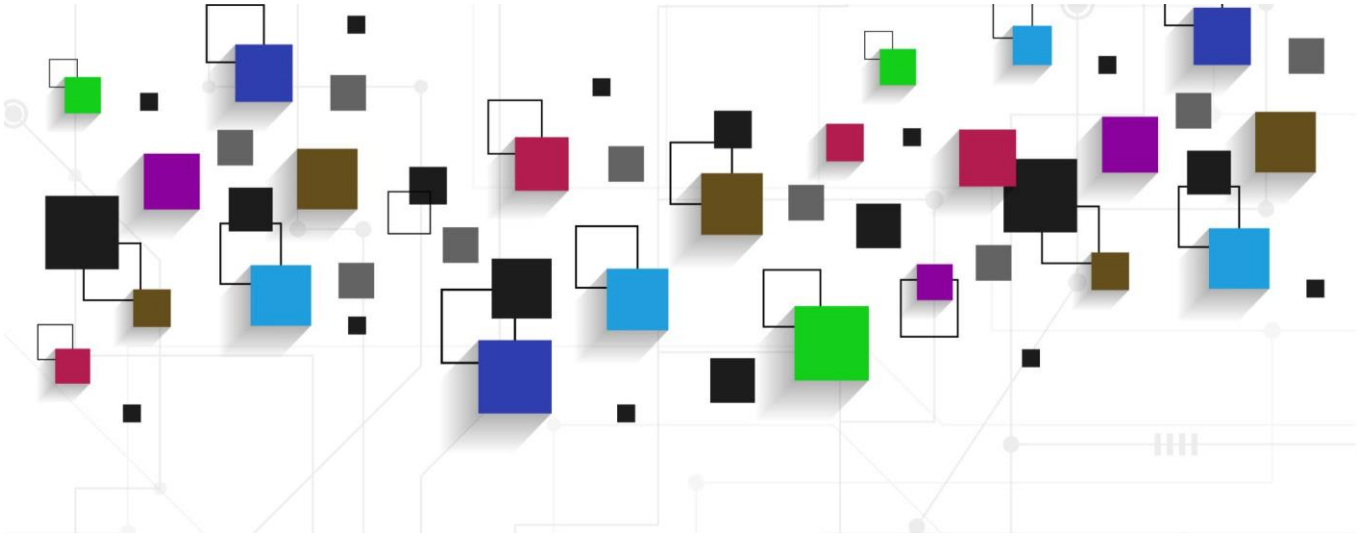
Set 2 (Inh 1)	With Inhibitor (1mM)									
C1 (mM)	Abs. for C2	ε (mM^-1 cm^-1	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	v = ΔC/t	1/[S]	1/V
0	0	14.2	1	1000	0.000	0	5	0.00		
50	0.008	14.2	1	1000	0.563	49	5	9.89	0.020	0.101
100	0.011	14.2	1	1000	0.775	99	5	19.85	0.010	0.050
150	0.034	14.2	1	1000	2.394	148	5	29.52	0.007	0.034
200	0.065	14.2	1	1000	4.577	195	5	39.08	0.005	0.026
250	0.069	14.2	1	1000	4.859	245	5	49.03	0.004	0.020


1/v vs 1/S
y = 5.0412x + 0.0002
R² = 1
0.12
0.1
0.08
0.06
0.04
0.02
0
1/v
0
0.005
0.01
0.015
0.02
0.025
1/S

With Inhibitor (10mM)

Set 3 (Inh 2)	With Inhibitor (10mM)									
C1 (mM)	Abs. for C2	ε (mM^-1 cm^-1	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	v = ΔC/t	1/[S]	1/V
0	0	14.2	1	1000	0.000	0	5	0.00		
50	0.015	14.2	1	1000	1.056	49	5	9.79	0.020	0.102
100	0.058	14.2	1	1000	4.085	96	5	19.18	0.010	0.052
150	0.023	14.2	1	1000	1.620	148	5	29.68	0.007	0.034
200	0.006	14.2	1	1000	0.423	200	5	39.92	0.005	0.025
250	-0.003	14.2	1	1000	-0.211	250	5	50.04	0.004	0.020


1/v vs 1/S
y = 5.1442x - 0.0004
R² = 0.9997
1/v
1/S
0.12
0.1
0.08
0.06
0.04
0.02
0
0
0.005
0.01
0.015
0.02
0.025



Thank you