


Kinetic Analysis of enzyme-catalyzed reaction (Determination of K_m and V_m)

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Background

- The experiment determines the kinetic parameters of an enzyme-catalyzed reaction using invertase enzyme and sucrose substrate.
- Kinetic parameters, K_m and V_{max} , are determined using two methods: Lineweaver-Burk plot and Eisenthal-Cornish-Bowden plot.
- The alkaline DNS assay is used to measure the reaction rate.
- Five different concentrations of sucrose are prepared and incubated with enzyme for 5 minutes at room temperature.
- Absorbance readings are taken at 540 nm, and enzyme activity is calculated using a standard curve.



Procedure

- Five different concentrations of sucrose (ranging from 0 to 300 mM) were prepared by diluting a 1 M sucrose stock solution.
- For each sucrose concentration, 5 μL of invertase enzyme was added to 50 μL of the freshly diluted sucrose solution.
- The mixture was incubated at room temperature for 5 minutes.
- After incubation, 200 μL of alkaline DNS was added to the mixture.
- The mixture was then incubated at 90°C for 5 minutes.
- Next, 200 μL of 50 mM sodium acetate buffer at pH 4.8 was added to the mixture.
- For the blank sample, 5 μL of Tris-Cl was added instead of the enzyme.
- The absorbance readings were taken at 540 nm using a spectrophotometer, and the enzyme activity was calculated using a standard curve.
- The data obtained from the experiment was used to prepare a Lineweaver-Burk plot to determine the kinetic parameters K_m and V_{max} of the enzyme.

Observance

Sample conc.	Abs.
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0	0
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200	0.688
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400	1.018
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600	1.402
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800	0.392
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1000	0.496
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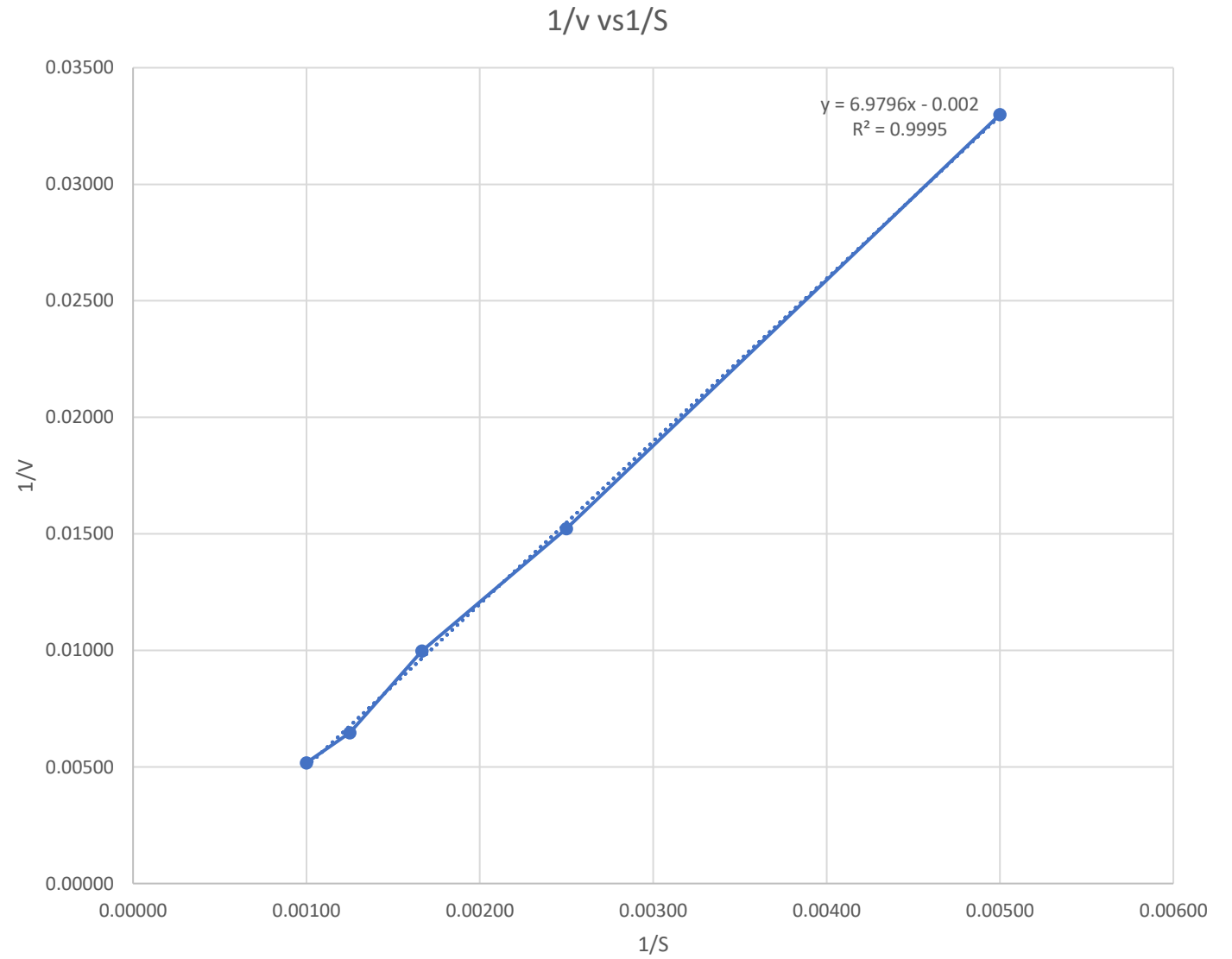
Reaction rate calculation

C1 (mM)	Abs. for C2	ϵ (mM ⁻¹ cm ⁻¹)	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	$v = \Delta C/t$
0	0	14.2	1	1000	0.000	0	5	0
200	0.688	14.2	1	1000	48.451	151.5492958	5	30.30985915
400	1.018	14.2	1	1000	71.690	328.3098592	5	65.66197183
600	1.402	14.2	1	1000	98.732	501.2676056	5	100.2535211
800	0.392	14.2	1	1000	27.606	772.3943662	5	154.4788732
1000	0.496	14.2	1	1000	34.930	965.0704225	5	193.0140845

$1/v$ vs $1/S$

$1/[S]$	$1/v$
0.00100	0.00518
0.00125	0.00647
0.00167	0.00997
0.00250	0.01523
0.00500	0.03299

Lineweaver-Burk plot



Calculations and result

The equation of the line is $y = 6.9796x - 0.002$.

The slope of the line is 6.9796 and the y-intercept is -0.002.

Based on the Lineweaver-Burk plot that was generated from the given data,

- the reciprocal of the y-intercept ($-1/0.002$) gives the value of **$V_{max} = 500 \text{ mM/min}$**
- The slope (6.9796) gives the value of K_m/V_{max} , which gives **$K_m = 3489.8 \text{ mM}$**
- Which is totally wrong, which signifies the errors in data collection and calculation using Lineweaver Burk plot.



Thank you