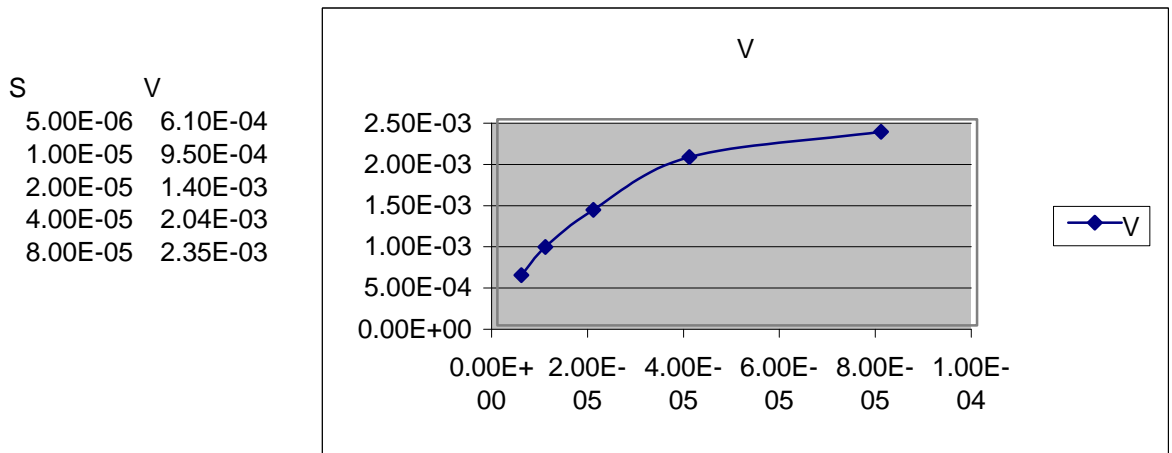
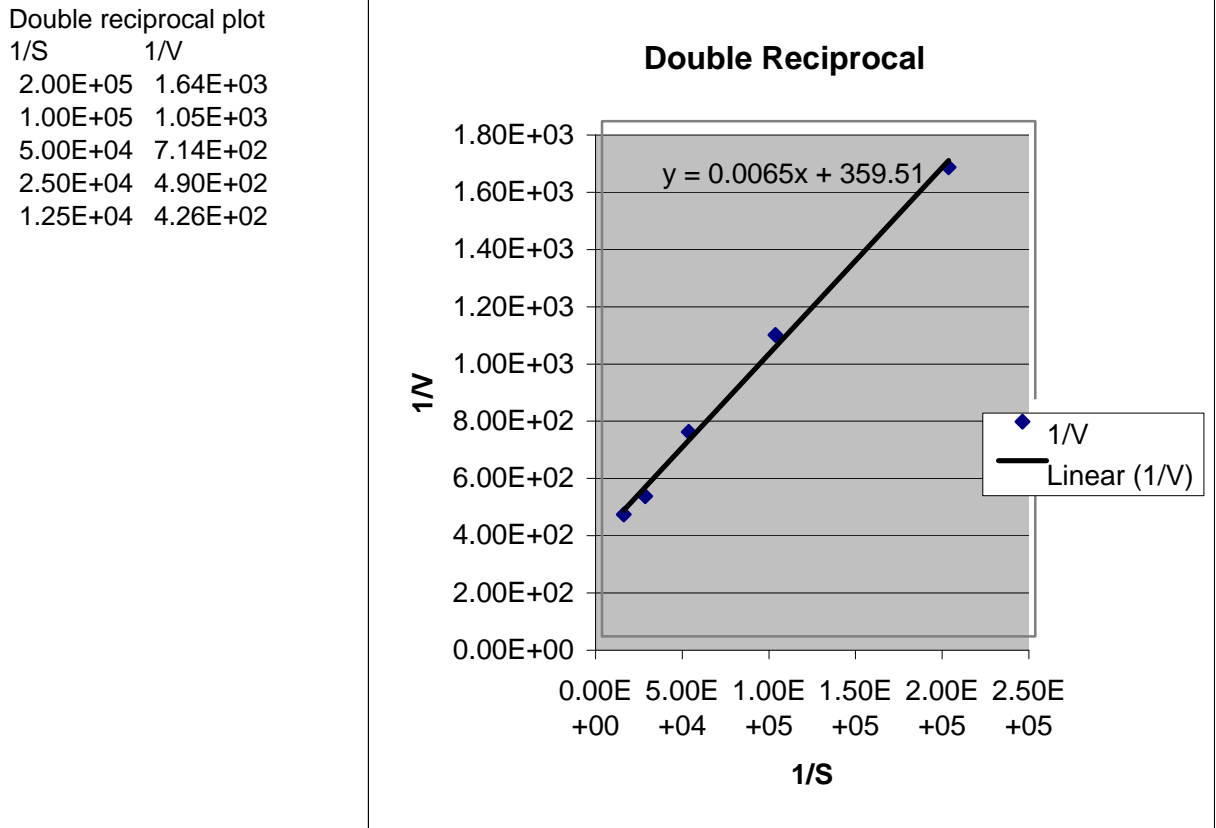


Plot of raw data - Shows standard Michaelis-Menten kinetics



From above data can estimate that V_{max} is about 2.5×10^{-3} mol/min and that K_m (S at $1/2 V_{max}$) is about 1.9×10^{-5} M

But you can get more accurate values using the double reciprocal plot



Y intercept = point where $X=0 = 1/V_{max}$

$$Y = .0065(0) + 359.51$$

$$Y = 359.51; \mathbf{V_{max} = 1/359.51 = 2.78 \times 10^{-3} \text{ mol/min}}$$

X intercept = point where $Y = 0 = -1/K_m$

$$0 = .0065X + 359.51$$

$$-359.51 = .0065X; X = -359.51/.0065 = -55310$$

$$-1/K_m = -55310; \mathbf{K_m = 1.8 \times 10^{-5} \text{ M}}$$

I will use $V_{max} = K_{cat}[E_0]$, but technically you can't make $K_3 = K_{cat}$ unless you prove that K_3 is rate limiting

$$2.78 \times 10^{-3} = K_{cat} (1 \times 10^{-6} \text{ M})$$

$$\mathbf{K_{cat} = 2780 \text{ min}^{-1}}$$

Specificity Constant = k_{cat}/K_m

$$2780 \text{ min}^{-1} / 1.8 \times 10^{-5} \text{ M}$$

$$\mathbf{1.54 \times 10^8 \text{ min}^{-1} \text{ M}^{-1}}$$

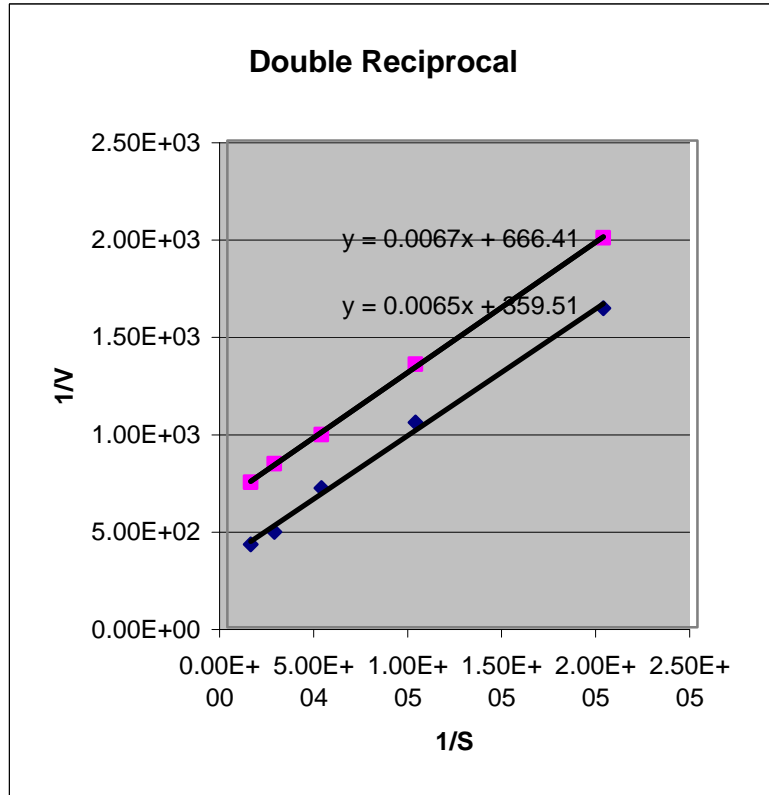
$$\mathbf{= 2.57 \times 10^6 \text{ sec}^{-1} \text{ M}^{-1}}$$

Part 2 Inhibition

S	V(no inhibit)	V(inhibition)
5.00E-06	6.10E-04	5.00E-04
1.00E-05	9.50E-04	7.40E-04
2.00E-05	1.40E-03	1.01E-03
4.00E-05	2.04E-03	1.19E-03
8.00E-05	2.35E-03	1.34E-03

Double reciprocal

1/S	NO	Inhibit
2.00E+05	1.64E+03	2.00E+03
1.00E+05	1.05E+03	1.35E+03
5.00E+04	7.14E+02	9.90E+02
2.50E+04	4.90E+02	8.40E+02
1.25E+04	4.26E+02	7.45E+02



Data indicates that this is a reversible inhibitor

2 parallel lines indicate that lactate is Uncompetitive Inhibitor

An Uncompetitive inhibitor binds at a site that is distinct from the active site, and only binds to the ES complex

For an uncompetitive inhibitor the equation is:

$$V_o = V_{max}[S] / (K_m + a[S])$$

You can plug in to this equation for any data point, so let's try the data point at $S = 8 \times 10^{-5}$, $V = 1.34 \times 10^{-3}$

$$1.34 \times 10^{-3} = (2.78 \times 10^{-3} \times 8 \times 10^{-5}) / (1.8 \times 10^{-5} + a[8 \times 10^{-5}])$$

$$1.34 \times 10^{-3} = (2.22 \times 10^{-7}) / (1.8 \times 10^{-5} + a[8 \times 10^{-5}])$$

$$1.34 \times 10^{-3} (1.8 \times 10^{-5} + a[8 \times 10^{-5}]) = 2.22 \times 10^{-7}$$

$$2.4 \times 10^{-8} + 1.072 \times 10^{-7} a = 2.22 \times 10^{-7}$$

$$a = (2.22 \times 10^{-7} - 2.4 \times 10^{-8}) / 1.072 \times 10^{-7}$$

$$a = 1.847014925$$

$$a = 1 + [I]/K_i$$

$$1.85 = 1 + [I]/K_i$$

$$1.85 - 1 = .5/X$$

$$X = .5/.85$$

$$X = 0.588235294$$

$$K_i = .59 \text{ M}$$

lex