ENZYME KINETICS:

- The **rate** of the reaction catalyzed by enzyme E

  $A + B \leftrightarrow P$

  is defined as

  $$\frac{-\Delta[A]}{\Delta t} \quad \text{or} \quad \frac{-\Delta[B]}{\Delta t} \quad \text{or} \quad \frac{\Delta[P]}{\Delta t}$$

  - A and B changes are negative because the substrates are disappearing
  - P change is positive because product is being formed.

- **Enzyme activity can be assayed in many ways**
  - disappearance of substrate
  - appearance of product

- **For example, you could measure**
  - appearance of colored product made from an uncolored substrate
  - appearance of a UV absorbent product made from a non-UV-absorbent substrate
  - appearance of radioactive product made from radioactive substrate

    - **Many other ways possible – Just need a way to distinguish the products from the substrates**

- The **VELOCITY** (reaction rate) (product formation of disappearance of substrate/time) of an enzyme catalyzed reaction is dependent upon the substrate concentration [S].

  Velocity related to [S]

Enzyme Kinetics: Velocity

The velocity (V) of an enzyme-catalyzed reaction is dependent upon the substrate concentration [S]

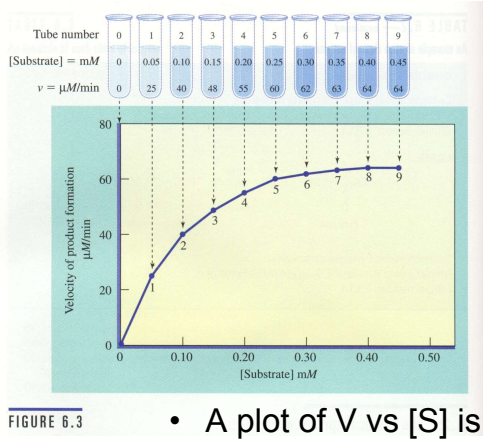
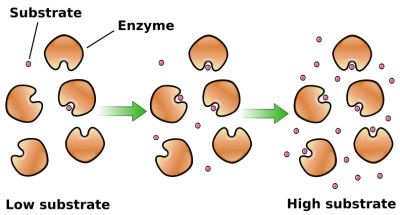


FIGURE 6.3



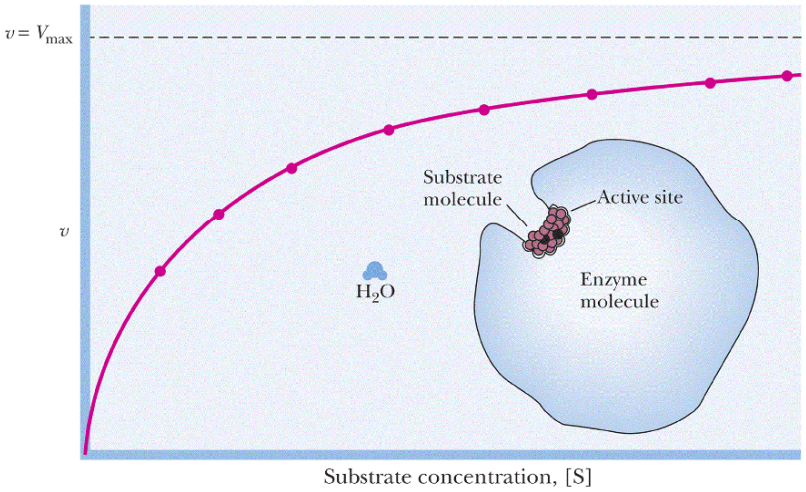
- A plot of V vs [S] is often hyperbolic

Michaelis-Menten plot

Graph is not a graph of product formation over time!!!

- **An example of how to do a kinetics experiment:**
  A. Take 9 tubes, add identical amount of enzyme (E) to each tube
  B. Each tube contains an **increasing** amount of substrate (S) starting with zero
  C. Measure the velocity by determining the rate of product formation
  D. Plot these values – **Velocity against substrate concentration**
  E. Generate the curve shown:
     i. Often the shape is hyperbolic – a characteristic of many enzymes – shape suggests that the enzyme physically combines with the substrate – ES complex
     ii. Called a **SATURATION PLOT or MICHAELIS-MENTEN PLOT** after the two biochemists that first described and explained the curve shape.
- Let's look at the various features of the plot:

Garrett/Grisham, Biochemistry with a Human Focus
Figure 10.7



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A. As [S] is first increased, the **initial rate or velocity (V_0)** increases with increasing substrate concentration
   i. V is proportional to [S]
B. As [S] increases, V increases less and less
   i. V is NOT proportional to [S] in this range
C. Finally, V doesn't increase anymore and velocity reaches its maximum (V_{max})
   i. Enzyme is working as fast as it can
D. Velocity won't change no matter how much substrate is present. At this point, the enzyme is **saturated** with substrate, **S**.

Two analogies:

1. **Toll Plaza (with 5 booths)**
   - Rate at which cars can get through the booths is not affected by the number of waiting cars, only by the available number of toll attendants.
2. **Paper Airplane Example**

http://www.wellesley.edu/Biology/Concepts/Html/initialvelocity.html

QUANTITATIVE EXPRESSION OF ENZYME BEHAVIOR:

- The **Michaelis-Menten** equation describes the kinetic behavior of many enzymes

- This equation is based upon the following reaction:

S → P

$$\mathbf{E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_2} E + P}$$

k_1, k_{-1} and k_3 are rate constants for each step

To derive the equation, they made 2 assumptions:

1. The reverse reaction (P → S) is not considered because the equation describes initial rates when [P] is near zero
2. The ES complex is a **STEADY STATE INTERMEDIATE**
   i.e. the concentration of ES remains relatively constant because it is produced and broken down at the same rate

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

Michaelis-Menten Equation
(equation for a hyperbola)

- **V is the reaction rate (velocity) at a substrate concentration [S]**

- $\mathbf{V_{max}}$ is the **maximum rate** that can be observed in the reaction
  - substrate is present in excess
  - enzyme can be **saturated** (zero order reaction)

- **K_M is the Michaelis constant**
  - a constant that is related to the affinity of the enzyme for the substrate
  - units are in terms of concentration
  - It is a combination of rate constants

$$K_M = \frac{k_2 + k_{-1}}{k_1}$$

Understanding K_m – the Michaelis Constant

- K_M is the Michaelis constant
  - K_M is constant for any given enzyme/substrate pair
    - Independent of substrate or enzyme concentration
  - units are in terms of concentration

  K_m is a constant derived from rate constants.

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

- K_m is a measure of ES binding; relative measure of the affinity of a substrate for an enzyme (how well it binds)
  - In the simplest assumption, the rate of ES breakdown to product (k_2) is the rate-determining step of the reaction

- Small K_m means tight binding; large K_m means weak binding.

- Since K_M has the same units as substrate concentration, this implies a relationship between K_M and [S]
- What happens when K_M = [S]

$$V = \frac{V_{max}[S]}{[S] + [S]} = V = \frac{V_{max}[S]}{2[S]} = \frac{V_{max}}{2}$$

- **K_M is also the substrate concentration at which the enzyme operates at one half of its maximum velocity**

K_M = [S] at ½ V_{max}

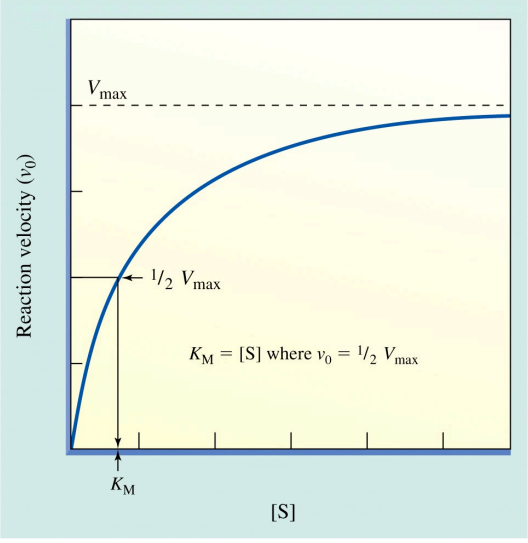

Figure 5-4 Concepts in Biochemistry, 3/e
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-

- Indicates how efficiently an enzyme selects its substrate and converts to product.

- So, if an enzyme has a **SMALL K_M** they it achieves maximal catalytic efficiency (V_{max}) at a low substrate concentration!

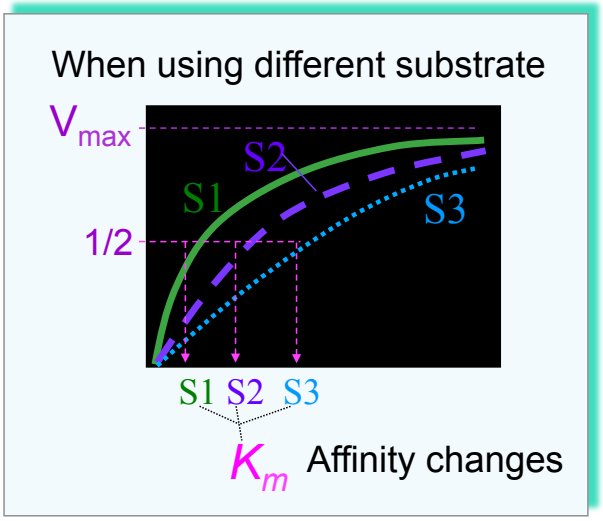
- **K_M** is unique for each enzyme/substrate pair

K_M = substrate concentration [S] when reaction velocity is ½ V_{max}

if [S] = K_M

$$V_0 = \frac{V_{max}[S]}{2[S]}$$

$$V_0 = \frac{V_{max}}{2}$$



Higher K_M = lower the affinity = higher [S] required to reach ½ V_{max}

- **For certain enzymes under certain conditions, K_M can also be a measure of affinity between E and S – approximates the dissociation constant of the ES complex**

- **If K_M is LOW (small number) = Substrate is held tightly (HIGH affinity)**
  1. **Reaches V_{max} at a lower [S]**
  2. **Small number means less than 10^{-3}M**

- **If K_M is HIGH (large number) = Substrate is held weakly (LOW affinity)**
  1. **Reaches V_{max} at a higher [S]**
  2. **Large number means 10^{-1} – 10^{-3}M**

Table 10.2 K_m Values for Some Enzymes

Enzyme	Substrate	K_m (mM)
Carbonic anhydrase	CO_2	12
Hexokinase	Glucose	0.15
	Fructose	1.5
β-Galactosidase	Lactose	4
Glutamate dehydrogenase	NH_4^+	57
	Glutamate	0.12
	α-Ketoglutarate	2
	NAD^+	0.025
	NADH	0.018
Aspartate aminotransferase	Aspartate	0.9
	α-Ketoglutarate	0.1
	Oxaloacetate	0.04
	Glutamate	4
Threonine deaminase	Threonine	5
Pyruvate carboxylase	HCO_3^-	1.0
	Pyruvate	0.4
	ATP	0.06
Penicillinase	Benzylpenicillin	0.05
Lysozyme	Hexa-*N*-acetylglucosamine	0.006

TURNOVER NUMBER (k_{cat}) – CATALYTIC CONSTANT

- How fast ES complex proceeds to E + P
- Number of catalytic cycles that each active site undergoes per unit time
- Rate constant of the reaction when enzyme is saturated with substrate
- First order rate constant (sec^{-1})

$$\textbf{turnover number} = k_{cat} = V_{max}/[E_T]$$

$[E_T]$ = total enzyme concentration

k_{cat}/K_M = catalytic efficiency

- Reflects both binding and catalytic events – indicates how the velocity varies according to how often the enzyme and substrate combine.
- Best value to represent the enzyme's overall ability to convert substrate to product
- Upper limit is diffusion controlled – 10^8 – 10^9 $M^{-1}s^{-1}$ - maximum rate at which two freely diffusion molecules can collide with each other in aqueous solution (E and S)

TABLE 7-1 | Catalytic Constants of Some Enzymes

Enzyme	k_{cat} (s^{-1})
Staphylococcal nuclease	95
Cytidine deaminase	299
Triose phosphate isomerase	4300
Cyclophilin	13,000
Ketosteroid isomerase	66,000
Carbonic anhydrase	1,000,000

[Data from Radzicka, A., and Wolfenden, R., *Science* 267, 90–93 (1995).]

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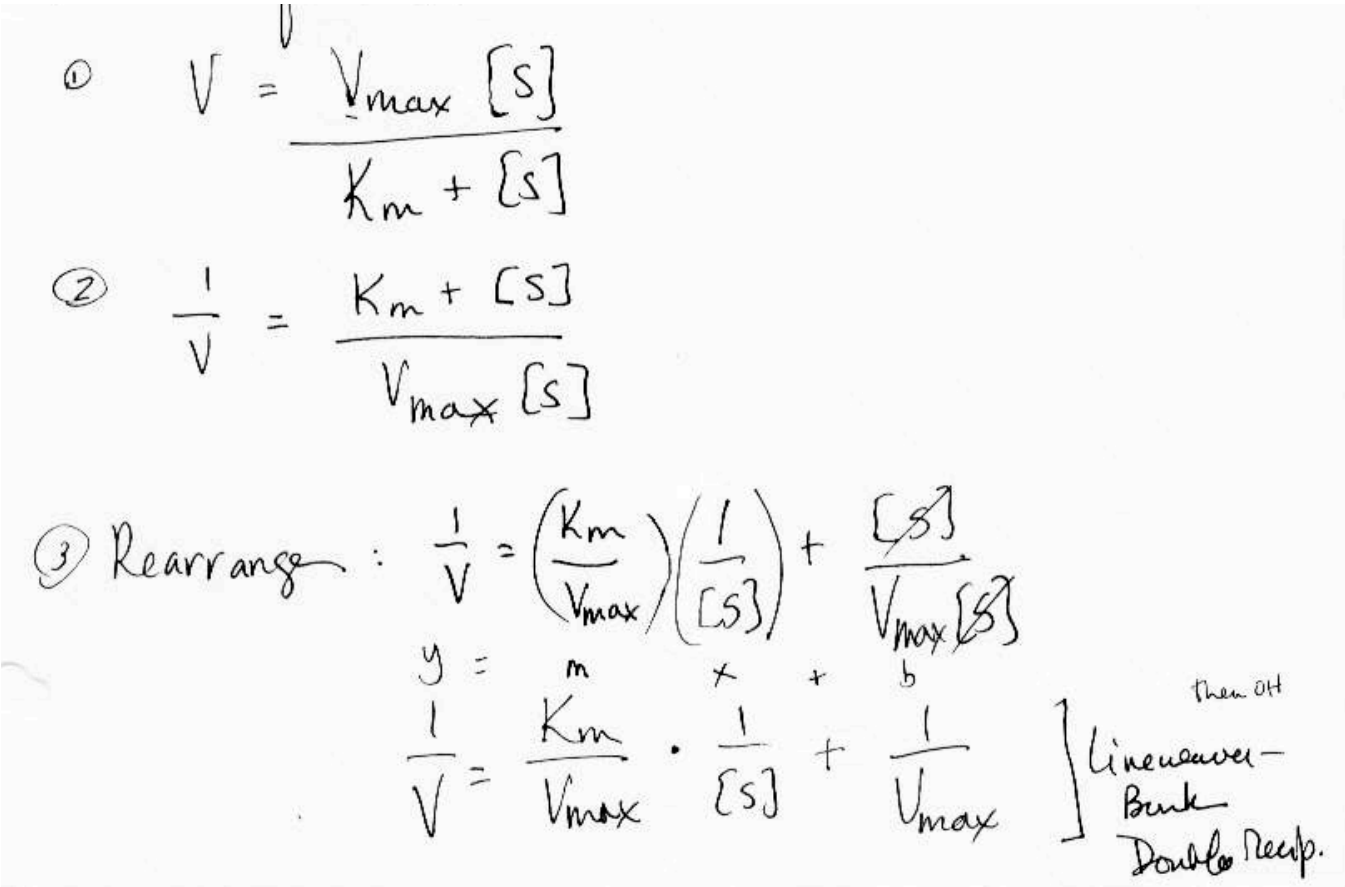
LINEAR TRANSFORMATION OF THE MICHAELIS – MENTEN EQUATION:

The Michaelis-Menten curve can be used to ESTIMATE V_{max} and K_M – although not exacting and we don't use it. Determine the values by a different version of the equation.

In 1934, **Lineweaver and Burk** devised a way to transform the hyperbolic plot into a linear plot.

- Actual values for K_M and V_{max} can then be easily determined from the graph.
- How can we do this:

We take the reciprocal of both sides of the Michaelis-Menten Equation:



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

Michaelis-Menten Equation

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

Lineweaver-Burk Equation

Same form as **y = mx + b:** equation for a straight line

y = m x + b

How do we use this equation:

1.) Plot y vs. x

• where $y = \frac{1}{V}$ & $x = \frac{1}{[S]}$

Take same data & plot the reciprocals of [S] & V

2.) $\frac{K_m}{V_{max}}$ is the slope (m) $\left(\frac{\Delta y}{\Delta x} \; \frac{\text{rise}}{\text{run}}\right)$

3.) y intercept is $\frac{1}{V_{max}}$ (b) (where x = 0, let x = 0 & solve for y)

4.) x intercept is $\frac{-1}{K_m}$ (where y = 0, let y = 0 & solve for x, $x = -\frac{b}{m}$)

(Extrapolated value)

- Experimentally:
  - Obtain data varying substrate concentration in different tubes and measure V at each concentration.

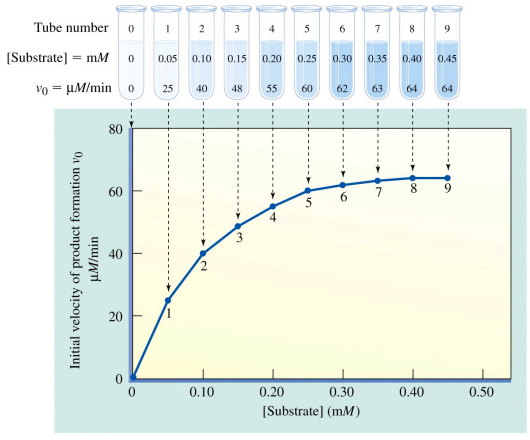


Figure 5-3 Concepts in Biochemistry, 3/e
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- Take reciprocal of S and V
- Plot the data - Use computer program (e.g. Excel) to generate the equation of the line
- Solve for K_M and V_{max}

Lineweaver-Burk Plot

- Michaelis-Menten plot is not useful for estimating K_M and V_{max}
- it is better to transform the Michaelis-Menten equation to a linear form
  - actual values for K_M and V_{max} determined from graph

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

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

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

same form as y = mx + b

plot is y vs x

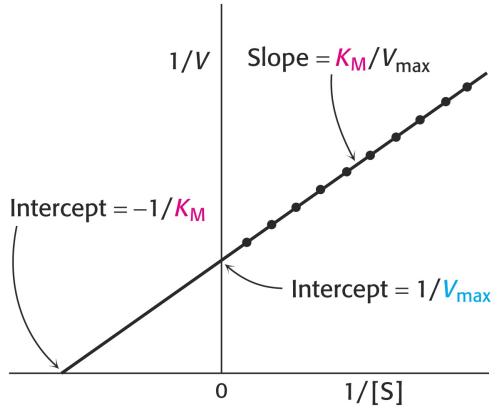
y is 1/V

x is 1/[S]

K_M/V_{max} is slope

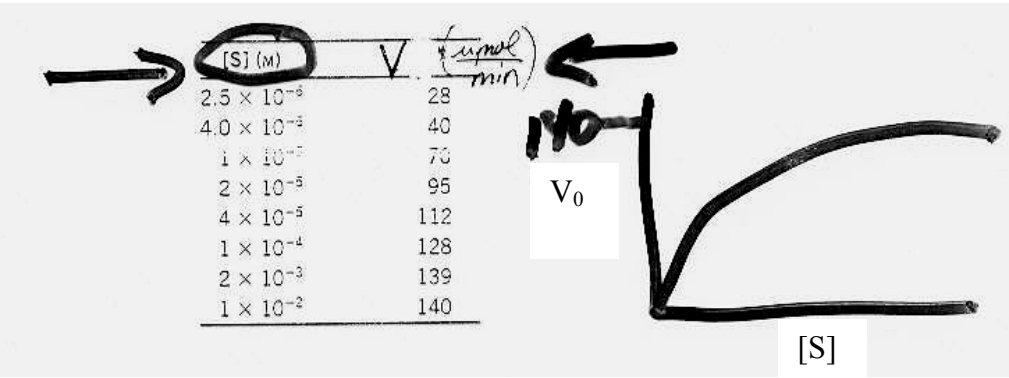
y intercept is $1/V_{max}$

x intercept is $-1/K_M$



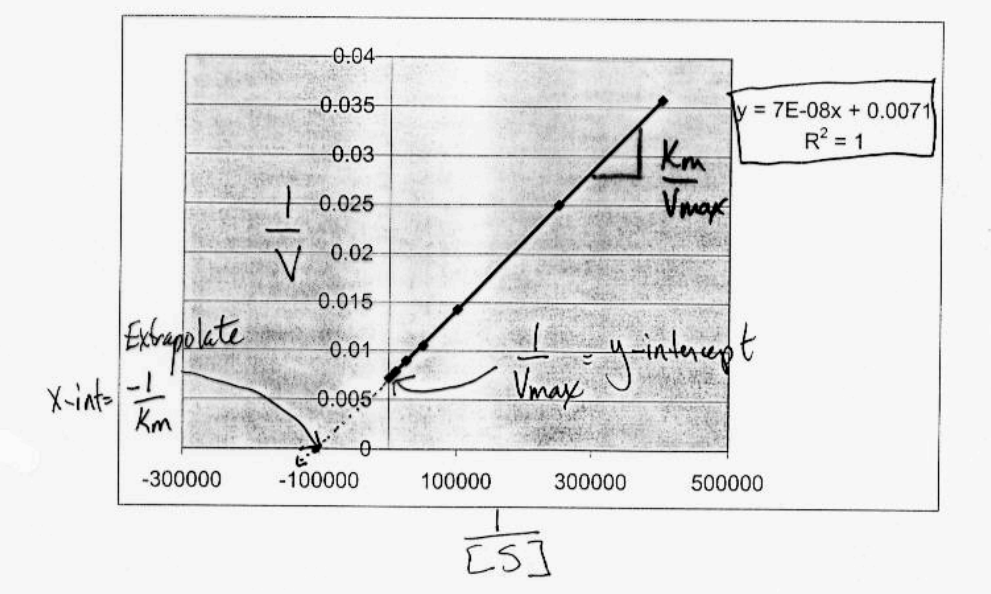
$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

For example:



1/[S]	1/V
400000	0.035714
250000	0.025
100000	0.01428
50000	0.010526
25000	0.008928
10000	0.007813
500	0.00719
100	0.00714

- Data from an experiment at 8 different concentrations of substrate. Enzyme kept constant.
- Velocity is in terms of μmol product X made per min
- Plot as Michaelis-Menten hyperbolic curve: Can only ESTIMATE K_m and V_{max}
- Take reciprocal of the data:



- Plot and generate equation of the line:
  - Calculate V_{max} and K_M

$y = 7 \times 10^{-8} x + 0.0071$

Remb: Lineweaver-Burk Equation: (double reciprocal of M-M)

$$\frac{1}{V} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Where slope "m" $= \frac{K_m}{V_{max}}$ & y-intercept "b" $= \frac{1}{V_{max}}$

So in our example: $m = \frac{K_m}{V_{max}} = 7 \times 10^{-8}$

$b = \frac{1}{V_{max}} = 0.0071$ (units of rate/velocity)

Solve for V_{max}: $\therefore V_{max} = 140 \frac{\mu mol}{min} \left(\frac{1}{0.0071}\right)$

Solve for K_m: $\frac{K_m}{V_{max}} = \frac{K_m}{140 \frac{\mu mol}{min}} = 7 \times 10^{-8}$ $\therefore K_m = \sim 1 \times 10^{-5}$ M