Enzyme Kinetics: Velocity

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

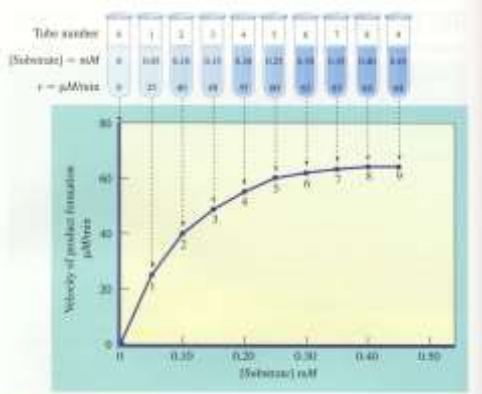
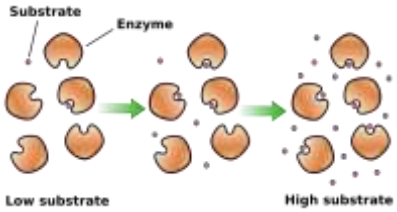


FIGURE 6.3



- 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 <u>zero</u>

  C. Measure the velocity by determining the rate of product formation

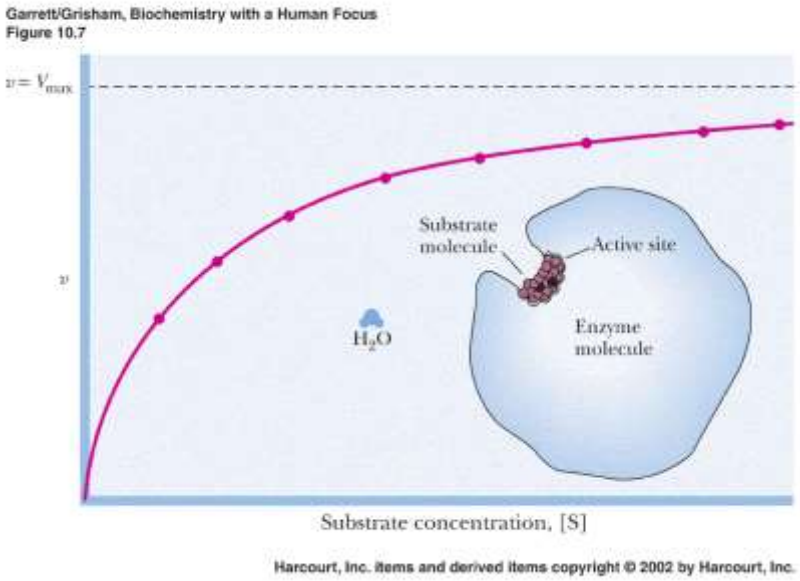
  D. Plot these values – **Velocity against substrate concentration**

  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:



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:

$$\mathbf{S \rightarrow P}$$

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

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 ($P \rightarrow S$) 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]**

- **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}

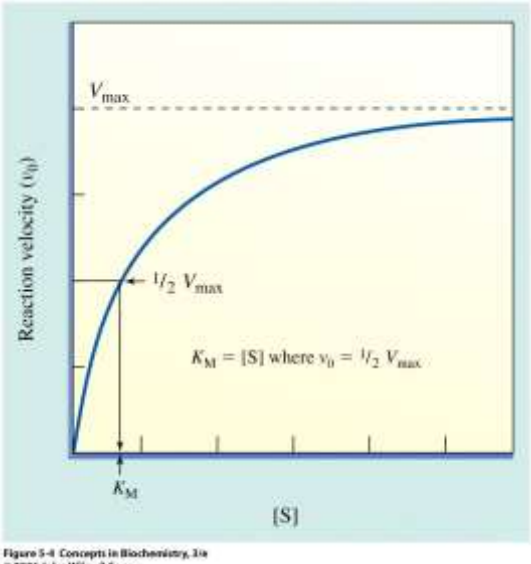


Figure 5-4 Concepts in Biochemistry, 3/e
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- Indicates how efficiently an enzyme selects its substrate and converts to product.

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

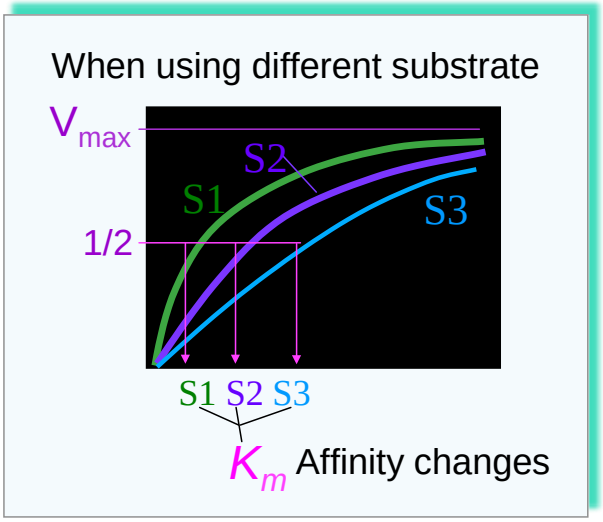
- **K_M** is unique for each enzyme/substrate pair

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)

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