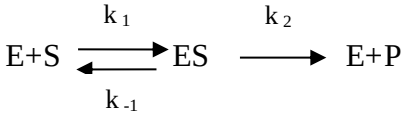
Chapter 2: Enzyme Kinetics of Single-Substrate Reactions and Inhibition

Enzyme kinetics studies are methods used to determine the **mode of action of enzymes**. In general, the reaction rate is proportional to the substrate concentration (when it is low) and to the amount of enzyme present. The progression of the reaction is studied using a **reaction mixture** containing a **very low concentration** of enzyme and a **large excess of substrate**.

1. Schematic of the enzymatic reaction

Michaelian enzymes function according to the following mechanism:



- **k_1:** Rate constant for the association of E + S.
- **k_{-1}:** Rate constant for the dissociation of the ES complex.
- **k_2 also called k_{cat}:** The catalytic constant of the enzymatic reaction.
- **ES:** The Michaelis complex.

A substrate (**S**) binds with the enzyme (**E**) to form a complex (**ES**), which then dissociates to yield a product (**P**) while regenerating the free enzyme (**E**).

N.B. To maintain **Michaelis conditions** at the start of the reaction, the amount of product formed is considered **negligible**; therefore, the reverse reaction **E + P → ES does not occur**.

2. Reaction rate (Michaelis-Menten-Henri Equation, 1913)

These authors quantitatively studied the variations in the **rate of an enzymatic reaction** as a function of **substrate concentration**. To do this, one must measure the **initial velocity (v_i)**, which represents the **rate of product (P) formation** from the enzyme–substrate complex **(ES)**:

$$\boldsymbol{v = \frac{d[P]}{dt} = k2.[ES]}$$

The **total enzyme concentration (E_t)** is defined as: E_t=E+ES. When the reaction reaches **maximum velocity**, all the quantity of enzyme is in the form of the complex: E_t=ES. Therefore the reaction velocity is: **Vmax =k_2.** [EtS]

Formation rate of the ES complex is:

$$v = \frac{d[ES]}{dt} = k1.[E][S] - (k - 1 + k2)[ES]$$

At equilibrium (steady state), the reaction rate is constant. We can then define the **Michaelis constant (K_M)**, which corresponds to the **dissociation constant (K_d)** of the ES complex under Michaelis-Menten assumptions:

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

K_M allows us to evaluate the **affinity ($1/K_M$) of the enzyme for its substrate**. Therefore, the lower the K_M value, is the higher the affinity of the enzyme for its substrate.

The final **Michaelis-Menten** equation describing the kinetic parameters is:

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

- **V_{max}:** maximum reaction velocity
- **K_M:** Michaelis constant

This equation describes the **kinetic parameters** (V_{max} and K_M) of an enzyme-catalyzed reaction. These parameters can be **determined experimentally**. The graphical representation of this equation (v as a function of [S]) is a **hyperbola** where the horizontal asymptote is V_{max}. Furthermore, **K_M corresponds to the substrate concentration at which the velocity is half of the maximum velocity ($V_{max}/2$) (Fig. 1)**.

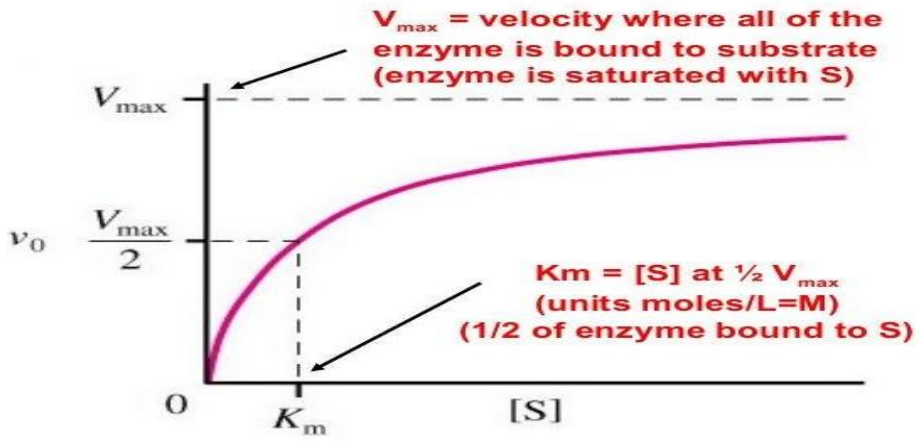


Figure 1. Representation of Michaelis–Menten Enzyme Kinetics

3. Linearization of the Michaelis-Menten equation

The **linearization** of the Michaelis–Menten graphical representation transforms the **hyperbolic curve into straight lines**, in order to **reduce errors** when estimating the kinetic parameters (V_{max} and K_M) that occur when trying to identify an asymptote on a curve.

3.1. Lineweaver-Burk plot (1934)

Also known as the **double-reciprocal plot**, this method expresses the Michaelis-Menten equation in its **reciprocal (inverse) form**, to create a **linear relationship**.

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

The curve is plotted with **1/v as a function of 1/[S]**. This results in is a straight line with a slope of K_M **/** V_{max}, and it intersects the **Y-axis** (ordinate) **at** $1/V_{max}$ and the **X-axis** (abscissa) **at** $-1/K_M$ **(Fig. 2)**.

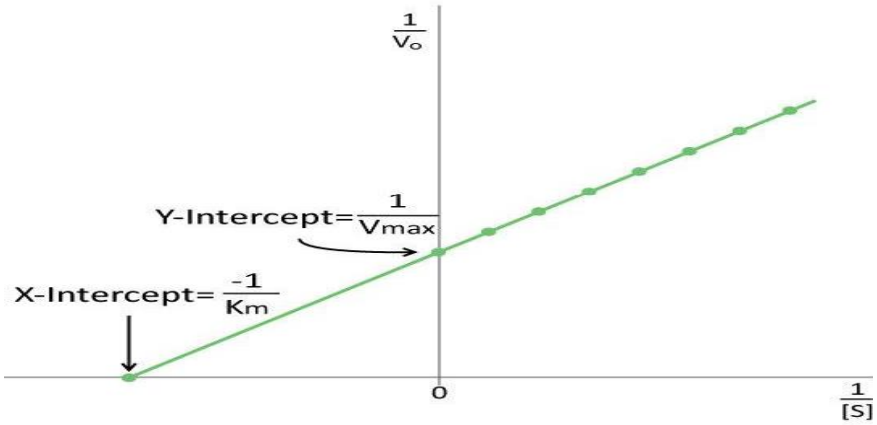


Figure 2. Representation of enzyme kinetics according to Lineweaver–Burk

3.2. Other Representations

There are other transformations such as the **Eadie–Hofstee (1952)** plot and the **Hanes (1952)** plot.

4. Effect of Inhibitors on enzymatic activity

An inhibitor is a substance that **slows down** or **stops** an enzymatic reaction. Inhibitors are generally classified into three main types based on how they affect the kinetic parameters (K_M and V_{max}).

1.1 Competitive inhibition

During the reaction, the **inhibitor (I)** binds to the enzyme at the **same active site as the substrate (S)**. This prevents the formation of the enzyme-substrate complex because the

substrate and the inhibitor share the same binding site. This results in the formation of either the active **ES complex** or the inactive **EI complex**.

- $E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E+P$
- $E+I \rightleftharpoons EI+S \rightarrow$ (no reaction)

K_i is the dissociation constant of the EI complex. It is defined as: $\boldsymbol{K_i = \frac{[E][I]}{[EI]}}$.

The inverse of K_i reflects the **affinity of the enzyme for the inhibitor**.

The reaction rate equation remains structurally unchanged, but the apparent Michaelis constant increases by a specific coefficient $\left(\mathbf{1 + \frac{[I]}{K_i}}\right)$.

The form of the velocity equation remains unchanged, but the $\mathbf{K_M}$ factor is increased

$$\boldsymbol{v = \frac{V_{max}[S]}{[S] + K'_M}}$$

Let $\boldsymbol{K'_M}$ be the **apparent Michaelis constant** in the presence of the inhibitor:

$$\boldsymbol{K'_M = K_M\left(1 + \frac{[I]}{K_i}\right)}$$

And the equation becomes:

$$\boldsymbol{\frac{1}{v} = \frac{K_M\left(1 + \frac{[I]}{K_i}\right)}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}}$$

According to the graphical representation shown in Figure 3, it can be observed that $\mathbf{V_{max}}$ **does not change** (the Y-intercept ($1/V_{max}$) remains the same), while $\mathbf{K_M}$ **increases** (The X-intercept ($-1/K'_M$) moves closer to zero), indicating a **decrease in affinity**.

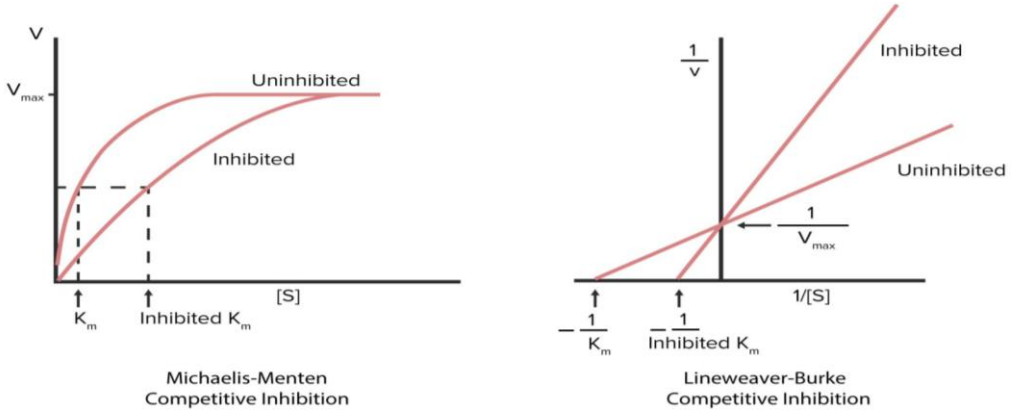


Figure 3. Enzyme kinetics in the presence of a competitive inhibitor

1.2 Non-competitive inhibition

In this case, the inhibitor binds to the enzyme at a **different site** than the substrate binding site. Consequently, there is no competition between the substrate (S) and the inhibitor(I).

- E+S⇌ES→E+P
- E+I⇌EI+S (no reaction)
- ES+I⇌ESI (no reaction)
- EI+S⇌ESI (no reaction)

In this type of inhibition, three enzymatic complexes are formed (**ES, EI, and ESI**), but **only the ES complex is active** and leads to the product.

Mathematical derivations show that the maximum velocity (V_{max}) is decreased by a specific coefficient $\left(\mathbf{1}+\frac{[I]}{K_i}\right)$. However, the **Michaelis constant (K_M) remains the same** as in the absence of the inhibitor (**Fig.4.)**.

Let **V'_{max}** be the **apparent maximum velocity**:

$$V'_{max} = \frac{V_{max}}{\left(1+\frac{[I]}{K_i}\right)}$$

The modified Michaelis-Menten equation becomes:

$$v = \frac{V'_{max}[S]}{K_M + [S]}$$

In a double-reciprocal plot, **the X-intercept (-1/K_M) is unchanged:** The affinity of the enzyme for its substrate remains identical. **The Y-intercept (1/V_{max}) increases,** Since **V_{max}** decreases, 1/ **V_{max}** becomes larger, causing the line to shift upwards.

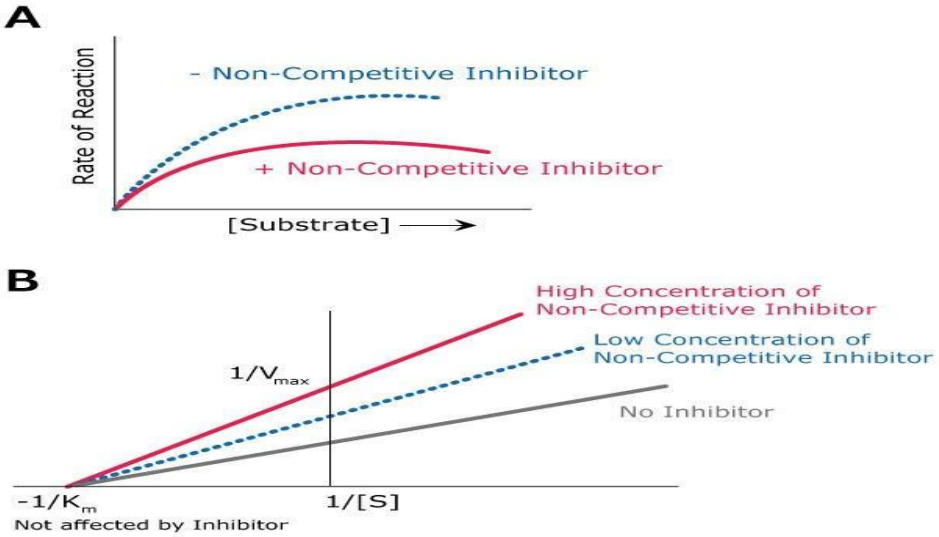


Figure 4. Enzyme kinetics in the presence of a non-competitive inhibitor

1.3 Uncompetitive Inhibition

In this type of inhibition, the inhibitor cannot bind to the free enzyme. It only binds to the **Enzyme-Substrate complex (ES)** that has already formed, preventing it from proceeding to the product.

- E+S⇌ES→E+P
- ES+I⇌ESI (no reaction)

The dissociation constant of the ESI complex is defined as:

$$K_i = \frac{[ES][I]}{[ESI]}$$

Kinetically, this type of inhibition is characterized by a **parallel decrease** in **both V_{max} and K_M**. Because the inhibitor effectively "removes" ES complexes from the reaction, it shifts the equilibrium in a way that appears to increase the enzyme's affinity (lowering **K_M**) while reducing its maximum capacity (**V_{max}**). The modified Michaelis-Menten equation is:

$$v = \frac{V'_{max}[S]}{K'_M + [S]}$$

Where:

$$V'_{max} = \frac{V_{max}}{\left(1 + \frac{I}{K_i}\right)} \quad \text{et} \quad K'_M = \frac{K_M}{\left(1 + \frac{I}{K_i}\right)}$$

In a double-reciprocal plot **(Fig. 5)**, uncompetitive inhibition is easy to recognize because it produces **parallel lines**. The **Slope (K_M/V_{max})** remains constant (since both parameters decrease by the same factor). The **Y-intercept ($1/V_{max}$)** increases (shifts upward) and the **X-intercept ($-1/K_M$)** moves further away from the origin (to the left).

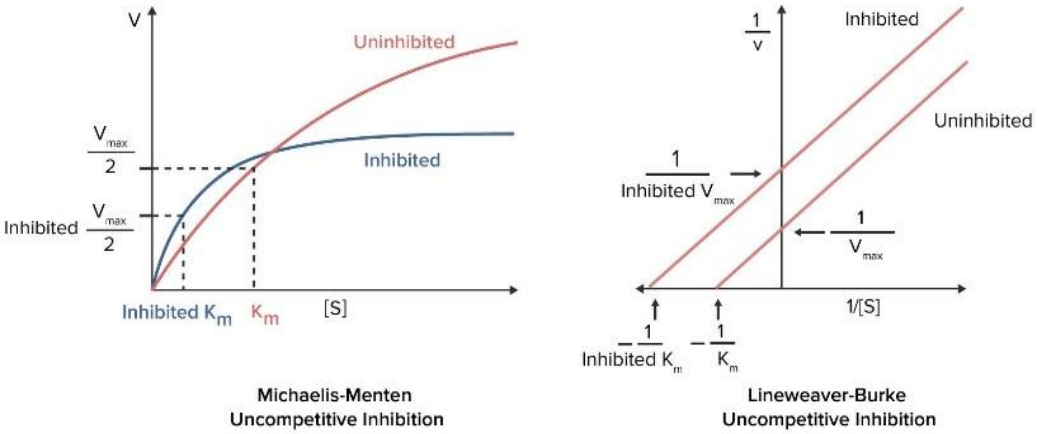


Figure 5. Enzyme kinetics in the presence of an uncompetitive inhibitor