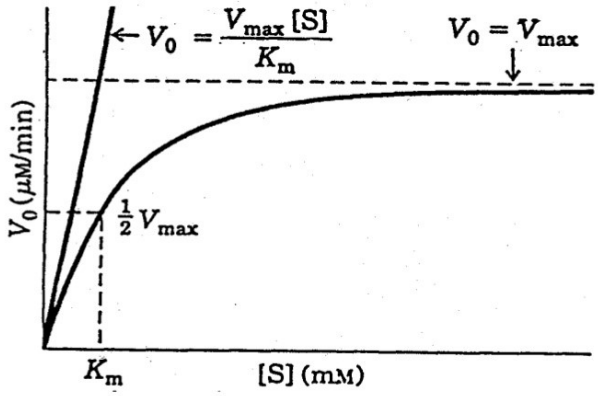

Figure 4: Michaelis-Menten curve for enzyme reaction.

Characteristics of K_m:

K_m (the Michaelis constant) is characteristic of an enzyme and its particular substrate, and reflects the affinity of the enzyme for that substrate. K_m is numerically equal to the substrate concentration at which the reaction velocity is equal to ½ V_{max}. K_m does not vary with the concentration of enzyme.

Small K_m: A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme, Figure 5.

Large K_m: A numerically large (high) K_m reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme, Figure 5.

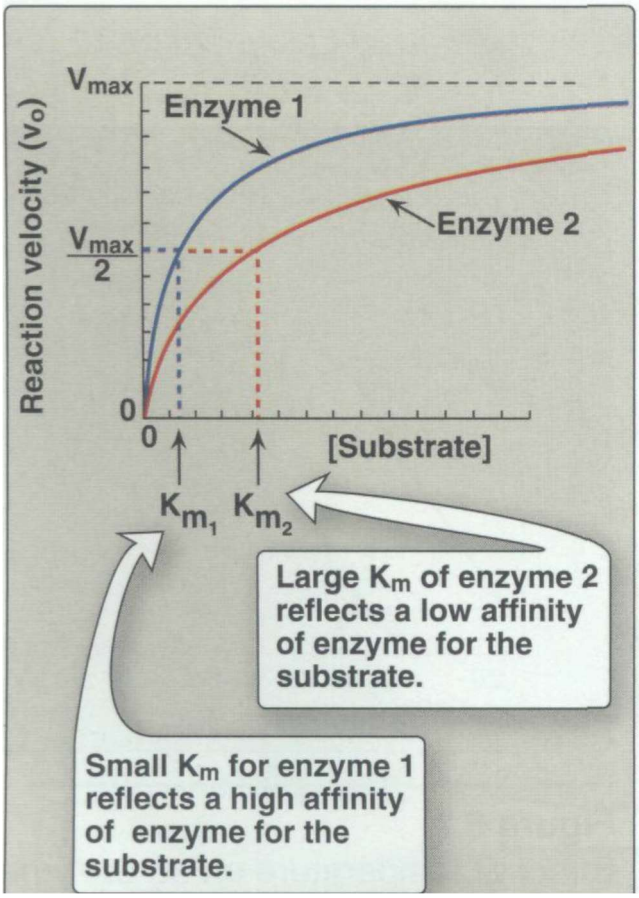

Figure 5: effect of substrate concentration on reaction velocities for two enzymes, enzyme 1 with a small K_m and enzyme 2 with a large K_m.

Lineweaver-Burke plot:

When V_0 is plotted against [S], it is not always possible to determine when V_{max} has been achieved, because of the gradual upward slope of the hyperbolic curve at high substrate concentrations. However, if $1/V_0$ is plotted versus 1/[S], a straight line is obtained (Figure 6). This plot, the Lineweaver-Burke plot (also called a double-reciprocal plot) can be used to calculate K_m and V_{max} as well as to determine the mechanism of action of enzyme inhibitors. The equation describing the Lineweaver-Burke plot is:

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

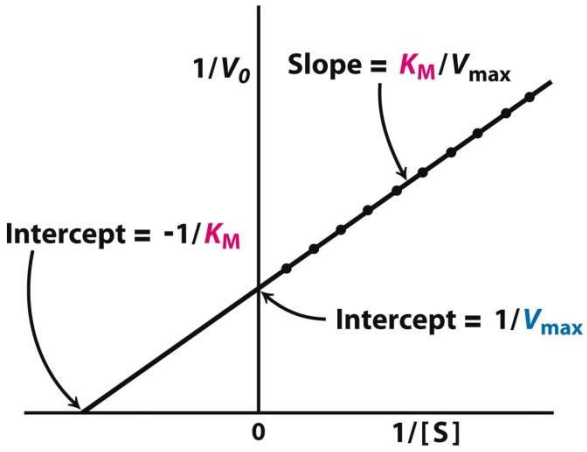

Figure 6: Lineweaver-Burke plot. Where the intercept on the x axis is equal to -1/ K_m and the intercept on the y axis is equal to 1/ V_{max}.

Inhibition of enzyme activity:

Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor. The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalyzing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically (e.g. via covalent bond formation). In contrast, reversible inhibitors bind non-covalently and different types of inhibition are produced depending on whether these inhibitors bind to the enzyme, the enzyme-substrate complex, or both. In fact, many drug molecules are enzyme inhibitors, so their discovery and improvement is an active area of research in biochemistry and pharmacology. Generally, there are three kinds of reversible enzyme inhibitors.

1- Competitive inhibitors: A competitive inhibitor molecule has a similar structure to the substrate molecule, and so it can fit into the active site of the enzyme. It therefore competes with the substrate for the active site, so the reaction is slower (the mechanism is illustrated in figure 7). Increasing the concentration of substrate restores the reaction rate and the inhibition is usually temporary and reversible. Competitive inhibitors increase K_m for the enzyme, but have no effect on V_{max} (figure 8), so the rate can approach a normal rate if the substrate concentration is increased high enough.

The equation describing the Lineweaver-Burke plot for this type of inhibition is:

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

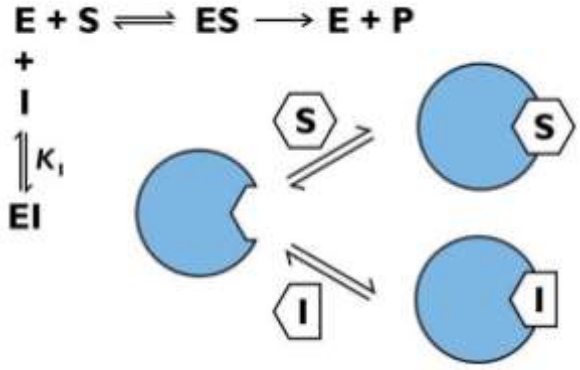

Figure 7: The scheme of competitive inhibition.

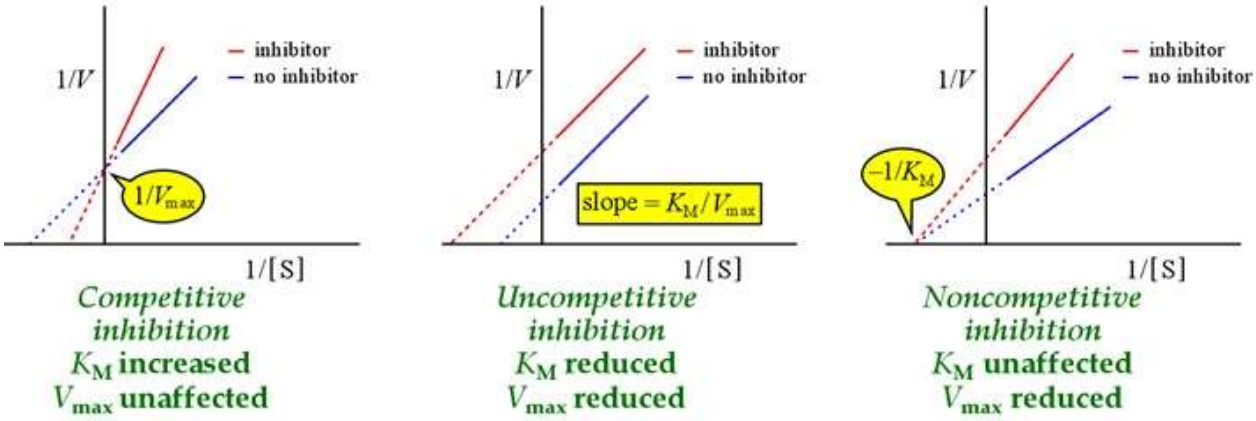

Figure 8: Lineweaver-Burk plots analysis for the three types of inhibition.

2- Non-competitive inhibitors: A non-competitive inhibitor molecule is quite different in structure from the substrate and does not fit into the active site. It binds to another part of the enzyme molecule, changing the shape of the whole enzyme, including the active site, so that it can no longer bind substrate molecules (the mechanism is illustrated in figure 9). Non-competitive inhibitors therefore simply reduce the amount of active enzyme. This is the same as decreasing the enzyme concentration, so they decrease V_{max}, but have no effect on K_m (figure 8). This kind of inhibitor tends to bind tightly and irreversibly, such as the poisons cyanide and heavy metal ions. Many nerve poisons (insecticides) work in this way too.

The equation describing the Lineweaver-Burke plot for non-inhibition is:

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

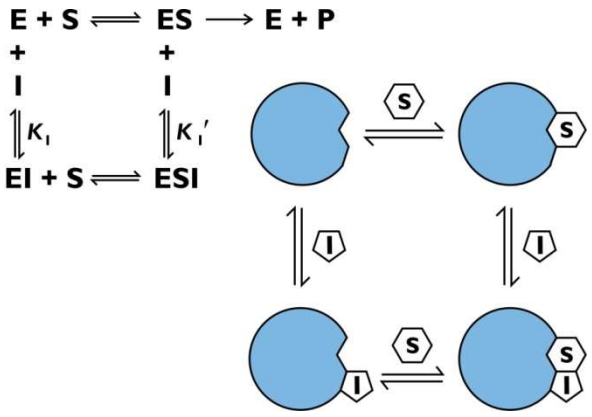

Figure 9: The scheme of non-competitive inhibition.

3- Uncompetitive inhibitors: An uncompetitive inhibitor is an inhibitor that only binds to the enzyme-substrate complex. The formation of its binding site only forms when the enzyme and the substrate have interacted amongst themselves (the mechanism is illustrated in figure 10). The uncompetitive inhibition does not work when additional substrates are trying to be involved. The enzyme-substrate-inhibitor complex does not produce any product. Uncompetitive inhibition

decreases the maximum velocity as well as the K_m. Both V_{max} and K_m are reduced by equal amounts (figure 8).

The equation describing the Lineweaver-Burke plot for uncompetitive inhibition is:

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

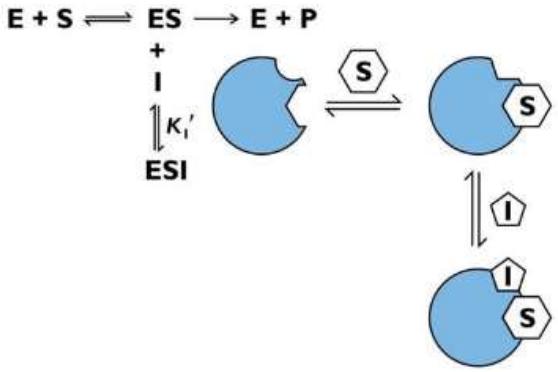

Figure 10: The scheme of uncompetitive inhibition.