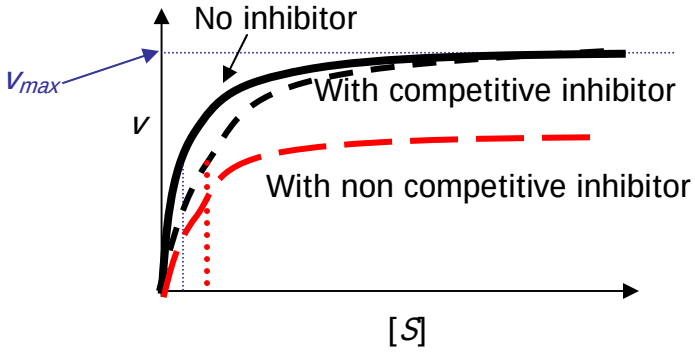
Experiment No. 5
Enzyme Kinetics and Inhibition Studies

Enzymes are protein catalysts that, like all catalysts, speed up the rate of a chemical reaction without being used up in the process. The study of the rate at which an enzyme works is called **enzyme kinetics**. Enzyme kinetics as a function of the **concentration of substrate [S]** available to the enzyme is shown in the figure.



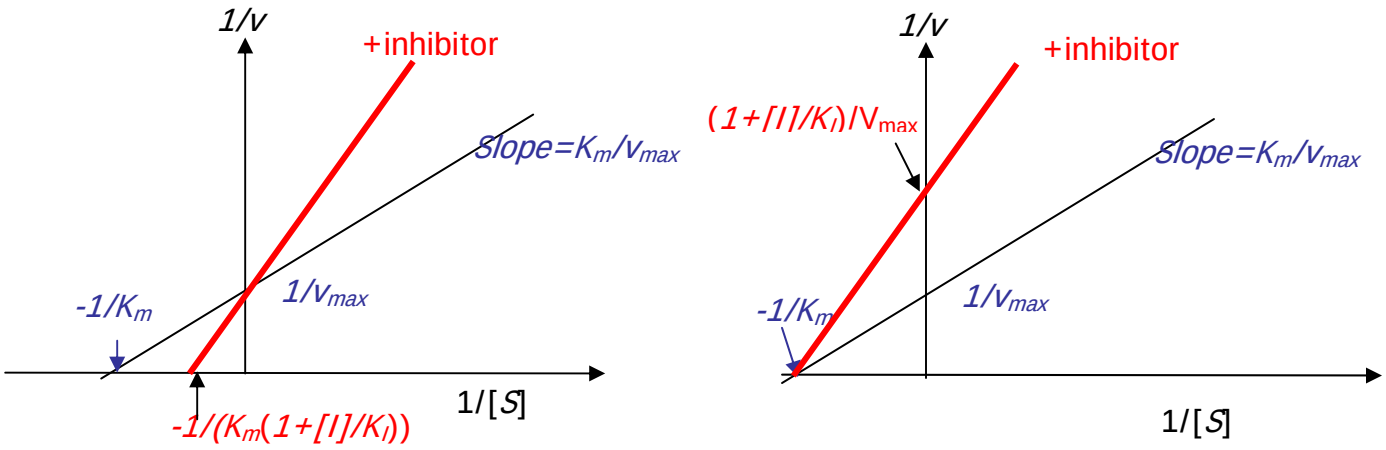
Plotting $\mathbf{V_i}$ as a function of **[S]**, we find that

- At low values of **[S]**, the initial velocity,$\mathbf{V_i}$, rises almost linearly with increasing **[S]**.
- The asymptote represents the maximum velocity of the reaction, designated $\mathbf{V_{max}}$
- The substrate concentration that produces a $\mathbf{V_i}$ that is one-half of $\mathbf{V_{max}}$ is designated the Michaelis-Menten constant, $\mathbf{K_m}$(named after the scientists who developed the study of enzyme kinetics).

$\mathbf{K_m}$ is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and its substrate. The lower the $\mathbf{K_m}$, the greater the affinity (so the lower the concentration of substrate needed to achieve a given rate).

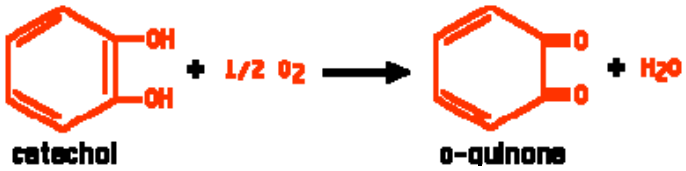
Enzymes can be inhibited **competitively**, when the substrate and inhibitor compete for binding to the same active site or **noncompetitively**, when the inhibitor binds somewhere else on the enzyme molecule reducing its efficiency.

In the presence of a competitive inhibitor (left panel), it takes a higher substrate concentration to achieve the same velocities that were reached in its absence. So while $\mathbf{V_{max}}$ can still be reached if sufficient substrate is available, one-half $\mathbf{V_{max}}$ requires a higher [S] than before and thus $\mathbf{K_m}$ is larger. With noncompetitive inhibition (right panel), enzyme rate (velocity) is reduced for all values of [S], including $\mathbf{V_{max}}$ and one-half $\mathbf{V_{max}}$ but $\mathbf{K_m}$ remains unchanged. A "double-reciprocal" or Lineweaver-Burk plot is usually plotted to determine the kinetic parameters $\mathbf{V_{max}}$ and $\mathbf{K_m}$



When a slice of apple is exposed to air, it quickly turns brown. This is because the enzyme **o-diphenol oxidase** catalyzes the oxidation of phenols in the apple to dark-colored products.

The $\mathbf{V_{max}}$ and the Michaelis-Menten constant ($\mathbf{K_m}$) for this enzyme are to be determined.



- With **catechol** as the substrate. The enzyme converts it into **o-quinone**, which is then further oxidized to dark products.
- when it acts in the presence of a **competitive inhibitor**
- when it acts in the presence of a **noncompetitive inhibitor**

Procedure:

- Grind up pieces of apple and centrifuge the resulting soup.
- The supernatant is your enzyme preparation.
- Because of the speed with which colored products are formed, we can use the intensity of the color as a measure of product formation.

No Inhibitor

1. Set up five tubes (Tubes A to D) with different concentrations of catechol (the substrate).
2. Add a fixed amount of enzyme preparation to Tube A and measure the change in absorbance (**O**ptical **D**ensity) at 540 nm at 1 minute intervals for several minutes.
3. Record the average **change** in OD_{540} per minute (Δ OD_{540}). Since the OD is directly proportional to the concentration of the products, we can use it as a measure of the rate or velocity of the reaction ($\mathbf{V_i}$).
4. Repeat with the other three tubes.

Effect of para-hydroxybenzoic benzoic acid and phenylthiourea

Repeat the above procedure adding a fixed amount of a solution of any one of the above compounds to each of the four tubes.

Plot Lineweaver-Burke plots to determine the kinetic parameters in each case.

Explain what you observe. What type of inhibitors are the two compounds?