

Enzyme kinetics with a one substrate

Introduction :

Enzyme kinetics studies are methods used to determine the mode of action of enzymes. In fact, studying the variation in speed as a function of substrate concentration allows enzymes to be characterized, as it highlights the existence of mathematical relationships between this concentration and speed, from which characteristic values for each enzyme can be derived.

1. Evolution of the enzymatic reaction :

The enzymatic reaction takes place in two steps :

1st step : corresponds to the formation of an enzyme-substrate complex according to a reversible and rapid reaction.

2nd step : corresponds to the decomposition of this complex into enzyme and product. This is the slowest step ; it determines the speed of the reaction.



1.1. Variation in the quantity of product formed over time :

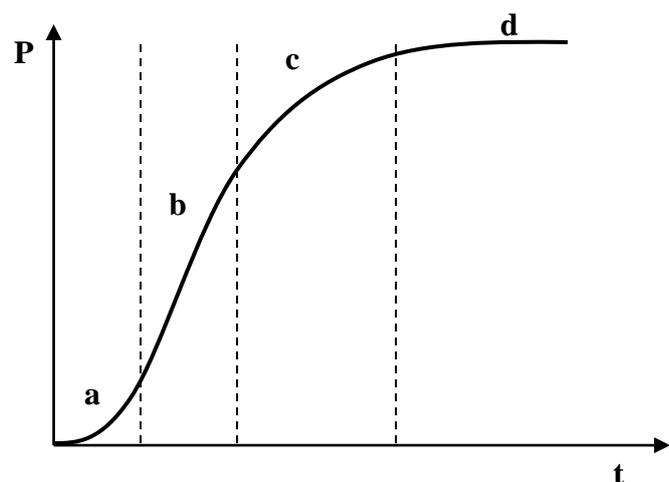
This kinetics shows several phases :

Pre-stationary phase (a) :

This is the shortest phase, during which the first ES complex molecules are formed until the concentration of this intermediate complex reaches a constant value (steady state).

Stationary phase (b) :

This is the phase during which the rate of appearance of product P is constant. According to Michaelis-Menten theory, the concentration of the ES complex is constant.



Inhibition phase by reaction products (c) :

This is the phase during which the concentration of the reaction products is no longer negligible and, as a result, the reverse reaction tends to decrease their concentration.

Equilibrium phase :

This is the phase during which equilibrium is reached. The amounts of S and P are constant. Under these conditions :

$$k_1 [S] = k_{-1} [P]$$

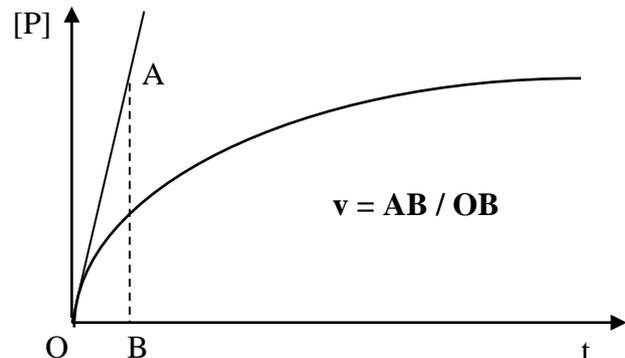
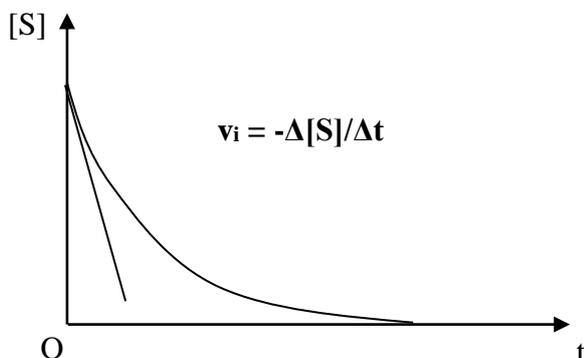
1. 2. Principle of enzymatic reaction rate measurement :

The speed of a reaction is measured by determining the concentration of substrate that disappears or the concentration of product that appears per unit of time. Various factors must be strictly controlled (pH and temperature). In fact, in enzyme catalysis, the speed of the reaction is called "enzyme activity".

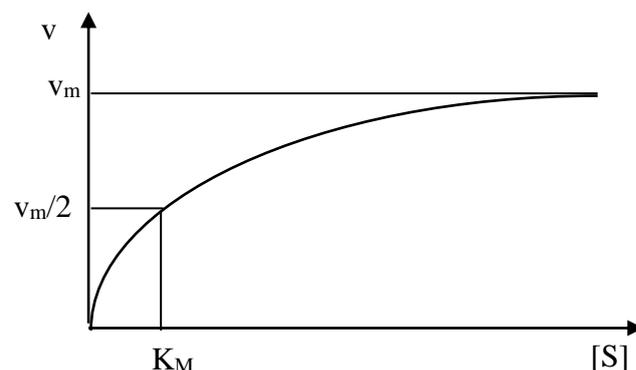
1. 3. Measuring the initial speed of a reaction :

In practice, after bringing the enzyme into contact with the substrate and measuring the concentration of the product formed at successive times, a curve is plotted showing the variation in speed over time.

The initial speed is measured by the slope of the tangent to this curve at time 0.

**1. 4. Speed variation as a function of substrate concentration :**

In practice, kinetic studies are carried out in the presence of a large excess of substrate (for example : 1000 times more substrate molecules than enzyme molecules).



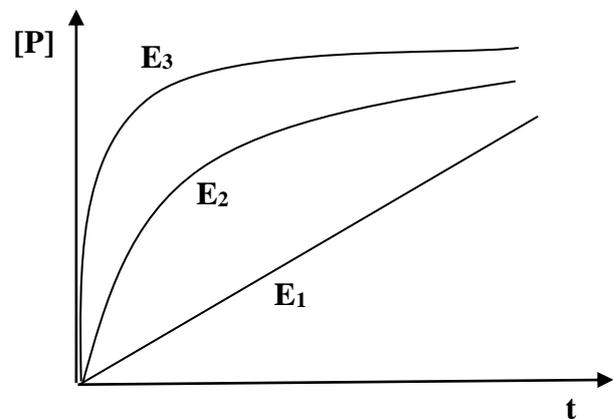
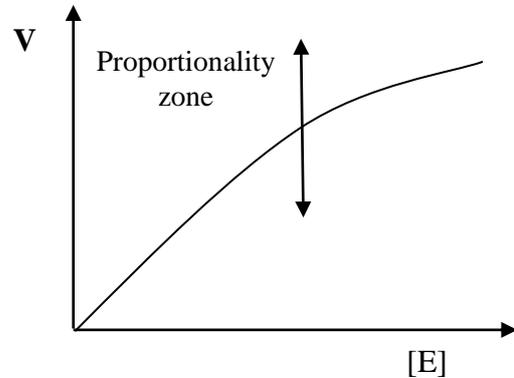
By tracing the curve of the initial speed as a function of the substrate concentration in the presence of the enzyme, we obtain a hyperbole, with a rapid rise and an asymptotic zone corresponding to the maximum speed (V_m).

1.5. Speed variation as a function of enzyme concentration :

The objective of studying the variation in initial speed as a function of enzyme concentration is to determine the zone of proportionality.

In this zone, the reaction is first order with respect to enzyme concentration, and the Michaelis equation is applicable.

It is important to know that, experimentally, we have to work under these conditions.



2. Michaelis-Menten-Henri equation (1913) :

The enzymatic reaction is written as :



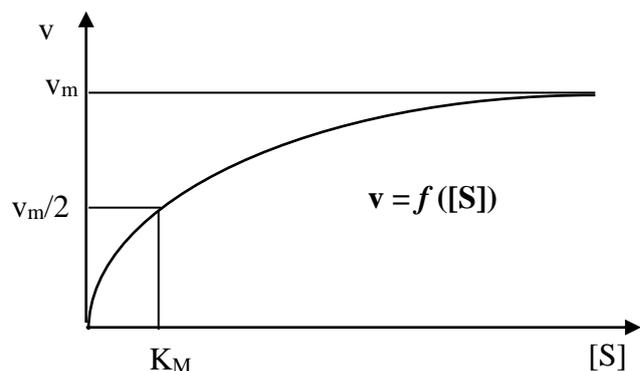
So :

$v = v_m [S] / (K_M + [S])$ is the **Michaelis-Menten-Henri equation**

3. Graphical representations :

3. 1. The Michaelian representation (hyperbolic, 1913) :

This type of curve is difficult to trace and use to measure V_m and K_M . The horizontal asymptote of the hyperbola, for large values of $[S_0]$, allows us to obtain the values of V_m and K_M .



3. 2. Hanse-Woolf representation (1932) :

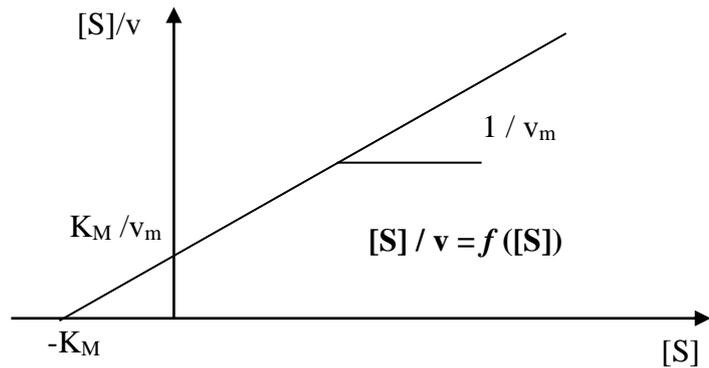
It uses the Michaelis-Menten equation.

$$\text{We have : } v = v_m [S] / (K_M + [S])$$

$$\text{From which : } K_M + [S] = v_m [S] / v$$

$$[S] / v = (K_M + [S]) / v_m$$

$$\boxed{[S] / v = [S] (1 / v_m) + K_M / v_m}$$



3. 3. Lineweaver-Burk representation (1935) :

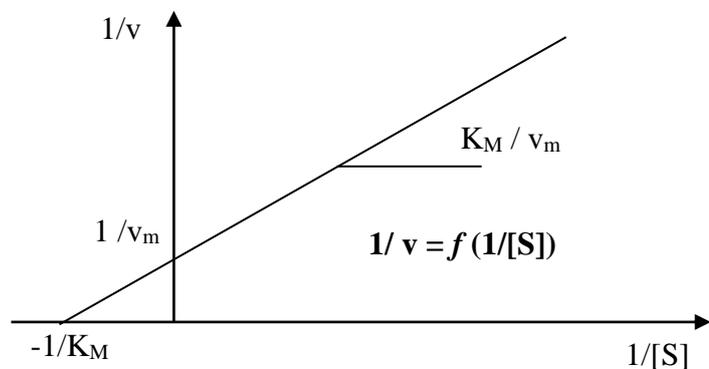
This is the representation of the double inverses. It uses the inverse function of the Michaelis-Menten equation.

$$\text{We have : } v = v_m [S] / (K_M + [S])$$

$$\text{From which : } 1/v = (K_M + [S]) / v_m [S]$$

$$1/v = K_M / v_m [S] + [S]/v_m [S]$$

$$\boxed{1/v = (K_M / v_m) (1/[S]) + 1/v_m}$$



Experimentally, 5 to 6 measurements of $1/v$ with different initial substrate concentrations are sufficient to trace the representative line.

3. 4. Eadie-Hofstee representation (1942-1959) :

Like the previous ones, this representation also uses the Michaelis-Menten equation.

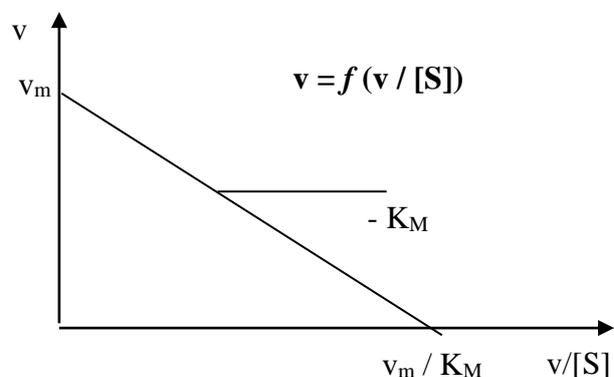
$$\text{We have : } v = v_m [S] / (K_M + [S])$$

$$\text{From which : } v (K_M + [S]) = v_m [S]$$

$$v K_M + v [S] = v_m [S]$$

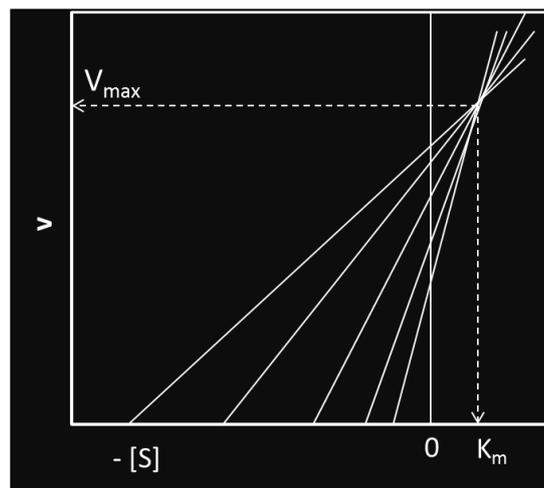
$$v [S] = v_m [S] - v K_M$$

$$\boxed{v = -K_M (v / [S]) + v_m}$$



3. 5. Eisenthal and Cornish-Bowden representation (1974) :

- ✓ It is performed by putting the values of the speed (v) on the y-axis and the negative values of the substrate concentration ($-[S]$) on the x-axis.
- ✓ Each couple of values v and $[S]$ is connected by a straight line.
- ✓ Extrapolating each line gives a single intersection point whose x-coordinate is equal to K_m and whose y-coordinate is equal to V_{max} .



4. Significance of kinetic constants :

4. 1. The maximum speed (v_m) :

The maximum speed is achieved when all enzyme molecules are saturated with the substrate (the active site of all enzyme molecules is occupied by the substrate). It is expressed by :

- Either the international unit (**IU**), which corresponds to the amount of enzyme that catalyzes the conversion of one micromole (μM) of substrate per minute (min).
- Or, the katal (**kat**), according to the International System (SI), which corresponds to the amount of enzyme that catalyzes the conversion of one mole (M) of substrate per second (S).

$$1 \text{ UI} = 1 \mu\text{M} \cdot \text{min}^{-1} = 10^{-6} / 60 \text{ M} \cdot \text{S}^{-1} = 16.67 \times 10^{-9} \text{ kat}$$

so :

$$\boxed{1 \text{ UI} = 16.67 \text{ nkat}}$$

4. 2. The Michaelis constant (K_M) :

By definition, K_M is the substrate concentration that causes the enzymatic reaction to proceed at a speed equal to half the maximum speed :

$$[S] = K_M \implies v = v_m/2$$

It expresses the affinity of the enzyme for the substrate. It varies from 10^{-8} to 10^{-2} . Therefore, the lower the K_M , the greater the affinity. In other words, only a low concentration of substrate is needed to achieve maximum speed. It has the dimension of a concentration (μM , M, ...).

4. 3. Catalytic constant (k_{cat}) :

This is the speed constant of the second step of the enzymatic reaction (catalytic step yielding the product) :

$$k_{cat} = k_2$$

It represents the frequency at which the enzyme performs the catalytic action when saturated with substrate (turnover) :

$$k_{\text{cat}} = V_m / [E_t]$$

It measures the efficiency of catalysis by the studied enzyme. In fact, k_{cat} is a first-order speed constant, so it is measured in (t^{-1}). ($1/k_{\text{cat}}$) is the duration of a catalytic cycle when the enzyme is saturated with substrate.

4. 4. The specificity constant (r_{sp}) :

This constant reflects the overall specificity of an enzyme with respect to a substrate. It is given by the following formula :

$$r_{\text{sp}} = k_{\text{cat}} / K_M$$

This constant allows the best substrate(s) for an enzyme to be selected, in this case, the highest value should be searched for.

5. Enzyme activities :

5. 1. Catalytic activity (Z) :

It is also called *catability*. It is the amount of enzyme capable of transforming one micromole of substrate per minute (IU) or one mole per second (katal).

5. 2. Specific catalytic activity (Z_{sp}) :

Also known as specific *catability*, this is the enzymatic activity relative to another quantity of the enzyme preparation, often the mass, sometimes the volume. In fact, the mass used is that of the pure enzyme protein or that of the protein contained in the reaction system.

This specific activity makes it possible to monitor the progress of enzyme purification (enrichment E). It is expressed in (IU.g⁻¹) or (kat.kg⁻¹).

$$Z_{\text{sp}} = Z / \text{Mass}_{\text{Protein}}$$

5. 3. Molar catalytic activity (Z_m) :

It is also known as molecular catalytic activity or turnover number. It is the catalytic activity relative to the amount of enzyme protein expressed in moles.

This quantity expresses the number of substrate molecules converted per unit of time and per enzyme molecule at saturation. It is expressed in (t^{-1}).

$$Z_m = Z / [\text{protein}] = Z / [E_t]$$