Kinetics and Enzymes

Elements of Biophysics

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Kinetics

Thermodynamics can be used to determine whether a reaction is spontaneous and how much energy is involved in the reaction.

However, answers are not determined to questions such as how long the process will take and which intermediate states are formed.

Kinetic studies of a process involve correlating the time evolution of each molecular species to a model of the mechanism of the reaction.

For reactions that proceed through a series of steps, the rates for each step can be determined and the slowest step, termed the rate-limiting step, can be identified. Examples in biological settings are the ability of enzymes to accelerate specific chemical processes in the cell.

The simplest case

The simplest model of reaction considers an irreversible reaction of molecule A converting to molecule B:

$$\mathrm{A} \rightarrow \mathrm{B}$$

In this case the rate of a reaction involving a molecule A, which at any time *t* has the concentration *A(t)*, is the change in the concentration or population of the molecule with time:

$$\frac{dA(t)}{A(t)} = -\,kdt \quad or \quad \frac{dA(t)}{dt} = -\,kA(t)$$

In this context, the instantaneous probability is equivalent to a first-order rate constant.

$$\int \frac{\mathrm{dA}(t)}{\mathrm{A}(t)} = -\int k\mathrm{d}t$$

$$\ln \mathrm{A}(t) = -kt + c$$

$$\mathrm{A}(t) = e^{-kt+c} = e^{c}e^{-kt}$$

$$\mathrm{A}(t = 0) = e^{c}e^{-k(0)} = e^{c}$$

The half-life time

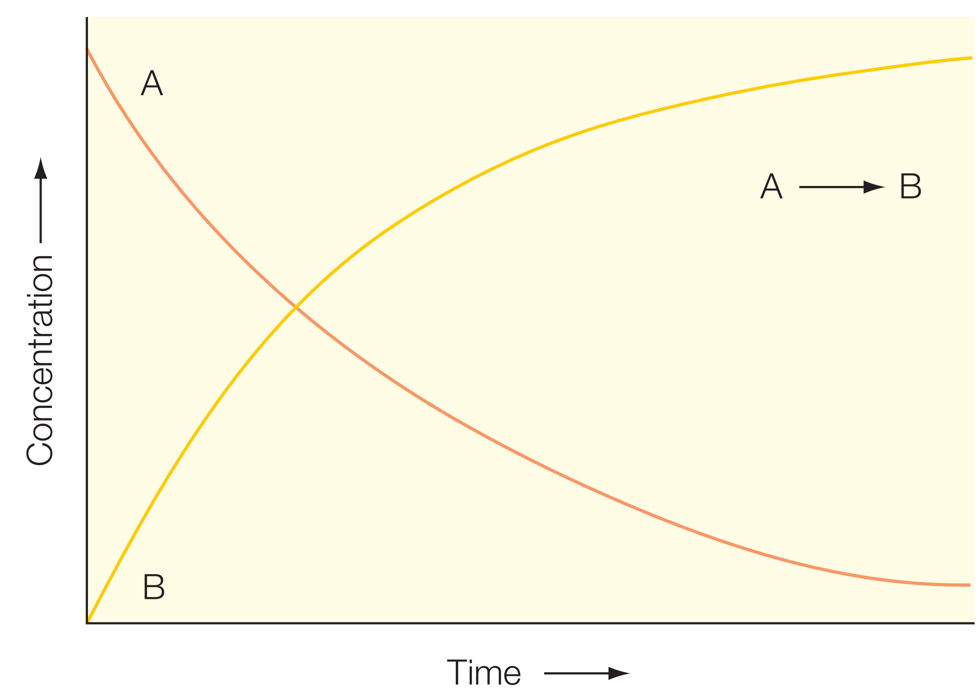
A plot of the time dependence of these two states shows an exponential decay of A and a corresponding increase of B. A classic example of a first-order process is radioactive decay in which the rate is often expressed in terms of the half-life, $t_{1/2}$, which represents the time required for molecule A to decay to half of its value.

$$A(t = 1/2) = \frac{A(t=0)}{2} = A(t = 0)e^{-kt_{1/2}}$$

$$\frac{1}{2} = e^{-kt_{1/2}} \text{ or } \ln(\frac{1}{2}) = -0.69 = -kt_{1/2}$$

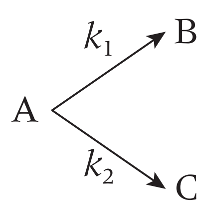
$$t_{1/2} = \frac{0.69}{k}$$

In the case of the previous reaction the half-life and rate constant are inversely related to each other



Parallel first-order reactions

In other cases, the decay may be possible by more than one pathway and the kinetics will reflect the possible formation of two or more different products with different rates. The mechanism of a particle which decays in two products with different k_1 and k_2:



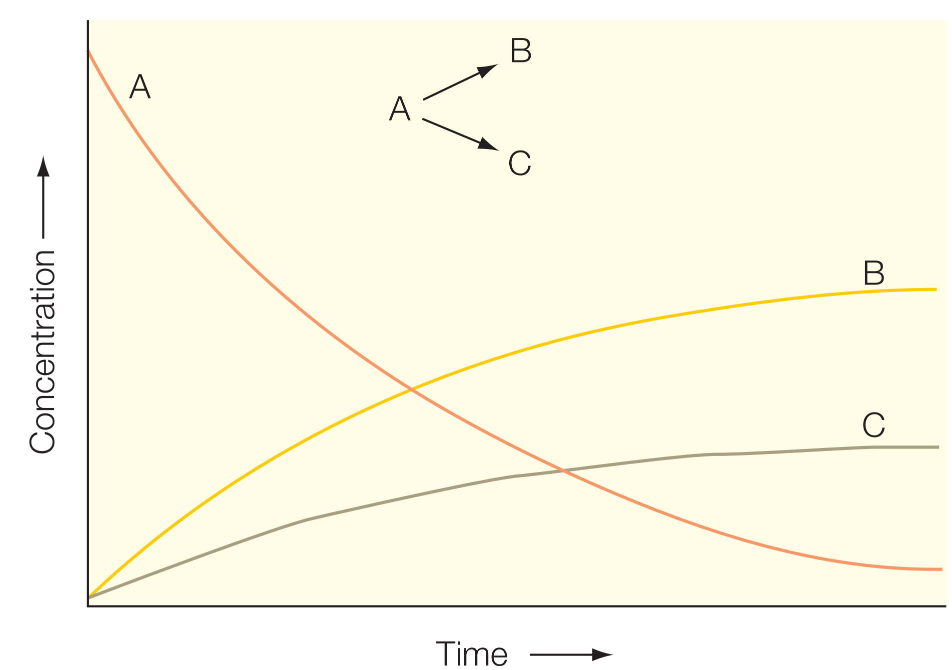
$$-\frac{d[A]}{dt} = k_1[A] + k_2[A] \qquad \frac{d[B]}{dt} = +k_1[A] \qquad \frac{d[C]}{dt} = +k_2[A]$$

For this reaction the rates can be calculated as a function of the the concentrations where we can replace k_1+k_2. with k_{obs}

$$-\frac{d[A]}{dt} = (k_1 + k_2)[A] = k_{obs}[A] \qquad A(t) = A(t=0)e^{-k_{obs}t}$$

$$\frac{d[B]}{dt} = +k_1[A] = +k_1[A(t=0)]e^{-k_{obs}t} \qquad [B(t)] = \frac{k_1[A(t=0)]}{k_{obs}}(1 - e^{-k_{obs}t})$$

$$\frac{d[C]}{dt} = +k_2[A] = +k_2[A(t=0)]e^{-k_{obs}t} \qquad [C(t)] = \frac{k_2[A(t=0)]}{k_{obs}}(1 - e^{-k_{obs}t})$$



The Order of a Reaction

A simple second-order reaction is usually considered to involve two steps: the two components, A and B, must first form a complex AB, and then the reaction proceeds to form the state C:

$$\mathrm{A} + \mathrm{B} \leftrightarrow \mathrm{AB} \rightarrow \mathrm{C} \qquad \frac{\mathrm{dC}}{\mathrm{d}t} = k[\mathrm{AB}] \qquad \frac{\mathrm{dC}}{\mathrm{d}t} = k[\mathrm{AB}] = kV_{\mathrm{B}}[\mathrm{A}][\mathrm{B}]$$

Although the reaction order may be considered from a molecular stand-point, in practice the order is an empirical quantity and may have a range of values. In general, the rate may depend upon the concentrations of the reactants according to:

$$\frac{\mathrm{dC}}{\mathrm{d}t} \propto k\,[\mathrm{A}]^{n}[\mathrm{B}]^{m}$$

where the values of n and m will depend upon the specifics of the reaction. For example, if the complex formation involves two molecules of A and one of B, then n = 2 and m = 1 overall the reaction is third order.

Reaction and Equilibrium

- Thermodynamics: A reaction reaches equilibrium when the ratio of the products and reactants is at the lowest Gibbs energy for the system

- Kinetics: the rate of the forward reaction is equal to the reverse reaction.

$$\mathrm{A} \underset{k_b}{\overset{k_f}{\leftrightarrow}} \mathrm{B}$$

$$\frac{\mathrm{dA}}{\mathrm{d}t} = \frac{\mathrm{dB}}{\mathrm{d}t} = 0$$

$$\frac{\mathrm{d[A]}}{\mathrm{d}t} = -k_f[\mathrm{A}] + k_b[\mathrm{B}] = 0$$

$$k_f[\mathrm{A}] = k_b[\mathrm{B}]$$

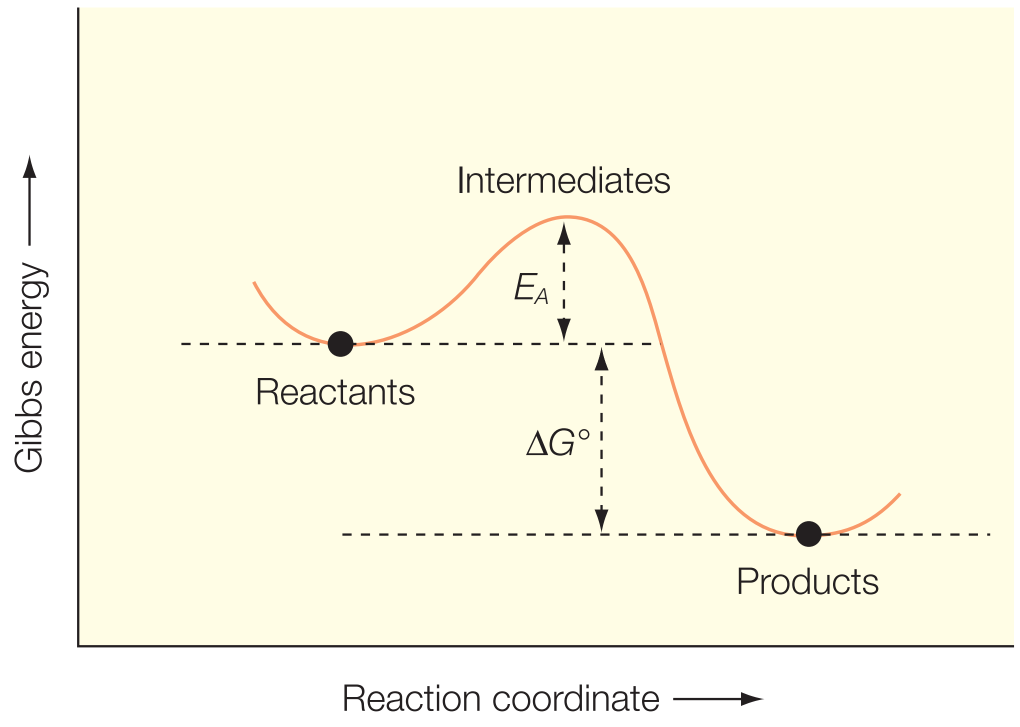
$$K_{eq} = \frac{[\mathrm{B}]}{[\mathrm{A}]} = \frac{k_f}{k_b}$$

Thus, the equilibrium constant for a reaction, K_{eq}, is equal to the ratio of the forward and backward rates for a reaction.

Activation Energy

For some reactions, the change in the Gibbs energy is a large negative (spontaneous) however, the rate of product formation may be slow. In these cases, the reaction usually requires the formation of an intermediate or transitional state that is energetically unfavorable.

$$A + B \leftrightarrow AB^{\ddagger} \rightarrow C$$



The intermediate state because the increase in Gibbs energy represents an energy barrier. The rate to overcome the energy difference between the initial and intermediate state, termed the activation energy, E_A, is given by:

$$k = Ae^{-E_A/k_BT}$$

How it is determined?

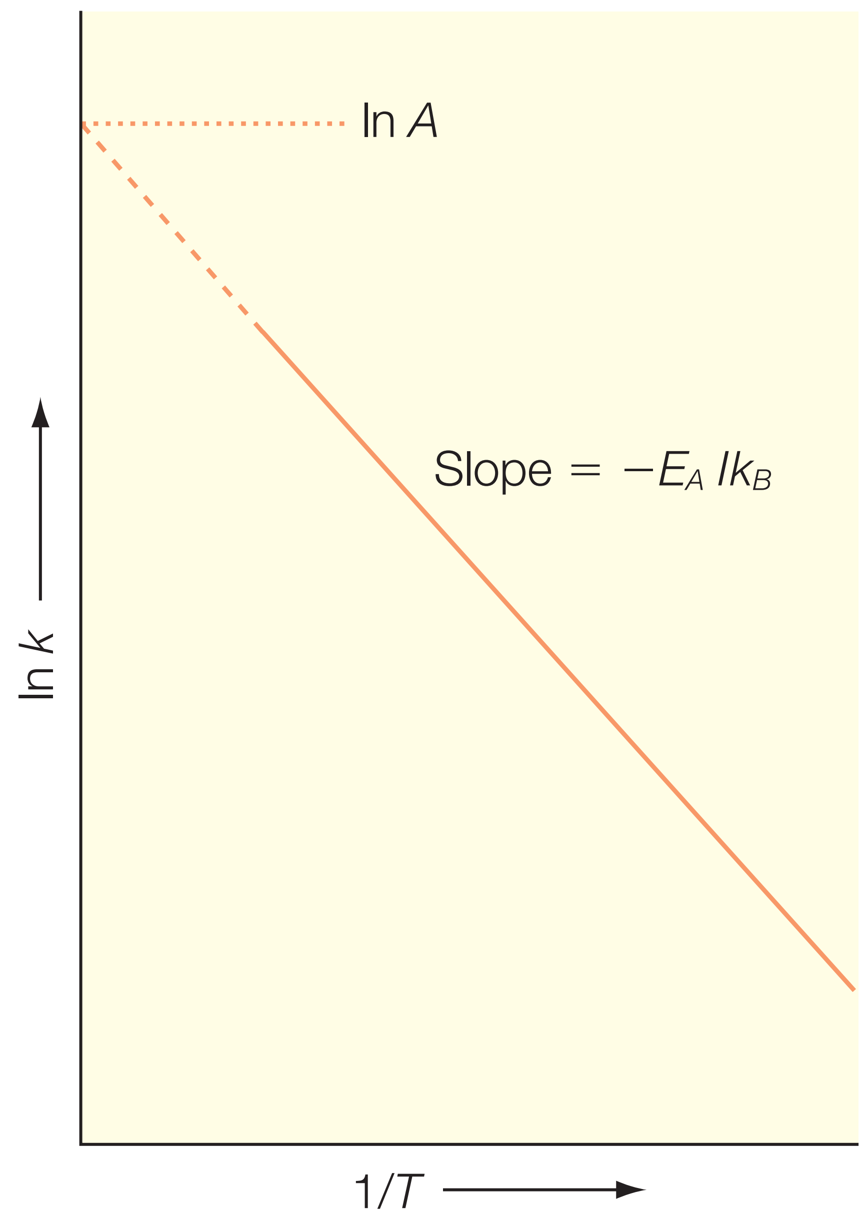
The activation energy can be determined by measurement of the temperature dependence of the reaction.

The temperature dependence, known as an Arrhenius equation, is most easily expressed as a linear equation by using the logarithm of the rate

$$\ln k = \ln A - \left(\frac{E_A}{k_B}\right)\frac{1}{T}$$

$$y = mx + b;$$

$$y = lnk; \quad m = \frac{E_A}{k_B}, \quad and \quad x = \frac{1}{T}$$



Enzymes

One of the fundamental conditions for life is that an organism must be able to catalyze chemical reactions efficiently and selectively. Such functions are performed in cells by highly specialized proteins called enzymes.

Enzymes not only have a remarkable degree of specificity for their substrates, but they also accelerate reactions tremendously under mild conditions of pH, temperature, and pressure.

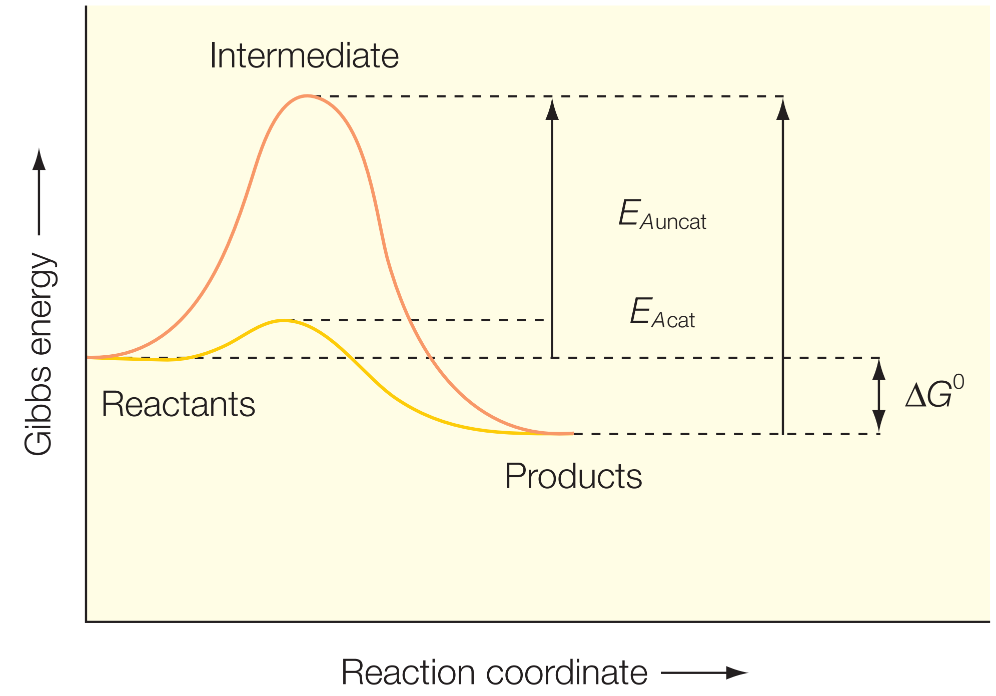
Classification of enzymes.

Enzyme class	Enzyme function
Oxidoreductase	Transfer of electrons
Transferase	Group-transfer reactions
Hydrolase	Hydrolysis reactions
Lyase	Addition of groups to double bonds or formation of double bonds by removal of groups
Isomerase	Transfer of groups within molecules to yield isomeric forms
Ligase	Formation of C–C, C–S, C–O, and C–N bonds coupled to ATP

Enzyme mechanism

Enzymes accelerate reactions that have a substantial activation energy by modifying the reaction rates. The Gibbs energy difference between the initial and final states is not altered and the equilibrium is not changed. Rather, enzymes alter the transitional state of the reaction such that the activation energy is significantly decreased

Although the specific mechanism by which enzymes stabilize the transitional state is unique for each protein, the enzyme promotes multiple weak interactions with the substrate that are specifically positioned such that binding is optimized for the intermediate state.



Michaelis-Menten Mechanism

The basis of the Michaelis–Menten mechanism is the transient formation of a enzyme–substrate complex, ES.

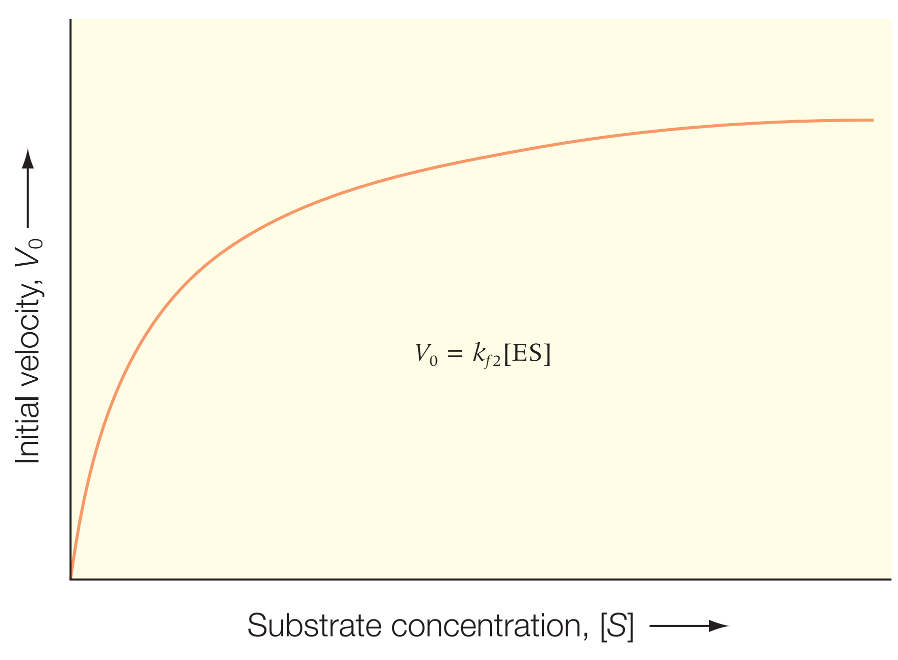
$$E + S \underset{k_{b1}}{\overset{k_{f1}}{\longleftrightarrow}} ES \underset{k_{b2}}{\overset{k_{f2}}{\longleftrightarrow}} E + P$$

The rate of the overall reaction is determined by the second step and is proportional to the concentration of the complex.

Experimentally, enzyme reactions are often probed by measuring the initial rate, or initial velocity, which is denoted by V_0, when the concentration of the substrate is much greater than the concentration of the enzyme.

The initial velocity is then determined by the product of the forward rate constant for the second step, k_{f2}, and the concentration of the complex, [ES]:

$$V_0 = k_{f2}[\mathrm{ES}]$$



Michaelis constant

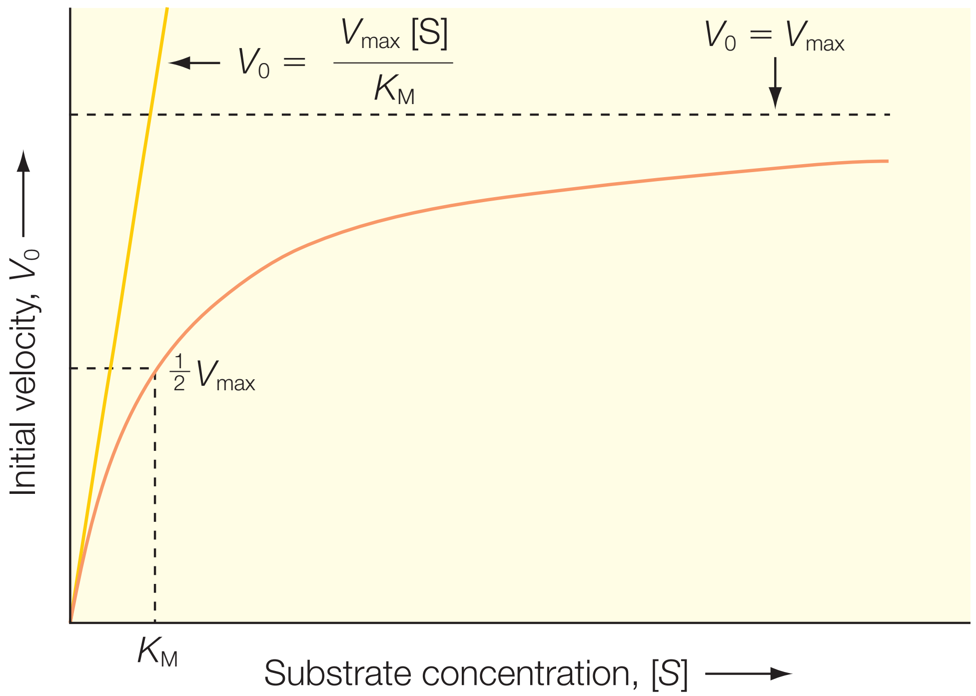
For the determination of V_0 we need to calculate the concentration of the complex ES, We describe the kinetics of each step and assume that the system is in the steady-state

$$\frac{d}{dt}[ES] = k_{f1}[E][S]$$

$$-\frac{d}{dt}[ES] = k_{b1}[ES] + k_{f2}[ES]$$

$$\frac{d}{dt}[ES] = -\frac{d}{dt}[ES]$$

$$k_{f1}[E][S] = k_{b1}[ES] + k_{f2}[ES] \quad [E] = [E_{total}] - [ES]$$



$$[ES] = \frac{k_{f1}[E_{total}][S]}{(k_{b1} + k_{f2} + k_{f1}[S])} = \frac{[E_{total}][S]}{(k_{b1} + k_{f2})/k_{f1} + [S]} = \frac{[E_{total}][S]}{K_M + [S]} \quad K_M = \frac{k_{b1} + k_{f2}}{k_{f1}}$$

Because the maximum velocity, V_{max}, occurs when the enzyme is saturated the maximum velocity defined in terms of the total enzyme concentration can substituted into the expression for the initial velocity:

$$V_{max} = k_{f2}[ES_{saturation}] = k_{f2}[E_{total}]$$

$$V_0 = k_{f2}[ES] = \frac{k_{f2}[E_{total}][S]}{K_M + [S]} = \frac{V_{max}[S]}{K_M + [S]}$$

Estimating K_M

The Michaelis–Menten equation can be transformed into a linear relationship by making use of parameters other than the initial velocity and substrate concentration for the graph.

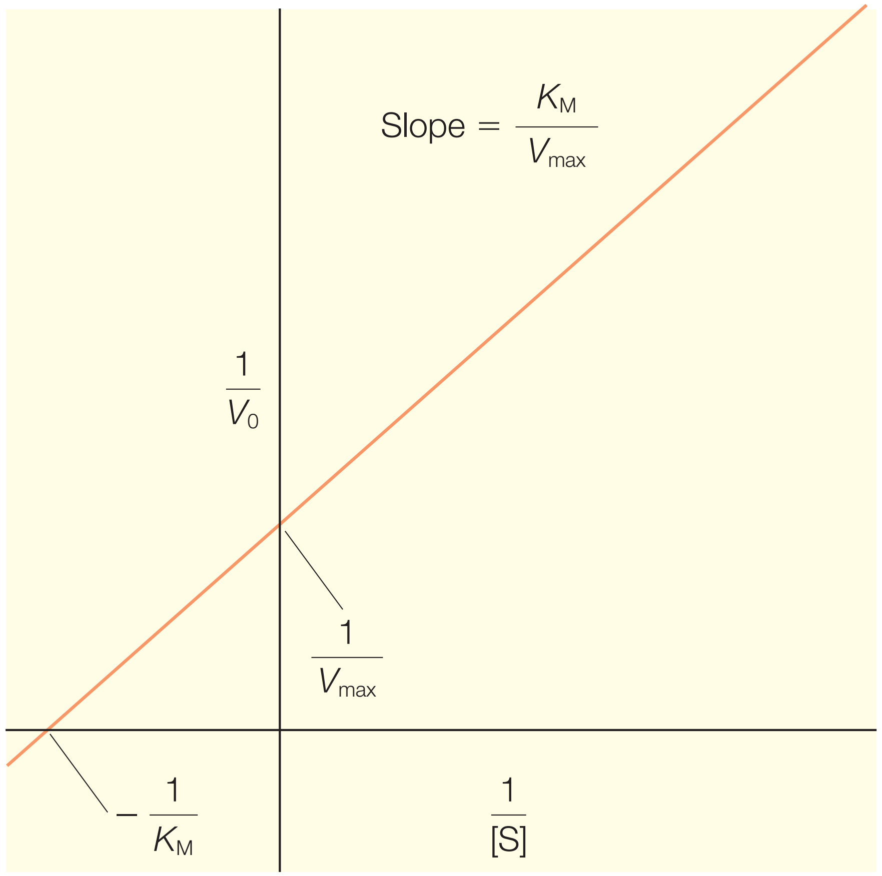
$$(V_0)^{-1} = \left(\frac{V_{\max}[\mathrm{S}]}{K_{\mathrm{M}} + [\mathrm{S}]}\right)^{-1}$$

$$\frac{1}{V_0} = \left(\frac{K_{\mathrm{M}} + [\mathrm{S}]}{V_{\max}[\mathrm{S}]}\right) = \frac{K_{\mathrm{M}}}{V_{\max}[\mathrm{S}]} + \frac{[\mathrm{S}]}{V_{\max}[\mathrm{S}]} = \frac{K_{\mathrm{M}}}{V_{\max}[\mathrm{S}]} + \frac{1}{V_{\max}}$$

Using the following transformation and fitting the curve K_M and V_{max} can be estimated

$$y = mx + b;$$

$$y = \frac{1}{V_0}; \quad m = \frac{K_M}{V_{max}}, \quad and \quad x = \frac{1}{S}$$



Exercise 1

Consider 1M solution of reactant *A* following second order reaction with a rate constant of 10^{-3} $M^{-1}s^{-1}$ what is the half-life time? What is the difference with respect to a first order reaction?

$$2A \rightarrow A_2$$

The differential equation for the kinetic of *A* is

$$\frac{dA(t)}{dt} = -kA^2$$

At the half-time

$$\frac{A(t)}{A(t_0)} = 0.5$$

Suggestion: Write the variation of the concentration of *A* ([*A*]) as a function of the square of the [*A*], transform the equation and use the integral below to calculate the half-life time.

$$-\int_{x_0}^{x} \frac{1}{x^2}\,dx = \left[\frac{1}{x}\right]_{x_0}^{x} = \frac{1}{x} - \frac{1}{x_0}$$

Exercise 2

Given the following points for an enzyme/substrate reaction following the Michaelis-Menten kinetics calculate the values of K_M and V_{max}.

[S] (μM)	V_0 (μM/min)
208	1.1
417	2.5
909	4.1
1429	5.1

Suggestion: Consider the Eq. 1 and use the transformations below to fit the points to a linear curve (Eq. 2).

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

$$y = mx + b; \qquad y = \frac{1}{V_0}; \quad m = \frac{K_M}{V_{max}}, \quad and \quad x = \frac{1}{S} \qquad [2]$$