

Enzyme Kinetics

CHEM 361A: Introduction to Physical Chemistry

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Lecture 19

Table of contents

- 1 Catalysts
 - Product Rate Law Expression
 - Lineweaver-Burk Plot
- 2 Enzymes
 - Michaelis-Menten Mechanism

Learning Objective: Quantify rate law expressions for enzyme catalysed reactions.

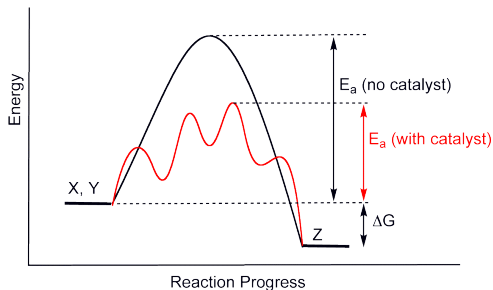
References:

- Atkins and de Paula Focus 6H
- Chang §13.1 and §13.2

Catalysts

A catalyst is a substance that is not consumed by a reaction and offers an alternative reaction pathway whose rate is faster than the original reaction.

- The activation energy of the catalysed pathway is lower than the uncatalysed pathway



The rate of the reaction with a catalyst is the sum of the uncatalysed and catalysed reaction rates.

Catalyst Reaction Mechanism

The simplest reaction mechanism involving a catalyst is



This reaction mechanism says

- 1 A substrate (S), and a catalyst (C), are initially present in a vessel.
- 2 Separated S and C form an equilibrium with a combined SC complex
- 3 The conversion of SC to the separated Product P and C is assumed to only occur in the forward direction

The Composite Constant

The differential rate law expression for the formation of product is

$$\frac{d[P]}{dt} = k_P[SC]$$

Need [SC] to quantify the rate of product production. Assuming $k_P \gg k_f, k_r$, then the rate law for [SC] is

$$\frac{d[SC]}{dt} = k_f[S][C] - k_r[SC] - k_P[SC] = 0; \quad \text{Steady State Approx}$$

$$[SC] = \frac{k_f}{k_r + k_P}[S][C] = \frac{[S][C]}{K_m}; \quad K_m = \frac{k_r + k_P}{k_f}$$

K_m is referred to as the **composite constant**.

Solving for the [SC] Complex

Now that we have an expression that relates [SC] to [S] and [C]
let's use the conservation of moles to relate it to [S]₀ and [C]₀.

$$[S]_0 = [S] + [SC] + [P] \quad \rightarrow \quad [S] = [S]_0 - [SC] - [P]$$

$$[C]_0 = [C] + [SC] \quad \rightarrow \quad [C] = [C]_0 - [SC]$$

So these two terms go into the SC Rate Law expression

$$K_m[SC] = [S][C] = ([S]_0 - [SC] - [P])([C]_0 - [SC])$$

$$K_m[SC] = [S]_0[C]_0 - [SC][C]_0 - [P][C]_0 - [S]_0[SC] + [SC]^2 + [P][SC]$$

$$0 = [S]_0[C]_0 - [P][C]_0 - [SC]([C]_0 + [S]_0 + K_m - [P]) + [SC]^2$$

Solving for the [SC] Complex (cont.)

For these types of reactions, we are typically only interested with the rate under steady state conditions which occur close to the start of the reaction. As a result, two assumptions can be made:

- ① The amount of Product formed is very small
- ② The amount of the intermediate complex, SC, is very small

So, [P] and [SC]² terms can be neglected:

$$0 = [S]_0[C]_0 - \cancel{[P][C]_0} \overset{0}{\rightarrow} - [SC]([C]_0 + [S]_0 + K_m - \cancel{[P]}) \overset{0}{\rightarrow} + \cancel{[SC]^2} \overset{0}{\rightarrow}$$

Meaning

$$[SC]([C]_0 + [S]_0 + K_m) = [S]_0[C]_0$$

$$[SC] = \frac{[S]_0[C]_0}{[C]_0 + [S]_0 + K_m}$$

Product Rate Law Expression

Returning to the Product Rate Law expression, we can substitute in our value for [SC]

$$rate = \frac{d[P]}{dt} = k_P \frac{[S]_0 [C]_0}{[C]_0 + [S]_0 + K_m}$$

- The Product Rate Law expression is a function of constants
- Only describes the rate at the start of the reaction

Case 1: $[S]_0 \ll [C]_0$

To determine rate constants, we would employ flooding to simplify the rate law expression. Let's examine the initial rate when $[S]_0 \ll [C]_0$. The rate law expression is

$$rate = \frac{d[P]}{dt} = k_P \frac{[S]_0 [C]_0}{[C]_0 + K_m}$$

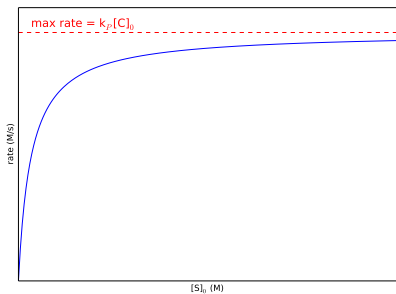
- First order in $[S]_0$
- Zeroth or first order in $[C]_0$ depending on its magnitude relative to K_m

This limit is avoided because catalysts are typically expensive and we can determine the rate constants using the following extreme case.

Case 2: $[C]_0 \ll [S]_0$

In this more common case $[C]_0 \ll [S]_0$. In this limit, the rate law expression is

$$\text{rate} = \frac{d[P]}{dt} = k_P \frac{[S]_0 [C]_0}{[S]_0 + K_m}$$



- First order in $[C]_0$
- Zeroth or first order in $[S]_0$ depending on its magnitude relative to K_m

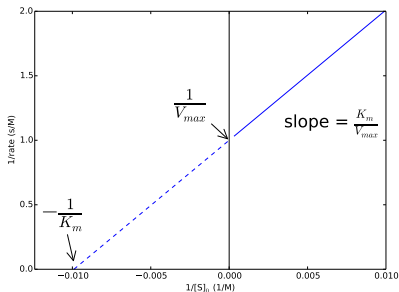
In the limit that $[S]_0 \gg K_m$ then

$$\text{max rate} = V_{max} = k_P [C]_0$$

Lineweaver-Burk Plot

In order to fit for K_m , V_{max} and the rate constants, it is common to plot $\frac{1}{rate}$ versus $\frac{1}{[S]_0}$

$$\underbrace{\frac{1}{rate}}_y = \frac{[S]_0 + K_m}{k_P[S]_0[C]_0} = \underbrace{\frac{K_m}{V_{max}}}_{\text{slope}} \underbrace{\frac{1}{[S]_0}}_x + \underbrace{\frac{1}{V_{max}}}_{\text{y-int}}$$

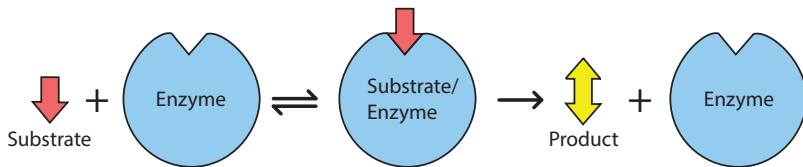


This double reciprocal plot is called a Lineweaver-Burk plot

Enzymes

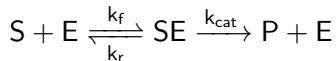
Enzymes are proteins or ribozymes that act like catalysts. They usually contain one or more active sites where reactions with very specific substrates take place.

The German biochemist Leonor Michaelis and the Canadian biochemist Maud L. Menten proposed a mechanism to explain the dependence of the initial rate of enzyme-catalysed reactions on the concentration of reagents using the following scheme:



Michaelis-Menten Mechanism

The Michaelis-Menten Mechanism can be written as



It can be assumed that the $[SE]$ complex quickly reaches a constant value, and that $[E]_0 \ll [S]_0$. Therefore

$$rate = \frac{d[P]}{dt} = k_{cat} \frac{[S]_0[E]_0}{[S]_0 + K_m}$$

This is the **Michaelis-Menten Rate Law** where K_m is the **Michaelis constant**.

The Significance of K_m , V_{max} , and k_{cat}

As was indicated before, V_{max} is the maximum rate attainable. If $[E]_0$ is known, then V_{max} can be used to determine the **turnover number**, k_{cat} . The turnover number, represents the number of substrate molecules that are converted to product per unit time when the enzyme is fully saturated with substrate.

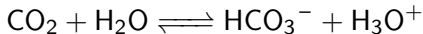
The Michaelis constant, K_m , is a ratio of rate constants, and also represents the substrate concentration at which half the enzyme active sites are filled by substrate molecules. From the rate law expression:

$$\frac{V_{max}}{2} = \frac{[S]_0 V_{max}}{[S]_0 + K_m} \rightarrow K_m = [S]_0$$

K_m varies greatly from enzyme to enzyme and within the same enzyme for different substrates. It also depends on temperature, pH, ionic strength, and other reaction conditions.

Michaelis-Menten Example

DeVoe and Kistiakowsky (from *Journal of the American Chemical Society* 83 (1961): 274) studied the kinetics of CO₂ hydration catalysed by the enzyme carbonic anhydrase:



The following initial reaction rates for the hydration reaction were obtained for $[\text{E}]_0 = 2.3 \text{ nM}$ and $T = 273.65 \text{ K}$:

Rate (M s ⁻¹)	[CO ₂] ₀ (mM)
2.78×10^{-5}	1.25
5.00×10^{-5}	2.5
8.33×10^{-5}	5.0
1.67×10^{-4}	20.0

Determine K_m and k_{cat} for the enzyme at this temperature.

Summary

- A catalyst is a substance that is not consumed by a reaction and offers an alternative reaction pathway whose rate is faster than the original reaction.
- An Enzyme is a protein or ribosome that acts like a catalyst on very specific substrates. Their initial reaction rate can be described using the Michaelis-Menten Rate Law

$$rate = k_{cat} \frac{[S]_0[E]_0}{[S]_0 + K_m}$$

- Determining the turnover number (k_{cat}) and Michaelis constant (K_m) can be achieved by fitting data using a Lineweaver-Burk plot