

A First Course on Kinetics and Reaction Engineering

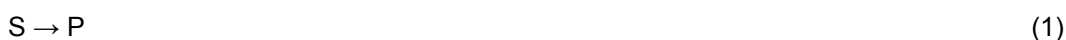
Example 9.3

Problem Purpose

This problem illustrates the generation of a rate expression for an enzymatic reaction involving an enzyme inhibitor.

Problem Statement

Suppose that enzyme E catalyzes the conversion of substrate S to product P, but it is inhibited by inhibitor I. The overall reaction is given by equation (1), and the mechanism is given by equations (2) through (4).



Assume that step (4) is effectively irreversible and derive a Michaelis-Menten type of rate expression for this system. You may assume that the concentration of the free inhibitor can be easily measured, so that its presence in the rate expression is acceptable.

Problem Analysis

This problem asks us to derive a rate expression from a mechanism. There is no rate-determining step in the mechanism, so the overall rate of production of P will be used as the rate expression for reaction (1) with respect to P. That rate expression will contain concentrations of non-complexed and complexed enzyme, which might be difficult to measure. These concentrations can be eliminated by applying the Bodenstein steady state approximation along with an expression for the conservation of the enzyme.

Problem Solution

Before deriving a rate expression, one should check that there is a linear combination of the mechanistic steps that is equal to the overall, macroscopically observed reaction. Here it is easy to see that reaction (1) is equal to reaction (2) plus reaction (4), so that requirement is met. Since no rate-determining step has been specified, we next choose a reactant or product of the overall reaction and generate an expression for the net rate of the overall reaction with respect to the chosen species that equals the sum of its rates of generation in the mechanistic steps. Here we will choose the product, P, but the exact same answer would be obtained if we chose the substrate, S. The rate of the overall reaction with respect to P is found using equation (5). Expanding the summation is trivial because the stoichiometric coefficient of P is zero in steps (2) and (3), so that the summation only contains one term corresponding to step (4). This leads to the rate expression given in equation (6)

$$r_{i,j} = \sum_{\substack{s=\text{all} \\ \text{steps}}} \nu_{i,s} \left(k_{s,f} \prod_{\substack{m=\text{all} \\ \text{reactants}}} [m]^{-\nu_{m,s}} - k_{s,r} \prod_{\substack{n=\text{all} \\ \text{products}}} [n]^{\nu_{n,s}} \right) \quad (5)$$

$$r_{P,1} = k_{4,f} [E - S] - k_{4,r} [E][P] \quad (6)$$

The problem statement also specifies that step (4) is irreversible. This means that the rate of step (4) in the reverse direction can be set equal to zero, resulting in the rate expression given in equation (7).

$$r_{P,1} = k_{4,f} [E - S] \quad (7)$$

This rate expression is not acceptable because it contains the concentration of a reactive intermediate (the enzyme-substrate complex). To eliminate this reactive intermediate concentration, the Bodenstein steady state approximation, equation (8), can be applied to each reactive intermediate.

$$0 = \sum_{\substack{s=\text{all} \\ \text{steps}}} \nu_{i,s} \left(k_{s,f} \prod_{\substack{m=\text{all} \\ \text{reactants}}} [m]^{-\nu_{m,s}} - k_{s,r} \prod_{\substack{n=\text{all} \\ \text{products}}} [n]^{\nu_{n,s}} \right) \quad i = \text{reactive intermediate} \quad (8)$$

In the present problem, there are three reactive intermediates, E, E-I and E-S, so equation (8) can be written three times, once for each of the reactive intermediates. However, based upon our experience (and the results of Example 9.1), we know that only two of the three resulting equations will be mathematically independent. Consequently, we will only apply the Bodenstein steady state approximation to E-S, leading to equation (10) and to E-I, leading to equation (12). In doing so, the term corresponding to the rate of reaction (4) in the reverse direction was dropped since the problem statement declares that step to be irreversible.

$$0 = (-1) \{ k_{2,f} [E][S] - k_{2,r} [E - S] \} + (1) k_{4,f} [E - S] \quad (9)$$

$$0 = -k_{2,f} [E][S] + k_{2,r} [E - S] + k_{4,f} [E - S] \quad (10)$$

$$0 = (1) \{ k_{3,f} [E][I] - k_{3,r} [E - I] \} \quad (11)$$

$$0 = k_{3,f} [E][I] - k_{3,r} [E - I] \quad (12)$$

These two equations clearly can't be solved to get expressions for the concentrations of the three reactive intermediates. An additional relationship is needed, and this comes from the conservation of catalyst or enzyme as expressed in equation (13). In this problem the non-complexed catalyst is E and the complexed forms are E-I and E-S. Thus, letting E^0 represent the equivalent concentration of enzyme before it was added to the system, equation (14) results.

$$C_{cat}^0 = C_{cat,free} + \sum_{\substack{i = \text{all} \\ \text{catalyst} \\ \text{complexing} \\ \text{species}}} v_{cat,i} C_{cat,i} \quad (13)$$

$$E^0 = [E] + [E - S] + [E - I] \quad (14)$$

Now equations (10), (12) and (14) can be solved to obtain expressions for the concentrations of the reactive intermediates in terms of constants and the concentrations of stable species. This can be done manually or using symbolic algebra software. In either case, the results are given in equations (15), (16) and (17).

$$[E] = \frac{k_{3,r}(k_{2,r} + k_{4,f})E^0}{k_{2,f}k_{3,r}[S] + (k_{2,r}k_{3,f} + k_{3,f}k_{4,f})[I] + (k_{2,r}k_{3,r} + k_{3,r}k_{4,f})} \quad (15)$$

$$[E - I] = \frac{k_{3,f}(k_{2,r} + k_{4,f})E^0[I]}{k_{2,f}k_{3,r}[S] + (k_{2,r}k_{3,f} + k_{3,f}k_{4,f})[I] + (k_{2,r}k_{3,r} + k_{3,r}k_{4,f})} \quad (16)$$

$$[E - S] = \frac{k_{2,f}k_{3,r}E^0[S]}{k_{2,f}k_{3,r}[S] + (k_{2,r}k_{3,f} + k_{3,f}k_{4,f})[I] + (k_{2,r}k_{3,r} + k_{3,r}k_{4,f})} \quad (17)$$

Finally, substituting equation (17) into equation (7) gives the desired result, equation (18).

$$r_{P,overall} = \frac{k_{2,f}k_{3,r}k_{4,f}E^0[S]}{k_{2,f}k_{3,r}[S] + (k_{2,r}k_{3,f} + k_{3,f}k_{4,f})[I] + (k_{2,r}k_{3,r} + k_{3,r}k_{4,f})} \quad (18)$$