where $K_m = \dfrac{k_{-1}+k_2}{k_1}$ and $K_p = \dfrac{k_{-1}+k_2}{k_{-2}}$ and $V_s = k_2[E_0]$, $V_p = k_{-1}[E_0]$.

**3.3.** The enzyme, fumarase, has the following kinetic constants:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

where $k_1 = 10^9\ M^{-1}\ s^{-1}$

$k_{-1} = 4.4 \times 10^4\ s^{-1}$

$k_2 = 10^3\ s^{-1}$

**a.** What is the value of the Michaelis constant for this enzyme?

**b.** At an enzyme concentration of $10^{-6}\ M$, what will be the initial rate of product formation at a substrate concentration of $10^{-3}\ M$?

[Courtesy of D. J. Kirwan from "Collected Coursework Problems in Biochemical Engineering" compiled by H. W. Blanch for 1977 Am. Soc. Eng. Educ. Summer School.]

**3.4.** The hydration of $CO_2$ is catalyzed by carbonic anhydrase as follows:

$$H_2O + CO_2 \overset{E}{\rightleftharpoons} HCO_3^- + H^+$$

The following data were obtained for the forward and reverse reaction rates at pH 7.1 and an enzyme concentration of $2.8 \times 10^{-9}\ M$.

| Hydration | | Dehydration | |
|---|---|---|---|
| $1/v$, $M^{-1}$ $(s \times 10^{-3})$ | $[CO_2]$ $(M \times 10^3)$ | $1/v$, $M^{-1}$ $(s \times 10^{-3})$ | $[HCO_3^-]$ $(M \times 10^3)$ |
| 36 | 1.25 | 95 | 2 |
| 20 | 2.5 | 45 | 5 |
| 12 | 5 | 29 | 10 |
| 6 | 20 | 25 | 15 |

$v$ is the *initial* reaction rate at the given substrate concentration. Calculate the forward and reverse catalytic and Michaelis constants.

[Courtesy of D. J. Kirwan from "Collected Coursework Problems in Biochemical Engineering" compiled by H. W. Blanch for 1977 Am. Soc. Eng. Educ. Summer School.]

**3.5.** An inhibitor (I) is added to the enzymatic reaction at a level of 1.0 g/l. The following data were obtained for $K_m = 9.2$ g S/l.

| $v$ | S |
|---|---|
| 0.909 | 20 |
| 0.658 | 10 |
| 0.493 | 6.67 |
| 0.40 | 5 |
| 0.333 | 4 |
| 0.289 | 3.33 |
| 0.227 | 2.5 |

**a.** Is the inhibitor competitive or noncompetitive?

**b.** Find $K_I$.

**3.6.** During a test of kinetics of an enzyme-catalyzed reaction, the following data were recorded:

| $E_0$ (g/l) | T (°C) | I (mmol/ml) | S (mmol/ml) | V (mmol/ml-min) |
|---|---|---|---|---|
| 1.6 | 30 | 0 | 0.1 | 2.63 |
| 1.6 | 30 | 0 | 0.033 | 1.92 |
| 1.6 | 30 | 0 | 0.02 | 1.47 |
| 1.6 | 30 | 0 | 0.01 | 0.96 |
| 1.6 | 30 | 0 | 0.005 | 0.56 |
| 1.6 | 49.6 | 0 | 0.1 | 5.13 |
| 1.6 | 49.6 | 0 | 0.033 | 3.70 |
| 1.6 | 49.6 | 0 | 0.01 | 1.89 |
| 1.6 | 49.6 | 0 | 0.0067 | 1.43 |
| 1.6 | 49.6 | 0 | 0.005 | 1.11 |
| 0.92 | 30 | 0 | 0.1 | 1.64 |
| 0.92 | 30 | 0 | 0.02 | 0.90 |
| 0.92 | 30 | 0 | 0.01 | 0.58 |
| 0.92 | 30 | 0.6 | 0.1 | 1.33 |
| 0.92 | 30 | 0.6 | 0.033 | 0.80 |
| 0.92 | 30 | 0.6 | 0.02 | 0.57 |

**a.** Determine the Michaelis–Menten constant for the reaction with no inhibitor present at 30°C and at 49.6°C.

**b.** Determine the maximum velocity of the uninhibited reaction at 30°C and an enzyme concentration of 1.6 g/l.

**c.** Determine the $K_I$ for the inhibitor at 30°C and decide what type of inhibitor is being used.

**3.7.** An enzyme ATPase has a molecular weight of $5 \times 10^4$ daltons, a $K_M$ value of $10^{-4}$ *M*, and a $k_2$ value of $k_2 = 10^4$ molecules ATP/min molecule enzyme at 37°C. The reaction catalyzed is the following:

$$\text{ATP} \xrightarrow{\text{ATPase}} \text{ADP} + \text{P}_i$$

which can also be represented as

$$\text{E} + \text{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{ES} \xrightarrow{k_2} \text{E} + \text{P}$$

where S is ATP. The enzyme at this temperature is unstable. The enzyme inactivation kinetics are first order:

$$\text{E} = \text{E}_0 e^{-k_d t}$$

where $E_0$ is the initial enzyme concentration and $k_d = 0.1$ min$^{-1}$. In an experiment with a partially pure enzyme preparation, 10 μg of total crude protein (containing enzyme) is added to a 1 ml reaction mixture containing 0.02 *M* ATP and incubated at 37°C. After 12 hours the reaction ends (i.e., $t \to \infty$) and the inorganic phosphate ($P_i$) concentration is found to be 0.002 *M*, which was initially zero. What fraction of the crude protein preparation was the enzyme? *Hint*: Since [S] >> $K_m$, the reaction rate can be represented by

$$\frac{d(\text{P})}{dt} = k_2[\text{E}]$$

**3.8.** Assume that for an enzyme immobilized on the surface of a nonporous support material the external mass transfer resistance for substrate is not negligible as compared to the reaction rate. The enzyme is subject to substrate inhibition (eq. 3.34).

a. Are multiple states possible? Why or why not?

b. Could the effectiveness factor be greater than one?

**3.9.** The following data were obtained for an enzyme-catalyzed reaction. Determine $V_{max}$ and $K_m$ by inspection. Plot the data using the Eadie–Hofstee method and determine these constants graphically. Explain the discrepancy in your two determinations. The initial rate data for the enzyme-catalyzed reaction are as follows:

| [S] mol/l | $v$ μmol/min |
|---|---|
| $5.0 \times 10^{-4}$ | 125 |
| $2.0 \times 10^{-4}$ | 125 |
| $6.0 \times 10^{-5}$ | 121 |
| $4.0 \times 10^{-5}$ | 111 |
| $3.0 \times 10^{-5}$ | 96.5 |
| $2.0 \times 10^{-5}$ | 62.5 |
| $1.6 \times 10^{-5}$ | 42.7 |
| $1.0 \times 10^{-5}$ | 13.9 |
| $8.0 \times 10^{-6}$ | 7.50 |

Do these data fit into Michaelis–Menten kinetics? If not, what kind of rate expression would you suggest? Use graphical methods.

**3.10.** a. H. H. Weetall and N. B. Havewala report the following data for the production of dextrose from corn starch using both soluble and immobilized (azo-glass beads) glucoamylase in a fully agitated CSTR system.

1. Soluble data: $T = 60°C$, $[S_0] = 168$ mg starch/ml, $[E_0] = 11{,}600$ units, volume = 1000 ml.
2. Immobilized data: $T = 60°C$, $[S_0] = 336$ mg starch/ml, $[E_0] = 46{,}400$ units initially, immobilized, volume = 1000 ml.

| | Product concentration (mg dextrose/ml) | |
|---|---|---|
| Time (min) | Soluble | Immobilized |
| 0 | 12.0 | 18.4 |
| 15 | 40.0 | 135 |
| 30 | 76.5 | 200 |
| 45 | 94.3 | 236 |
| 60 | 120.0 | 260 |
| 75 | 135.5 | 258 |
| 90 | 151.2 | 262 |
| 105 | 150.4 | 266 |
| 120 | 155.7 | 278 |
| 135 | 160.1 | 300 |
| 150 | 164.9 | 310 |
| 165 | 170.0 | 306 |
| 225 | — | 316 |
| 415 | — | 320 |

Determine the maximum reaction velocity, $V_m$ (mg/ml-min · unit of enzyme) and the saturation constant, $K_M$ (mg/ml).

b. The same authors studied the effect of temperature on the maximum rate of the hydrolysis of corn starch by glucoamylase. The results are tabulated next. Determine the activation energy ($\Delta E$ cal/g mole) for the soluble and immobilized enzyme reaction.

| | $V_{max}$ (m mol/min $10^6$) | |
|---|---|---|
| T, °C | Soluble | Azo-immobilized |
| 25 | 0.62 | 0.80 |
| 35 | 1.42 | 1.40 |
| 45 | 3.60 | 3.00 |
| 55 | 8.0 | 6.2 |
| 65 | 16.0 | 11.0 |

c. Using these results, determine if immobilized enzyme is diffusion limited.

[Courtesy of A. E. Humphrey from "Collected Coursework Problems in Biochemical Engineering" compiled by H. W. Blanch for 1977 Am. Soc. Eng. Educ. Summer School.]

**3.11.** Michaelis–Menten kinetics are used to describe intracellular reactions. Yet $[E_0] \approx [S_0]$. In in vitro batch reactors, the quasi-steady-state hypothesis does not hold for $[E_0] \approx [S_0]$. The rapid equilibrium assumption also will not hold. Explain why Michaelis–Menten kinetics and the quasi-steady-state approximation are still reasonable descriptions of intracellular enzyme reactions.

**3.12.** You are working for company A and you join a research group working on immobilized enzymes. Harry, the head of the lab, claims that immobilization improves the stability of the enzyme. His proof is that the enzyme has a half-life of 10 days in free solution, but under identical conditions of temperature, pH, and medium composition, the measured half-life of a packed column is 30 days. The enzyme is immobilized in a porous sphere 5 mm in diameter. Is Harry's reasoning right? Do you agree with him? Why or why not?

**3.13.** The following data were obtained from enzymatic oxidation of phenol by phenol oxidase at different phenol concentrations.

| S (mg/l) | 10 | 20 | 30 | 50 | 60 | 80 | 90 | 110 | 130 | 140 | 150 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $v$ (mg/l-h) | 5 | 7.5 | 10 | 12.5 | 13.7 | 15 | 15 | 12.5 | 9.5 | 7.5 | 5.7 |

**a.** What type of inhibition is this ?

**b.** Determine the constants $V_m$, $K_m$ and $K_{si}$.

**c.** Determine the oxidation rate at [S] = 70 mg/l.

**3.14.** Uric acid is degraded by uricase enzyme immobilized in porous Ca-alginate beads. Experiments conducted with different bead sizes result in the following rate data:

| Bead Diameter, Dp (cm) | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 |
|---|---|---|---|---|---|---|---|---|
| Rate, $v$ (mg/l.h) | 200 | 198 | 180 | 140 | 100 | 70 | 50 | 30 |

**a.** Determine the effectiveness factor for particle sizes Dp = 0.5 cm and Dp = 0.7 cm.

**b.** The following data were obtained for Dp = 0.5 cm at different bulk uric acid concentrations. Assuming negligible liquid film resistance, calculate $V_m$ and $K_s$ for the enzyme. Assume no substrate or product inhibition.

| $S_0$ (mg UA/l) | 10 | 25 | 50 | 100 | 200 | 250 |
|---|---|---|---|---|---|---|
| $v$ (mg UA/l.h) | 10 | 20 | 30 | 40 | 45 | 46 |