**Answer : 01**

**Part (A): Inhibitor Type:**

Based on the provided reaction mechanism, the inhibitor I exhibits characteristics resembling a **mixed inhibitor** rather than a purely competitive, uncompetitive, or non-competitive inhibitor. Here's why:

* **Not competitive:** It doesn't bind directly to the substrate binding site (ES + I ⇔ EIS).
* **Not uncompetitive:** It binds to the enzyme in both free (E + I ⇔ EI) and substrate-bound states (ES + I ⇔ EIS).
* **Not purely non-competitive:** While it can bind to the free enzyme, it also affects the substrate-bound form (ES + I ⇔ EIS), suggesting additional interaction beyond simply affecting the free enzyme's conformation.

Therefore, it exhibits aspects of both competitive and non-competitive inhibition, categorized as a **mixed inhibitor**.

**Part (B): Enzyme Balance Equation:**

The enzyme balance equation accounts for all possible forms of the enzyme (free, substrate-bound, inhibitor-bound, and both substrate and inhibitor-bound):

[E\_total] = [E] + [ES] + [EI] + [EAS]

**Part (C): Total Rate of Product Formation:**

The total rate of product formation (P) considers all pathways leading to its production:

v\_p = k\_2[ES] + k\_6[EAS]

However, expressing it solely in terms of measurable concentrations requires applying steady-state assumptions and solving for intermediate species concentrations.

**Part (D):Rapid Equilibrium and Quasi Steady-State Assumptions:**

**Rapid Equilibrium:**

* Assume rapid equilibrium for steps 1, 3, 4, and 7 as they involve small molecules and binding/unbinding processes.
* Apply the equilibrium constant expressions for each step.

**Quasi Steady-State:**

* Assume the concentrations of intermediate species (ES, EI, EAS) remain relatively constant during product formation.

d[ES]/dt ≈ 0

d[EAS]/dt ≈ 0

* Derive expressions for each intermediate concentration based on equilibrium constants and total enzyme concentration.

**Part (E): Relative Values of k2 and k6:**

* k2 represents the rate constant for product formation from the simple substrate-bound complex (ES).
* k6 represents the rate constant for product formation from the enzyme complex bound to both substrate and inhibitor (EAS)..

The relative values of *k*2​ and *k*6​ depend on the specific conditions of the reaction and the concentrations of substrate and activator. Generally, *k*6​ represents the rate of product formation when the activator is bound to the enzyme-substrate complex, while *k*2​ represents the rate of product formation without the activator. If the activator significantly enhances the catalytic activity of the enzyme, *k*6​ will be much larger than *k*2​. However, if the activator has minimal effect or if the substrate concentration is much higher than the activator concentration, *k*2​ may dominate. Therefore, the relative values of *k*2​ and *k*6​ depend on the specific circumstances of the reaction.

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**Answer : 02**

**Part (A):**

To determine Vmax and Km for the uninhibited reaction (no inhibitor), we can use the Michaelis-Menten equation:

where:

- V is the reaction velocity.

- V\_max is the maximum reaction velocity.

- [S] is the substrate concentration.

- K\_m is the Michaelis constant.

We can rearrange the equation to linearize it:

We can plot 1/V against 1/[S] and determine the slope and intercept to find K\_m and V\_max.

Let's perform the calculations:

From the linear plot, the slope (K\_m/V\_max) = 0.282, and the intercept (1/V\_max) = 5.88. Therefore,

Using any data point, we can find V\_max and then K\_m.

So, for the uninhibited reaction:

**Part (B):** To determine the type of inhibitor I, we can analyze the reaction velocities in the presence and absence of the inhibitor. If the inhibitor decreases V\_max but does not affect K\_m, it's a noncompetitive inhibitor. If it increases K\_m but does not affect V\_max, it's an uncompetitive inhibitor. If it affects both V\_max and K\_m, it's a mixed inhibitor.

Let's analyze the data:

Inhibited reaction:

From the data, we can observe that both V\_max and K\_m change, which indicates that the inhibitor I is a mixed inhibitor.

**Part (C):**

To determine V\_max,app and K\_m,app for the inhibited reaction, we can use the same process as for the uninhibited reaction, but with the data from the inhibited reaction.

Let's perform the calculations:

From the linear plot, the slope (K\_m,app/V\_max,app) = 0.364, and the intercept (1/V\_max,app) = 6.21. Therefore,

**Answer : 03**

**Part (A):**

To determine the initial reaction velocities for both reactions, we can calculate the average rate of product formation over the initial time period. Typically, the initial reaction velocity is determined by taking the slope of the tangent to the curve at the beginning of the reaction. Since the time intervals are not equal, we'll take the average rate over the first few time points.

For the reaction without the inhibitor:

Initial velocity = Change in product concentration / Change in time

For the reaction with the inhibitor, we'll use the same formula.

Let's calculate the initial reaction velocities:

**Reaction without inhibitor:**

**Reaction with inhibitor:**

**Part (B):**

To determine the type of inhibition occurring, we need to compare the reaction rates with and without the inhibitor at various substrate concentrations. From the provided data, we only have the reaction rates at a single substrate concentration.

To determine the type of inhibition, we would need to run the experiment at multiple substrate concentrations for both the inhibited and uninhibited reactions. Then, we could plot the data using Lineweaver-Burk plots or double reciprocal plots. Based on the patterns observed in the plots, we could determine whether the inhibition is competitive, non-competitive, or uncompetitive.

Since we only have data at one substrate concentration, we cannot definitively determine the type of inhibition occurring. To investigate further, additional experiments should be conducted at different substrate concentrations, and the data should be treated accordingly to analyze the inhibition type.

**Answer : 04**

**Part (A):**

To determine the type of inhibition occurring for this reaction, we need to examine the relationship between the reaction rate (v) and the substrate concentration ([S]). We can plot v against [S] and observe the pattern.

|  |  |
| --- | --- |
| **[S] (mg/L)** | **v (mg product/L-h)** |
| 30 | 2.1 |
| 60 | 3.8 |
| 90 | 5.7 |
| 120 | 7.4 |
| 150 | 8.5 |
| 200 | 7.1 |
| 250 | 4.5 |
| 300 | 2.5 |

Let's plot v vs. [S]:

Based on the plotted data, we can observe the relationship between v and [S] to determine the type of inhibition. If the plot shows a curve where the reaction rate starts to level off or decrease as the substrate concentration increases, it indicates inhibition. The type of inhibition can be determined by comparing the pattern observed with typical patterns for competitive, non-competitive, or uncompetitive inhibition.

**Part (B):**

To make a Lineweaver-Burk plot, we first need to calculate the reciprocal of v (1/v) and the reciprocal of [S] (1/[S]). Then, we can plot 1/v against 1/[S] to obtain a straight line.

Let's calculate 1/v and 1/[S] using the given data:

|  |  |  |  |
| --- | --- | --- | --- |
| **[S] (mg/L)** | **v (mg product/L-h)** | **1/v (h/L-mg)** | **1/[S] (L/mg)** |
| 30 | 2.1 | 0.476 | 0.033 |
| 60 | 3.8 | 0.263 | 0.017 |
| 90 | 5.7 | 0.175 | 0.011 |
| 120 | 7.4 | 0.135 | 0.008 |
| 150 | 8.5 | 0.118 | 0.007 |
| 200 | 7.1 | 0.141 | 0.005 |
| 250 | 4.5 | 0.222 | 0.004 |
| 300 | 2.5 | 0.400 | 0.003 |

Now, let's plot 1/v against 1/[S] and determine the slope and intercept to find Km and Vmax.

**Part (C):**

If the reaction reaches Vmax, it means that the enzyme is saturated with substrate, and further increases in substrate concentration do not increase the reaction rate. We can determine if the reaction reaches Vmax by observing the plotted data and checking if the reaction rate plateaus or levels off as the substrate concentration increases. If the reaction rate continues to increase linearly with substrate concentration, it suggests that Vmax has not been reached.

**Answer : 05**

**Part (A):**

The maximum reaction velocity (Vmax) with no inhibitor can be calculated using the Michaelis-Menten equation, which is expressed as:

Where:

- k2 is the rate constant for the conversion of the ES complex to product (F).

- [E]0 is the initial enzyme concentration.

Given:

- k2 = 0.72 s^-1

- [E]0 = 3.2 × 10^-5 M

We can plug in these values to find Vmax:

**Part (B):**

To determine the maximum reaction velocity (Vmax) with the uncompetitive inhibitor, we can use the equation for Vmax in the presence of an uncompetitive inhibitor, which is given by:

Where:

- Vmax' is the maximum reaction velocity in the presence of the inhibitor.

- Vmax is the maximum reaction velocity without the inhibitor.

- [I] is the concentration of the inhibitor.

- Ki is the inhibition constant.

Given:

- [I] = 0.0037 M

- Ki = 2.1 × 10^-3 M

- Vmax (from part A) = 2.304 × 10^-5 mol/L-s

We can plug in these values to find Vmax':

**Part (C):**

The Damkohler number (Da) is given by the ratio of the reaction rate (k2 × [E]0) to the mass transfer rate (kL × a), where a is the surface area over the reactor volume.

Given:

kL = 8.5 × 10^-4 cm/s

a = 750 cm^2

[E]0 = 3.2 × 10^-5 M (from part A)

k2 = 0.72 s^-1

We can calculate Da for both scenarios:

**(i) When there is no inhibitor:**

**(ii) When the uncompetitive inhibitor is present at [I] = 0.0037 M:**

**Part (D):**

To calculate KM using the Rapid Equilibrium approach, we first need to calculate KM for the uninhibited reaction, then adjust it for the presence of the inhibitor. Let's proceed with these calculations.

1. Use the Rapid Equilibrium approach to calculate Km:

Km = (k-1 + k2) / k1 = (0.0017 s^-1 + 0.72 s^-1) / 0.0085 M^-1 s^-1

Km ≈ 0.209 M

1. Calculate the actual reaction velocity (v) with the inhibitor and given substrate concentration (Sb):
   * Use the equation for an uncompetitive inhibitor with known Km and Ki:

v = (Vmax,app \* Sb) / (Km + Sb + Sb \* [I] / Ki)

1. Substitute the calculated values:
   * Vmax,app = 1.205 x 10^-5 mol/L-s
   * Sb = 0.073 M
   * Km = 0.209 M
   * Ki = 2.1 x 10^-3 M
   * [I] = 0.0037 M
2. Calculate v: v ≈ 1.00 x 10^-5 mol/L-s

* The inhibited reaction with immobilized enzyme remains **reaction-limited** due to Da < 1.
* The production rate of fructose can be calculated by multiplying the actual reaction velocity (v) by the reactor volume and converting units:

**Answer : 06**

To determine the effective diffusivity (Deff) for a substrate passing through each membrane, we can use the following equation:

Where:

- Ds,o is the free solution diffusivity of the substrate (given as 8.13 × 10^-6 cm²/s).

- Porosity is given as a percentage, so it needs to be converted to a fraction.

- Tortuosity is dimensionless.

Let's calculate Deff for each membrane:

**1. Cellulose membrane:**

**2. Methyl-Cellulose membrane:**

**3. Acrylic Fiber membrane:**

To avoid diffusion-limited reactions, we want a membrane with the highest effective diffusivity. Therefore, the Acrylic Fiber membrane would be recommended as it has the highest Deff among the three membranes.**Top of Form**

**Answer : 07**

To evaluate the immobilized enzyme reaction considering the presence of a non-competitive inhibitor and determine the rate of product formation, we will first analyze the reaction-limited and diffusion-limited scenarios and then apply the appropriate model.

1. Reaction-limited: In this case, the rate of product formation is limited by the enzyme's catalytic activity. The Michaelis-Menten equation can be used to describe the reaction rate:

However, we do not have the kinetic rate constants (k1, k-1, k2) to directly calculate Vmax and KM. Instead, we can use the % active enzyme (76 mol%) to estimate the effective concentration of active enzyme. Let's assume the total enzyme concentration in the beads is C\_total. Then, the active enzyme concentration is:

1. Diffusion-limited: In this case, the rate of product formation is limited by the diffusion of substrate to the enzyme active sites. The Thiele modulus (φ) can be used to determine whether the reaction is diffusion-limited:

Here, k\_cat is the catalytic rate constant, which is related to the kinetic rate constants (k1, k-1, k2). If φ > 1, the reaction is diffusion-limited.Now, let's consider the non-competitive inhibitor. The inhibition constant (Ki) is given, and the inhibitor concentration ([I]) is 0.0105 M. The reaction rate in the presence of the inhibitor can be described by the following equation:

* Interpret the Da value:
  + Da << 1: Reaction-limited
  + Da >> 1: Diffusion-limited
  + Da ≈ 1: Neither strongly limiting

Since we do not have the kinetic rate constants, we cannot directly calculate Vmax and KM. However, we can still analyze the reaction based on the given information.To determine whether the reaction is diffusion-limited, we need to calculate the Thiele modulus. Unfortunately, we do not have enough information to calculate k\_cat, so we cannot definitively determine whether the reaction is diffusion-limited or not.To estimate the rate of product formation, we can use the Michaelis-Menten equation with the inhibition term:

However, without the kinetic rate constants, we cannot calculate the exact rate of product formation.In conclusion, we cannot definitively determine whether the immobilized enzyme reaction is reaction- or diffusion-limited without the kinetic rate constants. We can estimate the rate of product formation using the Michaelis-Menten equation with the inhibition term, but the exact value would require the kinetic rate constants.

**Part (A):**

**Part (B):**

**Part (C):**

**Part (D):**

**Part (E):**

**END**