The background features a large, stylized blue and grey buffalo mascot. The buffalo is facing forward with its mouth open, showing its teeth. Below the buffalo's head, the word "BUFFALO" is written in a large, bold, white, italicized font with a grey outline. The text is centered horizontally and spans most of the width of the page.

A First Course on Kinetics and Reaction Engineering

Class 9 on Unit 9

Where We're Going

- Part I - Chemical Reactions
- Part II - Chemical Reaction Kinetics
 - ▶ A. Rate Expressions
 - 4. Reaction Rates and Temperature Effects
 - 5. Empirical and Theoretical Rate Expressions
 - 6. Reaction Mechanisms
 - 7. The Steady State Approximation
 - 8. Rate Determining Step
 - 9. Homogeneous and Enzymatic Catalysis
 - 10. Heterogeneous Catalysis
 - ▶ B. Kinetics Experiments
 - ▶ C. Analysis of Kinetics Data
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- Part IV - Non-Ideal Reactions and Reactors



Conservation of Catalyst and Charge

- Homogeneous catalysts and enzymes are not produced or consumed in the overall, macroscopically observed reaction
 - ▶ In the mechanism they typically appear in as complexes with one or more species
 - ▶ The concentration of these complexes and of the non-complexed catalyst can be difficult to measure; they should be treated like reactive intermediates and their concentrations eliminated
- The Bodenstein steady state equations and the quasi-equilibrium expressions very often cannot be solved to obtain expressions for reactive intermediates involving complexed catalysts or enzymes
 - ▶ Add an equation expressing conservation of catalyst and/or conservation of charge to generate a set of equations that can be solved for the concentrations of reactive intermediates

- Conservation of catalyst

- ▶
$$C_{cat}^0 = C_{cat,free} + \sum_{\substack{i = \text{all} \\ \text{catalyst} \\ \text{complexing} \\ \text{species}}} \nu_{cat,i} C_{cat,i}$$

- C_{cat}^0 is the equivalent concentration of the catalyst (or enzyme) before it is added to the system
 - $\nu_{cat,i}$ is the number of catalyst species in the form originally added to the system that are needed to create one complex of the catalyst with species i

- Conservation of charge

- ▶
$$\sum_{\substack{p = \text{all} \\ \text{positively} \\ \text{charged} \\ \text{species}}} C_p q_p = \sum_{\substack{n = \text{all} \\ \text{negatively} \\ \text{charged} \\ \text{species}}} C_n |q_n|$$



Michaelis-Menten Rate Expressions

- Michaelis-Menten kinetic expressions for enzymatic reactions are derived from mechanisms

- ▶ Often the product formation step is effectively irreversible
- ▶ The Bodenstein steady state approximation and the conservation of enzymes are used in generating the rate expression

- The simplest Michaelis-Menten expression for the reaction $S \rightarrow P$ is derived from a two step mechanism



- ▶
$$r = \frac{dC_P}{dt} = \frac{V_{\max} C_S}{K_m + C_S}$$

- The simple Michaelis-Menten rate expression can be linearized by taking its reciprocal

- ▶
$$\frac{1}{r} = \left(\frac{K_m}{V_{\max}} \right) \frac{1}{C_S} + \frac{1}{V_{\max}}$$

- ▶ A plot of the reciprocal of the rate versus the reciprocal of the substrate concentration should be linear
 - Called a Lineweaver-Burk plot
 - Useful when finding the best values for V_{\max} and K_m from experimental data

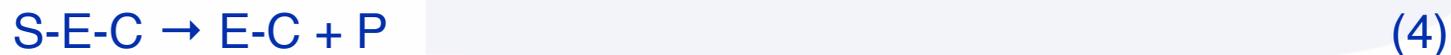
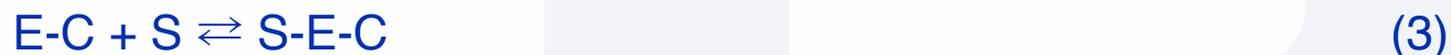
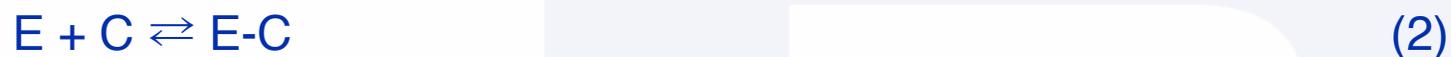
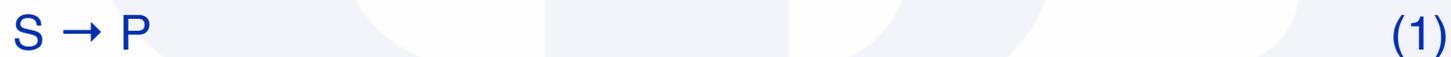


Questions?



Michaelis-Menten Kinetics with a Co-Factor

Consider the macroscopically observed reaction (1) which is catalyzed by the enzyme E. This enzyme will not complex with the substrate, S, unless it first complexes with the co-factor, C. The complete reaction mechanism is given by reactions (2) through (4), where step (4) is effectively irreversible. Derive a Michaelis-Menten type rate expression based upon this mechanism. You may assume that the concentration of the non-complexed cofactor is easy to measure, and therefore the concentration of non-complexed cofactor may appear in the rate expression.



Solution Procedure

- Check that the mechanism is valid
- Identify the stable species and the reactive intermediates
- Write an expression for the rate of generation of a reactant or product
- Write the Bodenstein steady state approximation for all but one reactive intermediate, simplifying it if any of the steps are kinetically insignificant or effectively irreversible.
- Write an expression for the conservation of enzyme
- Solve the steady state and enzyme conservation equations to get expressions for the concentrations of the reactive intermediates
- Substitute the expressions for the concentrations of the reactive intermediates into the rate expression



Solution Procedure

- Check that the mechanism is valid
 - ▶ Adding reactions (3) and (4) gives the overall reaction; the mechanism is valid
- Identify the stable species and the reactive intermediates
 - ▶ Stable species: S, C and P
 - ▶ Reactive intermediates: E, E-C and S-E-C
- Write an expression for the rate of generation of a reactant or product



Setting Up the Solution

- Write an expression for the rate of generation of a reactant or product
 - $r_{P,1} = r_4 = k_{4,f} [\text{S-E-C}]$
- Having identified E, E-C and S-E-C as reactive intermediates, apply the Bodenstein steady state approximation to two of them



Setting Up the Solution

- Write an expression for the rate of generation of a reactant or product
 - $r_{p,1} = r_4 = k_{4,f} [S-E-C]$
- Having identified E, E-C and S-E-C as reactive intermediates, apply the Bodenstein steady state approximation to two of them
 - $0 = -k_{2,f} [E][C] + k_{2,r} [E-C]$
 - $0 = k_{3,f} [E-C][S] - k_{3,r} [S-E-C] - k_{4,f} [S-E-C]$
- Write an expression for the conservation of enzyme



Setting Up the Solution

- Write an expression for the rate of generation of a reactant or product
 - $r_{p,1} = r_4 = k_{4,f} [S-E-C]$
- Identify E, E-C and S-E-C as reactive intermediates and apply the Bodenstein steady state approximation to two of them
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 - $0 = k_{3,f} [E-C][S] - k_{3,r} [S-E-C] - k_{4,f} [S-E-C]$
- Write an expression for the conservation of enzyme
 - $E^0 = [E] + [E-C] + [S-E-C]$
- Solve the steady state and enzyme conservation equations to get expressions for the concentrations of the reactive intermediates



Setting Up the Solution

- Write an expression for the rate of generation of a reactant or product
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- Write an expression for the conservation of enzyme
 - $E^0 = [E] + [E-C] + [S-E-C]$
- Solve the steady state and enzyme conservation equations to get expressions for the concentrations of the reactive intermediates

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

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



Simplifying the Rate Expression

- Substitute the expression for [S-E-C] into the rate expression



Simplifying the Rate Expression

- Substitute the expression for [S-E-C] into the rate expression

$$r_{P,1} = \frac{\left(\frac{k_{3,f}k_{4,f}}{(k_{3,r} + k_{4,f})} [S][C] \right) E^0}{\frac{k_{2,r}}{k_{2,f}} + [C] + \frac{k_{3,f}}{(k_{3,r} + k_{4,f})} [S][C]} = \frac{k_{4,f} E^0 [S][C]}{\frac{k_{2,r}(k_{3,r} + k_{4,f})}{k_{2,f}k_{3,f}} + \frac{(k_{3,r} + k_{4,f})}{k_{3,f}} [C] + [S][C]}$$

- Define

$$V_{\max} = k_{4,f} E^0 \quad K_m = \frac{k_{2,r}(k_{3,r} + k_{4,f})}{k_{2,f}k_{3,f}} \quad K_c = \frac{(k_{3,r} + k_{4,f})}{k_{3,f}}$$

- Michaelis-Menten form of the rate expression

$$r_{P,1} = \frac{V_{\max} [S][C]}{K_m + K_c [C] + [S][C]} = \frac{V_{\max} [S]}{\frac{K_m}{[C]} + K_c + [S]}$$



Lineweaver Burk Analysis

Inhibited Enzymatic Reaction

Suppose the enzyme-catalyzed reaction (1) is believed to obey Michaelis-Menten kinetics with inhibition, equation (2). This is the rate expression derived in Example 9.3. To test this, the rate of production of P was measured as a function of the concentrations of S and I using a 500 cm³ chemostat and 10.0 mg of enzyme. The temperature, pressure and solution volume were all constant over the course of the experiments. On the basis of the resulting data, presented in the Table, does equation (2) offer an acceptable description of the reaction rate? If so, what are the best values of V_{max} , K_I and K_m ?



$$r_{P,1} = \frac{V_{max} [S]}{K_m + K_I [I] + [S]} \quad (2)$$

C_S (M)	C_I (M)	r_P (M/min)
0.100	0.100	0.000798
0.086	0.050	0.000838
0.080	0.005	0.000912
0.075	0.001	0.000915
0.070	0.100	0.000745
0.063	0.050	0.000819
0.056	0.005	0.000898
0.048	0.001	0.000901
0.047	0.100	0.000689
0.041	0.050	0.000771
0.036	0.005	0.000896
0.030	0.001	0.000890
0.025	0.100	0.000563
0.021	0.050	0.000664
0.015	0.005	0.000846
0.010	0.001	0.000855



Solution Procedure

- Identify the known constants, variables and parameters in the model
- Linearize the model
- Compute the x and y values for the linearized model
- Fit the linear model to the data
- Calculate the “best” parameter values and their uncertainties



Linearize the Model and Fit to the Data

- Take the reciprocal of the rate expression

$$\frac{1}{r_{P,1}} = \left(\frac{K_m}{V_{\max}} \right) \frac{1}{C_S} + \left(\frac{K_I}{V_{\max}} \right) \frac{C_I}{C_S} + \frac{1}{V_{\max}}$$

- Define new variables to give a linear model

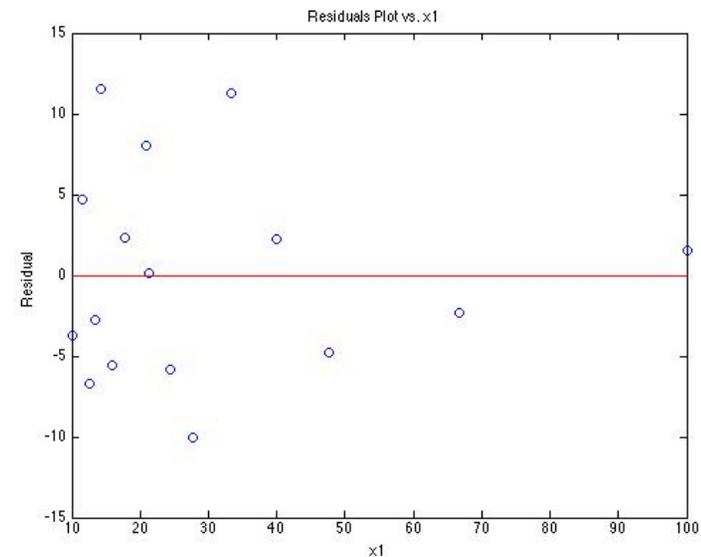
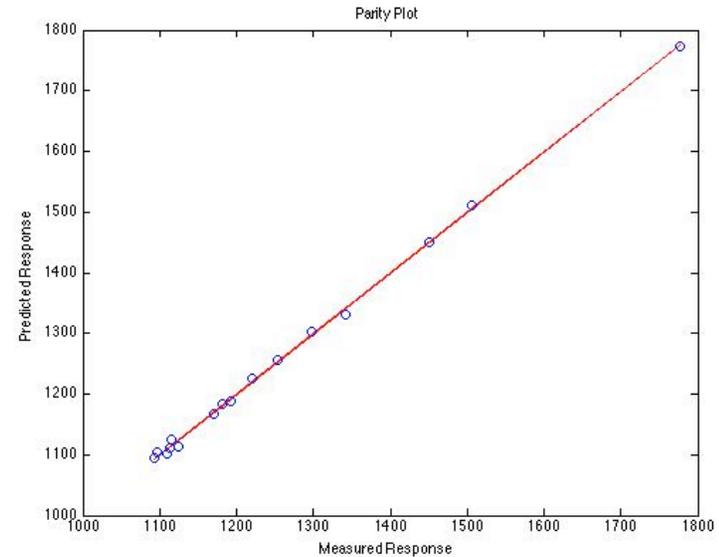
$$y = \frac{1}{r_{P,1}}; \quad x_1 = \frac{1}{C_S}; \quad x_2 = \frac{C_I}{C_S}$$

$$m_1 = \left(\frac{K_m}{V_{\max}} \right); \quad m_2 = \left(\frac{K_I}{V_{\max}} \right); \quad b = \frac{1}{V_{\max}}$$

$$y = m_1 x_1 + m_2 x_2 + b$$

- Calculate x_1 , x_2 and y for each data point and fit the linear model to the resulting data

- ▶ $r^2 = 0.999$
- ▶ $m_1 = 0.67 \pm 0.16 \text{ min}$
- ▶ $m_2 = 166 \pm 3 \text{ min M}^{-1}$
- ▶ $b = 1084 \pm 7 \text{ min M}^{-1}$



Calculate the Model Parameters

- The fit is acceptably accurate
- Calculate the model parameters and their uncertainties

$$V_{\max} = \frac{1}{b} ; K_m = m_1 V_{\max} = \frac{m_1}{b} ; K_I = m_2 V_{\max} = \frac{m_2}{b}$$

$$\lambda_{V_{\max}} = \frac{\lambda_b}{b^2}$$

$$\lambda_p = \sqrt{\sum_i \left(\frac{\partial f}{\partial m_i} \right)^2 \lambda_{m_i}^2 + \left(\frac{\partial f}{\partial b} \right)^2 \lambda_b^2} \Rightarrow$$

$$\lambda_{K_m} = \sqrt{\left(\frac{\lambda_{m_1}}{b} \right)^2 + \left(\frac{m_1 \lambda_b}{b^2} \right)^2}$$

$$\lambda_{K_I} = \sqrt{\left(\frac{\lambda_{m_2}}{b} \right)^2 + \left(\frac{m_2 \lambda_b}{b^2} \right)^2}$$

- $V_{\max} = (9.22 \pm 0.06) \times 10^{-4} \text{ M min}^{-1}$
- $K_m = (6.18 \pm 1.47) \times 10^{-4} \text{ M}$
- $K_I = 0.153 \pm 0.003$



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