Name: ___________________________________

Midterm Exam I

Chem Eng 170A: Biochemical Engineering

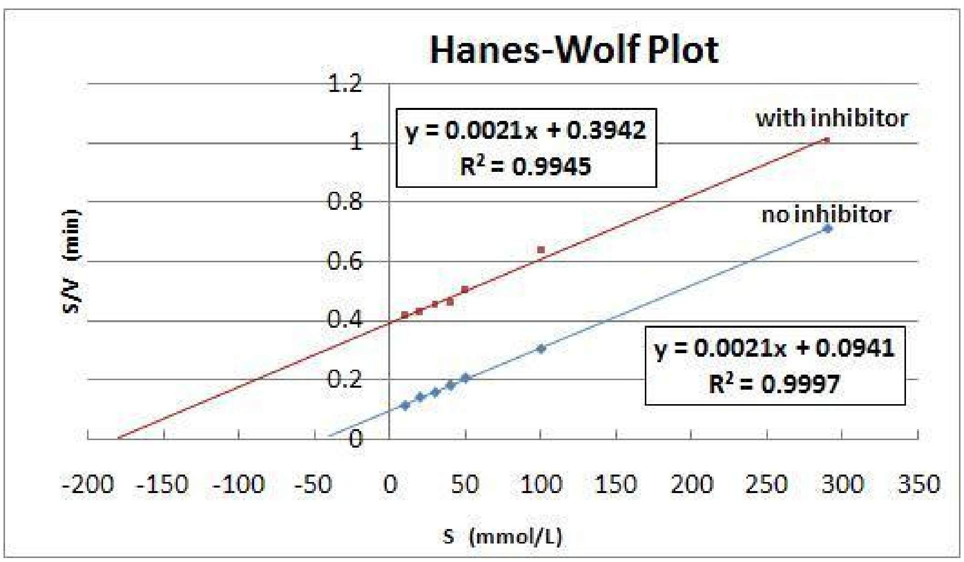
100 points

Question 1: Hanes-Wolf Plot (25 pts)

Much like the Lineweaver Burk plot and the Eadie Hofstee plot, the Hanes-Wolf plot is a graphical representation of enzyme kinetics. It is based on the rearrangement of the Michaelis Menten equation:

$$\frac{S}{V} = \frac{S}{V_{max}} + \frac{K_M}{V_{max}}$$

Shown below is the Hanes-Wolf plot of an enzyme from baker's yeast, with and without inhibitors:



(a) From the above plot, calculate the value of V_{max}

(b) From the plot, calculate the value of K_M

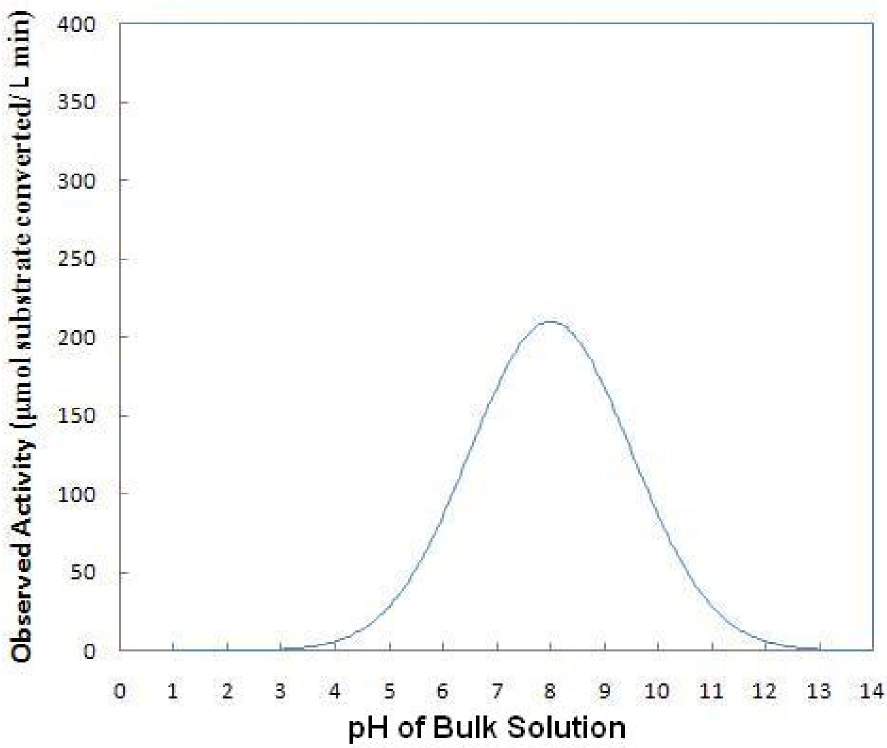
(c) What parameter is represented by the x-intercept in the absence of inhibitor? In the presence of inhibitor?

(d) What type of inhibition is shown? How do you know?

(e) Assume $[I] = 190mM$. What is the value of K_I?

Question 2: Charged Supports and Maximum Activity (10 pts)

Shown below is the activity vs pH profile for an enzyme at a concentration of 1mM:

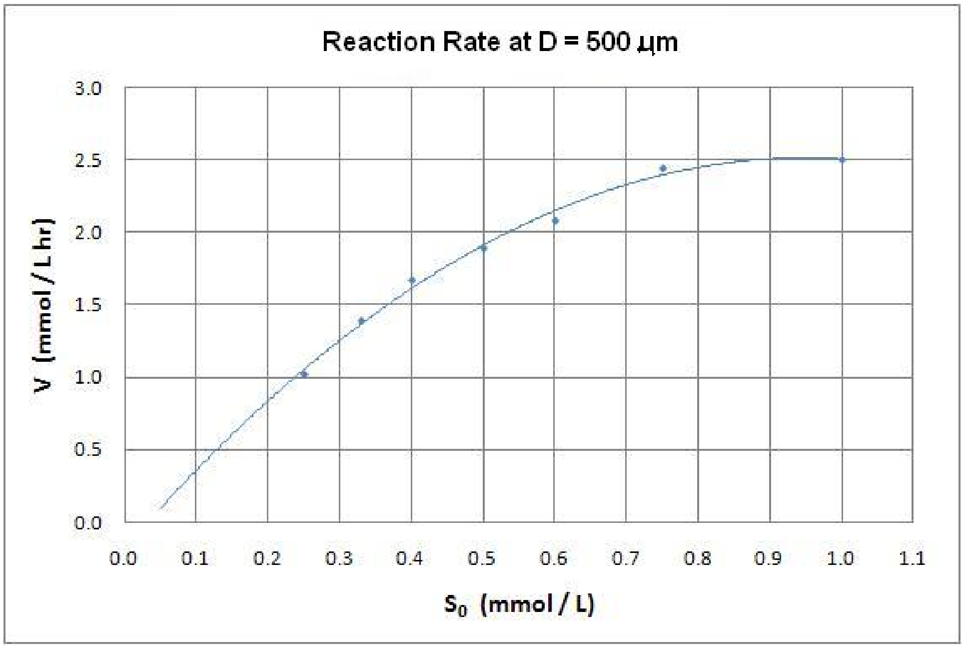


(a) Sketch on the graph above a possible activity vs pH profile for 1mM of the same enzyme immobilized onto a nonporous, positively charged support.

(b) Now assume that your bioreactor contains 1mM of immobilized enzyme and 1mM of free enzyme. Sketch on the same plot the activity vs pH profile that you would measure for this system.

Question 3: Internal Mass Transfer Effects (20 pts)

Enzyme is attached to an uncharged spherical support of diamater $500\mu m$. The kinetic data are shown below:



We suspect that our process is hindered by internal mass transfer limitations. We therefore decide to decrease the particle diameter. The reaction rate increased as the diameter decreased until the diameter reached $D = 50\mu m$, at which point further decreases in diameter did not increase the rate. What would the reaction rate be for the case of $D \leq 50\mu m$, if the bulk substrate concentration $S_0 = 0.54\ mmol/L$? Assume $\beta \cong 0$.

(Extra space for work)

Question 4: Damkohler Number (10 pts)

The following data were obtained for α-chymotrypsin attached to a porous support:

$$S_0 = 4.0 \times 10^{-9} mol/cm^3$$
$$D_e = 9.3 \times 10^{-7} cm^2/s$$
$$k_s = 2.1 \times 10^{-4} cm/s$$
$$v = 3.2 \times 10^{-12} mol/cm^2 \cdot s$$
$$Size = 90mm\ (particle\ diamater)$$

(a) Calculate $\overline{Da}$

(b) Is the calculated value of $\overline{Da}$ reasonable? Why or why not?

Question 5: Stoichiometry (20 pts)

The following describes the metabolism of an organism:

$$C_6H_{12}O_6 + aO_2 + bNH_3 \longrightarrow cC_\alpha H_{1.65}O_{0.273}N_{0.195} + dH_2O + 2.3CO_2$$

$$\gamma_b = 4.519 \ \frac{equiv\ electrons}{mol\ C}$$

(a) Calculate $Y_{X/S}$.

(b) Calculate the fraction of energy from the substrate that is converted to biomass.

Question 6: Transition State Theory

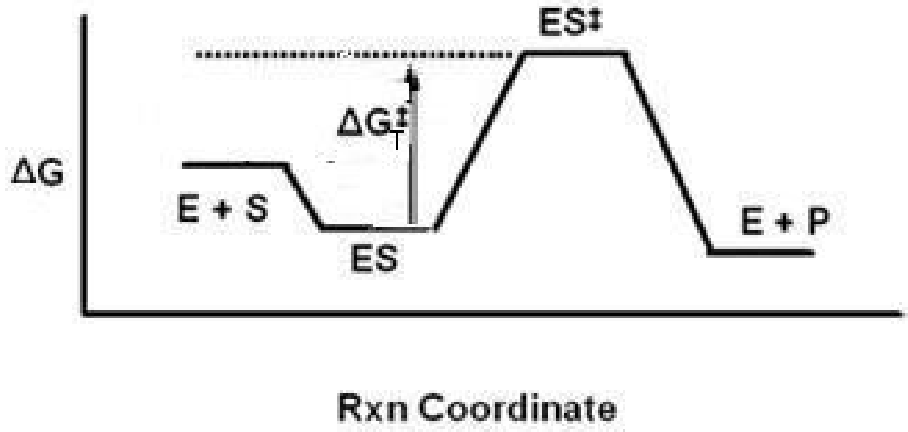
Consider a single-substrate enzymatic reaction. Assume rapid equilibrium between E, S, and ES. The measured value of the Michaelis constant, $K_M = 50\mu mol/L$.

A pharmaceutical company has synthesized a series of potent transition-state analogs for the above reaction, all of which function as competitive inhibitors. The inhibitiion constant, K_I, for the best transition-state analog is $25nmol/L$.

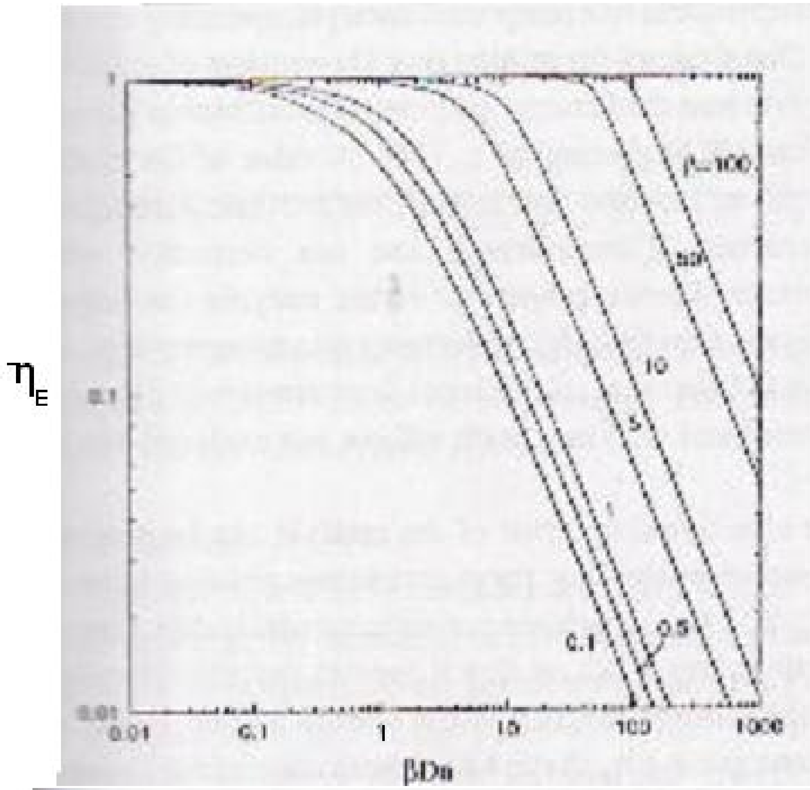
(a) Estimate the maximum possible rate enhancement (ratio of enzyme-catalyzed rate over the non-catalyzed rate) for the enzyme-catalyzed conversion of S to P.

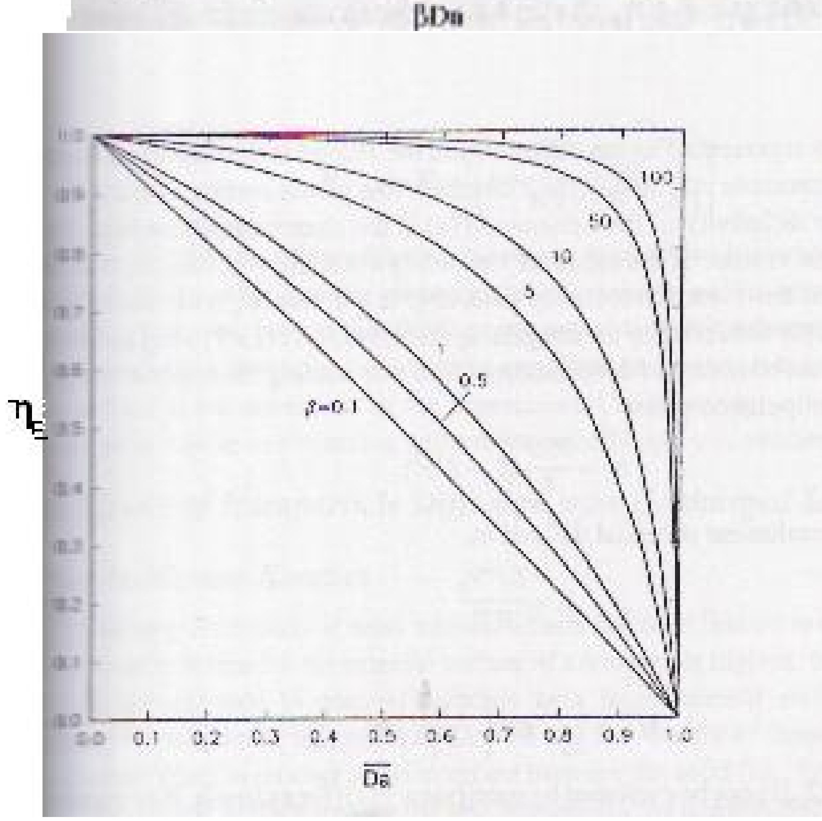
(b) The free energy of activation for this reaction, $\Delta G_T^{\ddagger}$, is shown on the diagram below. Use this value (and other parameters available to you, as needed) to calculate k_{cat} for the reaction (assume T = 25°C).

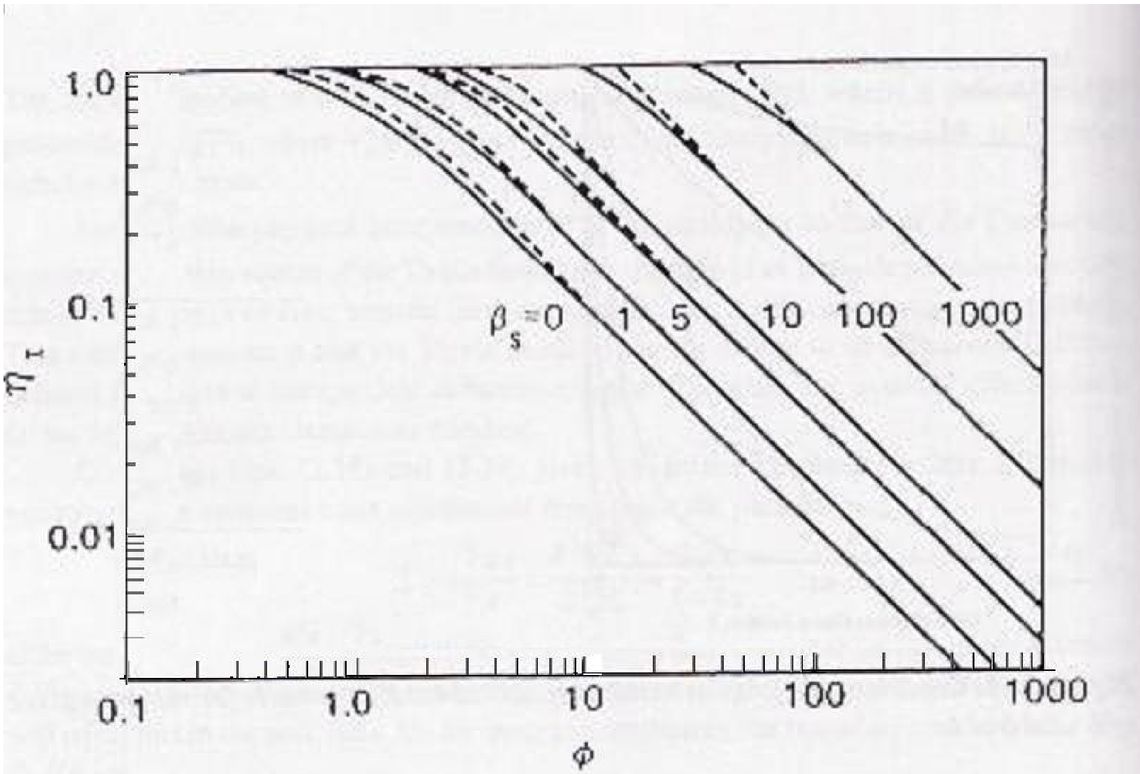
$$\Delta G_T^{\ddagger} = 30kJ/mol$$

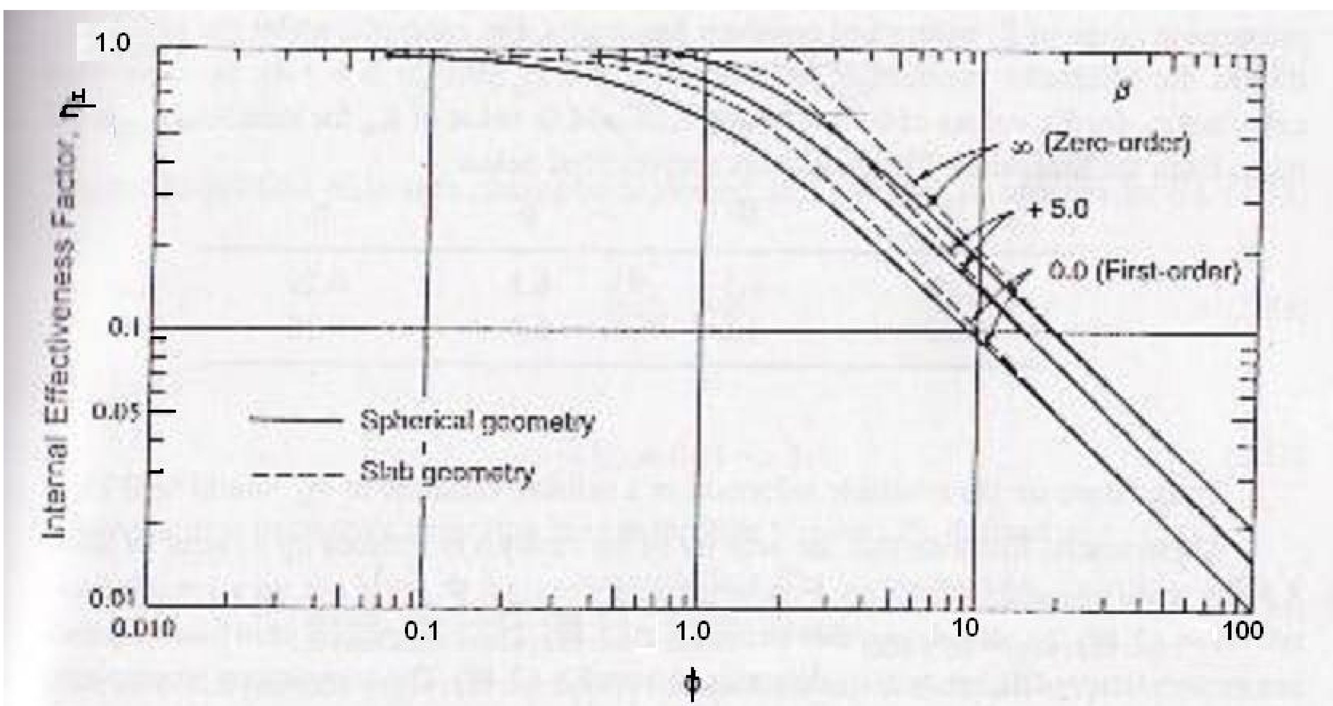


Effectiveness Factors






1.0
0.1
0.01
η_I
β_s = 0
1
5
10
100
1000
0.1
1.0
10
100
1000
ϕ


Internal Effectiveness Factor, η_I
1.0
0.1
0.05
0.01
β
∞ (Zero-order)
+ 5.0
0.0 (First-order)
Spherical geometry
Slab geometry
0.010
0.1
1.0
10
100
ϕ

Useful Equations

Constants

$k_B = 1.381 \times 10^{-23} J/K$

$h = 6.626 \times 10^{-34} J \cdot S$

$k_T \cong 1$

$R = 8.314 J/K \cdot mol$

Equations

$$k = k_T \left(\frac{k_B T}{h} \right) K^{\ddagger}$$

$$K^{\ddagger} = e^{\frac{-\Delta G^{\ddagger}}{RT}}$$

$$pH = pK_a + log \left(\frac{[A^-]}{[HA]} \right)$$

$$v = \frac{v_{max} S}{S + K_M}$$

Dimensionless numbers

$$x = S/S_0$$

$$\beta = S_0/K_M$$

$$Da = \frac{v_{max}^{'}}{k_S S_0}$$

$$\overline{Da} = \frac{v_{obs}^{'}}{k_S S_0}$$

$$\phi = \frac{R}{3} \left(\frac{v_{max}}{D_e K_M} \right)^{1/2}$$

$$\Phi = \left(\frac{R}{3} \right)^2 \left(\frac{v_{obs}}{D_e S_0} \right)$$

$$\eta_E = \frac{(1+\nu)(1-x^*)}{Da}$$

$$\eta_I = \frac{\frac{dx}{d\bar{r}}\Big|_{\bar{r}=1}}{3\phi^2(1/(1+\beta))}$$

$$Bi = \frac{k_S R}{D_e}$$