**Chapter 3**

Problem 3.1



a.) Equilibrium approach

\* r values are the same as k values all are rate constants

 (1)

 (2)

 (3)

from (2)  (4)

from (1) + (4)  (5)

Substitute (4) and (5) into (3):



b.) Quasi–steady–state approach

 (6)

 (7)

from (7)  (8)

from (6) + (8):  (9)

substitute (8) and (9) into (3):







where  and 

Problem 3.2

\* r values are the same as k values all are rate constants



 (1)

 (2)

 (3)

from (1):  (4)

Combine (3) + (4): 

 (5)

Substitute (3) and (5) into (2):



Substituting (ES) in terms of E0:



LetVS = r2 E0 and VP = r-1 E0



Divide top and bottom by (r-1 + r2) and let

 and 



Problem 3.3

a) 

b) 

but 



Problem 3.4.

At low substrate concentrations So< 150 mg/l substrate inhibition is negligible.

V0 = Vm0 So / (Km + So) or 1/V0 = 1/ Vm + Km/Vm (1/S0)

For S0 < 150 mg/l Plot 1/V0 versus 1/S0

Slope = Km/Vm0 = 13.8 y-intercept = 1/ Vm = 0.023

Then, Vm = 43.5 mg/l-h and Km = 600 mg/l

At high substrate concentrations above 150 mg/l , substrate inhibition is significant.

V0 = Vm0/ (1+ S0/Ksı) or 1/V0 = 1/Vm0 + S0/Vm Ksı

For S0 > 150 mg/l plot 1/V0 versus S0

Slope = 1/ Vm0Ksı = 2.59x 10-3 Then, Ksı = 8.9 mg/l

Low Ksı indicates severe substrate inhibition.

Problem 3.5.

Plot 1/V versus 1/S at different inhibitor concentrations

Since the lines intercept at the same point on y-axis inhibition is competitive (Constant Vm, increased Km).

For I = 0 , No inhibitor : From the intercept on y axis , 1/Vm = 0.2 and Vm = 5 mM/h

And from the intercept on X-axis, - 1/Km = -1.2 and Km = 0.83 mM

From 1/V versus 1/S plot for I = 1.3 mM and S0 = 0.50 mM V = 1.3 mM/h

Then, V = Vm S/ (Km(1+I/Ksı)) + S , 1.3 = 5(0.5)/ (0.83(1+1.3/Kı) + 0.5 )

Then Kı = 1.82 mM

Problem 3.6 .

a. V = Vm (1 + A/KA) S /(Ks + S) = Vm (1+A/KA) / (1+Ks/S)

Define Vm(1+ A/KA) = Vapp and plot Vapp versus activator (A or M0) concentration

Slope = 9.2x 10-3 = Vm/KA and y –intercept = Vm = 0.04 ml/h

Then, KA = 4.35 ug/l

b. V= Vm (1+ KA) = 0.04(1+60/4.35) = 0.59 ml/h

Problem 3.7.

a. V = KL (S0 - Ss) = Vm Ss / (Km+ Ss)

Ss = S0 – V/KL Calculate Ss at different RPMs

RPM 25 50 100 200 300 400

Ss(mg/l) 33.3 50 78.8 101.2 150 180

b. V = Vm Ss/ (Km+ Ss) or 1/V = 1/Vm + Km/Vm (1/Ss)

Plot 1/V versus 1/Ss at different RPMs

1/Ss 0.03 0.02 0.0127 0.0099 0.0067 0.005

1/V 0.033 0.0277 0.025 0.024 0.0227 0.022

y- intercept: 1/Vm = 0.02 Vm = 50 mg/l

Slope : Km/Vm = 0.42 Then, Km = 21 mg/l

Problem 3.8



Plot 1/V versus 1/S (Lineweaver – Burk plot)

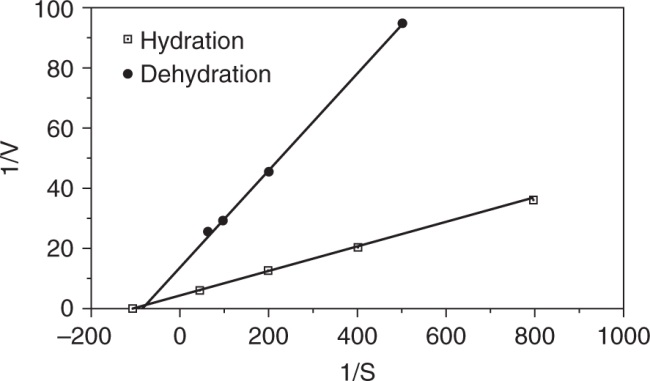
y – intercept = , x – intercept = 

Hydration: 



Dehydration: 



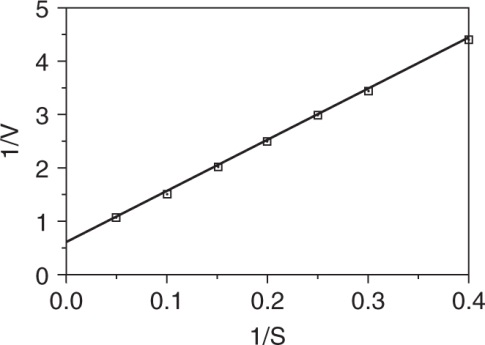


Problem 3.9

a.) Plot 1/V versus 1/S: 



SinceKm,app> Km the inhibitor is competitive.



b.)



Problem 3.10

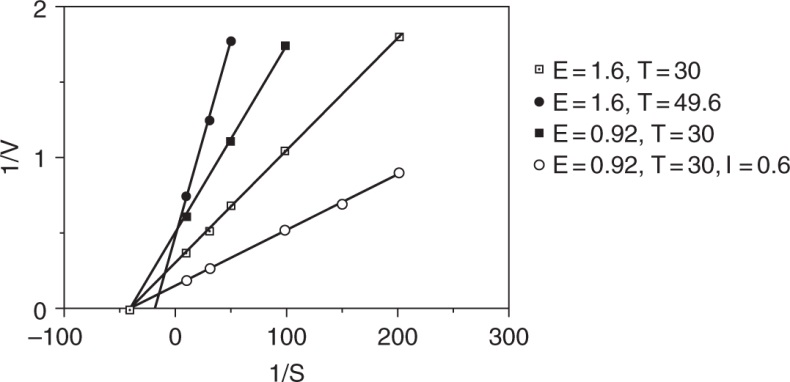
Plot 1/V versus 1/S

a.) For E0 = 1.6 g/L

Km = 0.0246 mmol/ml at 30°C

Km = 0.0238 mmol/mlat 49.6°C

b.) 



c.) The inhibitor is competitive.



Problem 3.11





Enzyme inactivation kinetics: E = E0e-rt, r = 0.1 min-1

Michaelis-Menten Kinetics: 

But 









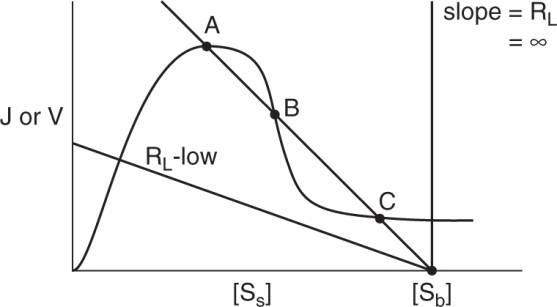
Fraction of pure enzyme 

10% of the crude protein was ATPase.

Problem 3.12

At steady – state: reaction rate = mass transfer rate





Solving graphically,

a.) For [Sb] when the enzyme is inhibited and kL has an intermediate value, multiple steady states are possible.

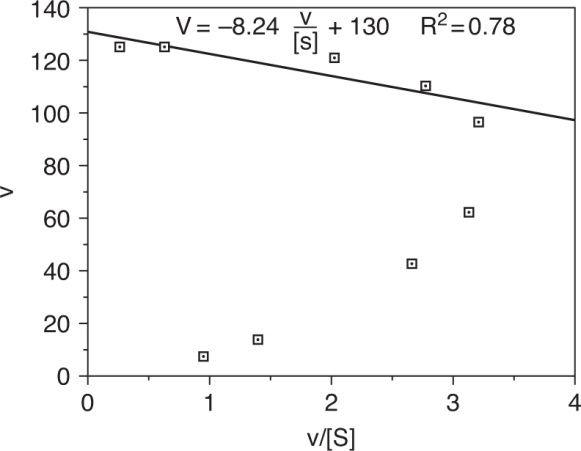
Multiple steady – states occur as the enzyme changes from being reaction controlled to being diffusionally controlled.

b.) Yes. Diffusional limitations can decrease the substrate concentration such that it is no longer inhibitory. Thus the apparent reaction rate will be greater than the intrinsic reaction rate for [SS] less than [Sb].

Problem 3.13

By inspection,Vmax = 125 μmol/min, Km = 20 μmol/L

Plot V versus V/[S](Eadie- Hofstee) where y-int = Vm, x-int. = Vm/Km and slope = - Km.



Plotting the date and using the data points for high substrate concentration,

Vm = 130 μmol/min, Km = 8.24 μmol/L

From the plot it is obvious that the data do not fit into Michaelis – Menten kinetics at low substrate concentrations.

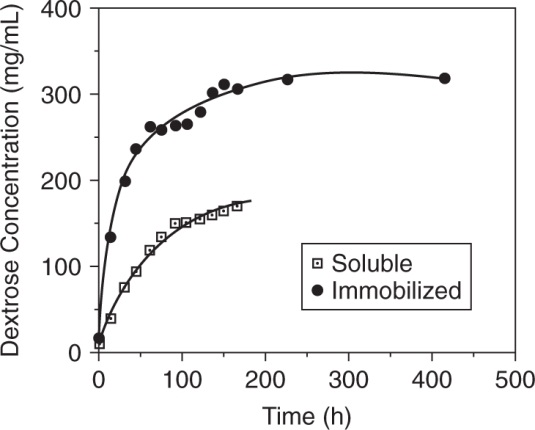
Some of the error may be attributed to the method since both axes contain v. However, there are two possible explanations for the deviations from Michaelis – Menten Kinetics:

(i) the enzyme is immobilized, thus diffusional effects become important

(ii) there might be unspecific binding of substrate to the enzyme thus requiring a critical substrate concentration for the reaction to follow Michaelis – Menten Kinetics.

Problem 3.14

a.) Plot [P] versus t data for both cases.



Obtain rates by taking tangents at specific time points.



Obtain the amount of substrate present for both cases by a mass balance.



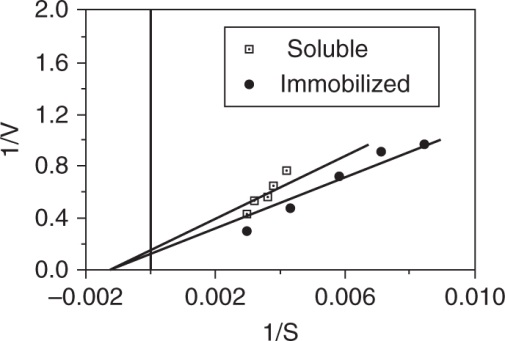
amount of dextrose formed (0.1) = amount of H2O used

(180) (0.1) = 18

starch remaining = S0 – (dextrose formed – H2O used)

= S0 – 0.9 ∆ P

Using initial time data, a plot of 1/V versus 1/S can be made.



Soluble:

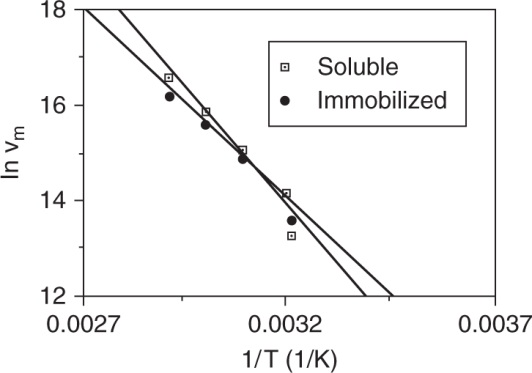


Immobilized:



b.) Plot In Vm versus 1/T for both cases.





Soluble:lnVm = 41.18 – 8299 (1/T)



Immobilized: lnVm = 35.65 – 6582 (1/T)



c.) Since Km (soluble) = Km (apparent) there is no diffusional limitation.

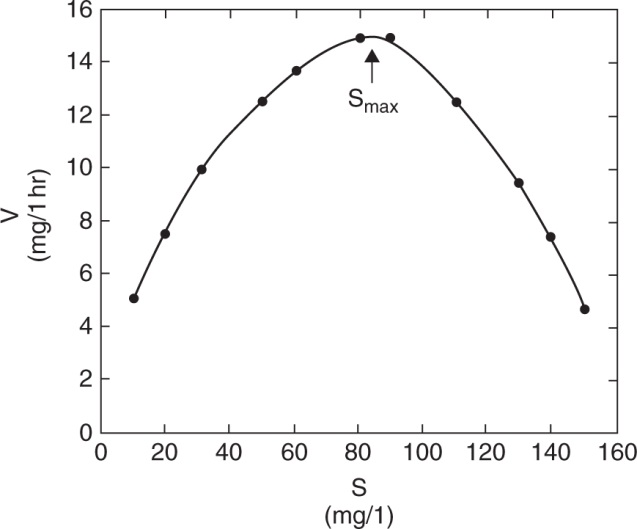
Problem 3.15

In vitro batch reactors represent a closed system of constant volume, thus Michaelis-Menten Kinetics and the quasi-steady-state approximation will not describe the system when E0 ≈ S0. However, intracellular enzyme reactions are open systems where there is a continuous supply and depletion of substrate and product provided by the interaction of cellular compartments and the intracellular and extracellular environments. E0 and S0 may be the same, but the concentrations may be orders of magnitude different either in different organelles or inside versus outside the cell.

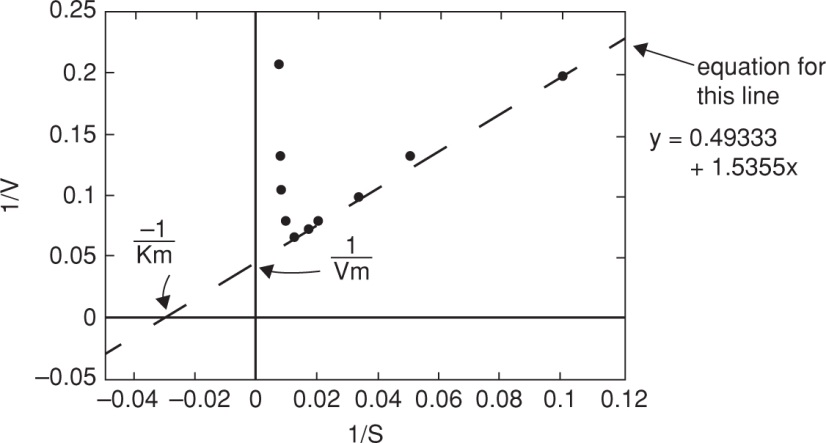
Problem 3.16

Harry’s reasoning is wrong. The soluble enzyme is reaction controlled while the immobilized enzyme may experience diffusional limitations. Thus the substrate is consumed more slowly giving rise to a larger apparent half-life. The large particle size may result in an unused or “reserved” catalytic capacity.

Problem 3.17



(a) Plot of V us. S indicates that this is substrate inhibition



(b) Vm andK'm are determined from low substrate concentration where inhibition is not ineffect:







Determine KSI from maximum reaction rate; which is determined by setting 



From plot,

(c) Rate of reaction at S = 70 mg/l?

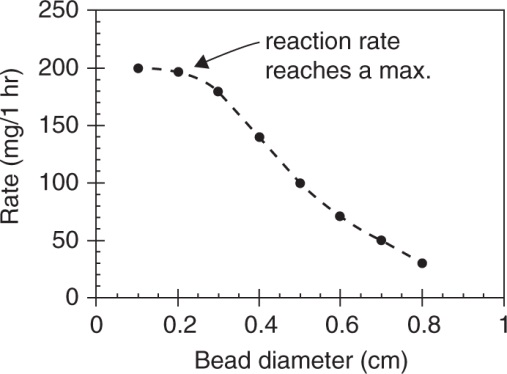
Since inhibition effect is observed at this substrate, we must use (eg) 3.34



Problem 3.18



We are given the reaction rate with diffusion limitation. To determine the reaction rate with no diffusion limitation, lets look at what happens to the rate as the particle gets smaller, and diffusional limitations are minimized. Aplot of Rate vs. particle size indicates that the rate reaches a maximum at small particle size (0.1cm). At this size there is no diffusion limitation, and the rate is N 200 mg/l hr



(a) Effectiveness factor at

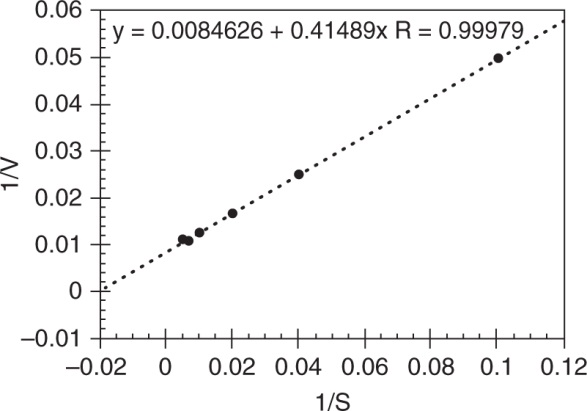


b. Assume negligible film resistance, so Sbulk = Ssurface Determine rate u/o diffusional limitation from effectiveness factor:



Plot 1/V vs 1/S to obtain the following:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S0 | 10 | 25 | 50 | 100 | 200 | 250 |
| V | 10 | 20 | 30 | 40 | 45 | 46 |
| Vno diff | 20 | 40 | 60 | 80 | 90 | 92 |





–1/Km is at y = 0: 0 =0.0084626 + 0.41489 (–1/Km)



Problem 3.19 a

 (1)

 (2)

 (3)

From Equ 1.  (1)

From Equ 2.  (2)

Multiply (1) × (2) = 



b.

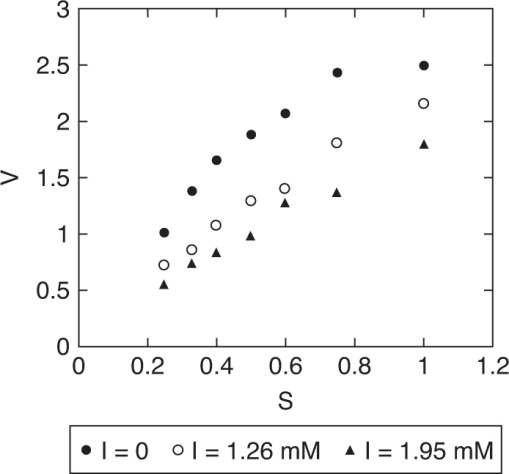


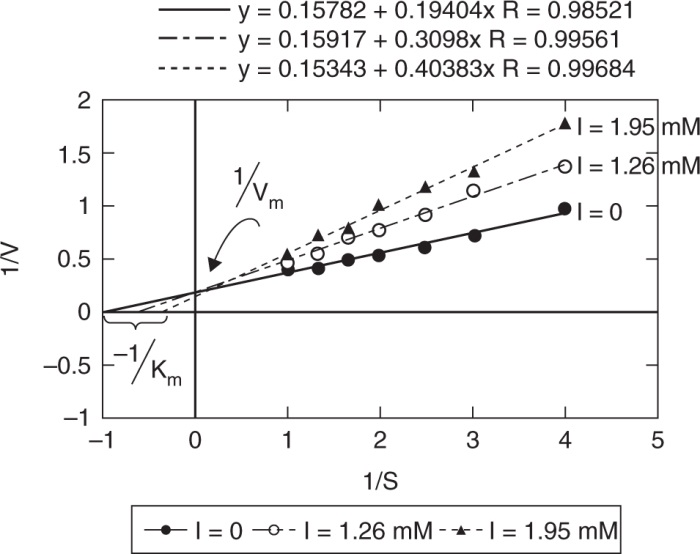
Since ϕ η ≈ 3, then, Vm ≈ 80.7 mmoles/L·h

η = 14/80.7 0.173; ϕ = 3/η = 17.3

Problem 3.20

(a) Plot 1/V vs 1/S for all cases to determine the type of inhibition





Vmax is the same, regardless of inhibition, but as I increases, –1/Km decreases (apparent), that is an increased value of Km,app, resulting in a reduction in reaction rate. This is characteristic of Competitive Inhibition

(b) Determine Vm, Km, KI

Vm, Km can be determined from the I = 0 case.@ I = 0 (1/V) = 0.15782 + 0.19404 (1/S)





KI must be determined from the I > 0 case













We would expect that KI is the same at different concentrations of inhibitor. The differences in the two values shown here are due to experimental error.

Problem 3.21



Then



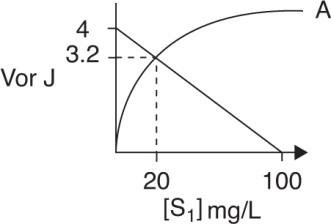
b.



Problem 3.22

a) Use the graphical technique:

Given



where A is a Function of surface concentration and is determined through rxn Kinetics.

SB = 100 mg/l , KL = 4 x 10 -5 cm/sec

use 

where



b)



Problem 3.23 a

Make total reaction rate curve →Vtot = 2.2.10 – 5 mg/cm2 S

b) 

c)  ratio of production rates

d) Sbulk = 370 mg/L

