

Analysis of Enzyme Kinetics: Stability of Steady State Solutions

PROJECT PRESENTATION

Dominic Essuman

Dr. George Yates (Advisor)

Youngstown State University
(YSU)

dessuman@student.ysu.edu

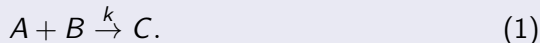
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- 3 Enzyme Kinetics: Reversible Reaction
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Definition: Chemical Reactions: law of Mass Action

Definition

Suppose two chemicals, say A and B react with each other to form product C .



The rate of reaction is the rate of accumulation of product C . This rate is proportional to the concentrations of the reactants A and B . The concentrations of the chemicals are denoted by $a = [A]$, $b = [B]$ and $c = [C]$.

$$\frac{dc}{dt} = kab. \quad (2)$$

This is called the law of mass reaction.

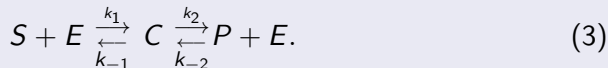
Definition: Enzyme Kinetics

Definition

- Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes.
- Enzymes are catalysts (generally protein) that help convert molecules called substrates into product.

Michaelis and Menten (1913) were the first to propose a derivation of this reaction scheme. In this scheme the enzyme converts the substrate S into the product P through a two-step process.

- Enzyme E first converts the substrate S into complex C
- Complex then breaks down into a product P , releasing the enzyme E in the process



Chemical Reaction Scheme (Irreversible Reaction)

Considering the irreversible reaction scheme;



and applying the law of mass action to this reaction scheme gives four differential equations for the rate of change of s , c , e and p .

$$\frac{ds}{dt} = k_{-1}c - k_1se \quad (5)$$

$$\frac{de}{dt} = k_{-1}c - k_1se + k_2c \quad (6)$$

$$\frac{dc}{dt} = k_1se - k_{-1}c - k_2c \quad (7)$$

$$\frac{dp}{dt} = k_2c. \quad (8)$$

Irreversible Reaction Cont'd

The following conservatives relation,

$$e_0 = e + c \quad (9)$$

$$s_0 = s + c + p \quad (10)$$

allow us to reduce the system of four differential equations to two differential equations.

$$\frac{ds}{dt} = k_{-1}c - k_1s(e_0 - c) \quad (11)$$

$$\frac{dc}{dt} = k_1s(e_0 - c) - k_{-1}c - k_2c \quad (12)$$

Now what do we do with this equations?

Equilibrium Approximation

Assumption

Michaelis and Menten assumed that the substrate is in instantaneous equilibrium with the complex. Thus the assumption was made that,

$$\frac{ds}{dt} = 0, \quad (13)$$

giving

$$k_{-1}c = k_1s(e_0 - c). \quad (14)$$

Solving for c , we have $c = \frac{e_0s}{K_1+s}$, where $K_1 = \frac{k_{-1}}{k_1}$

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Deriving the rate of product formed (velocity V) we recall $V = \frac{dp}{dt} = k_2c$. Thus,

$$V = \frac{dp}{dt} = k_2c = \frac{k_2se_0}{K_1 + s} = \frac{V_{max}s}{K_1 + s}, \quad (15)$$

Cont'd Equilibrium Approximation

The $k_2e_o = V_{max}$, which is the maximum velocity attained when all the enzyme is complexed with the substrate. For large substrate concentration the reaction rate saturates to V_{max}

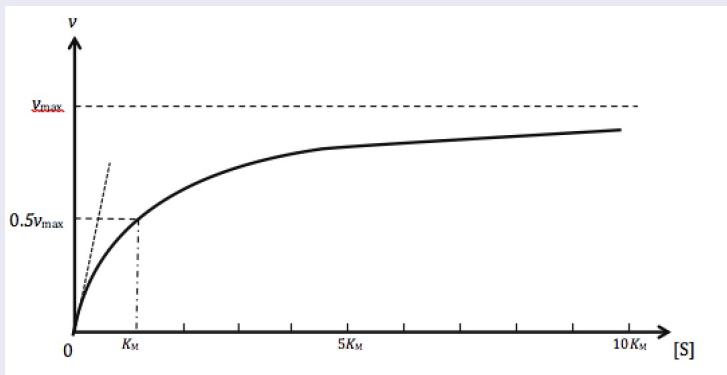


Figure: Equilibrium approximation

Quasi-Steady-State Approximation

Assumption

An alternative analysis of the system was proposed by Briggs and Haldane (1925). They assumed that the concentration of reaction does not change on the time scale of product formation. Thus, at quasi-steady-state the assumption is that,

$$\frac{dc}{dt} = 0.$$

From the differential equation,

$$\frac{dc}{dt} = k_1s(e_0 - c) - k_{-1}c - k_2c \quad (16)$$

we have

Cont'd of Quasi-Steady-State - Approximation

$$k_1 s(e_0 - c) - k_{-1}c - k_2c = 0 \quad (17)$$

Thus from equation (17) $c = \frac{k_1 s e_0}{k_1 s + k_{-1} + k_2}$, Recall $V = \frac{dp}{dt} = k_2 c$, thus

$$V = \frac{dp}{dt} = \frac{k_2 e_0 s}{s + K_m} = \frac{V_{max} s}{s + K_m} \quad (18)$$

where $K_m = \frac{k_{-1} + k_2}{k_1}$ is called the Michaelis-Menten constant and $V_{max} = k_2 e_0$

Cont'd of Quasi-Steady-State - Approximation

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The reaction rate for the equilibrium approximation $V = \frac{V_{max}}{s + K_1}$ is quite similar to the quasi-steady-state approximation. $V = \frac{V_{max}}{s + K_m}$. The only difference being the constants K_1 and K_m . this is due to different assumptions

Definition: Steady State

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Steady state of a reaction is where all the concentration of the reaction are at equilibrium, that is a state where there is no change in concentration with time

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Considering the system of differential equation,

$$\frac{ds}{dt} = k_{-1}c - k_1se_0 + k_1sc = f(s, c) \quad (19)$$

$$\frac{dc}{dt} = k_1se_0 - k_1sc - k_{-1}c - k_2c = g(s, c) \quad (20)$$

At steady state $f(s, c) = 0$ and $g(s, c) = 0$, hence

$$k_{-1}c - k_1se_0 + k_1sc = 0 \quad (21)$$

$$k_1se_0 - k_1sc - k_{-1}c - k_2c = 0 \quad (22)$$

Cont'd of Steady State and Stability Analysis

$$k_{-1}c - k_1se_0 + k_1sc = 0 \quad (23)$$

$$k_1se_0 - k_1sc - k_{-1}c - k_2c = 0 \quad (24)$$

Solving the system of differential equation at steady state we obtain,

$$c = 0 \quad \text{and} \quad se_0 = 0. \quad (25)$$

The latter condition, $se_0 = 0$ is satisfied when either $e_0 = 0$, $s = s_0$ or $s = 0$ and $e_0 \neq 0$. This also leads to the two steady state solutions

$$(s, c) = (0, 0) \quad \text{and} \quad (s, c) = (s_0, 0) \quad (26)$$

We now consider the stability of these two steady state.

Stability Analysis: Case 1: $e_0 \neq 0$

To do a linear stability analysis we need to find the Jacobian by Taylor series of the differential equation.

$$\frac{ds}{dt} = k_{-1}c - k_1se_0 + k_1sc = f(s, c) \quad (27)$$

$$\frac{dc}{dt} = k_1se_0 - k_1sc - k_{-1}c - k_2c = g(s, c) \quad (28)$$

let $s = s^* + u(t)$ and $c = c^* + v(t)$ Thus we obtain the following linear system of differential equation,

$$\begin{bmatrix} \frac{du}{dt} \\ \frac{dv}{dt} \end{bmatrix} \approx \begin{bmatrix} \frac{\partial f}{\partial s}(s^*, c^*) & \frac{\partial f}{\partial c}(s^*, c^*) \\ \frac{\partial g}{\partial s}(s^*, c^*) & \frac{\partial g}{\partial c}(s^*, c^*) \end{bmatrix} \begin{bmatrix} u(t) \\ v(t) \end{bmatrix} \quad (29)$$

Thus the Jacobian of the system is ,

$$J = \begin{bmatrix} -k_1e_0 + k_1c & k_{-1} + k_1s \\ k_1e_0 - k_1c & -k_1s - k_{-1} - k_2 \end{bmatrix}. \quad (30)$$

Cont'd Stability Analysis: Case 1: $e_0 \neq 0$ at steady state $c = 0, s = 0$

Evaluating the Jacobian at steady state the characteristics polynomial is,

$$\lambda^2 + (k_{-1} + k_2 + k_1 e_0) \lambda + k_1 e_0 k_2 = 0 \quad (31)$$

This polynomial is of the form,

$$\lambda^2 + b\lambda + d = 0 \quad (32)$$

Since the coefficients of the polynomial are both positive the roots of this polynomial are,

$$\lambda = \frac{1}{2}(-b \pm \sqrt{b^2 - 4d}). \quad (33)$$

Since d is positive, the quantity under the square root is either smaller than b^2 . Thus the square root must be smaller in absolute value than b so the two eigenvalues must be real and negative. Thus we conclude the steady state is stable.

Stability Analysis: Case 2: $e_0 = 0$

Considering the steady state at $(s, c) = (s_0, 0)$. Evaluating the Jacobian

$$J = \begin{bmatrix} -k_1 e_0 + k_1 c & k_{-1} + k_1 s \\ k_1 e_0 - k_1 c & -k_1 s - k_{-1} - k_2 \end{bmatrix}. \quad (34)$$

At steady state the characteristic polynomial is the form

$$(-\lambda)(-k_1 s_0 - k_{-1} - k_2 - \lambda) = 0 \quad (35)$$

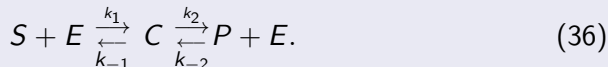
Thus,

$$\lambda_1 = 0, \quad \lambda_2 = -(k_1 s_0 + k_{-1} + k_2).$$

Since one of the eigenvalues is 0 we cannot conclude from linear stability whether the steady state is stable or not.

Reversible Reaction

Here we investigate the reversible reaction which includes formation and dissociation of the product. The reaction scheme is represented as;



The new feature here is the reverse reaction indicated by k_{-2} . Applying the law of mass action to this reaction scheme gives these differential equations.

$$\frac{ds}{dt} = k_{-1}c - k_1se \quad (37)$$

$$\frac{de}{dt} = k_{-1}c + k_2c - k_1se - k_{-2}pe \quad (38)$$

$$\frac{dc}{dt} = k_1se - k_{-1}c - k_2c + k_{-2}pe \quad (39)$$

$$\frac{dp}{dt} = k_2c - k_{-2}pe \quad (40)$$

Cont'd Reversible Reaction

The conservative relation

$$e_0 = e + c \quad (41)$$

$$s_0 = s + c + p \quad (42)$$

reduces the system of four ordinary differential equations in (37) to (40) to two ordinary differential equations

$$\frac{ds}{dt} = k_{-1}c - k_1s(e_0 - c) \quad (43)$$

$$\frac{dc}{dt} = k_1s(e_0 - c) - k_{-1}c - k_2c + k_{-2}(s_0 - s - c)(e_0 - c). \quad (44)$$

Find the rate of product formed (velocity of the reaction) using these differential equations.

Equilibrium Approximation

Assumption

Proceeding as in the irreversible case, we make the assumption that the substrate is in instantaneous equilibrium with the complex, that is $\frac{ds}{dt} = 0$, thus solving for c

$$c = \frac{e_0 s}{K_1 + s}, \quad (45)$$

which is the same as found for the irreversible case and $K_1 = \frac{k_{-1}}{k_1}$. Recall that $\frac{dp}{dt} = k_2 c - k_{-2} p e$ is the rate at which the product is formed (the velocity of the reaction). Substituting c

$$V = \frac{dp}{dt} = e_0 \frac{k_2 s - K_1 k_{-2} p}{K_1 + s} \quad (46)$$

$$V = \frac{dp}{dt} = \frac{V_{max} s - e_0 K_1 k_{-2} p}{K_1 + s} \quad (47)$$

Quasi-Steady-State Approximation

Assumption

Proceeding as in the irreversible case, we make the quasi-steady-state assumption that $\frac{dc}{dt} = 0$ which gives

$$k_1s(e_0 - c) - (k_{-1} + k_2)c + k_{-2}(s_0 - s - c)(e_0 - c) = 0. \quad (48)$$

$$c = e_0 \frac{k_1s + k_{-2}p}{k_1s + k_{-1} + k_2 + k_{-2}p} \quad (49)$$

Recall the reaction velocity $V = \frac{dp}{dt} = k_2c - k_{-2}pe$ Substituting c we have

$$V = \frac{dp}{dt} = e_0 \frac{sk_2 - K_1k_{-2}p}{s + K_m + \frac{k_{-2}p}{k_1}} \quad (50)$$

$$V = \frac{V_{max}s - K_1k_{-2}p}{s + K_m + \frac{k_{-2}p}{k_1}} \quad (51)$$

Steady State

Considering the system of differential equation,

$$\frac{ds}{dt} = k_{-1}c - k_1s(e_0 - c) = f(s, c) \quad (52)$$

$$\frac{dc}{dt} = k_1s(e_0 - c) - k_{-1}c - k_2c + k_{-2}(e_0 - c)(s_0 - s - c) = g(s, c) \quad (53)$$

At steady state $f(s, c) = 0$ and $g(s, c) = 0$, hence

$$k_{-1}c - k_1se_0 + k_1sc = 0 \quad (54)$$

$$k_1s(e_0 - c) - k_{-1}c - k_2c + k_{-2}(e_0 - c)(s_0 - s - c) = 0 \quad (55)$$

From equation (54),

$$s = \frac{k_{-1}c}{k_1(e_0 - c)} \quad (56)$$

Substituting equation (56) into equation (55), we have,

Cont'd of Steady State

Thus the steady state of the reaction is $(s, c) = (s^*, c^*)$ and $(s, c) = (s^{**}, c^{**})$ where

$$c^{*(*)} = \frac{1}{2} \left[\frac{k_{-1}}{k_1} + \frac{k_2}{k_{-2}} + e_0 + s_0 \pm \sqrt{\left(\frac{k_{-1}}{k_1} + \frac{k_2}{k_{-2}} + e_0 + s_0 \right)^2 - 4s_0e_0} \right] \quad (57)$$

$$s^{*(*)} = \frac{k_{-1}c^{*(*)}}{k_1(e_0 - c^{*(*)})} \quad (58)$$

Cont'd of Steady State

Nondimensionalizing the steady state solutions above by dividing the $c^{(*)}$ by e_0 and $s^{(*)}$ by s_0 we have the following system of steady state solutions

$$x^{(*)} = \frac{c^{(*)}}{e_0} = \frac{1}{2} \left[\beta + \eta + 1 \pm \sqrt{(\beta + \eta + 1)^2 - 4\beta} \right] \quad (59)$$

$$\sigma^{(*)} = \frac{k_1 s^{(*)}}{k_{-1}} = \frac{x^{(*)}}{1 - x^{(*)}}. \quad (60)$$

where

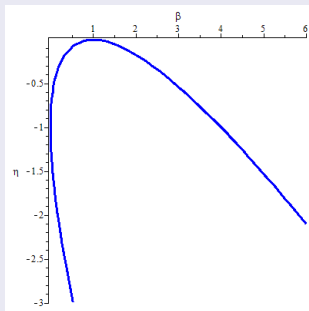
$$\eta = \frac{k_{-1}}{k_1 e_0} + \frac{k_2}{k_{-2} e_0} \quad \text{and} \quad \beta = \frac{s_0}{e_0} \quad (61)$$

Case: $x^* = x^{**}$

$$x^{*(*)} = \frac{c^{*(*)}}{e_0} = \frac{1}{2} \left[\beta + \eta + 1 \pm \sqrt{(\beta + \eta + 1)^2 - 4\beta} \right], \quad (62)$$

$$(\beta + \eta + 1)^2 - 4\beta = 0 \quad (63)$$

The region inside the curve(parabola) has no steady state solutions.



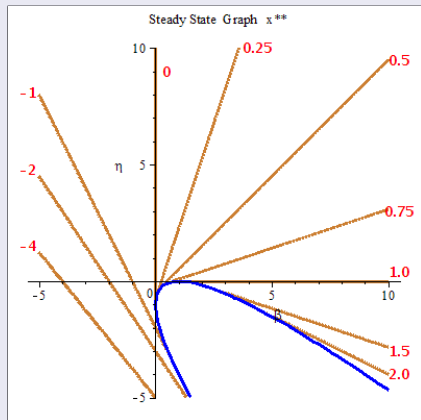
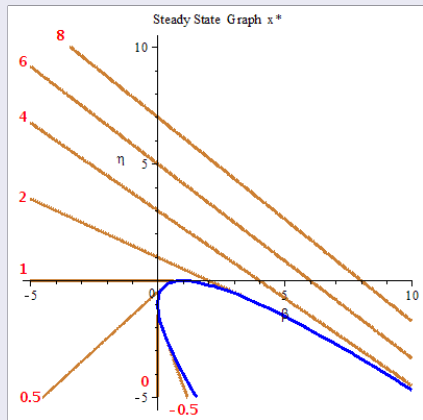


Figure: Nondimensionalize Enzyme concentration

Stability Analysis

The Jacobian associated with the system of differential equations for the reversible reaction is;

$$J^* = \begin{bmatrix} -k_1(e_0 - c) & k_{-1} + k_1s \\ (e_0 - c)(k_1 - k_{-2}) & -k_1s - k_{-1} - k_2 - k_{-2}(s_0 - s + e_0 - 2c) \end{bmatrix} \quad (64)$$

Evaluating the Jacobian J^* at the steady state, we have the characteristic equation of the Jacobian of the form,

$$\lambda^2 + b\lambda + d = 0 \quad (65)$$

where

$$b = \frac{1}{e_0 - c} [(k_1 + k_{-2})(e_0 - c)^2 + (k_{-1} + k_2)e_0] \quad (66)$$

$$d = k_1k_{-2}(e_0 - c) \left[\frac{k_{-1}}{k_1} + \frac{k_2}{k_{-2}} + s_0 + e_0 - 2c \right] \quad (67)$$

Stability Analysis

Solving for the eigenvalues of the characteristic polynomial we consider the following cases.

- Case 1: $(c < e_0) \dots (c^*(*), s^*(*)) \dots \text{STABLE}$
- Case 2: $(c > e_0) \dots (c^*(*), s^*(*)) \dots \text{UNSTABLE}$
- Case 3: $(c = e_0) \dots (c = 0, s = s_a) \dots \text{UNDETERMINED}$
- Case 4: (a) $(e_0 = 0) \dots (c = 0, s = s_a) \dots \text{UNDETERMINED}$
(b) $(e_0 = 0) \dots (c = \frac{k_2}{k_{-2}} + \frac{k_{-1}}{k_1} + s_0, s = -\frac{k_{-1}}{k_1}) \dots \text{UNSTABLE}$

$$c^{**} = x^{**} \text{ (STABLE)} \dots x < 1$$

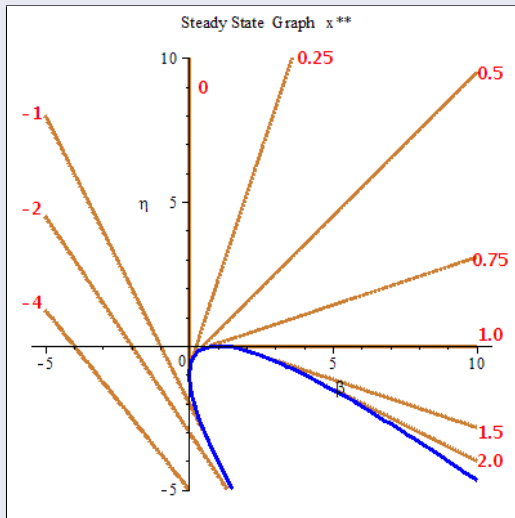


Figure: Nondimensionalize Enzyme concentration $x^{**} = \frac{c^{**}}{e_0}$

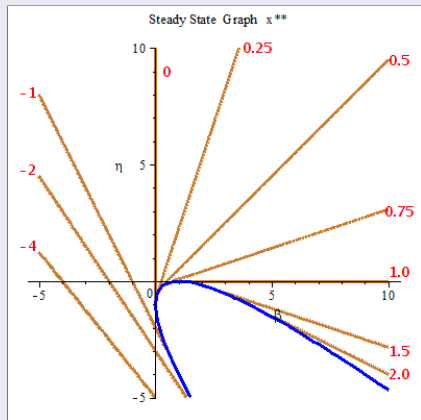
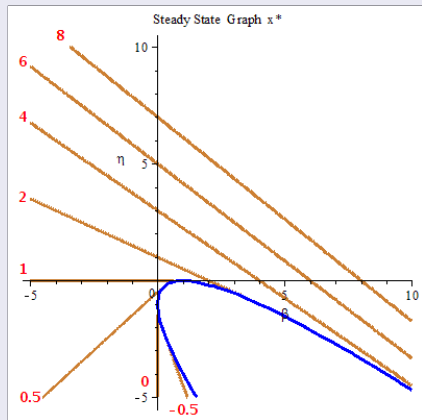


Figure: Nondimensionalize Enzyme concentration

Summary and Conclusion

- Techniques give vital information about the model without having to solve the differential equation directly.
- The steady state solution of the irreversible reaction scheme were all zero while for the reversible reaction the steady state were found to be non zero values.
- It was found that in some cases the concentration of the steady state is negative. This solution is not physically possible since negative concentration are not possible, however keeping in mind that it may never happen practically, mathematically a linear stability analysis was conducted on these solution to determine their stability.

Summary

	Irreversible	Reversible
EQUILB	$V = \frac{V_{max}s}{s + K_1}$	$V = \frac{V_{max}s - e_0 K_1 k_{-2} p}{K_1 + s}$

QSS	$V = \frac{V_{max}s}{s + K_m}$	$V = \frac{V_{max}s - e_0 K_1 k_{-2} p}{s + K_m + \frac{k_{-2} p}{k_1}}$
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Quasi-Steady-State

Assumption

An alternative analysis of the system was proposed by Briggs and Haldane (1925). They assumed that the concentration of reaction does not change on the time scale of product formation. Thus, at quasi-steady-state the assumption is that,

$$\frac{dc}{dt} = 0.$$

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$$\frac{dc}{dt} = 0.$$

Dimensionless variables were introduced to give this approximation a systematic mathematical basis.

$$\sigma = \frac{s}{s_0}, \quad x = \frac{c}{e_0}, \quad \kappa = \frac{k_{-1} + k_2}{k_1 s_0}, \quad \epsilon = \frac{e_0}{s_0}, \quad \alpha = \frac{k_{-1}}{k_1 s_0}, \quad \tau = k_1 e_0 t. \quad (68)$$

Cont'd of Quasi-Steady-State Approximation

The two system of equations can be written in dimensionless form as,

$$\frac{d\sigma}{d\tau} = -\sigma + x(\sigma + \alpha) \quad (69)$$

$$\epsilon \frac{dx}{d\tau} = \sigma - x(\sigma + \kappa) \quad (70)$$

The quasi-steady-state approximation $\epsilon \frac{dx}{d\tau} = 0$, is justified due to the different scaling of x and c and assuming ϵ is small. Thus it follows from (70) that,

$$x = \frac{\sigma}{\sigma + \kappa} \quad (71)$$

Substituting this into equation(69) we obtain,

$$\frac{d\sigma}{d\tau} = \frac{-\sigma q}{\sigma + \kappa}, \quad (72)$$

where $q = \kappa - \alpha$. Equation (72) is called the Michaelis-Menten Law.

Cont'd of Quasi-Steady-State Approximation

Rewriting the law with the original variables we have;

(73)

where $K_m = \frac{k_{-1} + k_2}{k_1}$ is called the Michaelis-Menten constant and $V_{max} = k_2 e_0$

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s^* (UNSTABLE).....

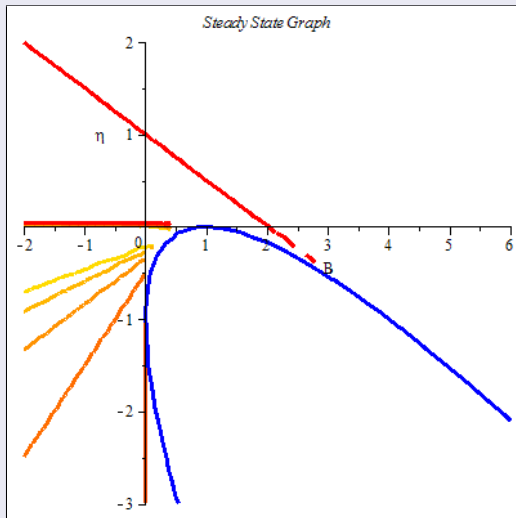


Figure: Nondimensionalize Enzyme concentration s^*

S^{**} ...STABLE.....

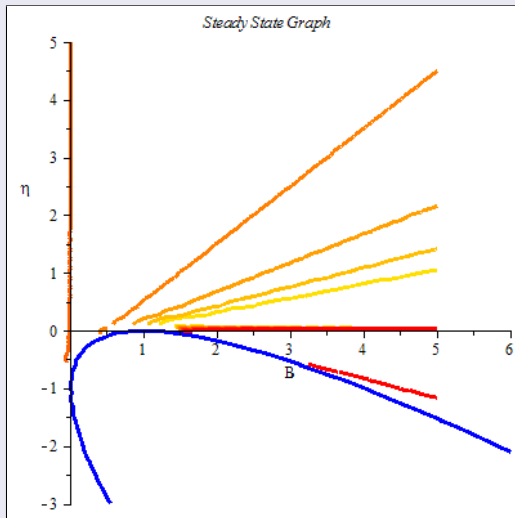


Figure: Nondimensionalize Enzyme concentration S^{**}

$$d = k_1 k_{-2} \left[(e_0 - c)^2 + \frac{k_2 e_0}{k_{-2}} + \frac{k_{-1} e_0}{k_1} \right] \quad (74)$$