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Analytical expression of concentrations in single-substrate enzyme kinetics

Meena V[1], Sriviveka saratha N[2]☼, Rajendran L[3]

1.Department of Mathematics, The Madura College, Madurai-625011, Tamilnadu, India; E–mail: meenakvishwa@gmail.com
2.Department of Mathematics, The Madura College, Madurai-625011, Tamilnadu, India; E–mail: saratha.2712@gmail.com
3.Department of Mathematics, The Madura College, Madurai-625011, Tamilnadu, India; E–mail: raj_sms@rediffmail.com

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ABSTRACT

In the present paper, the mathematical model of single- substrate enzyme kinetics is discussed. We employ the new Homotopy perturbation approach to solve the coupled non-linear differential equations containing a non-linear term related to basic Henri-Michaelis–Menten rate equation. The analytical expressions for concentration of substrate, enzyme- substrate complex, free enzyme and product have been derived for all values of rate constant. A numerical simulation is also reported using Matlab software program. Our analytical results are compared with our simulation results. A good agreement is noted between analytical and numerical results.

Keywords: Mathematical modeling, Henri-Michalies–Menten rate equation, Initial value problem, Rate constants of enzyme kinetics.

1. INTRODUCTION

The vast majority of chemical transformations inside cells are carried out by proteins called enzymes. Enzymes accelerate the rate of chemical reactions (both forward and backward) without being consumed in the process and tend to very selective, with a particular enzyme accelerating only a specific reaction. In biochemistry Michaelis–Menten kinetics is one of the simplest and best-known models of kinetics. This model contains three rate constant can be derived by temperature jump method or transient state kinetics,

but both methods need more complicated techniques and equipment [1]. Wei Chen and Cheng Zhu et al. [2] developed a kinetic model for a single-substrate trimolecular enzymatic system. The general kinetics of the trimolecular enzymatic system is more complex than the Michaelis-Menten kinetics. The Briggs–Haldane approximation of the irreversible Michaelis–Menten scheme of enzyme kinetics is cited in virtually every biochemistry text book and is widely considered the classic example of a quasi-steady-state approximation [3].

Many modeling approaches rely on ordinary differential equations (ODE) which are based on standard enzyme kinetics. Michaelis–Menten and Hill functions are indeed commonly used in dynamical models in systems and synthetic biology because they provide the necessary nonlinearity to make the dynamics nontrivial [4]. The most widely used rate expression for single-substrate enzyme catalyzed reactions, namely the Michaelis–Menten kinetics is based upon the assumption that enzyme concentration is in excess of the substrate in the medium and the rate is mainly limited by the substrate concentration according to saturation kinetics [5]. Alberto Maria et al. [6] described that a new asymptotic expansion valid in enzymatic reactions, where the total amount of enzyme exceeds greatly the total amount of substrate. In such a case, it is well known that the Michaelis–Menten approximation is no longer valid; therefore asymptotic expansion, which improves known results, is a new tool to approximate in a closed form the concentrations of the reactants in the presence of an enzyme excess. Time-dependent closed form solutions are derived for the three nominal cases of competition: even, slow and fast inhibitors, allowing for the first time the complete characterization of the reactions. The time-independent Michaelis–Menten approach is in accurate when a fast inhibitor is present [8-10].

Various aspects of the QSSA have been studied from time to time. An early study revealed that the condition is necessary for the applicability of the QSSA [11 - 14]. A few problems, however, remained unsolved. Thus, following an earlier work [15], Kargiet [5] mainly focused attention on the ratio $[S]_0/[E]_0$ at both ends and concluded again that QSSA can be implemented in either case, including the intermediate region. Bajzeret [7], on the other hand, maintained that QSSA is valid, if at all, only for a short time-interval when $[E]_0/[S]_0$ is large and that the region of validity is considerably larger for large $[S]_0/[E]_0$. The concentration profiles were calculated numerically and finding the steady state analytical expressions corresponding to the concentrations of substrate, enzyme- substrate complex, free enzyme and product. The purpose of this communication is to derive an approximate analytical expression for the concentrations in terms of rate constant using Homotopy perturbation method [16- 18].

2. MATHEMATICAL FORMULATION OF THE INITIAL VALUE PROBLEM

The two-step Michalies - Menten model is symbolized by the following reaction scheme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P \qquad (1)$$

Where $[S](t), [E](t), [ES](t)$ and $P(t)$ denote, the free substrate, free enzyme, the enzyme - substrate inter-mediate complex and product respectively. The parameters k_{-1}, k_1 and k_2 are positive constants for each reaction.

By applying the law of mass action, which states that reaction rates are proportional to the concentrations of the reactants, the time evolution of scheme (1) can be determined from the solution of the system of following coupled nonlinear differential equations: [7]

$$\frac{d[S]}{dt} = k_{-1}[ES] - k_1[E][S] \qquad (2)$$

$$\frac{d[E]}{dt} = (k_{-1} + k_2)[ES] - k_1[E][S] \qquad (3)$$

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_2)[ES] \qquad (4)$$

$$\frac{d[P]}{dt} = k_2[ES] \qquad (5)$$

with the initial conditions are:

$$At \quad t = 0 \; : \; [S] = [S]_0, \; [E] = [E]_0, \quad [ES] = 0 \quad and \quad [P] = 0 \qquad (6)$$

Since the enzyme *E* is a catalyst, its total concentration must be a constant. This conservation law is readily obtained by adding Eqns. (3) and (4):

$$\frac{d[E]}{dt}+\frac{d[ES]}{dt}=0 \qquad (7)$$

The integration of this equation leads to:

$$[E](t)+[ES](t)=[E]_0 \qquad (8)$$

Similarly from eqns. (2), (4) and (5) we get the following relations:

$$\frac{d[S]}{dt}+\frac{d[ES]}{dt}+\frac{d[P]}{dt}=0 \qquad (9)$$

The integration of this equation leads to:

$$[S](t)+[P](t)+[ES](t)=[S]_0 \qquad (10)$$

With this system of ordinary differential equations reduce to only two, for $[S]$ and $[ES]$, namely

$$\frac{d[S]}{dt}=-k_1([E_0]-[ES])[S]-k_{-1}[ES] \quad (11)$$

$$\frac{d[ES]}{dt}=k_1([E_0]-[ES])[S]-(k_{-1}+k_2)[ES] \qquad (12)$$

3. ANALYTICAL EXPRESSION OF CONCENTRATIONS USING HOMOTOPY PERTURBATION APPROACH

The analytical solution of non-linear equation is of great importance due to its wide application in scientific research. The Homotopy perturbation method (ref. Appendix A) [16-18] is used to give the approximate analytical solution of non-linear reaction/diffusion Eqns. (11) and (12) are

$$[S](t)=S_0\exp\left(\begin{array}{l}-\dfrac{k_1}{k_{-1}+k_2}-k_1E_0t+\\ \dfrac{k_1^2E_0}{k}\left(\dfrac{e^{-k_1E_0t}}{k_1E_0}-\dfrac{e^{-(k_{-1}+k_2)t}}{(k_{-1}+k_2)}\right)\end{array}\right) \qquad (13)$$

$$[ES](t)=\frac{k_1E_0\left(e^{-k_1E_0t}-e^{-(k_{-1}+k_2)t}\right)}{k}$$

where

$$k=k_{-1}+k_2-k_1E_0\,. \qquad (14)$$

We have derived Eqns. (8) and (10) using Eqns. (14) and(15) we get the approximate analytical solution respectively.

$$[E](t)=E_0-\frac{k_1E_0\left(e^{-k_1E_0t}-e^{-(k_{-1}+k_2)t}\right)}{k} \qquad (15)$$

$$[P](t)=S_0-S_0\exp\left(\begin{array}{l}-\dfrac{k_1}{k_{-1}+k_2}-k_1E_0t+\\ \dfrac{k_1^2E_0}{k}\left(\dfrac{e^{-k_1E_0t}}{k_1E_0}-\dfrac{e^{-(k_{-1}+k_2)t}}{(k_{-1}+k_2)}\right)\end{array}\right)-\left(\frac{k_1E_0\left(e^{-k_1E_0t}-e^{-(k_{-1}+k_2)t}\right)}{k}\right) \qquad (16)$$

4. NUMERICAL SIMULATION

The non-linear differential Eqns. (2-6) for the given initial conditions is being solved numerically. The function mat1, in Matlab software is used to solve the ordinary differential equations given in Appendix B. The numerical solution is compared with analytical solution in Figs. 1-3. Satisfactory agreement is noted between the analytical and numerical results.

5. DISCUSSION

Eqns. (13)-(16) are the closed and simple analytical expression of concentrations of the free substrate, enzyme - substrate intermediate complex, free enzyme, and product respectively. Figure (1-3) describes the dimensionless unsteady-state concentrations versus time t .The analytical expression of concentrations of the free enzyme $[E](t)$, free substrate $S(t)$, the enzyme - substrate

inter-mediate complex $[ES](t)$ and the product $[P](t)$ have been plotted for various values of the parameters k_{-1}, k_1 and k_2. From these figures it is inferred that the concentration of substrate $[S](t)$ follows a first order exponential decay and it is always decreasing function from the initial value. The concentration of enzyme-substrate complex $[ES](t)$ and product $[P](t)$ initially increases and attains its steady state value at short intervals of time for all values of parameters. Free enzyme $[E](t)$ increases slowly and reaches the steady state when time is very large. The time taken the steady value depends upon the values of parameters k_{-1}, k_1 and k_2. Our approximate analytical expressions of free substrate, enzyme substrate and free enzyme concentration are compared with simulation results in figs.1-3. A satisfactory agreement is noted.

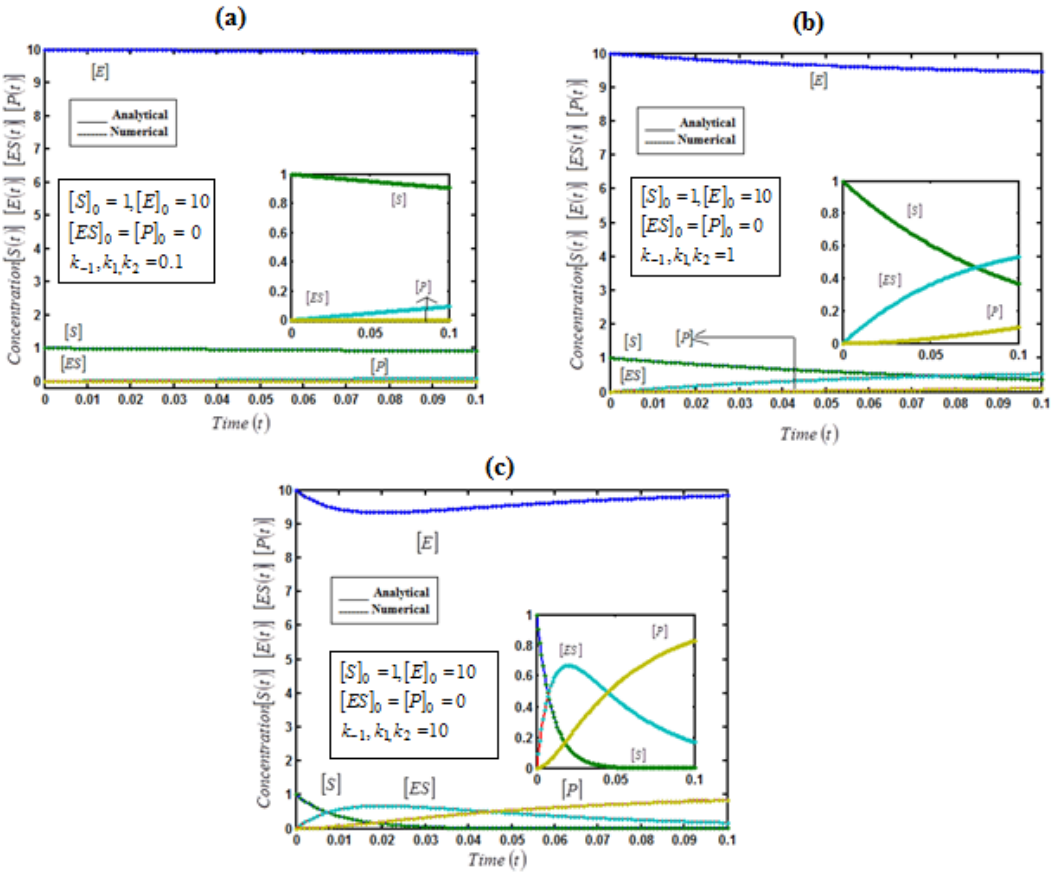

Figure 1 Plot of concentrations $[S(t)]$, $[E(t)]$,$[ES(t)]$ and $[P(t)]$ versus time t for different values of the parameters k_{-1}, k_2 and k_3 . The curves are plotted using Eqns. (13 - 16), for some fixed values of the other parameters:

$(a)\ [S]_0 = 1,\ [E]_0 = 10,\ [ES]_0 = [P]_0 = 0$ and $k_{-1}, k_2, k_3 = 0.1$

$(b)\ [S]_0 = 1,\ [E]_0 = 10,\ [ES]_0 = [P]_0 = 0$ and $k_{-1}, k_2, k_3 = 1$

$(C)\ [S]_0 = 1,\ [E]_0 = 10,\ [ES]_0 = [P]_0 = 0$ and $k_{-1}, k_2, k_3 = 10$

6. CONCLUSION

The times dependent non-linear equations have been solving analytically. The obtained results have a good agreement with those obtained using numerical method. The primary result of this work is simple, straight forward and a new method of estimating the concentrations of substrate, product, enzyme- substrate complex and enzyme are derived for all possible values of parameters k_{-1}, k_1 and k_2. The solution procedure can be easily extended to all kinds of system of coupled nonlinear equations with various complex boundary conditions in enzyme – substrate reaction diffusion processes. The analytical method is an extremely simple method and it is also promising method to solve other nonlinear equations.

Appendix A. The analytical solutions of non-linear Eqns. (11) and (12)

In this appendix, we derive the solution of non-linear equation Eqns. (11) and (12) using new approach of Homotopy perturbation method.

The Homotopy for the nonlinear equation (11) and (12) can be constructed as follows

$$(1-p)\left\{\frac{d[S]}{dt}+k_1[E_0][S]\right\} + p\left\{\frac{d[S]}{dt}+k_1([E_0]-[ES])[S]-k_{-1}[ES]\right\}=0 \quad \text{(A.1)}$$

$$(1-p)\left\{\frac{d[ES]}{dt}-k_1[E_0][S]+(k_{-1}+k_2)[ES]\right\}+ p\left\{\frac{d[ES]_1}{dt}-k_1([E_0]-[ES])[S]-(k_{-1}+k_2)[ES]\right\}=0 \quad \text{(A.2)}$$

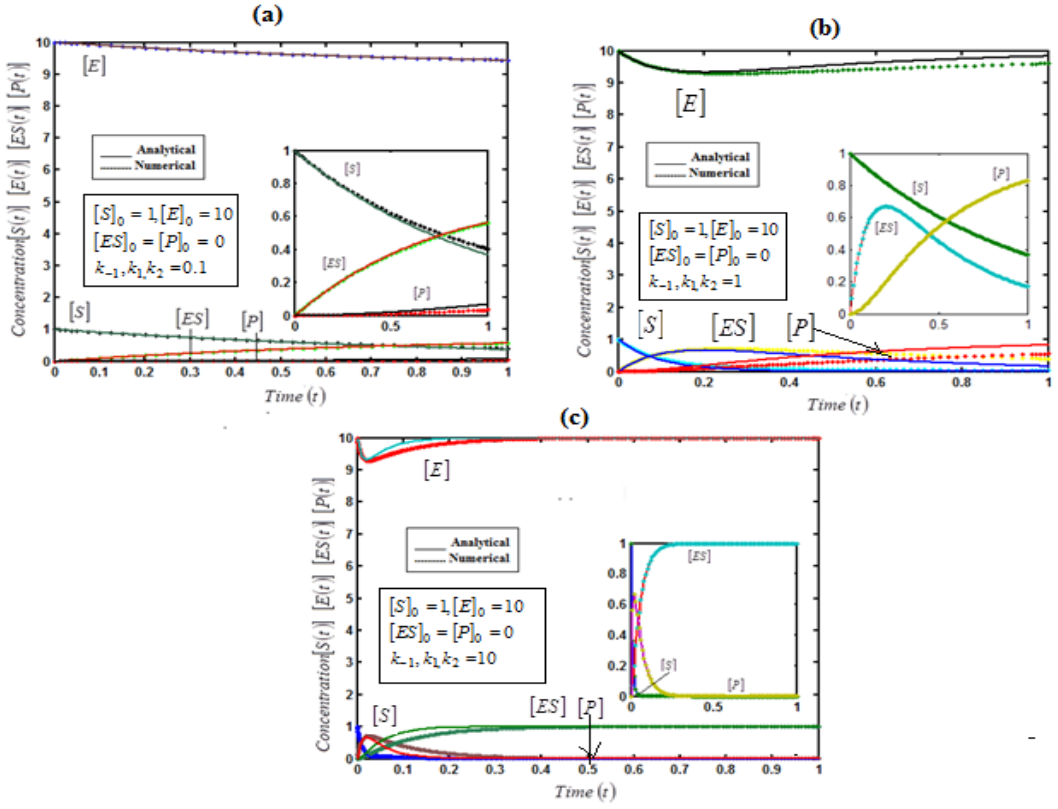

Figure 2 Plot of concentrations $[S(t)]$, $[E(t)]$,$[ES(t)]$ and $[P(t)]$ versus time t for different values of the parameters k_{-1}, k_2 and k_3 .The curves are plotted using Eqns. (13 - 16), for some fixed values of the other parameters:

$(a)\ [S]_0=1,\ [E]_0=10,\ [ES]_0=[P]_0=0$ and $k_{-1},k_2,k_3=0.1$

$(b)\ [S]_0=1,\ [E]_0=10,\ [ES]_0=[P]_0=0$ and $k_{-1},k_2,k_3=1$

$(C)\ [S]_0=1,\ [E]_0=10,\ [ES]_0=[P]_0=0$ and $k_{-1},k_2,k_3=10$

Suppose the approximate solutions of Eqns. (A.1) and (A.2) have the form

$$[S]=[s]_0+p[s]_1+p^2[s]_2+\ldots \quad \text{(A.3)} \qquad [ES]=[es]_0+p[es]_1+^2[es]_2+\ldots \quad \text{(A.4)}$$

Substituting Eqns. (A.3) and (A.4) into Eqn. (A.1) and Eqn. (A.2) respectively equate the terms with the identical powers of *p*, we obtain

$$p^0:\frac{d[s]_0}{dt}-k_1[E_0][s]_0=0 \quad \text{(A.5)}$$

$$p^0:\frac{d[es]_0}{dt}-k_1[E_0][s]_0+(k_{-1}+k_2)[es]_0=0 \quad \text{(A.6)}$$

The initial conditions are as follows:

$$t=0\ :\ [s]_0=[S]_0,\ \text{and}\ [es]_0=0 \qquad \text{(A.7)}$$

Solving the Eq. (A.5) using the boundary conditions Eq. (A.7), we get

$$[s]_0(t)=S_0e^{-k_1E_0t} \qquad \text{(A.8)}$$

Solving the Eq. (A.6), substitute Eqn. (A.8) in Eqn. (A.6) and using the boundary conditions Eq. (A.7), we get

$$[es]_0(t)=\frac{k_1E_0\left(e^{-k_1E_0t}-e^{-(k_{-1}+k_2)t}\right)}{k} \qquad \text{(A.9)}$$

where $k=k_{-1}+k_2-k_1E_0$

Substitute Eqns. (A.8) and (A.9) in Eqn. (11) we get

$$[S](t)=S_0\exp\left(-\frac{k_1}{k_{-1}+k_2}-k_1E_0t+\frac{k_1^2E_0}{k}\left(\frac{e^{-k_1E_0t}}{k_1E_0}-\frac{e^{-(k_{-1}+k_2)t}}{(k_{-1}+k_2)}\right)\right) \qquad \text{(A.10)}$$

where $k=k_{-1}+k_2-k_1E_0$

Higher order iteration will be considered to improve the accuracy of the results.

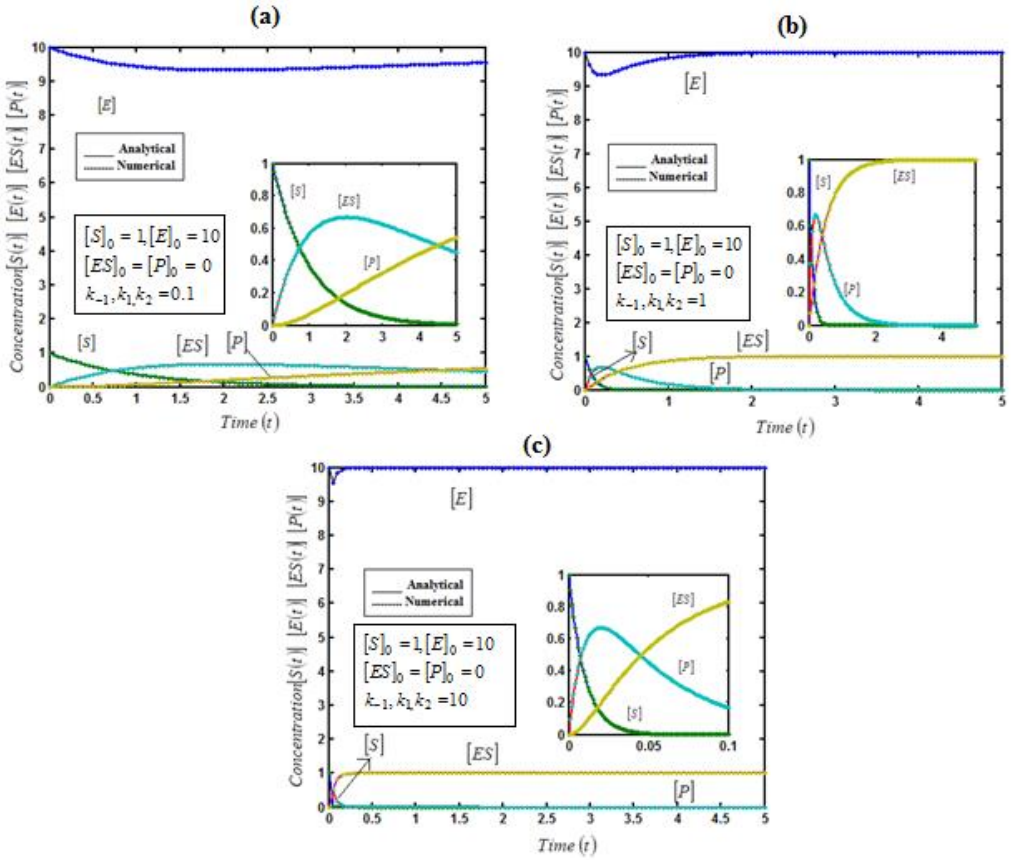

Figure 3 Plot of concentrations $[S(t)]$, $[E(t)]$,$[ES(t)]$ and $[P(t)]$ versus time t for different values of the parameters k_{-1}, k_2 and k_3 .The curves are plotted using Eqns. (13 - 16), for some fixed values of the other parameters:

$(a)\ [S]_0=1,\ [E]_0=10,\ [ES]_0=[P]_0=0$ and $k_{-1},k_2,k_3=0.1$

$(b)\ [S]_0=1,\ [E]_0=10,\ [ES]_0=[P]_0=0$ and $k_{-1},k_2,k_3=1$

$(C)\ [S]_0=1,\ [E]_0=10,\ [ES]_0=[P]_0=0$ and $k_{-1},k_2,k_3=10$

Appendix B: Scilab/Matlab program for the numerical solution of nonlinear differential equation (5)

```
function mat1
options= odeset('RelTol',1e-6,'Stats','on');
%initial conditions
x0 = [1;10;0;0];
tspan = [0,5];
tic
[t,x]=ode45(@TestFunction,tspan,x0,options);
toc
figure
holdon
plot(t, x(:,1))
plot(t, x(:,2))
plot(t, x(:,3))
plot(t, x(:,4))
plot(t, x(:,5))
legend('x1','x2','x3','x4','x5')
ylabel('x')
xlabel('t')
return
function [dx_dt]= TestFunction(t,x)
k1=1;k2=1;k3=1;
dx_dt(1)= k3*x(3)-k1*x(1)*x(2);
dx_dt(2) =(k3+k2)*x(3)-k1*x(1)*x(2);
dx_dt(3)=k1*x(1)*x(2)-(k3+k2)*x(3);
dx_dt(4)=k2*x(3);
dx_dt = dx_dt';
return
```

Appendix C

Nomenclature

Symbol	Meaning	Usual dimension
$[S]$	Concentration of free substrate	*mol/l*
$[E]$	Concentration of free Enzyme	$\mu molmin^{-1}$
$[ES]$	Concentration of free Enzyme – Substrate intermediate complex	*mol/l*
$[P]$	Concentration of Product	*mol/l*
$[S]_0$	Initial concentration of free substrate	*mol/l*
$[E]_0$	Initial concentration of free Enzyme	*mol/l*
$[ES]_0$	Initial concentration of free Enzyme – Substrate intermediate complex	*mol/l*
$[P]_0$	Initial concentration of Product	*mol/l*
k_{-1}, k_1, k_2	Positive rate constant for each reaction	$mol^{-1}\ min^{-1}$
t	Time factor	*min*

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