Applied Mathematics, **2011, 2, 1140-1147**
doi:10.4236/am.2011.29158 Published Online September 2011 (http://www.SciRP.org/journal/am)

Analytical Solutions of System of Non-Linear Differential Equations in the Single-Enzyme, Single-Substrate Reaction with Non-Mechanism-Based Enzyme Inactivation

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Received June 23, 2011; *revised July* 15, 2011; *accepted July* 23, 2011

Abstract

A closed form of an analytical expression of concentration in the single-enzyme, single-substrate system for the full range of enzyme activities has been derived. The time dependent analytical solution for substrate, enzyme-substrate complex and product concentrations are presented by solving system of non-linear differential equation. We employ He's Homotopy perturbation method to solve the coupled non-linear differential equations containing a non-linear term related to basic enzymatic reaction. The time dependent simple analytical expressions for substrate, enzyme-substrate and free enzyme concentrations have been derived in terms of dimensionless reaction diffusion parameters ε, λ_1, λ_2 and λ_3 using perturbation method. The numerical solution of the problem is also reported using SCILAB software program. The analytical results are compared with our numerical results. An excellent agreement with simulation data is noted. The obtained results are valid for the whole solution domain.

Keywords: Non-Linear Reaction Equations, Enzyme Inactivation, Homotopy Perturbation Method, Time Dependent Analytical Solution

1. Introduction

An enzyme is a biological catalyst that regulates the rate of chemical reaction in a living organism. Enzymes are very essential as most chemical reactions would occur too slowly or would lead to different product without the activity of enzymes. Enzymes bond with a substrate to form a transient state, an unstable intermediate complex that requires less energy for the reaction to proceed. Like any catalyst, the enzyme remains unaltered by the completed reaction and can therefore continue to interact with substrates. Enzymes may speed up reactions by a factor of many millions. Under temperature changes [1], diluted conditions, or changes in the reaction medium (pH or buffer) [2,3], enzyme can undergo progressive loss of activity. Actually enzyme inactivation can result in grievous errors in describing the behaviour of the system, such as incorrect estimation of the kinetic parameters. Therefore it is very important to be able to know when enzyme inactivation is affecting a reaction. Understanding the effects of enzyme inactivation is important for application such as predicting the behaviour of chemical reactions in the food, chemical, and pharmaceutical industries [4,5]. While there are numerous models available for mechanism-based inactivation systems or suicide substrates [6,7], there is currently no methodology that yields quantitative predictions for non-mechanism-based enzyme inactivation. In this paper we derive an expression for concentration of substrate, enzyme-substrate complex and product with non-mechanism-based enzyme inactivation, interms of dimensionless reaction diffusion parameters ε, λ_1, λ_2 and λ_3 using Homotopy perturbation method (HPM) and comparative study of the same with numerical simulation.

2. Mathematical Formulation of the Problem

The model of biochemical reaction was set forth by Michaelis and Menten in 1913 [8] and further developed by Briggs and Haldane in 1925 [9]. This formulation

considers a reaction where a substrate *S* binds an enzyme *E* reversibly to form a complex *C*. The complex can then decay irreversibly to a product *P* and the enzyme, which is then free to bind another molecule of the substrate. The enzyme is normally considered more stable while incorporated in *C* than when in free *E* form. From this, we can add to the Michaelis-Menten mechanism that free *E* decays into its inactive form E_i. This single enzyme-substrate reaction system is represented as follows [10]:

$$S + E \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} C \xrightarrow{k_{cat}} P + E \tag{1}$$

$$E \xrightarrow{k_3} E_i \tag{2}$$

In this mechanism, the enzyme inactivation is irreversible. This mechanism illustrates the binding of substrate *S* and release of product *P*. *E* is the free enzyme and *C* is the enzyme-substrate complex. The time evolution of reaction (1)-(2) are obtained by applying the law of mass action to yield the set of system of following non-linear differential equations.

$$\frac{\mathrm{d}s}{\mathrm{d}t} = k_1\left(-es + K_S c\right) \tag{3}$$

$$\frac{\mathrm{d}e}{\mathrm{d}t} = k_1\left(-es + K_M c - K_\delta e\right) \tag{4}$$

$$\frac{\mathrm{d}c}{\mathrm{d}t} = k_1\left(es - K_M c\right) \tag{5}$$

$$\frac{\mathrm{d}p}{\mathrm{d}t} = k_{cat} c \tag{6}$$

$$\frac{\mathrm{d}e_i}{\mathrm{d}t} = k_1 K_\delta e \tag{7}$$

Initial conditions at $t = 0$ is given by

$$s = s_0,\ \ e = e_0,\ \ c = 0,\ p = 0,\ \ e_i = 0. \tag{8}$$

In this system, K_S is the equilibrium dissociation constant for enzyme-substrate complex, K_M is the Michaelis-Menten constant and K_δ is the constant for enzyme inactivation. By imposing the following two conservation of laws,

$$e_0 = e + c + e_i \tag{9}$$

and

$$s_0 = s + c + p \tag{10}$$

We describe the reaction mechanism (3)-(7) using the following differential equations

$$\frac{\mathrm{d}s}{\mathrm{d}t} = -k_1 s\left(e_0 - c - e_i\right) + k_1 K_S c \tag{11}$$

$$\frac{\mathrm{d}c}{\mathrm{d}t} = k_1 s\left(e_0 - c - e_i\right) - k_1 K_M c \tag{12}$$

$$\frac{\mathrm{d}e_i}{\mathrm{d}t} = k_1 K_\delta\left(\ e_0 - c - e_i\ \right) \tag{13}$$

with initial conditions at

$$t = 0,\ \ s = s_0,\ \ c = 0,\ \ e_i = 0 \tag{14}$$

By introducing the following set of dimensionless parameters

$$\tau = \frac{k_1 e_0 t}{\varepsilon},\ \ u(\tau) = \frac{\mathrm{s}\ (t)}{s_0},\ \ \mathrm{v}(\tau) = \frac{c\ (t)}{e_0},$$

$$w(\tau) = \frac{e_i(t)}{e_0},\ \ \lambda_1 = \frac{K_S}{s_0},\ \lambda_2 = \frac{K_M \varepsilon}{e_0},$$

$$\lambda_3 = \frac{K_\delta \varepsilon}{e_0},\ \ \varepsilon = \frac{e_0}{s_0}$$

The system of Equations (11)-(13) with initial condition (14) can be represented in dimensionless form as follows:

$$\frac{\mathrm{d}u}{\mathrm{d}\tau} = -u\varepsilon + \varepsilon\ uv + \varepsilon\ uw + \lambda_1 \varepsilon\ v \tag{15}$$

$$\frac{\mathrm{d}v}{\mathrm{d}\tau} = u - uv - uw - \lambda_2 v \tag{16}$$

$$\frac{\mathrm{d}w}{\mathrm{d}\tau} = \lambda_3 - \lambda_3 v - \lambda_3 w \tag{17}$$

The boundary conditions are

$$u(0) = 1,\ v(0) = 0\ ,w(0) = 0 \tag{18}$$

3. Analytical Expressions of Concentrations under Non-Steady State and Steady State Condition

Nonlinear phenomena play a crucial role in applied mathematics and physics. Explicit solutions to the nonlinear equations are of fundamental importance. Various methods for obtaining explicit solution to nonlinear evolution equations have been proposed. Recently, many authors have used the HPM for solving various problems and demonstrated the efficiency of the HPM for solving non-linear structures and various physics and engineering problems [11-14]. This method is a combination of topology and classic perturbation techniques. Ji Huan He used the HPM to solve the Lighthill equation [15], the Duffing equation [16] and the Blasius equation [17]. The idea has been used to solve non-linear boundary value problems, integral equations and many other problems. In these methods [18-22], the homotopy perturbation method is applied and the obtained results show that the HPM is very effective and simple. The HPM is unique in its applicability, accuracy, efficiency and uses the imbedding parameter *p* as a small parameter and only a few iterations are needed to search for an asymptotic solution. Using this method, we can obtain the following solution to Equations (15)-(17) (Ref Appendix-A)

$$u(\tau)=e^{-\varepsilon\tau}+\frac{\lambda_1\varepsilon\tau e^{-\varepsilon\tau}}{\lambda_2-\varepsilon}+\frac{\lambda_1\varepsilon\left[\,e^{-\lambda_2\tau}-e^{-\varepsilon\tau}\,\right]}{(\lambda_2-\varepsilon)^2}+\varepsilon\ \tau\ e^{-\varepsilon\tau}+\frac{\varepsilon\left[\,e^{-(\lambda_3+\varepsilon)\tau}-e^{-\varepsilon\tau}\,\right]}{\lambda_3} \tag{19}$$

$$v(\tau)=\frac{e^{-\lambda_2\tau}}{\varepsilon(\lambda_2-\varepsilon)}-\frac{e^{-(\lambda_2+\varepsilon)\tau}}{\varepsilon(\lambda_2-\varepsilon)}+\frac{e^{-\lambda_2\tau}}{(\lambda_2-\varepsilon)(\lambda_2-2\varepsilon)}-\frac{e^{-2\varepsilon\tau}}{(\lambda_2-\varepsilon)(\lambda_2-2\varepsilon)}+\frac{\left[e^{-(\lambda_3+\varepsilon)\tau}-e^{-\lambda_2\tau}\right]}{\lambda_2-\lambda_3-\varepsilon} \tag{20}$$

$$w(\tau)=1-e^{-\lambda_3\tau}+\frac{\lambda_3 e^{-\lambda_3\tau}}{(\lambda_2-\varepsilon)(\lambda_3-\varepsilon)}-\frac{\lambda_3 e^{-\varepsilon\tau}}{(\lambda_2-\varepsilon)(\lambda_3-\varepsilon)}+\frac{\lambda_3 e^{-\lambda_2\tau}}{(\lambda_2-\varepsilon)(\lambda_3-\lambda_2)}-\frac{e^{-\lambda_3\tau}}{(\lambda_2-\varepsilon)(\lambda_3-\lambda_2)} \tag{21}$$

Equations (19)-(21) represent the analytical expression of the dimensionless substrate concentration $u(\tau)$, enzyme-substrate concentration $v(\tau)$ and free enzyme concentration $w(\tau)$ for all values of parameters ε, λ_1, λ_2 and λ_3.

For steady state condition, the differential Equations (15)-(17) become as follows:

$$-u\varepsilon+\varepsilon uv+\varepsilon uw+\lambda_1\varepsilon\ v=0 \tag{22}$$

$$u-uv-uw-\lambda_2 v=0 \tag{23}$$

$$\lambda_3-\lambda_3 v-\lambda_3 w=0 \tag{24}$$

Solving the above equations, we can obtain substrate concentration u, enzyme substrate concentration v and free enzyme concentration w, as follows:

$$u=0,\ v=0,\ w=1$$

When t tends to infinity, the analytical expression corresponding to the substrate concentration u, enzyme substrate concentration v and free enzyme concentration w from the Equations (19)-(21) confirms the validity of mathematical analysis.

4. Results and Discussion

The non-linear differential Equations (15)-(18), are also solved using numerical methods. The functionbvp4c in Scilab software which is a function of solving two-point boundary value problems (BVPs) for ordinary differential equations is used to solve this equation. Its numerical solution is compared with our analytical result in **Figures 1-5** and it gives a satisfactory agreement for all values of time τ. The Scilab program is also given in Appendix B.

Equations (19)-(21) are the closed and simple analytical expression of concentrations. **Figures 1-5** describes the dimensionless unsteady-state concentrations versus time τ. The analytical expression of concentrations of substrate $u(\tau)$, enzyme-substrate complex $v(\tau)$ and free enzyme $w(\tau)$ have been plotted for various values of dimensionless reaction parameters ε, λ_1, λ_2 and λ_3. Recently Schnell and Hanson [10] obtained the following analytical expression of concentration of enzyme substrate complex c and free enzyme e_i assuming

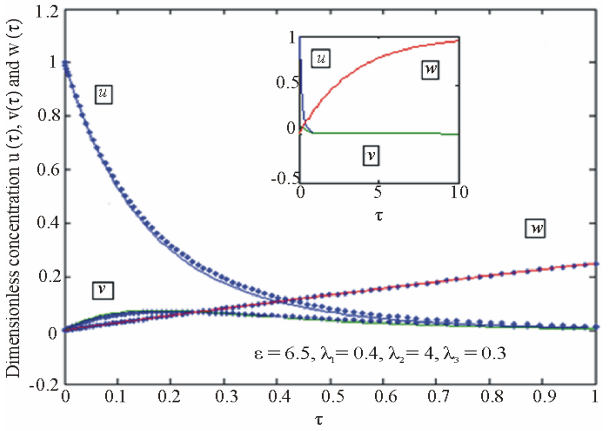

Figure 1. Time-dependent behaviour of the reactant concentration of the substrate $u(\tau)$, enzyme-substrate $v(\tau)$ and free enzyme $w(\tau)$ for ε = 6.5, λ_1 = 0.4, λ_2 = 4 and λ_3 = 0.3. The curves are plotted using Equations (19)-(21). The key to the graph (---) represents Equations (19)-(21) and (….) denotes simulation.

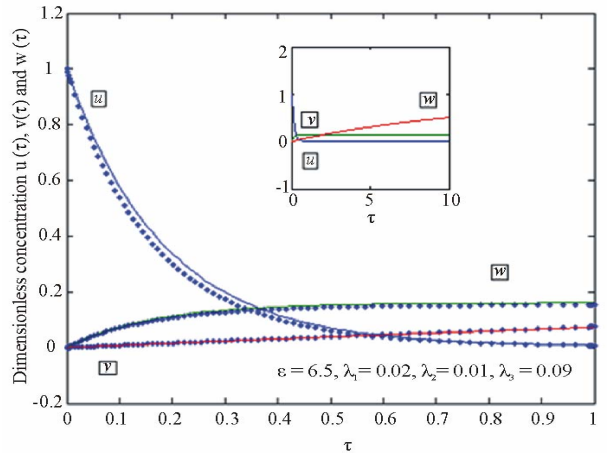

Figure 2. Time-dependent behaviour of the reactant concentration of the substrate $u(\tau)$, enzyme-substrate $v(\tau)$ and free enzyme $w(\tau)$ for ε = 6.5, λ_1 = 0.02, λ_2 = 0.01 and λ_3 = 0.09 .The curves are plotted using Equations (19)-(21). The key to the grap (----) represents Equations (19)-(21) and (….) denotes simulation.

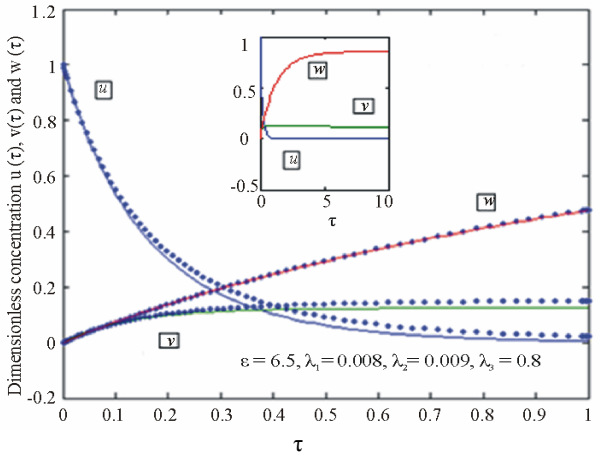

Figure 3. Time-dependent behaviour of the reactant concentration of the substrate $u(\tau)$, enzyme-substrate $v(\tau)$ and free enzyme $w(\tau)$ for $\varepsilon = 6.5$, $\lambda_1 = 0.008$, $\lambda_2 = 0.009$ and $\lambda_3 = 0.8$. The curves are plotted using Equations (19)-(21). The key to the graph (---) represents Equations (19)-(21) and (….) denotes simulation.

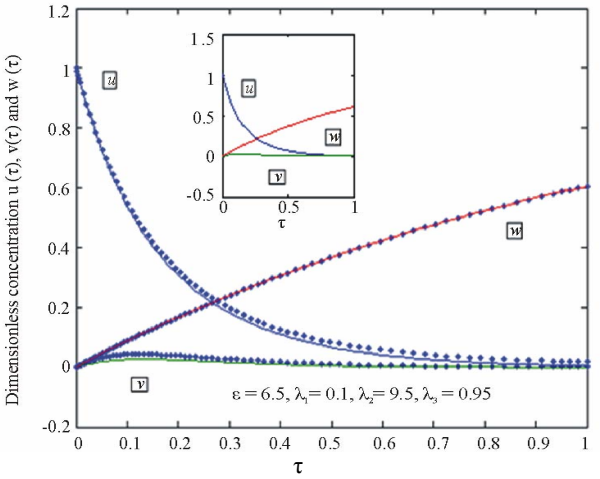

Figure 4. Time-dependent behaviour of the reactant concentration of the substrate $u(\tau)$, enzyme-substrate $v(\tau)$ and free enzyme $w(\tau)$ for $\varepsilon = 6.5$, $\lambda_1 = 0.1$, $\lambda_2 = 9.5$ and $\lambda_3 = 0.95$. The curves are plotted using Equations (19)-(21). The key to the graph (---) represents Equations (19)-(21) and (….) denotes simulation.

$$\frac{dc}{dt} \approx 0 \quad \text{for} \quad t > t_c \quad \text{and} \quad s \approx s_0 \text{ for } t \le t_c$$

$$c = ab\left[\frac{l_1 - l_2}{k_1 k_\delta e_0}\right]\left[\exp(l_2 t) - \exp(l_1 t)\right] \tag{25}$$

$$e_i = e_0 + a\exp(l_1 t) - b\exp(l_2 t) \tag{26}$$

where

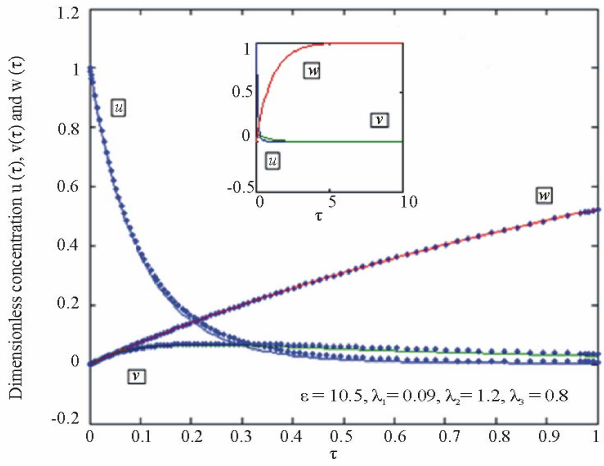

Figure 5. Time-dependent behaviour of the reactant concentration of the substrate $u(\tau)$, enzyme-substrate $v(\tau)$ and free enzyme $w(\tau)$ for $\varepsilon = 10.5$, $\lambda_1 = 0.09$, $\lambda_2 = 1.2$ and $\lambda_3 = 0.8$. The curves are plotted using Equations (19)-(21). The key to the graph (---) represents Equations (19)-(21) and (….) denotes simulation.

$$a = \frac{e_0\left(l_2 / l_1 + k_\delta\right)}{\left(l_1 - l_2\right)/k_1}, \quad b = \frac{e_0\left(l_1 / k_1 + k_\delta\right)}{\left(l_1 - l_2\right)/k},$$

$$l_1 = \frac{k_1 k_\delta k_M}{k_M^{app} + s_0} + O(t^2), \; l_2 = -k_1\left(k_M^{app} + s_0\right) + O\left(t^2\right)$$

(27)

Figures 1-5 represent the dimensionless concentration of substrate *u,* enzyme-substrate complex *v*, and free enzyme *w* for various values of parameter ε, λ_1, λ_2 and λ_3. From these figures it is inferred that the concentration of substrate *u* follows a first-order exponential decay and it is always decreasing function from the initial value. The concentration of enzyme-substrate complex *v* initially increases and attains its steady state value at short intervals of time for all values of rate constant. The value of free enzyme *w* increases slowly and reaches the steady state when time is very large. The time taken the steady value depends upon the values of parameters ε, λ_1, λ_2 and λ_3. Our approximate analytical expressions of substrate, enzyme substrate and free enzyme concentration are compared with simulation results in **Figures 1-5**. A satisfactory agreement is noted.

5. Conclusions

The time dependent non-linear reaction-diffusion equation has been formulated and solved analytically and numerically. Analytical expression of substrate, enzyme-substrate and free enzyme concentrations interms of dimensionless reaction diffusion parameters ε, λ_1, λ_2

and λ_3 are derived using the HPM. The primary result of this work is simple approximate calculations of concentrations for all values of dimensionless parameters ε, λ_1, λ_2 and λ_3. The HPM is an extremely simple method and it is also a promising method to solve other non-linear equations. This method can be easily extended to all kinds of system of coupled non-linear equations in multi-substrate systems and networks of coupled enzyme reactions.

6. Acknowledgements

This work was supported by the Council of Scientific and Industrial Research (CSIR No.01 (2442)/10/EMR-II), Government of India. The authors also thank Secretary, The Madura College Board and Principal, Head of the Department of Mathematics, The Madura College, Madurai, India for their constant encouragement.

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APPENDIX A

Solution of the Equations (15)-(18) using Homotopy perturbation method. In this Appendix, we indicate how Equations (19)-(21) in this paper are derived. Furthermore, a Homotopy was constructed to determine the solution of Equations (15)-(17).

$$(1-p)\left[\frac{\mathrm{d}u}{\mathrm{d}\tau} + u\varepsilon\right] + p\left[\frac{\mathrm{d}u}{\mathrm{d}\tau} + u\varepsilon - \lambda_1\varepsilon v - \varepsilon uv - \varepsilon uw\right] = 0 \tag{A1}$$

$$(1-p)\left[\frac{\mathrm{d}v}{\mathrm{d}\tau} + \lambda_2 v\right] + p\left[\frac{\mathrm{d}v}{\mathrm{d}\tau} + \lambda_2 v - u + uv + uw\right] = 0 \tag{A2}$$

$$(1-p)\left[\frac{\mathrm{d}w}{\mathrm{d}\tau} + \lambda_3 w\right] + p\left[\frac{\mathrm{d}w}{\mathrm{d}\tau} + \lambda_3 w - \lambda_3 + \lambda_3 v\right] = 0 \tag{A3}$$

The initial approximations are as follows:

$$u(0) = 1,\ v(0) = 0 \text{ and } w(0) = 0 \tag{A4}$$

Approximate solutions of (A1), (A2) and (A3) are

$$u = u_0 + pu_1 + p^2u_2 + p^3u_3 + \ldots\ldots\ldots\ldots \tag{A5}$$

$$v = v_0 + pv_1 + p^2v_2 + p^3v_3 + \ldots\ldots\ldots\ldots \tag{A6}$$

and

$$w = w_0 + pw_1 + p^2w_2 + p^3w_3 + \ldots\ldots\ldots\ldots \tag{A7}$$

Substituting Equations (A5)-(A7) in Equations (A1)-(A3) and comparing the coefficients of like powers of p, we can obtain the following differential equations.

$$p^0:\ \frac{\mathrm{d}u_0}{\mathrm{d}\tau} + \varepsilon u_0 = 0 \tag{A8}$$

$$p^1:\ \frac{du_1}{d\tau} + \varepsilon u_1 - \lambda_1\varepsilon v_0 - \varepsilon u_0v_0 - \varepsilon u_0w_0 = 0 \tag{A9}$$

$$p^2:\ \frac{\mathrm{d}u_2}{\mathrm{d}\tau} + \varepsilon u_2 + \varepsilon u_1 - \lambda_1\varepsilon v_1 - \varepsilon u_0w_1 - \varepsilon u_1w_0 = 0 \tag{A10}$$

and

$$p^0:\ \frac{\mathrm{d}v_0}{\mathrm{d}\tau} + \lambda_2 v_0 = 0 \tag{A11}$$

$$p^1:\ \frac{\mathrm{d}v_1}{\mathrm{d}\tau} + \lambda_2 v_1 - u_0 + u_0v_0 + u_0w_0 = 0 \tag{A12}$$

$$p^2:\ \frac{\mathrm{d}v_2}{\mathrm{d}\tau} + \lambda_2 v_2 - u_1 + u_0v_1 + u_1v_0 + u_0w_1 + u_1w_0 = 0 \tag{A13}$$

and

$$p^0:\ \frac{\mathrm{d}w_0}{\mathrm{d}\tau} + \lambda_3 w_0 = 0 \tag{A14}$$

$$p^1:\ \frac{\mathrm{d}w_1}{\mathrm{d}\tau} + \lambda_3 w_1 - \lambda_3 + \lambda_3 v_0 = 0 \tag{A15}$$

$$p^2:\ \frac{\mathrm{d}w_2}{\mathrm{d}\tau} + \lambda_3 w_2 + \lambda_3 v_1 = 0 \tag{A16}$$

Solving the Equations (A8)-(A16), and using the boundary conditions (A4) , we can find the following results.

$$u_0(\tau) = e^{-\varepsilon\tau} \tag{A17}$$

$$u_1(\tau) = 0 \tag{A18}$$

$$u_2(\tau) = \frac{\lambda_1\varepsilon\ \tau e^{-\varepsilon\tau}}{\lambda_2-\varepsilon} + \frac{\lambda_1\varepsilon\left[e^{-\lambda_2\tau} - e^{-\varepsilon\tau}\right]}{(\lambda_2-\varepsilon)^2} + \varepsilon\tau e^{-\varepsilon\tau} + \frac{\varepsilon\left[e^{-(\lambda_3+\varepsilon)\tau} - e^{-\varepsilon\tau}\right]}{\lambda_3} \tag{A19}$$

and

$$v_0(\tau) = 0 \tag{A20}$$

$$v_1(\tau) = \frac{e^{-\varepsilon\tau} - e^{-\lambda_2\tau}}{\lambda_2-\varepsilon} \tag{A21}$$

$$v_2(\tau) = \frac{1}{\lambda_2-\varepsilon}\left[\frac{e^{-\lambda_2\tau}}{\varepsilon} - \frac{e^{-(\lambda_1+\varepsilon)\tau}}{\varepsilon} + \frac{e^{-\lambda_2\tau}}{\lambda_2-2\varepsilon} - \frac{e^{-2\varepsilon\tau}}{\lambda_2-2\varepsilon}\right] + \left[\frac{e^{-(\lambda_3+\varepsilon)\tau} - e^{-\lambda_2\tau}}{\lambda_2-\lambda_3-\varepsilon}\right] + \left[\frac{e^{-\lambda_2\tau} - e^{-\varepsilon\tau}}{\lambda_2-\varepsilon}\right] \tag{A22}$$

and

$$w_0(\tau) = 0 \tag{A23}$$

$$w_1(\tau) = 1 - e^{-\lambda_3\tau} \tag{A24}$$

$$w_2(\tau) = \frac{\lambda_3 e^{-\lambda_3\ \tau}}{(\lambda_2-\varepsilon)(\lambda_3-\varepsilon)} - \frac{\lambda_3 e^{-\varepsilon\tau}}{(\lambda_2-\varepsilon)(\lambda_3-\varepsilon)} + \frac{\lambda_3 e^{-\lambda_2\tau}}{(\lambda_2-\varepsilon)(\lambda_3-\lambda_2)} - \frac{e^{-\lambda_3\tau}}{(\lambda_2-\varepsilon)(\lambda_3-\lambda_2)} \tag{A25}$$

According to the HPM, we can conclude that

$$u(\tau) = \lim_{p\to 1} u(\tau) = u_0 + u_1 + u_2 + \ldots\ldots\ldots \tag{A26}$$

$$v(\tau) = \lim_{p\to 1} v(\tau) = v_0 + v_1 + v_2 + \ldots\ldots\ldots \tag{A27}$$

$$w(\tau) = \lim_{p\to 1} w(\tau) = w_0 + w_1 + w_2 + \ldots\ldots\ldots \tag{A28}$$

Using Equations (A17)-(A19) in Equation (A26), Equations (A20)-(A22) in Equations (A27) and (A23)-(A25) in Equation (A28), we obtain the final results as described in Equations (19)-(21).

APPENDIX B

Scilab program to find the solutions of the Equations (15)-(18)

```
function main123456
options= odeset('RelTol',1e-6,'Stats','on');
%initial conditions
x0 = [1; 0;0];
tspan = [0 1];
tic
[t,x] = ode45(@TestFunction,tspan,x0,options);
toc
figure
hold on
plot(t, x(:,1))
plot(t, x(:,2),'.')
plot(t, x(:,3))
legend('x1','x2')
ylabel('x')
xlabel('t')
return
function [dx_dt]= TestFunction(t,x)
```



```
a=6.5;b=0.4;c=4;d=0.3;
dx_dt(1)=-a*x(1)+a*x(1)*x(2)+a*x(1)*x(3)+a*b*x(2);
dx_dt(2) =x(1)-x(1)*x(2)-x(1)*x(3)-c*x(2);
dx_dt(3) =d-d*x(2)-d*x(3);
dx_dt = dx_dt';
return
```

APPENDIX C

Nomenclature

u	Dimensionless Substrate concentation (None)
v	Dimensionless Enzyme-substrate concentration (None)
w	Dimensionless free enzyme concentration (None)
K_M	Michaelis-Menten constant (μ M)
K_S	Equlibrium dissociation constant (μ M)
K_δ	Enzyme inactivation constant (μ M)
k_{cat}	Unimolecular rate constant (μ M)
λ_1	Dimensionless Equlibrium dissociation constant (None)
λ_2	Dimensionless Michaelis-Menten constant (None)
λ_3	Dimensionless enzyme inactivation constant (None)
ε	Dimensionless reaction diffusion constant (None)
τ	Dimensionless time (None)

