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ANALYTICAL SOLUTIONS OF NON-LINEAR INITIAL VALUE PROBLEM IN BIOCHEMICAL SCIENCES

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ABSTRACT

The initial value problem in biochemical enzyme mechanism is discussed. The approximate closed analytical expression of enzyme (E, E_1, E_2, and E_3) concentrations of phenolic compound and H_2O_2complex are presented. Modified He's Homotopy perturbation method (HPM) is used to give approximate and analytical solutions of non-linear reaction equations containing a non-linear term related to enzymatic reaction.

***Keywords**: Mathematical modeling; Horseradish peroxidase; 4-Aminoantipyrine; Immobilization; Biosensors; Kinetic parameters; Modified HPM.*

1. INTRODUCTION

Horseradish peroxidase (HRP) is an intensively used enzyme in biochemical and medical analyses. It is also proposed for waste water processing .A bioprocess monitoring method based on co-oxidation of phenol-4-sulfonic acid (PSA) and 4-aminoantipyrine (4-AAP) by immobilized HRP was recently described. Azevedo, *et.al.* [1] describe that Horseradish peroxidase (HRP) is one of the most extensively studied enzymes for historical reasons, but also because of its availability, relatively easy extraction and purification, and growing number of applications. A large family of HRP is oenzymes has been identified. HRP is a single chain polypeptide glycosilated at eight specific sites, containing two CaII ions, and one heme group (which contains the Fe-protoporphyrin IX) [2].Several methods have been proposed for the treatment of halogenated phenolic compounds such as biodegradation [3], electrochemical oxidation [4], resin adsorption [5], and peroxidase polymerization [6]. In the presence of hydrogen peroxide, peroxidases can be used for the removal of phenolic compounds by oxidizing them to phenoxyl radicals, which then react to create less soluble hydrophobic polymeric products. This approach has been applied to the decontamination of waste waters containingtoxic phenols, anilines, hydroxyquinoline, and arylaminecarcinogens such as benzidines and naphthylamines [7].

Vojinovi´c, *et al.* [8] describe a set of reactions that occur in phenol/4-AAP/HRP system upon addition of hydrogen peroxide has been used for the evaluation of the PSA/4-AAP/HRP system by estimating and comparing the apparent kinetic parameters for different reaction systems. The parameters thus obtained were compared for three phenolic compounds (phenol, p-chlorophenol and PSA) and for immobilized HRP in the PSA/4-AAP system. The model was able to capture distinctive features of the system, such as increasing initial reaction rates for increasing concentrations of H_2O_2; a plateau upon reactant exhaustion; and decreasing initial reaction rates at high H_2O_2 concentrations. The performance of the PSA/4-AAP/HRP system expressed as the initial reaction rate was found to be less than 12% that of the conventional phenol/4-AAP/HRP system.

Modeling of the system of HRP-catalysed reactions allows the prediction of the behaviour of the system in order to optimize conversion, shorten reaction times and avoid waste. It is also useful for studies of mechanistic behaviour of HRP systems. Steady state models were previously described [9, 10] that successfully predict initial reaction rates, but do not allow evaluation of non-rate limiting rate constants. The purpose of this communications of phenolic compound and H_2O_2 complex using modified new Homotopy method.

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2. MATHEMATICAL FORMULATION OF PROBLEM

The HRP\phenol\$H_2O_2$systems [11], can be represented by the following reaction equations:

$$E + R \xrightarrow{k_1} E_1 + R \tag{1}$$

$$E_1 + R_1 \xrightarrow{k_2} E_2 + R_2 \tag{2}$$

$$E_2 + R_1 \xrightarrow{k_3} E + R_2 + R \tag{3}$$

$$2R_2 + R_3 + R \xrightarrow{k_r} R_4 + R_1 + 2R \tag{4}$$

$$E_2 + R_1 \xrightarrow{k_{app}} E_3 + R \tag{5}$$

$$E_3 \xrightarrow{k_a} E + R_5 + R_6 \tag{6}$$

$$E_3 + R_1 \xrightarrow{k_b} E_1 + R_2 + R \tag{7}$$

where $R=H_2O_2$, R_1=PhOH,R_2=PhO$^\bullet$,R_3=Am-NH2 , $R_4=\ ^\bullet O_2^-$ and $R_5=H^+$. E, E_1, E_2 and $E_3$are the enzyme. The model is derived from differential mass balances based on the set of reactions (1)–(7). Nicell and Wright have shown that the initial quantity of the native enzyme introduced in the assay will rapidly distribute among oxidation states, and that enzyme forms (E, E_1, E_2 and E3) can be considered to be in a pseudo-steady state [11]. A numerical simulation of time course of various enzymatic forms was undertaken (data not shown), in order to test the pseudo-steady state assumption for the concentration of the four HRP forms. The pseudo-steady-state assumption was thus considered valid. In the case of PSA, the steady state of the enzyme forms distribution is only achieved after 0.02 s, while the time course of the concentration of the chemical reactants is much slower. Therefore, as the time required for the system to assume steady state concentrations of the enzymatic forms can be neglected when compared to the time scale of the consumption of the reagents and formation of the product, the pseudo-steady-state assumption was considered acceptable. The balance equations for the enzyme reaction mechanism (Eqns. (1)–(7)) are given as follows:

$$\frac{dE}{dt} = -k_1 R\, E + k_2 R_1\, E_2 + k_a\, E_3 \tag{8}$$

$$\frac{dE_1}{dt} = k_1 R\, E - k_2 R_1\, E_1 + k_b R_1\, E_3 \tag{9}$$

$$\frac{dE_2}{dt} = k_2 R_1\, E_1 - k_3 R_1\, E_2 - k_{app} R\, E_2 \tag{10}$$

$$\frac{dE_3}{dt} = k_{app} R\, E_2 - k_a\, E_3 - k_b R_1\, E_3 \tag{11}$$

The Initial conditions are

t=0; $E=E_0$, $E1=E_{10}$, $E_2=E_{20}$ and $E_3=E_{30}$ (12)

Time derivatives of phenolic compound and H_2O_2 concentrations are calculated from differential mass balances Eqns. (1)–(7):

$$\frac{dR}{dt} = -k_1 R\, E - k_r R_1\, R_3\, R - k_{app}\, R\, E_2 \tag{13}$$

$$\frac{dR_1}{dt} = -k_2 R_1 E_1 - k_3 R_1 E_2 + k_r R^2{}_1 R_3 R - k_b R E_3 \tag{14}$$

$$\frac{dR_2}{dt} = k_2 R_1 E_1 + k_3 R_1 E_2 - 2k_r R^2{}_1 R_3 R + k_b R E_3 \tag{15}$$

$$\frac{dR_3}{dt} = -k_r R^2{}_2 R_3 R_1 \tag{16}$$

$$\frac{dS}{dt} = k_r R^2{}_2 R_3 R_1 \tag{17}$$

The Initial conditions are

t=0; R=R_0, R_1=R_{10}, R_2 = R_{20}, R_3=R_{30} and S=S_0 (18)

3. ANALYTICAL EXPRESSIONS FOR CONCENTRATIONS OF THE ENZYMES

Recently, many authors have applied the modified HPM to various problems and demonstrated the efficiency of the HPM for handling non-linear structures and solving various physics and engineering problems [12–17]. This method is a combination of Homotopy in topology and classic perturbation techniques. The modified HPM is unique in its applicability, accuracy and efficiency. The modified HPM 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 Eqns.8-11 for the given boundary conditions (Eqn.12).

$$E(t) = \left[\frac{E_0 k_1 R_0 - k_3 R_{10} E_{20} - k_a E_{30}}{k_1 R_{10}}\right] e^{-k_1 R_0 t} + \left[\frac{k_1 R_{10} E_{20} + k_a E_{30}}{k_1 R_{10}}\right] \tag{19}$$

$$E_1(t) = \left[\frac{E_{10} k_2 R_{10} - k_1 R_0 E_0 - k_b R_{10} E_{30}}{k_2 R_{10}}\right] e^{-k_2 R_{10} t} + \left[\frac{k_1 R_0 E_0 + k_b R_{10} E_{30}}{k_2 R_{10}}\right] \tag{20}$$

$$E_2(t) = \left[\frac{E_{20}(k_3 R_{10} + k_{app} R_0) - k_2 R_{10} E_{10}}{k_3 R_{10} + k_{app} R_0}\right] e^{-(k_3 R_{10} + k_{app} R_0)t} + \left[\frac{k_2 R_{20} E_{10}}{k_3 R_{10} + k_{app} R_0}\right] \tag{21}$$

$$E_3(t) = \left[\frac{E_{30}(k_a + k_b R_{10}) - k_{app} R_0 E_{20}}{k_a + k_b R_{10}}\right] e^{-(k_a + k_b R_{10})t} + \left[\frac{k_{app} R_0 E_{20}}{k_a + k_b R_{10}}\right] \tag{22}$$

4. ANALYTICAL EXPRESSIONS FOR CONCENTRATIONS OF PHENOLIC COMPOUND AND H_2O_2

In order to solve the initial value problem, Eqns. (8)-(12), we used the modified HPM [12-15]. As a result we have

$$\text{Obtained } R(t) = \left[\frac{R_0 (k_1 E + k_{app} E_2) - k_r R_{30} R_{20}^2 R_0}{k_1 E + k_{app} E_2}\right] e^{-(k_1 E + k_{app} E_2)t} + \left[\frac{k_r R_{30} R_{20}^2 R_0}{k_1 E + k_{app} E_2}\right] \tag{23}$$

$$R_1(t) = \left[\frac{R_{10}(k_2 E_1 + k_3 E_2 + k_b E_3) - k_r R_{30} R_{20}^2 R_0}{k_2 E_1 + k_3 E_2 + k_b E_3}\right] e^{-(k_2 E_1 + k_3 E_2 + k_b E_{33})t} + \left[\frac{k_r R_{30} R_{20}^2 R_0}{k_2 E_1 + k_3 E_2 + k_b E_3}\right] \tag{24}$$

$$R_3(t) = R_{30} + \left[k_r\, R_{30}\, R_{20}^2\, R_0 - R_{10}\right](e^{-(k_2 E_1 + k_3 E_2 + k_b E_{33})t} - 1) - 2k_r\, R_{30}\, R_{20}^2\, R_0\, t \tag{25}$$

$$R_3(t) = R_{30} - k_r R^2{}_{20}\, R_{30}\, R_{10}\, t \tag{26}$$

$$S(t) = S_0 + k_r R^2{}_{20}\, R_{30}\, R_{10}\, t \tag{27}$$

5. DISCUSSION

Eqns. (20)-(24) and Eqns. (25)-(26) are the new and simple analytical expressions of concentration of enzyme E, E_1, E_2 and E_3, phenolic compound and H_2O_2 calculated using modified homotopy perturbation method. Figure (1)-(4), analytical expressions of concentration enzyme E, E_1, E_2 and E_3 for various values of kinetic parameters $k_1, k_2, k_3, k_a, k_b, k_r$ and k_{app} versus time *t*are compared with numerical solution. From these figures, it is inferred that the value of the enzyme E, E_1, E_2 and E_3 attains steady state value when $t \geq 4$. Fig (5)-(6) indicates the concentration of phenolic compound and H_2O_2 using Eqns. (25) and (26) for various values of kinetic parameters. Upon careful evaluation of these figures, it is evident that concentrations is uniform when $t \geq 2$.

6. NUMERICAL SIMULATION

The non-linear differential equations in enzyme reaction mechanisms are solved by numerical method. To show that efficiency of the present method, our analytic result is compared with numerical solution in Figs.1-6 satisfactory agreement is noted. The Scilab/Matlab program is also give in Appendix A and Appendix B.

7. CONCLUSION

A nonlinear time dependent ordinary differential equation has been solved analytically and numerically. The primary result of this work is first appropriate calculation concentration of enzymeE, E_1, E_2 and E_3, phenolic compound and H_2O_2 for all values of kinetic parameter. The modified homotopy perturbation method is extremely simple and promising method to solve other non-linear equations. This work can be easily extended to find the solution for other enzymatic mechanism in biochemical and medical systems.

Appendix A

Matlab / Scilab program to find the numerical solution of Eqns. (8) - (12)

```
function
options= odeset ('RelTol',1e-6,'Stats','on');
%initial conditions
Xo = [1;1;1;1];
tspan = [0,10];
tic
[t,X] = ode45(@TestFunction,tspan,Xo,options);
toc
figure
hold on
plot(t, X(:,1),'-')
plot(t, X(:,2),'-')
plot(t, X(:,3),'-')
plot(t, X(:,4),'-')
legend('x1','x2','x3','x4')
ylabel('x')
xlabel('t')
return
function [dx_dt]= TestFunction(t,x)
R1=1; R2=1;k1=0.01;k2=1;k3=1;ka=0.01;kb=0.01;kapp=1;
dx_dt(1) = -k1*R1*x(1)+k3*R2*x(3)+ka*x(4);
dx_dt(2) = k1*R1*x(1)-k2*R2*x(2)+kb*R2*x(4);
dx_dt(3) = k2*R2*x(2)-(k3*R2+kapp*R1)*x(3);
dx_dt(4) = kapp*R1*x(3)-(ka+(kb*R2))*x(4);
dx_dt = dx_dt';
return
```


Appendix B

Matlab / Scilab program to find the numerical solution of Eqns. (13) - (18)

```
function
options= odeset ('RelTol',1e-6,'Stats','on');
%initial conditions
Xo = [1;1;1;1;1];
tspan = [0,1];
tic
[t,X] = ode45(@TestFunction,tspan,Xo,options);
toc
figure
hold on
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)
E=0.1;E1=0.1;E2=0.1;E3=1;k1=0.01;k2=1;k3=1;kr=0.01;kb=0.01;kapp=1;
dx_dt(1) = -k1*E*x(1)-kr*x(1)*x(3)*x(3)*x(4)-kapp*E2*x(2);
dx_dt(2) = -k2*E1*x(2)-k3*E2*x(2)+kr*x(1)*x(3)*x(3)*x(4)-kb*E3*x(2);
dx_dt(3) = k2*E1*x(2)+k3*E2*x(2)-2*kr*x(1)*x(3)*x(3)*x(4)+kb*E3*x(2);
dx_dt(4) = -kr*x(1)*x(3)*x(3)*x(4);
dx_dt(5) = -kr*x(1)*x(3)*x(3)*x(4);
dx_dt = dx_dt';
return
```

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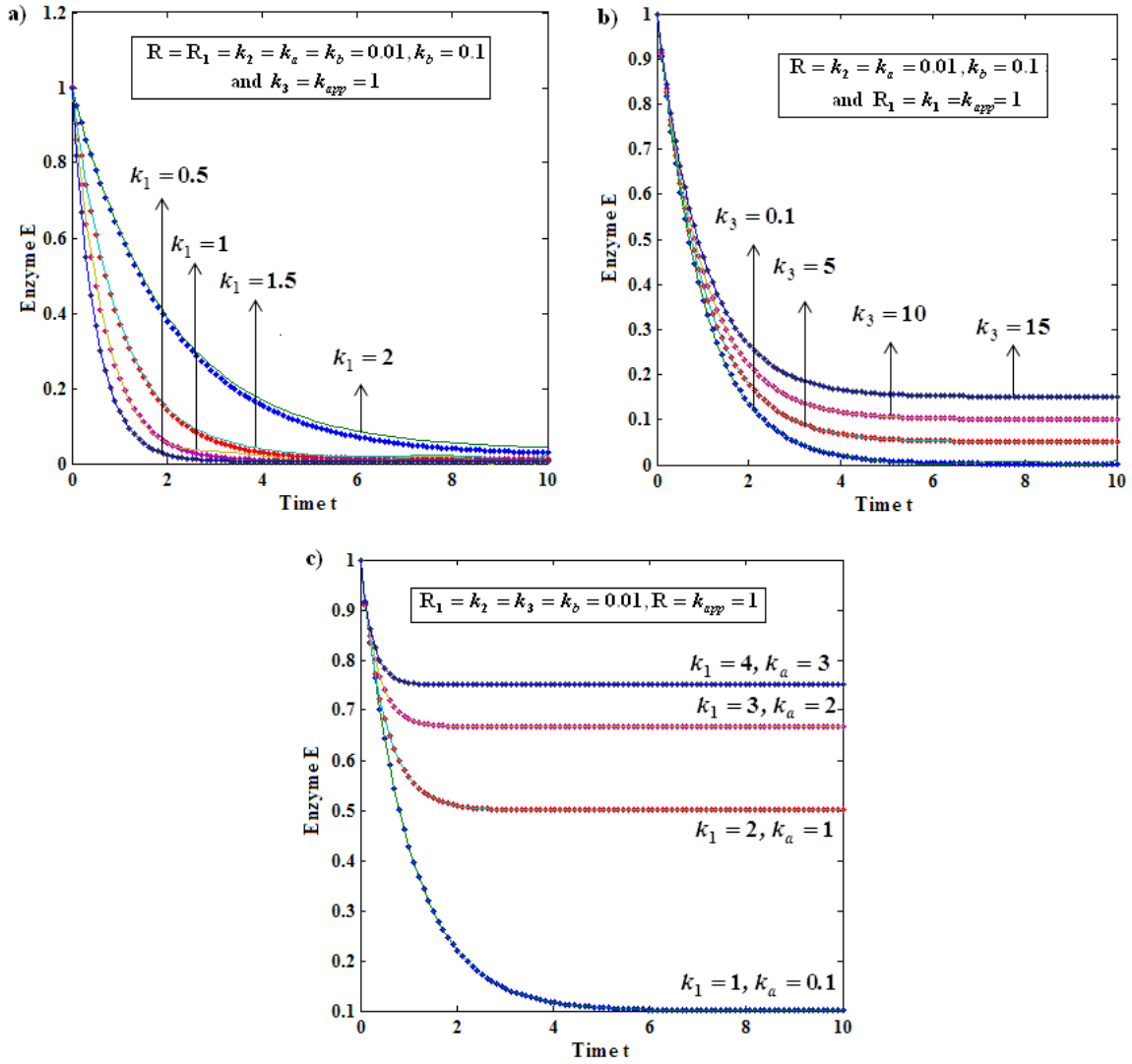
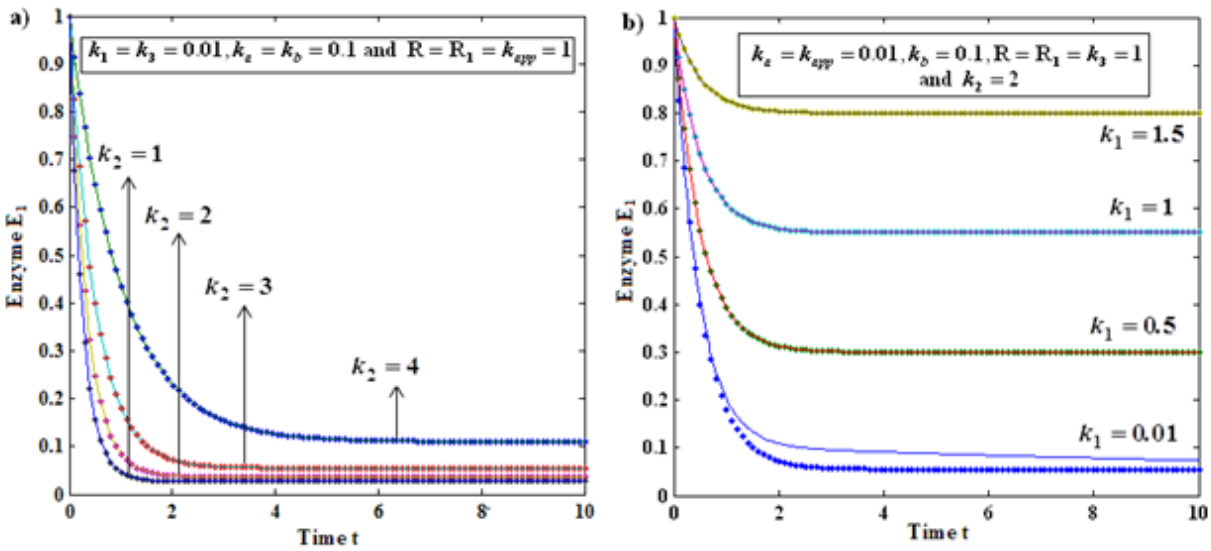


Fig. 1: Enzyme E versus time *t* is plotted using equation (19). (a) For various values of kinetic parameter k_1. (b) For various values of kinetic parameter k_3. (c) For various values of kinetic parameter k_1 and k_a.



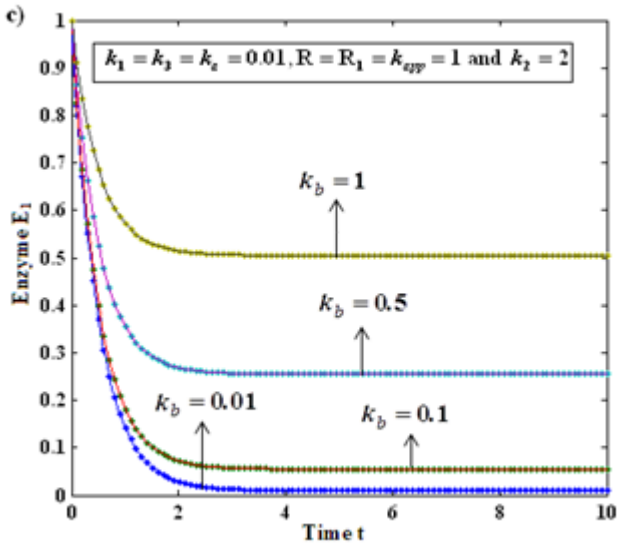


Fig. 2: Enzyme E_1 versus time t is plotted using equation (20). (a) For various values of kinetic parameter k_2. (b) For various values of kinetic parameter k_1. (c) For various values of kinetic parameter k_b.

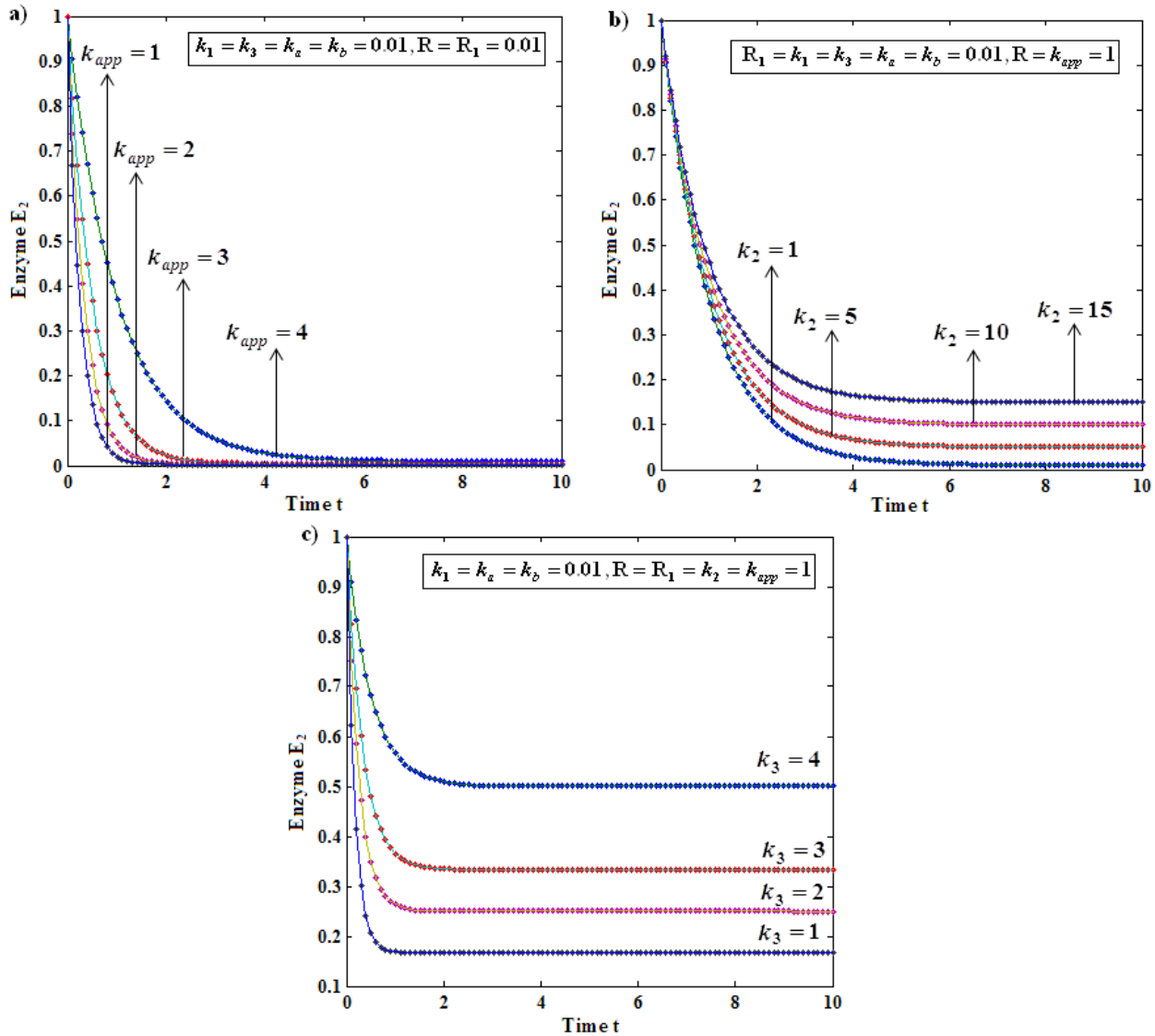


Fig. 3: Enzyme E_2 versus time t is plotted using equation (21). (a) For various values of kinetic parameter k_{app}. (b) For various values of kinetic parameter k_2. (c) For various values of kinetic parameter k_3.

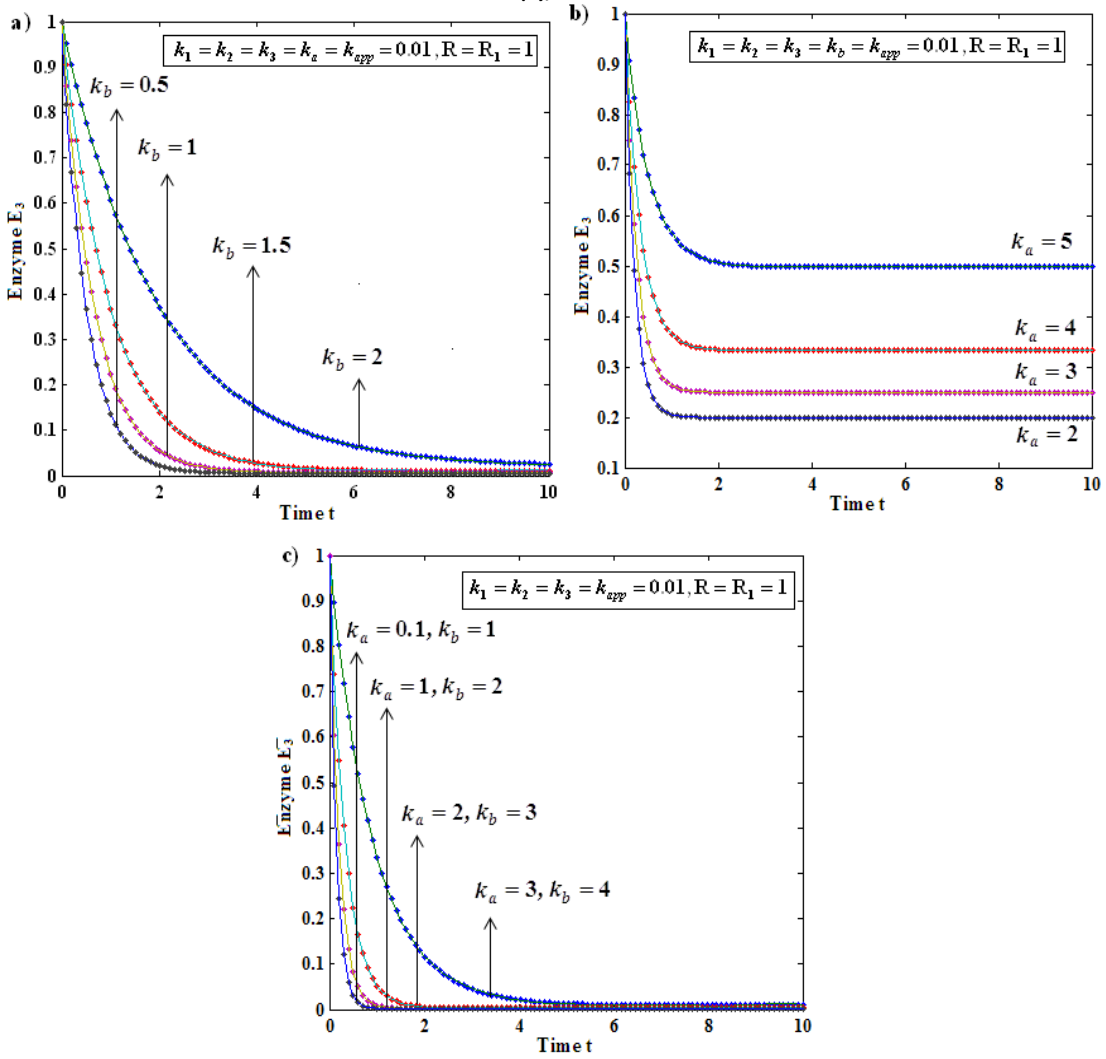


Fig. 4: Enzyme E_3 versus time *t* is plotted using equation (22). (a) For various values of kinetic parameter k_b. (b) For various values of kinetic parameter k_a. (c) For various values of kinetic parameter k_a and k_b.

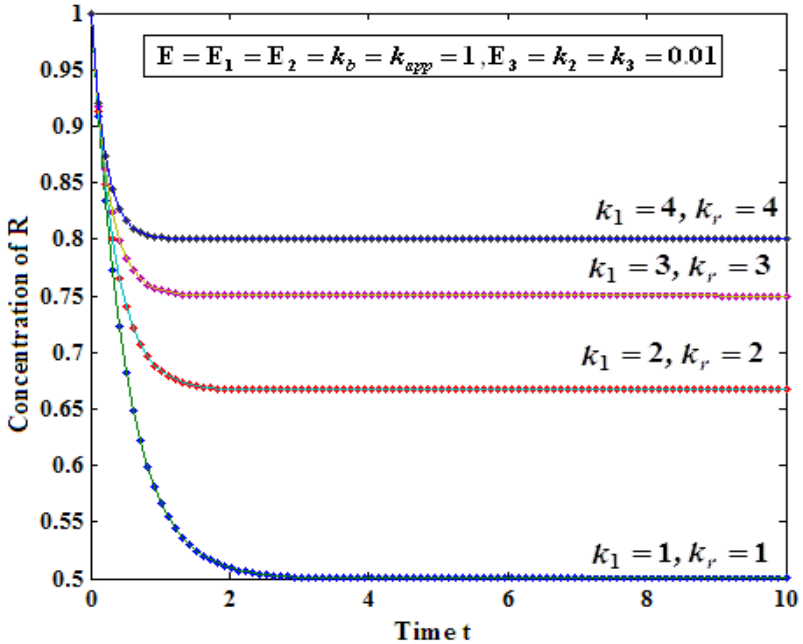


Fig. 5(a): Plot of concentration R versus time *t*. The concentration was computed for various values of the kinetic parameter k_1 and k_r. The curves are plotted using equation (23). (—) denotes the analytical results and (●●●) denotes the numerical simulations.

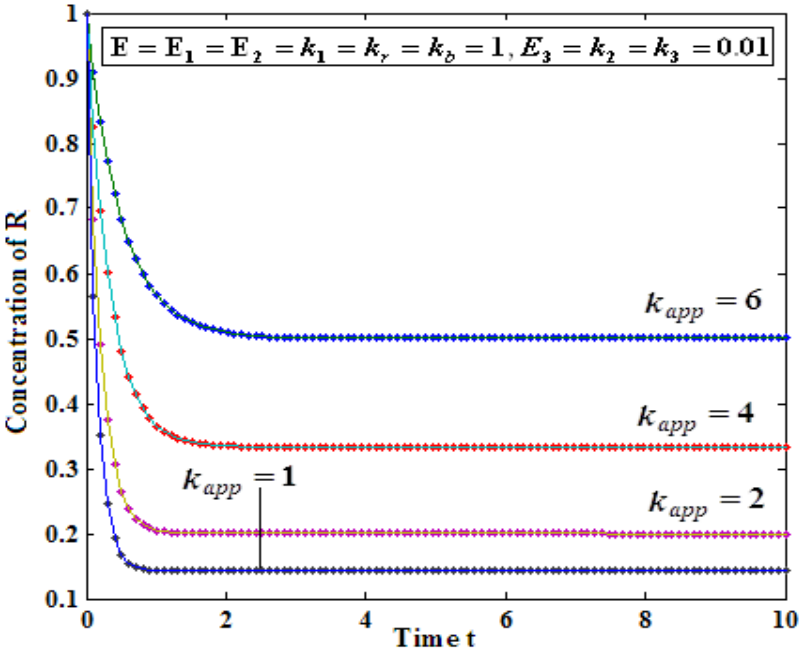


Fig. 5(b): Plot of concentration R versus time *t*. The concentration was computed for various values of the kinetic parameter k_{app}. The curves are plotted using equation (23). (—) denotes the analytical results and (●●●) denotes the numerical simulations.

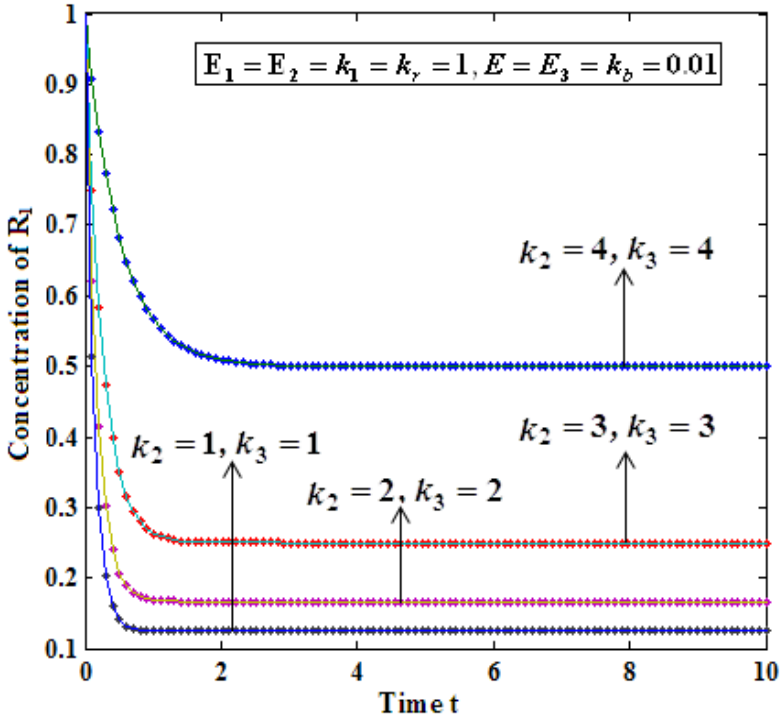


Fig. 6(a): Plot of concentration R_1 versus time *t*. The concentration was computed for various values of the kinetic parameter k_2 and k_3. The curves are plotted using equation (24). (—) denotes the analytical results and (●●●) denotes the numerical simulations.

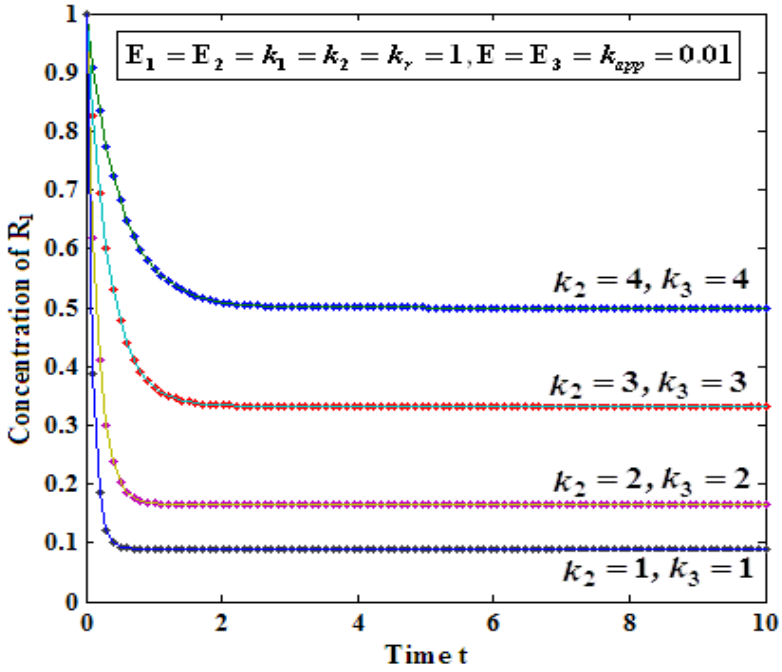


Fig. 6(b): Plot of concentration R_1 versus time *t*. The concentration was computed for various values of the kinetic parameter k_2 and k_3. The curves are plotted using equation (24). (—) denotes the analytical results and (●●●) denotes the numerical simulations.

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