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A Kinetic Model for Amperometric Biosensor at Mixed Oxidase Enzyme

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In this paper the response of an amperometric biosensor at mixed enzyme kinetics and diffusion limitation in the cases of both enzyme entrapped within the conducting polymer film and in bulk solution is discussed. The model is based on diffusion equations containing a non-linear term related to Michaelis–Menten kinetics of the enzymatic reaction processes. New Homotopy perturbation method is employed to solve the system of coupled non-linear diffusion equations for the non-steady-state condition. Simple and accurate general analytical expressions of concentration of substrate, hydrogen peroxide and flux are derived for all possible values of parameters. A numerical simulation was carried out using Matlab/Scilab program. The analytical results are compared with the numerical results and found to be in good agreement. The results obtained in this work are valid for the entire solution domain

Keywords: Amperometric biosensors, Kinetic model, Hydrogen peroxide, Non-linear equations, New Homotopy approach, Mathematical modelling.

1. INTRODUCTION

The development of amperometric biosensor is one of the major areas of interest concerning research in the detection of substances. Amperometric biosensors, commonly also referred to as enzyme electrodes, constitute a rapidly growing area of interest to biotechnologists. These devices combine the analytical power of electrochemistry with the specificity of biological catalysts for particular substrates. Amperometric biosensor is a type of biosensor which measures the change in the current of a working indicator electrode by direct electrochemical oxidation or reduction of the products of a biochemical reaction. In these types of biosensors, the potential at the electrode is made constant during the measurement of current. These are known to be reliable, cheap and highly sensitive for environment, clinical and industrial purposes.

Amperometric biosensors based on oxidase enzymes that generate H_2O_2 are the most widely used biosensors, the transduction path being the electrochemical oxidation of the peroxide formed in an enzyme reaction. In this case, the electrode response is dependent on oxygen concentration in the reaction medium [1-4]. Oxidase enzymes are commonly used in amperometric biosensors. This is due to wide commercial availability and because the oxidase reaction involves electroactive species. Wang et al. [5-7] have shown that cathodic detection can be made possible, by using a noble metal catalyst of microscopic dimensions. Somasundrum et al. [8,9] have shown that conducting polymer films can also be used for this purpose, following the electrodeposition of Rh microparticles.

Mathematical model relating the various experimental parameters (enzyme loading, film thickness, etc.) to the electrode response is useful for further understanding of the microparticle catalytic properties [10]. Currently, no such mathematical model exists. However related models have been reported both for an enzyme within a microparticle-free conducting polymer film [11], and for an enzyme-free microheterogenous coating [12]. Wang et al. [13,14] presented a concise discussion about carbon paste containing dispersed microparticles of metals such as rhodium or platinum can reduce H_2O_2 without significantly reducing O_2 . This observation is shown that the microparticles play a vital role in selective reduction of H_2O_2.

Romero et al. [15] presented a comprehensive numerical treatment of the diffusion and reaction within sandwich-type amperometric biosensor. Transient response of electrochemical biosensors with asymmetrical sandwich membranes [16] and enzyme electrode [17] are discussed. Baronas et al. [18] developed the mathematical model of amperometric biosensor. Pierre Gros and Alain Bergel [19] showed the experimental study of electrodes modified by entrapment of glucose oxidase in an insulating polypyrrole film.

Earlier, mathematical expressions pertaining to analytical concentration and current for limiting cases in a conducting polymer film were calculated by Bartlett and Whitaker [11]. Somasundrum et al. [20] suggested a mathematical model of the steady state current and the function and optimization of metal particles deposited in a conducting polymer for the limiting cases only (high and low substrate concentration). However, to the best of our knowledge, till date no simple analytical results for the concentrations of substrate and hydrogen peroxide for all values of the parameters have been reported. The purpose of this communication is to derive analytical expressions for concentrations of substrate and hydrogen peroxide in a conducting film containing metal microparticles using new Homotopy perturbation method. This method is very easy compared to all other asymptotic methods. This simple analytical expression of concentrations of substrate, hydrogen peroxide and flux is very much useful to the electrochemical scientists for the analysis of experimental data. The analytical results have been extended numerically and are to be in good agreement with each other.

2. MATHEMATICAL FORMULATION OF PROBLEMS AND ANALYSIS

2.1. Mathematical formulation

The governing parameters for the Michaelis-Menten kinetics of enzymatic reactions are the enzyme kinetic rate and the diffusion rate across the enzymatic layer. In Michaelis-Menten reaction

scheme, the enzyme E_1 converts the oxidation of substrate (S) into the product (P) through a two-step process. First E_1 combines with S to form a complex E_1S which then breaks down into the product P releasing E_2 in the process. The reaction scheme is represented schematically by

$$S + E_1 \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} [E_1S] \overset{k_{cat}}{\rightarrow} P + E_2 \quad (1)$$

Eq. (1) gives the reduction of the enzyme from E_1 to E_2. In this reaction scheme, the re-oxidation of the enzyme reacts with oxygen (A) producing hydrogen peroxide (B), and then B which reacts at a microparticle with a pseudo first-order rate constant (*k*) producing water (C).

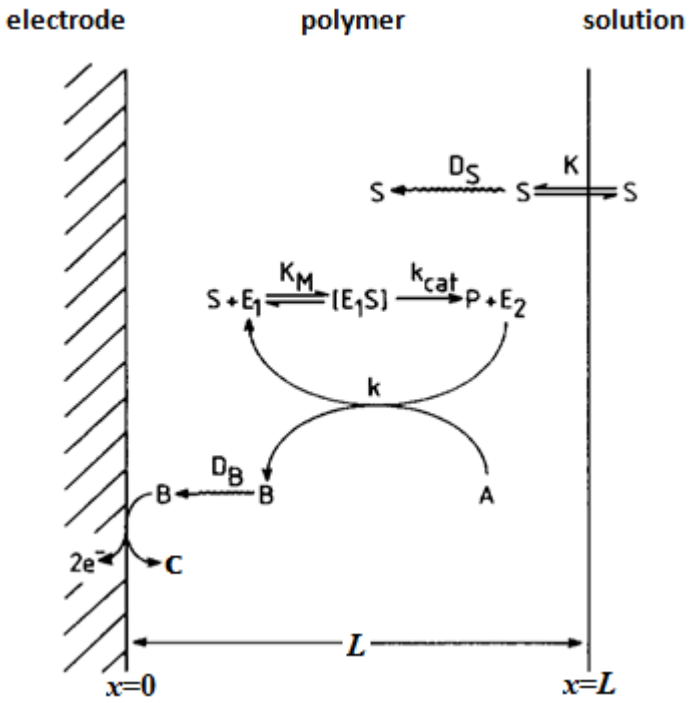

Figure 1. Schematic diagram of possible mechanisms occurring at an enzyme electrode.

A general scheme (Fig. 1) that represents the reaction occurring at a microparticle is shown below:

$$E_2 + A \overset{k_e}{\rightarrow} E_1 + B \quad (2)$$

$$B + 2e^- \overset{k}{\rightarrow} C \quad (3)$$

At steady-state mass balances within the film for the above enzymatic reaction, leads to the following system of non-linear differential equations [20]:

$$D_S \frac{d^2 s}{dx^2} - \frac{k_{cat} s e_\tau}{K_M + s} = 0 \quad (4)$$

$$D_B \frac{d^2 b}{dx^2} - kb + \frac{k_{cat} s e_\tau}{K_M + s} = 0 \quad (5)$$

with boundary conditions [11]

$$\text{At } x = 0,\ \frac{ds}{dx} = 0,\ b = 0 \tag{6}$$

$$\text{At } x = L,\ s = k_s s_\infty,\ b = 0 \tag{7}$$

Where x stands for space, $s(x)$ and $b(x)$ denote the concentration functions of the substrate and, hydrogen peroxide respectively. e_τ is the total enzyme concentration in the film $(e_\tau \approx e_1 + e_2)$, D_S is the diffusion coefficient of substrate into polymer film, D_B is the diffusion coefficient of hydrogen peroxide into polymer film, K_M is the Michaelis-Menten constant, k_{cat} is the catalytic reaction rate constant, k_s is the partition coefficient of substrate in the film,L is film thickness, k is the change transfer rate constant for oxidation of hydrogen peroxide and s_∞ denotes concentration of substrate in the bulk solution. The flux of hydrogen peroxide reacting at the electrode surface is

$$j_b = D_B \left(\frac{db}{dx} \right)_{x=0} \tag{8}$$

2.2. Normalized form

The governing differential equations are non-dimensionalized using the following appropriate normalizing parameters.

$$u = \frac{s}{k_s s_\infty};\ v = \frac{b}{k_s s_\infty};\ X = \frac{x}{L};\ \chi_s = \left(\frac{D_S K_M}{k_{cat} e_\tau} \right)^{1/2};\ \chi_b = \left(\frac{D_B}{k} \right)^{1/2};\ \alpha = \frac{L}{\chi_s};\ \alpha^1 = \frac{L}{\chi_b};\ \beta = \frac{k_s s_\infty}{K_M} \tag{9}$$

Using the above normalizing parameters, the equations (4) and (5) can be written as follows:

$$\frac{d^2 u}{dX^2} - \frac{\alpha^2 u}{1 + \beta u} = 0 \tag{10}$$

$$\frac{d^2 v}{dX^2} - \alpha^{1^2} v + \frac{\alpha^2 u}{1 + \beta u} = 0 \tag{11}$$

Where χ_s is the kinetic length of the substrate and χ_b is the kinetic length of the hydrogen peroxide and u and v represent the normalised concentrations of substrate and hydrogen peroxide. The boundary conditions in non-dimensional form for the studied cases are:

$$\text{when } X = 0,\ \frac{du}{dX} = 0,\ v = 0 \tag{12}$$

$$\text{when } X = 1,\ u = 1,\ v = 0 \tag{13}$$

The normalised flux is expressed as,

$$\psi = \frac{j_b L}{D_B k_s s_\infty} = \left(\frac{dv}{dX} \right)_{X=0} \tag{14}$$

3. ANALYTICAL EXPRESSION OF THE CONCENTRATION AND FLUX USING NEW HOMOTOPY APPROACH

Recently, Rajendran and Anitha [21] have applied the new Homotopy approach to non linear problems and demonstrated the efficiency of the new Homotopy approach for non-mathematicians. By using this method (Appendix – A), the concentrations of substrate and hydrogen peroxide can be obtained as follows:

$$u(X) = u_0(X) = \cosh\left(\frac{\alpha}{\sqrt{1+\beta}}X\right)\sec h\left(\frac{\alpha}{\sqrt{1+\beta}}\right) \qquad (15)$$

$$v(X) = v_0(X) = \frac{\alpha^2}{\left(\alpha^2 - \alpha^{1^2}(1+\beta)\right)}\left[\frac{\sinh\left(\alpha^1(1-X)\right)}{\sinh\left(\alpha^1\right)\cosh\left(\frac{\alpha}{\sqrt{1+\beta}}\right)} - \frac{\sinh\left(\alpha^1 X\right)}{\sinh\left(\alpha^1\right)} - \frac{\cosh\left(\frac{\alpha}{\sqrt{1+\beta}}X\right)}{\cosh\left(\frac{\alpha}{\sqrt{1+\beta}}\right)}\right]$$

(16)

Using Eq. (14), we can obtain the normalised flux as follows:

$$\psi = \frac{\alpha^2\alpha^1}{\left(\alpha^2 - \alpha^{1^2}(1+\beta)\right)}\left[\cos ech\left(\alpha^1\right) - \coth\left(\alpha^1\right)\sec h\left(\frac{\alpha}{\sqrt{1+\beta}}\right)\right] \qquad (17)$$

Eq. (15) and Eq. (16) represent the new simple analytical expression of the concentrations of the substrate and hydrogen peroxide for all values of parameters α, α^1 and β. Eq. (17) is the simple analytical expression of normalised flux for all values of parameters α, α^1 and β.

4. NUMERICAL SIMULATION

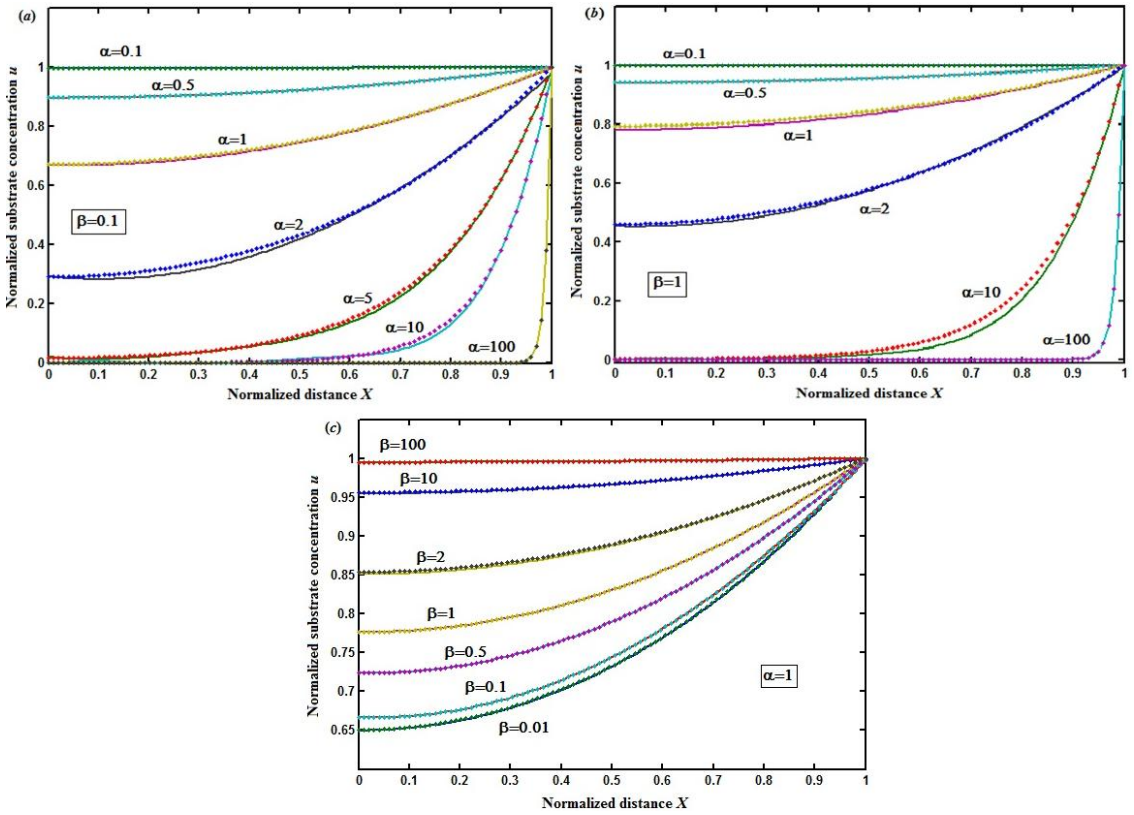

Figure 2. Normalized concentration profiles of substrate calculated from Eq. (15), for different values of α; α^1; β when (*a*) $\beta = 0.1$, (*b*) $\beta = 1$ and (*c*) $\alpha = 1$. Solid lines represent the analytical solution obtained in this work; dotted lines represent the numerical solution.

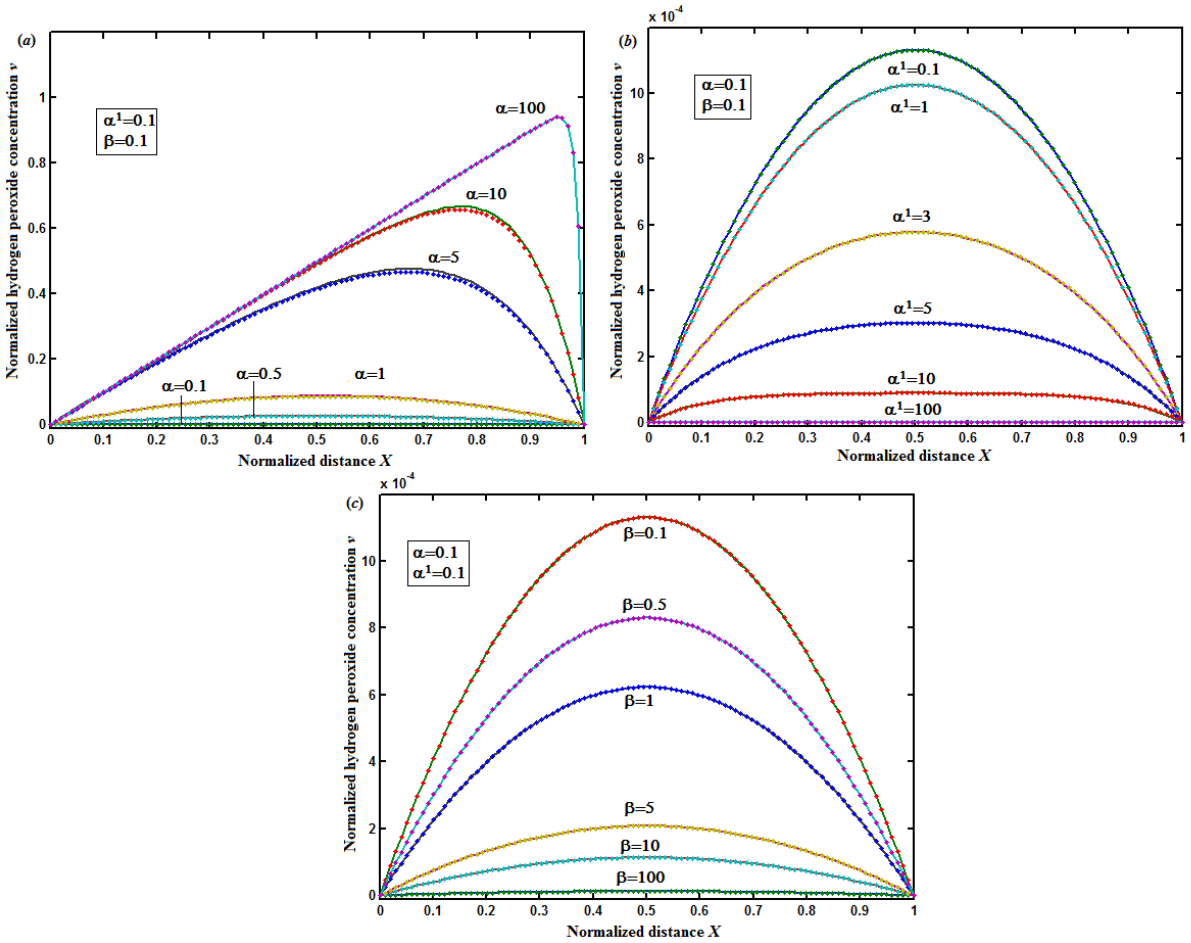

Figure 3. Normalized concentration profiles of hydrogen peroxide calculated from Eq. (16) when (*a*) $\alpha^1 = 0.1;\ \beta = 0.1$, (*b*) $\alpha = 0.1;\ \beta = 0.1$ and (*c*) $\alpha = 0.1;\ \alpha^1 = 0.1$. Solid lines represent the analytical solution obtained in this work; dotted lines represent the numerical solution.

The function pdex4 in Matlab/Scilab software which is a function of solving the initial boundary value problems for parabolic-elliptic partial differential equations is used to solve the equation (10) and (11).

Figures 2-4 illustrate the comparison of analytical result obtained in this work with the numerical result. Upon comparison, it is evident that both the results give satisfactory agreement. The Matlab/Scilab program is also given in Appendix -B.

5. DISCUSSION

Figs. 2-4 show the normalized concentration profiles of substrate and hydrogen peroxide in the film which are calculated from Eqs. (15) and (16) respectively. The distributions of substrate and hydrogen peroxide are shown to be dependent on the value of kinetic length $\alpha = L/\chi_s$ and $\alpha^1 = L/\chi_b$.

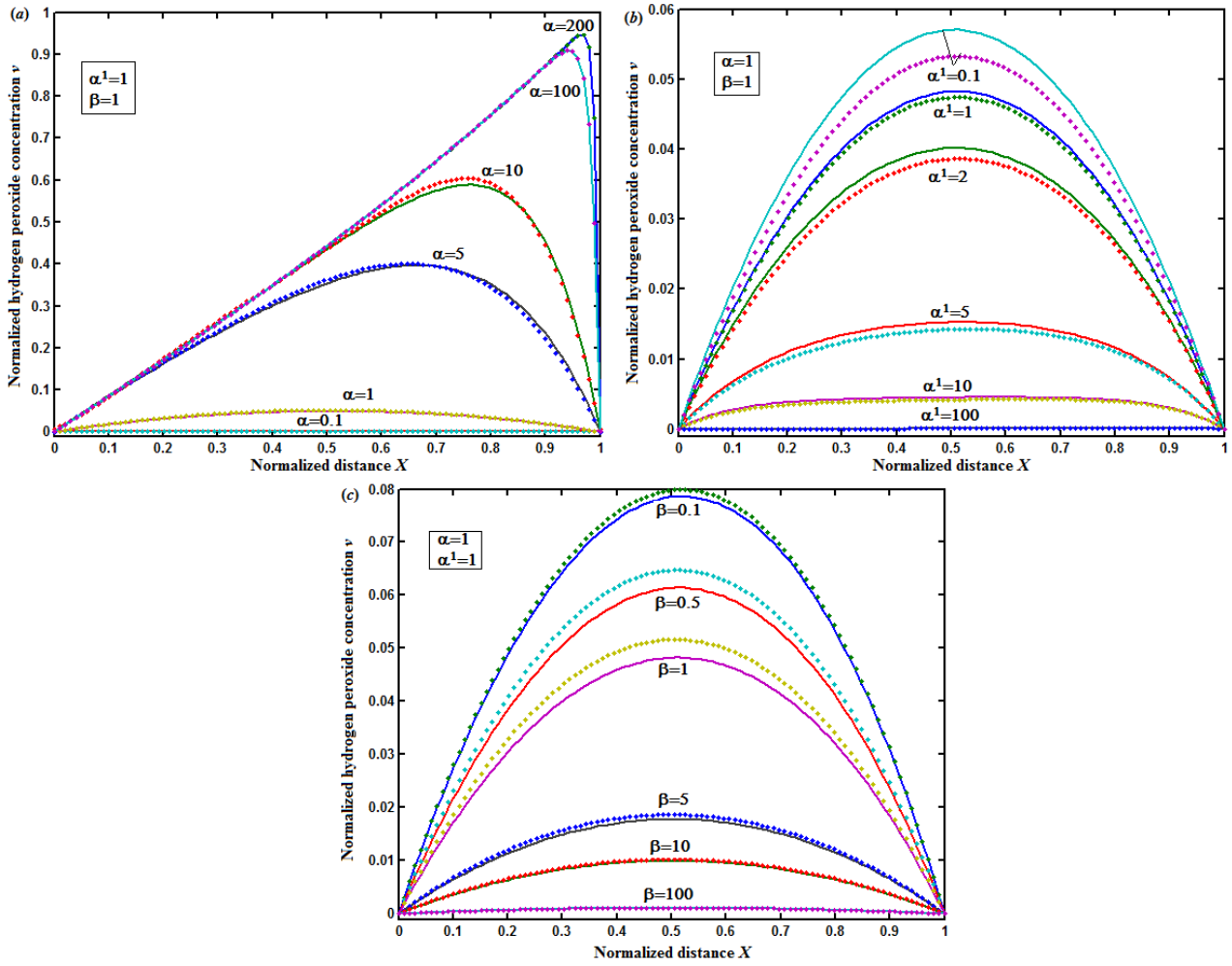

Figure 4. Plot of the normalized concentration profiles for hydrogen peroxide for various values of (*a*) normalised film thickness, L/χ_s, (*b*) the normalised film thickness, L/χ_b, and (*c*) the normalised parameter β. The curves are calculated from Eq. (16).

Concentration profiles in the film may be helpful to understand the present model in detail. Figs. 2(*a*)-(*c*) represent the concentration profiles of the substrate for various values of the parameters α and β. From figures 2(*a*)-(*b*), it is inferred that the value of the concentration *u* increases when $\alpha = L/\chi_s$ decreases. Concentration of the substrate is uniform when $\alpha = L/\chi_s < 0.5$ or width of the film is small. For small value of $\alpha = L/\chi_s$, substrate diffuses into the electrode surface.

Figs. 3-4 show the concentration profiles of hydrogen peroxide for several values of the parameters α, α^1 and β. From figs. 3(*a*) and 4(*a*), it is evident that initially the value of the concentration of hydrogen peroxide increases and attains the maximum value and then decreases gradually for all values of α. The value of hydrogen peroxide reaches the maximum value at X=0.5 for all values of α^1 and β in figs. 3(*b*)-(*c*) and 4(*b*)-(*c*).

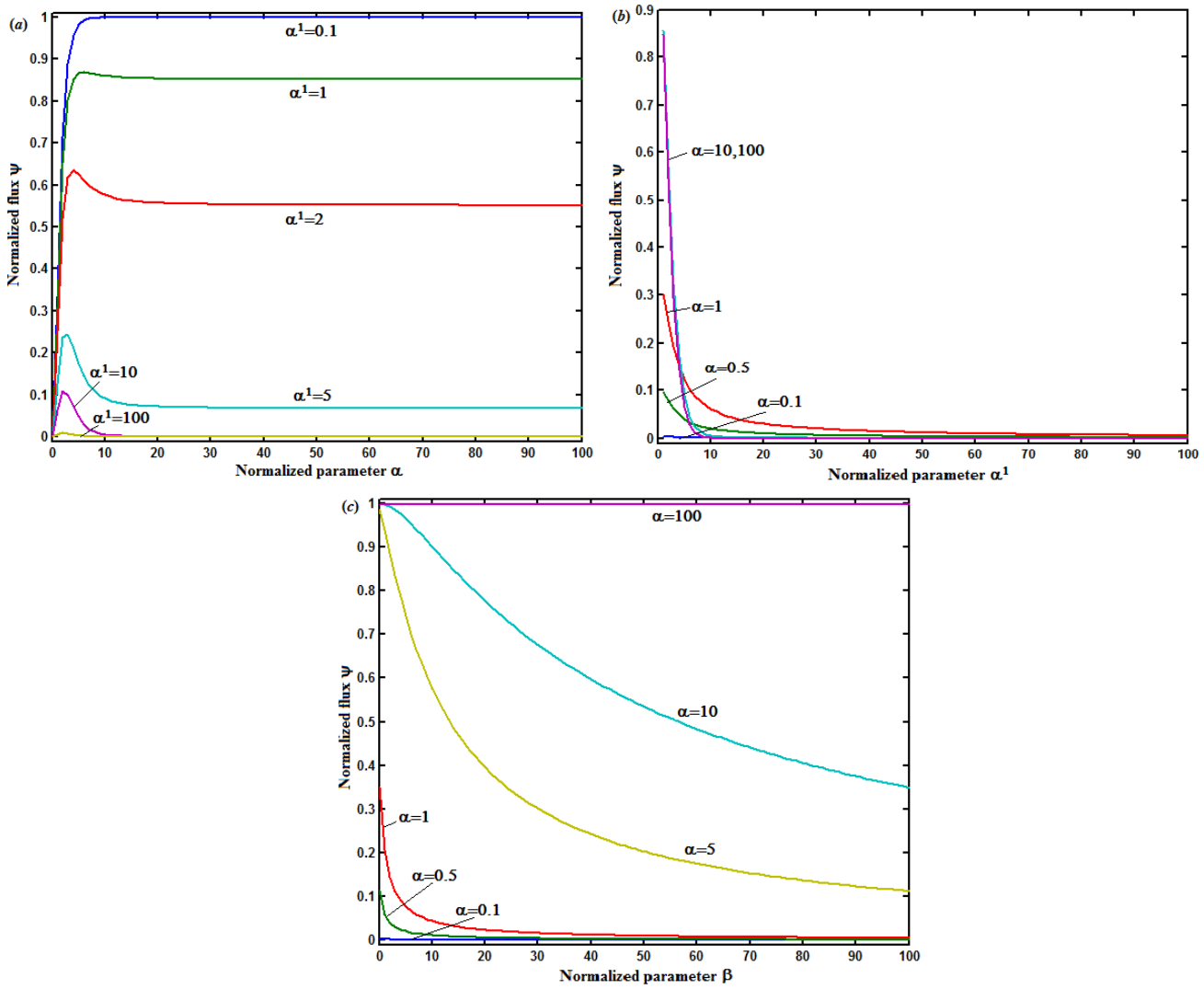

Figure 5. Plot of the normalised flux, ψ, against (*a*) the normalised parameter α and (*b*) the normalised parameter α^1 and (c) the normalised parameter β. The curves are calculated from Eq. (17).

The normalized flux ψ is plotted in Figs. 5(*a*)-(*c*). It illustrates that the flux for all values of $\alpha = L/\chi_s$ and $\alpha^1 = L/\chi_b$. In fig. 5(*a*), the flux increases when the parameter α^1 decreases. The flux reaches the steady state value when $\alpha = L/\chi_s \geq 20$, for all values of $\alpha^1 = L/\chi_b$. From fig. 5(*b*), it is observed that the flux increases as α increases and when $\alpha^1 \geq 20$, all the curves reach the steady state value. From fig. 5(*c*), it is known that the flux increases when α increases.

Recently Anitha et.al [22] studied a theoretical model for an amperometric enzyme electrodes based on an immobilized flavoprotein. When $D_S = D_B$, and $\alpha^1 = 0$ $(\chi_b \to \infty)$, Anitha et.al [22] obtained the analytical expression for the concentration of substrate and hydrogen peroxide as follows:

$$u(X) = \frac{\cosh(\alpha X)}{\cosh(\alpha)} + \frac{\beta\{\cosh(\alpha)[3-\cosh(2\alpha X)] - \cosh(\alpha X)[3-\cosh(2\alpha)]\}}{6\cosh(\alpha)^3} \qquad (18)$$

$$v(X)=\left|\begin{array}{l}\dfrac{[(1-\cosh(\alpha X))-(1-\cosh(\alpha))X]}{\cosh(\alpha)} \\ +\dfrac{\beta\{(3-\cosh(2\alpha))[\cosh(\alpha X)-1]-\cosh(\alpha)[1-\cosh(2\alpha X)]-[2\cosh(\alpha)+\cosh(2\alpha)-3]X\}}{6\cosh(\alpha)^3}\end{array}\right| \tag{19}$$

The expression of the normalized current becomes

$$\psi=1-\sec h(\alpha)+\frac{\beta[3-2\cosh(\alpha)-\cosh(2\alpha)]}{6\cosh(\alpha)^3} \tag{20}$$

The normalized current ψ in previous work (Eqn.(20)) is compared with our present work (Eq. (17)) in Figure-6 for $\beta \le 1$ and $\alpha^1 = 0.01$. From this figure, we conclude that the numerical value of current reported in previous work [22] (Eq. (20)) and our work Eq. (17) are same. From this figure, it is obvious that the values of the current reach the maximum value 1.

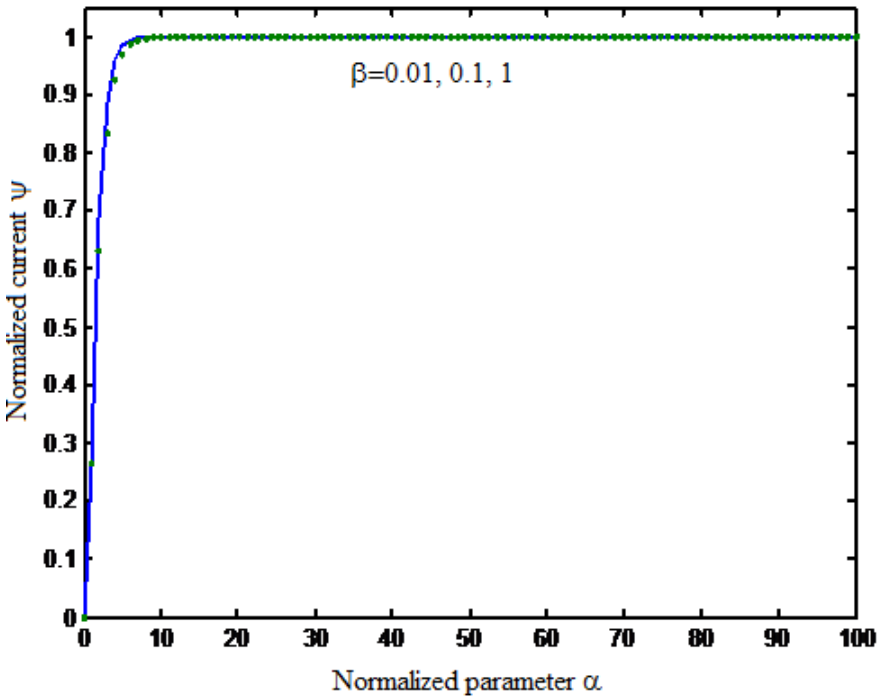

Figure 6. Plot of normalized current ψ versus α at mixed oxidase enzyme for $\beta \le 1$ when $\alpha^1 = 0.01$. Symbols: (—)Eq. (20); (• • •)Eq. (17). Solid lines are compared with points.

6. CONCLUSION

The mathematical model of an amperometric biosensor can be successfully used to investigate the response of biosensors when enzyme reacts with its substrate to produce hydrogen peroxide. The system of non-linear reaction diffusion equation in amperometric biosensor is solved analytically using new Homotopy perturbation method. This model can be used to investigate the regularities and

kinetics of the amperometric biosensor. This theoretical results will be used to investigate the biosensor response and current by altering this model parameters which influencing the enzyme kinetics as well as the mass transport by diffusion. Our study helps future researchers in better understanding of the application of an amperometric biosensor in suitable phase with the help of mathematical analysis.

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Appendix A:

Approximate analytical solution of the concentrations of the substrate and hydrogen peroxide using new Homotopy perturbation method.In this appendix, solution of non-linear system of equations (Eq. (10) and Eq. (11)) is derived using new Homotopy perturbation method.

$$`(1-p)\left\{\frac{d^2u}{dX^2}-\frac{\alpha^2 u}{\left(1+\beta u\left(X=1\right)\right)}\right\}+p\left\{u''+\beta uu''-\alpha^2 u\right\}=0` \quad \text{(A.1)}$$

$$(1-p)\left\{\frac{d^2v}{dX^2}-\alpha^{1^2}v+\frac{\alpha^2 u}{\left(1+\beta u\left(X=1\right)\right)}\right\}+p\left\{v''+\beta uv''-\alpha^{1^2}\beta uv+\alpha^2 u-\alpha^{1^2}v\right\}=0 \quad \text{(A.2)}$$

Supposing the approximate solutions of Eq. (A.1) and Eq. (A.2) have the form

$$\begin{cases} u=u_0+pu_1+p^2u_2+..... \\ v=v_0+pv_1+p^2v_2+..... \end{cases} \quad \text{(A.3)}$$

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

$$p^0:\frac{d^2u_0}{dX^2}-\frac{\alpha^2u_0}{\left(1+\beta\right)}=0 \quad \text{(A.4)}$$

$$p^1:\frac{d^2u_1}{dX^2}-\frac{\alpha^2u_1}{\left(1+\beta\right)}+\beta u_0\frac{d^2u_0}{dX^2}+\frac{\alpha^2u_0}{\left(1+\beta\right)}-\alpha^2u_0=0 \quad \text{(A.5)}$$

and

$$p^0:\frac{d^2v_0}{dX^2}-\alpha^{1^2}v_0+\frac{\alpha^2u_0}{\left(1+\beta\right)}=0 \quad \text{(A.6)}$$

$$p^1:\frac{d^2v_1}{dX^2}-\alpha^{1^2}v_1+\beta u_0\frac{d^2v_0}{dX^2}-\frac{\alpha^2u_0}{\left(1+\beta\right)}+\frac{\alpha^2u_1}{\left(1+\beta\right)}-\alpha^{1^2}u_0v_0+\alpha^2u_0=0 \quad \text{(A.7)}$$

The initial conditions are as follows:

$$du_0(X=0)/dX=0;\quad u_0(X=1)=1 \quad \text{(A.8)}$$

$$v_0(X=0)=0;\quad v_0(X=1)=0 \quad \text{(A.9)}$$

and

$$du_i(X=0)/dX=0;\ \ u_i(X=1)=0 \text{ for all } i=1,2,3,\ldots \quad \text{(A.10)}$$

$v_i(X=0)=0;\ \ v_i(X=1)=0$ for all i=1,2,3,… (A.11)

Solving the Eq. (A.4) and Eq. (A.6) and using the boundary conditions Eq. (A.8) and Eq. (A.9), we get

$$u_0(X)=\cosh\left(\frac{\alpha}{\sqrt{1+\beta}}X\right)\sec h\left(\frac{\alpha}{\sqrt{1+\beta}}\right) \quad \text{(A.12)}$$

$$v_0(X)=\frac{\alpha^2}{\left(\alpha^2-\alpha^{1^2}(1+\beta)\right)}\left[\frac{\sinh\left(\alpha^1(1-X)\right)}{\sinh\left(\alpha^1\right)\cosh\left(\frac{\alpha}{\sqrt{1+\beta}}\right)}-\frac{\sinh\left(\alpha^1X\right)}{\sinh\left(\alpha^1\right)}-\frac{\cosh\left(\frac{\alpha}{\sqrt{1+\beta}}X\right)}{\cosh\left(\frac{\alpha}{\sqrt{1+\beta}}\right)}\right] \quad \text{(A.13)}$$

Substituting the value of $u_0(X)$ in the Eq. (A.5) and solving the equations, using the boundary condition Eq. (A.10), we can obtain the value of $u_1(X)$. Similarly we can get the value of $v_1(X)$ by solving the Eq. (A.7). When p=1, the approximate solution Eq. (A.3) becomes

$$u(X)=u_0+u_1\approx u_0 \quad \text{(A.14)}$$

$$v(X)=v_0+v_1\approx v_0 \quad \text{(A.15)}$$

Using the above equations, we get equations. (15) and . (16) in the next.

Appendix B:

Matlab/Scilab program to find the numerical solution of equations (10)–(11)

```
function
m = 0;
x = linspace(0,1);
t = linspace(0,100);
sol = pdepe(m,@pdex4pde,@pdex4ic,@pdex4bc,x,t);
u1 = sol(:,:,1);
u2 = sol(:,:,2);
figure
plot(x,u1(end,:))
title('Solution at t = 2')
xlabel('Distance x')
ylabel('u1(x,2)')
figure
plot(x,u2(end,:))
title('Solution at t = 2')
xlabel('Distance x')
ylabel('u2(x,2)')
% --------------------------------------------------------------
function [c,f,s] = pdex4pde(x,t,u,DuDx);
h=0.1;
h1=0.1;
```

```
alpha=1;
beta=1;
c = [1;1];
f = [1; 1] .* DuDx;
F1 =-u(1)/(h^2*(1+alpha*u(1)));
F2 =-u(2)/(h1^2)+beta*u(1)/(h^2*(1+alpha*u(1)));
s =[F1; F2];
% --------------------------------------------------------------
function u0 = pdex4ic(x);
u0 = [1; 0];
% --------------------------------------------------------------
function [pl,ql;pr;qr] = pdex4bc(xl,ul,xr,ur,t);
pl = [0; 0];
ql = [1; 1];
pr = [ur(1)-1; ur(2)-1];
qr = [0; 0];
```

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