Analytical Expressions for an Electrochemical Biosensor employing the Enzymes Glucose Oxidase & Horseradish Peroxidase using the Homotopy Perturbation Method

*P. Felicia Shirly, ** V. Ananthaswamy, *** L. Rajendran

Abstract— A mathematical model of an electrochemical enzyme biosensor is analyzed. This model is based on the existence of a convection layer, where the glucose concentration is maintained constant and a diffusion layer. This article deals with approximate analytical expressions of the system of non–linear differential equations that describe the kinetics of the enzyme-substrate reactions, according to the Michaelis-Menten scheme. The analytical expressions for the enzymes, substrate and product have been derived for all values of the parameters. Satisfactory agreement is acquired in the comparison of approximate analytical solution and numerical simulation.

Keywords— Electrochemical enzyme biosensor; Michaelis-Menten kinetics; Glucose oxidase and horseradish peroxidase electro enzymatic model system; Non-linear differential equations; Homotopy perturbation method; Numerical simulation.

1 INTRODUCTION

A biosensor is an analytical device which uses a living organism or biological molecules, especially enzymes or antibodies, to detect the presence of chemicals. Enzymes are the most common bio-recognition components of biosensors. Many obstacles still lie in the way of the widespread commercialization of biosensor system.

Attempts have been made to get around the problem with enzyme-inhibition based systems [1]. Electrochemical biosensors have also been held back by the need for diffusional electron transfer mediators. These are low molecular weight molecules that can diffuse rapidly between the enzyme redox site and the electrode surface. In the original Clarke-type electrode, oxygen acted as the mediator [2]. An amperometric peroxide biosensor was prepared by electrochemical deposition of horseradish peroxidase (HRP) on a Pt disc electrode modified with polyaniline (PANI) film doped with polyvinyl sulphonate (PVS) [3]. This paper investigates a model biosensor system which consists of two enzymes namely glucose oxidase and horseradish peroxidase.

Glucose serves as the base of a series of enzymatic experiments. A generalized glucose enzyme electrode would Therefore consist of essential three layers: the outer diffusion limiting membrane, in contact with the bulk solution containing the analyze of interest ;the immobilized enzyme layer, within which substrate is depleted and product is formed; and the inner selective membrane in contact with the electrode [4]. Both the glucose oxidase and the horseradish peroxidase are stable enzymes. Glucose oxidase enzyme is an oxidoreductase that catalysis the oxidation of glucose to hydrogen peroxide. Glucose oxidase is widely used for the determination of free glucose in body fluids, vegetal raw material, and in the food industry. The peroxidase-glucose oxidase enzyme system involves a coupled reaction with glucose oxidase and peroxidase. Horseradish peroxidase is a heme-linked oxidase that catalysis the oxidation of various substrates with hydrogen peroxidase. One of the most basic enzymatic reactions was proposed more than a century ago by Michaelis and Menten. These substrates are produced at different rates and are subjected to different diffusion processes. To our knowledge no rigorous analyticalsolutions have been devised for first complex, second complex, second substrate (hydrogen peroxide) and final enzyme product for all values of parameters.

In this paper, we put forward the approximate analytical expressions for first complex, second complex, second substrate (hydrogen peroxide) and final enzyme product using Homotopy perturbation method (HPM) .Comparative study of the same with numerical simulation is shown.

2 MATHEMATICAL FORMULATION OF THE PROBLEM

The mathematical model is based on the existence of a diffusion layer and a convection layer, where the glucose concentration is maintained constant. The immobilized enzymes form a monolayer, so all reactions can be assumed to undergo, at the lower boundary of the diffusion domain. The equations are one-dimensional, where the variable x measures the distance from the electrode. These reactions are modeled by a standard Michaelis-Menten kinetics scheme which are given below [5]

* *Department of Mathematics, Lady Doak College, Madurai - 625002, Tamil Nadu, India.*
** *Department of Mathematics, Madura College, Madurai - 625011, Tamil Nadu, India.*
*** *Corresponding Author: raj_sms@rediffmail.com*

$$E_1 + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} E_1 + S_2 \quad (1)$$

$$E_2 + S_2 \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2 \xrightarrow{k_4} E_2 + P \quad (2)$$

where $E_1(t)$= first enzyme (glucose oxidase) concentration, $E_2(t)$=second enzyme (horseradish peroxidase) concentration, $S_1(x,t)$= first substrate (glucose), $S_2(x,t)$= second substrate (hydrogen peroxide), $C_1(t)$= first complex, $C_2(t)$= second complex, $P(x,t)$= final product, the differential equations governing the behavior of the relevant chemical substrates, glucose and hydrogen peroxide are as follows.

$$\frac{\partial S_1}{\partial t} = D_1 \frac{\partial^2 S_1}{\partial x^2}, \quad 0 \le x \le L, \quad t \ge 0 \quad (3)$$

$$\frac{\partial S_2}{\partial t} = D_2 \frac{\partial^2 S_2}{\partial x^2}, \quad 0 \le x \le L, \quad t \ge 0 \quad (4)$$

with the boundary conditions,

$$S_1(L,t) = S_0, \quad S_2(L,t) = 0, \quad t \ge 0 \quad (5)$$

The following boundary conditions hold on $x = 0$

$$D_1 \frac{\partial S_1}{\partial x} = k_1 E_1 S_1 - k_{-1} C_1 \quad (6)$$

$$D_2 \frac{\partial S_2}{\partial x} = k_3 E_2 S_2 - k_2 C_1 - k_{-3} C_2 \quad (7)$$

The following equations describe the kinetics of the enzyme-substrate reactions, according to the Michaelis-Menten scheme, taking place at the electrode.

$$\frac{dE_1}{dt} = -k_1 E_1 S_1 + (k_2 + k_{-1}) C_1 \quad (8)$$

$$\frac{dE_2}{dt} = -k_3 E_2 S_2 + (k_4 + k_{-3}) C_2 \quad (9)$$

$$\frac{dC_1}{dt} = k_1 E_1 S_1 - (k_2 + k_{-1}) C_1 \quad (10)$$

$$\frac{dC_2}{dt} = k_3 E_2 S_2 - (k_4 + k_{-3}) C_2 \quad (11)$$

$$\frac{dP}{dt} = k_4 C_2 \quad (12)$$

The initial conditions are

$$S_1(x,0) = S_0(x), \; S_2(x,0) = 0, \; P(x,0) = 0 \quad (13)$$

$$E_1(0) = \frac{\xi e}{1+\xi}, \; E_2(0) = \frac{e}{1+\xi}, \; C_1(0) = 0, \; C_2(0) = 0 \quad (14)$$

where D_1 and D_2 are diffusion constants of glucose and hydrogen peroxide, L is the depth of the diffusion layer, $k_1, k_2, k_3, k_4, k_{-1}, k_{-3}$ are reaction rate constants, ξ is the ratio of glucose oxidase to horseradish peroxidase on the electrode, e is the total amount of enzyme present on the electrode, S_O is the initial glucose concentration and $S_o(x) = S_o$ *if* $x = L$ and 0 otherwise. In order to obtain an analytical expression for the dependence of the optimal enzyme ratio on the system parameters, the following simplified model which focuses on the kinetic surface processes, while neglecting the movement of chemical species to and from the electrode is considered. With the assumption that the concentration of glucose is maintained constant at the reaction point, $S_1(t) = S_o$ for all $t \ge 0$ the above mathematical model now reduces to the following set of ordinary differential equation [6].

$$\frac{dC_1}{dt} = -(k_1 S_0 + k_{-1} + k_2) C_1 + \frac{\xi e k_1 S_0}{1+\xi} \quad (15)$$

$$\frac{dC_2}{dt} = \frac{e k_3 S_2}{1+\xi} - (k_{-3} + k_4) C_2 - k_3 S_2 C_2 \quad (16)$$

$$\frac{dS_2}{dt} = k_2 C_1 + k_{-3} C_2 - \frac{e k_3 S_2}{1+\xi} + k_3 S_2 C_2 \quad (17)$$

with the initial conditions $C_1(0) = C_2(0) = S_2(0) = 0$ (18)

The final product $P(x,t)$ can be calculated from the equation

$$\frac{dP}{dt} = k_4 C_2 \quad (19)$$

with the initial condition $P(0) = 0$ (20)

3 Solution of the non-linear differential Equations using the Homotopy perturbation Method

Linear and non-linear phenomena are of fundamental importance in various fields of science and engineering. Most models of real – life problems are still very difficult to solve. Therefore, approximate analytical solutions using Homotopy perturbation method (HPM) [7-18] was introduced. This method is the most effective and convenient ones for both linear and non-linear equations. Perturbation method is based on assuming a small parameter. The majority of non-linear problems, especially those having strong non-linearity, have no small parameters at all and the approximate solutions obtained by the perturbation methods, in most cases, are valid only for small values of the small parameter. Generally, the perturbation solutions are uniformly valid as long as a scientific system parameter is small. However, we cannot rely fully on the approximations, because there is no criterion on which the small parameter should exists. Thus, it is essential to check the validity of the approximations numerically and/or experimentally. To overcome these difficulties, HPM have been proposed recently.

Recently, many authors have applied the Homotopy perturbation method (HPM) to solve the non-linear boundary value problem in physics and engineering sciences [16-19]. This method is also used to solve some of the non-linear problem in physical sciences [11-23]. This method is a combination of Homotopy in topology and classic perturbation techniques. Ji-Huan He used the HPM to solve the Lighthill equation [11], the Diffusion equation [12] and the Blasius equation [13-14]. The HPM is unique in its applicability, accuracy and efficiency. The 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 approximate analytical solutions to (15)-(20) as follows:

$$C_1(t)=\left(\frac{a_2}{a_1}\right)\left(e^{a_1 t}-1\right) \tag{21}$$

$$C_2(t)=\left(\frac{a_2 a_6}{a_1 a_5}\right)\left(1-e^{a_5 t}\right)-\left(\frac{a_2 a_3 a_6\left(e^{a_1 t}-e^{a_5 t}\right)}{a_1(a_1-a_5)(a_1-a_3)}\right)+\left(\frac{a_2 a_6\left(e^{a_3 t}-e^{a_5 t}\right)}{(a_1-a_5)(a_3-a_5)}\right) \tag{22}$$

$$\begin{aligned}S_2(t)=&\left(\frac{2a_2a_6}{a_1a_3}\right)\left(1+\frac{a_3e^{a_1t}-a_1e^{a_3t}}{a_1-a_3}\right)+\left(\frac{a_2a_6}{a_1}\right)\left(\frac{e^{a_1t}}{a_1-a_3}+\frac{1}{a_3}\right)\\&+ke^{a_3t}-\left(\frac{a_2a_3a_6a_7}{a_1(a_1-a_5)(a_1-a_3)}\right)\left(\frac{e^{a_1t}}{a_1-a_3}-\frac{e^{a_5t}}{a_5-a_3}\right)\\&+\left(\frac{a_2a_6a_7}{(a_1-a_3)(a_3-a_5)}\right)\left(te^{a_3t}-\frac{e^{a_5t}}{a_5-a_3}\right)\\&+\left(\frac{a_2a_6a_7}{a_1a_5}\right)\left(\frac{-1}{a_3}-\frac{e^{a_5t}}{a_5-a_3}\right)\end{aligned} \tag{23}$$

$$\begin{aligned}P(t)=&\frac{a_2a_6k_4}{a_1a_5}\left(t-\frac{e^{a_5t}}{a_5}+\frac{1}{a_5}\right)\\&-\frac{a_2a_3a_6k_4}{a_1(a_1-a_3)}\left(\frac{e^{a_1t}}{a_1(a_1-a_5)}-\frac{e^{a_5t}}{a_5(a_1-a_5)}+\frac{1}{a_5a_1}\right)\\&+\frac{a_2a_6k_4}{(a_1-a_3)}\left(\frac{e^{a_3t}}{a_3(a_3-a_5)}-\frac{e^{a_5t}}{a_5(a_3-a_5)}+\frac{1}{a_3a_5}\right)\end{aligned} \tag{24}$$

Where

$$\begin{aligned}k=&-\left(\frac{a_2a_6}{a_3(a_1-a_3)}\right)\\&-\left(\frac{a_2a_6a_7}{a_3-a_5}\right)\left(\frac{1}{a_1a_3}+\frac{1}{(a_1-a_3)(a_3-a_5)}-\frac{a_3}{a_1\left(a_1-a_3^{\,2}\right)}\right)\end{aligned} \tag{25}$$

$$a_2=\frac{\xi\, e\, k_1S_0}{1+\xi},\ a_3=-\frac{ek_3}{1+\xi},\ a_4=-k_3, \tag{26}$$

$$a_1=-\left(k_1S_0+k_{-1}+k_2\right),\ a_5=-\left(k_{-3}+k_4\right),\ a_6=k_2,\ a_7=-k_3 \tag{27}$$

4 Numerical Simulation

The non-linear differential equations (15)-(20) are also solved numerically. We have used the function main in Matlab/Scilab software to solve the initial-boundary value problems for the non-linear differential equations numerically. This numerical solution is compared with our analytical results in Fig. 1- 5. Upon comparison, it gives a satisfactory agreement for all values of the parameters k_1, k_3, k_4, ξ and S_o. The Matlab/Scilab program is also given in Appendix C.

5 Results and Discussions

The equations. (21)-(27) represent the simple analytical expressions pertaining to the first complex $C_1(t)$, second complex $C_2(t)$, second substrate $S_2(t)$ and the final product $P(t)$ respectively. The main variables of interest in this study are initial glucose concentration S_o, the ratio of glucose oxidase to horseradish peroxidase on the electrode ξ and the reaction rate constants k_1, k_3 and k_4. In fig.1(a)-1(c) first complex $C_1(t)$ versus time t for various values of the initial glucose concentration S_0, the ratio of the glucose oxidase and horseradish peroxide on the electrode ξ and reaction rate constant k_1 is presented. From these figures, it is confirmed that the first complex $C_1(t)$ increases when initial glucose concentration S_o, the ratio of glucose oxidase to horseradish peroxidase on the electrode ξ and the reaction rate constants k_1 .increases with respect to time *t*.

In fig. 2(a)-2(e) second complex $C_2(t)$ versus time t for various values of the initial glucose concentration S_0, the ratio of the glucose oxidase and horseradish peroxide on the electrode ξ and reaction rate constants k_1, k_3, k_4 is plotted. From fig .2(a)-2(d) we conclude that the second complex $C_2(t)$ increases when the initial glucose concentration S_o, the ratio of the glucose oxidase and horseradish peroxidase on the electrode ξ □and the reaction rate constants k_1, k_3 increases with respect to time t. Fig. 2(e) shows that the second complex $C_2(t)$ increases when the reaction rate constant k_4 decreases with respect to time t. In fig. 3(a)-3(d), second substrate $S_2(t)$ versus time t for various values of the parameters is presented. From fig.3(a) -3(b) we conclude that second substrate $S_2(t)$ increases as the initial glucose concentration S_o and the ratio of the glucose oxidase and horseradish peroxide on the electrode ξ increases with respect to time t. .From Fig.3(c) it is inferred that second substrate $S_2(t)$ increases as the reaction rate constant k_1 .increases. Fig.3(d) illustrates that second substrate $S_2(t)$ increases as the reaction rate constant k_3 decreases.

Fig. 4(a) and 4(b) depict the analytical and numerical profiles of first complex $C_1(t)$ second complex $C_2(t)$ and second substrate $S_2(t)$ versus time t . The graph is plotted for (21)-(23). The obtained analytical results are verified. In fig.5(a)-5(d) final product $P(t)$ versus time t for various values of the initial glucose concentration S_0, the ratio of the glucose oxidase and horseradish peroxide on the electrode ξ and reaction rate constants k_1, k_4 is presented. From these figures it is observed that final product $P(t)$ increases as the initial glucose concentration S_o, the ratio of the glucose oxidase and horseradish peroxide on the electrode ξ and reaction rate constant k_1 and k_4 . In fig.6(a)-6(d) the time evolution of dP/dt (the rate of formation of final product) on the electrode (obtained after approximately 40s) is plotted using (19) for various values of the dimensionless parameters.

6 Conclusion

The time dependent non-linear differential equations for the electrochemical enzyme biosensor can be solved analytically and numerically. The approximate analytical expression of the first complex, second complex, second substrate and final product has been derived using the Homotopy perturbation method. These analytical results can be used to analyze the efficiency of electron transfer to the enzyme active site from the conducting
polymer surface, which is affected by the random orientation of enzyme on the surface, probably making much of the immobilized material completely inactive. The Homotopy perturbation method is not only an extremely simple method

but also a promising method to solve the strongly non-linear reaction-diffusion equations. Our results are compared with the numerical simulation and it gives satisfactory agreement .This analytical result helps us for the better understanding of the model.

APPENDIX: A

BASIC CONCEPTS OF THE HOMOTOPY PERTURBATION METHOD

To explain this method, let us consider the following function:

$$D_o(u) - f(r) = 0, \quad r \in \Omega \tag{A.1}$$

with the boundary conditions of

$$B_o(u, \frac{\partial u}{\partial n}) = 0, \quad r \in \Gamma \tag{A.2}$$

where D_o is a general differential operator, B_o is a boundary operator, $f(r)$ is a known analytical function and Γ is the boundary of the domain Ω. In general, the operator D_o can be divided into a linear part L and a non-linear part N. (A.1) can therefore be written as

$$L(u) + N(u) - f(r) = 0 \tag{A.3}$$

By the Homotopy technique, we construct a Homotopy $v(r, p) : \Omega \times [0,1] \to \Re$ that satisfies

$$H(v, p) = (1-p)[L(v) - L(u_0)] + p[D_o(v) - f(r)] = 0. \tag{A.4}$$

$$H(v, p) = L(v) - L(u_0) + pL(u_0) + p[N(v) - f(r)] = 0. \tag{A.5}$$

where $p \in [0, 1]$ is an embedding parameter, and u_0 is an initial approximation of (A.1) that satisfies the boundary conditions. From (A.4) and (A.5) we have

$$H(v,0) = L(v) - L(u_0) = 0 \tag{A.6}$$

$$H(v,1) = D_o(v) - f(r) = 0 \tag{A.7}$$

When p=0, (A.4) and (A.5) become linear equations. When p=1, they become non-linear equations. The process of changing p from zero to unity is that of $L(v) - L(u_0) = 0$ to $D_o(v) - f(r) = 0$. We first use the embedding parameter p as a small parameter and assume that the solutions of . (A.4) and (A.5) can be written as a power series in p:

$$v = v_0 + pv_1 + p^2 v_2 + \ldots \tag{A.8}$$

Setting $p = 1$ results in the approximate solution of (A.1):

$$u = \lim_{p \to 1} v = v_0 + v_1 + v_2 + \ldots \tag{A.9}$$

This is the basic idea of the HPM.

APPENDIX: B

SOLUTION OF THE NONLINEAR DIFFERENTIAL EOUATIONS (16) AND (17) USING THE HOMOTOPY PERTURBATION METHOD

In this appendix, we derive the approximate analytical solution of (16) and (17) using Homotopy perturbation method.

$$(1-p)\left[\frac{dC_2}{dt} - a_5 C_2\right] + p\left[\frac{dC_2}{dt} - a_5 C_2 + a_3 S_2 - a_5 S_2 C_2\right] = 0 \tag{B.1}$$

$$(1-p)\left[\frac{dS_2}{dt} - a_3 S_2\right] + p\left[\frac{dS_2}{dt} - a_3 S_2 - a_6 C_1 - a_7 C_2 + a_4 C_2 S_2\right] = 0 \tag{B.2}$$

The approximate solution of (B.1) and (B.2) be

$$C_2 = C_{20} + pC_{21} + p^2 C_{22} + p^3 C_{23} + \cdots. \tag{B.3}$$

$$S_2 = S_{20} + pS_{21} + p^2 S_{22} + p^3 S_{23} + \cdots. \tag{B.4}$$

Substituting (B.3) and (B.4) in (B.1) and (B.2) we get

$$(1-p)\left[\frac{d(C_{20} + pC_{21} + p^2 C_{22} + \cdots)}{dt} - a_5 (C_{20} + pC_{21} + p^2 C_{22} + \cdots)\right] + p\left[\begin{array}{l} \frac{d(C_{20} + pC_{21} + p^2 C_{22} + \cdots)}{dt} - a_5 (C_{20} + pC_{21} + p^2 C_{22} + \cdots) \\ + a_3 (S_{20} + pS_{21} + p^2 S_{22} + \cdots) \\ - a_5 (S_{20} + pS_{21} + p^2 S_{22} + \cdots)(C_{20} + pC_{21} + p^2 C_{22} + \cdots) \end{array}\right] = 0 \tag{B.5}$$

$$(1-p)\left[\frac{d(S_{20} + pS_{21} + p^2 S_{22} + \cdots)}{dt} - a_3 (S_{20} + pS_{21} + p^2 S_{22} + \cdots)\right] + p\left[\begin{array}{l} \frac{d(S_{20} + pS_{21} + p^2 S_{22} + \cdots)}{dt} - a_3 (S_{20} + pS_{21} + p^2 S_{22} + \cdots) \\ - a_6 C_1 - a_7 (S_{20} + pS_{21} + p^2 S_{22} + \cdots) \\ + a_4 (C_{20} + pC_{21} + p^2 C_{22} + \cdots)(S_{20} + pS_{21} + p^2 S_{22} + \cdots) \end{array}\right] = 0 \tag{B.6}$$

Comparing the coefficients of like powers of p, we can obtain the following differential equations :

$$p^0 : \frac{dC_{20}}{dt} - a_5 C_{20} = 0 \tag{B.7}$$

$$p^0 : \frac{dS_{20}}{dt} - a_3 S_{20} = 0 \tag{B.8}$$

$$p^1 : \frac{dC_{21}}{dt} - a_5 C_{21} + a_3 S_{20} - a_4 S_{20} C_{20} = 0 \tag{B.9}$$

$$p^1 : \frac{dS_{21}}{dt} - a_3 S_{21} - a_6 C_1 - a_7 C_{20} + a_4 S_{20} C_{20} = 0 \tag{B.10}$$

$$p^2 : \frac{dC_{22}}{dt} - a_5 C_{22} + a_3 S_{21} - a_4 S_{21} C_{21} = 0 \tag{B.11}$$

$$p^2 : \frac{dS_{22}}{dt} - a_3 S_{22} - a_6 C_1 - a_7 C_{21} + a_4 (S_{20} C_{21} + S_{21} C_{20}) = 0 \tag{B.12}$$

$$p^3 : \frac{dS_{23}}{dt} - a_3 S_{23} - a_6 C_1 - a_7 C_{22} + a_4\left(S_{20}C_{21} + S_{21}C_{21} + S_{21}C_{20}\right) = 0 \tag{B.13}$$

The initial approximations are as follows:

$$C_{20}(0) = 0, \quad S_{20}(0) = 0 \tag{B.14}$$

$$C_{2i}(0) = 0, \; S_{2i}(0) = 0 \text{ for } i = 1,2,3..... \tag{B.15}$$

Solving (B.7)-(B.13) and using the initial approximations (B.14) and (B.15), we get the following results:

$$C_{20}(t) = 0 \tag{B.16}$$

$$C_{21}(t) = 0 \tag{B.17}$$

$$C_{22}(t) = \left(\frac{a_2 a_6}{a_1 a_5}\right)\left(1 - e^{a_5 t}\right) - \left(\frac{a_2 a_3 a_6\left(e^{a_1 t} - e^{a_5 t}\right)}{a_1(a_1 - a_5)(a_1 - a_3)}\right) + \left(\frac{a_2 a_6\left(e^{a_3 t} - e^{a_5 t}\right)}{(a_1 - a_5)(a_3 - a_5)}\right) \tag{B.18}$$

$$S_{20}(t) = 0 \tag{B.19}$$

$$S_{21}(t) = \left(\frac{a_2 a_6}{a_1 a_3}\right)\left(1 + \frac{a_3 e^{a_1 t} - a_1 e^{a_3 t}}{a_1 - a_3}\right) \tag{B.20}$$

$$S_{22}(t) = \left(\frac{a_2 a_6}{a_1 a_3}\right)\left(1 + \frac{a_3 e^{a_1 t} - a_1 e^{a_3 t}}{a_1 - a_3}\right) \tag{B.21}$$

$$S_{23}(t) = \left(\frac{a_2 a_6}{a_1}\right)\left(\frac{e^{a_1 t}}{a_1 - a_3} + \frac{1}{a_3}\right) + \left(\frac{a_2 a_6 a_7}{a_1 a_5}\right)\left(\frac{-1}{a_3} - \frac{e^{a_5 t}}{a_5 - a_3}\right) + k e^{a_3 t} - \left(\frac{a_2 a_3 a_6 a_7}{a_1(a_1 - a_5)(a_1 - a_3)}\right)\left(\frac{e^{a_1 t}}{a_1 - a_3} - \frac{e^{a_5 t}}{a_5 - a_3}\right) + \left(\frac{a_2 a_6 a_7}{(a_1 - a_3)(a_3 - a_5)}\right)\left(t e^{a_3 t} - \frac{e^{a_5 t}}{a_5 - a_3}\right) \tag{B.22}$$

where $$k = -\left(\frac{a_2 a_6 a_7}{a_3 - a_5}\right)\left(\frac{1}{a_1 a_3} + \frac{1}{(a_1 - a_3)(a_3 - a_5)} - \frac{a_3}{a_1\left(a_1 - a_3^2\right)}\right) - \left(\frac{a_2 a_6}{a_3(a_1 - a_3)}\right) \tag{B.23}$$

According to the HPM, we can conclude that

$$C_2(t) = \lim_{p \to 1} C_2 = C_{20} + C_{21} + C_{22} \tag{B.24}$$

$$S_2(t) = \lim_{p \to 1} S_2 = S_{20} + S_{21} + S_{22} + S_{23} \tag{B.25}$$

Substituting (B.16) - (B.18) in (B.24) and (B.19) – (B.23) in. (B.25), we obtain the solutions (22) and (23) in the text.

APPENDIX: C

MATLAB/SCILAB PROGRAM TO FIND THE NUMERICAL SOLUTION OF THE NONLINEAR DIFFERENTIAL EQNS. (15)-(20)

```
function
main
options= odeset('RelTol',1e-6,'Stats','on');
%initial conditions
xo = [0;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))
plot(t, x(:,4))
legend('x1','x2','x3', 'x4')
y label('x')
x label('t')
return
function [dx_dt]= TestFunction(t,x)
k1=15;
So=1;
k-1 =0.1;
k2=1;
k3=5;
zeta=1;
e=2;
k-3=0.1;
k4=15;
dx_dt(1)= -((k1*So+k-1+k2))*x(1)+((So*zeta*e*k1)/(1+zeta));
dx_dt(2) =(e*k3/(1+zeta))*x(3)-k3*x(3)*x(2)-( k-3+k4)*x(2);
dx_dt(3) =k2*x(1)+k-3*x(2)-
e*k3/(1+zeta))*x(3)+k3*x(3)*x(2);
dx_dt(4)=k4*x(2);
dx_dt = dx_dt';
return
```

APPENDIX D:

NOMENCLATURE

SYMBOL	MEANING
L	Diffusion layer depth(m)
D_1	Diffusion constants (m^2/s) (Glucose)
D_2	Diffusion constants (m^2/s) (Hydrogen peroxide)
k_1, k_3	Reaction rate constants ($m^3/mol.s$)
k_2, k_4, k_{-1}, k_{-3}	Reaction rate constants(s^{-1})
S_0	Initial glucose concentration(mol/m^3)
ξ	The ratio of glucose oxidase to horseradish peroxidase on the electrode
e	The total amount of enzyme present on the electrode(mol/m^2)
$E_1(t)$	First enzyme(glucose oxidase concentration)
$E_2(t)$	Second enzyme (horseradish peroxidase) concentration
$S_1(x,t)$	First substrate (glucose)
$S_2(x,t)$	Second substrate (hydrogen peroxide)
$C_1(t)$	First complex
$C_2(t)$	Second complex
$P(x,t)$	Final product

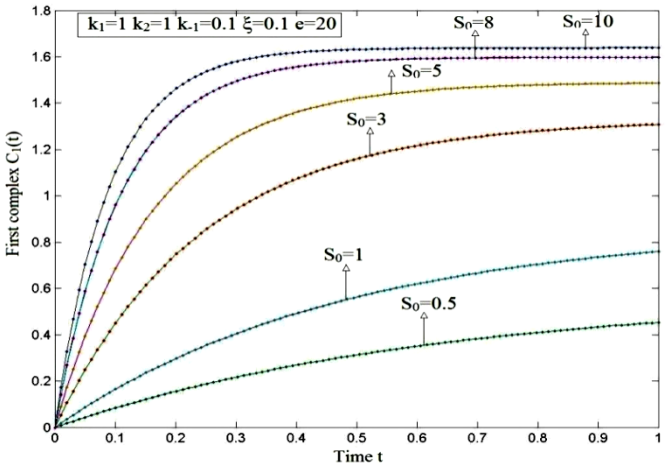

Fig.1(a): First complex $C_1(t)$ versus time t using (21) for various values initial glucose concentration S_o .The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

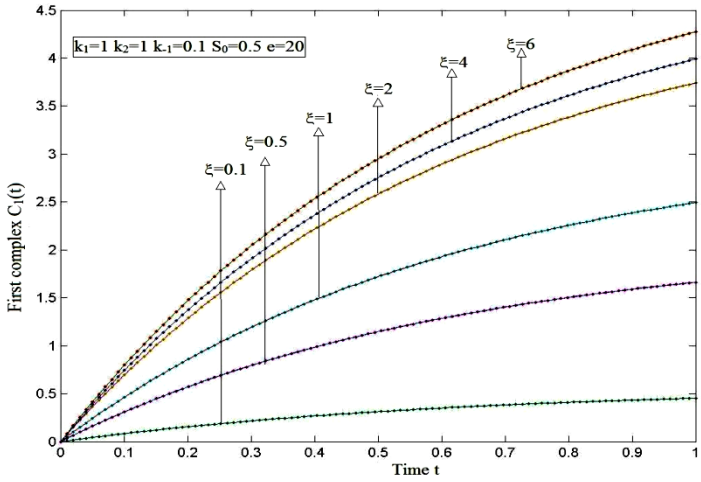

Fig.1(b): First complex $C_1(t)$ versus time t using (21) for various values of the ratio of glucose oxidase to horseradish peroxidase on the electrode ξ. The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

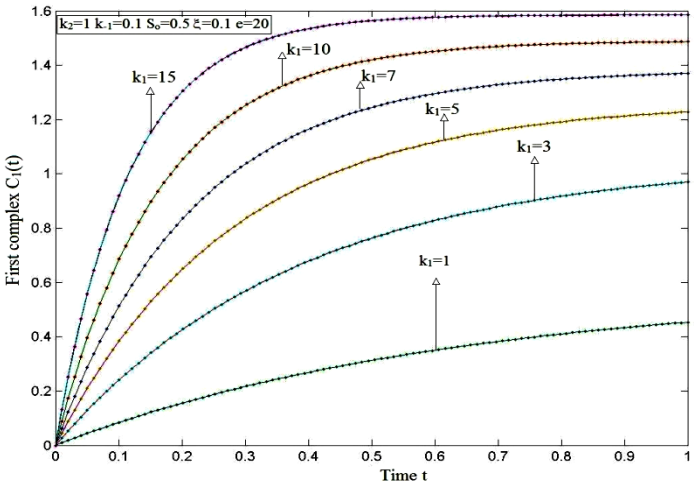

Fig.1(c): First complex $C_1(t)$ versus time t using (21) for various values of the reaction rate constants The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

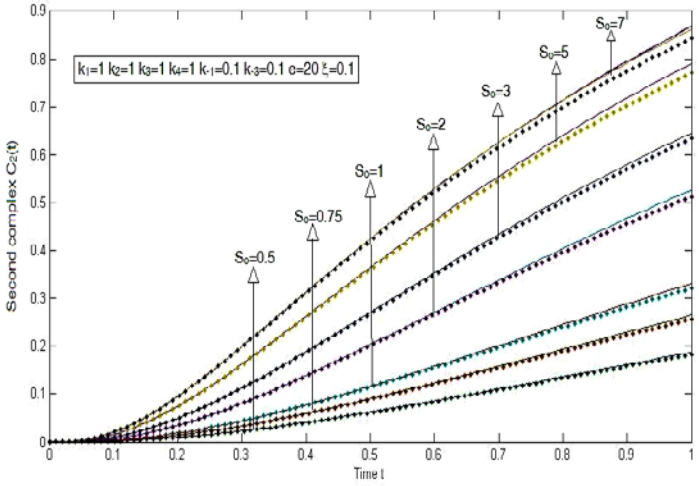

Fig. 2(a): Second complex $C_2(t)$ versus time t using (22) for various values initial glucose concentration S_0. The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

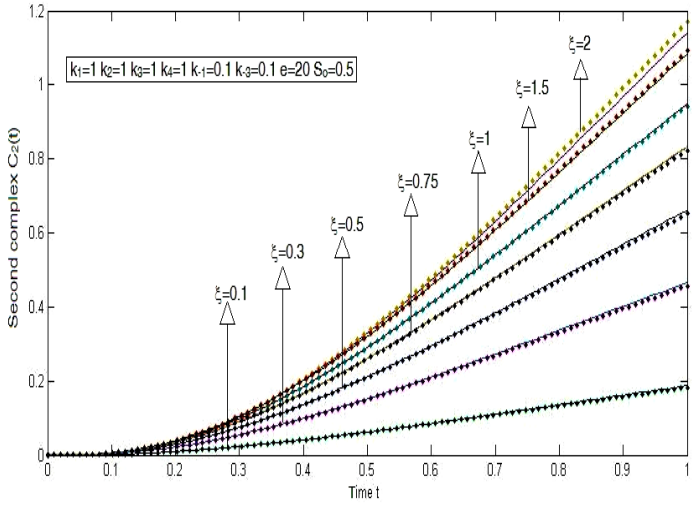

Fig. 2(b): Second complex $C_2(t)$ versus time t using (22) for various values of the ration of the glucose oxidase and horseradish peroxide on the electrode ξ. The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

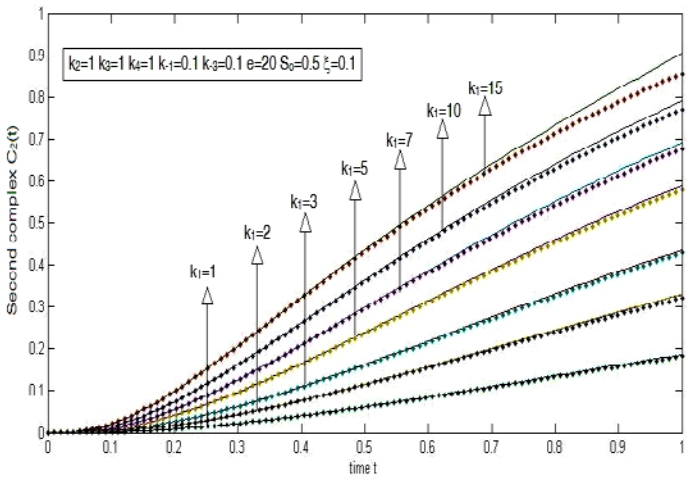

Fig. 2(c): Second complex $C_2(t)$ versus time t using (22) for various values of the reaction rate constants k_1. The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

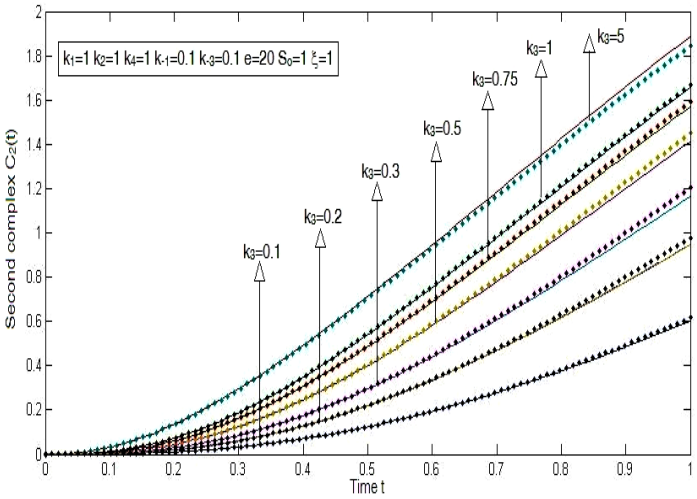

Fig.2(d): Second complex $C_2(t)$ versus time t using (22)for various values of the reaction rate constants k_3. The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

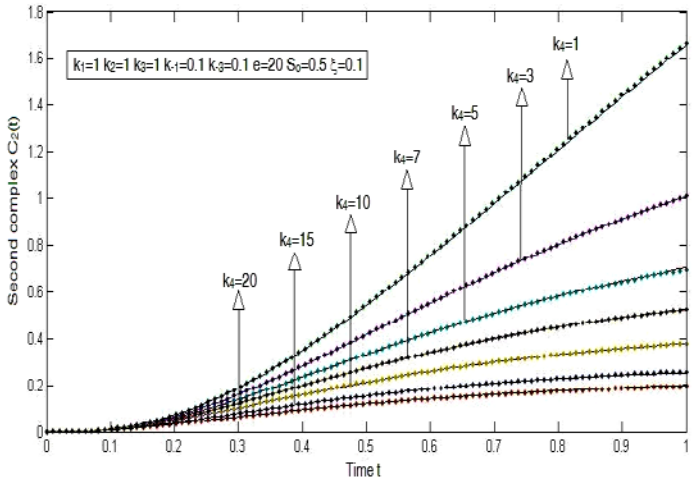

Fig. 2(e): Second complex $C_2(t)$ versus time t using (22) for various values of the reaction rate constants k_4 .The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

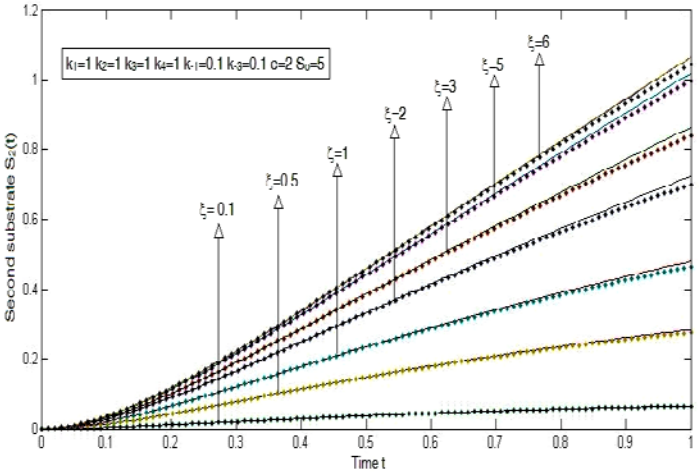

Fig. 3(b): Second substrate $S_2(t)$ versus time t using (23) for various values of the ratio of glucose oxidase to horseradish peroxidase on the electrode ξ . The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

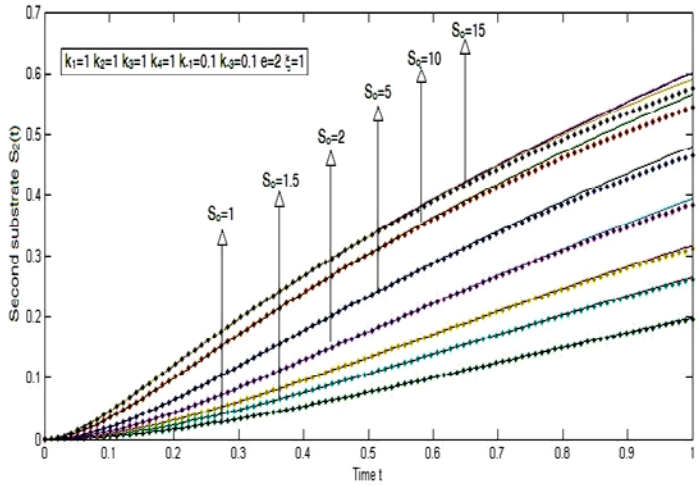

Fig. 3(a): Second substrate $S_2(t)$ versus time t using (23) for various values initial glucose concentration S_0 .The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

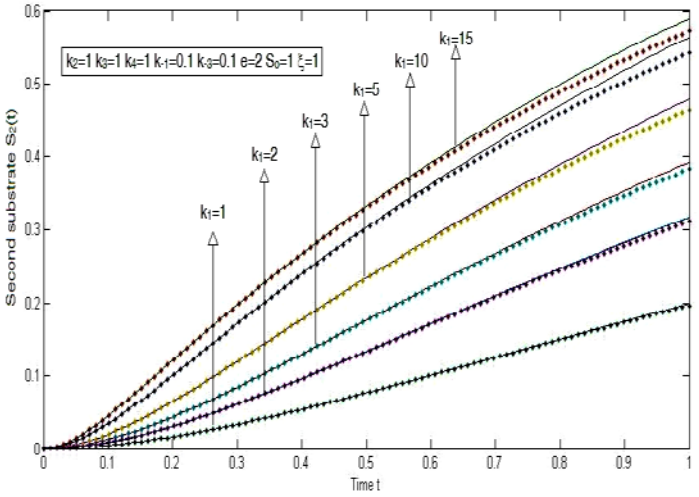

Fig.3(c): Second substrate $S_2(t)$ versus time t using (23) for various values reaction rate constants k_1 . The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

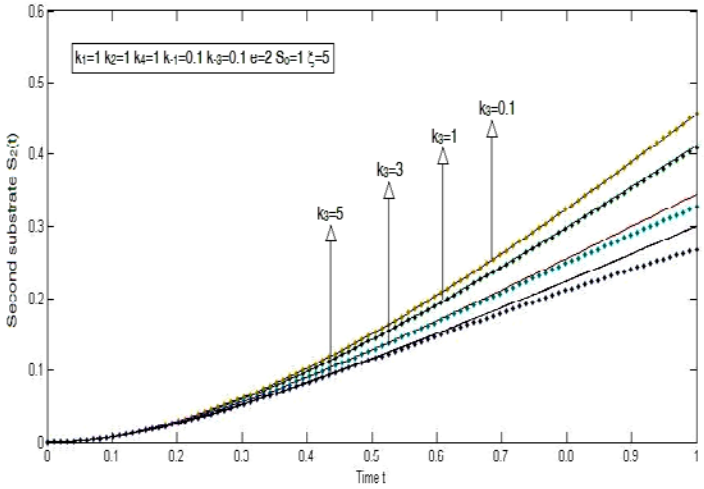

Fig. 3(d): Second substrate $S_2(t)$ versus time t using (23) for various values reaction rate constants k_3. The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

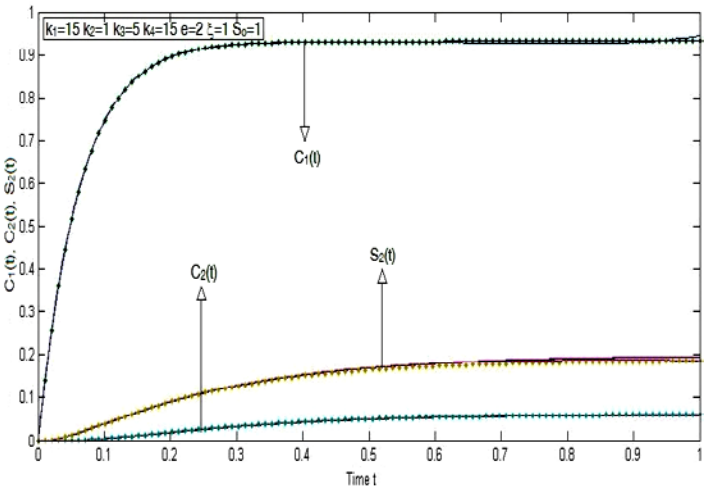

Fig. 4(b): First complex $C_1(t)$, Second complex $C_2(t)$ and Second substrate $S_2(t)$ versus Time t using (21), (22) and (23). The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

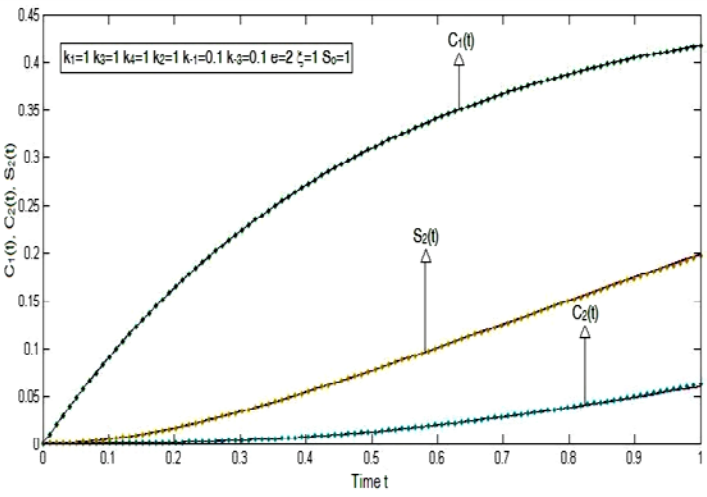

Fig.4(a): First complex $C_1(t)$, Second complex $C_2(t)$ and Second substrate $S_2(t)$ versus time t using. (21), (22) and (23). The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

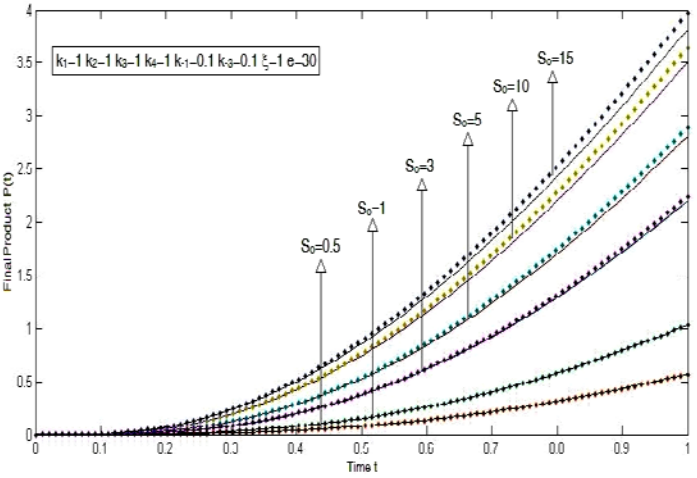

Fig. 5(a): Final product $P(t)$ versus time t using (24) for various values initial glucose concentration S_0. The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

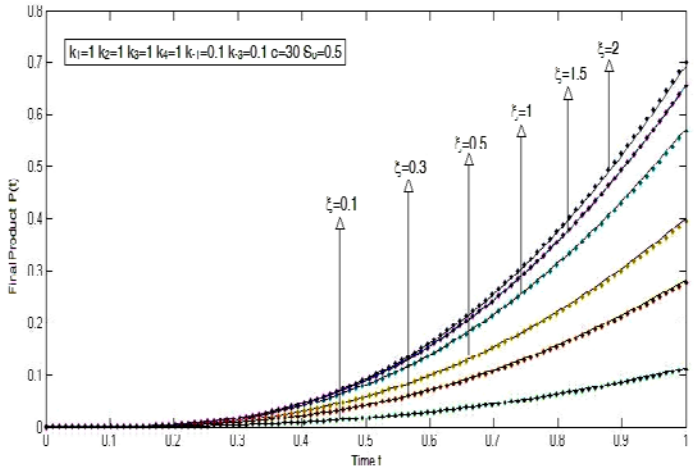

Fig. 5(b): Final product $P(t)$ versus time t using (24) for various values of the ratio of glucose oxidase to horseradish peroxidase on the electrode ξ . . The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

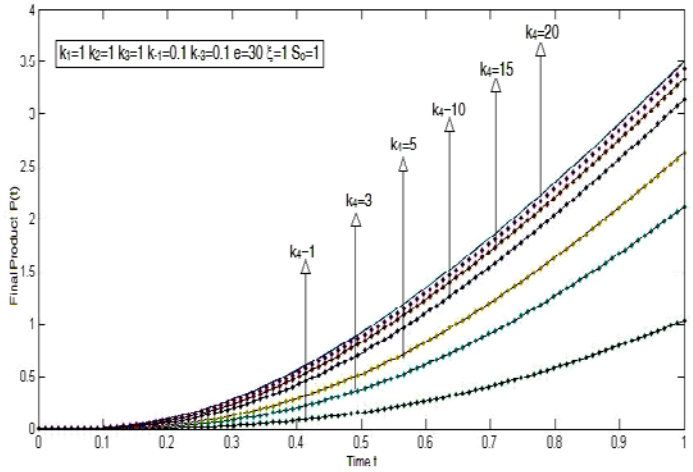

Fig. 5(d): Final product $P(t)$ versus time t using (24) for various values reaction rate constants k_4 .. The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

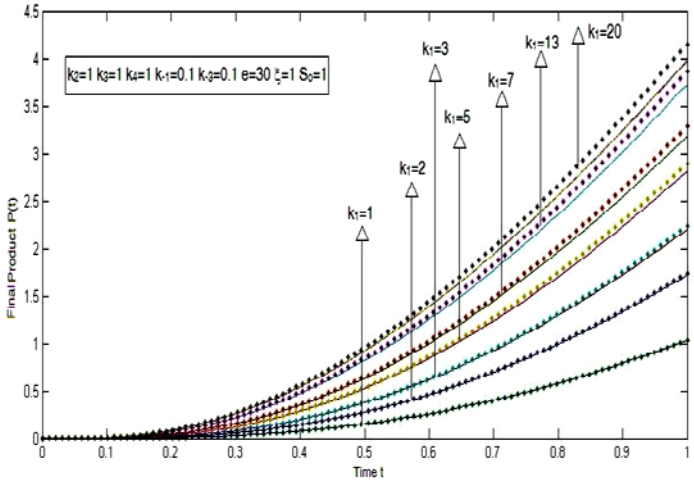

Fig. 5(c): Final product $P(t)$ versus time t using (24) for various values reaction rate constants k_1 . The solid lines (—) represent analytical solution and dotted lines (····) represent numerical simulation.

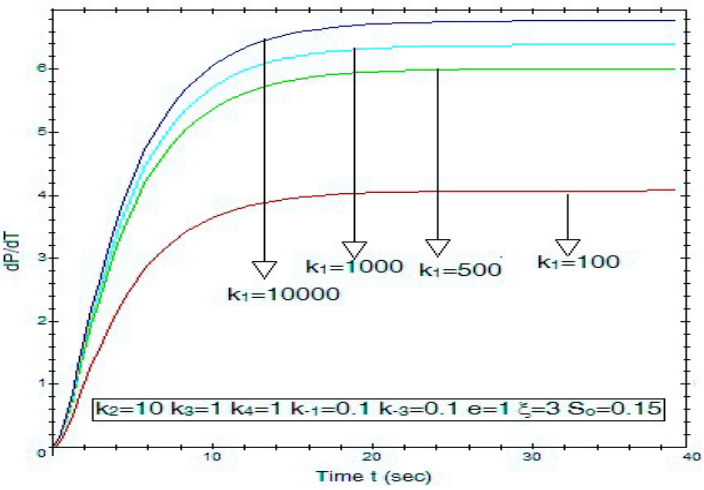

Fig. 6(a): The time revolution dP/dt versus time t using (19) for various values of reaction rate constants k_1.

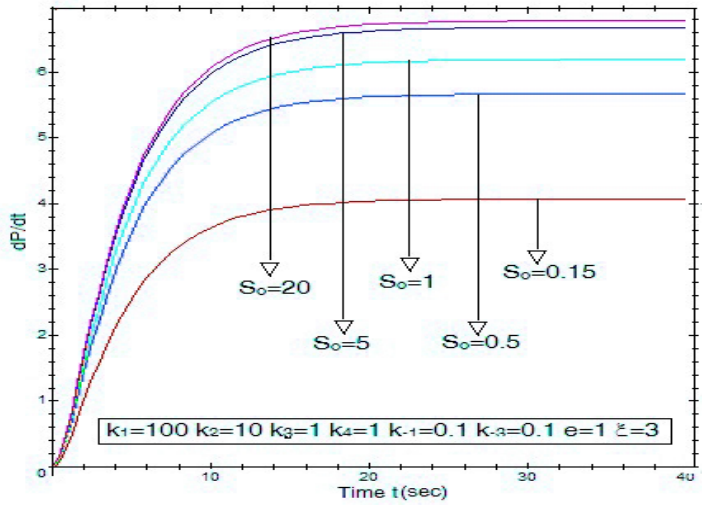

Fig.6(b): The time revolution dP/dt versus time t using (19) for various values of initial glucose concentration S_0.

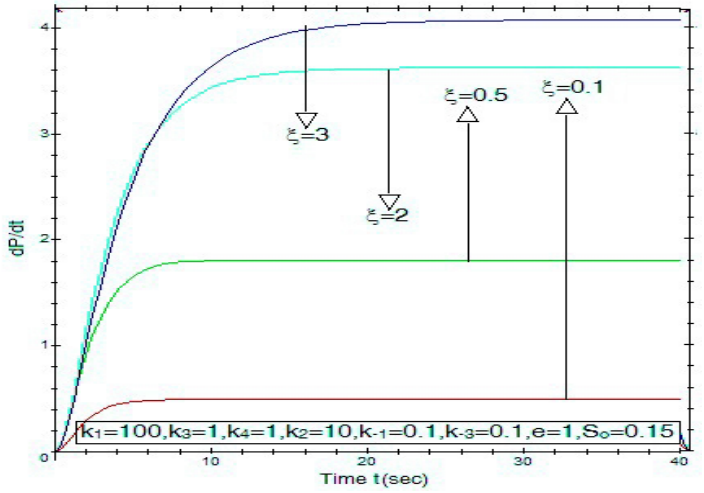

Fig. 6(c): The time revolution dP/dt versus time t using (19) for various values of the ratio of glucose oxidase to horseradish peroxidase on the electrode ξ.

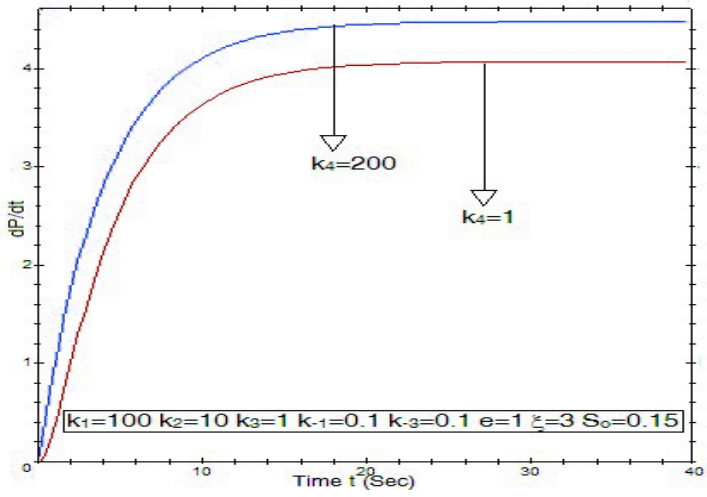

Fig.6(d): The time revolution dP/dt versus time t using. (19) for various values of the reaction rate constant k_4.

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