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ANALYSIS OF OXIDOREDUCTASE ENZYME KINETICS IN AN AMPEROMETRIC BIOSENSORS

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Abstract—A non-linear mathematical model based on Michaelis-Menten enzyme reaction system is analysed to study the effect of different parameters on the performance of amperometric biosensor. Numerical simulations of the problem are performed by applying finite difference method to a system of reaction-diffusion partial differential equation as a function of time and space. The limiting cases of catalytic site saturation and unsaturated site are considered with respect to steady state scenario and as well as only time dependent situation. The results of substrate, product and current response on diffusion moduli, enzyme thickness and saturation parameter are presented graphically and discussed quantitatively.

Keywords—Oxidoreductase, Enzyme, Kinetics, Amperometric, Biosensors

I. INTRODUCTION

A biosensor is any analytical integrated device that converts physico-chemical reactions (between the analyte and bio-sensing element) into analytical information which is proportional to the amount of analyte present in the sample [1]. The device uses specific bio recognition element such as enzyme, protein, antigen-antibody nucleic acid, cells, tissues, receptors and micro-organisms that selectively reacts with the targeted analyte from bulk sample [2], the application of the bio-recognition element enables high selectivity and specificity [3]. In recent years there have been increasingly demand of the device due to its application in different fields such as environment, food safety, health assessment and industrial monitoring as the result of their ability to detect trace quantities of biological analytes [4].

The device uses specific biochemical reactions catalysed by enzymes immobilized on electrodes catalyse the electrolytic oxidations or reductions of the substrates by using the redox compounds as electron transfer mediator [5], where the targeted analyte present in the substrate pass and become converted into product. The enzyme catalysed electrochemical oxidation/reduction of the substrate to yield product which interns gives rise to the catalytic current called bioelectrocatalysis [5]. For more than fifty years, the analyte and electrode reactions have been an attractive approach for the development of biosensors since 1962 the use of enzyme electrode was reported for the first time[6]. Various studies have been conducted on modelling the device, Kirthiga and Rajendran [5] reported the analytical solution of the nonlinear steady state differential equations by applying Homotopy analysis method. Rajendran and Anitha [7] applied Homotopy Perturbation method to the nonlinear reaction diffusion model. Analysis of the diffusion and kinetics in an enzyme-modified microcylinder was done and the system of differential equation solved by Homotopy perturbation method by Eswari and Rajendran [8] Despite the innovations in the improvement of biosensor devices, there still limited number of factors such as long-term stability, read-out time and miniaturization [9], therefore there is a need to continue the investigation of different parameter that affect the response of the device in order to have effective and reliable

devices for the measurement and analysis for samples. To improve the productivity and efficiency of biosensor design as well as to optimize the device configuration a model of the biosensor should be built and analysed [10].

The understanding of the kinetic peculiarities of the biosensors is of crucial significance for their design. Various mathematical models on enzyme kinetics have been formulated making the analysis on the performance of an amperometric biosensor and its optimization. Theoretical models give the information about the mechanism and kinetics operating in the biosensor. Unlike the experimental investigation of biosensors, where changing one parameter inevitably alters others, the influence of individual variable can be analysed in an idealized way when the process is translated into mathematical models. Thus the information gained from theoretical modelling can be useful in sensor design, optimization and prediction of biosensor's operation and response. This study therefore aims at making analysis of Oxidoreductase Enzyme Kinetics in an Amperometric Biosensor.

II. MODEL FORMULATION AND ANALYSIS

Fundamental understanding of sensor behaviour will enable efficient sensor optimization which is based on experimentation and validated model. This chapter thus presents a mathematical model that predicts the steady state and transient response of one dimensional biosensor model. The essential characteristic of enzyme activity is catalysis; it enhances or speeds up the rate of the reaction. The catalyst is neither consumed nor alters the equilibrium constants[11] and [12]. The following reaction scheme for immobilized enzyme in the presence of substrate is as follows

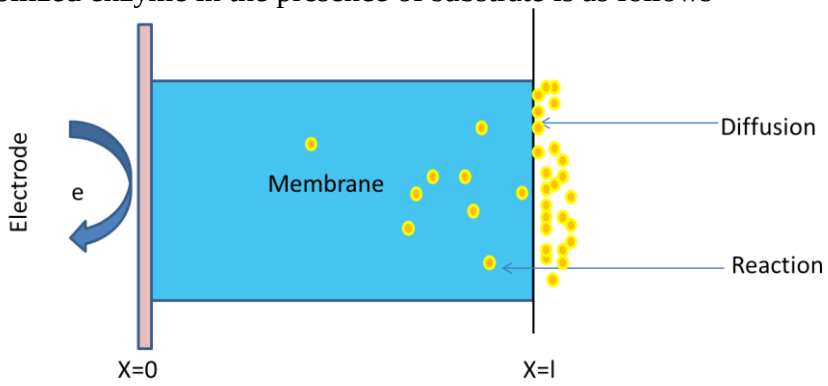

Figure 1. Schematic representation for an enzyme-membrane biosensor

$$S + E \underset{k_r}{\overset{k_f}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P \qquad (1)$$

The model equation (1) describes a single substrate-enzyme catalysed reaction. When the law of mass action is applied to this reaction scheme, it yields four differential equations for the rates of change of variables as seen in the system bellow.

$$\begin{aligned}
\frac{d[S]}{dt} &= k_r[ES] - k_f[E][S] \\
\frac{d[E]}{dt} &= (k_{cat} + k_r)[ES] - k_f[E][S] \\
\frac{d[ES]}{dt} &= k_f[E][S] - (k_{cat} + k_r)[ES] \\
\frac{d[P]}{dt} &= k_{cat}[ES]
\end{aligned} \qquad (2)$$

The concentration change of s and p is associated with the diffusion and the enzymatic reaction rate. The derivation of model is determined by both reaction and diffusion processes, the diffusion

term is achieved by applying Fick's second law, this law is the basis of most diffusion measurements and describes diffusion in a non-steady state condition when the concentration in the system varies with time. Coupling the enzyme catalysed reaction in enzyme layer with the one dimensional in space diffusion as described by Fick's law, the differential equations that quantify the diffusion and reaction within the film for $s = s(x,t)$ and $p = p(x,t)$, the enzyme catalysed reaction in the enzyme layer in one dimensional in space and time can be written a

$$\frac{\partial s(x,t)}{\partial t} = D_s \frac{\partial^2 s(x,t)}{\partial x^2} - \frac{V_{\max} s(x,t)}{K_M + s(x,t)}$$
$$\frac{\partial p(x,t)}{\partial t} = D_p \frac{\partial^2 p(x,t)}{\partial x^2} + \frac{V_{\max} s(x,t)}{K_M + s(x,t)} \qquad (3)$$

with the initial condition for the system given as,

$$s(x,0) = \begin{cases} 0, & 0 \le x < l, \\ s_0, & x = l, \end{cases} \qquad (4)$$
$$p(x,0) = 0, \quad 0 \le x \le l,$$

and the boundary conditions as

$$p(0,t) = 0, \quad \frac{ds}{dx}(0,t) = 0, \quad p(l,t) = 0, \quad s(l,t) = s_0 \qquad (5)$$

on the domain $0 < x < l$ and $0 < t \le T$ .This is the standard scheme of classical amperometric biosensor; the action of biosensor usually consists of alternating diffusion and enzyme layer reaction process [11]. It is assumed that; x=0 represents the electrode surface, while x=l represents the bulk solution membrane interface. For the biosensor to start operating requires some substrate to appear on the surface of the enzyme layer. The current at the anode $i_A(t)$ of the amperometric biosensor at time (t) can be obtained explicitly from Faraday's and Fick's laws [13].

$$i_A(t) = n_e F A D_p \left(\frac{\partial p}{\partial x} \right)_{x=0} \qquad (6)$$

The anodic current depends upon the flux of the electro active substances (analyte) at the electrode surface $x = 0$. That is the current directly proportional to the flux (concentration gradient) of the reaction product at the electrode surface. In the case of Biosensor, current is also directly proportional to surface area of the electrode. The corresponding steady state non-linear differential equations for the model are

$$D_s \frac{d^2 s(x)}{dx^2} = \frac{V_{\max} s(x)}{K_M + s(x)}, \quad D_p \frac{d^2 P(x)}{dx^2} = -\frac{V_{\max} s(x)}{K_M + s(x)}, \qquad (7)$$

with

$$p(0) = 0, \quad \text{s(0)=0}, \quad \frac{ds}{dx}(0) = 0, \quad p(l) = 0, \quad s(l) = s_0 \qquad (8)$$

We introduced the following dimension variables and parameters into the model equations as follows;

$$X = \frac{x}{l}, T = \frac{D_s t}{l^2}, S = \frac{s}{s_0}, P = \frac{p}{s_0}, \phi_i^2 = \frac{l^2 V_{\max}}{s_0 D_i}, \beta = \frac{K_M}{s_0}. \qquad (9)$$

and we obtained for the following

$$I = \lim_{t \to \infty} i(t) = n_e F D_p \left(\frac{dP}{dX} \right)_{X=0} \qquad (10)$$

For steady state scenario in equations (12)-(13), we obtain

$$\frac{d^2}{dX^2}\left(\frac{S(X)}{\phi_s^2}+\frac{P(X)}{\phi_p^2}\right)=0 \tag{11}$$

$$\frac{S(X)}{\phi_s^2}+\frac{P(X)}{\phi_p^2}=aX+b \tag{12}$$

$$P(X)=\phi_p^2\left(aX+b-\frac{S(X)}{\phi_s^2}\right) \tag{13}$$

The constants a and b are obtained by applying boundary conditions. The substrate and product concentrations are all related to the proces.

$$S(X)=1-\frac{1}{2}\left(\frac{\phi_s^2}{\beta+1}\right)\left[\left(1-X^2\right)+\frac{1}{12}\frac{\beta\phi_s^2}{(\beta+1)^2}\left(X^4-6X^2+5\right)\right] \tag{14}$$

$$P(X)=\phi_p^2\left(\begin{array}{l}\frac{1}{2}\left(\frac{1}{\beta+1}\right)\left[1+\frac{5\beta\phi_s^2}{12(\beta+1)^2}\right]X \\ +\frac{1}{\phi_s^2}\left[1-\frac{1}{2}\left(\frac{\phi_s^2}{\beta+1}\right)\left[1+\frac{5\beta\phi_s^2}{12(\beta+1)^2}\right]\right] \\ -\frac{1}{\phi_s^2}\left[1-\frac{1}{2}\left(\frac{\phi_s^2}{\beta+1}\right)\left[\begin{array}{l}\left(1-X^2\right) \\ +\frac{1}{12}\frac{\beta\phi_s^2}{(\beta+1)^2}\left(X^4-6X^2+5\right)\end{array}\right]\right]\end{array}\right) \tag{15}$$

A. For only time dependent corresponding model, we obtain

$$\begin{aligned} S(T)&=1-\frac{\phi_s^2}{\beta+1}\left[T+\frac{\beta\phi_s^2}{2(\beta+1)^2}T^2\right], \\ P(T)&=\frac{\phi_s^2}{\beta+1}\left[T+\frac{\beta\phi_s^2}{2(\beta+1)^2}T^2\right] \end{aligned} \tag{16}$$

As we have obtained the analytical solutions we now calculate the biosensor current response expressed as,

$$I=\frac{n_e F D_p \phi_p^2}{(\beta+1)}\left[1+\frac{5\beta\phi_s^2}{12(\beta+1)^2}\right]. \tag{17}$$

Following [8-10] the dimensionless parameter Dam Koehler numbers σ which essentially compares the rate of enzyme $\frac{V_{\max}}{K_M}$ with the diffusion through the enzyme layer $\frac{l^2}{D_s}$ can be obtain as

$$\sigma=\sqrt{\frac{V_{\max}l^2}{D_s K_M}}=\sqrt{\frac{\varphi_s^2}{\beta}} \tag{18}$$

The amperometric biosensor response considerably depends on the fact that either enzyme kinetics or the mass transport predominate the biosensor response[14]. The response is under diffusion control if $\sigma<1$, the enzyme predominate [14]. The overall kinetics is governed by the total active enzyme in the membrane. The response is under diffusion control if $\sigma>1$[15], which is observed at

high catalytic activity and active membrane thickness or low reaction rate constant or at low diffusion coefficient values [16]. At high substrate concentrations, biosensor response that is steady state current does not depend on initial concentration of substrate containing the analyte[17]. However in the intermediate case when initial substrate concentration is approximately equal to Michaelis-Menten constant, the analytical solutions are unknown and numerical methods are to be used to solve the problem [11].

III. GRAPHICAL RESULTS AND DISCUSSION

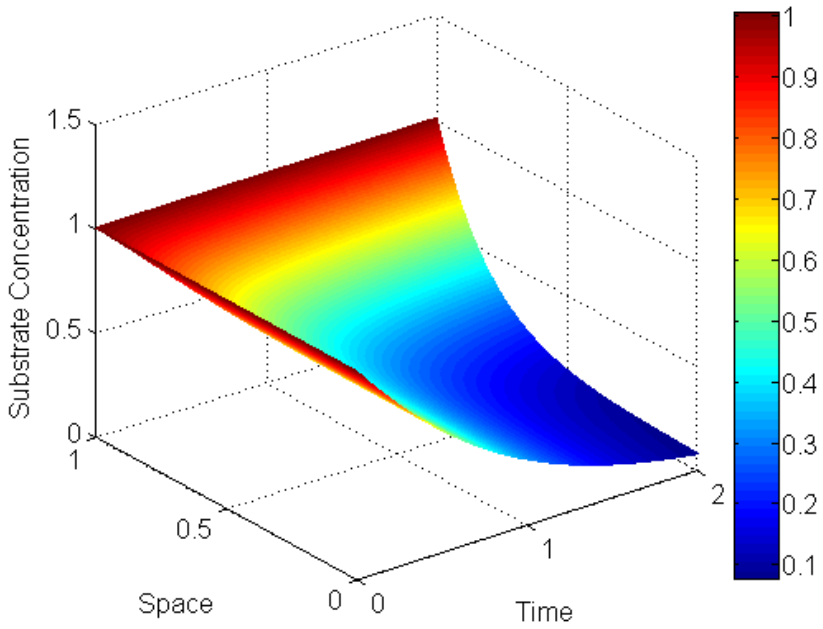

Figure 2. Profile of Substrate concentration function as of both time and space showing the variation of concentration with both variables

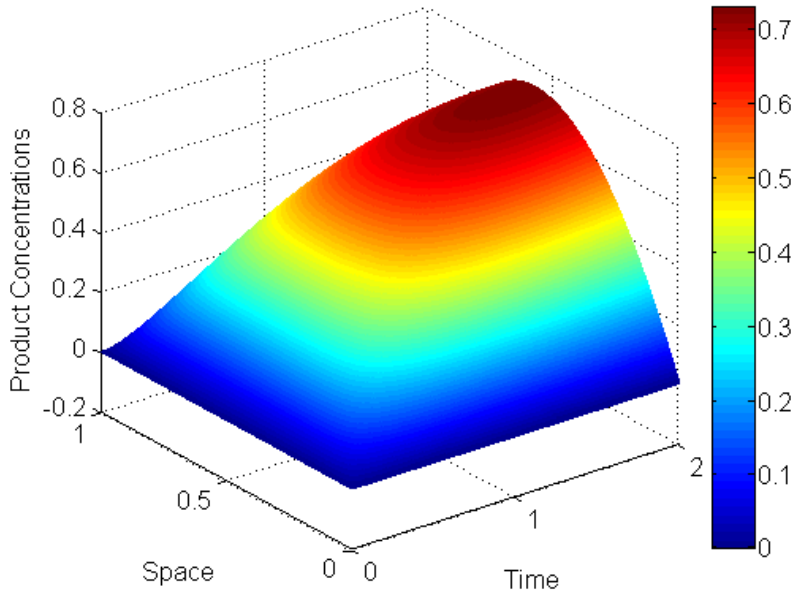

Figure 3. Profile of Product concentration as function of both time and space showing the variation of concentration with both variables

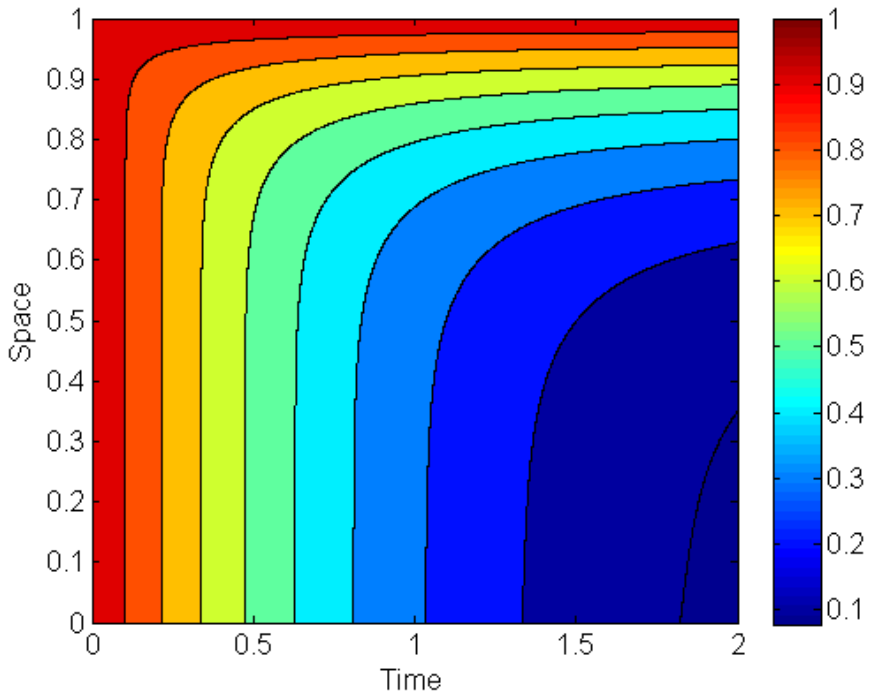

Figure 4. Contour plots of Substrate concentration as function of both time and space showing the variation of concentration with both variables.

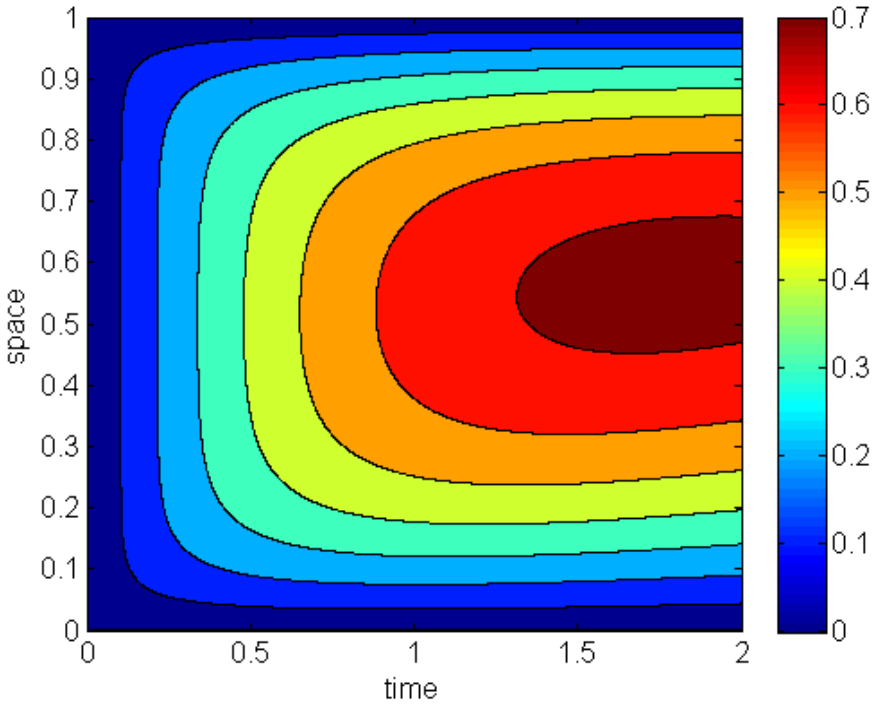

Figure 5. Contour plots of Product concentration as function of both time and space showing the variation of concentration with both variables.

Figure 4-5 show the normalized contour plots for substrate and product concentration respectively calculated using the reaction diffusion model equation, the contour plots clearly shows the boundaries of concentrations at different levels which could not be clearly seen from the three dimensional profiles Figure 2-3.

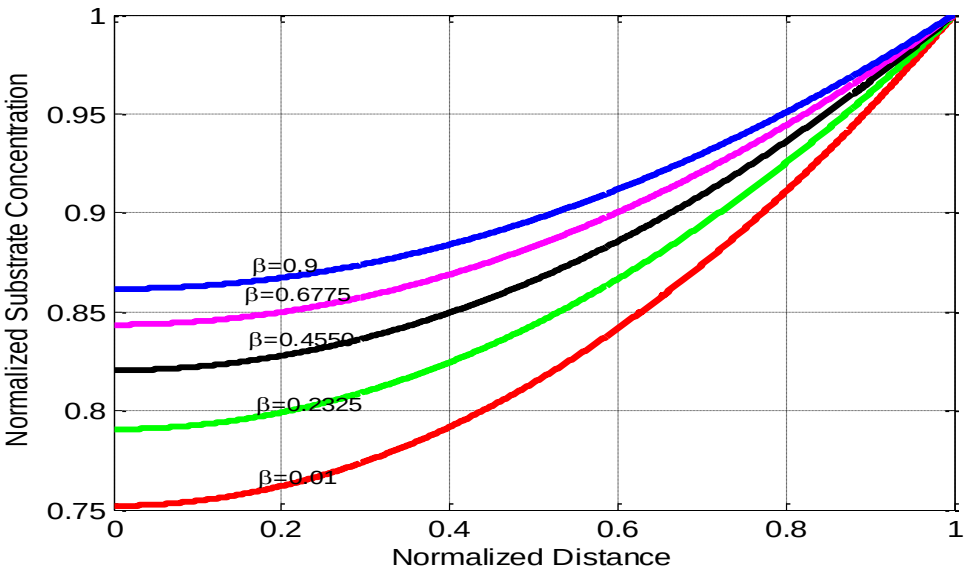

Figure 6. Profile of the normalized substrate concentration in the enzyme layer $X \in (0,1)$ ***at approximate steady state for different values of saturation parameter*** $\phi_s^2 = \phi_p^2 = 0.5.$

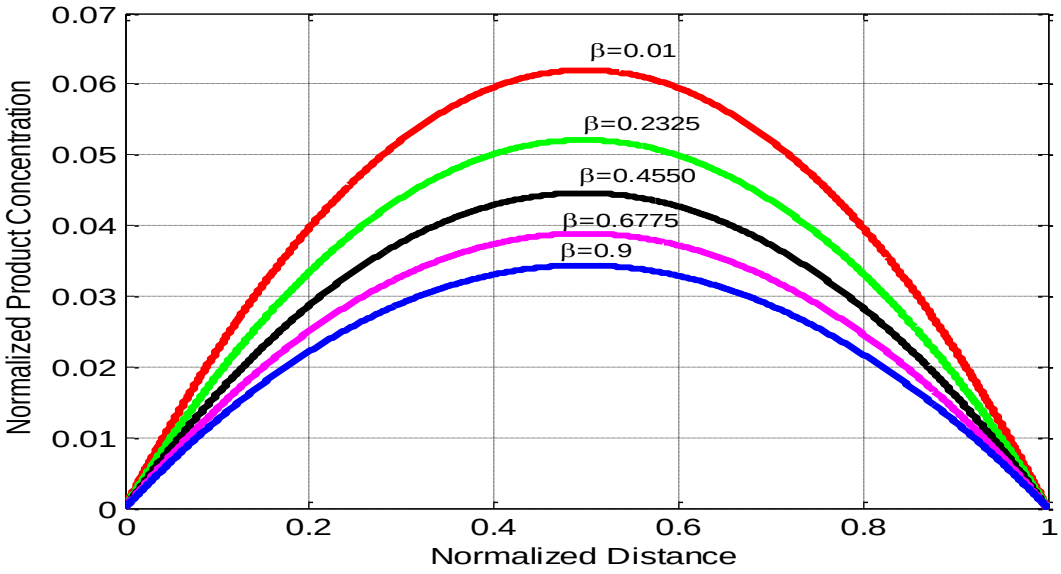

Figure 7. Profile of the normalized product concentration in the enzyme layer $X \in (0,1)$ ***at approximate steady state for different values of saturation parameter*** $\phi_s^2 = \phi_p^2 = 0.5.$

At small values for saturation parameter we observe high substrate conversion into product this can be observed from Figure 6-7 when the concentration are plotted against normalised distance while varying the saturation parameter. This gives us an idea of the type of enzyme to be used in the device, the enzyme to be used it is suggested that should have small K_M value.

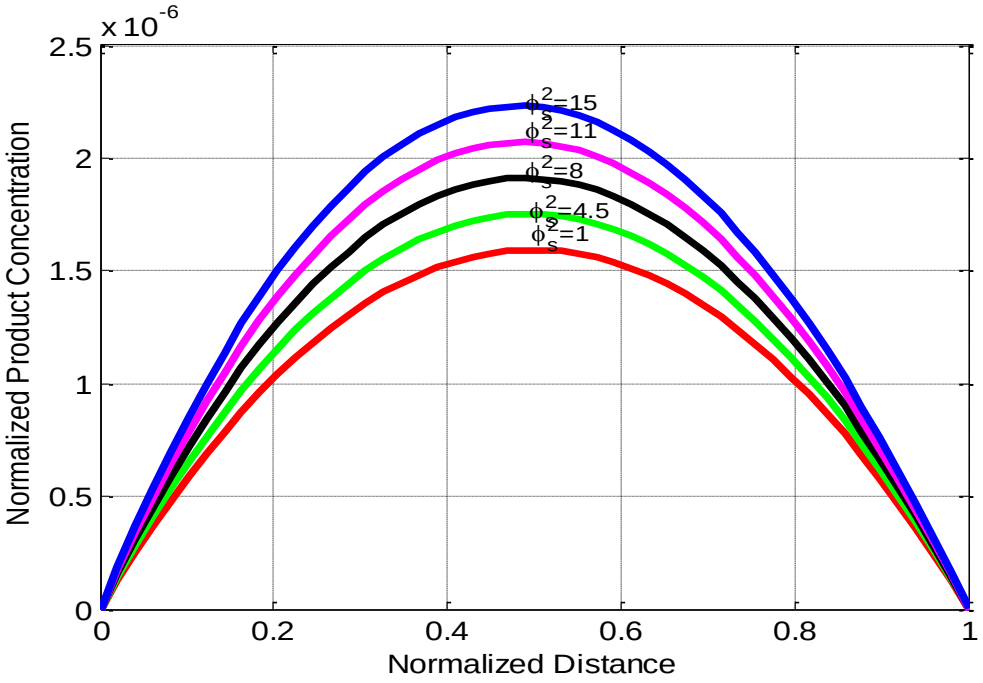

Figure 8. Profile of the normalized product concentration when substrate diffusion module is varied in the enzyme layer $X \in (0,1)$ ***at approximate steady state.***

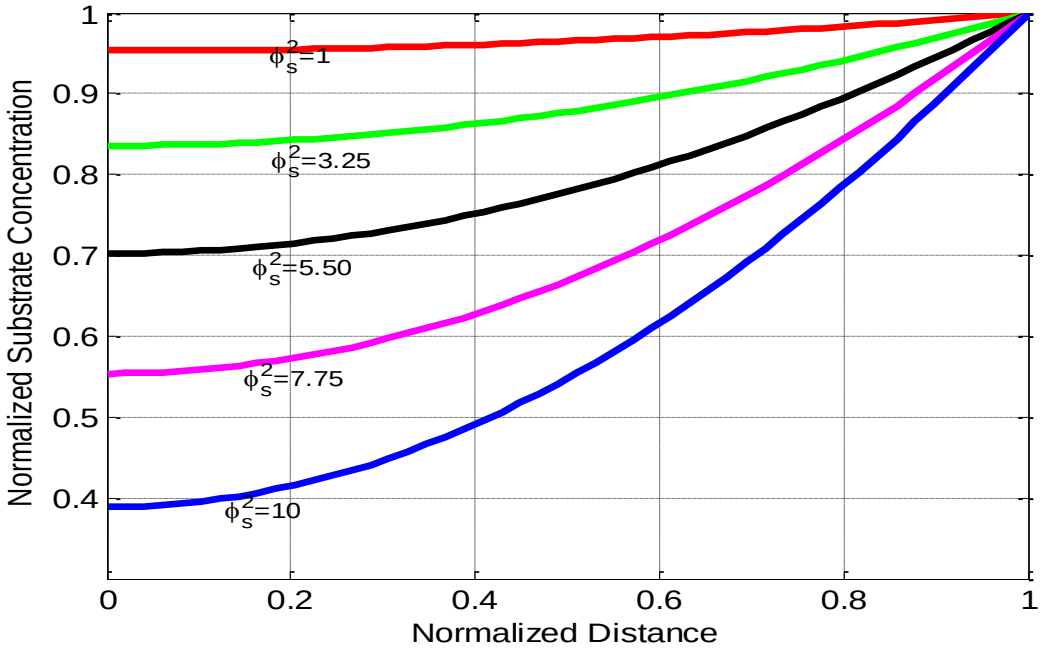

Figure 9. Profile of the normalized Substrate concentration when substrate diffusion module is varied in the enzyme layer $X \in (0,1)$ ***at approximate steady.***

From figure 9 it is confirmed that diffusion of substrate is dependent on the enzyme thickness at small values of substrate diffusion module large quantity of substrate seems to remain in the bulk solution, with increasing substrate diffusion module more substrate diffuse into the enzyme membrane.This is revealed in product formation from figure 8, with increasing substrate diffusion module. This confirms that the enzyme membrane thickness is important factor in designing of the device; large thickness of enzyme membrane enables high substrate diffusion. The increase in enzyme thickness consequently lead to increase in surface area, the high surface to volume ratio improves the sensitivity of the device thus from this model it evident that the higher the surface area,

the better the immobilisation and the higher the current response as result high flux of product at the electrode.

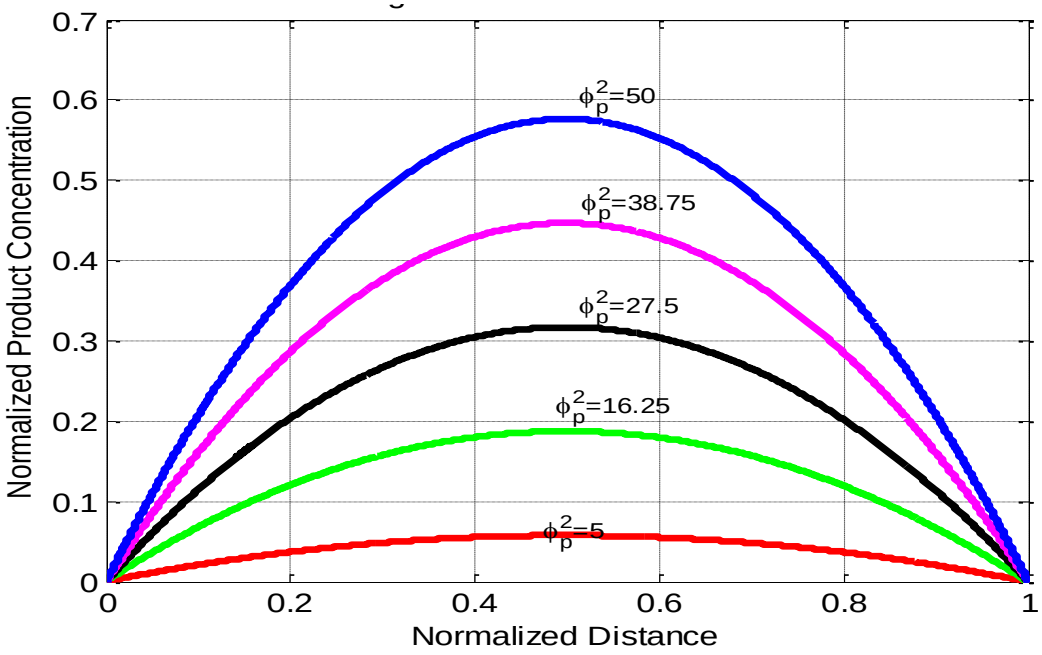

Figure 10. Profile of the normalized Product concentration when Product diffusion module is varied in the enzyme layer $X \in (0,1)$ ***at approximate steady.***

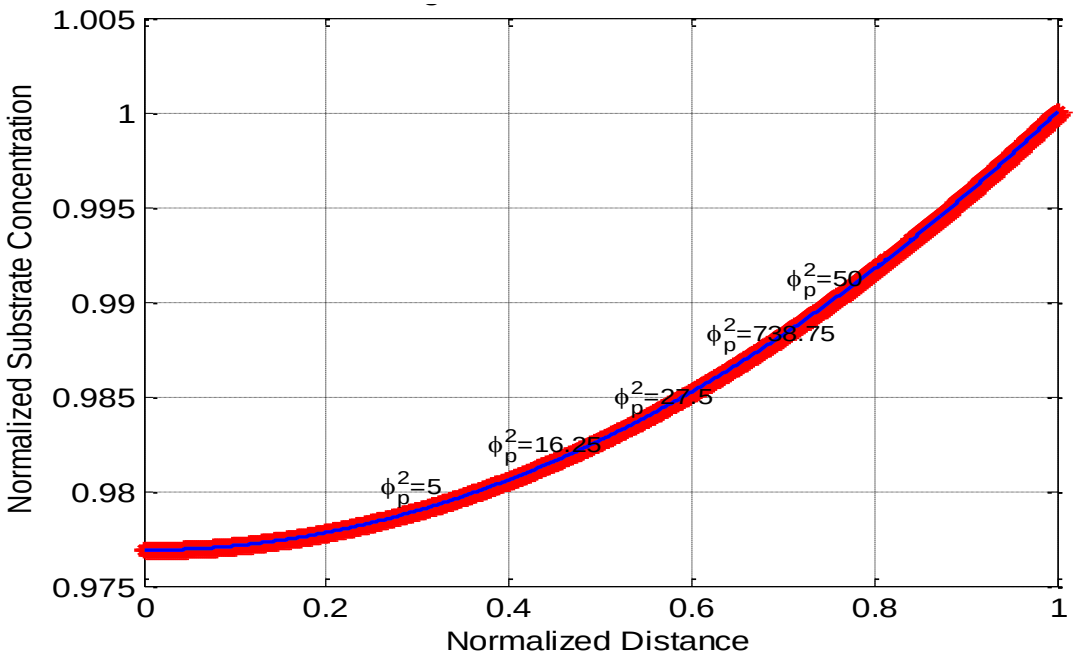

Figure 11. Profile of the normalized Substrate concentration when Product diffusion module is varied in the enzyme layer $X \in (0,1)$ ***at approximate steady.***

From Figure 10, It is observed that a change in the parameter ϕ_p^2 do not affect variation in Substrate concentration. Dimensionless substrate is maximum at $X = 1$ whereby $S(1) = 1$. Variation of product diffusion module leads to the change in the product concentration profiles can be noted from figure 11. The product concentration increases with increase in the product diffusion module.

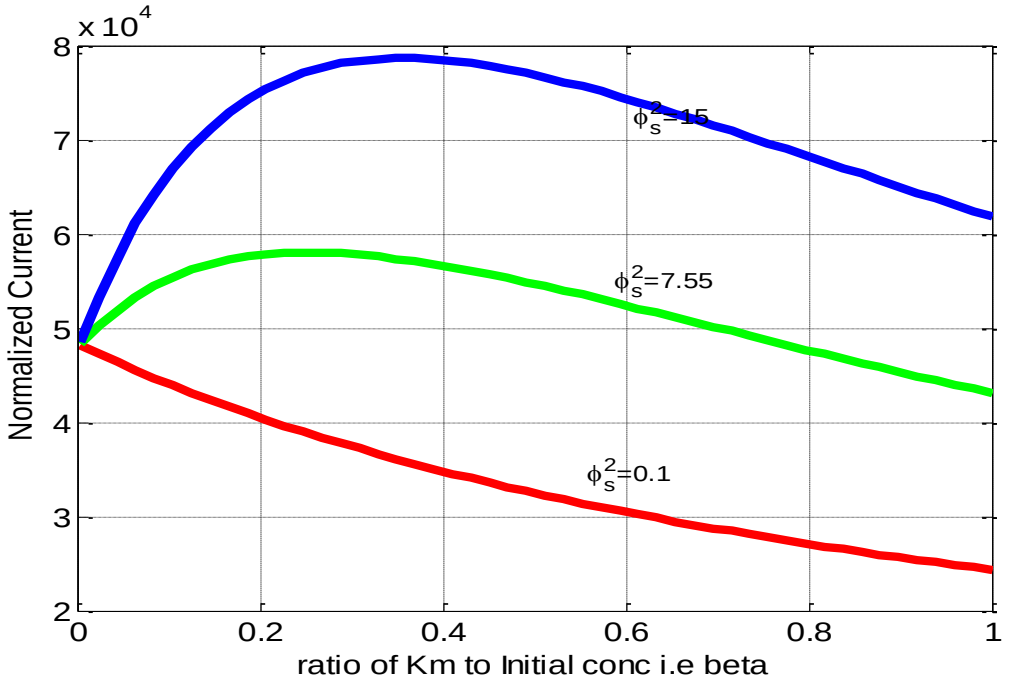

Figure 12. Dependence of steady state normalized current response on saturation for different parameter values of substrate diffusion module.

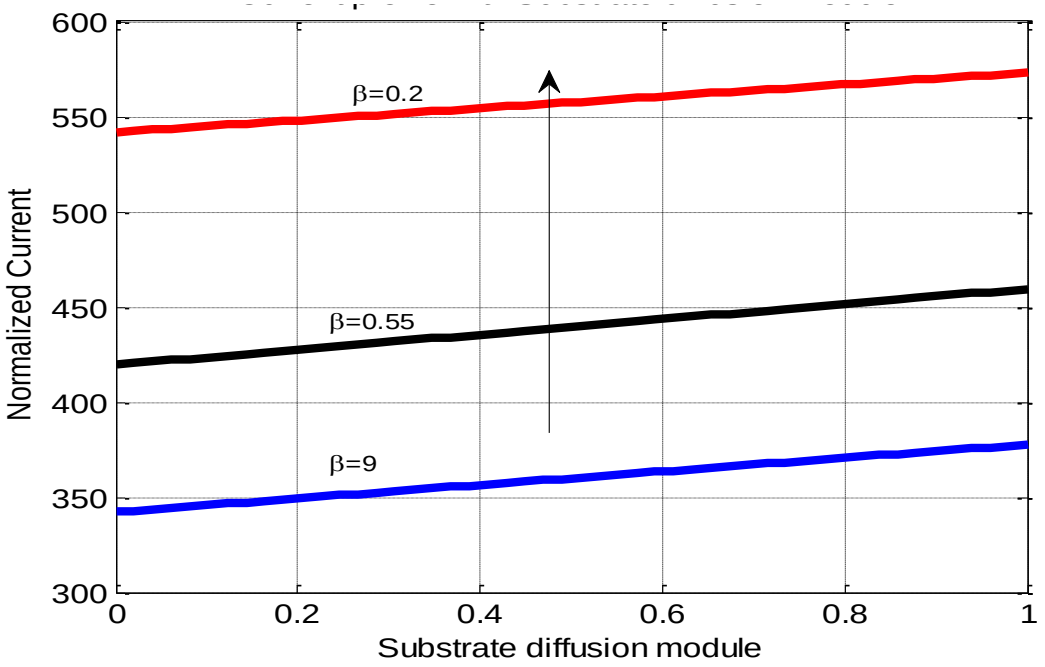

Figure 13. Dependence of steady state normalized current response on Substrate diffusion module $\left(\phi_s^2 < 1\right)$ ***for different values of saturation parameter*** β **.**

The electrochemical behaviour of the device was investigated by changing β parameter values. Figure 13 shows the linear relationship when the response current I is plotted against the substrate diffusion module. The response current of the device is increasing with decreasing β values. We observe that at high substrate affinity and large enzyme thickness the device shows high steady state current response, which implies that we can get response at relatively low concentrations of analytes in the sample. The response is low when the initial concentration is small and as the concentration

increases the reaction rate increases, until when it reaches the maximum enzymatic rate V_{max} (enzyme is full saturated with substrate).

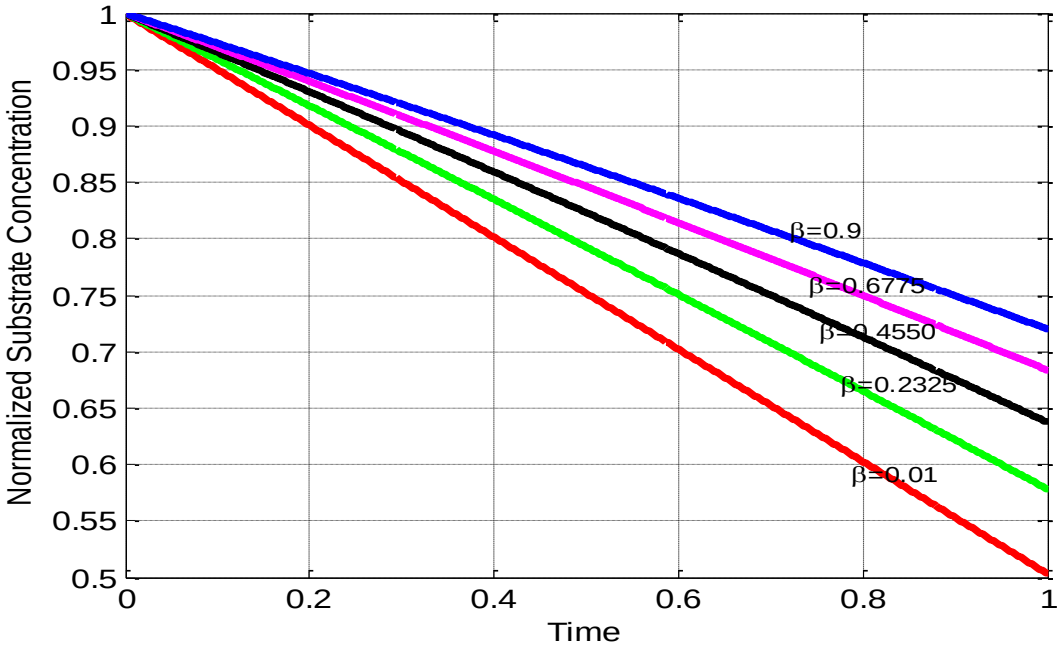

Figure 14. Substrate concentration as function time for different saturated parameters.

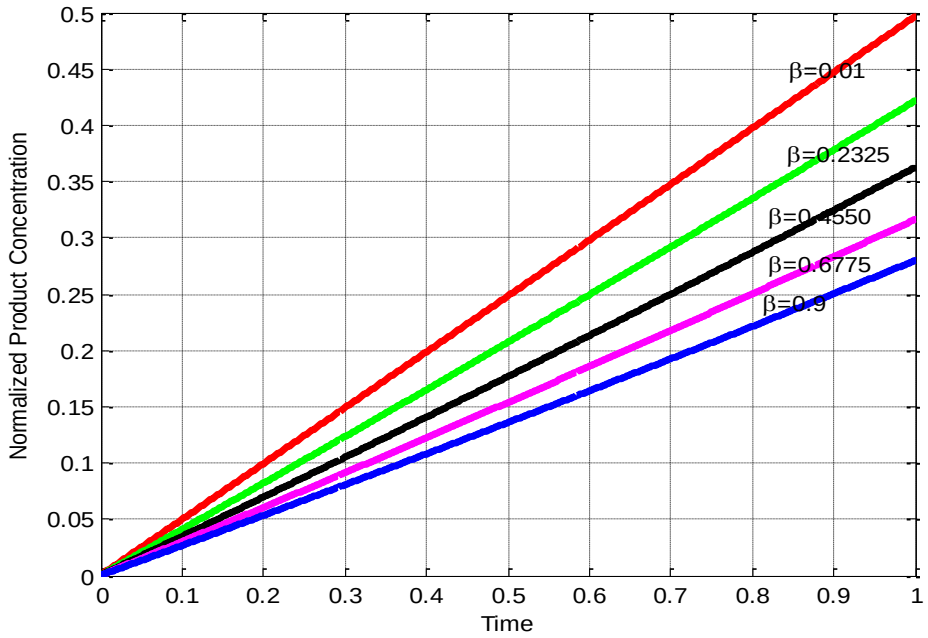

Figure 15. Product concentration as function time for different saturated parameters.

Figures 14-15 show the change in substrate and product concentrations respectively with time upon variation of saturation parameter. The concentration of substrate seems to be highly converted into product at small values of saturated parameter.

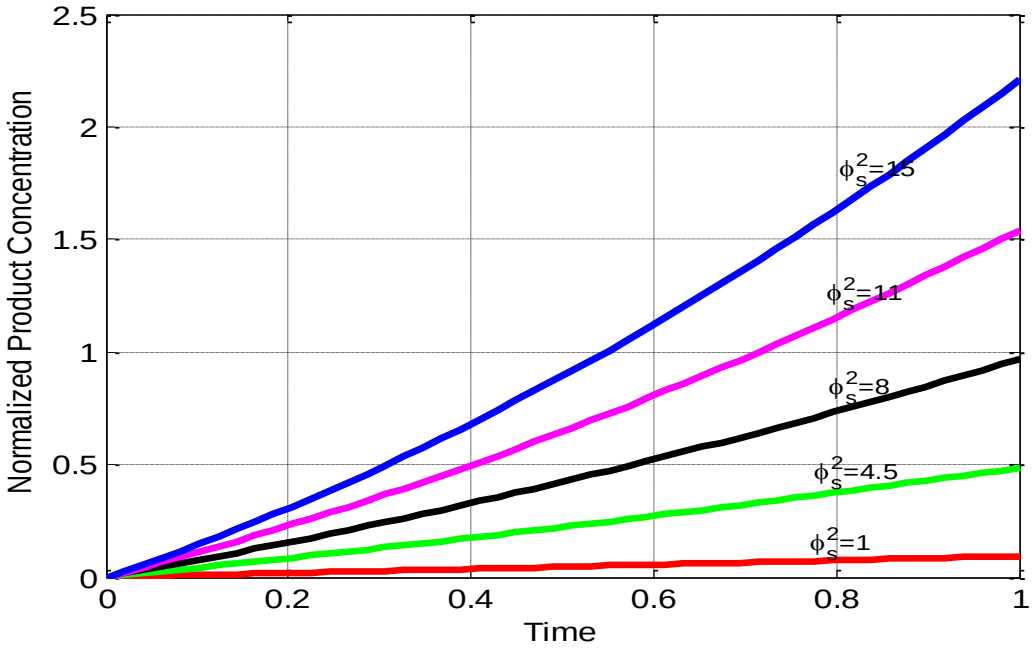

Figure 16. Product concentration as function time for different diffusion module.

Figure 16 represents the product concentration against time as the substrate diffusion module is varied. As it was expected product concentrations seems to increase with time. At t=0 the concentration product is zero, as the time goes on there is a formation of product. This formation of product is facilitated by a number of variable factors mainly Michaelis-Menten constant, initial substrate, enzyme thickness, maximum ferocity of the reaction and substrate diffusion constant which is reflected in the substrate diffusion. The product concentration is low at low values of substrate diffusion module. At higher values of substrate diffusion module we observe high values of product concentrations.

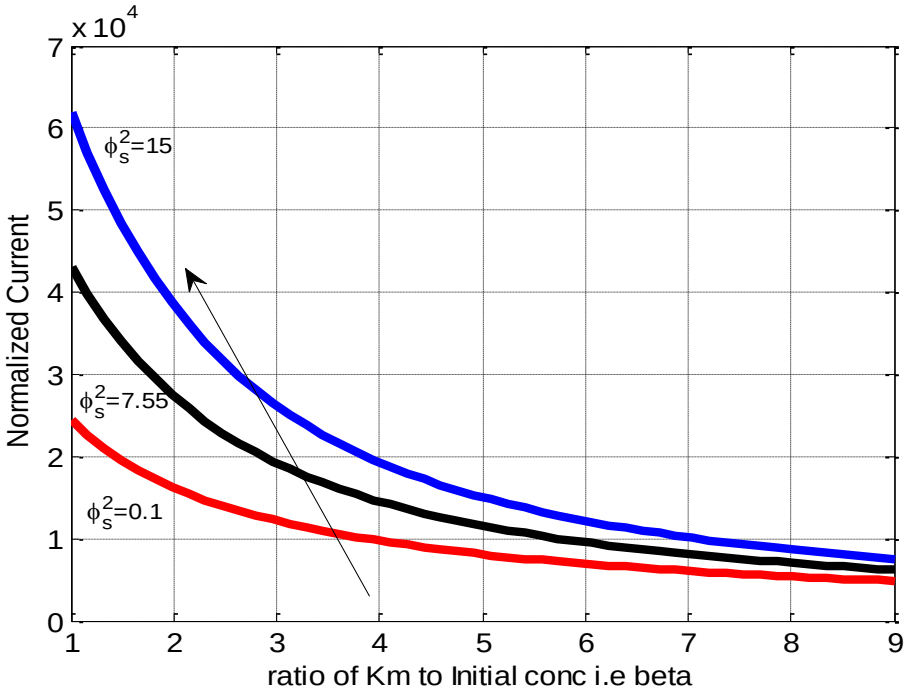

Figure 17. Normalized current versus saturation parameter for different values of substrate diffusion module.

Increasing the film thickness decreases the concentration of the substrate from the bulk solution which means that large quantity of substrate diffuse into the membrane to undergo the reaction and

hence reduction-oxidation occurs at the electrode surface. From this fact the observation is made from figure 17, we noted the increase of current density with increasing substrate diffusion module $\phi_s^2\left(s_0, l, V_{\max}, D_s\right)$. Also the response current decreases with increasing $\beta(k_M, s_0)$, this implies that the type of enzyme to be used in manufacturing of biosensor devises must have small K_M values which enables high substrate affinity and low values for diffusion coefficients for substrate.

IV. CONCLUSION

In this work mathematical model describing the amperometric biosensor has been presented and the analysis made. The model nonlinear PDE was tackled numerically using finite difference technique while the ODE special cases were solved analytically. The results obtained are used to analyse the effect of different parameters such as enzyme membrane thickness and enzyme loading in the membrane. The theoretical result is also useful for the optimization of the biosensor response.

REFERENCES

[1] Khor, B.H., et al., *A redox mediated UME biosensor using immobilized Chromobacterium violaceum strain R1 for rapid biochemical oxygen demand measurement.* Electrochimica Acta, 2015. **176**: p. 777-783.
[2] Ronkainen, N.J., H.B. Halsall, and W.R. Heineman, *Electrochemical biosensors.* Chemical Society Reviews, 2010. **39**(5): p. 1747-1763.
[3] Zhang, D. and Q. Liu, *Biosensors and bioelectronics on smartphone for portable biochemical detection.* Biosensors and Bioelectronics, 2016. **75**: p. 273-284.
[4] Zhang, M., et al., *Kinetic Studies of microfabricated Biosensors Using Local Adsorption Strategy.* Biosensors and Bioelectronics, 2015.
[5] Kirthiga, O. and L. Rajendran, *Approximate analytical solution for non-linear reaction diffusion equations in a mono-enzymatic biosensor involving Michaelis-Menten kinetics.* Journal of Electroanalytical Chemistry, 2015.
[6] Clark, L.C. and C. Lyons, *Electrode systems for continuous monitoring in cardiovascular surgery.* Annals of the New York Academy of sciences, 1962. **102**(1): p. 29-45.
[7] Rajendran, L. and S. Anitha, *Reply to "Comments on analytical solution of amperometric enzymatic reactions based on Homotopy perturbation method," by Ji-Huan He, Lu-Feng Mo [Electrochim. Acta (2013)].* Electrochimica Acta, 2013. **102**: p. 474-476.
[8] Eswari, A. and L. Rajendran, *Analytical solution of steady-state current an enzyme-modified microcylinder electrodes.* Journal of Electroanalytical Chemistry, 2010. **648**(1): p. 36-46.
[9] Scognamiglio, V., et al., *Synthetic biology and biomimetic chemistry as converging technologies fostering a new generation of smart biosensors.* Biosensors and Bioelectronics, 2015. **74**: p. 1076-1086.
[10] Eswari, A. and L. Rajendran, *Analytical solution of steady state current at a microdisk biosensor.* Journal of Electroanalytical Chemistry, 2010. **641**(1): p. 35-44.
[11] Gaidamauskait, E., *Computational modeling of complex reactions kinetics in biosensors*, 2011, Doctoral Dissertation, Physical Sciences, Informatics (09P), Vilnius.
[12] Muthukumar, S. and L. Rajendran, *Analytical expressions of the concentrations of substrate and product in enzyme inhibition process.* Natural Science, 2013. **5**(09): p. 1047.
[13] Liu, Y., *Mathematical and Computational Modelling for Biosensors: A Modular Approach.* 2012.
[14] Baronas, R., et al., *Computational modelling of a sensor based on an array of enzyme microreactors.* Nonlinear Anal. Model. Control, 2004. **9**: p. 203-218.
[15] Baronas, R., et al., *Mathematical Modeling of Plate− gap Biosensors with an Outer Porous Membrane.* Sensors, 2006. **6**(7): p. 727-745.
[16] Shunmugham, L. and L. Rajendran, *Analytical Expressions for Steady-State Concentrations of Substrate and Oxidized and Reduced Mediator in an Amperometric Biosensor.* International Journal of Electrochemistry, 2013. **2013**.
[17] Carr, P.W. and L.D. Bowers, *Immobilized enzymes in analytical and clinical chemistry*. 1980: Wiley.