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Theoretical treatment of diffusion and kinetics of osmium redox polymer mediated glucose oxidase enzyme electrodes: Analytical expression of current density for varying potential.

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Abstract

We present for the first time a mathematical model of osmium redox polymer mediated glucose oxidase enzyme electrodes. This model is based on a system of three coupled nonlinear reaction-diffusion equations under steady-state conditions for biochemical reactions occurring in the biofuel cells that describes the oxidized mediator, oxygen and substrate (Glucose) concentration within the biofuel cell. Simple analytical expressions for the concentration of oxidized mediator, oxygen and substrate and the corresponding current-potential response have been derived for all the values of reaction diffusion parameters using the new homotopy perturbation method (NHPM). The current-potential response in osmium redox polymer mediated glucose oxidase enzyme electrodes is discussed. The analytical results for the concentrations are also compared with numerical results and a satisfactory agreement is noted. The influence of diffusion coefficient of mediator, thickens of the film, turnover rate of Gluocose Oxidase and Michaelis-Menten constant on current-potential curve is also analyzed.

Keywords:

Mathematical modeling, Biofuel, Osmium redox polymer, Non-linear reaction diffusion equation,

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1. Introduction

Enzymatic fuel cell convert the chemical energy of biofuel into electrical energy. Enzymatic biofuel cells are the electricity generating devices that mimic this process by using enzyme-modified electrons using oxygen as the final electrodes acceptor [1-6]. These devices have the potential to provide a flexible, compact, and inexpensive micropower sources [7]. Glucose oxidizing enzyme electrodes have long been studied for their application to biosensors and, more recently, anodes in biofuel cells[8].

Theoretical models in biofuel cells are useful to identify and optimize important experimental parameters such as film thickness, diffusivity of mediators, loading of biocatalysts, kinetic parameters, amount of substrates, mediators and inhibitors etc. Mathematical models must incorporate consideration of various processes like electron and species transport, reaction mechanism and experimental techniques etc, which controls the overall performance[9]. Interesting approach for the theoretical modeling of enzymatic approach is discussed by Glykys *et al.* [10]. Paul Kavanagh *et al.* recently analyzed mediated electron transfer processes for glucose oxidizing enzyme electrodes based on anodes in a biofuel cell [8]. In the literature contains several modeling of enzymatic electrodes [10-13] and enzymatic biosensors [14], the modeling of enzymatic electrodes has been more attractive due to the consideration of the enzymatic reaction and the material balance of species which are participating in the enzymatic reaction. Andrieux and Saveant [15] have reported kinetics of electrochemical reactions mediated by redox polymer films for stationary voltammetry techniques. Bartlett *et al.* [16,17] presented the analysis by considering Michaelis-Menten enzyme kinetics and stated one dimensional catalytic film model for steady state conditions. Gallaway *et al.* [3] used this approach to obtain the kinetic information of oxygen reducing laccase-based electrodes, having different osmium redox polymers mediated through redox hydrogels. For the enzyme kinetics problem, approximate analytical solutions have been developed by Elaedel *et al.* [18], Kulys *et al.* [19] and Bartlett and Whitaker [20] only for the limiting cases (saturated and unsaturated). The applications of numerical and approximate analytical methods have been reported by Barlett and Pratt [16].

Senthamarai and Rajendran [21] derived the approximate analytical expressions for the concentration of substrate, mediator and current for the non-linear Michaelis–Menten kinetic scheme by solving a system of non-linear coupled reaction-diffusion using the variational iteration method. Logambal *et al.* [22] presented the approximate analytical expressions for the concentrations of the mediator and substrate using homotopy perturbation methods. Rasi *et al.* [23] presented a theoretical model describing the transient response of electroreduction of oxygen to water in the presence of laccase

enzyme, interacting via ping-pong kinetic scheme. Mathematical modelling of non-linear reaction and diffusion processes in a biofuel cell were also discussed [24]. A novel graphical procedure for estimating the Michaelis-Menten constants and turnover rate solely from the current-potential curve is suggested in this manuscript. Influence of the controllable parameters such as diffusion of the mediator, Michaelis-Menten constant for substrate, second-order rate constant, thickness of the film, turnover rate and initial substrate concentration on the current density are also presented. Bambhania [9] developed the kinetics of osmium redox polymer mediated glucose oxidase electrodes. To the best our knowledge, no analytical expression for the concentration of mediator, oxygen and substrate has been derived. In this paper we have derived for the first time the simple and closed-form of an approximate analytical expressions for the concentration of mediator, oxygen and substrate in terms of kinetic parameters. This modeling approach is useful to understand and optimize the kinetics behavior of the enzymatic fuel cells.

2. Analytical expressions of concentrations using a new approach to the new homotopy perturbation method

Figure 1 shows the general kinetic scheme of reaction rate in the redox hydrogel film-modified enzyme electrodes, which may be limited and affected by several factors such as: electron transport via mediator, enzyme kinetics, substrate transport and by the presence of oxygen for the GOx based electrodes. The one-dimensional steady state equations for the reduced form of GOx (Appendix A) can be written as follows [9]:

$$\frac{d^2a}{d\chi^2} = \frac{\kappa^2 as}{\gamma a\ (1+\mu s)+s} \tag{1}$$

$$\frac{d^2o}{d\chi^2} = \frac{\kappa^2 os\eta^{-1}\beta d}{\gamma a\ (1+\mu s)+s} \tag{2}$$

$$\frac{d^2s}{d\chi^2} = \frac{\kappa^2 as\eta^{-1}\gamma}{\gamma a\ (1+\mu s)+s} \tag{3}$$

Dimensionless boundary conditions for eqns. (1-3) are as follows:

$$\begin{aligned} &a = a_e;\ \frac{do}{d\chi} = 0;\quad \frac{ds}{d\chi} = 0; \qquad when\ \chi = 0 \\ &\frac{da}{d\chi} = 0\ ;\ o = 1-\sigma_2\left(\frac{do}{d\chi}\right);\quad s = 1-\sigma_1\left(\frac{ds}{d\chi}\right); \qquad when\ \chi = 1 \end{aligned} \tag{4}$$

Eqs(1-3) are non linear differential equations. Non linear differential equations play a crucial role in many branches of physical sciences. Solving systems of non linear differential equations have gained importance and popularity in recent years, mainly due to the necessity of analytical solutions in diverse fields of science and engineering.

Many authors have paid attention to study the solutions of non linear differential equations by using various advanced analytical methods such as Homotopy perturbation method [25], Homotopy analysis methods [26], variational iteration methods [27], Laplace Adomian decomposition methods [28], a new approach to Homotopy perturbation method [29,30] among others. Among these, a new approach to the Homotopy perturbation methods are employed to solve the non linear ordinary differential equations (1) – (3). The advantage of this method is that, the results are given in a simple form which is the zeroth iteration [29]. Recently, an analytical expression of the concentration of mediated bio-electrocatalysis for the steady and non-steady-state conditions have been derived using Danckwerts' expression and new approach to homotopy perturbation method [30]. Using the same method the concentration of mediator, substrate (glucose) and oxygen are obtained as follows (Appendix B):

$$a(\chi)=\frac{a_e}{\cosh\sqrt{\xi}}\cosh\sqrt{\xi}\,(1-\chi) \tag{5}$$

$$s(\chi)=\frac{\gamma}{\eta}\left\{a(\chi)+\left[\frac{\eta}{\gamma}\left(1-\frac{\gamma}{\eta}\frac{a_e}{\cosh\sqrt{\xi}}-(\sigma_1+1)\frac{\gamma}{\eta}\,a_e\sqrt{\xi}\tanh\sqrt{\xi}\right)\right]\right\} \tag{6}$$

$$o(\chi)=\frac{\cosh\sqrt{\varsigma}\,\chi}{\cosh\sqrt{\varsigma}+\sigma_2\sqrt{B}\sinh\sqrt{\varsigma}} \tag{7}$$

$$\text{where } \xi=\frac{\kappa^2\lambda}{\gamma\,a_e\left(\frac{1}{\lambda}+\mu\right)+1},\ \varsigma=\frac{\kappa^2\eta^{-1}\beta\,d\,\lambda}{(\gamma\,a_e+\beta)\,(1+\mu\lambda)+\lambda},\ \lambda=\frac{1}{\sigma_1\sinh(1)+\cosh(1)} \tag{8}$$

Concentration of the reduced enzyme is

$$\frac{E_{red}}{E_T}=\frac{s(\chi)}{\gamma\,a(\chi)[1+\mu\,s(\chi)]+s(\chi)} \tag{9}$$

where $a(\chi)$ is the mediator concentration, $s(\chi)$ is the concentration of substrate and $o(\chi)$ is the concentration of oxygen which are given in Eq.(5-7). The concentration of substrate at electrode surface is given by the following equation:

$$s(\chi)_{\chi=0} = \frac{\gamma}{\eta}\left\{a(\chi) + \left[\frac{\eta}{\gamma}\left(1 - \frac{\gamma}{\eta}\frac{a_e}{\cosh\sqrt{\xi}} - (\sigma_1 + 1)\frac{\gamma}{\eta}\, a_e \sqrt{\xi}\tanh\sqrt{\xi}\right)\right]\right\} \tag{10}$$

At the surface of the electrode the concentration of the reduced enzyme becomes

$$\left[\frac{E_{red}}{E_T}\right]_{\chi=0} = \left\{1 + \gamma\frac{a_e}{\cosh\sqrt{\xi}}\cosh\sqrt{\xi}\left[\left(\frac{\gamma}{\eta}\left\{\frac{a_e}{\cosh\sqrt{\xi}}\cosh\sqrt{\xi} + \left[\frac{\eta}{\gamma}\left(1 - \frac{\gamma}{\eta}\frac{a_e}{\cosh\sqrt{\xi}} - (\sigma_1 + 1)\frac{\gamma}{\eta}\, a_e\sqrt{\xi}\tanh\sqrt{\xi}\right)\right]\right\}\right)^{-1} + \mu\right]\right\}^{-1} \tag{11}$$

Using Eq. (5) the dimensionless current density is given by the following equation:

$$J_{obs} = \frac{L_{j_{obs}}}{D_m[M_T]} = -\left(\frac{d\,a}{d\chi}\right)_{x=0} = a_e\sqrt{\xi}\tanh\sqrt{\xi} \tag{12}$$

3. Discussion

Eqs. (5-7) are the new simple approximate analytical expressions for the concentrations of oxidized mediator, substrate and oxygen. Concentration profiles of mediator $a(\chi)$, substrate $s(\chi)$ and oxygen $o(\chi)$ against the normalized distance from the electrode interface χ, are shown in Figures 2-4 for various values of kinetic parameters.

3.1 Influence of kinetic parameters over the concentration of mediator

Figure 2 shows the concentration of mediator versus normalized distance from the electrode interface. The results obtained using NHPM is compared with numerical results in Fig. 2. Satisfactory agreement is noted. From Fig.2a, it is inferred that, a decrease in κ (hydrogel film thickness *L*) allows an increase in the mediator concentration. Also the concentration is uniform when $\kappa \leq 0.1$. From Fig. 2b, it is observed that the concentration of mediator does not differ significantly for various values of parameter μ or bulk concentration of mediator. From Figure-2(a-b) it is also observed that, oxidized mediator concentration increases, which is discernible from the fact that oxygen has been more efficient electron capturing agent due to its higher bimolecular rate constant with respect to GOx and higher diffusivity (D_O) as compared to that of the redox polymer (D_m). From the Fig. 2c, it reflects that the increased concentration of mediator increases the rate of turnover of the reduced enzyme back to its active oxidised form. From Fig. 2d, it is inferred that σ_1 is directly proportional to the concentration of

mediator. The distance from the electrode interface as a function of the hydrogel thickness is shown in Fig. 2e, for different concentrations of mediator present in the hydrogel film. The hydrogel film thickness depends upon concentrations of mediator and distance from electrode interface. It is clear from Fig. 2e, that the film growth rate is nearly the same for different mediator concentrations.

3.2 Influence of kinetic parameters over the concentration of substrate (glucose)

Figure 3 shows the concentration of substrate (glucose) versus distance from the electrode surface for various values of parameters γ, κ and μ. From this figure, it is observed that the concentration of substrate at the electrode surface is less than concentration of substrate at solution electrode interface for all values of parameters. This is due to depletion of the substrate concentration in the vicinity of the electrode when there is a high mediator concentration, high enzyme activity, and low substrate concentration. It is also noted that the concentration is uniform when γ, κ are very small and μ is very large.

From the Fig. 3a, it is observed that the concentration of substrate at electrode surface is small when bulk mediator concentration at electrode surface is higher or bulk substrate concentration at electrode surface is lower, this is because of glucose consumption. The experimental values are given in Table 1e. From the Fig. 3b, it is inferred that, when κ, or thickness of hydrogel decreases when the concentration of substrate is higher or less consumption of substrate. Concentration of substrate is directly proportional to bulk concentration of substrate (Fig. 3c).

3.3Influence of parameters over the concentration of substrate at electrode surface

The substrate concentration at the electrode surface is a crucial parameter in mass transfer. Figure 4 shows that the variation of the surface concentration of the substrate with potential for different system parameters is estimated using Eq.10. Hence, explicit equations for surface concentrations pertaining to electrochemical reactions are essential to comprehend reaction mechanisms.

From Figure 4, it is inferred that the concentration of substrate at electrode surface depends upon thickness of the hydrogel, bulk substrate concentration, diffusion layer thickness and ratio of diffusion coefficient of substrate and mediator. The substrate concentration at electrode surface increases when thickness of the hydrogel (Fig. 4a) and diffusion layer thickness (Fig. 4d) decreases, whereas it increases when bulk substrate concentration (Fig. 4c) and ratio of diffusion coefficient of substrate and mediator

increases (Fig. 4e) due to reoxidation of enzyme. The substrate concentration does not depends upon ratio of bulk concentration of mediator and substrate ($[M_T]_\infty/[S]_\infty$) or ration of Michaelis-Menten constant and turnover rate of GOx (K_S/k_{cat}).

3.4 Influence of parameter over the concentration of oxygen (Fig. 5)

The concentration profile of oxygen is shown in Fig. 5. Our analytical result is compared with numerical result in Fig. 5a, for the experimental values of parameters [9]. Satisfactory agreement is noted. From the Figs. 5a-5e, it is inferred that the concentration of oxygen at electrode surface decreases when γ, μ decreases and κ, β increases. This is due to removal of electrons by oxygen from the reduced active site of GOx.

3.5 Influence of parameters over the concentration reduced enzyme (Fig. 6)

The analytical result of concentration of reduced enzyme is compared with numerical result in Fig. 6a. The concentration-potential profile for reduced enzyme is shown in Figs. 6b-6f. The experimental values are given in Table 1f. Concentration increases with increase in k_{cat} (Fig. 6b), and decreases in k_m (Fig. 6c), K_S (Fig. 6e). No significant changes occurs when thickness of the hydrogel L (Fig. 6e) and diffusion coefficient D_m (Fig. 6f) changes. The concentration of reduced enzyme attains the steady state value when potential is greater than 2.

4. Effects of various parameter over the current density

4.1 Effects of all dimensionless parameters over the current-potential profile (Fig. 7)

Figure 7 depicts the dependence of the current density on the electrode potential using Eq.(12) for various values of ratio of Michaelis-Menten constant (K_S) and turnover rate of GOx (γ)(Fig. 7a), ratio of reaction rate constant between enzyme and mediator and diffusion coefficient of the mediator or thickness of hydrogel (κ) (Fig. 7b), bulk substrate concentration (μ) (Fig. 7c) and ratio between diffusion coefficient of the substrate and thickness of the film (σ_1) (Fig. 7d).

The current is produced at the electrode surface due to the oxidation of the glucose. From the Figs. 7a-7d it is observed that the current increases with increasing potential until the potential independent plateau current is reached. This plateau current depends on the mediator (Fig. 7a) and substrate concentration (Fig. 7c), enzyme concentration (Fig. 7b) and kinetics of the system (electron and

substrate transport in the film) (Fig. 7d). For large negative potential ($\varepsilon \leq -5$), the current density is negligible whereas at large positive potential, the current becomes steady state value which is equal to $\sqrt{\xi}\tanh\sqrt{\xi}$. Also when the potential is $-5 \leq \varepsilon \leq 5$, the current lies in exponential phase.

4.2 Influence of diffusion coefficient of the mediator, thickness of the redox polymer film, Michaelis constant for substrate, turnover rate on the current density

The kinetic parameters like diffusion coefficient of the mediator, thickness of the redox polymer film, Michaelis constant for substrate, turnover rate are used to characterize the catalytic activity of enzymes and biofilm transporters. The influences of the parameters on the current are plotted in Figures S1-S4. The experimental values used for these graphs are given in Table 1a-1d. The crucial current density is found to decreases as a function of increasing diffusion coefficient of the mediator (Figure S1). It is well known that the film thickness plays a crucial role in redox polymer electrodes since it can make the electron transfer to occur either at the electrode/film or film/solution interface [31]. Since, the thickness indicates the maximum loading of the redox centers, it is of interest to analyze its influence on the current–potential response of redox polymers for typical parameters of the enzyme. Here the thickness increases with increase in current density (Figure S2). This behavior is often associated with the enhanced loading of the enzyme which causes an increase in electron mobility [23].

The calculated steady state current density is shown as a function of turnover rate k_{cat} in Figure S3. Since the loading of active enzyme in the film is difficult to assess, the obtained turnover number can be compared with values from free solution by multiplying with nominal enzyme loading to yield the maximum enzyme velocity, $V_{max} = k_{cat}\, E_T$. At high turnover rate of GOx or maximum enzyme velocity, the current density increases. The system performance can also be improved by improving enzyme turnover number. Figure S4 depicts the current density as a function of Michaelis-Menten constant (K_S) for the substrate by using Eq. (9). It shows that current density decreases at high Michaelis-Menten constant. Increase in K_S value indicates that oxygen also binds with the enzyme.

5 Sensitivity analysis of parameters.

We have found the partial derivative of current density (dependent variable) with respect to the parameters D_m, K_S, k_{cat}, L. At some fixed experimental values of the parameters, numerical value of rate of change of current density *jobs* can be obtained [9]. From this value we can obtain the percentage of

change in current density with respect to the kinetic parameters D_m, K_S, k_{cat}, L. Sensitivity analysis of the parameters is given in Fig. 8. From this figure, it is inferred that the diffusion has more impact than Michaelis-Menten constant for the variation of the current density. The remaining parameters Michaelis–Menten constant, thickness of the film and turnover of GOx accounts for only small changes in current density. This result is also confirmed in the Fig. 8.

6 Estimation of kinetics parameters

From the Eq. (1) the mediator reaction rate (R_a) can be written as follows:

$$\frac{1}{R_a} = \frac{\gamma \mu}{\kappa^2} + \frac{1}{\kappa^2}\left(\frac{1}{a}\right) + \frac{\gamma}{\kappa^2}\left(\frac{1}{s}\right) \tag{13}$$

The equation 13 can be represented as the following equation form:

$$Y = l + mX + nZ \tag{14}$$

where $Y = 1/R_a, X = 1/a, Z = 1/s$ and kinetic parameters $l = \gamma\mu/\kappa^2, m = 1/\kappa^2, n = \gamma/\kappa^2$. Using the formula of least square coefficients we can obtain the parameters κ, γ and μ or kinetic parameters turnover rate (k_{cat}), Michaelis-Menten constant (K_S) for GOx and reaction rate mediator (k_m) from the Eq. (10).

7 Conclusion

A theoretical one-dimensional model of a redox polymer-mediated, enzyme electrode is analyzed. The time-independent nonlinear ordinary differential equations of concentration of mediator, substrate and oxygen have been solved analytically using a modified homotopy perturbation method. The effect of kinetic on parameters such as turnover rate of GOx (k_{cat}) reaction, rate constant (k_m), thickness of the film (L), Michaelis–Menten constant (K_S) and diffusion coefficients of mediator (D_m) on current density are discussed. According to our theoretical study, high current densities could be obtained by using high thickness of the hydrogel film glassy carbon electrode in the presence of a redox polymer by

increasing enzyme loading in the catalyst layer and enzyme turnover rate. Further, a graphical procedure is suggested for estimating the reaction rate constant, Michaelis–Menten constants and diffusion coefficients of mediator with the help of the rate equation.

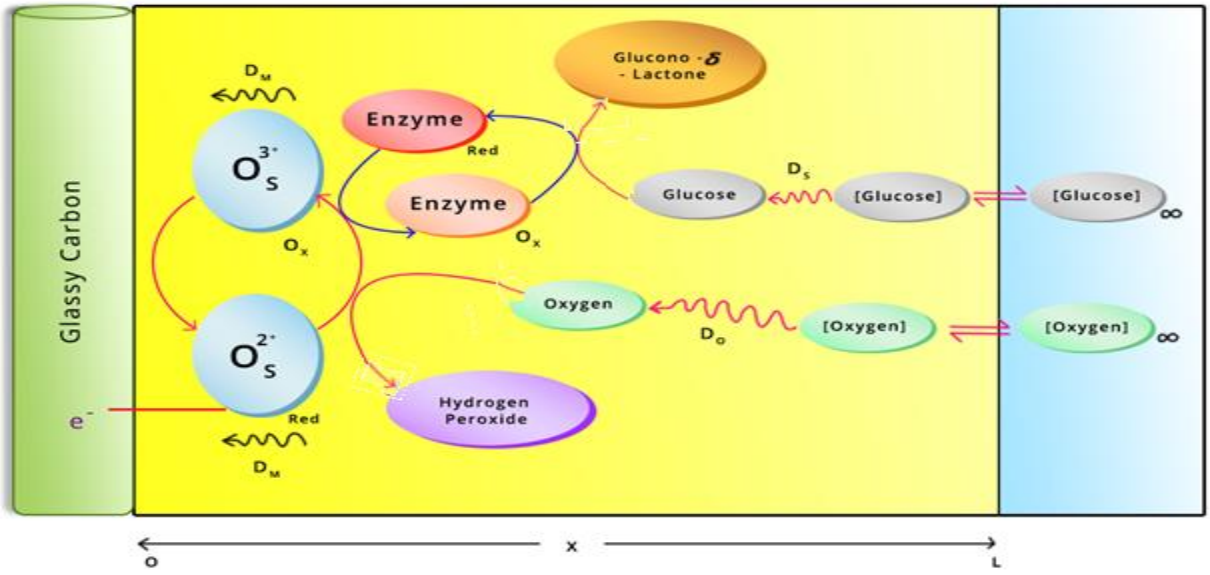

Fig 1. Systamatic diagram of osinum –based redoxc polymer mediator glucose oxidized electrodes

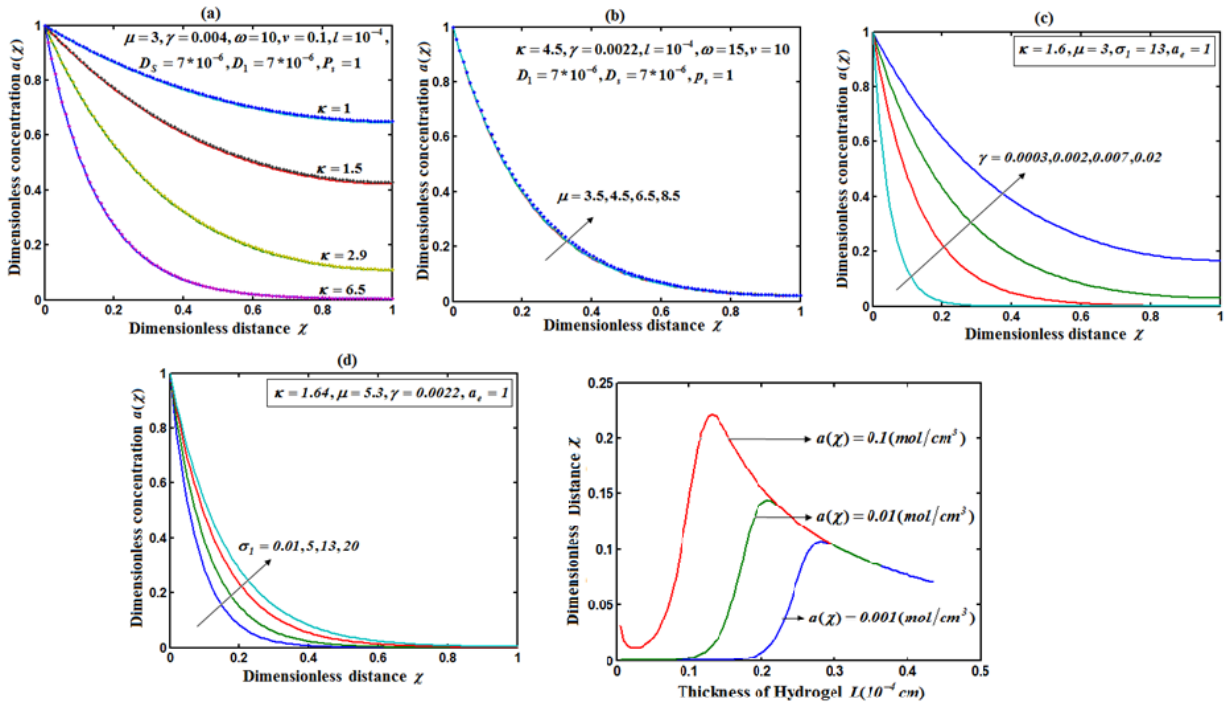

Fig 2. Comparison of dimensionless concentrations of mediator with numerical results for various values of parameter such as κ, μ, γ and σ_1. Solid line represent Eq. (5) and dotted line represent the numerical result. Unit of all symbols are given in the nomenclature. Fig. 2e is the distance-thickness profile.

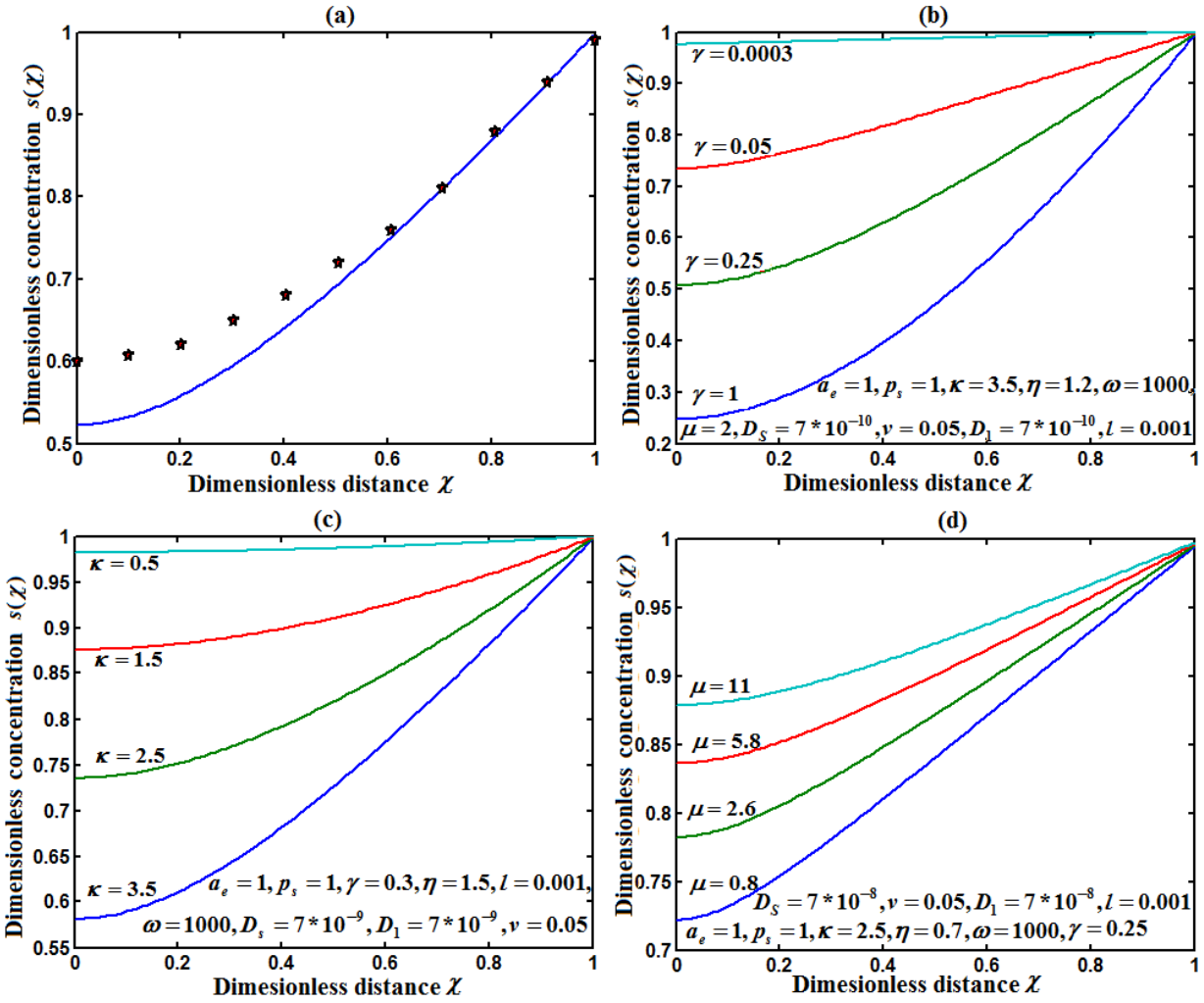

Fig. 3. Plot of dimensionless concentrations of substrate versus normalized distance from the electrode interface for various values of parameters such as γ, κ and μ using the Eq.(6). our analytical result is compared with numerical results in Fig. 3a for the experimental values of parameters which are given in Table 1f. Unit of all symbols are given in the nomenclature.

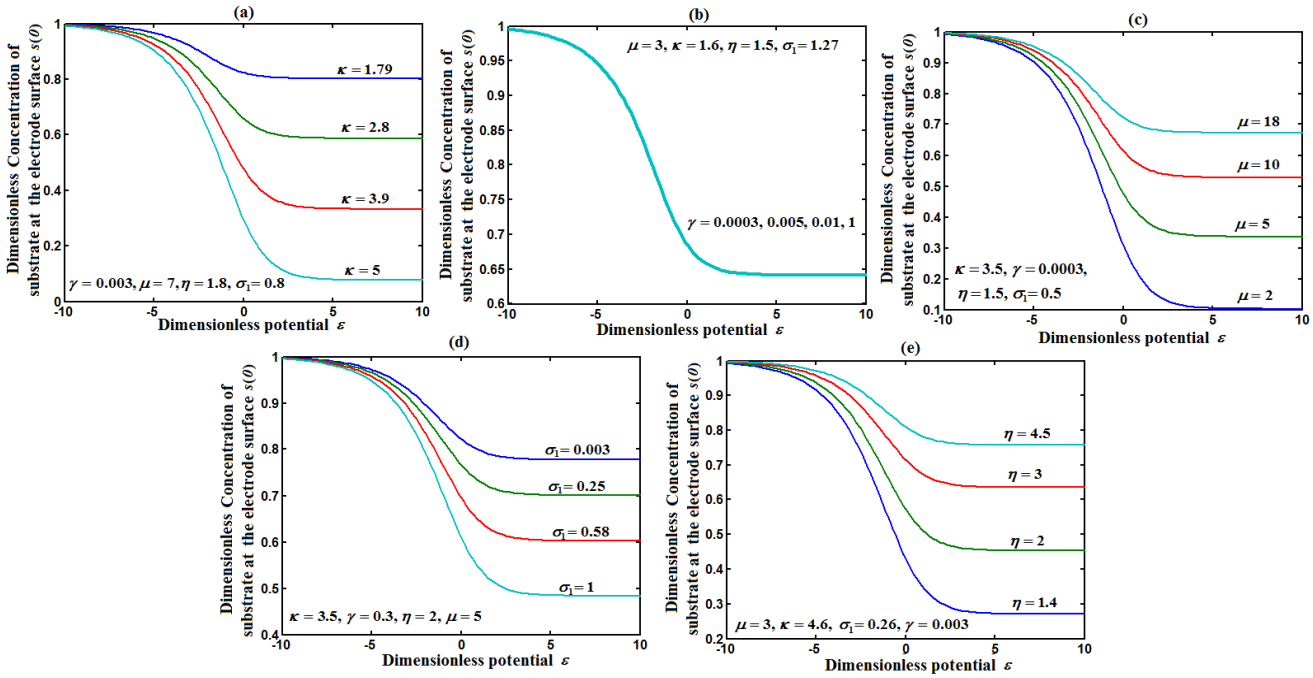

Fig.4. Dependence of the dimensionless concentration of substrate at electrode surface versus the potential estimated using the Eq.(10) under different values of parameters: (a)κ, (b)γ, (c) μ (d) σ (e) η

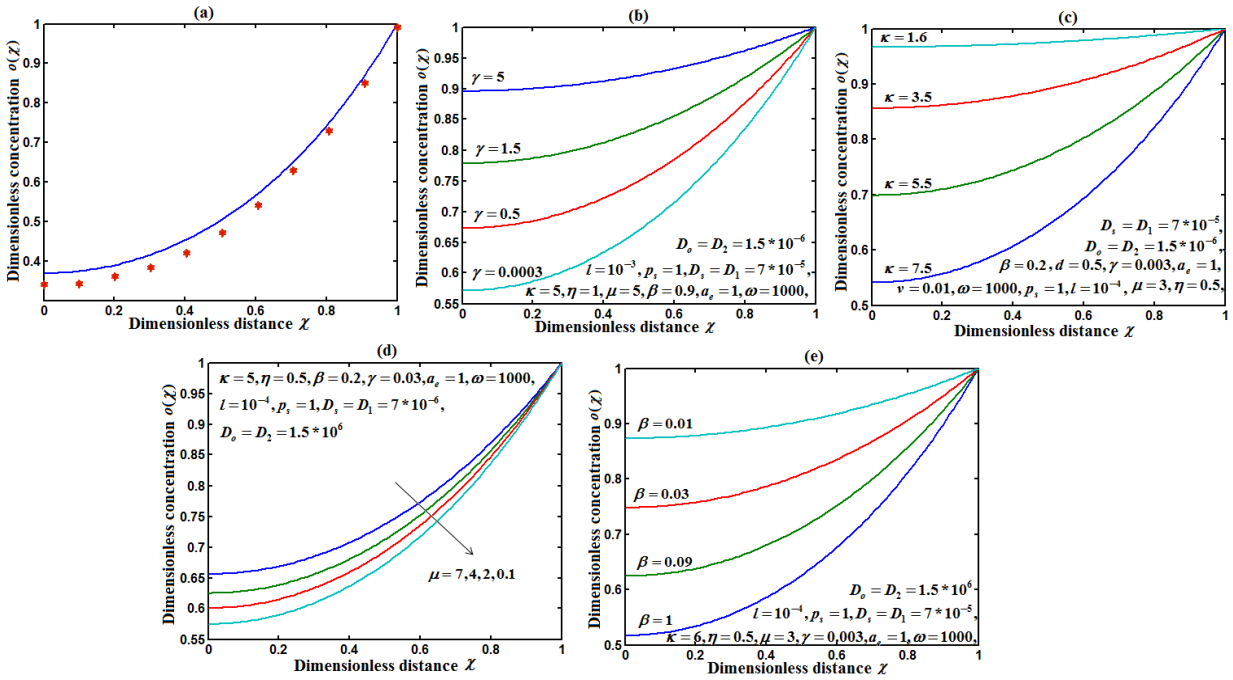

Fig. 5. Plot of dimensionless concentration of oxygen versus dimensionless distance from the electrode interface for various kinetic parameters using the Eq.(7). The analytical result is compared with numerical result in Fig. 5a. The values of the parameters used and unit of all symbols are given in Table 1f and nomenclature.

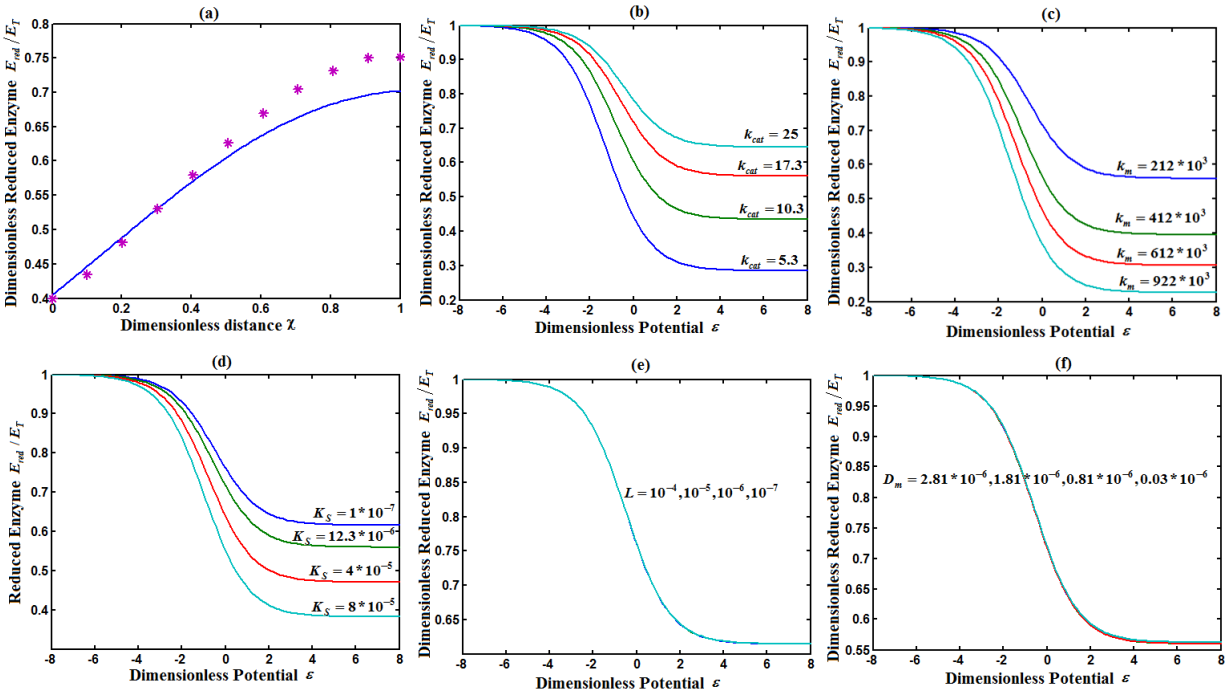

Fig. 6. Plot of dimensionless reduced enzyme in the film versus dimensionless distance (Fig. 5a) and dimensionless potential (Fig 5b-5f) for the various values of the parameters (Table 1f) using Eq.(9) and Eq.(11) respectively. In Fig. 6a, dotted line represents the numerical results and solid line Eq.(11). Unit of all symbols are given in the nomenclature.

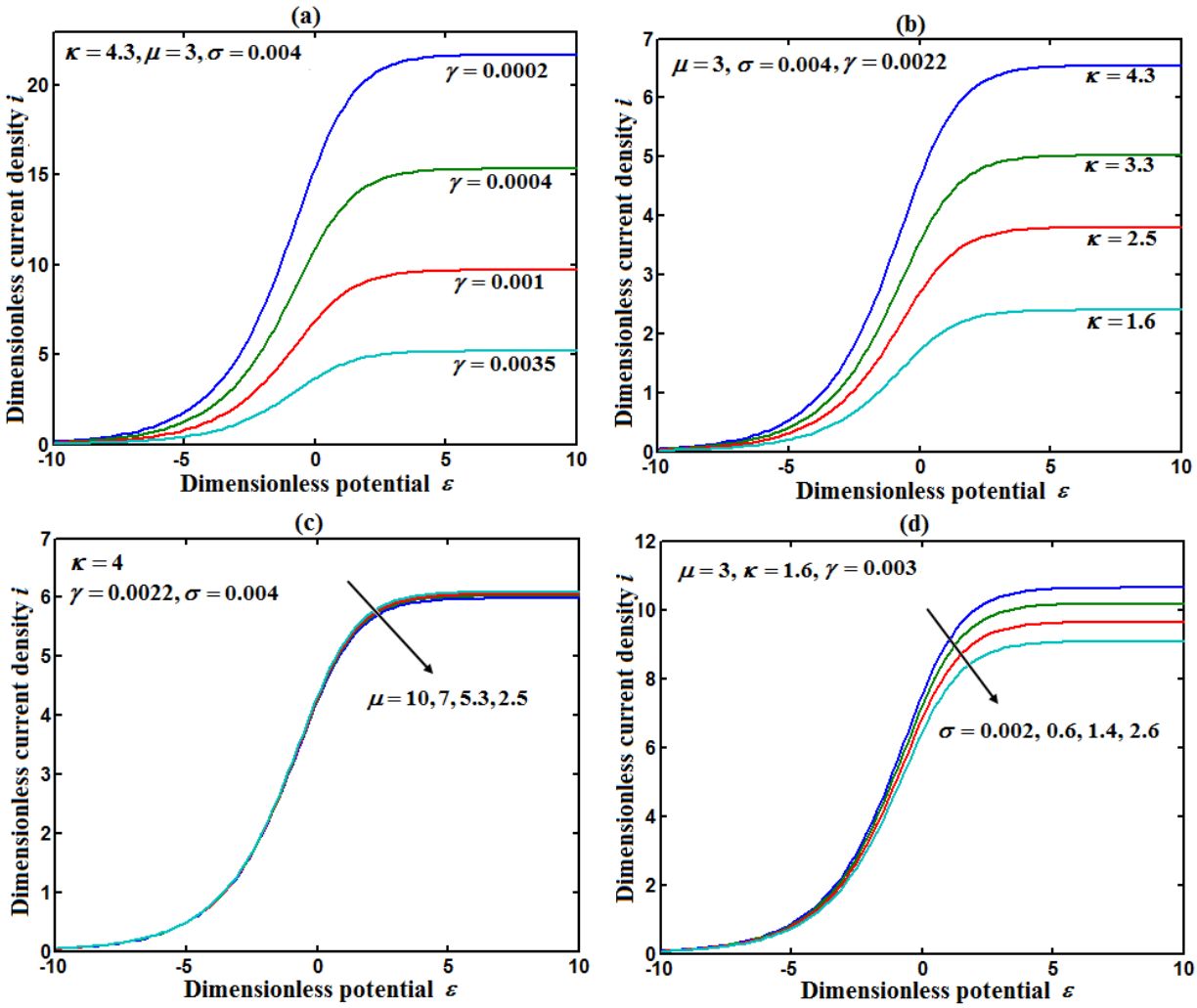

Fig.7. Variation of the steady state current on potential estimated using the Eq.(12) for different values of parameters: γ, κ, μ and σ.

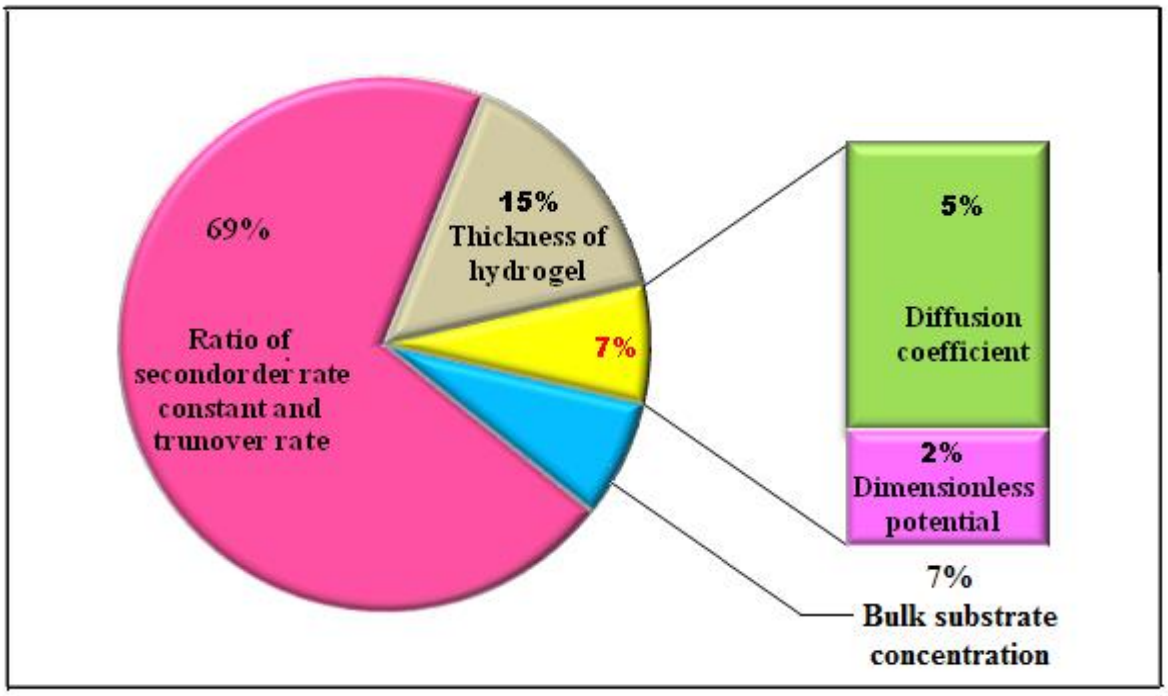

Fig. 8. The quantitative influence of the kinetic parameters in the current density from the sensitivity analysis using Eq.(12)

Nomenclature:

Symbol	Description	Symbol	Description
$[M_{ox}](molcm^{-3})$	Concentration of mediator	E_{red}	Reduced form of enzyme
$[S](molcm^{-3})$	Concentration of substrate	E	Potential at electrode surface
$[O_2](molcm^{-3})$	Concentration of oxygen	$E_T\ (molcm^{-3})$	Total concentration of immobilized enzyme
$[M_T]_\infty(molcm^{-3})$	Bulk Concentration of Oxidized mediator	E^0	Formal potential of mediator couple
$[S]_\infty(molcm^{-3})$	Bulk Concentration of substrate	N	Number of electrons transferred by mediator
$[O_2]_\infty(molcm^{-3})$	Bulk Concentration of oxygen	F	Faraday constant
$K_S(molcm^{-3})$	Michaelis constant of substrate	R	Universal gas constant
$k_o(cm^3\ mol^{-1}s^{-1})$	Second-order reaction rate constant between enzymeand oxygen	T	Absolute temperature
$L(cm)$	Thickness of the hydrogel	a	Dimensionless concentration of substrate
$k_m(cm^3\ mol^{-1}s^{-1})$	Second-order reaction rate constant between enzymeand mediator	s	Dimensionless concentration of mediator
$k_{cat}(s^{-1})$	Turnover number of enzyme GOx	o	Dimensionless concentration of oxygen
p_a	Partition coefficient of mediator	$d,\beta,\beta,\eta,\mu,\kappa$	Dimensionless parameter

p_S	Partition coefficient of substrate at the film solution interface	χ	Dimensionless distance
p_O	Partition coefficient of oxygen at the film solution interface	δ_1	Diffusion layer thickness of glucose at the film-solution interface
$D_m(cm^2s^{-1})$	Diffusivities of mediator	δ_2	Diffusion layer thickness of oxygen at the film-solution interface
$D_S(cm^2s^{-1})$	Diffusivities of substrate	σ_1	Dimensionless parameter
$D_O(cm^2s^{-1})$	Diffusivities of oxygen	σ_2	Dimensionless parameter
$x(cm)$	Distance	a_e	Dimensionless mediator concentration at electrode surface
$D_1(cm^2s^{-1})$	Glucose diffusion coefficient in bulk solution	ε	Dimensionless potential
$D_2(cm^2s^{-1})$	Oxygen diffusion coefficient in bulk solution	J_{obs}	Flux mediator at electrode surface
$v(cm^2s^{-1})$	Kinematic viscosity of electrolyte	j_{obs}	Dimensionless flux mediator at electrode surface
$\omega(rpm)$	Rotation of electrode	i	Current density per projected surface area

Appendix-A. Mathematical formulation of the problem is given in supplementary reader.

Appendix B. Approximate analytical solution of Eqs (1-3) using NHPM.

Eq.(1) is given as:

$$\frac{d^2a}{d\chi^2} = \frac{\kappa^2 as}{\gamma a\,(1+\mu s)+s} \tag{B.1}$$

In order to solve the above equation, we construct the homotopy as follows:

$$(1-p)\left\{\frac{da^2(\chi)}{d\chi^2} + \frac{\kappa^2 a s_0}{\gamma a\,(1+\mu s)+s_0}\right\} + p\left\{\gamma a\,\frac{da(\chi)}{d\chi^2} + \gamma a\,\frac{da(\chi)}{d\chi^2}\mu s + s_0\,\frac{da(\chi)}{d\chi^2}\kappa^2 as\right\} = 0 \tag{B.2}$$

The approximate solution of (B.1) is as follows:

$$a = a_{zeroth} + p\,a_{first} + p^2\,a_{second} + \ldots \tag{B.3}$$

Substituting (A.3) in Eq. (A.2) and equating the like powers of p

$$p^0 : \frac{d^2a_{zeroth}(\chi)}{d\chi^2} + \frac{\kappa^2 a_{zeroth}(\chi)s_0}{\gamma a_e(1+\mu\, s_0)+s_0} = 0 \tag{B.4}$$

The boundary condition for the above equation is

$$\text{At } \chi = 0,\ a_{zeroth}(0) = 1 \qquad \text{(B.5)}$$

Solving the Eq. (A.4), $a_{zeroth}(\chi)$ can be obtained as follows:

$$a_{zeroth}(\chi) = \frac{a_e}{\cosh\sqrt{\xi}} \cosh\sqrt{\xi}\,(1-\chi) \qquad \text{(B.6)}$$

$$\text{where, } \xi = \frac{\kappa^2 s_0}{\gamma a_e\left(1/s_0 + \mu\right) + 1} \qquad \text{(B.7)}$$

Substituting (B.6) in Eq. (B.2), and by taking $a(\chi) \approx a_{zeroth}(\chi)$, we obntined the Eq.(5) in the text. Similarly, we can find next iteration to improve the accuracy of the solution. Also similarly, the predited method can be followed to find the solution of concentration of substrate and concentration of oxygen Eq.(6-7) respectively.

Appendix C: MATLAB code to find the numerical solution of Eq.(5) (This is given in supplementary material)

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Supplementary material of the manuscript

Theoretical treatment of diffusion and kinetics of osmium redox polymer mediated glucose oxidase enzyme electrodes: Analytical expression of current density for varying potential

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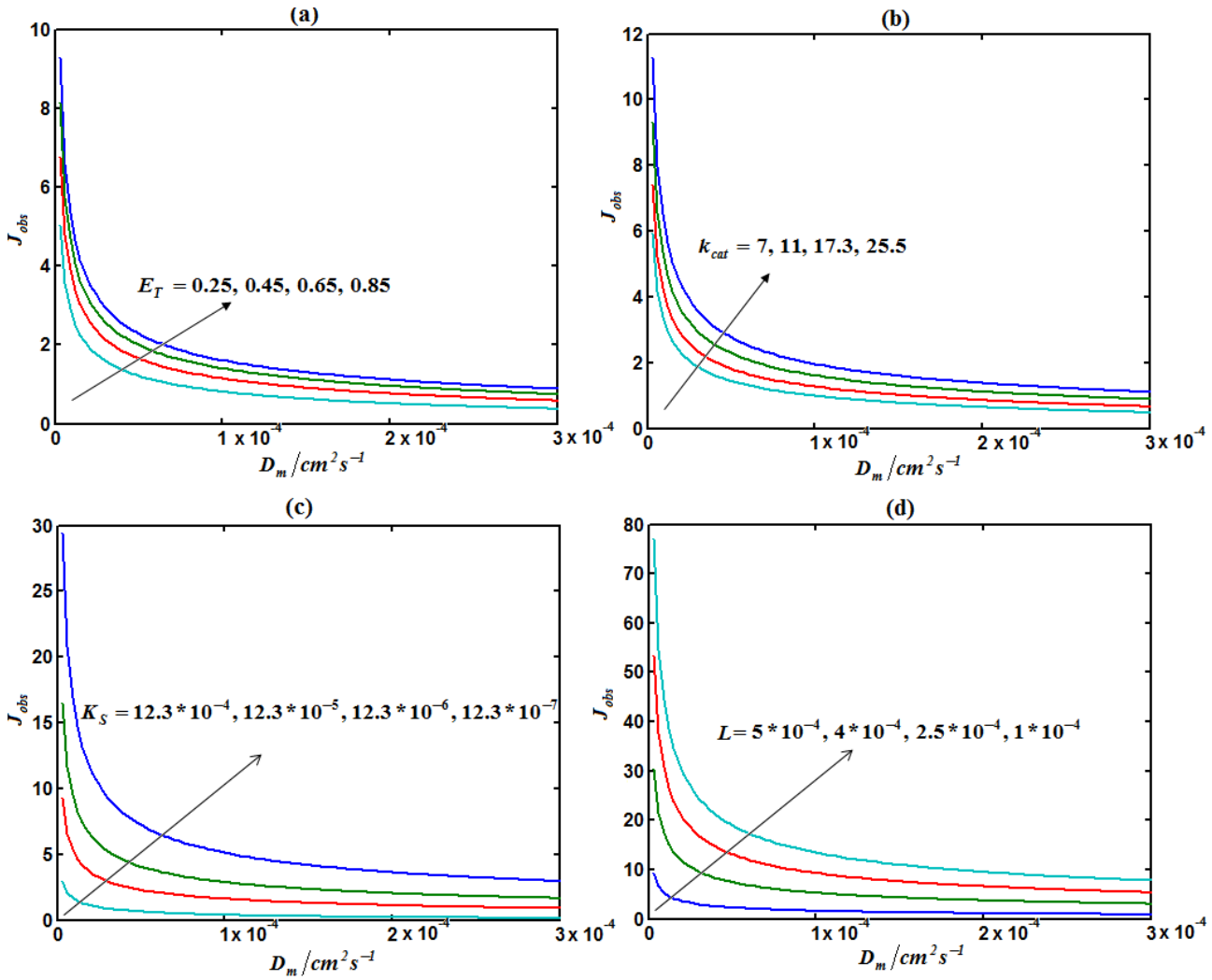

Fig. S1. Plot of the variation of the current density with diffusion coefficient of the mediator d_m for various values of E_T, k_{cat}, K_S and L. The parameters employed are given in Table 1.

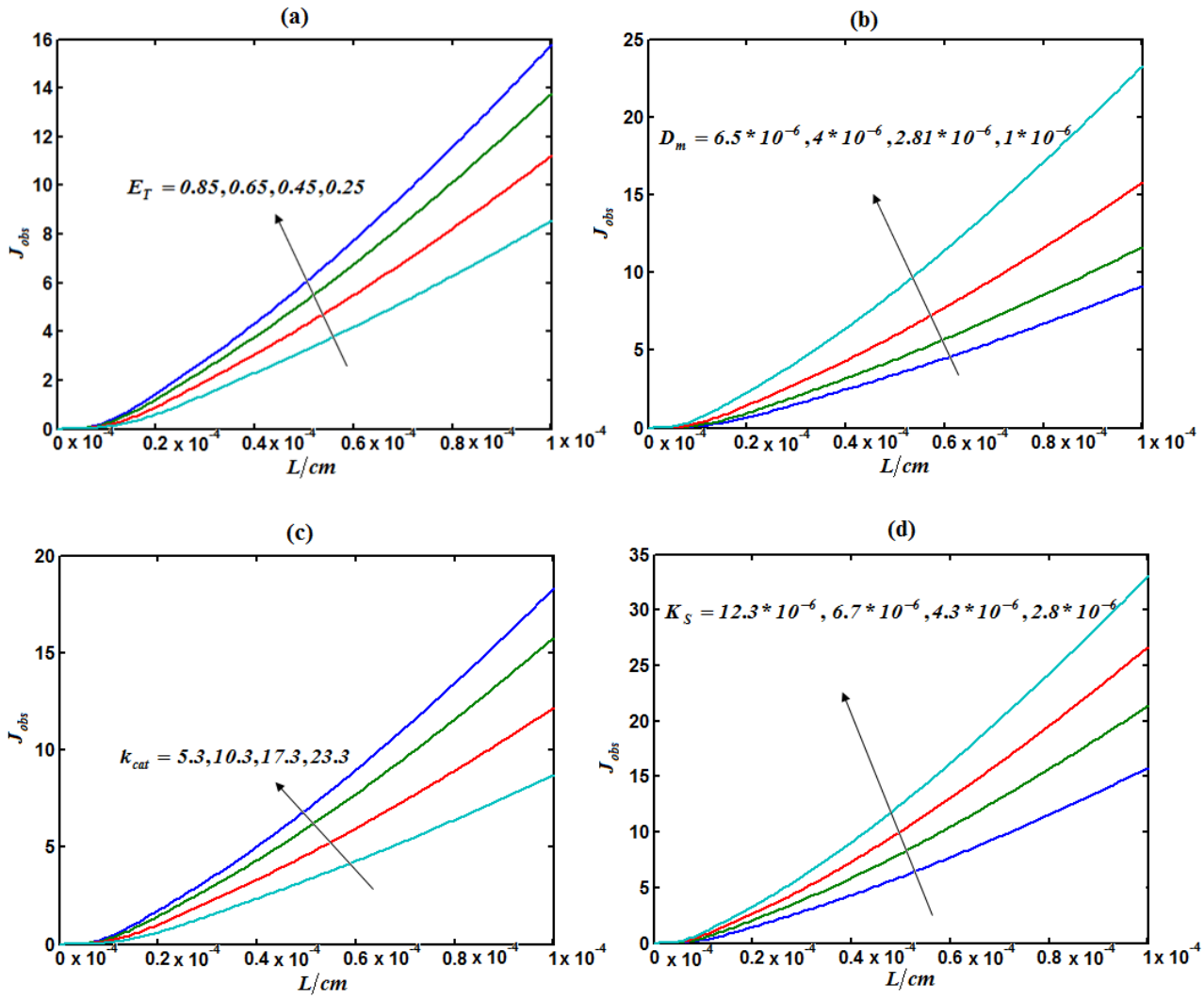

Fig. S2. The plot of current density versus thickness of the redox polymer film l for various values of E_T, d_m, k_{cat}, K_S and l. The parameters employed are given in Table 2.

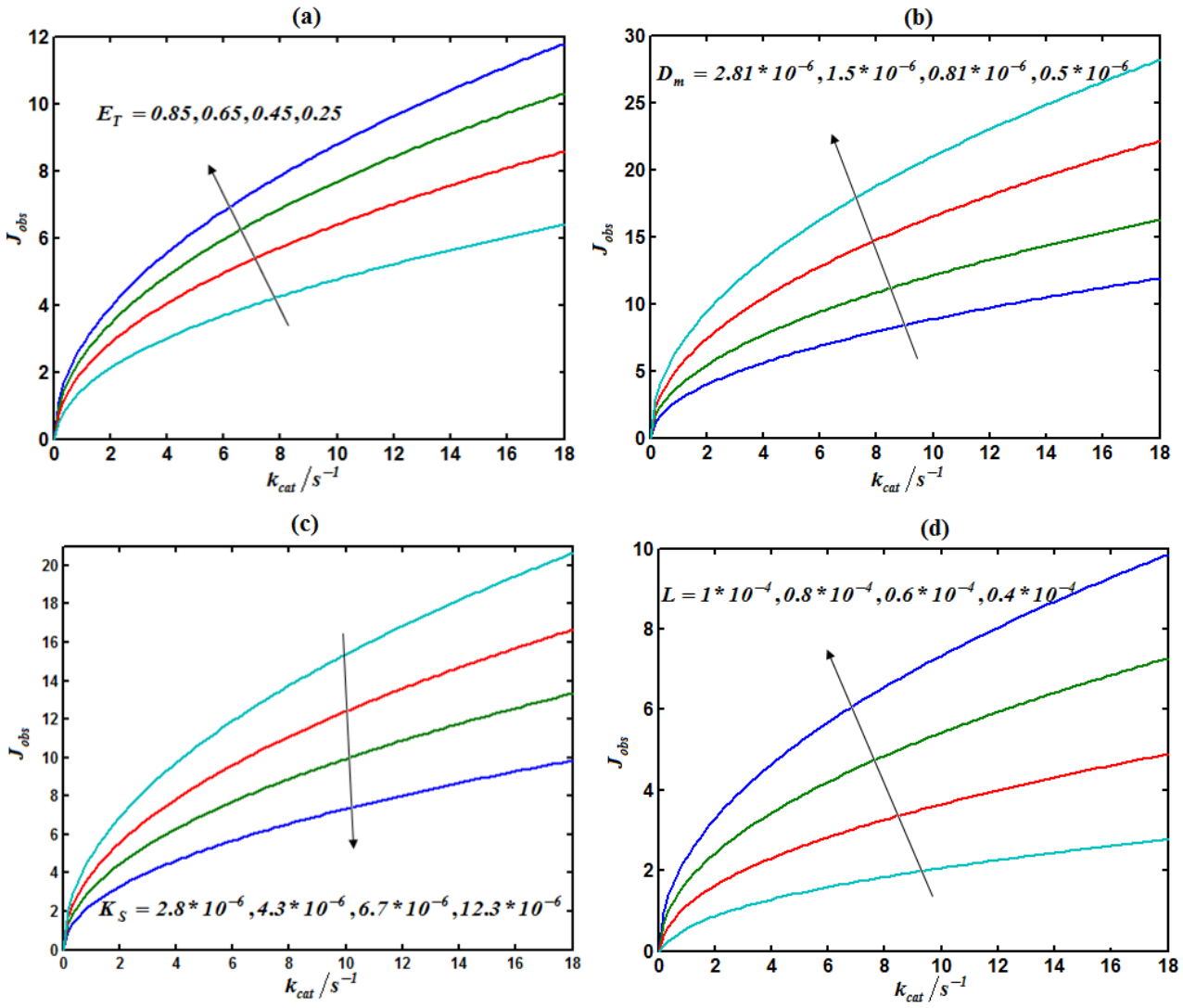

Fig. S3. Plot of current density versus turnover rate of GOx k_{cat} for various values of E_T, d_m, K_S, and l. The parameters employed are given in Table 4.

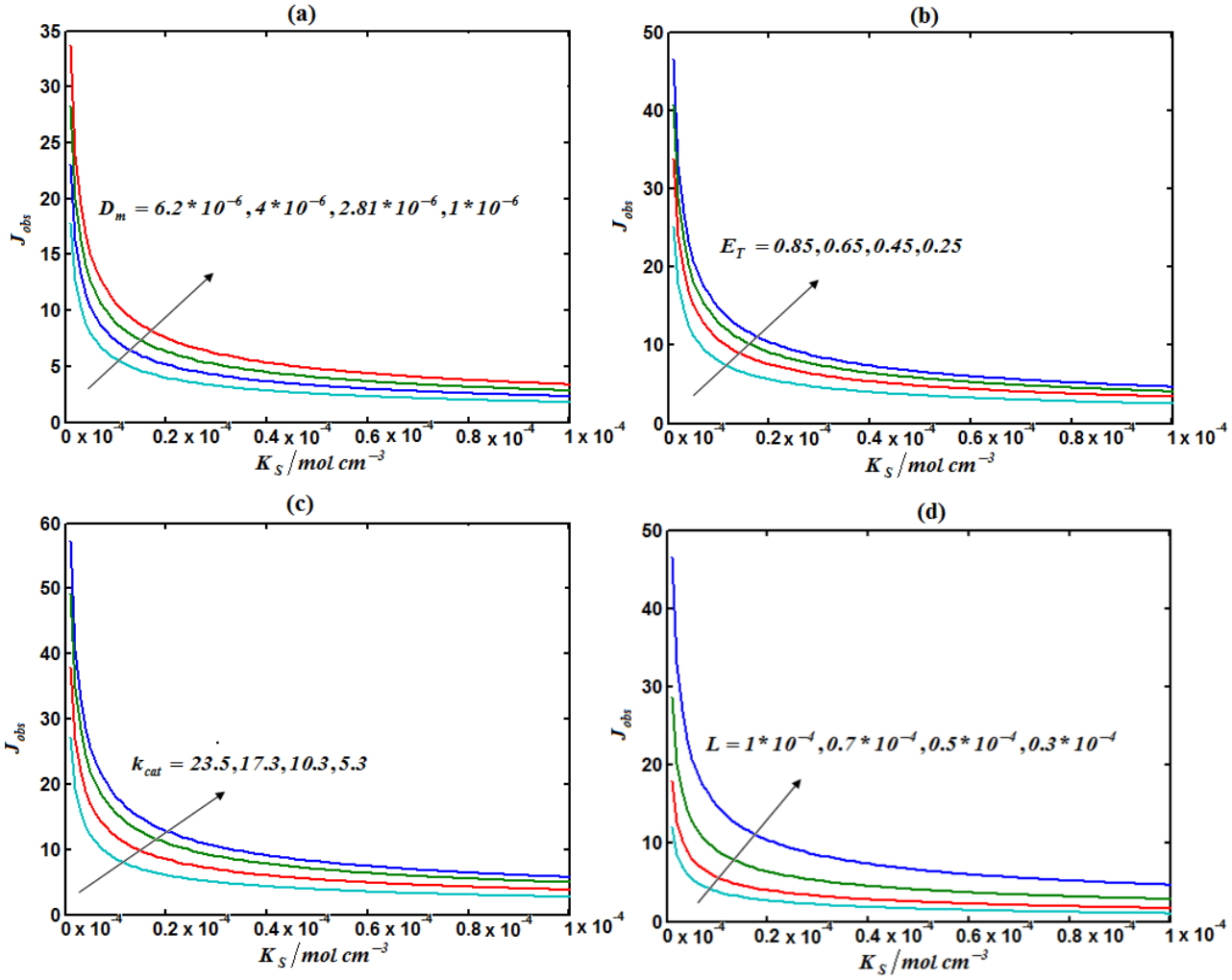

Fig. S4. The plot of variation of the current density with Michaelis constant for substrate K_S for various values of E_T, d_m, k_{cat} and l. The parameters employed are given in Table 3.

Appendix-A. Mathematical formulation of the problem.

Fig. 1 depicts ~~that~~ the general chemical kinetics reaction scheme for glucose oxidize enzyme electrodes which follows a Michaelis-Menten scheme [9]. Diffusion and kinetics of glucose oxidizing enzyme anode in the presence of oxygen has been analyzed by (original paper author). For the sake of self-consistency, the general reaction scheme for glucose oxidation can be represented as follows:

$$\underset{\text{(aq.)}}{v_s\ S} + E_{ox} \xrightarrow{k_\varepsilon} \underset{\text{(aq.)}}{v_s\ P} + E_{red}$$

$$\underset{\text{(aq.)}}{v_s\ M_{ox}} + E_{red} \xrightarrow{k_m} \underset{\text{(aq.)}}{v_s\ M_{red}} + E_{ox} \quad \text{(A1)}$$

$$\underset{\text{(aq.)}}{v_o\ O_2} + E_{red} \xrightarrow{k_o} \underset{\text{(aq.)}}{v_o\ H_2O_2} + E_{ox}$$

The symbols and abbreviations are listed in the nomenclature. The stoichiometric coefficients v_i, are: $v_s = 0.5,\ v_p = 0.5, v_m = 1$ and $v_o = 1$ [14].The one-dimension dimensionless reaction diffusion equations can be written in the following form [9]:

$$\frac{d^2a}{d\chi^2} = \frac{\kappa^2 as}{(\gamma a + \beta o)(1+\mu s) + s} \quad \text{(A2)}$$

$$\frac{d^2o}{d\chi^2} = \frac{\kappa^2 os\eta^{-1}\beta d}{(\gamma a + \beta o)(1+\mu s) + s} \quad \text{(A3)}$$

$$\frac{d^2s}{d\chi^2} = \frac{\kappa^2 as\eta^{-1}\gamma}{(\gamma a + \beta o)(1+\mu s) + s} + \frac{\kappa^2 os\eta^{-1}\beta}{(\gamma a + \beta o)(1+\mu s) + s} \quad \text{(A4)}$$

The boundary conditions are:

$$\frac{ds}{d\chi} = 0; \quad \frac{do}{d\chi} = 0; \quad a = a_e \quad \text{when } \chi = 0 \quad \text{(A5)}$$

$$s = 1 - \sigma_1\left(\frac{ds}{d\chi}\right); \quad o = 1 - \sigma_2\left(\frac{do}{d\chi}\right); \quad \frac{da}{d\chi} = 0 \qquad \text{when } \chi = 1 \quad \text{(A6)}$$

$$\text{where } \sigma_1 = \frac{\delta_1\, p_s}{D_S L}; \qquad \delta_1 = 4.98 D_S^{\,1/3} v^{1/6} \omega^{-1/2}; \sigma_2 = \frac{\delta_2\, p_o}{D_O L}; \qquad \delta_{21} = 4.98 D_O^{\,1/3} v^{1/6} \omega^{-1/2} \quad \text{(A7)}$$

$$\text{and} \quad \begin{array}{l} s = \dfrac{[S]}{p_s[S]_\infty}; \quad a = \dfrac{[M_{ox}]}{p_a[M_T]_\infty}; \quad o = \dfrac{[O_2]}{p_O[O_2]_\infty}; \quad \chi = \dfrac{x}{L}; \quad \kappa = L\sqrt{\dfrac{k_m E_T}{D_m}}; \\ \eta = \dfrac{D_S k_m K_S}{D_m k_{cat}}; \quad \gamma = \dfrac{k_m K_S [M_T]_\infty}{k_{cat}[S]_\infty}; \quad \beta = \dfrac{k_O K_S [O_2]_\infty}{k_{cat}[S]_\infty}; \quad \mu = \dfrac{[S]_\infty}{K_S}; \quad d = \dfrac{D_S}{D_O} \end{array} \quad \text{(A8)}$$

where *a, s* and *o* are the dimensionless concentration of mediator, glucose and oxygen respectively.

The dimensionless mediator concentration a_e at electrode surface is defined as in eqn. (A13):

$$a_e = \frac{1}{1+\exp(-\varepsilon)} \qquad \text{(A9)}$$

where $\varepsilon = \frac{(E-E^0)nF}{RT}$ is the dimensionless potential. The dimensionless flux mediator at electrode can be considered by the following equation [9] :

$$J_{obs} = \frac{L_{j_{obs}}}{D_m[M_T]} = -\left(\frac{da}{d\chi}\right)_{x=0} \qquad \text{(A10)}$$

The parameter β is similar to that of γ which describes the balance between two forms of the enzyme. Along with oxidized mediator, now oxygen also oxidizes the reduced form of GOx. When β >>1, GOx enzymes will be in the oxidized form and when β <<1, the reduced form of GOx predominates. But this situation is coupled with γ which also controls the oxidation state of GOx. The parameter *d* is the ratio of substrate diffusion to that of oxygen diffusion in the film. The system will be substrate limited when *d*<<1. Under this above condition (β <<1 and *d*<<1) the above Eq. (A2 – A4) can be reduced to the Eq.(1-3) which are given in the text.

Appendix C: MATLAB code to find the numerical solution of Eq.(5)

```
function [jobs,out]=N2
kappa=4;
eta=2;
gamma=0.007;
mu=3;
sigma1 = 0.01;
eps = 10;
ae = 1/(1+exp(-eps
x0=linspace(0,1,100);
solinit = bvpinit(x0,[.5 0 1 0]);
sol = bvp4c(@deriv, @bc, solinit);
x = sol.x;
a = sol.y(1,:);
dadx = sol.y(2,:);
s = sol.y(3,:);
dsdx = sol.y(4,:);
Ra= kappa^2*a.*s./(gamma*a.*(1+mu*s)+s) .* (a >= 0) .* (s >=0);
Rs= Ra.*(gamma/eta).*(Ra>=0);
e2=s./((gamma*a.*(1+mu*s)+s)).* (a >= 0) .* (s >= 0);
```

```
plot(x,a,'--g');figure(gcf)
axis([0,1,0,1]);
xlabel('\chi');
ylabel('Dimensionless concentration profile');
legend('a - Oxidized mediator','s - Substrate','R /Rmax','Reduced
enzyme','Location','Southwest');
functiondzdx=deriv(x,z)
a= z(1);
va=z(2);
s= z(3);
vs=z(4);
Ra= kappa^2*a*s/(gamma*a*(1+mu*s)+s) * (a >= 0) * (s >=0);
Rs= Ra*(gamma/eta)*(Ra>=0);
dvadx= Ra;
dadx= va;
dvsdx= Rs;
dsdx= vs;
dzdx= [dadx; dvadx; dsdx; dvsdx];
end
function res= bc(z0,z1)
a0= z0(1); a1= z1(1);
va0= z0(2); va1= z1(2);
s0= z0(3); s1= z1(3);
vs0= z0(4); vs1= z1(4);
res(1)= a0 - ae;
res(2)= vs0;
res(3)= va1;
res(4)= s1 - 1 + (sigma1*vs1);
res=res(:);
end
out=sol;
out.in=[kappa,eta,gamma,mu,ae,sigma1];
out.a = a;
out.dadx=dadx;
out.s = s;
out.dsdx=dsdx;
out.Ra = Ra;
out.Rs = Rs;
out.e2=e2;
end
```

Table 1a. Numerical values of the parameters in Eq. (12) for Fig. S1

S.No	E_T $(molcm^{-3})$	k_{cat} (s^{-1})	K_S $(molcm^{-3})$	$[M_T]_\infty$ $(molcm^{-3})$	$[S]_\infty$ $(molcm^{-3})$	ε	D_S (cm^2s^{-1})	D_1 (cm^2s^{-1})	ν (cm^2s^{-1})	ω (rpm)	L (cm)	p_S
Fig S1 a	--	17.3	12.3*10^(-6)	0.1	0.1	0.1	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig S1 b	0.85	10	--	0.01	0.01	0.1	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig S1 c	0.85	--	12.3*10^(-6)	0.1	0.1	0.1	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig S1 d											--	

Table 1b. Numerical values of the parameters in Eq. (12) for Fig. S2

S.No	E_T $(molcm^{-3})$	D_m (cm^2s^{-1})	K_S $(molcm^{-3})$	$[M_T]_\infty$ $(molcm^{-3})$	$[S]_\infty$ $(molcm^{-3})$	ε	D_S (cm^2s^{-1})	D_1 (cm^2s^{-1})	ν (cm^2s^{-1})	ω (rpm)	L (cm)	p_S
Fig S2 a		2.81*10^(-6)	12.3*10^(-6)	0.0001	0.0001	0.8	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig S2 b	0.85		12.3*10^(-6)	0.0001	0.0001	0.8	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
FigS 2 c	0.85	2.81*10^(-6)		0.0001	0.0001	0.1	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig S2 d	0.85	2.81*10^(-6)	12.3*10^(-6)	0.0001	0.0001	0.1	7*10^(-6)	7*10^(-6)	0.01	1000		1

Table 1c. Numerical values of the parameters in Eq. (12) for Fig. S3

S.No	E_T $(molcm^{-3})$	D_m (cm^2s^{-1})	k_{cat} (s^{-1})	$[M_T]_\infty$ $(molcm^{-3})$	$[S]_\infty$ $(molcm^{-3})$	ε	D_S (cm^2s^{-1})	D_1 (cm^2s^{-1})	ν (cm^2s^{-1})	ω (rpm)	L (cm)	p_S
Fig S3 a	---	2.81*10^(-6)	17.3	0.0001	0.0001	0.3	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
FigS 3 b	0.85	---	17.3	0.0001	0.0001	0.1	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig S3 c	0.95	2.81*10^(-9)	---	0.0001	0.0001	0.2	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig S3 d	0.95	2.81*10^(-9)	17.3	0.0001	0.0001	0.2	7*10^(-6)	7*10^(-6)	0.01	1000	---	1

Table 1d. Numerical values of the parameters in Eq. (12) for Fig. S4

S.No	E_T $(molcm^{-3})$	D_m (cm^2s^{-1})	k_{cat} (s^{-1})	K_S $(molcm^{-3})$	$[M_T]_\infty$ $(molcm^{-3})$	$[S]_\infty$ $(molcm^{-3})$	ε	D_S (cm^2s^{-1})	D_1 (cm^2s^{-1})	ν (cm^2s^{-1})	ω (rpm)	p_S
FigS 4 a	--	2.81*10^(-6)	17.3	12.3*10^(-6)	0.0001	0.0001	1	7*10^(-6)	7*10^(-6)	0.01	1000	1
Fig S4 b	0.85	--	17.3	--	0.0001	0.0001	1	7*10^(-6)	7*10^(-6)	0.01	1000	1
FigS 4 c	0.85	2.81*10^(-6)	--	12.3*10^(-6)	0.0001	0.0001	1	7*10^(-6)	7*10^(-6)	0.01	1000	1

Fig S4 d	0.85	2.81*10^(-6)	17.3	--	0.0001	0.0001	1	7*10^(-6)	7*10^(-6)	0.01	1000	1

Table 1e. Numerical values of the parameters in Eq. (9,11) for Fig. 6

S.No	E_T $(molcm^{-3})$	D_m $(cm^2 s^{-1})$	k_{cat} (s^{-1})	k_m $(cm^3 mol^{-1})$	K_S $(molcm^{-3})$	$[M_T]_\infty$ $(molcm^{-3})$	$[S]_\infty$ $(molcm^{-3})$	D_S $(cm^2 s^{-1})$	D_1 $(cm^2 s^{-1})$	ν $(cm^2 s^{-1})$	ω (rpm)	L (cm)	p_S
Fig 6b	0.0001	2.5*10^(-5)	--	212*10^(3)	12.3*10^(-6	0.0005	0.0005	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig 6 c	0.01	2.85*10^(-6)	17.3	212*10^(3)	--	0.0005	0.0005	7*10^(-6)	7*10^(-6)	0.05	1000	0.0001	1
Fig 6d	0.01	2.85*10^(-6)	17.3	--	12.3*10^(-6)	0.0001	0.0001	7*10^(-6)	7*10^(-6)	0.01	1000	0.0001	1
Fig 6e	0.01	--	17.3	212*10^(3)	12.3*10^(-6)	0.0005	0.0005	7*10^(-6)	7*10^(-6)	0.05	1000	0.0001	1
Fig 6f	0.01	2.85*10^(-6)	17.3	212*10^(3)	12.3*10^(-6)	0.0005	0.0005	7*10^(-6)	7*10^(-6)	0.05	1000	--	1

Table 1f. Experimental values of parameters used in Fig. (3a, 5a, 6a)

S.No.	a_e	p_s	β	κ	γ	η	μ	d	D_s $(cm^2 s^{-1})$	D_1 $(cm^2 s^{-1})$	D_o $(cm^2 s^{-1})$	D_2 $(cm^2 s^{-1})$	ω (rpm)	ν $(cm^2 s^{-1})$	L (cm)
Fig 3a	1	1.1	--	3.5	0.25	1.2	2.2		6*10^(-6)	6*10^(-6)	--	--	9000	0.01	0.01
Fig 5a	1	1	0.2	3	0.003	0.5	2	1	7*10^(-5)	7*10^(-5)	1.5*10^(-8)	1.5*10^(-8)	1000	0.01	0.01
Fig 6a	1	1.5	--	2.5	0.3	1.5	3		7*10^(-7)	7*10^(-7)	--	--	1500	0.01	0.001