Topic C Project

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The paper, *Analytical solution of amperometric enzymatic reactions based on Homotopy perturbation method* by Shanmugarajana et al uses a homotopy perturbation method to obtain a solution to a set of non-linear diffusion equations giving a general mathematical description the reaction of electrochemical polymerisation.

Using homotopy they get the system

$$(1-p)\left(\frac{d^2s}{dx^2} - \kappa s\right) + p\left(\frac{d^2s}{dx^2} + \alpha s\frac{d^2s}{dx^2} - \kappa s\right) = 0$$

$$(1-p)\left(\frac{d^2b}{dx^2} - \eta s\right) + p\left(\frac{d^2b}{dx^2} + \alpha s\frac{d^2b}{dx^2} - \eta s\right) = 0$$

With boundary conditions

$$x = 0; \quad \frac{ds}{dx} = 0; \quad b = 0$$

$$x = l; \quad s = Ks_\infty; \quad b = 0$$

Where l is the distance between the electrode and the solution.

Variables and parameters

- s is the substrate concentration

- b is the concentration of the mediator

- x is the position

- Ks_∞ is the surface concentration of substrate

- l is the film thickness

- α, κ, η are constant parameters

I will redo the *leading order solutions* using perturbation methods. Skipping to the point after the homotopy is applied, the system is Which simplifies to

$$\frac{d^2s}{dx^2} - \kappa s + p\alpha s\frac{d^2s}{dx^2} = 0$$

$$\frac{d^2b}{dx^2} + \eta s + p\alpha s\frac{d^2b}{dx^2} = 0$$

$$\alpha := \frac{k_{cat} + ka}{K_M ka}, \quad \kappa := \frac{k_{cat}e_\Sigma}{D_s K_M} \quad and \quad \eta := \frac{k_{cat}e_\Sigma}{D_B K_M}$$

Take the perturbation series

$$s = \sum_{n=0}^{\infty} p^n s_n = s_0 + p s_1 + \mathcal{O}(p^2)$$

And

$$b = \sum_{n=0}^{\infty} p^n b_n = b_0 + p b_1 + \mathcal{O}(p^2)$$

Sub into the system to order $\mathcal{O}(p)$ First equation:

$$\frac{d^2}{dx^2}(s_0 + p s_1 + \mathcal{O}(p^2)) - \kappa(s_0 + p s_1 + \mathcal{O}(p^2)) + p\alpha(s_0 + p s_1 + \mathcal{O}(p^2))\frac{d^2}{dx^2}(s_0 + p s_1 + \mathcal{O}(p^2)) = 0$$

$\mathcal{O}(p^0):\quad \dfrac{d^2 s_0}{dx^2} - \kappa s_0 = 0$

$\mathcal{O}(p):\quad \dfrac{d^2 s_1}{dx^2} - \kappa s_1 + \alpha s_0 \dfrac{d^2 s_0}{dx^2} = 0$

Second equation:

$$\frac{d^2}{dx^2}(b_0 + p b_1 + \mathcal{O}(p^2)) + \eta(s_0 + p s_1 + \mathcal{O}(p^2)) + p\alpha(s_0 + p s_1 + \mathcal{O}(p^2))\frac{d^2}{dx^2}(b_0 + p b_1 + \mathcal{O}(p^2)) = 0$$

$\mathcal{O}(p^0):\quad \dfrac{d^2 b_0}{dx^2} + \eta s_0 = 0$

$\mathcal{O}(p):\quad \dfrac{d^2 b_1}{dx^2} + \eta s_1 + \alpha s_0 \dfrac{d^2 b_0}{dx^2} = 0$

Solve for s_0:

$$\frac{d^2 s_0}{dx^2} - \kappa s_0 = 0$$
$$\implies s_0 = c_1 \cosh(\sqrt{\kappa}x) + c_2 \sinh(\sqrt{\kappa}x)$$

Applying boundary conditions: To leading order the BCs are identical: $\frac{ds_0}{dx}\big|_{x=0} = 0$ and $s_0|_{x=l} = K s_\infty$

$$\frac{ds_0}{dx} = c_2\sqrt{\kappa}\cosh(\sqrt{\kappa}x) + c_1\sqrt{\kappa}\sinh(\sqrt{\kappa}x)$$

$$\frac{ds_0}{dx}\bigg|_{x=0} = c_2\sqrt{\kappa} = 0$$

$$\implies c_2 = 0$$

And

$$s_0\bigg|_{x=l} = c_1 \cosh(\sqrt{\kappa}l) = K s_\infty$$

$$\implies c_1 = \frac{K s_\infty}{\cosh(\sqrt{\kappa}l)}$$

So

$$\boxed{s_0 = \frac{K s_\infty \cosh(\sqrt{\kappa}x)}{\cosh(\sqrt{\kappa}l)}}$$

b_0:

$$\frac{d^2 b_0}{dx^2} + \eta s_0 = 0$$

$$\frac{d^2 b_0}{dx^2} + \frac{\eta K s_\infty \cosh(\sqrt{\kappa}x)}{\cosh(\sqrt{\kappa}l)} = 0$$

$$b_0 = Ax + B - \frac{\eta K s_\infty \cosh(\sqrt{\kappa}x)}{\kappa \cosh(\sqrt{\kappa}l)}$$

$$b_0 \Big|_{x=0} = B - \frac{\eta K s_\infty \cosh(0)}{\kappa \cosh(\sqrt{\kappa}l)} = 0$$

$$\implies B = \frac{\eta K s_\infty}{\kappa \cosh(\sqrt{\kappa}l)}$$

$$b_0 \Big|_{x=l} = Al + \frac{\eta K s_\infty}{\kappa \cosh(\sqrt{\kappa}l)} - \frac{\eta K s_\infty \cosh(\sqrt{\kappa}l)}{\kappa \cosh(\sqrt{\kappa}l)} = 0$$

$$A = \frac{\eta K s_\infty}{l\kappa \cosh(\sqrt{\kappa}l)} \left(\cosh(\sqrt{\kappa}l) - 1\right)$$

Hence

$$b_0 = \frac{x\eta K s_\infty}{l\kappa \cosh(\sqrt{\kappa}l)} \left(\cosh(\sqrt{\kappa}l) - 1\right) + \frac{\eta K s_\infty}{\kappa \cosh(\sqrt{\kappa}l)} - \frac{\eta K s_\infty \cosh(\sqrt{\kappa}x)}{\kappa \cosh(\sqrt{k}l)}$$

$$b_0 = \frac{\eta K s_\infty}{\kappa \cosh(\sqrt{k}l)} \left(\frac{x}{l}(\cosh(\sqrt{\kappa}l) - 1) + 1 - \cosh(\sqrt{k}x)\right)$$

Which is the same as derived in the paper.

Analytical solution of amperometric enzymatic reactions based on Homotopy perturbation method

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ABSTRACT

Electrochemical polymerization is a simple and direct technique often employed for immobilizing redox enzymes at an electrode surface. Besides these, it allows precise control over the amount, spatial distribution and orientation of the enzymes. Analytical expressions pertaining to the immobilization of enzyme by electrochemical polymerization on the electrode surface were obtained by Homotopy perturbation method (HPM). This expression further distinguishes the product of the enzyme reaction at the electrode surface from those occurring alongside the polymer employed for immobilization. These analytical results are compared with the available limiting case results and they are found to be in good agreement.

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1. Introduction

Amperometric enzyme electrodes are routinely employed for the development of wide range of biosensors [1–5]. These electrodes incorporate the specificity of the enzyme together with the rapid analysis time of the electrochemical detection. Further, these electrodes can operate in one of the following mode: (i) the electrode can detect the product or the reactant or (ii) a mediator can be employed or (iii) the enzyme can undergo a redox reaction at the electrode surface [6–8]. The detection limit of the enzyme electrodes depends on the sensitivity of amperometric systems [9,10]. The sensitivity can be increased by cyclic conversion of the substrate and this cyclic conversion of the substrate and the regeneration of the analyte was performed by using a membrane containing two enzymes [11]. Amperometric enzyme electrodes were used to design amperometric biosensors and they are known to be reliable, cheap and highly sensitive for the environmental, clinical and industrial applications [11].

Recently, Meena and Rajendran [12] presented an analysis of system of coupled non-linear reaction diffusions within an electroactive polymer film deposited on an inlaid microdisc electrode. Approximate analytical expression for substrate concentration for

an amperometric biosensor at mixed enzyme kinetics have analyzed by Manimozhi et al. [13]. More recently, Meena and Rajendran [14] have proposed the approximate analytical expressions for the substrate concentration, product concentration and corresponding current response for amperometric and potentiometric biosensors. Loghambal and Rajendran [15] reported the diffusion and kinetics in amperometric immobilized enzyme electrodes for reactions of the enzyme and substrate.

Earlier, mathematical expressions pertaining to approximate analytical concentration and current for limiting cases at enzyme electrodes were calculated by Bartlett and Whitaker [16]. However, to the best of our knowledge, to date, no analytical expressions corresponding to the steady state substrate, oxidized mediator concentrations and current for all values of parameters k_{cat}, ka and K_M at the amperometric enzyme electrodes have been reported. The purpose of this communication is to derive an analytical expression for steady state concentration and current of electrodes for all values of parameters using "Homotopy perturbation method".

2. Mathematical formulation of the boundary value problem

Fig. 1 represents a general kinetic scheme of possible mechanisms occurring at an enzyme electrode. The differential equations that quantify the diffusion and reaction within the film may be
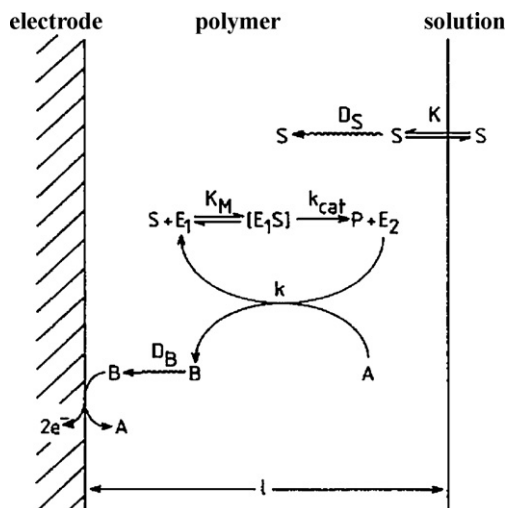
Fig. 1. Schematics of possible mechanisms occurring at an enzyme electrode.

written as follows [16]

$$\frac{de_2}{dt} = \frac{k_{cat}s(e_\sum - e_2)}{K_M + s} - kae_2 \tag{1}$$

$$D_S\frac{d^2s}{dx^2} - \frac{k_{cat}s(e_\sum - e_2)}{K_M + s} = 0 \tag{2}$$

$$D_B\frac{d^2b}{dx^2} + kae_2 = 0 \tag{3}$$

where D_S is the diffusion coefficient of substrate into polymer film, D_B is the diffusion coefficient of oxidized mediator into polymer film, K_M is the Michaelis–Menten constant, k_{cut} is the catalytic reaction rate constant, ka is the regeneration of the oxidized enzyme, x is the distance from the electrode/polymer interface and $e_\sum = e_1 + e_2$ is the total enzyme concentration in the film. Applying the steady state condition to Eq. (1) and on substituting into Eqs. (2) and (3),

$$D_S\frac{d^2s}{dx^2} - \frac{k_{cat}kase_\sum}{k_{cat}s + K_M ka + kas} = 0 \tag{4}$$

$$D_B\frac{d^2b}{dx^2} + \frac{k_{cat}kase_\sum}{k_{cat}s + K_M ka + kas} = 0 \tag{5}$$

Using the following parameters

$$\alpha = \frac{k_{cat} + ka}{K_M ka}; \qquad \kappa = \frac{k_{cat}e_\Sigma}{D_S K_M}; \qquad \eta = \frac{k_{cat}e_\Sigma}{D_B K_M} \tag{6}$$

we obtained the following non-linear reaction diffusion equations:

$$\frac{d^2s}{dx^2} - \frac{\kappa s}{1 + \alpha s} = 0 \tag{7}$$

$$\frac{d^2b}{dx^2} + \frac{\eta s}{1 + \alpha s} = 0 \tag{8}$$

The boundary conditions reduce to

$$x = 0; \quad \frac{ds}{dx} = 0; \quad b = 0 \tag{9a}$$

$$x = l; \quad s = Ks_\infty; \quad b = 0 \tag{9b}$$

where l is the film thickness and Ks_∞ is the surface concentration of the substrate in the film. The fluxes j_S and j_B are given by

$$j_S = D_S\left(\frac{ds}{dx}\right)_{x=l} \tag{10}$$

and

$$j_B = D_B\left(\frac{db}{dx}\right)_{x=0} \tag{11}$$

3. Analytical solution of the concentrations and currents using Homotopy

3.1. Perturbation method

Recently, many authors have applied the HPM to various problems and demonstrated the efficiency of the HPM for handling non-linear structures and solving various physics and engineering problems [17–22]. This method is a combination of Homotopy in topology and classic perturbation techniques. Using this Homotopy perturbation method [23–31] (refer Appendix A), we obtain the normalized concentrations of the substrate ϕ_s and oxidized mediator ϕ_B as follows:

$$\phi_S = \frac{s}{Ks_\infty} \approx \frac{\cosh(x/X_K)}{\cosh(l/X_K)} + \frac{\alpha(Ks_\infty)}{6\cosh(l/X_K)^3}\left\{\cosh\left(\frac{l}{X_K}\right)\left[3 - \cosh\left(\frac{2x}{X_K}\right)\right] - \cosh\left(\frac{x}{X_K}\right)\left[3 - \cosh\left(\frac{2l}{X_K}\right)\right]\right\} \tag{12}$$

and

$$\phi_B = \frac{bD_B}{D_S(Ks_\infty)} \approx \left|\frac{[(1 - \cosh(x/X_K)) - (1 - \cosh(l/X_K))x/l]}{\cosh(l/X_K)}\right.$$

$$\left. + \frac{\alpha(Ks_\infty)\left\{(3 - \cosh(2l/X_K))[\cosh(x/X_K) - 1] - \cosh(l/X_K)[1 - \cosh(2x/X_K)] - [2\cosh(l/X_K) + \cosh(2l/X_K) - 3]x/l\right\}}{6\cosh(l/X_K)^3}\right| \tag{13}$$

Eqs. (12) and (13) represent the new dimensionless concentration of substrate ϕ_S and mediator ϕ_B for all values of α and l/X_K. Here X_K describes the distance over which the substrate can diffuse in the film before undergoing reaction and l denotes the film thickness. The expression of the normalized current becomes

$$\psi_S = \frac{j_S X_K}{D_S(Ks_\infty)} \approx \tanh\left(\frac{l}{X_K}\right) - \frac{\alpha(Ks_\infty)}{6\cosh(l/X_K)^2}\left[2\sinh\left(\frac{2l}{X_K}\right)\right.$$

$$\left. - \left\{3 - \cosh\left(\frac{2l}{X_K}\right)\right\}\tanh\left(\frac{l}{X_K}\right)\right] \tag{14}$$

and

$$\psi_B = \frac{j_B X_K}{D_B(Ks_\infty)} \approx \frac{X_K}{l}\left[1 - \text{sech}\left(\frac{l}{X_K}\right)\right]$$

$$+ \frac{\alpha X_K(Ks_\infty)}{6l\cosh(l/X_K)^3}\left[3 - 2\cosh\left(\frac{l}{X_K}\right) - \cosh\left(\frac{2l}{X_K}\right)\right] \tag{15}$$

where

$$X_K = \sqrt{1/\kappa} \tag{16}$$

Eqs. (14) and (15) represent the new approximate closed form of analytical expressions of normalized current of substrate and mediator for all values of parameter α. Bartlett and Whitaker

Table 1
Analytical expressions of concentration of substrate, mediator and current for all values of parameters.

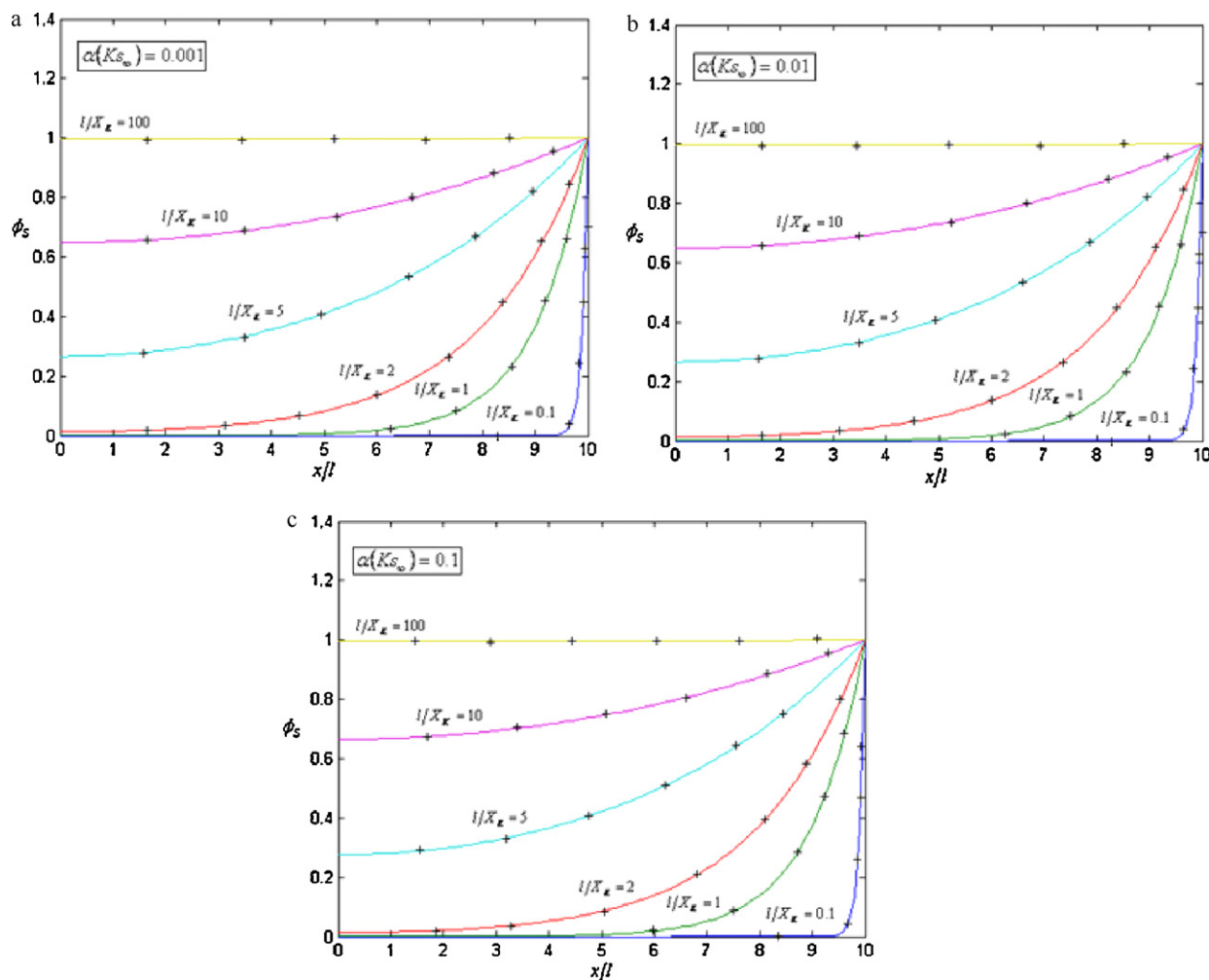| Conditions	Concentrations and currents	Figures and tables
For all values of α (this work)	$\phi_S = \dfrac{\cosh(x/X_K)}{\cosh(l/X_K)} + \dfrac{\alpha(Ks_\infty)}{6\cosh(l/X_K)^3}\left\{\cosh\left(\dfrac{l}{X_K}\right)\left[3-\cosh\left(\dfrac{2x}{X_K}\right)\right] - \cosh\left(\dfrac{x}{X_K}\right)\left[3-\cosh\left(\dfrac{2l}{X_K}\right)\right]\right\}$	Fig. 2a–c and Table 2a–c
	$\phi_B = \left\|\dfrac{\left[(1-\cosh(x/X_K))-(1-\cosh(l/X_K))x/l\right]}{\cosh(l/X_K)} + \dfrac{\alpha(Ks_\infty)}{6\cosh(l/X_K)^3}\left\{\left(3-\cosh\left(\dfrac{2l}{X_K}\right)\right)\left[\theta\cosh\left(\dfrac{x}{X_K}\right)-1\right]\right.\right.$ $\left.\left. -\cosh\left(\dfrac{l}{X_K}\right)\left[\theta 1 - \cosh\left(\dfrac{2x}{X_K}\right)\right] - \left[2\cosh\left(\dfrac{l}{X_K}\right)+\cosh\left(\dfrac{2l}{X_K}\right)-3\right]x/l\right\}\right\|$	Fig. 3a–c and Table 3a–c
	$\psi_S = \tanh\left(\dfrac{l}{X_K}\right) - \dfrac{\alpha(Ks_\infty)}{6\cosh(l/X_K)^2}\left[2\sinh\left(\dfrac{2l}{X_K}\right) - \left\{3-\cosh\left(\dfrac{2l}{X_K}\right)\right\}\tanh\left(\dfrac{l}{X_K}\right)\right]$	Fig. 4
	$\psi_B = \dfrac{X_K}{l}\left[1-\mathrm{sech}\left(\dfrac{l}{X_K}\right)\right] + \dfrac{\alpha X_K(Ks_\infty)}{6l\cosh(l/X_K)^3}\left[3-2\cosh\left(\dfrac{l}{X_K}\right)-\cosh\left(\dfrac{2l}{X_K}\right)\right]$	Fig. 5
$\alpha_S < 1$ (or) $(K_{cat}+ka)s < K_Mka$ (Bartlett and Whitaker work [16])	$\phi_S = \dfrac{\cosh(x/X_K)}{\cosh(l/X_K)}$	Fig. 2a–c
	$\phi_B = \dfrac{(1-\cosh(x/X_K))-(1-\cosh(l/X_K))x/l}{\cosh(l/X_K)}$	Fig. 3a–c
	$\psi_S = \tanh(l/X_K)$	Fig. 4
	$\psi_B = \dfrac{X_K}{l}[1-\mathrm{sech}(l/X_K)]$	Fig. 5
$\alpha s > 1$ (or) $(K_{cat}+ka)s > K_Mka$ (Bartlett and Whitaker work [16])	$\phi_S = 1 + \dfrac{l^2}{2X_K^2\alpha(Ks_\infty)}\left(\dfrac{x^2}{l^2}-1\right)$	Table 2a–c
	$\phi_B = \dfrac{l^2}{2X_K^2\alpha(Ks_\infty)}\left(\dfrac{x}{l}-\dfrac{x^2}{l^2}\right)$	Table 3a–c
	$\psi_S = \dfrac{l}{X_K\alpha(Ks_\infty)}$	–
	$\psi_B = \dfrac{l}{2X_K\alpha(Ks_\infty)}$	–

Fig. 2. A plot of normalized substrate concentration ϕ_S in the film as a function of x/l. Values of ϕ_S are calculated from Eq. (12). The key to the graph (−) represents Eq. (12) and (+) represents Eq. (17). (*a*) $\alpha(Ks_\infty) = 0.001$, (*b*) $\alpha(Ks_\infty) = 0.01$, and (*c*) $\alpha(Ks_\infty) = 0.1$.

[16] derived the approximate analytical solutions of the substrate and the mediator concentrations and current for different limiting cases only. Various analytical expressions for the concentrations of the substrate and mediator for $(K_{cat} + ka)s < K_M ka$ (or $\alpha s < 1$) and $(K_{cat} + ka)s < K_M ka$ (or $\alpha s < 1$) are given in Table 1. The analytical expressions for the dimensionless current derived by Bartlett and Whitaker [16] for various limiting cases are also given in Table 1.

4. Discussion and comparison with Bartlett and Whitaker work [16]

4.1. Substrate – limited kinetics $((K_{cat} + ka)s < K_M ka)$

In this case, the reaction of substrate with enzyme is rate limiting. The rate of reaction depends upon the concentration of *S*. The approximate expression corresponding to the concentration of substrate (Eq. (17)), mediator (Eq. (18)) and current (Eqs. (19) and (20)) was provided by Bartlett and Whitaker [16] (refer Table 1). The first term of our results (Eqs. (12)–(15)) compares well with the Bartlett and Whitaker [16] results (Eqs. (17)–(20)) demonstrating the accuracy of our work.

Fig. 2a–c represents a series of normalized substrate concentration ϕ_S using Eq. (12) for various values of l/X_K and $\alpha(Ks_\infty)$. Here X_K describes the distance over which the substrate can diffuse in the film before undergoing reaction and l denotes the film thickness. From these figures, it is evident that the value of substrate

concentration $\phi_S \approx 1$ for all values of $\alpha(Ks_\infty)$ and $l/X_K \geq 100$. Furthermore, Fig. 2a–c compares the normalized substrate concentration ϕ_S obtained in this work with Bartlett and Whitaker work [16]. Upon comparison, it is evident that both results are identical. From this Fig. 2a–c, it is inferred that the concentration of substrate ϕ_S increases when normalized film thickness l/X_K (or enzyme loading) increases. Also when the film thickness is much less than the kinetic length ($l/X_K \leq 0.1$), the normalized concentration of substrate $\phi_S = 0$ when $x/l \leq 9.5$. Increasing the film thickness increases the concentration of substrate ϕ_S because the reaction of substrate is occurring uniformly throughout the film.

Fig. 3a–c indicates the dimensionless concentration profile of oxidized mediator ϕ_B using Eq. (13) for various values of l/X_K and $\alpha(Ks_\infty)$. Upon careful evaluation of these figures, it is evident that there is a simultaneous decrease in the values of concentration of oxidized mediator ϕ_B and reaches the steady-state value ($\phi_B = 0$) when $l/X_K \geq 100$. In addition, Fig. 3a–c represents the comparison of normalized mediator concentration ϕ_B of this work and Bartlett and Whitaker work [16]. On conclusion, it is evident that the results are identical. From this Fig. 3a–c, it is evident that, the concentration of mediator ϕ_B increases when thickness of the film l/X_K decreases for all small values of $\alpha(Ks_\infty)$. Also the concentration ϕ_B increases slowly and reach the maximum value and then suddenly decreases. The normalized concentration of mediator ϕ_B at the electrode surface ($x = 0$) and the bulk ($x = l$) is equal. Increasing the film thickness decreases the concentration of mediator ϕ_B, because *B* diffuse all the way through the film to react at the electrode surface.
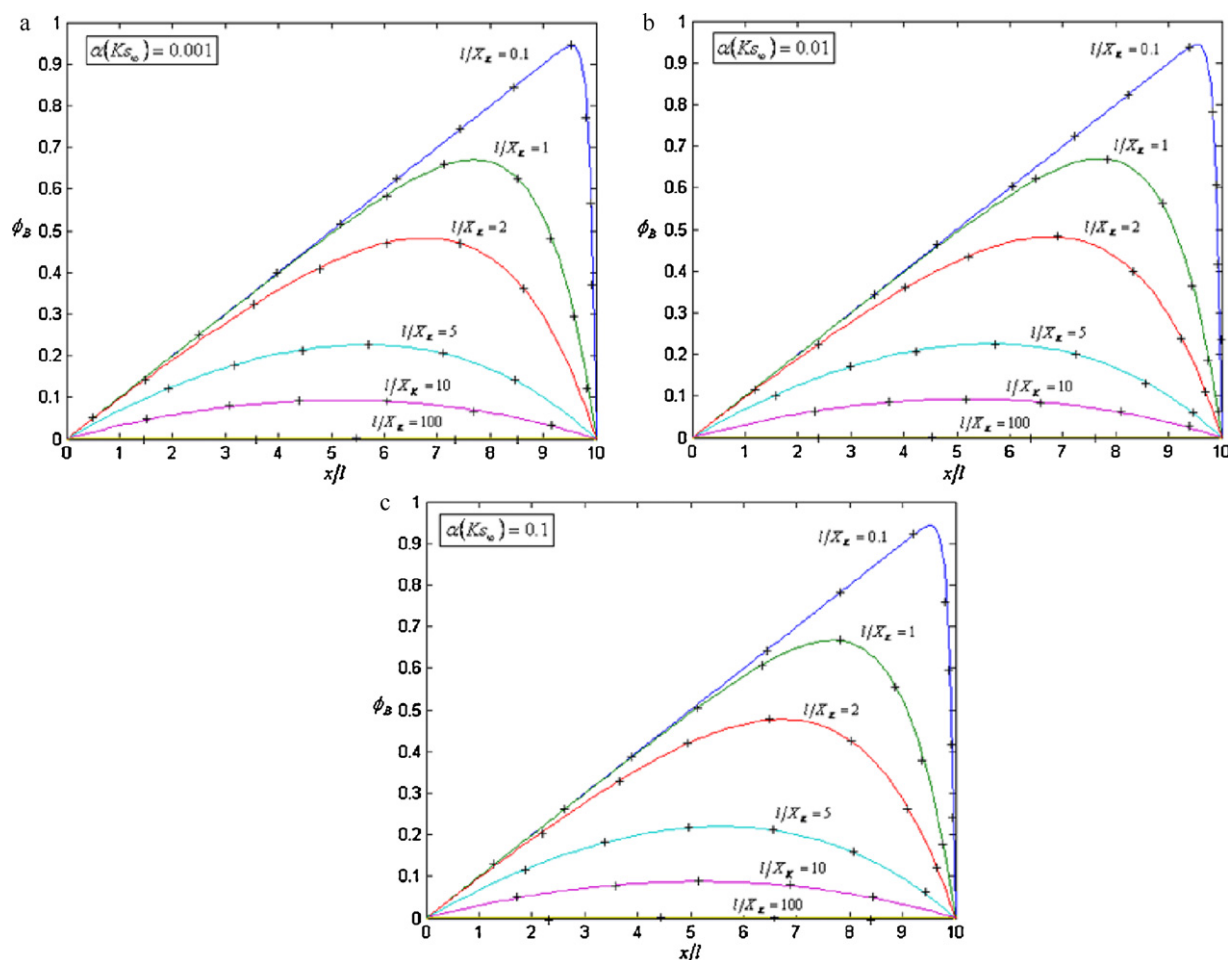
Fig. 3. A plot of normalized oxidized mediator concentration ϕ_B in the film as a function of x/l. The values of ϕ_B are calculated from Eq. (13). The key to the graph (–) represents Eq. (13) and (+) represents Eq. (18). (a) $\alpha(Ks_\infty) = 0.001$, (b) $\alpha(Ks_\infty) = 0.01$, and (c) $\alpha(Ks_\infty) = 0.1$.

Fig. 4 shows the dimensionless current of substrate ψ_S for all values of l/X_K and $\alpha(Ks_\infty)$ [=0.001, 0.01, 0.1] using Eqs. (19) and (14). From this figure, it is evident that as ψ_S decreases, when $\alpha(Ks_\infty)$ increases. Also when $l/X_K \geq 2$, all the curves reach the steady state value.
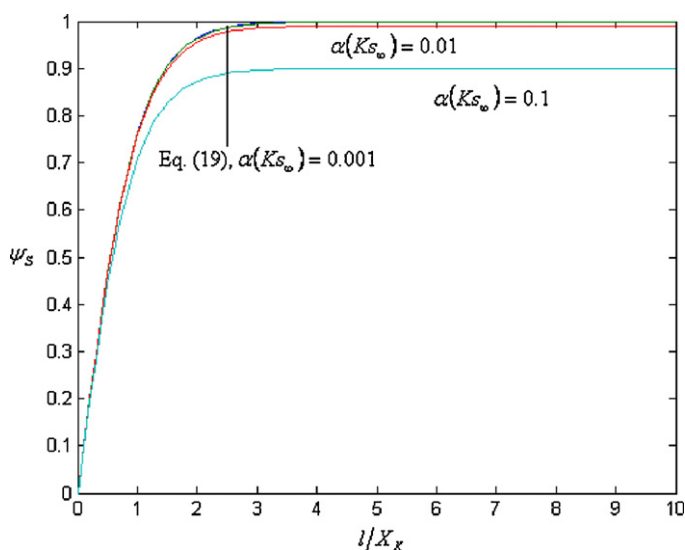
Fig. 5 shows the dimensionless current of mediator ψ_B for all values of l/X_K using Eqs. (20) and (15). From this figure, it is inferred that, initially the value of the current increases and reaches the maximum value when $l/X_K = 2.4$ for all values $\alpha(Ks_\infty)$. The value of the current increases when the film

Fig. 4. A plot of dimensionless current, ψ_S, versus the normalized film thickness, l/X_K. Current values were calculated using Eqs. (19) and (14) for various values of $\alpha(Ks_\infty)$ [=0.001, 0.01, 0.1].
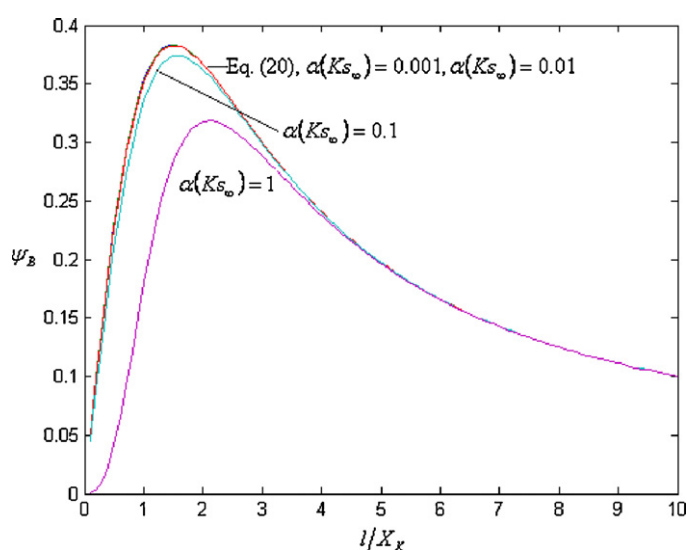

Fig. 5. A plot of the dimensionless current, ψ_B, versus the normalized film thickness, l/X_K. The current were calculated using Eqs. (20) and (15) for various values of $\alpha(Ks_\infty)$ [=0.001, 0.01, 0.1, 1].

Table 2

Comparison of normalized substrate concentration ϕ_S between Eqs. (21) and (12) for various values of (a) l/X_K and $\alpha(Ks_\infty) = 1$, (b) l/X_K and $\alpha(Ks_\infty) = 2$, and (c) l/X_K and $\alpha(Ks_\infty) = 5$.

x/l	$l/X_K = 0.001$		$l/X_K = 0.01$		$l/X_K = 0.1$		$l/X_K = 1$	
	Bartlett and Whitaker Eq. (21)	This work Eq. (12)	Bartlett and Whitaker Eq. (21)	This work Eq. (12)	Bartlett and Whitaker Eq. (21)	This work Eq. (12)	Bartlett and Whitaker Eq. (21)	This work Eq. (12)
(a) l/X_K and $\alpha(Ks_\infty) = 1$								
0.0	1.0000	1.0000	0.9999	1.0000	0.9950	0.9999	0.5000	0.8226
0.2	1.0000	1.0000	0.9999	1.0000	0.9952	0.9999	0.5200	0.8306
0.4	1.0000	1.0000	0.9999	1.0000	0.9958	0.9999	0.5800	0.8543
0.6	1.0000	1.0000	0.9999	1.0000	0.9968	0.9999	0.6800	0.8925
0.8	1.0000	1.0000	0.9999	1.0000	0.9982	0.9999	0.8200	0.9426
1.0	1.0000	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000
(b) l/X_K and $\alpha(Ks_\infty) = 2$								
0.0	1.0000	1.0000	0.9999	1.0000	0.9975	1.0049	0.7500	0.9971
0.2	1.0000	1.0000	0.9999	1.0000	0.9976	1.047	0.7600	1.0002
0.4	1.0000	1.0000	0.9999	1.0000	0.9979	1.0041	0.7900	1.0081
0.6	1.0000	1.0000	0.9999	1.0000	0.9984	1.0031	0.8400	1.0167
0.8	1.0000	1.0000	0.9999	1.0000	0.9991	1.0018	0.9100	1.0184
1.0	1.0000	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000
(c) l/X_K and $\alpha(Ks_\infty) = 5$								
0.0	1.0000	1.0000	0.9999	1.0002	0.9990	1.0197	0.9000	1.5209
0.2	1.0000	1.0000	0.9999	1.0002	0.9990	1.0189	0.9040	1.5090
0.4	1.0000	1.0000	0.9999	1.0002	0.9992	1.0166	0.9160	1.4693
0.6	1.0000	1.0000	0.9999	1.0002	0.9994	1.0126	0.9360	1.3894
0.8	1.0000	1.0000	0.9999	1.0001	0.9996	1.0071	0.9640	1.2458
1.0	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Table 3

Comparison of normalized mediator concentration ϕ_B between Eqs. (22) and (13) for various values of (a) l/X_K and $\alpha(Ks_\infty) = 1$, (b) l/X_K and $\alpha(Ks_\infty) = 2$, and (c) l/X_K and $\alpha(Ks_\infty) = 5$.

x/l	$l/X_K = 0.01$		$l/X_K = 0.1$		$l/X_K = 1$		$l/X_K = 2$	
	Bartlett and Whitaker Eq. (22)	This work Eq. (13)	Bartlett and Whitaker Eq. (22)	This work Eq. (13)	Bartlett and Whitaker Eq. (22)	This work Eq. (13)	Bartlett and Whitaker Eq. (22)	This work Eq. (13)
(a) l/X_K and $\alpha(Ks_\infty) = 1$								
0.0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.2	0.0000	0.0000	0.0008	0.0000	0.0800	0.3200	0.3200	0.1032
0.4	0.0000	0.0000	0.0012	0.0000	0.1200	0.4800	0.4800	0.1570
0.6	0.0000	0.0000	0.0012	0.0000	0.1200	0.4800	0.4800	0.1573
0.8	0.0000	0.0000	0.0008	0.0000	0.0800	0.3200	0.3200	0.1012
1.0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
(b) l/X_K and $\alpha(Ks_\infty) = 2$								
0.0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.2	0.0000	0.0000	0.0004	0.0008	0.0400	0.0025	0.1600	0.0811
0.4	0.0000	0.0000	0.0006	0.0012	0.0600	0.0098	0.2400	0.1101
0.6	0.0000	0.0000	0.0006	0.0012	0.0600	0.0178	0.2400	0.0895
0.8	0.0000	0.0000	0.0004	0.0008	0.0400	0.0189	0.1600	0.0344
1.0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
(c) l/X_K and $\alpha(Ks_\infty) = 5$								
0.0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.2	0.0000	0.0000	0.0002	0.0032	0.0160	0.0923	0.0640	0.0147
0.4	0.0000	0.0000	0.0002	0.0047	0.0240	0.1568	0.0960	0.0308
0.6	0.0000	0.0000	0.0002	0.0047	0.0240	0.1813	0.0960	0.1140
0.8	0.0000	0.0000	0.0002	0.0032	0.0160	0.1416	0.0640	0.1660
1.0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

thickness is much less than the kinetic length ($l \leq X_K$), because the reaction of substrate is occurring uniformly throughout the film.

4.2. Mediator – limited kinetics ($(K_{cat} + ka)s > K_M ka$)

In this case, the breakdown of the enzyme substrate complex (K_{cat}) or the regeneration of the oxidized enzyme is the rate limiting step. For this condition, Bartlett and Whitaker [16] reported the approximate analytical expression of concentration of substrate (Eq. (21)), mediator (Eq. (22)) and current (Eqs. (23) and (24)). Table 2a–c represents a series of normalized substrate concentration ϕ_S using Eqs. (12) and (21) for various values of l/X_K and $\alpha(Ks_\infty)$. From these tables, it is evident that as $\phi_S \approx 1$ when $l/X_K \leq 1$ and $\alpha(Ks_\infty) \leq 5$. Table 3a–c represents a series of normalized substrate concentration ϕ_B using Eqs. (13) and (22) for various values of l/X_K and $\alpha(Ks_\infty)$.

From these tables, it is inferred that $\phi_B \approx 0$ when $l/X_K \leq 2$ and $\alpha(Ks_\infty) \leq 5$.

5. Conclusions

This paper presents a mathematical treatment for analyzing amperometric enzymatic reactions. In this paper, we have re-studied a theoretical model for an amperometric enzyme electrodes based on an immobilized flavoprotein. We have solved two non-linear differential equations analytically. The approximate analytical expressions for the steady state substrate concentration and oxidized mediator concentration profiles for all values of k_{cat}, ka and K_M at the amperometric enzyme electrodes were obtained using Homotopy perturbation method. Furthermore, an analytical expression corresponding to the steady state current response is also presented. A satisfactory agreement with the existing limiting cases results is noted.

Acknowledgements

Appendix A. Solution of Eqs. (7) and (8) using Homotopy perturbation method

In this appendix, we indicate how Eqs. (12) and (13) in this paper are derived. Furthermore, a Homotopy was constructed to determine the solution of Eqs. (7) and (8).

$$(1-p)\left[\frac{d^2s}{dx^2}-\kappa s\right]+p\left[\frac{d^2s}{dx^2}+\alpha s\frac{d^2s}{dx^2}-\kappa s\right]=0. \tag{A1}$$

$$(1-p)\left[\frac{d^2b}{dx^2}+\eta s\right]+p\left[\frac{d^2b}{dx^2}+\alpha s\frac{d^2b}{dx^2}+\eta s\right]=0 \tag{A2}$$

and the initial approximations are as follows:

$$x=0; \quad \frac{ds_0}{dx}=0; \quad b_0=0 \tag{A3a}$$

$$x=l; \quad s_0=Ks_\infty; \quad b_0=0 \tag{A3b}$$

The approximate solutions of (A1) and (A2) are

$$s=s_0+ps_1+p^2s_2+p^3s_3+\cdots \tag{A4}$$

and

$$b=b_0+pb_1+p^2b_2+p^3b_3+\cdots \tag{A5}$$

Substituting Eqs. (A4) and (A5) into Eqs. (A1) and (A2) and comparing the coefficients of like powers of p

$$p^0: \quad \frac{d^2s_0}{dx^2}-\kappa s_0=0 \tag{A6}$$

and

$$p^1: \quad \alpha s_0\frac{d^2s_0}{dx^2}+\frac{d^2s_1}{dx^2}-\kappa s_1=0 \tag{A7}$$

$$p^0: \quad \frac{d^2b_0}{dx^2}+\eta s_0=0 \tag{A8}$$

and

$$p^1: \quad \alpha s_0\frac{d^2b_0}{dx^2}+\frac{d^2b_1}{dx^2}+\eta s_1=0 \tag{A9}$$

Solving Eqs. (A6)–(A9), and using the boundary conditions (A3a)

and (A3b), we can find the following results

$$s_0(x)=\frac{(Ks_\infty)\cosh\left(\sqrt{\kappa}x\right)}{\cosh\left(\sqrt{\kappa}l\right)} \tag{A10}$$

and

$$s_1(x)=\frac{\alpha(Ks_\infty)^2}{6\cosh\left(\sqrt{\kappa}l\right)^3}\left\{\cosh\left(\sqrt{\kappa}l\right)\left[3-\cosh\left(2\sqrt{\kappa}x\right)\right]\right.$$
$$\left.-\cosh\left(\sqrt{\kappa}x\right)\left[3-\cosh\left(2\sqrt{\kappa}l\right)\right]\right\} \tag{A11}$$

$$b_0(x)=\frac{\eta(Ks_\infty)}{\kappa\cosh\left(\sqrt{\kappa}l\right)}\left[\left(1-\cosh\left(\sqrt{\kappa}x\right)\right)-\left(1-\cosh\left(\sqrt{\kappa}l\right)\right)x/l\right] \tag{A12}$$

and

$$b_1(x)=\frac{\alpha\eta(Ks_\infty)^2}{2\kappa\cosh\left(\sqrt{\kappa}l\right)^3}\left[\cosh\left(\sqrt{\kappa}x\right)-\cosh\left(\sqrt{\kappa}l\right)x/l\right]$$
$$+\left[\frac{\alpha\eta(Ks_\infty)^2\cosh\left(2\sqrt{\kappa}l\right)}{6\kappa\cosh\left(\sqrt{\kappa}l\right)^3}-\frac{\alpha\eta(Ks_\infty)^2}{6\kappa\cosh\left(\sqrt{\kappa}l\right)^2}\right.$$
$$\left.-\frac{\alpha\eta(Ks_\infty)^2}{2\kappa\cosh\left(\sqrt{\kappa}l\right)^3}\right]\left(1-\frac{x}{l}\right)-\frac{\alpha\eta(Ks_\infty)^2}{6\kappa\cosh\left(\sqrt{\kappa}l\right)^3}$$
$$\left[\cosh\left(2\sqrt{\kappa}l\right)\cosh\left(\sqrt{\kappa}x\right)-\cosh\left(\sqrt{\kappa}l\right)\cosh\left(2\sqrt{\kappa}x\right)\right] \tag{A13}$$

According to the HPM, we can conclude that

$$s(x)=\lim_{p\to 1}s(x)=s_0+s_1 \tag{A14}$$

and

$$b(x)=\lim_{p\to 1}b(x)=b_0+b_1 \tag{A15}$$

Using Eqs. (A10) and (A11) in Eq. (A14) and Eqs. (A12) and (A13) in Eq. (A15), we obtain the final results as described in Eqs. (12) and (13).

Appendix B. Nomenclature and units

Symbol	Meaning	Usual dimension
S	Concentration of the substrate	mole cm^{-3}
b	Concentration of the oxidized (reduced) mediator	mole cm^{-3}
e_Σ	Total enzyme concentration in the film	mole cm^{-3}
K_M	Michaelis–Menten constant	mole cm^{-3}
k_{cat}	Catalytic reaction rate constant	s^{-1}
ka	Regeneration of the oxidized enzyme	s^{-1}
Ks_∞	Surface concentration of the substrate in the film	mole cm^{-3}
D_S	Diffusion coefficient of the substrate	cm^2 s^{-1}
D_B	Diffusion coefficient for the mediator	cm^2 s^{-1}
l	Film thickness	cm
x	Distance to the electrode	cm
X_K	Distance to which substrate can diffuse in the film before undergoing reaction	cm
j_S	Flux of substrate reacting in the film	mole cm^{-2} s^{-1}
j_B	Flux of the mediator reacting at the electrode	mole cm^{-2} s^{-1}
ϕ_S	Normalised concentration parameter for the substrate	–
ϕ_B	Normalised concentration parameter for the mediator	–
ψ_S	Dimensionless current of the substrate	–
ψ_B	Dimensionless current of the oxidized (reduced) mediator	–
α	Parameter	mole^{-1} cm^3
κ	Parameter	cm^{-2}
η	Parameter	cm^{-2}

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