Voltammetric and Mathematical Analysis of Adsorption of Enzymes at Rotating Disk Electrode

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The steady-state model of an enzyme monolayer adsorbed onto a rotating disc electrode is explored. This model is predicated on a nonlinear system of equations that consists of a nonlinear term related to the reaction rate. A new homotopy perturbation method and Taylor's series approach are employed to derive two efficient and reliable analytical expressions for the concentration of adsorbed enzymes are obtained. The validity of the obtained closed-form results is established by direct comparison with numerical simulations generated by popular and highly accurate MATLAB routines. The derived analytical expression is then used to determine when concentrations attain their steady-state position.

1. INTRODUCTION

The voltammetry method at a rotating disk has been intensively used to study electrode processes at solid electrodes due to many desirable features such as the steady-state [1-2]. Of other desirable features is that diffusion of the substrate is linear, and hence the use of RDE provides control of the flux of substrate, and linear sweep voltammetry implies steady-state currents [3].

Many studies have investigated the rotating disk enzyme since the 60s of the past century. For example, Shu et al. conducted theoretical and experimental studies on rotating ring-disk enzyme electrodes (RRDEE) for surface catalysis [4]. Karmin et al. implemented and tested several RRDEE for kinetic performance and immobilized enzyme layer characterization [5]. Albery et al. investigated the reduction and oxidation of cytochrome at a gold electrode using RDDE [6].

Obtaining analytical solution are the ultimate goal of researchers to obtain practical incite of the model and then derive a meaningful interpretation of experimental data. In addition, a closed-form solution of RDDE allows for exploring limiting cases by varying critical kinetic and thermodynamic parameters within the simulation. Although the process is assumed to produce a steady-state system, rotating disc voltammetry is developed to study enzyme catalysis [7].

Though still in premature stages, some notable efforts have been carried out to determine analytical and numerical rotating disk electrode voltammetry. Earles et al.[8] presented an analytical investigation of an RDE voltammetric measurement of dopamine transporter activity. A closed-form of analytical expression of the enzyme for the steady-state was reported by Heering et al. [3] for various (mass transport, electron transfer, and enzymatic) limiting cases. Saravanakumar et al. [9] obtained the concentration and current at a rotating disc electrode controlled by diffusion, convection, and migration using an analytical method. King and Altman employed a graphical approach to derive rate laws for enzyme-catalyzed reactions [10].

This paper presents theoretical methods for the analysis of the nonlinear differential equation in rotating disk voltammetry of adsorbed enzymes**.** Analytical solution of the underlined nonlinear system may be sought by well-established effective methods such as variation iteration, homotopy analysis and Green's function based methods [11-16]. However, we chose, in this article, to employ two simple, reliable and efficient mathematical approaches using Taylor series method and a modified form of the homotopy perturbation method to derive accurate analytical expressions for the concentration of absorbed enzymes and current at RDE. The simplicity of these approaches make them widely accessible to researchers to further investigate the kinetic parameters and hence attain optimal designs

NOMENCLATURE:

Symbol	Description	Unit
Γ_i	The surface concentration of form i of the enzyme	mol cm^{-2}
k_j	Rate constant for enzyme catalysis	s^{-1}
$k_j^{F/B}$	Forward or backward electrochemical rate	s^{-1}
$k_{S,j}$	The electrochemical standard rate constant for the jth reaction	s^{-1}
α	Transfer coefficient	-----
Γ_t	Total electroactive surface concentration	1.0×10^{-11} (mol cm^{-2})
x_i	Fractional coverage	-----
x_i	Fractional coverage	-----
ω	Angular electrode rotation rate	rad s^{-1}
ν	Kinematic viscosity	mV s^{-1}
$H(\gamma)$	Dimensionless variable	-----
$x_{OX}(t)$	Steady state concentration of OX	(mol cm^{-2})
$x_{HR}(t)$	Steady state concentration of HR	(mol cm^{-2})
$x_{Red}(t)$	Steady state concentration of Red	(mol cm^{-2})
$x_{Red.S}(t)$	Steady state concentration of Red.S	(mol cm^{-2})
$c_S(t)$	Concentration of the substrate	(mol cm^{-2})
D_S	Diffusion coeffecent of substrate	Cm^2 s^{-1}
t	Time	s

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2. FORMULATION OF THE INITIAL VALUE PROBLEM.

The system of nonlinear rate equations is summarized by [7],

$$\frac{d\Gamma_{OX}}{dt} = -k_{E1}^F \Gamma_{OX} + k_{E1}^B \Gamma_{HR} + k_2 \Gamma_{Red.S} \tag{1}$$

$$\frac{d\Gamma_{HR}}{dt} = k_{E1}^F \Gamma_{OX} - (k_{E1}^B + k_{E2}^F)\Gamma_{HR} + k_{E2}^B \Gamma_{Red} \tag{2}$$

$$\frac{d\Gamma_{Red}}{dt} = k_{E2}^F \Gamma_{HR} - \left(k_{+1}c_S(0,t) + k_{E2}^B\right)\Gamma_{Red} + k_{-1}\Gamma_{Red.S} \tag{3}$$

$$\frac{d\Gamma_{Red.S}}{dt} = k_{+1}c_S(0,t)\Gamma_{Red} - (k_{-1} + k_2)\Gamma_{Red.S} \tag{4}$$

where Γ_i is the surface concentration (mol cm^{-2}), i stands for enzyme OX, HR, Red, or Red.S. The parameter k_j is a rate constant for enzyme catalysis and $k_j^{F/B}$ is a forward or backward electrochemical rate given by the Butler-Volmer kinetics. The total electroactive surface concentration Γ_t, is assumed to be constant, that is,

$$\sum_i \Gamma_i = \Gamma_T \tag{5}$$

Dividing Eqs. (1)–(4) by Γ_T gives

$$\frac{dx_{OX}(t)}{dt} = -k_{E1}^F x_{OX}(t) + k_{E1}^B x_{HR}(t) + k_2 x_{Red.S}(t), \tag{6}$$

$$\frac{dx_{HR}(t)}{dt} = k_{E1}^F x_{OX}(t) - (k_{E1}^B + k_{E2}^F)x_{HR}(t) + k_{E2}^B x_{Red}(t), \tag{7}$$

$$\frac{dx_{Red}(t)}{dt} = k_{E2}^F x_{HR}(t) - \left(k_{+1}c_S(0,t) + k_{E2}^B\right)x_{Red}(t) + k_{-1}x_{Red.S}(t), \tag{8}$$

$$\frac{dx_{Red.S}(t)}{dt} = k_{+1}c_S(0,t)x_{Red}(t) - (k_{-1} + k_2)x_{Red.S}(t). \tag{9}$$

The initial conditions are given by

$$x_{OX}(t) = (x_{OX})_0, x_{HR}(t) = (x_{HR})_0, x_{Red}(t) = (x_{Red})_0, x_{Red.S}(t) = (x_{Red.S})_0 \quad \text{at} \quad t = 0 \tag{10}$$

where x_i is the fractional coverage. The mass transport equation for the substrate (coupled to the rate equations for the enzyme) is given by

$$\frac{\partial c_S(z,t)}{\partial t} = D_S \frac{\partial^2 c_S(z,t)}{\partial z^2} - v_z \frac{\partial c_S(z,t)}{\partial z} \tag{11}$$

where v_z is the velocity of the fluid normal to the electrode and is given by

$$v_z = -0.51023 v^{-1/2}\Omega^{3/2}z^2 + \frac{1}{3}v^{-1}\Omega^2 z^3 + \cdots \tag{12}$$

Th2 series in (12) is usually truncated at the first term. The initial and boundary conditions are

$$c_S(z,0) = c_S^*, c_S(\infty,0) = c_S^* \tag{13}$$

where c_S^* is the concentration of substrate in the bulk solution.

The concentration at the electrode surface is [16]

$$c_S(0, t) = \frac{e^{\eta}}{1 + e^{\eta}}. \tag{14}$$

When the scan rate is low, $c_S(0, t)$ is assumed constant. The catalytic current density (A cm^{-2}) is

$$D_S \left(\frac{\partial c_S(z, t)}{\partial z} \right)_{z=0} = k_{+1} c_S(0, t) \Gamma_{Red}(t) - k_{-1} \Gamma_{Red.S}(t). \tag{15}$$

Using Eq. (10), we obtain

$$x_{OX}(t) = 1 - x_{HR}(t) - x_{Red}(t) - x_{Red.S}(t). \tag{16}$$

Fig.1 shows a schematic representation of the reaction rate in the scheme of electrochemically driven catalytic systems with an enzyme in this layer [10].
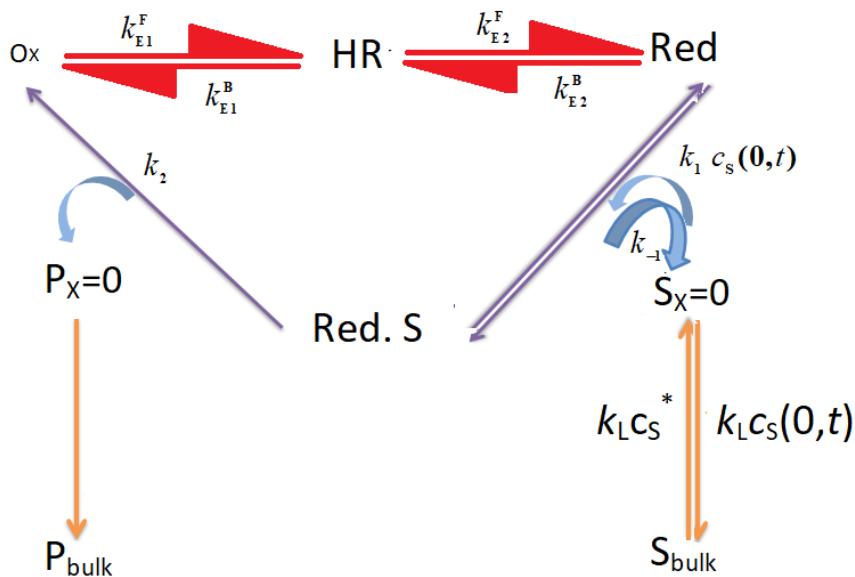

Figure 1. Scheme of enzyme catalysis within electrode-immobilized enzyme layer.

By substituting Eq. (15) into Eqs. (6)–(9) and simplifying, we obtain the following one-dimensional steady-state equations for the reduced form of enzymes (see Appendix A for details)

$$\frac{dx_{HR}(t)}{dt} = k_{E1}^F - k_0 x_{HR}(t) + k_1 x_{Red}(t) - k_{E1}^F x_{Red.S}(t), \tag{17}$$

$$\frac{dx_{Red}(t)}{dt} = k_{E2}^F x_{HR}(t) - k_{0\eta} x_{Red}(t) + k_{-1} x_{Red.S}(t), \tag{18}$$

$$\frac{dx_{Red.S}(t)}{dt} = k_{1\eta} x_{Red}(t) - k_{21} x_{Red.S}(t) \tag{19}$$

with initial conditions

$$x_{HR}(t = 0) = (x_{HR})_0, \; x_{Red}(t = 0) = (x_{Red})_0, \quad x_{Red.S}(t = 0) = (x_{Red.S})_0 \tag{20}$$

3. ANALYTICAL EXPRESSION OF THE CONCENTRATIONS OF ENZYMES

As articulated in the introduction, analytical solutions are more important in understanding the qualitative features and processes of physical systems than numerical solutions. In this section, we employ effective and easily accessible mathematical approaches to obtain approximate analytical solutions of the nonlinear system (17)–(20).

3.1 A modified homotopy perturbation method

The homotopy perturbation method (HPM) first presented by J. He in 1999 [17] has shown to be very powerful in solving a wide range of problems arising in many science and engineering applications [18, 19]. Over the past three decades, the HPM has been modified to become even more efficient in solving nonlinear dynamical systems [20-23].

By employing a new version of the HPM for solving the governing system (17)–(20) (details in Appendix B), we obtain the following approximate analytical expressions of concentrations of the enzymes

$$x_{\text{HR}}(t) = -\left(\frac{k_3 + k_9}{k_0}\right) + \frac{k_1 k_7 e^{-k_{0\eta}t}}{k_0 - k_{0\eta}} - \frac{k_{E1}^{\text{F}} k_8 e^{-k_{21}t}}{k_0 - k_{21}} \tag{21}$$

$$- \left\{\frac{k_9}{k_0} + \frac{k_1 k_7}{k_0 - k_{0\eta}} + \frac{k_{E1}^{\text{F}} k_8}{k_0 - k_{21}} - k_6\right\} e^{-k_0 t}$$

$$x_{\text{Red}}(t) = -\left(\frac{k_5 - k_{10}}{k_{0\eta}}\right) + \frac{k_{E2}^{\text{F}} k_6 e^{-k_0 t}}{k_{0\eta} - k_0} + \frac{k_{-1} k_8 e^{-k_{21}t}}{k_{0\eta} - k_{21}} \tag{22}$$

$$- \left\{\frac{k_{10}}{k_{0\eta}} + \frac{k_{E2}^{\text{F}} k_6}{k_{0\eta} - k_0} + \frac{k_{-1} k_8}{k_0 - k_{21}} - k_7\right\} e^{-k_{0\eta}t}$$

$$x_{\text{Red.S}}(t) = \frac{k_{1\eta}(x_{\text{Red.S}})_i + k_{11}}{k_{21}} + k_8 e^{-k_{21}t} - \frac{k_{1\eta}(x_{\text{Red.S}})_i e^{-k_{0\eta}t}}{k_{21} - k_{0\eta}} \tag{23}$$

$$- \left\{\frac{k_{11}}{k_{21}} + \frac{k_{1\eta}(x_{\text{Red.S}})_i}{k_{21} - k_{0\eta}}\right\} e^{-k_{21}t}$$

$$x_{\text{OX}}(t) = 1 - x_{\text{HR}}(t) - x_{\text{Red}}(t) - x_{\text{Red.S}}(t) \tag{24}$$

where,

$$k_0 = k_{E1}^{\text{F}} + k_{E1}^{\text{B}} + k_{E2}^{\text{F}}, k_1 = k_{E2}^{\text{B}} - k_{E1}^{\text{F}}, k_{0\eta} = \frac{k_{+1} e^\eta}{1 + e^\eta} + k_{E2}^{\text{B}}, k_{1\eta} = \frac{k_{+1} e^\eta}{1 + e^\eta}, \; k_{21} = k_{-1} + k_2 \tag{25}$$

and the parameters k_3 to k_{11} are given in Eq. (A28). The catalytic current density is given by

$$\frac{\text{i}(t)}{\text{nFD}_0 \text{A}} = k_{+1} c_{\text{S}}(0, t) x_{\text{Red}}(t) - k_{-1} x_{\text{Red.S}}(t). \tag{26}$$

3.2 Taylor's series approach

In a revolutionary approach, J. He has resorted to one of the most basic mathematical approaches for solving some of the most challenging mathematical models. For example, He successfully employed Taylor's series method coupled with Padé approximation to derive a highly accurate approximate solution for the famous Lane-Emden equation [24]. The powerful Taylor's series approach was also proved successful in fractional calculus [25]. The Taylor series was recently used to investigate the chronoamperometric limiting flux produced by an electrochemical reaction in a rotating disc electrode [26].

In this article, we employed Taylor's series method to solve the governing system (17)–(20) to obtain the following analytical expressions for concentrations of enzymes (see details in Appendix C)

$$x_{\text{HR}}(t) = 10^{-4}(990t - 64.83t^2 - 2.966774t^3 + 415.525253t^4 + 72.541373t^5 \tag{27}$$

$$+ 1.233305t^6 + 0.175627t^7 + 0.021965t^8 \qquad + \cdots)$$

$$x_{\text{Red}}(t) = 10^{-4}(t + 4.8834975t^2 - 0.550937t^3 + 0.040296t^4 + 0.081049t^5 \tag{28}$$

$$+ 0.009273t^6 + 0.001502t^7 + 0.000181t^8 + \cdots)$$

$$x_{\text{Red.S}}(t) = 10^{-4}(10 - 1.1t + 0.11075t^2 + 0.157911t^3 - 0.018184t^4 + 0.001210t^5 \tag{29}$$

$$+ 0.001335t^6 + 0.000112t^7 + 0.000017t^8 + \cdots)$$

4. NUMERICAL SIMULATION

To examine the accuracy of the derived analytical solutions, the nonlinear system (17)–(20) is solved numerically by using the widely used and highly accurate MATLAB function pdex4 (see Appendix B).
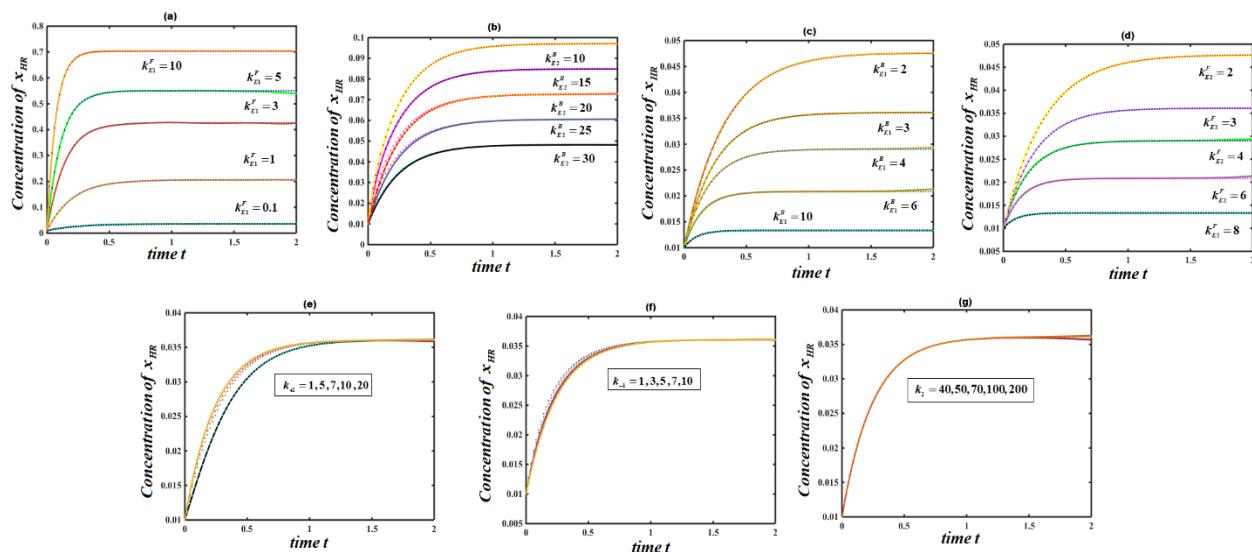

Figure 2. Comparison between analytical (Eq. (20)) and numerical results for the concentration of enzymes x_{HR} for various values of parameters.
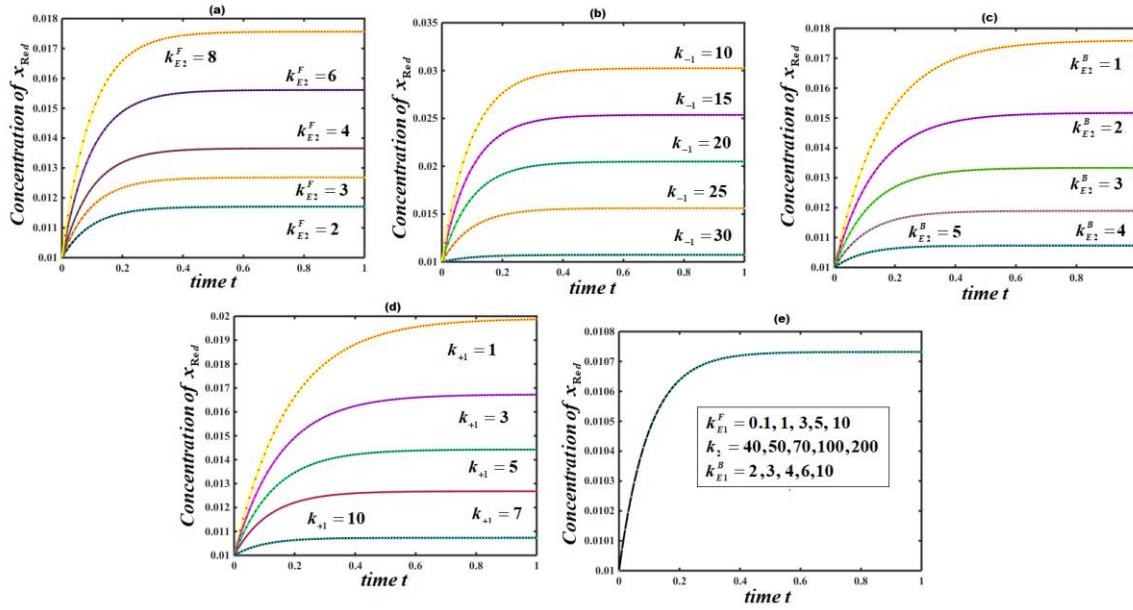
Figure 3. Comparison between analytical (Eq. (21)) and numerical results for the concentration x_{Red} for various values of parameters.
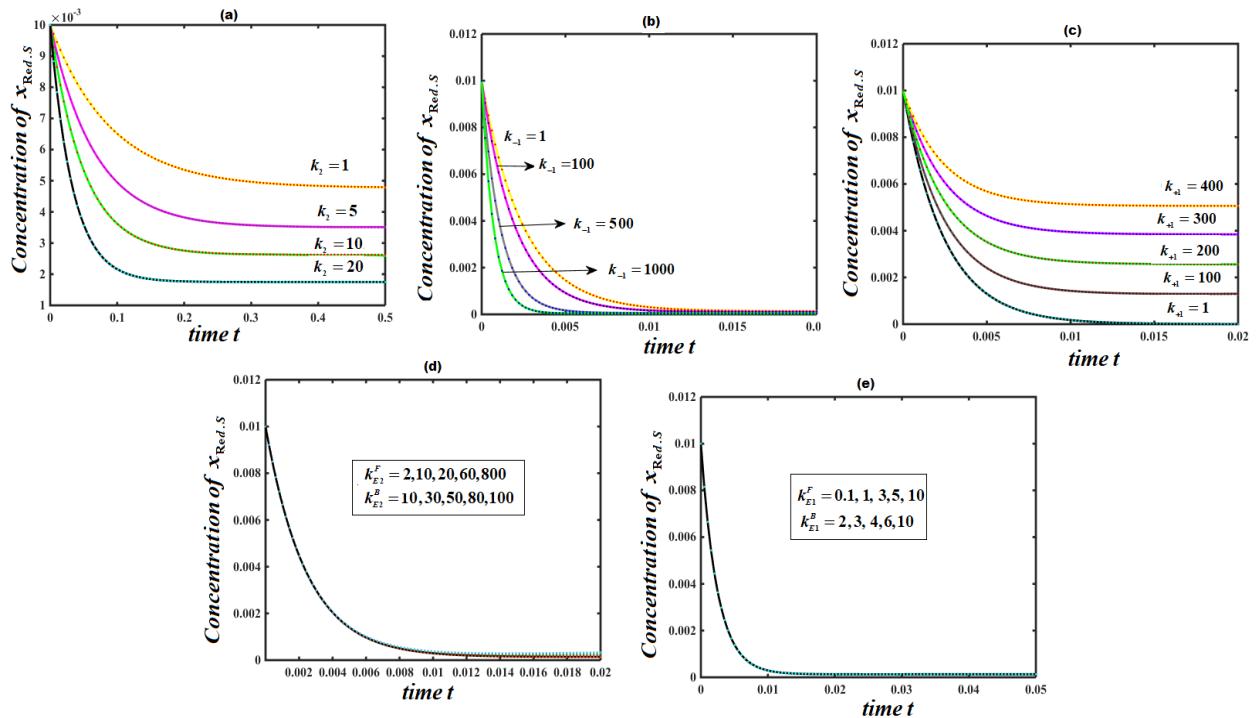
Figure 4. Comparison between analytical (Eq. (22)) and numerical results for the concentration enzymes $x_{\text{Red.S}}$ for various values of parameters.

The derived analytical solutions obtained by a new form of the homotopy perturbation method (NHPM) and Taylor series (Taylor) are compared to the numerical results in Figures (2)–(5) and Tables 1-3, where satisfactory agreements are noted. Using Taylor's, the average relative errors in the concentration of HR, Red and Red.S were 1.83%, 0.48%, 2.31%, respectively whereas the relative errors

using NHPM were 1.39% 0.34% 0.08%. These errors can be further reduced by using more terms of the Taylor series and the NHPM series.
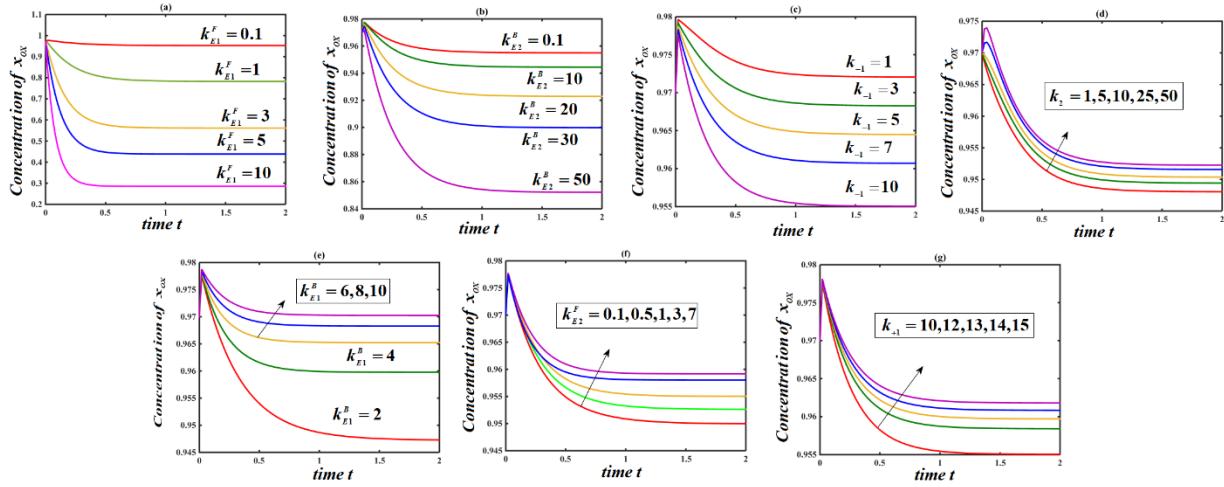
Figure 5. The analytical expression for the concentration of enzyme x_{OX} (Eq. (23)) for various values of parameters.

5. DISCUSSION
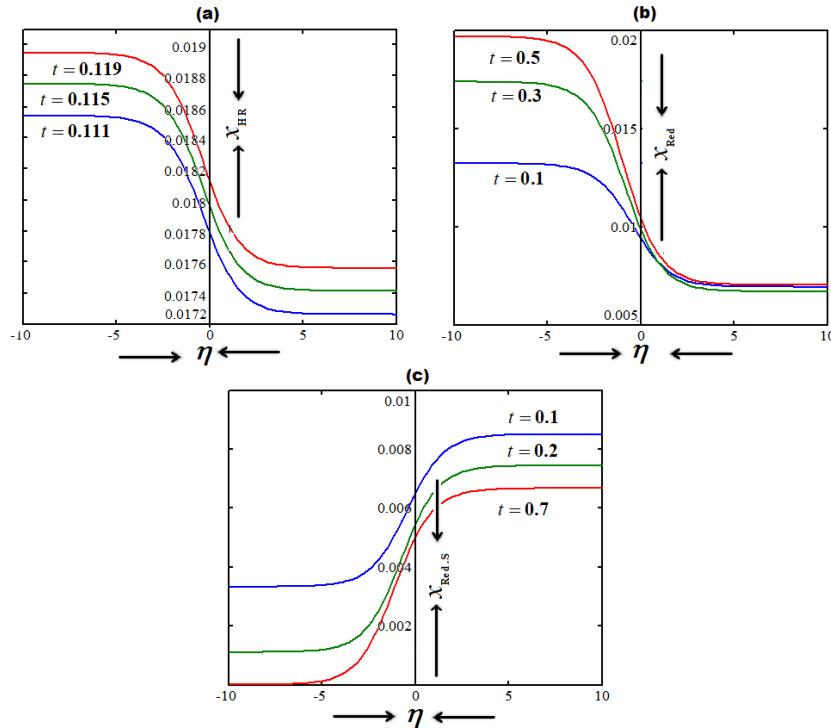
Figure 6. Plot of concentration x_{HR}, x_{Red} and $x_{Red.S}$ (Eqs. (20)–(22)) versus potential η for various values of time.
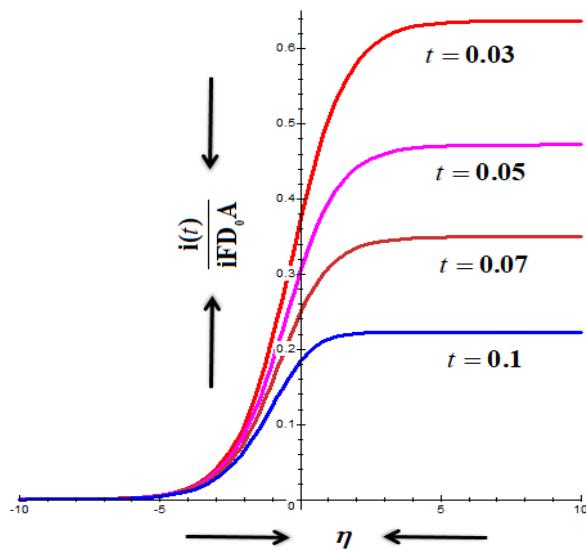
Figure 7. Plot of current (Eq. (25)) versus potential η for various values of the time.

Eqs. (21)–(23) and (27)–(29) represent the new derived analytical expressions of the concentrations of adsorbed enzymes x_{HR}, x_{Red} and $x_{Red.S}$, obtained by NHPM and Taylor, respectively. In addition, Eq. (25) represents the derived analytical expression for the steady-state current at rotating disc electrode. Figures 2 (a)–(g) represent the concentration of adsorbed enzyme x_{HR} for all values of rate constant. From these figures, it is observed that the value of concentration increases from its initial value to reach the steady-state value at $t = 2$. Figures 3 (a)–(e) show the concentration of the adsorbed enzyme x_{Red} for all values of other parameters. It is observed from these figure that the value of enzyme concentration increases from its initial concentration value and gets the steady-state when $t = 1$.

Similarly, Figures 4 (a)–(e) and 5 (a)–(e) show the concentrations of enzymes $x_{Red.S}$ and x_{OX}, respectively, for all rate constant values. It is inferred that concentrations of enzymes $x_{Red.S}$ and x_{OX} decrease from their initial concentration values until they reach their steady-state at $t < 1$ and $t > 1$, respectively. Figure 6 shows the behavior of the concentration of all enzymes versus the potential for different times, while Figure 7 shows the current versus the potential for the rotating disk electrode for different times.

6. CONCLUSIONS

Approximate analytical expressions for the concentration of absorbed enzymes in the rotating disc electrode are obtained using a new approach of the homotopy perturbation method and the Taylors series method. The effects of various parameters on the concentration of enzymes are also discussed and the time at which concentrations attain their steady-state position are determined. The derived analytical expressions are compared to numerical simulation obtained by the MATLAB function pdex4, where strong agreements are noted.

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Appendix A. Solution of Eqs. (16)–(18) using a new approach of the homotopy perturbation method

The constructed homotopies for Eqs. (16)–(18) are given by [12]

$$
(1-p)\left\{\frac{\mathrm{d}}{\mathrm{d}t}[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots] + k_0[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots] - k_1(x_{\mathrm{Red}})_i \right. \tag{A1}
$$

$$
\left. + k_{\mathrm{E1}}^{\mathrm{F}}(x_{\mathrm{Red.S}})_i - k_{\mathrm{E1}}^{\mathrm{F}}\right\}
$$

$$
+ p\left\{\frac{\mathrm{d}}{\mathrm{d}t}[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots] + k_0[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots]\right.
$$

$$
\left. - k_1[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots] + k_{\mathrm{E1}}^{\mathrm{F}}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] - k_{\mathrm{E1}}^{\mathrm{F}}\right\}
$$

$$
(1-p)\left\{\frac{\mathrm{d}}{\mathrm{d}t}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots] + k_{0\eta}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots] - k_{-1}(x_{\mathrm{Red.S}})_i \right. \tag{A1}
$$

$$
\left. - k_{\mathrm{E2}}^{\mathrm{F}}(x_{\mathrm{HR}})_i\right\}
$$

$$
+ p\left\{\frac{\mathrm{d}}{\mathrm{d}t}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots] + k_{0\eta}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots]\right.
$$

$$
\left. - k_{-1}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] - k_{\mathrm{E2}}^{\mathrm{F}}[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots]\right\}
$$

$$
(1-p)\left\{\frac{\mathrm{d}}{\mathrm{d}t}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] + k_{21}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] - k_{1\eta}(x_{\mathrm{Red}})_i\right\} \tag{A1}
$$

$$
+ p\left\{\frac{\mathrm{d}}{\mathrm{d}t}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] + k_{1\eta}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots]\right.
$$

$$
\left. + k_{21}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots]\right\}
$$

And the approximate solutions of the equations (A1)–(A3), expressed in series forms, are given by

$$
x_{\mathrm{HR}} = (x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots, \tag{A4}
$$

$$
x_{\mathrm{Red}} = (x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots, \tag{A5}
$$

$$
x_{\mathrm{Red.S}} = (x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots. \tag{A6}
$$

Substituting Eqs. (A1)–(A3) into Eqs. (A4)–(A6) gives

$$
(1-p)\left\{\frac{\mathrm{d}}{\mathrm{d}t}[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots] + k_0[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots] - k_1(x_{\mathrm{Red}})_i\right. \tag{A7}
$$

$$
\left. + k_{\mathrm{E1}}^{\mathrm{F}}(x_{\mathrm{Red.S}})_i - k_{\mathrm{E1}}^{\mathrm{F}}\right\}
$$

$$
+ p\left\{\frac{\mathrm{d}}{\mathrm{d}t}[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots] + k_0[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots]\right.
$$

$$
\left. - k_1[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots] + k_{\mathrm{E1}}^{\mathrm{F}}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] - k_{\mathrm{E1}}^{\mathrm{F}}\right\}
$$

$$(1-p)\left\{\frac{\mathrm{d}}{\mathrm{dt}}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots] + k_{0\eta}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots] - k_{-1}(x_{\mathrm{Red.S}})_i \right.$$

$$\left. - k_{E2}^{\mathrm{F}}(x_{\mathrm{HR}})_i \right\} \tag{A8}$$

$$+ p\left\{\frac{\mathrm{d}}{\mathrm{dt}}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots] + k_{0\eta}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots]\right.$$

$$\left. - k_{-1}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] - k_{E2}^{\mathrm{F}}[(x_{\mathrm{HR}})_0 + p(x_{\mathrm{HR}})_1 + \cdots] \right\}$$

$$(1-p)\left\{\frac{\mathrm{d}}{\mathrm{dt}}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] + k_{21}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] - k_{1\eta}(x_{\mathrm{Red}})_i \right\} \tag{A9}$$

$$+ p\left\{\frac{\mathrm{d}}{\mathrm{dt}}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] + k_{1\eta}[(x_{\mathrm{Red}})_0 + p(x_{\mathrm{Red}})_1 + \cdots]\right.$$

$$\left. + k_{21}[(x_{\mathrm{Red.S}})_0 + p(x_{\mathrm{Red.S}})_1 + \cdots] \right\}.$$

Comparing the coefficient of p, up to first power, on both sides of Eqs. (A7)–(A9) yields the system

$$p^0: \frac{\mathrm{d}(x_{\mathrm{HR}})_0}{\mathrm{dt}} + k_0(x_{\mathrm{HR}})_0 + k_3 = 0, \tag{A10}$$

$$p^0: \frac{\mathrm{d}(x_{\mathrm{Red}})_0}{\mathrm{dt}} + k_{0\eta}(x_{\mathrm{Red}})_0 + k_5 = 0, \tag{A11}$$

$$p^0: \frac{\mathrm{d}(x_{\mathrm{Red.S}})_0}{\mathrm{dt}} + k_{21}(x_{\mathrm{Red.S}})_0 - k_{1\eta}(x_{\mathrm{Red}})_i = 0 \tag{A12}$$

$$p^1: \frac{\mathrm{d}(x_{\mathrm{HR}})_1}{\mathrm{dt}} + k_0(x_{\mathrm{HR}})_1 - k_1(x_{\mathrm{Red}})_0 + k_{E1}^{\mathrm{F}}(x_{\mathrm{Red.S}})_0 + k_4 = 0 \tag{A13}$$

$$p^1: \frac{\mathrm{d}(x_{\mathrm{Red}})_1}{\mathrm{dt}} + k_{0\eta}(x_{\mathrm{Red}})_1 - k_{-1}(x_{\mathrm{Red.S}})_0 - k_{E2}^{\mathrm{F}}(x_{\mathrm{HR}})_0 + k_5 = 0 \tag{A14}$$

$$p^1: \frac{\mathrm{d}(x_{\mathrm{Red.S}})_1}{\mathrm{dt}} + k_{21}(x_{\mathrm{Red.S}})_1 - k_{1\eta}(x_{\mathrm{Red.S}})_0 + k_{1\eta}(x_{\mathrm{Red.S}})_i = 0 \tag{A15}$$

Subject to the following initial conditions:

$$x_{\mathrm{HR}}(t=0) = 0.01, x_{\mathrm{Red}}(t=0) = 0.01, x_{\mathrm{Red.S}}(t=0) = 0.01 \tag{A16}$$

$$x_{\mathrm{HR}}(t=1) = 0, x_{\mathrm{Red}}(t=1) = 0, x_{\mathrm{Red.S}}(t=1) = 0 \tag{A17}$$

the solution of system (A10)–(A17) is given by

$$(x_{\mathrm{HR}})_0(t) = -\frac{k_3}{k_0} + k_6 e_0^{-k_0 t} \tag{A18}$$

$$(x_{\mathrm{Red}})_0(t) = -\frac{k_5}{k_2} + k_7 e_0^{-k_2 t} \tag{A19}$$

$$(x_{\mathrm{Red.S}})_0(t) = \frac{k_{1\eta}(x_{\mathrm{Red.S}})_i}{k_{21}} + k_8 e_0^{-k_{21} t} \tag{A20}$$

$$(x_{\text{HR}})_1(t) = \frac{k_9}{k_0} + \frac{k_1 k_7 e^{-k_{0\eta}t}}{k_0 - k_{0\eta}} - \frac{k_{E1}^F k_8 e^{-k_{21}t}}{k_0 - k_{21}} - \left[\frac{k_9}{k_0} + \frac{k_1 k_7}{k_0 - k_{0\eta}} + \frac{k_{E1}^F k_8}{k_0 - k_{21}}\right] e^{-k_0 t} \tag{A21}$$

$$(x_{\text{Red}})_1(t) = \frac{k_{10}}{k_{0\eta}} + \frac{k_{E2}^F k_6 e^{-k_0 t}}{k_{0\eta} - k_0} + \frac{k_{-1} k_8 e^{-k_{21}t}}{k_{0\eta} - k_{21}} - \left[\frac{k_{10}}{k_{0\eta}} + \frac{k_{E2}^F k_6}{k_{0\eta} - k_0} + \frac{k_{-1} k_8}{k_{0\eta} - k_{21}}\right] e^{-k_{0\eta}t} \tag{A22}$$

$$(x_{\text{Red.S}})_1(t) = \frac{k_{11}}{k_{21}} + \frac{k_{1\eta}(x_{\text{Red.S}})_i e^{-k_{0\eta}t}}{k_{21} - k_{0\eta}} - \left[\frac{k_{11}}{k_{21}} + \frac{k_{1\eta}(x_{\text{Red.S}})_i}{k_{21} - k_{0\eta}}\right] e^{-k_{21}t} \tag{A23}$$

Setting $p = 1$ in Eqs. (A4)–(A6) gives the approximate homotopy analytical solutions

$$x_{\text{HR}}(t) = \frac{-k_3 + k_9}{k_0} + \frac{k_1 k_7 e^{-k_{0\eta}t}}{k_0 - k_{0\eta}} - \frac{k_{E1}^F k_8 e^{-k_{21}t}}{k_0 - k_{21}} \tag{A24}$$

$$- \left[\frac{k_9}{k_0} + \frac{k_1 k_7}{k_0 - k_{0\eta}} + \frac{k_{E1}^F k_8}{k_0 - k_{21}} - k_6\right] e^{-k_0 t}$$

$$x_{\text{Red}}(t) = \frac{-k_5 + k_{10}}{k_{0\eta}} + \frac{k_{E2}^F k_6 e^{-k_0 t}}{k_{0\eta} - k_0} + \frac{k_{-1} k_8 e^{-k_{21}t}}{k_{0\eta} - k_{21}} \tag{A25}$$

$$- \left[\frac{k_{10}}{k_{0\eta}} + \frac{k_{E2}^F k_6}{k_{0\eta} - k_0} + \frac{k_{-1} k_8}{k_{0\eta} - k_{21}} - k_7\right] e^{-k_{0\eta}t}$$

$$x_{\text{Red.S}}(t) = \frac{k_{1\eta}(x_{\text{Red.S}})_i + k_{11}}{k_{21}} + k_8 e_0^{-k_{21}t} + \frac{k_{1\eta}(x_{\text{Red.S}})_i e^{-k_{0\eta}t}}{k_{21} - k_{0\eta}} \tag{A26}$$

$$- \left[\frac{k_{11}}{k_{21}} + \frac{k_{1\eta}(x_{\text{Red.S}})_i}{k_{21} - k_{0\eta}}\right] e^{-k_{21}t}$$

where

$$k_0 = k_{E1}^F + k_{E1}^B + k_{E2}^F, \qquad k_1 = k_{E2}^B - k_{E1}^F, \qquad k_{0\eta} = \frac{k_{+1} e^\eta}{1 + e^\eta} - k_{E2}^B, \qquad k_{1\eta} = \frac{k_{+1} e^\eta}{1 + e^\eta} \tag{A27}$$

$$k_3 = -k_{E1}^F - k_1(x_{\text{Red}})_i + k_{E1}^F(x_{\text{Red.S}})_i, \qquad k_4 = k_1(x_{\text{Red}})_i + k_{E1}^F(x_{\text{Red.S}})_i$$

$$k_5 = -k_{E2}^F(x_{\text{HR}})_i - k_{-1}(x_{\text{Red.S}})_i, \qquad k_6 = (x_{\text{HR}})_i + \frac{k_3}{k_0}, \qquad k_7 = (x_{\text{Red}})_i + \frac{k_5}{k_{0\eta}}$$

$$k_8 = (x_{\text{Red.S}})_i + \frac{k_{1\eta}(x_{\text{Red.S}})_i}{k_{21}}, \qquad k_9 = -\frac{k_1 k_5}{k_{0\eta}} - \frac{k_{E1}^F k_{1\eta}(x_{\text{Red.S}})_i}{k_{21}} + k_4$$

$$k_{10} = -\frac{k_{E2}^F k_3}{k_0} + \frac{k_{-1} k_{1\eta}(x_{\text{Red.S}})_i}{k_{21}}, \qquad k_{11} = -\frac{k_{1\eta} k_5}{k_{0\eta}} - k_{1\eta}(x_{\text{Red}})_i, \qquad k_{21} = k_{-1} + k_2$$

Appendix B. Matlab program for numerical solution of Eqs. (17)–(20)

function main1

options= odeset('RelTol',1e-6,'Stats','on');

Xo = [0.01;0.01;0.01];

tspan = [0,0.8];

tic

[t,X] = ode45(@TestFunction,tspan,Xo,options);

toc

figure

holdon

%plot(t, X(:,1))

%plot(t, X(:,2))

plot(t, X(:,3))

return

function [dx_dt]= TestFunction(t,x)

k1=0.1;k2=1;k6=10;k7=10;k4=1;k3=3;k5=5;eta=1;

lm=(exp(eta)/(1+(exp(eta))));

a=k1+k3+k4;b=k5-k1;c=(k6*lm)+k5;f=(k6*lm);g=k7+k2;

dx_dt(1)=k1-(a*x(1))+(b*x(2))-(k1*x(3));

dx_dt(2)=(k4*x(1))-(c*x(2))+(k7*x(3));

dx_dt(3)=(f*x(2))-(g*x(3));

dx_dt = dx_dt';

return

Appendix C. Approximate analytical solution of Eqs. (17)–(20) using Taylor's series method

Taylor's series expansion of $y(t)$ about $t = 0$ is given by

$$y(t) = y(0) + ty'(t) + \frac{t^2}{2!}y''(0) + \frac{t^3}{3!}y'''(0) + \cdots \tag{C1}$$

For the experimental values $k_{E1}^F = 0.1, k_2 = 0.01, k_{E1}^B = 0.02, k_{E2}^F = 0.01, k_{E2}^B = 0.2, k_{+1} = 0.02, k_{-1} = 0.1, \eta = 0.01$, the nonlinear system (17)–(20) becomes

$$\frac{dx_{HR}(t)}{dt} = 0.1 - 0.13x_{HR}(t) + 0.1x_{Red}(t) - 0.1x_{Red.S}(t) \tag{C2}$$

$$\frac{dx_{Red}(t)}{dt} = 0.01x_{HR}(t) - 0.21005x_{Red}(t) + 0.1x_{Red.S}(t) \tag{C3}$$

$$\frac{dx_{Red.S}(t)}{dt} = 0.01005x_{Red}(t) - 0.11x_{Red.S}(t) \tag{C4}$$

Using the initial condition $x_{HR}(t = 0) = x_{Red}(t = 0) = 0$ and $x_{Red.S}(t = 0) = 0.001$, we obtaine

$$x'_{\text{HR}}(0) = 0.0999, x'_{\text{Red}}(0) = 0.0001, x'_{\text{Red.S}}(0) = -0.00011 \tag{C5}$$

Computation of higher differentiation of (C2)–(C4) at $t = 0$ gives

$$x''_{\text{HR}}(0) = -0.12966, x''_{\text{Red}}(0) = 0.000967, x''_{\text{Red.S}}(0) = 0.000022 \tag{C6}$$

$$x'''_{\text{HR}}(0) = -0.00178, x'''_{\text{Red}}(0) = -0.000331, x'''_{\text{Red.S}}(0) = 0.000095$$

$$x^{(4)}_{\text{HR}}(0) = 0.099726, x^{(4)}_{\text{Red}}(0) = 0.000967, x^{(4)}_{\text{Red.S}}(0) = -0.000436$$

$$x^{(5)}_{\text{HR}}(0) = 0.087050, x^{(5)}_{\text{Red}}(0) = 0.000973, x^{(5)}_{\text{Red.S}}(0) = 0.000145$$

$$x^{(6)}_{\text{HR}}(0) = 0.887793, x^{(6)}_{\text{Red}}(0) = 0.000668, x^{(6)}_{\text{Red.S}}(0) = 0.000961$$

$$x^{(7)}_{\text{HR}}(0) = 0.088516, x^{(7)}_{\text{Red}}(0) = 0.007572, x^{(7)}_{\text{Red.S}}(0) = 0.000057$$

$$x^{(8)}_{\text{HR}}(0) = 0.088563, x^{(8)}_{\text{Red}}(0) = 0.000732, x^{(8)}_{\text{Red.S}}(0) = 0.000070$$

Using Taylor series expansion (C1) for $x_{\text{HR}}, x_{\text{Red}},$ and $x_{\text{Red.S}}$, we obtain the explicit forms

$$x_{\text{HR}} = 10^{-4}(990t - 64.83t^2 - 2.966774t^3 + 415.525253t^4 + 72.541373t^5 \tag{C7}$$
$$+ 1.233305t^6 + 0.175627t^7 + 0.021965t^8 + \cdots)$$

$$x_{\text{Red}} = 10^{-4}(t + 4.8834975t^2 - 0.550937t^3 + 0.040296t^4 + 0.081049t^5 \tag{C8}$$
$$+ 0.009273t^6 + 0.001502t^7 + 0.000181t^8 + \cdots)$$

$$x_{\text{Red.S}} = 10^{-4}(10 - 1.1t + 0.11075t^2 + 0.157911t^3 - 0.018184t^4 + 0.001210t^5 \tag{C8}$$
$$+ 0.001335t^6 + 0.000112t^7 + 0.000017t^8 + \cdots)$$

Table 1. Comparison of analytical (NHPM and Taylor's) and numerical results for concentration enzyme x_{HR} with simulation result.

Time	Numerical	Taylor's (Eq. (27))	Taylor's Relative Error (%)	NHPM (Eq. 21)	NHPM Relative Error (%)
0.001	9.99935E-05	9.98935E-05	0.100	9.98953E-05	0.098
0.005	0.000499838	0.000499338	0.100	0.000499376	0.092
0.01	0.000999353	0.000998352	0.100	0.000998502	0.085
0.06	0.005977248	0.00597078	0.108	0.005976101	0.019
0.08	0.007959936	0.007950833	0.114	0.007960202	0.003
0.1	0.009937999	0.00992589	0.122	0.009940351	0.024
0.6	0.058306602	0.058271376	0.060	0.058184624	0.209
0.8	0.07737161	0.077898481	0.681	0.076821554	0.711
1	0.096489351	0.093713677	2.877	0.095092954	1.447
1.5	0.145446943	0.136264536	6.313	0.139222667	4.279
2	0.197834938	0.176241419	10.915	0.181231656	8.392
Average Error (%)			1.829		1.391

Table 2. Comparison of analytical (NHPM and Taylor's) and numerical results for concentration enzyme x_{Red} with simulation result.

Time	Numerical	Taylor's (Eq. (28))	Taylor's Relative Error (%)	NHPM (Eq. 22)	NHPM Relative Error (%)
0.001	1.00479E-07	1.00483E-07	0.009	1.00483E-07	0.009
0.005	5.11993E-07	5.12081E-07	0.017	5.12081E-07	0.017
0.01	1.04794E-06	1.04829E-06	0.029	1.04829E-06	0.029
0.06	7.71617E-06	7.72875E-06	0.163	7.72871E-06	0.163
0.08	1.1044E-05	1.10664E-05	0.199	1.10662E-05	0.199
0.1	1.47454E-05	1.47804E-05	0.237	1.47801E-05	0.237
0.6	0.000221386	0.000223359	0.889	0.000222589	0.542
0.8	0.000360499	0.000362881	0.662	0.000362603	0.583
1	0.000528528	0.000532433	0.739	0.000531758	0.611
1.5	0.001061825	0.001072328	0.989	0.001068747	0.652
2	0.00173391	0.001757714	1.372	0.00174554	0.670
Average Error (%)			0.482		0.338

Table 3. Comparison of analytical (NHPM and Taylor's) and numerical results for concentration enzyme $x_{\text{Red.S}}$ with simulation result.

Time	Numerical	Taylor's (Eq. (29))	Taylor's Relative Error (%)	NHPM (Eq. 23)	NHPM Relative Error (%)
0.001	0.001	0.001	0.000	0.001	0.000
0.005	0.000999	0.000999	0.000	0.000999	0.000
0.01	0.000999	0.000999	0.000	0.000999	0.000
0.05	0.000995	0.000995	0.000	0.000995	0.000
0.08	0.000991	0.000991	0.000	0.000991	0.000
0.1	0.000989	0.000989	0.000	0.000989	0.000
0.6	0.000937	0.000941	0.427	0.000937	0.000
0.8	0.000917	0.000927	1.090	0.000917	0.000
1	0.000897	0.000915	2.007	0.000898	0.111
1.5	0.000852	0.000907	6.455	0.000854	0.235
2	0.000811	0.000936	15.413	0.000815	0.493
Average Error (%)			2.308		0.076

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