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Analytical Expression of the Substrate Concentration in Different Part of Particles with Immobilized Enzyme and Substrate Inhibition Kinetics

Vincent Michael Raj Margret PonRani[1], Lakshmanan Rajendran[1,*], and Raju Eswaran[2]

[1]*Department of Mathematics,The Madura College, Madurai 625 011, Tamil Nadu, India*
[2]*Department of Zoology, the Madura College, Madurai 625 011, Tamil Nadu, India*

*Corresponding Author, Tel: +91-9442228951; Fax: +91-0452-2675238
E-Mail: raj_sms@rediffmail.com

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Abstract- A mathematical model of enzyme flow calorimetry for the monitoring of hysteresis behaviour of immobilized enzyme is developed. The model is based on diffusion equations containing a non linear term related to substrate inhibition kinetics of the enzymatic reaction. This paper presents an approximate analytical method (He’s Homotopy perturbation method) to solve the non-linear differential equations for spherical, cylindrical and planar particles. Closed and simple analytical expressions for substrate concentration have been derived for all possible values of parameters. These results are compared with numerical results and are found to be in good agreement. The obtained results are valid for the whole solution domain.

Keywords- Immobilized Enzyme, Spherical Particle, Cylindrical Particle, Planar Particle, Homotopy Perturbation Method and Numerical Simulation

1. INTRODUCTION

Immobilization of enzyme is limitation of enzyme mobility achieved by different approaches. Some examples are chemical or physical immobilization on some surface (particles, plates etc.), chemical or physical immobilization inside particle, immobilization on soluble polymers, cross-linking of enzymes by bi-functional reagents without any carrier and retardation of enzyme in membrane reaction .

There are two cases when enzyme activity is limited by diffusion of substrate to enzyme, such as reaction limitation by external diffusion and reaction limitation by internal diffusion. The second is discussed in this paper.

Enzyme reactions taking part in metabolic pathways, regulation processes, or in "in vitro" conditions often exhibit complicated dynamic behaviour in terms of the relation between the reaction rate and reaction. It can be studied by kinetic nonlinearities resulting from allosteric interactions, by autocatalytic mechanisms, or by combination of enzyme reaction with mass transfer conditions [1]. This can lead to one of typical effects, the so-called hysteresis, when a retardation of the response between reaction rate and concentration of substrate or other compounds is observed. When the system is repeatedly wiggled back and forth (the concentration is cycled up and down), a hysteresis loop can appear in the reaction rate concentration diagram.

It has been recommended that hysteresis effects in biological systems are adequate to account for short-term memory [2] and the existence of hysteresis was described simply by coupling the enzyme kinetics and diffusion transport. It was also shown that in some cases the changes in conformation of enzyme molecule may give rise to hysteresis [3]. Kernevz and co workers [2] were developed a mathematical model to study hysteresis loop when bulk substrate concentration was progressively increased and decreased and it was explained by combination of the substrate inhibitory effect with diffusion limitation in the membrane. The numerical solution for spherical particles surrounded by a substrate solution with a constant concentration was studied by Malik & Stefuca [4]. However, to the best of our knowledge, till date no general analytical expressions of substrate concentrations in different part of particles with immobilized enzyme for spherical, cylindrical and planar particles have been reported. The purpose of this communication is to derive approximate analytical expression of concentrations for spherical, cylindrical and planar particles by solving the non-linear differential equations using He's Homotopy perturbation method.

2. EXPERIMENTAL

2.1. Mathematical formulation of the problem and analysis

We consider the non-linear differential equation described by the concentration within the enzymatic layer at steady state conditions as follows [4]:

$$\nabla^2 c_S - \frac{V_m c_S}{K_m + c_S + \frac{c_S{}^2}{K_i}} = 0 \tag{1}$$

where c_s is the substrate concentration, K_m is the Michaelis constant, K_i is substrate inhibition constant, V_m is the maximum reaction rate. Here ∇^2 stands for Laplace operator. Eqn. (1) was combined with the boundary conditions

$$\frac{\partial c_S}{\partial r} = 0 \quad \text{when} \quad r = 0 \tag{2}$$

$$c_S = c_{S0} \quad \text{when} \quad r = R_p \tag{3}$$

where c_{S0} is the substrate concentration on the particle surface. We make the non-linear PDE (Eq.1) dimensionless by defining the following parameters:

$$c = \frac{c_S}{K_m},\ x = \frac{r}{R_p},\ \Phi = R_P \sqrt{\frac{V_m}{K_m D_e}},\ \beta = \frac{K_i}{K_m},\ m = \frac{c_{S0}}{K_m} \tag{4}$$

where c is the dimensionless substrate concentration, x is the dimensionless particle radial coordinate, Φ is the Thiele modulus , β is the dimensionless kinetic parameter and m is the dimensionless substrate concentration on the particle surface.

2.1.1. For spherical particle

For this case the Eq.(1) was reduces to the following dimensionless form

$$\left(\frac{\partial^2 c}{\partial x^2} + \frac{2}{x}\frac{\partial c}{\partial x}\right) - \Phi^2 \frac{c}{1 + c + \frac{c^2}{\beta}} = 0 \tag{5}$$

whereas the boundary condition reduces to

$$x = 0 \ , \ \frac{\partial c}{\partial x} = 0 \tag{6}$$

$$x = 1 \ , \quad c = m \tag{7}$$

2.1.2. For cylindrical particle

For the case of cylindrical particle, the Eq.(1) becomes in dimensionless form as follows:

$$\left(\frac{\partial^2 c}{\partial x^2}+\frac{1}{x}\frac{\partial c}{\partial x}\right)-\Phi^2\frac{c}{1+c+\dfrac{c^2}{\beta}}=0 \tag{8}$$

2.1.3. For planar particle

For the case of planar particle, the Eq.(1) becomes in dimensionless form as follows:

$$\frac{\partial^2 c}{\partial x^2}-\Phi^2\frac{c}{1+c+\dfrac{c^2}{\beta}}=0 \tag{9}$$

The boundary conditions for cylindrical and planar particle are same as Eq. (6) and Eq. (7).

3. ANALYTICAL SOLUTION OF SUBSTRATE CONCENTRATION USING HOMOTOPY PERTURBATION METHOD (HPM)

Recently, many authors have applied the Homotopy perturbation method (HPM) to various problems and demonstrated the efficiency of the HPM for handling non-linear structures and solving various physics and engineering problems [5-8]. This method is a combination of homotopy in topology and classic perturbation techniques. Ji-Huan He used the HPM to solve the Lighthill equation [9], the Duffing equation [10] and the Blasius equation [11]. The idea has been used to solve non-linear boundary value problems [12], integral equations [13-15], Klein–Gordon and Sine–Gordon equations [16], Emden-Flower type equations [17] (Chowdhury, & Hashim, 2007) and many other problems. In this paper, the homotopy perturbation method [18-24] is applied and the obtained results show that the HPM is very effective and simple.

3.1. For spherical particle

We can obtain the substrate concentration by solving the Eq. (5) using Homotopy perturbation method (see Appendix A).

$$c=m+\frac{\Phi^2\beta\, m}{3\left(\beta+\beta m+m^2\right)}\left(x^2-1\right) \tag{10}$$

Eq. (10) is the new and simple analytical expression for the dimensionless substrate concentration as a function dimensionless distance x, dimensionless kinetic parameter β , Thiele modulus Φ and dimensionless substrate concentration at particle surface m.

3.2. For cylindrical particle

We can obtain the dimensionless substrate concentration for the case of cylindrical particle by solving the Eq. (8):

$$c = m + \frac{\Phi^2 \beta\, m}{2(\beta + \beta m + m^2)}\left(x^2 - 1\right) \tag{11}$$

Eq. (11) is the analytical expressions for the dimensionless substrate concentrations as a function dimensionless distance x for all possible values of parameters. The dimensionless substrate concentration for the case of cylindrical particle differ from the spherical particle only by a constant term in the denominator. The equations (10) and (11) are approximate expression of substrate concentration for spherical and cylindrical particle provided $\Phi^2 \beta\, m < 3\ (\beta + \beta m + m^2)$ and $\Phi^2 \beta\, m < 2\ (\beta + \beta m + m^2)$

3.3. For planar particle

We can obtain the dimensionless substrate concentration for the case of planar particle by solving the Eq. (9):

$$\begin{aligned} c = {} & \frac{m \cosh \Phi x}{96\beta \cosh^4 \Phi}\left(16\beta \cosh \Phi \cosh 2\Phi - 48\beta \cosh \Phi + 3m \cosh 3\Phi + 36 m \Phi \sinh \Phi\right) \\ & - \frac{m^2 \Phi^2}{4\beta \cosh^3 \Phi}\left(\frac{\cosh 3\Phi x}{8\Phi^2} + \frac{3x \sinh \Phi x}{2\Phi}\right) - \frac{m\Phi^2}{2\cosh^2 \Phi}\left(\frac{\cosh 2\Phi x}{3\Phi^2} - \frac{1}{\Phi^2}\right) + \frac{m \cosh \Phi x}{\cosh \Phi} \end{aligned} \tag{12}$$

Eq. (12) is the analytical expressions for the dimensionless concentration as a function dimensionless distance x for all small values of parameters.

4. NUMERICAL SIMULATION

The diffusion equations (Eq. (5), (8) and (9)) for the boundary conditions (Eqs. (6)-(7)) are solved by numerical methods. The function pdex4 in Scilab software which is a function of solving the initial-boundary value problems for partial differential equation was used to solve these equations. The Scilab program is also given in APPENDIX B. The numerical results are also compared with our analytical results in Tables 1–2 for spherical and cylindrical electrode. In all the cases the average relative error between analytical and numerical result is less than 3%.

Table 1. Comparison of normalized substrate concentration c for various values of β when $m = 800$, $\Phi = 20$ (spherical particle)

X	$\beta = 20$			$\beta = 50$			$\beta = 100$		
	Eq.(10)	Numericl	% deviation of Eq. (10)	Eq. (10)	Numerical	% deviation	Eq. (10)	Numerical of Eq. (10)	% deviation of Eq. (10)
0	792.15	796.00	0.4860	758.18	792.40	0.9195	798.35	799.20	0.1064
0.2	792.47	796.10	0.4454	785.77	792.70	0.8819	798.41	799.20	0.0989
0.4	793.41	796.70	0.4146	787.55	793.70	0.7809	798.61	799.30	0.0864
0.6	794.98	797.50	0.3169	790.51	795.20	0.5932	798.94	799.50	0.0700
0.8	797.17	798.60	0.1793	797.30	797.30	0.3322	799.40	799.70	0.0375
1	800	800	0	800	800	0	800	800	0
Average deviation			0.3070	Average deviation		0.5846	Average deviation		0.0665

Table 2. Comparison of normalized substrate concentration c for various values of β when $m = 800$, $\Phi = 20$ **(**cylindrical particle**)**

X	$\beta = 20$			$\beta = 50$			$\beta = 100$		
	Eq. (11)	Numerical	% deviation of Eq. (11)	Eq. (11)	Numerical	% deviation of Eq. (11)	Eq. (11)	Numerical	% deviation of Eq. (10)
0	795.12	798.30	0.1106	788.23	796.00	0.9857	777.78	792.40	1.8797
0.2	795.31	798.40	0.0993	788.70	796.10	0.9832	778.66	792.70	1.8031
0.4	795.90	798.60	0.1005	790.11	796.70	0.8340	781.33	793.70	1.5832
0.6	796.87	799.00	0.0878	792.47	797.50	.6347	785.77	795.20	1.2001
0.8	798.24	799.40	0.0501	795.76	798.60	0.3568	792.00	797.30	0.6691
1	800	800	0	800	800	0	800	800	0
	Average deviation		0.0747	Average deviation		0.6249	Average deviation		1.1892

5. RESULT AND DISCUSSION

Eq.(10)-(12) are the new and simple approximate analytical expressions of concentrations of substrate $c(m,\phi^2,\beta)$ for spherical, cylindrical and planar particle respectively. The values of Thiele modulus Φ were in the range 20-400 with standard value 160 , kinetic parameter β were in the range 10-300 with standard value 128 and substrate concentration on the particle surface m were in the range 100-1000 (Malik & Stefuca 2002).

The concentration of substrate c depends upon the three parameters ϕ, β and m. The Thiele modulus Φ can be varied by changing either the thickness of the enzyme layer or the amount of enzyme immobilized in the matrix. This parameter describes the relative importance of diffusion and reaction in the enzyme layer. When Thiele modulus Φ is small, the kinetics is the dominant resistance and the overall uptake of substrate is kinetically controlled. Under these conditions, the substrate concentration profile is essentially uniform. The overall kinetics is governed by the total amount of active enzyme. When is Φ large, diffusion limitations are the principal determining factor .

Fig. 1(a) and 2(a) represent the dimensionless steady state substrate concentration for different values of dimensionless Thiele modulus Φ for spherical and cylindrical particle. From these figures, it is obvious that the substrate concentration reaches the maximum value m (Here $m=800$), when $x=1$. When Thiele modulus $\Phi < 20$, the substrate concentration profile is essentially uniform (refer Fig. 1(a) and 2 (a)).

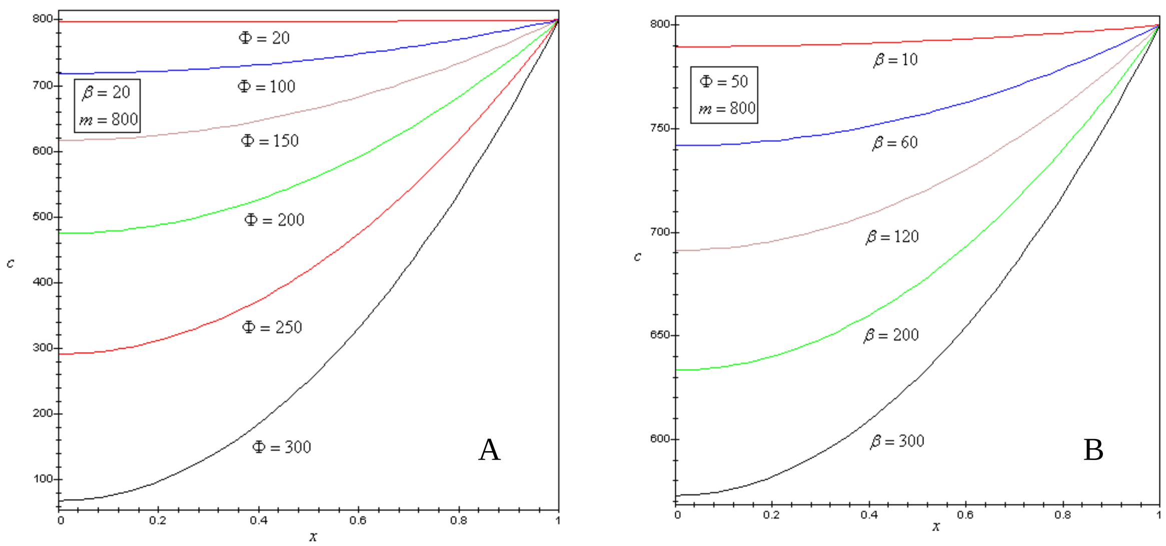

Fig. 1. Plot of normalized concentration profiles for substrate c versus the normalized distance x for the spherical particle. The concentrations are computed using Eq. (10) for (a) various values of Φ and some fixed values of m and Φ ($m=800, \beta=20$)
b) various values of β and some fixed value of Φ and β ($\Phi=50$, $m=800$).

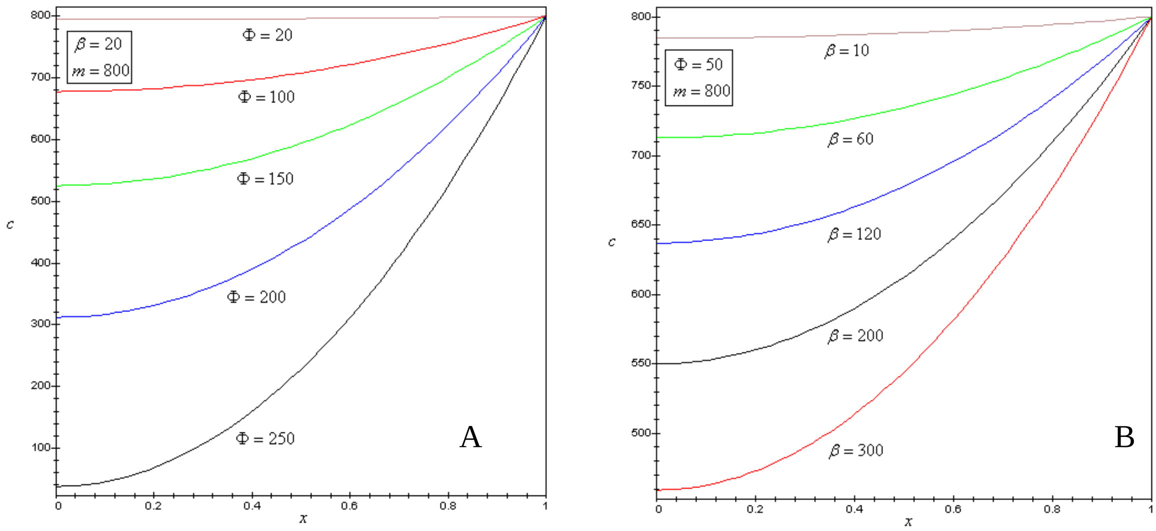

Fig. 2. Plot of normalized concentration profiles for substrate c *vs*. the normalized distance x for the cylindrical particle. The concentrations are computed using Eq. (11) for
(a) various values of Φ and some fixed values of m and Φ ($m = 800$, $\beta = 20$)
(b) various values of β and some fixed value of Φ and β ($\Phi = 50$, $m = 800$)

Fig. 1(b) and 2(b) represent the spherical and cylindrical particle substrate concentration c for different values of dimensionless kinetic parameter β. From these figures it is inferred that the concentration of the substrate c increases when kinetic parameter $\beta = K_i / K_m$ decreases. The normalized three-dimensional substrate concentration profiles for spherical, cylindrical and planar particle are plotted in Fig. 3 where the data given by previous figures are verified. The dimensionless substrate concentrations for the spherical, cylindrical and planar particle are plotted in Fig. 4. From these Figures it is inferred that the dimensionless substrate concentration for spherical particle is greater than planar and cylindrical particle**.**

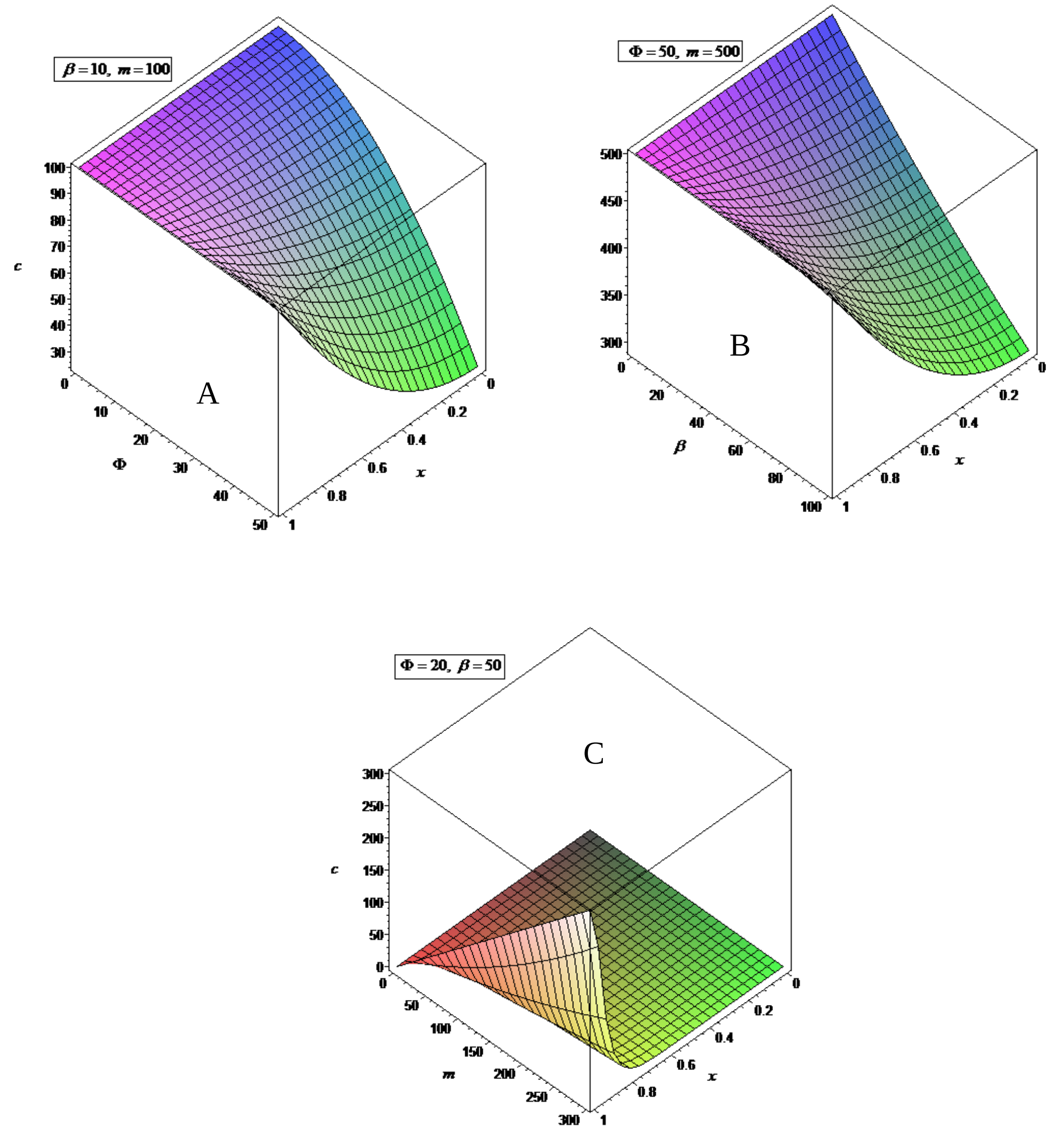

Fig. 3. The three-dimensional normalized substrate concentrations *c* for (a) spherical particle (Eq. (10)) when $0 \le \Phi \le 50$, $\beta = 10$ (b)cylindrical particle(Eq.(11)) when $0 \le \beta \le 100$, $\Phi = 50$ (c) planar particle (Eq. (12)) when $0 \le m \le 300$, $\beta = 50$

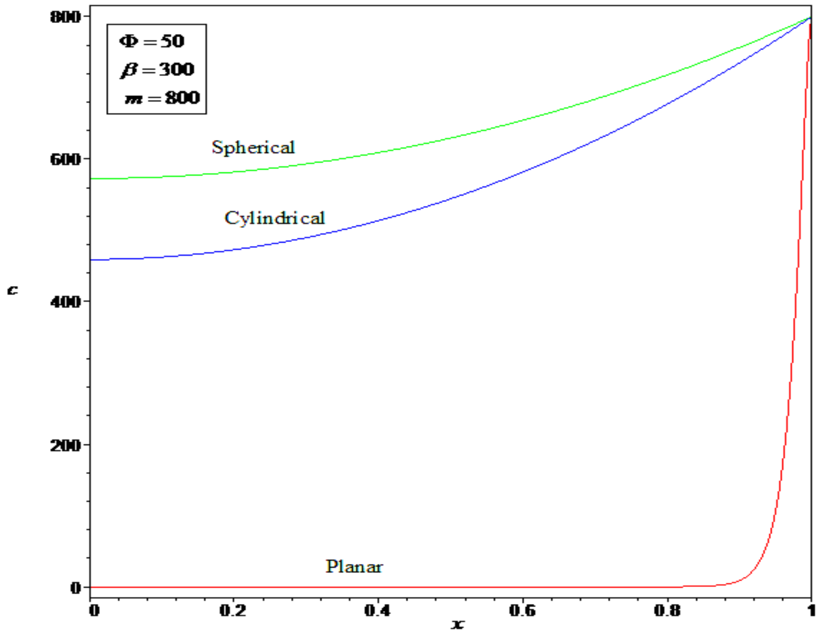

Fig. 4. Plot of the two-dimensional case diagram of the normalized substrate concentration c versus the normalized distance x in the case of spherical, cylindrical, and planar particles. The normalized substrate concentrations were computed using Eq. (10), (11) and (12) when the parameters $\Phi = 50$, $m = 800$ and $\beta = 300$

APPENDIX A

Solution of the Eq. (5) using Homotopy perturbation method

In this Appendix, we indicate how Eq. (5) in this paper is derived. To find the solution of Eq. (10) ,we first construct a Homotopy as follows:

$$(1-p)\left[\frac{\partial^2 c}{\partial x^2}+\frac{2}{x}\frac{\partial c}{\partial x}\right]+p\left[\frac{\partial^2 c}{\partial x^2}+\frac{2}{x}\frac{\partial c}{\partial x}-\frac{\Phi^2 \beta\, c}{\left(\beta+\beta c+\beta c^2\right)}\right]=0 \qquad \text{(A1)}$$

The boundary conditions are

$$x=0 \quad \frac{\partial c}{\partial x}=0 \qquad \text{(A2)}$$

$$x=1 \quad c=m \qquad \text{(A3)}$$

The approximate solutions of (A1) is

$$c=c_0+pc_1+p^2c_2+p^3c_3+....... \qquad \text{(A4)}$$

substituting Eq. (A4) into Eq.(A1) and comparing the coefficients of like powers of p

$$p^0: \ \frac{\partial^2 c_0}{\partial x^2}+\frac{2}{x}\frac{\partial c_0}{\partial x}=0 \qquad \text{(A5)}$$

The boundary conditions are

$$\frac{\partial c}{\partial x} = 0 \text{ when } x = 0 \tag{A6}$$

$$c = m \quad \text{when } x = 1 \tag{A7}$$

$$p^1 : \frac{\partial^2 c_1}{\partial x^2} + \frac{2}{x}\frac{\partial c_1}{\partial x} - \frac{\Phi^2 \beta c_0}{\left(\beta + \beta c_0 + \beta c_0^{\,2}\right)} = 0 \tag{A8}$$

$$\frac{\partial c}{\partial x} = 0 \text{ when } x = 0 \tag{A9}$$

$$c = 0 \text{ when } x = 1 \tag{A10}$$

According to the HPM, we can conclude that

$$c = c_0 + c_1 + ... \tag{A11}$$

Solving the Eq. (A5), and using the boundary conditions Eqs. (A6) - (A7) we get

$$c_0 = m \tag{A12}$$

Solving the Eq. (A8), and using the boundary conditions Eqs. (A9) - (A10) we get

$$c_1 = \frac{\Phi^2 \beta m}{6\left(\beta + \beta m + m^2\right)}\left(x^2 - 1\right) \tag{A13}$$

Adding Eq. (A12) and (A13), we get final results and it can be described in Eq. (10) in the text.

APPENDIX B

Scilab program to find the analytical solutions for Eq. (5):

```
function pdex4
m = 2;
x = linspace(0,1);
t=linspace(0,100000);
sol = pdepe(m,@pdex4pde,@pdex4ic,@pdex4bc,x,t);
u1 = sol(:,:,1);
figure
plot(x,u1(end,:))
title('u1(x,t)')
xlabel('Distance x')
ylabel('u1(x,2)')
%-----------------------------------------------------------------

function [c,f,s] = pdex4pde(x,t,u,DuDx)
c = 1;
f = DuDx;
q=0.1;
```

```
B=10;
F=-q^2*B*u(1)/(B+B*u(1)+u(1)^2);
s=F;
% ------------------------------------------------------------
function u0 = pdex4ic(x);                                %create a initial conditions
u0 = 1;
% ------------------------------------------------------------
function [pl,ql,pr,qr]=pdex4bc(xl,ul,xr,ur,t)            %create a boundary conditions
pl = 0;
ql = 1;
pr = ur-1;
qr = 0;
```

APPENDIX C

Nomenclature

Symbols

A_P	Particle surface area (m^2)
c	Dimensionless substrate concentration
c_S	Substrate concentration $(mole\ m^{-3})$
c_{S0}	Substrate concentration at particle surface $(mole\ m^{-3})$
D_e	Particle effective diffusion coefficient $(m^2\ s^{-1})$
K_m	Michaelis constant $(mole\ m^{-3})$
K_i	Substrate inhibition constant $(mole\ m^{-3})$
r	Particle radial coordinate (m)
R_P	Particle diameter (m)
v_{obs}	Observed reaction rate $(mole\ m^{-3}\ s^{-1})$
v_p	Particle volume $(mole\ m^{-3}\ s^{-1})$
V_m	Maximum reaction rate $(mole\ m^{-3}\ s^{-1})$
V_p	Particle volume (m^3)
x	Dimensionless particle radial coordinate
i	Dimensionless current
m	Dimensionless substrate concentration at particle surface.

Greek symbols

β	Dimensionless kinetic parameter
Φ	Thiele modulus

6. CONCLUSION

A mathematical model for an immobilized enzyme electrode has been described. In this present paper the particle balance equation have been formulated and solved under steady state conditions subject to defined boundary conditions. In this work, we obtained an analytical expression of the normalized substrate concentration for all possible values of dimensionless kinetic parameters β and the Thiele modulus Φ for spherical, cylindrical, and planar particle. The simple closed forms of analytical solutions have been proposed using Homotopy perturbation method. Furthermore, it gives good agreement with simulation results.

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REFERENCES

[1] J. P. Kernevez, Enzyme Mathematics; North-Holland Publishing Company (1980).

[2] J. P. Kernevez, G. Joly, M. C. Duban, B. Bunow, and D. Thomas, J. Math. Biol. 7 (1979) 41.

[3] E. E. Selkov, and S. N. Dynnik, Biofizika 21(1976) 214.

[4] F. Malik, and V. Stefuca, Chem. Pap. 56 (2002) 406.

[5] Q. K. Ghori, M. Ahmed, and A. M. Siddiqui, Int. J. Nonlinear. Sci. Numer. Simulat. 8 (2007) 179.

[6] T. Ozis, and A. Yildirim, Int. J. Nonlinear. Sci. Numer. Simulat. 8 (2007) 243.

[7] S. J. Li, and Y. X. Liu, Int. J. Nonlinear. Sci. Numer. Simulat. 7 (2006) 177.

[8] M. M. Mousa, and S. F. Ragab, Z. Naturforsch 63 (2008) 140.

[9] J. H. He, Comp Meth. Appl. Mech. Eng. 178 (1999) 257.

[10] J. H. He, Appl. Math. Comput. 135 (2003) 73.

[11] J. H. He, Appl. Math. Comput. 140 (2003) 217.

[12] J. H. He, Phys. Lett. A. 350 (2006) 87.

[13] M. Ghasemi, M. Tavassoli Kajani, and E. Babolian, Appl. Math. Comput. 188 (2007) 446.

[14] A. Golbabai, and B. Keramati, Chaos solitons fractals 37 (2008) 1528.

[15] J. Biazar, and H. Ghazvini, Chaos Soliton. Fract. 39 (2009) 770.

[16] Z. Odibat, and S. Momani, Phys. Lett. A. 365 (2007) 351.
[17] M. S. H. Chowdhury, and I. Hashim, Phys. Lett. A. 368 (2007) 305.
[18] X. C. Cai, W. Y. Wu, and M. S. Li, Int. J. Nonlinear. Sci. Numer. Simulat. 7 (2006) 109.
[19] J. H. He, Int. J. Mod. Phys. B. 20 (2006) 1141.
[20] P. Donald Ariel, Nonlinear Sci. Lett. A. 1 (2010) 43.
[21] S. Loghambal, and L. Rajendran, Electrochim. Acta 55 (2010) 5230.
[22] A. Meena, and L. Rajendran, J. Electroanal. Chem. 644 (2010) 50.
[23] A. Eswari, and L. Rajendran, J. Electroanal. Chem. 648 (2010) 36.
[24] V. Margret and L. Rajendran, Nat. Sci. 2 (2010) 1318.