Solution of non-steady-state substrate concentration in the action of biosensor response at mixed enzyme kinetics

R Senthamarai* and R Jana Ranjani
Department of Mathematics, SRM Institute of Science and Technology, Kattankulathur-603 203, Tamil Nadu, India
Email: rsenthamarai@rediffmail.com

Abstract: In this paper, a mathematical model of an amperometric biosensor at mixed enzyme kinetics and diffusion limitation in the case of substrate inhibition has been developed. The model is based on time dependent reaction diffusion equation containing a non-linear term related to non-Michaelis-Menten kinetics of the enzymatic reaction. Solution for the concentration of the substrate has been derived for all values of parameters using the homotopy perturbation method. All the approximate analytic expressions of substrate concentration are compared with simulation results using Scilab/Matlab program. Finally, we have given a satisfactory agreement between them.

1. Introduction

The biosensors response is determined by an enzyme activity and mass transport [1]. Catalytic biosensors are sensors that use enzymes which catalyse a specific conversion of analyte [2]. Amperometric biosensors measure the faradic response that arises on a working indicator electrode due to direct electrochemical oxidation or reduction of products of biocatalytic reaction. In amperometric biosensors, the potential at the electrode is held constant while the response is measured as a current. The response of amperometric biosensors is determined by enzyme activity and mass transport [3]. Proportional to the concentration of the measured analyte, the biosensors produce a signal. These devices have been widely used in environmental, medical applications because of their simplicity and low cost [4]. In the literature, many mathematical models have been developed and they are used as an important tool to study and optimize the analytical characteristic of actual biosensors [5]. We consider a system where a membrane biosensor is used for the analysis of a continuously flowing analyte over the membrane surface.

2. Mathematical formulation

The modelling of biosensors utilizing simple Michaelis-Menten kinetics in different regimes was carried out using digital integration [6, 7]. Very often the kinetics of an enzyme action is much more complicated. Inhibition, activation, allostery and other types of non Michaelis-Menten kinetics determinate the diversity of the enzymes, and finally a life [8]. The simplest scheme of non Michaelis-Menten kinetics, for example, may be produced by addition into Michaelis-Menten scheme (equation (1)) a stadium of the interaction of the enzyme substrate complex (ES) with other substrate molecule (S) (equation (2)) following the generation of non active complex (ES$_2$):

\[
E + S \leftrightarrow ES \rightarrow E + P
\]  

(1)
ES + S ↔ ES$_2$  \hspace{1cm} (2)

The enzyme kinetics in biochemical systems has usually been modelled by ordinary differential equations which are based only on reaction without spatial dependence of the various concentrations. Recent attention has been given to the effect of diffusion in the process of interactions [9, 10]. When considering this effect, the various concentrations in the reaction process are spatially dependent and the equations governing these concentrations become partial differential equations of parabolic type [11].

The rate of change of substrate concentration $S = S(X, t)$ at time $t$, position $X \in \Omega$ is equal to the sum of the rate due to reaction and diffusion, and is given by Pao[9].

$$\frac{\partial S}{\partial t} = D_s \nabla \cdot (\nabla S) - v(X, t)$$

where $D_s$ is the substrate diffusion coefficient, $\nabla S$ is the gradient operation and $v$ is the so-called “initial reaction velocity”. Various models regarding the expression for $v(X, t)$ are formulated by researchers in this field [9, 12]. Based on the non-Michaelis–Menten hypothesis, the velocity function $v$ for the simple reaction process without competitive inhibition is given by Pao [8] and Baronas et al. [9].

$$v = \frac{K_i \left[ E_o \right] \left[ S \right]}{K_M + [S] + [S]^2 / K_i} = \frac{V_{max} \left[ S \right]}{K_M + [S] + [S]^2 / K_i}$$

in which the constants $V_{max} = K_i \left[ E_o \right], K_M$ and $K_i$ are Michaelis–Menten and inhibition constant respectively. In Eq. (4), the rate passes through a maximum as the concentration increases, and there is said to be inhibition by substrate, and the constant $K_i$ which has the dimensions of a concentration, is called the substrate inhibition constant. In this model, the equation for $S$ becomes

$$\frac{\partial S}{\partial t} = D_s \frac{\partial^2 S}{\partial X^2} - \frac{K_i E_o S}{K_M + S + S^2 / K_i}$$

Introducing a pseudo-first-order rate constant $K = K_i E_o / K_M$ we can write the above equation as:

$$\frac{\partial S}{\partial t} = D_s \frac{\partial^2 S}{\partial X^2} - \frac{K S}{1 + S / K_M + S / K_i K_M}$$

The equation must be solved subject to the following initial and boundary conditions:

$t = 0, S = 0$

$X = 0, \frac{\partial S}{\partial X} = 0$

$X = 1, S = 1$

The system governs the substrate concentration $S$ when there is no competitive inhibition in the reaction. The non-linear PDE (Eq. (6)) is made dimensionless by defining the following parameters:

$$u = \frac{S}{Ks^\alpha}, x = \frac{X}{L}, \tau = \frac{Dt}{L^2}, k = \frac{KL^2}{D_s}, \phi = \frac{Ks^\alpha}{K_M}, \alpha = \frac{Ks^\alpha}{K_M}, \beta = \frac{Ks^\alpha}{K_M}$$

where $u, x$ and $\tau$ represent dimensionless concentration, distance and time respectively. Here $\alpha$ denotes a saturation parameter and $k$ denotes reaction diffusion parameter. Now Eq. (6) reduces to the following dimensionless form:

$$\frac{\partial u}{\partial \tau} = \frac{\partial^2 u}{\partial x^2} - \frac{ku}{1 + \alpha u + \beta u^2}, 0 < u \leq 1$$

with initial and boundary conditions

$\tau = 0, u = 0$

$x = 0, \frac{\partial u}{\partial x} = 0$

$x = 1, u = 1$
To the best of the authors knowledge, no general analytical expression of substrate concentration for all values of parameters [13] results have been published for the above non-linear reaction–diffusion equation. The work describes the results of biosensors modeling at mixed enzyme kinetics and external diffusion limitation.

3. Analytical expression for the substrate concentration using homotopy perturbation method

By using Homotopy perturbation method (APPENDIX A), we can obtain the concentration of substrate is as follows:

$$u_o = \frac{\cosh(\sqrt{A}x)}{\cosh \sqrt{A}} - \sum_{m=0}^{\infty} (-1)^m \pi (2m+1)e^{-\gamma} \cos((2m+1)\pi x/2)$$

where $$l = \frac{\pi^2(2m+1)^2 + 4A}{4}$$, $$A = \frac{k}{1 + \alpha + \beta}$$

$$\alpha = 0.001 \quad \beta = 0.001$$

(a) (b)

Figure1: Plot of dimensionless non steady concentration profiles of the substrate $$u$$ versus dimensionless distance $$x$$ for various values of the parameters $$k$$ and $$\alpha$$ and $$\beta$$, when $$\tau = 100$$. Solid lines represent the analytical solution in (12) and the dotted lines represent the numerical simulation for (9).
4. Numerical simulation

The diffusion equation (9) for the corresponding boundary conditions (10) is solved by numerical method. The function pde4 in Matlab software, which is a function of solving the initial boundary value problems for partial differential equations, is used to solve this equation numerically (Appendix B). The numerical solutions are compared with our analytical results (MATLAB codes given in Appendix C) as shown in Figures 1 and this comparison gives satisfactory agreement for some possible values of the reaction diffusion parameters.

5. Discussion and results

Equation (12) is the new analytical expression of substrate concentration for all values of parameters $k$ and $\alpha$ and $\beta$. Figure 1 shows the time dependent evolution of normalised concentration profiles for the substrate $u$. Figure 1(a), (b) show dimensionless concentration $u$ versus the dimensionless distance $x$. The reaction diffusion parameter $k$ is an indicator of the competition between the reaction and diffusion. When $k$ is small, the kinetics dominates and the uptake of the substrate is kinetically controlled. From Figure1 (a), it is evident that the value of the substrate concentration $u$ decreases when the reaction diffusion parameter $k$ increases. Figure1(b) illustrates that, when $k$ increases, the concentration of the substrate $u$ decreases even though the values of $\alpha$ and $\beta$ are increased. Figure 2(a), (b) represent the dimensionless concentration of substrate with dimensionless distance $x$ and dimensionless time $\tau$ with two different sets of values of $k$ and $\alpha$ and $\beta$. From these Figures, it is inferred that the concentration increases when the distance increases and it remains same for all values of $\tau$. 
6. Conclusion
A non-linear non-steady state partial differential equation in the action of biosensor response at mixed enzyme kinetics has been solved analytically and numerically. Moreover, we have obtained analytical expression for the substrate concentration using homotopy perturbation method. The primary result of the work is the first accurate calculation of substrate concentration. These analytical results will be helpful in determining the kinetic characteristics of the biosensor and provide a better understanding of the non-steady state. It gives good agreement with simulation results.

7. Appendices

appendix A
In this Appendix, we have shown how eqn. (11) is derived. Using Homotopy perturbation method [14, 15] can be written as

\[
\frac{\partial u}{\partial \tau} = \frac{\partial^2 u}{\partial x^2} + \frac{ku}{1+\alpha u[x=1] + \beta u^2[x=1]} , \quad 0 < u \leq 1
\]

(A.1)
The equation must be solved subject to the initial and boundary conditions given in (10). Now by constructing the homotopy, we get,

\[
(1 - p) \left[ \frac{\partial^2 u}{\partial x^2} - \frac{ku}{1+\alpha + \beta} \cdot \frac{\partial u}{\partial \tau} \right] + p \left[ \frac{\partial^2 u}{\partial x^2} - \frac{ku}{1+\alpha u + \beta u^2} - \frac{\partial u}{\partial \tau} \right] = 0
\]

(A.2)
Supposing the approximate solution of the eqn. (A.1) has the form

\[
u = u_0 + pu_1 + p^2u_2 + ...
\]

(A.3)
Substituting eqn. (A.3) into eqn. (A.2), and equate the terms with the identical powers of p, we obtain

\[
p^0 = \frac{\partial^2 u_0}{\partial x^2} - \frac{ku_0}{1+\alpha + \beta} \frac{\partial u_0}{\partial \tau} = 0
\]

(A.4)
\[
p_1 = \frac{\partial^2 u_1}{\partial x^2} - \frac{ku_1}{1+\alpha + \beta} \frac{\partial u_1}{\partial \tau} + \frac{ku_0}{1+\alpha u_0} - \frac{ku_0}{1+\alpha u_0 + \beta u_0^2} = 0
\]

(A.5)
Solving (A.4) by using Laplace transform technique, we obtain

\[
u_0 = \frac{\cosh(\sqrt{s + A})x}{s \cosh \sqrt{s + A}}
\]

(A.6)
Then by using inverse Laplace transform technique and complex inversion formula, we obtain the concentration of substrate eqn. (11) given in the text.

appendix B
The Matlab program for finding the numerical solution for (9) is as follows:

```matlab
function pdex4
m = 0;
x = linspace(0,1);
t = linspace(0,1);
sol = pdepe(m,@pdex4pde,@pdex4ic,@pdex4bc,x,t);
u1 = sol(:,:,1);
u2 = sol(:,:,2);
figure
```
```matlab
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;1];
f=[1;1].*DuDx;
K=10;
a=0.001;
b=0.001;
F1=-(r*A^2*u(1))/(1+(a*u(1))+(b*(u(1)^2)));
s=[F1;F1];

function u0=pdex4ic(x)
u0=[0;0];

function [pl,ql,pr,qr]=pdex4bc(xl,ul,xr,ur,t)
pl=[0;0];
ql=[1;1];
pr=[ur(1)-1;ur(2)];
qr=[0;0];

appendix C
Matlab program for finding the summation of the series in (12) is as follows:
k = 10;
a = 0.001;
b = 0.001;
A = k / (1+a+b);
x = linspace (0,1, 100);
t = 100;
s0 = 0;
```

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;1];
f=[1;1].*DuDx;
K=10;
a=0.001;
b=0.001;
F1=-(r*A^2*u(1))/(1+(a*u(1))+(b*(u(1)^2)));
s=[F1;F1];

function u0=pdex4ic(x)
u0=[0;0];

function [pl,ql,pr,qr]=pdex4bc(xl,ul,xr,ur,t)
pl=[0;0];
ql=[1;1];
pr=[ur(1)-1;ur(2)];
qr=[0;0];

appendix C
Matlab program for finding the summation of the series in (12) is as follows:
k = 10;
a = 0.001;
b = 0.001;
A = k / (1+a+b);
x = linspace (0,1, 100);
t = 100;
s0 = 0;
N = 100;
for n = 0 : 1:N+1;
    l = ((pi ^ 2 *(((2 * n) + 1) ^ 2) + (4 * A))) / 4;
    s = s0 + (((-1) ^ n) * pi * ((2 * n) + 1) * exp (-1 * t) * cos(((2 * n) + 1) * pi * x ) / 2 ) / l;
end
u = (cosh (( sqrt ( A )). *x ) . / (cosh ( sqrt ( A ))) - s;
plot (x, u, 'r')

References
[1] Scheller F, schubert F, 1988, Biosensors, vol. 7, Elseiver, Amsterdam.
[2] Kulys J, 1981, Analytical systems based on immobilized enzymes, Mokslas, Vilnius, .
[3] Kulys J, 1981, The development of new analytical systems based on biocatalysts. Anal. Lett. . 14, 377-397.
[4] Wollenberger U, Lisdat F, Scheller F W, 1997, Enzymatic Substrate Recycling Electrodes. Frontiers in Biosensors. B and II, Practical Applications, BirkhauserVerlag, Basel, 45–70.
[5] Schulmeister T, 1990, Mathematical modeling of the dynamic behavior of ampero-metric enzyme electrodes, Selective Electrode Rev. 12, 203–260.
[6] Baronas R, Ivanauskas F, Kulys J, 1998, Modelling of a microreactor on heterogeneous surface and an influence of geometry to microreactor operation, Nonlinear Analysis: Modelling and Control, 3, 19–30.
[7] Baronas R, Kulys J, Ivanauskas F, 2006, Computational Modelling of Biosensors with Perforated and Selective Membranes, Journal of Mathematical Chemistry, 39(2), 345–362,
[8] Gufreund H, 1995, Kinetics for the life sciences, Cambridge University Press.
[9] Pao C V, 1979, Mathematical analysis of enzyme-substrate reaction diffusion in some biochemical systems, Nonlinear Anal. Theor., 4 (2), 369–392.
[10] Baronas R, Ivanauskas F, Kulys J, Sapagovas M, 2003, Modeling of amperometric biosensors with rough surface of the enzyme membrane, J. Math. Chem., 34, 227–242.
[11] Kulys J, Baronas R, 2006, Modelling of amperometric biosensors in the case of substrate inhibition, Sensors . 6, 1513–1522.
[12] Senthamaraiv R, Rajendran L, 2010, System of coupled non-linear reaction diffusion processes at conducting polymer ultramicroelectrodes, Electrochimica Acta, Vol. 55, .3223-3235.
[13] Manimozhi P, Subbiah A, Rajendran L, 2010, Solution of steady-state substrate concentration in the action of biosensor response at mixed enzyme kinetics, Sensors and Actuators ,B 147 ,290-297.
[14] He J H, 2005, Application of homotopy perturbation method to nonlinear wave equations, Chaos Solitons Fractals, 26 ,695–700.
[15] Hamed A, 2012, Homotopy perturbation method for solving systems of non linear coupled equations, Appl. Math. Sci., 6, 4787–4800.