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MATHEMATICAL MODELLING OF ENZYME KINETICS REACTION MECHANISMS AND ANALYTICAL SOLUTIONS OF NON-LINEAR REACTION EQUATIONS USING VARIATIONAL ITERATION METHOD

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ABSTRACT

T*he boundary value problem in basic enzyme reactions is discussed and approximate expressions for substrates, enzyme, substrate-enzyme and product concentrations are presented. He's variational iteration method is used to give approximate and analytical solutions of non-liner reaction equations containing a non-linear term related to enzymatic reaction. The relevant analytical solutions for the substrate, enzyme, substrate-enzyme and product concentration profiles are discussed in terms of dimensionless reaction diffusion parameters. Numerical solutions are also presented using Matlab software. Our analytical results are compared with numerical solution and satisfactory agreement is noted.*

Keywords: *Initial value problems, Enzyme kinetics, Non-linear reaction equations, Variational iteration method, Mathematical modelling.*

INTRODUCTION

Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme. Enzymes are usually protein molecules that manipulate other molecules - the enzyme's substrates. These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism. These mechanisms can be divided into single-substrate and multiple-substrate mechanisms. Kinetic studies on enzymes that only bind one substrate, such as triosephosphate isomerase, aim to measure the affinity with which the enzyme binds this substrate and the turnover rate. Some other examples of enzymes are phosphofructokinase and hexokinase, both of which are important for cellular respiration (glycolysis). When enzymes bind multiple substrates, such as dihydrofolate reductase, enzyme kinetics can also show the sequence in which these substrates bind and the sequence in which products are released. An example of enzymes that bind a single substrate and release multiple products are proteases, which cleave one protein substrate into two polypeptide products. Others join two substrates together, such as DNA polymerase linking anucleotide to DNA.

Enzymes accelerate the rate of chemical reactions (both forward and backward) without being consumed in the process and tend to be very selective, with a particular enzyme accelerating only a specific reaction. Enzymes are important in regulating biological processes, for example, as activators or inhibitors in a reaction. To understand the role of enzyme kinetics, the researcher has to study the rates of reactions, the temporal behaviours of the various reactants and the conditions which influence the enzyme kinetics

Hogan and Woodley [1] developed a model to describe the interaction between two enzymes in a stirred vessel. Using the model, cofactor (NADPH) recycle has been investigated by simultaneous solution of the rate equations, solved with the aid of a numerical solution [1]. The theoretical analysis of the steady-state amperometric oxidase enzyme–membrane electrode is developed. The model is based on diffusion equations containing a non-linear term related to Michaelis–Menten kinetics of the enzymatic reaction. Logambal and Rajendran solved the system of coupled non-linear diffusion equations in amperometric oxidase enzyme-membrane electrodes for the steady-state condition using the Homotopy perturbation method (HPM) [2]. Krishna and co- workers [3] described the mathematical model of a glucose sensor based on the amperometric detection of hydrogen peroxide using immobilized glucose oxidase (GOD). In this sensor GOD is immobilized on Stöber glass beads that are attached to a platinum electrode. The model describes approximate analytical solutions for the behavior of the system, which is assumed to follow the Michaelis-Menten scheme of reaction [3].

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Ramin *et.al* [4] introduced a simple mathematical procedure for obtaining explicit overall rate expressions of reactants/products molecules involved in various elementary reactions. Multi-dimensional steady state and dynamic models for an enzymatic fuel cell are developed [5]. Karel *et. al* [6] applied the model of multiphase system to reversible mono- substrate and bi- substrate reactions catalyzed by membrane- bound enzymes. Mathematical modeling of enzyme reaction mechanisms are discussed in the books by Rubinow [7], Murray [8], Segel [9] and Roberts [10]. The purpose of this communication is to derive asymptotic approximate expressions for the substrate, product, enzyme and enzyme-substrate concentrations using variational iteration method for all values of dimensionless reaction diffusion parameters.

MATHEMATICAL FORMULATION AND SOLUTIONS OF THE PROBLEM

The enzyme kinetics in biochemical system have traditionally been modeled by ordinary differential which are based solely on reactions without spatial dependence of the various concentrations. The model for an enzyme action, first elucidated by Michaelis and Menten suggested the binding of free enzyme to the reactant forming and enzyme-reactant complex. A cascade reaction is a sequence of biochemical reactions which have the property that the product of one reaction is reactant in the following reaction. We will consider on a cascade scheme which consists of two enzyme-substrate reactions described by the Michaelis-Menten kinetic models

$$E_1 + S_1 \underset{k_5}{\overset{k_1}{\leftrightarrow}} C_1 \xrightarrow{k_2} E_1 + S_2, \tag{1}$$

Where E_1 is first enzyme, E_2 is the second enzyme, S_1 is the first substrate, S_2 is the second substrate, C_1 and C_2 are the complexes and P is the final product, while k_1, k_2, k_3, k_4, k_5 and k_6 are constant parameters which represent the rate of the reactions. The concentration of the reactants in the Eq. (1) is denoted by lower case letters.

$$e_1 = [E_1], \quad e_2 = [E_2],\ s_1 = [S_1],\ s_2 = [S_2],\ c_1 = [C_1],\ c_2 = [C_2],\ p = [P] \tag{2}$$

The differential equations governing the evolution of these concentrations are

$$\frac{de_1}{dt} = -k_1 e_1^0 (k_2 + k_5) c_1 \tag{3}$$

$$\frac{de_2}{dt} = -k_3 e_2^0 s_2 + (k_4 + k_6) c_2 \tag{4}$$

$$\frac{ds_1}{dt} = -k_1 e_1^0 s_1 + k_5 c_1 \tag{5}$$

$$\frac{ds_2}{dt} = k_2 c_1 - k_3 e_2^0 s_2 + k_6 c_2 \tag{6}$$

$$\frac{dc_1}{dt} = k_1 e_1^0 s_1 - (k_2 + k_5) c_1 \tag{7}$$

$$\frac{dc_2}{dt} = k_3 e_2^0 s_2 - (k_4 + k_6) c_2 \tag{8}$$

$$\frac{dp}{dt} = k_4 c_2 \tag{9}$$

The initial conditions are

$$e_1(0) = e_1^0,\ e_2(0) = e_2^0,\ s_1(0) = s_0,\ s_2(0) = 0, c_1(0) = 0,\ c_2(0) = 0,\ p(0) = 0 \tag{10}$$

where e_1^0 and e_2^0 are constants. Note that the conservation laws for this system are.

$$e_1 - c_1 = e_1^{\,0} \tag{11}$$

$$e_2 - c_2 = e_2^{\,0} \tag{12}$$

$$s_1 + c_1 + s_2 + c_2 + p = s_0 \tag{13}$$

An experimental problem involving a cascade reaction of the type in which two enzymes are immobilised on an electrode at the bottom of a flow cell. It is assumed that the two enzymes fully cover the surface of the electrode and it is only the total concentration, e, that can be measured experimentally, rather than the individual concentrations, e_1^0 and e_2^0. From the two conservation laws given by equations (11) to (13), and taking into account the fact that the product is uncoupled from all the other chemical reactants, we can reduce equations (3) to (9) to the following four equations.

$$\frac{ds_1}{dt} = -k_1(e_1 - c_1)s_1 + k_5 c_1 \tag{14}$$

$$\frac{ds_2}{dt} = k_2 c_1 - k_3(e_2 - c_2)s_2 + k_6 c_2 \tag{15}$$

$$\frac{dc_1}{dt} = k_1(e_1 - c_1)s_1 - (k_2 + k_5)c_1 \tag{16}$$

$$\frac{dc_2}{dt} = k_3(e_2 - c_2)s_2 - (k_4 + k_6)c_2 \tag{17}$$

The initial conditions are

$$s_1(0) = s_0,\ s_2(0) = 0,\ c_1(0) = 0,\ c_2(0) = 0 \tag{18}$$

The above system of nonlinear equations (14-17) can be solved analytically in a simple and closed form using variational iteration method(Appendix-A). The solutions of the above equations become

$$s_1(t) = \alpha_0 + \alpha_1 e^{-k_1 e_1 t} + \alpha_2 e^{-bt} + \alpha_3 e^{-(k_1 e_1 + b)t} \tag{18}$$

$$s_2(t) = \beta_0 + \beta_1 e^{-at} + \beta_2 e^{-k_2 t} + \beta_3 e^{-ct} + \beta_4 e^{-2k_2 t} + \beta_5 e^{-(c+k_2)t} + \beta_6 e^{-(d+k_2)t} + \beta_7 e^{-(c+d)t} + \beta_8 e^{-2ct} + \beta_9 e^{-dt} \tag{19}$$

$$c_1(t) = \gamma_0 e^{-at} + \gamma_1 e^{-bt} + \gamma_2 e^{-2at} \tag{20}$$

$$c_2(t) = \delta_0 e^{-k_2 t} + \delta_1 e^{-dt} + \delta_2 e^{-ct} + \delta_3 e^{-2k_2 t} + \delta_4 e^{-2ct} + \delta_5 e^{-(c+k_2)t} + \delta_6 e^{-(d+k_2)t} + \delta_7 e^{-(c+d)t} \tag{21}$$

where $$a = k_1 e_1,\ b = (k_2 + k_5),\ c = k_3 e_2,\ d = (k_4 + k_6) \tag{22}$$

$$\alpha_0 = \frac{k_5 a}{b k_1 e_1} - s_0,\ \alpha_1 = s_0 + \frac{k_1 a s_0 t}{b} - \frac{k_1 a s_0}{b} - \frac{k_5 a}{b k_1 e_1} + \frac{k_5 a}{b(k_1 e_1 - b)},\ \alpha_2 = \frac{-k_5 a}{b(k\ e_1 - b)},\ \alpha_3 = \frac{-k_1 a s_0}{b} \tag{23}$$

$$\beta_0 = \frac{-k_2 a s_0}{(b-a)c},\ \beta_1 = \frac{k_2 a s_0}{(b-a)(c-a)},\ \beta_2 = s_0 + \frac{k_2 s_0}{(c-k_2)} - \frac{c s_0}{(c-k_2)} + \frac{k_6}{(d-k_2)(c-k_2)}, \tag{24}$$

$$\begin{aligned}\beta_3 = {} & -s_0 + \frac{k_2 s_0}{(c-k_2)} - ct - \frac{k_2 a s_0}{(b-a)(c-a)} + \frac{k_2 a s_0}{c(b-a)} + \frac{c s_0}{(c-k_2)} + cts_0 - \frac{k_3 c {s_0}^2}{(d-k_2)(c-2k_2)} \\ & - \frac{k_3 c {s_0}^2}{k_2(d-k_2)} - \frac{k_3 c {s_0}^2}{(d-k_2)(d+k_2-c)} + \frac{k_3 c {s_0}^2}{k_2(d-c)} - \frac{k_3 c {s_0}^2}{c(d-c)} + \frac{k_3 c {s_0}^2}{(d-c)(d+k_2-c)} - \frac{k_3 c {s_0}^2}{d(d-c)} \\ & - \frac{k_6}{(d-k_2)(c-k_2)} + \frac{k_6}{(d-k_2)(c-d)} - \frac{k_6 t}{(d-c)} - \frac{k_6}{(d-c)(c-d)}\end{aligned} \tag{25}$$

$$\beta_4 = \frac{k_3 c {s_0}^2}{(d-k_2)(c-2k_2)},\ \beta_5 = \frac{k_3 c {s_0}^2}{k_2(d-k_2)} + \frac{k_3 c {s_0}^2}{k_2(d-c)}, \tag{26}$$

$$\beta_6 = \frac{k_3 c s_0^{\,2}}{(d-k_2)(d+k_2-c)} - \frac{k_3 c s_0^{\,2}}{(d-c)(d+k_2-c)}, \beta_7 = -\frac{k_3 c s_0^{\,2}}{d(d-k_2)} + \frac{k_3 c s_0^{\,2}}{d(d-c)}, \beta_8 = \frac{k_3 c s_0^{\,2}}{c(d-c)}, \tag{27}$$

$$\beta_9 = \frac{k_6}{(d-k_2)(c-d)} + \frac{k_6}{(d-c)(c-d)}, \gamma_0 = \frac{2as_0}{(b-a)} + \frac{a^2 s_0}{(b-a)^2} + \frac{k_1 a s_0^{\,2}}{(b-a)^2} - \frac{abs_0}{(b-a)^2}, \tag{28}$$

$$\gamma_1 = \frac{-a^2 s_0}{(b-a)^2} - \frac{2as_0}{(b-a)} + \frac{k_1 a s_0^{\,2}}{(b-a)(b-2a)} - \frac{k_1 a s_0^{\,2}}{(b-a)^2} + \frac{abs_0}{(b-a)^2}, \gamma_2 = \frac{-k_1 a s_0^{\,2}}{(b-a)(b-2a)} \tag{29}$$

$$\delta_0 = \frac{2cs_0}{(d-k_2)} + \frac{k_2 c s_0}{(d-k_2)^2} - \frac{cds_0}{(d-k_2)^2}, \tag{30}$$

$$\begin{aligned}\delta_1 = {} & \frac{-2cs_0}{(d-k_2)} + \frac{2cs_0}{(d-c)} - \frac{k_2 c s_0}{(d-k_2)^2} + \frac{c^2 s_0}{(d-c)^2} + \frac{k_3 c s_0^{\,2}}{(d-k_2)(d-2k_2)} + \frac{k_3 c s_0^{\,2}}{k_2(d-k_2)} - \frac{k_3 c s_0^{\,2}}{k_2(d-c)} \\ & + \frac{k_3 c s_0^{\,2}}{(d-c)(c+k_2-d)} + \frac{k_3 c s_0^{\,2}}{(d-k_2)(c+k_2-d)} - \frac{k_3 s_0^{\,2}}{(d-k_2)} + \frac{k_3 c s_0^{\,2}}{(d-c)(d-2c)} + \frac{k_3 s_0^{\,2}}{(d-c)} \\ & + \frac{cds_0}{(d-k_2)^2} - \frac{cds_0}{(d-c)^2}\end{aligned} \tag{31}$$

$$\delta_2 = \frac{c^2 s_0}{(d-c)^2} + \frac{cds_0}{(d-c)^2}, \delta_3 = \frac{-k_3 c s_0^{\,2}}{(d-k_2)(d-2k_2)}, \delta_4 = \frac{-k_3 c s_0^{\,2}}{(d-c)(d-2c)}, \tag{32}$$

$$\delta_5 = \frac{-k_3 c s_0^{\,2}}{(d-c)(c+k_2-d)} - \frac{k_3 c s_0^{\,2}}{(d-k_2)(c+k_2-d)}, \delta_6 = \frac{-k_3 c s_0^{\,2}}{(d-k_2)k_2} + \frac{-k_3 c s_0^{\,2}}{(d-c)k_2}, \tag{33}$$

$$\delta_7 = \frac{k_3 s_0^{\,2}}{(d-k_2)} - \frac{k_3 s_0^{\,2}}{(d-c)} \tag{34}$$

NUMERICAL SIMULATION

The non- linear differential equations are solved by numerical methods. The function pdex4 in MATLAB/SCILAB software which is a function of solving the boundary value problem for differential equation is used to solve this equation. Its numerical solution is compared with variational iteration method in Figures1-4.

RESULTS AND DISCUSSION

Equations (18) to (21) are new and simple approximate analytical expression for the concentrations of the substrate and the enzyme-substrate-complex calculated using variational iteration method. Fig.1 represent the concentration of the first substrate s_1 versus time t for some fixed values of other parameters. From the Fig.1, it is inferred that concentration of substrate decreases when time increases. Also concentration of substrate s_1 increases when k_1 increases. Fig. 2 represent the concentration of the second substrate s_2 versus time t for some fixed values of other parameters. From the Fig.2, it is noted that concentration of substrate decreases when time increases. Also concentration of substrate s_2 increases when k_4, e_1, s_0 increases. Fig. 3 represent the concentration of the first complex c_1 versus time t for some fixed values of other parameters. From the Fig.3, it is infored that concentration of substrate decreases when time increases. Also concentration of complex c_1 increases when k_1, e_1, s_0 increases. Fig.4 represent the concentration of the first complex c_2 versus time t are calculated for various values of other parameters. From the Fig.4, it is observed that the concentration is linear with time t.

CONCLUSION

Approximate analytical solutions to the non-linear reaction equations are presented using variational iteration method. A simple, straight forward and a new method of estimating the concentrations of substrate, product, enzyme-substrate complex and enzyme are derived. This solution procedure can be easily extended to all kinds of system of non-linear equations with various complex boundary conditions in enzyme-substrate reaction diffusion processes.

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APPENDIX: A

Solution of non-linear equations using variational iteration method (VIM)

In this appendix, the general solution of non-linear reaction using He's variational method is derived. To illustrate the basic concepts of variational method (VIM), the following non-linear partial differential equation is considered.

$$L[u(x)] + N[u(x)] = g(x) \tag{A.1}$$

Where L is a linear operator, N is a nonlinear operator, and $g(x)$ is a given continuous function According to the variational method, we can construct a correct functional as follows

$$u_{n+1}(x) = u_n(x) + \int_0^x \lambda[L[u_n(\tau)] + N[\tilde{u}_n(\tau)] - g(\tau)]d\tau \tag{A.2}$$

Where λ is a general Lagrange multiplier which can be identified optimally via variational theory.

$$(s_1)_{n+1}(t) = (s_1)_n(t) + \int_0^t \lambda_1 \left[(s_1)_n'(\xi) + k_1 e_1 (s_1)_n(\xi) - \overbrace{k_1 (c_1)_n(\xi)}^{\approx} (s_1)_n(\xi) - \overbrace{k_5 (c_1)_n(\xi)} \right] d\xi \tag{A.3}$$

$$(s_2)_{n+1}(t) = (s_2)_n(t) + \int_0^t \lambda_2 \left[\begin{array}{l} (s_2)_n'(\xi) - \overbrace{k_2 (c_1)_n(\xi)} + k_3 e_2 (s_2)_n(\xi) - \overbrace{k_3 (c_2)_n(\xi)} (s_2)_n(\xi) \\ - \overbrace{k_6 (c_2)_n(\xi)} \end{array} \right] d\xi \tag{A.4}$$

$$(c_1)_{n+1}(t) = (c_1)_n(t) + \int_0^t \lambda_3 \left[(c_1)_n'(\xi) - \overbrace{k_1 e_1 (s_1)_n(\xi)} + \overbrace{k_1 (c_1)_n(\xi)} (s_1)_n(\xi) + (k_2 + k_5)(c_1)_n(\xi) \right] d\xi \tag{A.5}$$

$$(c_2)_{n+1}(t) = (c_2)_n(t) + \int_0^t \lambda_4 \left[(c_2)_n'(\xi) - \overbrace{k_3 e_2 (s_2)_n(\xi)} + \overbrace{k_3 (c_2)_n(\xi)} (s_2)_n(\xi) + (k_4 + k_6)(c_2)_n(\xi) \right] d\xi \tag{A.6}$$

Taking variation with respect to the independent variable $(s_1)_n, (s_2)_n, (c_1)_n, (c_2)_n$, we get

$$\delta(s_1)_{n+1}(t) = \delta(s_1)_n(t) + \delta\int_0^t \lambda_1 \left[(s_1)_n'(\xi) + k_1 e_1 (s_1)_n(\xi) - \overbrace{k_1 (c_1)_n(\xi)}^{\approx} (s_1)_n(\xi) - \overbrace{k_5 (c_1)_n(\xi)} \right] d\xi \tag{A.7}$$

$$\delta(s_2)_{n+1}(t) = \delta(s_2)_n(t) + \delta\int_0^t \lambda_2 \left[\begin{array}{l} (s_2)_n'(\xi) - \overbrace{k_2 (c_1)_n(\xi)} + k_3 e_2 (s_2)_n(\xi) - \overbrace{k_3 (c_2)_n(\xi)} (s_2)_n(\xi) \\ - \overbrace{k_6 (c_2)_n(\xi)} \end{array} \right] \tag{A.8}$$

$$\delta(c_1)_{n+1}(t) = \delta(c_1)_n(t) + \delta\int_0^t \lambda_3 \left[\begin{array}{l} (c_1)_n{}'(\xi) - \overbrace{k_1 e_1 (s_1)_n(\xi)} + \overbrace{k_1 (c_1)_n(\xi)(s_1)_n(\xi)} \\ + (k_2 + k_5)(c_1)_n(\xi) \end{array} \right] d\xi \tag{A.9}$$

$$\delta(c_2)_{n+1}(t) = \delta(c_2)_n(t) + \delta\int_0^t \lambda_4 \left[\begin{array}{l} (c_2)_n{}'(\xi) - \overbrace{k_3 e_2 (s_2)_n(\xi)} + \overbrace{k_3 (c_2)_n(\xi)(s_2)_n(\xi)} \\ + (k_4 + k_6)(c_2)_n(\xi) \end{array} \right] d\xi \tag{A.10}$$

where $\lambda_1, \lambda_2, \lambda_3 \; and \; \lambda_4$ are general Lagrangian multipliers, $(s_1)_0, (s_2)_0, (c_1)_0 \; and (c_2)_0$ are initial approximations or trial functions.

$$\begin{array}{l} \delta(s_1)_n : 1 + \lambda_1(\xi)\big|_{\xi=\tau} = 0 \\ \delta(s_1)_n : -\lambda_1'(\xi)\big| + k_1 e_1 \lambda_1(\xi)\big|_{\xi=\tau} = 0 \end{array}$$

$$\begin{array}{l} \delta(s_1)_n : 1 + \lambda_2(\xi)\big|_{\xi=\tau} = 0 \\ \delta(s_1)_n : -\lambda_2'(\xi)\big| + k_3 e_2 \lambda_1(\xi)\big|_{\xi=\tau} = 0 \end{array} \tag{A.11}$$

$$\begin{array}{l} \delta(s_1)_n : 1 + \lambda_3(\xi)\big|_{\xi=\tau} = 0 \\ \delta(s_1)_n : -\lambda_3'(\xi)\big| + (k_2 + k_5)\lambda_3(\xi)\big|_{\xi=\tau} = 0 \end{array}$$

$$\begin{array}{l} \delta(s_1)_n : 1 + \lambda_4(\xi)\big|_{\xi=\tau} = 0 \\ \delta(s_1)_n : -\lambda_4'(\xi)\big| + (k_4 + k_6)\lambda_4(\xi)\big|_{\xi=\tau} = 0 \end{array}$$

The above equations are called Lagrangian – Euler equations. The Lagrangian multipliers, can be identified as

$$\begin{array}{l} \lambda_1(\xi) = -e^{k_1 e_1 (\xi - \tau)} \\ \lambda_2(\xi) = -e^{k_3 e_2 (\xi - \tau)} \\ \lambda_3(\xi) = -e^{(k_2 + k_5)(\xi - \tau)} \\ \lambda_4(\xi) = -e^{(k_4 + k_6)(\xi - \tau)} \end{array} \tag{A.12}$$

Substituting the Lagrangian multipliers and n=0 in the iteration formula, (A.3-A.6), we obtain

$$(s_1)_1(t) = (s_1)_0(t) - \int_0^t e^{k_1 e_1 (\xi - \tau)} \left[(s_1)_0{}'(\xi) + k_1 e_1 (s_1)_0(\xi) - k_1 (c_1)_0(\xi)(s_1)_0(\xi) - k_5 (c_1)_0(\xi) \right] d\xi \tag{A.13}$$

$$(s_2)_1(t) = (s_2)_0(t) - \int_0^t e^{k_3 e_2 (\xi - \tau)} \left[\begin{array}{l} (s_2)_0{}'(\xi) - k_2 (c_1)_0(\xi) + k_3 e_2 (s_2)_0(\xi) \\ - k_3 (c_2)_0(\xi)(s_2)_0(\xi) - k_6 (c_2)_0(\xi) \end{array} \right] d\xi \tag{A.14}$$

$$(c_1)_1(t) = (c_1)_0(t) - \int_0^t e^{(k_2 + k_5)(\xi - \tau)} \left[\begin{array}{l} (c_1)_0{}'(\xi) - k_1 e_1 (s_1)_0(\xi) + k_1 (c_1)_0(\xi)(s_1)_0(\xi) \\ + (k_2 + k_5)(c_1)_0(\xi) \end{array} \right] d\xi \tag{A.15}$$

$$(c_2)_1(t) = (c_2)_0(t) - \int_0^t e^{(k_4 + k_6)(\xi - \tau)} \left[\begin{array}{l} (c_2)_0{}'(\xi) - k_3 e_2 (s_2)_0(\xi) + k_3 (c_2)_0(\xi)(s_2)_0(\xi) \\ + (k_4 + k_6)(c_2)_0(\xi) \end{array} \right] d\xi \tag{A.16}$$

Substituting the initial conditions $s_i(0) = s_0$, $s_i(0) = 0$, $c_i(0) = 0$, $c_i(0) = 0$ for all i in the above equations, we obtain equations (18) to (21) in the text. Here we can consider the first iteration only. Higher order iteration may be considered for better accuracy.

APPENDIX B: NUMERICAL PROGRAM FOR SOLVING EQUATIONS (14)-(17)

MATLAB Program

```
function graphmain1
options= odeset('RelTol',1e-6,'Stats','on');
%initial conditions
Xo = [1; 0; 0; 0];
tspan = [0 3];
tic
[t,X]=ode45(@TestFunction,tspan,Xo,options);
toc
figure
hold on
%plot(t, X(:,1))
%plot(t, X(:,2))
%plot(t, X(:,3))
plot(t, X(:,4))
legend('x1','x2','x3','x4')
ylabel('x')
xlabel('t')
return
function [dx_dt]= TestFunction(t,x)
k1=50;k2=.1;k3=.195;k4=1.13;k5=.001;k6=.0002;e1=1;e2=1;
dx_dt(1)=-k1*(e1-x(3))*x(1)+k5*x(3);
dx_dt(2)=k2*x(3)-k3*(e2-x(4))*x(2)+k6*x(4);
dx_dt(3)=k1*(e1-x(3))*x(1)-(k2+k5)*x(3);
dx_dt(4)=k3*(e2-x(4))*x(2)-(k4+k6)*x(4);
dx_dt = dx_dt';
return
```

APPENDIX C

Nomenclature and units

Symbol	Meaning	Usual dimension
s_1	Concentration of first substrate	molecm^{-3}
s_2	Concentration second substrate	molecm^{-3}
c_1	Concentration of first enzyme –substrate complex	molecm^{-3}
c_2	Concentration of second enzyme-substrate complex	molecm^{-3}
e	Concentration of the enzyme	molecm^{-3}
p	Concentration of the product	molecm^{-3}
s_0	Bulk concentration of the substrate	molecm^{-3}
e_0	Bulk concentration of the enzyme	molecm^{-3}
k_1,k_2, k_3, k_4, k_5, k_6	Reaction rate	s^{-1}
t	Time	s^{-1}
a,b,c,d	constant	none
$\alpha_0 \ldots \alpha_3, \beta_0, \ldots \beta_9, \gamma_0 \ldots \gamma_2, \delta_0 \ldots \delta_7$	constant	none

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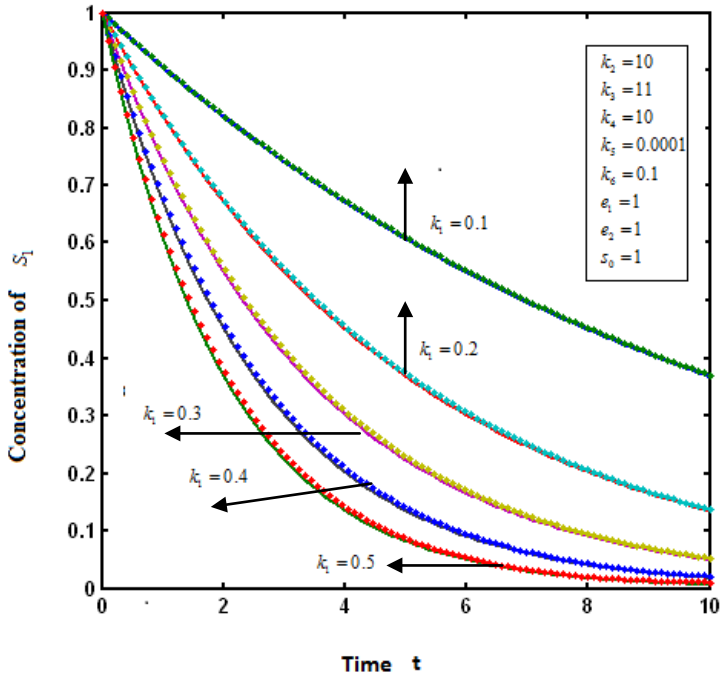

Fig.1. The concentrations of the first substrate s_1 versus the time t are calculated using Eq. (18) for various values of k_1 when $k_2 = 10, k_3 = 11, k_4 = 10, k_5 = 0.0001, k_6 = 0.1, e_1 = 1, e_2 = 1, s_0 = 1$. Dotted line represents numerical simulation and bold line represents analytical expression.

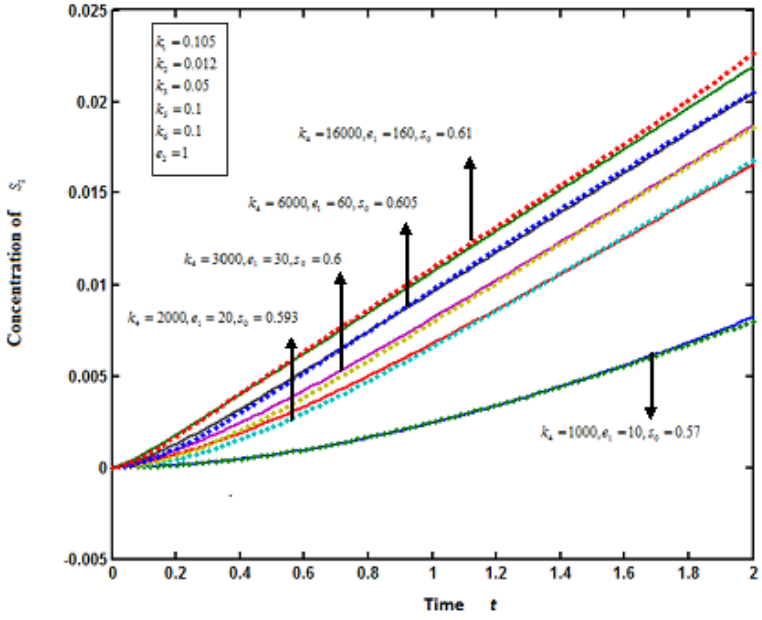

Fig.2. The concentration of the second substrate s_2 versus the time t are calculated using Eq. (19) for various values of k_4, e_1, s_0 when $k_1 = 0.105,\ k_2 = 0.012,\ k_3 = 0.05,\ k_5 = 0.1,\ k_6 = 0.1, e_2 = 1$. Dotted line represents numerical simulation and bold line represents analytical expression.

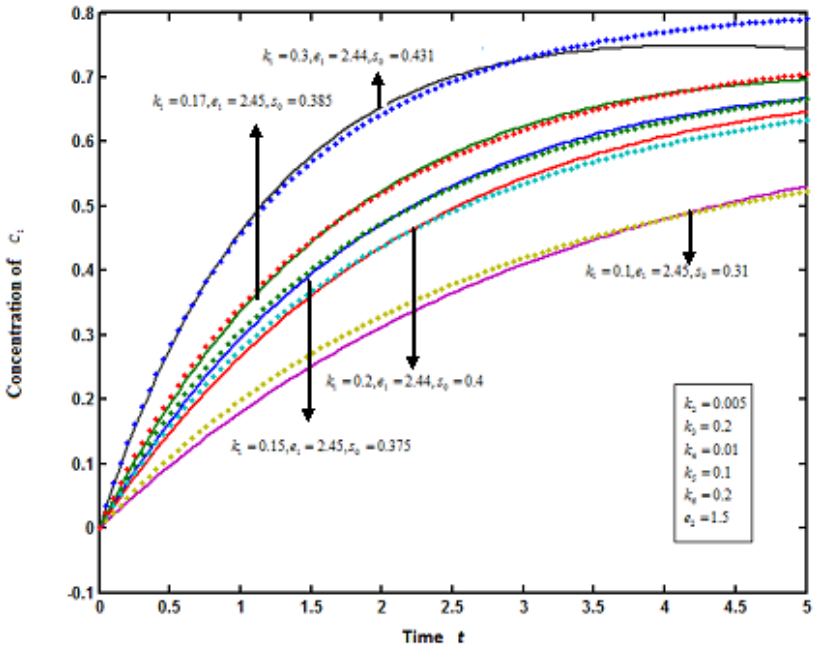

Fig.3. The concentration of the first complex c_1 versus the time t are calculated using Eq. (20) for various values of k_1, e_1, s_0 when $k_3 = 0.2,\ k_4 = 0.01, k_5 = 0.1, k_6 = 0.2, e_2 = 1.5$. Dotted line represents numerical simulation and bold line represents analytical distance.

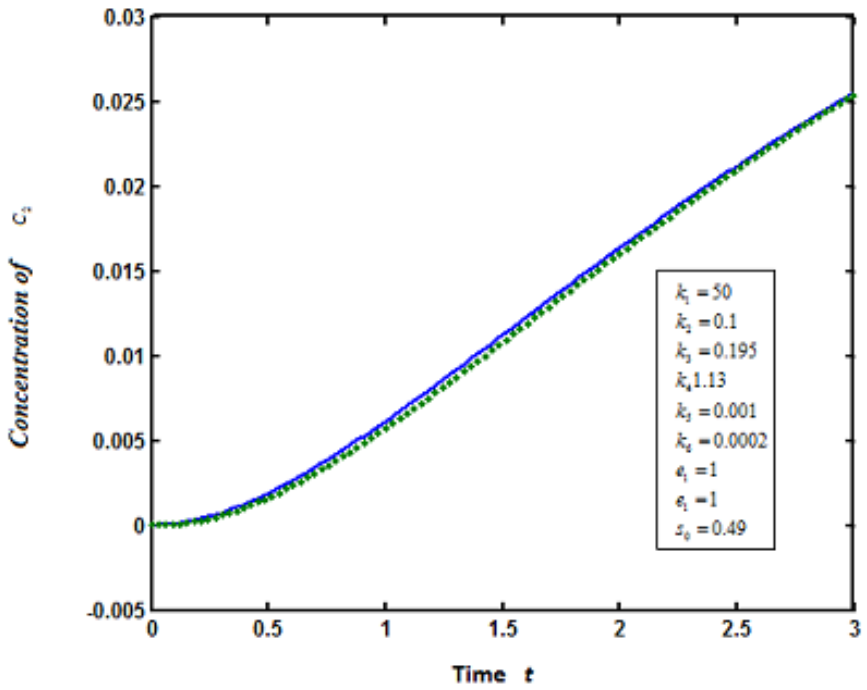

Fig.4. The concentration of the second complex C_2 versus the time t are calculated using Eq. (21) for values of $k_1 = 50, k_2 = 0.1,\ k_3 = 0.195, k_4 = 1.13,\ k_5 = 0.001,\ k_6 = 0.0002 \quad e_1 = 1, e_2 = 1, s_0 = 0.49$. Dotted line represents numerical simulation and bold line represents analytical expression.

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