APPLICATION OF A NEW ITERATIVE METHOD TO ANALYSIS OF KINETICS OF THERMAL INACTIVATION OF ENZYME

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In this paper, a new iterative method proposed by Temimi and Ansari is applied to analyze the kinetic of thermal inactivation of jack bean urease (EC3.5.1.5). The analyzed kinetics or reaction mechanisms consist of three-reaction steps and included the Arrhenius equation for the temperature dependence of rate constants as well as the temperature change in the initial heating period. The obtained solutions are used to study the model parameters on the kinetics of thermal inactivation of enzyme. The analytical solutions are verified with numerical solutions using Runge – Kutta with shooting method and good agreements are established between the solutions. The information given in this theoretical investigation will assist in the kinetic analysis of the experimental results over handling rate constants and molar concentrations.

Keywords: Kinetics; Thermal activation; Jack bean urease; Enzyme; Temini and Ansari's method

Nomenclature

c_N: Molar concentration of the native enzyme form (mole/cm)
c_D: Molar concentration of the denatured enzyme form (mole/cm
k_{-1},k_{+1},k_2,k_3: Rate constants of individual reaction (s^{-1})
k'_{-1}, k'_2, k'_3: Modified rate constants (s^{-1})
K: Coefficient in the enthalpy balance (s)
T_B: Bath temperature (K)
T: Temperature (K)
t: Time

1. Introduction

The study of Urease (urea amino hydrolase E.C.3.5.1.5) as part of the superfamily of amidohydrolases and phosphotriesterases has in recent times attracted research interests in last few decades. This is due to its high efficiency as catalyst that catalyzes the hydrolysis of urea into carbon dioxide and ammonia. It

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catalyzes at a rate approximately 10^{14} times faster than the rate of the non-catalyzed reaction [1]. The hydrolysis of urea is catalyzed by urease to produce ammonia and carbamate and the carbamate produced is subsequently degraded by spontaneous hydrolysis to produce another ammonia and carbonic acid. Urease activity tends to increase the pH of its environment as it produces ammonia.

Jack bean urease, which is the most widely used plant urease, is a nickel containing oligomeric enzyme exhibiting a high degree of specifity to urea [2]. The importance and applications of the urease as a good catalyst for hydrolysis of urea has attracted several research interests especially in biotechnology and biomedical engineering studies. Winquist et al. [3] presented trace level analysis for mercury using urease in combination with an ammonia gas sensitive semiconductor structure. Few years after, Miyagawa et al. [4] submitted a study on purification, characterization and application of an acid urease. Meanwhile, Cullen et al [5] analyzed the multi-analyte miniature conductance biosensor. Also, Alonso et al. [6] examined enzyme immobilization on an epoxy matrix through the determination of l-arginine by flow-injection techniques. In another study, Sansubrino and Mascini [7] developed an optical fibre sensor for ammonia, urea, urease and IgG. Godjevargova and Dimov [8] investigated the immobilization of urease onto membranes of modified acrylonitrile copolymer. Rejikumar and Devi [9] presented a work on the preparation and characterization of urease bound on crosslinked poly (vinyl alcohol) while Chen and Chiu [10] considered poly(n-sopropylacrylamide-co-Nacroloxysuccinimide-co-2-hydroxyetyl methacrylate) composite hydrogel membrane for urease immobilization to enhance urea hydrolysis rate by temperature swing. Lencki et al. [11] investigated the effect of subunit dissociation, denaturation, aggregation, coagulation, and decomposition on enzyme inactivation kinetics. Omar and Bauregard [12] examined the dissociation and unfolding of jack bean urease studied by fluorescence emission spectroscopy. Series mechanism of enzyme deactivation was studied by Gianfreda et al. [13]. Characterization of intermediate forms. Henley and Sadana [14] categorized the enzyme deactivations using series-type mechanism while Grego and Gianfreda [15] presented an experimental technique for the discrimination between series and parallel mechanisms of enzyme deactivation. Sadana and Henley [16] carried out mechanistic analysis of complex enzyme deactivations and examined influence of various parameters on series-type inactivations. Significance, regulation, and molecular characterization of microbial ureases was presented by Mobley and Hausinger [17]. Summner [18] scrutinised the isolation and crystallization of the enzyme urease. Dixon [19] examined the biological role of Jack Bean urease (EC 3.5.1.5) for nickel while Polacco and Havir [20] compared of soybean urease isolated from seed and tissue culture. Prakash and Bhushan [21] analyzed the isolation, purification and partial characterisation of urease from seeds of water melon. Hirai and Ueki [22] explored the structural

change of jack bean urease induced by addition of surfactants. Garcia et al. [23] presented the kinetics of thermal inactivation of horseradish peroxidase:stabilizing effect of methoxypoly(ethylene glycol). Also, in some other studies, the thermostability of jack bean urease has often been a subject of investigation [24-27]. However, there are few studies where the temporal loss of enzyme activity and the kinetic analysis of heat induced decay of enzyme activity were presented. Moreover, none of these studies involved consistent evaluation of kinetics of the urease inactivation. Most of the past studies described the complex mechanisms of thermal deactivation of enzymes as a “one step - two states” process where the native (active) form is transformed in the denaturated (inactive) form by a first order unimolecular irreversible reaction [26]. This unifying simplification is of interest for people focusing attention to phenomenological rather than mechanistic description of the kinetics of heat induced enzyme deactivation. However, the multitemperature evaluation revealed that an adequate kinetic model had to incorporate at least three reaction steps [26]. Although, three-step mechanism model of inactivation of the enzyme has been developed by Illeova et al. [26], there is no provision of analytical solutions (except by Ananthi *et al*, [28], Sobamowo and Adeleye [29, 30]) for the predictions of model concentrations of the native enzyme, denature enzyme and temperature for thermal inactivation of urease. Ananthi *et al*, [28] applied homotopy analysis method to develop approximate analytical solutions for the analysis of kinetic and thermal inactivation of the enzyme. Although, the homotopy analysis method is a reliable and efficient semi-analytical technique, but it suffers from a number of limiting assumptions such as the requirements that the solution ought to conform to the so-called rule of solution expression and the rule of coefficient ergodicity. Also, the use of homotopy analysis method (HAM) in developing analytical solution to linear and nonlinear equations requires the determination of auxiliary parameter which will increase the computational cost and time. Also, the lack of rigorous theories or proper guidance for choosing initial approximation, auxiliary linear operators, auxiliary functions, auxiliary parameters limit the applications of homotopy analysis method. Moreover, such method requires high skill in mathematical analysis and the solution comes with large number of terms. In practice, analytical solutions with large number of terms and conditional statements for the solutions are not convenient for use by designers and engineers [28].The determination of Adomian polynomials as carried out in Adomian decomposition method (ADM), the need for small perturbation parameter as required in traditional perturbation methods, the rigour of the derivations of differential transformations or recursive relation as carried out in differential transformation methods, the lack of rigorous theories or proper guidance for choosing initial approximation, auxiliary linear operators, auxiliary functions, auxiliary parameters, and the requirements of conformity of the solution to the

rule of coefficient ergodicity as done in homotopy analysis method, the search Langrange multiplier as carried in variational iterative method (VIM), and the challenges associated with proper construction of the approximating functions for arbitrary domains or geometry of interest as in Galerkin weighted residual method (GWRM), least square method (LSM) and collocation method (CM) are some of the difficulties that are not experienced by homotopy perturbation method (HPM). Furthermore, in the class of the newly developed approximate analytical methods, homotopy perturbation method is considered to relatively simple with fewer requirements for mathematical rigour or skill. Also, the search for a particular value that will satisfy second the boundary condition in DTM, HAM, ADM, and VIM necessitated the use of software and such could result in additional computational cost in the generation of solution to the problem.

As a means of overcoming the drawbacks in the other approximation analytical methods, recently, Temimi and Ansari [31] introduced the semi-analytical iterative technique in 2011 for solving nonlinear problems. The new iterative method has been used to solve many differential equations, such as nonlinear second order multi-point boundary value problems [32], nonlinear ordinary differential equations [31], Korteweg–de vries equations [33]. The results obtained in these studies indicate that the Temimi and Ansari method (TAM) provides excellent approximations to the solution of non-linear equation with high accuracy, low computational time and high order of convergence. From the previous studies, it was established that the TAM can solve nonlinear differential and integral equations without linearization, discretization, closure, restrictive assumptions, perturbation, approximations, round-off error and discretization that could result in massive numerical computations. The method is derivative-free, and does not require the calculations of Adomian polynomials, Lagrange multiplier, developments, derivations of differential transforms, construction of homotopy as found in ADM, VIM, DTM and HPM, respectively [34-35]. Therefore, in this work, Temini and Ansari method is applied to the kinetics analysis of thermal inactivation of enzyme. The developed analytical solutions are used to study the effects of the models parameters on the molar concentration of the native and denatured enzyme.

2. Model Formulation

The three – step mechanism of inactivation with a dissociation reaction of the native form of the enzyme, N, into a denatured form, D, and with two parallel association reactions of the native and denatured forms into irreversible denatured enzymes forms $I_1$and I_2, respectively.

$$N \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} 2D \qquad 2D \overset{k_2}{\rightarrow} I_1 \qquad 2N \overset{k_3}{\rightarrow} I_2 \tag{1}$$

where k_{+1}, k, k_2 and k_3 represent the rate constants of individual reactions. The material balances equations for N, D and temperature are given as follows [28, 29]:

$$\frac{dc_N}{dt} = -k_{+1}c_N + k'_{-1}c_D^2 - 2k'_3c_D^2 \tag{2a}$$

$$\frac{dc_D}{dt} = 2k_{+1}c_N - 2\left(k'_{-1} + k'_2\right)c_D^2 \tag{2b}$$

$$\frac{dT}{dt} = K\left(T - T_B\right) \tag{2c}$$

Initial conditions are

$$t = 0, \quad c_N = 1, \quad c_D = 0\,, \; T = 30 + T_B, \tag{3}$$

The kinetic model was formed by the set of nonlinear ordinary differential equations (Eqs. (2a)–(2c)). The core of the kinetic model was formed by the material balances of the forms N and D (Eqs. (2a) and (2b)). The third equation of the model was the enthalpy balance (Eq. (2c)) describing the initial heating period

Let c_N, c_D, k_{+1}, k_{-1}, k_2 and k_3 by X, Y, a, b, c and d, respectively, Equ. 2a and 2b become

$$\frac{dX}{dt} = -aX + bY^2 - 2dX^2 \tag{4a}$$

$$\frac{dY}{dt} = 2aX - 2\left(b + c\right)Y^2 \tag{4b}$$

$$t = 0, \quad X = 1, Y = 0 \tag{5}$$

while the exact solution of Eq. (2c) is given as

$$T\left(t\right) = T_B + 30e^{-Kt} \tag{6}$$

3.0 Approximate Analytical Methods of Solution: Temini and Ansari method

The nonlinearities in the above Eqs. (4a) and (4b) makes it very difficult to generate closed form solutions to the equations. Therefore; in this work, recourse is made to an approximation analytical method, Temini and Ansari method.

3.1 Principle of Temini and Ansari method

The principle of the method is described as follows. The general system of nonlinear equation is in the form

$$L(u(x)) + N(u(x)) + g(x) = 0 \tag{7}$$

with the boundary conditions

$$B\left(u, \frac{du}{dx}\right) = 0 \tag{8}$$

where x denotes the independent variable, $u(x)$ represents an unknown function, $g(x)$ is a known function, L is a linear operator, N is a nonlinear operator and B is a boundary operator. Since L is taken as the linear (highest order derivative) part of the DE, it is possible to take some or the remaining linear parts of the DE and add them to N as needed. The procedure of the proposed TAM is as follows. Assuming that $u(x)$ is an initial guess of the solution to the problem $u(x)$ and is the solution of the equation

$$L(u_0(x)) + g(x) = 0 \quad B\left(u_0, \frac{du_0}{dx}\right) = 0 \tag{9}$$

In order to generate the next improvement to the solution, Eq. (10) is solved

$$L(u_1(x)) + g(x) + N(u_0(x)) = 0, \; B\left(u_1, \frac{du_1}{dx}\right) = 0 \tag{10}$$

Following the above procedure, the Temini and Ansari method gives the possibility to write the solution of the general nonlinear equation (7) in the iterative formula

$$L(u_{n+1}(x)) + g(x) + N(u_n(x)) = 0 \quad B\left(u_{n+1}, \frac{du_{n+1}}{dx}\right) = 0 \tag{11}$$

From Eqs. (4a) and (4b), it is clear that

$$L_1\left(X(t), Y(t)\right) = \frac{dX}{dt}, \;\; N_1\left(X(t), Y(t)\right) = aX - bY^2 + 2dX^2 \tag{12a}$$

$$L_2\left(X(t), Y(t)\right) = \frac{dY}{dt}, \;\; N_2\left(X(t), Y(t)\right) = -2aX + 2\left(b + c\right)Y^2 \tag{12b}$$

The initial problem is

$$L_1\left(X_0, Y_0\right) = 0, \quad L_2\left(X_0, Y_0\right) = 0 \tag{13}$$

with initial conditions

$$X_0(0) = 1, \quad Y_0(0) = 0 \tag{14}$$

and subsequent problems can be obtained from the iterative problem generating relation

$$L_1(X_{n+1}(t), Y_{n+1}(t)) + g(t) + N_1(X_n(t), Y_n(t)) = 0 \tag{15a}$$

$$L_2((X_{n+1}(t), Y_{n+1}(t))) + g(t) + N_2((X_n(t), Y_n(t))) = 0 \tag{15b}$$

with initial conditions

$$X_{n+1}(0) = 1, \quad Y_{n+1}(0) = 0 \tag{16}$$

On solving the initial problem, one arrives at

$$X_0 = 1, \quad Y_0 = 0 \tag{17}$$

Using the iterative schemes in Eqs. (15a) and (15b), give

$$\frac{dX_1}{dt} + aX_0 - bY_0^2 + 2dX_0^2 = 0 \tag{18a}$$

$$\frac{dY_1}{dt} - 2aX_0 + 2\left(b + c\right)Y_0^2 = 0 \tag{18b}$$

with initial conditions

$$X_1(0) = 1, \quad Y_1(0) = 0 \tag{19}$$

Solving the above Eqs. (18a) and (18b), we have

$$X_1 = -\left(a + 2d\right)t \tag{20a}$$

$$Y_1 = 2at \tag{20b}$$

Also, for the second iteration, using the iterative schemes in Eqs. (15a) and (15b), give

$$\frac{dX_2}{dt} + aX_1 - bY_1^2 + 2dX_1^2 = 0 \tag{21a}$$

$$\frac{dY_2}{dt} - 2aX_1 + 2\left(b+c\right)Y_1^2 = 0 \tag{21b}$$

with initial conditions

$$X_2(0) = 1, \quad Y_2(0) = 0 \tag{22}$$

The solution of Eqs. (21a) and (21b) are

$$X_2 = 1 + \frac{1}{6}\left(4abt + 3a\left(a+2d\right) - 4d\left(a+2d\right)^2 t\right)t^2 \tag{23a}$$

$$Y_2 = -\frac{a}{3}\left[3\left(a+2d\right) + 8a\left(b+c\right)t\right]t^2 \tag{23b}$$

And now for the third iteration, using Eqs. (15a) and (15b), give

$$\frac{dX_3}{dt} + aX_2 - bY_2^2 + 2dX_2^2 = 0 \tag{24a}$$

$$\frac{dY_3}{dt} - 2aX_2 + 2\left(b+c\right)Y_2^2 = 0 \tag{24b}$$

with initial conditions

$$X_3(0) = 1, \quad Y_3(0) = 0 \tag{25}$$

On solving Eq. (24a) and (24b) using the initial conditions in Eq. (25), one arrives at

$$X_3 = 1 + \frac{a^2 b}{63}\left[8a(b+c)^2 t^2 + 56a(b+c)(a+2d)t + 63a^2(a+2d)\right]t^5 - \frac{at}{6}\left[\left(ab - 4ad^2 - a^2 d - 4d^3\right)t^3 + a(a+2d)t^2 + 6\right] - 2dt\left\{\begin{array}{l} \frac{1}{63}\left(\begin{array}{l} 4a^4 d^2 - 8a^3 bd + 32a^3 d^3 + 4a^2 b^2 - 32a^2 bd^2 \\ +96a^2 d^4 - 32abd^3 + 128ad^5 \end{array}\right)t^6 \\ +\frac{1}{9}\left(ba^3 - 6a^3 d^2 - a^4 d - 12a^2 d^3 + 2a^2 bd - 8ad^4\right)t^5 \\ +\frac{1}{20}\left(a^4 + 4a^3 d + 4a^2 d^2\right)t^4 + \frac{1}{3}\left(ba - 4ad^2 - a^2 d - 4d^3\right)t^3 \\ +\frac{a}{20}(a+2d)t^2 + 1 \end{array}\right\} \quad (26a)$$

$$Y_3 = \frac{a^2 t}{3}\left[\left(ab - 4ad^2 - a^2 d - 4d^3\right)t^3 + a(a+2d)t^2 + 6\right] - \frac{2a^2(b+c)t^5}{9}\left[8at(b+c)(a+2d) + \frac{64a^2 t^2(b+c)}{7} + \frac{1}{5}\right] \quad (26b)$$

Similarly, X_4, Y_4, X_5, Y_5, X_6, Y_6, X_7, Y_7, X_8, Y_8, X_9, Y_9, X_{10}, Y_{10}... are determined using the iterative schemes.

$$\frac{dX_{n+1}}{dt} + aX_n - bY_n^2 + 2dX_n^2 = 0 \quad (27a)$$

$$\frac{dY_{n+1}}{dt} - 2aX_n + 2(b+c)Y_n^2 = 0 \quad (27b)$$

with initial conditions

$$X_{n+1}(0) = 1, \quad Y_{n+1}(0) = 0 \quad (28)$$

The solutions of X_n and Y_n form the approximate analytical solutions of concentrations of native and denatured enzyme. The analytical solutions are simulated and the results are shown below

4. Results and Discussion

Tables 1 and 2 show the comparison between the results of Temimi and Ansari method (TAM) and numerical method (NM) using Fourth-order Runge-Kutta method coupled with shooting method. The obtained results of velocity distributions using TAM as compared with the numerical procedure are in good agreements. The high accuracy of TAM gives high confidence about validity of the method in providing solutions to the problem. It should be noted that a, b, c and d represent the rate constants of individual reactions which are k_{+1}, k_{-1}, k_2 and k_3, respectively.

Table 1: Comparison of results

The results of TAM and Numerical methods for X(t) for $a = 1,\ b = 0.01,\ c = 0.001,\ d = 0.05$

	X(t)	
X	**TAM**	**NUM**	**Residue of TAM**
0.00	1.000000	1.000000	0.000000
0.10	0.896320	0.896320	0.000000
0.20	0.804239	0.804239	0.000000
0.30	0.722362	0.722362	0.000000
0.40	0.649479	0.649479	0.000000
0.50	0.584542	0.584542	0.000000
0.60	0.526638	0.526637	0.000001
0.70	0.474968	0.474965	0.000003
0.80	0.428836	0.428824	0.000012
0.90	0.387641	0.387599	0.000042
1.00	0.350878	0.350748	0.000130

Table 2: Comparison of results

The results of HPM and Numerical methods for X(t) for $a = 1,\ b = 0.01,\ c = 0.001,\ d = 0.05$			
Y(t)			
X	**TAM**	**NUM**	**Residue of TAM**
0.00	0.000000	0.000000	0.000000
0.10	0.189399	0.189399	0.000000
0.20	0.359101	0.359101	0.000000
0.30	0.511178	0.511178	0.000000
0.40	0.647477	0.647477	0.000000
0.50	0.769644	0.769644	0.000000
0.60	0.879150	0.879150	0.000000
0.70	0.977312	0.977311	0.000001
0.80	1.065300	1.065300	0.000000
0.90	1.144180	1.144170	0.000010
1.00	1.214880	1.214840	0.000040

Fig.1 shows variation of the molar concentration of native and denatured enzyme with time when $k_{-1} = 1,\ k_{+1} = 0.01,\ k_2 = 0.001,\ k_3 = 0.05$. As depicted in the figure, the molar concentration of native enzyme decreases as the time increases while the molar concentration of the denatured enzyme increases as the time increases. The time taken to reach the maximum value of the molar concentration of native enzyme is the same as the time taken to reach the minimum value of the molar concentration of the denature enzyme. The steady values of molar concentrations of native and denatured enzyme depend upon the rate constants.

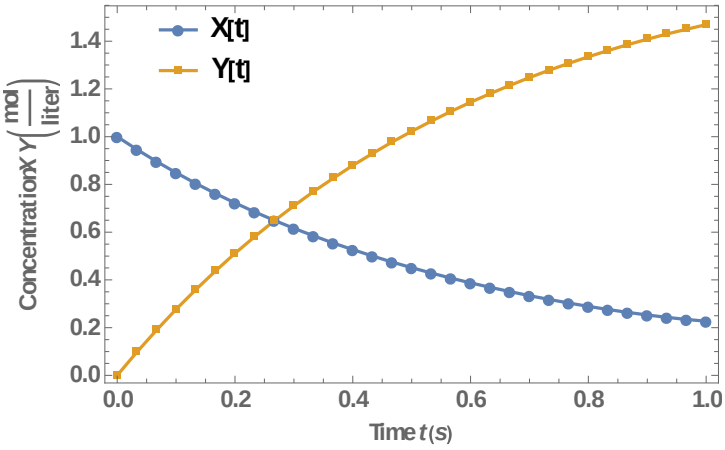

Figure 1: Molar concentrations of native and denatured enzyme when $k_{-1}=1,\ k_{+1}=0.01,\ k_2=0.001,\ k_3=0.05$

Fig. 2 show the effects of dissociation native rate constant (k_{-1}) on molar concentration of denatured enzyme while Fig. 3 depict the effects of dissociation native rate constant (k_{-1}) on molar concentration of native enzyme when $k_{+1}=0.01,\ \ k_2=0.001,\ \ k_3=0.001$. From these figures, it is found that, the value of molar concentration of the denatured enzyme initially increases and reaches the steady state value when $t\geq 5$. Also, the molar concentration of the denatured enzyme increases when k increases and the molar concentration becomes zero when $k_{+1} \leq 0.01\text{s}^{-1}$.

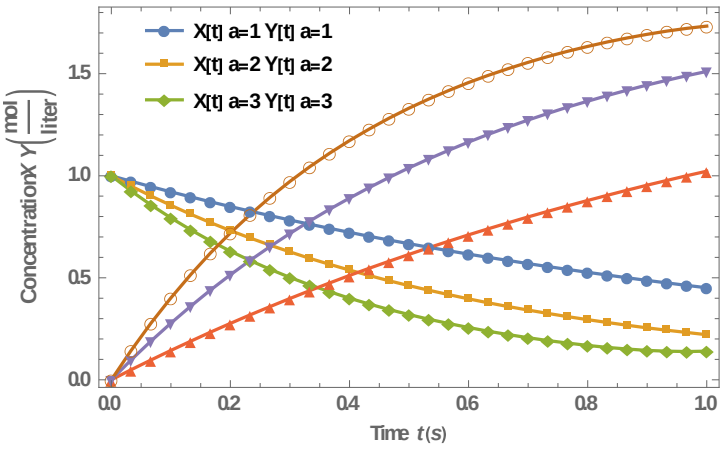

Figure 2: Effects of dissociation native rate constant (k_{-1}) on molar concentration of denatured enzyme

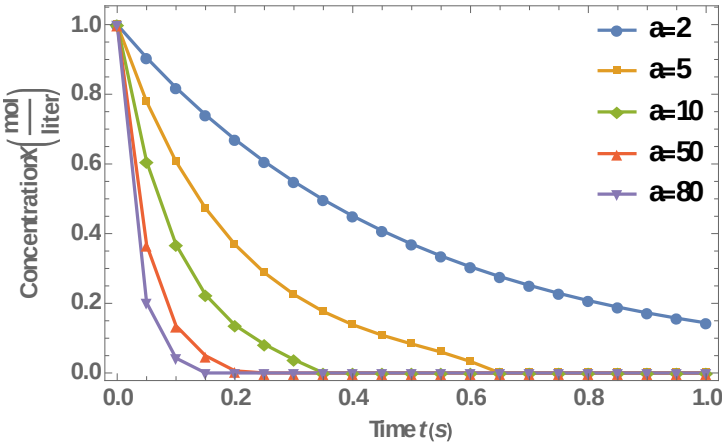

Figure 3: Effects of dissociation native rate constant (k_{-1}) on mmolar concentration of native enzyme when $k_{+1} = 0.01,\quad k_2 = 0.001,\quad k_3 = 0.001$

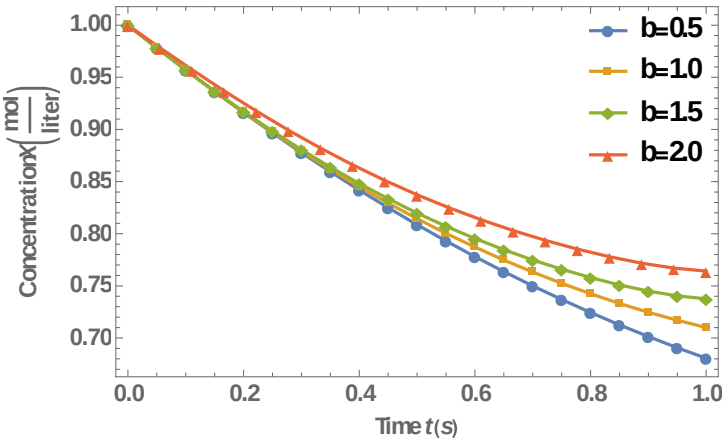

Figure 4: Effects of dissociation native rate constant (k_{+1}) on molar concentration of native enzyme When $k_{-1} = 0.88,\quad k_2 = 0.001,\quad k_3 = 0.00028$

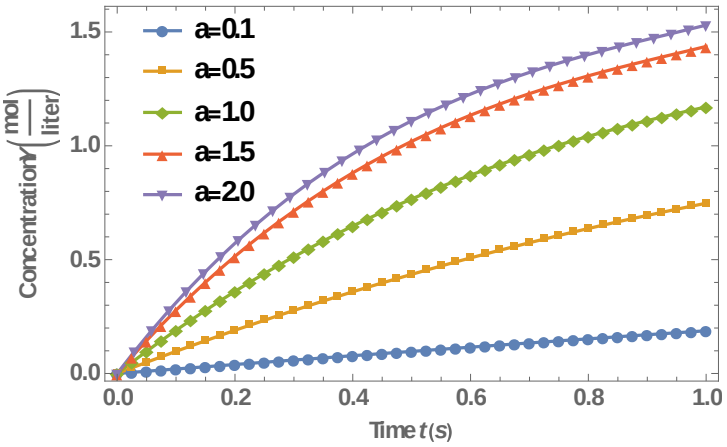

Figure 5: Effects of dissociation native rate constant (k_{-1}) on molar concentration of denaturedenzyme when $k_{+1} = 0.1,\quad k_2 = 0.00026,\quad k_3 = 0.001$

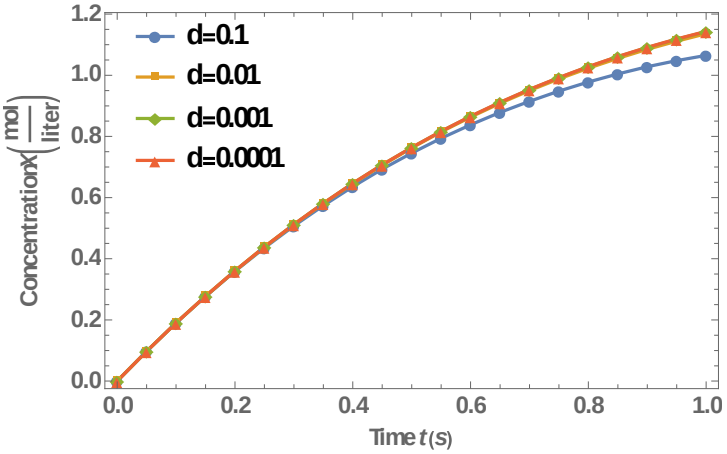

Figure 6: Effects of dissociation native rate constant (k_{-1}) on molar concentration of native enzyme when $k_{-1} = 1,\quad k_2 = 0.1,\quad k_3 = 0.001$

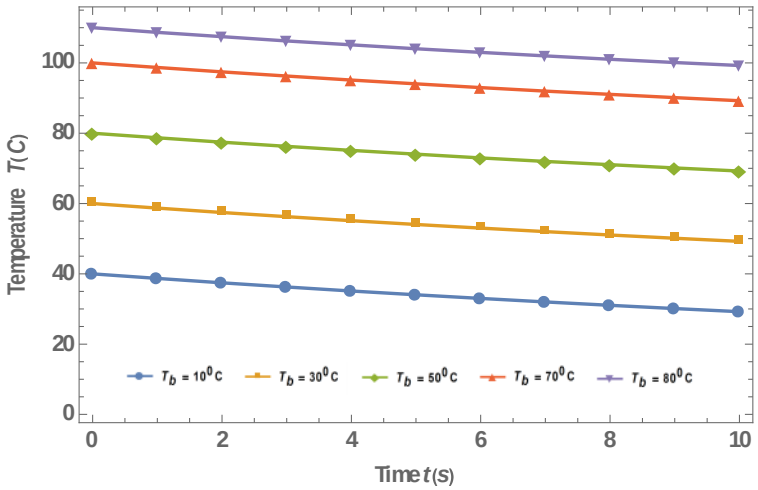

Figure 7: Temperature variation with time of the enzyme when $k_{-1}=1,\ k_2=0.1,\ k_3=0.001$

Fig. 4 presents the effects of dissociation native rate constant (k_{+1}) on molar concentration of native enzyme when $k_{-1}=0.88,\ k_2=0.001,\ k_3=0.00028$ while Fig. 5 shows the effects of dissociation native rate constant (k_{-1}) on molar concentration of denatured enzyme when $k_{+1}=0.1,\ k_2=0.00026,\ k_3=0.001$. Effects of dissociation native rate constant (k_{-1}) on molar concentration of native enzyme when $k_{-1}=1,\ k_2=0.1,\ k_3=0.001$ are shown in Fig. 6.
Fig. 7 show the temperature history of the enzyme when $k_{-1}=1,\ k_2=0.1,\ k_3=0.001$. Also, effects of bath temperature on the temperature history are depicted in the figure. The temperature of the enzyme decreases linearly with time. It could be seen that as the bath temperature, T_B increases, the temperature of the enzyme increase.

4. Conclusion

In this work, approximate analytical solutions for the analysis of kinetic model of thermal inactivation of the jack bean urease (E.C.3.5.1.5) have been developed using Temini and Ansari method method. The analytical solutions are verified with numerical solution using Runge – Kutta with shooting method and good agreements are established. The information given in this theoretical investigation will assist in the kinetic analysis of the experimental results over handling rate constants and molar concentrations.

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