Picard Iteration Method to Kinetic Analysis of Thermal Inactivation of Enzyme as Applied in Biotechnology

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Abstract: In this work, Picard iteration method is used to obtain analytical expressions for the prediction of molar concentration of native and denatured jack bean urease (EC 3.5.1.5) through the three-reaction steps kinetic model of thermal inactivation of the urease. The obtained solutions are used to study the kinetics of thermal inactivation of the enzyme as applied in biotechnology. The analytical solutions are verified with numerical solutions using Runge –Kutta with shooting method and good agreements are established between the solutions. From the parametric studies using the iterative method, 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 information given in this theoretical investigation will assist in the kinetic analysis of the experimental results over handling rate constants and molar concentrations.

Keywords: Enzyme, Iteration Method, Jack Bean Urease, Kinetics, Picard, Thermal Activation

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1 INTRODUCTION

Urease (urea amino hydrolase E.C.3.5.1.5) is a part of the superfamily of amidohydrolases and phosphotriesterases. It catalyzes the hydrolysis of urea to produce ammonia and carbamate. The produced carbamate is subsequently degraded by spontaneous hydrolysis to produce another ammonia and carbonic acid [1]. Consequently, the pH of its environment increases as ammonia is produced. Ureases catalyzes at a rate approximately 10^{14} times faster than the rate of the non-catalyzed reaction [2]. As a nickel-containing metalloenzymes of high molecular weight [3], it can be found in numerous bacteria, fungi, algae, plants and some invertebrates, as well as in soils, as a soil enzyme. Jack bean urease is a nickel containing oligomeric enzyme exhibiting a high degree of specifity to urea [4]. It is the most widely used plant urease [4]. The importance and applications of the urease as a good catalyst for hydrolysis of urea has attracted several research interests [1-19] especially in biotechnology and biomedical engineering studies. Also, the thermostability of jack bean urease has often been a subject of investigation. In chemical reactions, the knowledge of transient phase is crucial for the determination of various system parameters such as the rate constants and for distinguishing among the different mechanisms of enzyme catalysis. The development of symbolic solution is very important as it shows the relationship between the various parameters of the kinetic models. 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 [18]. 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 multi-temperature evaluation revealed that an adequate kinetic model had to incorporate at least three reaction steps [18]. Although, three-step mechanism model of inactivation of the enzyme has been developed by Illeova et al. [18], there is no provision of analytical solutions (except by Ananthi *et al.* [19]) for the predictions of model concentrations of the native enzyme, denature enzyme and temperature for thermal inactivation of urease. Ananthi *et al.* [19] 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 HAM in the analysis of 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, and auxiliary parameters limits the applications of HAM. Moreover, such method requires high skill in mathematical analysis and the solution comes with large number of terms. Although, there various approximate analytical methods in literature for solving linear and nonlinear equations, the recent development in iterative techniques such variational iteration method, variation parameter method, Picard iteration method, perturbation iteration method, Temini and Ansari method, etc. has increased their applications in nonlinear analysis. Picard iteration method is one of the iterative techniques for solving a large class of linear or nonlinear differential equations without the tangible restriction of sensitivity to the degree of the nonlinear term and also it reduces the size of calculations besides, its interactions are direct and straightforward. Therefore, in this work, Picard iteration 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.

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

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]:

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

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

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

Initial conditions are:

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

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:

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

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

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

While the exact solution of "Eq. (2c)" is given as:

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

3 APPROXIMATE ANALYTICAL METHODS OF SOLUTION: PICARD ITERATION 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, Picard iteration method.

3.1. Principle of Picard iteration method

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

$$Lu = Ru + Nu + F\left(u\right) \tag{7a}$$

$$Lv = Rv + Nv + F\left(v\right) \tag{7b}$$

The linear terms are decomposed into *L* + *R*, with *L* taken as the highest order derivative which is easily invertible and R as the remainder of the linear bounded operator of less order than *L*. where *F* is the coupled nonlinear term and *u* is the system output, *Nu* represents the nonlinear terms, which is assumed to be analytic.

The perturbation iteration method gives the possibility to write the solution of the general nonlinear equation (7) in the iterative formula:

$$u_n(t) = u\left(0\right) + \int_0^t \left[R_1 u_{n-1} + N\left(u_{n-1}\right) + F\left(u_{n-1}\right)\right]dt, \quad n \geq 1 \tag{8a}$$

$$v_n(t) = v\left(0\right) + \int_0^t \left[R_2 v_{n-1} + N\left(v_{n-1}\right) + F\left(v_{n-1}\right)\right]dt, \quad n \geq 1 \tag{8b}$$

The successive approximations $u_{n,}\ v_n \quad n \geq 1$, of the solutions will be readily obtained upon using any of selective function u_o *and* v_o. Consequently, the exact solutions may be obtained by using:

$$u = \lim_{n\to\infty} u_n \qquad v = \lim_{n\to\infty} v_n \tag{9}$$

From "Eqs. (4a) and (4b)", using the Picard iteration method, one can write:

$$X_{n+1}(t) = X\left(0\right) + \int_0^t \left[bY_n^2 - aX_n - 2dX_n^2\right]dt, \quad n \geq 1 \tag{10a}$$

$$Y_{n+1}(t) = Y\left(0\right) + \int_0^t \left[2aX_n - 2\left(b + c\right)Y_n^2\right]dt, \quad n \geq 1 \tag{10b}$$

From the initial condition:

$$X_0 = 1, \quad Y_0 = 0 \tag{11}$$

Using the iterative scheme in "Eqs. (10a) and (10b)", we have:

$$X_1 = -\left(a + 2d\right)t \tag{12a}$$

$$Y_1 = 2at \tag{12b}$$

$$X_2 = 1 + \frac{1}{6}\left(4abt + 3a\left(a + 2d\right) - 4d\left(a + 2d\right)^2 t\right)t^2 \tag{13a}$$

$$Y_2 = -\frac{a}{3}\left[3\left(a + 2d\right) + 8a\left(b + c\right)t\right]t^2 \tag{13b}$$

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$$X_3 = 1 + \frac{a^2 b}{63}\left[8a(b+c)^2 t^2 + 56a(b+c)(a+2d)t + 63a^2(a+2d)\right]t^5 - \frac{at}{6}\left[(ab - 4ad^2 - a^2 d - 4d^3)t^3 + a(a+2d)t^2 + 6\right] - 2dt\left\{\begin{array}{l} \frac{1}{63}\left(\begin{array}{l} 4a^4 d^2 - 8a^3 bd + 32a^3 d^3 + 4a^2 b^2 - 32a^2 bd^2 \\ +96a^2 d^4 - 32abd^3 + 128ad^5 \end{array}\right)t^6 \\ +\frac{1}{9}(ba^3 - 6a^3 d^2 - a^4 d - 12a^2 d^3 + 2a^2 bd - 8ad^4)t^5 \\ +\frac{1}{20}(a^4 + 4a^3 d + 4a^2 d^2)t^4 + \frac{1}{3}(ba - 4ad^2 - a^2 d - 4d^3)t^3 \\ +\frac{a}{20}(a+2d)t^2 + 1 \end{array}\right\} \quad (14a)$$

$$Y_3 = \frac{a^2 t}{3}\left[(ab - 4ad^2 - a^2 d - 4d^3)t^3 + a(a+2d)t^2 + 6\right] - \frac{2a^2(b+c)t^5}{9}\left[8at(b+c)(a+2d) + \frac{64a^2 t^2 (b+c)}{7} + \frac{1}{5}\right] \quad (14b)$$

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 in "Eqs. (9)". 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

The comparisons of the results of PIM and NM are shown in "Table 1 and 2". The obtained results of the concentrations using PIM as compared with the numerical procedure using Runge-Kutta method coupled with shooting method are in good agreements. The high accuracy of PIM gives high confidence about validity of the method in providing solutions to the problem.

Table 1 Comparison of results

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

X(t)

t	PIM	NUM
0.00	1.000000	1.000000
0.10	0.896320	0.896320
0.20	0.804239	0.804239
0.30	0.722363	0.722362
0.40	0.649475	0.649479
0.50	0.584542	0.584542
0.60	0.526639	0.526637
0.70	0.474967	0.474965
0.80	0.428821	0.428824
0.90	0.387599	0.387599
1.00	0.350848	0.350748

Table 2 Comparison of results

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

Y(t)

t	PIM	NUM
0.00	0.000000	0.000000
0.10	0.189399	0.189399
0.20	0.359101	0.359101
0.30	0.511178	0.511178
0.40	0.647477	0.647477
0.50	0.769644	0.769644
0.60	0.879150	0.879150
0.70	0.977312	0.977311
0.80	1.065300	1.065300
0.90	1.144173	1.144170
1.00	1.214842	1.214840

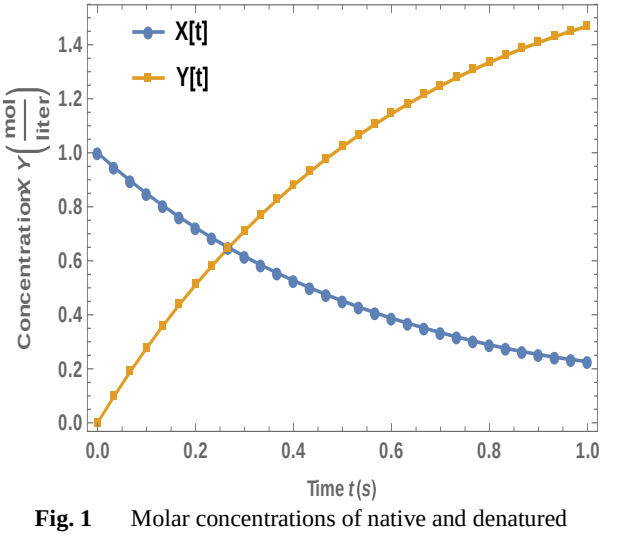

Fig. 1 Molar concentrations of native and denatured enzyme when $k_{-1} = 1,\ k_{+1} = 0.01,\ k_2 = 0.001,\ k_3 = 0.05$.

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Figure 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 2 shows the effects of dissociation native rate constant (k_{-1}) on molar concentration of denatured enzyme while "Fig. 3" depicts 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 $k_{+1} \leq 0.01 s^{-1}$.

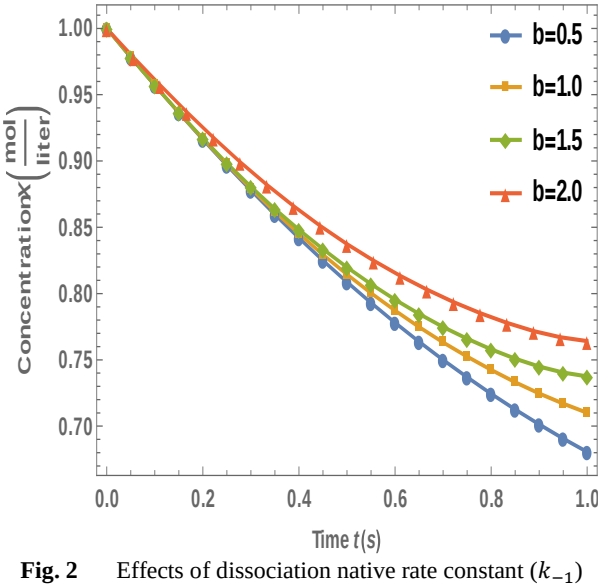
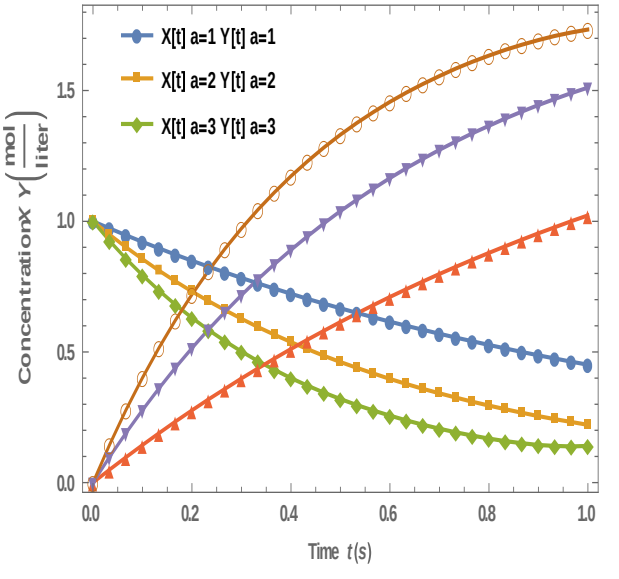


Fig. 2 Effects of dissociation native rate constant (k_{-1}) on molar concentration of denatured enzyme.



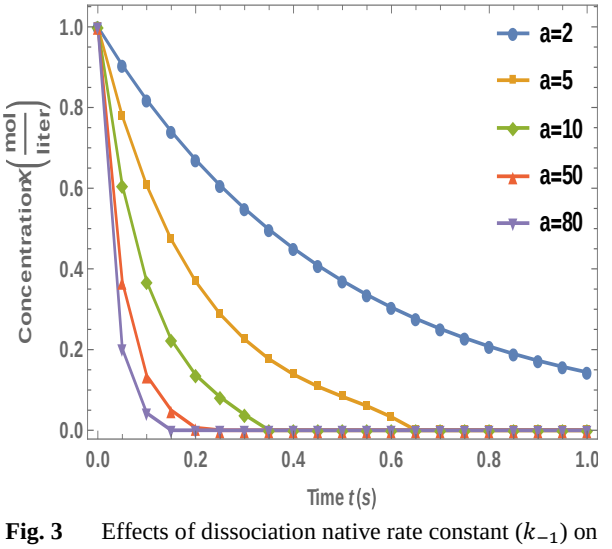


Fig. 3 Effects of dissociation native rate constant (k_{-1}) on molar concentration of native enzyme when $k_{+1} = 0.01$, $k_2 = 0.001$, $k_3 = 0.001$

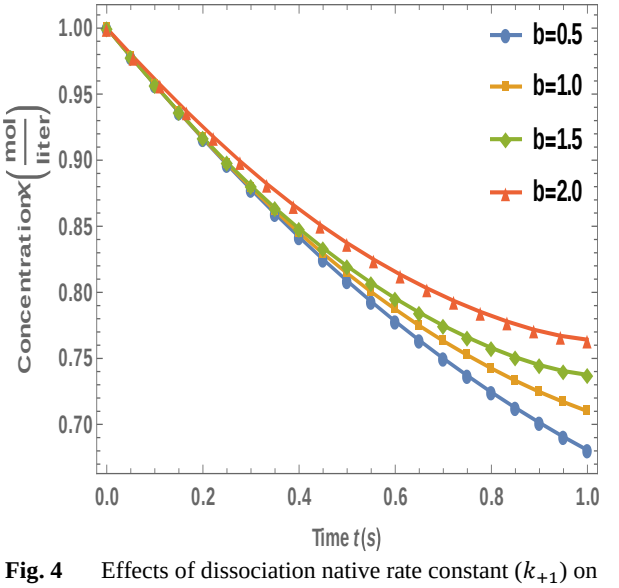


Fig. 4 Effects of dissociation native rate constant (k_{+1}) on molar concentration of native enzyme when $k_{-1} = 0.88$, $k_2 = 0.001$, $k_3 = 0.00028$.

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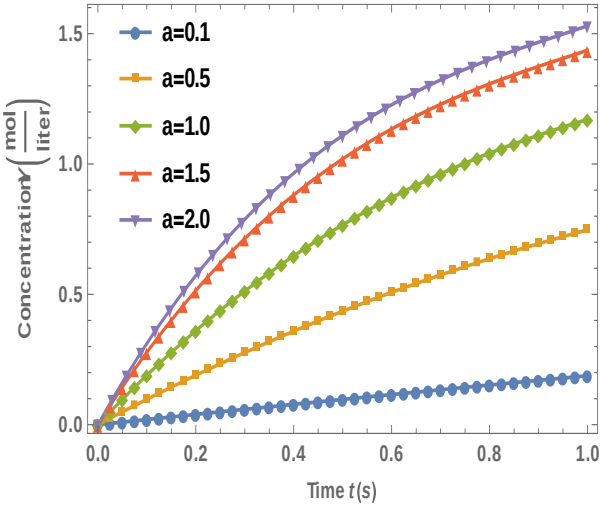

Fig. 5 Effects of dissociation native rate constant (k_{-1}) on molar concentration of denatured enzyme when $k_{+1} = 0.1,\quad k_2 = 0.00026,\quad k_3 = 0.001.$

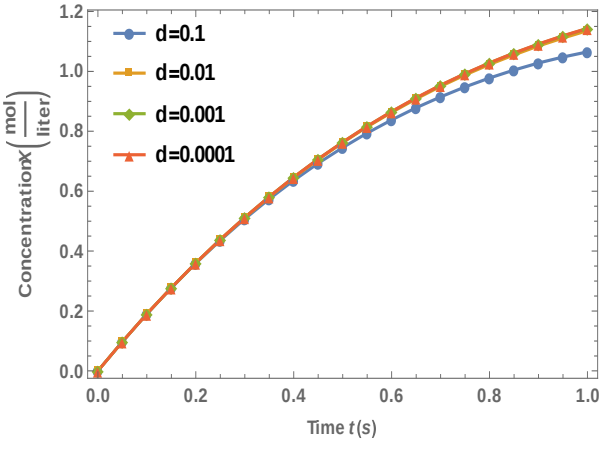

Fig. 6 Effects of dissociation native rate constant (k_{-1}) on molar concentration of native enzyme when $k_{-1} = 1,\quad k_2 = 0.1,\quad k_3 = 0.001.$

Figure 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 $k_{+1} = 0.1,\quad k_2 = 0.00026, k_3 = 0.001$. Effects of dissociation native rate constant (k_{-1}) on molar concentration of native enzyme when $k_{-1} = 1,\quad k_2 = 0.1,\quad k_3 = 0.001$ are shown in "Fig. 6".
Figure 7 shows the temperature history of the enzyme when $k_{-1} = 1,\ k_2 = 0.1,\quad k_3 = 0.001$. 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.

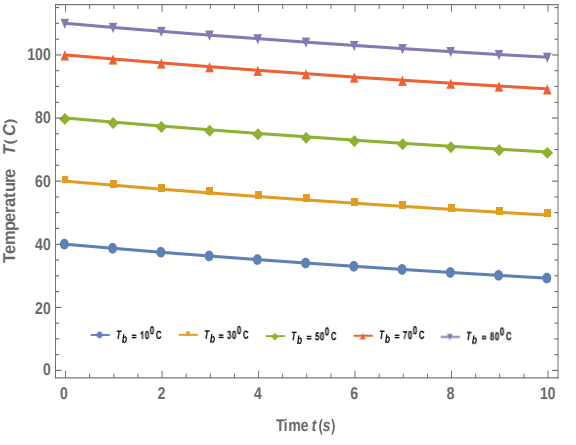

Fig. 7 Temperature variation with time of the enzyme when $k_{-1} = 1,\quad k_2 = 0.1,\quad k_3 = 0.001.$

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 Picard iterative 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.

5 NOMENCLATURE

c_N: Molar concentration of the native enzyme form (mole/cm)
c_D: Molar concentration of the denaturedenzyme 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

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