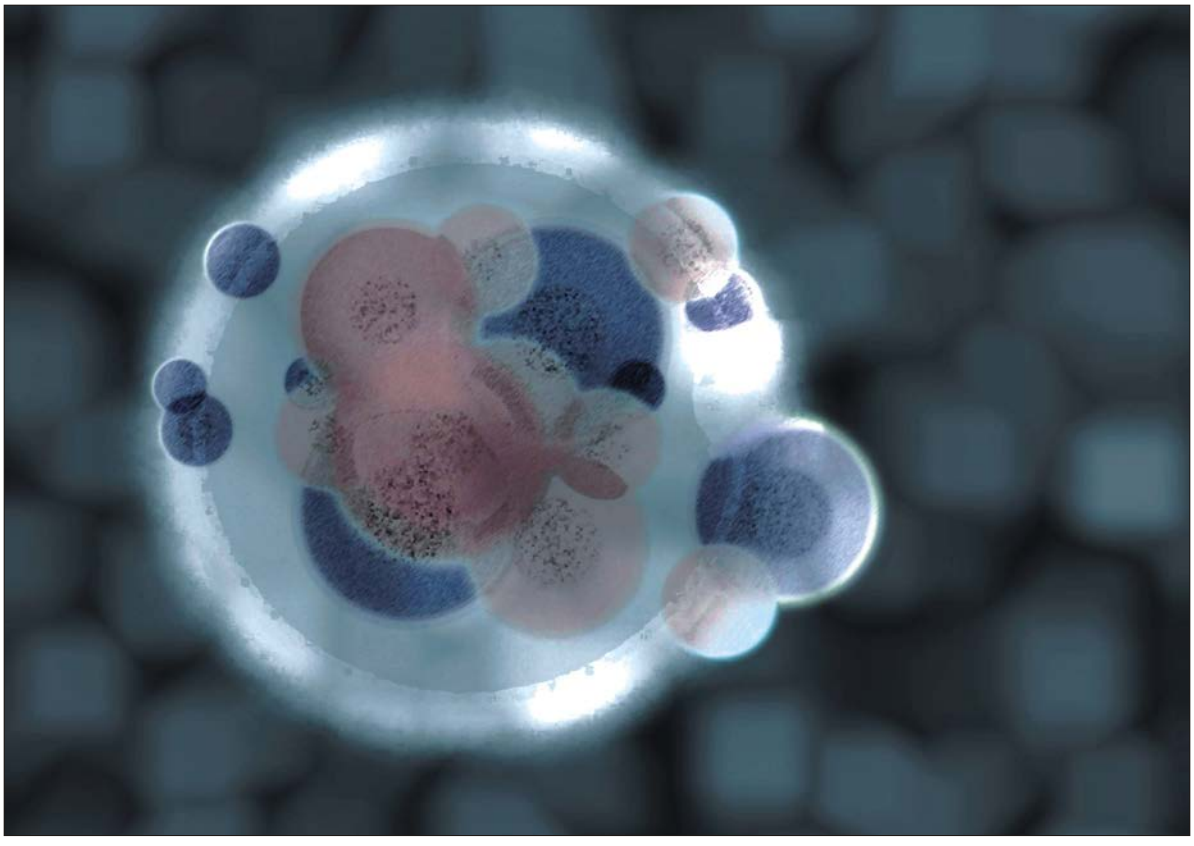

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Nonlinear Optimization of Enzyme Kinetic Parameters

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Abstract: In the analysis of enzyme kinetics data, K_m and V_{max} play a very important role. Linearization of kinetic equation is still a common practice for determining these parameters. Although graphical methods help in understanding the kinetic behavior of enzymes, they have certain shortcomings associated with them due to which they sometimes lead to an anomalous estimation of the kinetic parameters. In order to yield a more accurate estimate of parameters, minimization of least square error can be quite useful. However, since the least square error determination is a non linear function, the usual methods may not be fruitful. This research recommends the use of two simple and fast evolutionary optimization techniques such as Genetic Algorithms (GA) and Particle Swarm Optimization (PSO) which may be applied for the determination of Michaelis Menten (MM) enzyme analysis. We have shown the working of these methods on a set of six enzymes taken from literature along with a unique enzyme, geraniol acetyltransferase (GAAT), purified from the aromatic grass palmarosa. The entire study shows that GA and PSO can be used efficiently for determining accurate values for K_m and V_{max}.

Key words: Genetic algorithms, global optimization, kinetic parameter, Michaelis-Menten enzymes, nonlinear regression, particle swarm optimization

INTRODUCTION

Enzymes are proteinaceous biocatalysts that accelerate the rate of biochemical reactions without being used up in the process. Understanding the kinetics of enzyme catalyzed reactions in pathway enables comprehension of the pace of the biological processes in an organism. Mathematically, enzyme kinetics are governed by several reaction constants and parameters like Michaelis-Menten constant (K_m), V_{max}, K_{cat} etc. Accurate determination of the essential kinetic parameters in the kinetic study of various Michaelis-Menten enzymes within a minimum time scale and handling of huge amount of data is a challenge encountered by the present-day biochemists and molecular biologists when screening an incredible number of mutants for stumbling on an improved strain that produces a kinetically perfect enzyme.

Three most common methods available in literature for determining the parameters of Michaelis-Menten equation are the linearization method of Lineweaver-Burke involving construction of a double reciprocal plot, Hanes plot (Hanes, 1932) and Eadie-Hofstee plot (Eadie, 1942).

In the method of Lineweaver and Burk (1934), a plot is generated from 1/v versus 1/[S] data. The linear equation used to determine the parameters is given by:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$

The slope of the line equals K_m/V_{max}, the x intercept represents $1/K_m$ in second quadrant and the y intercept is $1/V_{max}$. In Eadie-Hofstee plot, v/[S] values are plotted against v. The linear equation is given by:

$$v = V_{max} - K_m \frac{v}{[S]}$$

where, the intercept is V_{max} and the slope denotes K_m.

In Hanes plot [S]/v is plotted against [S]. Here, the linear equation is of the form:

$$\frac{[S]}{v} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}}[S]$$

where, the intercept is K_m/V_{max} and the slope is $1/V_{max}$.

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In all these methods, a linear regression is used to estimate the slope and intercept, K_m and V_{max} are then approximated from the straight line parameters (intercepts in first and second quadrants). Although, the graphical illustrations obtained by these methods are useful in analyzing the behavior of enzymes, there are certain disadvantages associated with these methods. For instance; Lineweaver-Burke plot, the most favoured plot by researchers, has two distinct advantages over the Michaelis-Menten plot, in that it gives a more accurate estimate of V_{max} and more accurate information about inhibition. It increases the precision by linearizing the data. The values for K_m and V_{max} can thus be determined precisely and the error can be estimated quantitatively. However, one major disadvantage of plotting data in the Lineweaver-Burke mode is that the data points in one region are tightly clustered and tend to propagate small differences, which can be overwhelmed by small random errors, thus giving a non-uniform distribution of error. The V-intercept in Lineweaver-Burke plot is equivalent to the inverse of V_{max} due to which the experimental error gets magnified. Similarly the Eadie-Hofstee plot has the disadvantage that v appears on both axes. Experimentally, this quantity is generally subject to more error than substrate concentration.

In order to reduce the errors due to the linearization of parameters Wong (1975) suggested the use of nonlinear optimization techniques for more accurate approximation of Michaelis-Menten parameters (Wong, 1975). With the availability of fast computers and different nonlinear optimization techniques, this task has become fairly simple. In this study we have chosen two simple nonlinear optimization techniques; GA and PSO for determining the kinetic parameters K_m and V_{max} of some selected Michaelis-Menten enzymes. We compared the performance of aforementioned nonlinear optimization techniques with nonlinear regression (Motulsky and Ransnas, 1987) method and Lineweaver-Burke and Hanes plots on a set of six enzymes including a unique enzyme geraniol acetyltransferase (GAAT), purified from the aromatic grass palmarosa (Sharma *et al*., 2005). The present study shows that nonlinear optimization methods like GA and PSO can be used as an alternative for determination of MM enzyme kinetics.

MATERIALS AND METHODS

The estimation of kinetic parameters is an important aspect of enzyme mathematical evaluation of the enzyme catalyzed biochemical reaction (unit) and its pertinent metabolic pathway as a system.

Mathematical model: The mathematical model of the problem is designed using the least square approach. The kinetic parameters K_m and V_{max} should minimize the quadratic error between observed value and the theoretical value. The only constraints associated with the problem are the positive restrictions on the kinetic parameters. The objective function therefore becomes:

$$\text{Min } z = \sum_{i}^{N} \delta_i^2 \quad (1)$$

Where:

$\delta_i = \left(v_i - \frac{V_{max} S_i}{k_m + S_i} \right)$

v_i = Observed value

$\frac{V_{max} S_i}{k_m + S_i}$ = Theoretical value

Subject to: $K_m \geq 0$, $V_{max} \geq 0$ (non negativity conditions).

EVOLUTIONARY ALGORITHMS USED FOR COMPARISON

Evolutionary Algorithms (EAs) are general purpose algorithms for solving optimization problems. Each EA is assisted with special operators that are based on some natural phenomenon. These algorithms are iterative in nature and in each iteration the operators are invoked to reach to optimal (or near optimal) solution. A brief description of the two EAs used in this study is given in the following subsections:

Genetic algorithms: Genetic Algorithms (GAs) are perhaps the most commonly used EA for solving optimization problems. The natural phenomenon which forms the basis of GA is the concept of survival of the fittest. GAs were first suggested by Holland (1975). The main operators of GA are selection, reproduction and mutation. GAs work with a population of solutions called chromosomes. The fitness of each chromosome is determined by evaluating it against an objective function. The chromosomes then exchange information through crossover or mutation. More detail on the working of GAs may be obtained from (Goldberg, 1989). In the present study, a steady state GA using single point crossover and Roulette wheel selection was used.

Particle Swarm Optimization: Particle Swarm Optimization (PSO) was first suggested by Kennedy and Eberhart (1995). The mechanism of PSO is inspired from the complex social behavior shown by the natural species (Kennedy and Eberhart, 1995; Eberhart and Shi, 2001). In PSO, the particles or members of the swarm fly through multidimesional search looking for a potential

solution for solving numerical optimization problems. For a D-dimensional search space the position of the ith particle is represented as $X_i = (x_{i1}, x_{i2},..x_{iD})$. Each particle maintains a memory of its previous best position $P_i = (p_{i1}, p_{i2}... p_{iD})$ and a velocity $V_i = (v_{i1}, v_{i2},...v_{iD})$ along each dimension. During each iteration, the P vector of the particle with best fitness in the local neighborhood was designated as g and the P vector of the current particle were combined to adjust the velocity along each dimension and a new position of the particle is determined using that velocity. The basic equations which direct the working of PSO are:

$$v_{id} = \omega v_{id} + c_1 r_1 (p_{id} - x_{id}) + c_2 r_2 (p_{gd} - x_{id}) \quad (2)$$

$$x_{id} = x_{id} + v_{id} \quad (3)$$

where, c_1, c_2 are acceleration constants, ω is inertia weight predefined by the user and r_1, r_2 are the uniformly generated random numbers in the range of [0, 1].

EXPERIMENTAL SETTINGS

Software used for GAAT enzyme: The M-M plots for the GAAT enzyme from palmarosa are constructed using Sigma Plot software, version 9.0 and K_m and V_{max} are calculated by application of non-linear regression using the same software.

Parameter settings for GA and PSO:
Population size = 10 (for both algorithms).

For GA: The crossover and mutation rates are fixed at 0.5 and 0.05, respectively

For PSO: A linearly decreasing (0.9- 0.4) inertia weight w is taken and acceleration constants c_1, c_2 are fixed as 2.0. Both the algorithms are executed on a PIV PC, using DEV C++

ENZYME DATA SETS

We have carefully chosen a set of six enzymes for the present study. Besides taking enzymes from literature, we have included kinetics data for the purified acetyl CoA: geraniol acetyltransferase (GAAT), an important enzyme involved in volatile ester biosynthesis in the aromatic grass palmarosa (*Cymbopogon martinii* var. *motia*) grown in glasshouse conditions at Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India (Sharma *et al*., 2005). For the GAAT enzyme, one unit is equivalent to $10^{-3} \times$ IU mg^{-1} protein. AATs (Alcohol acetyltransferases) are an important class of plant secondary metabolic enzymes catalyzing non-volatile as well as volatile ester biosynthesis (Walker *et al*., 1999; Beekwilder *et al*., 2004).

RESULTS AND DISCUSSION

In this study, we have used the data from the literature as well as unpublished experimental kinetic data related to a unique plant secondary metabolism enzyme from Palmarosa leaves, named acetyl CoA: geraniol acetyltransferase (GAAT), to test the comparative performance of the conventional versus the two stochastic methods (GA and PSO) (Table 1). Table 2 shows the K_m and V_{max} values obtained from hyperbolic plot, nonlinear regression plot, the Lineweaver plot, Hanes plot, GA and PSO.

In order to check the viability of the algorithm chosen, we initially executed the GA for the two data sets comprising of two enzymes and compared those data with the nonlinear regression and the Lineweaver-Burke results. The kinetic parameters for an enzyme were calculated in presence and absence of enzyme inhibitors (David and Cox, 2004) whiles those for PI-3 kinase were determined in presence and absence of sodium chloride (Michaelis and Menten, 1913). From the numerical results,

Table 1: Input experimental kinetic data sets for palmarosa geraniol acetyl transferase (GAAT) and other enzymes (II to IV)

Geraniol	S (μM)	50, 100, 250, 500, 1000, 2500, 5000, 7500, 10000
	V (unit)	0.663, 1.657, 4.31, 6.962, 8.619, 11.935, 14.255, 14.255, 15.581
Acetyl Co A	S (μM)	50, 100, 250, 500, 750, 1000, 1250
	V (unit)	3.647, 6.962, 11.272, 16.244, 16.907, 16.907, 16.907
Nonanol	S (μM)	25, 50, 100, 250, 500, 1000, 2500, 5000, 7500, 10000
	V (unit)	0.583, 1.360, 2.331, 4.856, 6.021, 7.187, 10.1, 13.402, 14.762, 14.179
(–)-β-citronellol	S (μM)	25, 50, 100, 250, 500, 1000, 2500, 5000, 7500, 10000
	V (unit)	1.360, 2.525, 3.302, 5.050, 6.798, 8.352, 10.683, 10.683, 10.877,10.877
(+)-β-citronellol	S (μM)	25, 50, 100, 250, 500, 1000, 2500, 5000, 7500, 10000
	V (unit)	1.554, 2.525, 4.079, 6.798, 8.935, 11.071, 13.791, 17.093, 17.093, 17.093
Enzyme II	S (μM)	0.08 0.12 0.54 1.23 1.82 2.72 4.24 10
	V (unit)	0.15 0.21 0.70 1.10 1.30 1.50 1.70 1.80
Enzyme III	S (μM)	5 10 20 50 100 200
	V (unit)	22 39 65 102 120 135
Enzyme IV	S (μM)	1600 800 400 200 100
	V (unit)	1.39 1.13 0.83 0.53 0.32

[1 unit of enzyme activity = $10^{-3} \times$IU mg^{-1} protein, where, IU refers to International Units]. For geraniol to (+)-β-citronellol; S = Substrate concentration and V = V_o

Table 2: Values of K_m and V_{max} of GAAT and other enzymes (II to IV) calculated by different methods

Data set	Parameters	V/s hyperbolic plot	Non-linear regression	Lineweaver-Burke plot	Hanes plot	GA	PSO
Geraniol	K_m (μM)	650.0	775.10	727.00	700.00	718.62	775.04
	V_{max}	13.8	16.20	14.29	16.39	16.29	16.19
	Z_{min}*	--	--	--	--	2.46	1.62
Acetyl CoA	K_m (μM)	145.0	190.60	200.00	155.00	190.00	190.00
	V_{max}	17.0	20.52	20.00	20.33	20.50	20.50
	Z_{min}	--	--	--	--	3.66	3.66
Nonanol	K_m (μM)	750.0	853.30	800.00	825.00	860.00	853.26
	V_{max}	13.3	15.38	16.00	15.52	15.38	15.38
	Z_{min}	--	--	--	--	6.18	6.87
(–)-β-citronellol	K_m (μM)	286.0	275.80	200.00	200.00	275.76	277.60
	V_{max}	10.2	11.23	11.76	10.90	11.25	11.23
	Z_{min}	--	--	--	--	1.72	1.73
(+)-β-citronellol	K_m (μM)	410.00	456.20	190.00	400.00	446.57	456.18
	V_{max}	15.86	17.68	12.50	18.25	17.54	17.67
	Z_{min}	--	--	--	--	5.66	5.71
Enzyme II	K_m	1.00	1.007	1.00	--	0.93	1.007
	V_{max}	2.00	2.032	2.00	--	1.98	2.030
	Z_{min}	--				0.028	0.0063
Enzyme III	K_m	26.80	27.94	32.30	--	27.93	27.9400
	V_{max}	161.50	154.70	164.00	--	154.65	154.6500
	Z_{min}			--		14.53	14.5300
Enzyme IV	K_{min}	--	470.500	470.00	--	403.20	470.5400
	V_{max}	--	1.798	1.75	--	1.685	1.7990
	Z_{min}				--	0.00316	8.3e-05

*Z_{min} (function value) is the minimum error detected by two methods

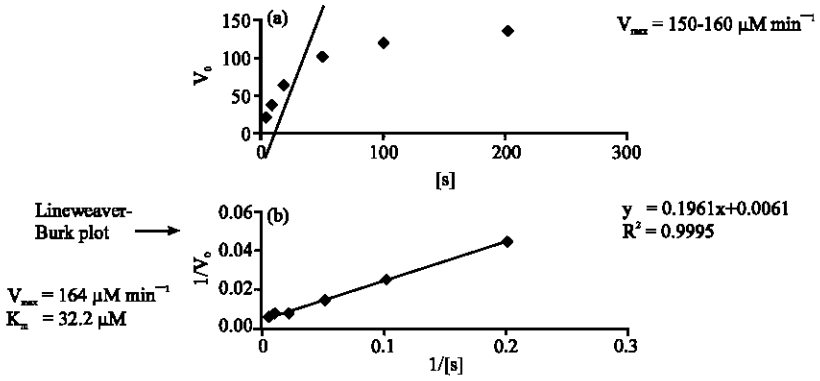

Fig. 1: The distribution of output data of enzyme III. (a) V_{max} values and (b) Calculations of K_m and V_{max} using the Lineweaver-Burke plot

it was evident that the stochastic methods can be conveniently used to determine the K_m and V_{max} of the enzymes.

The applicability of the stochastic methods was then tested by using the experimental data obtained for an enzyme GAAT. The Lineweaver-Burke (L-B) plot estimated values of K_m and V_{max} were 727 μM and 14.29 units for geraniol and 200 μM and 20 units for acetyl CoA, respectively (Table 2). The Michaelis-Menten (M-M) plot showed the K_m and V_{max}, respectively as 775.1 μM and 16.20 units for geraniol and 190.6 μM and 20.52 units for K_m and V_{max} for acetyl CoA. On critical observation and comprehension of the values, it was revealed that GA and PSO analyses, especially the latter, showed similar results: 718.62, 16.29 μM (GA) and 775.04 and 16.19 μM (PSO) for geraniol; 190 μM, 20.50 units (GA) and 190 μM and 20.50 units (PSO) for acetyl CoA, respectively. However, in case of nonanol and (+)-β-citronellol, the kinetic values i.e., K_m and V_{max} obtained by the PSO method (853.26 μM, 15.38 units for nonanol and 456.18 μM, 17.67 units for (+)-β-citronellol) were highly comparable and much closer to the values obtained by the M-M plots (853.30 μM, 15.38 units for nonanol and 456.20 μM, 17.68 units for (+)-β-citronellol, respectively) than the GA method (860 μM, 15.38 units and 446.57 μM, 17.54 units), especially the K_m values (Table 2). Quite interestingly the L-B values (K_m 190 μM, V_{max} 12.5 units) obtained for the substrate (+)-β-citronellol were nowhere nearer the values obtained by the Hanes, GA and the PSO methods.

If we analyze this data (Enzyme II, Table 2) we would observe that as a result of the distribution of the output data of the enzyme (Enzyme III) shown in Fig. 1, the V_{max}

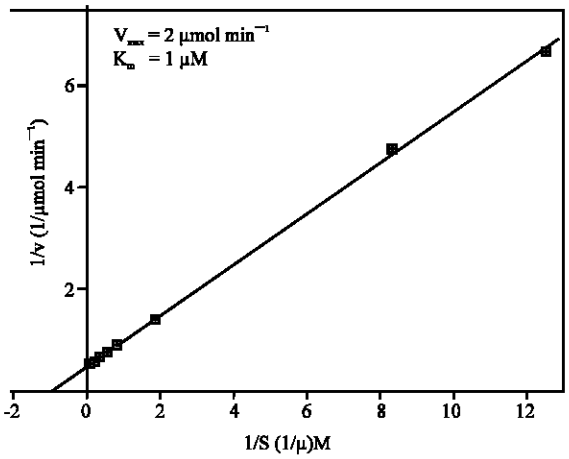

Fig. 2: Plotting the reciprocals of [S] and Vo yields a line whose slope and intercepts yield values of K_m and V_{max}

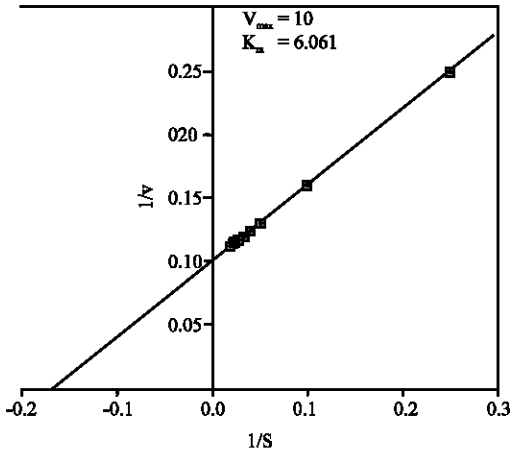

Fig. 3: Calculation of K_m and V_{max} by Lineweaver-Burke plot (data set 3)

values can fall between 150 and 160 µM min^{-1} and as per the L-B plot the value of the V_{max} and the K_m are 164 µM min^{-1} and 32.2 µM, respectively. But the GA and PSO are able to minimize the errors yielding the much improved results where, the V_{max} is 154.65 µM min^{-1} and the K_m is 27.94 µM, respectively

Similarly when considering the values of Enzyme IV we would observe that as per the LB plot the K_m and the V_{max} are estimated to be 470.50 µM and 1.75 µM min^{-1}. When GA and PSO were used, the kinetic data gave 450 µM as estimates of K_m and 1.75 µM min^{-1} as V_{max} and by PSO the corresponding values of K_m and V_{max} were 470.54 µM and 1.79 µM min^{-1}, respectively.

When we took another set of values (Enzyme II, Table 2) the L-B plot determined the K_m as 1 µM and V_{max} as 2 µmol min^{-1}. Inspection of M-M plot allowed reasonable estimate of V_{max} in the range of 1.8 to 2.0 µM min^{-1}. As indicated in the L-B plot (Fig. 2) the y intercept is 0.5 ($1/V_{max}$) and the x intercept is −1.0 ($-1/K_m$). Thus, V_{max} is computed to be 2.0 µmol min^{-1} and the K_m as 1.0 µM. In this case, some data points are very tightly clustered or grouped near the origin end of the line while a few data points falling at other end are too distant and slope of the line seems to be influenced by the low and usually less precisely determined rates at low substrate concentrations. It is this visual uncertainty in this plot Fig. 3 that advises to choose an alternate method that obviates the problem. However, when the PSO and the GA methods were applied, the kinetic parameters were estimated as 1.007 µM (K_m) and 2.03 µM min^{-1} (V_{max}) and 0.93 µM (K_m) and 1.98 µM min^{-1} (V_{max}), respectively. This first set of data obtained with the PSO approach was found almost identical with the results of the nonlinear regression by GraphPad Prism 6.0 i.e., K_m and the V_{max} as 1.007 µM and 2.03 µM min^{-1}, respectively.

Numerically all the values for each enzyme look similar to each other, however with the help of the least square error method we can see the values of parameters for which the error is minimum. Thus from Table 2, we may say that best values obtained are for the Enzyme IV, for which the minimum value (Z_{min}) is 8.3×10^{-5} (as obtained by PSO), this shows that observed values are more or less similar to the theoretical values. However, the poorest value obtained is 14.53 for the Enzyme III. In all other cases the minimum error is less than 10.00. This shows the credibility of the least square method and also of evolutionary algorithms. If we compare the two evolutionary algorithms, we find that both the algorithms gave almost similar values for Z_{min} (the objective function).

CONCLUSION

In the present study, we considered the option of using two evolutionary algorithms GA and PSO for calculating the MM enzyme parameters K_{max} and V_{max} on a set of five enzymes from literature and the unpublished data set one unique plant enzyme. We do not claim the superiority of GA and PSO, over the conventional methods, nor do we totally reject the use of conventional techniques. The aim of this study is to discuss the use nonlinear optimization methods as an alternative for determining MM parameters. The choice of GA and PSO in the present study is purely the authors' decision and any other evolutionary optimization technique may be used. However, we would like to add that the mathematical model of the problem do gives a better picture of how much the error is minimized.

Also, the algorithms used in the paper are simple and easy to apply and even researchers with little or no mathematical background can use them with ease, which may help to solve more complex problems.

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