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Jeričević, Željko

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Non-linear Optimization of Parameters in Hill Kinetics

Željko Jeričević

^a Department of Computer Engineering, Technical Faculty, Vukovarska 58, HR-51000 Rijeka, Croatia

^b Department of Biology and Medical Genetics, Medical Faculty, Braće Branchetta 20, HR-51000 Rijeka, Croatia

Author's e-mail address: zeljko.jericevic@riteh.hr

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THIS PAPER IS DEDICATED TO THE LOVING MEMORY OF IVANA WEYGAND-ĐURAŠEVIĆ (1952 – 2014)

Abstract: A novel least squares optimization of parameters in Hill enzyme kinetics has been developed. The method is based on transformation into "Michaelis-Menten like" type of equation with guaranteed convergence and the initial guess equal to zero. By using this approach, a three parameters non-linear optimization problem is transformed into a one-dimensional search for Hill's coefficient (exponential parameter n). Numerical experiments illustrating feasibility of the method and quality of the solution are presented.

Keywords: Hill, enzyme kinetics, non-linear, optimization, data processing, least squares.

INTRODUCTION

LINEARIZATION of kinetic equations in order to determine kinetic parameters by the linear least squares is still in use for chemical and enzyme kinetics. However, linear optimization has serious drawbacks like redistribution of points and changing error statistics. Moreover, for the Hill model^[1] linearization requires an initial guess for the parameter V_{max} , which is an added complication.

THEORY

There are multiple forms of Hill kinetics. We will analyze the one described by the following equation which resembles Micheaelis-Menten equation but for the parameter n :

$$v = \frac{V_{max} S^n}{K_d + S^n} \quad (1)$$

where S is a concentration of substrate, v is velocity of reaction, V_{max} is saturation velocity, K_d is apparent dissociation constant, and n is Hill coefficient. Another form of Equation (1) has a K_A , which is ligand concentration at half saturation. The relationship between these two constants is $K_d = (K_A)^n$.

In order to determine V_{max} , K_d , and n from a collected series of measurements, v as a function of S is fitted to the model. Linearization transforms Equation (1) as follows:

$$vK_d + vS^n = V_{max} S^n \quad (2)$$

$$v = \frac{(V_{max} - v)}{K_d} S^n \quad (3)$$

$$\log v = n \log S + \log(V_{max} - v) - \log K_d \quad (4)$$

$$\log \frac{v}{V_{max} - v} = n \log S - \log K_d \quad (5)$$

The Equation (5) shows that the intercept in this plot $\log(v/(V_{max}-v))$ vs. $\log(S)$ is $-\log(K_d)$ with slope n . The construction of the plot requires an initial value for V_{max} .

It is well known that linearization does not yield a correct estimate of parameters and strongly recommends the use of non-linear optimization techniques (see Wong^[2] for extensive discussion of this fact for Michaelis-Menten kinetics). However, he recommends general non-linear optimization routines which require a good initial guess and careful balancing of parameters. Jeričević and Kušter^[3] proposed a specialized nonlinear estimation method valid

only for the Michaelis-Menten model. This proposed alternative method has guaranteed convergence with an initial guess of K_M equal to zero and has no problem balancing the parameters. The main idea of the approach is to first reduce the dimensionality of the problem (by eliminating the linear parameter V_{\max} from the normal equations) and then find the root of resulting non-linear equation. The work presented here is an application of a similar idea to the Hill equation.

Normal equations^[4] are developed using the least squares formalism:

$$\sum_{i=1}^N \delta_i^2 = \sum_{i=1}^N (v_i - {}^{th}v_i)^2 = \sum_{i=1}^N \left(v_i - \frac{V_{\max} S_i^n}{K_d + S_i^n} \right)^2 \quad (6)$$

where δ_i is error between the experimental v_i and theoretical ${}^{th}v_i$ value of dependent variable for experimental point i . The associated value of independent variable is S_i and N is number of experimental points.

Taking the derivative of the Equation (6) with respect to V_{\max} (knowing it will be zero at the minimum) yields:

$$\frac{\partial}{\partial V_{\max}} \sum_{i=1}^N \delta_i^2 = -2 \sum_{i=1}^N \left(v_i - \frac{V_{\max} S_i^n}{K_d + S_i^n} \right) \frac{S_i^n}{K_d + S_i^n} = 0 \quad (7)$$

Taking the derivative of the Equation (6) with respect to K_d (again knowing it will be zero at the minimum) yields:

$$\frac{\partial}{\partial K_d} \sum_{i=1}^N \delta_i^2 = 2 \sum_{i=1}^N \left(v_i - \frac{V_{\max} S_i^n}{K_d + S_i^n} \right) \frac{V_{\max} S_i^n}{(K_d + S_i^n)^2} = 0 \quad (8)$$

Taking the derivative of the Equation (6) with respect to n (again knowing it will be zero at the minimum) yields:

$$\frac{\partial}{\partial n} \sum_{i=1}^N \delta_i^2 = 2 \sum_{i=1}^N \left(v_i - \frac{V_{\max} S_i^n}{K_d + S_i^n} \right) \cdot \left(\left(\frac{S_i^n}{K_d + S_i^n} - 1 \right) \frac{V_{\max} S_i^n \ln S_i}{K_d + S_i^n} \right) = 0 \quad (9)$$

Combining the Equations (7) with (8), and Equation (8) with (9) removes linear parameter V_{\max} and yields two nonlinear equation in K_d and n . By this procedure three parameters non-linear optimization problem is transformed into finding the solution of the system of two nonlinear equations: $f(K_d, n)$ shown as Equation (11) and $g(K_d, n)$ shown as Equation (13).

$$V_{\max} = \frac{\sum_{i=1}^N \frac{v_i S_i^n}{K_d + S_i^n}}{\sum_{i=1}^N \frac{S_i^{2n}}{(K_d + S_i^n)^2}} = \frac{\sum_{i=1}^N \frac{v_i S_i^n}{(K_d + S_i^n)^2}}{\sum_{i=1}^N \frac{S_i^{2n}}{(K_d + S_i^n)^3}} \quad (10)$$

$$f(K_d, n) = \sum_{i=1}^N \frac{v_i S_i^n}{K_d + S_i^n} \sum_{i=1}^N \frac{S_i^{2n}}{(K_d + S_i^n)^3} - \sum_{i=1}^N \frac{v_i S_i^n}{(K_d + S_i^n)^2} \sum_{i=1}^N \frac{S_i^{2n}}{(K_d + S_i^n)^2} = 0 \quad (11)$$

$$V_{\max} = \frac{\sum_{i=1}^N \frac{v_i S_i^n}{K_d + S_i^n}}{\sum_{i=1}^N \frac{S_i^{2n}}{(K_d + S_i^n)^2}} = \frac{\sum_{i=1}^N \left(\frac{S_i^n}{K_d + S_i^n} - 1 \right) \frac{v_i S_i^n \ln S_i}{K_d + S_i^n}}{\sum_{i=1}^N \left(\frac{S_i^n}{K_d + S_i^n} - 1 \right) \frac{S_i^{2n} \ln S_i}{(K_d + S_i^n)^2}} \quad (12)$$

$$g(K_d, n) = \sum_{i=1}^N \frac{v_i S_i^n}{K_d + S_i^n} \sum_{i=1}^N \left(\frac{S_i^n}{K_d + S_i^n} - 1 \right) \frac{S_i^{2n} \ln S_i}{(K_d + S_i^n)^2} - \sum_{i=1}^N \left(\frac{S_i^n}{K_d + S_i^n} - 1 \right) \frac{v_i S_i^n \ln S_i}{K_d + S_i^n} \cdot \sum_{i=1}^N \frac{S_i^{2n}}{(K_d + S_i^n)^2} = 0 \quad (13)$$

The root can be found iteratively using Newton-Raphson method^[5] and Jacobian matrix $J(K_d, n)$ shown as Equation (14):

$$J(K_d, n) = \begin{bmatrix} \frac{\partial f(K_d, n)}{\partial K_d} & \frac{\partial f(K_d, n)}{\partial n} \\ \frac{\partial g(K_d, n)}{\partial K_d} & \frac{\partial g(K_d, n)}{\partial n} \end{bmatrix} \quad (14)$$

$$(K_d)_{new} = K_d - \frac{1}{|J(K_d, n)|} \begin{vmatrix} f(K_d, n) & \frac{\partial f(K_d, n)}{\partial n} \\ g(K_d, n) & \frac{\partial g(K_d, n)}{\partial n} \end{vmatrix} \quad (15)$$

$$n_{new} = n - \frac{1}{|J(K_d, n)|} \begin{vmatrix} \frac{\partial f(K_d, n)}{\partial x} & f(K_d, n) \\ \frac{\partial g(K_d, n)}{\partial x} & g(K_d, n) \end{vmatrix} \quad (16)$$

Although the above system consisting of Equations (15) and (16) with good initial guess can be solved this way, it is much more complicated than the single equation used in Michaelis-Menten kinetics, repeated here for convenience:

$$f(K_M) = \sum_{i=1}^N \frac{v_i S_i}{K_M + S_i} \sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^3} - \sum_{i=1}^N \frac{v_i S_i}{(K_M + S_i)^2} \sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^2} = 0 \quad (17)$$

Besides of complexity for Hill equation, the more serious problem is a requirement for good initial guess for two parameters: K_d , and n . For that reason, we also developed a simpler approach, namely, for each guess of n , the system was transformed into Michaelis-Menten like one,

using simple substitution $X=S^n$. That transforms the problem into systematic, stepwise one-dimensional search for optimal n , using guaranteed convergence of Michaelis-Menten in each step for different guesses of n .

$$X = S^n$$

$$f(K_d) = \sum_{i=1}^N \frac{v_i X_i}{K_d + X_i} - \sum_{i=1}^N \frac{X_i^2}{(K_d + X_i)^3} \quad (18)$$

$$-\sum_{i=1}^N \frac{v_i X_i}{(K_d + X_i)^2} + \sum_{i=1}^N \frac{X_i^2}{(K_d + X_i)^2} = 0$$

RESULTS AND DISCUSSION

Simulated data were constructed using different levels (0, 1, 5, and 10 percent) absolute error of V_{max} value. The noise was normally distributed with zero mean and added to the theoretical data. Each noise level was generated independently and then used to illustrate our procedure (see Table 1). The recovered and true values for parameters K_d and V_{max} with error statistics^[6] are shown in Table 2. Graph of model data and simulated points with the corresponding noise levels used in Table 1 and 2 are shown in Figure 1.

Table 1. demonstrates that transforming Hill equation into Michaelis-Menten allows the least squares estimation of parameters K_d and V_{max} for selected n . In other words, doing one-dimensional search for n by optimizing Michaelis-Menten with guaranteed convergence in each step will yield the optimal value for n . The purist still may wish to use Newton-Raphson method for the few final improvement iterations. The Newton-Rapson method for the Hill equation as outlined in Equations (14–16) will converge if parameters n and K_d from one-dimensional search are used as initial guesses. At that point, the closeness to the root will assure the convergence of Newton-Raphson method.

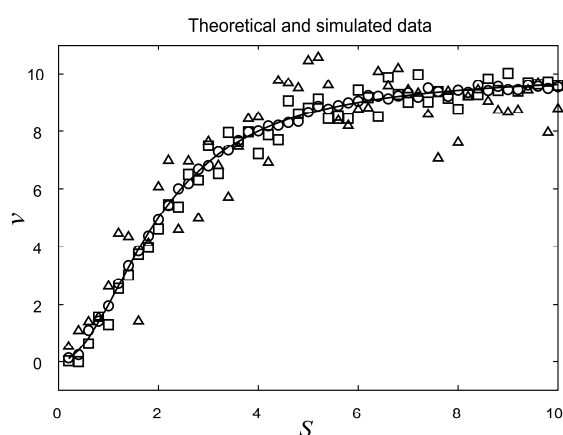


Figure 1. Plot of data summarized in Tables 1 and 2. Line is theoretical data ($n=2$, $K_d=4$, $V_{max}=10$). Circles are data with 1 % error, squares are data with 5 % error and triangles are data with 10 % error.

Table 1. The least squares sum for simulated data with various error levels. Three guesses for parameter n are shown, true value is $n=2$)

% error	$n=1.5$	$n=2$	$n=2.5$
0	2.846	4×10^{-10}	1.905
1	0.996	0.4906	2.423
5	11.153	7.007	7.674
10	57.202	53.886	55.462

Table 2. Recovered parameters for $n=2$. True values are $K_d=4$ and $V_{max}=10$

% error	K_d	error K_d	V_{max}	error V_{max}	Graph symbol
0	4.000	2×10^{-6}	10.000	7×10^{-7}	line
1	4.001	0.061	9.995	0.027	circle
5	4.432	0.252	10.009	0.105	square
10	3.262	0.544	9.623	0.263	triangle

By combining a previously published^[3] method that has guaranteed convergence for optimizing Michaelis-Menten kinetics with one dimensional search for the Hill coefficient n , our results show it is possible to construct a very robust and accurate non-linear optimization targeted to the Hill equation. The method also has the advantage of simplicity and speed. Guaranteed convergence algorithms are particularly important in analyzing large data sets from data intensive methods like sensor data.^[7]

CONCLUSION

A specialized least squares algorithm for determining the parameters in the Hill equation using experimental measurement in enzyme kinetics was developed. The algorithm is simple, fast, and numerically stable. It transforms the nonlinear problem of optimizing three parameters into an efficient one dimensional search for Hill coefficient n .

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