

Non-linear optimization of parameters in Michaelis-Menten kinetics*

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A novel least squares optimization of parameters in Michaelis-Menten enzyme kinetics has been developed. The method is based on elimination of linear parameter from so called normal equations which transforms a non-linear, two-parameter optimization problem into one of finding a root of non-linear equation. The algorithm is simple and has guaranteed convergence with initial guess of zero for K_M . Data set from literature is used to illustrate feasibility of the method and accuracy of the solution in comparison with linearization methods and general optimization technique.

INTRODUCTION

In order to determine kinetic parameters by the linear least squares method, linearization of kinetic equations is still an established practice in chemical and enzyme kinetics. Although simplicity of linear optimization is tempting, that approach has some pitfalls, for example changing error statistics. For the Michaelis-Menten model three different linearization models are available: Lineweaver-Burk plot ($1/v$ versus $1/S$), Eadie plot (v versus v/S) and Hanes plot (S/v versus S). Generally, the linearization models will give a different estimation of model parameters and all of them will also differ from the result of nonlinear optimization.

We propose a variant of nonlinear optimization based on elimination of linear parameter from so called normal equations which transforms a non-linear, two-parameter optimization problem into one of finding a root of non-linear equation.

THEORY

Michaelis-Menten kinetics is described by the following equation:

$$v = \frac{V_{\max} S}{K_M + S} \quad (1)$$

where S is a concentration of substrate, v is the initial velocity of reaction, V_{\max} is the saturation velocity and K_M is Michaelis-Menten constant.

In order to determine V_{\max} and K_M from a collected series of measurements of v as function of S the measurements are fitted to the model (Eq. (1)). Linearized models transform the above equation into the form which can be graphed as a straight line. Lineweaver-Burk¹ plot ($1/v$ versus $1/S$) is defined by the following equation:

$$\frac{1}{v} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \frac{1}{S} \quad (2)$$

* Dedicated to Dr. Edward C. Kirby in happy celebration of his 70th birthday.

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The intercept in this plot is $1/V_{\max}$ and slope is K_M/V_{\max} . Eadie² plot (v versus v/S) has the following form:

$$v = V_{\max} - K_M \frac{v}{S} \quad (3)$$

The intercept in Eadie plot is V_{\max} and slope is K_M . Hanes³ plot (S/v versus S) has the following form:

$$\frac{S}{v} = \frac{K_M}{V_{\max}} + \frac{1}{V_{\max}} S \quad (4)$$

The intercept in Hanes plot is K_M/V_{\max} and slope is $1/V_{\max}$.

The above described linear transformations allow use of linear regression to estimate slope and intercept of the straight line by the linear least squares method, and afterwards the K_M and V_{\max} are calculated from the straight line parameters. They were developed and widely used before the age of computers. However, they are still heavily entrenched, probably because the biochemistry courses typically do not assume knowledge of non-linear regression, and the linear plots can be very illustrative in teaching basic enzyme kinetics. It is worth to point out that in computer age the linear transformations are not proper way for analyzing this type of data in spite of their visual appeal.

The common problem with these transformations is the fact that transformed data usually do not satisfy the assumptions of linear regression, namely that the scatter of points around the straight line follows a Gaussian distribution, and that the standard deviation is equal at every value of the independent variable. Wong⁴ points out that the linearization does not yield correct estimate of the parameters and strongly recommends use of non-linear optimization techniques. The whole chapter 11 in his book⁴ is dedicated to general non-linear optimization methods. However, non-linear optimization methods require a good initial guess, careful balancing of parameters and there is no guarantee of convergence to the global minimum. These facts may explain continuing study and use of linearization methods. Hoppe and Cumme⁵ performed series of numerical experiments to find a proper weighting in linearization methods. Page⁶ used linear regression results as initial guess for non-linear optimization. Marszalek *et al.*⁷ constructed specialized program (LEHM) for fitting Michaelis-Menten and Hill^{8,4} models and they also use linearization results for initial guess and Marquardt⁹ algorithm for nonlinear optimization, which is more complicated than the approach proposed here. Modern bioinformatics package EMBOSS¹⁰ uses Line-weaver-Burk plot.

In order to avoid these difficulties and complications we developed a specialized nonlinear estimation method valid only for Michaelis-Menten model (with possible extension to Hill's model) which has a guaranteed con-

vergence with initial guess zero for K_M and has no problem of balancing the parameters. The main idea of our approach is to reduce the dimensionality of the problem by eliminating linear parameter V_{\max} from normal equations and find the root of a resulting non-linear equation.

So called normal equations¹¹ are developed using the well-known least squares formalism:

$$\begin{aligned} \sum_{i=1}^N \delta_i^2 &= \sum_{i=1}^N (v_i - {}^t v_i)^2 \\ &= \sum_{i=1}^N \left(v_i - \frac{V_{\max} S_i}{K_M + S_i} \right)^2 \end{aligned} \quad (5)$$

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

Taking the derivative of the above equation with respect to V_{\max} (knowing it has to be zero at the minimum) one gets:

$$\begin{aligned} \frac{\partial}{\partial V_{\max}} \sum_{i=1}^N \delta_i^2 &= -2 \sum_{i=1}^N \left(v_i - \frac{V_{\max} S_i}{K_M + S_i} \right) \frac{S_i}{K_M + S_i} \\ &= 0 \end{aligned} \quad (6)$$

Taking the derivative of the same equation with respect to K_M (again knowing it has to be zero at the minimum) yields:

$$\begin{aligned} \frac{\partial}{\partial K_M} \sum_{i=1}^N \delta_i^2 &= 2 \sum_{i=1}^N \left(v_i - \frac{V_{\max} S_i}{K_M + S_i} \right) \frac{V_{\max} S_i}{(K_M + S_i)^2} \\ &= 0 \end{aligned} \quad (7)$$

Combining the two normal equations above removes linear parameter V_{\max} and yields nonlinear equation in K_M . This is the key point of our approach: by this procedure two parameters non-linear optimization problem is simply transformed into one of finding the root of nonlinear equation in single independent variable (K_M).

$$V_{\max} = \frac{\sum_{i=1}^N \frac{v_i S_i}{K_M + S_i}}{\sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^2}} \quad (8a)$$

$$= \frac{\sum_{i=1}^N \frac{v_i S_i}{(K_M + S_i)^2}}{\sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^3}} \quad (8b)$$

TABLE I. Input data set taken from Wong⁴ (p. 245)

$S / \text{mmol dm}^{-3}$	0.25	0.30	0.40	0.50	0.70	1.00	1.40	2.00
$v / \mu\text{mol dm}^{-3} \text{min}^{-1}$	2.4	2.6	4.2	3.8	6.2	7.4	10.2	11.4

Transforming the above function into $f(x) = 0$ form yields:

$$f(K_M) = \left(\sum_{i=1}^N \frac{v_i S_i}{K_M + S_i} \right) \left[\sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^3} \right] - \left[\sum_{i=1}^N \frac{v_i S_i}{(K_M + S_i)^2} \right] \left[\sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^2} \right] \quad (9)$$

$$= 0$$

which is non-linear function in K_M and its solution is optimized value for K_M as determined from experimental data. The original two-parameter, non-linear optimization problem is transformed into one dimensional problem of finding the root of nonlinear equation. Among the methods that could be applied, Newton-Raphson method seems to be quite appropriate:

$$f(x_{j+1}) = x_j - \frac{f(x_j)}{f'(x_j)} \quad (10)$$

where j is iterations index and prime denotes first derivative. In our case $f'(x)$ is:

$$f'(K_M) = \left[\sum_{i=1}^N \frac{v_i S_i}{(K_M + S_i)^2} \right] \left[\sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^3} \right] +$$

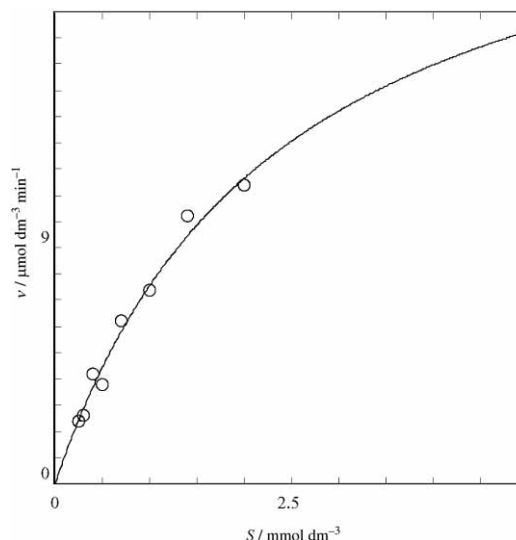


Figure 1. Points from Wong's data sets are shown as circles. The curve is Michaelis-Menten model whose parameters were determined by our method.

$$+ 2 \left[\sum_{i=1}^N \frac{v_i S_i}{(K_M + S_i)^3} \right] \left[\sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^2} \right] - 3 \left[\sum_{i=1}^N \frac{v_i S_i}{K_M + S_i} \right] \left[\sum_{i=1}^N \frac{S_i^2}{(K_M + S_i)^4} \right] \quad (11)$$

RESULTS AND DISCUSSION

The aim of the calculation was to illustrate the algorithm of our procedure and compare results with ones from lit-

TABLE II. Computer output of iteration process for our method with Wong's data set (shown in Table I). Two values for V_{\max} are coming from formulas 8a and 8b, respectively. At root point, these values become identical, as illustrated by the values in the last column

iter	$K_M/\text{mmol dm}^{-3}$	$f(K_M)$	$f'(K_M)$	${}^1V_{\max}(\partial/\partial V_{\max})$	${}^2V_{\max}(\partial/\partial K_M)$	$\Sigma\delta^2({}^1V_{\max})$	$\Sigma\delta^2({}^2V_{\max})$	ΔV_{\max}
1	0.0	221.08	-2747.1	6.0250	4.2394	81.395	106.90	1.7856
2	0.08047	91.432	-893.37	7.1459	5.4749	59.887	76.942	1.6709
3	0.18282	37.448	-293.92	8.3768	6.8877	42.538	52.943	1.4892
4	0.31023	15.180	-97.856	9.7271	8.4584	29.186	35.015	1.2688
5	0.46536	6.0891	-32.975	11.208	10.171	19.367	22.384	1.0371
6	0.65002	2.4156	-11.253	12.830	12.014	12.458	13.909	0.81509
7	0.86468	0.94654	-3.8953	14.597	13.982	7.8172	8.4638	0.61525
8	1.1077	0.36527	-1.3730	16.503	16.059	4.8584	5.1233	0.44339
9	1.3737	0.13793	-0.49677	18.515	18.214	3.0944	3.1917	0.30090
10	1.6514	5.0261 10^{-2}	-0.18764	20.562	20.374	2.1394	2.1700	0.18703
11	1.9192	1.7112 10^{-2}	-7.6716 10^{-2}	22.499	22.399	1.6973	1.7047	0.10073
12	2.1423	5.0095 10^{-3}	-3.6606 10^{-2}	24.093	24.052	1.5429	1.5440	4.1782 10^{-2}
13	2.2791	9.9953 10^{-4}	-2.2977 10^{-2}	25.064	25.054	1.5120	1.5121	1.0193 10^{-2}
14	2.3226	7.2133 10^{-5}	-1.9738 10^{-2}	25.372	25.371	1.5101	1.5101	7.8201 10^{-4}
15	2.3263	6.0598 10^{-7}	-1.9485 10^{-2}	25.398	25.398	1.5101	1.5101	5.7220 10^{-6}
16	2.3263	1.3388 10^{-7}	-1.9483 10^{-2}	25.398	25.398	1.5101	1.5101	1.9073 10^{-6}
17	2.3263	-1.5637 10^{-8}	-1.9482 10^{-2}	25.398	25.398	1.5101	1.5101	0.0

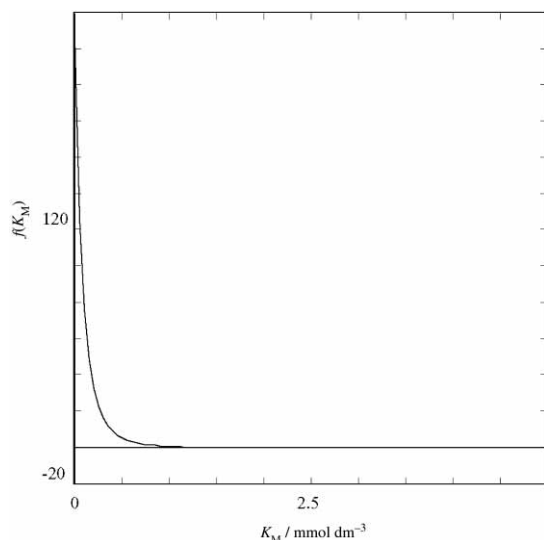


Figure 2. Outline of $f(K_M)$ in interval 0–5 for Wong's data set. Note that initial guess zero for Newton-Raphson method will converge toward the root.

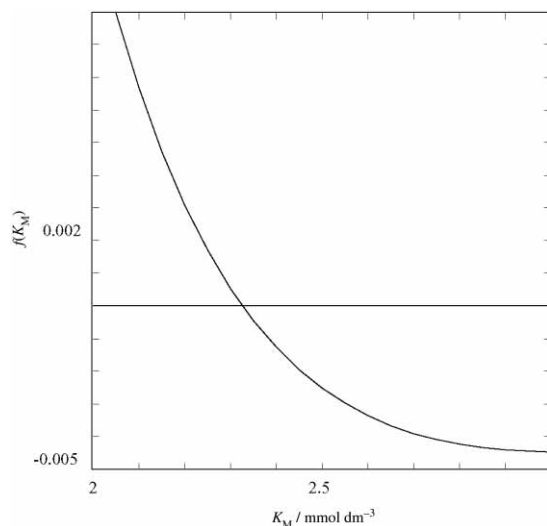


Figure 3. Detail from Figure 2. The shape of $f(K_M)$ in the neighborhood of the root. Note that approaching the root from left side results in convergence.

erature. Therefore, test data set from Wong⁴ is used (see Table I). Graph of data points from Table I and experimental model determined by our optimization are shown in Figure 1.

Table II is computer output showing progress of our iterative optimization using Newton-Raphson method starting with initial guess $K_M = 0$. During the iteration process, two different values of V_{\max} are obtained from the formulas (8a) and (8b). At root point for $f(K_M)$, these two values become identical. The final results for the parameters K_M and V_{\max} are in row 17, printed **bold**. The outline of $f(K_M)$ is shown in Figure 2 and more detailed graph of the same function in the neighborhood of the root is shown in Figure 3.

TABLE III. Comparison of linearization, general non-linear regression and our method. Except for the last row, data are from Wong.⁴ Errors are computed according to Bard¹²

Method	V_{\max}
	$\mu\text{mol dm}^{-3} \text{ min}^{-1}$
Lineweaver-Burk	29.5 ± 14
Eadie	20.3 ± 4.9
Hanes	27.4 ± 5.4
Non-linear regression	25.4 ± 4.0
Our method	25.4 ± 4.0

For the initial guess of K_M (zero), Eq. (9) becomes:

$$f(K_M) = \left(\sum_{i=1}^N v_i \right) \left(\sum_{i=1}^N \frac{1}{S_i} \right) - N \sum_{i=1}^N \frac{v_i}{S_i} \quad (12)$$

If for any two points from the data set we have

$$S_i < S_{i+1} \Rightarrow v_i < v_{i+1} \quad (13)$$

then it can be shown that for set of N points $f(K_M)$ from Eq. (12) is always greater than zero. In that case, the convergence of Eq. (9) is achieved only when $f'(K_M) < 0$. Hence, slope of $f(K_M)$ is negative and it indicates approaching to the root from the left side, as can be seen from the shape of $f(K_M)$ (Figure 3).

Contour plot showing logarithm of a sum of squared residuals $\log_{10} \left(\sum_{i=1}^N \delta_i^2 \right) = g(K_M, V_{\max})$ as a function of

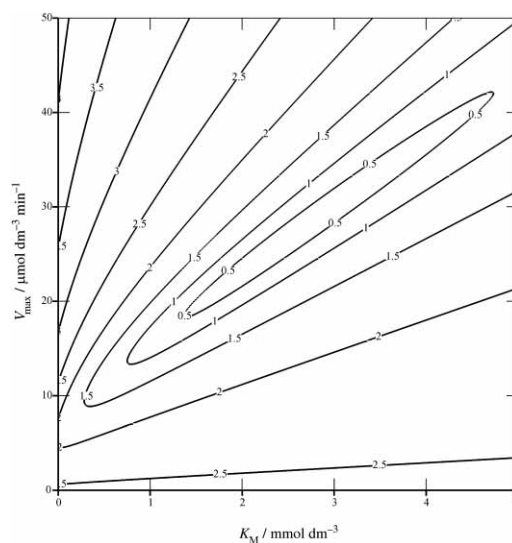


Figure 4. Contour plot for the least squares surface for Michaelis-Menten model and Wong's data set. Log scale is used (z-axis) and contours are spaced for 0.5. The global minimum is in the middle of the plot. General non-linear optimization routine must find a region on the surface which satisfies the convergence criteria. Note that range is different for each parameter and that the search on the surface will be more sensitive for changes in K_M .

K_M and V_{max} for Wong's data set is in Figure 4. Global minimum is inside innermost ellipsoid in the middle of the plot. Horizontal axis of the image is change in K_M in range from 0 to 5 mmol dm⁻³ and vertical is change in V_{max} from 0 to 50 μ mol dm⁻³ min⁻¹. This illustrates that parameters are not well balanced, namely the results of two parameter optimization will be more sensitive to the changes in K_M than in V_{max} . Our method does not have such a potential problem because iterative search is for the point along K_M axis instead of a region on three dimensional surface.

Results of linear and non-linear optimization are summarized in Table III. As expected, our results are the same (within number of decimal places provided) as Wong's using general non-linear optimization. However the method presented here has distinct advantage of speed, simplicity and guaranteed convergence.

CONCLUSION

A specialized non-linear least squares algorithm for determining the parameters in Michaelis-Menten equation using experimental measurement in enzyme kinetics was developed. The proposed algorithm is fast, numerically

stable and has guaranteed convergence with initial guess of zero for K_M . Initial guess for linear parameter V_{max} is not required.

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SAŽETAK

Nelinearna optimizacija parametara u Michaelis-Mentenovoj kinetici

Željko Jeričević i Željko Kušter

Razvijena je nova optimizacijska metoda za određivanje parametara u Michaelis-Mentenovoj kinetici enzimskih reakcija. Metoda se temelji na eliminiranju linearnoga parametra iz normalnih jednadžbi, što transformira nelinearni optimizacijski problem u problem nalaženja korijena nelinearne jednadžbe. Algoritam je učinkovit i ima zajamčenu konvergenciju s početnom vrijednošću nula za parameter K_M .

Skup podataka iz literature obrađen je za ilustraciju upotrebljivosti i točnosti rješenja predložene metode u usporedbi s metodama linearizacije i općom metodom nelinearne optimizacije.