CROATICA CHEMICA ACTA
CCACAA **78** (4) 519–523 (2005)
ISSN-0011-1643
*CCA*-3043
*Original Scientific Paper*

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

**Željko Jeričević[a,**] and Željko Kušter[b]**

[a]*Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA*

[b]*Department of Nuclear Medicine, University Hospital Dubrava, Zagreb, Croatia*

RECEIVED MAY 6, 2005; REVISED JUNE 27, 2005; ACCEPTED JUNE 28, 2005

*Key words*
Michaelis-Menten
enzyme kinetics
non-linear
optimization
data processing
least squares

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

Micheaelis-Menten kinetics is described by the following equation:

$$v = \frac{V_{max} S}{K_M + S} \tag{1}$$

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-Burk[1] plot (1/*v versus* 1/*S*) is defined by the following equation:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{S} \tag{2}$$

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

** Author to whom correspondence should be addressed. (E-mail: zeljkoj@bcm.tmc.edu)

The intercept in this plot is $1/V_{max}$ and slope is $K_M/V_{max}$. Eadie[2] plot (*v versus v/S*) has the following form:

$$v = V_{max} - K_M \frac{v}{S} \tag{3}$$

The intercept in Eadie plot is $V_{max}$ and slope is $K_M$. Hanes[3] plot (*S/v versus S*) has the following form:

$$\frac{S}{v} = \frac{K_M}{V_{max}} + \frac{1}{V_{max}} S \tag{4}$$

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. Wong[4] 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 book[4] 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 Cumme[5] performed series of numerical experiments to find a proper weighting in linearization methods. Page[6] 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 Marquardt[9] algorithm for nonlinear optimization, which is more complicated than the approach proposed here. Modern bioinformatics package EMBOSS[10] uses Lineweaver-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 convergence 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 equations[11] are developed using the well-known least squares formalism:

$$\sum_{i=1}^{N} \delta_i^2 = \sum_{i=1}^{N} (v_i - {}^{th}v_i)^2 = \sum_{i=1}^{N} \left( v_i - \frac{V_{max} S_i}{K_M + S_i} \right)^2 \tag{5}$$

where $\delta_i$ is error – the difference 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 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:

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

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

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

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 ($K_M$).

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

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

TABLE I. Input data set taken from Wong[4] (p. 245)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $S$ / mmol dm$^{-3}$ | 0.25 | 0.30 | 0.40 | 0.50 | 0.70 | 1.00 | 1.40 | 2.00 |
| $v$ / μmol dm$^{-3}$ 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:

$$f(K_M) = \left(\sum_{i=1}^{N} \frac{v_i S_i}{K_M + S_i}\right)\left[\sum_{i=1}^{N} \frac{S_i^2}{(K_M + S_i)^3}\right] - \left[\sum_{i=1}^{N} \frac{v_i S_i}{(K_M + S_i)^2}\right]\left[\sum_{i=1}^{N} \frac{S_i^2}{(K_M + S_i)^2}\right] = 0 \quad (9)$$

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:

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

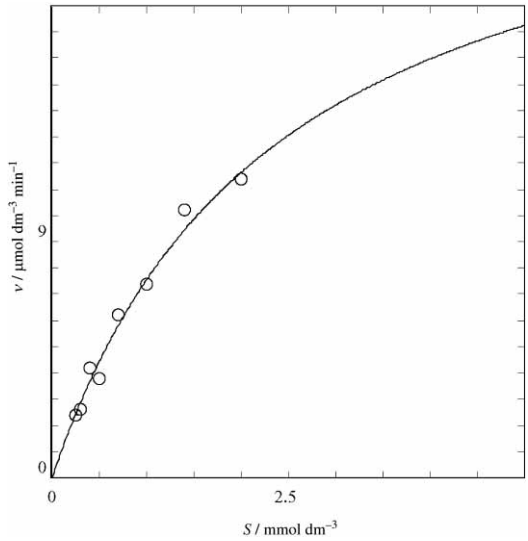


Figure 1. Points from Wong's data sets are shown as circles. The curve is Michaelis-Menten model whose parameters were determined by our method.

## 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 | $K_M$/mmol dm$^{-3}$ | $f(K_M)$ | $f'(K_M)$ | $^1V_{max}(\partial/\partial V_{max})$ | $^2V_{max}(\partial/\partial K_M)$ | $\Sigma\delta^2(^1V_{max})$ | $\Sigma\delta^2(^2V_{max})$ | $\Delta V_{max}$ |
|---|---|---|---|---|---|---|---|---|
| 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 |

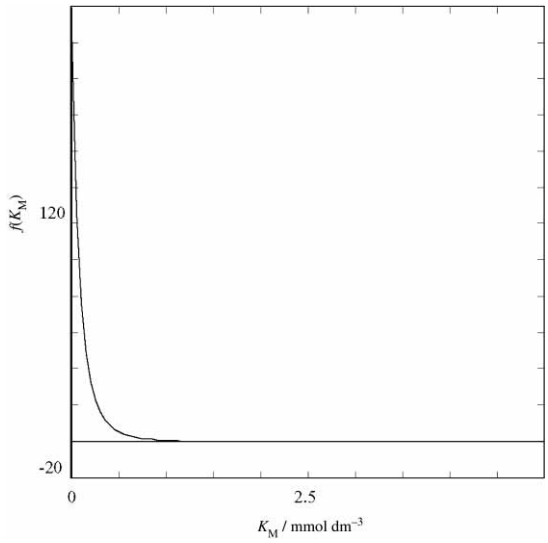


Figure 2. Outline of $f(K_M)$ in interval 0–5 for Wong's data set. Note that initial guess zero for Newton-Raphson method will converge toward the root.

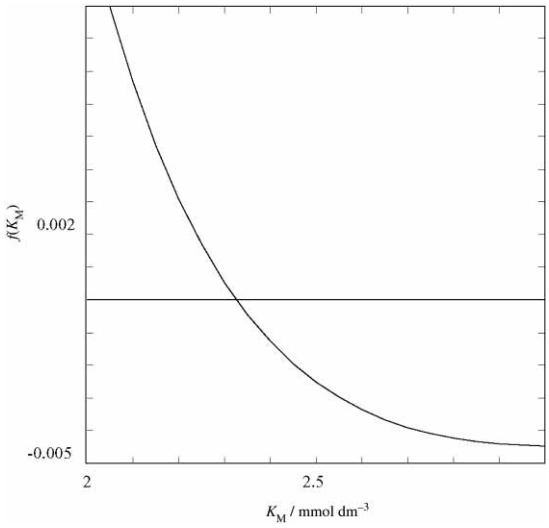


Figure 3. Detail from Figure 2. The shape of $f(K_M)$ in the neighborhood of the root. Note that approaching the root from left side results in convergence.

erature. Therefore, test data set from Wong[4] 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 Bard[12]

| Method | $V_{max}$ / μmol dm$^{-3}$ 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:

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

If for any two points from the data set we have

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

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

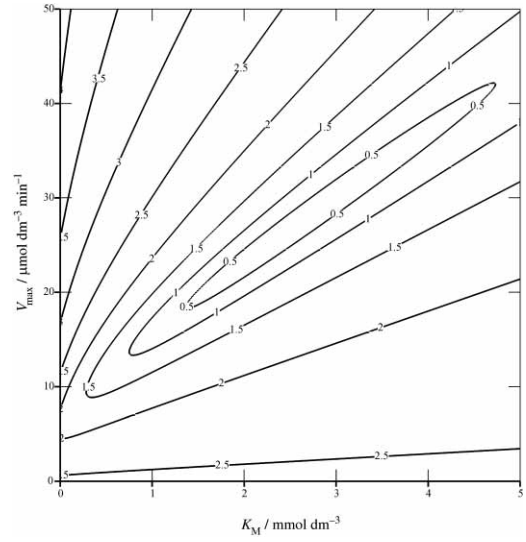


Figure 4. Contour plot for the least squares surface for Michaelis-Menten model and Wong's data set. Log scale is used (z-axis) and contours are spaced for 0.5. The global minimum is in the middle of the plot. General non-linear optimization routine must find a region on the surface which satisfies the 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 μmol 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.

## REFERENCES


1. H. Lineweaver and D. Burk, *J. Am. Chem. Soc.* **56** (1934) 658–666.
2. G. S. Eadie, *J. Biol. Chem.* **146** (1942) 85–93.
3. C. S. Hanes, *Biochem. J.* **26** (1932) 1406–1421.
4. J. T.-F. Wong, *Kinetics of Enzyme Mechanisms*, Academic Press, New York, 1975.
5. H. Hoppe and G. A. Cumme, *Acta. Biol. Med. Ger.* **37** (1978) 1177–1184.
6. A. Page, *Comput. Appl. Biosci*. **3** (1987) 49–51.
7. J. Marszalek, J. Kostrowicki, and J. Spychala, *Comput. Appl. Biosci*. **5** (1989) 239–240..
8. A. V. Hill, *J. Physiol.* **40** (1910) 4–6.
9. D. W. Marquardt, *SIAM J. App. Math.* **11** (1963) 431–441.
10. P. Rice, I. Longden, and A. Bleasby, *Trends Genet.* **16** (2000) 276–277.
11. S. D. Conte and C. de Boor, *Elementary Numerical Analysis*, 3rd Ed., McGraw-Hill, New York, 1980.
12. Y. Bard, *Nonlinear Parameter Estimation*, Academic Press, New York, 1974.


---

## 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 upotrebljivost i točnosti rješenja predložene metode u usporedbi s metodama linearizacije i općom metodom nelinearne optimizacije.