

AN ANALYSIS OF A FRACTAL KINETICS CURVE OF SAVAGEAU

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Abstract

The fractal kinetics curve derived by Savageau is analysed to show that its parameters are not uniquely determined given four appropriately situated data points. Comparison is made with an alternate fractal Michaelis-Menten equation derived by Lopez-Quintela and Casado.

1. Introduction

The Michaelis-Menten equation

$$V_p = \frac{V_m S}{S + K_m}$$

has long been the standard framework for biochemical kinetics [4], describing the reaction of a substrate S on a free enzyme E to form a product P . Here $V_p = dP/dt$ and V_m and K_m are empirically determined parameters. It is convenient to rewrite this equation in the form

$$\left(\frac{S}{K_m}\right) = \left(\frac{V_p}{V_m}\right) \left(1 - \frac{V_p}{V_m}\right)^{-1}. \quad (1.1)$$

If the reaction is allosteric (cooperative) so that n molecules of S bind to E , the kinetics are described by the Hill equation [5]

$$V_p = \frac{V_m S^n}{K_m + S^n},$$

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which can be written

$$\left(\frac{S^n}{K_m}\right) = \left(\frac{V_p}{V_m}\right) \left(1 - \frac{V_p}{V_m}\right)^{-1}. \quad (1.2)$$

Recently, Savageau [6] has shown that both the Michaelis-Menten and Hill equations are special cases of a more general fractal power law mechanism which produces the kinetic equation

$$\left(\frac{S}{K_f}\right)^{g_s} = \left(\frac{V_p}{V_m}\right) \left(1 - \frac{V_p}{V_m}\right)^{-g_e}. \quad (1.3)$$

Clearly, (1.3) includes (1.1) and (1.2) as special cases where g_s and g_e can be interpreted as generalised cooperativity exponents. In fact, $g_e = 1$ reduces (1.3) to the Hill equation (1.2).

Note that (1.1) has two parameters V_m and K_m , (1.2) has three parameters V_m , K_m and n while (1.3) has four parameters V_m , K_f , g_s and g_e . Recently, the authors [1] have made a data-fitting analysis of (1.1) and (1.2). For the Michaelis-Menten equation (1.1) let (V_{p_1}, S_1) and (V_{p_2}, S_2) be two points on the hyperbolic (concave down) response curve described by (1.1). Then these two pieces of data uniquely determine the parameters V_m and K_m .

For the Hill equation (1.2) three data points (V_{p_1}, S_1) , (V_{p_2}, S_2) and (V_{p_3}, S_3) either determine the three parameters V_m , K_m and n uniquely or there may be no solution for the three parameters. It depends on a precisely determined but complicated relationship between the three data points [1].

The purpose of this paper is to carry out a similar analysis for the more general equation (1.3) with four parameters. It turns out that there will always be two, infinitely many, or no solutions for the four parameters, given four data points. That is, it is impossible to have a single unique solution for the four parameters regardless of the specified data.

Before beginning the analysis of (1.3), another fractal generalisation of the Michaelis-Menten equation should be mentioned. By assuming that the reaction rate constants K_i are scale dependent, $K_i = A_i S^{1-D}$ where $D \leq 1$ is the fractal dimension of the scaling variable S , Lopez-Quintela and Casado [3] were led to the equation

$$V_p = \frac{V_m^{\text{eff}} S^{2-D}}{K_m^{\text{eff}} + S}, \quad (1.4)$$

which is distinct from (1.2) and (1.3) above. The data-fitting problem for (1.4) has been analysed in [2]. It turns out that for this three-parameter equation there can also be either one, two, or no solutions depending in a complicated way on the alignment of the data points. However, a unique solution exists only if the three data points lie

on a single special curve separating the region of no solutions from the regions of two solutions ([2, Curve (11) in Figure 7]).

Thus the two papers, [3] and [6], propose different models for fractal reaction kinetics. In one model there exists a single data curve producing a unique solution for the parameters, which is not robust. In the other model, there is never a unique solution for the system parameters. Thus data analysis of the two models uncovers shortcomings in each one and raises the question as to whether either is an adequate model of reality.

This is a theoretical analysis, assuming only a minimal amount of data is available to determine the parameters of the model. Such an analysis is a first step in evaluating the practical utility of a model. It is clearly important to know whether or not the model parameters are uniquely determined by data even if only in a theoretical setting. Then the problems of parameter estimation and sensitivity analysis can be carried out later if the model is applied to specific sets of data.

2. Results

We now turn to the analysis of (1.3). For ease of notation we use V_i instead of V_{p_i} .

THEOREM 1. *Given four data points (V_i, S_i) , $i = 1, 2, 3, 4$, where $0 < V_1 < \dots < V_4$ and $0 < S_1 < \dots < S_4$, then there either exists one, two, infinitely many, or no solutions for V_m , K_f , g_s and g_e , where V_m and K_f are assumed to be positive.*

PROOF. Substitute (V_i, S_i) , $i = 1, 2, 3, 4$, into (1.3) for (V_i, S_i) and take logarithms of both sides to obtain

$$g_s(\ln S_i - \ln K_f) = \ln V_i - (1 - g_e) \ln V_m - g_e \ln(V_m - V_i).$$

Solving for $\ln K_f$ gives

$$\ln K_f = \frac{1}{g_s} \ln \left(\frac{V_m}{V_i} \right) + \frac{g_e}{g_s} \ln \left(\frac{V_m - V_i}{V_m} \right) + \ln S_i.$$

Subtracting the first two of these four equations (that is, for $i = 1$ and 2) eliminates the K_f term and gives

$$\frac{1}{g_s} \ln \left(\frac{V_2}{V_1} \right) + \frac{g_e}{g_s} \ln \left(\frac{V_m - V_1}{V_m - V_2} \right) + \ln \left(\frac{S_1}{S_2} \right) = 0.$$

Similarly using $i = 1$ and 3 gives

$$\frac{1}{g_s} \ln \left(\frac{V_3}{V_1} \right) + \frac{g_e}{g_s} \ln \left(\frac{V_m - V_1}{V_m - V_3} \right) + \ln \left(\frac{S_1}{S_3} \right) = 0.$$

These two equations can be solved for g_e and g_s to give

$$g_e = \frac{\ln\left(\frac{S_1}{S_2}\right) \ln\left(\frac{V_2}{V_3}\right) - \ln\left(\frac{V_2}{V_1}\right) \ln\left(\frac{S_3}{S_2}\right)}{\ln\left(\frac{S_1}{S_2}\right) \ln\left(\frac{V_m - V_2}{V_m - V_3}\right) + \ln\left(\frac{V_m - V_1}{V_m - V_2}\right) \ln\left(\frac{S_3}{S_2}\right)}$$

and

$$g_s = \frac{\ln\left(\frac{V_m - V_1}{V_m - V_2}\right) \ln\left(\frac{V_3}{V_1}\right) - \ln\left(\frac{V_m - V_1}{V_m - V_3}\right) \ln\left(\frac{V_2}{V_1}\right)}{\ln\left(\frac{S_1}{S_2}\right) \ln\left(\frac{V_m - V_2}{V_m - V_3}\right) + \ln\left(\frac{V_m - V_1}{V_m - V_2}\right) \ln\left(\frac{S_3}{S_2}\right)}.$$

Thus K_f , g_e and g_s are all expressed in terms of V_m and the data points (V_i, S_i) . Since we haven't yet used ($i = 1$ and 4)

$$\frac{1}{g_s} \ln\left(\frac{V_4}{V_1}\right) + \frac{g_e}{g_s} \ln\left(\frac{V_m - V_1}{V_m - V_4}\right) + \ln\left(\frac{S_1}{S_4}\right) = 0$$

we can substitute into this the expressions for g_e and g_s to obtain

$$\begin{aligned} & \frac{\ln\left(\frac{S_1}{S_2}\right) \ln\left(\frac{V_m - V_2}{V_m - V_3}\right) + \ln\left(\frac{V_m - V_1}{V_m - V_2}\right) \ln\left(\frac{S_3}{S_2}\right)}{\ln\left(\frac{V_m - V_1}{V_m - V_2}\right) \ln\left(\frac{V_3}{V_1}\right) - \ln\left(\frac{V_m - V_1}{V_m - V_3}\right) \ln\left(\frac{V_2}{V_1}\right)} \ln\left(\frac{V_4}{V_1}\right) \\ & + \frac{\ln\left(\frac{S_1}{S_2}\right) \ln\left(\frac{V_2}{V_3}\right) - \ln\left(\frac{V_2}{V_1}\right) \ln\left(\frac{S_3}{S_2}\right)}{\ln\left(\frac{V_m - V_1}{V_m - V_2}\right) \ln\left(\frac{V_3}{V_1}\right) - \ln\left(\frac{V_m - V_1}{V_m - V_3}\right) \ln\left(\frac{V_2}{V_1}\right)} \ln\left(\frac{V_m - V_1}{V_m - V_4}\right) + \ln\left(\frac{S_1}{S_4}\right) = 0. \end{aligned}$$

This equation can be rewritten as

$$\alpha_1 \ln(V_m - V_1) + \alpha_2 \ln(V_m - V_2) + \alpha_3 \ln(V_m - V_3) + \alpha_4 \ln(V_m - V_4) = 0$$

or

$$(V_m - V_1)^{\alpha_1} (V_m - V_2)^{\alpha_2} (V_m - V_3)^{\alpha_3} (V_m - V_4)^{\alpha_4} = 1, \quad (2.1)$$

where

$$\begin{aligned} \alpha_1 &= \ln\left(\frac{S_3}{S_4}\right) \ln(V_2) + \ln\left(\frac{S_4}{S_2}\right) \ln(V_3) + \ln\left(\frac{S_2}{S_3}\right) \ln(V_4), \\ \alpha_2 &= \ln\left(\frac{S_4}{S_3}\right) \ln(V_1) + \ln\left(\frac{S_1}{S_4}\right) \ln(V_3) + \ln\left(\frac{S_3}{S_1}\right) \ln(V_4), \\ \alpha_3 &= \ln\left(\frac{S_2}{S_4}\right) \ln(V_1) + \ln\left(\frac{S_4}{S_1}\right) \ln(V_2) + \ln\left(\frac{S_1}{S_2}\right) \ln(V_4), \\ \alpha_4 &= \ln\left(\frac{S_3}{S_2}\right) \ln(V_1) + \ln\left(\frac{S_1}{S_3}\right) \ln(V_2) + \ln\left(\frac{S_2}{S_1}\right) \ln(V_3) \end{aligned}$$

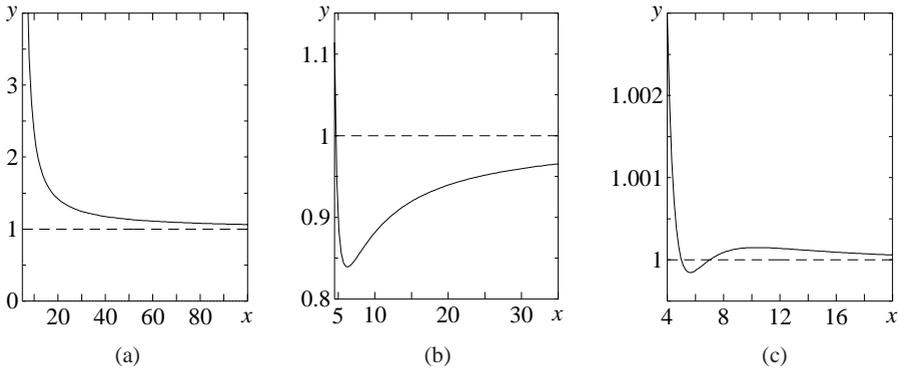


FIGURE 1. Graph of $h(V_m)$ with $h(V_4^+) = \infty$ where the line $y = 1$ is intersected (a) zero times, (b) once or (c) twice.

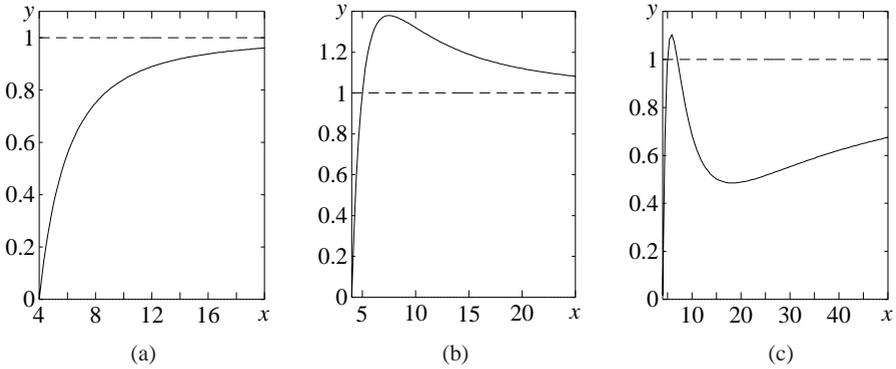


FIGURE 2. Graph of $h(V_m)$ with $h(V_4^+) = 0$ where the line $y = 1$ is intersected (a) zero times, (b) once or (c) twice.

and $\alpha_1 + \alpha_2 + \alpha_3 + \alpha_4 = 0$.

We note that V_m represents the maximum velocity in (1.1)–(1.3). Then with $V_m > V_i$ for $i = 1, 2, 3, 4$ and using the fact that $\alpha_4 = -\alpha_1 - \alpha_2 - \alpha_3$, we set

$$h(V_m) \equiv \left(\frac{V_m - V_1}{V_m - V_4}\right)^{\alpha_1} \left(\frac{V_m - V_2}{V_m - V_4}\right)^{\alpha_2} \left(\frac{V_m - V_3}{V_m - V_4}\right)^{\alpha_3}$$

and note that (2.1) is equivalent to finding a V_m such that $h(V_m) = 1$. We also note that $h(V_m) \equiv 1$ if and only if $\alpha_1 = \alpha_2 = \alpha_3 = \alpha_4 = 0$, in which case there are infinitely many solutions.

It is easy to show that

$$h'(V_m) = \frac{h(V_m)Q(V_m)}{(V_m - V_4)(V_m - V_3)(V_m - V_2)(V_m - V_1)},$$

where $Q(V_m)$ is a quadratic in V_m whose coefficients are combinations of V_i 's and α_i 's. Since $h(V_m) > 0$ on its domain (V_4, ∞) and assuming $h(V_m) \neq 1$, $\lim_{V_m \rightarrow V_4} h(V_m) = h(V_4^+) = \infty$ or 0 and $\lim_{V_m \rightarrow \infty} h(V_m) = h(\infty) = 1$, then $h'(V_m)$ changes sign at most twice and the graph of $h(V_m)$ has one of the forms shown in Figure 1 for $h(V_4^+) = \infty$ and in Figure 2 for $h(V_4^+) = 0$.

It is thus clear that $h(V_m)$ intersects the line $y = 1$ either once, twice, or no times and the theorem is proved.

To proceed further, we need to know the coefficient of the squared term in $Q(V_m)$. It is (found by MAPLE)

$$\begin{aligned} \alpha &= (V_1 - V_4)\alpha_1 + (V_2 - V_4)\alpha_2 + (V_3 - V_4)\alpha_3 \\ &= (V_1 - V_4) \left(\ln \left(\frac{S_3}{S_4} \right) \ln(V_2) + \ln \left(\frac{S_4}{S_2} \right) \ln(V_3) + \ln \left(\frac{S_2}{S_3} \right) \ln(V_4) \right) \\ &\quad + (V_2 - V_4) \left(\ln \left(\frac{S_4}{S_3} \right) \ln(V_1) + \ln \left(\frac{S_1}{S_4} \right) \ln(V_3) + \ln \left(\frac{S_3}{S_1} \right) \ln(V_4) \right) \\ &\quad + (V_3 - V_4) \left(\ln \left(\frac{S_2}{S_4} \right) \ln(V_1) + \ln \left(\frac{S_4}{S_1} \right) \ln(V_2) + \ln \left(\frac{S_1}{S_2} \right) \ln(V_4) \right). \end{aligned}$$

It turns out that the question of the existence of a unique solution of (2.1), that is, a unique V_m such that $h(V_m) = 1$, can be resolved completely using only α , the coefficient of the quadratic term in $Q(V_m)$. It is clear from Figure 1 that when $h(V_4^+) = \infty$, there is a unique solution if $\alpha > 0$, and either two solutions or no solution if $\alpha < 0$. In a similar manner it is clear from Figure 2 that when $h(V_4^+) = 0$, there is a unique solution if $\alpha < 0$ and either two solutions or no solution if $\alpha > 0$.

Of course, the expression for α involves α_1, α_2 and α_3 . It turns out that the sign of each of the α_i has a simple and useful geometric interpretation.

LEMMA 1. *The parameter $\alpha_1 > 0$ ($= 0$), (< 0) if and only if $(\ln V_3, \ln S_3)$ lies above (on), (below) the line from $(\ln V_2, \ln S_2)$ to $(\ln V_4, \ln S_4)$.*

PROOF. Suppose that

$$\begin{aligned} 0 = \alpha_1 &= (\ln S_3 - \ln S_4) \ln V_2 + (\ln S_4 - \ln S_2) \ln V_3 + (\ln S_2 - \ln S_3) \ln V_4 \\ &= \ln \frac{V_2}{V_4} \ln S_3 + \ln \frac{S_4}{S_2} \ln V_3 + \ln S_2 \ln V_4 - \ln S_4 \ln V_2. \end{aligned}$$

Thus

$$\ln S_3 - \ln V_3 \frac{\ln S_4 - \ln S_2}{\ln V_4 - \ln V_2} = \frac{\ln S_2 \ln V_4 - \ln S_4 \ln V_2}{\ln V_4 - \ln V_2}$$

or

$$\ln S_3 = \frac{\ln S_4 - \ln S_2}{\ln V_4 - \ln V_2} \ln V_3 + \ln S_2 - \frac{\ln S_4 - \ln S_2}{\ln V_4 - \ln V_2} \ln V_2,$$

which is the line $y = mx + b$ with $y = \ln S_3$ and $x = \ln V_3$ between $(\ln V_2, \ln S_2)$ and $(\ln V_4, \ln S_4)$.

Since we have divided by a positive number, the two inequalities follow directly.

In a completely analogous manner the following geometric interpretation of α_2 , α_3 and α_4 can also be established.

LEMMA 2. *The parameter $\alpha_2 > 0$ ($= 0$), (< 0) if and only if $(\ln V_3, \ln S_3)$ lies above (on), (below) the line from $(\ln V_1, \ln S_1)$ to $(\ln V_4, \ln S_4)$.*

LEMMA 3. *The parameter $\alpha_3 > 0$ ($= 0$), (< 0) if and only if $(\ln V_2, \ln S_2)$ lies above (on), (below) the line from $(\ln V_1, \ln S_1)$ to $(\ln V_4, \ln S_4)$.*

LEMMA 4. *The parameter $\alpha_4 > 0$ ($= 0$), (< 0) if and only if $(\ln V_2, \ln S_2)$ lies above (on), (below) the line from $(\ln V_1, \ln S_1)$ to $(\ln V_4, \ln S_4)$.*

We are now able to establish the main result of this paper.

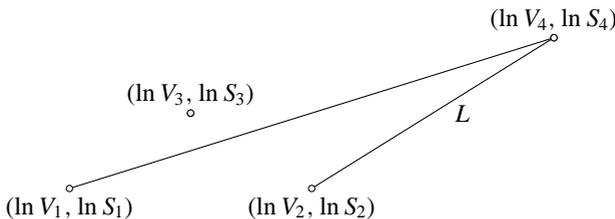
THEOREM 2. *There is never a unique set of parameters for (1.3). Otherwise stated, (2.1), $h(V_m) = 1$, never has a unique solution V_m .*

PROOF. We adopt the notation $(+, -, +, -)$, for example, to indicate that $\alpha_1 > 0$, $\alpha_2 < 0$, $\alpha_3 > 0$ and $\alpha_4 < 0$. Similarly a $+$ or $-$ replaced by a zero indicates that the corresponding component is zero.

First suppose that $\alpha_1 + \alpha_2 + \alpha_3 > 0$ so that $h(V_4^+) = \infty$ and Figure 1 applies. Thus also $\alpha_4 < 0$ since $\alpha_1 + \alpha_2 + \alpha_3 + \alpha_4 = 0$. We now have to consider various cases for the sign possibilities for α_1 , α_2 and α_3 (with $\alpha_4 < 0$).

Case 1: $(+, -, -, -)$. Since $\alpha_1 + \alpha_2 + \alpha_3 > 0$ and therefore $\alpha_1 > -\alpha_2 - \alpha_3 > 0$, then $\alpha = (V_1 - V_4)\alpha_1 + (V_2 - V_4)\alpha_2 + (V_3 - V_4)\alpha_3 < 0$ and (2.1) has either two solutions or no solution.

Case 2: $(-, +, -, -)$. This case like many others can be ruled out by using the geometric interpretation of the α_i 's provided by the lemmas. In this case consider the points and lines below.



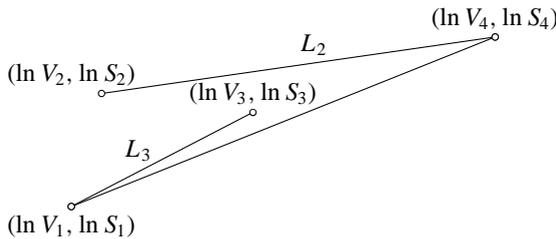
The first minus sign means that $(\ln V_3, \ln S_3)$ must be below L , a contradiction.

Case 3: $(-, -, +, -)$. This case is ruled out in a completely similar manner to Case 2.

Case 4: $(+, +, -, -)$. This case is ruled out in a completely similar manner to Case 1.

Case 5: $(+, -, +, -)$. This case is ruled out either by the reasoning of Case 1 or by the reasoning of Case 2.

Case 6: $(-, +, +, -)$. In a similar manner to Case 2, consider the points and lines shown below.



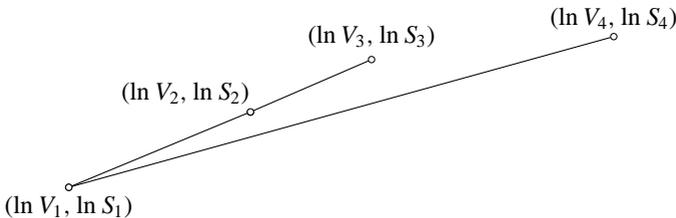
The first minus sign says that L_3 is below L_2 but the last minus sign says that L_2 is below L_3 , a contradiction.

Case 7: $(+, +, +, -)$. Here the same approach as in Case 1 leads to a contradiction.

Case 8. If any one of the first three α_i 's is zero, consistent with $\alpha_1 + \alpha_2 + \alpha_3 > 0$, the very same arguments as above still apply. If any two of the first three α_i 's are zero then all α_i 's are zero (using the geometric argument) and therefore $\alpha = 0$ and $h = 1$ which gives an infinity of solutions.

We now suppose that $\alpha_1 + \alpha_2 + \alpha_3 = 0 = \alpha_4$ and again consider several sub cases.

Case 9: $\alpha_2 > 0$. Using the geometric approach we consider the points and lines shown below.



Thus we also have $\alpha_1 > 0$ and hence $\alpha_3 < 0$, a contradiction.

Case 10: $\alpha_2 < 0$. In a completely similar manner we find that $\alpha_1 < 0, \alpha_3 > 0$ also gives a contradiction.

Case 11: $\alpha_2 = 0$. Again by the geometric argument we find that $\alpha_1 = \alpha_2 = \alpha_3 = \alpha_4 = 0$ and $h(V_m) = 1$ has an infinity of solutions.

This disposes of all cases with $\alpha_1 + \alpha_2 + \alpha_3 \geq 0$. If we have $\alpha_1 + \alpha_2 + \alpha_3 < 0$ (Figure 2) similar arguments to the above show that there can't be a unique solution here either. The theorem is proved.

3. Conclusions

The lack of a unique solution for the parameters V_m , K_f , g_s and g_e given any four regular (that is, monotonically arranged) data points (V_i, S_i) , $i = 1, 2, 3, 4$, suggest that Savageau's fractal kinetics model, (1.3), may be deficient as a model of reality.

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