AN ANALYSIS OF A FRACTAL

KINETICS CURVE OF SAVAGEAU

by

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Running Head Fractal Kinetics Curve Analysis

Abstract

The fractal kinetics curve

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

derived by Savageau [6], is analyzed to show that the parameters $V_{m,}\, K_{f\,,}\, g_s$ *and* g_e are not uniquely determined given four appropriately situated data points $\left(V_{p_i}, S_i\right)$, i=1,2,3,4. Comparison is made to an alternate fractal Michaels-Menten equation derived by Lopez-Quintela and Casado, J. [3].

Key words: **f**ractal kinetics, biochemical kinetics, data analysis, parameter uniqueness

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 $V_p = \frac{dP}{dt}$ and V_m *and* K_m are empirically determined parameters. It is convenient to rewrite this equation in the form

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

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

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

which can be written

$$\left(\frac{S^n}{K_m}\right) = \left(\frac{V_p}{V_m}\right)\left(1 - \frac{V_p}{V_m}\right)^{-1}. \tag{2}$$

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

$$\left(\frac{S}{K_f}\right)^{g_s} = \left(\frac{V_p}{V_m}\right)\left(1 - \frac{V_p}{V_m}\right)^{-g_e}. \tag{3}$$

Clearly, (3) includes (1) and (2) as special cases where g_s *and* g_e can be interpreted as generalized cooperativity exponents. In fact, $g_e = 1$ reduces (3) to the Hill equation (2).

Note that equation (1) has two parameters V_m *and* K_m, equation (2) has three parameters $V_{m,}\ K_m$ *and* n while equation (3) has four parameters $V_{m,}\ K_{f\,,}\ g_s$ *and* g_e. Recently, the authors [1] have made a data fitting analysis of equations (1) and (2). For the Michaelis-Menten equation (1) let $\left(V_{p1,}\ S_1\right)$ and $\left(V_{p2,}\ S_2\right)$ be two points on the hyperbolic (concave down) response curve described by (1). Then these two pieces of data uniquely determine the parameters V_m *and* K_m.

For the Hill equation (2) three data points $\left(V_{p1,}\ S_1\right)$, $\left(V_{p2,}\ S_2\right)$, and $\left(V_{p3,}\ S_3\right)$ 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 (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 solutions for the four parameters regardless of the specified data.

Before beginning the analysis of (3), another fractal generalization 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

$$V_p = \frac{V_m^{eff}\, S^{2-D}}{K_m^{eff} + S} \tag{4}$$

which is distinct from equations (2) and (3) above. The data-fitting problem for (4) has been analyzed in [2]. It turns out that for this three-parameter equation there can also be either one, two, or no solutions depending on the alignment of the data points in a complicated way. 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 (Curve (11) in figure 7 of [2]).

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 the 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 equation (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 $0 < V_1 < V_2 < V_3 < V_4$ and $0 < S_1 < S_2 < S_3 < S_4$, 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 (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:

$$\frac{\ln\left(\frac{S_1}{S_2}\right)\ln\left(\frac{V_m-V_2}{V_m-V_3}\right)+\ln\left(\frac{V_m-V_1}{V_m-V_2}\right)\ln\left(\frac{S_3}{S_2}\right)}{\ln\left(\frac{V_m-V_1}{V_m-V_2}\right)\ln\left(\frac{V_3}{V_1}\right)-\ln\left(\frac{V_m-V_1}{V_m-V_3}\right)\ln\left(\frac{V_2}{V_1}\right)}\ln\left(\frac{V_4}{V_1}\right)+$$

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

This equation can be rewritten as:

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

or

$$\left(V_m - V_1\right)^{\alpha_1}\left(V_m - V_2\right)^{\alpha_2}\left(V_m - V_3\right)^{\alpha_3}\left(V_m - V_4\right)^{\alpha_4} = 1 \qquad (5)$$

where:

$$\alpha_1 = \ln\left(\frac{S_3}{S_4}\right)\ln\left(V_2\right) + \ln\left(\frac{S_4}{S_2}\right)\ln\left(V_3\right) + \ln\left(\frac{S_2}{S_3}\right)\ln\left(V_4\right)$$

$$\alpha_2 = \ln\left(\frac{S_4}{S_3}\right)\ln\left(V_1\right) + \ln\left(\frac{S_1}{S_4}\right)\ln\left(V_3\right) + \ln\left(\frac{S_3}{S_1}\right)\ln\left(V_4\right)$$

$$\alpha_3 = \ln\left(\frac{S_2}{S_4}\right)\ln\left(V_1\right) + \ln\left(\frac{S_4}{S_1}\right)\ln\left(V_2\right) + \ln\left(\frac{S_1}{S_2}\right)\ln\left(V_4\right)$$

$$\alpha_4 = \ln\left(\frac{S_3}{S_2}\right)\ln\left(V_1\right) + \ln\left(\frac{S_1}{S_3}\right)\ln\left(V_2\right) + \ln\left(\frac{S_2}{S_1}\right)\ln\left(V_3\right)$$

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

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

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

and note that (5) 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:

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

where $Q(V_m)$ is a quadratic in V_m whose coefficients are combinations of the V_i's and α_i's. Since $h(V_m) > 0$ on its domain (V_4, ∞) and assuming $h(V_m) \not\equiv 1$, $\lim\limits_{V_m \to V_4} h(V_m) = h\left(V_4^+\right) = \infty\ or\ 0$, and $\lim\limits_{V_m \to \infty} h(V_m) = h(\infty) = 1$, then $h'(V_m)$ changes sign at most twice, and the graph of $h(V_m)$ has one of the forms:

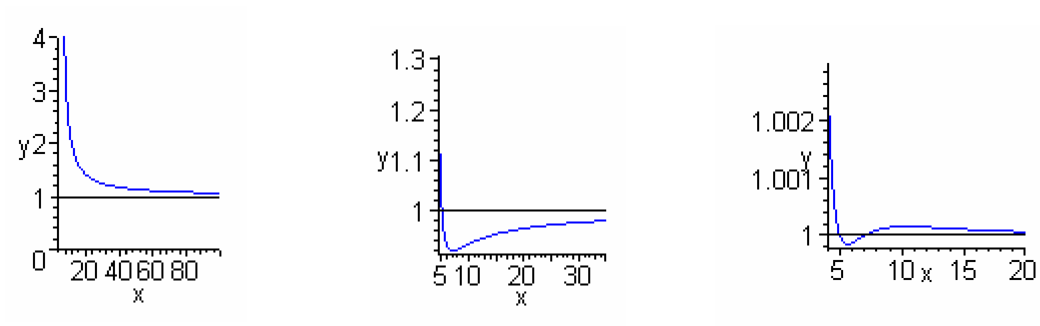


figure 1

with $h\left(V_4^+\right) = \infty$ and for $h\left(V_4^+\right) = 0$

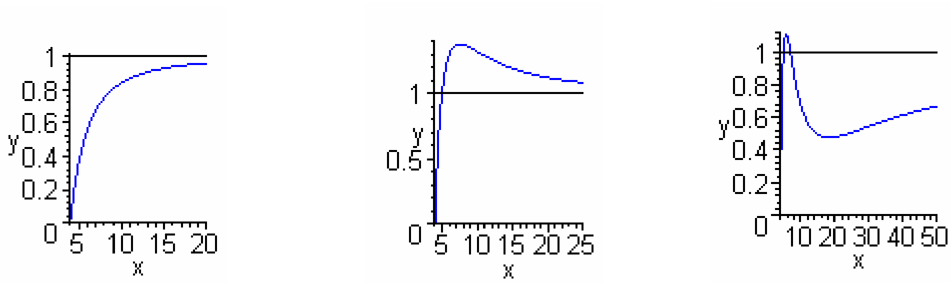


figure 2

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):

$$\alpha = (V_1 - V_4)\alpha_1 + (V_2 - V_4)\alpha_2 + (V_3 - V_4)\alpha_3$$

$$=\left(V_1-V_4\right)\left(\ln\left(\frac{S_3}{S_4}\right)\ln V_2+\ln\left(\frac{S_4}{S_2}\right)\ln V_3+\ln\left(\frac{S_2}{S_3}\right)\ln V_4\right)$$

$$+\left(V_2-V_4\right)\left(\ln\left(\frac{S_4}{S_3}\right)\ln V_1+\ln\left(\frac{S_1}{S_4}\right)\ln V_3+\ln\left(\frac{S_3}{S_1}\right)\ln V_4\right)$$

$$+\left(V_3-V_4\right)\left(\ln\left(\frac{S_2}{S_4}\right)\ln V_1+\ln\left(\frac{S_4}{S_1}\right)\ln V_2+\ln\left(\frac{S_1}{S_2}\right)\ln V_4\right).$$

It turns out that the question of the existence of a unique solution of (5), ie a unique V_m such that $h\left(V_m\right)=1$, can be resolved completely using only α, the coefficient of the quadratic term in $Q\left(V_m\right)$. It is clear from figure 1 that when $h\left(V_4+\right)=\infty$ 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 $h\left(V_k+\right)=0$, 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 $\alpha_1, \alpha_2,$ and α_3. It turns out that the sign of each of the α_i has a simple and useful geometric interpretation.

<u>**Lemma 2**</u> $\alpha_1>0,(=0),(<0)$ if and only if $\left(\ln V_3, \ln S_3\right)$ lies above, (on), (below) the line from $\left(\ln V_2, \ln S_2\right)$ to $\left(\ln V_4, \ln S_4\right)$.

<u>**Proof**</u>

Suppose that

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

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 $\alpha_2, \alpha_3,$ and α_4 can also be established.

Lemma 3 $\alpha_2 > 0, (=0), (<0)$ 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 4 $\alpha_3 > 0, (=0), (<0)$ 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 5 $\alpha_4 > 0, (=0), (<0)$ 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 the equation (3). Otherwise stated, equation (5), $h(V_m) = 1,$ never has a unique solution V_m.

Proof

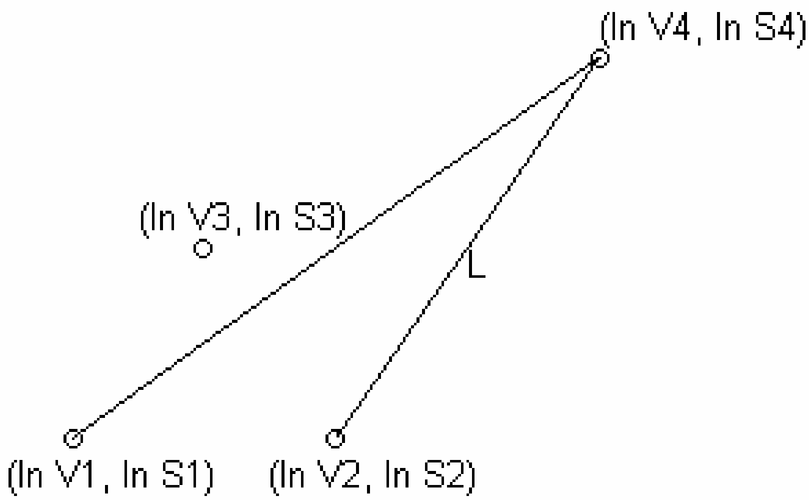
We adopt the notation $(+,-,+,-)$, for example, to indicate that $\alpha_1 > 0, \alpha_2 < 0, \alpha_3 > 0,$ and $\alpha_4 < 0.$ Similarly a $+$ or $-$ replaced by a zero indicates that the corresponding component is zero.

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 $\alpha_1, \alpha_2,$ 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 (5) has either two solutions or no solution.

<u>Case 2</u> $(-,+,-,-)$. 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:



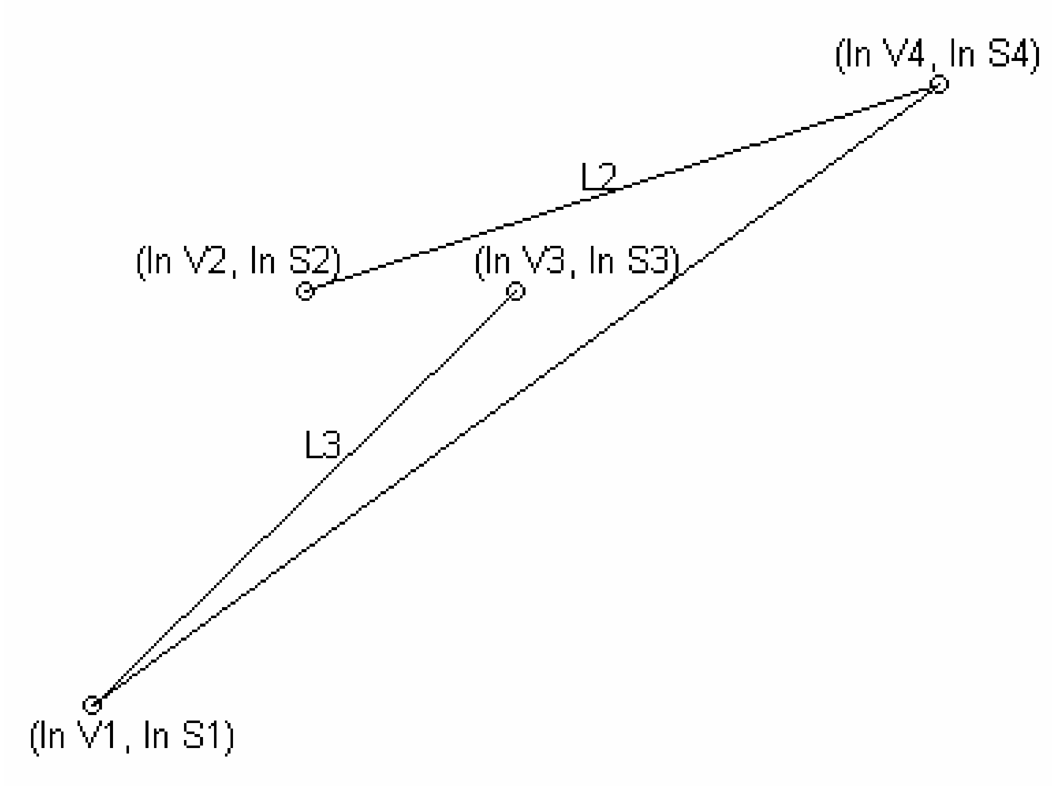
The first minus sign means that $(\ln V_3, \ln S_3)$ must be below L, a contradiction.

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

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

<u>Case 5</u> $(+,-,+,-)$. This case is ruled out either by the same reasoning as case 1 or by the same reasoning of case 2.

<u>Case 6</u> $(-,+,+,-)$. In a similar manner to case 2, consider the points and lines



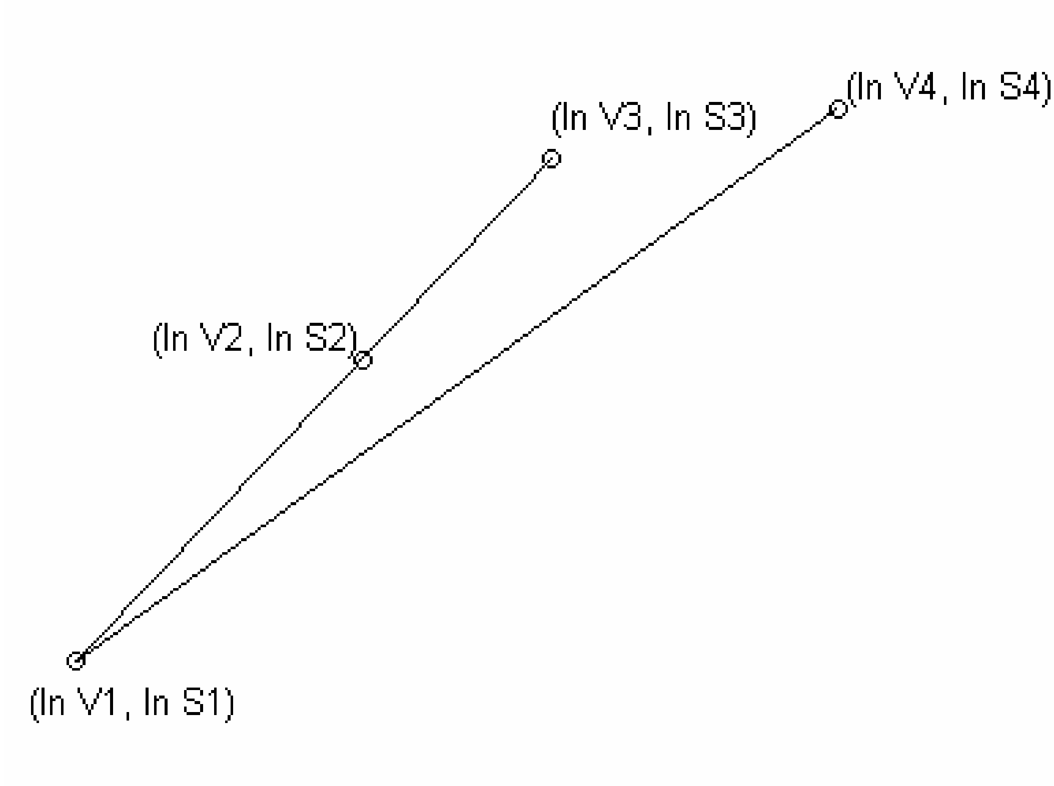
The first minus sign says that L3 is below L2 but the last minus sign says that L2 is below L3, a contradiction.

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

Case 8 If any one 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:



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

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

<u>Case 11</u> $\alpha_2 = 0$. 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 $\alpha_1 + \alpha_2 + \alpha_3 < 0$ we have $h(V_m +) = 0$ (figure 2). Similar arguments to the above show that there can't be a unique solution here either. The theorem is proved.

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

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