STUDIES ON THE DIFFERENTIAL EQUATIONS OF ENZYME KINETICS.
II. BIMOLECULAR REACTION:
THE FITTING OF EXPERIMENTAL DATA TO THE SIMPLIFIED MODEL.

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ABSTRACT. Efficient and unbiased estimates of the parameters of the differential sys-
tem, as well as simultaneous fiducial limits, are obtained through an (eventually
weighted) least-squares fitting to a Taylor expansion of the concentration of the
products of the reaction.

1. INTRODUCTION.

Let us consider a reaction in which an enzyme E reacts with a substrate S;
this results in the formation of an activated complex ES^* which, in the simplified
model under investigation, splits irreversibly into the enzyme and the products of
the reaction P. The corresponding chemical scheme is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES^* \overset{k_2}{\longrightarrow} E + P \quad ,$$
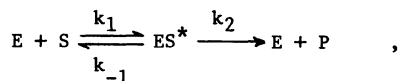
where the k's are velocity constants. Let e, s and x be the concentration of free
enzyme, free substrate and activated complex respectively. Then the differential sys-
tem which describes the kinetics of the reaction is

$$ds/dt = -k_1 e_o s + k_{-1} x + k_1 sx$$
$$dx/dt = k_1 e_o s - (k_{-1} + k_2)x - k_1 sx \quad , \tag{1.1}$$

where e_o denotes the initial concentration of enzyme and with the initial conditions

$$s(0) = s_o \quad , \qquad x(0) = 0 \quad . \tag{1.2}$$

This system is non-linear and no close-form solution is available. It has been

shown in [1] that of the two classical approximations of (1.1)-(1.2), that due to

Haldane and Briggs and known as the steady-state approximation is preferable, but its

domain of validity is limited to very small values of e_o or s_o, which points to the

need of a more direct approach. Anyway this approximation allows one to estimate only

the two combinations of parameters $k_2 e_o$ (the v_{max} of classical enzyme kinetics theo-

ry) and the Michaelis constant $K_M = (k_{-1} + k_2)/k_1$. Thus, even assuming that the

Briggs-Haldane scheme would be used in conjunction with the extrapolation technique

suggested in [1] and therefore made more reliable, it remains that it is intrinsi-

cally limited and leads to discard much of the information contained in a kinetic

curve.

In practice the interest is focused on the initial portion of this curve

because – a feature not restricted to experiments carried out with enzymes – parasite

reactions may occur later and mask the phenomenon under scrutiny. In the Briggs-

Haldane method (and in the Michaelis-Menten one as well) one uses exclusively the

linear segment of the graph reaction velocity versus time. However the information

sought, i.e. the numerical value of the parameters of the differential system is con-

tained in part in the non-linear portion of the curve and it is there generally

speaking – that is whatever may be the dependent variable selected – that the anal-

ysis must be extended.

Thus the purpose of this paper is to describe a technique suitable for the

fitting of a set of experimental data to the solution of (1.1)-(1.2) and for obtain-

ing estimates, with their fiducial limits, of the parameters e_o and k's. The discus-

sion is conducted in terms of quantities easily evaluated by a digital computer.

2. THE FUNDAMENTAL REGRESSION.

Let there be t_i (i = 1,2,...,n) the times at which a certain variable is mon-

itored in order to define the kinetic curve of an enzymatic reaction. As usual we

take t as the (statistically) independent variable; we select as dependent variable

the concentration of the products of the reaction p, which satisfies

$$dp/dt = -(ds/dt + dx/dt) = k_2 x \quad . \tag{2.1}$$

Taking into account the initial conditions $p(0) = x(0) = 0$ one obtains, near

the origin, p in the form

$$p = k_2 [x_o' t^2/2! + x_o'' t^3/3! + \ldots + x_o^{(i)} t^{i+1}/(i+1)! + \ldots] \quad ,$$

where the ' indicate derivatives with respect to time, and the problem is reduced to

the evaluation of $x_o^{(i)}$ (i = 2,3,...). The graph of p versus t starts tangentially to

the time axis (at the origin), with an upward concavity (as at the origin dx/dt =

$k_1 e_o s_o > 0$) and later exhibits an inflexion point (at the time T of [1]). Thus, at the beginning of the reaction at least, the function $p(t)$ is strongly non-linear and therefore presents the features required for the analysis under consideration.

In order to facilitate the computer processing of the equations to be developed, we relabel the parameters as follows,

former notation: e_o k_1 k_{-1} k_2

new notation   : K_1 K_2 K_3 K_4 .

Then (1.1) becomes

$$ds/dt = K_3 x - K_2(K_1 - x)s$$
$$dx/dt = K_2(K_1 - x)s - (K_3 + K_4)x \qquad . \qquad (2.2)$$

The solution $p = p(t; K_1, K_2, K_3, K_4)$ is obtained by means of the standard Taylor expansion method. The calculations are much simplified if, taking into account (2.1), one rewrites (2.2) in the non-canonical form

$$s' = K_3 x - K_2(K_1 - x)s$$
$$x' = -K_4 x - s'.$$

Putting

$$a = K_1 K_2 + K_2 s_o + K_3$$
$$b = a + K_4$$
$$c = K_1 K_2 (2K_2 s_o - K_4)$$
$$d = b^2 + 2c + 4K_1^2 K_2 s_o \qquad ,$$

one obtains a Taylor approximation in the form

$$p = K_1 K_2 K_4 s_o [t^2/2! - bt^3/3! + (b^2 + c)t^4/4! - (bd - 3K_1 K_2^2 K_4 s_o)t^5/5!] \qquad . \quad (2.3)$$

Assume now that we know a set of approximate values K_i (i = 1,2,3,4) of the parameters. The effect of small variations ΔK_i around these values on p considered as a function of the K_i's is given by

$$p(t; K_1 + \Delta K_1, K_2 + \Delta K_2, K_3 + \Delta K_3, K_4 + \Delta K_4) = p(t; K_1, K_2, K_3, K_4) + \sum_{i=1}^{4} \phi_i \Delta K_i + \sum_{i=1}^{4} 0(\Delta K_i^2)$$

in the domain D discussed in Section 5 and with $\phi_i = \dfrac{\partial p}{\partial K_i}$ (i = 1,2,3,4). We have

$$\phi_1/K_2 K_4 s_o = t^2/2! - (b + K_1 K_2)t^3/3! + (b^2 + 2bK_1 K_2 + 2c)t^4/4!$$
$$- [(b + K_1 K_2)d + 2b(bK_1 K_2 + c + 2K_1 K_2^2 s_o) - 6K_1 K_2^2 K_4 s_o]t^5/5!$$

$$\phi_2/K_1 K_4 s_o = t^2/2! - (a + b - K_3)t^3/3! + [b^2 + 2b(a - K_3) + 2c + 2K_1 K_2^2 s_o]t^4/4!$$
$$- \{(a + b - K_3)d + 2b[(a - K_3)b + c + 6K_1 K_2^2 s_o] - 9K_1 K_2^2 K_4 s_o\}t^5/5!$$

$$\phi_3/K_1 K_2 K_4 s_o = -t^3/3! + 2bt^4/4! - (2b^2 + d)t^5/5!$$

$$\phi_4/K_1 K_2 s_o = t^2/2! - (b + K_4)t^3/3! + (b^2 + 2bK_4 + c - K_1 K_2 K_4)t^4/4!$$
$$- [(b + K_4)d + 2bK_4(b - K_1 K_2) - 6K_1 K_2^2 K_4 s_o]t^5/5!$$

Given a certain set of experimental values p_i^{exp} ($i = 1,2,\ldots,n$), one can apply the classical Gauss linearization technique and compute, within the domain D, corrections ΔK_i ($i = 1,2,3,4$) to the estimates K_i by means of the quadrilinear regression

$$p^{exp} = p(t;K_1,K_2,K_3,K_4) + \sum_{i=1}^{4} \phi_i \Delta K_i \qquad . \qquad (2.4)$$

This is a constrained regression as the coefficient of p is equal to unity. In order to remove this constraint we define the new variable $z \equiv p^{exp} - p$ and relation (2.4) becomes

$$z = \sum_{i=1}^{4} \phi_i \Delta K_i \qquad . \qquad (2.5)$$

The preceding regression constitutes the fundamental regression of the problem. It will be noticed that it is mathematically constrained (as opposed to statistically) to pass through the origin. Hence there remain n - 4 degrees of freedom.

3. ESTIMATES AND FIDUCIAL LIMITS: BASIC THEORY.

We first assume that p^{exp} is normally distributed. Then a least-squares solution of (2.5) or more generally of a N-linear regression of the same type will furnish estimates of ΔK_i ($i = 1,2,\ldots,N$) which are both efficient and unbiased. Hence by solving iteratively the regression until the increments are made sufficiently small, one will obtain efficient and unbiased estimates of the K_i's. Denoting by ϕ_{ik} and z_k respectively the value of ϕ_i and z for the k-th experimental point, the normal equations of the problem can be written as $T\vec{\Delta} = \vec{q}$, where T is the real symmetric matrix of general element

$$t_{ij} = \sum_{k=1}^{n} \phi_{ik}\phi_{jk} \qquad (i,j = 1,2,\ldots,N) \qquad ,$$

$\vec{\Delta}$ the vector of general element ΔK_i and $\vec{q}$ the vector of general element

$$q_i = \sum_{k=1}^{4} \phi_{ik}z_k \qquad (i = 1,2,\ldots,N) \qquad .$$

Then one formally obtains $\vec{\Delta}$ from $\vec{\Delta} = T^{-1}\vec{q}$.

The total sum of squares is Σz_k^2 and the sum of squares accounted for by the regression is $\vec{\Delta} \cdot \vec{q}$. Hence the variance S^2 is estimated from

$$(n - N)S^2 = \sum_{k=1}^{n} z_k^2 - \vec{\Delta} \cdot \vec{q}. \qquad (3.1)$$

Let t^{ij} be the general element of T^{-1}. Then the variance of K_i is $S^2 t^{ii}$ and as K_i is normally distributed, fiducial limits for this quantity (and consequently for K_i) can be obtained as

$$K_i + St\sqrt{t^{ii}} \qquad (i = 1,2,\ldots,N) \qquad , \qquad (3.2)$$

where t is the value of the Student t-distribution at the selected level of confidence.

One could use the fiducial limits (3.2) to test statistically the concordance between the set of experimental data and the computed values of the parameters, but

such a test is inefficient because these limits are not simultaneous: the probability that they be reached simultaneously is smaller than the probability corresponding to the selected t point[4].

The determination of simultaneous fiducial limits has already been treated in [2] and [3] but we give here a more streamlined derivation. The sum of squares accounted for by the regression $\vec{\Delta}\cdot\vec{q}$, used in (3.1) to estimate the variance, can be written as

$$Q = \vec{\Delta}'T\vec{\Delta} \qquad , \qquad (3.3)$$

where a ' denotes a transposition; thus Q is a quadratic form which is distributed as χ^2 with N degrees of freedom. On the other hand S^2 is χ^2-distributed with n - N degrees of freedom. Hence the ratio $(Q/N) \div [S^2/(n - N)]$ is distributed as F with N and n - N degrees of freedom and the relation

$$Q = [N/(n - N)]FS^2 \qquad (3.4)$$

defines simultaneous limits for the ΔK_i's at the level of confidence selected for F.

In many situations however, the interest is focused on the relative variations $\Delta K_i/K_i$ rather than on the magnitude of the K_i's. Therefore we define

$$\alpha_i = \Delta K_i/K_i \qquad (i = 1,2,...,N)$$

and Q becomes

$$Q = \sum_{i=1}^{N} \sum_{j=1}^{N} t_{ij}\alpha_i\alpha_j K_i K_j \qquad (3.5)$$

In the space of the α_i's the solutions of (3.4)-(3.5), that is feasible simultaneous limits, are hyperellipsoids and this makes in most cases for unnecessarily complicated calculations. Now amongst all these ellipsoids there is, at a given level of confidence, one hypersphere whose radius x satisfies

$$x^2(\vec{K}'T\vec{K}) = [N/(n - N)]FS^2 \qquad , \qquad (3.6)$$

where $\vec{K}$ is the vector of general element K_i. Let us define

$$Q_K = \vec{K}'T\vec{K} \qquad ;$$

it will be noticed that Q_K is computed from quantities present in memory at the end of the iteration. Then (3.6) takes the form

$$x^2 Q_K = [N/(n - N)]FS^2 \qquad .$$

Let x_1 be the value of x for the critical value F = 1. Thus

$$x_1^2 Q_K = [N/(n - N)]S^2 \qquad ,$$

and simultaneous relative departures of the parameters from the computed values K_i by an amount no greater than x_1 are meaningless, whatever may be the level of confidence. This is to say that x_1 is a statistic which measures the degree of concordance between say the solution (2.3) of the differential system and the (normally dis-

tributed) set of experimental data.

Now (3.6) can be rewritten as $x^2 = x_1^2 F$. Hence once x_1 is known, one can compute simultaneous fiducial limits at a specified level of confidence.

When the variance of p^{exp} is not homogeneous, it is sufficient to divide both sides of (2.5) by the weight factor $w = Var(p^{exp})$. The introduction of w is quite essential: for example when the course of the reaction is followed spectrophotometrically, as in that case the variance of p^{exp} exhibits dramatic variations on the domain of absorbancies most used[5]. For the calculation of w in situations of practical interest, see [3].

Finally it must be emphasized that even when one exclusively analyzes sets of normally distributed p^{exp}, it is advisable to introduce formally the weight factors w (all equal to 1 when the variance is homogeneous) because the scanning technique described in [3] and used to detect inhomogeneities in a set of data analyzed with respect to a steady-state approximation formula is directly applicable to the case at hand. Assume that the k-th point is aberrant: if one depresses in turn the influence of each point by increasing its associated w factor, the x_1 values calculated will be more or less of the same magnitude, except for the k-th one which will be markedly smaller. One can then test the hypothesis that the k-th point is the only aberrant one of the set in the following way: the influence of that point is depressed or quasi-eliminated by increasing its w factor and the remaining points are scanned as previously. If the sole k-th point stands apart from the others, the x_1 values recorded now will have more or less the same magnitude; etc.

4. THE ILL-CONDITIONED CASE.

By construction the matrix T is positive definite, but barely so in fact, and it is nearly singular (as can be verified, e.g., on the system $s_o = 100$, $K_1 = K_2 = .01$, $K_3 = .005$, $K_4 = .008$). On the other hand it must be emphasized that to some extent the condition of a linear system is relative, in the sense that, as in any linear algebra computation, the machine word size and the presence or the absence of a double-length accumulator are of paramount importance. Furthermore, even if the matrix of the complete system, that is to say with N = 4 variables, cannot be inverted, it may happen that through the exclusion of one variable, the matrix of order 3 so obtained will prove readily invertible on the particular computer used. For instance the condition of the matrix T associated with the system mentioned improves when one excludes either K_1 or K_2. Such a feature could obviously be used in special circumstances to bracket by successive approximations satisfactory estimates of the 4 para-

meters but in general it is advisable to handle directly the 4-parameters problem and because from a practical standpoint the most useful discussion refers to a worst case situation, we shall examine the analysis of the solution (2.3) of the same example within the domain D (see Section 5), with a machine using mantissae corresponding to 13-14D (a value typical of many a microprocessor-based system); all the operations were performed in single length mode.

The most striking feature of the matrix T is that it loses easily its positive definiteness, for example in the course of a Cholevsky triangularization. In fact the inversion by all the classical techniques involving a triangularization fails. Thus one must abandon any idea of inverting en bloc the matrix T; as a matter of fact this is of little consequence because the only computation in which the t^{ij} - strictly speaking the t^{ii} (i = 1,2,3,4) - are involved in an essential way, is that of the variance of the parameters. But these variances are not much used as x_1 is a more convenient and realistic statistic. Thus one can dispense with the inversion of T and we consider now the lesser problem of solving once the normal equations by an iterative technique which does not require a preliminary deep transformation of the matrix of the coefficients. The standard techniques for solving a linear system with a positive and non-singular matrix, such as the conjugate gradient method or the steepest descent method, all failed.

An obvious way to deal with the evanescence of the positive definiteness of T is to multiply on the left the normal equations by T but the multiplication (here the squaring) of ill-conditioned matrices is usually deemed undesirable. This formal calculation of T^2 is avoided in a variant described by Hestenes and Stiefel [6]. This procedure proved unsuitable for our needs because huge oscillations of the terms b_i of formulae (10:2) of [6] were recorded, although the stability of the coefficients a_i was acceptable. This suggests to modify the method and to recalculate at each step the residual vector $\vec{r}$ from its definition.

This is to say that we use the following algorithm:

$$\vec{r} = \vec{q} - T\vec{\Delta}$$

$$\vec{u} = T\vec{r}$$

$$a = |T\vec{r}|^2 / |T\vec{u}|^2 \qquad\qquad (4.1)$$

$$\vec{\Delta}_{new} = \vec{\Delta}_{old} + a\vec{u}$$

with the initial value $\vec{\Delta} = 0$. No stability problem was encountered.

This procedure bears little resemblance to a conjugate gradient technique, which is essentially an orthogonalization method. On the other hand the first step of

the conjugate gradient method and that of the steepest descent method are identical
[7]; thus the algorithm (4.1) is best classified as that of an iterated steepest des-
cent and the general theory of the steepest descent method [7] is applicable to each
step individually.

The iterated steepest descent method shares with all iterative techniques one
feature, viz. there comes a time when the limiting accuracy is reached, which implies
in turn the existence of a limiting accuracy for the estimates of the parameters. The
limiting accuracy of the normal equations routine is easily observable in the case at
hand; hence an efficient strategy (worst case situation) is to subordinate the whole
computation, including the exit from the fundamental regression iteration, primarily
to the limiting accuracy exhibited by the normal equations routine rather than set
beforehand strict escape clauses of the type $|\Delta K_i|$ and/or $|\Delta K_i|/K_i < 10^{-3}$-$10^{-6}$,
$(i = 1,2,3,4)$.

Needless to say the exclusion of one variable may result in an appreciable
improvement of the limiting accuracy.

5. DISCUSSION.

There remains to characterize the domain D. It will be noticed that K_1, K_2 or
K_4 must not vanish as then the matrix T becomes singular. Furthermore negative values
of the K's are meaningless and therefore we take as first conditions for defining D:

$$K_1, K_2, K_4 > 0 \qquad , \qquad K_3 \geqq 0 \qquad . \qquad (5.1)$$

The conditions of validity of the expansion (2.3) are the same as those of
$x(t)$ and $s(t)$. In order to study the latter we consider system (2.2) in the rectangle

$$|s - s_o| \leqq B \qquad , \qquad |x| \leqq B \qquad , \qquad (5.2)$$

where $B = \max(s_o, K_1)$. Let $f_1(s,x)$ and $f_2(s,x)$ denote the right-hand sides of (2.2);
then we have [in (5.2)] the following estimates:

$$|f_1(s,x)| \leqq K_3 B + K_2(K_1 + B)(s_o + B)$$

$$|f_2(s,x)| \leqq K_2(K_1 + B)(s_o + B) + (K_3 + K_4)B \quad .$$

Putting

$$M = K_2(K_1 + B)(s_o + B) + (K_3 + K_4)B \qquad ,$$

we have in (5.2)

$$|f_1(s,x)| \leqq M \qquad , \qquad |f_2(s,x)| \leqq M \qquad .$$

Therefore the problem (1.2)-(2.2) has a unique solution defined on the inter-
val

$$|t| < B/M \qquad (5.3)$$

Since $f_1(s,x)$ and $f_2(s,x)$ are analytic (and even entire) functions, the solution

$\{s(t), x(t)\}$ is analytic on (5.3). Hence an upper bound of the maximum sampling time allowed $t_{max} = B/M$ is obtained as

$$t_{max} = B/[K_2(K_1 + B)(s_o + B) + (K_3 + K_4)B]$$.

Whatever may be the largest of the two quantities s_o and K_1, one obtains

$$t_{max} = [2K_2(s_o + K_1) + K_3 + K_4]^{-1}$$.

In other words t_{max} satisfies

$$(a + b - K_3)t_{max} = 1$$, (5.4)

and the domain D is characterized by (5.1)-(5.4).

The practical implementation of the techniques discussed in this paper and their implications regarding the experimental design of enzyme kinetics measurements will be described elsewhere. Suffice it to point out here that an immediate consequence and a major advantage of this numerical procedure is that it furnishes an estimate of e_o (= K_1) in the same units as those used for s_o. In other words, through the analysis of a kinetic curve one can obtain directly the value of the initial concentration of an enzyme in well-defined physical units.

REFERENCES.

1.   MARMASSE, C. and WIENER, J., Studies on the differential equations of enzyme
           kinetics. I. Bimolecular reaction: simplified model, Internat. J. Math.
           & Math. Sci., 1986, in print.

2.   MARMASSE, C., Structure analysis of physical data. I. General theory, Rev.
           Mex. Fis., 20 (1971), 239-52.

3.   MARMASSE, C., Enzyme kinetics - Physical bases, data analysis and uses, Gordon
           and Breach, New York, 1977.

4.   WILLIAMS, E., Regression analysis, John Wiley & Sons, New York, 1959.

5.   MARMASSE, C., On the numerical exploitation of spectrometric data, Appl. Opt.,
           4 (1965), 1632-3.

6.   HESTENES, M.R. and STIEFEL, E., Methods of conjugate gradients for solving
           linear system, J. Res. Nat. Bur. Standards, 49 (1952), 409-38.

7.   HOUSEHOLDER, A.S., Principles of numerical analysis, McGrawHill, New York,
           1953.