BIOMETRICS 38, 907–919
December 1982

Estimating Michaelis–Menten Parameters: Bias, Variance and Experimental Design[1]

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SUMMARY

The accuracy and precision with which the parameters of the Michaelis–Menten function can be estimated are known to depend upon the technique used to fit the function to experimental data. In this paper it is demonstrated that the design matrix (or choice of values for the independent variable) is equally important. The efficiency of parameter estimation by three fitting techniques is compared by Monte Carlo simulations, and the sensitivity of each technique to changes in the design matrix is examined. Minimum variance unbiased estimates of the parameters are obtained by taking half the observations at exactly K and the other half as high as possible, and then fitting the data by a maximum likelihood technique. Some designs which spread the observations across the range of substrate concentrations produce biased, high-variance parameter estimates. A geometric sequence of observations, fitted by either a maximum likelihood technique or Eisenthal and Cornish-Bowden's (1974, *Biochemical Journal* **139,** 721–730) nonparametric technique, yields acceptable estimates.

1. Introduction

The rectangular hyperbola has been used to describe saturation functions for a number of physical and biological phenomena. The form encountered most often in biology is the familiar Michaelis–Menten enzyme kinetic function,

$$v = \frac{Vs}{K+s},$$

where v is the reaction velocity, V the maximum velocity of that reaction, s the concentration of substrate, and K the half-saturation constant, the value of s at which v is half-maximal.

Given any function which is nonlinear in its parameters, such as Michaelis–Menten, the estimation of those parameters presents two distinct difficulties. The first is that the variance of the parameter estimates can be shown to depend upon the design matrix, **D**—the matrix of values chosen for the independent variable(s)—and upon the values of the parameters themselves (Box and Lucas, 1959). In the case of the Michaelis–Menten parameters, this means that the location of observations with respect to K is critical, and it will be necessary to have some preliminary estimate of K before designing an experiment. For example, in order to determine K accurately, it seems appropriate that several substrate concentrations should be included which will produce subsaturated reaction velocities, and that a few nearly saturated points should be included for determination of

[1] This paper forms a contribution to the Lake Memphremagog Project, Limnology Research Group.
Key words: Michaelis–Menten; Experimental design; Enzyme kinetics; Nonlinear regression; Bias.

V. On this sort of intuitive basis, Cleland (1967) suggests a range of substrate concentrations from $\frac{1}{5}K$ to $5K$, arranged such that their reciprocals form a linear sequence. He provides no indication, however, of the particular advantages to this arrangement, nor of the consequences of departing from it. Although little other work examining the choice of **D** appears to have been done, a recent symposium examines related aspects of the problem (Endrenyi, 1981).

The second difficulty is that a fitting technique must be chosen which produces unbiased, low-variance estimates of the parameters. Many techniques for fitting the Michaelis–Menten equation to experimental data have been proposed, including least squares fits to linear transformations of the data, iterative nonlinear techniques, and nonparametric techniques (see references in Atkins and Nimmo, 1975). In the absence of experimental error, any of these methods will yield exact values for the parameters K and V. However, the methods differ greatly in their treatment of experimental error, and can produce badly biased estimates of K and V. The various linearizing transformations have been examined exhaustively in this regard, and are generally more prone to bias the parameter estimates than are the techniques which fit untransformed data (Dowd and Riggs, 1965; Atkins and Nimmo 1975; Cornish-Bowden and Eisenthal, 1974).

Greater differences among methods of estimation, regarding the bias and variance of parameter estimates, emerge when the error is not homoscedastic (Atkins and Nimmo, 1975). The weighting of observations according to their variance can remedy the problem of heteroscedastic error (Cleland, 1967), but in practice it rarely seems to be the case that sufficient data are collected to justify this procedure (Reich, 1970). A preferable alternative to variance weighting may often be to identify a technique which is insensitive to the nature of experimental error.

The purpose of this study is to demonstrate how the variance and the bias of experimental estimates of K and V depend upon the combined effects of the choice of substrate concentrations, the fitting technique, and the nature of the experimental error. In consideration of these factors, I will suggest practical experimental protocols (**D** and fitting technique) based upon provisional estimates of K and V. These are chosen to minimize bias and variance in the parameter estimates, and to be insensitive to heterogeneity of experimental error and to poor preliminary estimates of the parameters.

2. Methods

2.1 *An Overview*

A simulation study was undertaken to determine the accuracy and variance of estimates of K and V, as three factors were varied: the design matrix, the fitting technique, and the homogeneity of the experimental error. Sets of simulated reaction-velocity data were constructed according to the function

$$v_i = \frac{Vs_i}{K + s_i} + \varepsilon_i, \qquad i = 1, 2, \ldots, n,$$

for a total of n observations, where the parameters V and K were set by definition to 1.0 and 0.75, respectively. The exact numerical values chosen for the parameters do not affect the generality of the argument, since they are in arbitrary units of measurement. The substrate levels s_i were provided in one of the design matrices described below. To this function, a stochastic error term ε_i was added. The Michaelis–Menten function was then fitted to each set of simulated data, by use of one of the fitting techniques discussed below.

This procedure produces parameter estimates $\hat{K}$ and $\hat{V}$. With each set of conditions, 250 simulations were done in order to generate distributions of $\hat{K}$ and $\hat{V}$. The mean, variance and skewness of these distributions were then examined. In a few cases, these were compared with the results obtained from 2500 simulations, and were found to be nearly identical.

2.2 *The Design Matrix*

Each design matrix **D** examined in this study was constructed to contain $n = 7$ values of s_i, at each of which v_i was evaluated. The results obtained with seven observations are easily generalizable to other values of n. Obviously, there is an infinite number of possible choices for the elements of **D**. I shall concentrate on three types of design matrix.

The minimum-variance or optimal design. The optimal design would be that which will yield minimum variance, unbiased parameter estimates. These would be provided by an ordinary least squares regression, assuming that the independent variable is error-free and the error in the dependent variable is homoscedastic. Although it is not possible to estimate the Michaelis–Menten parameters directly by these means (because one cannot write explicit solutions for the normal equations), it is a simple matter to construct the variance–covariance matrix for the parameter estimates. The determinant of this matrix is proportional to the area of any ellipsoidal joint probability contour around $\hat{K}$ and $\hat{V}$ (Box and Lucas, 1959). As described in the Appendix, the elements in the design matrix may be manipulated to minimize either the generalized joint variance of the parameter estimates, or the variance of either parameter singly.

The design matrix **D** which would yield the minimum-variance estimates of $\hat{K}$ and $\hat{V}$, given a least squares regression, has half of its elements at exactly K, and the other half as large as possible, at saturation. This is true with any number of observations in **D**. If instead, **D** is chosen to minimize the value of var($\hat{K}$), the same general arrangement of s_i results, with the lower group of s_i at 0.8 K, while var($\hat{V}$) always decreases as any s_i increases. Following this general design, a **D** in which $s_1 = s_2 = s_3 = s_4 = K$, and $s_5 = s_6 = s_7 = 10\,000$ was used for the curve-fitting simulations, and will be referred to as the optimal design.

The linear design and the geometric design. In practical experimental situations, it is usually the case that one wishes not only to estimate the parameters, but also to verify the agreement with the model. Although the optimal **D** does yield the lowest-variance parameter estimates, it affords no means of detecting departure from the Michaelis–Menten model. In practice, the s_i are usually spread in some fashion over the range of v: in a sample of papers containing Michaelis–Menten figures in the *Journal of Biological Chemistry* **255**(7) and **255**(8), the majority used a linear sequence of s_i at low concentrations, with a few s_i sprinkled at higher concentrations. A few papers used geometric sequences of s_i, or reciprocal linear sequences. The positioning of s_i with respect to $\hat{K}$ varied from all $s_i > \hat{K}$, to nearly all $s_i < \hat{K}$. It is of practical interest to examine the variance and degree of bias which results from these types of **D**, and to identify the most efficient among them.

In this context, two general types of **D** were examined: a family of **D** containing a linear sequence of s_i in which $s_i = ai$ for $i = 1, 2, \ldots, 7$ (e.g. for $a = 0.5$, **D** contains substrate concentrations 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5), and a geometric family of **D**, where $s_i = a2^{i-1}$ for $i = 1, 2, \ldots, 7$ (e.g. for $a = 0.5$, **D** contains 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0). In both cases the single constant a, which is equal to s_1, entirely defines the sequence. Varying a serves to compress or expand the sequence of s_i relative to the fixed K.

2.3 *The Experimental Error*

From each design matrix, a corresponding series of error-free reaction velocities was calculated. To each of these, a random, normally distributed error term ε_i, was added in order to produce simulated experimental data. The ε_i were produced by a McGill University Computing Centre Library program, using a combination of a congruential multiplicative, and a shift-register pseudorandom number generator.

Two special assumptions about variances were used in developing the model: (i) homogeneous variance, in which $\varepsilon \sim N(0, 0.1)$; (ii) a heteroscedastic condition in which error was dependent on s_i, and $\varepsilon_i \sim N(0, z)$ where $z = 0.05 + 0.1 s_i/(K + s_i)$. When negative values of v_i were produced, they were arbitrarily set to +0.01. As a result, some error distributions are not strictly normal, but they are all unbiased.

2.4 *Fitting Technique*

The Michaelis–Menten function was fitted to each set of simulated data, by one of the following three techniques, selected because each appeared to offer certain advantages in comparison with the array of other possible alternatives.

The Woolf linearizing transformation. The Woolf transformation,

$$\frac{s_i}{v_i} = \frac{K}{V} + \frac{1}{V} s_i,$$

has been shown by Dowd and Riggs (1965) to be the most efficient of the three common reciprocal transformations. An unweighted, least squares regression was used to fit a straight line to the transformed data.

It is worth noting that virtually all of the papers examined in the *Journal of Biological Chemistry* used the Lineweaver–Burke double-reciprocal transformation, despite the fact that it is more prone to produce biased $\hat{K}$ than any other commonly used technique (Dowd and Riggs, 1965; Atkins and Nimmo, 1975).

The iterative nonlinear procedure. This technique, described by Bliss and James (1966) and programmed by Hanson, Lung and Havir (1967), provides maximum likelihood parameter estimates, given the assumptions of least squares regression. Provisional parameter estimates, provided by the experimenter, are used to fit a bilinear, least squares regression of v_i on the Michaelis–Menten function plus its first derivative. The K is iteratively modified until the derivative becomes vanishingly small. The procedure is very similar to that of Wilkinson (1961).

The nonparametric procedure. This procedure is an algorithmic version of the 'direct linear plot' technique, originated by Eisenthal and Cornish-Bowden (1974). Each of the n observations (s_i, v_i) are taken pairwise in two simultaneous equations to solve for the parameters of the Michaelis–Menten function, so that $n(n-1)$ estimates of the parameters are generated. The medians of these estimates are taken as the final parameter estimates. The authors describe their technique as making no assumptions about the nature of experimental error.

3. Results

3.1 *The Optimal Design*

The optimal design should yield the most precise estimates of K and V, given a least squares fit. A maximum likelihood solution found by the iterative nonlinear technique

yielded a positively skewed distribution of $\hat{K}$ whose mean was slightly, but not significantly, greater than K [mean $\hat{K} = 1.04K$; var$(\hat{K}) = 0.038$]. The parameters can also be estimated by the Woolf transformation, which produces positively biased estimates of K [mean $\hat{K} = 1.10K$; var$(\hat{K}) = 0.048$]. In this case and in all those discussed below, var$(\hat{V})$ is very much smaller than var$(\hat{K})$, is markedly less sensitive to the fitting technique, and is invariably monotone decreasing as any s_i increases. For this reason, the discussion below will be confined primarily to $\hat{K}$.

Given the optimal design, the simplest procedure to estimate K and V is to average the replicates in the two groups of observations, and calculate the parameters directly in a pair of simultaneous equations. For example, for (s_i, v_i) and (s_i, v_i),

$$\hat{V} = (s_i - s_j)\left(\frac{s_i}{v_i} - \frac{s_j}{v_j}\right)$$

and

$$\hat{K} = \frac{s_j(V - v_j)}{v_j},$$

where v_j and v_i are the means of the two groups of reaction velocities, and s_j and s_i are the corresponding substrate concentrations. This procedure yields parameter estimates identical to those from the iterative technique, and can be done with paper and pencil. Among the unbiased estimators of K, this estimator had the smallest variance of all those examined, but it did not achieve the Cramér–Rao lower bound.

The minimum generalized variance is obtained with this **D** only when the first half of the observations are taken at exactly K, requiring one's preliminary estimate of K to be precisely correct. The effect of varying s_1 (where $s_1 = s_2 = s_3 = s_4$) with respect to K is shown in Fig. 1; this is essentially a demonstration of the loss of precision resulting from the departure of the estimated K from the true K. Over a range of $\frac{1}{2}K < s_1 < 2K$, $|(\mathbf{X}'\mathbf{X})^{-1}|$ increases by approximately 50% above its optimum value, and var$(\hat{K})$ behaves similarly. As $s_1, s_2, \ldots, s_{\frac{1}{2}n}$ depart from K, the $\hat{K}$ estimated with the nonlinear procedure becomes increasingly positively biased, although the bias is slight near K.

The number of observations in each of the two groups must be the same if the generalized variance is to be minimized; an odd number of observations may be split either way. Given a constant total number of observations in **D**, placing more in the lower group leads to improved var$(\hat{K})$ at the cost of increased var$(\hat{V})$ and cov$(\hat{K}, \hat{V})$. Conversely, var$(\hat{V})$ is improved as more observations are taken at a higher level.

3.2 *The Geometric Design and the Linear Design*

Using these more orthodox designs, the theoretical least squares variance of the parameters, calculated in the manner described in the Appendix, as a function of the constant a, is shown in Fig. 2, A and B. The values of var$(\hat{K})$ and the joint parameter variance $|(\mathbf{X}'\mathbf{X})^{-1}|\,\sigma^2$ depend strongly upon the location of the s_i with respect to the true value of K. The minimum value of $|(\mathbf{X}'\mathbf{X})^{-1}|$ found among all of these sequences is approximately twice the optimum $|(\mathbf{X}'\mathbf{X})^{-1}|$. It occurs with a geometric design where $s_1 = \frac{2}{3}K$. Note that $s_2, s_3, \ldots, s_7$ are all greater than K. With the linear sequences of s_i, the variances are still greater than those of the geometric sequences. Again, in order to minimize the variance of the parameter estimates only one of the seven observations was taken below K.

The Woolf transformation. Depending upon the **D** and the fitting technique chosen, the variance of the parameter estimates may be considerably greater than the theoretical least

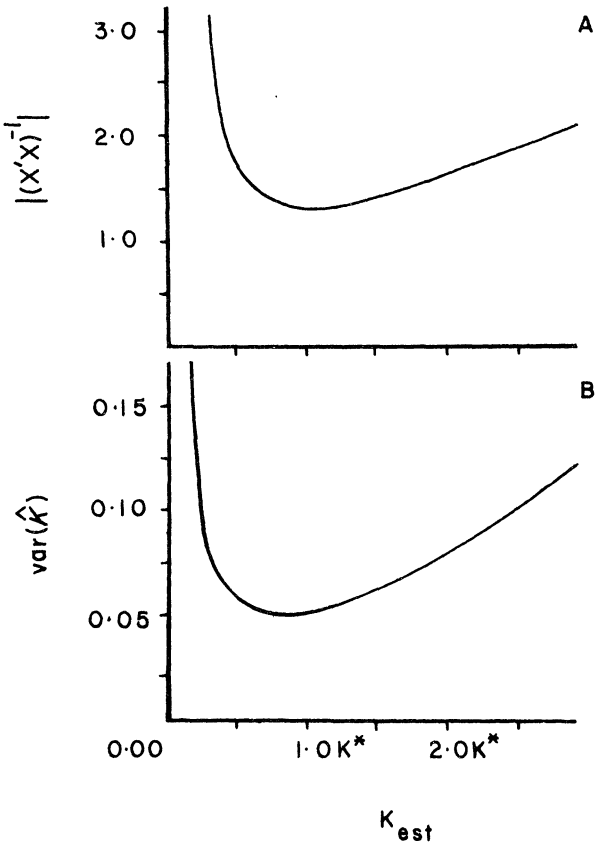

Figure 1. The loss of precision resulting from the departure of a preliminary estimate K_{est}, from the true parameter K^*, given an optimized design matrix (where $s_1 = s_2 = s_3 = s_4 = K_{est}$ and $s_5 = s_6 = s_7 \gg K^*$).

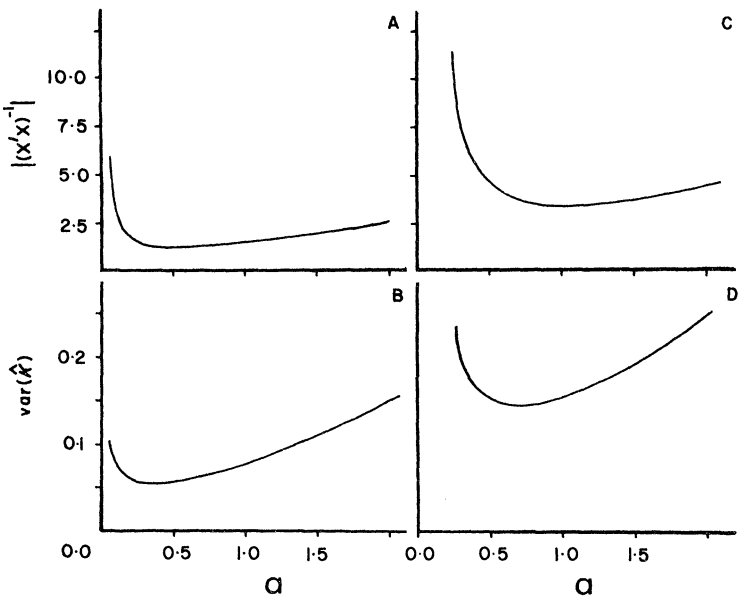

Figure 2. The response of the theoretical maximum likelihood generalized parameter variance $|(\mathbf{X}'\mathbf{X})^{-1}|$, and the variance of the estimated K, to the spacing of observations in the design matrix with respect to a fixed $K = 0.75$. In A and B a geometric sequence was used, with $s_i = a2^{i-1}$ for $i = 1, 2, \ldots, 7$; in C and D, a linear sequence with $s_i = ai$.

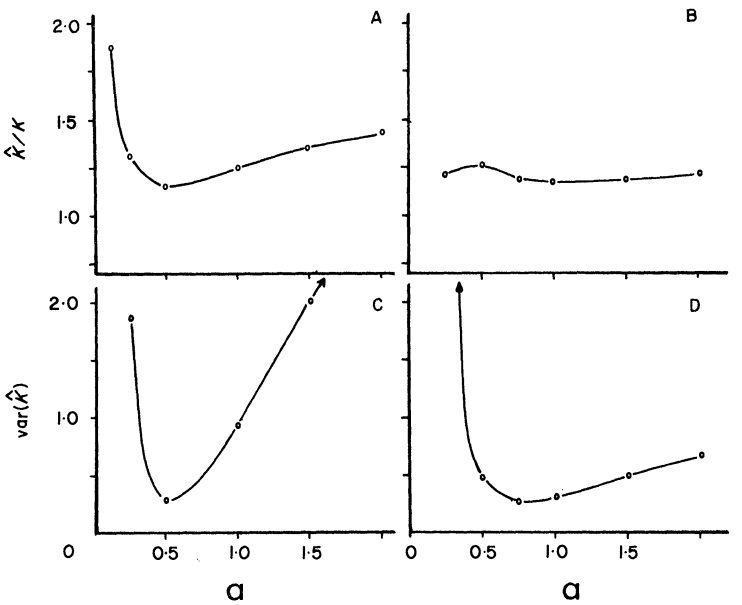

Figure 3. The response of the bias (mean $\hat{K}/K$) and var($\hat{K}$) to geometric sequences of observations (A and B) and to linear sequences of observations (C and D), when the Woolf transformation is used.

squares variance given above, and the estimates may be biased. Using the same families of **D** as before, the variance and bias of $\hat{K}$ provided by the Woolf transformation are shown in Fig. 3. Unbiased estimates of $\hat{K}$ are never obtained with either linear or geometric sequences of s_i, and the $\hat{K}$ becomes more positively biased as the observations are concentrated below K. The value of var($\hat{K}$) also depends very strongly upon a: at its minimum it is three times the optimum var($\hat{K}$), and increases very rapidly as a departs from $\frac{2}{3}K$.

The Woolf transformation actually performs somewhat better with heteroscedastic error increasing proportionally to the mean, inasmuch as the reciprocal transformation serves to improve the stability of the variance. The bias of $\hat{K}$ varies with a in a manner similar to Fig. 3, but is slightly diminished.

The iterative nonlinear procedure. The iterative nonlinear technique of Bliss and James is very much less sensitive to **D** than is the Woolf transformation, provided that a geometric sequence of s is used. The method then produces an estimator $\hat{K}$ which is very slightly positively biased (Fig. 4). The value of var($\hat{K}$) has a minimum at $a = \frac{1}{3}K$, with a variance approximately $2\frac{1}{2}$ times greater than the efficient var($\hat{K}$), and increases very little over a range of $\frac{1}{5}K < a < 1\frac{1}{2}K$. However, a linear design can badly overestimate $\hat{K}$. Even though one of the assumptions of this technique is homoscedastic error, the $\hat{K}$ obtained with the heteroscedastic error described above differed very little from the $\hat{K}$ obtained with homoscedastic error.

The nonparametric procedure. The nonparametric technique of Eisenthal and Cornish-Bowden (Fig. 5) is also relatively insensitive to **D**. It yields an estimator $\hat{K}$ which is no more than slightly biased, with any of the geometric sequences of s_i examined. The value of var($\hat{K}$) is considerably less dependent upon **D** and has a minimum variance at $a = \frac{1}{3}K$, about 2.3 times greater than the calculated least squares var($\hat{K}$). All of the linear

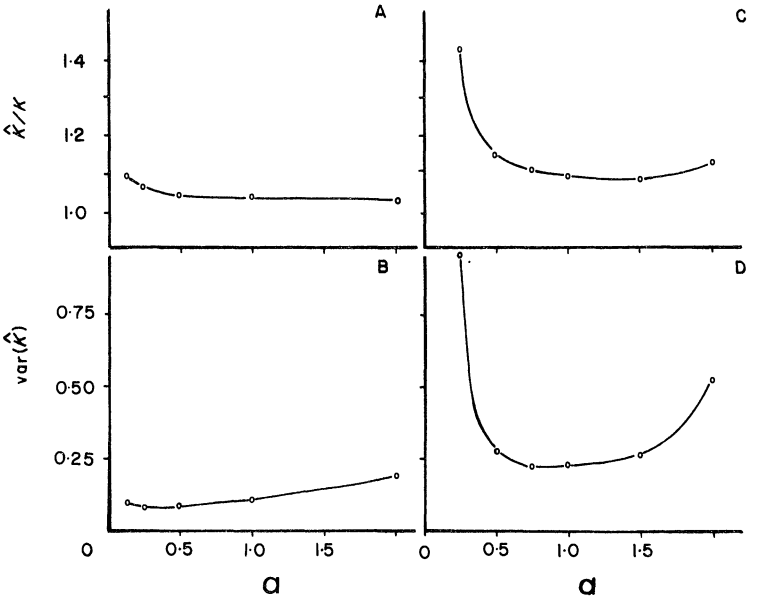

Figure 4. As in Fig. 3, for the maximum likelihood, iterative nonlinear technique of Bliss and James.

sequences of s_i, however, produced badly negatively biased estimates of K, especially when the observations are concentrated below K, i.e. when $a \ll K$.

A most attractive feature of Eisenthal and Cornish-Bowden's technique is that it is described as being independent of the nature of experimental error. Given a geometric sequence of observations, this is quite true: the bias in $\hat{K}$ is virtually identical with either of the two types of error investigated, and var($\hat{K}$) is only slightly greater with the

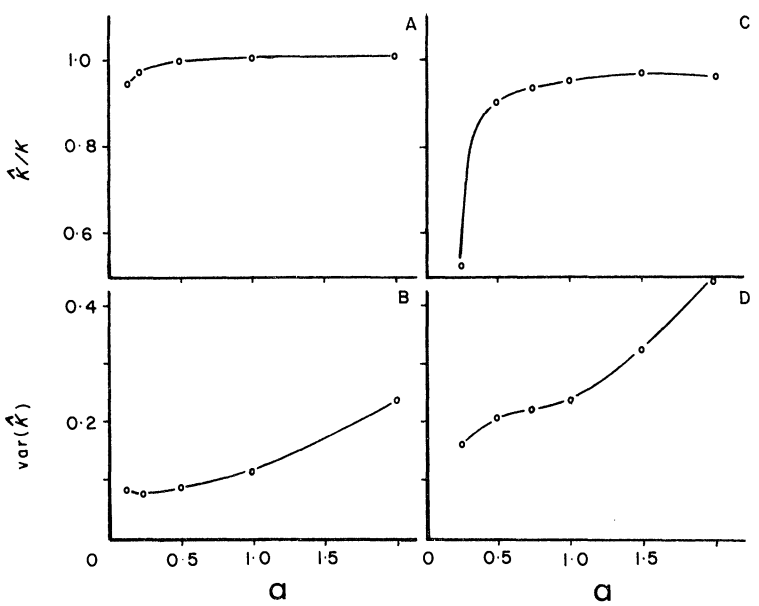

Figure 5. As in Fig. 3, for the nonparametric procedure of Eisenthal and Cornish-Bowden.

Table 1
For a sequence of n observations of the form $s_i = ar^{i-1}$, the values of a and r which minimize the generalized parameter variance $|(\mathbf{X}'\mathbf{X})^{-1}|$

n	a	r	$\lvert(\mathbf{X}'\mathbf{X})^{-1}\rvert$	$\text{var}(\hat{K})/\sigma^2$
5	0.712 K	4.56	$4.07(K/V)^2$	$14.67(K/V)^2$
6	0.601 K	3.45	$2.85(K/V)^2$	$12.17(K/V)^2$
7	0.544 K	2.88	$2.11(K/V)^2$	$10.45(K/V)^2$
8	0.508 K	2.52	$1.62(K/V)^2$	$9.16(K/V)^2$
9	0.482 K	2.27	$1.28(K/V)^2$	$8.15(K/V)^2$
10	0.462 K	2.09	$1.04(K/V)^2$	$7.33(K/V)^2$
12	0.434 K	1.84	$0.72(K/V)^2$	$6.12(K/V)^2$
15	0.408 K	1.63	$0.46(K/V)^2$	$4.90(K/V)^2$

heteroscedastic error. However, with linear sequences of s_i, K is even more severely underestimated when the error is not homoscedastic.

A modification of this technique was more recently published (Cornish-Bowden and Eisenthal, 1978) which involved using a double-reciprocal transformation, solved for K/V and $1/V$ rather than K and V. Although this procedure is supposed to produce 'median-unbiased' parameter estimates, it appears to yield parameter estimates which are often more badly biased than those of the original technique; (work in course of preparation).

3.3 *Other Designs*

It is possible that many designs **D** other than those considered thus far would provide equally accurate and precise parameter estimates. Any geometric sequence, $s_i = ar^{i-1}$, $i = 1, 2, \ldots, n$, with r between 2 and 5, behaved similarly to the geometric sequence used to produce Fig. 2, with about the same minimum $|(\mathbf{X}'\mathbf{X})^{-1}|$. Table 1 shows the constants a and r which minimize $|(\mathbf{X}'\mathbf{X})^{-1}|$ for design matrices of varying size. An experiment designed according to this sequence should produce unbiased, reasonably low-variance $\hat{K}$ and $\hat{V}$. A series of s_i such that the v_i will be linearly spaced is also intuitively appealing; it is slightly more efficient than a geometric series when fitted by the iterative method, but $\hat{K}$ is negatively biased if the Eisenthal and Cornish-Bowden technique is used.

3.4 *The Magnitude of the Experimental Error*

In all the simulations reported in earlier sections, a constant error term of $\sigma^2 = 0.1$ was used. Both the bias and the variance of the parameter estimates increase as simple monotonic functions of σ^2 when the Woolf transformation is used to fit the data, as shown in Fig. 6. The shape of this function is essentially the same, regardless of the geometric sequence of s_i used. The iterative nonlinear technique behaves similarly, except that at very low σ^2 the direction of the bias may be reversed (see Fig. 6). The magnitude of the effect is so small, however, that it is not significant. Essentially, this means that the magnitude of the error will not affect the relative efficiency of the various geometric designs when either of these fitting techniques is used.

The nonparametric technique, however, behaves very differently, as shown in Fig. 7. Although $\text{var}(\hat{K})$ is a simple function of σ^2, the bias is not even monotonic. Strictly, if the nonparametric technique is to be used one must have an *a priori* estimate of σ^2 before designing an experiment, in order to obtain an unbiased $\hat{K}$. In practice, however, unless σ^2 is quite large, the values of $\text{var}(\hat{K})$ and the bias of $\hat{K}$ will be nearly minimal, given a geometric sequence with a between $\frac{1}{3}K$ and $2K$.

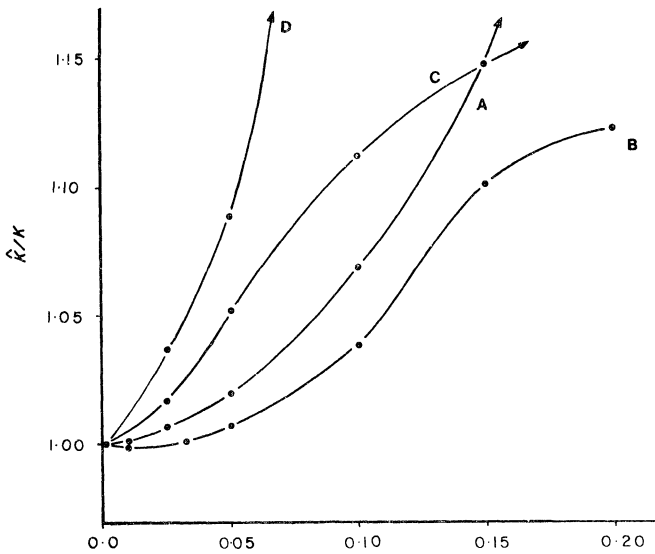

Figure 6. The bias (mean $\hat{K}/K$) as a function of increasing error variance σ^2, with fixed geometric design matrices. A, iterative nonlinear fit, $a = 0.25$; B, iterative nonlinear fit, $a = 1.0$; C, Woolf transformation, $a = 0.25$; D, Woolf transformation, $a = 1.0$.

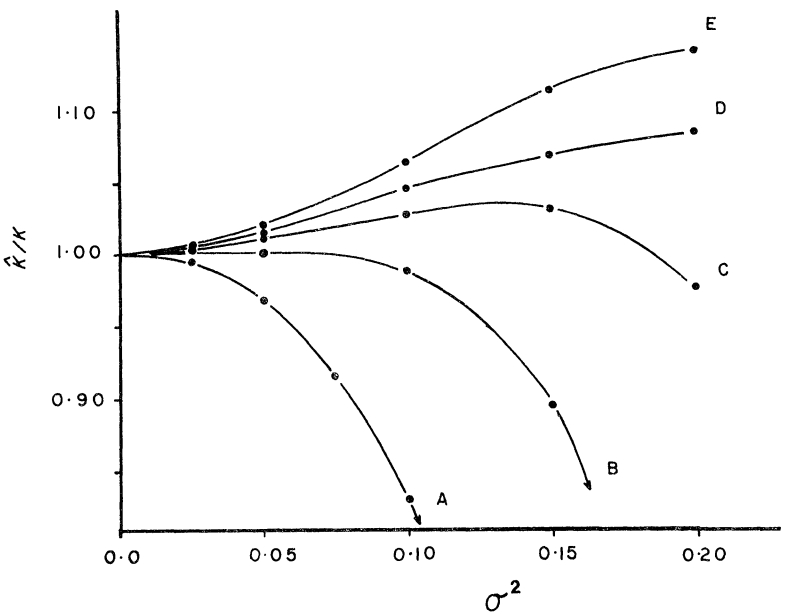

Figure 7. The bias (mean $\hat{K}/K$) as a function of increasing error variance σ^2, with fixed design, and the nonparametric fit. A, $a = 0.125$; B, $a = 0.25$; C, $a = 0.50$; D, $a = 1.00$; E, $a = 2.00$.

4. Conclusions

It has been well demonstrated by several investigators that the choice of fitting technique can strongly influence the accuracy and precision of estimates of the Michaelis–Menten parameters. The results presented here demonstrate that the choice of the design matrix is equally important.

Given a provisional estimate of K, the most precise and accurate parameter estimates are obtained by taking half the observations at the estimated K, the other half at the highest practical limit of s, and fitting with either the simple averaging method discussed above, or by the iterative nonlinear technique. Provided that one's preliminary estimate of K is between one-half and two times the true K, very little efficiency is lost from a poor preliminary estimate (Fig. 1).

The design matrices commonly found in the literature yield parameter estimates which vary considerably in accuracy and precision. The best among them produce unbiased parameter estimates with variances two to three times greater than the optimal design. In general, linear sequences of observations yield much poorer estimates than do geometric sequences. The worst estimates occur when more than about 10% of the observations are taken below K. However, these designs have the distinct advantage of allowing one to observe the agreement of observations with the Michaelis–Menten model.

A geometric sequence of substrates constructed according to Table 1 will provide unbiased, relatively low-variance parameter estimates, if the observations are fitted by either the iterative nonlinear procedure of Bliss and James (1966), or the nonparametric technique of Eisenthal and Cornish-Bowden. On the basis of the evidence presented in this paper, there is little ground to choose between the two techniques; the Eisenthal and Cornish-Bowden nonparametric procedure is probably preferable when the error is known to be heteroscedastic, and the Bliss and James iterative nonlinear technique when it is homoscedastic. There are, however, several other techniques for obtaining maximum likelihood parameter estimates, such as that of Wilkinson (1961), the Generalized Linear Models (GLIM) statistical package of the Royal Statistical Society, or a more general nonlinear curve-fitting algorithm such as that of Marquardt (1963). Any of these techniques should yield results comparable to those of the iterative technique used in this study.

Despite the extremely widespread use of linear fits to reciprocal transformations of the Michaelis–Menten function, it again appears that this is the worst procedure. The Woolf transformation, the best among the linear transformation (Dowd and Riggs, 1965; Atkins and Nimmo, 1975), provides unreliable parameter estimates which depend strongly upon **D**. Transformations should not be used, except in cases where they stabilize the error.

Finally, it is well to note that many other fitting techniques have been proposed in the literature. A number of these techniques have been shown to provide unreliable parameter estimates under certain circumstances; the Lineweaver–Burke transformation is the prime example (Dowd and Riggs, 1965). Often the efficiency of a proposed technique is not tested at all. It should now be apparent that even the techniques which have been demonstrated to provide good parameter estimates at one particular **D** will not necessarily be equally appropriate for any other design.

Acknowledgements

I am very grateful to John Downing and Bruce Lazerte for their many helpful discussions and suggestions, and to two referees who provided valuable comments. This work was supported by grants to the author from the McGill University Computing Centre, and the Faculty of Graduate Studies, and by Natural Sciences and Engineering Research Council of Canada grants to Dr J. Kalff.

Résumé

La précision avec laquelle les paramètres de la fonction de Michaelis–Menten sont estimés dépendent de la technique utilisée pour ajuster la fonction aux données. Dans cet article, on démontre que la matrice du plan d'expérience (ou bien le choix des valeurs de la variable

indépendante a également de l'importance. L'efficacité de l'estimation des paramètres selon trois méthodes d'estimation est étudiée ainsi que la robustesse de chaque technique à une modification de la matrice du plan d'expérience à l'aide de simulations. On obtient des estimateurs sans biais de variance minimum en prenant la moitié des observations à K, et l'autre moitié aussi haute que possible puis en ajustant les données à l'aide d'une technique du maximum de vraisemblance. Avec quelques plans d'expérience où les concentrations de substrats sont distribuées plus largement, les estimateurs des paramètres peuvent être fortement biaisés et imprécis. Une suite géométrique des observations, ajustée soit à l'aide d'une technique du maximum de vraisemblance soit avec la technique non-paramètrique de Eisenthal et Cornish–Bowden (1974, *Biochemical Journal* **139**, 721–730), produit des estimateurs acceptables.

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Received February 1981; *revised September* 1981

Appendix

Optimization of Design Matrix

In an ordinary least squares regression, the variance–covariance matrix of the parameter estimates is approximated by $(\mathbf{X}'\mathbf{X})^{-1}\sigma^2$, where $\mathbf{X}$ is the Jacobian matrix of the model in question, and σ^2 is the expected variance of the dependent variable (see, for example, Draper and Smith, 1966). Consider the Michaelis–Menten function:

$$v_i = f(s_i, \theta_1, \theta_2) = \frac{\theta_2 s_i}{\theta_1 + s_i} + \varepsilon_i,$$

where the parameters V and K are represented by θ_1 and θ_2, respectively, and $\sigma^2 = \mathrm{E}(\varepsilon^2)$. The elements g_{ij} of the Jacobian are given by the partial derivatives of the function with respect to each of the parameters, for the ith element s_i in $\mathbf{D}$, and evaluated at the true values of the parameters θ^*:

$$g_{ij} = \left\{\frac{\partial f(s, \theta)}{\partial \theta_j}\right\}_{\theta = \theta^*,\, s = s_i}$$

The determinant of the matrix $(\mathbf{X}'\mathbf{X})^{-1}$ is proportional to the area of any ellipsoidal joint probability contour around θ_1 and θ_2 (Box and Lucas, 1959). It is a function only of the values of θ_1 and θ_2, and of $\mathbf{D}$, the matrix of values of s_i. Thus, a design matrix may be optimized by finding that combination of s_i which minimizes $|(\mathbf{X}'\mathbf{X})^{-1}|$, unless the particular experimental situation requires minimization of $\text{var}(\hat{K})$ or $\text{var}(\hat{V})$. Although the optimal $\mathbf{D}$ can be found analytically for the Michaelis–Menten function if the $\mathbf{D}$ contains few s_i, it is necessary to employ numerical techniques for larger matrices. In this study, I used the quasi-Newtonian algorithm ZXMIN, from the International Mathematical and Statistical Libraries, to find the $\mathbf{D}$ which minimizes $|(\mathbf{X}'\mathbf{X})^{-1}|$, $\text{var}(\hat{K})$ or $\text{var}(\hat{V})$.