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Statistical Analysis of the Michaelis–Menten Equation

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SUMMARY

An application of the method of maximum likelihood (ML) is described for analysing the results of enzyme kinetic experiments in which the Michaelis–Menten equation is obeyed. Accurate approximate solutions to the ML equations for the parameter estimates are presented for the case in which the experimental errors are of constant relative magnitude. Formulae are derived that approximate the standard errors of these estimates. The estimators are shown to be asymptotically unbiased and the standard errors observed in simulated data rapidly approach the theoretical lower bound as the sample size increases. The results of a large-scale Monte Carlo simulation study indicate that for data with a constant coefficient of variation, the present method is superior to other published methods, including the conventional transformations to linearity and the nonparametric technique proposed by Eisenthal and Cornish-Bowden (1974, *Biochemical Journal* **139**, 715–720). Finally, the present results are extended to the analysis of simple receptor binding experiments using the general approach described by Munson and Rodbard (1980, *Analytical Biochemistry* **107**, 220–239).

1. Introduction

In many biochemical and pharmacological experiments the data are assumed to conform to a two-parameter rectangular hyperbola, i.e.,

$$v = \frac{\alpha s}{(s + \beta)}, \tag{1}$$

where v is the dependent variable, s the independent variable, and α and β are parameters or constants. The aim of the experiment is to determine the value of these constants. For example, in enzyme kinetic studies, v refers to the velocity of an enzyme reaction and s refers to the substrate concentration. In this case one wishes to estimate $\alpha = V$ or V_{max}, the maximal velocity, and $\beta = K_m$, the Michaelis constant. In this context, equation (1) is known as the *Michaelis–Menten equation.* Similarly, in receptor binding studies, the dependent variable is the amount of ligand bound (usually denoted by B), which is a function of the free concentration F of that ligand. In this case, α corresponds to B_{max}, the maximal binding capacity, and β to K_d, the dissociation constant. One important difference between the latter case and the application in enzyme kinetics studies is that in binding studies the experimentally controlled variable is not s, but $T = s + v$, the total ligand concentration. Hence, any method for the analysis of the Michaelis–Menten equation will require some changes in order to adapt it to this procedural difference. In this article, we will focus on the application to enzyme kinetic studies. The adaptation to binding studies will be discussed in a separate section.

Several methods have been proposed for the estimation of the parameters α and β. Most of these are based on transformations of equation (1) to a linear plot of the form

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$Y = a + bX$, e.g.,

$$\frac{1}{v} = \frac{1}{\alpha} + \frac{(\beta/\alpha)}{s}, \tag{2}$$

$$\frac{s}{v} = \frac{\beta}{\alpha} + \frac{s}{\alpha}, \tag{3}$$

$$\frac{v}{s} = \frac{\alpha}{\beta} - \frac{v}{\beta}, \tag{4}$$

$$v = \alpha - \frac{\beta v}{s}. \tag{5}$$

Estimates for the parameters are then obtained from the slope and intercept, which are estimated by applying ordinary linear regression techniques. The oldest and still most widely used procedure is based on equation (2), which is known as the Lineweaver–Burk plot. Based on a comparison of the number of citations, Glick, Landman, and Roufogalis (1979) conclude that this analysis procedure still appears to be widely used by enzyme researchers, despite the fact that the simulation analyses by Dowd and Riggs (1965) and Colquhoun (1969) showed that this procedure is by far the worst of all proposed estimation methods. Better results may be obtained using equation (3) (the so-called Woolf transformation) which is, however, seldom used in practice.

Equations (4) and (5) are based on the same transformation of the data. Both are based on the fact that the Michaelis–Menten equation predicts a linear relationship between v and v/s. The difference between the two methods is that in equation (4), v/s is considered as a function of v, while in equation (5), v is assumed to be a function of v/s. Hence, the difference between these two methods is a result of the fact that the slope of the regression line of Y on X (b_{yx}) is generally not equal to $1/b_{xy}$, where b_{xy} is the slope of the regression line of X on Y. The analysis based on equation (4) is frequently used by pharmacologists in receptor binding studies and is known as a Scatchard analysis (the corresponding plot of B/F vs B is called a Scatchard plot) after Scatchard (1949), who introduced this estimation method. The alternative procedure based on equation (5) was introduced by Eadie and Hofstee (see Zivin and Waud, 1982).

Since each of these procedures gives different estimates for the parameters α and β (except in the case of errorless data), the obvious question is which of these methods, if any, is correct. Many analyses have been made to answer this question. It should be noted that the answer depends crucially on the assumptions one wishes to make concerning the error variance. That is, the analysis of the statistical model corresponding to the mathematical equation (1),

$$v = \frac{\alpha s}{s + \beta} + \varepsilon, \tag{6}$$

should depend on the distribution that one assumes for ε.

It can be easily shown that each of the so-called linear methods described above implicitly introduces transformations of ε that invalidate the conventional regression analysis. A number of researchers (e.g., Dowd and Riggs, 1965; Colquhoun, 1969; Atkins and Nimmo, 1975) have examined the extent of the bias and the variance of the parameter estimates that are obtained with these linear methods. Other techniques have been developed that do not involve a transformation of the data (e.g., Eisenthal and Cornish-Bowden, 1974; Wilkinson, 1961). Cornish-Bowden and Eisenthal (1974) and Atkins and Nimmo (1975) have compared these and other methods for fitting the Michaelis–Menten equation. None

of the methods seems to be uniformly superior, and the extent of the bias with these methods depends on the exact nature of the error and the design of the experiment (i.e., which values of s have been employed; see Currie, 1982).

In this paper, I will present a new solution to this problem that is based on the method of maximum likelihood. It will be shown that this method leads to simple, analytic formulae for the parameter estimates. Moreover, it enables us to derive large-sample equations for the standard errors.

2. The Maximum Likelihood Solution

As mentioned earlier, the optimal solution to the estimation problem depends on the distribution assumed for the error component. The method of maximum likelihood (ML) has been used previously to fit a rectangular hyperbola, but only under the assumption of a constant *absolute* error. In that case, the solution is equivalent to that of the direct least squares method (see Wilkinson, 1961) and may be obtained with any of the several programs that are on the market. These programs make use of an iterative procedure. However, the assumption of a constant absolute error does not seem to be appropriate for most applications. According to Zivin and Waud (1982), the standard deviation of the dependent variable (v) is usually roughly proportional to the mean value, i.e., it has a constant coefficient of variation. This assumption leads to the following statistical model:

$$v_i = \frac{\alpha s_i}{s_i + \beta} + \frac{s_i}{s_i + \beta}\varepsilon_i, \quad i = 1, \ldots, n, \tag{7}$$

where ε_i is assumed to be normally distributed with mean 0 and variance σ^2.

Although the traditional methods do not take the particular error structure into account, a number of authors have developed methods and/or computer programs that do allow the specification of a variety of assumptions on the error structure. Many of these approaches use a weighted least squares approach. It can be shown by simple algebraic manipulation that under the present assumption with respect to the errors, a weighted least squares approach in the original coordinate system leads to a solution that is equivalent to the analysis based on equation (5), the Eadie–Hofstee plot.

Let SS be the weighted least squares function that is to be minimized:

$$SS = \sum_{i=1}^{n} \frac{[v_i - \alpha s_i/(s_i + \beta)]^2}{\sigma^2[s_i/(s_i + \beta)]^2}.$$

This is equivalent to

$$SS = \sum_{i=1}^{n} \frac{(v_i + \beta v_i/s_i - \alpha)^2}{\sigma^2}.$$

Apart from the irrelevant constant σ^2, this is equal to the sums-of-squares function that is minimized when the parameters are estimated using equation (5). This equivalence is not surprising since the transformation has in this case a variance-equalizing effect (see Zivin and Waud, 1982). Hence, the performance of such a weighted least squares approach as compared to the present ML solution may be evaluated by using equation (5) as a substitute.

In order to derive the ML estimators, we have to differentiate the likelihood function, $L(X \mid \alpha, \beta, \sigma^2)$, the likelihood of the data as a function of the true parameter values. In this case, the likelihood is equal to

$$L = \prod_{i=1}^{n} \left[\frac{\sqrt{2\pi\sigma^2}\, s_i}{s_i + \beta}\right]^{-1} \exp\left\{\frac{-[v_i - \alpha s_i/(s_i + \beta)]^2}{2\sigma^2[s_i/(s_i + \beta)]^2}\right\}.$$

As is usual, it turns out to be easier to maximize $\ln(L)$ instead of L itself. Since $\ln(X)$ is a monotonic function of X, $\ln(L)$ attains its maximum value for the same values of α, β, and σ^2 as the likelihood function itself. After some algebraic simplification, the following result is obtained:

$$-\ln(L) = \left(\frac{n}{2}\right)\ln(2\pi) + \left(\frac{n}{2}\right)\ln(\sigma^2) - \sum_{i=1}^{n} \ln\left[\frac{s_i + \beta}{s_i}\right] + \sum_{i=1}^{n} \frac{(v_i + \beta v_i/s_i - \alpha)^2}{(2\sigma^2)}.$$

The maximum likelihood solution is obtained by setting the derivatives of $\ln(L)$ with respect to the parameters equal to 0. It turns out that the equations for these estimators may be simplified by substituting $X_i = v_i/s_i$ and $Y_i = v_i$, i.e., the ML estimators may be written in terms of the variables involved in a Scatchard or Eadie–Hofstee plot. Denoting the ML estimators by $\hat{\alpha}$, $\hat{\beta}$, and $\hat{\sigma}^2$, we arrive at the following result:

$$\hat{\alpha} = \bar{Y} + \hat{\beta}\bar{X}, \tag{8}$$

$$\hat{\sigma}^2 = \frac{S_{yy} + 2\hat{\beta}S_{xy} + \hat{\beta}^2 S_{xx}}{n}, \tag{9}$$

$$S_{xy} + \hat{\beta}S_{xx} = (S_{yy} + 2\hat{\beta}S_{xy} + \hat{\beta}^2 S_{xx}) \sum_{i=1}^{n} \frac{X_i/(Y_i + \hat{\beta}X_i)}{n}, \tag{10}$$

where S_{yy}, S_{xx}, and S_{xy} are the sums of squares and cross-products of the deviations $Y_i - \bar{Y}$ and $X_i - \bar{X}$. From equation (10), $\hat{\beta}$ may be obtained using an algorithm for the solution of a nonlinear equation.

Equation (10) may be simplified further by the following approximation:

$$\left(\frac{1}{n}\right) \sum_{i=1}^{n} \frac{X_i}{Y_i + \hat{\beta}X_i} \approx \frac{\bar{X}}{\bar{Y} + \hat{\beta}\bar{X}}.$$

That is, we equate the mean of the ratios to the corresponding ratio of means. This approximation is quite accurate, as will be documented in the next section. This simplification leads to the following estimator for β:

$$\hat{\beta} = \frac{\bar{X}S_{yy} - \bar{Y}S_{xy}}{\bar{Y}S_{xx} - \bar{X}S_{xy}}. \tag{10a}$$

$\hat{\alpha}$ and $\hat{\sigma}^2$ may be obtained by substituting the value of $\hat{\beta}$ given by equation (10a) into equations (8) and (9). As in the conventional linear regression model, an unbiased estimator for σ^2 is given by

$$\hat{\sigma}^2 = \frac{S_{yy} + 2\hat{\beta}S_{xy} + \hat{\beta}^2 S_{xx}}{n - 2}.$$

One of the advantages of the ML method is that it may be used to derive a large-sample approximation to the variance–covariance matrix of the parameter estimates (Kendall and Stuart, 1967), assuming the values of s_i are drawn from a fixed distribution, independent of the sample size. These variances are equal to the Cramér–Rao lower bound and may be calculated from the second derivatives of $\ln(L)$. Let $\mathbf{D}$ be the matrix whose elements are equal to

$$d_{ij} = -\mathrm{E}\left[\frac{\partial^2}{\partial\theta_i\partial\theta_j} \ln(L)\right],$$

where θ_i and θ_j are two arbitrary parameters, i.e., $-\mathbf{D}$ contains the expected values of the second partial derivatives of the log-likelihood function with respect to the parameters. The

variance–covariance matrix $\boldsymbol{\Sigma}$ of the parameter estimates is given by the inverse $\mathbf{D}^{-1}$ of $\mathbf{D}$. This leads to the following large-sample formula for the variance of $\hat{\beta}$:

$$\text{var}(\hat{\beta}) \approx \frac{\sigma^2}{[(1 + 2\sigma^2/\alpha^2) \sum_{i=1}^{n} (U_i - \bar{U})^2]}, \tag{11}$$

where U_i is defined as $U_i = \alpha/(s_i + \beta)$, i.e., the true score corresponding to v_i/s_i. Similarly, the large-sample variance of $\hat{\alpha}$ and the covariance of $\hat{\alpha}$ and $\hat{\beta}$ are given by

$$\text{var}(\hat{\alpha}) \approx \frac{\sigma^2}{n} + \bar{U}^2 \text{var}(\hat{\beta}), \qquad \text{cov}(\hat{\alpha}, \hat{\beta}) = \bar{U}\, \text{var}(\hat{\beta}). \tag{12}$$

Sample estimates for these variances and covariance are obtained by substituting the estimates for α, β, and σ^2 (corrected for bias) into the above equations. These estimates may be used to compute confidence intervals for α and β, since, for example, $\hat{\beta}$ will be approximately normally distributed with mean β and variance $\text{var}(\hat{\beta})$. In the next section, we will present the results of a Monte Carlo investigation designed to evaluate the adequacy of this method in comparison to other published analyses of the Michaelis–Menten equation. We will also investigate whether the variances of the estimates indeed attain the Cramér–Rao lower bound, and if so, how fast.

3. Monte Carlo Evaluation

A number of data sets were simulated by generating values of v_i using equation (7). Normally distributed values of ε_i were obtained using the method described in Box and Muller (1958). Seven different sample sizes were used—namely, $n = 5$, 10, 20, 50, 100, 200, and 500. The large sample sizes were included to evaluate the correctness of the large-sample approximations to the parameter variances. In each case, only five different values of s were used, however: $s = 1$, 4, 16, 64, and 256. For larger sample sizes these values were used repeatedly (e.g., in the case $n = 100$, each s-value was used 20 times). In all simulations, the true value of α was equal to 25 and β was equal to 10. The error variance σ^2 was either small ($\sigma = 1.25$) or relatively large ($\sigma = 6.25$), i.e., the coefficient of variation (CV) was either 5% or 25%. For each combination of sample size and error variance, 10,000 sets of data were simulated, i.e., each reported mean and standard deviation is based on 10,000 values.

The basic results are presented in Table 1. This table includes for each simulation the mean value of the estimates for α and β [using the approximation given in equation (10a)], their observed standard deviations, and the predicted standard deviations based on equations (11) and (12). For each set of data, we also obtained the exact ML estimates for β based on equation (10) using Newton's method of successive approximations (Abramowitz and Stegun, 1968, Formula 3.9.5). For CV = 5%, the exact ML estimates were virtually identical to the approximate estimates: the mean absolute deviation for the different sample sizes varied between .000009 and .000053 and the correlation between the exact and approximate solutions was always 1.0 (up to six decimal places). For the larger error variance (CV = 25%), the mean absolute deviation varied between .005 and .041 and the correlation was always .9994 or larger. Hence, even in that case the approximation was quite accurate, especially in comparison to the standard errors of the estimates. More important, the mean values and the standard deviations of the exact estimates were virtually identical to those given in Table 1. Hence, the simple solution given by equation (10a) may be used in practice as a substitute for the exact ML solution.

It is evident that the results are quite good. The bias in the parameter estimates is quite low and the predicted standard deviations correspond reasonably well to the observed

Table 1
Mean bias and standard deviations of the parameter estimates as a function of sample size and error variance

	CV = 5%		CV = 25%	
n	Bias	s.d.	Bias	s.d.
		Parameter α		
5	.014	.910 (.920)	.384	4.571 (4.444)
10	.010	.655 (.651)	.140	3.208 (3.143)
20	.008	.460 (.460)	.122	2.242 (2.222)
50	.001	.290 (.291)	.024	1.410 (1.405)
100	.000	.207 (.206)	.026	1.007 (.994)
200	.003	.145 (.146)	.005	.699 (.703)
500	.000	.092 (.092)	.007	.449 (.444)
		Parameter β		
5	.022	.671 (.671)	.619	3.647 (3.169)
10	.009	.477 (.474)	.262	2.382 (2.241)
20	.009	.336 (.335)	.150	1.641 (1.585)
50	.004	.211 (.212)	.041	1.001 (1.002)
100	.001	.149 (.150)	.030	.719 (.709)
200	.001	.106 (.106)	.014	.494 (.501)
500	.000	.067 (.067)	.007	.321 (.317)

Note: The values in parentheses are the predicted standard deviations according to the large-sample approximation.

values, even when the sample size is as small as $n = 5$. Of particular interest is the fact that, as sample size increases, the bias becomes vanishingly small and the standard deviation attains the theoretical lower bound. This is shown more clearly in Table 2, where the observed values of $\sqrt{n}\sigma_{\hat{\beta}}$ are given. The predicted values of this quantity depend only on the size of the error and not on the sample size, n. For large sample sizes the fit to the theoretical normal distribution is very good (see Figure 1). For $n = 500$, the observed distributions of $\hat{\beta}$ do not differ significantly from the expected distribution. This was tested using the Kolmogorov test statistic $D_{\max}$, which measures the maximum deviation of the observed from the expected distribution (Conover, 1971). For $\sigma = 1.25$, $D_{\max}$ was equal to .0112 ($P > .10$) and for $\sigma = 6.25$, $D_{\max}$ was equal to .0086 ($P > .20$). For the smaller error variance (CV = 5%) the fit to the normal distribution was already quite good for a sample size as small as $n = 5$ (see Figure 2).

Table 2
Normalized index of adequacy of large-sample approximation to standard error as a function of sample size and error variance

n	CV = 5%	CV = 25%
5	1.50	8.16
10	1.51	7.53
20	1.50	7.34
50	1.49	7.08
100	1.49	7.19
200	1.50	6.99
500	1.50	7.18
Theoretical value	1.50	7.09

Note: Values given are equal to $\sqrt{n}\sigma_{\hat{\beta}}$.

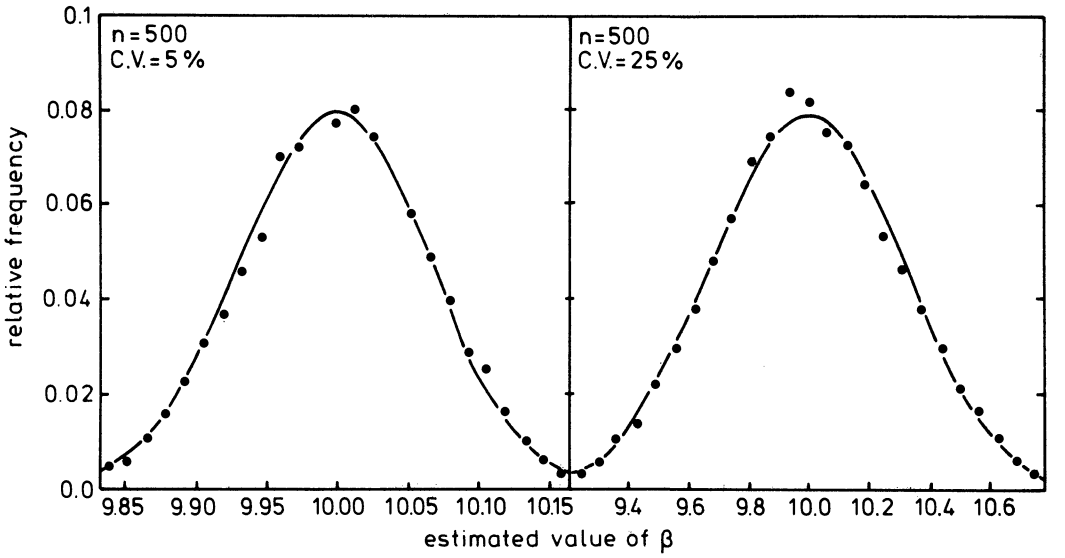

Figure 1. Observed and predicted distribution of $\hat{\beta}$ for large sample sizes. Continuous lines correspond to the normal distribution predicted on the basis of the large-sample approximation.

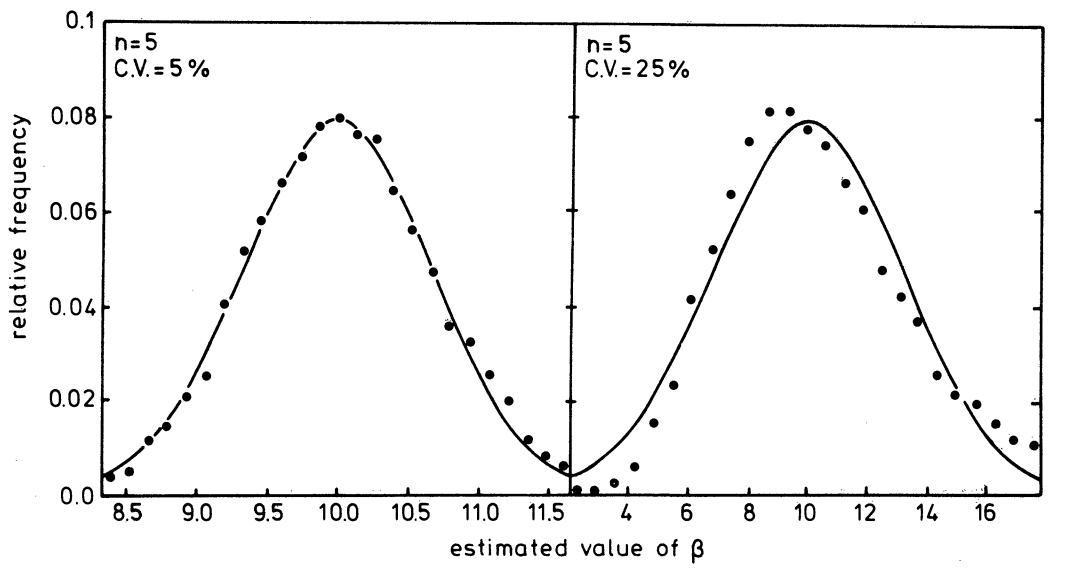

Figure 2. Observed and predicted distribution of $\hat{\beta}$ for small sample sizes. Continuous lines correspond to the normal distribution predicted on the basis of the large-sample approximation.

How does the present method compare with other published methods of analysis? Colquhoun (1969) compared the efficiency of the three linearity transformations corresponding to equations (2), (3), and (5) to that of a direct least squares method (Wilkinson, 1961). In case of a constant relative error (which is assumed in our method), the best results were obtained with the analysis based on equation (5). As had been previously observed by Dowd and Riggs (1965), the most common method of analysis, based on the Lineweaver–Burk plot [equation (2)], performed quite badly. We will therefore compare our method with respect to the estimation of β with the analysis based on equations (4) (which was not

included in the Colquhoun study) and (5). As mentioned earlier, the latter analysis is equivalent to a weighted least squares method (in the original coordinate system). Since the estimates of β and α are highly correlated, similar results hold for the estimation of α. An additional reason for making this particular comparison is that these methods are based on the same variables, v and v/s, that are used in the proposed maximum likelihood method.

In our simulations we observed that the estimates for β based on equation (4) (the Scatchard plot) were severely biased as well as more variable than those obtained with the present ML method. The standard deviation of the estimates for β obtained from the analysis based on equation (5) (the Eadie–Hofstee plot) was slightly smaller than that of the ML method. These estimates were, however, also severely biased, the net result being a larger mean squared error (MSE, the mean of the squared deviations of the estimates from their true values). Moreover, the bias in the estimates resulting from the two transformations *increased* with increasing sample size, an additional sign of their theoretical inadequacy. Table 3 gives for each condition the MSE for the ML method and these two linearity transformations.

Table 3
Mean squared error for different methods of estimation of the parameter β

CV	n	ML method	v vs v/s	v/s vs v	CV	n	ML method	v vs v/s	v/s vs v
5%	5	.451	.448	.469	25%	5	13.684	11.507	816.124
	10	.228	.230	.244		10	5.743	6.842	22.436
	20	.113	.118	.131		20	2.715	5.328	16.869
	50	.045	.052	.062		50	1.004	4.750	13.541
	100	.022	.031	.039		100	.518	4.586	12.887
	200	.011	.021	.028		200	.244	4.513	12.419
	500	.004	.014	.021		500	.103	4.456	12.077

In case of $n = 5$, we also investigated the adequacy of the nonparametric technique proposed by Eisenthal and Cornish-Bowden (1974). In this method, each pair of observations is used to solve for the parameters α and β. The final estimates are equal to the medians of these $n(n - 1)/2$ estimates. This method makes no assumptions about the nature of the error. On the basis of their results, Atkins and Nimmo (1975) recommended this method as the one to use. In case of the smaller error variance (CV = 5%), the estimates produced by the Eisenthal and Cornish-Bowden method were slightly more biased and had a larger standard deviation than those of the ML method. Hence, the mean squared error was larger than that obtained with our method ($MSE_\beta = .524$ for their method compared to .451 for our method). For the larger error variance (CV = 25%), their estimates were slightly less biased, but more variable than ours, the net result being a larger mean squared error ($MSE_\beta = 14.816$ vs 13.684).

Cornish-Bowden and Eisenthal (1978) proposed a modification of the original method in order to eliminate certain bias problems that may arise in the final estimates if any pairs lead to negative values for α. In this modification, one first calculates the medians of $1/\alpha$ and β/α instead of the medians of α and β. The final estimates are obtained by applying the inverse transformation to these medians. However, this modification did not lead to better results than the original method. The bias was not affected much but the standard deviation was even larger, especially in case of the larger error variance ($MSE_\beta = .581$ and 23.117 for CV = 5% and 25%, respectively). Considering the fact that our method is much simpler to use and leads to analytic expressions for the standard errors of the parameters,

it should be concluded that the present ML method is to be favoured over the nonparametric technique proposed by Eisenthal and Cornish-Bowden.

4. Application to Binding Studies

As mentioned in the introduction, the present method cannot be applied directly to binding studies. The reason for this is that in such studies the experimentally controlled variable is the total concentration $T (= v + s)$. Hence, s can only be estimated from the observed data and these estimates will contain errors. Our proposed method of analysis assumes, however, that s is measured without (noticeable) error. From a statistical point of view the presence of measurement error in the independent variable changes the situation quite radically [see, e.g., the large statistical and econometric literature on the so-called errors-in-variables model (Anderson, 1984)].

Munson and Rodbard (1980) have described a general procedure for the analysis of data from ligand-binding experiments that utilizes total ligand concentration as the independent variable. Their method uses a weighted least squares approach. It is possible, however, to adapt their general procedure to the present ML approach. The total concentration T_i is equal to

$$T_i = s_i + \alpha s_i/(s_i + \beta). \tag{13}$$

Given T_i and starting values for α and β, this equation may be solved for s_i. (It reduces to a quadratic equation whose positive root is s_i.) These s_i-values may then be used to generate new estimates for α and β using equations (8) and (10a). The new values may then be inserted again in equation (13) to get new estimated values for s_i. This iterative process continues until the values for α and β stabilize. It should be noted that this procedure still generates ML estimates for the parameters since the likelihood given α, β, and T_i equals the likelihood given α, β, and s_i.

Given the superiority of the present ML method to the weighted least squares approach in the enzyme kinetic model, we may conclude that it will also lead to better results in the adaptation to binding studies.

5. Conclusions

We have shown that the application of the method of maximum likelihood to the estimation of the parameters of the Michaelis–Menten equation leads to a simple analytical solution for the parameter estimates for the case of errors with constant coefficient of variation. Moreover, formulae were derived that approximate the observed standard errors of the estimates reasonably well. It should be emphasized that the adequacy of the present method hinges on the correctness of the assumed distribution of the errors. We do feel, however, that the assumption of a constant relative error describes the observed error distributions reasonably well. At least, the present assumption seems more appropriate than the principal alternative that assumes an error of constant magnitude, independent of the mean. In case one does have reason for assuming that the error is homoscedastic, the direct least squares method proposed by Wilkinson (1961) should be used. This method corresponds in that case to the maximum likelihood solution.

Application of the method of maximum likelihood has a number of advantages over other methods of parameter estimation. First of all, the commonly used transformations to linearity are dubious from a statistical point of view since they do not take into account the effect of such a transformation on the error component. This leads to a relatively large bias in the estimates, as was shown in the present study as well as in previous investigations. Second, the ML method is firmly rooted in statistical theory and has a number of optimum large-sample properties (see, e.g., Mood, Graybill, and Boes, 1974). Finally, the present

method leads to simple formulae for the standard errors of the parameters, which is of obvious importance in practical situations.

We have presented results from a simulation study designed to evaluate the adequacy of the present method. It was observed that the results were quite favourable and superior to those of the other major candidates. In particular, the mean squared error was smaller than that of the nonparametric technique proposed by Eisenthal and Cornish-Bowden (1974), which is generally regarded as one of the best of the available methods of analysis (Currie, 1982; Atkins and Nimmo, 1975). It should be noted that our results were obtained using a specific set of parameter values and a specific experimental design (i.e., a particular set of values for s). While it is not likely that the choice of parameter values matters much, it is conceivable that the adequacy of the present method relative to other methods is dependent on the experimental design (Currie, 1982). Although maximum likelihood theory guarantees that the estimates will have optimum large-sample properties, this may not be true for small samples. Notwithstanding these reservations, our data show that the present method is at least preferable to the other methods in designs similar to the one employed in this study. These results indicate that extension of the present method to more complex situations (e.g., involving multiple-substrate reactions) promises to be a worthwhile effort. However, such an extension has not yet been made.

Résumé

L'article décrit une application de la méthode du maximum de vraisemblance (ML) pour analyser les résultats d'expériences de cinétique enzymatique obéissant à l'équation de Michaelis–Menten. Il présente de bonnes solutions approchées des équations du ML pour estimer les paramètres dans le cas où les erreurs expérimentales ont une valeur relative constante. Il donne des formules approchées des écarts-type des estimateurs. Il montre que ces estimateurs sont asymptotiquement sans biais et que les écarts-type observés sur des données simulées approchent rapidement la borne inférieure théorique quand la taille de l'échantillon augmente. Les résultats d'une étude de simulation de Monte-Carlo importante, indiquent que pour les données ayant un coefficient de variation constant, la présente méthode est supérieure aux autres déjà publiées, y compris celles utilisant les transformations pour linéariser, conventionnelles, et la technique non paramétrique proposée par Eisenthal et Cornish-Bowden (1974, *Biochemical Journal* **139,** 715–720). Enfin, les résultats sont étendus à l'analyse d'expériences à un seul récepteur liant, utilisant l'approche générale décrite par Munson et Rodbard (1980, *Analytical Biochemistry* **107,** 220–239).

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