Biochem. J. (1974) **141**, 913–914
Printed in Great Britain

A Comparison of Two Methods for Fitting the Integrated Michaelis–Menten Equation

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(*Received* 10 *May* 1974)

The methods of Atkins & Nimmo (1973) and Fernley (1974) for fitting the integrated Michaelis–Menten equation were compared by using the same sets of simulated experimental data. The method of Fernley (1974) is to be preferred because it gives precise and unbiased estimates of the Michaelis–Menten parameters over a wide range of substrate concentrations. However, the estimates may not be symmetrically distributed, especially at low substrate concentrations.

The Michaelis–Menten parameters of an enzyme-catalysed reaction (K_m and V) can be determined by fitting the integrated form of the Michaelis–Menten equation to the progress curve of the reaction (Laidler, 1958; Lowe & Williams, 1965; Cornish-Bowden, 1972). For the simplest type of reaction the relevant equation is:

$$V \cdot t = p - K_m \cdot \ln\left(1 - \frac{p}{s_0}\right) \qquad (1)$$

where s_0 is the initial concentration of substrate, and p is the concentration of product at time t ($p = 0$ when $t = 0$).

Recently two methods have been described for fitting eqn. (1) to data. Atkins & Nimmo (1973) calculated K_m and V from a weighted least-squares regression of p/t on $\frac{1}{t}\ln\left(1 - \frac{p}{s_0}\right)$. Since the regression is not strictly valid (both its variables being functions of the error-containing quantity p) the method was tested on simulated experimental data and found to give unbiased estimates of the parameters only when s_0 was greater than K_m.

The second method fits eqn. (1) directly to the data by an iterative technique (Fernley, 1974), and has two theoretical advantages. First, as it minimizes the sum of squares of deviations (S.S.D.) of the dependent variable (p), the final estimates of the parameters may be unbiased (provided that the error in p is normally distributed). Secondly, approximate standard errors of these final estimates can be calculated. However, non-linear regressions of this sort may fail either because the S.S.D. oscillates and does not converge to a stable minimum, or because the final estimates depend on the starting values chosen for them.

At present there are no data to show which of these two methods gives the 'better' estimates of K_m and V. (In this context the 'best' estimates are unbiased, symmetrically distributed and precise. Since either method may give answers with one or two but not all three of these attributes, the decision as to which is 'better' may to some extent be subjective.) We have therefore attempted to compare the two methods, by applying the iterative one to the same sets of simulated data already analysed by the linear method. We have also used extensions of both methods to analyse progress curves whose origins have to be determined because they have in effect been displaced an unknown distance p_0 perpendicular to the time axis [see Atkins & Nimmo (1973) for a more detailed description of the problem]. Essentially this means fitting the equation:

$$V \cdot t = (p - p_0) - K_m \cdot \ln\left[1 - \frac{(p - p_0)}{s_0}\right] \qquad (2)$$

where p_0 is a third parameter to be estimated.

Methods

The simulated data are described in Atkins & Nimmo (1973). For each error-free curve $K_m = V = 1$, $p_0 = 0$ and s_0 was either 0.5, 0.7, 1.0, 1.4 or 2.0. The standard deviation of p was either 0.005 or 0.01. Forty different experimental curves were simulated at each substrate concentration and error level.

The data were first analysed assuming that $p_0 = 0$. In the iterative method predicted values of p ($\hat{p}$) are found by the Newton–Raphson procedure from eqn. (1) with initial estimates of K_m and V. The initial estimates are then adjusted by adding ΔK_m and ΔV, which are computed from the regression:

$$(p - \hat{p}) = \frac{\Delta K_m}{D} \cdot \ln\left(1 - \frac{\hat{p}}{s_0}\right) + \frac{\Delta V \cdot t}{D}$$

where

$$D = 1 + \frac{K_m}{(s_0 - \hat{p})}$$

The whole process is repeated until ΔK_m and ΔV become trivial, and the standard errors of the final estimates of K_m and V are equal to those of ΔK_m and ΔV at this point.

The data were then re-analysed by assuming that p_0 was unknown. In this instance $\hat{p}$ is found from eqn.

Table 1. *Mean estimates of K_m and V for progress curves of unknown origin*

Values are means ± S.D. ($n = 40$).

	Linear method		Iterative method	
s_0	K_m	V	K_m	V
0.5	0.60±0.13*	0.70±0.11*	1.11±0.40†	1.09±0.35†
0.7	0.84±0.17*	0.89±0.13*	1.03±0.25†	1.02±0.18†
1.0	0.94±0.14*	0.97±0.10	1.01±0.15	1.01±0.10†
1.4	1.02±0.09	1.01±0.06	1.03±0.07*	1.02±0.04
2.0	1.00±0.06	1.00±0.03	1.01±0.04	1.00±0.02

* $P < 0.04$ that there is no difference between median and theoretical value of 1.00 (Campbell, 1967).
† $P < 0.02$ that the distribution is symmetrical (Snedecor & Cochran, 1967).

(2) with initial estimates of p_0 as well as of K_m and V, and the adjustment regression is:

$$(p-\hat{p}) = \frac{\Delta K_m}{D} \ln\left[1-\frac{(\hat{p}-p_0)}{s_0}\right] + \frac{\Delta V \cdot t}{D} + \Delta p_0$$

where

$$D = 1 + \frac{K_m}{[s_0-(p-p_0)]}$$

In the linear method the weighted regression is computed after p_0 has been estimated from a third-order polynomial fitted to the progress curve.

Results and discussion

Table 1 summarizes the values of the parameters determined from the low-error progress curves when p_0 was assumed to be unknown; similar results were obtained from the high-error curves and when p_0 was set to zero. The iterative method gave unbiased answers at nearly all substrate concentrations, whereas the linear one did not. On the other hand, at the lower substrate concentrations the iterative method's answers were asymmetrically distributed, whereas the linear method's were symmetrically distributed. The iterative method was the less precise of the two at the lower substrate concentrations, but the more precise at the higher ones. The standard errors of K_m and V calculated by the iterative method from the individual progress curves were on average equal to the corresponding standard deviations in Table 1 and therefore seem to be good approximations. But they must be interpreted with caution because the distributions of both K_m and V may be skewed.

The iterative method sometimes failed to converge when the starting values of K_m and V were outside the range 0.5–2.0; it appeared to be more vulnerable when they were too high than when they were too low. On the other hand, when the method did converge, the final estimates of the parameters were independent of the starting values. Thus the point at which the S.S.D. is a minimum seems to be unique.

We have concluded that the iterative method is to be preferred, because it usually converges and gives unbiased estimates. It is less satisfactory than the linear method in that these estimates may not be symmetrically distributed; this implies that their confidence limits (derived from their standard errors) may also be asymmetric. A similar situation exists when K_m and V are determined from initial velocities: the iterative technique of Wilkinson (1961) gives better answers (judged by all three criteria) than the double-reciprocal linear plot (Colquhoun, 1971). Such linear plots should be used only for deriving starting values of the parameters and for showing whether the hypothesis is consistent with the data.

We thank Dr. H. N. Fernley for showing us the proofs of his paper and for valuable discussions, and Miss Caroline Thompson for her cheerful assistance.

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