Short Communications

A Comparison of Seven Methods for Fitting the Michaelis–Menten Equation

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The Michaelis–Menten equation was fitted to simulated data containing different sorts of error by using the three linear transformations, and the methods of S. R. Cohen [*Anal. Biochem.* (1968) 22, 549–552], R. Eisenthal & A. Cornish-Bowden [*Biochem. J.* (1974) 139, 715–720], F. de M. Merino [*Biochem. J.* 143, 93–95] and G. N. Wilkinson [*Biochem. J.* (1961) 80, 324–332]. The best methods were those of Eisenthal & Cornish-Bowden (1974) and Wilkinson (1961).

There are numerous methods for fitting the Michaelis–Menten equation to data (i.e. substrate concentrations, s , and initial velocities, v) and thus estimating the K_m and V of the reaction (e.g. Lineweaver & Burk, 1934; Eadie, 1942; Hofstee, 1952; Wilkinson, 1961; Cohen, 1968; Colquhoun, 1971; Eisenthal & Cornish-Bowden, 1974; Merino, 1974). The methods will give different estimates of K_m and V unless the data are error-free, because each one makes a different set of assumptions about the distribution of any error. The best method to use in any given situation will be the one whose assumptions most nearly match the actual distribution of error.

Dowd & Riggs (1965) compared the three transformations ($1/v$ versus $1/s$, s/v versus s and v versus v/s) by using simulated data with different sorts of normally distributed error, and demonstrated that in every instance the Lineweaver–Burk ($1/v$ versus $1/s$) plot gave the worst answers. Colquhoun (1971) extended the comparison to include a direct least-squares method similar to that of Wilkinson (1961), and found that it was the most reliable when v had a constant variance, whereas if the relative error in v was constant the v versus v/s or s/v versus s plots were the best. As the distribution of error is not usually known, Eisenthal & Cornish-Bowden (1974) have developed a non-parametric method which makes a minimum of assumptions about error. They tested their method with simulated data incorporating error that was either normally distributed or contained outliers, and showed that it was more robust than that of Wilkinson (1961), which assumes the errors are normally distributed and of constant variance (Cornish-Bowden & Eisenthal, 1974).

So far as we are aware, no attempts have been made to assess either the iterative method of Cohen (1968), which is specifically designed for data with normally

distributed error of constant relative magnitude, or the method of Merino (1974). We have therefore used simulated data to test these two methods, together with those of Eisenthal & Cornish-Bowden (1974) and Wilkinson (1961), and the three linear transformations. The simulated data had error of constant absolute magnitude, with or without outliers, or of constant relative magnitude, again with or without outliers.

Methods

A set of ‘perfect’ (i.e. error-free) data was formed by setting $K_m = V = 1$ and $s = 0.25, 0.50, 0.75, 1.00, 1.25, 1.50$ and 1.75 , and then calculating v from the Michaelis–Menten equation (v therefore ranged from 0.20 to 0.64). Sets of ‘experimental’ data (i.e. containing error in v) were constructed from the perfect set by using series of normally distributed pseudo-random numbers of known mean and standard deviation, generated by the Edinburgh Regional Computing Centre program Random. Four sorts of error were incorporated.

AN: normally distributed error of constant absolute magnitude. Random numbers of mean zero and standard deviation 0.01, 0.02 and 0.05 were added to the values of v .

AO: error of constant magnitude, with outliers. Identical with AN, except that for one in ten of the values of v , chosen at random, the standard deviation of the random numbers was 0.05.

RN: normally distributed error of constant relative magnitude. Each v was multiplied by a number of mean unity and standard deviation 0.01, 0.02, 0.05 or 0.10.

RO: error of constant relative magnitude, with outliers. Identical with RN, except that for one in ten

Table 1. Mean estimates of K_m for data with different sorts of error

Values are means $\pm$ s.d. In parentheses: number of instances that $0.05 > K_m > 5.00$; these estimates were excluded from the means. Otherwise $n = 50$. AN: constant absolute magnitude, s.d. = 0.05. AO: constant absolute magnitude, s.d. = 0.02, with 10% outliers of s.d. = 0.05. RN: constant relative magnitude, coefficient of variation (C.V.) = 10%. RO: constant relative magnitude, C.V. = 10%, with 10% outliers of C.V. = 50%.

Method	Type of error	... AN	AO	RN	RO
1/ v versus 1/ s		1.13 $\pm$ 0.82 (4)	1.12 $\pm$ 0.47 (1)	1.09 $\pm$ 0.43 (—)	1.07 $\pm$ 0.57 (1)
s/v versus s		1.05 $\pm$ 0.46 (1)	1.02 $\pm$ 0.20 (—)	1.05 $\pm$ 0.33 (—)	1.24 $\pm$ 0.73 (—)
v versus v/s		0.93 $\pm$ 0.46 (2)*	0.95 $\pm$ 0.22 (—)*	0.88 $\pm$ 0.31 (—)*	0.81 $\pm$ 0.31 (2)*
Cohen (1968)		1.11 $\pm$ 0.50 (6)	1.05 $\pm$ 0.24 (—)	1.08 $\pm$ 0.31 (—)	1.15 $\pm$ 0.61 (—)
Cornish-Bowden & Eisenthal (1974)		0.79 $\pm$ 0.31 (1)*	1.00 $\pm$ 0.17 (—)	0.94 $\pm$ 0.29 (—)*	0.92 $\pm$ 0.40 (—)*
Merino (1974)		0.90 $\pm$ 0.73 (16)*	1.03 $\pm$ 0.66 (4)*	1.08 $\pm$ 0.83 (8)*	1.23 $\pm$ 1.00 (10)
Wilkinson (1961)		0.99 $\pm$ 0.43 (—)	0.99 $\pm$ 0.17 (—)	1.06 $\pm$ 0.36 (—)	1.14 $\pm$ 0.45 (—)

* $P < \text{about } 0.05$ that there is no difference from theoretical value of 1.00, calculated from interval estimate of median (Campbell, 1967).

of the values of v , chosen at random, the standard deviation of the random numbers was increased fivefold.

Fifty different sets of data of each sort and error level were simulated.

The Michaelis–Menten equation was fitted to the data by using the unweighted version of each of the methods tested: the three linear transformations, and the methods of Cohen (1968), Eisenthal & Cornish-Bowden (1974), Merino (1974) and Wilkinson (1961). The programs were run on an ICL 4–75 computer at the Edinburgh Regional Computing Centre.

Results and discussion

The results for K_m obtained at the highest level of each of the four sorts of error are summarized in Table 1. Although this level of error is higher than is likely in practice, the differences between the seven methods are seen most clearly at it. The results obtained at the lower error levels were qualitatively the same (with one exception, mentioned below) and so have not been included. The values of V have also been omitted, as K_m is usually of more interest to enzymologists and is the more difficult parameter to estimate reliably. The expected value of K_m is 1.00, and values outside the range 0.05–5.00 have been excluded from the means in Table 1, on the grounds that the Michaelis–Menten equation could not have been said to fit the data.

The acceptability of the results for K_m given by the different methods can be judged by either of two criteria: their lack of bias (i.e. accuracy) or the size of their coefficient of variation (i.e. precision). Which is the more important will depend on the reasons for finding K_m . For instance, if estimates obtained under different conditions are to be compared a small coefficient of variation may be the prime consideration, whereas if accurate answers are

required (e.g. for simulating multi-enzyme systems) the possibility of bias must be minimized.

It is immediately apparent from Table 1 that Merino's (1974) method gave answers that were always imprecise and usually inaccurate, and is therefore unlikely to be useful. As expected from the work of Dowd & Riggs (1965) and Colquhoun (1971), the Lineweaver–Burk plot was the least precise of the linear transformations (except when the error was of constant relative magnitude with outliers). The s/v versus s plot was the most precise when the error was of constant absolute magnitude, but tended to overestimate K_m , whereas the v versus v/s plot was the most precise when the error was of constant relative magnitude but markedly underestimated K_m .

The method of Cohen (1968) was less satisfactory than that of Eisenthal & Cornish-Bowden (1974), except perhaps when the error was of constant absolute magnitude. Thus there seems to be no compelling reason for using Cohen's (1968) method, even when the error distribution is likely to fit its assumptions. Wilkinson's (1961) method gave the best answers of all the methods tested when the error was of constant absolute magnitude, and performed fairly well when the error followed the other three sorts of distribution. The non-parametric method of Eisenthal & Cornish-Bowden (1974) was the most reliable in these three other instances, and only gave poor answers when the error was of constant absolute magnitude. However, at the lower levels of this type of error its answers were almost as good as those given by Wilkinson's (1961) method (this is the one instance, mentioned above, in which the results at the different error levels were not qualitatively the same). Further, the bias in the results given by the non-parametric method was only apparent at the highest error level.

We have therefore concluded that, unless the error is definitely known to be normally distributed and of

constant magnitude, Eisenthal & Cornish-Bowden's (1974) method is the one to use. We also strongly recommend that new methods for fitting equations to data be rigorously tested before they are published.

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