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LETTER TO THE EDITOR

Parameter Estimating Procedures for the Michaelis–Menten Model: Reply to Tseng and Hsu

In a recent account of methods of obtaining best-fit parameters of the parameters V and K_m of the Michaelis-Menten equation, Tseng & Hsu (1990) appear to have misunderstood the method used by Lineweaver & Burk (1934), and to have overlooked much of the work done since 1934. The Michaelis-Menten equation represents the observed rate of v of an enzyme-catalysed reaction in terms of the two parameters V and K_m, and the substrate concentration s,

$$v = \frac{Vs}{K_m + s} + \varepsilon \tag{1}$$

As written here [as well as by Tseng & Hsu (1990)] this equation assumes that the rate is subject to an additive error ε.

To some extent the article by Tseng & Hsu (1990) simply repeats widely known information (Wilkinson, 1961; Johansen & Lumry, 1961; Cleland, 1967). In particular, their finding that for the error structure they assumed better results are obtained by direct fitting than by an unweighted fit to the double-reciprocal form of eqn (1) is neither novel nor surprising. Unfortunately, their paper also introduces some new ideas and repeats some old and seriously misleading misconceptions.

The methods that Tseng & Hsu (1990) call LB and ECB purport to be those of Lineweaver & Burk (1934) and of Eisenthal & Cornish-Bowden (1974) respectively, but in reality they bear little relation to them. In the case of Lineweaver & Burk (1934) the misconception is almost universal, apparently extending even to the original authors (see Burk, 1984). What Lineweaver & Burk (1934) actually did (see Lineweaver *et al.*, 1934), was to fit the data to the double-reciprocal form of eqn (1) with each v value given a weight equal to v^4: this gives a close approximation to a direct fit of eqn (1) with equal weight to each rate, and for the primitive computing equipment available in 1934, it is as good an approximation as one could expect. More recently, Cleland (1967) suggested using weights of v^4 as a first approximation but refining the fit iteratively with weights of $\hat{v}^4$, where $\hat{v}$ is the value of v calculated with the values of V and K_m for the previous approximation, whereas I suggested refining with weights of $v^2\hat{v}^2$ (Cornish-Bowden, 1976). Actually, these two suggestions differ from the ideal solution in opposite directions by about the same amount: as I have discussed elsewhere (Cornish-Bowden, 1982), refining with weights of $v\hat{v}^3$ leads iteratively to *exactly* the same result as direct minimization of $\sum \varepsilon^2$ by non-linear regression.

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The misrepresentation by Tseng & Hsu (1990) of the method of Eisenthal & Cornish-Bowden (1974) apparently derives from an attempt to put a precise mathematical interpretation on a textbook summary of the method. Their proposal to minimize the sum of distances (squared? absolute values?) between the point (V, K_m) in parameter space and set of lines $V=(v/s)K_m+v$ generated by the data owes nothing to Eisenthal & Cornish-Bowden (1974). It is not even fully defined, because it ignores the fact that V and K_m have different dimensions: in consequence, not only the "distances", but also, more seriously, their relative magnitudes among themselves, are arbitrarily dependent on the scales used for plotting. What Eisenthal & Cornish-Bowden (1974) actually proposed was to take the median of all values of V given by the intersection points of the lines as the best value of V, and likewise for K_m. The statistical justification for doing this was set out in detail in an accompanying paper (Cornish-Bowden & Eisenthal, 1974). The fact that Tseng & Hsu (1990) have found that an aribtrary procedure of their own gives less than ideal results tells us nothing about the behaviour of the real method.

Likewise, while it is true that their own method ECB provides no information about the precision of the fitted parameters, it is not correct to suppose that the method on which it purports to be based is similarly deficient: as discussed in this Journal (Cornish-Bowden *et al.*, 1978), the median method not only gives satisfactory parameter estimates with a much wider range of error structures than regression methods, but it is also capable of giving confidence intervals that are at least as reliable as those given by regression methods.

A further problem with the paper of Tseng & Hsu (1990) is that the results of their simulation are valid for real experiments only to the extent that the particular error structure that they assume is the one that actually underlies the data. There is no physical necessity to assume that ε in eqn (1) has a normal distribution with mean zero and constant variance. As various authors have realized (Burk, 1934; Storer *et al.*, 1975; Siano *et al.*, 1975; Askelöf *et al.*, 1976; Nimmo & Mabood, 1979; Mannervik *et al.*, 1986), the distribution of ε in real experiments is accessible, at least in part, to experimental investigation. The earliest of these studies (Burk, 1934) gave results that agree with the assumption of Tseng & Hsu (1990); however, one may question how relevant it is to modern experimental practice, as all modern studies have indicated that although the assumption of a normal distribution may be an acceptable approximation to reality the assumption of a uniform variance is rarely if ever acceptable.

Tseng & Hsu (1990) attribute their random search method to their earlier paper (Hsu & Tseng, 1989), but in neither paper do they specify how many trial points they calculated in each iteration, how they generated these, or how many of them they considered to constitute the set giving the smallest values of the sum of squares. None the less, a method along the lines suggested ought to be capable of arriving at the same parameter values as the non-linear regression method of Wilkinson (1961), or indeed regression of the double-reciprocal equation with weights $v\hat{v}^3$ as discussed above. In this sense, therefore, it appears to be valid. However, it is not obvious that it has any practical advantage over these more systematic ways of arriving at the result [or over other search methods, such as that suggested by Bannister *et al.* (1976)] and it seems likely to be much slower.

As the error structure of experimental data is normally unknown *a priori*, and few experimenters undertake the necessary experiments to determine it, it is as a general rule unwise to assume that any one method will always yield good results. However, the median method of Eisenthal & Cornish-Bowden (1974) has proved in numerous studies (e.g. Atkins & Nimmo, 1975) to be quite robust. Its main disadvantage is that it is not easily generalizable to models of more than two parameters: in such cases one needs more elaborate robust methods, such as the "biweight" (Mosteller & Tukey, 1977) coupled with some method of estimating heteroscedasticity from internal evidence in the data (Cornish-Bowden & Endrenyi, 1986). Such methods are tedious and difficult to apply manually, but are available as computer programs (e.g. Cornish-Bowden, 1985).

Tseng & Hsu (1990) do not comment on the remarkable concordance between the results they reported for the ECB method and those for linear regression of the plot of v against v/s, which they refer to as E-H. In the relative errors of K_m in their Table 1, for example, the ratios of values for E-H/ECB range from 0·986-0·994, not only very close to 1 but also far more uniform than one finds in comparisons between other pairs of methods; for example, the corresponding ratios between E-H and L-B (the double-reciprocal plot) range from 1·091-1·882. Similar correspondences in all of the data reported suggest that in practice E-H and ECB are almost equivalent. They cannot be exactly equivalent of course, because E-H exactly gives the parameter values $\hat{V}$ and $\hat{K}_m$ that minimize the sum of squares $SSE_{\text{E-H}}$ defined by

$$SSE_{\text{E-H}} = \sum [v - \hat{V} + (v/s)\hat{K}_m]^2, \tag{2}$$

whereas ECB is arbitrarily dependent on the units in which v and s are measured. None the less, the numerical agreement suggests that one should look for a sense in which they approach equivalence. Assuming that by "sum of distances" Tseng & Hsu (1990) mean the sum of squares of distances measured along the perpendicular, with scales chosen such that numerically equal s and v ranges correspond to equal distnces, then their method ECB gives the parameter values $\hat{V}$ and $\hat{K}_m$ that minimize the sum of squares of SSE_{ECB} defined by

$$SSE_{\text{ECB}} = \sum \frac{[v - \hat{V} + (v/s)\hat{K}_m]^2}{1 + (v/s)^2}. \tag{3}$$

Although this is not the same as eqn (2), it differs only by the weighting factor $[1 + (v/s)^2]^{-1}$, which is essentially constant if the v values are all numerically small compared with the s values. As this condition appears to have been met in the simulations reported by Tseng & Hsu (1990), the agreement between apparently different methods is explained. This analysis also shows that it was unnecessary for them to use their random search algorithm to obtain their ECB results: linear regression with weights $[1 + (v/s)^2]^{-1}$ would have been sufficient.

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