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WEIGHTING OF LINEAR PLOTS IN ENZYME KINETICS

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Abstract

IF the rates of an enzyme-catalysed reaction are equally weighted in least-squares analysis, the corresponding reciprocal rates $1/v_i$ *should be given weights of* $v_i^2\hat{v}_i^2$*, where* $\hat{v}_i$ *is the calculated rate, in order to give the same sum of squares as for the untransformed Michaelis-Menten equation. However, these weights are functions of the parameter values and therefore cannot be treated as constants in partial differentiation with respect to the parameters. Consequently, linear regression with these wieghts does not give the same parameter estimates as non-linear regression of the untransformed equation. The error can be exactly corrected by using weights of* $v_i\hat{v}_i^3$ *in the linear regression.*

Introduction

"It was found upon analysis of the data that equal weighting was to be assigned to each measured v value, which means that the weighting of 1/v is proportional to v^4"

Dean Burk, 1934[1]

IN the folklore of biochemistry Lineweaver and Burk[2] are given the blame for introducing a method for estimating the parameters of the Michaelis-Menten equation quite different from the one they actually used. Although their commonly cited paper does not itself give details of how they did their calculations, it contains references to two others[1,3] that do. We can see from the statement that I have quoted at the beginning of this paper that they were aware of three important points about least-squares analysis: (a) least-squares estimation requires weights; (b) these should be based on observation, not on surmise; (c) equal weighting of rates implies grossly unequal weighting of their reciprocals. The first and third of these points did not begin to penetrate the general biochemical consciousness until a quarter of a century later, with the publication of papers by Johansen and Lumry[4] and Wilkinson[5]. The second point is still little recognized, and even now review articles[6] continue to be written that advocate weighting assumptions that take no account of published investigations of actual error behaviour in enzyme kinetics[7-10].

In the present paper I want to examine an inconsistency in the way supposedly equivalent ways of analysing the Michaelis-Menten equation behave. Although it is in most cases a numerically trivial inconsistency it is nonetheless worthy of study, because even a numerically trivial inconsistency casts doubt on the validity of a mathematical method if it cannot be explained.

I. Weighting of the Double-Reciprocal Plot

THE Michaelis-Menten equation expresses the dependence of an observed initial rate v_i on the substrate concentration s_i, and contains two parameters, V and K_m, that need to be estimated:

$$v_i = \frac{Vs_i}{K_m + s_i} + e_i \tag{1}$$

The error term e_i in this equation expresses the fact that real experiments cannot be carried out without experimental error, and so v_i is not in general exactly equal to $Vs_i/(K_m + s_i)$.

The least-squares method of estimating V and K_m requires us to define a weighted sum of squares SS,

$$SS = \sum w_i e_i^2 \tag{2}$$

in which the summation is for i=1 to n, i.e., it is carried out over all of n observations, and the weight w_i to be assigned to each v_i should be inversely proportional to the variance of that v_i. In the present paper I shall not consider how these weights should be defined (but see refs.(7-12)), but will treat them as known constants. We now define the best-fit values $\hat{V}$ and $\hat{K}_m$ as the values of V and K_m respectively that make SS a minimum. I shall also define

$$\hat{v}_i = \frac{Vs_i}{K_m + s_i} \tag{3}$$

as the calculated rate corresponding to the observed rate v_i*.

Non-linear regression[4-5] provides one method of minimizing SS. Here, however, I shall discuss the alternative method of using a properly weighted linear regres-

* *Although it might seem more logical to use the best-fit parameter values $\hat{V}$ and $\hat{K}_m$ on the right-hand side of eqn.(3), it is considerably more useful to define $\hat{v}_i$ as shown, and so I shall sacrifice strict logical consistency for convenience.*

sion[3-5]. If correctly carried out this gives exactly the same parameter estimates as non-linear regression and is computationally just as convenient; but it has the added advantages that it is (in my view) easier to understand and it sheds light on the widely used straight-line plots of the Michaelis-Menten equation. The best known of these is the double-reciprocal plot, based on the following form of the Michaelis-Menten equation:

$$1/v_i = (K_m/V)(1/s_i) + (1/V) + e_i' \tag{4}$$

This equation cannot be derived from Eq.(1) simply by taking reciprocals of both sides, because the error term e_i in Eq.(1) prevents its right-hand side from having a simple reciprocal resembling the right-hand side of Eq.(4). It follows that e_i' is not the same as e_i, but simple algebra shows that they are related as follows:

$$e_i' = -e_i(K_m + s_i)/v_i V s_i = -e_i/v_i \hat{v}_i \tag{5}$$

Consequently, if we define the sum of squares SS' for the double-reciprocal plot as follows:

$$SS' = \sum w_i' e_i'^2 \tag{6}$$

it will be different from the original sum of squares SS unless the new weights w_i' are defined in a way that takes account of Eq.(5):

$$w_i' = w_i v_i^2 \hat{v}_i^2 \tag{7}$$

II. Minimization of the Sum of Squares

FOR a straight line $y_i = a + bx_i + e_i'$ the formulae for minimizing the weighted sum of squares $SS' = \Sigma w_i' e_i'^2$ are as follows[13]:

$$\hat{b} = \frac{\sum w_i' \sum w_i' x_i y_i - \sum w_i' x_i \sum w_i' y_i}{\sum w_i' \sum w_i' x_i^2 - (\sum w_i' x_i)^2} \tag{8}$$

$$\hat{a} = (\sum w_i' y_i - \hat{b} \sum w_i' x_i)/\sum w_i' \tag{9}$$

We can apply these formulae to Eq.(4) by means of the substitutions $\hat{V} = 1/\hat{a}$, $\hat{K}_m = \hat{b}/\hat{a}$, $x_i = 1/s_i$, $y_i = 1/v_i$, $w_i' = w_i v_i^2 \hat{v}_i^2$. Initially there are no values of V and K_m for use in calculating $\hat{v}_i$, but as a first approximation we can assume that the measurements are accurate enough to replace $\hat{v}_i$ by v_i, i.e., to define $w_i' = w_i v_i^4$. Then Eqs.(8-9) allow the calculation of first-approximation values of $\hat{V}$ and $\hat{K}_m$, which can be used to calculate $\hat{v}_i$, and the cycle of calculations can be repeated until $\hat{V}$ and $\hat{K}_m$ do not change from one cycle to the next.

Now this argument, which is essentially as I have presented it previoursly[13], may seem plausible. But plausible or not, it is incorrect, as can be seen from the results shown in Table 1. For the data used as an illustration by Wilkinson[5], and with $w_i=1$ for all i, $\hat{V}$ and $\hat{K}_m$ have been calculated by a direct non-linear fit to Eq.(1), as described by Wilkinson[5], and also by linear regression of Eq.(4) with weights w_i' defined in various ways. Not surprisingly, the preliminary results with $w_i' = v_i^4$ are different from those given by non-linear regression, but in addition the results from the iterative approach I have described, with $w_i' = v_i^2\hat{v}_i^2$, are also different, as are those obtained with $w_i' = \hat{v}_i^4$, as suggested by Cleland[14]. Only a calculation with the apparently arbitrary compromise of $w_i' = v_i\hat{v}_i^3$ gives the same parameter estimates as direct non-linear regression. These variations in results are not a consequence of numerical problems, such as rounding error; they reflect the fact that the argument given above for carrying out the linear regression with $w_i' = v_i^2\hat{v}_i^2$ is invalid, for reasons that I shall now discuss.

Table 1. Results of fitting the same data with different weights. The data used were those given by Wilkinson[5] for illustration, and consisted of the following six (s_i, v_i) pairs: (0.138, 0.148),(0.220, 0.171), (0.291, 0.234), (0.560, 0.324), (0.766, 0.390), (0.146,0.493). Values of s_i and $\hat{K}_m$ are in mM, v_i and $\hat{V}$ in arbitrary units.

Weighting	$\hat{K}_m$	$\hat{V}$	Reference
Weight for $v_i = 1$	0.59655	0.69040	[5]
Weight for $1/v_i = v_i^4$	0.57097	0.67986	[1,3-5]
Weight for $1/v_i = v_i^2\hat{v}_i^2$	0.58770	0.68672	[13]
Weight for $1/v_i = v_i\hat{v}_i^3$	0.59655	0.69040	This paper
Weight for $1/v_i = \hat{v}_i^4$	0.60553	0.69416	[14]

III. The Correct Weighting Function

THE derivation of Eqs.(8-9) for fitting a straight line is made with the assumption that the weights w_i' are independent of the parameter values, so that they can be treated as constants during partial differentiation. As defined by Eq.(7), however, they are not constants because $\hat{v}_i$ is a function of V and K_m.

Partial differentiation of Eq.(6) with respect to K_m gives

$$\frac{\partial SS'}{\partial K_m} = 2\sum w_i'\varepsilon_i'\frac{\partial \varepsilon_i'}{\partial K_m} + \sum \varepsilon_i'^2\frac{\partial w_i'}{\partial K_m} \tag{10}$$

The second term in this equation is simply a consequence of the dependence of $\hat{v}_i$, and hence w'_i, on K_m. Consideration of Eqs.(3), (5) and (7), with some rearrangement, allows this derivative to be written as follows:

$$\frac{\partial SS'}{\partial K_m} = 2\sum w'_i e'_i (\hat{v}_i/v_i)\frac{\partial e'_i}{\partial K_m} \tag{11}$$

Comparison with Eq.(10) shows that this is exactly the expression we should have obtained if we had replaced w'_i by $w'_i\hat{v}_i/v_i$ initially but treated it as a constant in the partial differentiation. The corresponding result may be shown by an analogous argument to apply to partial differentiation with respect to V. If follows, therefore, that the error of ignoring the dependence of the weights on the parameter values can be exactly corrected by carrying out the linear regression with refined weights $w'_i = w_i v_i \hat{v}_i^3$ instead of $w'_i = w_i v_i^2 \hat{v}_i^2$. Thus the results of Table 1 are explained.

It is perhaps worth adding that the need to replace $w'_i = v_i^2\hat{v}_i^2$ with $w'_i = v_i\hat{v}_i^3$ applies only to the iterative process for estimating the parameters V and K_m. For calculation of the sum of squares at the end of the computation the original weights $w'_i = v_i^2\hat{v}_i^2$ must be used, because the conclusion that for SS'as defined in Eq.(6) to be identical with SS as defined in Eq.(2) requires these weights be independent of any considerations of partial differentiation.

IV. Discussion

THE variation in results given by the various weighting schemes shown in Table 1 is not great, and is certainly trivial compared with the error incurred by fitting data to a double-reciprocal plot by eye or by unweighted linear regression**. On many occasions there is likely to be very little advantage in proceeding beyond the first-approximation fit obtained with $w'_i = v_i^4$. If one does decide to go to the appreciable extra computational labour of using refined weights, however, it is obviously better to use the correct formula $w'_i = v_i\hat{v}_i^3$, which takes the calculation 100% of the way to the correct solution, rather than either $w'_i = v_i^2\hat{v}_i^2$, which will take it only 67% of the way, or $w'_i = \hat{v}_i^4$, which will take it 33% too far.

I have concentrated in this paper on the weighting of the double-reciprocal plot, because it is the best known and most widely used plot of the Michaelis-Menten equation. However, essentially the same kind of analysis can be applied to the alternative plot of s_i/v_i against s_i, which has the advantage that the weights are must less steeply dependent on v_i [5]. For this plot the first-approximation weights are $w'_i = w_i v_i^4/s_i^2$ and the refined weights are $w'_i = w_i v_i \hat{v}_i^3/s_i^2$.

** If the same data as in Table 1 are fitted with $w'_i = 1$ for each value of $1/v_i$ the results are $\hat{K}_m = 0.44062$, $\hat{V} = 0.58532$.

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