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USE OF MODIFIED DOUBLE-RECIPROCAL PLOT FOR ENZYME KINETIC PARAMETERS

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Summary

The modified double-reciprocal plot suggested by Lee and Wilson (Lee, H.J. and Wilson, I.B. (1971) Biochim. Biophys. Acta 242, 519—522) has until now been accepted as giving a simple and accurate method for the determination of enzyme kinetic parameters V and K_m, which can be used with little error even when as much as 50% of the substrate is consumed. In this paper it has been shown with analytical proof that the errors in the application of their method can in fact be much higher and that the integrated Michaelis-Menten equation should be used in its complete form.

It has been a common practice to determine the intrinsic kinetics of enzymatic reactions with the help of double-reciprocal plots of which the Lineweaver-Burk plot is the most common. However, these plots require initial-reaction rate measurements. In cases where it is not practical to limit the change in substrate concentration to low values of 1—2% Lee and Wilson [1] reported that a simple modification of the integrated Michaelis-Menten equation enabled the double-reciprocal form to be retained even when as much as 50% of the substrate is utilized. This method has also been recognized as simple and fairly accurate [2,3] for the simple Michaelis-Menten kinetics without inhibition effects. However, Atkins and Nimmo [4] pointed out that when the reaction is competitively inhibited by one of the products,

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the above procedure ought not to be used. They showed that the method of Lee and Wilson would give systematically increasing error in initial velocity with increasing fraction of substrate converted, especially at high degrees of product inhibition. This drawback has also been mentioned by Cornish-Bowden [2].

We shall show in this paper that though the conclusion of Lee and Wilson [1] appears to be correct on the face of it and indicates that large conversions can be handled with little loss of accuracy, in fact the limitation on the extent of reaction is much more severe even in the case of simple Michaelis-Menten kinetics. Lee and Wilson observed that even if the extent of reaction is 50%, the error in K_m will be only 4%. It is shown in this communication that the error in K_m and V can be much higher and the maximum extent of reaction must be limited to very low values to retain the accuracy of the parameter estimation.

The integrated Michaelis-Menten equation for a simple uninhibited enzymic reaction is given by

$$\frac{1}{\bar{v}} = \frac{1}{V} + \frac{K_m}{V} \frac{1}{S_o - S_t} \ln\left(\frac{S_o}{S_t}\right) \tag{1}$$

where $\bar{v} = \dfrac{S_o - S_t}{t}$.

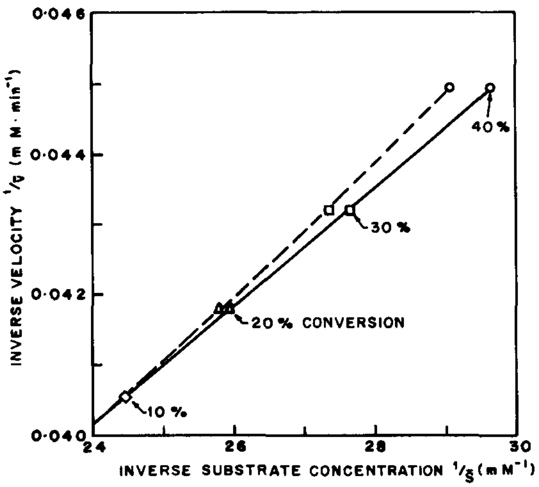
The modified Lineweaver-Burk equation given by Lee and Wilson [1] is

$$\frac{1}{\bar{v}} = \frac{1}{V} + \frac{K_m}{V} \frac{1}{\bar{S}} \tag{2}$$

where $\bar{S}$ is the arithmetic mean substrate concentration AMSC given by

$$\bar{S} = \frac{S_o + S_t}{2} \tag{3}$$

which replaces the log mean substrate concentration LMSC = $(S_o - S_t)/\ln(S_o/S_t)$ in Eqn. 1. They went on to calculate the error in approximating the LMSC by AMSC and assumed that this is also the error in the final kinetic parameter values calculated. However, by actual plotting, the error in K_m and V is seen to be much higher, as shown in Figs. 1 and 2. In Fig. 1, the solid line is the one given by Eqn. 2, where $\bar{S}$ is the LMSC and the broken curve is given by the same equation, where $\bar{S}$ is the AMSC. By taking two points on the broken curve corresponding to 40% and 30% conversions, and assuming linearity, the slope and intercept and thus the K_m and V values can be determined. Comparing these with the true values and calculating the respective errors for the average conversion of 35%, the error in K_m and V values exceeds 50% and 25%, respectively, which is quite high. In fact, it is observed that for the error to be less than 5%, the conversions should not exceed about 7%.



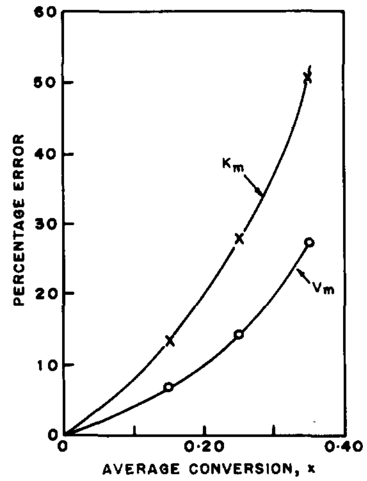


Fig. 1. Modified double-reciprocal plot. ------, AMSC used for $\bar{S}$; ——, LMSC used for $\bar{S}$. True parameter values used: $K_m = 0.042$ mM; $V = 50$ nM·mg^{-1}·m^{-1}; $S_o = 0.043$ mM.

Fig. 2. Percentage error in the calculation of the kinetic parameters K_m and V, as a function of average conversion.

The above findings can be proved analytically as follows: Eqn. 1 can be rewritten as

$$\frac{1}{v} = \frac{1}{V} + \frac{K_m}{V}(1+\alpha)\frac{1}{\bar{S}} \tag{4}$$

where $\bar{S}$ is the AMSC and $1 + \alpha = \dfrac{\bar{S}}{S_o - S_t} \ln \dfrac{S_o}{S_t}$.

The slope m of the curve on the modified double-reciprocal plot (broken curve in Fig. 1) is given by

$$m = \frac{d\,(1/\bar{v})}{d\,(1/\bar{S})} = \frac{K_m}{V}\,\frac{d}{d(1/\bar{S})}\left[(1+\alpha)\frac{1}{\bar{S}}\right] \tag{5}$$

Expressing α and $\bar{S}$ in Eqn. 5 in terms of conversion,

$$1 + \alpha = \left(\frac{1}{x} - \frac{1}{2}\right)\ln\frac{1}{1-x} \quad \text{and} \tag{6}$$

$$\bar{S} = S_o(1 - x/2) \tag{7}$$

yields

$$m = \frac{K_m}{V}\cdot E \tag{8}$$

where $E = \left(\dfrac{1}{x} - \dfrac{1}{2}\right)\left[\dfrac{2-x}{1-x} + \left(1 - \dfrac{2}{x}\right)\ln\dfrac{1}{1-x}\right]$

TABLE I

x	0.1	0.2	0.3	0.4
E	1.037	1.088	1.155	1.246

Table I gives the values of E calculated for different conversions x and it is clear that the error in calculating the slope can be as high as 24% for $x = 0.4$. It can be proved that as the conversion level falls to a very low value, the error given by E tends to zero [i.e. value of E tends to unity].

The ultimate error in K_m and V by actual plotting are even higher as shown in Fig. 2.

In view of the demonstrated [5] superiority of other plots such as Hane's plot and the direct-linear plot of Eisenthal and Cornish-Bowden [6], over that of Lineweaver and Burk, it is perhaps of interest to examine whether the modification of Lee and Wilson will be applicable with good accuracy in those types of plots. However, actual investigations by us showed no improved results. In the case of Hane's plot, modified according to Lee and Wilson ($\bar{S}/\bar{v}$ vs. $\bar{S}$) to handle reaction progress curve data, similar errors in K_m and V to those of the modified Lineweaver-Burk plot were evident. Application to the direct linear plot of Eisenthal and Cornish-Bowden [6], suffers from the drawback that the point of intersection of lines of slope $\bar{v}/\bar{S}$ and intercept $\bar{v}$ becomes ill-defined, making it difficult to meaningfully extract the kinetic constants from the plot.

In conclusion, the arguments presented in this paper should make clear the limitations of the modified double-reciprocal plot suggested by Lee and Wilson. In view of this, the accuracy quoted by them for an experimental system appears to be fortuitous. Therefore, in order to keep the error to a minimum while using the progress curve for the kinetics determination, it is necessary to use the integrated equation in the complete form for simple Michaelis-Menten kinetics.

References

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