This Week's Citation Classic®

Wilkinson G N. Statistical estimations in enzyme kinetics.
Biochemical J. 80:324-32, 1961.
[Division of Mathematical Statistics, CSIRO, and University of Adelaide, South Australia]

Simple iterative statistical methods for estimating the parameters of enzyme-kinetic reactions are described. The methods avoid the subjective biases of graphical methods and produce reliable and more accurate estimates of their standard errors. To facilitate the methods' applications to a range of problems, the paper emphasizes the explanation of basic principles. [The *SCI*® indicates that this paper has been cited in over 2,615 publications.]

Graham N. Wilkinson
Division of Mathematics and Statistics
CSIRO
Private Bag No. 2
Glen Osmond, South Australia 5064
Australia

April 10, 1987

Before this paper appeared, graphical methods (most often the Lineweaver-Burk reciprocal plot[1]) were widely used for enzyme-kinetic determinations. By showing how simply proper statistical calculations could be carried out, the paper had a major impact on the field;[2] it was listed as one of the most-cited papers of the 1960s in biochemistry and molecular biology, having 1,171 citations at that time. It was also 1 of 39 articles in that study with more than 1,000 citations. The list included a paper by W.W. Cleland,[3] who subsequently developed computer programs for statistical calculations.

The paper arose from my collaboration as a statistician with M.R. Atkinson and R.K. Morton (both now deceased) at the Waite Agricultural Research Institute in South Australia. Their work with J.F. Jackson[4] involved estimating two dissociation constants from a series of K_m determinations at varying pH for the adenylation of nicotinamide mononucleotide by adenosine triphosphate. It was important not only to estimate the constants with maximum accuracy but also to determine accurate standard errors for them; for this the use of optimal statistical methods was essential, as illustrated in the paper. The success of the paper is due to expository simplicity and practical importance, as well as to its publication in the prestigious *Biochemical Journal*; it was the first such paper to appear there. (I am grateful to Morton for persuading the editor to publish it.)

The paper advocated direct least-squares fitting of enzyme-kinetic relations such as the Michaelis-Menten law.[5] As these relations will be nonlinear in some parameters (such as K_m), the calculations need to be iterated to improve initial approximate parameter estimates. Convergence is usually rapid, however, and the iterative calculations are easily programmed on handheld, programmable calculators. Nowadays, in fact, most biochemical laboratories are equipped with personal computers, so it is somewhat disappointing to see a continuing spate of papers on fitting the Michaelis-Menten law in one of its linear graphical forms such as the Lineweaver-Burk plot, the de Woolf plot of s/v against s, or the Hofstee plot of v/s against v.[6]

Least-squares fitting is not appropriate for any of these plots because it produces non-optimal and biased results. In two of the three cases, these results are due to the skewness induced in the error distribution for reaction rates (v) by transformation to reciprocals (1/v or s/v). Fitting should be done on a scale with homogeneous and symmetrically distributed errors. For the Michaelis-Menten law this usually implies fitting the law either to v or in logarithmic form to log v, depending on whether the standard error for v is roughly constant or varying in proportion to the theoretical value for v (so that the standard error of log v is constant). It is important to note that K_m estimates from small data sets tend to be positively skewed whereas their logarithms pK_m are much more normally distributed and more homogeneous in variance under varying conditions such as changing pH. It is then that further statistical analysis should be applied to pK_m values.

1. **Lineweaver H.** Citation Classic. Commentary on *J. Amer. Chem. Soc.* 56:658-66, 1934. *Current Contents/Life Sciences* 28(11):19, 18 March 1985. (Cited 8,560 times since 1955.)
2. **Garfield E.** Most-cited articles of the 1960s. 2. Biochemistry and molecular biology. *Essays of an information scientist.* Philadelphia: ISI Press, 1981. Vol. 4. p. 254-63.
3. **Cleland W W.** The kinetics of enzyme-catalyzed reactions with two or more substrates or products. I. Nomenclature and rate equations. *Biochim. Biophys. Acta* 67:104-37, 1963. (Cited 1,690 times.) [See also: **Cleland W W.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 2: the molecules of life.* Philadelphia: ISI Press, 1986. p. 195.]
4. **Atkinson M R, Jackson J F & Morton R K.** The effects of nicotinamide mononucleotide concentration and pH on dinucleotide synthesis. *Biochemical J.* 80:318-23, 1961.
5. **Michaelis L & Menten M L.** Die Kinetik der Invertinwirkung (Kinetics of invertase action). *Biochem. Z.* 49:333-69, 1913. (Cited 985 times since 1955.)
6. **Currie D J.** Estimating Michaelis-Menten parameters: bias, variance and experimental design. *Biometrics* 38:907-19, 1982.