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This Week's Citation Classic™

Cleland W W. The statistical analysis of enzyme kinetic data. *Advan. Enzymol. Relat. Areas Mol.* **29**:1-32, 1967. [Dept. Biochemistry, Univ. Wisconsin, Madison, WI]

Equations are given for applying the least-squares method of curve fitting to enzyme kinetic data. Experimental design and evaluation of the results of statistical analysis are discussed. A FORTRAN program for fitting data to the Michaelis-Menton equation is given and explained. [The *Social Sciences Citation Index*® (*SSCI*®) and the *Science Citation Index*® (*SCI*®) indicate that this paper has been cited in over 1,205 publications since 1967.]

W. Wallace Cleland
Department of Biochemistry
University of Wisconsin
Madison, WI 53706

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I became an enzyme kineticist by accident when, as a young assistant professor, I was asked to teach a first-year graduate-level course in biochemistry. Kinetics was one of the topics, and I rapidly discovered that the field was in a very unsatisfactory state. I began to play with equations, and this led to the theoretical papers in *Biochimica et Biophysica Acta* in 1963, the first of which was an earlier *Citation Classic*.[1] As we began to do experimental kinetics, it became obvious that statistical analysis of the data was needed, and I wrote FORTRAN programs that made least-squares fits to the rate equations using the method of Wilkinson.[2] An announcement of the availability of these programs in *Nature* in 1963[3] instituted many requests for them and has received 620 citations since then.

I have never had a course in statistics and what little I know is self-taught, so when I was asked to write an article for *Advances in Enzymology and Related Areas of Molecular Biology* in 1967 on the statistical analysis of enzyme kinetic data, I was placed in the position of the blind leading the blind. I think this article has been successful for several reasons. First, the FORTRAN program for fitting data to the basic equation, $v = VA/(K + A)$, was included, and people who used the program felt obliged to cite the article. Second, I believe that, because of my limited knowledge of statistics, I was able to express the principles in language that an ordinary biochemist could understand. Certainly no statistician I have known can write so a nonstatistician can understand him! Third, I think that the time was ripe. People had begun to feel the need for more rigorous data analysis, but few could write computer programs, and fewer still could understand nonlinear least-squares analysis.

Numerous articles have been written since 1967 on statistical analysis of enzyme kinetic data, but most are, like other statistical literature, unintelligible to the average biochemist. The simple analysis presented in 1967 still is the easiest to use, and is valid as long as proper attention is paid to weights of the experimental velocities. The early programs I wrote assumed that the errors in the velocities were constant. I have since written programs to fit data with proportional error (that is, where the equation is expressed in the log form) and even one where the fit is to the square-root form of the equation (this assumes that the variances of velocities are proportional to velocity). I have about 110 programs in the drawer, and 18 of these were published in *Methods in Enzymology* in 1979.[4] It feels good to know that programs written in a 1963 version of FORTRAN are still useful and are the standard tools for statistical analysis of enzyme kinetic data over 20 years later.

1. **Cleland W W.** The kinetics of enzyme-catalyzed reactions with two or more substrates or products. 1. Nomenclature and rate equations. *Biochim. Biophys. Acta* 67:104-37, 1963. [See also: **Cleland W W.** Citation Classic. *Current Contents* (28):8, 11 July 1977.]
2. **Wilkinson G N.** Statistical estimations in enzyme kinetics. *Biochemical J.* **80**:324-32, 1961. (Cited 2,175 times.)
3. **Cleland W W.** Computer programmes for processing enzyme kinetic data. *Nature* **198**:463-5, 1963. (Cited 620 times.)
4. ------------------. Statistical analysis of enzyme kinetic data. *Meth. Enzymology* **63**:103-38, 1979. (Cited 210 times.)

