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Enzyme Kinetics Calculations—The Direct Linear Plot Procedure

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The availability of low-cost computing facilities makes it possible to ask students to explore a greater number and wider range of problems in enzyme kinetics without making excessive demands on their limited time resources. Students should be required to perform some manual data manipulation and analysis, in order for them to develop a better understanding of the principles and procedures involved. From this experience they will also discover something about the advantages of the application of computers to biochemical problems.

The Michaelis-Menten equation can be rearranged to three forms that give linear plots, from which K_m and V_{max} can be evaluated. Cornish-Bowden has discussed the relative merits of these commonly used straight-line plots in terms of the way they reflect errors in initial velocity (*22*). A different approach to plotting the Michaelis-Menten equation, the direct linear plot, has been described by Eisenthal and Cornish-Bowden (*23*, *24*) (see also (*22*) pp. 25–30). This procedure for obtaining estimates of K_m and V_{max} is based on distribution-free (or nonparametric) statistics and is much less dependent on assumptions than the least-squares approach to data fitting (*22*).

We have written a computer program to calculate estimates of $(K_m/V_{max})_{ij}$ and $(1/V_{max})_{ij}$ using pairs of initial velocities (v_i and v_j) measured at different substrate concentrations (s_i and s_j). Although these will, in general, be poor estimates subject to large random errors, there are a large number of them, and, if they are arranged in rank order, the median (middle) values are statistically satisfactory estimates from which the other parameters K_m, V_{max}, V_{max}/K_m, and $1/K_m$ may be readily calculated. The most striking advantage of this approach (one that is of special importance in analyzing student experiments!) is that occasional very bad experimental points have little effect on the determination of a median, though they can have a devastating effect on a least-squares calculation.

The vast majority of textbooks (even the most recent ones) continue the "romance" with the double-reciprocal plot, in spite of the severe way it is affected by experimental errors. Two exceptions are Rawn, who briefly mentions the direct linear plot procedure (*25*) and Wood, Wilson, Benbow and Hood, who, in the second edition of their book, point out that it is strongly preferred (*26*).

The program MM-DLPLOT, written for the Commodore PET microcomputer with 16K RAM, fits experimental data to the Michaelis-Menten equation according to the method of Cornish-Bowden and Eisenthal (*23*, *24*). The user is prompted to input the number of data points and substrate concentration-initial velocity pairs. For completeness, and especially for students, the data are given a title and the units of concentration and velocity are also entered. The program calculates the 95% confidence limits for the parameters by the method of Porter and Trager (*27*), modified as described by Cornish-Bowden, Porter, and Trager (*28*). The lower, median and upper values of K_m, V_{max}, V_{max}/K_m, $1/K_m$, $1/V_{max}$, and K_m/V_{max} are printed. Errors can be printed on request and a table of substrate concentration, observed velocity, calculated velocity, and error is presented. Finally a summary table of the median values of K_m and V_{max} (with associated units) is printed, and the program provides the option for the user to plot v against s on the screen.

The program MM-DLPLOT is available from K. A. H. Adams, Department of Chemistry, Mount Allison University, Sackville, New Brunswick, Canada E0A 3C0 on cassette tape for $15, PET 4040 diskette for $25, or print-out for $4. It is also available on PET 8050 diskette from Project SERAPHIM. Send a check for $4 (U.S.) made out to Project SERAPHIM, Acct. 20350, to John W. Moore, Department of Chemistry, Eastern Michigan University, Ypsilanti, MI 48197. Although the program has been written and tested for a Commodore PET, it requires only minor alterations to allow it to be run on other microcomputers that use extended Dartmouth BASIC. The original FORTRAN version (*28*), intended for batch processing on mainframe computers, is still available from A. Cornish-Bowden, Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham, England B15 2TT.

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