Constructing nonlinear Scatchard plots

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Table 1. Calculated and Experimental Values of the Internal Rotation Barrier for the Studied Molecules (kcal/mole) [a]

Molecule	CNDO/2 (this paper)	ab-initio (20)	experimental values
$H_3C—OH$	0.95	1.12	1.07 (*14*)
$H_3C—CH_3$	2.18	3.26	2.93 (*16*)
$H_3C—NH_2$	1.27	2.13	1.98 (*17*)
$H_3C—CH_2CH_3$	2.37	3.70	3.33 (*18*)
$H_3C—CH_2F$	2.00	3.63	3.30 (*19*)

[a] The numbers within parentheses refer to the references listed in the cited literature.

technique makes the interpolation in each interval using a third-order polynomial, which reduces the errors occurring in other interpolation rules. The program is reasonably simple. The results show that there is a good correlation between the data obtained by the interpolation with those from the QM calculations (CNDO/2 method) (Fig. 6).

The results of Table 1 show that the experimental and theoretical data of the IRB are qualitatively comparable. On one hand, this series of molecules exhibits the tendency of having their barrier decreased by the electron-withdrawing groups bonded to the methyl group. The obtained results also show that the experimental values of IRB are higher than those calculated by the CNDO/2 and INDO methods from the experimental geometries. They are also lower than those calculated by the *ab-initio* method, whose values are dependent on the choice of the basis functions. When the experimental bond distances are optimized the calculated results are closer to the experimental values (16). The direct use of the experimental geometry results in lower barriers.

In summary the CNDO/2—INDO program described here provides easy access to a semi-empirical method. With this program one is able to study molecular properties at the theoretical level even in cases where only a mini-computer is available. A program listing is available from Project SERAPHIM. Send a check for $2.00 made out to Project SERAPHIM, acct. 20350, to John W. Moore, Department of Chemistry, Eastern Michigan University, Ypsilanti, MI 48197.

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.

Constructing Nonlinear Scatchard Plots

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The interactions of macromolecules and smaller ligands are often treated in advanced undergraduate biochemistry courses or in qualifying graduate courses. The well-studied Scatchard binding isotherm (*29*) is usually included, with cases of both linear and nonlinear plots. Often examples are given to illus-

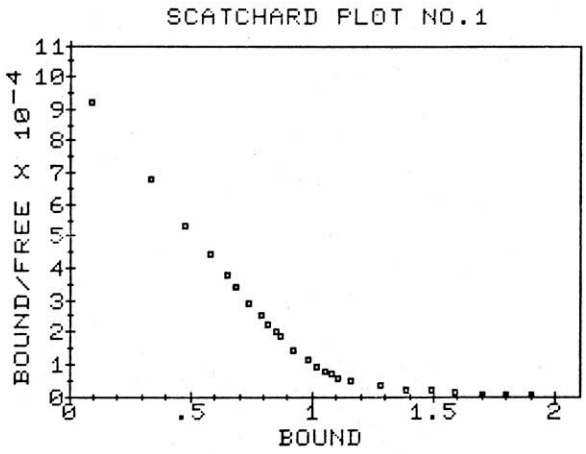

Figure 7. $k_1 = 1 \times 10^5$, $n_1 = 1$, $k_2 = 1 \times 10^3$, $n_2 = 1$. This plot examines a high and low affinity site for a single ligand. This case is likened to the association of a protein to a drug which allows for increased binding due to higher drug concentration.

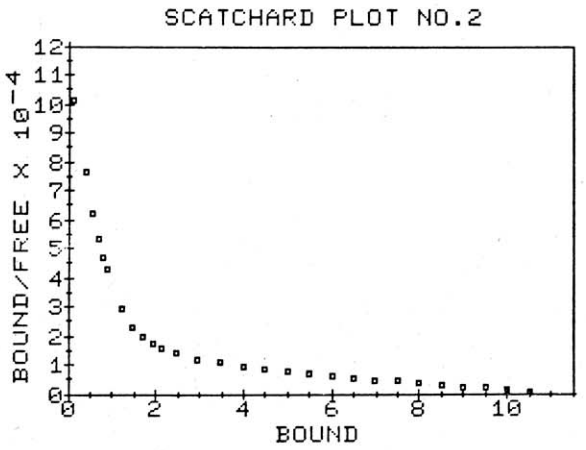

Figure 8. $k_1 = 1 \times 10^5$, $n_1 = 1$, $k_2 = 1 \times 10^3$, $n_2 = 10$. Scatchard plot of a single high affinity site with a number of nonspecific sites at higher ligand concentration. This might be illustrative of a protein-steroid or protein-lipid interaction.

trate the effects of variation in k, the association constant, and n, the number of binding sites, on the Scatchard line shape.

In this discussion it is usually pointed out that a linear Scatchard plot results when binding involves a single class of equivalent and independent sites. Such a plot has a slope of $-k$, an intercept on the bound axis of n and an intercept on the bound/free axis of nk. In contrast, a nonlinear Scatchard plot is produced when two or more non-equivalent binding sites exist. In this type of plot, the intercept on the bound axis becomes the sum of the n values, $(n_1 + n_2 + \ldots n_n)$ and the intercept on the bound/free axis is now the sum of the nk products $(n_1k_1 + n_2k_2 + \ldots n_nk_n)$.

The simplest example of a nonlinear Scatchard plot involves the case of two classes of independent sites. To produce this type of plot precisely, a small program, SCATSUM in Applesoft Basic, is offered below. SCATSUM uses the curve peeling method of Rosenthal (*30*) as a model to construct the resultant Scatchard curve from two straight Scatchard lines. This task is achieved by vector addition of points on each straight line. Rosenthal offers a full explanation of this procedure which she uses graphically to deconvolute a curved Scatchard plot into its straight line components.

In SCATSUM the binding line with the larger k association value is identified as line 1. Line 2 has the lower k value. The

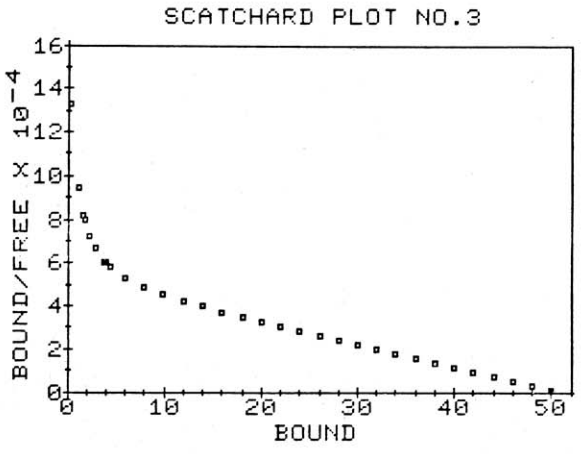

Figure 9. $k_1 = 1 \times 10^5$, $n_1 = 1$, $k_2 = 1 \times 10^3$, $n_2 = 50$. This is an extreme case of a single site with massive nonspecific sites at high ligand concentration.

program requires the input of k_1,n_1,k_2,n_2 values, then asks for values on the bound axis of line 2. These values are used in the program to produce the appropriate points on line 1 and then on the final curved line. SCATSUM output includes the input k and n values as well as three columns of numbers. Column one lists the generated values on the bound axis of line two as a reference. Columns two and three list bound and bound/free values on the composite curve. Plotting the values in columns three versus two gives the resultant nonlinear Scatchard plot, for class demonstration. The line shapes of three plots of different k and n values are given in Figures 7–9. The plots were produced on the Warme program Scientific Plotter (*31*). When invariant k values are given, the program will produce a linear plot as expected.

Because this program produces precise values along the curved Scatchard plot, the student can use the generated data to determine the k and n values. This exercise on the student's part will be an informative homework problem. SCATSUM is available as Apple Disk #16 from Project SERAPHIM. Send a check for $4 made out to Project SERAPHIM, acct. 20350, to John W. Moore, Department of Chemistry, Eastern Michigan University, Ypsilanti, MI 48197.

Computer Simulation of Mass Spectral Envelopes of Polyisotopic Elements

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In work dealing with organotin compounds, we found it necessary to examine isotopic abundance ratios in order to identify the compounds responsible for spectral envelopes. We sought to compare peak envelopes with profiles predicted from the isotopic abundances of the constituents of the cluster ions produced in the spectrometer (*32*). While the calculation of such abundances is straightforward (*33–36*), the presence of tin with 10 stable isotopes along with other polyisotopic elements in our reactions made the calculation of the expected peak profiles lengthy and tedious, and, therefore, we sought computer software for the purpose. Despite indications that such software exists (*37*) none that was compatible with hardware available to us was located.

Therefore, we undertook to write a program that would calculate the mass numbers and relative abundances expected for a cluster ion given the number of atoms, the mass numbers, and the abundances of the isotopes of each atom. The mathematical model described by Hugentobler and Löliger (*36*) was chosen as the basis for the program because of the general approach employed and the flexibility it permits. The abun-