LEAST SQUARES FIT OF DATA TO HYPERBOLIC DOSE-RESPONSE CURVES USING A PROGRAMMED MINICALCULATOR (TI-59)

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Equations of the Michaelis-Menten form are frequently encountered in a number of areas of biochemical and pharmacological research. A program is presented for use on the programmable TI-59 calculator with added printer which performs an iterative least-squares fit of up to 80 data pairs to this equation and estimates the standard deviations and standard errors of the determined parameters. The program assigns equal weights to errors over the entire data range and is thus appropriate for situations in which data precision is independent of amplitude.

Introduction

Equations of the form

$$y = Ax/(x + K) \tag{1}$$

are frequently encountered in biochemical and pharmacological research: in the former, as the Michaelis-Menten equation, it describes first-order enzyme kinetics; in the latter, it expresses a simple dose-response relationship in the absence of cooperativity or inhibition. Because of the frequent need to fit experimental data to equations of this form, and in view of the limited availability and high cost of minicomputers capable of performing such a fit, the present program, which is capable of being run on a programmable calculator with attached printer (TI-59 and PC-100C, Texas Instruments, Lubbock, TX), was developed.

Methods

Because of the limited storage capacity of the TI-59, a number of methodological simplifications had to be made in order to shorten the program and thereby permit maximum utilization of the calculator's available memory

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for data. The version presented here, which utilizes a separate data input program to compact (x, y) data pairs into a single memory register, permits the analysis of up to 80 data points and determines the values of A and K that minimize the sum of the squares of the deviations from the calculated y_i values:

$$\sum [y_i - Ax_i/(x_i + K)]^2 \tag{2}$$

It also computes the standard errors and standard deviations of A and K.

For any value of K, the value of A that will minimize expression [2] is found by setting equal to 0 the derivative of that expression with respect to A:

$$d[2]/dA = 0 = -2 \sum [x_i y_i/(x_i + K)] + 2A \sum [x_i/(x_i + K)]^2 \tag{3}$$

This yields, by rearrangement,

$$A = \sum [x_i y_i/(x_i + K)]/\sum [x_i/(x_i + K)]^2 \tag{4}$$

Unfortunately, there is no corresponding analytical expression for the optimal value of K.

The program determines the optimal values for A and K by an iterative method. For a starting value of K, the program calculates A from equation (4) and the summed squared deviation from expression (2) using the calculated A. It then changes K, repeats the calculations, and compares the new error value with that produced with the previously used value of K. If the new error is smaller, it again steps K and repeats the process; if the new error is larger, it steps K in the opposite direction in steps half the size of the previous steps. Thus, by a process of bisection, successive repetitions of the calculations converge on the desired value of K and its associated value of A.

There is no built-in termination of the process of iteration: the program continues to produce closer and closer approximations to the correct values of K and A, printing the result of each trial on the PC-100C printer. A procedure is provided whereby the user, on deciding that the estimated values are sufficiently close to the end point, can manually terminate this phase of computation and start the calculation of standard deviations and standard errors.

The calculation is relatively slow, with a set of 10 data points requiring about 1 min per iteration and a set of 80 data points requiring nearly 10 min per iteration. Nevertheless, since calculators such as the TI-59 are often single-user instruments, this should not be a serious drawback.

```
000  LBL    010  R/S    020  R/S    030  =      040  D
      E            LBL          LBL        ST*       STO
     CMs           A            B          09        05
      9            +            OP         R/S       RCL
     STO           3            28         LBL       08
      09          INV           OP         C         STO
     INV           LOG          29         STO       07
     LOG           =            +          04   047  R/S
     STO           STO          RCL        R/S
009   02    019    00    029    00    039  LBL
```

Fig. 1. Data Input program. This compacts the (x, y) data pairs into single registers.

Data input

Data are entered into the memory of the calculator using the Data Input program listed in Fig. 1. After initializing by pressing [E], which clears the data memory, sets the data pointer in register 09, and places an arbitrary large number (10^9) in register 02 to serve as the "old" error term, the user then enters (x_i, y_i) data points with the sequence: x_i [A], y_i, [B]. If several y_i values have the same x_i, they can be entered without repeatedly entering the x_i; e.g., x_1, [A], y_1, [B], y_2, [B], y_3, [B]. After the last data value is entered, a starting approximation of K is entered, followed by [C], and the size of the steps by which K is to be changed is entered, followed by [D].

In order to conserve storage space in the calculator, the data are stored in the format YYYY . XXX; it is therefore necessary that: (a) values of y_i be integers; (b) values of x_i be less than 1000; and (c) all data be non-negative. Changes of scale may be needed to insure this.

At the end of the input phase, register 02 (R_{02}) contains the value 10^9, R_{04} contains K, R_{05} contains ΔK, R_{07} and R_{08} contain N (the number of data points), R_{09} contains the value $N + 9$, and registers 10 through $N + 9$ hold the data.

Calculations

The values of A and K that provide the best least-squares fit to the data are then calculated by the program listed in Fig. 2. Steps 000–012 and 027–034 are subroutines that retrieve x and y values, respectively, from the compact data storage format used; register 09 is used as a pointer to select the appropriate data. Steps 013–026 are a subroutine that calculates the value of $x/(x + K)$, an expression that arises frequently. The segment 035–043 clears registers that are to be used for summations. The main part of the program, steps 044–137, calculates A from equation [4], calculates the summed squared error (expression [2]), and prints, in order, A, K, and the error term [2]; it then steps K and repeats the procedure.

000	LBL	050	x^2	100	RCL	150	SBR	200	=
	x		SUM		00		CLR		√x
	(		00		PRT		LBL		PRT
	RC*		RCL		RCL		SUM		÷
	09		06		04		RCL		RCL
	INV		x		PRT		07		03
	INT		SBR		RCL		÷		√x
	x		x^2		03		SBR		=
	3		=		PRT		x		PRT
	INV		SUM		x⇌t		+		RCL
010	LOG	060	01	110	RCL	160	1	210	02
	)		OP		03		=		÷
	RTN		39		EXC		x		(
	LBL		DSZ		02		SBR		RCL
	1/x		08		GE.		x^2		03
	(		LNx		EE		-		-
	SBR		RCL		2		RCL		1
	x		01		+/-		00		)
	÷		÷		INV		=		=
	(		RCL		PRD		x⇌t		√x
020	CE	070	00	120	05	170	RCL	220	PRT
	+		=		LBL		00		÷
	RCL		STO		EE		÷		RCL
	04		00		RCL		SBR		03
	)		RCL		05		x^2		√x
	)		07		SUM		-		=
	RTN		SUM		04		1		PRT
	LBL		09		RCL		=		R/S
	x^2		STO		07		x		
	(		08		SUM		SBR		
030	RC*	080	LBL	130	09	180	x		
	09		CLR		STO		-		
	INT		SBR		08		RCL		
	)		1/x		INV		07		
	RTN		x		Iff		=		
	LBL		RCL		1		Σ+		
	C		00		C		OP		
	CLR		-		R/S		39		
	STO		SBR		LBL		DSZ		
	00		x^2		D		08		
040	STO	090	=	140	RCL	190	SUM		
	01		x^2		04		RCL		
	STO		SUM		-		05		
	03		03		RCL		÷		
	LBL		OP		05		(		
	LNx		39		=		RCL		
	SBR		DSZ		STO		03		
	1/x		08		07		-		
	STO		CLR		PGM		1		
049	06	099	ADV	149	01	199	)		

Fig. 2. Least-squares data-fitting program for equations of the form $y = Ax/(x + K)$.

To terminate the iterative process when the estimates of A and K are sufficiently close to the end point, the user presses the following keystroke sequence at any time:

[R/S] [2nd] [Stflg] [1] [R/S]

This sequence sets flag 1 which is checked at steps 133–135 of each iteration; with the flag set, the program halts in step 137 at the end of the loop in progress at the time the flag was set. At this time, the user can initiate additional single iterative loops by pressing [C] for each desired step.

Pressing [D] initiates the error analysis calculation, steps 138–227, and, in sequence, SD(A), SEM(A), SD(K), and SEM(K) are calculated and printed.

The standard deviation (SD) of A is estimated by rearranging equation [1] to yield an expression for A_i, the A determined by data point (x_i, y_i) and the determined value of K

$$A_i = y_i[1 + (K/x_i)] \tag{5}$$

and then evaluating the standard deviation as

$$\mathrm{SD}(A) = \left[\sum (A_i - A)^2/(N-1)\right]^{1/2} \tag{6}$$

and the standard error of the mean as

$$\mathrm{SEM}(A) = \mathrm{SD}(A)/N^{1/2} \tag{7}$$

Similarly, SD(K) is determined from the values of K_i given by the following rearrangement of [1]:

$$K_i = x_i[(A/y_i) - 1] \tag{8}$$

$$\mathrm{SD}(K) = \left[\sum (K_i - K)^2/(N-1)\right]^{1/2} \tag{9}$$

$$\mathrm{SEM}(K) = \mathrm{SD}(K)/N^{1/2} \tag{10}$$

Procedure for use

The following is the sequence of operations to be carried out by the user of the programs listed:

1. Repartition the memory of the calculator by entering [9] [2nd] [op] [17].
2. Enter the Data Input program (Fig. 1) from the keyboard or magnetic card.
3. Initialize with [E].
4. Enter data with the sequence x_i, [A], y_i, [B].
5. Enter initial values of K (followed by [C]) and ΔK (followed by [D]).*
6. Enter the Main Program (Fig. 2) from the keyboard or magnetic card.
7. Initiate the calculation with [C].
8. When the iterative process has converged sufficiently close to the desired values of A and K, key in [R/S] [2nd] [stflg] [1] [R/S]. The calculations will terminate at the end of the iteration then in progress.
9. Additional single iterative loops may be initiated at this time by pressing [C].
10. Press [D] to initiate the error analysis.

Illustration of use

Potassium-depolarized rat ileal smooth muscle undergoes a transient relaxation when the osmolality of its bathing medium is increased by addition of sucrose (Schiff *et al.*, 1980). The raw data are shown in Table 1. The program

TABLE 1

PERCENT RELAXATION OF DEPOLARIZED RAT ILEAL SMOOTH MUSCLE IN RESPONSE TO AN INCREASE IN OSMOLALITY BY ADDITION OF SUCROSE TO THE BATHING MEDIUM (RAW DATA)

Means ± S.E.M.s of these data (n = 48) have been published previously (Schiff *et al.*, 1980).

Increase in osmolality (mOsmol/kg)					
19.3	58.0		116.0	174.0	290.0
5	21	18	24	26	25
7	24	19	39	41	45
8	14	15	48	20	29
13	19	16	16	22	35
7	15	13	26	37	34
8	17	19	17	32	20
	16	14	27		35
	23	18	23		28
	17	28	30		
	24				

* At this point, the data may be recorded on magnetic cards.

yielded $A = 40.16 \pm 1.56$ (SEM) % and $K = 65.8 \pm 8.4$ (SEM) mOsmol/kg. With 48 data points, the time per iteration and the time for the calculation of standard errors were each 5–6 min. The number of iterations depends, of course, on the accuracy of the starting estimate of K.

For comparison, a double-inverse plot (Lineweaver *et al.*, 1934) of the same data yielded values of A and K of 44.7% and 95.5 mOsmol/kg, respectively, which are quite poor fits to the data. Indeed, it is apparent that the Lineweaver-Burk plot is even a poor choice as a source of the seed value of K with which to initiate the iterative calculation.

Discussion

The programs illustrated in Figs. 1 and 2 provide the parameters with which to fit data, by least-squares analysis, to a curve of the form of equation [1]. It assigns equal weights to deviations of measured y_i values from the computed curve at every x_i and is thus appropriate for situations in which the precision of data measurement is uniform over a range of data amplitudes.

The technique used to compact data pairs into single registers makes it possble for the calculator to analyze up to 80 data pairs. This is in contrast to, for example, the pharmacokinetics program of Nielsen-Kudsk (1981) which can analyze 18 data pairs and is comparable to more recent programs for minicomputers such as ROSFIT (Greco *et al.*, 1982) which can manage 75 data pairs at a time (although ROSFIT can store several such sets of 75 data points on magnetic tape).

The goal of maximizing data storage capacity and the space thereby needed for subroutines for retrieval of the compacted data made it necessary to simplify the actual calculation program as much as possible. Therefore, the bisection approach was used instead of Cleland's (1967) differential equation and matrix technique of optimizing the convergence of successive approximations. As a consequence of this choice, a large number of iteration loops are often necessary to attain a sufficiently close estimate of the parameters A and K. However, since the TI-59 is frequently a one-user calculator which can be left running for an hour or two if necessary, it was felt that the slow convergence of the algorithm would not be a significant disadvantage. Furthermore, a reasonably accurate estimate of the initial value of K can greatly reduce the number of iterations required and thus the total running time.

Because of the relatively widespread use of minicalculators such as the TI-59 and in view of the frequent need of biochemists, pharmacologists, and others to fit data to curves of the Michaelis-Menten form, the programs presented here should find wide application.

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