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A COMPARISON OF METHODS FOR ESTIMATING THE KINETIC PARAMETERS OF TWO SIMPLE TYPES OF TRANSPORT PROCESS

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Sets of experimental data, with known characteristics and error structures, have been simulated for the Michaelis-Menten equation plus a second term, either for linear transport or for competitive inhibition. The Michaelis-Menten equation plus linear term was fitted by several methods and the accuracy and the precision of the parameter estimates from the several methods were compared. The model-fitting methods were: three for least-squares non-linear regression, computer versions of two graphical methods and of two non-parametric methods. The most precise and accurate method was that of D.W. Marquardt (J. Soc. Ind. Appl. Math. 11 (1963) 431–441). The Michaelis-Menten equation with competitive inhibition was also fitted by several methods, viz., two for least-squared non-linear regression, a non-parametric method and four variants of the Preston-Schaeffer-Curran plot (Preston, R.L. et al. (1974) J. Gen. Physiol. 64, 443–467). The most precise and accurate of these was the non-linear regression method of W.W. Cleland (Adv. Enzymol. 29 (1967) 1–32). For both these models, the various graphical methods and non-parametric methods gave poor results and are not recommended.

Introduction

Kinetic analysis is now well established as a means for studying membrane transport processes. Of the several types of transport process known to be present in membranes, two are of particular interest in this study. The first of these consists of two components: one saturable and having the characteristics described by a Michaelis-Menten equation, the other non-saturable and apparently linear with respect to substrate concentration (see Ref. 1). The second type consists of a single saturable component, but the transport of one substrate is inhibited competitively by the presence of a second [2]. Analysis of such transport processes therefore requires the fitting of a Michaelis-Menten equation plus either a linear term or a term for competitive inhibition to the data in order to calculate the three parameters defining the transport process.

Graphical methods have been extensively used for fitting both these models to experimental data. The most common method for the first model is still that of Neame and Richards [3], although Matthews et al. have recommended an alternative method based on the Preston-Schaeffer-Curran plot [2,4]. More recently, the use of least-squares non-linear regression has been advocated [5,6]. The second model is usually fitted by a graphical method [7], although non-linear regression methods have been available for many years [8,9]. The advantages of non-linear regression include the elimination of statistical bias, which is inevitably introduced on transformation of data into a linear form, and the elimination of subjectivity inherent in drawing straight lines by eye. Over the past few

years there has also developed an interest in 'robust' methods (i.e., methods insensitive to wrong weighting or the presence of outliers) and, of these, non-parametric ones have been used most frequently [10]. However, this approach has not, until now, been used for fitting the Michaelis-Menten equation plus either a linear term or a term for competitive inhibition.

It is well known from other studies (cf. Refs. 11 and 12) on fitting various models to biological data that many of the available methods can give biased and inaccurate parameter estimates. This paper describes a similar comparison in which the two models described above were fitted to artificially generated data of known characteristics and error structure. For the first model the methods used were the graphical methods of Neame and Richards [3] and of Matthews [4], three different least-squares methods and two non-parametric methods. For the second model the methods were two different least-squares methods, a non-parametric method and four variants of the Preston-Schaeffer-Curran plot [2].

The results of this study are in general agreement with work done on various other biological equations, namely that, assuming one of these models is appropriate for a particular type of experiment on a transport process, least-squares non-linear regression will give the most accurate and precise values for the parameters, whereas graphical methods using linear regression will give poor values.

Methods

Michaelis-Menten plus linear term

Simulated data. Two different sets, A and B, of error-free data for (s, v) were used. They were both generated from the equation

$$v = \frac{V \cdot s}{(K_m + s)} + k \cdot s \qquad (1)$$

with $V = K_m = 1.0$ and $k = 0.2$. Both series had eight datum points. For series A, $s = 0.2$, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 and 4.0 and for series B, $s = 0.25$, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0. Previous experience with fitting this equation indicates that, in order to estimate all three parameters accurately and precisely, a well-designed experiment should produce data in which s and v show an approximately linear relationship over a large portion of the range of s. The results of this study support this view. Fig. 1 shows that series A was curved over its entire length and therefore represents a poorly designed experiment, whereas series B was approximately linear for three-quarters of its length and would represent a well-designed experiment.

'Experimental' data (i.e., data containing error) were simulated as previously described, using series of pseudo-random normally distributed numbers of known mean and S.D. [11]. The only minor change was that the subroutines GO5CCF and GO5DDF from the NAG Library were used to generate the pseudo-random numbers. Two types of error were present and they were chosen to represent the two extremes of the sort of error likely to be found in practice:

(i) N5: normally distributed relative error (i.e., error which is proportional to the dependent variable) with a coefficient of variation of 5%.

(ii) O5: as for N5, except that 10% of the points (chosen at random) were outliers with a coefficient of variation of 10%.

Thus, four series of data sets were generated and each contained 1000 sets of data.

Curve-fitting methods. Three least-squares methods were used: the methods of Davidon [13] and Fletcher and Powell [14], as described by Atkins [15]; Marquardt's method [16]; and the Taylor expansion method described by Cleland [9]. Since, in the field of transport kinetics, the error structure of the data [17] is often unknown, the common practice of weighting the data equally was followed (but see Ref. 18). Three computer programs were written for two commonly used graphical procedures. The first was the curve-peeling method described by Neame and Richards [3] for which I used Wilkinson's method for fitting the Michaelis-Menten equation [19]. The second method was that adapted by Matthews [4] from the one described by Preston et al. [2]. In addition, a program for an interative version of the Neame and Richards method was written and used. Two versions of a non-parametric method (i.e., a method that does not assume any type of distribution for the error) were also used. They are based on solving sets of linear algebraic equations as follows.

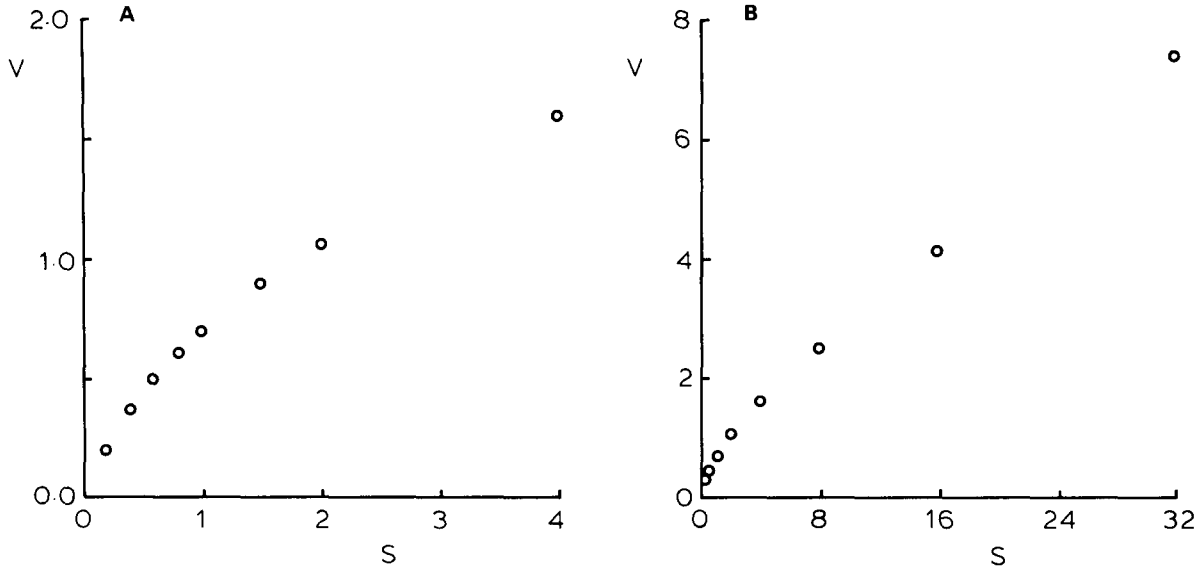

Fig. 1. Perfect values for the data sets used to test the fitting of a Michaelis-Menten equation plus linear term. B represents a well-designed experiment with many points on a linear portion, whereas in A the points lie on a curve and represent a poor experiment.

Eqn. 1 can be written as

$$V \cdot s - K_m \cdot v + k \cdot s^2 + K_m \cdot k \cdot s = v \cdot s$$

or

$$\theta \cdot s - K_m \cdot v + k \cdot s^2 = v \cdot s \qquad (2)$$

where

$$\theta = V + K_m \cdot k$$

If three datum points (s_1, v_1; s_2, v_2; s_3, v_3) are inserted into Eqn. 2 in turn, the set of three linear algebraic equations so formed can be solved for θ, K_m and k. From these parameters, V can also be calculated: $V = (\theta - K_m \cdot k)$. By taking all the possible combinations of three datum points out of eight, 56 estimates of V, K_m and k are obtained. This is an extension of the non-parametric method described by Eisenthal and Cornish-Bowden [20] for fitting the Michaelis-Menten equation. In the first version of the technique, the individual values of V, K_m and k were each ranked in ascending order and the median of each then found. For the second version, a 20% trimmed mean of the individual estimates was calculated. To do this, the lowest 20% and the highest 20% of the ranked estimates were discarded and a mean was calculated from the remainder.

Each method was used with the 1000 data sets within each series. The mean with its S.E. and the median with its 95% confidence limits were calculated for V, K_m and k.

Michaelis-Menten equation with competitive inhibition

Simulated data. Three sets, C, D and E, of error-free data for (s, i, v) were generated using the equation

$$v = V \cdot s / (K'_m + s)$$

where

$$K'_m = K_m \cdot (1 + i/K_i) \qquad (3)$$

For the data in series C, $V = K_m = 1.0$ and $K_i = 5.0$; $s = 0.6$, 1.4, 2.2, 3.0 and 3.8; and $i = 0.0$, 2.0, 2.5, 3.33, 5.0 and 10.0.

For series D, $V = K_m = K_i = 1.0$; $s = 1.0$, 3.0, 5.0, 7.0 and 9.0; and $i = 0.0$, 10.0, 12.5, 16.67, 25.0 and 50.0.

For series E, $V = K_m = 1.0$ and $K_i = 0.2$; $s = 0.25$,

0.5, 1.0, 2.0 and 4.0; and $i = 0.0$, 0.11, 0.14, 0.2, 0.33 and 1.0.

A wide range of parameter sets is possible and, since it is impracticable to study a large number, three representative sets were chosen. All had $V = K_m$, but the ratios for K_i/K_m were 5.0, 1.0 and 0.2. Series C and E correspond to good experimental designs for estimating the parameters by least-squares methods, whereas series D corresponds to a good experimental design for analysis by the Preston-Schaeffer-Curran method. This gave 30 datum points for each data set. 500 data sets were generated for each series.

Curve fitting. Two least-squares methods were used, those of Marquardt [16] and Cleland [9] mentioned above. Eqn. 3 was fitted to all 30 datum points at once, rather than by replot methods (for example, Ref. 7). A non-parametric method was also developed as follows. Eqn. 3 can be rearranged to give:

$$s \cdot v \cdot K_i + v \cdot K_m \cdot K_i + v \cdot i \cdot K_m - s \cdot V \cdot K_i = 0$$

and then to

$$V \cdot s - K_m \cdot v - \eta \cdot v \cdot i - s \cdot v = 0 \qquad (4)$$

where $\eta = K_m/K_i$. Thus if three datum points are inserted into Eqn. 4 in turn, a set of three linear algebraic equations is obtained. These can be solved for V, K_m and η; and from these $K_i = K_m/\eta$. Again, all the possible combinations of three datum points out of 30 were used to obtain a large number of estimates of V, K_m and K_i; the median was used as the final answer. In this particular example of a non-parametric method, many of the solutions are singular and were therefore not attempted (e.g., when $s_i = s_j$, etc.).

The graphical method of Preston et al. [2] requires two linear plots. The first is for the equation

$$v_0/(v_\psi - v_i) - 1.0 = (K_i(K_m + s)/K_m) \cdot (1/i) \qquad (5)$$

where v_0 is the initial velocity without inhibitor and v_i is the initial velocity in the presence of inhibitor at concentration i. From a plot with $v_0/(v_0 - v_i)$ as the dependent variable and $(1/i)$ as the independent variable, the slope (ξ) gives an estimate of $K_i(K_m + s)/K_m$, whence

$$\xi = K_i + (K_i/K_m) \cdot s$$

A secondary plot of ξ vs. s allows K_i and K_m (but not V) to be calculated. Four variants of this method were programmed for the computer. In each of them, both straight lines were fitted by linear regression. In two variants the primary plot was constrained to pass through the origin as in Eqn. 5 and in the other two a non-zero intercept was allowed. Weighting factors for both linear regressions were calculated (see Cleland [9]) and they are of the form $(v_0 - v_i)^4/(v_0 \cdot v_i)^2$ for the primary plot (constant relative error) and $1.0/(\mathrm{SE}(\xi))^2$ for the secondary plot. Two variants used the correct weighting as described above and the other two used equal weighting for both plots. Thus, four variants of the basic method were used.

Each method was used with the 500 data sets within each series, except that for the non-parametric program, where the amount of computer time needed is relatively much longer, only 100 data sets were used. The mean and its S.E., and the median and its 95% confidence limits were calculated for V (where calculated), K_m and K_i.

Results

Tables I and II present the results of this study in concise form. They show the mean values of the estimated parameters (V, K_m, k and K_i as appropriate) together with their standard errors and their median values. In the calculation of these values, estimates which fell outside certain limits were discarded because the method was deemed to have failed. The ranges were: for V and K_m, 0.25–4.0; for k, 0.05–0.8; and for K_i, 1.25–20.0, 0.25–4.0, or 0.05–0.8. The acceptability of the results were assessed by three criteria: the precision (as measured by the S.E.), the accuracy (as measured by the lack of bias) and the number of failures. Bias is assessed by how close the median is to the known 'true' value.

Michaelis-Menten equation plus linear term

Table I shows the results when this equation was fitted to simulated experimental data by several methods. Comparison of the results from the three least-squares methods shows that the method of Davidon, Fletcher and Powell gave the most precise estimates (i.e., the smallest S.E.s), whereas the methods of Marquardt and Cleland

TABLE I

VALUES OF V, K_m AND k CALCULATED FROM FOUR TYPES OF SIMULATED EXPERIMENTAL DATA REPRESENTING MEMBRANE TRANSPORT BY A SATURABLE AND A NON-SATURABLE PROCESS USING EIGHT DIFFERENT PARAMETER ESTIMATION METHODS

The values given for each parameter are the mean, its S.E., the number of estimates within the acceptable range and the median. See text for further details.

Parameter	Data set				Total number of failures
	AN5	AO5	BN5	BO5	
(i) Least-squares: Davidon, Fletcher and Powell					
V	0.984 ± 0.005(1000), 1.029 [a]	0.990 ± 0.005(1000), 1.033 [a]	1.019 ± 0.007(1000), 1.006	1.003 ± 0.008(1000), 0.989	0
K_m	0.936 ± 0.005(1000), 0.978 [a]	0.942 ± 0.005 (998), 0.984 [a]	1.031 ± 0.014 (997), 0.961 [a]	0.996 ± 0.014 (991), 0.948 [a]	14
k	0.199 ± 0.001(1000), 0.192 [a]	0.199 ± 0.001 (994), 0.192 [a]	0.199 ± 0.001(1000), 0.199	0.200 ± 0.001(1000), 0.200	6
(ii) Least-squares: Marquardt					
V	1.104 ± 0.015 (994), 0.976	1.171 ± 0.017 (983), 1.059 [a]	1.083 ± 0.011 (995), 1.008	1.103 ± 0.015 (990), 0.993	38
K_m	1.085 ± 0.016 (992), 0.972	1.156 ± 0.018 (982), 1.056 [a]	1.167 ± 0.020 (980), 0.999	1.157 ± 0.021 (965), 0.973	81
k	0.203 ± 0.002 (925), 0.207 [a]	0.196 ± 0.002 (895), 0.199	0.197 ± 0.001 (998), 0.199	0.197 ± 0.001 (997), 0.199	185
(iii) Least-squares: Cleland					
V	1.130 ± 0.017 (982), 0.976	1.212 ± 0.020 (953), 1.060 [a]	0.954 ± 0.008 (964), 0.971 [a]	1.080 ± 0.013 (975), 0.989	126
K_m	1.107 ± 0.018 (980), 0.972	1.021 ± 0.021 (958), 1.057 [a]	0.954 ± 0.009 (958), 0.969 [a]	1.147 ± 0.021 (960), 0.965	144
k	0.202 ± 0.002 (912), 0.207 [a]	0.195 ± 0.002 (862), 0.199	0.206 ± 0.001 (968), 0.209	0.198 ± 0.001 (977), 0.200	281
(iv) Graphical: Neame and Richards					
V	0.799 ± 0.007(1000), 0.763 [a]	0.835 ± 0.009 (998), 0.781 [a]	1.007 ± 0.006(1000), 0.989	0.998 ± 0.006(1000), 0.974 [a]	2
K_m	0.855 ± 0.011(1000), 0.789 [a]	0.905 ± 0.013 (994), 0.812 [a]	1.030 ± 0.010(1000), 0.981	1.015 ± 0.011 (999), 0.961 [a]	7
k	0.277 ± 0.001(1000), 0.278 [a]	0.274 ± 0.001(1000), 0.275 [a]	0.203 ± 0.001(1000), 0.202 [a]	0.203 ± 0.001(1000), 0.203 [a]	0
(v) Graphical: Neame and Richards, iterative					
V	0.801 ± 0.007 (565), 0.794 [a]	0.806 ± 0.007 (532), 0.807 [a]	1.031 ± 0.006(1000), 1.010	1.022 ± 0.007(1000), 0.996	903
K_m	0.746 ± 0.008 (565), 0.755 [a]	0.749 ± 0.008 (531), 0.758 [a]	1.061 ± 0.011(1000), 1.006	1.047 ± 0.012 (999), 0.084	905
k	0.232 ± 0.002 (565), 0.235 [a]	0.229 ± 0.002 (532), 0.230 [a]	0.199 ± 0.001(1000), 0.199	0.200 ± 0.001(1000), 0.200	903
(vi) Graphical: Matthews					
V	1.102 ± 0.043 (399), 0.762 [a]	1.217 ± 0.049 (386), 0.863 [a]	1.284 ± 0.025 (832), 1.046 [a]	1.245 ± 0.024 (776), 1.040	1607
K_m	1.173 ± 0.051 (296), 0.926	1.323 ± 0.060 (281), 0.961	1.206 ± 0.033 (678), 0.951	1.181 ± 0.033 (628), 0.920	2117
k	0.296 ± 0.006 (439), 0.305 [a]	0.294 ± 0.006 (381), 0.300 [a]	0.192 ± 0.001 (888), 0.197 [a]	0.192 ± 0.001 (857), 0.196 [a]	1435
(vii) Non-parametric					
V	0.808 ± 0.009 (998), 0.755 [a]	0.791 ± 0.010 (992), 0.741	1.010 ± 0.005(1000), 0.997	1.006 ± 0.005(1000), 0.995	10
K_m	0.621 ± 0.009 (855), 0.570 [a]	0.617 ± 0.010 (766), 0.568 [a]	0.993 ± 0.007(1000), 0.989	0.978 ± 0.007 (999), 0.971 [a]	380
k	0.286 ± 0.002 (999), 0.284 [a]	0.304 ± 0.003 (995), 0.297 [a]	0.200 ± 0.000(1000), 0.200	0.201 ± 0.001(1000), 0.201	6
(viii) Non-parametric, 20% trimmed mean					
V	0.975 ± 0.012 (998), 0.896 [a]	0.970 ± 0.013 (998), 0.890 [a]	1.038 ± 0.005(1000), 1.026 [a]	1.034 ± 0.005(1000), 1.022 [a]	4
K_m	0.635 ± 0.009 (853), 0.595 [a]	0.634 ± 0.010 (767), 0.595 [a]	1.021 ± 0.007(1000), 1.016	1.007 ± 0.007 (999), 0.994	381
k	0.310 ± 0.002 (998), 0.310 [a]	0.328 ± 0.003 (997), 0.327 [a]	0.199 ± 0.000(1000), 0.200	0.200 ± 0.000(1000), 0.200	5

[a] $P <$ about 0.05 that the median differs from its theoretical value.

TABLE II

VALUES FOR V, K_m AND K_i CALCULATED FROM THREE TYPES OF SIMULATED EXPERIMENTAL DATA REPRESENTING THE COMPETITIVE INHIBITION OF AN ENZYME-CATALYSED REACTION USING SEVEN DIFFERENT PARAMETER ESTIMATION METHODS

The values given for each parameter are the mean, its S.E., the number of estimates within the acceptable range and the median. See text for further details.

Parameter	Data set			Total number of failures
	CN2	DN2	EN2	
(i) Least-squares: Marquardt				
V	1.000 ± 0.001(500), 0.999	0.992 ± 0.007(500), 0.995 [a]	0.999 ± 0.001(500), 0.998	0
K_m	1.001 ± 0.002(500), 1.001	1.205 ± 0.027(471), 1.017 [a]	0.999 ± 0.002(500), 0.997	29
K_i	5.014 ± 0.011(500), 5.002	1.138 ± 0.028(423), 1.006	0.200 ± 0.000(500), 0.200	77
(ii) Least-squares: Cleland				
V	1.000 ± 0.001(500), 0.999	1.001 ± 0.001(500), 0.999	0.999 ± 0.001(500), 0.998	0
K_m	1.001 ± 0.002(500), 1.001	1.003 ± 0.003(500), 0.996	0.999 ± 0.002(500), 0.997	0
K_i	5.014 ± 0.011(500), 5.002	1.002 ± 0.002(500), 1.000	0.200 ± 0.000(500), 0.200	0
(iii) Non-parametric				
V	1.235 ± 0.003(100), 1.231 [a]	1.600 ± 0.009(100), 1.591 [a]	1.150 ± 0.002(100), 1.150 [a]	0
K_m	0.997 ± 0.005(100), 0.995	1.057 ± 0.009(100), 1.060 [a]	1.003 ± 0.003(100), 1.005	0
K_i	4.976 ± 0.029(100), 4.962	1.040 ± 0.007(100), 1.041 [a]	0.201 ± 0.001(100), 0.201	0
(iv) PSC unweighted				
K_m	1.251 ± 0.036(419), 1.120 [a]	1.000 ± 0.009(500), 0.994	1.033 ± 0.020(476), 0.994	105
K_i	5.485 ± 0.101(430), 5.526 [a]	0.994 ± 0.007(500), 0.995	0.194 ± 0.002(487), 0.197	83
(v) PSC weighted				
K_m	1.720 ± 0.056(303), 1.607 [a]	1.112 ± 0.023(464), 1.055	1.093 ± 0.022(486), 1.035	247
K_i	7.481 ± 0.182(364), 7.316 [a]	1.056 ± 0.019(468), 1.039	0.200 ± 0.003(493), 0.203	175
(vi) PSC (unconstrained) unweighted				
K_m	1.479 ± 0.050(336), 1.266 [a]	1.002 ± 0.013(500), 0.990	1.173 ± 0.031(446), 1.048 [a]	218
K_i	6.363 ± 0.149(387), 6.170 [a]	0.988 ± 0.010(500), 0.986	0.198 ± 0.003(466), 0.205	147
(vii) PSC (unconstrained) weighted				
K_m	1.619 ± 0.088(141), 1.327 [a]	1.250 ± 0.032(426), 1.151 [a]	1.447 ± 0.045(403), 1.289 [a]	530
K_i	10.16 ± 0.29 (302), 10.41 [a]	1.156 ± 0.025(431), 1.130 [a]	0.235 ± 0.005(439), 0.235 [a]	328

[a] $P <$ about 0.05 that the median differs from its theoretical value.

both gave less precise values and resulted in more failures. On the other hand, Marquardt's and Cleland's methods gave less biased (i.e., more accurate) answers than the Davidon, Fletcher and Powell method. All three methods appear not to have been sensitive to outliers.

The three graphical methods all gave strikingly poor results with the data of type series A. In the majority of cases for this type, the medians were significantly different from the theoretical value. This was not unexpected, because there were insufficient data at high values of s to define sufficiently well a linear portion to the curve. With the data from series B, the method of Neame and Richards gave precise and accurate values, except that the presence of outliers caused the answers to be biased. When the method was used in an iterative manner, the precision was unchanged but now unbiased estimates were obtained when outliers were present. Matthews' method gave poor results with all the series of data. The precision was poor (i.e., the standard errors were large), there was an unacceptably large number of failures, and biased values were often obtained even when no outliers were present.

The non-parametric methods also gave poor results with data of series A. With data of series B, the unmodified version was precise, gave only one failure and indeed gave the best results of any method with this series of data. The modified version was less satisfactory because some parameter estimates were biased. Neither version was sensitive to outliers.

Michaelis-Menten equation with competitive inhibition

Table II shows the results when this equation was fitted by several methods. The results from the two least-squares methods show that with two of the series of data, both methods gave accurate and precise estimates. However, with series D, although Cleland's method gave very good results, that of Marquardt gave less precise (and some biased) values. With data from series C and E, the non-parametric method gave good results for K_m and K_i but poor results for V. However, for series D all the results were poor.

The results obtained by using the various methods based on that of Preston et al. [2] are also given in Table II. None of the methods gave good results with data of series C. With data of series D (and to a lesser extent series E) the published method worked well. However, the results from methods which tried to improve upon the original method gave either less precise values or less accurate values, or both. Why the use of correct weighting should yield results which are worse is not known.

Discussion

At present, most experiments on transport kinetics are not designed in such a manner that standard deviations can be calculated at each data point. Therefore any curve fitting procedures that are used, in general, assume equal weighting of the data. Experience from other fields suggests that the error structure of most experimental data is complex [21]. Although in the area of transport kinetics it is known that error in the data increases in some fashion with the rate of absorption, no quantitative analysis has been done to determine an algebraic relationship. During the simulation of 'experimental' data for use in this study I have caused the error to be relative, i.e., the magnitude of the error was proportional to the absorption rate. Normally during any curve-fitting procedure, the weighting given to the data should be related to the type of error present in the data. The weighting I have used, where appropriate, was not correct for the simulated data, but I believe this approach is justified because it conforms to current practice.

Michaelis-Menten equation plus linear term

Data of series A represented a poorly designed experiment in that there were insufficient data at high values of s to give a linear section in a plot of v vs. s. It is apparent that none of the methods for fitting Eqn. 1 gave accurate and precise estimates for the parameters V and K_m. The two best methods (equally effective) were those using least squares, and it might be that if the data had been correctly weighted (as they theoretically ought to have been) they would have given better results. Data of series B represented a well-designed experiment and, as expected, all the methods performed better than with series A. Marquardt's

method was the best of the least squares methods because it not only produced more accurate and precise values, but also was resistant to outliers and produced fewer failures. The method of Neame and Richards [3] was marginally better, but only if used iteratively, i.e., the graphical version itself was inadequate.

It is difficult to give guidance as to the best method to use in future work where a wide range of data types will be encountered. In this survey, the least-squares method of Marquardt was the most successful of those investigated. It might perform better than predicted here if experiments are designed to allow the curve fitting to be correctly weighted – for example, if it were possible to obtain sufficient replicates at each datum point. On the other hand, the various graphical methods and non-parametric methods gave poor results. The least successful was that of Matthews. Although his principle of 'self-inhibition' may be a useful approach to the kinetic study of transport processes, his graphical method is unsuitable because the graphical plot of Preston et al. [2] is a poor method for analysis of the data.

Michaelis-Menten equation with competitive inhibition

The results in Table II show that the method of Preston et al. [2] did not give good parameter estimates with any of the types of data. Although it gave accurate and precise estimates of K_m and K_i, the method only did so when the 'experiment' was designed with this method of analysis in mind, i.e., with all $s > K_m$ and all $i > K_i$. It is also apparent that modifications to this graphical method did not lead to improved performance. Hence, it is unlikely that the method of Matthews can be altered to give more precise and accurate estimates for V and K_m over a wide range of experimental designs. A puzzling feature is that weighting the Preston-Schaeffer-Curran plot gave worse, not better, results. The reason may be that in the derivation of weighting factors an approximate method [19] is used and perhaps the assumptions used in this approximation are not valid. It is also obvious from Table II that, as for the competitive inhibition of an enzyme-catalysed reaction, the most suitable method for estimating V, K_m and K_i is to fit Eqn. 3 by the non-linear regression method of Cleland using all the data at once.

The results in both tables demonstrate that when an experiment was well designed with a particular method of data analysis in mind, i.e., the substrate concentrations were spread over a suitable range, the parameters were more precisely and more accurately estimated than for less well-designed experiments. It must be emphasized that good statistical analysis will not compensate for poorly designed expeirments or poor quality data. Although good kinetic analysis is essential, it must be stressed that there can be limitations to its use [6]. For example, two or more quite different models often appear to fit well to the same data and it is impossible to distinguish between them even with very precise data [22]. Also, kinetic analysis is a very indirect method and transport is a complex multi-stage process, so that considerable caution is needed in attributing physical interpretations to kinetic parameters. In particular, K_m and K_i must not automatically be regarded as inverse affinity constants for carrier-substrate binding.

Conclusion

This survey shows that non-linear regression using a least-squares method is of most general value when the Michaelis-Menten equation plus a linear term is to be fitted to experimental data for transport kinetics. The three methods tested gave slightly different results and a decision as to which would be the better one to use depends on the type of data set. Overall, Marquardt's method was the best. It is probable that if the linear section of the v vs. s curve could be reasonably well defined and if the S.E. of the data points could be determined (so that weighting factors could be used in the non-linear regression), then all three least-squares methods would give better, and possibly similar, results.

This survey shows that the non-linear regression method of Cleland, correctly weighted if possible, should be used when fitting the Michaelis-Menten equation plus a term for competitive inhibition. This is consistent with advice given in the field of enzyme kinetics.

The results of using the non-parametric method, the method of Matthews and the Preston-

Schaeffer-Curran plot for these two models were disappointing. Neither precise nor accurate estimates were consistently obtained for the three parameters of each model.

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References

1 Debnam, E.S. and Levin, R.J. (1975) J. Physiol. 246, 181–196
2 Preston, R.L., Schaeffer, J.F. and Curran, P.F. (1974) J. Gen. Physiol. 64, 443–467
3 Neame, K.D. and Richards, T.G. (1972) Elementary Kinetics of Membrane Carrier Transport, pp. 53–55, Blackwell, Oxford
4 Matthews, D.M., Gandy, R.H., Taylor, E. and Burston, D. (1979) Clin. Sci. 56, 15–23
5 Atkins, G.L. and Gardner, M.L.G. (1977) Biochim. Biophys. Acta 468, 127–145
6 Gardner, M.L.G. and Atkins, G.L. (1982) Clin. Sci. 63, 405–414
7 Cornish-Bowden, A. (1976) Principles of Enzyme Kinetics, pp. 57–61, Butterworths, London
8 Cleland, W.W. (1963) Nature 198, 463–465
9 Cleland, W.W. (1967) Adv. Enzymol. 29, 1–32
10 Atkins, G.L. (1982) Comput. Biol. Med. 12, 201–215
11 Atkins, G.L. and Nimmo, I.A. (1975) Biochem. J. 149, 775–777
12 Nimmo, I.A., Atkins, G.L., Strange, R.C. and Percy-Robb, I.W. (1977) Biochem. J. 165, 107–110
13 Davidon, W.C. (1959) Variable Metric Method for Minimisation, ANL-5990 (Rev), Argonne National Laboratory, Argonne, IL
14 Fletcher, R. and Powell, M.J.D. (1963) Comput. J. 6, 163–168
15 Atkins, G.L. (1971) Biochim. Biophys. Acta 252, 405–420
16 Marquardt, D.W. (1963) J. Soc. Ind. Appl. Math. 11, 431–441
17 Nimmo, I.A. and Atkins, G.L. (1979) Trends Biochem. Sci. 4, 236–239
18 Paterson, J.Y.F., Sepulveda, F.V. and Smith, M.W. (1979) J. Physiol. 292, 339–350
19 Wilkinson, G.N. (1961) Biochem. J. 80, 324–332
20 Eisenthal, R. and Cornish-Bowden, A. (1974) Biochem. J. 139, 715–720
21 Atkins, G.L. and Nimmo, I.A. (1980) Anal. Biochem. 104, 1–9
22 Atkins, G.L. and Gardner, M.L.G. (1980) Clin. Sci. 59, 301–303