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Estimation of the Reliability of Parameters Obtained by Non-linear Regression

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Four methods for estimating the reliability of parameters obtained by non-linear regression are compared. Matrix inversion demands the least computational effort, but can be unreliable for over-determined models. The 'jack-knife' technique was found to give results of the right magnitude, but some unexplained discrepancies suggest that this method should be used with caution. A Monte Carlo method and the method of support planes were found to be in good agreement with matrix inversion, but both involve a substantial computational investment. The method of support planes is preferred as it gives information on the degree of non-linearity of the equation which is fitted to the data.

In a recent article Cornish-Bowden and Wong [1] have applied the statistical tool known as the 'jack-knife' to the analysis of a set of enzyme-kinetic data. The motivation for using this procedure was that the conventional matrix-inversion method [2] for obtaining standard errors was unreliable when a complex rate equation was fitted to their data. Subsequently Duggleby [3] criticised this work and suggested that the failure of the matrix-inversion method may have resulted from an arithmetic problem which could be overcome by using a different computer program, a conjecture which has now been confirmed (R. G. Duggleby, unpublished results).

It should not be imagined that the arithmetic problems involved in inverting large matrices can be dismissed lightly. Complex rate equations almost invariably lead to matrices which are difficult to invert with any accuracy, when using computational methods in which all numbers are rounded (or worse, truncated) to a finite number of digits. The application of the 'jack-knife' to a problem in enzyme kinetics is welcome as it provides an alternative when the conventional method fails. The purpose of the present paper is to describe, in simple terms, two other methods for obtaining standard errors: the Monte Carlo method and the support plane method. Each of these methods has been described by Chandler et al. [4] but are not well known to biochemists.

It is shown that these two methods give results which agree with those obtained by matrix inversion, and that the 'jack-knife' also gives similar results. The data employed for this study are the well-known results of Roughton et al. [5] on the binding of oxygen by haemoglobin.

THEORY

It is axiomatic that experimental data contain uncertainties. For each datum the measured value of the dependent variable (y) differs from the 'true' value by an unknown amount. Regression analysis involves fitting an equation or model to a set of data by manipulating the values of the parameters of the equation in such a way as to minimize the weighted sum of the squares of the residuals. The residual is the difference between the experimental value of y and that predicted by the equaton, while the weight given to each residual in the summation is inversely related to the estimated uncertainty in y. Since each measurement contributes to some extent to the determination of the value of a particular parameter (θ_j), it must also contribute some uncertainty to the determination of θ_j. It is this uncertainty that error analysis attempts to quantify.

Any particular set of data is a sample of an infinite number of potential data sets which could have been obtained. Each of these hypothetical data sets would yield a slightly different set of values for the parameters and the standard error of a parameter value is a measure of this variability. Naturally the variability could be assessed by repeating the experiment several times but the effort involved make this method unattractive. Moreover, it fails to make full use of the information contained in a single set of data. If we are prepared to accept that the data set actually obtained

is representative of all possible sets, then we may simulate other data sets with similar statistical properties. This is the basis of the Monte Carlo method. Each simulated data set will yield parameter values which differ from those obtained from the experimental data by an amount ϕ. The standard error for a particular parameter is then calculated as the root mean square of the ϕ values, i.e. $(\Sigma\phi^2/M)^{1/2}$ where M is the number of Monte Carlo simulations. If required, the covariance of a pair of parameters θ_j and θ_k may be calculated as $\Sigma\phi_j\phi_k/M$.

Regression analysis involves locating the lowest point on a sum-of-squares surface plotted in parameter space (e.g. see Fig. 1 of Hoare [6]). If this surface is relatively flat in a particular direction which is parallel to one of the parameter axes, then there is substantial uncertainty in the value of this parameter; a range of values give an equally good fit. Thus, a reasonable definition of the standard error of a parameter might be the distance from the minimum to the limits of a contour in parameter space which connects all points where the sum of squares equals some particular value. For equations which are linear in the parameters, it can be shown [7] that the contour which corresponds to the normal definition of a standard error connects the points where the sum of squares is greater than that at the global minimum (S_0) by the factor $1 + 1/\nu$ where ν is the number of degrees of freedom. We can use this same definition for non-linear models: if a parameter is increased or decreased by one standard error and the sum of squares minimized with respect to the remaining parameters, then this new minimum value will equal $S_0(1 + 1/\nu)$. A procedure which locates the values which satisfy this criterion will be a method for calculating standard errors. This is the method of support planes.

METHODS

The data were taken from Table 2A of Roughton et al. [5] and consist of ten measurements of the percentage saturation (y) of haemoglobin solutions at specified oxygen pressures (p). The data were weighted as suggested by these authors and were analyzed according to Eqn (1), in which

$$y = \frac{25\,(a_1p + 2a_2p^2 + 3a_3p^3 + 4a_4p^4)}{1 + a_1p + a_2p^2 + a_3p^3 + a_4p^4} \qquad (1)$$

$a_1 - a_4$ are parameters to be estimated. This equation was fitted to the data using a program which is based on Marquardt's algorithm and which was obtained as part of QCPE307 from the Quantum Chemistry Program Exchange, Indiana University. Standard errors are calculated by a matrix-inversion method and experience has shown this program to be fairly accurate. The partial derivatives of Eqn (1) were obtained numerically, using a first-order approximation [4].

Support-plane calculations were performed using an adaptation of the CURVFIT program, which is part of QCPE307, using STEPIT as the optimizing algorithm. Consider the parameter a_1: the best-fit value was perturbed by a small amount (d_i) and Eqn (1) was fitted to the data, maintaining a_1 at this new value. The sum of squares (S_i) was calculated and a new perturbation (d_{i+1}) calculated from a parabolic interpolation or extrapolation: $d_{i+1} = d_i/[\nu(S_i/S_0 - 1)]^{1/2}$, where S_0 is the minimum sum of squares from the best fit, and ν is the number of associated degrees of freedom. This process was repeated using the new perturbation and continued until $S_i \approx S_0(1 + 1/\nu)$ when the standard error is equal to the current perturbation. A similar series of calculations was performed for each of the parameters. Monte Carlo calculations were performed as follows. Simulated data were generated by calculating the theoretical values of y from the best fit to the experimental data and adding to these a normally distributed random number. These random numbers were drawn from a population with a mean of zero and a standard deviation equal to the standard deviation of each data point as indicated by Roughton et al. [5], multiplied by the residual standard error obtained from the fit to the actual data (0.91735). These simulated data were fitted using the CURVFIT program and after 20 such simulations the standard errors of the parameters were calculated as described in the Theory section.

The 'jack-knife' calculations were performed by fitting Eqn (1) to ten subsets of the data formed by omitting each of the original points in turn. The 'jack-knife' was applied as described by Cornish-Bowden and Wong [1], both with and without the logarithmic transformation that they employ.

RESULTS

The best fit of Eqn (1) to the data was obtained with the values $a_1 = 9.61 \times 10^{-2}$, $a_2 = 9.82 \times 10^{-3}$, $a_3 = 3.39 \times 10^{-3}$ and $a_4 = 3.11 \times 10^{-3}$, in good agreement with those reported by Roughton et al. [5] (9.61×10^{-2}, 10.0×10^{-3}, 3.35×10^{-3} and 3.10×10^{-3}). No difficulties were encountered in matrix inversion and the values obtained for standard errors agree with those reported by Roughton to within a few percent (Table 1). The 'jack-knife' gave comparable standard errors for a_1 and a_4 but the errors for a_2 and a_3 are larger by 37% and 65% respectively. When the logarithmic transformation recommended by Cornish-Bowden and Wong [1] was omitted, the error for a_2 is reduced to a value near to that obtained by matrix inversion but the error for a_3 is barely affected.

The Monte Carlo method (20 simulations) gave errors which were between 3% and 11% too high, but

Table 1. *Estimation of standard errors by four different methods*
Eqn (1) was fitted to the data of Roughton et al. [5] and errors determined by four methods described in Theory

Method	$10^4 \times$ error in parameter			
	a_1	a_2	a_3	a_4
Matrix inversion				
Present work	47.5	24.6	2.35	0.51
Roughton et al.	49.1	24.6	2.30	0.51
'Jack-knife'				
log transformed	45.5	33.8	3.87	0.49
Untransformed	45.7	27.1	3.77	0.49
Monte Carlo	51.4	25.4	2.60	0.56
Support planes				
Positive	47.8	23.8	2.39	0.51
Negative	47.8	24.8	2.30	0.51

Table 2. *Convergence path for the positive support plane of each parameter*
Calculations were performed as described in the Methods section. For the first iteration the perturbation (d_i) in the parameter was equal to one-tenth of the parameter value: subsequent perturbations were calculated by parabolic interpolation or extrapolation. Iteration was continued until the sum of squares (S_i) was equal to $S_0\,(1 + x/v)$, where S_0 is the minimum sum of squares (= 5.049), v is the number of associated degrees of freedom (= 6) and x is between 0.95 and 1.05. That is to say, iteration was terminated when a value of S_i between 5.848 and 5.933 was found

Parameter	Iteration	$10^4 \times a$	$10^4 \times d_i$	S_i
a_1	0	961	0	5.049
	1	1057	96.11	8.448
	2	1009	47.82	5.886
a_2	0	98.2	0	5.049
	1	108.0	9.82	5.193
	2	121.9	23.77	5.857
a_3	0	33.87	0	5.049
	1	37.26	3.387	6.743
	2	36.26	2.388	5.883
a_4	0	31.12	0	5.049
	1	34.23	3.112	33.779
	2	31.65	0.533	5.956
	3	31.63	0.514	5.893

it is to be anticipated that statistical fluctuations in the simulated data will lead to some variation from the expected values. Thus, groups of five simulations gave an error of a_1 of 4.68×10^{-3}, 5.61×10^{-3}, 5.97×10^{-3} and 4.09×10^{-3}, which were pooled to give the value of 5.14×10^{-3} reported in Table 1. Support planes were almost exactly symmetrical about the minimum and gave values very close to those obtained by matrix inversion. This suggests that the model is fairly linear around the minimum and this is confirmed by efficiency of the parabolic search method, which was used to locate the support planes. The convergence paths are shown in Table 2.

DISCUSSION

The data of Roughton et al. [5] were chosen for this study for three reasons: (a) the raw data are reported in tabulated form and can be obtained without any subjective estimation from a graphical representation; (b) the data have been analyzed quantitatively, both by Roughton and by others [8,9]; (c) the data obey a fairly complex model [Eqn (1)], which is known to be highly redundant [9] and, therefore, difficult to analyze.

The matrix-inversion method for obtaining standard errors gave satisfactory results with these data, and it might be argued that they are not a very stringent test of the value of the alternative methods presented here. On the other hand, it is necessary that there be some basis for comparison and the errors obtained by matrix inversion are most likely to be acceptable for this purpose. Roughton et al. [5] reported standard errors which are in good agreement with those reported here.

The 'jack-knife' gave reliable results for the errors of a_1 and a_4, whereas the errors in a_2 and a_3 were overestimated by about one-half. Elimination of the logarithmic transformation led to a more reliable result for a_2 but the error in a_3 was still overestimated. These results certainly confirm that the 'jack-knife' can be useful, but the discrepancies noted above give some cause for concern. In this particular example the difference between the 'jack-knife' and matrix inversion is not large, but it is unexplained. Much larger discrepancies may occur in other situations and for this reason the 'jack-knife' is less attractive than the other methods described.

The Monte Carlo method gave results which differed slightly from those obtained by matrix inversion, but this is an entirely natural consequence of the use of random numbers. There is no reason to believe that the method is biased, but it must be admitted that it is rather time-consuming. Fewer simulations would reduce the computational cost, but with a corresponding reduction in the reliability of the results. Roughton et al. [5] have considered a different formulation of Eqn (1), in which a_1 is replaced with K_1, a_2 with K_1K_2, a_3 with $K_1K_2K_3$ and a_4 with $K_1K_2K_3K_4$, where $K_1 - K_4$ represent association constants. We may calculate (for example) K_4 as a_4/a_3 and obtain its standard error from the covariance matrix using the formula given by Cleland [2], but when the matrix inversion fails then this method for obtaining the standard error of a combination of parameters is not available. Cornish-Bowden and Wong [1] have pointed out that the 'jack-knife' may be applied to the individual determinations of a_4/a_3 to obtain a value for the standard error. Equally we may use the results from the Monte Carlo method by calculating the root mean square of the deviation (ϕ) for a_4/a_3. Applica-

tion of this method gave $K_4 = 0.919 \pm 0.069$, which agrees well with Roughton's value of 0.926 ± 0.066.

The method of support planes is unusual in that it does not involve any assumptions regarding the approximate linearity of the model around the minimum and will reveal the extent of any non-linearity in two ways. First the support planes are not symmetrical about the minimum unless the model is exactly linear; thus, the asymmetry can be used as a guide to the extent of non-linearity. Second, non-linearity can reveal itself as a difference between matrix-inversion errors and those obtained using support planes. (That is to say, a cross-section through the sum-of-squares surface need not be parabolic, even if it is symmetrical about the minimum. Haarhoff [10] has described a related method of assessing non-linearity.) Table 1 does not reveal any sign of non-linearity by either criterion, which agrees with the parabolic convergence path, which was observed in support plane calculations (Table 2). When the method of support planes gives different results from matrix inversion, the former values are a more realistic guide to the precision of parameter estimates.

The cheapest method for determining standard errors is the matrix-inversion method; all other methods involve multiple refits of the data, subsets of it or of simulated sets. If computer time is readily available then the other methods described here can be useful. On the basis of the results presented in this paper, the support plane method is preferred as it gives the most complete information and is independent of the amount of non-linearity in the model.

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