Comparison of several non-linear-regression methods for fitting the Michaelis–Menten equation

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The known jackknife methods (i.e. standard jackknife, weighted jackknife, linear jackknife and weighted linear jackknife) for the determination of the parameters (as well as of their confidence regions) were tested and compared with the simple Marquardt's technique (comprising the calculation of confidence intervals from the variance–co-variance matrix). The simulated data corresponding to the Michaelis–Menten equation with defined structure and magnitude of error of the dependent variable were used for fitting. There were no essential differences between the results of both point and interval parameter estimations by the tested methods. Marquardt's procedure yielded slightly better results than the jackknives for five scattered data points (the use of this method is advisable for routine analyses). The classical jackknife was slightly superior to the other methods for 20 data points (this method can be recommended for very precise calculations if great numbers of data are available). The weighting does not seem to be necessary in this type of equation because the parameter estimates obtained with all methods with the use of constant weights were comparable with those calculated with the weights corresponding exactly to the real error structure whereas the relative weighting led to rather worse results.

INTRODUCTION

One of the most important steps in the analysis of a series of steady-state enzymological experiments is the fitting of a proposed function to the obtained data (i.e. concentrations of substrates, coenzymes, effectors etc. and the corresponding initial reaction rates). Assuming that the proposed function is suitable for the description of the data, the estimation of the parameters of this function is the main goal of the analysis. The simplest method for obtaining the parameters is the graphical plotting (or linear regression) of the transformed data. However, this method is applicable only in the case of simple linearizable functions, and it does not yield sufficiently correct results in some cases (Wilkinson, 1961; Colquhoun, 1969). The non-linear-regression methods for fitting the kinetic equations are more versatile and usually give more reliable parameter estimates (together with their confidence intervals) (Atkins & Nimmo, 1975). There are numerous non-linear-regression methods; none of them can, however, be regarded as the best in all instances known from enzyme kinetics (Lam, 1981). Each method has specific requirements as regards the experimental values themselves and especially as regards the type of their error (Draper & Smith, 1981). In practice, it is usually unknown (*a priori* as well as *a posteriori*) which requirements are fulfilled best, and therefore the choice of the method (and the type of weights) is often rather fortuitous. There is an experimental way of choosing the optimal method for a set of data of a specific kind, and this is based on testing their behaviour towards the simulated data with defined type and magnitude of error.

The aim of the present paper is to test the most frequently used methods for estimation of parameters of non-linear functions by using the simulated data with defined errors that have the structure corresponding to that of the errors of the enzymological data obtained by

usual techniques of the initial-rate measurements (Storer *et al.*, 1975; Askelöf *et al.*, 1976; Nimmo & Mabood, 1979). We chose the Michaelis–Menten equation as the model function, since it is the simplest non-linear equation occurring in enzyme kinetics. Even in this case the optimal method is unknown (Atkins & Nimmo, 1980), though it seems that the direct linear plot (Eisenthal & Cornish-Bowden, 1974) fulfils most requirements (Atkins & Nimmo, 1975). However, this method, belonging to the class of distribution-free methods, is not applicable to more complicated dependences occurring in enzyme kinetics. In choosing the regression methods for our testing we preferred the methods of universal application to non-linear dependences of various types. The basic method for parameter estimation is represented by Marquardt's (1963) modification of the Gauss–Newton gradient method, and the more complicated (and perhaps more robust) methods are all described jackknife methods (Mosteller & Tukey, 1977; Hinkley, 1977; Duncan, 1978; Fox *et al.*, 1980). The chosen methods were tested especially with regard to their sensitivity to the number of data points and to the structure and magnitude of the errors of the data.

METHODS AND THEORY

Model function

The Michaelis–Menten equation in the non-linear representation is:

$$v_i = \frac{V \cdot [S]_i}{K_m + [S]_i} + e_i, \quad i = 1, 2, \dots, n \quad (1)$$

where $[S]_i$ (substrate concentrations) and v_i (initial reaction rates) are the data obtained from a steady-state kinetic experiment, V (maximal reaction rate) and K_m (Michaelis constant) are the parameters whose values (and standard errors) are to be estimated, the e values are

the random error components and n denotes the sample size (i.e. the number of $[S]_i, v_i$ pairs).

Marquardt's method

The estimation of the best values of parameters ($P_1 \equiv V, P_2 \equiv K_m$) in the least-squares sense can be formulated as finding the minimum of the weighted sum of squares (SS) of the residuals:

$$SS = \sum_{i=1}^n w_i \left(v_i - \frac{P_1 \cdot [S]_i}{P_2 + [S]_i} \right)^2 \quad (2)$$

where w_i are the weights. The estimation of parameters in the $(k+1)$ th iterative step of Marquardt's procedure can be formulated as:

$$p^{(k+1)} = p^{(k)} - \alpha^{(k)} (J^T W J + \mu^{(k)} I)^{-1} \cdot J^T W R \quad (3)$$

where

$$J_{i1} = \frac{\partial}{\partial P_1} \frac{P_1^{(k)} \cdot [S]_i}{P_2^{(k)} + [S]_i}, \quad J_{i2} = \frac{\partial}{\partial P_2} \frac{P_1^{(k)} \cdot [S]_i}{P_2^{(k)} + [S]_i},$$

$$R_i = v_i - \frac{P_1^{(k)} \cdot [S]_i}{P_2^{(k)} + [S]_i}, \quad i = 1, 2, \dots, n$$

and $W_{ij} = w_i^2$ (for $i = j$) and $W_{ij} = 0$ (for $i \neq j$), $i, j = 1, 2, \dots, n$. $\alpha^{(k)}$ and μ are the parameters of Marquardt's algorithm.

The standard errors of the parameters of the fitted function can be calculated from the corresponding variance-co-variance matrix (Draper & Smith, 1981). The standard errors (S.E.) are then given by:

$$S.E.(P_j) = \sqrt{c_{jj}}, \quad j = 1, 2 \quad (4)$$

where C represents the variance-co-variance matrix of parameters, which, after approximation and substitution, is given by:

$$C = (SS/\nu J^T W J)^{-1} \quad (5)$$

and ν is the number of degrees of freedom ($\nu = n - 2$ in our case).

Jackknife methods

The original jackknife technique was introduced by Quenouille (1956) and Tukey (1958) and belongs to the robust methods of parameter estimation. If large numbers of experimental data are available, the method eliminates bias in the parameter values. However, its behaviour is rather ambiguous in the case of small series of data (Duncan, 1978). Hinkley (1977) proposed a modification of this method, introducing the design factors (so-called weights, also); another modification is the linear jackknife (Fox *et al.*, 1980). These variants of the jackknife method have found only few applications in biochemistry (e.g. Oppenheimer *et al.*, 1981).

The standard jackknife requires $(n+1)$ non-linear estimates of parameters (i.e. of $P_1 \equiv V$ and $P_2 \equiv K_m$ in our case) by some least-squares method (e.g. by the above-mentioned Marquardt's method) (Cornish-Bowden & Wong, 1978); n estimates ($\hat{P}_{1,-i}, \hat{P}_{2,-i}$) are obtained from $(n-1)$ data points with the i th point deleted, the last estimate ($\hat{P}_{1,0}, \hat{P}_{2,0}$) is obtained from all data points. The method uses pseudo-values of parameters defined as:

$$\hat{P}_{j,i} = n \cdot \hat{P}_{j,0} - (n-1) \hat{P}_{j,-i}, \quad j = 1, 2, \quad i = 1, 2, \dots, n \quad (6)$$

The final jackknife estimate of parameters is given by:

$$\tilde{P}_{j,0} = \frac{1}{n} \cdot \sum_{i=1}^n \hat{P}_{j,i} \quad (7)$$

the standard errors of the parameters being:

$$S.E.(\tilde{P}_{j,0}) = \frac{1}{n(n-1)} \cdot \sum_{i=1}^n (\hat{P}_{j,i} - \tilde{P}_{j,0})^2 \quad (8)$$

Instead of the elimination of one experimental point, the whole group of data can be eliminated (Cornish-Bowden & Wong, 1978). It was shown by Duncan (1978), however, that the standard jackknife loses most of its robustness in this case.

The linear jackknife requires only one non-linear fit of the proposed function to the data (Fox *et al.*, 1980). The parameter estimates ($\hat{P}_{1,-i}, \hat{P}_{2,-i}$) in the standard jackknife procedure are calculated iteratively to minimize the modified sum of squares:

$$\sum_{j=1}^n w_j \left(v_j - \frac{P_1 \cdot [S]_i}{P_2 + [S]_i} \right)^2, \quad j = 1, 2, \dots, n \quad (9)$$

If this non-linear expression is substituted by the corresponding Taylor expansion, the following formula can be obtained (after some re-arrangements and neglecting the higher derivatives) for the pseudo-values of parameters:

$$\hat{P}_{j,i} = \hat{P}_{j,0} + n(Z^T Z)^{-1} \cdot z_i \left(\frac{v_i - \frac{\hat{P}_{1,0} \cdot [S]_i}{\hat{P}_{2,0} + [S]_i}}{1 - w_i} \right), \quad i = 1, 2, \dots, n \quad (10)$$

where

$$z_i = \left(\frac{\partial}{\partial P_1} \frac{\hat{P}_{1,0} \cdot [S]_i}{\hat{P}_{2,0} + [S]_i}, \frac{\partial}{\partial P_2} \frac{\hat{P}_{1,0} \cdot [S]_i}{\hat{P}_{2,0} + [S]_i} \right)^T$$

$$Z^T = (z_1, z_2, \dots, z_n), \quad w_i = z_i^T (Z^T Z)^{-1} z_i, \quad i = 1, 2, \dots, n \quad (11)$$

The parameter estimates defined by eqn. (10) are then treated in the same way as was the case of the standard jackknife (eqns. 7 and 8).

The weights, introduced into the jackknife procedure by Hinkley (1977), represent the distances of single design points from the centre of the design. In this way, the experimental design is involved into the calculation. The aim of such a weighting is to eliminate the influence of the extreme values of the independent variable ($[S]$) on the parameter estimates. These weights, defined by eqn. (11), make it possible to introduce two other jackknife methods, namely the weighted jackknife and the weighted linear jackknife. In the case of weighted jackknife eqn. (6) is modified to eqn. (6'):

$$\hat{P}_{j,i} = [n(1 - w_i) + 1] \hat{P}_{j,0} - n(1 - w_i) \hat{P}_{j,-i}, \quad i = 1, 2, \dots, n \quad (6')$$

and for weighted linear jackknife eqn. (10) turns into eqn. (10'):

$$\hat{P}_{j,i} = \hat{P}_{j,0} + n(Z^T Z)^{-1} \cdot z_i \left(v_i - \frac{\hat{P}_{1,0} \cdot [S]_i}{\hat{P}_{2,0} + [S]_i} \right), \quad i = 1, 2, \dots, n \quad (10')$$

The parameter estimates obtained by weighted linear jackknife are identical with the simple $\hat{P}_{j,0}$ values calculated by Marquardt's method provided that the final estimate obtained by Marquardt's method is the stationary point of the sum of the squares of residuals (eqn. 2) (Fox *et al.*, 1980). Therefore we used weighted linear jackknife for variance estimation only. In using the jackknife procedures one is to be aware of their dependence on the underlying non-linear-regression technique used; the results obtained by the tested jackknife variants are influenced by the behaviour of Marquardt's procedure.

'Experimental' data

The set of 'perfect' (i.e. error-free) values was obtained from eqn. (1) by setting $K_m = 10$, $V = 1$ and $e_i = 0$ for all i ; the 'substrate' concentrations ($[S]$) were distributed equidistantly in the interval from $0.1 K_m$ to $10 K_m$ depending on the number of 'experimental' values (5, 10, 15 and 20).

Several empirical functions were used to describe the relationship between the variance of error (e) in eqn. (1) and the magnitude of the dependent variable (Storer *et al.*, 1975; Askelöf *et al.*, 1976; Nimmo & Mabood, 1979). We chose eqn. (12) because it is one of the most satisfactory functions (Askelöf *et al.*, 1976) and it has a simple physical interpretation (Cornish-Bowden & Endrenyi, 1981; Little *et al.*, 1982):

$$\text{var}(e) = \sigma_v^2 = \sigma_0^2 + \sigma_2^2 v_{\text{per}} \quad (12)$$

where σ_0^2 can be considered as the constant part of the total error whereas σ_2^2 determines the relative part of the total error and v_{per} is the value of the dependent variable (initial rate) from the 'perfect' set. 'Experimental' values were obtained from the series of the 'perfect' values as:

$$v_{\text{exp}} = N(v_{\text{per}}, \sigma_v^2) \quad (13)$$

where $N(\mu, \sigma^2)$ represents a pseudo-random number chosen from normal distribution with the mean of μ and the standard deviation equal to σ^2 .

Weighting

For the correct calculation of the parameter value by Marquardt's method the variability of the dependent variable (i.e. of initial reaction rates in our case) has to be taken into account. This variability is introduced into the calculation in the form of weights. If the variance of each 'experimental' value σ_i^2 is known then the weight can be defined as (Draper & Smith, 1981):

$$w_i = \frac{1}{\sigma_i} \quad (14)$$

The variance σ_i^2 is unknown in usual enzymological practice, since the experiments are carried out without repetition or they are repeated only two or three times, which is not sufficient for the determination of σ_i^2 . It is therefore necessary to accept some assumptions about the value and structure of this variance in most real experiments. On the basis of such assumptions the weights are assessed. The most frequent assumptions are as follows.

(a) The variance σ_i^2 does not depend on the value of the dependent variable (this corresponds to $\sigma_2^2 = 0$ in eqn. 12); then:

$$w_i = 1, \quad i = 1, 2, \dots, n \quad (15)$$

(b) The variance σ_i^2 is directly proportional to the square of the dependent variable and the variance of the data point with zero value of the dependent variable is zero (this corresponds to $\sigma_0^2 = 0$ in eqn. 13); then:

$$w_i = \frac{1}{|v_i|}, \quad i = 1, 2, \dots, n \quad (16)$$

As the error structure and value were known exactly in our simulated data, it was possible to test the above-mentioned regression methods by using both the correct weights (eqn. 14) and the simple weights defined by eqns. (15) and (16).

Processing of the data

Each series of simulations was formed by 1000 independent 'experiments' with the same error structure. The individual estimates of K_m and V obtained by each of the tested methods were collected and the mean value ($\pm$ variance) of these sets were calculated. As these values should belong to a skew distribution (Colquhoun, 1969), we also calculated the 10% trimmed means (Eadie *et al.*, 1971) and sample medians. The tested methods were compared mutually (by the method of one-way classification, by Tukey's method of multiple comparison on the 95% confidence level and by the distribution-free Kruskal-Wallis's tests) and with the nominal (correct) parameter values (by the two-sided one-sample tests on the 95% confidence level) (Anděl, 1978). We also computed the skewness and kurtosis parameters for each set of the obtained data and calculated the probability that skewness and kurtosis belong to a normal (Gaussian) distribution (Anděl, 1978).

The real confidence levels corresponding to the nominal 95% level were computed in the case of methods for interval estimation. The confidence intervals for each parameter calculated by all methods were tested to determine whether they cover nominal values of parameters. In this way the number of successful coverages in each series of independent 'experiments' with the same error structure and the coverage probabilities corresponding to the nominal value of 95% were found. The coverage probabilities for joint confidence intervals (corresponding to the nominal value of 90.25%) were found in the same way.

The S.E. values for each parameter from each method were also collected and their means were calculated for each series of independent 'experiments' with the same error structure. These means (denoted as M.S.E., mean square error) were compared with the variances of 1000 values of the corresponding parameters.

RESULTS AND DISCUSSION

We performed 48 series of simulations in accordance with Table 1 comprising the most interesting combinations of the absolute and relative components of the total error (eqn. 12), acquiring the values of 0, 0.05 and 0.1, the number of 'experimental' data points being 5, 10, 15 and 20. Each combination of σ_0^2 , σ_2^2 and n in Table 1 corresponds to 1000 simulations. The data were generated as described in the Methods and theory section. These 'experimental' data were analysed by the tested methods of non-linear regression and the results of calculations were evaluated as described in the Methods and theory section. We investigated both the influence of

Table 1. Types of error and numbers of 'experimental' points of the simulated data analysed by the tested regression methods

The total error is given by eqn. (12). Numbers of 'experimental' points analysed in individual series (n) as functions of error structure and magnitude are given.

Relative error (σ_2^2)	Absolute error (σ_0^2)...	n		
		0	0.05	0.1
0	—	—	5, 20	5, 20
0.05	5, 20	5, 20	5, 10, 15, 20	—
0.1	5, 20	5, 20	—	5, 10, 15, 20

Table 2. Comparison of the methods for centre estimation

The correct weights calculated according to eqn. (14) with n 'experimental' points were used ($\sigma_0^2 = \sigma_2^2 = 0.1$). The following methods served for the regression: Marquardt's modification of the Gauss–Newton method (M), jackknife (JK), weighted jackknife (WJK), and linear jackknife (LJK). The results for the linear parameter V (with the correct value equal to 1) calculated as arithmetic means (μ), 10% censored means (μ_c) and sample medians (m) are presented. The asterisks at the values of skewness (γ_3) and kurtosis (γ_4) denote the probability that these values are obtained from a normal distribution (*, ** and *** correspond to $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; $\gamma_3 = 0$ and $\gamma_4 = 3$ for a normal distribution).

n	M	JK	WJK	LJK
10 μ	1.009	0.978	1.032	0.988
μ_c	1.004	0.975	1.034	0.984
m	0.996	0.968	1.042	0.977
γ_3	−0.72***	−0.35***	0.18*	−0.53***
γ_4	3.91***	3.73***	3.47***	3.72***
20 μ	1.008	0.998	1.010	0.999
μ_c	1.005	0.996	1.008	0.997
m	1.000	0.993	1.009	0.994
γ_3	−0.68***	−0.53***	−0.23***	−0.53***
γ_4	4.71***	4.00***	3.37*	3.95***

the number of 'experimental' points (n) and the effect of different weighting on the results obtained by the tested methods. The above-mentioned types of weights [i.e. the correct weights (eqn. 14), constant weights (eqn. 15) and relative weights (eqn. 16)] were applied in the calculations by all tested methods. In the case of a great absolute component (and a zero relative component) of the error the relative weights were completely incorrect, and, vice versa, with great relative component (and the zero absolute component) the constant weights were entirely incorrect. The results of the methods for parameter estimation and of those for interval estimation are presented and discussed separately.

Point estimation

As the values of both parameters obtained by all methods belong to a skew distribution (see the Methods and theory section) we used three ways of calculating the centres of parameter distributions (Table 2). Although our data confirm that the distributions are far from a non-deformed normal distribution, there is no significant difference between the distribution centres calculated as arithmetic means, 10% censored means and sample medians. Similar results were obtained for all error structures, and therefore arithmetic means and their variances are given throughout.

Typical results obtained by the analysis of different series of simulations with an increasing number of 'experimental' points (for $\sigma_0^2 = \sigma_2^2 = 0.1$, i.e. for the

highest error of the tested error structures; Table 1) are presented in Table 3. Table 3 is confined to K_m values because the estimations of this non-linear parameter are more biased and have less accuracy in comparison with the linear parameter (V). Moreover, the exact value of K_m is of more practical importance. On increasing the number of 'experimental' points the bias of the determined parameters decreases, and the accuracy increases irrespective of the method and weight used. Although five points with $\sigma_0^2 = \sigma_2^2 = 0.1$ are insufficient for good estimates, the results are satisfactory even for this small number of 'experimental' points when the error in v decreases (i.e. for $\sigma_0^2 = \sigma_2^2 = 0.05$ and the other error structures given in Table 1).

In the case of five 'experimental' points (for $\sigma_0^2 = \sigma_2^2 = 0.1$), all jackknife methods give nonsense values of both parameters with unreasonable scatters, and the Marquardt's method gives biased results. On increasing the number of points ($n \geq 10$) the situation is altered markedly; all tested methods yield sufficiently correct estimates for both parameters (this effect is more pronounced if correct or absolute weights are used; cf. below). The weighted jackknife is the most sensitive method to the number of 'experimental' points (for the same improvement of bias and accuracy more points are required than in the case of the other methods tested). There is no difference between the linear and classical jackknives and Marquardt's method if medium numbers of 'experimental' points (10 or 15) are used. Only for 20

Table 3. Results of parameter estimations by the tested regression methods with different numbers of ‘experimental’ points

Error structure and regression methods are the same as in Table 2, W denotes weight (0, correct weight; 1, absolute weight; 2, relative weight), P stands for the value of the non-linear parameter (K_m) with the correct value of 10, var denotes the parameter variance and M.S.E. is the mean standard error (the arithmetic mean of standard errors computed by each method in each run).

<i>n</i>	W	M			JK			WJK			LJK		
		P	var	M.S.E.	P	var	M.S.E.	P	var	M.S.E.	P	var	M.S.E.
5	0	15.4	17	11	−74	701	98	15.3	24	20	−22	296	49
	1	15.4	18	12	−88	782	112	16.4	24	21	−35	408	63
	2	20.7	29	27	−145	1066	166	−4.0	163	53	−63	600	109
10	0	10.8	5.6	5.6	8.7	5.7	6.4	10.6	6.0	6.7	9.4	5.2	5.0
	1	11.3	6.0	6.3	9.0	5.7	7.0	11.3	6.1	7.2	9.6	5.4	5.6
	2	14.0	12.2	7.2	11.5	12.2	10.0	3.1	17	10.8	13.8	13.8	9.7
15	0	10.7	4.3	4.4	9.7	4.0	4.6	10.7	4.5	4.8	9.6	3.9	4.0
	1	10.7	4.6	4.9	9.7	4.1	4.8	10.8	4.5	4.9	9.9	4.1	4.2
	2	11.8	8.8	4.3	10.1	10.2	7.3	7.2	7.4	6.3	11.1	10.5	7.0
20	0	10.5	3.6	3.7	9.9	3.3	3.7	10.5	3.8	3.9	10.0	3.3	3.3
	1	10.5	3.6	4.1	9.8	3.5	3.8	10.6	3.7	3.9	9.9	3.5	3.5
	2	11.3	6.5	3.1	10.0	8.3	5.6	8.5	4.8	4.7	10.6	8.4	5.5

(and probably more) ‘experimental’ points the classical jackknife performs slightly better than its linear approximation and the simple Marquardt’s method.

The results in Table 3 also suggest that there is no difference between correct and absolute weighting for the Michaelis–Menten equation. The bias and accuracy for both parameters (and all error structures tested) are nearly the same whenever correct or absolute weights are used. The only exception is the case when the error of v has a zero absolute part ($\sigma_0^2 = 0$) and the relative part is high ($\sigma_2^2 = 0.1$). But even in this case the results are not changed essentially by use of the wrong absolute weight (e.g. Marquardt’s method gives for 20 points with this error structure $K_m = 10.1 \pm 1.4$ with constant weights and $K_m = 10.0 \pm 0.5$ with correct weights). The usage of relative weights, however, leads to a very different pattern. All results calculated for the data with a non-zero absolute part of error are biased and have a great variance. Although the bias can be removed to some extent by increasing the number of ‘experimental’ points the variances are always 2–3-fold in comparison with those obtained with correct or absolute weights. The weighted jackknife seems to be the method most sensitive to the type of weight used.

The values of the mean standard deviation for each parameter (M.S.E.) are also shown in Table 3. These values (calculated for each regression method) are closely related to the respective confidence intervals (which are discussed below). All M.S.E. values are in good agreement with the variances of each parameter; this confirms the suitability of all tested methods for the estimation of Michaelis–Menten parameters. The small differences in the obtained values of M.S.E. are in accord with the observations that were mentioned for the values of parameters and their variances.

Although all four tested methods provide similar results, some conclusions might be stated. The jackknife methods lose all their robustness if only small numbers of scattered ‘experimental’ points are used. This conclusion is in accord with that of Duncan (1978). It is conceivable that the logarithmic transformation (Cornish-

Bowden & Wong, 1978) would eliminate the nonsense negative values of parameters; however, we only intended to compare the non-transformed methods of non-linear regression. Moreover, it is known that the logarithmic transformation does not solve all problems connected with the jackknife method (Duggleby, 1978). Marquardt’s method of non-linear regression (or similar gradient methods) can be recommended for routine calculations, especially in view of its short computational time and low complexity (Table 6). The use of 10–15 data points depending on the supposed absolute value of error is advisable. When very great accuracy of results is required the classical jackknife with 20 (or more) data points seems to be preferable. Our results indicate that no weighting is needed (this conclusion is at variance with that of Atkins & Nimmo, 1980).

Interval estimation

Our data show that the situation in the interval estimation is not so clear as in the case of point estimation (see Tables 4 and 5, which comprise the data for the most complex error structure, $\sigma_0^2 = \sigma_2^2 = 0.1$). It can be seen that the coverage probabilities of the matrix-inversion method are close to the nominal value of 95% and that there is almost no improvement of the results for both parameters if the number of ‘experimental’ points is increased from 5 to 20. This is true for both correct and absolute weights, which yield very similar results. The picture changes markedly if relative weights are used. All results (except for 5 ‘experimental’ points) are very distinct from the nominal value of 95% in this case. There even occurs some deterioration of results with the increasing number of data points. The behaviour of the classical jackknife is nearly the same. The coverage probabilities for 20 ‘experimental’ points are even better (as measured by the distance from the nominal value) than those obtained by matrix inversion. The results for correct and absolute weights are almost equal. The obtained values are worse with relative weights, and a deteriorating trend can be seen with the increasing number of data points, as in the case of matrix inversion.

Table 4. Results of interval estimations of the linear parameter (V) by the tested methods

Error structure, numbers of points (n) and weights (W) are the same as in Table 3. The following methods were tested: calculation from the variance-co-variance matrix (M), jackknife (JK), weighted jackknife (WJK), linear jackknife (LJK) and weighted linear jackknife (WLJK). The results are presented as coverage probabilities at the nominal 95% probability level.

n	W	Coverage probability				
		M	JK	WJK	LJK	WLJK
5	0	96.2	97.5	94.7	100	97.9
	1	95.6	97.1	93.1	100	98.0
	2	93.7	96.2	94.9	98.7	98.0
10	0	94.7	95.4	93.2	100	99.0
	1	95.2	95.4	93.9	100	99.0
	2	86.7	92.3	88.8	86.2	100
15	0	95.3	95.0	93.8	100	100
	1	97.0	95.3	94.5	100	100
	2	80.9	88.9	86.5	77.2	99.8
20	0	96.6	95.9	95.3	100	100
	1	95.9	95.4	94.5	100	100
	2	81.2	88.2	86.1	77.2	100

Table 5. Results of interval estimations of the non-linear parameter (K_m) by the tested methods

The same symbols as in Table 4 are used.

n	W	Coverage probability				
		M	JK	WJK	LJK	WLJK
5	0	93.2	95.1	84.5	96.2	82.0
	1	95.3	95.7	85.6	96.4	80.8
	2	68.5	92.3	81.4	89.0	84.4
10	0	94.3	91.2	87.3	94.2	88.7
	1	96.5	91.7	89.2	96.1	88.7
	2	63.9	83.3	76.5	88.2	82.1
15	0	94.9	92.2	90.1	94.6	91.2
	1	97.3	93.0	90.5	96.5	92.0
	2	63.0	82.4	77.4	89.8	78.7
20	0	96.9	93.4	91.2	96.3	93.0
	1	97.1	93.2	91.8	95.8	92.2
	2	66.9	81.5	78.1	92.2	79.5

The coverage probabilities for the weighted jackknife are worse than those for both above-mentioned methods, the values of the non-linear parameter (K_m) especially being quite far from the nominal value. Both linear and weighted jackknife methods give too great coverage probabilities for the linear parameter (V) (see Table 4). Linear jackknife yields very good results for the non-linear parameter (K_m); they are very close to those obtained by matrix inversion and better than those calculated by the classical jackknife. The relative weighting gives too small values of coverage probabilities for both parameters. The coverage probabilities provided by the weighted jackknife for the non-linear parameter (K_m) are too small compared not only with the nominal value but also with the results of all other methods. The behaviour of coverage probabilities of both linear weighted jackknife methods with relative weights is similar to that observed in the case of all other methods.

Table 6. Comparison of relative computational times of the tested methods

Computational time of Marquardt's procedure is regarded as unity, n denotes the number of 'experimental' points, and the other symbols are the same as in Table 4.

Method	Relative computational time
M	1
JK	n
WJK	$n+1$
LJK	2
WLJK	2

The conclusions that can be derived from the analysis of the data on interval estimation are very similar to those stated for point estimation. On the basis of the results given in Tables 4 and 5 and considering the computational time and complexity of the tested methods (Table 6), it is possible to recommend the interval estimations with constant weights with the use of the calculations from the variance–co-variance matrix (obtained by Marquardt's non-linear gradient method). This procedure can be recommended even if the errors of the dependent variable (initial reaction rate) are unknown. The required number of data points is between 10 and 15, depending on the absolute values of errors. If very precise interval estimates are needed, the use of the classical jackknife with 20 (or more) data points is to be recommended.

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