Error structure as a function of substrate and inhibitor concentration in enzyme kinetic experiments

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Optimal design of experiments as well as proper analysis of data are dependent on knowledge of the experimental error. A detailed analysis of the error structure of kinetic data obtained with acetylcholinesterase showed conclusively that the classical assumptions of constant absolute or constant relative error are inadequate for the dependent variable (velocity). The best mathematical models for the experimental error involved the substrate and inhibitor concentrations and reflected the rate law for the initial velocity. Data obtained with other enzymes displayed similar relationships between experimental error and the independent variables. The new empirical error functions were shown superior to previously used models when utilized in weighted non-linear-regression analysis of kinetic data. The results suggest that, in the spectrophotometric assays used in the present study, the observed experimental variance is primarily due to errors in determination of the concentrations of substrate and inhibitor and not to error in measuring the velocity.

INTRODUCTION

Knowledge of the variance of experimental data is fundamental to optimal design and proper analysis in enzyme kinetics, as well as to many other areas of investigation (Mannervik, 1981, 1982). In most enzyme kinetic studies published it would appear that the data have been treated as if the variance were constant, and in some studies it has been assumed that the experimental error was proportional to the velocity (i.e. constant relative error). In a few investigations the experimental variance has been studied (cf. Storer *et al.*, 1975; Siano *et al.*, 1975; Askelöf *et al.*, 1976; Mannervik *et al.*, 1979; Nimmo & Mabood, 1979), and the results of these studies suggest that each experimental system may have its own characteristic error structure.

An empirical error function in which the estimated variance, Var or s^2 , is expressed as a function of velocity, v , was formulated:

$$\text{Var}(v) = K_1 \cdot v^\alpha \quad (1)$$

where K_1 and α are empirical constants (Askelöf *et al.*, 1976). An equivalent expression is:

$$s = c \cdot v^{\alpha/2} \quad (2)$$

where the absolute value of the experimental error, s , is equal to $\sqrt{\text{Var}}$ and $c = \sqrt{K_1}$. The two most common assumptions about experimental error, i.e. constant absolute error and constant relative error, correspond to $\alpha = 0$ and $\alpha = 2$ respectively. The constants in eqns. (1) or (2) can be estimated by examination of the experimental error as a function of v . Such estimates may be obtained from replicate experiments (Askelöf *et al.*, 1976) or by analysis of residuals (Mannervik *et al.*, 1979).

In spite of the successful application of expressions of experimental error as a function of v , it has been noted that v in itself is a stochastic variable and that the true independent variables in enzyme kinetics are substrate and inhibitor concentrations and other predetermined

variables (Mannervik, 1981). It is also clear that a given velocity value will usually not uniquely define the experimental conditions, because it is possible that the same value may arise from different settings of the independent variables. In the present paper, such cases have been studied experimentally and the results show that, indeed, the experimental error is more accurately and precisely described as a function of the true independent variables than by eqn. (1) or eqn. (2). A preliminary report of this work has been presented (Mannervik *et al.*, 1982).

MATERIALS AND METHODS

Chemicals

GSH, 1,2-dichloro-4-nitrobenzene, 5,5'-dithiobis-(2-nitrobenzoic acid) and *S*-n-octyl-GSH were obtained as previously described (Askelöf *et al.*, 1975). Acetylthiocholine iodide and decamethonium bromide were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Enzymes and assays

Acetylcholinesterase from electric eel was obtained from Sigma Chemical Co. and was assayed by the method outlined by Wiedmer *et al.* (1979). The enzyme analyses were performed on an Aminco DW-2 u.v.-visible-region spectrophotometer. Glutathione transferase 3–3 from rat liver was obtained and assayed as earlier described (Askelöf *et al.*, 1975; Jakobson *et al.*, 1977). (The enzyme was earlier referred to as glutathione transferase A; see Jakoby *et al.*, 1984).

Analysis of kinetic data

Non-linear-regression analysis of initial-velocity data was performed as previously described (Mannervik, 1981, 1982). The weighting factors were based on estimates of the experimental variance (Askelöf *et al.*, 1976; Mannervik *et al.*, 1979).

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RESULTS

Experimental error as a function of substrate concentration

Acetylcholinesterase was chosen for the experiments because the steady-state kinetics of this enzyme show substrate inhibition. Therefore selection of pairs of substrate concentrations that yield the same velocity could be made within a defined range of substrate concentrations. By making replicate experiments in each of a pair of such experimental points, it can be established whether or not the experimental variance is the same for a given v at two different settings of the substrate concentration. Replicate measurements (ten for each experimental point) in several pairs of such points demonstrated conclusively that, in most cases, the estimate of the variance for a given v at low substrate concentration was significantly larger than that obtained for the same v at high substrate concentration. The variance estimates were compared by the F statistic (Box *et al.*, 1978). Fig. 1 shows a similar experiment in which the curve of the plot of velocity versus substrate concentration was constructed by replicate measurements ($n = 10$) for each substrate concentration. The estimated error, s , in each point was then plotted versus velocity (Fig. 2) and substrate concentration (Fig. 3) and fitted by weighted non-linear regression (Mannervik, 1982) to two types of mathematical models (Table 1). One type expresses the error as a function of velocity. The second type relates the error to substrate concentration and included the 'best' of the models tried:

$$s = \frac{K_1 \cdot [A]}{K_2 + [A] + K_3 \cdot [A]^2} \quad (3)$$

Fig. 1. Initial velocity of the acetylcholinesterase reaction as a function of acetylthiocholine concentration $[A]$

The error bars show the standard deviation, s , in each experimental point as estimated by replicate measurements ($n = 10$). The curve was fitted by weighted non-linear regression analysis by using the rate equation (cf. Table 3):

$$v = \frac{K_1 \cdot [A]}{K_2 + [A] + K_3 \cdot [A]^2}$$

and the weighting factors (cf. Table 1):

$$w_i = \left(\frac{0.025 + [A] + 0.137 \cdot [A]^2}{0.206 \cdot [A]} \right)^2$$

Fig. 2. Estimated experimental error (s) for the kinetics of acetylcholinesterase plotted versus initial velocity

The points represent the data displayed in Fig. 1. The trajectory shows the relationship between s and velocity as calculated on the basis of the best fits of velocity versus substrate concentration (Fig. 1) and of s versus substrate concentration (Fig. 3). This trajectory is the best representation of the experimental error in the s /velocity space. Points corresponding to substrate concentrations greater than 1 mm are marked by $\bullet$ symbols.

Fig. 3. Estimated experimental error (s) for the kinetics of acetylcholinesterase plotted versus substrate concentration

The continuous curve (—) was fitted by weighted non-linear regression by using the equation:

$$s = \frac{K_1 \cdot [A]}{K_2 + [A] + K_3 \cdot [A]^2}$$

and the inverse values of estimated experimental variance, s^{-2} , in each point as weighting factors. The broken curve (---) is reproduced from Fig. 1. Comparison of the two curves shows that the coefficient of variation changes with substrate concentration.

where K_1 , K_2 and K_3 are empirical constants. Addition of a constant term to this equation gave a high standard deviation for the corresponding parameter (K_4) and did not significantly improve the fit (Table 1). Various models involving power functions of the substrate concentration were also tested in a search for the simplest expression, since earlier studies had demonstrated that only a small number of parameters can be fitted precisely (Askelöf *et al.*, 1976). However, these models were inferior to the rational functions. The weighting factors

Table 1. Parameter values of alternative models of the experimental-error structure for kinetic data obtained with acetylcholinesterase and different concentrations of the substrate acetylthiocholine

Units of parameters can be deduced on the basis of values of velocity, v , in $\mu\text{mol}/\text{min}$ per ml and substrate concentration, $[A]$, in mM. The experimental data are given in Fig. 1.

Model	Residual sum of squares	Parameter values $\pm$ s.d.				
		K_1	K_2	K_3	K_4	K_5
$s = K_1 + K_2 \cdot v$	3.452	0.013 ± 0.004	0.030 ± 0.004			
$s = K_1 + K_2 \cdot v^2$	3.872	0.018 ± 0.004	0.008 ± 0.001			
$s = K_1 \cdot v^{K_2}$	3.620	0.057 ± 0.006	0.547 ± 0.081			
$s = K_1 \cdot [A]^{K_2}$	8.919	0.055 ± 0.011	0.145 ± 0.042			
$s = \frac{K_1 \cdot [A]^{K_2}}{K_3 + [A]^{K_4}}$	7.638	1.153 ± 0.0	0.703 ± 0.177	2.666 ± 0.768	1.971 ± 0.689	
$s = \frac{K_1 \cdot [A]^{K_2}}{K_3 + [A]^{K_4}} + K_5$	5.937	1.125 ± 0.0	0.743 ± 0.246	2.564 ± 0.731	2.185 ± 1.118	0.0094 ± 0.0085
$s = \frac{K_1 \cdot [A]}{K_2 + [A] + K_3 \cdot [A]^2}$	1.497	0.206 ± 0.026	0.025 ± 0.006	0.137 ± 0.043		
$s = \frac{K_1 \cdot [A]}{K_2 + [A] + K_3 \cdot [A]^2 + K_4}$	1.414	0.213 ± 0.030	0.033 ± 0.013	0.162 ± 0.060	0.005 ± 0.004	

in the regression were inversely proportional to the estimated experimental variance, s^2 . The plot of the estimated experimental error versus velocity (Fig. 2) shows that each velocity value in a certain range corresponds to two different errors (obtained under different experimental conditions). Hence it is evident that the error cannot be treated as a mathematical function of velocity in this experimental system. Fig. 3, on the other hand, represents a mathematically adequate expression of error as a function of substrate concentration. In the absence of information about the origin of the points in Fig. 2, it might have been assumed that eqn. (1) was applicable. However, the projection of the fitted curve from Fig. 3 on to the error/velocity space depicted in Fig. 2 makes it evident that this assumption is invalid. The trajectory in Fig. 2 is the graph of the fit of eqn. (3) to the points in Fig. 3. The fit of eqn. (2) [or the equivalent eqn. (1)] to the same data set is inferior (Table 1).

Experimental error as a function of both inhibitor and substrate concentration

A certain enzymic velocity may be obtained by different combinations of the settings of the experimental variables. For example, low substrate and low inhibitor concentrations will give the same velocity as a suitable combination of higher substrate and inhibitor concentrations. Accordingly, the experimental variance was examined by making replicate ($n = 8$) measurements of initial velocities at different fixed concentrations of substrate and variable inhibitor concentrations. Fig. 4(a) shows the experimental error estimated from replicates plotted versus velocity. The results show clearly that the velocity does not uniquely define an experimental error. Accordingly, the error cannot be considered a function of velocity. Fig. 4(b) shows a plot of s versus $s \cdot [I]$ for three different substrate concentrations. This plot corresponds to a plot of v versus $v \cdot [I]$ earlier used to analyse the kinetics of inhibition (Jakobson *et al.*, 1979) and should give straight lines if the inverse function is linearly dependent on $[I]$. The non-linear graphs in Fig. 4(b)

demonstrate that the relationship of s to $[I]$ is of higher order.

Table 2 summarizes the results of fitting alternative mathematical models of the error structure to the data. As in the experiments analysed in Table 1, it is obvious that the models expressing the error as a function of velocity are inferior to models based on substrate and inhibitor concentrations as independent variables. The lowest residual sum of squares was obtained with:

$$s = \frac{K_1 \cdot [A] + K_5 \cdot [I]}{K_2 + [A] + K_3 \cdot [A]^2 + K_4 \cdot [I]} \quad (4)$$

where K_1 , K_2 , K_3 , K_4 and K_5 are empirical constants. Inclusion of an $[I]^2$ term in the denominator did not improve the fit. Eqn. (4) can be considered as an extension of eqn. (3).

Effect of weighting factors on parameter estimation

Kinetic data were fitted by using different weighting procedures in order to evaluate their effect on the parameter estimation. Table 3 shows the results obtained when the kinetic data displayed in Fig. 1 were analysed by using a rate equation expressing inhibition by excess of substrate. Unweighted data ($w_i = 1$) were compared with data fitted by using weighting factors inversely proportional to expressions derived according to eqns. (2) and (3). The effects of the different weighting procedures on the estimates of the kinetic constants were small, but the decrease in the residual sum of squares shows that the fit is best when weighting factors based on substrate concentrations are used. Table 4 shows a similar comparison of different weighting procedures in the regression analysis of the kinetic data displayed in Fig. 4. In this case the estimates of several of the parameter values in the rate equation change more markedly when different weighting factors are used. Consequently it appears that the weighting procedure may have a large or a small effect on the parameter estimation depending on the particular data set analysed.

Fig. 4. Dependence of experimental error (s) for acetylcholinesterase on inhibitor concentration at three fixed substrate concentrations

(a) Plot of s versus initial velocity. (b) Plot of s versus $s/[I]$. The substrate acetylthiocholine was used at 0.1 mM ($\square$), 1.0 mM ($\bullet$) and 4.0 mM ($\blacktriangle$) concentration. The inhibitor decamethonium bromide was used in the concentration range 0.1–20 μ M. Replicate measurements ($n = 8$) were made for each experiment point.

Table 2. Parameter values of alternative models of the experimental-error structure for kinetic data obtained with acetylcholinesterase with decamethonium bromide as inhibitor

Units of parameters can be deduced on the basis of values of velocity in μ mol/min per ml, acetylthiocholine concentration, $[A]$, in mM and decamethonium bromide concentration, $[I]$, in μ M. The experimental data are given in Fig. 4.

Model	Residual sum of squares	Parameter values $\pm$ s.d.					
		K_1	K_2	K_3	K_4	K_5	K_6
$s = K_1 + K_2 \cdot v$	5.604	0.011 ± 0.009	0.040 ± 0.007				
$s = K_1 + K_2 \cdot v^2$	5.948	0.036 ± 0.007	0.008 ± 0.002				
$s = K_1 \cdot v^{K_2}$	5.779	0.050 ± 0.007	0.889 ± 0.136				
$s = K_1 \cdot v^{K_2} + K_3$	5.453	0.024 ± 0.017	1.315 ± 0.425	0.023 ± 0.015			
$s = \frac{K_1 \cdot [A]^{K_2}}{[A]^{K_3} + K_4 \cdot [I]^{K_5}}$	2.159	0.259 ± 0.050	0.529 ± 0.107	0.699 ± 0.041	0.561 ± 0.266	0.505 ± 0.075	
$s = \frac{K_1 \cdot [A]^{K_2}}{[A]^{K_3} + K_4 \cdot [I]^{K_5}} + K_6$	2.129	0.258 ± 0.064	0.556 ± 0.131	0.747 ± 0.042	0.548 ± 0.269	0.528 ± 0.130	0.003 ± 0.016
$s = \frac{K_1 \cdot [A]}{K_2 + [A] + K_3 \cdot [A]^2 + K_4 \cdot [I]}$	2.609	0.308 ± 0.093	0.063 ± 0.060	0.273 ± 0.198	0.095 ± 0.037		
$s = \frac{K_1 \cdot [A]}{[A] + K_2 \cdot [A]^2 + K_3 \cdot [I]}$	3.063	0.176 ± 0.017	0 ± 0	0.053 ± 0.014			
$s = \frac{K_1 \cdot [A] + K_5 \cdot [I]}{K_2 + [A] + K_3 \cdot [A]^2 + K_4 \cdot [I]}$	1.527	0.380 ± 0.107	0.054 ± 0.052	0.376 ± 0.213	0.302 ± 0.136	0.007 ± 0.004	
$s = \frac{K_1 \cdot [A] + K_5 \cdot [I]}{K_2 + [A] + K_3 \cdot [A]^2 + K_4 \cdot [I] + K_6 \cdot [I]^2}$	1.527	0.380 ± 0.107	0.054 ± 0.052	0.376 ± 0.213	0.302 ± 0.136	0.007 ± 0.004	0 ± 0

Determination of error structure on the basis of residual analysis

It has previously been shown (Mannervik *et al.*, 1979) that the local experimental variance can be approximated by determination of the mean of squared residuals in the region in question. A precondition was that an adequate fit had been obtained by regression analysis and that at least five residuals were used for each estimate. Fig. 5

shows the distribution of the experimental points (design matrix) in a kinetic inhibition experiment in which the concentration of one substrate and one inhibitor were used as variables. The residuals obtained after fitting a suitable rate equation (see Fig. 5 in Jakobson *et al.*, 1979) were divided into groups of six (as indicated by boxes in Fig. 5 of the present paper) and the local variance was calculated for each group of points. The estimates of the error were then analysed as shown in Table 5. The

Table 3. Effect of using different weighting factors in parameter estimation on the kinetics of acetylcholinesterase

The kinetic data for acetylcholinesterase displayed in Fig. 1 were fitted by using the following equation (cf. Table 1):

$$v = \frac{K_1 \cdot [A]}{K_2 + [A] + K_3[A]^2}$$

Weighting factor	Residual sum of squares	Parameter values $\pm$ s.d.		
		K_1	K_2	K_3
1	0.00906	5.553 ± 0.079	0.086 ± 0.004	0.080 ± 0.004
$\frac{1}{v^{1.1}}$	0.00120	5.553 ± 0.094	0.089 ± 0.004	0.079 ± 0.005
$\left(\frac{0.025 + [A] + 0.137 \cdot [A]^2}{0.206 \cdot [A]} \right)^2$	0.00096	5.473 ± 0.108	0.089 ± 0.005	0.074 ± 0.004

Table 4. Effect of using different weighting factors in parameter estimation

The kinetic data for inhibition of acetylcholinesterase with decamethonium bromide displayed in Fig. 4 were fitted by using the following equation (cf. Table 2):

$$v = \frac{K_1 \cdot [A]}{[A] + K_2 \cdot [A]^2 + K_3 \cdot [I]}$$

Weighting factor	Residual sum of squares	Parameter values $\pm$ s.d.		
		K_1	K_2	K_3
1	1.342	5.279 ± 0.821	0.364 ± 0.183	0.099 ± 0.048
$\frac{1}{v^{1.78}}$	0.1397	4.048 ± 0.664	0.757 ± 0.227	0.038 ± 0.013
$\left(\frac{0.054 + [A] + 0.376 \cdot [A]^2 + 0.302 \cdot [I]}{0.38 \cdot [A] + 0.007 \cdot [I]} \right)^2$	0.1459	4.310 ± 0.616	0.429 ± 0.203	0.047 ± 0.013
$\left(\frac{[A]^{0.70} + 0.56 \cdot [I]^{0.51}}{0.26 \cdot [A]^{0.53}} \right)^2$	0.1495	4.914 ± 1.101	0.389 ± 0.222	0.060 ± 0.014

experimental error is adequately described by a simple function of substrate and inhibitor concentration.

Finally, the error structure of kinetic data for butyrylcholinesterase earlier analysed according to eqn. (1) on the basis of replicates as well as residuals was re-investigated. The results given in Table 6 demonstrate that also for this experimental system a model based on error expressed as a function of substrate concentration gives the best description.

DISCUSSION

The results of the present investigation show unambiguously that the experimental-error structure for several different data sets obtained in kinetic experiments is best described by a model involving substrate and/or inhibitor concentration as the independent variable (cf. Tables 1, 2, 5 and 6). In each case the error structure was more complex than the classical assumptions of constant absolute or constant relative error. Also, models of the experimental variance composed as the sum of a constant term and a term increasing with velocity, earlier tried by Askelöf *et al.* (1976) and Cornish-Bowden (1981), were inferior to the best models involving the substrate and/or inhibitor concentration (cf. Tables 1, 2 and 6).

In the data sets obtained with acetylcholinesterase it can be stated that models expressing error as a function of velocity are mathematically inadequate, since one velocity value may correspond to more than one value of the error (Figs. 2 and 4).

The results indicate that the error structure is a reflection of the mathematical model describing the kinetics of the enzyme studied. Thus, when the substrate concentration is varied, the error can be described by an equation similar to the rate law (Fig. 3 and Table 1). Similarly, when the concentration of an inhibitor is varied, the error structure reflects the dependence of velocity on this variable (Figs. 4 and 5 and Tables 2, 4 and 5). It may therefore seem necessary to establish the rate equation before the model of the experimental error can be defined. However, the error is a stochastic variable and is less precisely defined than the velocity in kinetic experiments. Therefore the information content in the data may only permit expression of the error as a degenerate form of the rate equation. For example, the kinetics of glutathione transferase 3-3 are much more complex (Jakobson *et al.*, 1979) than what can be described by the equation that gives a satisfactory representation of the experimental error (Table 5). In general, degenerate forms of a complex rate law, which

Fig. 5. Estimated experimental error for rat glutathione transferase 3-3 as a function of substrate and inhibitor concentrations

The local experimental error in the substrate/inhibitor concentration space was estimated by the use of the means of squared residuals obtained after non-linear regression analysis (cf. Mannervik *et al.*, 1979). The ○ symbols mark the design points used in the kinetic experiments; the X symbols show the location of the points for which the local experimental variance has been estimated by using residuals from the six adjacent points. The diagonal lines show iso-variance contours, calculated on the basis of the residual analysis, by using the equation (cf. Table 5):

$$s = \frac{0.82 \cdot [A]}{[A] + 13.1 \cdot [I]}$$

The diagonal contour lines from left to right correspond to $s = 0.05, 0.1, 0.2, 0.4, 0.6, 0.7$ and $0.8 \mu\text{M}/\text{min}$.

Table 5. Parameter values of alternative models of the experimental-error structure for kinetic data obtained with rat glutathione transferase 3-3

Means of squared residuals ($n = 6$) were used as estimates of the local experimental variance (Mannervik *et al.*, 1979). The residual analysis was based on a set of kinetic data for glutathione transferase 3-3 obtained by varying the GSH (A) and S-octyl-GSH (I) concentrations (cf. Fig. 5 in Jakobson *et al.*, 1979). Proper units of the parameters can be deduced by considering that the standard error is expressed in $\mu\text{M}/\text{min}$ and the reactant concentrations in mM (cf. Fig. 5).

Model	Residual sum of squares	Parameter values $\pm$ s.d.						
		K_1	K_2	K_3	K_4	K_5	K_6	K_7
$s = \frac{K_1 \cdot [A]}{[A] + K_2 \cdot [I]}$	0.353	0.822 ± 0.109	13.108 ± 2.926					
$s = \frac{K_1 \cdot [A]}{[A] + K_2 \cdot [A]^2 + K_3 \cdot [I]}$	0.353	0.822 ± 0.109	0 ± 0	13.108 ± 2.926				
$s = \frac{K_1 \cdot [A]^{K_2}}{K_3 + [A]^{K_4} + K_5 \cdot [I]^{K_6}}$	0.803	0.663 ± 1.381	1.216 ± 0.858	0.052 ± 0	1.132 ± 0.143	1.571 ± 4.952	0.557 ± 0.676	
$s = \frac{K_1 \cdot [A]^{K_2}}{K_3 + [A]^{K_4} + K_5 \cdot [I]^{K_6}} + K_7$	0.485	0.582 ± 0.280	1.141 ± 0.275	0 ± 0	1.018 ± 0.048	2.853 ± 3.619	0.768 ± 0.629	0 ± 0

accounts for the major kinetic features, may in many cases prove sufficient to express the error structure.

A limited study of the error structure of acetylcholinesterase has previously been made (Nimmo & Mabood, 1979). No regular relationship between experimental error and substrate concentration was found, but only few points in a limited velocity range were investigated.

On the other hand, proportionality between error and enzyme concentration was obtained. The latter result is consistent with the concepts put forward in the present paper, since the rate law expresses proportionality between velocity and enzyme concentration, i.e. the error reflects the dependence of velocity on the independent variable.

Table 6. Parameter values of alternative models of the experimental-error structure for kinetic data obtained with butyrylcholinesterase (cf. Fig. 3 in Mannervik *et al.*, 1979)

The experimental error was estimated by replicate measurements ($n = 10$) at various concentrations of butyrylthiocholine iodide ($1 \mu\text{M}$ – 20 mM). Values are in arbitrary units.

Model	Residual sum of squares	Parameter values $\pm$ s.d.			
		K_1	K_2	K_3	K_4
$s = K_1 + K_2 \cdot v$	1.379	6.151 ± 1.903	956.6 ± 206.9		
$s = K_1 + K_2 \cdot v^2$	2.872	8.622 ± 2.568	9711 ± 3931		
$s = K_1 \cdot v^{K_2}$	0.800	317.7 ± 99.9	0.582 ± 0.069		
$s = K_1 \cdot v^{K_2} + K_3$	0.800	317.7 ± 99.9	0.582 ± 0.069	0 ± 0	
$s = \frac{K_1 \cdot [A]}{K_2 + [A]}$	1.202	61.90 ± 13.88	2.666 ± 1.168		
$s = \frac{K_1 \cdot [A] + K_2 \cdot [A]^2}{K_3 + [A] + K_4 \cdot [A]^2}$	0.696	4.405 ± 1.825	13.13 ± 4.70	0 ± 0	0.186 ± 0.086

Thus, when the error structure shall be determined for an investigation in enzyme kinetics, estimates of the local experimental variance have to be obtained for all regions of the space of independent variables (such as substrate and inhibitor concentrations). This can be achieved by replicate measurements ($n \geq 5$) or by analysis of residuals from an adequate fit of a rate equation as described previously (Mannervik *et al.*, 1979). The final selection of the best model for the error structure may have to await the establishment of the best rate equation for the system. Since the latter goal to some extent depends on the prior choice of the weighting factors in the regression analysis, which in turn are based on the error structure, an iterative procedure may be needed. However, in the cases investigated in the present study, the best equation for the error structure was a rational function related to the Michaelis–Menten equation or predictable derivatives of this rate law. The kinetic data give information about the rate law, and we have experienced no difficulties in finding an adequate equation for the error structure by using the similarities to the rate law.

It may be noted that many of the error models used previously by other investigators and by ourselves (cf. Askelöf *et al.*, 1976) can be regarded as simple approximations of the models proposed in the present work. For example, at low substrate concentrations eqn. (3) degenerates to:

$$s = \frac{K_1[A]}{K_2}$$

which is equivalent to eqn. (2) with $\alpha = 2$, since v becomes proportional to $[A]$ at low values of the latter. This corresponds to constant relative error, one of the typical assumptions about experimental error. The condition of constant absolute error ($\alpha = 0$) will prevail under such experimental conditions that the error function is approximately constant, for example at high substrate concentrations for the models in Table 6. Intermediate concentrations would correspond to $0 < \alpha < 2$. It is also possible that for some data sets the error function should contain a constant term independent of the dependent and the independent variables (Askelöf *et al.*, 1976; Cornish-Bowden, 1981), but in no case investigated here (cf. Tables 1, 2 and 6) was the inclusion of such a term warranted. In models that do contain a constant term,

this may become dominant under certain conditions and yield an approximately constant absolute error. Thus the models based on substrate and inhibitor concentrations proposed in the present paper have increased generality as well as improved accuracy and include previously used models as special cases.

In summary, the experimental data presented here counter the general view of the error, and show that it is not governed by the measured variable (i.e. velocity in enzyme kinetic experiments), but by the independent variables. This new finding may indicate that the error in the spectrophotometric enzyme assays used in the present work is small in comparison with errors arising in the determination of the added reactant concentrations. Temperature, pH and pressure may also have to be considered in modelling the error, when they are used as experimental variables.

Even if it is not known to what extent the present findings are generally applicable, the results and conclusions emphasize strongly the necessity of examining the error structure for each experimental system studied (Mannervik, 1982). We expect, further, that experimental systems outside the field of enzyme kinetics, in which a dependent variable is studied as a function of one or several independent variables, will show a similar dependence of experimental error on the settings of the independent variables.

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