A single-parameter family of adjustments for fitting enzyme kinetic models to progress-curve data

Ronald G. DUGGLEBY* and John C. NASH†

*Department of Biochemistry, University of Queensland, St. Lucia, Queensland 4067, Australia, and

†Faculty of Administration, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

Current methods for fitting integrated rate equations to enzyme progress curves treat each observation as if it were an independent measurement. When the data are obtained by taking several successive readings from each of a series of progress curves, the data will not be truly independent and will exhibit autocorrelation. Here we propose a simple pragmatic extension of integrated rate equations which takes account of first-order autocorrelations. The value of the method is assessed when applied to five sets of experimental data.

INTRODUCTION

The progress curve for an enzyme-catalysed reaction records the extent of product formation or substrate depletion at increasing times after initiation of the reaction. Since the seminal work of Fernley (1974), the analysis of measured progress-curve data conventionally employs non-linear regression techniques (Darvey *et al.*, 1975; Duggleby & Morrison, 1977, 1978; Markus *et al.*, 1981; Kellershohn & Laurent, 1985; Duggleby, 1986; Boeker, 1987) to estimate kinetic parameters from several progress curves. The sequential nature of the measurements for each progress curve, together with the possibility that the conditions for an individual curve, may be slightly different from the intended conditions may, however, result in deviations between fitted and observed data which display some pattern for an individual progress curve within the set under analysis. This problem is illustrated in Fig. 1, which shows some results obtained for the fumarase-catalysed conversion of fumarate to malate. The lines are theoretical curves obtained by an overall fit of a one-substrate one-product reversible model to these three, and seven other, progress curves. It is clear that there are systematic deviations between the data and the fitted lines.

A number of reasons can be suggested for the systematic deviations seen in Fig. 1. First, the data might not be corrected properly relative to the origin; that is, each whole curve is improperly displaced on the concentration axis. Although great care was taken to avoid this possibility in collecting the data shown in Fig. 1, this is a problem of which one must always be conscious in progress-curve analysis (Atkins & Nimmo, 1973; Duggleby & Morrison, 1977). Secondly, the quantity of enzyme added may differ from that intended, which would have the effect of stretching or contracting the time axis. Thirdly, the concentrations of substrates and products may differ from their nominal values, which would affect the final amount of product formed and the shape of the curve approaching this final amount. Finally, other experimental variables (pH, temperature, co-substrates, activators, inhibitors, contaminants and so on) may vary from curve to curve, which could again result in systematic deviations.

A correction for the first of these, displacement on the

concentration axis, could be achieved by including in the analysis a group of extra parameters representing the unknown displacements for each of the experimental curves. Similarly, correction for errors in the quantity of enzyme could be made by including an extra abscissa normalization parameter for each curve. Extra parameters could also be included to account for variations in the concentrations of added substrates (Newman *et al.*, 1974) and products. Clearly, when some combination of the factors outlined above is operating, the number of extra parameters would be so large that the analysis would become extraordinarily cumbersome and, in all probability, the results practically meaningless.

The present paper proposes a simple pragmatic adjustment procedure to improve the fit of the models generated. The procedure avoids the use of one or more adjustment parameter per progress curve, adding only a single parameter to any model. As such, this adjustment yields models which are parsimonious in the statistical sense.

THEORY

Many enzymic reactions have an integrated rate equation which can be written in the form:

$$\rho t = z + \gamma z^2 - \delta \ln(1 - z/z_\infty) \quad (1)$$

where t is the time after initiation of the reaction, z is the amount of product produced by the reaction, with z_∞ its value at completion (i.e. when $t = \infty$). The quantities ρ , γ and δ are functions of the reaction conditions and the usual enzyme kinetic constants, but are independent of time. Duggleby (1986) has discussed the solution of eqn. (1) for z if the other quantities (ρ , γ , δ and t) are given. Such a z is the model value, which can be compared with observed value(s) for given time t . Altering the kinetic parameters will change the computed z , and various procedures exist for systematizing the alterations so that some measure of fit between the set of model and observed values of z is optimized. In the present work we have chosen the widely accepted criterion that the optimized values of the parameters are those which minimize a weighted sum of squares of the residuals:

$$r_{kj} = z(\text{model})_{kj} - z(\text{observed})_{kj} \quad (2)$$

where the index k selects one of the progress curves, and the index j selects a particular point along that curve.

The unadjusted model and residual for datum j on curve k will be referred to using the symbols $z(\text{raw})_{kj}$ and $r(\text{raw})_{kj}$. The modifications proposed to the integrated-rate-equation model are additive, and take the form:

$$z(\text{model})_{kj} = z(\text{raw})_{kj} + \alpha \text{corrector}_{kj} \quad (3)$$

where α is an adjustment parameter in the new model which will be determined in the same way, and through the same computer codes, as the parameters within the integrated rate equation.

We have considered seven correctors:

1. $z(\text{model})_{k1}$, i.e. the first adjusted model value from the k th progress curve
2. $z(\text{model})_{k\infty}$, i.e. the limiting value from each progress curve
3. $z(\text{model})_{k(j-1)}$, i.e. the previous model value for curve k . The model value preceding the first point on each curve is taken to be zero
4. $z(\text{raw})_{k(j-1)}$, i.e. the previous uncorrected model value
5. r_{k1} , i.e. the first residual for the series
6. $r(\text{raw})_{k(j-1)}$, i.e. the previous unadjusted residual value
7. $r_{k(j-1)}$, i.e. the previous residual value

There are various heuristic justifications for the forms of the corrections above. The last three, however, should be particularly helpful:

- (i) Because they use residual information, they correct the model by amounts which are proportional to the deviations from observed data rather than amounts proportional to the data itself
- (ii) For a given progress curve, systematic errors will usually be similar across the whole curve
- (iii) The final two adjustments are similar to forms used in time-series analysis to model autocorrelative processes

The last point raises the issue of correlated errors in observations. In kinetics generally the usual assumption made is that errors in observation follow a Gaussian distribution. A scaling of the errors may be assumed (in the present work we assume the variance of the errors is proportional to z_{∞} for each progress curve), but correlation structure between errors is generally ignored. Since we have sequential observations, an assumption of no autocorrelation may require justification. Although the incorporation of autocorrelation into kinetic models is uncommon, it is not unknown (Watts & Bacon, 1974; Matis & Wehrly, 1979).

Further refinement of the corrections described above are possible. For example, adjustments 3, 4, 6 and 7 all involve the previous model or residual value, but do not take account of the fact that the spacing of the data on the time axis may vary. Indeed, this is the case in Fig. 1, where the data were collected so as to be approximately evenly spaced in concentration rather than in time. For such situations, and following the example of Watts & Bacon (1974), the adjustment parameter (α) could be replaced with α' where τ is the time interval between the current and previous observations. We have not yet tested such refinements.

Fig. 1. Progress curves for the fumarase reaction

Reactions were conducted as described in the Methods section, using initial fumarate concentrations of 0.318 (●), 0.249 (■) and 0.199 (▲) mM. The lines represent overall fits of the integrated rate equation for a one-substrate one-product reversible reaction to these, and seven other, progress curves.

METHODS

Test data

Although it is useful to have a theoretical motivation for a model or its adjustment, the practical utility of our proposal is its principal justification. To test the conjecture that adjustments of the kind proposed above would improve models of enzyme progress-curve kinetics, we estimated the eight models (unadjusted plus seven adjusted models) for each of five data sets which are described below.

The data set designated 'PDH' are for the dehydration of prephenate by *Escherichia coli* prephenate dehydratase and are identical with those described by Duggleby & Morrison (1977). The data consist of five progress curves, each of nine observations, and are described by an integrated rate equation with three parameters: V_m (maximum velocity), K_a and K_p (Michaelis and product-inhibition constants). The relationship between these parameters and those in eqn. (1) is: $z_{\infty} = A_0$; $\rho = V_m / (1 - K_a/K_p)$; $\gamma = 0$; and $\delta = K_a[1 + (A_0 + P_0)/K_p] / (1 - K_a/K_p)$, where A_0 and P_0 are the initial concentrations of substrate and product in the assay.

The 'PAP' data concern the hydrolysis of *p*-nitrophenyl phosphate by potato acid phosphatase and, apart from small rounding errors, are identical with those described by Duggleby & Morrison (1977). There are eight progress curves, with 27, 36, 27, 24, 25, 24, 27 and 24 (total 224) observations; the integrated rate equation is the same as for the PDH data set.

The data designated 'FFM' describe the hydration of fumarate to malate by pig heart fumarase and consist of ten progress curves, seven with 18 observations and three with 19 observations (total 183 observations). This reversible reaction is described by four parameters (V_f , K_f , V_r and K_r), the maximum velocity and Michaelis constants for the forward (fumarate to malate) and reverse (malate to fumarate) reactions. Eqn. (1) is defined by the following relationships: $z_{\infty} = A_0 V_f / Q1 K_f$; $\rho = Q1 / Q2$; $\gamma = 0$; and $\delta = 1/Q2 + A_0(V_f/Q1 + 1/Q2)/K_r$, where $Q1 = V_f/K_f + V_r/K_r$ and $Q2 = 1/K_f - 1/K_r$.

The 'FMF' data are also for pig heart fumarase, but in the opposite direction, that is, dehydration of malate to fumarate. The data consist of nine progress curves, six with 18 observations, two with 19 observations and one with 17 observations (total 163 observations). The integrated rate equation is as for data set FFM, but in comparing the results from the two experiments it should be remembered that the definitions of parameters are different, as the forward reaction now refers to the conversion of malate to fumarate.

Fumarase progress curves were collected at 30 °C and at pH 7.5 in mixed 50 mM-Tris/acetate and 25 mM-sodium phosphate buffer. Reactions were started by adding the enzyme to solutions of fumarate (0.050 to 0.744 mM) for the FFM data set, or to solutions of malate (0.199 to 2.472 mM) for the FMF data set. The reaction was monitored by the change in absorbance at 240 nm due to fumarate.

The final data set ('LDH') are for the oxidation of NADH by pyruvate, catalysed by rabbit muscle lactate dehydrogenase; these data are taken from the same original recorder tracings as, but are not exactly identical with, those described by Duggleby & Morrison (1977). They consist of 20 progress curves of 13, 7, 14, 7, 11, 6, 10, 7, 14, 8, 16, 8, 13, 8, 10, 7, 11, 16 and 9 (total 202) observations. Since pyruvate was held at a constant, high concentration, the integrated rate equation for an ordered Bi Bi reaction is simplified and contains only five estimatable parameters, namely V_m , K_a , K_{ip} , K_p^* and K_{iq} . Relating these to the coefficients of eqn. (1) gives: $z_\infty = A_0$; $\rho = V_m/Q1$; $\gamma = (Q2/Q1)/2$ and $\delta = Q3/Q1$, where $Q1 = 1 + P_0/K_{ip} - K_a$; $Q2 = 1/K_{ip} + K_a/(K_p^*K_{iq})$; $Q3 = K_a(1 + A_0/Q4)$; and $Q4 = [1 + (A_0 + P_0)/K_p^*]/K_{iq}$.

Measures of fit

The principal measure of fit of model to data used in estimating parameters of the integrated rate equation was the scaled sum of squared residuals:

$$SSQ(\text{scaled}) = \sum_k \left[\sum_j (r_{kj})^2 / z_{k\infty} \right]$$

However, we have also looked at two other measures of fit, namely (i) the unscaled sum of squared residuals:

$$SSQ(\text{unscaled}) = \sum_k \left[\sum_j (r_{kj})^2 \right]$$

and (ii) the sum of squares of the first-order autocorrelations for the progress curves:

$$SSQ(\text{acf}) = \sum_k (\text{acf}_k)^2$$

where for an individual progress curve:

$$\text{acf} = \frac{\sum_{j=2}^K (r_j r_{(j-1)})}{\sum_{j=1}^K (r_j)^2} \quad (4)$$

and K is the number of observations in the individual progress curve.

As a reference by which to judge the improvement of the fit of the adjusted models, we have also calculated the scaled and unscaled sums of squares which result from subtracting the mean of residuals for a single progress curve from those residuals. That is, we define:

$$r(\text{best})_{kj} = r_{kj} - r(\text{mean})_k \quad (5)$$

where for each individual progress curve:

$$r(\text{mean})_k = \left(\sum_{j=1}^K r_{kj} \right) / K$$

This de-meaning of the residuals for a progress curve provides the 'best possible' adjustment of each series that can be obtained purely by constant vertical displacements of the model for each curve and will be referred to as the 'benchmark' adjustment. We have calculated the percentage improvement toward this benchmark for our adjusted models in terms of both scaled and unscaled sum of squares. It is possible, however, that, by addressing the issue of autocorrelation, the adjusted models may surpass this benchmark adjustment, and indeed we note some examples where this occurs. Moreover, the benchmark adjustment requires as many additional parameters, $r(\text{mean})$, to be used as there are progress curves.

Model fitting

Minimization of the SSQ(scaled) measure of fit described above was carried out using the Nash (1977) modification of the Marquardt (1963) algorithm, as coded in the software system of Nash & Walker-Smith (1987). Scaling was applied within our programs to avoid estimated parameters having widely differing magnitudes. Standard errors for the parameters have been estimated in a conventional manner using the Jacobian inner product matrix inverse (Nash & Walker-Smith, 1987). Such dispersion estimates are the subject of ongoing research into statistical methods [see Donaldson & Schnabel (1987) and references therein].

RESULTS AND DISCUSSION

The sums-of-squares measures of fit (Table 1) show that the adjustments do, in almost every instance, achieve the goal of improved alignment of model with observation. In particular, adjustment 6 succeeds in achieving the greatest improvement for four of the five data sets, and offers a respectable improvement in fit for the PDH data set. The results for the FFM data set were representative of the overall pattern and these are shown in more detail below.

The effect of the various adjustments on the residuals are shown in Fig. 2, where in each panel the residuals are plotted against the initial concentration of fumarate. The unadjusted model is in the upper-left panel, where the tendency for all data points to fall either above or below the fitted line is again seen (cf. Fig. 1). The benchmark adjustment is in the top-centre panel, and the relationship between this and the unadjusted model is clear; each group of residuals is moved up or down in a block, so that the mean is zero. The remaining seven panels in Fig. 2 show adjustments 1–7, and the patterns seen correspond to the conclusions which may be drawn from Table 1. Adjustment 6 (bottom-centre panel) clearly stands out as the best; the spread of residuals is the smallest as is expected, since the sum of squares is smallest (Table 1). Moreover, each group of residuals is scattered around zero, indicating that there is no tendency for the data from each curve to be arranged systematically on one side or the other of the fitted line. This is more clearly illustrated in Fig. 3, which shows the residuals for the three curves given in Fig. 1 for the unadjusted model (open symbols) and for adjustment 6 (closed symbols).

Table 1. Performance of adjusted models under different criteria

An arrow ($\rightarrow$) has been placed beside the adjusted model which has the lowest sum of squares under each measure. The arrow is preceded by an asterisk (*) if this adjusted model has a measure of fit inferior to that found by de-meaning the residuals for each progress curve (benchmark adjustment method; eqn. 5). The percentage value for the unscaled and scaled sum of squares is calculated by setting the unadjusted fit at 0% and the benchmark adjustment at 100%.

Data set	Adjustment method	Unscaled		SSQ(acf)	Scaled	
		SSQ	(%)		SSQ	(%)
PDH	Benchmark	1.71×10^{-3}	100.0	0.671	9.76×10^{-4}	100.0
	Unadjusted	2.81×10^{-3}	0.0	3.199	2.42×10^{-3}	0.0
	1	2.62×10^{-3}	17.7	2.826	2.23×10^{-3}	13.1
	2	2.58×10^{-3}	21.1	3.198	2.24×10^{-3}	12.6
	3	2.69×10^{-3}	11.3	2.825	2.18×10^{-3}	16.6
	4	2.69×10^{-3}	11.3	2.825	2.18×10^{-3}	16.5
	5	* $\rightarrow 1.99 \times 10^{-3}$	74.3	1.819	* $\rightarrow 1.15 \times 10^{-3}$	87.9
	6	2.05×10^{-3}	68.8	$\rightarrow 0.668$	1.18×10^{-3}	86.0
PAP	Benchmark	7.10×10^{-2}	100.0	6.179	2.94×10^{-2}	100.0
	Unadjusted	1.51×10^{-1}	0.0	7.439	7.53×10^{-2}	0.0
	1	1.50×10^{-1}	1.0	7.430	7.51×10^{-2}	0.3
	2	1.21×10^{-1}	37.3	6.667	6.40×10^{-2}	24.5
	3	4.13×10^{-2}	137.1	6.876	3.15×10^{-2}	95.3
	4	4.12×10^{-2}	137.2	6.807	3.15×10^{-2}	95.4
	5	4.11×10^{-2}	137.4	5.223	2.46×10^{-2}	110.4
	6	$\rightarrow 2.20 \times 10^{-3}$	186.0	$\rightarrow 3.673$	$\rightarrow 1.37 \times 10^{-3}$	161.0
FFM	Benchmark	7.21×10^{-4}	100.0	5.282	2.26×10^{-3}	100.0
	Unadjusted	4.58×10^{-3}	0.0	9.038	1.44×10^{-2}	0.0
	1	4.53×10^{-3}	1.4	7.997	1.32×10^{-2}	9.8
	2	4.58×10^{-3}	0.1	9.023	1.44×10^{-2}	0.4
	3	4.57×10^{-3}	0.4	8.990	1.43×10^{-2}	1.1
	4	4.57×10^{-3}	0.3	8.979	1.43×10^{-2}	0.9
	5	2.07×10^{-3}	65.0	6.914	7.02×10^{-3}	60.8
	6	$\rightarrow 1.90 \times 10^{-4}$	113.8	$\rightarrow 2.140$	$\rightarrow 5.17 \times 10^{-4}$	114.3
FMF	Benchmark	1.86×10^{-3}	100.0	3.765	1.66×10^{-3}	100.0
	Unadjusted	6.77×10^{-3}	0.0	6.037	9.09×10^{-3}	0.0
	1	5.40×10^{-3}	28.0	3.713	7.35×10^{-3}	23.5
	2	6.74×10^{-3}	0.6	6.049	9.08×10^{-3}	0.1
	3	6.53×10^{-3}	4.9	6.222	8.96×10^{-3}	1.7
	4	6.59×10^{-3}	3.6	6.177	9.00×10^{-3}	1.2
	5	5.35×10^{-3}	28.9	4.957	5.53×10^{-3}	47.9
	6	$\rightarrow 1.39 \times 10^{-3}$	109.6	$\rightarrow 0.719$	$\rightarrow 9.25 \times 10^{-4}$	109.9
LDH	Benchmark	6.60×10^{-5}	100.0	2.301	6.13×10^{-4}	100.0
	Unadjusted	2.61×10^{-4}	0.0	9.538	2.37×10^{-3}	0.0
	1	2.59×10^{-4}	0.9	9.224	2.36×10^{-3}	0.8
	2	2.60×10^{-4}	0.4	9.592	2.37×10^{-3}	0.3
	3	2.58×10^{-4}	1.6	9.655	2.34×10^{-3}	1.8
	4	2.58×10^{-4}	1.6	9.647	2.34×10^{-3}	1.8
	5	2.60×10^{-4}	0.6	8.293	2.27×10^{-3}	5.7
	6	* $\rightarrow 7.70 \times 10^{-5}$	94.1	* $\rightarrow 2.381$	* $\rightarrow 7.15 \times 10^{-4}$	94.2
	7	1.55×10^{-4}	54.4	4.499	1.44×10^{-3}	53.0

This adjustment markedly reduces the autocorrelations, resulting in residuals which are close to, and roughly equally scattered around, zero.

A quantitative measure of the extent of autocorrelation is the first-order autocorrelation function (eqn. 4). This measures the extent to which each residual is similar to the previous one from the same curve and has an expected value of zero for independent observations. Fig. 4 shows the value of the autocorrelation function for each of the ten curves in the FFM data set, with the unadjusted

model, the benchmark adjustment and adjustment 6. For the unadjusted model, the values are quite high, in all cases greater than 0.9; the sum of squares of these values leads to the high SSQ(acf) for the ten curves of 9.04 given in Table 2. The benchmark adjustment reduces the autocorrelation function for each curve to values between 0.43 and 0.88, and adjustment 6 achieves further reduction to -0.06 to 0.70 .

Thus adjustment 6 of the model results in a better fit (as judged by the reductions in the size of the residuals)

Fig. 2. Residual plots for the scaled fit to the FFM data set

In each of the nine panels, the residual (eqn. 2) is plotted against the initial fumarate concentration in this data set. The dotted line in each indicates a residual of zero where there is exact agreement between the model and the data. The labels 1–7 indicate adjustments 1–7, 0 indicates the unadjusted model, and B indicates the benchmark adjustment.

Fig. 3. Residual plots for the fumarase data

The residual (eqn. 2) was calculated for the three curves shown in Fig. 1, using the unadjusted model (open symbols) and adjustment 6 (closed symbols). The type of symbol used for the curves is as described in Fig. 1.

Fig. 4. Autocorrelations for the scaled fit to the FFM data set

The first-order autocorrelation function (eqn. 4) was calculated for each separate progress curve for the unadjusted model ($\square$), the benchmark adjustment of the model (hatched) and adjustment 6 (striped). The curve numbers are in the order from lowest (0.050 mM, curve 1) to highest (0.744 mM, curve 10) initial fumarate concentrations.

and leaves less pattern in the residuals (as judged by the first-order autocorrelation between the residuals).

Including these adjustments will, of course, change the estimates of the remaining parameters and their standard errors. In the discussion below we will not address the issue of the statistical significance of parameter changes, because there is a degree of uncertainty surrounding estimates of parameters and their standard errors in the presence of non-linearity in the model and correlation of the residuals.

For the specific examples considered here, the changes

in the parameter values are in most cases only a few per cent (Table 2). Since we regard adjustment 6 as the most useful of those considered here, attention will be focused on this adjustment. For three of the five data sets, adjustment 6 results in changes in the parameter values which are quite small, so the adjustment is causing a substantial improvement in the fit without excessive distortion of the parameter estimates. The two cases (PAP and FFM) where we observe somewhat larger changes in the parameter estimates are those that yield improvements in measures of fit that exceed those

Table 2. Parameters values obtained as a result of model adjustment

Adjustment methods 1–7 are as described in the text; method 0 represents the unadjusted fit; in all cases, the scaled sum of squares is minimized. For the FFM data set, the fitted parameters are shown, together with the standard error (where applicable) for each method. The values shown in parentheses after the values for parameters and their standard errors represent the percentage change from the unadjusted fit. For the remaining four data sets, only the results for methods 0 and 6 are shown.

Data set	Method	Parameter ...	V_t (units/mg)	K_t (mM)	V_r (units/mg)	K_r (mM)	α
FFM	0		531.5 ±19.0	0.3114 ±0.0177	347.4 ±136.6	0.8237 ±0.2454	0
	1		623.7 (17) ±35.5 (86)	0.3463 (11) ±0.0223 (26)	153.1 (–56) ±47.3 (–65)	0.4369 (–47) ±0.0824 (–66)	–0.471 ±0.120
	2		546.6 (3) ±28.6 (51)	0.3184 (2) ±0.0206 (16)	291.4 (–16) ±121.7 (–11)	0.7145 (–13) ±0.2220 (–10)	–0.004 ±0.005
	3		495.3 (–7) ±34.3 (80)	0.3233 (4) ±0.0235 (33)	448.2 (29) ±204.7 (50)	0.7617 (–8) ±0.2825 (15)	0.110 ±0.085
	4		494.5 (–7) ±36.9 (94)	0.3213 (3) ±0.0229 (30)	460.0 (32) ±219.6 (61)	0.7887 (–4) ±0.2949 (20)	0.117 ±0.099
	5		479.9 (–11) ±11.0 (–42)	0.2406 (–23) ±0.0102 (–42)	296.7 (–15) ±71.1 (–48)	0.6057 (–26) ±0.1068 (–56)	–3.111 ±0.228
	6		485.6 (–9) ±3.1 (–84)	0.2449 (–21) ±0.0027 (–85)	226.4 (–35) ±13.2 (–90)	0.4773 (–42) ±0.0190 (–92)	–1.008 ±0.015
	7		485.6 (–9) ±10.3 (–46)	0.2449 (–21) ±0.0090 (–49)	226.4 (–35) ±44.2 (–68)	0.4773 (–42) ±0.0637 (–74)	–1.008 ±0.088
		Parameter ...	V_m (units/mg)	K_a (mM)	K_p (mM)	α	
	PDH	0	18.03 ±0.19	0.4718 ±0.0082	4.587 ±0.617	0	
	6		18.02 (0) ±0.14 (–30)	0.4785 (1) ±0.0060 (–28)	5.375 (17) ±0.588 (–5)	–0.755 ±0.115	
	PAP	0	117.1 ±8.3	2.116 ±0.200	0.2624 ±0.0109	0	
	6		137.3 (17) ±1.7 (–80)	3.980 (88) ±0.060 (–70)	0.4909 (87) ±0.0034 (–69)	–1.023 ±0.010	
		Parameter ...	V_t (units/mg)	K_t (mM)	V_r (units/mg)	K_r (mM)	α
FMF	0		591.8 ±50.0	1.221 ±0.149	457.5 ±146.7	0.2704 ±0.0746	0
	6		606.2 (2) ±16.8 (–66)	1.237 (1) ±0.049 (–67)	408.8 (–11) ±38.7 (–74)	0.2418 (–11) ±0.0194 (–74)	–0.974 ±0.026
		Parameter ...	V_m (units/mg)	K_a (μ M)	K_{ip} (mM)	K_p^* (mM)	K_{iq} (μ M)
LDH	0		1177 ±8	11.81 ±0.32	442.9 ±49.3	32.00 ±4.65	109.2 ±9.0
	6		1182 (0) ±4 (–44)	11.96 (1) ±0.18 (–44)	438.6 (–1) ±27.0 (–45)	32.79 (2) ±2.55 (–45)	103.8 (–5) ±4.6 (–50)
							–0.874 ±0.041

obtained from the benchmark vertical translation of the residuals. Thus the explicit accounting for autocorrelation causes the model to be adjusted. Although this might be thought to be a defect of adjustment 6, it is in fact the most compelling reason for using it. To assume wrongly that the observational errors are independent of one another in the analysis of kinetic data guarantees that the fitted parameters are biased. The model adjustments described here, and adjustment 6 in particular, offer a simple and economical means of correcting for correlations in the data.

Conclusions

A simple family of adjustments, and in particular the adjustment by use of the last uncorrected residual (adjust-

ment 6), to integrated rate equations for enzyme-catalysed reactions has been demonstrated by a set of examples to offer considerable improvements in the fit and first-order autocorrelation properties of the models. Although our motivation for using such adjustments is based on these improvements, we hope to stimulate others to find a theoretical basis for these corrections to integrated rate equations.

The fumarase experiments were performed by Chris Wood. This work was supported by grants from the Australian Research Grants Scheme (to R. G. D.) and the Natural Sciences and Engineering Research Council of Canada (to J. C. N.). Some computations were performed on computers belonging to the Department of Mathematics, University of Queensland,

and Applied Mathematics Division, Department of Scientific and Industrial Research, Mt. Albert Research Centre, Auckland, New Zealand.

REFERENCES

- Atkins, G. L. & Nimmo, I. A. (1973) *Biochem. J.* **135**, 779–784
- Boeker, E. A. (1987) *Biochem. J.* **245**, 67–74
- Darvey, I. G., Shrager, R. & Kohn, L. D. (1975) *J. Biol. Chem.* **250**, 4696–4701
- Donaldson, J. R. & Schnabel, R. B. (1987) *Technometrics* **29**, 67–82
- Duggleby, R. G. (1986) *Biochem. J.* **235**, 613–615
- Duggleby, R. G. & Morrison, J. F. (1977) *Biochim. Biophys. Acta* **481**, 297–312
- Duggleby, R. G. & Morrison, J. F. (1978) *Biochim. Biophys. Acta* **526**, 398–409
- Fernley, H. N. (1974) *Eur. J. Biochem.* **43**, 377–378
- Kellershohn, N. & Laurent, M. (1985) *Biochem. J.* **231**, 65–74
- Markus, M., Plesser, T. & Kohlmeier, M. (1981) *J. Biochem. Biophys. Methods* **4**, 81–90
- Marquardt, D. W. (1963) *J. Soc. Ind. Appl. Math.* **11**, 431–441
- Matis, J. H. & Wehrly, T. E. (1979) *Biometrics* **35**, 199–220
- Nash, J. C. (1977) *J. Inst. Math. Appl.* **19**, 231–237
- Nash, J. C. & Walker-Smith, M. (1987) *Nonlinear Parameter Estimation: An Integrated System in BASIC*, Marcel Dekker, New York
- Newman, P. F., Atkins, G. L. & Nimmo, I. A. (1974) *Biochem. J.* **143**, 779–781
- Watts, D. G. & Bacon, D. W. (1974) *Technometrics* **16**, 369–373

Received 13 May 1988/28 June 1988; accepted 14 July 1988