Enzyme kinetic studies from progress curves

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A method is described for fitting the velocities obtained from progress curves to a steady-state rate equation. It is based on the method of Markus & Plesser [(1981) in *Kinetic Data Analysis: Design and Analysis of Enzyme and Kinetic Data* (Edrenyi, ed.), pp. 317–339, Plenum Press, New York]. The obstacle of needing good initial estimates of kinetic parameters is removed by using the parameters provided graphically by a minor modification of the method of Yun & Suelter [(1977) *Biochim. Biophys. Acta* **480**, 1–13]. This progress-curve-based method allows the same discrimination among rival models as do the initial-velocity-based methods, with a great saving of experimental time. The BASIC and FORTRAN77 programs are deposited as Supplementary Publication SUP 50132 (17 pages) at the British Library (Lending Division), Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1986) **233**, 5–6.

INTRODUCTION

Progress curves are a powerful tool for parameter estimation and model discrimination in enzyme kinetics. Their big advantage is that since each curve contains all information concerned with the rate law, much experimentation time may be saved. The information contained in the progress curves can be treated in two different manners. The more direct way is to fit the concentration of the product formed (dependent variable) at each time (independent variable) to the integrated rate law. The second form is to fit the velocities of product formation (dependent variable) for each concentration of substrates and products (independent variables) to the rate equation.

Both methods have a common problem: the errors of the data obtained from progress curves are correlated, and hence the least-squares method (or more robust one) used for fitting the data to the corresponding function is not correct. This correlation is due to the error associated in initial substrate concentration that is unique for each curve and is propagated along all the curve. This fact is readily observed in the Michaelis–Menten equation:

$$v = \frac{V([S]_0 - \Delta[P])}{K + [S]_0 - \Delta[P]}$$

where v is a function of V , K , $[S]_0$ and $\Delta[P]$. Error in $[S]_0$ affects the values of v throughout the curve.

This problem has been solved by Markus & Plesser (1981), who devised a weighting matrix from which a transformation matrix is obtained, which transforms the data with systematic errors and uncorrelated errors into a new data set with uncorrelated errors. The computation of this matrix requires more computer effort, but the reliability of the parameter estimates increases.

Although the computation sequence of both progress curve-based methods is analogous, the time span in fitting product-formed–time data to the integrated rate equation is longer, since the computation of estimates of the dependent variable (product formed at each time) is performed by numerical integration of the rate equation, whereas when fitting initial-velocity–product-formed data the estimates of the dependent variable (velocities) are computed by means of a formula (rate equation).

Despite the large amount of information available from progress curves, they have rarely been used. This is probably due to the difficulties encountered in model diagnostics, implementation on computers and longer time for computation.

In the present paper a program for fitting velocities and the product formed, from progress curves, to the rate equation is given. The program, which uses the weighting matrix devised by Markus & Plesser (1981), is available in BASIC and FORTRAN77. Several approximations for obtaining initial parameter estimates and suitable model (e.g. reversible or irreversible reactions, type of product inhibition) are also propounded.

THEORY AND COMPUTATIONAL METHOD

The Michaelis–Menten equation along a progress curve can be expressed as eqn. (1):

$$v = \frac{V([S]_0 - \Delta[P])}{K + [S]_0 - \Delta[P]} \quad (1)$$

where $\Delta[P]$ is the concentration of product formed at any given time, to which the velocity is v , and $[S]_0$ is the initial concentration of the substrate. Thus, since $v = f([S]_0, K, V, \Delta[P])$, for each progress curve can be defined the function:

$$SS_i = \{v_{\text{exp.}} - f([S]_0, K, V, \Delta[P])\}' W \{v_{\text{exp.}} - f([S]_0, K, V, \Delta[P])\}$$

where $v_{\text{exp.}}$ is the computed v from the progress curve, and the prime (') denotes the transposed matrix; in this case $v_{\text{exp.}} - f([S]_0, K, V, \Delta[P])'$ corresponds to the transposed vector of $v_{\text{exp.}} - f([S]_0, K, V, \Delta[P])$. W is a square weighting matrix whose dimensions are $n \times n$, n being the number of points taken from the progress curve. For parameter estimation $\sum SS_i$ should be minimized.

According to Markus & Plesser (1981) the matrix W should be computed by using:

$$W = [I - H(H'H + I\sigma^2)^{-1}H]/\sigma^2$$

H being the matrix defined by $H_{ij} = \partial v(i)/\partial [S]_{0j}$.

The matrix W can be transformed into the product $W = T'T$, T being a square matrix of dimensions identical with those of W . T is computed by a minor modification of Cholesky's method, and then the function SS_i becomes:

$$SS_i = \{v_{\text{exp.}} - f([S]_0, K, V, \Delta[P])\} T'T \{v_{\text{exp.}} - f([S]_0, K, V, \Delta[P])\}$$

and the matrix $T\{v_{\text{exp.}} - f([S]_0, K, V, \Delta[P])\}$ corresponds to the uncorrelated residuals for each progress curve.

It should be noted that for each progress curve the Jacobian matrix of the residuals and the product of $J' \times J$ are computed; gradient is also computed. All information matrices and gradient vectors (corresponding to each progress curve) are computed, and then the sum is obtained. From this point the minimization is carried out by using the method previously described by Canela (1984).

The program described in the present paper needs more iterations than does the analogous program (Canela, 1984) without the weighting matrix propounded by Markus & Plesser (1981). The difference is probably due to the fact that the transformed data define a error surface more curved than that defined by the untransformed data.

The method described has been implemented in BASIC and FORTRAN77. Both programs have been extensively used, on a Tektronix 4051 and on a VAX 11/750 computer respectively. In the present version, the BASIC program needs the corresponding Matrix ROM package.

The memory required for implementation and execution in BASIC is less than 32 kilobytes. When the requirement is greater the time span for minimization also increases, and then the FORTRAN77 version on a computer is suggested.

The time required for execution depends on the model complexity, number of parameters, number of curves, number of products and data points (see the Results section).

The simulation of absorbance-time curves was carried out by means of a BASIC program of numerical

by fitting the data to a polynomial equation. For choosing the best we have simulated several curves with known velocities for each time and have computed these velocities by the three methods. The results showed that the best method is to fit the absorbance values on a fifth-degree polynomial equation and then to obtain the velocities by means of spline differentiation (the program in BASIC for this purpose is available on request). The fitting of the absorbance values to the polynomial equation allows one to avoid the 'noise' in the progress curve due to the device used in the experiment.

Selecting the model

A graphical method is propounded in order to establish the model and the best estimates of the parameters to start the regression program. We have studied the different classical plots (i.e. v versus $[S]$, $1/v$ versus $1/[S]$, $v/[S]$ versus v and $[S]/v$ versus $[S]$), $[S]$ being obtained from the difference between the initial substrate concentration and the formed product (this assumption implies considering correct the initial concentration of the substrate). We have studied the more classical cases [competitive (eqn. 2), uncompetitive (eqn. 3) and mixed (eqn. 4) inhibition of the enzyme by the product in irreversible reactions] and have found that the best plot for this purpose is $1/v$ versus $1/[S]$ (Fig. 1). This choice is justified by two reasons. The first is the fact that errors in $[S]_0$ produce deviations in the plots, these deviations being more important in the zone of low substrate concentration; however, in the extrapolations corresponding to the plot of $1/v$ versus $1/[S]$ they have a lesser effect and therefore the variations of the initial estimates of the parameters are hardly significant. The second reason is that the extrapolations (asymptotes and tangents) necessary to obtain the parameters are more easily obtained with this plot than with the remaining ones. Likewise, the plot allows discrimination among the different inhibition patterns.

$$v = \frac{V([S]_0 - \Delta[P])}{K\{1 + ([P]_0 + \Delta[P])/K_{p(s)}\} + [S]_0 - \Delta[P]} \quad (2)$$

$$v = \frac{V([S]_0 - \Delta[P])}{K + ([S]_0 - \Delta[P])\{1 + ([P]_0 + \Delta[P])/K_{p(i)}\}} \quad (3)$$

$$v = \frac{V([S]_0 - \Delta[P])}{K\{1 + ([P]_0 + \Delta[P])/K_{p(s)}\} + ([S]_0 - \Delta[P])\{1 + ([P]_0 + \Delta[P])/K_{p(i)}\}} \quad (4)$$

integration of differential equations (Franco & Canela, 1984a).

RESULTS

Processing of the data

From progress curves, pairs of values (product formed-time) are obtained. Nevertheless for the program described here pairs of values (velocity-product formed) are required. The procedure used was to determine the best method for obtaining the initial velocities corresponding to each point on the progress curve. Logically the only exact data are the time values; however, the increments of absorbance at each time also are sufficiently exact. The increments of absorbance correspond to the product formed at each time. Three methods could be used for computing the velocities: from tangents of the curves (graphically), by means of a spline function and

When product inhibits competitively, we have also considered the presence of an inhibitor (competitive, uncompetitive and mixed). It was observed that discrimination among models and estimation of the kinetic constants can be graphically obtained (Fig. 2; competitive case).

Also, we have studied the reversible transformation of S into P (eqn. 5) showing that the plots of v versus $[S]$ and $1/v$ versus $1/[S]$ allow good estimates of the kinetic parameters to be obtained (Fig. 3). The graphical method suggested here enables one to obtain all the kinetic parameters of the reversible equation without knowing previously K_{eq} or any other kinetic parameter, in contrast with that indicated by Yun & Suelter (1977).

$$v = \frac{V_r([S]_0 - \Delta[P]) - V_r([P]_0 + \Delta[P])/K_{\text{eq}}}{K_s + ([S]_0 - \Delta[P]) + V_r([P]_0 + \Delta[P])/(V_r \cdot K_{\text{eq}})} \quad (5)$$

Fig. 1. Product inhibition in irreversible reactions

The pairs of values v and $\Delta[P]$ were calculated from product-formed-time simulated progress curves, obtained from the rate equation by means of a program of numerical integration of differential equations (Franco & Canela, 1984a). The plotted values of $[S]$ were obtained subtracting the value of $\Delta[P]$ from the exact value of the initial substrate concentration. In all the cases the initial substrate concentration was 1.0×10^{-4} M. (a) Competitive inhibition. The exact parameters were: $V = 1.0$ $\mu\text{mol/min}$; $K_m = 5.0 \times 10^{-5}$ M; $K_{p(s)} = 2.0 \times 10^{-4}$ M. (b) Uncompetitive inhibition. The exact parameters were: $V = 1.0$ $\mu\text{mol/min}$; $K_m = 5.0 \times 10^{-5}$ M; $K_{p(i)} = 1.0 \times 10^{-4}$ M. (c) Mixed inhibition. The exact parameters were: $V = 1.0$ $\mu\text{mol/min}$; $K_m = 5.0 \times 10^{-5}$ M; $K_{p(s)} = 3.0 \times 10^{-4}$ M; $K_{p(i)} = 1.0 \times 10^{-4}$ M.

Fig. 2. External competitive inhibition in irreversible reactions

The pairs of values v and $\Delta[P]$ were calculated from product-formed-time simulated progress curves, obtained from the rate equation by means of a program of numerical integration of differential equations (Franco & Canela, 1984a). The plotted values of $[S]$ were obtained subtracting the value of $\Delta[P]$ from the exact value of the initial substrate concentration. The initial substrate concentration was 1.0×10^{-4} M, and reaction product inhibited competitively. The exact parameters were: $V = 1.0 \mu\text{mol/min}$; $K_m = 5.0 \times 10^{-5}$ M; $K_{p(s)} = 2.0 \times 10^{-4}$ M; $K_i = 1.3 \times 10^{-4}$ M.

Fitting the data

In order to fit the data to the rate equation by using the program we have simulated several cases of the above models assuming different random errors in the initial concentrations of the products. The initial velocities were computed by means of the fit of the progress-curve data to a polynomial equation. The calculated velocities and substrate concentrations were plotted as described above (see Figs. 1 and 2). The initial parameters to be estimated were determined. With the use of these data the pairs of values velocity-product formed were fitted to the rate equation by using the described program in the FORTRAN77 version. A maximum of ten pairs of values evenly distributed along each progress curve were used. The results appear in Table 1. It is shown that good estimate values of the parameters (V , K_m and K_i for product inhibition) can be obtained with this program even with a considerable error in the initial substrate concentration. In general, it is observed that better parameter values are obtained with an initial substrate concentration higher than the real value than with a lower concentration.

In order to compare the simulated cases with an experimental one we have studied the transformation of

adenosine into inosine catalysed by adenosine deaminase (EC 3.5.4.4). This reaction is well characterized: it is irreversible, and inosine inhibits competitively with regard to adenosine. The results obtained with two progress curves and eight pairs of values per curve agree with the previous data (obtained by the initial-velocities method; results not shown) and demonstrated the usefulness of this method (Table 2). The curves were obtained in an HP 8450A UV/VIS spectrophotometer provided with a thermostatically controlled cell holder (89100A temperature controller) and a 7470A plotter. Time zero was considered to be when 0.1 ml of enzymic solution was added to 2.9 ml of the substrate solution. This was possible, since the HP 8450A UV/VIS spectrophotometer allows stirring of the reaction mixture without delay. If a less sophisticated spectrophotometer is used we recommend starting the recording with a delay of 15 or 30 s after the addition of the enzyme solution. Afterwards an extrapolation to time zero should be carried out if the reaction products inhibit the enzyme. It should be noted that the K_m obtained is greater than the value published by Spector (1984); however, that author fitted the data to the Michaelis-Menten equation without considering the inhibition by inosine; operating in this manner we have obtained a similar value for K_m , but this differs from the real behaviour of this enzyme.

Designing the experiment

We have studied the number of curves necessary to obtain a good fit. The result showed that for the above cases three curves should often be sufficient (see below). It is important to use a curve with the maximum $[S]_0$ possible. If the error in $[S]_0$ is not very large and curves are well selected, no further improvement is achieved on increasing the number of curves. However, it should be noted that the number of curves depends on the enzyme model.

Another question is the number of pairs of values from each curve that should be fitted. In general a number of points ranging between seven and 12 would be suitable. The minimum number of points of each curve has to be equal to or greater than the number of parameters plus the number of the reactants (substrates and products added into the cell reaction).

In order to illustrate this aspect we have studied the uncompetitive-inhibition case ($V = 1.00 \mu\text{mol/min}$, $K_m = 5.00 \times 10^{-5}$ M and $K_{ii} = 1.00 \times 10^{-4}$ M). We have assumed that the maximum substrate concentration ($[S]_0$) allowable is 2.50×10^{-4} M, and have studied different possible sets of curves and series of points, measuring the value of the determinant of the information matrix, which is a criterion of optimal design of experiments (Box & Lucas, 1959; Fedorov, 1972; Markus & Plessner, 1976; Canela, 1985). The maximum determinant value of the information matrix is obtained when the number of curves is maximum; however, the increment of the determinant value when increasing the number of curves is asymptotic, i.e. the increment is not proportional to the number of curves. In arbitrary units, with five curves and 15 points each ($[S]_0 = 2.50 \times 10^{-4}$, 2.00×10^{-4} , 1.00×10^{-4} , 0.50×10^{-4} and 0.10×10^{-4} M) the determinant value is 2.4×10^{15} , with four curves (1, 2, 3 and 4) is 2.3×10^{15} , with three curves (1, 2 and 4) is 1.2×10^{15} and with two curves (1 and 4) is 3.6×10^{14} . When the number of points taken from the curves is lower, the value of the determinant increases in similar form, and thus for the

Fig. 3. Reversible reaction

The pairs of values v and $\Delta[P]$ were calculated from product-formed-time simulated progress curves, obtained from the rate equation by means of a program of numerical integration of differential equations (Franco & Canela, 1984a). The plotted values of $[S]$ were obtained subtracting the value of $\Delta[P]$ from the exact value of the initial substrate concentration. The initial substrate concentration was $1.0 \times 10^{-4} \text{ M}$. The exact parameters were: $V_f = 10.0 \mu\text{mol/min}$; $V_r = 2.0 \mu\text{mol/min}$; $K_s = 5.0 \times 10^{-5} \text{ M}$; $K_p = 2.5 \times 10^{-4} \text{ M}$; $K_{eq} = 25$. (a) Plot of v versus $[S]$. (b) Plot of $1/v$ versus $1/[S]$.

three-curves case (1, 2 and 4) and ten points each the value is 7.3×10^{14} and with seven points each the value is 3.6×10^{14} . From these results it follows that four curves should be an ideal optimum between experimentation time and information; however, if the experimental error is not great three curves might be sufficient. Another question is the number of points taken from each curve: increasing the number of points does not increase the experimentation time, but the computation time increases. We suggest the use of ten points evenly distributed, from which accurate results should be obtained.

DISCUSSION

It has been pointed out that progress curves can be used in kinetic studies when the enzyme remains completely stable during at least 85% of the reaction, when the reaction is irreversible, and when product(s) is (are) non-inhibitory (Spector, 1984). The first statement is essentially correct and the stability must be confirmed by the Selwyn (1965) test. Given this fact, the other statements are not correct. Thus in this and in other papers (Bates & Frieden, 1973a,b; Philo & Selwyn, 1973; Darvey *et al.*, 1975; Duggleby & Morrison, 1977; Orsi & Tipton, 1979; Markus *et al.*, 1980; Markus & Plessner, 1981; Boiteaux *et al.*, 1981, 1983; Boeker, 1982) it has been demonstrated that, even when the reaction is

reversible and when the product inhibits, which often occurs, the progress curves are an excellent method for obtaining good parameter estimates with an important saving of experimental time.

Likewise this method should be a previous step in order to study complex systems by using the integrated-rate-law method (Duggleby, 1983); for example, in a coupled system each reaction can be studied by this method so as to obtain the kinetic parameters and mechanism of each enzyme in a short time, and then with the integrated-rate-law method to study the coupled model. Moreover, when individual enzymes are characterized the results can be extended by using the integrated law to very complex systems that at present can only be studied by means of computer simulation (Franco & Canela, 1984b).

The purpose of this method is not to replace that based on the integrated equation but to make possible the use of the information contained in the progress curves for those enzymologists having a microcomputer, since the BASIC version of this method would be easily implemented. The only problem of using this is the large amount of time consumed by the BASIC version; however, the times given (Table 2) have been obtained with a relatively slow microcomputer, and with the Tektronix 4052 the time should be shortened 6-7-fold.

We believe that the method propounded here should be useful, and much improvement should be achieved

Table 1. Calculated parameters from different initial substrate concentrations

Product concentrations at each time were obtained with real parameters and initial substrate concentrations. From these progress curves velocities were calculated (see the text). Velocities and product concentrations were fitted to the corresponding rate equation assuming error in the initial substrate concentration. The parameters obtained with real substrate concentrations are not shown because they were fully exact.

(a) Competitive inhibition			(b) Uncompetitive inhibition			(c) Mixed inhibition		
Real parameters	Real substrates		Real parameters	Real substrates		Real parameters	Real substrates	
$V = 1.00 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.00 \times 10^{-4} \text{ M}$		$V = 1.00 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.00 \times 10^{-4} \text{ M}$		$V = 1.00 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.00 \times 10^{-4} \text{ M}$	
$K_m = 5.00 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.50 \times 10^{-4} \text{ M}$		$K_m = 5.00 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.50 \times 10^{-4} \text{ M}$		$K_m = 5.00 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.50 \times 10^{-4} \text{ M}$	
$K_{is} = 1.00 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.50 \times 10^{-4} \text{ M}$		$K_{is} = 1.00 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.50 \times 10^{-4} \text{ M}$		$K_{is} = 3.00 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.50 \times 10^{-4} \text{ M}$	
Calculated parameters			Calculated parameters			Calculated parameters		
$V = 1.00 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.10 \times 10^{-4} \text{ M}$		$V = 1.00 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.10 \times 10^{-4} \text{ M}$		$V = 1.00 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.10 \times 10^{-4} \text{ M}$	
$K_m = 5.00 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.65 \times 10^{-4} \text{ M}$		$K_m = 4.90 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.65 \times 10^{-4} \text{ M}$		$K_m = 5.20 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.65 \times 10^{-4} \text{ M}$	
$K_{is} = 1.00 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.75 \times 10^{-4} \text{ M}$		$K_{is} = 1.04 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.75 \times 10^{-4} \text{ M}$		$K_{is} = 4.51 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.75 \times 10^{-4} \text{ M}$	
Assumed substrates			Assumed substrates			Assumed substrates		
$V = 1.00 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.20 \times 10^{-4} \text{ M}$		$V = 1.01 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.20 \times 10^{-4} \text{ M}$		$V = 1.02 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.10 \times 10^{-4} \text{ M}$	
$K_m = 5.00 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.80 \times 10^{-4} \text{ M}$		$K_m = 4.86 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.80 \times 10^{-4} \text{ M}$		$K_m = 5.20 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.65 \times 10^{-4} \text{ M}$	
$K_{is} = 1.02 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 3.00 \times 10^{-4} \text{ M}$		$K_{is} = 1.10 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 3.00 \times 10^{-4} \text{ M}$		$K_{is} = 4.51 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.75 \times 10^{-4} \text{ M}$	
Calculated parameters			Calculated parameters			Calculated parameters		
$V = 1.00 \mu\text{mol/min}$	Curve 1 $[S]_0 = 0.90 \times 10^{-4} \text{ M}$		$V = 1.19 \mu\text{mol/min}$	Curve 1 $[S]_0 = 0.90 \times 10^{-4} \text{ M}$		$V = 1.02 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.10 \times 10^{-4} \text{ M}$	
$K_m = 5.00 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.35 \times 10^{-4} \text{ M}$		$K_m = 9.03 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.35 \times 10^{-4} \text{ M}$		$K_m = 5.20 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.65 \times 10^{-4} \text{ M}$	
$K_{is} = 1.01 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.25 \times 10^{-4} \text{ M}$		$K_{is} = 1.08 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.25 \times 10^{-4} \text{ M}$		$K_{is} = 4.51 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.75 \times 10^{-4} \text{ M}$	
Assumed substrates			Assumed substrates			Assumed substrates		
$V = 1.01 \mu\text{mol/min}$	Curve 1 $[S]_0 = 0.80 \times 10^{-4} \text{ M}$		$V = 1.19 \mu\text{mol/min}$	Curve 1 $[S]_0 = 0.90 \times 10^{-4} \text{ M}$		$V = 1.02 \mu\text{mol/min}$	Curve 1 $[S]_0 = 1.10 \times 10^{-4} \text{ M}$	
$K_m = 5.03 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.20 \times 10^{-4} \text{ M}$		$K_m = 9.03 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.35 \times 10^{-4} \text{ M}$		$K_m = 5.20 \times 10^{-5} \text{ M}$	Curve 2 $[S]_0 = 1.65 \times 10^{-4} \text{ M}$	
$K_{is} = 1.08 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.00 \times 10^{-4} \text{ M}$		$K_{is} = 1.08 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.25 \times 10^{-4} \text{ M}$		$K_{is} = 4.51 \times 10^{-4} \text{ M}$	Curve 3 $[S]_0 = 2.75 \times 10^{-4} \text{ M}$	

Table 2. Calculated parameters for adenosine deaminase

Product concentrations at each time were obtained from two progressive curves, whose initial substrate concentrations are indicated. Product concentrations and calculated velocities (see the text) were fitted to the rate equation corresponding to competitive inhibition. The experiment was performed with commercial adenosine deaminase in 0.1 M-Tris/HCl buffer, pH 7.4, in 1 cm-pathlength cells, in a HP 8450A UV/VIS spectrophotometer provided with a thermostatically controlled ($30 \pm 0.1^\circ \text{C}$) cell holder (89100A temperature controller) and a 7470A plotter.

Curve 1	$[\text{Adenosine}]_0 = 1.0 \times 10^{-4} \text{ M}$
Curve 2	$[\text{Adenosine}]_0 = 5.0 \times 10^{-5} \text{ M}$
V	$0.0359 \pm 0.0005 \mu\text{mol/min}$
K_m	$1.95 \times 10^{-5} \pm 0.10 \times 10^{-5} \text{ M}$
K_{is}	$2.13 \times 10^{-4} \pm 0.74 \times 10^{-4} \text{ M}$
Time span	
BASIC (Tektronix 4051)	8 min
FORTAN77 (VAX 11/750)	7 s

with an intelligent management; for instance, Duggleby & Morrison (1978) devised a technique of product recycling, in order to study the reaction catalysed by aspartate aminotransferase, that allows saving of time and easier computing in complex models.

CONCLUSIONS

The program described in this paper easily allows the use of the information contained in the progress curve for calculating estimates of the kinetic parameters. The program uses pairs of values velocity-product formed, and in this sense is not beyond the classical kinetic treatments. Furthermore, with the values of velocity-substrate concentration along the progress curve a classical Lineweaver-Burk plot ($1/v$ versus $1/[S]$) allows discrimination among rival models and estimation of the parameter values. The whole kinetic treatment can be done with two to four cuvettes in a single spectrophotometric assay.

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