Progress-curve analysis in enzyme kinetics

Numerical solution of integrated rate equations

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Progress curves of enzyme-catalysed reactions are described by equations of a type that precludes direct calculation of the extent of reaction at any time. Previously, such equations have been solved by the Newton–Raphson method, but this procedure may fail when based upon the usual formulae. An alternative formulation is proposed that is both quicker and more robust.

INTRODUCTION

The rapid growth in the electronics industry has had an enormous impact on many laboratory instruments. If it is not already, a spectrophotometer either containing or connected to a microcomputer will soon be standard equipment in a biochemical laboratory. It seems probable that the availability of such equipment will promote interest in the analysis of the progress curves of enzyme-catalysed reactions as a means to determine enzyme kinetic parameters (see, e.g., Hasinoff, 1985).

Fernley (1974) proposed that progress curves could be analysed by fitting the integrated Michaelis–Menten equation to the data. Although Fernley's (1974) method can only be applied directly to irreversible single-substrate reactions that do not exhibit product inhibition, later developments (Darvey *et al.*, 1975; Duggleby & Morrison, 1977, 1978) now permit a much wider range of reactions to be analysed in this way.

For single-substrate reactions, or two-substrate reactions that are conducted in such a way that the concentration of one of the substrates does not change appreciably during the reaction, the progress curve is described by eqn. (1):

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

In this equation z represents the amount of product formed by reaction at any time (t) and z_∞ is the amount formed at equilibrium. The constants ρ , γ , δ and z_∞ are related to the usual kinetic parameters and the initial concentrations of substrates and products. In the simplest case of a single-substrate irreversible reaction that is not inhibited by products, ρ is the maximum velocity, δ is the Michaelis constant, γ is zero and z_∞ equals the initial substrate concentration.

Fitting of an integrated rate equation necessarily involves repeated calculation of the expected value of z for particular values of ρt , γ , δ and z_∞ . This is not a straightforward task, since eqn. (1) is an implicit function of z . Even in the simplest case where γ is zero, the presence of both linear and logarithmic terms in z precludes rearrangement of eqn. (1) into a form where z is expressed as a function of the remaining quantities; consequently, z must be approximated by using an iterative procedure.

Fernley (1974) and later workers (Darvey *et al.* 1975; Duggleby & Morrison, 1977; Kellershohn & Laurent, 1985) have all employed the Newton–Raphson method, which involves taking an initial estimate of z that

approximates the true value and from this calculating a revised, and hopefully better, value. Under certain circumstances the Newton–Raphson method will fail because the revised value of z exceeds z_∞ , whereupon the logarithmic term becomes undefined. In the present paper I briefly describe the Newton–Raphson method as it is usually formulated and show why it sometimes fails, then propose an alternative formulation that is virtually immune to failure.

THEORY, RESULTS AND DISCUSSION

Calculating a value of z that satisfies eqn. (1) is equivalent to finding a value for which F_1 , as defined by eqn. (2), equals zero:

$$F_1 = z + \gamma z^2 - \delta \ln(1 - z/z_\infty) - \rho t \quad (2)$$

Fig. 1. Dependence of F upon z for two versions of the Newton–Raphson method

The function F , which represents (a) F_1 as defined by eqn. (2) or (b) F_2 as defined by eqn. (7), is plotted against z for the case where $\rho t = 4.2$, $\gamma = 0$, $\delta = 2.5$ and $z_\infty = 1.0$; the solution, where $F = 0$, is at $z = 0.748566$. In (a), one iteration of the Newton–Raphson method is illustrated; starting with $z^+ = 0.9$, F_1 is calculated (indicated by the length of the ----- line) from eqn. (2) and the slope (F'_1 ; — line) is found by using eqn. (3). A revised value ($z^* = 0.805521$) is then obtained by using eqn. (4).

Table 1. Performance of two variants of the Newton-Raphson method

The Newton-Raphson method, as defined by either eqns. (2) and (3) or by eqns. (6)–(8), was applied using initial estimates as shown for the case where $\rho t = 4.2$, $\gamma = 0$, $\delta = 2.5$ and $z_\infty = 1.0$. The correct solution is $z = 0.748566$; values of z^* after each iteration are shown. Where convergence was achieved in two iterations and a third was not required, this is indicated by N.R.

Method	Iteration	Initial estimate (z^+)					
		-1.0	0.1	0.5	0.9	0.9999	2.0
Eqns. (2) and (3)	1	2.08127	1.11557	0.827855	0.805521	0.999107	Failure
	2	Failure	Failure	0.761732	0.755062	0.993982	
	3			0.748886	0.748643	0.970984	
	4			0.748566	0.748566	0.906496†	
Eqns. (6)–(8)	1	0.785828	0.755183	0.749632	0.749000	0.749789	0.786545
	2	0.748591	0.748567	0.748566	0.748566	0.748566	0.748592
	3	0.748566	0.748566	N.R.	N.R.	N.R.	0.748566

† Converged after a total of eight iterations.

The subscript 1 is used to indicate that eqn. (2) contains a logarithmic term. It is shown below that an alternative function (F_e) can be defined that contains an exponential term instead. As can be seen from Fig. 1(a), F_1 is a curved function of z , reaching $-\rho t$ when z equals zero and approaching infinity as z tends to z_∞ . That eqn. (2) will always describe a curve of this general shape can easily be appreciated by noting that it is simply an ordinary progress curve (z versus time) that has been reflected, rotated through 90° and then rescaled on the new ordinate. The slope (F'_1) of the curve in Fig. 1(a) at any point can be found by differentiating F_1 with respect to z , yielding eqn. (3):

$$F'_1 = 1 + 2\gamma z + \delta/(z_\infty - z) \quad (3)$$

The basis of the Newton-Raphson method is illustrated by the two broken lines in Fig. 1(a); a revised value (z^*) is obtained from an initial estimated value (z^+) by calculating F_1 and F'_1 at this estimate and using the ratio as a correction as shown by eqn. (4):

$$z^* = z^+ - F_1/F'_1 \quad (4)$$

If the initial estimate is now replaced by z^* , the process can be repeated until correction makes no appreciable difference to z^* .

From simple geometry, it is clear that, if z^+ is greater than the correct value of z but less than z_∞ , then z^* will be a better estimate of, but still greater than, the true value. It follows that the Newton-Raphson method will converge towards a close approximation of the solution under these circumstances. On the other hand, if z^+ is less than the true value, then z^* will be greater than the solution and possibly further from it than was z^+ . In the worst case, and the one with which the present paper is concerned, when z^+ is below a critical value then z^* will be greater than z_∞ . This revised value cannot be used to start a second iteration because F_1 becomes undefined owing to the presence of the term containing the logarithm of a negative number. One cannot avoid this situation by the simple expedient of choosing z^+ such that it is greater than the true value, since the latter is unknown.

It was noted in the preceding discussion that the Newton-Raphson method requires a function to be defined such that it will equal zero when a solution is found. For integrated rate equations, this is normally done by subtracting ρt from both sides of eqn. (1), but it has just been shown that this leads to a method that is by no means foolproof. The problem arises because of the logarithmic term, and this term may be eliminated as follows. First, eqn. (1) is rearranged to give eqn. (5):

$$\ln(1 - z/z_\infty) = \beta \quad (5)$$

where

$$\beta = (z + \gamma z^2 - \rho t)/\delta \quad (6)$$

Exponentiation of eqn. (5) yields a new function (F_e) and its derivative (F'_e), which are given as eqns. (7) and (8):

$$F_e = z/z_\infty - 1 + \exp(\beta) \quad (7)$$

$$F'_e = 1/z_\infty + (1 + 2\gamma z) \cdot \exp(\beta)/\delta \quad (8)$$

This definition of F_e (eqns. 7 and 6) is much preferable to eqn. (2) because F_e can be calculated for any value of z , including values above z_∞ and negative values (Fig. 1b).

The performance of the original method and that proposed in the present paper are compared in Table 1. The original method fails when z^+ is below the critical value (0.2442 in this example) and makes poor progress when z^+ is close to z_∞ (1.0). This latter result is of some importance, because it means that one cannot choose z^+ so that it is close to z_∞ in an attempt to select an initial estimate that is greater than the solution. By contrast, the method based on eqns. (6)–(8) successfully converges for all values of z^+ , including absurd values such as $-z_\infty$ or $2z_\infty$. Moreover, it converges rapidly to the solution requiring no more than two or three iterations, whereas the original method requires at least twice as many. This increase in speed, which was seen in almost all cases that were examined, is a direct consequence of the fact that F_e is close to a linear function of z (Fig. 1b) whereas F_1 is strongly curved (Fig. 1a). Thus the Newton-Raphson method proposed here is not only substantially faster than the original one, but it is also more robust and has failed on only three occasions in over 50000 trials.

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