An easy method for the determination of initial rates

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When the Michaelis–Menten equation is obeyed, the rate near the beginning of an enzyme-catalysed reaction (or of an experiment on transport) can be found accurately from the slope of a chord joining two points on the progress curve. This slope gives the rate at an intermediate concentration. Exact values of this intermediate concentration are easily calculated from equations in the text, and a number of values have also been tabulated. Methods of using two chords to find the initial rate are given. A mid-point formula for numerical differentiation is advocated when the Michaelis–Menten equation does not hold.

An estimate of the rate at (or near) the start of the experiment is generally required in enzyme-catalysed reactions and studies on transport into cells. The usual method, determination of the slope of the tangent at the origin of the progress curve, is even more unreliable than might be expected (Walter & Barrett, 1970; Philo & Selwyn, 1973; Nimmo & Mabood, 1979; Atkins & Nimmo, 1980), but has yet to be supplanted. It goes on being used owing to the absence of an easy alternative. The method to be described is based on the form of the integrated Michaelis–Menten equation. Fortunately, the form of this equation describes the course of progress curves for a wide variety of mechanisms (Orsi & Tipton, 1979), and indeed the equation has been recommended as a basis for estimating the initial rate (Cornish-Bowden, 1975; Nimmo & Atkins, 1978). An exact method was used by these workers when the results were being processed by computer. The advantage claimed for the procedure now to be described is that it combines the simplicity of an approximate method with the accuracy of an exact one.

Basis of the method

Chords and 'corresponding' concentrations

The integrated Michaelis–Menten equation is:

$$t = A(s_0 - s) + B \ln \left(\frac{s_0}{s} \right) \quad (1)$$

where s is the concentration of substrate at time t , and s_0 is the initial substrate concentration; A and

B are parameters. Eqn. (1) holds for an (effectively) irreversible one-substrate reaction (and then $A = 1/V_{\max}$, and $B = K_m/V_{\max}$), or where there are two or more substrates, as long as all but one are at saturating concentrations, or when an inhibitor is present, or when the product is an inhibitor (as long as it is a competitive one) (Orsi & Tipton, 1979). Eqn. (1) describes the progress curve for the disappearance of substrate; when the appearance of product is being monitored, eqn. (2) is more convenient:

$$t = Ap - B \ln [1 - (p/s_0)] \quad (2)$$

where p is the concentration of product at time t . If the reaction is reversible, eqn. (2) holds when s_0 is replaced by p_e , the concentration of product at equilibrium. Now, although it is hard to draw a tangent, it is easy to draw a chord (Fig. 1). The slope of a chord joining the points (s_1, t_1) and (s_2, t_2) on a progress curve of substrate concentration against time is $(s_1 - s_2)/(t_1 - t_2)$, and this slope is equal to the rate (i.e. the slope of the tangent) at the 'corresponding' concentration s_3 , given by:

$$s_3 = \frac{s_1 - s_2}{\ln \left(\frac{s_1}{s_2} \right)} \quad (3)$$

The basis for eqn. (3) is as follows. At the corresponding concentration of substrate, the reciprocal rate is:

$$\frac{dt}{ds} = -A - \frac{B}{s_3}$$

and the slope of the chord is:

$$\frac{t_2 - t_1}{s_2 - s_1} = \frac{A(s_2 - s_1) + B \ln \left(\frac{s_2}{s_1} \right)}{s_2 - s_1}$$

$$= -A - B \frac{\ln \left(\frac{s_2}{s_1} \right)}{s_2 - s_1}$$

These two expressions become equal when the corresponding concentration has the value given by eqn. (3). When the reaction is being monitored by the change in absorbance, it may be convenient to find the corresponding concentration of substrate from eqn. (4):

$$s_3 = \left(\frac{s_0}{a_0 - a_\infty} \right) \frac{a_1 - a_2}{\ln \left(\frac{a_1 - a_\infty}{a_2 - a_\infty} \right)} \quad (4)$$

where a_0 , a_1 , a_2 and a_∞ are the absorbances at the times denoted by the suffixes, and the chord in question joins the points (a_1, t_1) and (a_2, t_2) .

When the concentration of product is being measured, eqn. (2) is used, and the slope of the chord

joining (p_1, t_1) and (p_2, t_2) gives the rate at the corresponding concentration of product, p_3 , which is found from eqn. (5):

$$p_3 = s_0 - \frac{p_2 - p_1}{\ln \left(\frac{s_0 - p_1}{s_0 - p_2} \right)} \quad (5)$$

Use of the method

Rates

A chord is drawn, the slope is measured, and the corresponding concentration then found from eqns. (3), (4) or (5), or from Table 1. For example, the slope of a chord drawn between 5% reaction and 25% reaction gives the rate at 15.39% reaction (Table 1). This is exact, as long as eqn. (1) is obeyed.

Single-point assays are sometimes used, particularly in measurements of transport. Atkins & Nimmo (1978) recommended the use of two datum points and the procedure of Cornish-Bowden (1975). The use of one datum point, however, suffices if the present method is used. Thus, if the fractional extent of reaction, f , is measured ($f = p/s_0$) after time t , the corresponding fractional extent of reaction (g) is:

$$g = 1 + \frac{f}{\ln(1-f)}$$

Fig. 1. Progress curve showing the 'corresponding' concentration

The continuous curve is drawn from eqn. (2) in the text, with $s_0 = 5$, $A = 1$, $B = 10$ (in arbitrary units). The line (---) joining the points at 0 and 50% is the chord, whose slope gives the rate at 27.87% reaction (Table 1), the 'corresponding' concentration (marked with an arrow). At this point, the tangent (---) is parallel to the chord.

Kinetic parameters

The values for the rates and corresponding concentrations of substrates may be further processed by the direct linear plot (Eisenthal & Cornish-Bowden, 1974; Cornish-Bowden, 1979; Atkins & Nimmo, 1980). If the likelihood of error in both variables (velocity and substrate concentration) is regarded as a serious drawback, then, as described by Cornish-Bowden (1975), the values of initial rate and corresponding substrate concentration can be used to obtain provisional values for A and B , which are then used to calculate the rate (v_0) at the well-defined initial substrate concentration, from $v_0 = (A + B/s_0)^{-1}$. There are other occasions when the initial rate has to be calculated. Perhaps the commonest is when the product inhibits competitively. The method described gives the exact rate at the corresponding concentration of product, but it is necessary to calculate the rate when the concentration of product is zero to obtain the correct values for $V_{\max}$ and K_m (their quotient, however, is correctly obtained). Two chords are required (e.g. one from 0 to 20% reaction and the other from 0 to 40% reaction): if their slopes are v and v' , and the corresponding concentrations of substrate are s and

Table 1. *Values of 'corresponding' concentrations*

The 'corresponding' concentration is the concentration of substrate or product at which the rate of reaction is equal to the slope of the chord drawn between two 'straddling' points on the progress curve. The values below are given as percentage extents of formation of product, calculated from eqn. (5) in the text, in the form:

$$100 - \frac{P_2 - P_1}{\ln \left(\frac{100 - P_1}{100 - P_2} \right)}$$

where P_1 and P_2 are the extents of formation of product (%) at the start and end of the chord. For example, if the chord is from 0 to 30% reaction, its slope gives the rate at 15.89% reaction.

Percentage extent of formation of product at start of chord (%)	Percentage extent of formation of product at end of chord (%) ...	'Corresponding' concentration									
		5	10	15	20	25	30	35	40	45	50
0		2.521	5.088	7.703	10.37	13.10	15.89	18.75	21.70	24.73	27.87
5			7.523	10.09	12.71	15.39	18.14	20.95	23.84	26.81	29.89
10				12.52	15.10	17.73	20.42	23.18	26.01	28.93	31.95

s' , then it may be shown that the initial rate v_0 is given by:

$$v_0 = \frac{s_0 (s - s')}{ss' \left(\frac{1}{v'} - \frac{1}{v} \right) + s_0 \left(\frac{s}{v} - \frac{s'}{v'} \right)} \quad (6)$$

If the product is a powerful inhibitor (e.g. $K_i < 0.5K_m$) the use of eqn. (6) is recommended. Otherwise the subtractions may introduce appreciable errors (whose magnitude will depend on the precision of the data), and it may be better to use the following method of obtaining the initial rate from the two chords. In a plot of v against p the intercept at $p = 0$ of the line through the points v, v' and p, p' (where p and p' are the corresponding concentrations of product) is the initial rate v_0 . The errors in v_0 are less than 1% if $K_i > K_m$, for the chords given above. The same empirical procedure is probably the best easy method to use when the enzyme is being inactivated during the assay and the initial rate is required, and may also be the best method if the product inhibits other than competitively.

The ratio of the slopes of two chords can be useful. For the two chords mentioned above, v/v' lies between 1 and 1.145 when there is no product inhibition or enzyme inactivation. The value depends on the substrate concentration, and the upper bound is the quotient of the corresponding concentrations of substrate. This quotient is the lower bound if there is product inhibition, the upper bound being approx. 2.2. Unexpectedly, it is thus possible to detect product inhibition in a single experiment.

Comparison with other methods

The chords method does not seem to have been described before. Lee & Wilson (1971) used $(s_1 + s_2)/2$ as an approximation to the corresponding

substrate concentration. This, however, is little simpler, and, moreover, the approximation is sometimes not good enough (Atkins & Nimmo, 1978). A related procedure has been used to obtain values of the kinetic parameters from a single progress curve (Yun & Suelter, 1977); there are, however, good reasons for favouring initial velocities over progress curves for the determination of kinetic parameters (Atkins & Nimmo, 1980). Progress curves can be successfully used to estimate initial rates (Jennings & Niemann, 1955), but the method is relatively laborious.

Numerical differentiation

When eqn. (1) does not hold, a differentiation rule can be useful. A mid-point rule is likely to be more accurate than one in which the slope at the origin of the progress curve is estimated (the chords method described above resembles a mid-point rule). One procedure is as follows. The concentrations of product ($p_0 - p_4$) are measured at regular intervals of time (e.g. 0.5 min), the times being $t_0 - t_4$. The rate at the mid-point, p_2 , is then (to a good approximation):

$$[4(p_3 - p_1)/(t_3 - t_1) - (p_4 - p_0)/(t_4 - t_0)]/3 \quad (\text{Phillips \& Taylor, 1973})$$

The accuracy in practice will depend on the smoothness and shape of the progress curve; the point p_4 might be chosen at about 20% reaction. If the origin is uncertain, as may happen when a pH-stat is used (see, e.g., Atkins & Nimmo, 1973), either the origin can be transposed to a point on the smooth progress curve (Nicholls *et al.*, 1974), or the regular curve can be extrapolated back to $t = 0$.

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