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Initial rates

A new plot

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Excellent estimations of initial rates can be obtained from plots of $\Delta P/t$ versus product formed (where P is the instantaneous concentration of the product). $\Delta P/t$ is the chord from P_0,t_0 to P,t on an ordinary P-versus-t plot. When the chord is plotted as a function of product, the intercept at P_0 of the resulting curve is necessarily $\mathrm{d}P/\mathrm{d}t_0$. This curve approximates to a straight line extremely closely in all cases tested thus far. If $\Delta P/t$ versus product is calculated from the integrated rate equation for a first-order reaction, and if a straight line is fitted through points representing the first 50% of the reaction, the discrepancy between the true initial rate and $\mathrm{d}P/\mathrm{d}t_0$ estimated from the plot is 0.68%. For the most common form of the integrated rate equation for catalysed reactions the discrepancy varies between 0 and 0.90%. Because of the complexities of the integrated rate equations, catalysed second-order reactions have not been evaluated directly; uncatalysed reactions have been done instead. For a reaction with one reactant and two products, the discrepancy varies from 0.68 to 2.02%. For two reactants and one product, it varies from 0 to 0.68%; for two and two, 0 to 2.02%. The larger discrepancies occur only when unfavourable equilibrium constants are being overcome by the initial conditions.

The integrated rate equation for uncatalysed first-order reactions is:

$$\ln[1-\Delta P/(P_e-P_0)] = -k_1(1+1/K_e)t.$$

Rewritten in order to display the dependence on the initial rate, the equation is:

$$\ln[1-\Delta P/(P_e-P_0)] = -(\mathrm{d}P/\mathrm{d}t_0)t/(P_e-P_0)$$

The logarithm in this equation, $\ln[1-\Delta P/(P_e-P_0)]$, can be approximated by $\Delta P/[-(P_e-P_0)+\Delta P/2]$. This approximation is accurate to within 4% when $\Delta P \leq 0.5(P_e-P_0)$ (Cornish-Bowden, 1975), i.e., when the reaction is up to 50% complete. Substituting and rearranging gives:

$$\Delta P/t = (\mathrm{d}P/\mathrm{d}t_0)[1-\Delta P/2(P_e-P_0)]$$

This is the equation of a straight line if $\Delta P/t$, the average rate over time t, is plotted as a function of ΔP, the total product formed. The intercept is $\mathrm{d}P/\mathrm{d}t_0$, the initial rate.

Abbreviations used: A, B, S, instantaneous concentrations of reactants; P and Q, instantaneous concentrations of products. The subscripts 0 and e indicate initial and equilibrium concentrations; ΔP is $P-P_0$; K_a is the apparent and K_m is the true Michaelis constant; and V_a is the apparent and V_m is the true maximum velocity.

The integrated rate equation for an irreversible reaction between two reactants A and B is:

$$\Delta P = (k_1A_0B_0 - k_1B_0\Delta P)t$$

if A is the limiting reactant. Rearranging gives:

$$\Delta P/t = dP/\mathrm{d}t_0 - k_1B_0\Delta P$$

which is again the equation of a straight line.

The integrated Michaelis–Menten equation can be very closely approximated by:

$$\Delta P/t = V_m/(1+K_m/S_0) - \Delta P V_m K_m/2(K_m+S_0)^2$$

(Goldenberg, 1954; Morgan, 1972)

This is also the equation of a straight line, and the first term is, of course, $\mathrm{d}P/\mathrm{d}t_0$.

These simple considerations suggest that a plot of $\Delta P/t$ versus product formed might prove to be a general method of estimating initial rates. $\Delta P/t$ is the chord from P_0,t_0 to P,t on an ordinary P-versus-t plot. As t approaches zero, $\Delta P/t$ necessarily approaches $\mathrm{d}P/\mathrm{d}t_0$. If it can be shown that this plot is linear for the general run of enzyme-catalysed reactions, it could be used as a simple, less judgmental alternative to tangent analysis; the

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standard evaluation techniques for Michaelis constants, and substrate dependence and product inhibition patterns would remain unchanged.

In order to evaluate the linearity of this plot for a particular type of reaction, it is necessary to know the integrated rate equation. In the present paper I evaluate the plot for those catalysed reactions whose integrated equation has the form:

$$V_a t = \Delta P - K_a \ln[1 - \Delta P/(P_e - P_0)]$$

This is true for all one-substrate–one-product cases, including those where product inhibition is significant and those which are reversible (Huang & Niemann, 1951; Schønheyder, 1952; Alberty & Koerber, 1957). It also applies to a two-substrate–one-product ternary-complex reaction in which the first substrate is limiting (Laidler & Bunting, 1973). Integrated equations for other catalysed reactions with two substrates, two products, or both, are complex (Darvey & Williams, 1964); the progress curves depend on the equilibrium constant, all of the Michaelis constants, the initial concentrations of substrates and products, and the type of product inhibition. In order to avoid the complexities inherent in such an analysis, I have used uncatalysed second-order reactions to model the catalysed reactions. It is then possible to consider all the possible initial conditions in a systematic but reasonably economic fashion.

The integrated rate equation for catalysed first-order reactions consists of terms for a zero-order and a first-order reaction. Since a plot of $\Delta P/t$ versus product is necessarily linear for a zero-order reaction, it is not surprising that, as I will show, the uncatalysed reactions, with only first-order terms, model the catalysed ones quite well. Similarly, the integrated equations for uncatalysed second-order reactions have first- and second-order terms; for many of the commonly encountered second-order catalysed reactions (identified in the Discussion section), the equations have zero-, first- and second-order terms. The uncatalysed reactions should, again, model their catalysed counterparts well.

A preliminary report of this work has appeared (Boeker, 1981).

Table 1. *Rate equations*

$\mathrm{d}P/\mathrm{d}t$	$\dfrac{\Delta P/t}{\mathrm{d}P/\mathrm{d}t_0}$
$k_1A - k_2P$	$\dfrac{\Delta P}{P_e - P_0} \cdot \dfrac{1}{-\ln\left(1 - \dfrac{\Delta P}{P_e - P_0}\right)}$
$k_1A - K_2PQ$	$\dfrac{\Delta P}{K_eA_0} \cdot \dfrac{1}{1 - \dfrac{P_0Q_0}{K_eA_0}} \cdot \dfrac{2(P_e - P_0) + P_0 + Q_0 + K_e}{\ln\left(1 + \dfrac{\Delta P}{(P_e - P_0) + P_0 + Q_0 + K_e}\right) - \ln\left(1 - \dfrac{\Delta P}{P_e - P_0}\right)}$
$k_1AB - k_2P$	$\dfrac{\Delta P}{A_0B_0} \cdot \dfrac{1}{1 - \dfrac{P_0}{K_eA_0B_0}} \cdot \dfrac{A_0 + B_0 - 2(P_e - P_0) + 1/K_e}{\ln\left(1 + \dfrac{\Delta P}{P_e - P_0 - A_0 - B_0 - 1/K_e}\right) - \ln\left(1 - \dfrac{\Delta P}{P_e - P_0}\right)}$
$k_1AB - k_2PQ$	$\dfrac{\Delta P}{A_0B_0} \cdot \dfrac{1}{1 - \dfrac{P_0Q_0}{K_eA_0B_0}} \cdot \dfrac{A_0 + B_0 + P_0 + Q_0 - (1 - 1/K_e)[2(P_e - P_0) + P_0 + Q_0]}{\ln\left[1 + \dfrac{\Delta P}{(P_e - P_0) + P_0 + Q_0 - \dfrac{A_0 + B_0 + P_0 + Q_0}{1 - 1/K_e}}\right] - \ln\left(1 - \dfrac{\Delta P}{P_e - P_0}\right)}$
$\dfrac{V_a}{1 + \dfrac{K_a}{P_e - P_0}} = \dfrac{V_a}{1 + \dfrac{K_a}{S_0}(1 + 1/K_e)}$	$\dfrac{1 + \dfrac{K_a}{P_e - P_0}}{1 - \dfrac{K_a}{\Delta P}\ln\left(1 - \dfrac{\Delta P}{P_e - P_0}\right)} = \dfrac{1 + \dfrac{K_a}{S_0}(1 + 1/K_e)}{1 - \dfrac{P_e - P_0}{\Delta P} \cdot \dfrac{K_a}{S_0}(1 + 1/K_e)\ln\left(1 - \dfrac{\Delta P}{P_e - P_0}\right)}$

Methods

Four uncatalysed reactions were evaluated. For each, an exact equation for $\Delta P/t$ was obtained from the integrated rate equation. These equations are shown in Table 1. They take into account the possibility that, as is frequently the case in enzyme-kinetic studies, P_0 may not be zero.

In the integrated rate equation used for catalysed reactions shown in Table 1, P_0 has not been constrained to be zero, and the dependence on K_e has been made explicit. The transformations shown in Table 1 are true if $P_0/K_eS_0 \ll 1$. This is nothing more than the condition required to obtain hyperbolic kinetics from a reversible reaction, i.e., to ignore the velocity term due to the reverse reaction.

In order to plot the results in a dimensionless form, $\Delta P/t$ was expressed as a fraction of the initial rate (see Table 1) and the product formed was expressed as a fraction of the product formed at equilibrium, $\Delta P/(P_e - P_0)$. Experimental data would, of course, be plotted directly, not as the fractions. It is not necessary to know P_e in order to use this plot. The Figures shown in the present paper were drawn by a Burroughs B6800 computer on a CalComp plotter. The lines shown on the progress curves are accurate regression lines rather than estimates.

Each of the equations in Table 1 was used to generate exact curves for $\Delta P/t$ versus product formed. Essentially all initial conditions were examined for each equation. Since each such curve is in fact error-free, it was treated as if it deviated from a straight line only on the vertical ($\Delta P/t$) axis, and a best-fit line was obtained for the first 50% of the reaction by carrying out a linear regression on ten points spaced at equal intervals of product. The difference between the intercept of this line and the actual value of dP/dt_0 was used as an empirical measure of the discrepancy inherent in estimating the initial rate for that particular set of initial conditions.

This procedure is not meant to suggest that a simple regression should be used when experimental data are plotted. As shown in the discussion, this can be justified only under certain specific circumstances.

Rather than express the degree to which each curve resembles a straight line in some statistical fashion, I have presented actual curves which include the poorest fit. In most cases, the correlation coefficient calculated for the linear regression was so high that it did not give any reasonable idea of the precision of the fit.

Results

The expected progress curve for a first-order reaction is shown in Fig. 1(*a*). The intercept of the regression for the first 50% of the reaction is 1.0068 dP/dt_0.

Unlike most of the calculations in the present paper, the one shown in Fig. 1(*a*) was done at equal intervals of time, since experiments will inevitably be done this way. The difference between results collected at equal intervals of time and product is shown in Fig. 1(*b*). Either method gives a very small discrepancy for the first 50% of the reaction. Beyond this, however, the error accumulates at equal time intervals. The reason can be clearly seen in Fig. 1(*a*); the frequency of the points increases substantially at longer times. Since these are the points that also deviate most from a straight line, the estimate of dP/dt_0 becomes increasingly biased.

A compromise of 50% completion has been chosen. The difference between equal time and equal product intervals is small; the experimental design will not be critical. The absolute discrepancy is also small, yet much of the available progress curve can still be used. If the reaction has a finite equilibrium constant, 50% completion of course refers not to the initial reactant concentration, but to $P_e - P_0$, the total change in product at equilibrium.

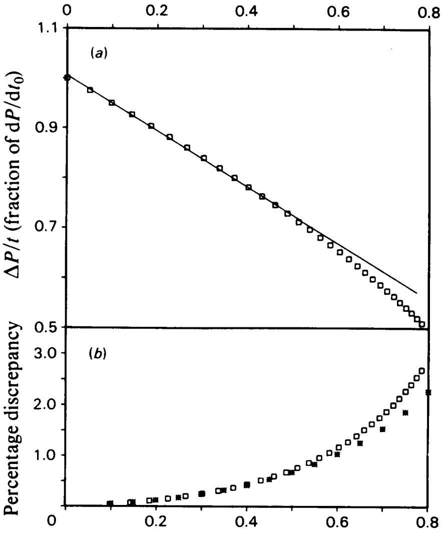

Fig. 1. *Linearity of $\Delta P/t$ versus product for an uncatalysed, reversible first-order reaction*

(*a*) $\Delta P/t$ versus product. The line shown is the regression for the first 50% of the reaction. (b) Increase in the discrepancy between the estimated and the true value of the initial rate as more of the progress curve is used to estimate the intercept. The data are calculated for equal intervals of time (□) or product (*); see the text for details. In this and all other plots, $\Delta P/t$ is expressed as a fraction of dP/dt_0, and product formed is expressed as a fraction of $P_e - P_0$.

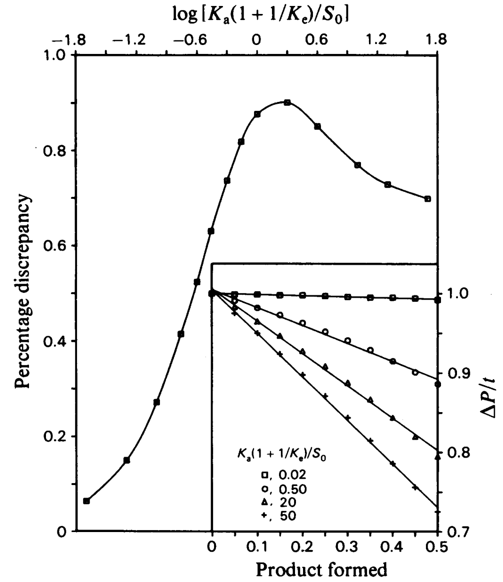

Fig. 2. *Evaluation of the plot for a reversible catalysed reaction with one substrate and one product*

In order to cover a velocity range from 2 to 98% of the apparent maximum velocity, the effective Michaelis constant, $K_a(1+1/K_e)/S_0$, was varied from 0.02 to 50. It is plotted as the logarithm for convenience. Predicted progress curves and the regression lines that result are shown in the inset.

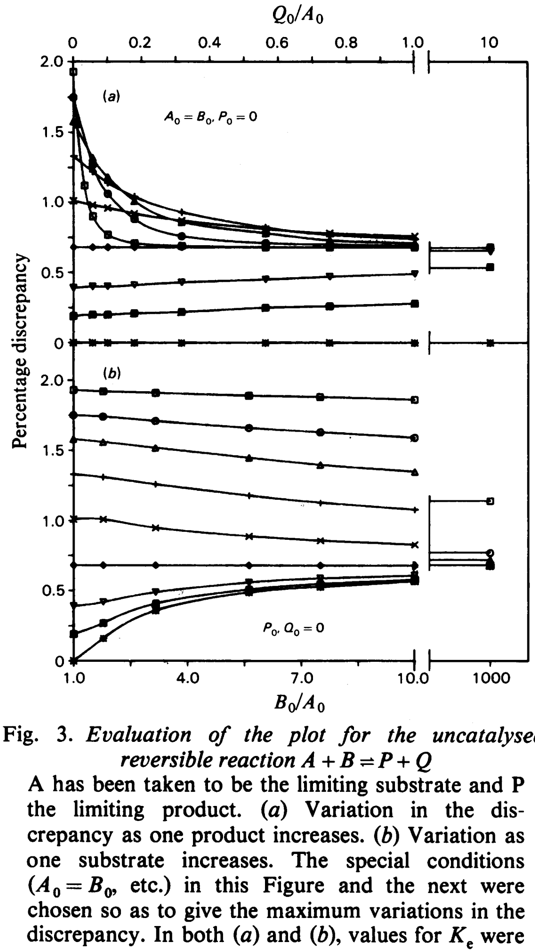

Fig. 3. *Evaluation of the plot for the uncatalysed, reversible reaction* $A + B \rightleftharpoons P + Q$

A has been taken to be the limiting substrate and P the limiting product. (*a*) Variation in the discrepancy as one product increases. (*b*) Variation as one substrate increases. The special conditions ($A_0 = B_0$, etc.) in this Figure and the next were chosen so as to give the maximum variations in the discrepancy. In both (*a*) and (*b*), values for K_e were as follows: □, 10^{-3}; ○, 0.010; △, 0.032; +, 0.100; ×, 0.316; ◇, 1.00; ▽, 3.16; ⊠, 10.0; *, 10^4.

The results for simple enzyme kinetics are shown in Fig. 2. The integrated rate equation used takes into account reversibility, but not competitive product inhibition, in first-order reactions (see the 'Note added in proof' below). For the velocity range shown, which is 2–98% of V_a, the discrepancy varies from 0.15% at the lowest S_0 to 0.90% at twice the effective Michaelis constant. For a velocity range from 10–90% of V_a, the discrepancy varies from 0.34–0.90%.

From Fig. 2 it is apparent that, as is to be expected, the catalysed reaction approaches zero-order at high substrate concentrations (□ in the inset of Fig. 2; no dependence on *P*) and first-order at low concentrations (+). In the vicinity of the Michaelis constant, the discrepancies are very close to that for a first-order reaction.

For the uncatalysed second-order reaction $A + B \rightleftharpoons P + Q$, the effect on the discrepancy of increasing one initial product concentration is shown in Fig. 3(*a*), the effect of increasing one initial substrate concentration is shown in 3(*b*), and that of increasing two or more of these together in Fig. 4. $P_0Q_0/K_eA_0B_0$ has been used for the ordinate of Fig. 4 because it represents the full range of initial conditions while varying only from 0 to 1; if $P_0Q_0/K_eA_0B_0$ is greater than 1, the reaction is proceeding from products to reactants. The maximum discrepancy for a second-order reaction is 2.02%. The progress curve for this worst case is among those shown in the inset of Fig. 4 (◇).

When this reaction is irreversible (* in the inset of Fig. 4 and * on the ordinate elsewhere in Figs. 3 and 4), $\Delta P/t$ versus product is a straight line and the intercept is dP/dt_0, as pointed out in the introduction. In all cases where K_e is greater than 1, the discrepancy is less than 0.68%, i.e., less than that for a first-order reaction. Only when the initial conditions are such as to overcome an unfavourable equilibrium do discrepancies greater than 0.68% appear.

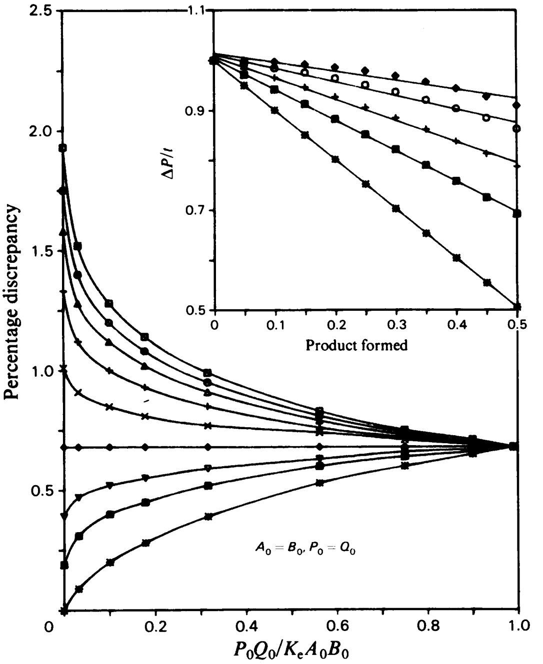

Fig. 4. *Continued evaluation of the plot for $A + B \rightleftharpoons P + Q$ (see Fig. 3)*

The main Figure shows the variation in the discrepancy as the initial concentration ratio, P_0Q_0/A_0B_0, approaches the equilibrium ratio, K_e. The inset shows the variation in the predicted progress curves, as well as the resulting regression lines. In the main Figure the following values for K_e apply: □, 10^{-3}; ○, 0.010; △, 0.032; +, 0.100; ×, 0.316; ◇, 1.00; ▽, 3.16; ⊠, 10.0; *, 10^4. In the inset, the following table applies:

	K_e	B_0/A_0	P_0/A_0	Q_0/A_0
◇	10^{-6}	1	0	0
○	0.01	1	0	0.01
+	0.1	10	0	0
⊠	10	1	1.78	1.78
*	10^4	1	0	10

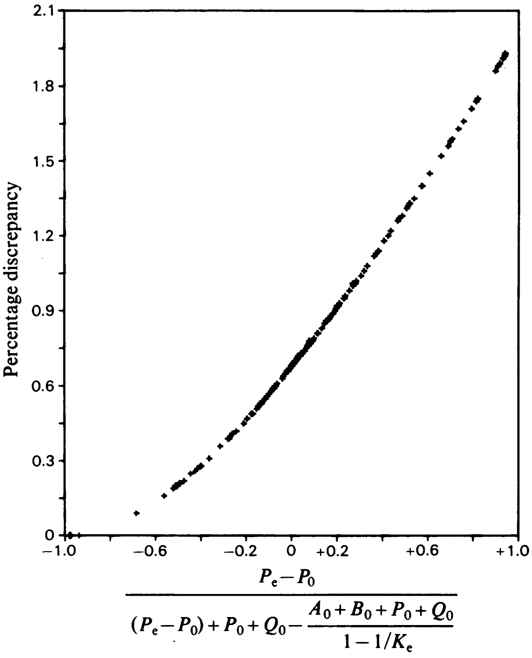

Fig. 5. *Correlation between the discrepancy and the initial conditions for the uncatalysed reaction $A + B \rightleftharpoons P + Q$*

See the text for an explanation of the abscissa. All the points in Figs. 3 and 4 are represented here, except in the vicinty of 0.68% discrepancy, where a number have been omitted for clarity.

This observation, together with the very regular patterns of Figs. 3 and 4, suggests that some direct correlation may exist between the initial conditions and the discrepancy itself. Such a correlation is shown in Fig. 5. The abscissa is derived from the first logarithm in the integrated equation in Table 1. This logarithm is the fundamental difference between a first- and a second-order reaction. In order to calculate it as the reaction progresses, the variable term must be separated into $\Delta P/(P_e-P_0)$ and $(P_e-P_0)\,/\,[(P_e-P_0)+P_0+Q_0-(A_0+B_0+P_0+Q_0)\,/\,(1-1/K_e)]$. This second term is the abscissa in Fig. 5. An alternate formulation, using equilibrium instead of initial concentrations, is $-(P_e-P_0)(1-1/K_e)/[A_0+B_e+(P_e+Q_0)/K_e]$. This makes clear the dependence on K_e in Figs. 3 and 4. It can readily be shown that this term will approach 1 only when $K_e(A_0+B_0)+P_0+Q_0$ approaches 0. This is the initial condition that produces the maximum discrepancy.

For the uncatalysed reaction $A + B \rightleftharpoons P$, the total variation in the discrepancy is from 0 to 0.68%; the reaction approaches first-order as one substrate becomes limiting. $A + B \rightleftharpoons P$ is in effect a special case of $A + B \rightleftharpoons P + Q$. If A_0/B_0 is varied at $P_0 = 0$, the observed discrepancies show a pattern very similar to that in the lower portion of Fig. 3(*b*). Similarly, if $P_0/K_eA_0B_0$ (the dimensionless quantity for this reaction) is varied at $A_0 = B_0$, the results resemble the lower portion of Fig. 4. The correlation in Fig. 5 is true from 0 to 0.68% if the abscissa, now $(P_e-P_0)/(P_e-P_0-A_0-B_0-1/K_e)$ or $-(P_e-P_0)/(A_0+B_e+1/K_e)$, varies from −1 to 0.

The uncatalysed reaction $A \rightleftharpoons P + Q$ is also a special case of $A + B \rightleftharpoons P + Q$. Here the variation in discrepancy is from 0.68 to 2.02%. If Q_0/A_0 is varied at $P_0 = 0$, the results resemble the upper portion of Fig. 3(*a*), and if P_0Q_0/K_eA_0 is varied at $P_0 = Q_0$, they resemble the upper portion of Fig. 4. The correlation (Fig. 5) is true from 0.68 to 2.02% if the abscissa, $(P_e - P_0)/(P_e + Q_0 + K_e)$, varies from 0 to 1.

Discussion

The need for a good method for obtaining initial rates has been recognized for many years. The practice of characterizing enzymes in terms of a hyperbolic rate equation (where applicable) is so widespread as to be almost universal; initial rates are almost always employed for these purposes. A number of methods have been proposed, but only the simple graphical procedure of estimating a tangent by eye has gained wide acceptance. This appears to be due either to the theoretical and computational complexities of the other methods or to their limited applicability. In addition to tangent estimation, two types of methods have been proposed: polynomial fitting (Booman & Niemann, 1956; Elmore *et al.*, 1963) and, specifically for enzyme kinetics, methods based on an integrated rate equation (Jennings & Niemann, 1955; Cornish-Bowden, 1975).

The plotting methods of Jennings & Niemann (1955) and Cornish-Bowden (1975) are based on an equation, related to the one in Table 1, that takes into account reversibility and product inhibition, but only for one-substrate–one-product reactions. With $\mathrm{d}P/\mathrm{d}t_0$ eliminated, the equation shown becomes:

$$\Delta P/t = V_a/\{1 - K_a \ln[1 - \Delta P/(P_e - P_0)]/\Delta P\}$$

which has the same form as the Michaelis–Menten equation. Jennings & Niemann (1955) suggest using one of the three linear transformations of the Michaelis–Menten equation, plotting $\Delta P/t$ in place of velocity and $-\Delta P/\ln[1 - \Delta P/(P_e - P_0)]$ in place of substrate concentration. Cornish-Bowden (1975) suggests using the direct linear plot. In either method, extrapolation of $-\Delta P/\ln[1 - \Delta P/(P_e - P_0)]$ to S_0 gives the initial rate. If the logarithm in these equations is replaced by the approximation suggested in the introduction, then:

$$\Delta P/t = V_a/[1 + K_a/(P_e - P_0 - \Delta P/2)]$$

Lee & Wilson (1971) suggest a reciprocal plot based on this equation.

Although perfectly sound in principle, none of these methods is in common use. It seems likely that the ideas behind them, and the limitations of the integrated rate equation on which they are based, are not widely understood. It is also true that, when a reaction is reversible, these methods require some knowledge of P_e (as opposed to S_0), although they are fortunately not very sensitive to errors in it.

Polynomial fitting has theoretical limitations that have been thoroughly discussed by Cornish-Bowden (1975). In general, it appears that a polynomial which is of a high enough order to estimate the initial rate well is so flexible that it is unable to smooth the data. In practice, the principal objection to polynomials is undoubtedly the start-up time required. Unless a computer program is actually running locally, polynomial fitting is not worthwhile for a few kinetic experiments.

Tangent estimation by eye relies extremely heavily on data taken in the initial phase of a reaction, precisely the time when artefacts and errors are most likely to occur. This is a serious difficulty, particularly when a reaction must be monitored by discrete measurements.

The method proposed here has three important features: (1) the plot is linear, and therefore reasonably objective, but is very simple. (2) It can make use of 50% of the progress curve of a reaction, if that much information is available. This is perhaps five to ten times as much information as is used for tangent estimation. (3) It appears to be general. It applies to all first- and second-order uncatalysed reactions and many one-substrate–one-product catalysed reactions. As the following discussion shows, it seems likely that it also applies to more complex catalysed reactions.

It is clear from the results presented here that an uncatalysed first-order reaction provides a good model for analysing a plot of $\Delta P/t$ versus product for a catalysed, one-substrate–one-product reaction. The discrepancy between true and estimated initial rates is 0.68% in the one case, and the range is from 0 to 0.90% in the other. The form of the integrated equation for a reversible first-order reaction is

$$(\text{Constant})t = \ln[1 - \Delta P/(P_e - P_0)]$$

For a corresponding catalysed reaction, it is:

$$C_1t = \Delta P - C_2 \ln[1 - \Delta P/(P_e - P_0)]$$

where C_1 and C_2 are constants.

The integrated equations for reversible, uncatalysed second-order reactions and certain catalysed reactions bear a similar relationship. For $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P} + \mathrm{Q}$, the form is:

$$\begin{aligned}(\text{Constant})t = &-\ln[1 - \Delta P/(P_e - P_0)]\\ &+ \ln[1 - \Delta P(1 - 1/K_e)/(A_0 + B_e + P_0/K_e + Q_e/K_e)]\end{aligned}$$

This form does not change if there is only one reactant or one product, but the term in the second logarithm does (see Table 1). In order to integrate the rate equation for a catalysed reaction of this type, the derivative equation term $V_m(1 - PQ/K_eAB)$ must not be reduced to V_m; it becomes significant as the reaction proceeds. Many second-order catalysed reactions then have integrated equations of the form:

$$C_1t = \Delta P - C_2 \ln[1-\Delta P/(P_e-P_0)] + C_3 \ln[1-\Delta P \times (1-1/K_e)/(A_0+B_e+P_0/K_e+Q_e/K_e)]$$

(Darvey & Williams, 1964; E. A. Boeker, unpublished work).

A mechanism will have an integrated equation of this form if the derivative equation, written in coefficient form, has no substrate–product terms higher than second order, e.g., *AB*, *AP*, not *ABP*. This is of course true for all two-substrate reactions in the absence of products. In their presence, the only ordered mechanism producing a more complex equation is the ternary-complex mechanism, $A+B \rightleftharpoons P+Q$, when Q is present. Most (rapid-equilibrium) random mechanisms have the form above as well; the two exceptions are ordered substrates/random products and vice-versa, if P is present. If a stable enzyme species isomerizes, all second-order reactions have a more complex form under at least some circumstances.

On the basis of this analysis, uncatalysed second-order reactions should model the corresponding catalysed reactions quite well. Furthermore, the correlation established in the Results section suggests that the larger discrepancies occur only under fairly unusual initial conditions. For $A+B \rightleftharpoons P+Q$, K_e must be less than 1 and $K_e(A_0+B_0)+P_0+Q_0$ must approach zero. In other words, addition of even a small amount of product to this reaction will improve the estimation of the initial rate, contrary to the usual situation. For $A+B \rightleftharpoons P$, the discrepancies are all less than 0.68%. For $A \rightleftharpoons P+Q$, $P_0+Q_e+K_e$ must approach zero before discrepancies greater than 1.5% occur. The detailed behaviour of plots of $\Delta P/t$ versus product for complex catalysed reactions still needs to be investigated.

The discrepancies calculated in the present paper should not be used as correction factors. The actual discrepancy in any experimental situation will be a function of the number and spacing of the data points.

If the equation for this plot is written as $\Delta P/t = \alpha - \alpha\beta\Delta P$, where $\alpha = dP/dt_0$ and β depends on the reaction, it is easy to see that it corresponds to the Eadie transformation of the Michaelis–Menten equation. There are then two additional linear forms, corresponding to a Lineweaver–Burk plot and a Hanes plot: $1/\Delta P = \beta + 1/\alpha t$, and $t/\Delta P = 1/\alpha + \beta t$. Of the three forms, a plot of $\Delta P/t$ versus product appears to be the best choice, for two reasons. First, neither new form bears any obvious relationship to ordinary reaction rates, whereas $\Delta P/t$ does. And second, even though the plot is meant to be analysed simply, with nothing more than a ruler, it seems inevitable that regression analysis will be applied to obtain 'best-fit' values of the initial rates. Although it is not theoretically sound to apply such analysis to plots with dependent variables on each axis, it is also true that application of least squares to either of the other plots does not correspond to any reasonable sort of experimental error (see, for example, Cornish-Bowden, 1979) unless proper weights are used. If each plot is drawn with error bars corresponding to the two most likely types of experimental error, a constant amount of product, and a percentage of ΔP_i, it can be seen that a plot of $\Delta P/t$ versus product has more nearly constant error bars than either of the others, although the Hanes-type plot is a close second. The error in applying unweighted least squares should then be the least for the $\Delta P/t$-versus-product plot.

Note added in proof

The equation for catalysed first-order reactions (see the introduction) includes competitive product inhibition. However, K_a in this equation depends on S_0; this was not taken into account in the calculations. The effect of this is that, strictly, Fig. 2 applies only in the absence of competitive product inhibition. If this is taken into account, the discrepancy depends on the ratio of K_m to the competitive-product-inhibition constant, and varies between 0.90 and −1.25% for all values of this ratio ≤2.

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