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Integrated rate equations for irreversible enzyme-catalysed first-order and second-order reactions

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Integrated rate equations are presented that describe irreversible enzyme-catalysed first-order and second-order reactions. The equations are independent of the detailed mechanism of the reaction, requiring only that it be hyperbolic and unbranched. The results should be directly applicable in the laboratory.

In two previous papers (Boeker, 1984*a*,*b*) I have shown that the general integrated rate equation for enzyme-catalysed reactions that are second-order in substrates and/or products is:

$$e_0 t = C_f\left[-\ln\left(1-\frac{\Delta P}{P_e-P_0}\right)\right] + C_s\cdot\ln\left(1-\frac{\Delta P}{D+P_e-P_0}\right) + C_1\Delta P + \tfrac{1}{2}C_2(\Delta P)^2 + \tfrac{1}{3}C_3(\Delta P)^3 \quad (1)$$

In this equation, P_e-P_0 is a concentration expressing the conversion of substrate into product at equilibrium. This quantity is one root of the quadratic equation for P_e-P_0 in terms of K_e and the initial concentrations of the substrates and the products. The second, physically impossible, root of this equation is $D+P_e-P_0$, which appears in the second logarithmic term of eqn. (1), and represents a concentration greater than the amount of substrate initially present. For any given reaction, the coefficients C_f, C_s, C_1 etc. in eqn. (1) depend on the macroscopic constants (K_A, $k_{cat.}$ etc.) and the equilibrium concentrations of the substrates and products. They do not depend on the enzyme concentration. The exact dependences (Boeker, 1984*b*) are a function of the stoichiometry of the reaction.

Abbreviations used: A, B, P, and Q are the instantaneous concentrations of substrates and products; the subscripts 0 and e indicate the initial and equilibrium concentrations respectively. ΔP is $P-P_0$, the net change in product concentration at time t. K_e is the equilibrium constant, k_1 is the forward rate constant for uncatalysed reactions, V_f is the maximum velocity in the forward direction, $k_{cat.}$ is the catalytic constant or turnover number, e_0 is the enzyme concentration, and K_A is the Michaelis constant for the substrate. For $A \rightleftharpoons P+Q$, $D=-(P_e+Q_e+K_e)$; for $A+B \rightleftharpoons P$, $D=A_e+B_e+1/K_e$; for $A+B \rightleftharpoons P+Q$, $D=[K_e(A_e+B_e)+P_e+Q_e]/(K_e-1)$ (Boeker, 1984*a*). The coefficients J are collections of microscopic rate constants whose specific definition, for any particular mechanism, can be obtained directly from a derivation in accordance with King & Altman (1956). Definitions of the coefficients C for reversible catalysed reactions are given in Boeker (1984*b*).

Eqn. (1) describes all linear hyperbolic mechanisms, including those with dead-end inhibition, but is strictly valid for random (i.e. branched) mechanisms only if the rapid-equilibrium assumption holds. If the reaction is first-order in both substrate and product, C_s and C_3 are necessarily zero. If the reaction is uncatalysed, but second-order, C_1, C_2 and C_3 are zero and C_f and C_s are equal; if it is first-order, C_s is also zero. As the equilibrium constant in eqn. (1) increases, P_e-P_0 approaches the initial concentration of the limiting substrate, and $D+P_e-P_0$ approaches the initial concentration of the other substrate.

In principle, eqn. (1) should be applicable experimentally, but the analysis will be complicated greatly by the need either to know or to fit the equilibrium constant. This problem can be avoided by examining irreversible reactions, i.e. by examining reactions where the limit of eqn. (1) as $1/K_e \rightarrow 0$ is applicable.

Obtaining this limit by starting with eqn. (1) and letting $1/K_e \rightarrow 0$ is not a straightforward process. A simpler approach, in fact, is to begin with a form of the derivative rate equation appropriate for irreversible reactions and repeat the integration. In the present paper I give the results of that process for the stoichiometries $A \rightarrow P$, $A \rightarrow P+Q$, $A+B \rightarrow P$ and $A+B \rightarrow P+Q$. In addition, results are given for two experimentally likely but mathematically unique special cases, one where the initial substrate con-

centrations are equal, and one where one substrate concentration greatly exceeds the other.

Integrated rate equations specifically for irreversible first-order reactions have been presented previously by a number of authors (Henri, 1902; Huang & Niemann, 1951; Schønheyder, 1952; Laidler & Bunting, 1973; Orsi & Tipton, 1979). These equations have been derived for reactions that follow a particular mechanism; with this limitation, they are consistent with the general form presented here. As far as I am aware, equations specifically for irreversible second-order reactions do not appear in the literature. First-order equations have been applied to second-order reactions under pseudo-first-order conditions (Duggleby & Morrison, 1977, 1978).

Derivations

The steady-state rate equations used in this paper have been described previously (Boeker, 1984*b*). For a reversible reaction with stoichiometry $A+B \rightleftharpoons P$, for example, the general derivative equation is:

$$\frac{dP}{dt} = \frac{e_0 k_{cat.} J_{AB}(AB - P/K_e)}{J_0 + J_A A + J_B B + J_P P + J_{AB} AB + J_{AP} AP + J_{BP} BP + J_{ABP} ABP} \quad (2)$$

For $A = P+Q$, the numerator term in parentheses is $A - PQ/K_e$; the denominator lacks terms in B but has additional terms in Q, AQ, PQ and APQ. For $A+B \rightleftharpoons P+Q$, the numerator term is $AB - PQ/K_e$ and the denominator contains all 16 terms in substrates and products. For a first-order reaction, the numerator term is $A - P/K_e$ and the denominator has terms in J_0, J_A, J_P and J_{AP}. As these equations are written, the coefficients J are not all independent; division by J_{AB} (two substrates) or J_A (one substrate) is normally used to accomplish this. The equations become mechanism-specific when the appropriate coefficients J are set equal to zero.

If the reaction is irreversible, the second term in the parentheses in the numerator is zero. The necessary conditions are discussed rigorously in the Results section. It is important to note that irreversibility does not imply the absence of product inhibition; these terms still appear in the denominator.

The method of integration is a modification of that described for reversible reactions (Boeker, 1984*b*). Of the several concentration variables available, the most economical (in terms of the complexity of the resulting derivation) appears to be the instantaneous concentration of one substrate, e.g. A. The other concentrations, B, P and Q, are eliminated from the derivative equations with the substitutions $B = B_0 - A_0 + A$, $P = A_0 + P_0 - A$ and $Q = A_0 + Q_0 - A$. For the special case where $B_0 = A_0$, $B = A$ is used, and, where $B_0 \gg A_0$, $B = B_0$ is used. The denominators are then sorted to give a polynomial in A.

For reactions with one substrate, the result of this process is:

$$\frac{-dA}{dt} = \frac{e_0 k_{cat.} J_A A}{\alpha + \beta A + \gamma A^2 + \delta A^3}$$

α, β, γ and δ are collections of concentrations and J coefficients that are defined in the sorting process. The same equation is obtained for two-substrate reactions when $B_0 \gg A_0$ except that J_A is replaced by $B_0 J_{AB}$. It can be re-arranged and integrated in a straightforward fashion. If, after integration, $A_0 - A$ is replaced by ΔP, the result is:

$$k_{cat.} J_A e_0 t = -\alpha \cdot \ln\left(1 - \frac{\Delta P}{A_0}\right) + (\beta + \gamma A_0 + \delta A_0^2)\Delta P - \tfrac{1}{2}(\gamma + 2\delta A_0)(\Delta P)^2 + \tfrac{1}{3}\delta(\Delta P)^3$$

The coefficients α, β, γ and δ must now be replaced with the use of the definitions obtained when the original denominator was sorted. Many terms cancel, substantially simplifying the final equation. The results are described in the next section.

The process is more complex when there are two substrates. After the concentration substitutions have been carried out, the equation has the form:

$$\frac{-dA}{dt} = \frac{e_0 k_{cat.} J_{AB} A (B_0 - A_0 + A)}{\alpha + \beta A + \gamma A^2 + \delta A^3 + \varepsilon A^4} \quad (3)$$

Before integration, $\alpha + \beta A + \gamma A^2 + \delta A^3 + \varepsilon A^4$ must be divided by $A(B_0 - A_0 + A)$. This can be accomplished by algebraic long division in which the final two terms are obtained by the method of partial fractions. One of these terms will depend on A^{-1} and the second on $(B_0 - A_0 + A)^{-1}$. Integration is then straightforward, and replacing $A_0 - A$ by ΔP gives:

$$k_{cat.} J_{AB} e_0 t = -\frac{\left\{\begin{array}{l}\alpha - \beta(B_0 - A_0) + \gamma(B_0 - A_0)^2 \\ \quad - \delta(B_0 - A_0)^3 + \varepsilon(B_0 - A_0)^4\end{array}\right\}}{A_0 - B_0} \cdot \ln\left(1 - \frac{\Delta P}{B_0}\right) - \frac{\alpha}{B_0 - A_0} \cdot \ln\left(1 - \frac{\Delta P}{A_0}\right) + (\gamma + 2\delta A_0 - \delta B_0 + 3\varepsilon A_0^2 - 3\varepsilon A_0 B_0 + \varepsilon B_0^2)\Delta P - \tfrac{1}{2}(\delta + 3\varepsilon A_0 - \varepsilon B_0)(\Delta P)^2 + \tfrac{1}{3}\varepsilon(\Delta P)^3$$

For two-substrate reactions where $B_0 = A_0$, the equation that results from the concentration substitutions is eqn. (3) above except that $B_0 - A_0 = 0$. The algebraic long division is now greatly simplified; of the last two terms, one is βA^{-1} and the second is αA^{-2}, thus changing the form of the final result. After re-arrangement and integration:

$$k_{cat.}J_{AB}e_0t = \frac{\alpha}{A_0}\left(\frac{\Delta P}{A_0 - \Delta P}\right) - \beta \cdot \ln\left(1 - \frac{\Delta P}{A_0}\right) + (\gamma + \delta A_0 + \varepsilon A_0^2)\Delta P - \tfrac{1}{2}(\delta + 2\varepsilon A_0)(\Delta P)^2 + \tfrac{1}{3}\varepsilon(\Delta P)^3$$

Results

The general form of the integrated equation for these reactions is:

$$e_0t = C_f\left[-\ln\left(1 - \frac{\Delta P}{A_0}\right)\right] + C_s\left[-\ln\left(1 - \frac{\Delta P}{B_0}\right)\right] + C_1\Delta P + \tfrac{1}{2}C_2(\Delta P)^2 + \tfrac{1}{3}C_3(\Delta P)^3 \qquad (4)$$

Definitions of the coefficients C in terms of the fundamental kinetic constants are given in Tables 1–4. The information in these Tables has been ordered according to initial concentrations. Thus, for example, for the reaction A→P+Q in Table 1, the first terms shown are those that depend only on A_0. Next are the additional terms that occur when one product is present initially, then those for the second product, and finally those that occur only with both. When the initial concentrations of products are zero, the expressions in Tables 1–4 simplify greatly. Again, with A→P+Q as an example, C_f is now simply a quadratic in A_0, and C_1 a linear function.

An interesting aspect of the results in Tables 1–4 is the relationship between the C and the J coefficients. This is most clearly seen when the numbers of substrates and products are balanced. For the stoichiometry A→P, the highest-order C coefficient is C_2, which depends only on J_{AP}/J_A, a 'two-way' constant. C_1 depends on this constant and on the one-way constants J_A/J_A and J_P/J_A, whereas C_f depends on these three plus J_0/J_A. An identical trend can be seen for A+B→P+Q: C_3 contains only J_{ABPQ}/J_{AB}, C_2 contains all the three-way constants as well, and C_1 adds the two-way constants. C_s contains all the constants except J_B, and C_f omits only J_A. This trend in the C coefficients is completely obscured by the usual kinetic notation: in A→P, for example, J_{AP}/J_A and J_P/J_A are the uncompetitive and competitive product inhibition constants, and might be written as K_{iP} and K_P. J_A/J_A is of course 1, and J_0/J_A is K_A.

In comparing the integrated equations for reversible (Boeker, 1984*b*) and irreversible reactions, one feature stands out. When a reaction of stoichiometry A⇌P+Q approaches irreversibility, the logarithmic term characteristic of second-order equations drops out, and the order of the polynomial in ΔP increases by one. In other words, this stoichiometry is correctly treated as second-order when reversible, but as first-order when irreversible.

One additional result can be obtained from Tables 1–4. For reactions with one substrate, the sum $C_1 + C_f/A_0$ can, simply by adding the terms in Tables 1 and 3, be shown to be the reciprocal of the initial velocity; compare the appropriate version of eqn. (2). For two substrates, $C_1 + C_f/A_0 + C_s/B_0$ is the reciprocal of the initial velocity. This result immediately suggests that complete time courses could be analysed with the same plots etc. as are used for initial-rate studies. However, this does not make good use of the information in each progress curve; in the Discussion section I suggest better ways to obtain the macroscopic kinetic constants.

That a particular sum of the coefficients should give the reciprocal of the initial velocity may at first glance seem surprising, but there is a straightforward reason for it. One of the series expansions for $-\ln(1 - \Delta P/A_0)$ is $\Delta P/A_0 + \frac{1}{2}(\Delta P/A_0)^2 + \frac{1}{3}(\Delta P/A_0)^3 + \ldots$. If the logarithmic terms in eqn. (4) are each expanded, and if the equation is then sorted according to dependence on ΔP, the resulting coefficient of the first-power term is either $C_1 + C_f/A_0$ or $C_1 + C_f/A_0 + C_s/B_0$, depending on the stoichiometry. This is the first virial coefficient when t is a function of ΔP. Its reciprocal must be the first virial coefficient when ΔP is a function of t, and this virial coefficient is the initial rate. Techniques in which initial rates are extracted from progress curves by obtaining a first virial coefficient are based on this principle.

The results shown in Tables 1–4 depend on the assumption of irreversibility in the derivative equation. The conditions for irreversibility depend on the stoichiometry, and can be formulated explicitly. The simplest is A→P, where, when the reaction is irreversible, the numerator term $A - P/K_e$ reduces to A. $A - P/K_e$ can be rewritten as $(1 + 1/K_e)(P_e - P)$ (see Boeker, 1984*a*). This reduces to A when $K_e \gg 1$ and when $P_e - P$ approaches A, i.e. when A_e approaches 0. To determine when $P_e - P$ approaches A, let X be the minimum desired ratio between $P_e - P_0$ and A_0. Eliminate P_e from this expression in favour of K_e, A_0 and P_0, and solve the result for K_e. This gives:

$$K_e \geqslant \frac{X + \dfrac{P_0}{A_0}}{1 - X}$$

Table 1. *Interpretations of* C_f
The units of C_f are those of (enzyme concentration)(time), e.g. $\mu g \cdot ml^{-1} \cdot min$.

Stoichiometry	$C_f k_{cat.} J_{AB}$*
A → P	$J_0 + J_P A_0 + J_P P_0$
A → P + Q	$J_0 + (J_P + J_Q)A_0 + J_{PQ}A_0^2 + (J_P + J_{PQ}A_0)P_0 + (J_Q + J_{PQ}A_0)Q_0 + J_{PQ}P_0Q_0$
A + B → P	$\frac{J_0}{B_0 - A_0} + J_B + \left(\frac{J_P}{B_0 - A_0} + J_{BP}\right)(A_0 + P_0)$
A + B → P, $B_0 \gg A_0$	$\frac{J_0}{B_0} + J_B + J_{BP}A_0 + \left(\frac{J_P}{B_0} + J_{BP}\right)P_0$
A + B → P, $B_0 = A_0$	$J_A + J_B - J_P + (J_{AP} + J_{BP})(A_0 + P_0)$
A + B → P + Q	$\frac{J_0}{B_0 - A_0} + J_B + \left(\frac{J_P + J_Q}{B_0 - A_0} + J_{BP} + J_{BQ}\right)A_0 + \left(\frac{J_{PQ}}{B_0 - A_0} + J_{BPQ}\right)A_0^2 + \left[\frac{J_P}{B_0 - A_0} + J_{BP} + \left(\frac{J_{PQ}}{B_0 - A_0} + J_{BPQ}\right)A_0\right]P_0 + \left[\frac{J_Q}{B_0 - A_0} + J_{BQ} + \left(\frac{J_{PQ}}{B_0 - A_0} + J_{BPQ}\right)A_0\right]Q_0 + \left(\frac{J_{PQ}}{B_0 - A_0} + J_{BPQ}\right)P_0Q_0$
A + B → P + Q, $B_0 \gg A_0$	$\frac{J_0}{B_0} + J_B + (J_{BP} + J_{BQ})A_0 + \left(\frac{J_{PQ}}{B_0} + J_{BPQ}\right)A_0^2 + \left(\frac{J_{PQ}}{B_0} + J_{BPQ}\right)P_0Q_0 + \left[\frac{J_P}{B_0} + J_{BP} + \left(\frac{J_{PQ}}{B_0} + J_{BPQ}\right)A_0\right]P_0 + \left[\frac{J_Q}{B_0} + J_{BQ} + \left(\frac{J_{PQ}}{B_0} + J_{BPQ}\right)A_0\right]Q_0$
A + B → P + Q, $B_0 = A_0$	$J_A + J_B - (J_P + J_Q) + (J_{AP} + J_{AQ} + J_{BP} + J_{BQ} - 2J_{PQ})A_0 + (J_{APQ} + J_{BPQ})A_0^2 + [J_{AP} + J_{BP} - J_{PQ} + (J_{APQ} + J_{BPQ})A_0]P_0 + [J_{AQ} + J_{BQ} - J_{PQ} + (J_{APQ} + J_{BPQ})A_0]Q_0 + (J_{APQ} + J_{BPQ})P_0Q_0$

* $C_f k_{cat.} J_A$ when there is only one substrate.

Since this condition also requires that $K_e \gg 1$, it is both necessary and sufficient.

For A+B→P or A+B→P+Q, the numerator term $AB - P/K_e$ or $AB - PQ/K_e$ is equal to $(P_e - P)(D + P_e - P)$ (Boeker, 1984*a*). $P_e - P$ must again approach A, and $D + P_e - P$ must approach B. For A→P+Q, $A - PQ/K_e = (P_e - P)(D + P_e - P)(-1/K_e)$; $P_e - P$ must approach A and $(D + P_e - P)(-1/K_e)$ must approach 1. Explicit conditions for these limits are shown in Table 5.

Discussion

A fundamental requirement of progress-curve analysis is that the enzyme is not subject to inactivation during the experiment. The equations presented in this paper have been written in terms of $e_0 t$, rather than t alone, in order to make explicit a test for enzyme instability (Selwyn, 1965; see also Cornish-Bowden, 1979, for relevant early literature). Selwyn (1965) pointed out that, regardless of the actual quantities in the integrated rate equation, the value of $e_0 t$ depends only on ΔP and the initial substrate and product concentrations. This is clear from eqns. (1) and (4). If no inactivation occurs, two progress curves at different values of e_0 will be superimposable if plotted as product formed versus $e_0 t$, even though a much longer time is required to reach a given ΔP at the lower e_0. If inactivation is occurring, for example as a first-order process, there will be more inactivation at the lower value of e_0 (where t will be longer) and

Table 2. *Interpretations of* C_s

The units of C_s are the same as C_f (see Table 1).

Stoichiometry*	$C_s k_{cat.} J_{AB}$
A + B → P	$\frac{J_0}{A_0-B_0}+J_A+\left(\frac{J_P}{A_0-B_0}+J_{AP}\right)(B_0+P_0)$
A + B → P† $B_0=A_0$	$\frac{J_0}{A_0}+J_P+\frac{J_P}{A_0}P_0$
A + B → P + Q	$\frac{J_0}{A_0-B_0}+J_A+\left(\frac{J_P+J_Q}{A_0-B_0}+J_{AP}+J_{AQ}\right)B_0+\left(\frac{J_{PQ}}{A_0-B_0}+J_{APQ}\right)B_0^2$ $+\left[\frac{J_P}{A_0-B_0}+J_{AP}+\left(\frac{J_{PQ}}{A_0-B_0}+J_{APQ}\right)B_0\right]P_0$ $+\left[\frac{J_Q}{A_0-B_0}+J_{AQ}+\left(\frac{J_{PQ}}{A_0-B_0}+J_{APQ}\right)B_0\right]Q_0+\left(\frac{J_{PQ}}{A_0-B_0}+J_{APQ}\right)P_0Q_0$
A + B → P + Q† $B_0=A_0$	$\frac{J_0}{A_0}+J_P+J_Q+J_{PQ}A_0+\left(\frac{J_P}{A_0}+J_{PQ}\right)P_0+\left(\frac{J_Q}{A_0}+J_{PQ}\right)Q_0+\frac{J_{PQ}}{A_0}P_0Q_0$

* C_s is 0 for A → P or P + Q and when $B_0 \gg A_0$ for A + B → P.
† C_s is the coefficient of $\Delta P/(A_0-\Delta P)$ rather than $-\ln(1-\Delta P/B_0)$.

Table 3. *Interpretations of* C_1

The units of C_1 are those of (enzyme concentration)(time)/(substrate concentration), e.g. $\mu g \cdot ml^{-1} \cdot min \cdot mM^{-1}$.

Stoichiometry	$C_1 k_{cat.} J_{AB}$*
A → P	$J_A-J_P+J_{AP}P_0$
A → P + Q	$J_A-(J_P+J_Q)-J_{PQ}A_0+(J_{AP}-J_{PQ})P_0+(J_{AQ}-J_{PQ})Q_0+J_{APQ}P_0Q_0$
A + B → P†	$J_{AB}-(J_{AP}+J_{BP})+J_{ABP}P_0$
A + B → P $B_0 \gg A_0$	$\frac{J_A-J_P}{B_0}+J_{AB}-J_{BP}+\left(\frac{J_{AP}}{B_0}+J_{ABP}\right)P_0$
A + B → P + Q†	$J_{AB}+J_{PQ}-(J_{AP}+J_{AQ}+J_{BP}+J_{BQ})-J_{BPQ}A_0-J_{APQ}B_0+(J_{ABP}-J_{APQ}-J_{BPQ})P_0$ $+(J_{ABQ}-J_{APQ}-J_{BPQ})Q_0+J_{ABPQ}P_0Q_0$
A + B → P + Q $B_0 \gg A_0$	$\frac{J_A-(J_P+J_Q)}{B_0}+J_{AB}-(J_{BP}+J_{BQ})-\left(\frac{J_{PQ}}{B_0}+J_{BPQ}\right)A_0+\left(\frac{J_{AP}-J_{PQ}}{B_0}+J_{ABP}-J_{BPQ}\right)P_0$ $+\left(\frac{J_{AQ}-J_{PQ}}{B_0}+J_{ABQ}-J_{BPQ}\right)Q_0+\left(\frac{J_{APQ}}{B_0}+J_{ABPQ}\right)P_0Q_o$

* $C_1 k_{cat.} J_A$ if the reaction has but one substrate.
† C_1 is unchanged when $B_0=A_0$.

Table 4. *Interpretations of C_2 and C_3*

The dimensions of C_2 and C_3 are those of (enzyme concentration) (time)/(substrate concentration)^2 and (substrate concentration)^3 respectively. For C_2, for example, this might be $\mu g \cdot ml^{-1} \cdot min \cdot mM^{-2}$.

Stoichiometry	$C_3 k_{cat.} J_{AB}$*	$C_2 k_{cat.} J_{AB}$†
A → P	0	J_{AP}
A → P + Q	J_{APQ}	$J_{AP} + J_{AQ} - J_{PQ} + J_{APQ}(P_0 + Q_0)$
A + B → P‡	0	J_{ABP}
A + B → P, $B_0 \gg A_0$	0	$\frac{J_{AP}}{B_0} + J_{ABP}$
A + B → P + Q‡	J_{ABPQ}	$J_{ABP} + J_{ABQ} - (J_{APQ} + J_{BPQ}) + J_{ABPQ}(P_0 + Q_0)$
A + B → P + Q, $B_0 \gg A_0$	$\frac{J_{APQ}}{B_0} + J_{ABPQ}$	$\frac{J_{AP} + J_{AQ} - J_{PQ}}{B_0} + J_{ABP} + J_{ABQ} - J_{PBQ} + \left(\frac{J_{APQ}}{B_0} + J_{ABPQ}\right)(P_0 + Q_0)$

* $C_3 k_{cat.} J_A$ if the is only one substrate.
† $C_2 k_{cat.} J_A$ if there is only one substrate.
‡ C_2 and C_3 are unchanged when $B_0 = A_0$.

Table 5. *Conditions for irreversibility*

X is the desired ratio of $P_e - P_0$ to A_0.

A → P	$K_e \geqslant \frac{X + \frac{P_0}{A_0}}{1 - X}$
A → P + Q	$\frac{K_e}{A_0} \geqslant \frac{X^2 + \left(\frac{P_0 + Q_0}{A_0}\right)X + \frac{P_0 Q_0}{A_0^2}}{1 - X}$
A + B → P	$K_e A_0 \geqslant \frac{X + \frac{P_0}{A_0}}{(1 - X)\left(\frac{B_0}{A_0} - X\right)}$
A + B → P + Q	$K_e \geqslant \frac{X^2 + \left(\frac{P_0 + Q_0}{A_0}\right)X + \frac{P_0 Q_0}{A_0^2}}{(1 - X)\left(\frac{B_0}{A_0} - X\right)}$

the curves will separate. Progress curves at two values of e_0 can therefore provide a test for enzyme inactivation as well as duplicate data.

For a given set of initial concentrations, the progress curve will be described by the set of coefficients C that gives the best fit to eqn. (4). It would at first seem reasonable to obtain these best-fit coefficients by a multiple regression of $e_0 t$ on the terms in eqn. (4). However, this procedure minimizes the error in $e_0 t$, i.e. it predicts values of $e_0 t$ given values of ΔP. In fact, we wish to predict ΔP, given $e_0 t$; the major source of experimental error lies in the measurement of ΔP.

The straightforward solution to this problem would be to solve eqn. (4) for ΔP in terms of t, and then to perform a regression that predicts ΔP for a given t. Since eqn. (4) is complex, this is not possible; what can be done is to minimize the errors in ΔP by using a non-linear-regression technique to fit eqn. (4). The principles of non-linear regression, as applied to enzyme kinetics, were first discussed by Wilkinson (1961) and Johansen & Lumry (1961). The technique has been employed by Fernley (1974), Darvey *et al.* (1975) and Duggleby & Morrison (1977, 1978) to fit first-order integrated rate equations. A computer program has been published for one of these applications (Duggleby, 1981).

Once the coefficients C have been extracted from a set of progress curves, the kinetic constants must be obtained from them. This is less difficult than it first appears. If the stoichiometry A + B → P is used as an example, we have, from Table 1:

$$C_f k_{cat.} J_{AB} = \frac{J_0}{B_0 - A_0} + J_B + \left(\frac{J_P}{B_0 - A_0} + J_{BP}\right)(A_0 + P_0)$$

From this it is apparent that, given a set of progress curves in which the initial substrate concentrations are varied with a constant difference between them, a plot of C_f versus A_0 (or A_0+P_0 if desired) will be linear and will have:

$$\text{Intercept} = \frac{1}{k_{cat.}}\left(\frac{J_0/J_{AB}}{B_0-A_0}+\frac{J_B}{J_{AB}}\right)$$

$$\text{Slope} = \frac{1}{k_{cat.}}\left(\frac{J_P/J_{AB}}{B_0-A_0}+\frac{J_{BP}}{J_{AB}}\right)$$

A second set of curves with a different value of B_0-A_0 will allow separation of the two sums into their components.

From Table 2 it can be seen that, if C_s from the same set of progress curves is plotted against B_0, the intercept term in J_B is replaced by one in J_A, and the slope term in J_{BP} by one in J_{AP}. Two additional constants can then be calculated. The only constants that remain are $k_{cat.}$ and J_{ABP}/J_{AB}, and both of these can be obtained from C_1. Similar analyses can be applied to the other stoichiometries.

In principle, for a reaction with a total of three substrates plus products, it is possible to obtain a complete set of kinetic constants from as few as three progress curves, done under different initial conditions. For a reaction with four substrates plus products, four curves are needed. How well each of the constants is estimated of course depends on its magnitude and the accuracy of the data. Three or four curves will probably never be an adequate number in practice, but does serve to show the power of the technique.

A further advantage of integrated rate equations is that product inhibition constants can be obtained even when a product is for some reason unavailable. For reactions with one product, the product inhibition constants can be obtained simply by varying A_0 and (if there are two substrates) B_0, i.e. without the need for any product at all. If there are two products, only one or the other is required.

The equations presented here are limiting cases of the complete equations for reversible reactions (Boeker, 1984*b*). They are reliable only if applied to reactions that are truly irreversible. The mathematical requirements for this are shown in Table 5. If we assume that a 1% error in measuring the product formed is within the limits of experimental error, a reaction that proceeds to 99% of completion is essentially irreversible. For the stoichiometry A→P, it can be calculated from Table 5 that $\Delta G^{0\prime}$ must then be less than -11.3kJ/mol (-2.7kcal/mol), assuming that no product has been added to the reaction initially. For A+B→P+Q, $\Delta G^{0\prime}$ must be less than -22.6kJ/mol (-5.4kcal/mol), if the initial substrate concentrations are equal. If one concentration is at least twice the other, $\Delta G^{0\prime}$ must be less than -11.3kJ/mol. A goodly number of reactions of biochemical interest have standard free-energy changes of this magnitude.

The situation is more complex when the substrates and products are unbalanced. For A→P+Q, again assuming that products are not added initially, the ratio of K_e to A_0 must be 98 or more. This corresponds to $\Delta G^{0\prime}$ of 5.9kJ/mol (1.4kcal/mol) or less, but this value assumes the (rather unrealistic) standard state, where the substrate concentration is 1 M. For a substrate concentration near 1 mM, K_e must be 0.1 M or greater for 99% reaction.

For the stoichiometry A+B→P, the situation is less favourable. The dimensionless quantity K_eA_0 must be greater than 9900 if the two substrates are present at equal concentrations. This corresponds to a $\Delta G^{0\prime}$ less than -39.7kJ/mol (-9.5kcal/mol), or, at an initial substrate concentration of 1 mM, an equilibrium constant of 10^7M^{-1}. However, this equilibrium constant is decreased by a factor of 100-fold if one substrate is twice the concentration of the other, and by an additional factor of 10-fold if the ratio is 11.

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