Expressions of the Michaelis–Menten Equation when Studying Enzyme Reactions in a Variable-Volume Medium

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Expressions of integrated rate equations for enzyme reactions studied in a system where the volume of the medium changes, e.g. with a pH-stat device, are given.

Instead of considering only the initial velocities of enzyme-catalysed reactions, the full time-course is sometimes followed (Bizzozero *et al.*, 1973; Schönheyder, 1951; Wolthers, 1969). In this instance use is often made of the pH-stat device, which records the amount of base or acid delivered to maintain constant pH in the course of an enzyme reaction that releases or consumes protons (Jacobsen *et al.*, 1957). The consideration of the classical expressions of the reaction rates, such as the Michaelis–Menten equation

$$-\frac{d[S]}{dt} = k_2[E]_{\text{total}} \frac{[S]}{K_m + [S]}$$

for the simplest reaction

may lead to erroneous values of the calculated kinetic parameters when the increase in volume of the medium as the reaction progresses is not negligible compared with the initial volume. I give in this communication new expressions which are appropriate in such a case.

Expression of the reaction rate: integration

When the reaction volume V is changing, the definition of the reaction rate v is simplified if the molar quantities (P) are substituted for the molar concentrations (Jungers, 1958):

$$[P] = (P)/V \quad (2)$$

i.e.

$$v = (1/V)d(P)/dt$$

If V_0 is the initial volume and c the concentration of the added basic (or acid) solution, we have:

$$V = V_0 + n(H)^+/c$$

or, taking into account the buffering capacity (assumed to be constant) of the medium:

$$V = V_0 + p[(P) - (P)_0]$$

where p is a constant factor and $(P)_0$ the amount of product P initially present.

For reaction (1), the steady-state approximation (Laidler & Bunting, 1973)

$$d(ES)/dt \ll k_{+1}(E)_{\text{total}}(S)$$

where $(E)_{\text{total}}$ is the total amount of enzyme E , allows us to write:

$$V = V_0 + p[(S)_0 - (S)] \quad (3)$$

and

$$-\frac{d(S)}{dt} = \frac{k_2(E)_{\text{total}}}{\frac{k_{-1} + k_2}{k_{+1}[S]} + 1} \quad (4)$$

If V_f is the final volume when all the initial amount $(S)_0$ of the substrate S has been transformed, we have from eqn. (3):

$$p = (V_f - V_0)/(S)_0 \quad (5)$$

According to eqn. (3), the reaction volume varies linearly with the conversion factor $f = 1 - (S)/(S)_0$. Further, eqn. (4) shows that the Michaelis–Menten equation cannot easily be expressed in terms of the concentration $[S]$ of the substrate alone.

Insertion of eqns. (2), (3) and (5) into eqn. (4) followed by integration with the boundary conditions $t = 0$, $(P)_0 = 0$, gives the (S) –time relationship:

$$k_2(E)_{\text{total}} t = \left(1 - K_m \frac{V_f - V_0}{(S)_0}\right) [(S)_0 - (S)] + K_m V_f \cdot \ln \frac{(S)_0}{(S)} \quad (6)$$

If the increase in volume is negligible compared with V_0 , then $V_f = V_0$, and eqn. (6) reduces, after division by V_0 , to the Victor Henri equation:

$$k_2[E]_{\text{total}} t = [S]_0 - [S] + K_m \ln \left(\frac{[S]_0}{[S]}\right) \quad (7)$$

If not, determination of the parameters k_2 and K_m from eqn. (7) with $[S] = (S)/V_0$ gives the apparent values (see Table 1):

$$k_2^* = k_2 \frac{1}{1 - \frac{K_m}{(S)_0} \left(\frac{V_f}{V_0} - 1\right)} \quad (8a)$$

Table 1. Calculated k_2^*/k_2 and K_m^*/K_m values for different V_f/V_0 ratios [eqns. (8a) and (8b)](1) $K_m/[S]_0 = 0.5$; (2) $K_m/[S]_0 = 0.1$.

V_f/V_0	k_2^*/k_2		K_m^*/K_m	
	(1)	(2)	(1)	(2)
1.01	1.00	1.00	1.02	1.01
1.02	1.01	1.00	1.03	1.02
1.1	1.05	1.01	1.16	1.11
1.5	1.33	1.05	2	1.58
2	2	1.11	4	2.22

and

$$K_m^* = K_m \frac{k_2^*}{k_2} \cdot \frac{V_f}{V_0} \quad (8b)$$

According to eqn. (6), when $(S)_0/(V_f - V_0) = K_m$, the kinetics become first-order with respect to the substrate during the entire reaction, with:

$$\frac{d(S)}{dt} = -\frac{k_2(E)_{\text{total}}}{(S)_0 + V_0 K_m} \cdot (S)$$

In this case eqns. (8a) and (8b) are no longer applicable.

This treatment of reaction (1) leads formally to the same eqn. (6) (with molar quantities instead of concentrations, however) as in the case of pure competitive product inhibition at constant volume (Laidler & Bunting, 1973; Sch6nheyder, 1951); in the present paper, $1/K_p = p$.

Generalized expression

In complex reactions involving several catalytic sites on the enzyme or various ligands S, I, A, ..., the kinetic models give rise to an equation of the form:

$$\frac{1}{(E)_{\text{total}}} \cdot \frac{d(P)}{dt} = \frac{N([P], [S], [I], [A], \dots)}{D([P], [S], [I], [A], \dots)}$$

where N and D are polynomials in $[P]$, $[S]$ etc.

Thus, in the case of substrate inhibition, the equation [see Laidler & Bunting (1973), eqn. (15), p. 179]

$$\frac{d(P)}{dt} = \frac{k_2(E)_{\text{total}} [S]}{K_s + (1+q)[S] + K[S]^2}$$

integrates as follows:

$$k_2(E)_{\text{total}} t = \left[1 + q - K_s \frac{V_f - V_0}{(S)_0} \right] (P) + K_s V_f \cdot \ln \left[\frac{(S)_0}{(S)_0 - (P)} \right] + K \frac{(S)_0}{V_f - V_0} \left\{ \frac{V_f}{V_f - V_0} (S)_0 \cdot \ln \left[1 + \frac{V_f - V_0}{V_0} \cdot \frac{(P)}{(S)_0} \right] - (P) \right\}$$

When $(P) = (S)_0$, the two first terms of the expansion of the last bracket in terms of $x = (V_f - V_0)/V_0$, $x < 1$, give:

$$K \frac{(S)_0^2}{2V_0} \left(1 - \frac{x}{3} \right)$$

Accordingly an approximation of the relative error made on K when neglecting $V_f - V_0$ is given by:

$$\frac{K^*}{K} - 1 = -\frac{1}{3} \left(\frac{V_f - V_0}{V_0} \right)$$

For mixed product inhibition, integration of the general equation [see Laidler & Bunting (1973), eqn. (89), p. 94]

$$\frac{d(P)}{dt} = \frac{k_2(E)_{\text{total}} [S]}{K_s + [S] + \frac{K_s}{K_p^i} [P] + \frac{1}{K_p^i} [P][S]}$$

gives:

$$k_2(E)_{\text{total}} t = \left[1 - K_s \frac{V_f - V_0}{(S)_0} \right] (P) + K_s V_f \cdot \ln \left[\frac{(S)_0}{(S)_0 - (P)} \right] + \frac{K_s}{K_p^i} \left\{ (S)_0 \cdot \ln \left[\frac{(S)_0}{(S)_0 - (P)} \right] - (P) \right\} + \frac{1}{K_p^i} \cdot \frac{(S)_0}{V_f - V_0} \left\{ (P) - \frac{V_0}{V_f - V_0} (S)_0 \cdot \ln \left[1 + \frac{V_f - V_0}{V_0} \cdot \frac{(P)}{(S)_0} \right] \right\}$$

An approximation of the relative error made on K_p^i when neglecting $V_f - V_0$ is given by:

$$\frac{K_p^{i*}}{K_p^i} - 1 = \frac{2}{3} \left(\frac{V_f - V_0}{V_0} \right)$$

Applications

To give an example, a pH-stat device provides a set of burettes delivering total volumes of 0.25, 2.5 or 25 ml of titrated solutions into an initial reaction medium of about 20 ml. Expressions such as eqns. (4) and (6) can be introduced into computer programs of iteration calculus similar to those of Bizzozero *et al.* (1973) or Atkins & Nimmo (1973). They permit kinetic parameters of a given reaction from several time-curves $V^i(t) = g[V_0^i, (E)_{\text{total}}^i, (S)_0^i, (P)_0^i, p^i, t]$, where in particular the concentrations of the added solutions and the volume of the employed burettes are varied, to be determined both for simulated and experimental data fittings. Table 1 shows that classical treatment may lead to erroneous conclusions when the increase in volume with the progress of the reaction can no longer be neglected when compared with the expected precision of the chosen method for determining the parameters.

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