Biochem. J. (1974) **143**, 93–95
Printed in Great Britain

A New Method for Determining the Michaelis Constant

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(*Received* 11 *March* 1974)

A new method is described for evaluating the parameters K_m and V in the Michaelis–Menten equation, and is illustrated with experimental data from the literature.

Many methods have been published for determining K_m and V in the Michaelis–Menten equation (e.g. Michaelis & Menten, 1913; Hanes, 1932; Lineweaver & Burk, 1934; Eadie, 1942; Walker & Schmidt, 1944; Johansen & Lumry, 1961; Wilkinson, 1961). Commonly the two parameters are determined jointly, though this is not necessarily advantageous; for partially purified or otherwise ill-characterized enzymes V is of little significance, whereas K_m ought to be reproducible and meaningful. Moreover, saturation functions similar to the Michaelis–Menten equation appear in some non-kinetic contexts, such as drug–receptor studies in pharmacology, where there is a useful quantity corresponding to K_m, but none corresponding to V. So there would be some value in a method that treated the determination of K_m and V as separate problems. Such a method is described in this paper.

Theory

The Michaelis–Menten equation may be formulated as follows:

$$v = \frac{Vs}{K_m + s} \qquad (1)$$

where v is the velocity at a substrate concentration s. A plot of v against s is a rectangular hyperbola through the origin with asymptotes $s = -K_m$ and $v = V$. The point of intersection of the asymptotes may be found by an interesting projection of Tamarit (unpublished work), in which straight lines are drawn through the points $(s, 0)$ and $(0, v)$ for each of several (s, v) pairs, as illustrated in Fig. 1. This plot has several useful characteristics: it displays the relationship between the curve and its asymptotes; it provides a rapid method for drawing a rectangular hyperbola with any chosen asymptotes; and it permits the determination of K_m and V from any two experimental points. This last point will be developed in the remainder of this paper.

In principle, lines may be drawn as shown in Fig. 1 for any number of experimental points, but in practice the lines do not all intersect at a unique point on account of experimental error. So a computational method is necessary for evaluating K_m from more than two experimental points. Eqn. (1) may be written as follows:

$$V = v + \frac{K_m v}{s} \qquad (2)$$

and differentiated with respect to s to give:

$$0 = v' - \frac{K_m(v - sv')}{s^2} \qquad (3)$$

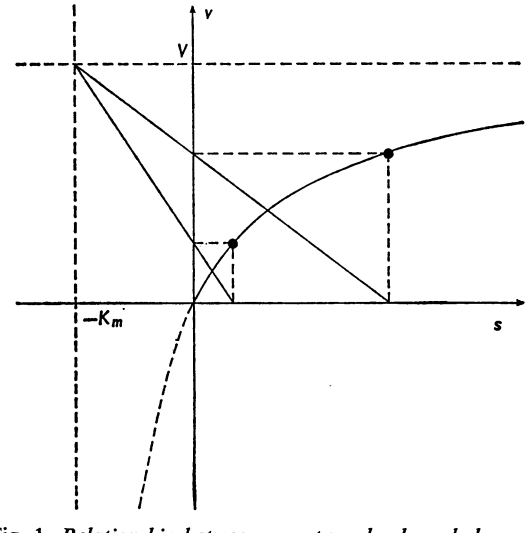

Fig. 1. *Relationship between a rectangular hyperbola and its asymptotes*

For a rectangular hyperbola through the origin, given by $v = Vs/(K_m+s)$, the asymptotes are $s = -K_m$ and $v = V$, and they intersect at the point $(-K_m, V)$ in the second quadrant. Any line through this point intersects the s and v axes at points $(s_i, 0)$ and $(0, v_i)$ that correspond to a point (s_i, v_i) on the hyperbola. This plot is based on unpublished work of Tamarit.

Table 1. *Values of R for data for the invertase-catalysed hydrolysis of sucrose*

The table shows values of s_t (M) and v_t (from time-courses of change of optical rotation) for the invertase-catalysed hydrolysis of sucrose, given by Kuhn (1923). Values of R_t were calculated from eqn. (5), and the means of the various columns are given. The standard deviations of the R_t are given in the bottom line.

s_t	0.1370	0.0995	0.0676	0.0262	0.0136	0.0100	0.0079
v_t	22.0	20.5	19.0	12.5	9.0	7.0	6.0
R_t		3.015	2.715	0.873	0.524	0.360	0.296
	4.151		3.382	0.888	0.539	0.366	0.302
	5.502	4.977		0.790	0.518	0.349	0.291
	4.564	3.371	2.039		0.718	0.405	0.343
	5.282	3.943	2.574	1.382		0.191	0.257
	4.927	3.641	2.360	1.062	0.260		0.470
	5.128	3.798	2.488	1.138	0.443	0.595	
$\bar{R}_t$	4.926	3.791	2.593	1.022	0.500	0.378	0.327
S.D.	0.50	0.67	0.45	0.22	0.15	0.13	0.08

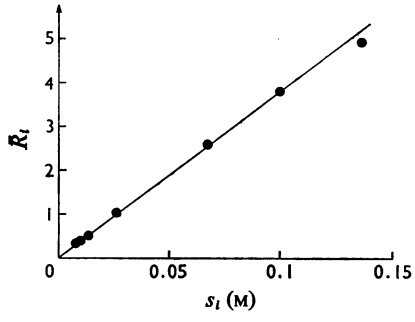

Fig. 2. *Determination of K_m for the invertase-catalysed hydrolysis of sucrose*

Values of $\bar{R}_t$ from Table 1 are plotted against s_t. The line passes through the origin and has a slope of $1/K_m$.

where $v' = dv/ds$. Rearranging, we have:

$$R = s/K_m \qquad (4)$$

where $R = v/sv' - 1$. R is a dimensionless number that goes from zero to infinity as s goes from zero to infinity. Moreover, a plot of R against s is a straight line through the origin, with slope $1/K_m$, and so it provides a convenient way of determining K_m, provided that R can be evaluated for each value of s.

For any substrate concentration s_t, with a corresponding velocity v_t, any other experimental point (s_j, v_j), where $s_j \neq s_t$, provides a value R_t given by:

$$R_t = \frac{v_j(s_t - s_j)}{s_j(v_t - v_j)} - 1 \qquad (5)$$

For a total of n experimental points, at n different substrate concentrations, $(n-1)$ such values of R_t may be calculated for each value of i, and the mean $\bar{R}_t$ of these may be used for plotting against s_t. In some cases, obviously erratic R_t values (e.g. negative values, which are physically impossible) may need to be eliminated before calculating the means. As mentioned above, the slope of the plot is $1/K_m$, and may be used to evaluate K_m, and the precision of the result may be judged from the scatter of points about the line. For computation, we may use the mean slope, and calculate K_m from:

$$K_m = n/\Sigma(R_t/s_t) \qquad (6)$$

If a value of V is required, it may be found by substituting the value of K_m into eqn. (2) for any experimental point. More accurately, this may be done for all n observations, and V taken as the mean.

An Example

To illustrate the method we may evaluate K_m from some values of s and v given by Kuhn (1923) for the hydrolysis of sucrose catalysed by invertase. The data are given in Table 1, together with the R values calculated from them. We may note that the values of R_t in each column are not all equal, as they would be in an error-free experiment. Moreover, the scatter in each column, as estimated by the standard deviations, is not constant, but decreases fairly steadily with s_t. In other words the $\bar{R}_t$ values are not of uniform variance (homoscedastic); but $\bar{R}_t/s_t$ does appear to be reasonably homoscedastic, and so the formula given in eqn. (6) is correct. It gives a value of $K_m = 0.0262$M for these data. The values of $\bar{R}_t$ are plotted against s_t in Fig. 2, and give a good straight line through the origin. This indicates a high degree of precision in the results.

I am especially grateful to Dr. Bliss and Dr. Tamarit for their help and advice. I am also most grateful to the Referee, for his very considerable help in improving the presentation of the ideas expressed in this paper.

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