

## BBA Report

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### ESTIMATION OF MICHAELIS CONSTANT AND MAXIMUM VELOCITY FROM THE DIRECT LINEAR PLOT

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#### Summary

When estimates of Michaelis-Menten parameters are obtained from kinetic observations taken in pairs, as in the direct linear plot, bias can arise in the final estimates if any pairs lead to negative values of the maximum velocity  $V$ . This bias can be removed by treating such negative values as if they were large and positive, and by treating the corresponding values of  $K_m$  in the same way.

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The direct linear plot [1] provides a method of estimating the parameters of the Michaelis-Menten equation that not only avoids all calculation but is also only weakly dependent on implied statistical assumptions [2,3]. It has been found useful in widely different branches of biochemistry [4–8], and is capable of being applied to models more complex than simple saturation phenomena [9]. However, it has become clear that appreciable improvement on the original method is possible without loss of its essential simplicity. In practice intersections sometimes occur in the third quadrant of the direct linear plot, i.e. they give negative estimates of both  $K_m$  and  $V$ , and these can lead to bias in the final estimates if taken at face value. This bias can be removed by treating  $1/V$  and  $K_m/V$  as the primary parameters of the Michaelis-Menten equation, as we shall show in this paper.

#### Theory

Consider two observations  $(s_i, v_i)$  and  $(s_j, v_j)$  such that  $s_i, s_j, v_i$  and  $v_j$  are all positive and  $s_j$  is greater than  $s_i$ , and assume that apart from experi-

mental error each observation obeys the Michaelis-Menten equation,  $v = Vs/(K_m + s)$ , with true parameter values  $K_m$  and  $V$  that are both positive. There is a single pair of estimates of  $K_m$  and  $V$  that satisfies both observations exactly [1], given by

$$V_{(ij)} = (s_j - s_i)/((s_j/v_j) - (s_i/v_i)) \text{ and}$$

$$K_{m(ij)} = s_i((V_{(ij)}/v_i) - 1).$$

With error-free data both expressions would necessarily be both finite and positive, but in practice they need not be either.

The dependence of  $V_{(ij)}$  on  $v_j$ , for constant  $s_i$ ,  $s_j$  and  $v_i$ , is shown in Fig. 1. Initially  $V_{(ij)} = 0$  for  $v_j = 0$ , and it increases smoothly with  $v_j$  until  $v_j = s_j v_i / s_i$ , whereupon it 'flips' from large positive to large negative values and then approaches  $-v_i(1 - s_i/s_j)$  smoothly as  $v_j$  increases further. Provided that our original assumption is correct, i.e. that the Michaelis-Menten equation is the correct model, and provided that  $s_i$  and  $s_j$  are sufficiently different that a meaningful intersection point can reasonably be expected,  $v_j$  is likely to approach or exceed  $s_j v_i / s_i$  only if  $s_i$  and  $s_j$  are both much smaller than  $V$ . In this case the true values of  $s_i/v_i$  and  $s_j/v_j$  are almost equal and the observed values may be in fact equal or incorrectly ranked ( $s_i/v_i$  greater than  $s_j/v_j$ ). For example, if  $s_i$  and  $s_j$  are  $0.05 \times K_m$  and  $0.1 \times K_m$  respectively,  $V_{(ij)}$  will be negative if  $v_i$  is 2.5% too small and  $v_j$  is 2.5% too big. It follows that, although  $K_{m(ij)}$  and  $V_{(ij)}$  may be poor estimates of  $K_m$  and  $V$ , they are not

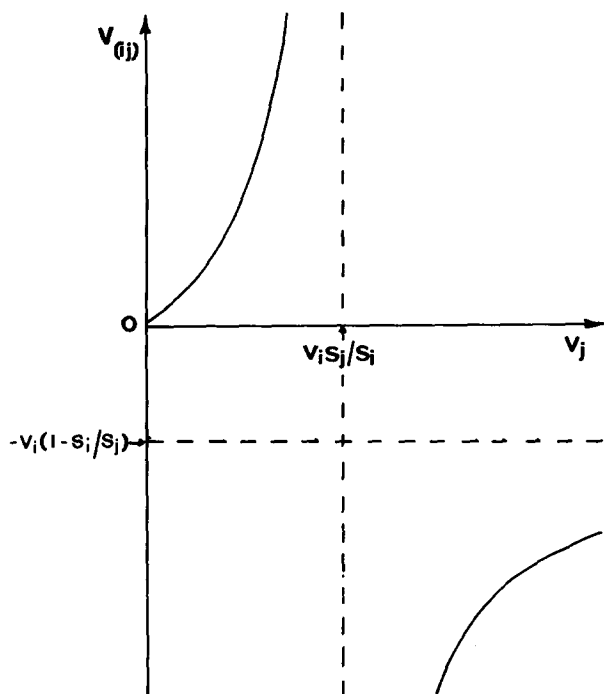


Fig. 1. Dependence of  $V_{(ij)}$  on  $v_j$ . The estimate  $V_{(ij)}$  is defined as  $V_{(ij)} = (s_j - s_i)/((s_j/v_j) - (s_i/v_i))$ , and is plotted against  $v_j$  for arbitrary constant values of  $s_i$ ,  $s_j$  and  $v_i$ .

empty of information as they indicate that  $K_m$  is much larger than  $s_j$  and that  $V$  is much larger than  $v_i$  and  $v_j$ . So in finding the median estimate of  $V$  one should treat negative  $V_{(ij)}$  values as very large positive estimates of  $V$ . The interpretation of negative  $K_{m(ij)}$  values is a little more complex.

If  $V_{(ij)}$  is negative,  $K_{m(ij)}$  must also be negative. So, for the reason we have just considered, a negative  $K_{m(ij)}$  value should be treated as a very large positive estimate of  $K_m$  if the corresponding  $V_{(ij)}$  value is also negative. However,  $K_{m(ij)}$  can also be negative in quite different circumstances when the corresponding  $V_{(ij)}$  is positive, and in this case  $K_{m(ij)}$  should be treated as a small or negative value of  $K_m$ . This is because negative  $K_{m(ij)}$  values can occur if  $s_i$  and  $s_j$  are both large compared with the true value of  $K_m$ ; then the true values of  $v_i$  and  $v_j$  are almost equal and the observed values may be in fact equal or incorrectly ranked ( $v_i$  greater than  $v_j$ ). So a negative  $K_{m(ij)}$  value with a corresponding positive  $V_{(ij)}$  value indicates that the true value of  $K_m$  is much smaller than  $s_i$  and that the true value of  $V$  is of the same order of magnitude as  $V_{(ij)}$ .

These complexities may be avoided entirely by replacing  $K_m$  and  $V$  as primary parameters of the Michaelis-Menten equation with  $1/V$  and  $K_m/V$ , because  $1/V_{(ij)}$  and  $K_{m(ij)}/V_{(ij)}$  show no discontinuities when plotted against positive  $v_i$  or  $v_j$  but are instead smooth monotonic functions over the whole physically meaningful range. Accordingly, if the direct linear plot is drawn as a plot of  $1/V$  against  $K_m/V$  the interpretation is simple and no special rules are required. In such a plot, which is illustrated in Fig. 2, each observation is represented as a straight line with intercepts  $1/v$  on the  $1/V$  axis and  $s/v$  on the  $K_m/V$  axis.

One can arrive at essentially the same conclusion in a different way by considering the effects of errors in  $v_i$  and  $v_j$  on the values of  $1/V_{(ij)}$  and  $K_{m(ij)}/V_{(ij)}$ . To simplify the discussion we shall use the term "median-unbiased" [11] to describe any quantity for which the experimental error is as likely to be positive as to be negative. If  $v_i$  and  $v_j$  are both median-unbiased, with errors that are not so large as to make either of the observed values negative, and if  $s_i$  and  $s_j$  are error-free, then both  $s_i/v_i$  and  $s_j/v_j$  are median-unbiased. So also is their difference and hence also  $1/V_{(ij)}$ , which is simply  $((s_j/v_j) - (s_i/v_i))$  divided by a known quantity,  $(s_j - s_i)$ . Thus, by adding the very weak assumption that the absolute errors in  $v_i$  and  $v_j$  do not exceed 100% to the ordinary non-parametric assumption of median-unbiased observations, one can show that each  $1/V_{(ij)}$  value is median-unbiased. Consequently the median of all the  $1/V_{(ij)}$  must be a median-unbiased estimator of  $1/V$ . One can apply a similar argument to show that the median of all the  $K_{m(ij)}/V_{(ij)}$  is median-unbiased. One cannot, however, apply similar arguments to  $V_{(ij)}$ ,  $K_{m(ij)}$ ,  $V_{(ij)}/K_{m(ij)}$  or  $1/K_{m(ij)}$ , because  $v_i$  and  $v_j$  appear in the denominators of the expressions for all of these parameters; and, in fact, none of them is median-unbiased.

Dr. I.A. Nimmo (personal communication) and Porter and Trager [10] have pointed out an omission from our original description of the direct linear plot [1,2], in that we offered no guidance as to the treatment of replicate observations, with two or more values of  $v$  at one value of  $s$ . The values of  $1/V_{(ij)}$  and  $K_{m(ij)}/V_{(ij)}$  obtained from duplicate observations are infinite (or

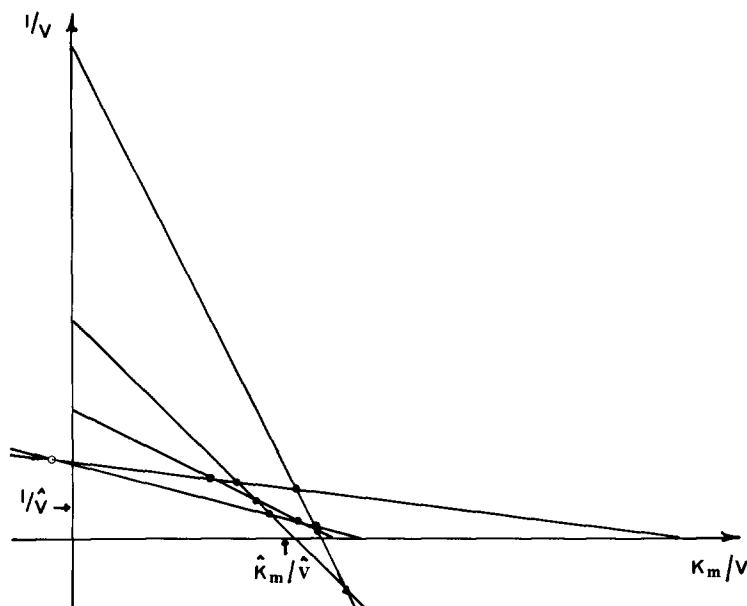


Fig. 2. Direct linear plot of  $1/V$  against  $K_m/V$ . Each observation is plotted as a straight line with intercept  $1/v$  on the  $1/V$  axis and  $s/v$  on the  $K_m/V$  axis. Each intersection point provides an estimate of  $K_m/V$  and an estimate of  $1/V$ . The best-fit values  $\hat{K}_m/V$  and  $1/\hat{V}$  are taken as the medians of the two series respectively, and are indicated on the axes. Intersection points that do not occur in the first quadrant ( $\circ$   $\nabla$ ) require no special treatment.

indefinite if the  $v$  values happen to be equal). They therefore have no sign and provide no information about the magnitudes of  $1/V$  and  $K_m/V$ . This is entirely reasonable, as one cannot estimate the parameters of a two-parameter equation from observations at a single value of the independent variable. However, each  $K_m(ij)$  obtained from duplicate observations has the value  $-s_i$ , and each corresponding  $V(ij)$  has the value zero. Thus although they purport to provide information about  $K_m$  and  $V$  the information is spurious and leads to bias if accepted at face value. In practice we have always omitted such estimates from the determination of the medians, and this is also true of the computer program that we have circulated [2].

The main conclusion to be drawn from this analysis is that better estimates of the Michaelis-Menten parameters can be expected if  $1/V$  and  $K_m/V$  are estimated first, and other parameters, such as  $V$  and  $K_m$ , are calculated from them. The most direct way of achieving this is by the method shown in Fig. 2, in which each observation is drawn as a straight line with intercepts  $1/v$  on the  $1/V$  axis and  $s/v$  on the  $K_m/V$  axis. The median estimates of  $1/V$  and  $K_m/V$  can then be found as described previously for  $K_m$  and  $V$  [1]. This plot has the added advantage over the original direct linear plot that most of the intersection points appear without extrapolation of the lines representing the observations.

The plot of  $1/V$  against  $K_m/V$  requires a small amount of calculation, but this can be avoided by using the original plot of  $V$  against  $K_m$  [1], with a slight modification to the interpretation of intersection points in the third

quadrant: these should be regarded as giving very large positive estimates of both  $V$  and  $K_m$ , not negative estimates. In effect one must regard the coordinates of points in the third quadrant as 'beyond infinity' rather than as 'below zero'. Intersections in the second quadrant should be taken at face value. The procedure is easier to apply than to describe in the abstract, and is illustrated in Fig. 3.

The proposal that negative numbers should in some contexts be treated as 'beyond infinity' rather than as 'below zero' may seem novel and objectionable. However, there is a well-established precedent in thermodynamics: when negative absolute temperatures occur in nuclear spin systems they are not cold but very hot, in the sense that they lose heat to systems at positive absolute temperatures [12,13]. For example, Purcell and Pound [12] observed such a system cooling from  $-350$  K to  $+300$  K, via infinite temperature, not via absolute zero.

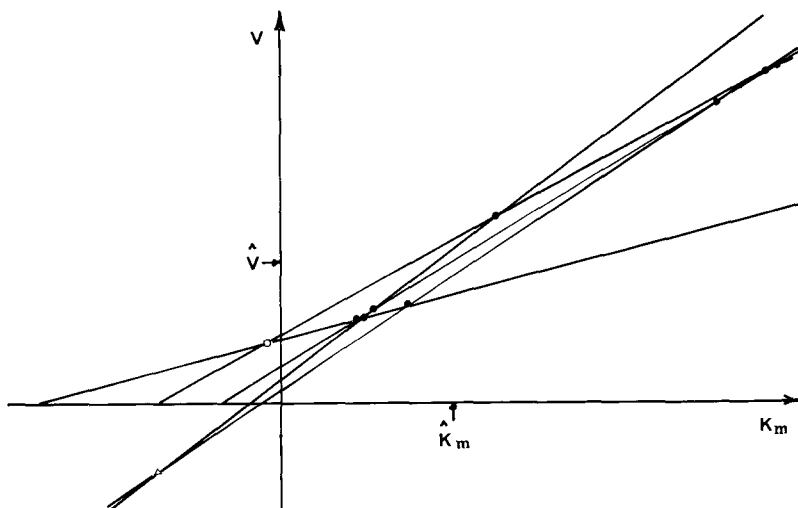


Fig. 3. Direct linear plot of  $V$  against  $K_m$ . The same observations as in Fig. 2 are shown but are plotted according to the procedure of Eisenthal and Cornish-Bowden [1]. In finding the median estimates the intersection point in the second quadrant (○) is taken at face value, but the intersection point in the third quadrant (⊗) is treated as giving very large positive estimates of both  $K_m$  and  $V$ . The medians found with this convention are indicated on the axes as  $\hat{K}_m$  and  $\hat{V}$ .

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